Crude Drugs and Related Drugs

Acacia

Gummi Arabicum

アラビアゴム

Acacia is the secretions obtained from the stems and branches of *Acacia senegal* Willdenow or other species of the same genus (*Leguminosae*).

Description Colorless or light yellow-brown, translucent or somewhat opaque spheroidal tears, or angular fragments with numerous fissures on the surface; very brittle; the fractured surface glassy and occasionally iridescent.

Odorless; tasteless, but produces a mucilaginous sensation on the tongue.

Pulverized Acacia (1.0 g) dissolves almost completely in 2.0 mL of water, and the solution is acid.

It is practically insoluble in ethanol (95).

Identification To 1 g of pulverized Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg each of D-galactose, L-arabinose and L-rhamnose monohydrate in 1 mL water separately, add methanol to make 10 mL, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100) and water (12:3:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: the three spots obtained from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose obtained from the standard solution in the color tone and the *R*f value, respectively.

Purity **(1)** Insoluble residue—To 5.0 g of pulverized Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Use the sample solution obtained in the Identification as the sample solution. Separately, dissolve 10 mg of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: any spot at the *R*f value corresponding to glucose from the standard solution does not appear from the sample solution.

Loss on drying <*5.01*> Not more than 17.0% (6 hours).

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Powdered Acacia

Gummi Arabicum Pulveratum

アラビアゴム末

Powdered Acacia is the powder of Acacia.

Description Powdered Acacia occurs as a white to light yellowish white powder. It is odorless, tasteless, but produces a mucilaginous sensation on the tongue.

Under a microscope <*5.01*>, Powdered Acacia, immersed in olive oil or liquid paraffin, reveals colorless, angular fragments or nearly globular grains. Usually starch grains or vegetable tissues are not observed or very trace, if any.

Powdered Acacia (1.0 g) dissolves almost completely in 2.0 mL of water, and the solution is acid.

It is practically insoluble in ethanol (95).

Identification To 1 g of Powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg each of D-galactose, L-arabinose and L-rhamnose monohydrate in 1 mL water, add methanol to make 10 mL, and use these solutions as the standard solutions, (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100) and water (12:3:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: the three spots obtained from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose obtained from the standard solution in the color tone and the *R*f value, respectively.

Purity **(1)** Insoluble residue—To 5.0 g of Powdered Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Powdered Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Use the sample solution obtained in the Identification as the sample solution. Separately, dissolve 10 mg of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: any spot at the *R*f value corresponding to glucose from the standard solution does not appear from the sample solution.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Achyranthes Root

Achyranthis Radix

ゴシツ

Achyranthes Root is the root of *Achyranthes fauriei* Leveillé et Vaniot or *Achyranthes bidentata* Blume (*Amaranthaceae*).

Description Main root or main root with some lateral roots, with or without short remains of rhizome at the crown; main root, long cylindrical and sometimes somewhat tortuous, 15 – 90 cm in length, 0.3 – 0.7 cm in diameter; externally grayish yellow to yellow-brown, with numerous longitudinal wrinkles, and with scattering scars of lateral roots. Fractured surface is flat; grayish white to light brown on the circumference, and with yellowish white xylem in the center. Hard and brittle, or flexible.

Odor, slight; taste, slightly sweet, and mucilaginous.

Under a microscope <*5.01*>, a transverse section reveals a rather distinct cambium separating the cortex from the xylem; small protoxylem located at the center of the xylem, and surrounded by numerous vascular bundles arranged on several concentric circles; parenchyma cells containing sand crystals of calcium oxalate; starch grains absent.

Identification Shake vigorously 0.5 g of pulverized Achyranthes Root with 10 mL of water: a lasting fine foam is produced.

Purity **(1)** Stem—When perform the test of foreign matter <*5.01*>, the amount of stems contained in Achyranthes Root does not exceed 5.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Achyranthes Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Achyranthes Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than stems contained in Achyranthes Root does not exceed 1.0%.

Loss on drying <*5.01*> Not more than 17.0% (6 hours).

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Agar

Agar

カンテン

Agar is the solid residue obtained by freezing dehydration of a mucilage derived from *Gelidium elegans* Kuetzing, other species of the same genus (*Gelidiaceae*), or other red algae (*Rhodophyta*).

Description White, translucent rectangular column, string or flakes. Rectangular column about 26 cm in length, 4 cm square in cross section; a string of about 35 cm in length and about 3 mm in width; flakes about 3 mm in length; externally, with wrinkles and somewhat lustrous, light and pliable.

Odorless; tasteless and mucilagenous.

It is practically insoluble in organic solvents.

A boiling solution of Agar (1 in 100) is neutral.

Identification **(1)** To a fragment of Agar add dropwise iodine TS: a dark blue to reddish purple color develops.

(2) Dissolve 1 g of Agar in 65 mL of water by boiling for 10 minutes with constant stirring, and add a sufficient amount of hot water to make up the water lost by evaporation: the solution is clear. Cool the solution between 30°C and 39°C: the solution forms a firm, resilient gel, which does not melt below 85°C.

Purity **(1)** Sulfuric acid—Dissolve 1.0 g of Agar in 100 mL of water by boiling: the solution is not acidic.

(2) Sulfurous acid and starch—To 5 mL of the solution obtained in (1) add 2 drops of iodine TS: the solution is not decolorized immediately, and does not show a blue color.

(3) Insoluble matter—To 7.5 g of Agar add 500 mL of water, boil for 15 minutes, and add water to make exactly 500 mL. Measure exactly 100 mL of the solution, add 100 mL of hot water, heat to boiling, filter while hot through a tared glass filter (G3), wash the residue with a small amount of hot water, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 15.0 mg.

(4) Water absorption—To 5.0 g of Agar add water to make 100 mL, shake well, allow to stand at 25°C for 24 hours, and filter through moistened glass wool in a 100-mL graduated cylinder: the volume of the filtrate is not more than 75 mL.

Loss on drying <*5.01*> Not more than 22.0% (6 hours).

Total ash <*5.01*> Not more than 4.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Powdered Agar

Agar Pulveratum

カンテン末

Powdered Agar is the powder of Agar.

Description Powdered Agar appears as a white powder, is odorless, and is tasteless and mucilagenous.

Under a microscope <*5.01*>, Powdered Agar, immersed in olive oil or liquid paraffin, reveals angular granules with striations or nearly spheroidal granules 5 to 60 μm in diameter.

It becomes transparent in chloral hydrate TS.

It is practically insoluble in organic solvents.

A boiling solution of Powdered Agar (1 in 100) is neutral.

Identification **(1)** To a part of Powdered Agar add dropwise iodine TS: a dark blue to reddish purple color develops.

(2) Dissolve 1 g of Powdered Agar in 65 mL of water by boiling for 10 minutes with constant stirring, and add a sufficient amount of hot water to maintain the original volume lost by evaporation: the solution is clear. Cool the solution between 30°C and 39°C: the solution forms a firm, resilient gel, which does not melt below 85°C.

Purity **(1)** Sulfuric acid—Dissolve 1.0 g of Powdered Agar in 100 mL of water by boiling: the solution is not acid.

(2) Sulfurous acid and starch—To 5 mL of the solution obtained in (1) add 2 drops of iodine TS: the solution is not decolorized immediately, and does not show a blue color.

(3) Insoluble matter—To 7.5 g of Powdered Agar add 500 mL of water, boil for 15 minutes, and add water to make exactly 500 mL. Take exactly 100 mL of the solution, add 100 mL of hot water, heat to boiling, filter while hot through a tared glass filter (G3), wash the residue with a small amount of hot water, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 15.0 mg.

(4) Water absorption—To 5.0 g of Powdered Agar add water to make 100 mL, shake well, allow to stand at 25°C for 24 hours, and filter through moistened glass wool in a 100-mL graduated cylinder: the volume of the filtrate is not more than 75 mL.

Loss on drying <*5.01*> Not more than 22.0% (6 hours).

Total ash <*5.01*> Not more than 4.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Akebia Stem

Akebiae Caulis

モクツウ

Akebia Stem is the climbing stem of *Akebia quinata* Decaisne or *Akebia trifoliata* Koidzumi (*Lardizabalaceae*), usually cut transversely.

Description Circular or ellipsoidal sections 0.2 – 0.3 cm in thickness, and 1 – 3 cm in diameter; phloem on both fractured surfaces is dark grayish brown; xylem reveals light brown vessel portions and grayish white medullary rays lined alternately and radially; pith light grayish yellow, and distinct; flank grayish brown, and with circular or transversely elongated elliptical lenticels.

Almost odorless; slightly acrid taste.

Under a microscope <*5.01*>, a transverse section reveals ring layers mainly consisting of fiber bundles with crystal cells and stone cell groups and surrounding the outside of the phloem in arc shape. Medullary rays of the phloem consisting of sclerenchyma cells containing solitary crystals; portion near cambium is distinct; cells around the pith remarkably thick-walled; xylem medullary rays and parenchyma cells around the pith contain solitary crystals of calcium oxalate and starch grains less than 8 μm in diameter.

Identification To 0.5 g of pulverized Akebia Stem add 10 mL of water, boil, allow to cool, and shake vigorously: lasting fine foams are produced.

Total ash <*5.01*> Not more than 10.0%.

Containers and storage Containers—Well-closed containers.

Alisma Tuber

Alismatis Tuber

タクシャ

Alisma Tuber is the tuber of *Alisma orientale* Juzepczuk (*Alismataceae*), from which periderm has been usually removed.

Description Spherical or conical tubers, 3 – 8 cm in length, 3 – 5 cm in diameter, sometimes a 2- to 4-branched irregular tuber; externally light grayish brown to light yellow-brown, and slightly annulate; many remains of root appearing as small warty protrusions; fractured surface nearly dense, the outer portion grayish brown, and the inner part white to light yellow-brown in color; rather light in texture and difficult to break.

Slight odor and slightly bitter taste.

Identification To 1.0 g of pulverized Alisma Tuber add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Use alisma tuber triterpenes TS for identification as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 1 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with a spot among the three spots obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Alisma Tuber according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Alisma Tuber according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed contain-

ers.

Powdered Alisma Tuber

Alismatis Tuber Pulveratum

タクシャ末

Powdered Alisma Tuber is the powder of Alisma Rhizome.

Description Powdered Alisma Tuber occurs as a light grayish brown powder, and has a slight odor and a slightly bitter taste.

Under a microscope <*5.01*>, Powdered Alisma Tuber reveals mainly starch grains, fragments of parenchyma containing them, parenchyma cells containing yellow contents, and fragments of vascular bundles. Starch grains, spheroidal to ellipsoidal simple grains, 3 – 15 μm in diameter.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Alisma Tuber according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Alisma Tuber according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Aloe

Aloe

アロエ

Aloe is the dried juice of the leaves mainly of *Aloe ferox* Miller, or of hybrids of the species with *Aloe africana* Miller or *Aloe spicata* Baker (*Liliaceae*).

It contains not less than 4.0% of barbaloin, calculated on the basis of dried material.

Description Aloe occurs as blackish brown to dark brown, irregular masses; sometimes the external surface covered with a yellow powder; the fractured surface smooth and glassy.

Odor, characteristic; taste, extremely bitter.

Identification **(1)** Dissolve 0.5 g of pulverized Aloe in 50 mL of water by warming. After cooling, add 0.5 g of siliceous earth, and filter. Perform the following tests using the filtrate as the sample solution.

(i) Dissolve 0.2 g of sodium tetraborate decahydrate in 5 mL of the sample solution by warming in a water bath. Add a few drops of this solution into 30 mL of water, and shake: a green fluorescence is produced.

(ii) Shake 2 mL of the sample solution with 2 mL of nitric acid: a yellow-brown color which changes gradually to green is produced. Then warm this colored solution in a water bath: the color of the solution changes to red-brown.

(2) To 0.2 g of pulverized Aloe add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (20:5:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Resin—Warm 0.5 g of pulverized Aloe with 10 mL of diethyl ether on a water bath, and filter. Wash the residue and the filter paper with 3 mL of diethyl ether. Combine the filtrate and the washing, and evaporate the diethyl ether solution: the mass of the residue is not more than 5.0 mg.

(2) Ethanol-insoluble substances—Boil 1.0 g of pulverized Aloe with 50 mL of ethanol (95) on a water bath for 30 minutes under a reflux condenser. Filter the warm mixture through a tared glass filter (G4), and wash the residue on the filter with ethanol (95) until the last washing becomes colorless. Dry the residue at 105°C for 5 hours, and weigh: the mass of the residue is not more than 0.10 g.

Loss on drying <*5.01*> Not more than 12.0%.

Total ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Water-soluble extract: not less than 40.0%.

Assay Weigh accurately about 0.1 g of pulverized Aloe, add 40 mL of methanol, and heat under a reflex condenser on a water bath for 30 minutes. After cooling, filter, and add methanol to the filtrate to make exactly 50 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of barbaloin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of barbaloin in each solution.

$$\text{Amount (mg) of barbaloin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of barbaloin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (74:26:1).

Flow rate: Adjust so that the retention time of barbaloin is about 12 minutes.

System suitability—

System performance: Dissolve 10 mg of barbaloin for assay add 40 mg of oxalic acid dihydrate, in methanol to

make 100 mL. To 5 mL of the solution add 1 mL of a solution of ethenzamide in methanol (1 in 2000) and methanol to make 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions except the wavelength of 300 nm, barbaloin and ethenzamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of barbaloin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Aloe

Aloe Pulverata

アロエ末

Powdered Aloe is the powder of Aloe.

It contains not less than 4.0% of barbaloin, calculated on the basis of dried material.

Description Powdered Aloe occurs as a dark brown to yellowish dark brown powder. It has a characteristic odor and an extremely bitter taste.

Under a microscope <*5.01*>, Powdered Aloe, immersed in olive oil or liquid paraffin, reveals greenish yellow to reddish brown, angular or rather irregular fragments.

Identification (1) Dissolve 0.5 g of Powdered Aloe in 50 mL of water by warming. After cooling, add 0.5 g of siliceous earth, and filter. Perform the following tests with the filtrate as the sample solution.

(i) Dissolve 0.2 g of sodium tetraborate decahydrate in 5 mL of the sample solution by warming in a water bath. Add a few drops of this solution into 30 mL of water, and shake: a green fluorescence is produced.

(ii) Shake 2 mL of the sample solution with 2 mL of nitric acid: a yellow-brown color which changes gradually to green is produced. Then warm this colored solution in a water bath: the color of the solution changes to red-brown.

(2) To 0.2 g of Powdered Aloe add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (20:5:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution has the same color tone and the same *R*f value with the red fluorescent spot from the standard solution.

Purity (1) Resin—Warm 0.5 of Powdered Aloe with 10 mL of diethyl ether on a water bath, and filter. Wash the residue and the filter paper with 3 mL of diethyl ether. Combine the filtrate and the washing, and evaporate the diethyl ether: the mass of the residue does not exceed 5.0 mg.

(2) Ethanol-insoluble substances—Boil 1.0 g of Powdered Aloe with 50 mL of ethanol (95) on a water bath for 30 minutes under a reflux condenser. Filter the warm mixture through a tared glass filter (G4), and wash the residue on the filter with ethanol (95) until the last washing becomes colorless. Dry the residue at 105°C for 5 hours, and weigh: the mass of the residue is not more than 0.10 g.

Loss on drying <*5.01*> Not more than 12.0%.

Total ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Water-soluble extract: not less than 40.0%.

Assay Weigh accurately about 0.1 g of Powdered Aloe, add 40 mL of methanol, and heat under a reflex condenser on a water bath for 30 minutes. After cooling, filter, and add methanol to the filtrate to make exactly 50 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of barbaloin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of barbaloin in each solution.

$$\text{Amount (mg) of barbaloin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of barbaloin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (74:26:1).

Flow rate: Adjust so that the retention time of barbaloin is about 12 minutes.

System suitability—

System performance: To about 10 mg of barbaloin for assay add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make 100 mL. To 5 mL of the solution add 1 mL of a solution of ethenzamide in methanol (1 in 2000) and methanol to make 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions except the wavelength of 300 nm, barbaloin and ethenzamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of barbaloin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Alpinia Officinarum Rhizome

Alpiniae Officinari Rhizoma

リョウキョウ

Alpinia Officinarum Rhizome is the rhizome of *Alpinia officinarum* Hance (*Zingiberaceae*).

Description Alpinia Officinarum Rhizome is a slightly curved and cylindrical rhizome, sometimes branched; 2 – 8 cm in length, 0.6 – 1.5 cm in diameter; externally red-brown to dark brown with fine striped lines, grayish white nodes and several traces of rootlet; hard to break; fracture surface, light brown in color and thickness of cortex is approximately the same as that of stele.

Odor, characteristic; taste, extremely pungent.

Under a microscope <*5.01*>, a transverse section reveals epidermal cells often containing oil-like substances; cortex, endodermis and stele present beneath the epidermis; cortex and stele divided by endodermis; vascular bundles surrounded by fibers, scattered throughout the cortex and stele, cortex and stele composed of parenchyma interspersed with oil cells; parenchyma cells containing solitary crystals of calcium oxalate and starch grains, starch grains generally simple (sometimes 2- to 8-compound), narrowly ovate, ellipsoidal or ovate, 10 – 40 μm in diameter and with an eccentric navel.

Identification To 0.5 g of pulverized Alpinia Officinarum Rhizome add 5 mL of acetone, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of cyclohexane, ethyl acetate and acetic acid (100) (12:8:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots appear at an *R*f value of about 0.4.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Alpinia Officinarum Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Alpinia Officinarum Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 7.5%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-extract: not less than 14.0%.

Containers and storage Containers—Well-closed containers.

Aluminum Silicate Hydrate with Silicon Dioxide

Kasseki

カッセキ

Aluminum Silicate Hydrate with Silicon Dioxide is a mineral substance, mainly composed of aluminum silicate hydrate and silicon dioxide.

It is not the same substance with the mineralogical talc.

Description Aluminum Silicate Hydrate with Silicon Dioxide occurs as white to light red powdered crystalline masses, which becomes easily fine powder on crushing. The powder is roughish and easily adheres to skin, and becomes slightly darken and obtains plasticity when moisten with water.

It has a characteristic odor and almost tasteless. It feels like as sand of fine grains by chewing.

Under a microscope <*5.01*>, the powder of Aluminum Silicate Hydrate with Silicon Dioxide, thoroughly grained between a slide glass and a cover glass together with mounting medium, shows numbers of round to polygonal crystals not smaller than 10 μm in diameter.

Identification To 0.5 g of powdered Aluminum Silicate Hydrate with Silicon Dioxide add 3 mL of diluted sulfuric acid (1 in 3), heat until white vapors evolve, then after cooling add 20 mL of water, and filter. The filtrate neutralized to be a weak acidity with ammonia TS responds to the Qualitative Tests <*1.09*> (1), (2) and (4) for aluminum salt.

Purity **(1)** Heavy metals <*1.07*>—To 1.5 g of Aluminum Silicate Hydrate with Silicon Dioxide add 50 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes while thorough shaking. After cooling, centrifuge, and separate the supernatant liquid. Wash the precipitate twice with 10 mL portions of water, centrifuging each time, and combine the supernatant liquids. Add ammonia solution (28) dropwise to the combined liquid until a slight precipitate form, then add, while shaking vigorously, dilute hydrochloric acid dropwise to dissolve the precipitate. Add 0.45 g of hydroxylammonium chloride to this solution, heat, then after cooling add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, and add water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution by adding to 2.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 40 ppm).

(2) Arsenic <*1.11*>—To 1.0 g of Aluminum Silicate Hydrate with Silicon Dioxide add 5 mL of dilute hydrochloric acid, heat gently until boiling begins while shaking thoroughly, then cool quickly, and centrifuge. To the precipitate add 5 mL of dilute hydrochloric acid, shake thoroughly, and centrifuge. Repeat this operation with 10 mL of water, combine all extracts, and concentrate the extract to make 5 mL by heating on a water bath. Perform the test using this solution as the test solution (not more than 2 ppm).

Containers and storage Containers—Well-closed containers.

Amomum Seed

Amomi Semen

シュクシャ

Amomum Seed is the seed mass of *Amomum xanthioides* Wallich (*Zingiberaceae*).

Description Approximately spherical or ellipsoidal mass, 1 – 1.5 cm in length, 0.8 – 1 cm in diameter; externally grayish brown to dark brown, and with white powder in those dried by spreading lime over the seeds; the seed mass is divided into three loculi by thin membranes, and each loculus contains 10 to 20 seeds joining by aril; each seed is polygonal and spherical, 0.3 – 0.5 cm in length, about 0.3 cm in diameter, externally dark brown, with numerous, fine protrusions; hard tissue; under a magnifying glass, a longitudinal section along the raphe reveals oblong section, with deeply indented hilum and with slightly indented chalaza; white perisperm covering light yellow endosperm and long embryo.

Characteristic aroma when cracked, and taste acrid.

Identification To 1.0 g of coarse powdered Amomum Seed add 20 mL of hexane, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, use a mixture of hexane and borneol acetate (1000:1) as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Total ash <*5.01*> Not more than 9.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Essential oil content <*5.01*> Perform the test with 30.0 g of pulverized Amomum Seed: the volume of essential oil is not less than 0.6 mL.

Containers and storage Containers—Well-closed containers.

Powdered Amomum Seed

Amomi Semen Pulveratum

シュクシャ末

Powdered Amomum Seed is the powder of Amomum Seed.

Description Powdered Amomum Seed occurs as a grayish brown powder, and has a characteristic aroma and an acrid taste.

Under a microscope <*5.01*>, Powdered Amomum Seed reveals fragments of wavy perisperm cells filled with starch grains and containing in each cell a calcium oxalate crystal; yellow and long epidermal cells of seed coat and fragments of thin-walled tissue perpendicular to them; fragments of groups of brown, thick-walled polygonal stone cells.

Identification To 2.0 g of Powdered Amomum Seed add 20 mL of hexane, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, use a mixture of hexane and borneol acetate (1000:1) as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Total ash <*5.01*> Not more than 9.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Essential oil content <*5.01*> Perform the test with 30.0 g of Powdered Amomum Seed: the volume of essential oil is not less than 0.4 mL.

Containers and storage Containers—Tight containers.

Anemarrhena Rhizome

Anemarrhenae Rhizoma

チモ

Anemarrhena Rhizome is the rhizome of *Anemarrhena asphodeloides* Bunge (*Liliaceae*).

Description Rather flat and cord-like rhizome, 3 – 15 cm in length, 0.5 – 1.5 cm in diameter, slightly bent and branched; externally yellow-brown to brown; on the upper surface, a longitudinal furrow and hair-like remains or scars of leaf sheath forming fine ring-nodes; on the lower surface, scars of root appearing as numerous round spot-like hollows; light and easily broken. Under a magnifying glass, a light yellow-brown transverse section reveals an extremely narrow cortex; stele porous, with many irregularly scattered vascular bundles.

Odor, slight; taste, slightly sweet and mucous, followed by bitterness.

Identification **(1)** Shake vigorously 0.5 g of pulverized Anemarrhena Rhizome with 10 mL of water in a test tube: a lasting fine foam is produced. Filter the mixture, and to 2 mL of the filtrate add 1 drop of iron (III) chloride TS: a dark green precipitate is produced.

(2) To 1 g of pulverized Anemarrhena Rhizome add 10 mL of 1 mol/L hydrochloric acid TS, and heat under a reflex condenser on a water bath for 30 minutes. After cooling, centrifuge, and remove the supernatant liquid. To the residue add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of sarsasapogenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone

(7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Anemarrhena Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Anemarrhena Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of fiber, originating from the dead leaves, and other foreign matters contained in Anemarrhena Rhizome is not more than 3.0%.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.5%.

Containers and storage Containers—Well-closed containers.

Angelica Dahurica Root

Angelicae Dahuricae Radix

ビャクシ

Angelica Dahurica Root is the root of *Angelica dahurica* Bentham et Hooker filius ex Franchet et Savatier (*Umbelliferae*).

Description Main root from which many long roots are branched out and nearly fusiform and conical in whole shape, 10 – 25 cm in length; externally grayish brown to dark brown, with longitudinal wrinkles, and with numerous scars of rootlets laterally elongated and protruded. A few remains of leaf sheath at the crown and ring-nodes closely protruded near the crown. In a transverse section, the outer region is grayish white in color, and the central region is sometimes dark brown in color.

Odor, characteristic; taste, slightly bitter.

Identification To 0.2 g of pulverized Angelica Dahurica Root add 5 mL of ethanol (95), shake for 5 minutes, and filter. Examine the filtrate under ultraviolet light (main wavelength: 365 nm): a blue to blue-purple fluorescence develops.

Purity **(1)** Leaf sheath—When perform the test of foreign matter <*5.01*>, the amount of leaf sheath contained in Angelica Dahurica Root does not exceed 3.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Angelica Dahurica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Angelica Dahurica Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than leaf sheath contained in Angelica Dahurica Root is not more than 1.0%.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Apricot Kernel

Armeniacae Semen

キョウニン

Apricot Kernel is the seed of *Prunus armeniaca* Linné, *Prunus armeniaca* Linné var. *ansu* Maximowicz or *Prunus sibirica* Linné (*Rosaceae*).

It contains not less than 2.0% of amygdalin, calculated on the basis of dried material.

Description Flattened, somewhat asymmetric ovoid seed, 1.1 – 1.8 cm in length, 0.8 – 1.3 cm in width, 0.4 – 0.7 cm in thickness; sharp at one end and rounded at the other end where chalaza situated; seed coat brown and its surface being powdery with rubbing easily detachable stone cells of epidermis; numerous vascular bundles running from chalaza throughout the seed coat, appearing as thin vertical furrows; seed coat and thin semitransparent white albumen easily separate from cotyledon when soaked in boiling water; cotyledon, white in color.

Almost odorless; taste, bitter and oily.

Under a microscope <*5.01*>, surface of epidermis reveals stone cells on veins protruded by vascular bundles, forming round polygon to ellipse and approximately uniform in shape, with uniformly thickened cell walls, and 60 – 90 μm in diameter; in lateral view, stone cell appearing obtusely triangular and its cell wall extremely thickened at the apex.

Identification **(1)** When Apricot Kernel is knocked and ground together with water, the odor of benzaldehyde is produced.

(2) To 1.0 g of ground Apricot Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a spot with a bluish white fluorescence appears at an *R*f value of about 0.7. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution.

Purity **(1)** Rancidity—Grind Apricot Kernel with hot water: no unpleasant odor of rancid oil is perceptible.

(2) Foreign matter <*5.01*>—When perform the test with not less than 250 g of Apricot Kernel, it contains not more than 0.10% of fragments of endocarp.

Loss on drying <*5.01*> Not more than 7.0% (6 hours).

Assay Weigh accurately 0.5 g of ground Apricot Kernel,

add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Apricot Kernel Water

キョウニン水

Apricot Kernel Water contains not less than 0.09 w/v% and not more than 0.11 w/v% of hydrogen cyanide (HCN: 27.03).

Method of preparation Prepare by one of the following methods.

(1) To Apricot Kernels, previously crushed and pressed to remove fixed oils as much as possible, add a suitable amount of Water, Purified Water or Purified Water in Containers, and carry out steam distillation. Determine the amount of hydrogen cyanide in the distillate by the method as directed in the Assay, and carry on the distillation until the content of hydrogen cyanide in the distillate is about 0.14 w/v%. To the distillate add Ethanol in about 1/3 of the volume of the distillate, and dilute with a mixture of Purified Water or Purified Water in Containers and Ethanol (3:1) until the content of hydrogen cyanide meets the specification.

(2) Dissolve 7.5 mL of freshly prepared mandelonitrile in 1000 mL of a mixture of Purified Water or Purified Water in Containers and Ethanol (3:1), mix well, and filter. Determine the amount of hydrogen cyanide in the solution as directed in the Assay, and, if the amount is more than that specified above, dilute the solution to the specified concentration by the addition of the mixture of Purified Water or Purified Water in Containers and Ethanol (3:1).

Description Apricot Kernel Water is a clear, colorless or pale yellow liquid. It has an odor of benzaldehyde and a characteristic taste.

pH: 3.5 – 5.0

Identification To 2 mL of Apricot Kernel Water add 1 mL of ammonia TS, and allow to stand for 10 minutes: a slight turbidity is produced. Allow to stand for 20 minutes: the turbidity is intensified.

Specific gravity <*2.56*> d_{20}^{20}: 0.968 – 0.978

Purity **(1)** Sulfate <*1.14*>—Add a few drops of 0.1 mol/L sodium hydroxide VS to 5.0 mL of Apricot Kernel Water to make slightly alkaline, evaporate on a water bath to dryness, and ignite between 450°C and 550°C. Dissolve the residue in 1.0 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.005%).

(2) Heavy metals <*1.07*>—Evaporate 50 mL of Apricot Kernel Water on a water bath to dryness, ignite between 450°C and 550°C, dissolve the residue in 5 mL of dilute acetic acid with warming, add water to make exactly 50 mL, and filter. Remove the first 10 mL of the filtrate, dilute the subsequent 20 mL to 50 mL with water, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 1 ppm).

(3) Free hydrogen cyanide—To 10 mL of Apricot Kernel Water add 0.8 mL of 0.1 mol/L silver nitrate VS and 2 to 3 drops of nitric acid at 15°C, filter, and add 0.1 mol/L silver nitrate VS to the filtrate: no change occurs.

(4) Residue on evaporation—Evaporate 5.0 mL of Apricot Kernel Water to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Measure exactly 25 mL of Apricot Kernel Water, add 100 mL of water, 2 mL of potassium iodide TS and 1 mL of ammonia TS, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS until a yellow turbidity persists.

Each mL of 0.1 mol/L silver nitrate VS
= 5.405 mg of HCN

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Aralia Rhizome

Araliae Cordatae Rhizoma

ドクカツ

Aralia Rhizome is usually the rhizome of *Aralia cordata* Thunberg (*Araliaceae*).

Description Aralia Rhizome is curved, irregular cylindrical to masses occasionally with remains of short roots. 4 – 12 cm in length, 2.5 – 7 cm in diameter, often cut crosswise or lengthwise. 1 to several, enlarged dents by remains of stems on the upper part or rarely 1.5 – 2.5 cm in diameter, remains of short stem. The outer surface is dark brown to yellow-brown, with longitudinally wrinkles, bases or dents of root. The transverse section of rhizome reveals dark brown to yellow-brown, scattered brownish small spots with oil canals, and with numerous splits.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of rhizome reveals the outermost layer to be cork layer, rarely composed of cork stone cells, followed these appeared several layers of collenchyma. Vascular bundle and medullary rays is distinct, pith broad. Phloem fibre bundles are sometimes observed at the outer portion of phloem. Oil canals composed of schizogenous intercellular space in cortex and pith. Cortex composed of vessels, xylem fibres, and occasionally thick-wall xylem parenchyma. Vascular bundles scattered on the pith. And, parenchymatous cells observed rosette aggregates of calcium oxalate. Starch grains composed of simple grains, 2- to 6- compound grains.

Identification To 1 g of pulverized Aralia Rhizome add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (30:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a purple spot appears at an *R*f value of about 0.5.

Purity Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Aralia Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <*5.01*> Not more than 12.0%.

Total ash <*5.01*> Not more than 9.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Areca

Arecae Semen

ビンロウジ

Areca is the seed of *Areca catechu* Linné (*Palmae*).

Description Rounded-conical or flattened nearly spherical seed 1.5 – 3.5 cm high and 1.5 – 3 cm in diameter; hilum at the center of its base and usually forming a dent; externally grayish red-brown to grayish yellow-brown, with a network of pale lines; hard in texture; cross section dense in texture, exhibiting a marbly appearance of grayish brown seed coat alternating with white albumen; center of the seed often hollow.

Odor, slight; taste, astringent and slightly bitter.

Identification To 1.0 g of pulverized Areca add 5 mL of 0.01 mol/L hydrochloric acid TS and 5 mL of ethyl acetate, shake for 15 minutes, centrifuge, and remove the upper layer. To the water layer add 1 mL of sodium hydroxide TS and 5 mL of ethyl acetate, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of arecoline hydrobromide for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and acetic acid (100) (10:6:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS, air-dry, then spray evenly sodium nitrite TS: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the brown spot obtained from the standard solution. The color of this spot fades immediately and then disappears after air-drying.

Purity **(1)** Pericarp—When perform the test of foreign matter <*5.01*>, the amount of pericarp contained in Areca is not more than 2.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than the pericarp contained in Areca does not exceed 1.0%.

Total ash <*5.01*> Not more than 2.5%.

Containers and storage Containers—Well-closed containers.

Artemisia Capillaris Flower

Artemisiae Capillaris Flos

インチンコウ

Artemisia Capillaris Flower is the capitulum of *Artemisia capillaris* Thunberg (*Compositae*).

Description Capitulum of ovoid to spherical, capitula, about 1.5 – 2 mm in length, about 2 mm in diameter, with linear leaves, peduncles, and thin stem. Outer surface of capitulum, light green to light yellow-brown in color; outer surface of leaf, green to green-brown in color; peduncle, green-brown to dark brown in color. Under a magnifying glasses, the capitulum; involucral scale, in 3 – 4 succubous

rows, outer scale of ovate with obtuse, inner scale of elliptical, 1.5 mm in length, longer than outer one, with keel midrib and thin membranous margin. Floret; tubular, marginal flower of female, disk flower of hermaphrodite. Achene of obovoid, 0.8 mm in length. Light in texture.

Odor, characteristic, slight; taste, slightly acrid, which gives slightly numbing sensation to the tongue.

Identification To 0.5 g of pulverized Artemisia Capillaris Flower add 10 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a principal spot with a blue fluorescence appears at an *R*f value of about 0.5.

Purity Stem—When perform the test of foreign matter <*5.01*>, Artemisia Capillaris Flower does not contain any stem more than 2 mm in diameter.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 9.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Artemisia Leaf

Artemisiae Folium

ガイヨウ

Artemisia leaf is the leaf and twig of *Artemisia princeps* Pampanini or *Artemisia montana* Pampanini (*Compositae*).

Description Wrinkled leaves and their fragments, frequently with thin stems. The upper surface of leaf dark green, the lower surface covered densely with grayish white cotton-like hairs. When smoothed by immersion in water, unfolded laminas 4 – 15 cm long, 4 – 12 cm wide, 1- to 2-pinnately cleft or pinnately parted. Segments in 2 to 4 pairs, oblong-lanceolate to oblong, apex acuminate sometimes obtuse, margins irregularly lobed or entire. Small sized leaves tri-cleft or entire, lanceolate.

Order, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of leaf reveals several-cells-layered collenchyma beneath epidermis of midvein; vascular bundles at the central portion of midvein, occasionally fiber bundles adjacent to phloem and xylem; laminas composed of upper epidermis, pallisad tissue, spongy tissue and lower epidermis, long soft hairs, T-shaped hairs and glandular hairs on epidermis of laminas; epidermal cells contain tannin-like substances, parenchyma cells contain oil-like substances and tannin-like substances.

Identification To 0.5 g of pulverized Artemisia Leaf (the parts like a floccose substance which are not easily pulverized may be removed) add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg each of umbelliferone for thin-layer chromatography and scopoletin for thin-layer chromatography in 10 mL each of methanol, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL each of the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (20:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): two of the spots among the several spots obtained from the sample solution have the same color tone and *R*f value with the corresponding bluish white fluorescent spot obtained respectively from the standard solutions (1) and (2). *System suitability*—(Ultraviolet light (main wavelength: 365 nm)).

To 1 mL of the standard solution (1) add methanol to make 10 mL. Confirm that when perform the test with 1 μL of this solution under the above conditions, a bluish white fluorescent spot is detectable.

Purity *Artemisia argyi*—To 0.5 g of powdered Artemisia Leaf (the parts like a floccose substance which are not easily pulverized may be removed) add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.5 g of artemisia·argyi for purity test add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (20:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): no spot appears from the sample solution at the position of the green fluorescent spot (*R*f value of about 0.5) obtained from the standard solution.

Loss on drying <*5.01*> Not more than 14.0%.

Total ash <*5.01*> Not more than 13.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 16.0%.

Asiasarum Root

Asiasari Radix

サイシン

Asiasarum Root is the root with rhizome of *Asiasarum sieboldii* F. Maekawa or *Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa (*Aristolochiaceae*).

Description Asiasarum Root is a nearly cylindrical rhizome with numerous thin and long roots, externally light brown to dark brown. The root, about 15 cm in length, about 0.1 cm in diameter, with shallow longitudinal wrinkles on the surface, and brittle. The rhizome, 2 – 4 cm in length, 0.2 – 0.3 cm in diameter, often branched, with longitudinal wrinkles

on the surface; internode short; each node has several scars of petiole and peduncle, and several thin and long roots.

Odor, characteristic; taste, acrid, with some sensation of numbness on the tongue.

Identification To 1 g of pulverized Asiasarum Root add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of asarinin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Asiasarum Root according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Asiasarum Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Terrestrial part—When perform the test of foreign matter <*5.01*>, any terrestrial parts are not found.

(4) Foreign matter <*5.01*>—The amount of foreign matter other than terrestrial part contained in Asiasarum Root is not more than 1.0%.

(5) Aristolochic acid I—To exactly 2.0 g of pulverized Asiasarum Root add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve exactly 1.0 mg of aristolochic acid I for crude drugs purity test in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, according to the following conditions: the sample solution shows no peak at the retention time corresponding to aristolochic acid I from the standard solution. If the sample solution shows such a peak, repeat the test under different conditions to confirm that the peak in question is not aristolochic acid I.

Operating conditions—

Detector: An ultraviolet or visible absorption photometer (wavelength: 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate and 2 mL of phosphoric acid in water to make 1000 mL and acetonitrile (11:9).

Flow rate: Adjust so that the retention time of aristolochic acid I is about 15 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add diluted methanol (3 in 4) to make exactly 10 mL. Confirm that the ratio, S/N, of the signal (S) and noise (N) of aristolochic acid I obtained from 20 μL of this solution is not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aristolochic acid I is not more than 5.0%.

(6) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Essential oil content <*5.01*> Perform the test with 30.0 g of pulverized Asiasarum Root: the volume of essential oil is not less than 0.6 mL.

Containers and storage Containers—Well-closed containers.

Asparagus Root

Asparagi Radix

テンモンドウ

Asparagus Root is the root of *Asparagus cochinchinensis* Merrill (*Liliaceae*), from which most of the cork layer is removed after being passed through hot water or steamed.

Description Asparagus Root is a fusiform to cylindrical tuber, 5 – 15 cm in length, 5 – 20 mm in diameter; externally light yellow-brown to light brown, translucent and often with longitudinal wrinkles; flexible, or hard and easily broken in texture; fractured surface, grayish yellow, glossy and horny.

Odor, characteristic; taste, sweet at first, followed by a slightly bitter aftertaste.

Under a microscope <*5.01*>, a transverse section of Asparagus Root reveals stone cells and bundles of them on outer layer of cortex; mucilaginous cells containing raphides of calcium oxalate in the parenchyma cells of cortex and stele; no starch grains.

Identification To 1 g of the coarse cutting of Asparagus Root add 5 mL of a mixture of 1-butanol and water (40:7), shake for 30 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (10:6:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 2 minutes: the spot of a red-brown at first then changes to brown color appears at an *R*f value of about 0.4.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Asparagus Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Asparagus Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 18.0% (6 hours).

Total ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Well-closed contain-

ers.

Astragalus Root

Astragali Radix

オウギ

Astragalus Root is the root of *Astragalus membranaceus* Bunge or *Astragalus mongholicus* Bunge (*Leguminosae*).

Description Nearly cylindrical root, 30 – 100 cm in length, 0.7 – 2 cm in diameter, with small bases of lateral root dispersed on the surface, twisted near the crown; externally light grayish yellow to light yellow-brown, and covered with irregular, dispersed longitudinal wrinkles and horizontal lenticel-like patterns; difficult to break; fractured surface fibrous. Under a magnifying glass, a transverse section reveals an outer layer composed of periderm; cortex light yellowish white, xylem light yellow, and zone near the cambium somewhat brown in color; thickness of cortex from about one-third to one-half of the diameter of xylem; white medullary ray from xylem to cortex in thin root, but often appearing as radiating cracks in thick root; usually pith unobservable.

Odor, slight; taste, sweet.

Identification To 1 g of pulverized Astragalus Root add 5 mL of potassium hydroxide TS and 5 mL of acetonitrile in a glass-stoppered centrifuge tube. After shaking this for 10 minutes, centrifuge, and use the upper layer as the sample solution. Separately, dissolve 1 mg of astragaloside IV for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellowish brown fluorescent spot obtained from the standard solution.

Purity **(1)** Root of *Hedysarum* species and others—Under a microscope <*5.01*>, a vertical section of Astragalus Root reveals no crystal fiber containing solitary crystals of calcium oxalate outside the fiber bundle.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Astragalus Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Astragalus Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Atractylodes Lancea Rhizome

Atractylodis Lanceae Rhizoma

ソウジュツ

Atractylodes Lancea Rhizome is the rhizome of *Atractylodes lancea* De Candolle, *Atractylodes chinensis* Koidzumi or their interspecific hybrids (*Compositae*).

Description Irregularly curved, cylindrical rhizome, 3 – 10 cm in length, 1 – 2.5 cm in diameter; externally dark grayish brown to dark yellow-brown; a transverse section nearly orbicular, with light brown to red-brown secretes as fine points.

Often white cotton-like crystals produced on its surface.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section usually reveals periderm with stone cells; parenchyma of cortex, usually without any fiber bundle; oil sacs, containing light brown to yellow-brown substances, located at the end region of medullary rays; xylem exhibits vessels surrounded by fiber bundles and arranged radially on the region adjoining the cambium; pith and medullary rays exhibit the same oil sacs as in the cortex; parenchyma cells contain spherocrystals of inulin and fine needle crystals of calcium oxalate.

Identification To 2.0 g of pulverized Atractylodes Lancea Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a grayish green spot appears at an *R*f value of about 0.5.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Atractylodes Lancea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Atractylodes Lancea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Atractylodes Lancea Rhizome: the volume of essential oil is not less than 0.7 mL.

Containers and storage Containers—Well-closed containers.

Powdered Atractylodes Lancea Rhizome

Atractylodis Lanceae Rhizoma Pulveratum

ソウジュツ末

Powdered Atractylodes Lancea Rhizome is the powder of Atractylodes Lancea Rhizome.

Description Powdered Atractylodes Lancea Rhizome occurs as a yellow-brown powder. It has a characteristic odor, and a slightly bitter taste.

Under a microscope ⟨*5.01*⟩, Powdered Atractylodes Lancea Rhizome reveals mainly parenchyma cells, spherocrystals of inulin, fragments of parenchyma cells containing fine needle crystals of calcium oxalate as their contents; and further fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels, and small yellow-brown secreted masses or oil drops; starch grains absent.

Identification To 2.0 g of Powdered Atractylodes Lancea Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a grayish green spot appears at an *R*f value of about 0.5.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of Powdered Atractylodes Lancea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Powdered Atractylodes Lancea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 7.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Essential oil content ⟨*5.01*⟩ Perform the test with 50.0 g of Powdered Atractylodes Lancea Rhizome: the volume of essential oil is not less than 0.5 mL.

Containers and storage Containers—Tight containers.

Atractylodes Rhizome

Atractylodis Rhizoma

ビャクジュツ

Atractylodes Rhizome is the rhizome of 1) *Atractylodes japonica* Koidzumi ex Kitamura (*Compositae*) (Wa-byakujutsu) or 2) *Atractylodes macrocephala* Koidzumi (*Atractylodes ovata* De Candolle) (*Compositae*) (Kara-byakujutsu).

Description **1)** Wa-byakujutsu—Periderm-removed rhizome is irregular masses or irregularly curved cylinder, 3 – 8 cm in length, 2 – 3 cm in diameter; externally light grayish yellow to light yellowish white, with scattered grayish brown parts. The rhizome covered with periderm is externally grayish brown, often with node-like protuberances and coarse wrinkles. Difficult to break, and the fractured surface is fibrous. A transverse section, with fine dots of light yellow-brown to brown secrete.

Odor, characteristic; taste, somewhat bitter.

Under a microscope ⟨*5.01*⟩, a transverse section reveals periderm with stone cell layers; fiber bundles in the parenchyma of the cortex, often adjoined to the outside of the phloem; oil sacs containing light brown to brown substances, situated at the outer end of medullary rays; in the xylem, radially lined vessels, surrounding large pith, and distinct fiber bundle surrounding the vessels; in pith and in medullary rays, oil sacs similar to those in cortex, and in parenchyma, crystals of inulin and small needle crystals of calcium oxalate.

2) Kara-byakujutsu—Irregularly enlarged mass, 4 – 8 cm in length, 2 – 5 cm in diameter; externally grayish yellow to dark brown, having sporadic, knob-like small protrusions. Difficult to break; fractured surface has a light brown to dark brown xylem remarkably fibrous.

Odor, characteristic; taste, somewhat sweet, but followed by slight bitterness.

Under a microscope ⟨*5.01*⟩, a transverse section usually reveals periderm with stone cells, absence of fibers in the cortex; oil sacs containing yellow-brown contents in phloem ray and also at the outer end of it; xylem with radially lined vessels surrounding large pith, and distinct fiber bundle surrounding the vessels; pith and medullary ray exhibit oil sacs as in cortex; parenchyma contains crystals of inulin and small needle crystals of calcium oxalate.

Identification To 2.0 g of pulverized Atractylodes Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.6.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of pulverized Atractylodes Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Atractylodes lancea rhizome—When proceed as directed in the Identification, using exactly 5 mL of hexane, any grayish green spot does not appear at an *R*f value of about 0.5, immediately below the red-purple spot appeared at an *R*f value of about 0.6.

Total ash ⟨*5.01*⟩ Not more than 7.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Essential oil content ⟨*5.01*⟩ Perform the test with 50.0 g of pulverized Atractylodes Rhizome: the volume of essential oil is not less than 0.5 mL.

Containers and storage Containers—Well-closed containers.

Powdered Atractylodes Rhizome

Atractylodis Rhizoma Pulveratum

ビャクジュツ末

Powdered Atractylodes Rhizome is the powder of Atractylodes Rhizome.

Description Powdered Atractylodes Rhizome occurs as a light brown to yellow-brown powder, and has a characteristic odor and a slightly bitter or slightly sweet taste, followed by a slightly bitter aftertaste.

Under a microscope <*5.01*>, Powdered Atractylodes Rhizome reveals mainly parenchyma cells, crystals of inulin and fragments of parenchyma cells containing small needle crystals of calcium oxalate; fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels; small yellow-brown secrete masses or oil droplets; starch grains absent.

Identification To 2.0 g of Powdered Atractylodes Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Atractylodes Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Atractylodes lancea rhizome—When proceed as directed in the Identification, using exactly 5 mL of hexane, any grayish green spot does not appear at an *R*f value of about 0.5, immediately below the red-purple spot appeared at an *R*f value of about 0.6.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of Powdered Atractylodes Rhizome: the volume of essential oil is not less than 0.4 mL.

Containers and storage Containers—Tight containers.

Bakumondoto Extract

麦門冬湯エキス

Bakumondoto Extract contains not less than 1.2 mg of ginesenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)
Ophiopogon Root	10 g
Pinellia Tuber	5 g
Brown Rice	5 g
Jujube	3 g
Ginseng	2 g
Glycyrrhiza	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1), using the crude drugs shown above.

Description Bakumondoto Extract occurs as a light yellow to light brown powder or blackish brown viscous extract. It has a slight odor, and a sweet taste.

Identification **(1)** Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of 1-butanol, shake, centrifuge, and use the water layer as the sample solution. Separately, to 3.0 g of ophiopogon root add 50 mL of water, and heat under a reflux condenser for 1 hour. After cooling, take 20 mL of the extract, add 5 mL of 1-butanol, shake, centrifuge, and use the water layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL of the sample solution and 5 μL of the standard solution as bands on the original line of a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water and acetic acid (100) (120:80:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark blue-green spot (*R*f value: about 0.3) obtained from the standard solution (Ophiopogon Root).

(2) Shake 5.0 g of dry extract (or 15 g of the viscous extract) with 15 mL of water, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of cycloartenyl ferulate for thin-layer chromatography in 1 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 30 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, acetone and acetic acid (100) (50:20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution. Or examine under ultraviolet light (main wavelength: 365 nm) after spraying evenly a mixture of sulfuric acid and ethanol (99.5) (1:1) and heating at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Brown Rice).

(3) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the

standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(4) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract: Not more than 7.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Ginsenoside Rb_1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to about 2 g of dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine all of the supernatant liquid, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column [about 10 mm in inside diameter, packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), and washed just before using with methanol and then diluted methanol (3 in 10)], and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <2.48> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rb_1\ (C_{54}H_{92}O_{23}) = M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl group bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (400:100:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb_1 is about 16 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb_1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5%, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Bear Bile

Fel Ursi

ユウタン

Bear Bile is the dried bile of *Ursus arctos* Linné or allied animals (*Ursidae*).

Description Indefinite small masses; externally yellow-brown to dark yellow-brown; easily broken; fractured surface has a glassy luster, and is not wet.

Usually in a gall sac, occasionally taken out, the gall sac consists of a fibrous and strong membrane, 9 – 15 cm in length and 7 – 9 cm in width; externally dark brown and translucent.

Odor, slight and characteristic; taste, extremely bitter.

Identification To 0.1 g of pulverized Bear Bile, add 5 mL of methanol, warm in a water bath for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of sodium tauroursodeoxycholate for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetic acid (100), toluene and water (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Purity Other animal biles—Use the sample solution obtained in the Identification as the sample solution. Separately, dissolve 10 mg of sodium glycocholate for thin-layer chromatography and 20 mg of powdered porcine bile for thin-layer chromatography in 5 mL each of methanol, and use these solutions as the standard solution (1) and (2), respectively. Perform the test with these solutions as directed in the Identification: Spots from the sample solution correspond to neither the spot of glycocholic acid from the standard solution (1) nor the grayish brown to black spot of powdered porcine bile at an *R*f value of about 0.3 from the standard solution (2).

Containers and storage Containers—Well-closed containers.

Bearberry Leaf

Uvae Ursi Folium

ウワウルシ

Bearberry Leaf is the leaf of *Arctostaphylos uva-ursi* Sprengel (*Ericaceae*).

It contains not less than 7.0% of arbutin.

Description Obovate to spatulate leaves, 1 – 3 cm in length, 0.5 – 1.5 cm in width; upper surface yellow-green to dark green; lower surface light yellow-green; margin entire; apex obtuse or round, sometimes retuse; base cuneate; petiole very short; lamina thick with characteristic reticulate venation, and easily broken.

Odor, slight; taste, slightly bitter and astringent.

Under a microscope <*5.01*>, the transverse section reveals thick cuticule; parenchyma cells of palisade tissue and sponge tissue being similar in form; in the vascular bundle, medullary ray consisting of 2 to 7 rows of one-cell line, appearing as bones of Japanese fan; polygonal solitary crystals and clustered crystals of calcium oxalate present sparsely in cells on both outer and inner sides of the vascular bundle, but no crystals in mesophyll.

Identification **(1)** Macerate 0.5 g of pulverized Bearberry Leaf with 10 mL of boiling water, shake the mixture for a few minutes, allow to cool, and filter. Place 1 drop of the filtrate on filter paper, and add 1 drop of iron (III) chloride TS: a dark purple color appears.

(2) To 0.2 g of pulverized Bearberry Leaf add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of arbutin for thin-layer chromatography in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, water and formic acid (8:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution and the spot from the standard solution show a yellow-brown to blackish brown color and the same *R*f value.

Purity **(1)** Twig—When perform the test of foreign matter <*5.01*>, the amount of twigs contained in Bearberry Leaf does not exceed 4.5%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than twigs contained in Bearberry Leaf does not exceed 2.0%.

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Assay Weigh accurately about 0.5 g of pulverized Bearberry Leaf in a glass-stoppered centrifuge tube, add 40 mL of water, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of water, and proceed in the same manner. To the combined extracts add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of arbutin for assay, previously dried for 12 hours (in vacuum, silica gel), dissolve in water to make exactly 100 mL,

and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_T and A_S, of arbutin in each solution.

$$\text{Amount (mg) of arbutin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of arbutin for assay taken

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 280 nm).

Column: A stainless steel column 4 – 6 mm in inside diameter and 15 – 25 cm in length, packed with octadecylsilanized silica gel (5 – 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, methanol and 0.1 mol/L hydrochloric acid TS (94:5:1).

Flow rate: Adjust so that the retention time of arbutin is about 6 minutes.

Selection of column: Dissolve 0.05 g each of arbutin for assay, hydroquinone and gallic acid in water to make 100 mL. Proceed with 10 μL of this solution under the above operating conditions, and calcutate the resolution. Use a column giving elution of arbutin, hydroquinone and gallic acid in this order, and clearly dividing each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of arbutin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Beef Tallow

Sevum Bovinum

牛脂

Beef Tallow is a purified fat obtained by wet steam rendering from the fresh fatty tissues of *Bos taurus* Linné var. *domesticus* Gmelin (*Bovidae*).

Description Beef Tallow occurs as a white, uniform mass. It has a characteristic odor and a mild taste.

It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

It is breakable at a low temperature, but softens above 30°C.

Melting point: 42 – 50°C

Acid value ⟨*1.13*⟩ Not more than 2.0.

Saponification value ⟨*1.13*⟩ 193 – 200

Iodine value ⟨*1.13*⟩ 33 – 50 (When the sample is insoluble in 20 mL of cyclohexane, dissolve it by shaking a glass-stoppered flask in warm water. Then, if insoluble, increase the volume of solvent.)

Purity **(1)** Moisture and coloration—Beef Tallow (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkalinity—To 2.0 g of Beef Tallow add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Beef Tallow add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the turbidity of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of an ethanolic solution of silver nitrate (1 in 50).

Containers and storage Containers—Well-closed containers.

White Beeswax

Cera Alba

サラシミツロウ

White Beeswax is bleached Yellow Beeswax.

Description White Beeswax occurs as white to yellowish white masses. It has a characteristic odor. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

It is slightly soluble in diethyl ether, and practically insoluble in water and in ethanol (99.5).

Acid value ⟨*1.13*⟩ 5 – 9 or 17 – 22 Weigh accurately about 6 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value ⟨*1.13*⟩ 80 – 100 Weigh accurately about 3 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS and 50 mL of ethanol (95), heat for 4 hours on a water bath under a reflux condenser, and proceed as directed in the Saponification value.

Melting point ⟨*1.13*⟩ 60 – 67°C

Purity Paraffin, fat, Japan wax or resin—Melt White Beeswax at the lowest possible temperature, drip the liquid into a vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.

Yellow Beeswax

Cera Flava

ミツロウ

Yellow Beeswax is the purified wax obtained from honeycombs such as those of *Apis mellifera* Linné or *Apis cerana* Fabricius (*Apidae*).

Description Yellow Beeswax occurs as light yellow to brownish yellow masses. It has a characteristic odor, which is not rancid.

It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

Acid value ⟨*1.13*⟩ 5 – 9 or 17 – 22 Weigh accurately about 6 g of Yellow Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value ⟨*1.13*⟩ 80 – 100 Weigh accurately about 3 g of Yellow Beeswax, place in a 250-mL glass-stoppered flask, and add 25 mL of 0.5 mol/L potassium hydroxide-ethanol and 50 mL of ethanol (95), insert a reflux condenser, heat for 4 hours on a water bath, and proceed as directed in the Saponification value.

Melting point ⟨*1.13*⟩ 60 – 67°C

Purity Paraffin, fat, Japan wax or resin—Melt Yellow Beeswax at the lowest possible temperature, drip the liquid into a glass vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.

Belladonna Root

Belladonnae Radix

ベラドンナコン

Belladonna Root is the root of *Atropa belladonna* Linné (*Solanaceae*).

When dried, it contains not less than 0.4% of hyoscyamine ($C_{17}H_{23}NO_3$: 289.37).

Description Cylindrical root, usually 10 – 30 cm in length, 0.5 – 4 cm in diameter; often cut crosswise or lengthwise; externally grayish brown to grayish yellow-brown, with longitudinal wrinkles; periderm often removed; fractured surface is light yellow to light yellow-brown in color and is powdery.

Almost odorless; taste, bitter.

Identification Place 2.0 g of pulverized Belladonna Root in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate RS or atropine sulfate hydrate for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the principal spot from the sample solution is the same in color tone and *R*f value with a yellow-red spot from the standard solution.

Purity **(1)** Stem and crown—When perform the test of foreign matter ⟨*5.01*⟩, the amount of stems and crowns contained in Belladonna Root does not exceed 10.0%.

(2) Foreign matter ⟨*5.01*⟩—The amount of foreign matter other than stems and crowns contained in Belladonna Root does not exceed 2.0%.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 4.0%.

Assay Weigh accurately about 0.7 g of pulverized Belladonna Root, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (previously determine the loss on drying ⟨*2.41*⟩ under the same conditions as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of hyoscyamine (atropine), to that of the internal standard.

$$\text{Amount (mg) of hyoscyamine } (C_{17}H_{23}NO_3) = M_S \times Q_T/Q_S \times 1/5 \times 0.855$$

M_S: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside

diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to pH 3.5, and add water to make 1000 mL, and mix this solution with acetonitrile (9:1).

Flow rate: Adjust so that the retention time of atropine is about 14 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and determine the resolution. Use a column giving elution of atropine and the internal standard in this order with the resolution between these peaks being not less than 4.

Containers and storage Containers—Well-closed containers.

Belladonna Extract

ベラドンナエキス

Belladonna Extract contains not less than 0.85% and not more than 1.05% of hyoscyamine ($C_{17}H_{23}NO_3$: 289.37).

Method of preparation To 1000 g of a coarse powder of Belladonna Root add 4000 mL of 35 vol% Ethanol, and digest for 3 days. Press the mixture, add 2000 mL of 35 vol% Ethanol to the residue, and digest again for 2 days. Combine all the extracts, and allow to stand for 2 days. Filter, and prepare the viscous extract as directed under Extracts. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 35 vol% Ethanol.

Description Belladonna Extract has a dark brown color, a characteristic odor and a bitter taste.

Identification Mix 0.5 g of Belladonna Extract with 30 mL of ammonia TS in a flask, transfer the mixture to a separator, then add 40 mL of ethyl acetate, and shake the mixture. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in the Identification under Belladonna Root.

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Belladonna Extract as directed under the Extracts (4), and perform the test (not more than 30 ppm).

Assay Weigh accurately about 0.4 g of Belladonna Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make exactly 25 mL. Proceed as directed under Belladonna Root.

$$\text{Amount (mg) of hyoscyamine } (C_{17}H_{23}NO_3) = M_S \times Q_T/Q_S \times 1/5 \times 0.855$$

M_S: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Belladonna Total Alkaloids

ベラドンナ総アルカロイド

Belladonna Total Alkaloids contains not less than 95.0% and not more than 99.0% of hyoscyamine ($C_{17}H_{23}NO_3$: 289.37), not less than 1.3% and not more than 3.9% of scopolamine ($C_{17}H_{21}NO_4$: 303.35), and not less than 99.0% and not more than 102.0% of the total alkaloids (hyoscyamine and scopolamine), calculated on the dried basis.

Method of preparation Belladonna Total Alkaloids is prepared by purification of the extract from Belladonna Root with water or aqueous ethanol.

Description Belladonna Total Alkaloids occurs as white, crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), and slightly soluble in water.

Identification Dissolve 2 mg of Belladonna Total Alkaloids in 1 mL of ethanol (95), and use this solution as the sample solution. Then proceed as directed in the Identification under Belladonna Root.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −18.5 – −22.0° (after drying, 1 g, ethanol (99.5), 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Place 1.0 g of Belladonna Total Alkaloids in a porcelain crucible, and mix with 1.2 mL of dilute hydrochloric acid. Mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and after evaporating the solvent on a boiling water bath, carbonize by gradual heating. Then proceed according to Method 4, and perform the test. The control solution is prepared as follows: Mix 1.2 mL of dilute hydrochloric acid with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and evaporate the solvent on a boiling water bath. After cooling, add 1 mL of sulfuric acid, then proceed according to Method 4, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Belladonna Total Alkaloids according to Method 4, and perform the test (not more than 1 ppm).

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, 60°C, 6 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 25 mg of Belladonna Total Alkaloids, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 3 mL of the internal standard solution and the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (separately determine the loss on drying <*2.41*> under the same condi-

tions as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as the standard stock solution (1). Also, weigh accurately about 25 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying <2.41> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in the mobile phase to make exactly 25 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard stock solution (2). Take exactly 5 mL of standard stock solution (1), add exactly 2 mL of the standard stock solution (2), and add exactly 3 mL of the internal standard solution. To this solution add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{TA} and Q_{SA}, of the peak area of hyoscyamine (atropine) to that of the internal standard and the ratios, Q_{TS} and Q_{SS}, of the peak area of scopolamine to that of the internal standard. Then calculate the amounts of hyoscyamine and scopolamine using the following equations. The amount of the total alkaloids is obtained as the sum of them.

The amount (mg) of hyoscyamine ($C_{17}H_{23}NO_3$)
$= M_{SA} \times Q_{TA}/Q_{SA} \times 0.855$

The amount (mg) of scopolamine ($C_{17}H_{21}NO_4$)
$= M_{SS} \times Q_{TS}/Q_{SS} \times 6/125 \times 0.789$

M_{SA}: The amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

M_{SS}: The amount (mg) of Scopolamine Hydrobromide RS taken, calculated on the dried basis

Internal standard solution: A solution of brucine *n*-hydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of around 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, add 10 mL of triethylamine, adjust to pH 3.5 with phosphoric acid, and add water to make 1000 mL. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust so that the retention time of atropine is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order, and the resolutions between scopolamine and atropine, and atropine and the internal standard are not less than 11 and not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of scopolamine to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Benincasa Seed

Benincasae Semen

トウガシ

Benincasa seed is the seed of 1) *Benincasa cerifera* Savi or 2) *Benincasa cerifera* Savi forma *emarginata* K. Kimura et Sugiyama (*Cucurbitaceae*).

Description **1)** *Benincasa cerifera* origin—Flattened, ovate to orbicular ovate seed, 10 – 13 mm in length, 6 – 7 mm in width, about 2 mm in thickness; slightly acute at base; hilum and germ pore form two protrusions; externally light grayish yellow to light yellowish brown; prominent band along with marginal edge of seed; under a magnifying glass, surface of the seed is with fine wrinkles and minute hollows.

Odorless; bland taste and slightly oily.

Under a microscope <5.01>, a transverse section reveals the outermost layer of seed coat composed of a single-layered and palisade like epidermis, the epidermis obvious at prominent band along with marginal edge of seed; hypodermis composed of slightly sclerified parenchyma beneath epidermis; inside of the parenchyma several layers of stone cells lie; the innermost layer of seed coat composed of parenchyma several cells thick; perisperm coated with cuticle, composed of parenchyma several cells thick; endosperm composed of a row of compressed cells; cotyledon contains oil drops and aleurone grains, occasionally starch grains.

2) *Benincasa cerifera* forma *emarginata* origin—Flattened, ovate to ellipsoidal seed, 9 – 12 mm in length, 5 – 6 mm in width, about 2 mm in thickness; hilum and germ pore form two protrusions as in 1); externally light grayish yellow, smooth, no prominent band along with marginal edge of seed.

Odorless; bland taste and slightly oily.

Under a microscope <5.01>, a transverse section reveals the outermost layer composed of a single-layered epidermis coated with cuticle, often detached; hypodermis composed of slightly sclerified parenchyma beneath epidermis; inside of the parenchyma several layers of stone cells lie; the innermost layer of seed coat composed of parenchyma several cells thick; perisperm coated with cuticle, composed of parenchyma several cells thick; endosperm composed of a row of compressed cells; cotyledon contains oil drops and aleurone grains, occasionally starch grains.

Identification To 0.5 g of pulverized Benincasa Seed add 10 mL of a mixture of methanol and water (4:1), shake for 10 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (8:6:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): two bluish white fluorescent spots appear at an *R*f value of about 0.4, and the spot having the smaller *R*f value shows more intense fluoresence.

Purity Foreign matter <5.01>—It contains not more than 2.0%.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 3.0%.

Containers and storage Containers—Well-closed containers.

Benzoin

Benzoinum

アンソッコウ

Benzoin is the resin obtained from *Styrax benzoin* Dryander or other species of the same genus (*Styracaceae*).

Description Benzoin occurs as grayish brown to dark red-brown blocks varying in size; the fractured surface exhibiting whitish to light yellow-red grains in the matrix; hard and brittle at ordinary temperature but softened by heat.

Odor, characteristic and aromatic; taste, slightly pungent and acrid.

Identification **(1)** Heat a fragment of Benzoin in a test tube: it evolves an irritating vapor, and a crystalline sublimate is produced.

(2) Digest 0.5 g of Benzoin with 10 mL of diethyl ether, decant 1 mL of the diethyl ether into a porcelain dish, and add 2 to 3 drops of sulfuric acid: a deep red-brown to deep red-purple color develops.

Purity Ethanol-insoluble substances—Boil gently 1.0 g of Benzoin with 30 mL of ethanol (95) on a water bath for 15 minutes under a reflux condenser. After cooling, collect the insoluble substances through a tared glass filter (G3), and wash with three 5-mL portions of ethanol (95). Dry the residue at 105°C for 4 hours: the mass of the residue does not exceed 0.30 g.

Total ash ⟨*5.01*⟩ Not more than 2.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Bitter Cardamon

Alpiniae Fructus

ヤクチ

Bitter Cardamon is the fruit of *Alpinia oxyphylla* Miquel (*Zingiberaceae*).

Description Spherical to fusiform fruit, with both ends somewhat pointed; 1 – 2 cm in length, 0.7 – 1 cm in width; externally brown to dark brown, with numerous longitudinal, knob-like protruding lines; pericarp 0.3 – 0.5 mm in thickness, closely adhering to the seed mass, and difficult to separate; inside divided vertically into three loculi by thin membranes, each loculus containing 5 to 8 seeds adhering by aril; seeds irregularly polygonal, about 3.5 mm in diameter, brown to dark brown in color, and hard in texture.

Odor, characteristic; taste, slightly bitter.

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 2.5%.

Essential oil content ⟨*5.01*⟩ Perform the test with 50.0 g of pulverized Bitter Cardamon: the volume of essential oil is not less than 0.4 mL.

Containers and storage Containers—Well-closed containers.

Bitter Orange Peel

Aurantii Pericarpium

トウヒ

Bitter Orange Peel is the pericarp of the ripe fruit of *Citrus aurantium* Linné or *Citrus aurantium* Linné var. *daidai* Makino (*Rutaceae*).

Description Usually quartered sections of a sphere, sometimes warped or flattened, 4 – 8 cm in length, 2.5 – 4.5 cm in width and 0.5 – 0.8 cm in thickness; the outer surface is dark red-brown to grayish yellow-brown, with numerous small dents associated with oil sacs; the inner surface is white to light grayish yellow-red, with irregular indented reticulation left by vascular bundles; light and brittle in texture.

Odor, characteristic aroma; taste, bitter, somewhat mucilaginous and slightly pungent.

Identification To 1.0 g of Bitter Orange Peel add 10 mL of ethanol (95), allow to stand for 30 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: one of the spot among the several spots from the sample solution and a grayish green spot from the standard solution show the same color tone and the same *R*f value.

Loss on drying ⟨*5.01*⟩ Not more than 14.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 5.5%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Essential oil content ⟨*5.01*⟩ Perform the test with 50.0 g of pulverized Bitter Orange Peel provided that 1 mL of silicon resin is previously added to the test sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage Containers—Well-closed containers.

Bitter Tincture

Tinctura Amara

苦味チンキ

Method of preparation

Bitter Orange Peel, in coarse powder	50 g
Swertia Herb, in coarse powder	5 g
Japanese Zanthoxylum Peel, in coarse powder	5 g
70 vol% Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 70 vol% Ethanol.

Description Bitter Tincture is a yellow-brown liquid. It has a characteristic aroma and a bitter taste.

Specific gravity d_{20}^{20}: about 0.90

Identification **(1)** To 1 mL of Bitter Tincture add 5 mL of methanol, then add 0.1 g of magnesium in ribbon form and 1 mL of hydrochloric acid, and allow to stand: the solution is red-purple in color.

(2) Use Bitter Tincture as the sample solution. Separately, to 5.0 g of pulverized Bitter Orange Peel add 100 mL of diluted ethanol (7 in 10), stopper the vessel tightly, shake for 30 minutes, filter, and use the filtrate as the standard solution (1). Proceed with 0.5 g each of pulverized Swertia Herb and Japanses Zanthoxylum Peel in the same manner, and use the solutions so obtained as the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1), (2) and (3) on the plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (broad spectrum wavelength): three of the several spots from the sample solution show the same color tone and *R*f value as those of the upper spot of the two bright blue to purple spots among the several spots from the standard solution (1), appearing close to each other at an *R*f value of about 0.4, and a bright red spot from the standard solution (2), appearing at an *R*f value of about 0.35, and a bright grayish red to red spot from the standard solution (3), appearing at an *R*f value of about 0.7.

Alcohol number <*1.01*> Not less than 6.9 (Method 2).

Containers and storage Containers—Tight containers.

Bofutsushosan Extract

防風通聖散エキス

Bofutsushosan Extract contains not less than 9 mg and not more than 36 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 4 mg and not more than 12 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 54 mg and not more than 162 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 16 mg and not more than 48 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)	6)
Japanese Angelica Root	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Peony Root	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Cnidium Rhizome	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Gardenia Fruit	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Forsythia Fruit	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Mentha Herb	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Ginger	0.3 g	0.3 g	0.4 g	0.4 g	1.2 g	0.3 g
Schizonepeta Spike	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Saposhnikovia Root and Rhizome	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	—
Glehnia Root and Rhizome	—	—	—	—	—	1.2 g
Ephedra Herb	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g	1.2 g
Rhubarb	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Sodium Sulfate	—	1.5 g	—	1.5 g	—	—
Anhydrous Sodium Sufate	0.7 g	—	0.75 g	—	1.5 g	0.75 g
Atractylodes Rhizome	2 g	2 g	2 g	2 g	2 g	2 g
Platycodon Root	2 g	2 g	2 g	2 g	2 g	2 g
Scutellaria Root	2 g	2 g	2 g	2 g	2 g	2 g
Glycyrrhiza	2 g	2 g	2 g	2 g	2 g	2 g
Gypsum	2 g	2 g	2 g	2 g	2 g	2 g
Aluminum Silicate Hydrate with Silicon Dioxide	3 g	3 g	3 g	3 g	3 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 6), using the crude drugs shown above.

Description Bofutsushosan Extract is a yellow-brown to brown powder or blackish brown viscous extract. It has a slightly odor and a sweet and slightly bitter taste.

Identification (1) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, separate the diethyl ether layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a

mixture of butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 1 minute: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to purple spot obtained from the standard solution (Peony Root).

(3) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfric acid TS on the plate, and heat at 105°C for 1 minute: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to purple spot obtained from the standard solution (Gardenia Fruit).

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 1.0 g of pulverized forsythia fruit add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution as bands on the original line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (10:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple spot (*R*f value: about 0.4) obtained from the standard solution (Forsythia Fruit).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, shake 0.2 g of pulverized mentha herb with 10 mL of diluted phosphoric acid (1 in 30), add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water, and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-brown spot (*R*f value: around 0.4) obtained from the standard solution (Mentha Herb).

(6) Perform the test according to the following (i) or (ii) (Ginger).

(i) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, allow to cool, and spray water: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution.

(ii) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, allow to cool, and spray water: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution.

(7) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of 0.1 mol/L hydrochloric acid TS, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 1 mL of methanol, and use the solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (60:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS: one of the spot among the several

spots obtained from the sample solution has the same color tone and *R*f value with the greenish brown spot obtained from the standard solution (Schizonepeta Spike; Mentha Herb).

(8) For preparation prescribed Saposhnikovia Root and Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glycosyl-5-*O*-methylvisamminol in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 2 minutes, then examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Saposhnikovia Root and Rhizome).

(9) For preparation prescribed Glehnia Root and Rhizome—To 0.5 g of the dry extract (or 1.5 g of the viscous extract) add 5 mL of ethyl acetate, and heat on a water bath under a reflux condenser for 30 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of scopoletin for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly diluted sulfuric acid, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Glehnia Root and Rhizome).

(10) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 15 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at about 0.5 of *R*f value (Ephedra Herb).

(11) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

(12) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air- dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-purple spot obtained from the standard solution (Atractylodes Rhizome).

(13) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium carbonate TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 2.0 g of pulverized platycodon root add 10 mL of sodium carbonate TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot (*R*f value: about 0.4) obtained from the standard solution (Platycodon Root).

(14) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, centrifuge, then add 25 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot obtained from the standard solution (Scutellaria Root).

(15) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use

this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes, then examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot obtained from the standard solution (Glycyrrhiza).

(16) Place 2.0 g of the dry extract (or 6.0 g of the viscous extract) in a porcelain crucible, ignite to incinerate at 550°C, then to the residue add 60 mL of water, shake, centrifuge, and use the supernatant as the sample solution. Add ammonium oxalate TS to the sample solution: a white precipitate is formed. The precipitate does not dissolve in diluted acetic acid, but dissolve on the addition of diluted hydrochloric acid (Gypsum).

(17) Place 2.0 g of the dry extract (or 6.0 g of the viscous extract) in a porcelain crucible, ignite to incinerate at 550°C. To the residue add 60 mL of water, shake well, centrifuge, and use the supernatant as the sample solution. The sample solution responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sulfate (Gypsum; Sodium Sulfate or Anhydrous Sodium Sulfate).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying ⟨*2.41*⟩ The dry extract: Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash ⟨*5.01*⟩ Not less than 10.0% and more than 22.0%, calculated on the dried basis.

Assay **(1)** Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, elute through a column packed with 2 g of polyamide for column chromatography using 20 mL of water, then add 1 mL of acetic acid (100), add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$)
$= M_S \times A_T/A_S \times 5/8$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(2) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine all the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drug, previously dried at 105°C for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained with the sample solution, and the peak area, A_S, of ephedrine obtained with the standard solution.

Amount (mg) of total alkaloids [ephedrine($C_{10}H_{15}NO$) and pseudoephedrine($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drug taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drug and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(3) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(4) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, and centrifuge. Separate the supernatant liquid, combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Boiogito Extract

防已黄耆湯エキス

Boiogito Extract contains not less than 4 mg and not more than 16 mg of shinomenine, and not less than 12 and not more than 36 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Sinomenium Stem and Rhizome	5 g	5 g	5 g
Astragalus Root	5 g	5 g	5 g
Atractylodes Rhizome	3 g	3 g	—
Atractylodes Lancea Rhizome	—	—	3 g
Ginger	0.8 g	1 g	1 g
Jujube	3 g	3 g	3 g
Glycyrrhiza	1.5 g	1.5 g	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above. Or, prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive, prepared as directed under Extracts, according to the prescription 3), using the crude drugs shown above.

Description Boiogito Extract is a light yellow-brown to reddish brown powder or blackish brown viscous extract. It has a slightly odor, and a sweet taste at first and then a slight hot and bitter taste later.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To this liquid add 10 mL of 1-butanol, shake, centrifuge, and separate 1-butanol layer. To this liquid add 10 mL of water, shake, centrifuge, separate the 1-butanol layer, then evaporate the solvent under reduced pressure, dissolve the residue in 1 mL of methanol, and use the solution as the sample solution. Separately, dissolve 1 mg of sinomenine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-brown spot obtained from the standard solution (Sinomenium Stem and Rhizome).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, centrifuge, and separate the supernatant liquid. To this liquid add 10 mL of 1-butanol, shake, centrifuge, and separate 1-butanol layer. To the aqueous layer add 10 mL of 1-butanol, and proceed in the same manner as above. Combine the 1-butanol layers, add 10 mL of water, shake, centrifuge, separate the 1-butanol layer, and evaporate the solvent under reduced pressure. Dissolve the residue in exactly 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of astragaloside IV for thin-layer chromatography in exactly 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-brown spot obtained from the standard solution, and the spot is larger and more ihtense than the spot from the standard solution (Astragalus Root).

(3) For preparation prescribed Atractylodes Rhizome—To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of Atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-purple spot obtained from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under reduced pressure, then dissolve the residue in 0.5 mL of hexane, and use the solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark violet spot is observed at an *R*f value of about 0.4, and this spot exhibits greenish brown when the plate is sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, allow to cool, and spray water: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution (Ginger).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with

these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract: Not more than 11.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.01> Not less than 8.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 9.0% and 18.0%.

Assay **(1)** Sinomenine—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 5.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes, centrifuge, and remove the upper layer. Add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 5.0 mL of diluted sodium hydroxide TS (1 in 10) and 10 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of sinomenine for assay, previously dried in a desiccator (silica gel) for 24 hours or more, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of sinomenine in each solution.

$$\text{Amount (mg) of sinomenine} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of sinomenine for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: To 3 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention time of sinomenine is about 18 minutes).

System suitability—

System performance: When the procedure is run according to the conditions above with 10 μL each of the sample solution, the standard solution of sinomenine, and the standard solution of glycyrrhizic acid obtained in Assay (2), peaks of sinomenine and glycyrrhizic acid are observed in the sample solution, glycyrrhizic acid and sinomenine are eluted in this order, and the resolution between these peaks is not less than 4.5. Furthermore, except for the peak of glycyrrhizic acid, distinct peaks are observed before and after of the peak of sinomenine, and the resolutions between sinomenine and these peaks are respectively not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sinomenine is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Brown Rice

Oryzae Fructus

コウベイ

Brown Rice is the caryopsis of *Oryza sativa* Linné (*Gramineae*).

Description Brown Rice occurs as ellipsoidal, slightly flattened, 4 – 6 mm in length; externally translucent, light yellowish white to light brown. Slightly cave in and a white embryo at one end; a brown small dent of scar of style at the other end; few longitudinally striates on the surface.

Odor, slight; taste, slightly sweet.

Under a microscope <*5.01*>, a transverse section of the caryopsis reveals the outermost layer composed of pericarp; vascular bundles in the pericarp; seed coat adhering closely to the pericarp; in the interior, 1 or 2 aleuron layers; parenchymatous cells of endosperm contain simple or compound starch grains.

Identification **(1)** To 0.1 g of pulverized Brown Rice add 50 mL of water, and heat in a water bath for 5 minutes. After cooling, add 1 drops of iodine TS, and shake: a blue-purple color develops.

(2) To 1 g of pulverized Brown Rice add 5 mL of ethyl acetate, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of cycloartenyl ferulate for thin-layer chromatography in 1 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (5:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple fluorescent spot obtained from the standard solution.

Total ash <*5.01*> Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Bupleurum Root

Bupleuri Radix

サイコ

Bupleurum Root is the root of *Bupleurum falcatum* Linné (*Umbelliferae*).

It contains not less than 0.35% of the total saponin (saikosaponin a and saikosaponin d), calculatd on the basis of dried material.

Description Single or branched root of long cone or column shape, 10 – 20 cm in length, 0.5 – 1.5 cm in diameter; occasionally with remains of stem on the crown; externally light brown to brown and sometimes with deep wrinkles; easily broken, and fractured surface somewhat fibrous.

Odor, characteristic, and taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals the thickness of cortex reaching 1/3 ~ 1/2 of the radius, tangentially extended clefts in cortex; and cortex scattered with a good many oil canals 15 – 35 μm in diameter; in xylem, vessels lined radially or stepwise, and fiber groups scattered; in the pith at the crown, the same oil canals as in the cortex; parenchyma cells containing starch grains and oil droplets. Starch grains composed of simple grains, 2 – 10 μm in diameter, or compound grains.

Identification **(1)** Shake vigorously 0.5 g of pulverized Bupleurum Root with 10 mL of water: lasting fine foams are produced.

(2) To 1.0 g of the pulverized Bupleurum Root, add 10 mL of methanol, and boil gently under a reflux condenser on a water bath for 15 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of saikosaponin a for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the gray-brown spot from the standard solution, accompanied by the adjacent yellow-red spot above.

Purity **(1)** Stem and leaf—When perform the test of foreign matter <*5.01*>, the amount of the stems and leaves contained in Bupleurum Root does not exceed 10.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Bupleurum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Bupleurum Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than stems and leaves contained in Bupleurum Root does not exceed 1.0%.

Loss on drying <*5.01*> Not more than 12.5% (6 hours).

Assay Weigh accurately about 1 g of pulverized Bupleurum Root, transfer in a glass-stoppered centrifuge tube, add 20 mL of diluted methanol (9 in 10), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Perform the same procedure with the precipitate using two 15-mL potions of diluted methanol (9 in 10), combine whole supernatant liquids, and add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add 2.5 mL of dilute sodium hydroxide TS, heat in a water bath at 50°C for 1 hour, and add 7.5 mL of phosphate buffer solution for assay of bupleurum root. Allow this solution to flow through a chromatographic column [about 10 mm inside diameter containing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μm in particle diameter), conditioned with 10 mL of methanol then 10 mL of water just before use]. Wash the column with 10 mL of diluted methanol (7 in 20), then flow with methanol to get exactly 10 mL of effluent solution, and use this as the sample solution. Separately, weigh accurately each about 10 mg of saikosaponin a for assay and saikosaponin d for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and

standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TA} and A_{SA}, of saikosaponin a and A_{TD} and A_{SD}, of saikosaponin d. Calculate the amount of saikosaponin a and saikosaponin d by the following equation.

Amount (mg) of saikosaponin a = $M_{SA} \times A_{TA}/A_{SA} \times 1/2$

M_{SA}: Amount (mg) of saikosaponin a for assay taken

Amount (mg) of saikosaponin d = $M_{SD} \times A_{TD}/A_{SD} \times 1/2$

M_{SD}: Amount (mg) of saikosaponin d for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 206 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of saikosaponin a is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order, and the numbers of theoretical plates and the symmetry factors of their peaks are not less than 4000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of saikosaponin a and saikosaponin d are not more than 1.5%, respectively.

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 11.0%.

Containers and storage Containers—Well-closed containers.

Burdock Fruit

Arctii Fructus

ゴボウシ

Burdock Fruit is the fruit of *Arctium lappa* Linné (*Compositae*).

Description Burdock Fruit is slightly curved, long obovate achene, 5 – 7 mm in length, 2.0 – 3.2 mm in width, 0.8 to 1.5 mm in thickness; externally grayish brown to brown, with black spots; hollow about 1 mm in diameter at one broad end; flat, indistinct, longitudinal ridge at the other narrow end. 100 fruits weigh 1.0 – 1.5 g.

Practically odorless; taste, bitter and oily.

Under a microscope <5.01>, transverse section reveals an exocarp of single-layered epidermal tissue, mesocarp of slightly sclerified parenchyma, and endocarp of a single layer of stone cells; seed coat composed of radially elongated, sclerified epidermis, and parenchyma several cells thick; parenchymatous cells of the mesocarp contain a brown substance; stone cells of endocarp contain solitary, discrete crystals of calcium oxalate; cotyledons with starch grains, oil drops, aleurone grains, and minute crystals of calcium oxalate.

Identification To 0.5 g of pulverized Burdock Fruit add 20 mL of methanol, shake for 10 minutes, filter, and use filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate and water (15:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.4.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Cacao Butter

Oleum Cacao

カカオ脂

Cacao Butter is the fat obtained from the seed of *Theobroma cacao* Linné (*Sterculiaceae*).

Description Cacao Butter occurs as a yellowish white, hard, brittle mass. It has a slight, chocolate-like odor, and has no odor of rancidity.

It is freely soluble in diethyl ether and in petroleum ether, soluble in boiling ethanol (99.5), and very slightly soluble in ethanol (95).

Congealing point of the fatty acids: 45 – 50°C

Melting point 31 – 35°C (Cram the sample into a capillary tube without melting the sample).

Specific gravity <1.13> d_{20}^{40}: 0.895 – 0.904

Acid value <1.13> Not more than 3.0.

Saponification value <1.13> 188 – 195

Iodine value <1.13> 35 – 43

Containers and storage Containers—Well-closed containers.

Calumba

Calumbae Radix

コロンボ

Calumba is the cross-sectioned root of *Jateorhiza columba* Miers (*Menispermaceae*).

Description Disk-like slices, 0.5 – 2 cm in thickness, 3 – 8 cm in diameter; mostly with concave center and slightly waved; side surface grayish brown in color, with irregular

wrinkles; cut surface light yellow and powdery, with pale and dark radiating stripes; cortex rather yellowish; cambium and its neighborhood light grayish brown, warty protrusions in the center; hard in texture, but brittle.

Odor characteristic; taste, bitter.

Identification To 3 g of pulverized Calumba add 30 mL of water, allow to stand for 5 minutes with occasional shaking, and filter. To 2 mL of the filtrate add gently 1 mL of sulfuric acid, and after cooling, add carefully chlorine TS to make two layers: a light red to red color develops at the zone of contact.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Calumba according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Calumba according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 7.5%.

Containers and storage Containers—Well-closed containers.

Powdered Calumba

Calumbae Radix Pulverata

コロンボ末

Powdered Calumba is the powder of Calumba.

Description Powdered Calumba occurs as a grayish yellow powder, and has a characteristic odor and a bitter taste.

Under a microscope <*5.01*>, Powdered Calumba reveals numerous starch grains, fragments of parenchyma cells containing them; fragments of cork cells, stone cells, fibers, substitute fibers, vessels, tracheids, and also solitary crystals of calcium oxalate; starch grains consisting of solitary grains or 2- to 3-compound grains; hilum, unevenly scattered, usually 25 – 50 μm, but up to 90 μm in diameter.

Identification To 3 g of Powdered Calumba add 30 mL of water, allow to stand for 5 minutes with occasional shaking, and filter. To 2 mL of the filtrate add gently 1 mL of sulfuric acid, and after cooling, add carefully chlorine TS to make two layers: a light red to red color develops at the zone of contact.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Calumba according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Calumba according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 7.5%.

Containers and storage Containers—Well-closed containers.

Camellia Oil

Oleum Camelliae

ツバキ油

Camellia Oil is the fixed oil obtained from the peeled seeds of *Camellia japonica* Linné (*Theaceae*).

Description Camellia Oil is a colorless or pale yellow, clear oil. It is nearly odorless and tasteless.

It is miscible with diethyl ether and with petroleum ether.

It is slighthy soluble in ethanol (95).

It congeals partly at −10°C, and completely at −15°C.

Specific gravity d_{25}^{25}: 0.910 – 0.914

Identification To 2 mL of Camellia Oil add dropwise 10 mL of a mixture of fuming nitric acid, sulfuric acid, and water (1:1:1), previously cooled to room temperature: a bluish green color develops at the zone of contact.

Acid value <*1.13*> Not more than 2.8.

Saponification value <*1.13*> 188 – 194

Unsaponifiable matters <*1.13*> Not more than 1.0%.

Iodine value <*1.13*> 78 – 83

Containers and storage Containers—Tight containers.

Capsicum

Capsici Fructus

トウガラシ

Capsicum is the fruit of *Capsicum annuum* Linné (*Solanaceae*).

It contains not less than 0.10% of total capsaicins ((*E*)-capsaicin and dihydrocapsaicin), calculated on the basis of dried material.

Description Elongated conical to fusiform fruit, often bent, 3 – 10 cm in length, about 0.8 cm in width; outer surface lustrous and dark red to dark yellow-red; interior of pericarp hollow and usually divided into two loculi, containing numerous seeds nearly circular and compressed, light yellow-red, about 0.5 cm in diameter.

Usually it remains of calyx and peduncle.

Odor, slight and characteristic; taste, hot and acrid.

Identification To 1.0 g of pulverized Capsicum add 5 mL of ethanol (95), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-capsaicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and formic acid (10:9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to an ammonia vapor: a spot obtained from the sample solution and a blue spot obtained from the standard solution show the same color tone and the same *R*f value.

Purity Foreign matter <5.01>—The amount of foreign matter contained in Capsicum does not exceed 1.0%.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 1.2%.

Assay Weigh accurately about 0.5 g of moderately fine powder of Capsicum in a glass-stoppered centrifuge tube, add 30 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of methanol, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure again, combine the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-capsaicin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TC} and A_{TD}, of (*E*)-capsaicin and dihydrocapsaicin (the relative retention time to (*E*)-capsaicin is about 1.3) obtained with the sample solution, and the peak area, A_S, of (*E*)-capsaicin obtained with the standard solution.

$$\text{Amount (mg) of total capsaicins} = M_S \times (A_{TC} + A_{TD})/A_S \times 0.08$$

M_S: Amount (mg) of (*E*)-capsaicin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of (*E*)-capsaicin is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of (*E*)-capsaicin for assay and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and (*E*)-capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (*E*)-capsaicin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Capsicum

Capsici Fructus Pulveratus

トウガラシ末

Powdered Capsicum is the powder of Capsicum.

It contains not less than 0.10% of total capsaicins ((*E*)-capsaicin and dihydrocapsaicin), calculated on the basis of dried material.

Description Powdered Capsicum occurs as a yellow-red powder. It has a slight, characteristic odor and a hot, acrid taste.

Under a microscope <5.01>, Powdered Capsicum reveals fragments of parenchyma containing oil droplets and yellow-red chromoplasts; fragments of epidermis from outer surface of pericarp with thick cuticle; fragments of stone cells from inner surface of pericarp, with wavy curved side walls; fragments of thin vessels; fragments of seed coat with thick wall, and fragments of parenchyma consisting of small cells of endosperm containing fixed oil and aleuron grains.

Identification To 1.0 g of Powdered Capsicum add 5 mL of ethanol (95), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-capsaicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and formic acid (10:9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to an ammonia vapor: a spot obtained from the sample solution and blue spot obtained from the standard solution show the same in color tone and *R*f value.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 1.2%.

Assay Weigh accurately about 0.5 g of Powdered Capsicum in a glass-stoppered centrifuge tube, add 30 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of methanol, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure again, combine the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-capsaicin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TC} and A_{TD}, of (*E*)-capsaicin and dihydrocapsaicin (the relative retention time to (*E*)-capsaicin is about 1.3) obtained with the sample solution, and the peak area, A_S, of (*E*)-capsaicin obtained with the standard solution.

$$\text{Amount (mg) of total capsaicins} = M_S \times (A_{TC} + A_{TD})/A_S \times 0.08$$

M_S: Amount (mg) of (*E*)-capsaicin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of (*E*)-capsaicin is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of (*E*)-capsaicin for assay and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and (*E*)-capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (*E*)-capsaicin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Capsicum Tincture

トウガラシチンキ

Capsicum Tincture contains not less than 0.010 w/v% of total capsaicins ((*E*)-capsaicin and dihydrocapsaicin).

Method of preparation

Capsicum, in moderately fine cutting	100 g
Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Tinctures, with the above ingredients.

Description Capsicum Tincture is a yellow-red liquid. It has a burning, pungent taste.

Specific gravity d_{20}^{20}: about 0.82

Identification Proceed as directed in the Identification under Capsicum, using Capsicum Tincture as the sample solution. Spot 20 μL each of the sample solution and the standard solution.

Alcohol number <*1.01*> Not less than 9.7 (Method 2).

Assay Pipet 2 mL of Capsicum Tincture, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-capsaicin for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TC} and A_{TD}, of (*E*)-capsaicin and dihydrocapsaicin (the relative retention time to (*E*)-capsaicin is about 1.3) obtained with the sample solution, and the peak area, A_S, of (*E*)-capsaicin obtained with the standard solution.

$$\text{Amount (mg) of total capsaicins} = M_S \times (A_{TC} + A_{TD})/A_S \times 0.032$$

M_S: Amount (mg) of (*E*)-capsaicin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of (*E*)-capsaicin is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of (*E*)-capsaicin for assay and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and (*E*)-capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of (*E*)-capsaicin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Capsicum and Salicylic Acid Spirit

トウガラシ·サリチル酸精

Method of preparation

Capsicum Tincture	40 mL
Salicylic Acid	50 g
Liquefied Phenol	20 mL
Castor Oil	100 mL
aromatic substance	a suitable quantity
Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Spirits, with the above ingredients.

Description Capsicum and Salicylic Acid Spirit is a light brown-yellow liquid.

Specific gravity d_{20}^{20}: about 0.84

Identification **(1)** Shake 10 mL of Capsicum and Salicylic Acid Spirit with 15 mL of sodium hydrogen carbonate TS and 10 mL of diethyl ether, and separate the water layer. To 1 mL of the solution add hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(2) To 0.5 mL of Capsicum and Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 20 mL of diethyl ether, wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and then extract with 20 mL of dilute sodium hydroxide TS. To 1 mL of the extract add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and allow to stand for 10 minutes. Add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(3) To 0.2 mL of Capsicum and Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution. Dissolve 0.01 g of salicylic acid and 0.02 g of phenol in 5 mL and 25 mL of chloroform, respectively, and use both solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same *R*f values as those from standard solution (1) and standard solution (2). Spray evenly iron (III) chloride TS upon the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Alcohol number <*1.01*> Not less than 8.1 (Method 2). Prepare the sample solution as follows: Pipet 5 mL of Capsicum and Salicylic Acid Spirit at 15 ± 2°C into a glass-stoppered, conical flask containing exactly 45 mL of water while shaking vigorously, allow to stand, and filter the lower layer. Discard the first 15 mL of the filtrate. Pipet 25 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, and add water to make exactly 100 mL.

Containers and storage Containers—Tight containers.

Cardamon

Cardamomi Fructus

ショウズク

Cardamon is the fruit of *Elettaria cardamomum* Maton (*Zingiberaceae*). The capsules are removed from the seeds before use.

Description Nearly ellipsoidal, 1 – 2 cm in length, 0.5 – 1 cm in diameter; externally, light yellow with three blunt ridges and many longitudinal lines; 0.1 – 0.2-cm beak at one end; pericarp thin, light and fibrous; interior longitudinally divided into three loculi by thin membranes, each loculus containing 3 to 7 seeds joining by aril; seed irregularly angular ovoid, 0.3 – 0.4 cm in length, dark brown to blackish brown; the dorsal side convex, the ventral side longitudinally grooved; external surface coarsely tuberculated.

Seed has a characteristic aroma, and pungent, slightly bitter taste; pericarp, odorless and tasteless.

Total ash <*5.01*> Not more than 6.0% (seed).

Acid-insoluble ash <*5.01*> Not more than 4.0% (seed).

Essential oil content <*5.01*> Perform the test with 30.0 g of the pulverized seeds of Cardamon: the volume of essential oil is not less than 1.0 mL.

Containers and storage Containers—Well-closed containers.

Carnauba Wax

Cera Carnauba

カルナウバロウ

Carnauba Wax is the wax obtained from the leaves of *Copernicia cerifera* Mart (*Palmae*).

Description Carnauba Wax occurs as light yellow to light brown, hard and brittle masses or white to light yellow powder. It has a slight, characteristic odor. It is tastelss.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in xylene.

Specific gravity d_{20}^{20}: 0.990 – 1.002

Melting point: 80 – 86°C

Acid value <*1.13*> Not more than 10.0. Use a mixture of xylene and ethanol (95) (2:1) as solvent.

Saponification value <*1.13*> 78 – 95 Weigh accurately about 3 g of Carnauba Wax in a 300-mL flask, add 25 mL of xylene, and dissolve by warming. To this solution add 50 mL of ethanol (95) and exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and proceed as directed in the Saponification value. The time of heating should be 2 hours and the titration should be done by warming.

Iodine value <*1.13*> 5 – 14 (Dissolve the sample by shaking a glass-stoppered flask in warm water.)

Containers and storage Containers—Well-closed containers.

Cassia Seed

Cassiae Semen

ケツメイシ

Cassia Seed is the seed of *Cassia obtusifolia* Linné or *Cassia tora* Linné (*Leguminosae*).

Description Short cylindrical seed, 3 – 6 mm in length, 2 – 3.5 mm in diameter; acuminate at one end and flat at the other; externally green-brown to brown and lustrous, with light yellow-brown longitudinal lines or bands on both sides; hard in texture; cross section round or obtuse polygonal; under a magnifying glass, albumen enclosing a bent, dark-colored cotyledon.

When ground, characteristic odor and taste.

Identification Place 0.1 g of pulverized Cassia Seed, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both internal diameter and height on it, then cover with moistened filter paper, and heat gently the slide glass over a small flame. Take off the filter paper when a yellow color has developed on the upper surface of it, and place 1 drop of potassium hydroxide TS on the surface of the filter paper where a sublimate is present: a red color appears.

Purity Foreign matter <*5.01*>—The amount of foreign mat-

ter contained in Cassia Seed does not exceed 1.0%.

Total ash ⟨*5.01*⟩ Not more than 5.0%.

Containers and storage Containers—Well-closed containers.

Castor Oil

Oleum Ricini

ヒマシ油

Castor Oil is the fixed oil obtained by compression from the seeds of *Ricinus communis* Linné (*Euphorbiaceae*).

Description Castor Oil is a colorless or pale yellow, clear, viscous oil. It has a slight, characteristic odor, and has a bland at first, and afterwards slightly acrid taste.

It is miscible with ethanol (99.5) and with diethyl ether.

It is freely soluble in ethanol (95), and practically insoluble in water.

When cooled to 0°C, it becomes more viscous, and turbidity is gradually formed.

Identification To 3 g of Castor Oil add 1 g of potassium hydroxide, and heat the mixture carefully to fuse: a characteristic odor is perceptible. Dissolve the fused matter in 30 mL of water, add an excess of magnesium oxide, and filter. Acidify the filtrate with hydrochloric acid: white crystals is produced.

Specific gravity ⟨*1.13*⟩ d_{25}^{25}: 0.953 – 0.965

Acid value ⟨*1.13*⟩ Not more than 1.5.

Saponification value ⟨*1.13*⟩ 176 – 187

Hydroxyl value ⟨*1.13*⟩ 155 – 177

Iodine value ⟨*1.13*⟩ 80 – 90

Purity Adulteration—Shake to mix 1.0 g of Castor Oil with 4.0 mL of ethanol (95): it dissolves clearly. Add 15 mL of ethanol (95): no turbidity is produced.

Containers and storage Containers—Tight containers.

Aromatic Castor Oil

加香ヒマシ油

Method of preparation

Castor Oil	990 mL
Orange Oil	5 mL
Mentha Oil	5 mL
To make	1000 mL

Mix the above ingredients.

Description Aromatic Castor Oil is a colorless or yellowish, clear, viscous liquid. It has an aromatic odor.

Identification To 3 g of Aromatic Castor Oil add 1 g of potassium hydroxide, and heat the mixture carefully to fuse: a characteristic odor is perceptible. Dissolve the fused matter in 30 mL of water, add an excess of magnesium oxide, and filter. Acidify the filtrate with hydrochloric acid: white crystals are produced.

Containers and storage Containers—Tight containers.

Catalpa Fruit

Catalpae Fructus

キササゲ

Catalpa Fruit is the fruit of *Catalpa ovata* G. Don or *Catalpa bungei* C. A. Meyer (*Bignoniaceae*).

Description Slender stick-like fruit, 30 – 40 cm in length and about 0.5 cm in diameter; externally, dark brown; inner part contains numerous seeds; seed compressed or semitubular, about 3 cm in length and about 0.3 cm in width, externally grayish brown; hairs, about 1 cm in length, attached to both ends of seed; pericarp, thin and brittle.

Odor, slight; taste, slightly astringent.

Identification To 1.0 g of pulverized Catalpa Fruit add 20 mL of water, warm on a water bath for 5 minutes, and filter immediately. Transfer the filtrate to a separator, and extract with two 20-mL portions of 1-butanol. Combine the extracts, evaporate to dryness under reduced pressure on a water bath, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of parahydroxybenzoic acid in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (20:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultra-violet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value. Prescribe that the moving distance of the spot corresponding to parahydroxybenzoic acid from the sample solution is 1: a dark purple spot develops at the relative moving distance of about 0.3.

Purity Peduncle—When perform the test of foreign matter ⟨*5.01*⟩, the amount of peduncles contained in Catalpa Fruit does not exceed 5.0%.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Cherry Bark

Pruni Cortex

オウヒ

Cherry Bark is the bark of *Prunus jamasakura* Siebold ex Koidzumi or *Prunus verecunda* Koehne (*Rosaceae*).

Description Flat or semi-tubular pieces of bark; 3 – 6 mm thick, externally light brown to brown, internal surface

smooth, grayish brown to brown, occasionally periderm peeled off; the bark with periderm externally rough and lenticels observed; internal surface with many fine longitudinal lines; transversely cut surface grayish brown to brown, fibrous.

Odor, slightly characteristics; taste, slightly bitter and astringent.

Under a microscope <*5.01*>, a transverse section reveals cork layer containing solitary crystals and rosette aggregates of calcium oxalate in the bark with periderm; in cortex many stone cells and idioblasts arranged irregularly and parenchyma cells containing solitary crystals and rosette aggregates of calcium oxalate dotted; groups of phloem fibers lined alternately with the other tissue of phloem between rays.

Identification Shake 1 g of pulverized Cherry Bark with 10 mL of dilute hydrochloric acid, and heat in a boiling water bath for 10 minutes. After cooling, add 5 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the diethyl ether layer as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (20:20:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a crimson spot appears at an *R*f value of about 0.5.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Chotosan Extract

釣藤散エキス

Chotosan Extract contains not less than 24 mg and not more than 72 mg of hesperidin, not less than 8 mg and not more than 24 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 0.3 mg of the total alkaloid (rhyncophylline and hirsutine), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Uncaria Hook	3 g	3 g
Citrus Unshiu Peel	3 g	3 g
Pinellia Tuber	3 g	3 g
Ophiopogon Root	3 g	3 g
Poria Sclerotium	3 g	3 g
Ginseng	2 g	3 g
Saposhnikovia Root and Rhizome	2 g	3 g
Chrysanthemum Flower	2 g	3 g
Glycyrrhiza	1 g	1 g
Ginger	1 g	1 g
Gypsum	5 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Chotosan Extract is a light brown to yellow-brown powder or blackish brown viscous extract. It has a slight odor, and has a pungent and slightly sweet first, then bitter taste.

Identification **(1)** Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 20 mL of water and 2 mL of ammonia TS, and then shake with 20 mL of diethyl ether, separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg each of rhyncophylline for thin-layer chromatography and hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with one of the two dark purple spots obtained from the standard solution (Uncaria Hook).

(2) Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, allow to stand in an ammonia gas: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue spot obtained from the standard solution (Citrus Unshiu Peel).

(3) Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-butanol and shake, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the sample solution. Separately, heat 3.0 g of ophiopogon root in 50 mL of water under a reflux condenser for 1 hour. After cooling, shake 20 mL of the extract with 5 mL of 1-butanol, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL of the sample solution and 5 μL of the standard solution as bands on original line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water and acetic acid (100) (120:80:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark blue-green spot (around *R*f value 0.3) obtained from the standard solution (Ophiopogon Root).

(4) Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginseno-

side Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(5) Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glycosyl-5-*O*-methylvisamminol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue spot obtained from the standard solution (Saposhnikovia Root and Rhizome).

(6) Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 20 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 3 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and formic acid (5:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Chrysanthemum Flower).

(7) Shake 2.0 g of a dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(8) Shake 1.0 g of a dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple spot obtained from the standard solution (Ginger).

(9) Shake 1.0 g of a dry extract (or 3.0 g of the viscous extract) with 30 mL of methanol, centrifuge, and separate the supernatant liquid. Shake the residue with 30 mL of water, centrifuge, and separate the supernatant liquid. Add ammonium oxalate TS to this solution: a white precipitate is formed, and it does not dissolve by addition of dilute acetic acid, but it dissolve by addition of dilute hydrochloric acid. (Gypsum)

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 7.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 15.0%, calculated on the dried basis.

Assay **(1)** Hesperidin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of hesperidin in each solution.

$$\text{Amount (mg) of hesperidin} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of hesperidin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hespeidin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hesperidin is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glychrrhizic Acid RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glychrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glychrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glychrrhizic acid is not more than 1.5%.

(3) Total alkaloid (rhyncophylline and hirsutine)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of dried substance), add 20 mL of diethyl ether, shake, add 3 mL of 1 mol/L hydrochloric acid TS and 7 mL of water, shake for 10 minutes, centrifuge, and separate the ether layer. To the aqueous layer add 20 mL of diethyl ether, and repeat the above process. To the aqueous layer add 10 mL of sodium hydroxide TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat the above process twice more with the residue using 20 mL portion of diethyl ether. Combine all supernatant liquids, evaporate to dryness under reduced pressure at not more than 40°C, and dissolve the residue in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhyncophylline for assay and about 5 mg of hirsutine for assay, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of rhyncophylline and hirsutine, A_{TR} and A_{TH}, and A_{SR} and A_{SH}, in each solution.

Amount (mg) of the total alkaloid (rhyncophylline and hirsutine)
$= M_{SR} \times A_{TR}/A_{SR} \times 1/50 + M_{SH} \times A_{TH}/A_{SH} \times 1/50$

M_{SR}: Amount (mg) of rhyncophylline for assay taken
M_{SH}: Amount (mg) of hirsutine for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 5 g of sodium lauryl sulfate in 1150 mL of acetonitrile and 1350 mL of water, mix with 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention time of rhyncophylline is about 12 minutes and that of hirsutine is about 27 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rhyncophylline and hirsutine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of rhyncophylline and hirsutine are not more than 1.5%, respectively.

Containers and storage Containers—Tight containers.

Chrysanthemum Flower

Chrysanthemi Flos

キクカ

Chrysanthemum Flower is the capitulum of 1) *Chrysanthemum morifolium* Ramatulle or 2) *Chrysanthemum indicum* Linné (*Compositae*).

Description 1) *Chrysanthemum morifolium* origin—Capitulum, 15 – 40 mm in diameter; involucre consisting of 3 to 4 rows of involucral scales; the outer involucral scale

linear to lanceolate, inner involucral scale narrow ovate to ovate; ligulate flowers are numerous, white to yellow; tubular flowers in small number, light yellow-brown; tubular flowers occasionally degenerate; outer surface of involucre green-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

2) *Chrysanthemum indicum* origin—Capitulum, 3 – 10 mm in diameter; involucre consisting of 3 to 5 rows of involucral scales; the outer involucral scale linear to lanceolatae, inner involucral scale narrow ovate to ovate; ligulate flower is single, yellow to light yellow-brown; tubular flowers in numerous, light yellow-brown; outer surface of involucre yellow-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

Identification To 1 g of pulverized Chrysanthemum Flower add 20 mL of methanol, shake for 10 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (25:3:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark green spot obtained from the standard solution.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 8.5%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Cimicifuga Rhizome

Cimicifugae Rhizoma

ショウマ

Cimicifuga Rhizome is the rhizome of *Cimicifuga simplex* Turczaninow, *Cimicifuga dahurica* Maximowicz, *Cimicifuga foetida* Linné or *Cimicifuga heracleifolia* Komarov (*Ranunculaceae*).

Description Knotted, irregularly shaped rhizome, 6 – 18 cm in length, 1 – 2.5 cm in diameter; externally dark brown to blackish brown, with many remains of roots, often with scars of terrestrial stems; the center of the scar dented, and the circumference being pale in color and showing a radial pattern; fractured surface fibrous; pith dark brown in color and often hollow; light and hard in texture.

Almost odorless; taste, bitter and slightly astringent.

Identification Dissolve 1 g of pulverized Cimicifuga Rhizome add 5 mL of dilute hydrochloric acid and 5 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Use (*E*)-isoferulic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (30:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue fluorescent spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Cimicifuga Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Cimicifuga Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Rhizome of *Astilbe thunbergii* Miquel—Under a microscope <*5.01*>, pulverized Cimicifuga Rhizome does not contain crystal druses in the parenchyma.

Total ash <*5.01*> Not more than 9.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 18.0%.

Containers and storage Containers—Well-closed containers.

Cinnamon Bark

Cinnamomi Cortex

ケイヒ

Cinnamon Bark is the bark of the trunk of *Cinnamomum cassia* Blume (*Lauraceae*), or such bark from which a part of the periderm has been removed.

Description Usually semi-tubular or tubularly rolled pieces of bark, 0.1 – 0.5 cm in thickness, 5 – 50 cm in length, 1.5 – 5 cm in diameter; the outer surface dark red-brown, and the inner surface red-brown and smooth; brittle; the fractured surface is slightly fibrous, red-brown, exhibiting a light brown, thin layer.

Characteristic aroma; taste, sweet and pungent at first, later rather mucilaginous and slightly astringent.

Under a microscope <*5.01*>, a transverse section of Cinnamon Bark reveals a primary cortex and a secondary cortex divided by an almost continuous ring consisting of stone cells; nearly round bundles of fibers in the outer region of the ring; cell wall of each stone cell often thickened in a U-shape; secondary cortex lacking stone cells, and with a small number of sclerenchymatous fibers coarsely scattered; parenchyma scattered with oil cells, mucilage cells and cells containing starch grains; medullary rays with cells containing fine needles of calcium oxalate.

Identification To 2.0 g of pulverized Cinnamon Bark add 10 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica

gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot develops at an *R*f value of about 0.4. Spray evenly 2,4-dinitrophenylhydrazine TS upon the spot: a yellow-orange color develops.

Purity Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 15.5% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Cinnamon Bark provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.5 mL.

Containers and storage Containers—Well-closed containers.

Powdered Cinnamon Bark

Cinnamomi Cortex Pulveratus

ケイヒ末

Powdered Cinnamon Bark is the powder of Cinnamon Bark.

Description Powdered Cinnamon Bark is red-brown to brown in color. It has a characteristic aroma and a sweet, pungent taste with a slightly mucilaginous and astringent aftertaste.

Under a microscope <*5.01*>, Powdered Cinnamon Bark reveals starch grains, fragments of parenchyma cells containing them; fragments of fibers, oil cells containing yellow-brown oil droplets, stone cells, cork stone cells, cork tissue, and fine crystals of calcium oxalate. Starch grains are simple and compound grains 6 to 20 μm in diameter.

Identification To 2.0 g of Powdered Cinnamon Bark add 10 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot develops at an *R*f value of about 0.4. Spray 2,4-dinitrophenylhydrazine TS upon the spot: a yellow orange color develops.

Purity **(1)** Petiole—Under a microscope <*5.01*>, Powdered Cinnamon Bark does not reveal epidermal cells, hairs, cells containing chlorophyll granules, and fragments of vascular bundle.

(2) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of Powdered Cinnamon Bark provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.35 mL.

Containers and storage Containers—Tight containers.

Cinnamon Oil

Oleum Cinnamomi

ケイヒ油

Cinnamon Oil is the essential oil distilled with steam from the leaves and twigs or bark of *Cinnamomum cassia* Blume or from the bark of *Cinnamomum zeylanicum* Nees (*Lauraceae*).

It contains not less than 60 vol% of the total aldehydes.

Description Cinnamon Oil is a yellow to brown liquid. It has a characteristic, aromatic odor and a sweet, pungent taste.

It is clearly miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

It is weakly acidic. Upon aging or long exposure to air, it darkens and becomes viscous.

Specific gravity d_{20}^{20}: 1.010 – 1.065

Identification Shake 4 drops of Cinnamon Oil with 4 drops of nitric acid: the mixture forms white to light yellow crystals at a temperature below 5°C.

Purity **(1)** Rosin—Mix 1.0 mL of Cinnamon Oil with 5 mL of ethanol (95), then add 3 mL of freshly prepared, saturated ethanol solution of lead (II) acetate trihydrate: no precipitate is produced.

(2) Heavy metals <*1.07*>—Proceed with 1.0 mL of Cinnamon Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Pipet 5.0 mL of Cinnamon Oil into a cassia flask, add 70 mL of sodium hydrogensulfite TS, and heat the mixture in a water bath with frequent shaking to dissolve completely. To this solution add sodium hydrogensulfite TS to raise the lower level of the oily layer within the graduate portion of the neck. Allow to stand for 2 hours, and measure the volume (mL) of the separated oily layer.

Total aldehydes (vol%)
= {5.0 − (volume of separated oily layer)} × 20

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cistanche Herb

Cistanchis Herba

ニクジュヨウ

Cistanche Herb is stout stem of 1) *Cistanche salsa* G. Beck, 2) *Cistanche deserticola* Y. C. Ma or 3) *Cistanche tubulosa* Wight (*Orobanchaceae*), spadix removed in case flowers open.

Description **1)** *Cistanche salsa* origin—Flatly cylindrical, 5 – 25 cm in length, 1 – 2.5 cm in diameter; the one end mostly slightly narrow and curved; external surface brown to blackish brown, covered with thick scales; fleshy and solid,

slightly soft and oily, hardly broken; fractured surface yellow-brown to brown, vascular bundles light brown and arranged in a wavy ring.

Odor, characteristic; taste, slightly sweet, followed by slight bitterness.

Under a microscope <*5.01*> a transverse section of middle part reveals the outermost part is a single layered epidermis coated with cuticle; cortex composed of parenchyma; collateral vascular bundles fusiform or rhombic and arranged in a wavy ring in the inner portion of cortex; groups of cells with slightly thickened cell walls sometimes attached outside of phloem of collateral vascular bundles, and exhibit tail like form; pith composed of parenchyma; parenchyma contains starch grains or gelatinized starch.

2) *Cistanche deserticola* origin—Flatly cylindrical, and approximate to 1), but large in size, 5 – 50 cm in length, 1 – 8 cm in diameter.

Odor, characteristic; taste, slightly sweet, followed by slight bitterness.

Under a microscope <*5.01*> a transverse section of middle part reveals, approximate to 1).

3) *Cistanche tubulosa* origin—Flatly fusiform to cylindrical, slightly curved, 5 – 25 cm in length, 2 – 9 cm in diameter; external surface brown to blackish brown, covered with thick scales; solid in texture and firm, hardly broken; fractured surface light grayish brown to yellow-brown, vascular bundles yellow-white and scattered throughout the surface.

Odor, characteristic; taste, slightly sweet, followed by slight bitterness.

Under a microscope <*5.01*> a transverse section of middle part reveals, approximate to 1) and 2), but collateral vascular bundles distributed throughout the parenchyma from marginal region to the center of transverse section; cells with slightly thickened cell walls observed sometimes around collateral vascular bundles, but exhibit no tail like form;

Identification To 1 g of pulverized Cistanche Herb add 5 mL of water and 5 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of verbascoside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chlolo-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Cistanche Herb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Cistanche Herb according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 20.0%.

Total ash <*5.01*> Not more than 11.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Well-closed containers.

Citrus Unshiu Peel

Citri Unshiu Pericarpium

チンピ

Citrus Unshiu Peel is the pericarp of the ripe fruit of *Citrus unshiu* Marcowicz or *Citrus reticulata* Blanco (*Rutaceae*).

It contains not less than 4.0% of hesperidin, calculated on the basis of dried material.

Description Irregular pieces of pericarp, about 2 mm in thickness; externally yellow-red to dark yellow-brown, with numerous small dents associated with oil sacs; internally white to light grayish yellow-brown; light and brittle in texture.

Odor, characteristic aroma; taste, bitter and slightly pungent.

Identification To 0.5 g of pulverized Citrus Unshiu Peel add 10 mL of methanol, warm on a water bath for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon-form and 1 mL of hydrochloric acid, and allow to stand: a red-purple color develops.

Purity Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 4.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 30.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Citrus Unshiu Peel provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Assay Weigh accurately about 0.1 g of pulverized Citrus Unshiu Peel, add 30 mL of methanol, heat under a reflux condenser on a water bath for 15 minutes, centrifuge after cooling, and separate the supernatant liquid. To the residue add 20 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of hesperidin in each solution.

$$\text{Amount (mg) of hesperidin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of hesperidin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica

gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in 10 mL of methanol, and add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hespiridin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Clematis Root

Clematidis Radix

イレイセン

Clematis Root is the root with rhizome of *Clematis chinensis* Osbeck, *Clematis mandshurica* Ruprecht, or *Clematis hexapetala* Pallas (*Ranunculaceae*).

Description Clematis Root consists of short rhizome and numerous slender roots. The root, 10 – 20 cm in length, 1 – 2 mm in diameter, externally brown to blackish brown, with fine longitudinal wrinkles, brittle. The cortex easily separable from central cylinder; root, grayish white to light yellow-brown in the transverse section, light grayish yellow to yellow in the central cylinder; under a magnifying glass, central cylinder almost round, slight 2 – 4 sinuses on xylem. The rhizome, 2 – 4 cm in length, 5 – 20 mm in diameter, externally light grayish brown to grayish brown; cortex peeled off and fibrous, often with rising node; apex having the residue of lignified stem.

Odor, slight; practically tasteless.

Under a microscope, <*5.01*> transverse section of root reveals a uni-layered epidermis in the outermost layer; with exodermis lying just inside of the epidermis; cortex and stele divided by endodermis; cortex composed of parenchymatous tissue; xylem with 2 – 4 small concavities where phloem is present; parenchymatous cells contain both simple and 2- to 8-compound starch grains.

Identification **(1)** To 0.5 g of pulverized Clematis Root add 10 mL of water, and boil for 2 to 3 minutes. After cooling, shake vigorously: lasting fine foams appear.

(2) To 0.5 g of pulverized Clematis Root add 3 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To the filtrate add 1 mL of sulfuric acid gently: a brown color appears at the zone of contact.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Clematis Root according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Clematis Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 8.5%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Clove

Caryophylli Flos

チョウジ

Clove is the flowering bud of *Syzygium aromaticum* Merrill et Perry (*Eugenia caryophyllata* Thunberg) (*Myrtaceae*).

Description Dark brown to dark red buds, 1 – 1.8 cm in length, consisting of slightly compressed and four-sided receptacle, crowned by 4 thick sepals and 4 nearly spherical, membranous, imbricated petals, enclosing numerous stamens and a single style.

Odor, strong and characteristic; taste, pungent, followed by a slight numbness of the tongue.

Identification Mix 0.1 mL of the mixture of essential oil and xylene, obtained in the Essential oil content, with 2 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green to blue color develops.

Purity **(1)** Stem—When perform the test of foreign matter <*5.01*>, the amount of the stem contained in Clove does not exceed 5.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than the stem contained in Clove does not exceed 1.0%.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Essential oil content <*5.01*> Perform the test with 10.0 g of pulverized Clove: the volume of essential oil is not less than 1.6 mL.

Containers and storage Containers—Well-closed containers.

Powdered Clove

Caryophylli Flos Pulveratus

チョウジ末

Powdered Clove is the powder of Clove.

Description Powdered Clove occurs as a dark brown powder. It has a strong, characteristic odor and a pungent taste, followed by slight numbness of the tongue.

Under a microscope <*5.01*>, Powdered Clove reveals epidermal tissue with stomata, collenchyma, parenchyma with oil sacs, and spongy parenchyma or its fragments; fur-

thermore, a few fusiform thick-walled fibers, spiral vessels 6 – 10 μm in diameter, anther and pollen grains, and rosette aggregates of calcium oxalate 10 – 15 μm in diameter. Epidermis of anther shows characteristically reticulated walls; pollen grains tetrahedral 10 – 20 μm in diameter; rosette aggregates of calcium oxalate arranged in crystal cell rows, or contained in collenchyma cells and parenchyma cells.

Identification Mix 0.1 mL of a mixture of essential oil and xylene, obtained in the Essential oil content, with 2 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green to blue color develops.

Purity Foreign matter ⟨*5.01*⟩—Under a microscope, Powdered Clove does not contain stone cells or starch grains.

Total ash ⟨*5.01*⟩ Not more than 7.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Essential oil content ⟨*5.01*⟩ Perform the test with 10.0 g of Powdered Clove: the volume of essential oil is not less than 1.3 mL.

Containers and storage Containers—Tight containers.

Clove Oil

Oleum Caryophylli

チョウジ油

Clove Oil is the volatile oil distilled with steam from the flower buds or leaves of *Syzygium aromaticum* Merrill et Perry (*Eugenia caryophyllata* Thunberg) (*Myrtaceae*).

It contains not less than 80.0 vol% of total eugenol.

Description Clove Oil is a colorless or light yellow-brown, clear liquid. It has a characteristic aroma and a burning taste.

It is miscible with ethanol (95) and with diethyl ether.

It is slightly soluble in water.

It acquires a brown color upon aging or by air.

Identification **(1)** To 5 drops of Clove Oil add 10 mL of calcium hydroxide TS, and shake vigorously: the oil forms a flocculent mass, and a white to light yellow color develops.

(2) Dissolve 2 drops of Clove Oil in 4 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green color is produced.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.527 – 1.537

Specific gravity ⟨*1.13*⟩ d_{20}^{20}: 1.040 – 1.068

Purity **(1)** Clarity of solution—Dissolve 1.0 mL of Clove Oil in 2.0 mL of diluted ethanol (7 in 10): the solution is clear.

(2) Water-soluble phenols—To 1.0 mL of Clove Oil add 20 mL of boiling water, shake vigorously, filter the aqueous layer after cooling, and add 1 to 2 drops of iron (III) chloride TS: a yellow-green, but no blue or violet, color develops.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 mL of Clove Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

(4) Optical rotation ⟨*2.49*⟩ α_D^{20}: 0 – −1.5° (100 mm).

Assay Take 10.0 mL of Clove Oil in a Cassia flask, add 70 mL of sodium hydroxide TS, shake for 5 minutes and warm for 10 minutes in a water bath with occasional shaking, add sodium hydroxide TS to the volume after cooling, and allow to stand for 18 hours. Measure the volume (mL) of the separated oily layer.

Total eugenol (vol%)
= {10 − (volume of separated oily layer)} × 10

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cnidium Monnieri Fruit

Cnidii Monnieris Fructus

ジャショウシ

Cnidium Monnieri Fruit is the fruit of *Cnidium monnieri* Cusson (*Umbelliferae*).

Description Elliptical cremocarp, often each mericarp separated; 2 – 3 mm in length, 1 – 2 mm in width; externally light brown to brown, each mericarp usually with five winged longitudinal ridges; inner surface of mericarp almost flat.

Odor, characteristic; it gives characteristic aroma, later a slight sensation of numbness on chewing.

Under a microscope ⟨*5.01*⟩, a transverse section reveals one oil canal between longitudinal ridges, usually two oil canals in the inner part of mericarp facing to gynophore; longitudinal ridges composed of slightly lignified parenchymatous cells, with vascular bundles in the base; epidermal cells and parenchymatous cells of longitudinal ridges contain solitary crystals of calcium oxalate; parenchymatous cells of albumen contain oil drops and aleurone grains, and occasionally starch grains.

Identification To 1 g of pulverized Cnidium Monnieri Fruit add 10 mL of ethyl acetate, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of osthole for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and the *R*f value with the bluish white fluorescent spot from the standard solution.

Loss on drying ⟨*5.01*⟩ Not more than 12.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 17.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 6.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Cnidium Rhizome

Cnidii Rhizoma

センキュウ

Cnidium Rhizome is the rhizome of *Cnidium officinale* Makino (*Umbelliferae*), usually passed through hot water.

Description Irregular massive rhizome, occasionally cut lengthwise; 5 – 10 cm in length, and 3 – 5 cm in diameter; externally grayish brown to dark brown, with gathered nodes, and with knobbed protrusions on the node; margin of the vertical section irregularly branched; internally grayish white to grayish brown, translucent and occasionally with hollows; dense and hard in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope ⟨*5.01*⟩, a transverse section reveals cortex and pith with scattered oil canals; in the xylem, thick-walled and lignified xylem fibers appear in groups of various sizes; starch grains usually gelatinized, but rarely remaining as grains of 5 – 25 μm in diameter; crystals of calcium oxalate not observable.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Cnidium Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Cnidium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Powdered Cnidium Rhizome

Cnidii Rhizoma Pulveratum

センキュウ末

Powdered Cnidium Rhizome is the powder of Cnidium Rhizome.

Description Powdered Cnidium Rhizome occurs as a gray to light grayish brown powder. It has a characteristic odor and a slightly bitter taste.

Under a microscope ⟨*5.01*⟩, Powdered Cnidium Rhizome reveals colorless and gelatinized starch masses, and fragments of parenchyma containing them; fragments of scalariform and reticulate vessels 15 – 30 μm in diameter; fragments of thick-walled and lignified xylem fibers 20 – 60 μm in diameter; fragments of yellow brown cork tissue; fragments of secretory tissue.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of Powdered Cnidium Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Powdered Cnidium Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope ⟨*5.01*⟩, Powdered Cnidium Rhizome does not contain a large quantity of starch grains, stone cells, crystals of calcium oxalate or other foreign matter.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Coconut Oil

Oleum Cocois

ヤシ油

Coconut oil is the fixed oil obtained from the seeds of *Cocos nucifera* Linné (*Palmae*).

Description Coconut Oil is a white to light yellow mass or a colorless or light yellow, clear oil. It has a slight, characteristic odor and a mild taste.

It is freely soluble in diethyl ether and in petroleum ether. It is practically insoluble in water.

At a temperature below 15°C, it congeals to a hard and brittle solid.

Melting point: 20 – 28°C

Acid value ⟨*1.13*⟩ Not more than 0.2.

Saponification value ⟨*1.13*⟩ 246 – 264

Unsaponifiable matter ⟨*1.13*⟩ Not more than 1.0%.

Iodine value ⟨*1.13*⟩ 7 – 11

Containers and storage Containers—Tight containers.

Codonopsis Root

Codonopsis Radix

トウジン

Codonopsis Root is the root of *Codonopsis pilosula* Nannfeldt or *Codonopsis tangshen* Oliver (*Campanulaceae*).

Description Codonopsis Root nearly cylindrical, 8 – 30 cm in length, 0.5 – 2.5 cm in diameter; gradually slender to the apex, often branched; outer surface light yellow to grayish brown; from the base to central part with ring-like wrinkles, and longitudinal wrinkles entirely obvious; numerous projections composed of scars of stems at the crown, with a round dent at the distal end; blackish brown and tremellose secretion often at the scars of lateral roots; flexible and easily bendable or hard and easily breakable in texture; in transverse section yellowish white to light brown in cortex, light yellow in xylem, sometimes with slit in cortex.

Odor, slight and characteristic; taste, slightly sweet.

Under a microscope ⟨*5.01*⟩, a transverse section reveals cork layer at the outermost portion, outer 1- to 10-layer consisting of cork stone cells; groups of laticifers containing light yellow substances arranged radially in phloem, intercellular spaces usually observed; vessels of xylem arranged radially; starch grains and crystals of inulin usually contained in phloem parenchyma cells.

Identification To 2.0 g of pulverized Codonopsis Root add 50 mL of water, and heat in a water bath for 1 hour. After cooling, filter, and wash the filtrate with two 20-mL portions of ethyl acetate. Separate the aqueous layer, extract with two 30-mL portions of water saturated 1-butanol. Combine the 1-butanol layers, and evaporate to dryness in a water bath under reduced pressure. Dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and ethyl acetate (6:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly naphthoresorcin-phosphoric acid TS on the plate, and heat at 105°C for 10 minutes: an orange to red-purple spot at an *R*f value of about 0.5 is observed.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Codonopsis Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Codonopsis Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 23.0% (6 hours).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Coix Seed

Coicis Semen

ヨクイニン

Coix Seed is the seed of *Coix lachryma-jobi* Linné var. *mayuen* Stapf (*Gramineae*), from which the seed coat has been removed.

Description Ovoid or broad ovoid seed, about 6 mm in length, and about 5 mm in width; with a slightly hollowed apex and base; dorsal side distended; ventral side longitudinally and deeply furrowed in the center; dorsal side mostly white in color and powdery; in the furrow on the ventral surface, attached brown, membranous pericarp and seed coat. Under a magnifying glass, the cross section reveals light yellow scutellum in the hollow of the ventral side. Hard in texture.

Odor, slight; taste, slightly sweet; adheres to the teeth on chewing.

Identification To a cross-section of Coix Seed add iodine TS dropwise: a dark red-brown color develops in the endosperm, and a dark gray color develops in the scutellum.

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Powdered Coix Seed

Coicis Semen Pulveratum

ヨクイニン末

Powdered Coix Seed is the powder of Coix Seed.

Description Powdered Coix Seed occurs as a brownish, grayish white to grayish yellow-white powder, and has a slight odor and a slightly sweet taste.

Under a microscope <*5.01*>, Powdered Coix Seed reveals starch grains, and fragments of endosperm containing them; fragments of tissue accompanied with epidermal cells of pericarp composed of yellowish and oblong cells, and fragments of parenchyma cells containing fixed oil, aleuron grains and starch grains; a very few fragments of spiral vessels. Starch grains are simple and 2-compound grains, simple grain nearly equidiameter to obtuse polygon, 10 – 20 μm in diameter, and have a stellate cleft-like hilum in the center. Spherical starch grains, coexisting with aleuron grains, are spherical simple grains, 3 – 7 μm in diameter.

Identification Place a small amount of Powdered Coix Seed on a slide glass, add dropwise iodine TS, and examine under a microscope <*5.01*>: nearly equidiameter and obtuse polygonal simple starch grains, usually 10 – 15 μm in diameter, and compound starch grains have a reddish brown color. Small spheroidal starch grains, coexisting with fixed oil and with aleuron grains in parenchymatous cells, have a blue-purple color.

Purity Foreign matter—Under a microscope <*5.01*>, Powdered Coix Seed reveals no fragments of tissue having silicified cell wall, no stone cells, no fragments of other thick-walled and lignified cells, no fragments of reticulate, scalariform and pitted vessels, no fragments of fibers and hairs, and no large starch grains, more than 10 μm in diameter, appearing blue-purple upon addition of iodine TS.

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Tight containers.

Condurango

Condurango Cortex

コンズランゴ

Condurango is the bark of the trunk of *Marsdenia cundurango* Reichenbach filius (*Asclepiadaceae*).

Description Tubular or semi-tubular pieces of bark, 0.1 – 0.6 cm in thickness, 4 – 15 cm in length; outer surface grayish brown to dark brown, nearly smooth and with numerous lenticels, or more or less scaly and rough; inner surface light grayish brown and longitudinally striate; fractured surface fibrous on the outer region and generally granular in the inner region.

Odor, slight; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals a cork layer composed of several layers of thin-walled cells; primary cortex with numerous stone cell groups; scondary cortex with phloem fiber bundles scattered inside the starch

sheath consisting of one-cellular layer; articulate latex tubes scattered in both cortices; parenchyma cells containing starch grains or rosette aggregates of calcium oxalate; starch grain 3 – 20 μm in diameter.

Identification Digest 1 g of pulverized Condurango in 5 mL of water, and filter: the clear filtrate becomes turbid on heating, but becomes clear again upon cooling.

Purity Foreign matter ⟨*5.01*⟩—The xylem and other foreign matter contained in Condurango do not exceed 2.0%.

Total ash ⟨*5.01*⟩ Not more than 12.0%.

Containers and storage Containers—Well-closed containers.

Condurango Fluidextract

コンズランゴ流エキス

Method of preparation Take moderately fine powder of Condurango, and prepare the fluidextract as directed under Fluidextracts using a suitable quantity of a mixture of Purified Water or Purified Water in Containers, Ethanol and Glycerin (5:3:2) as the first solvent, and a suitable quantity of a mixture of Purified Water or Purified Water in Containers and Ethanol (3:1) as the second solvent.

Description Condurango Fluidextract is a brown liquid. It has a characteristic odor and a bitter taste.

Identification Mix 1 mL of Condurango Fluidextract with 5 mL of water, filter, if necessary, and heat the clear solution: turbidity is produced. However, it becomes almost clear upon cooling.

Purity Heavy metals ⟨*1.07*⟩—Prepare the test solution with 1.0 g of Condurango Fluidextract as direct under the Fluidextracts (4), and perform the test (not more than 30 ppm).

Containers and storage Containers—Tight containers.

Coptis Rhizome

Coptidis Rhizoma

オウレン

Coptis Rhizome is the rhizome of *Coptis japonica* Makino, *Coptis chinensis* Franchet, *Coptis deltoidea* C. Y. Cheng et Hsiao or *Coptis teeta* Wallich (*Ranunculaceae*), from which the roots have been removed practically.

It contains not less than 4.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

For Coptis Rhizome used only for extracts or infusions and decoctions, the label states the restricted utilization forms.

Description Irregular, cylindrical rhizome, 2 – 4 cm, rarely up to 10 cm in length, 0.2 – 0.7 cm in diameter, slightly curved and often branched; externally grayish yellow-brown, with ring nodes, and with numerous remains of rootlets; generally remains of petiole at one end; fractured surface rather fibrous; cork layer light grayish brown, cortex and pith are yellow-brown to reddish yellow-brown, xylem is yellow to reddish yellow in color.

Odor, slight; taste, extremely bitter and lasting; it colors the saliva yellow on chewing.

Under a microscope ⟨*5.01*⟩, a transverse section of Coptis Rhizome reveals a cork layer composed of thin-walled cork cells; cortex parenchyma usually exhibiting groups of stone cells near the cork layer and yellow phloem fibers near the cambium; xylem consisting chiefly of vessels, tracheids and xylem fibers; medullary ray distinct; pith large; in pith, stone cells or stone cells with thick-walled and lignified cells are sometimes recognized; parenchyma cells contain minute starch grains.

Identification **(1)** To 0.5 g of pulverized Coptis Rhizome add 10 mL of water, allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid and 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) To 0.5 g of pulverized Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS or berberin chloride hydrate for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution and a yellow to yellow-green fluorescence spot obtained from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of pulverized Coptis Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). When the decision is difficult by this method, perform the test as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩. Put 5.0 g of pulverized Coptis Rhizome in a platinum, quartz or porcelain crucible, heat gently, and then incinerate by ignition between 450°C and 550°C. After cooling, add a small amount of 2 mol/L nitric acid TS, filter if necessary, and wash the crucible and filter several times with small portions of 2 mol/L nitric acid TS. Combine the filtrate and the washings, add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, to 2.5 mL of Standard Lead Solution add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 5 ppm).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: A lead hollow-cathode lamp.
Wavelength: 283.3 nm.

The procedure and permissible limit for Coptis Rhizome labeled to be used for extracts or infusions and decoctions are as follows.

To 4.0 g of moderately fine cuttings of Coptis Rhizome add 80 mL of water, and heat until the amount becomes about 40 mL with occasional stirring. After cooling, filter, and proceed with the filtrate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of

Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 11.0% (6 hours).

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of pulverized Coptis Rhizome, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (previously determine the water <*2.48*> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of berberine in each solution.

Amount (mg) of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$)]
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Coptis Rhizome

Coptidis Rhizoma Pulveratum

オウレン末

Powdered Coptis Rhizome is the powder of Coptis Rhizome.

It contains not less than 4.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

Description Powdered Coptis Rhizome occurs as a yellow-brown to grayish yellow-brown powder. It has a slight odor and an extremely bitter, lasting taste, and colors the saliva yellow on chewing.

Under a microscope <*5.01*>, almost all elements are yellow in color; it reveals mainly fragments of vessels, tracheids and xylem fibers; parenchyma cells containing starch grains; polygonal cork cells. Usually, round to obtuse polygonal stone cells and their groups, and phloem fibers, 10 – 20 μm in diameter, and fragments of their bundles. Sometimes, polygonal and elongated epidermal cells, originated from the petiole, having characteristically thickened cell walls. Starch grains are single grains 1 – 7 μm in diameter.

Identification **(1)** To 0.5 g of Powdered Coptis Rhizome add 10 mL of water, allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid and 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) To 0.5 g of Powdered Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS or berberine chloride hydrate for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution and a yellow to yellow-green fluorescence spot from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Phellodendron bark—Under a microscope <*5.01*>, crystal cell rows or mucilage masses are not observable. Stir 0.5 g of Powdered Coptis Rhizome with 2 mL of water: the solution does not become gelatinous.

(2) Curcuma—Place Powdered Coptis Rhizome on a filter paper, drop diethyl ether on it, and allow to stand. Remove the powder from the filter paper, and drop 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope <*5.01*>, Powdered Coptis Rhizome does not contain gelatinized starch or secretory cells containing yellow-red resin.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Coptis Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). When the decision is difficult by this method, perform the test as directed under Atomic Absorption Spectrophotometry <*2.23*>. Put 5.0 g of Powdered Coptis Rhizome in a platinum, quartz or porcelain crucible, heat gently, and then incinerate by igni-

tion between 450°C and 550°C. After cooling, add a small amount of 2 mol/L nitric acid TS, filter if necessary, and wash the crucible and filter several times with small portions of 2 mol/L nitric acid TS. Combine the filtrate and the washings, add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, to 2.5 mL of Standard Lead Solution add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 5 ppm).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: A lead hollow-cathode lamp.
Wavelength: 283.3 nm.

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 11.0% (6 hours).

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of Powdered Coptis Rhizome, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (previously determine the water <*2.48*> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of berberine in each solution.

Amount (mg) of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$)]
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 mm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Corn Oil

Oleum Maydis

トウモロコシ油

Corn Oil is the fixed oil obtained from the embryo of *Zea mays* Linné (*Gramineae*).

Description Corn Oil is a clear, light yellow oil. It is odorless or has a slight odor, and a mild taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95), and practically insoluble in water.

At −7°C, it congeals to an unguentary mass.

Specific gravity d_{25}^{25}: 0.915 – 0.921

Acid value <*1.13*> Not more than 0.2.

Saponification value <*1.13*> 187 – 195

Unsaponifiable matter <*1.13*> Not more than 1.5%.

Iodine value <*1.13*> 103 – 130

Containers and storage Containers—Tight containers.

Cornus Fruit

Corni Fructus

サンシュユ

Cornus Fruit is the pulp of the pseudocarp of *Cornus officinalis* Siebold et Zuccarini (*Cornaceae*).

It contains not less than 0.4% of loganin, calculated on the basis of dried material.

Description Flattened oblong, 1.5 – 2 cm in length, about 1 cm in width; externally dark red-purple to dark purple, lustrous, and with coarse wrinkles; a crack-like scar formed by removal of true fruit; a scar of calyx at one end, and a scar of peduncle at the other; soft in texture.

Odor, slight; taste, acid and slightly sweet.

Identification To 1 g of coarse cuttings of Cornus Fruit add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with a red-purple spot obtained from the stand-

ard solution. Further, a spot, slightly different in color tone from the above-mentioned spot, is found immediately below of the spot.

Purity **(1)** Foreign matter <*5.01*>—The amount of its peduncles and other foreign matter contained in Cornus Fruit does no exceed 2.0%.

(2) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 35.0%.

Assay Weigh accurately about 1 g of fine cuttings of Cornus Fruit (separately determine the loss on drying <*5.01*>), put in a glass-stoppered centrifuge tube, suspend in 30 mL of diluted methanol (1 in 2), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 30 mL of diluted methanol (1 in 2), and repeat the above process twice more. Combine all the extracts, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of loganin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: Adjust so that the retention time of loganin is about 25 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Corydalis Tuber

Corydalis Tuber

エンゴサク

Corydalis Tuber is the tuber of *Corydalis turtschaninovii* Basser forma *yanhusuo* Y. H. Chou et C. C. Hsu (*Papaveraceae*), usually after being passed through hot water.

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

Description Nearly flattened spherical, 1 – 2 cm in diameter, and with stem scar at one end; externally grayish yellow to grayish brown; hard in texture; fractured surface is yellow and smooth or grayish yellow-green in color and granular.

Almost odorless; taste, bitter.

Identification To 2 g of pulverized Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of dehydrocorydaline nitrate for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution, and a yellow fluorescent spot appears at the lower side of the spot. Separately, spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an *R*f value of about 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Corydalis Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Corydalis Tuber according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 15.0%.

Total ash <*5.01*> Not more than 3.0%.

Assay Weigh accurately about 1 g of pulverized Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser on a water bath for 30 minutes, and filter after cooling. To the residue add 15 mL of a mixture of methanol and dilute hydrochloric acid (3:1), and repeat the above procedure. Combine the filtrates, add a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for assay, previously dried in a desiccator (silica gel) for not less than 1 hour, dissolve in a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as

directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of dehydrocorydaline in each solution.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate ($C_{22}H_{24}N_2O_7$)]
$= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of dehydrocorydaline nitrate for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate dodecahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. To this solution add 14.05 g of sodium perchlorate, dissolve, and add water to make exactly 1000 mL. To this solution add 450 mL of acetonitrile, then dissolve 0.20 g of sodium lauryl sulfate.

Flow rate: Adjust so that the retention time of dehydrocorydaline is about 24 minutes.

System suitability—

System performance: Dissolve 1 mg each of dehydrocorydaline nitrate for assay and berberine chloride hydrate in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of dehydrocorydaline is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Corydalis Tuber

Corydalis Tuber Pulveratum

エンゴサク末

Powdered Corydalis Tuber is the powder of Corydalis Tuber.

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

Description Powdered Corydalis Tuber occurs as a greenish yellow to grayish yellow powder. Almost odorless; taste, bitter.

Under a microscope <5.01>, Powdered Corydalis Tuber reveals mainly, masses of gelatinized starch or light yellow to colorless parenchymatous cells containing starch grains, fragments of cork layers, light yellow stone cells, sclerenchymatous cells, reticulate vessels, spiral vessels and ring vessels; starch grains observed simple grains and 2- to 3-compound grains.

Identification To 2 g of Powdered Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of dehydrocorydaline nitrate for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution, and a yellow fluorescent spot appears at the lower side of the spot. Separately, spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an *R*f value of about 0.6.

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of Powdered Corydalis Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Corydalis Tuber according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 15.0%.

Total ash <5.01> Not more than 3.0%.

Assay Weigh accurately about 1 g of Powdered Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser on a water bath for 30 minutes, and filter after cooling. To the residue add 15 mL of the mixture of methanol and dilute hydrochloric acid (3:1), and proceed in the same way as above. Combine the filtrates, add the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for assay, previously dried in a desiccator (silica gel) for not less than 1 hour, dissolve in the mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of dehydrocorydaline in each solution.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate ($C_{22}H_{24}N_2O_7$)]
$= M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of dehydrocorydaline nitrate for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate dodecahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. To this solution add 14.05 g of sodium perchlorate, dissolve, and add water to make exactly 1000 mL. Add 450 mL of acetonitrile, and dissolve 0.20 g of

sodium lauryl sulfate in this solution.

Flow rate: Adjust so that the retention time of dehydrocorydaline is about 24 minutes.

System suitability—

System performance: Dissolve 1 mg of dehydrocorydaline nitrate for assay and 1 mg of berberine chloride hydrate in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μL of this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dehydrocorydaline is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Crataegus Fruit

Crataegi Fructus

サンザシ

Crataegus Fruit is the pseudocarp of **1)** *Crataegus cuneata* Siebold et Zuccarini or **2)** *Crataegus pinnatifida* Bunge var. *major* N. E. Brown (*Rosaceae*) without any treatment or cut crosswise or lengthwise.

Description

1) *Crataegus cuneata* origin—Nearly spherical fruits, 8 – 14 mm in diameter; externally yellow-brown to grayish brown, with fine reticulated wrinkles, remained dent of 4 – 6 mm in diameter at one end, often the base of calyx around the dent, short peduncle or scar at the other end. True fruits, usually five loculus, often split five, mericarp, 5 – 8 mm in length, light brown, usually, containing one seed into each mericarp.

Almost odorless; taste, slightly acid.

Under a microscope <*5.01*>, a transverse section of central parts reveals in the outermost layer composed of epidermis to be covered with comparatively thick cuticle layer, cuticle intrude into lateral cell walls of epidermis, and reveal wedge-like. Cell of the epidermis or 2- to 3-layer of parenchyma cells beneath these observed contents of yellow-brown to red-brown in color followed these appeared parenchyma. Vascular bundles and numerous stone cells appear single or gathered 2 to several cells scattered on the parenchyma, and observed solitary crystals and clustera crystals of calcium oxalate. Pericarp of true fruits composed of mainly sclerenchyma cells, seed covered with seed coats, perisperm, endosperm, cotyledon observed inside seed coats; sclerenchyma cells of true fruits and cells of seed coats containing solitary crystals of calcium oxalate.

2) *Crataegus pinnatifida* var. *major* origin—Approximate to 1), but it is large in size, 17 – 23 mm in diameter, the outer surface red-brown and lustrous, spot-like scars of hairs are distinct. At one end remained dent, 7 – 9 mm in diameter, mericarp, 10 – 12 mm in length, yellow-brown in color, usually ripe seeds are absent.

Odor, characteristic; taste, acid.

Under a microscope <*5.01*>, a transverse section of the central parts approximate to 1), but it contains a few stone cells in parenchyma.

Identification

1) *Crataegus cuneata* origin—To 1.0 g of pulverized Crataegus Fruit add 5 mL of methanol, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rutin for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green fluorescent spot obtained from the standard solution, and one or two similar green fluorescent spots are found at an *R*f value of about 0.5. These spots disappear gradually by allowing to cool, and appear again by heating.

2) *Crataegus pinnatifida* var. *major* origin—To 1 g of pulverized Crataegus Fruit add 5 mL of methanol, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hyperoside for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green fluorescent spot obtained from the standard solution, and a similar fluorescent spot is found just above the spot. These spots disappear gradually by allowing to cool, and appear again by heating.

Loss on drying <*5.01*> Not more than 17.0%.

Total ash <*5.01*> Not more than 4.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Cyperus Rhizome

Cyperi Rhizoma

コウブシ

Cyperus Rhizome is the rhizome of *Cyperus rotundus* Linné (*Cyperaceae*).

Description Fusiform rhizome, 1.5 – 2.5 cm in length, 0.5 – 1 cm in diameter; externally grayish brown to grayish blackish brown, with 5 to 8 irregular ring nodes, and with hair-like fiber bundles on each node; hard in texture. The transverse section red-brown to light yellow in color, with waxy luster; thickness of cortex approximately equal to or slightly smaller than the diameter of stele. Under a mag-

nifying glass, a transverse section reveals fiber bundles as brown spots lined in rings along circumference; here and there in the cortex, vascular bundles appear as red-brown spots, and numerous secretory cells scattered as minute yellow-brown spots; in the stele, numerous vascular bundles scattered as spots or lines.

Characteristic odor and taste.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Cyperus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Cyperus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 3.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Cyperus Rhizome, provided that 1 mL of silicon resin is previously added on the sample in the flask: the volume of essential oil is not less than 0.3 mL.

Containers and storage Containers—Well-closed containers.

Powdered Cyperus Rhizome

Cyperi Rhizoma Pulveratum

コウブシ末

Powdered Cyperus Rhizome is the powder of Cyperus Rhizome.

Description Powdered Cyperus Rhizome occurs as a light red-brown powder, and has a characteristic odor and taste.

Under a microscope <*5.01*>, Powdered Cyperus Rhizome reveals fragments of polygonal parenchyma cells, scalariform vessels, and seta-like fibers; a large quantity of starch, mostly gelatinized; an extremely small number of stone cells.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Cyperus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Cyperus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Cyperus Rhizome does not show extremely lignified cells, except stone cells, and crystals.

Total ash <*5.01*> Not more than 3.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Essential oil content <*5.01*> Perform the test with 50.0 g of Powdered Cyperus Rhizome provided that 1 mL of silicon resin is previously added on the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage Containers—Tight containers.

Daiokanzoto Extract

大黄甘草湯エキス

Daiokanzoto Extract contains not less than 3.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74), and not less than 9 mg and not more than 27 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 18 mg and not more than 54 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Rhubarb	4 g	4 g
Glycyrrhiza	1 g	2 g

Prepare a dry extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Daiokanzoto Extract occurs as a brown powder. It has a characteristic odor and an astringent first then slightly sweet taste.

Identification **(1)** To 1.0 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

(2) To 0.5 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Daiokanzoto Extract as directed under Extract (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Daiokanzoto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> Not more than 7.0% (1 g, 105°C,

5 hours).

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Assay (1) Sennoside A—Weigh accurately about 0.2 g of Daiokanzoto Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, and remove the upper layer. To the water layer add 20 mL of ethyl acetate, shake for 10 minutes, centrifuge, and remove the upper layer. To the water layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Sennoside A RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (2460:540:1).

Flow rate: 1.0 mL per minute (the retention time of sennoside A is about 14 minutes.)

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sennoside A are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

(2) Glycyrrhizic acid—Use the sample solution obtained in the Assay (1) as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Daisaikoto Extract

大柴胡湯エキス

Daisaikoto Extract contains not less than 1.8 mg and not more than 7.2 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 26 mg and not more than 78 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)
Bupleurum Root	6 g	6 g	6 g	6 g	6 g
Pinellia Tuber	4 g	4 g	4 g	3 g	4 g
Scutellaria Root	3 g	3 g	3 g	3 g	3 g
Peony Root	3 g	3 g	3 g	3 g	3 g
Jujube	3 g	3 g	3 g	3 g	3 g
Immature Orange	2 g	2 g	2 g	2 g	2 g
Ginger	1 g	1 g	2 g	1 g	1.5 g
Rhubarb	1 g	2 g	1 g	1 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 5), using the crude drugs shown above.

Description Daisaikoto Extract occurs as light yellow-brown to brown powder or blackish brown viscous extract, having a slightly order, and a hot first, then a bitter taste.

Identification (1) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of

ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot obtained from the standard solution (Scutellaria Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzoaldehyde-sulfuric acid TS on the plate, heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to purple spot obtained from the standard solution (Peony Root).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 1.0 g of pulverized immature orange add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: two consecutive spots at *R*f values of about 0.7 obtained from the sample solution have respectively the same color tone and *R*f value with the blue-green spot and blue spot underneath obtained from the standard solution (Immature Orange).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution (Ginger).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 11.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S,

of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, add 1 mL of acetic acid (100), to the effluent, then add water to make exactly 25 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 5/8$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Digenea

Digenea

マクリ

Digenea is the whole algae of *Digenea simplex* C. Agardh (*Rhodomelaceae*).

Description Rounded, string-like algae, 2 – 3 mm in diameter; externally, dark red-purple to dark grayish red or grayish brown; a few branched rods irregularly forked,

covered with short hairy twigs; calcified weeds and other small algae often attached.

Odor, seaweed-like; taste, disagreeable and slightly salty.

Identification To 2 g of pulverized Digenea add 10 mL of dilute ethanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of kainic acid in 10 mL of dilute ethanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, water and formic acid (5:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-red spot obtained from the standard solution.

Purity Foreign matter <*5.01*>—The amount of other algae in Digenea does not exceed 20.0%.

Loss on drying <*5.01*> Not more than 22.0%.

Acid-insoluble ash <*5.01*> Not more than 8.0%.

Containers and storage Containers—Well-closed containers.

Dioscorea Rhizome

Dioscoreae Rhizoma

サンヤク

Dioscorea Rhizome is the rhizome (rhizophore) of *Dioscorea japonica* Thunberg or *Dioscorea batatas* Decaisne (*Dioscoreaceae*), from which the periderm has been removed.

Description Cylindrical or irregular cylindrical rhizome, 5 – 15 cm in length, 1 – 4 cm in diameter, occasionally longitudinally split or transversely cut; externally whitish to yellowish white; fractured surface, whitish, smooth and powdery; hard in texture but breakable.

Practically odorless and tasteless.

Identification **(1)** To the cut surface of Dioscorea Rhizome add dilute iodine TS dropwise: a dark blue color develops.

(2) To 0.2 g of pulverized Dioscorea Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add 0.5 mL of sulfuric acid carefully to make two layers: a red-brown to purple-brown color appears at the zone of contact.

(3) To 1 g of pulverized Dioscorea Rhizome add 4 mL of a mixture of methanol and water (4:1), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of allantoin for thin-layer chromatography in 2 mL of a mixture of methanol and water (4:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly a solution of 0.2 g of 4-dimethylaminocinnamaldehyde in 10 mL of 6 mol/L hydrochloric acid TS and 10 mL of ethanol (99.5) on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the light red spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Dioscorea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Dioscorea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Powdered Dioscorea Rhizome

Dioscoreae Rhizoma Pulveratum

サンヤク末

Powdered Dioscorea Rhizome is the powder of Dioscorea Rhizome.

Description Powdered Dioscorea Rhizome occurs as nearly yellowish white to white; odorless and tasteless.

Under a microscope <*5.01*>, Dioscorea rhizome powder reveals starch grains; fragments of parenchyma cells containing starch grains; raphides of calcium oxalate, 100 to 200 μm in length and its containing mucilage cells; ring and scalariform vessels, 15 to 35 μm in diameter; starch grain isosceles deltoid or oblong, solitary, 18 to 35 μm, hilum and striation being distinct.

Identification **(1)** To 0.2 g of Powdered Dioscorea Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown to purple-brown color develops at the zone of contact.

(2) To 1 g of Powdered Dioscorea Rhizome add 4 mL of a mixture of methanol and water (4:1), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of allantoin for thin-layer chromatography in 2 mL of a mixture of methanol and water (4:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly a solution of 0.2 g of 4-dimethylaminocinnamaldehyde in 10 mL of 6 mol/L hydrochloric acid TS and 10 mL of ethanol (99.5) on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the light red spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Dioscorea Rhizome according to Method 3, and

perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Dioscorea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Dolichos Seed

Dolichi Semen

ヘンズ

Dolichos Seed is the seed of *Dolichos lablab* Linné (*Leguminosae*).

Description Flattened ellipsoidal to flattened orbicular-ovate seed, 9 – 14 mm in length, 6 – 10 mm in width, 4 – 7 mm in thickness; externally light yellowish white to light yellow, smooth and somewhat lustrous; caruncle white, like a half-moon, protrudent at one side; hard in texture.

Almost odorless; taste, slightly sweet and acid.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer of seed coat composed of a single layer of palisade like epidermal cells coated with cuticle; beneath epidermis a single layer of sclerenchymatous and sandglass like cells; inside of the layer mentioned above parenchyma lie, the innermost portion of the parenchyma decayed; cotyledons occur inside of the seed coat; the outermost layer of cotyledon composed of a single layer of epidermal cells, inner part of cotyledon mainly parenchyma, containing aleurone grains and oil drops, and occasionally starch grains.

Identification To 3 g of pulverized Dolichos Seed add 30 mL of methanol, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the solvent of the supernatant liquid, add 30 mL of water and 50 mL of ethyl acetate to the residue, shake, and take the ethyl acetate layer. To the ethyl acetate add 10 g of anhydrous sodium sulfate, shake, and filter. Evaporate the solvent of the filtrate, add 1 mL of ethyl acetate to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and acetic acid (100) (100:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at an *R*f value of about 0.4.

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 4.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 9.0%.

Containers and storage Containers—Well-closed containers.

Eleutherococcus Senticosus Rhizome

Eleutherococci senticosi Rhizoma

シゴカ

Eleutherococcus Senticosus Rhizome is the rhizome of *Eleutherococcus senticosus* Maximowicz (*Acanthopanax senticosus* Harms) (*Araliaceae*), often with root.

Description Slightly curved subcolumnar rhizome, 15 – 30 cm in length, 1 – 2.5 cm in diameter; externally grayish brown and slightly rough; transversely cut surface light brown, cortex thin, xylem thick with a pith in center; extremely hard in texture.

Odor, slightly characteristics; tasteless or slightly sweet, astringency.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer consisting of a cork layer 3 – 7 cells thick; oil canals scattered in parenchyma; fiber bundles lined stepwise in phloem; phloem and xylem separated clearly by cambium; xylem composed of vessels, xylem fibers and xylem parenchyma; ray composed of 2 – 6 rows of cells; pith composed of parenchyma; parenchyma of cortex and ray contain aggregate crystals of calcium oxalate; occasionally starch grains in ray, parenchyma of cortex and xylem.

Identification To 0.5 g of pulverized Eleutherococcus Senticosus Rhizome add 20 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of eleutheroside B for liquid chromatography in diluted methanol (1 in 2) to make 20 mL. To 2 mL of this solution add diluted methanol (1 in 2) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the peak corresponding to eleutheroside B in the chromatogram obtained from the sample solution shows the same retention time with the peak of eleutheroside B in the chromatogram obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (9:1).

Flow rate: Adjust so that the retention time of eleutheroside B is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eleutheroside B are not less than 5000 and not more than 1.5, respectively.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Eleutherococcus Senticosus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Eleutherococcus Senticosus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 2.5%.

Containers and storage Containers—Well-closed containers.

Ephedra Herb

Ephedrae Herba

マオウ

Ephedra Herb is the terrestrial stem of *Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C.A. Meyer or *Ephedra equisetina* Bunge (*Ephedraceae*).

Ephedra Herb contains not less than 0.7% of total alkaloids [as ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], calculated on the basis of dried material.

Description Thin cylindrical or ellipsoidal cylinder, 0.1 – 0.2 cm in diameter; 3 – 5 cm in length of internode; light green to yellow-green; numerous parallel vertical furrows on the surface; scaly leaves at the node portion; leaves, 0.2 – 0.4 cm in length, light brown to brown in color, usually being opposite at every node, adhering at the base to form a tubular sheath around the stem. Under a magnifying glass, the transverse section of the stem appears as circle and ellipse, the outer portion grayish green to yellow-green in color, and the center filled with a red-purple substance or hollow. When fractured at internode, the outer part is fibrous and easily split vertically.

Odor, slight; taste, astringent and slightly bitter, giving a slight sensation of numbness on the tongue.

Identification To 0.5 g of pulverized Ephedra Herb add 10 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying, and heat the plate at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.35.

Purity **(1)** Woody stem—When perform the test of foreign matter <*5.01*>, the amount of the woody stems contained in Ephedra Herb does not exceed 5.0%.

(2) Foreign matter <*5.01*>—Ephedra Herb does not contain stems of *Equisetaceae* or *Gramineae* plants, or any other foreign matter.

Loss on drying <*5.01*> Not more than 12.5% (6 hours).

Total ash <*5.01*> Not more than 11.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of moderately fine powder of Ephedra Herb, place in a glass-stoppered centrifuge tube, add 20 mL of diluted methanol (1 in 2), shake for 30 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 20-mL portion of diluted methanol (1 in 2). Combine all the extracts, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine (the relative retention time to ephedrine is about 0.9) obtained from the sample solution, and the peak area, A_S, of ephedrine obtained from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: Adjust so that the retention time of ephedrine is about 27 minutes.

System suitability—

System performance: Dissolve 1 mg of ephedrine hydrochloride for assay of crude drugs and 1 mg of pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Epimedium Herb

Epimedii Herba

インヨウカク

Epimedium Herb is the terrestrial part of *Epimedium pubescens* Maximowicz, *Epimedium brevicornu* Maximowicz, *Epimedium wushanense* T. S. Ying, *Epimedium sagittatum* Maximowicz, *Epimedium koreanum* Nakai, *Epimedium grandiflorum* Morren var. *thunbergianum* Nakai or *Epimedium sempervirens* Nakai (*Berberidaceae*).

Description Epimedium Herb is composed of a stem and a ternate to triternate compound leaf; leaflet ovate to broadly ovate or ovate-lanceolate, 3 – 20 cm in length, 2 – 8 cm in width, petiolule 15 – 70 mm in length, apex of leaflet acuminate, needle hair on margin 0.1 – 0.2 cm in length, base of leaflet cordate to deeply cordate, lateral leaflet asymmetry; upper surface green to green-brown, sometimes lustrous, lower surface light green to grayish green-brown, often pilose, especially on vein densely pilose, papery or coriaceous; petiole and stem cylindrical, light yellowish brown to slightly purplish and light green-brown, easily broken.

Odor, slight; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of the leaf reveals 3 – 6 vascular bundles in midvein; mesophyll composed of upper epidermis, single-layered palisade, spongy tissue and lower epidermis; leaf margins orbicular or oblong, sclerenchymatous; multi-cellular hairs on epidermis; 8 – 20 vascular bundles in petiole and 6 – 15 vascular bundles in petiolule. Under a microscope <*5.01*>, a transverse section of the stem reveals a single to several-layered hypodermis, cortex of 4 – 10 layers of sclerenchymatous cells, vascular bundle 13 – 30 in number, oblong to obovate.

Identification To 2 g of pulverized Epimedium Herb add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of icariin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Loss on drying <*5.01*> Not more than 12.5% (6 hours).

Total ash <*5.01*> Not more than 8.5%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 17.0%.

Containers and storage Containers—Well-closed containers.

Eucalyptus Oil

Oleum Eucalypti

ユーカリ油

Eucalyptus Oil is the essential oil distilled with steam from the leaves of *Eucalyptus globulus* Labillardière or allied plants (*Myrtaceae*).

It contains not less than 70.0% of cineol ($C_{10}H_{18}O$: 154.25).

Description Eucalyptus Oil is a clear, colorless or pale yellow liquid. It has a characteristic, aromatic odor and a pungent taste.

It is neutral.

Identification Shake 1 mL of Eucalyptus Oil vigorously with 1 mL of phosphoric acid, and allow to stand: the solution congeals within 30 minutes.

Refractive index <*2.45*> n_D^{20}: 1.458 – 1.470

Specific gravity <*1.13*> d_{20}^{20}: 0.907 – 0.927

Purity **(1)** Clarity of solution—Mix 1.0 mL of Eucalyptus Oil with 5 mL of diluted ethanol (7 in 10): the solution is clear.

(2) Heavy metals <*1.07*>—Proceed with 1.0 mL of Eucalyptus Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Weigh accurately about 0.1 g of Eucalyptus Oil, and dissolve in hexane to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add hexane to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of cineol for assay, proceed as directed in the sample solution, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of cineol to that of the internal standard of each solutions, respectively.

$$\text{Amount (mg) of cineol } (C_{10}H_{18}O) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of cineol for assay taken

Internal standard solution—A solution of anisol in hexane (1 in 250).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 5 m in length, having alkylene glycol phthalate ester for gas chromatography coated at the ratio of 10% on silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 120°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of cineol is about 11 minutes.

Selection of column: Dissolve 0.1 g each of cineol and limonene in 25 mL of hexane. To 1 mL of this solution add hexane to make 20 mL. Proceed with about 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of limonene and

cineol in this order with the resolution between these peaks being not less than 1.5.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Eucommia Bark

Eucommiae Cortex

トチュウ

Eucommia Bark is the bark of *Eucommia ulmoides* Oliver (*Eucommiaceae*).

Description Eucommia Bark is a semi-tubular or plate-like bark, 2 – 6 mm in thickness; externally pale grayish brown to grayish brown, and rough in texture, sometimes reddish-brown due to the cork layer falling off; internally dark violet, smooth and covered with a linear pattern that runs longitudinally, silk-like threads of gutta-percha (a thermoplastic rubber-like substance) appearing when broken.

It has a faint but characteristic odor and taste.

Under a microscope <*5.01*>, transverse section reveals parenchymatous cells containing gutta-percha; phloem with stone-cell and fiber layers; rays in rows of 2 – 3 cells; calcium oxalate crystals absent.

Identification Put 1 g of pulverized Eucommia Bark in a glass-stoppered centrifuge tube, add 10 mL of water and 20 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer so obtained, evaporate the diethyl ether on a water bath, and add 1 mL of ethanol (99.5) to the residue: colloidal substances appear.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 8.0%.

Acid-insoluble ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 7.0%.

Containers and storage Containers—Well-closed containers.

Euodia Fruit

Euodiae Fructus

ゴシュユ

Euodia Fruit is the fruit of *Euodia ruticarpa* Hooker filius et Thomson (*Evodia rutaecarpa* Bentham), *Euodia officinalis* Dode (*Evodia officinalis* Dode) or *Euodia bodinieri* Dode (*Evodia bodinieri* Dode) (Rutaceae).

Description Flattened spheroidal or globular fruit, 2 – 5 mm in diameter; externally dark brown to grayish brown, with many oil sacs appearing as hollow pits, and often with peduncle, 2 – 5 mm in length, covered densely with hairs; matured pericarp split to reveal five loculi, and each loculus containing obovoid or globular seeds of a lustrous brown to blackish brown or bluish black color.

Odor, characteristic; taste, acrid, followed by a lasting bitterness.

Identification To 1.0 g of pulverized Euodia Fruit add 20 mL of methanol, heat for 5 minutes on a water bath, cool, and filter. Evaporate the filtrate to dryness, add 3 mL of dilute acetic acid to the residue, warm for 2 minutes on a water bath, cool, and filter. Perform the following tests using the filtrate as the sample solution.

(1) Spot one drop of the sample solution on a filter paper, air-dry, spray Dragendorff's TS for spraying, and allow to stand: a yellow-red color develops.

(2) To 0.2 mL of the sample solution add 0.8 mL of dilute acetic acid. To this solution add gently 2 mL of 4-dimethylaminobenzaldehyde TS, and warm in a water bath: a purple-brown ring develops at the zone of contact.

Purity (1) Peduncle—The amount of peduncles contained in Euodia Fruit does not exceed 5.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than peduncles contained in Euodia Fruit does not exceed 1.0%.

Total ash <*5.01*> Not more than 8.0%.

Containers and storage Containers—Well-closed containers.

Fennel

Foeniculi Fructus

ウイキョウ

Fennel is the fruit of *Foeniculum vulgare* Miller (*Umbelliferae*).

Description Cylindrical cremocarp, 3.5 – 8 mm in length, 1 – 2.5 mm in width; externally grayish yellow-green to grayish yellow; two mericarps closely attached with each other, and with five longitudinal ridges; cremocarp often with pedicel 2 – 10 mm in length.

Characteristic odor and taste.

Under a microscope <*5.01*>, ridges near the bentral side are far protruded than those on the dorsal side; one large oil canal between each ridge, and two oil canals on the bentral side.

Identification To 0.5 g of pulverized Fennel add 10 mL of hexane, allow to stand for 5 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with a dark purple color appears at an *R*f value of about 0.4.

Purity (1) Peduncle—When perform the test of foreign matter <*5.01*>, the amount of peduncles contained in Fennel does not exceed 3.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than the peduncle contained in Fennel does not exceed 1.0%.

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Fennel: the volume of essential oil is not less than

0.7 mL.

Containers and storage Containers—Well-closed containers.

Powdered Fennel

Foeniculi Fructus Pulveratus

ウイキョウ末

Powdered Fennel is the powder of Fennel.

Description Powdered Fennel occurs as a greenish light brown to greenish brown, and is a characteristic odor and taste.

Under a microscope <*5.01*>, Powdered Fennel reveals fragments of parenchyma cells of perisperm containing aleurone grain, fragments of parenchyma cells of endosperm containing fatty oil, fragments of sclerenchyma with characteristic simple pits, fragments of oil canal within yellow-brown material, fragments of endocarp shown scalariform, spiral vessels, fragments of epidermis or epidermis with stomata.

Identification To 0.5 g of Powdered Fennel add 10 mL of hexane, allow to stand for 5 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate prepared with silica gel with fluorescent indicator for thin-layer chromatography. Then develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with dark purple color appears at an *R*f value of about 0.4.

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Essential oil content <*5.01*> Perform the test with 50.0 g of Powdered Fennel: the volume of essential oil is not less than 0.45 mL.

Containers and storage Containers—Tight containers.

Fennel Oil

Oleum Foeniculi

ウイキョウ油

Fennel Oil is the essential oil distilled with steam from the fruit of *Foeniculum vulgare* Miller (*Umbelliferae*) or of *Illicium verum* Hooker filius (*Illiciaceae*).

Description Fennel Oil is a colorless to pale yellow liquid. It has a characteristic, aromatic odor and a sweet taste with a slight, bitter aftertaste.

It is miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

When cold, white crystals or crystalline masses may often separate from the oil.

Identification Dissolve 0.30 g of Fennel Oil in 20 mL of hexane, pipet 1 mL of this solution, add hexane to make exactly 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with a dark purple color appears at the *R*f value of about 0.4.

Refractive index <*2.45*> n_D^{20}: 1.528 – 1.560

Specific gravity <*1.13*> d_{20}^{20}: 0.955 – 0.995

Purity **(1)** Clarity of solution—To 1.0 mL of Fennel Oil add 3 mL of ethanol (95): the solution is clear. To this solution add 7 mL of ethanol (95): the solution remains clear.

(2) Heavy metals <*1.07*>—Proceed with 1.0 mL of Fennel Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Foeniculated Ammonia Spirit

アンモニア·ウイキョウ精

Method of preparation

Ammonia Water	170 mL
Fennel Oil	30 mL
Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Spirits, with the above ingredients. A sufficient quantity of ammonia solution (28) and Purified Water or Purified Water in Containers may be used in place of Ammonia Water.

Description Foeniculated Ammonia Spirit is a colorless to yellow liquid, having a characteristic odor. It has a slightly sweet, pungent taste.

Specific gravity d_{20}^{20}: about 0.85

Alcohol number <*1.01*> Not less than 7.8 (Method 2).

Containers and storage Containers—Tight containers.

Forsythia Fruit

Forsythiae Fructus

レンギョウ

Forsythia Fruit is the fruit of *Forsythia suspensa* Vahl (*Oleaceae*).

Description Ovoid to long ovoid capsule, 1.5 – 2.5 cm in length, 0.5 – 1 cm in width, with acute apex, and sometimes with a peduncle at the base; externally light gray to dark brown, scattered with light gray and small ridged dots, and with two longitudinal furrows; a capsule dehiscing along the longitudinal furrows has the apexes bent backward; the inner surface of dehisced pericarp is yellow-brown in color, with a longitudinal partition-wall in the middle; seeds, slender and oblong, 0.5 – 0.7 cm in length, and usually with a wing.

Odor, slight; taste, slightly bitter.

Identification To 1.0 g of pulverized Forsythia Fruit add 10

mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: a red-purple to red-brown spot is observed at an *R*f value of about 0.3.

Purity **(1)** Branchlet—When perform the test of foreign matter <*5.01*>, the amount of branchlets contained in Forsythia Fruit does not exceed 5.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than branchlets contained in Forsythia Fruit does not exceed 1.0%.

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 10.0%.

Containers and storage Containers—Well-closed containers.

Fritillaria Bulb

Fritillariae Bulbus

バイモ

Fritillaria Bulb is the bulb of *Fritillaria verticillata* Willdenow var. *thunbergii* Baker (*Liliaceae*).

Description Fritillaria Bulb is a depressed spherical bulb, 2 – 3 cm in diameter, 1 – 2 cm in height, consisting of 2 thickened scaly leaves often separated; externally and internally white to light yellow-brown in color; inside base is in a slightly dark color; the bulb sprinkled with lime before drying is dusted with white powder; fractured surface, white in color and powdery.

Odor, slight and characteristic; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer (epidermis) to be composed of a single layer of cells; numerous vascular bundles scattered throughout the parenchyma inside of the epidermis; parenchyma filled with starch grains; starch grains are mainly simple (rarely 2- to 3-compound), 5 – 60 μm in diameter, narrowly ovate to ovate or triangular to obovate, stratiform figure obvious; epidermal cells and parenchyma cells near the vessels contain solitary crystals of calcium oxalate.

Identification Put 2 g of pulverized Fritillaria Bulb in a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of a mixture of ethyl acetate and diethyl ether (1:1), shake for 20 minutes, and centrifuge. Take the upper layer, add 20 g of anhydrous sodium sulfate to the layer, shake, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: spots of a yellow-red color appear at *R*f values of about 0.4 and about 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Fritillaria Bulb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Fritillaria Bulb according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 16.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Gambir

Gambir

アセンヤク

Gambir is the dried aqueous extract prepared from the leaves and young twigs of *Uncaria gambir* Roxburgh (*Rubiaceae*).

Description Brown to dark brown, brittle mass; inside light brown.

Odor, slight; taste, extremely astringent and bitter.

Identification **(1)** To 0.2 g of pulverized Gambir add 10 mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2) Shake 0.1 g of pulverized Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1 mL of vanillin-hydrochloric acid TS: a light red to red-brown color develops.

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 70.0%.

Containers and storage Containers—Well-closed containers.

Powdered Gambir

Gambir Pulveratum

アセンヤク末

Powdered Gambir is the powder of Gambir.

Description Powdered Gambir occurs as a red-brown to dark brown powder. It has a slight odor, and an extremely astringent and bitter taste.

Under a microscope <*5.01*>, Powdered Gambir, immersed in olive oil or liquid paraffin, consists of masses of needle

crystals or yellow-brown to red-brown angular fragments, and reveals epidermal tissue and thick-walled hairs.

Identification **(1)** To 0.2 g of Powdered Gambir add 10 mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2) Shake 0.1 g of Powdered Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1 mL of vanillin-hydrochloric acid TS: a light red to red-brown color develops.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 70.0%.

Containers and storage Containers—Well-closed containers.

Gardenia Fruit

Gardeniae Fructus

サンシシ

Gardenia Fruit is the fruit of *Gardenia jasminoides* Ellis (*Rubiaceae*).

It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

Description Nearly long ovoid to ovoid fruit, 1 – 5 cm in length, 1 – 1.5 cm in width; usually having 6, rarely 5 or 7, markedly raised ridges; calyx or its scar at one end, and sometimes peduncle at the other end; inner surface of pericarp yellow-brown, smooth and lustrous; internally divided into two loculi, containing a mass of seeds in yellow-red to dark red placenta; seed nearly circular, flat, about 0.5 cm in major axis, blackish brown or yellow-red.

Odor, slight; taste, bitter.

Identification **(1)** To 1.0 g of pulverized Gardenia Fruit, previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate trihydrate in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

(2) To 1.0 g of pulverized Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution.

Loss on drying ⟨*5.01*⟩ Not more than 13.0%.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Assay Weigh accurately about 0.5 g of pulverized Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of geniposide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for assay and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Gardenia Fruit

Gardeniae Fructus Pulveratus

サンシシ末

Powdered Gardenia Fruit is the powder of Gardenia Fruit.

It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

Description Powdered Gardenia Fruit occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope <5.01>, Powdered Gardenia Fruit reveals fragments of yellow-brown epidermis consisting of polygonal epidermal cells in surface view; unicellular hairs, spiral and ring vessels, stone cells often containing crystals of calcium oxalate; fragments of thin-walled parenchyma containing yellow pigments, oil drops and rosette aggregates of calcium oxalate (the above elements from fruit receptacle and pericarp); fragments of large and thick-walled epidermis of seed coat, containing a red-brown substance; fragments of endosperm filled with aleuron grains (the above elements from seed).

Identification **(1)** To 1.0 g of Powdered Gardenia Fruit, previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate trihydrate in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

(2) To 1.0 g of Powdered Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution.

Loss on drying <5.01> Not more than 13.0%.

Total ash <5.01> Not more than 6.0%.

Assay Weigh accurately about 0.5 g of Powdered Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of geniposide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for assay and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Gastrodia Tuber

Gastrodiae Tuber

テンマ

Gastrodia Tuber is the steamed tuber of *Gastrodia elata* Blume (*Orchidaceae*).

Description Gastrodia Tuber is an irregularly curved and flattened cylindrical to flattened fusiform tuber, 5 – 15 cm in length, 2 – 5 cm in diameter, 1 – 2 cm in thickness; externally light yellow-brown to light yellowish white; with ring nodes, and irregular longitudinal wrinkles; hard in texture; fractured surface, dark brown to yellow-brown in color, with luster, horny and gluey.

Odor, characteristic; practically tasteless.

Under a microscope <5.01>, a transverse section reveals parenchyma cells containing needle raphides of calcium oxalate; starch grain absent.

Identification To 1 g of pulverized Gastrodia Tuber add 5 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: a red-purple to light brown spot appears at an *R*f value of about 0.4.

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Gastrodia Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Gastrodia Tuber according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 16.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 16.0%.

Containers and storage Containers—Well-closed containers.

Gentian

Gentianae Radix

ゲンチアナ

Gentian is the root and rhizome of *Gentiana lutea* Linné (*Gentianaceae*).

Description Nearly cylindrical pieces, 10 – 50 cm in length, 2 – 4 cm in diameter; externally dark brown; the rhizome short, with fine, transverse wrinkles, and sometimes with buds and remains of leaves at the upper edge. The root longitudinally and deeply wrinkled, and more or less twisted; fractured surface yellow-brown and not fibrous, and a cambium and its neighborhood tinged dark brown.

Odor, characteristic; taste, sweet at first, later persistently bitter.

Under a microscope <*5.01*>, a transverse section of the root reveals several layers of collenchyma adjoined internally to 4 to 6 layers of thin-walled cork; secondary cortex of the parenchyma with irregularly distributed phloem; xylem consisting chiefly of parenchyma, with individual or clustered vessels and tracheids, and exhibiting some sieve tubes of xylem; parenchyma of the xylem and the cortex containing oil droplets, minute needle crystals of calcium oxalate and very rarely starch grains 10 – 20 μm in diameter.

Identification **(1)** Place 0.1 g of pulverized Gentian, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide, and heat gently and gradually: pale yellow crystals are sublimed on the upper slide. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

(2) To 0.5 g of pulverized Gentian add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Gentian according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Gentian according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Powdered Gentian

Gentianae Radix Pulverata

ゲンチアナ末

Powdered Gentian is the powder of Gentian.

Description Powdered Gentian occurs as a yellow-brown powder, and has a characteristic odor. It has a sweet taste at first, which later becomes persistently bitter.

Under a microscope <*5.01*>, Powdered Gentian reveals parenchyma cells containing oil droplets and minute needle crystals, vessels, tracheids, cork tissues, and crystals of calcium oxalate. Vessels are chiefly reticulate vessels and scalariform vessels, 20 – 80 μm in diameter. Starch grains are observed very rarely, in simple grains about 10 – 20 μm in diameter.

Identification **(1)** Place 0.1 g of Powdered Gentian, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide glass, and heat gently and gradually: light yellow crystals are sublimed on the upper glass. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

(2) To 0.5 g of Powdered Gentian add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Gentian according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Gentian according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, stone cell and fiber are not observed.

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Tight containers.

Gentian and Sodium Bicarbonate Powder

ゲンチアナ·重曹散

Method of preparation

Powdered Gentian	300 g
Sodium Bicarbonate	700 g
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Gentian and Sodium Bicarbonate Powder occurs as a light yellow-brown powder, and has a bitter taste.

Identification **(1)** To 2 g of Gentian and Sodium Bicarbonate Powder add 10 mL of water, stir, and filter: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for bicarbonate.

(2) To 1.5 g of Gentian and Sodium Bicarbonate Powder add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R*f value.

Containers and storage Containers—Well-closed containers.

Geranium Herb

Geranii Herba

ゲンノショウコ

Geranium Herb is the terrestrial part of *Geranium thunbergii* Siebold et Zuccarini (*Geraniaceae*).

Description Stem with leaves opposite; stem, slender and long, green-brown; stem and leaf covered with soft hairs; leaf divided palmately into 3 to 5 lobes, and 2 – 4 cm in length, grayish yellow-green to grayish brown; each lobe oblong to obovate, and its upper margin crenate.

Odor, slight; taste, astringent.

Identification Boil 0.1 g of Geranium Herb with 10 mL of water, filter, and to the filtrate add 1 drop of iron (III) chloride TS: a blackish blue color develops.

Purity Foreign matter ⟨*5.01*⟩—The amount of the root and other foreign matter contained in Geranium Herb does not exceed 2.0%.

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Powdered Geranium Herb

Geranii Herba Pulverata

ゲンノショウコ末

Powdered Geranium Herb is the powder of Geranium Herb.

Description Powdered Geranium Herb occurs as a grayish green to light yellow-brown powder. It has a slight odor and an astringent taste.

Under a microscope ⟨*5.01*⟩, Powdered Geranium Herb reveals mainly fibers, spiral vessels, pitted vessels, and unicellular hairs; furthermore, multicellular glandular hairs, epidermis with stomata, fragments of palisade tissue, rosette aggregates of calcium oxalate, and starch grains. Fiber is thick-walled, with somewhat distinct pits; unicellular hair shows small point-like protrusions on the surface; palisade tissue consisting of circular parenchyma cells in surface view, each cell containing one rosette aggregate of calcium oxalate which is about 20 μm in diameter. Starch grains consisting of simple grains but rarely of 2-compound grains, ovoid to spherical, 5 – 30 μm in diameter, with distinct hilum.

Identification Boil 0.1 g of Powdered Geranium Herb with 10 mL of water, filter, and to the filtrate add 1 drop of iron (III) chloride TS: a dark blue color develops.

Purity Foreign matter—Under a microscope ⟨*5.01*⟩, Powdered Geranium Herb reveals no stone cells.

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Ginger

Zingiberis Rhizoma

ショウキョウ

Ginger is the rhizome, with (unpeeled) or without (peeled) the periderm, of *Zingiber officinale* Roscoe (*Zingiberaceae*).

It contains not less than 0.3% of [6]-gingerol ($C_{17}H_{26}O_4$: 294.39), calculated on the basis of dried material.

Description Irregularly compressed and often branched massive rhizome or a part of it; the branched parts are slightly curved ovoid or oblong-ovoid, 2 – 4 cm in length, and 1 – 2 cm in diameter; external surface grayish white to light grayish brown, and often with white powder; fractured surface is somewhat fibrous, powdery, light yellowish brown; under a magnifying glass, a transverse section reveals

cortex and stele distinctly divided; vascular bundles and secretes scattered all over the surface as small dark brown dots.

Odor, characteristic; taste, extremely pungent.

Under a microscope <*5.01*>, a transverse section reveals cork layer, cortex, endodermis and stele in this order from the outside, cork layer often peeled off; cortex and stele, divided by a single-layered endodermis, composed of parenchyma; vascular bundles surrounded by fibers scattered in cortex and stele; oil cells contain yellow oily substances, scattered in parenchyma; parenchyma cells contain solitary crystals of calcium oxalate; starch grains in parenchyma cells mainly simple, ovoid, triangular ovoid, ellipsoidal or spherical, with abaxial hilim, usually 10 – 30 μm in long axis.

Identification To 2 g of pulverized Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution and the spot from the standard solution show the same color tone and *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Ginger according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Ginger according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 8.0%.

Assay Weigh accurately about 1 g of pulverized Ginger (separately determine the loss on drying <*5.01*>, at 105°C for 5 hours), place in a centrifuge tube, add 30 mL of a mixture of methanol and water (3:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of a mixture of methanol and water (3:1), and repeat the extraction twice more. To the combined all extracts add a mixture of methanol and water (3:1) to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-gingerol for assay, dissolve in a mixture of methanol and water (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-gingerol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile and phosphoric acid (3800:2200:1).

Flow rate: Adjust so that the retenton time of [6]-gingerol is about 19 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Ginger

Zingiberis Rhizoma Pulveratum

ショウキョウ末

Powdered Ginger is the powder of Ginger.

It contains not less than 0.20% of [6]-gingerol ($C_{17}H_{26}O_4$: 294.39), calculated on the basis of dried material.

Description Powdered Ginger occurs as a light grayish brown to light grayish yellow powder. It has a characteristic odor and an extremely pungent taste.

Under a microscope <*5.01*>, Powdered Ginger reveals mainly starch grains and parenchyma cells containing them; also, parenchyma cells containing yellow-brown to dark brown oily substances or single crystals of calcium oxalate; fragments of fibers with distinct pits; fragments of spiral, ring and reticulate vessels, and rarely fragments of cork tissue; starch grains composed of simple, compound or half-compound grains, ovoid, triangular ovoid, ellipsoidal or spherical, with abaxial hilum, usually 10 – 30 μm in long axis.

Identification To 2 g of Powdered Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution and the spot from the standard solution show the same color tone and *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Ginger according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Ginger according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Ginger does not show stone cells, lignified parenchyma cells and other foreign matter.

Total ash <5.01> Not more than 8.0%.

Assay Weigh accurately about 1 g of Powdered Ginger (separately determine the loss on drying <5.01>, at 105°C for 5 hours), place in a centrifuge tube, add 30 mL of a mixture of methanol and water (3:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of a mixture of methanol and water (3:1), and repeat the extraction twice more. To the combined all extracts add a mixture of methanol and water (3:1) to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-gingerol for assay, dissolve in a mixture of methanol and water (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-gingerol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile and phosphoric acid (3800:2200:1).

Flow rate: Adjust so that the retenton time of [6]-gingerol is about 19 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Ginseng

Ginseng Radix

ニンジン

Ginseng is the root of *Panax ginseng* C. A. Meyer (*Panax schinseng* Nees) (*Araliaceae*), from which rootlets have been removed, or the root that has been quickly passed through hot water.

It contains not less than 0.10% of ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$: 801.01) and not less than 0.20% of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), calculated on the basis of dried material.

Description Thin and long cylindrical to fusiform root, often branching 2 to 5 lateral roots from the middle; 5 – 20 cm in length, main root 0.5 – 3 cm in diameter; externally light yellow-brown to light grayish brown, with longitudinal wrinkles and scars of rootlets; sometimes crown somewhat constricted and with short remains of rhizome; fractured surface practically flat, light yellow-brown in color, and brown in the neighborhood of the cambium.

Odor, characteristic; taste, at first slightly sweet, followed by a slight bitterness.

Identification (1) On a section of Ginseng add dilute iodine TS dropwise: a dark blue color is produced on the surface.

(2) To 2.0 g of pulverized Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (14:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of pulverized Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of pulverized Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Foreign matter <5.01>—The amount of stems and other foreign matter contained in Ginseng does not exceed 2.0%.

(4) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 4.2%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 14.0%.

Assay (1) Ginsenoside Rg_1—Weigh accurately about 1.0 g of pulverized Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg_1 RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rg_1 in each solution.

Amount (mg) of ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Ginsenoside Rg_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of ginsenoside Rg_1 is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg_1 RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg_1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg_1 is not more than 1.5%.

(2) Ginsenoside Rb_1—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

Amount (mg) of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of ginsenoside Rb_1 is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rb_1 RS and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb_1 and ginsenoside Rc are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Ginseng

Ginseng Radix Pulverata

ニンジン末

Powdered Ginseng is the powder of Ginseng.

It contains not less than 0.10% of ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$: 801.01) and not less than 0.20% of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), calculated on the basis of dried material.

Description Powdered Ginseng occurs as a light yellowish white to light yellowish-brown powder. It has characteristic odor and is a slight sweet taste followed by a slight bitterness.

Under a microscope <*5.01*>, Powdered Ginseng reveals round to rectangular parenchyma cells containing starch grains, occasionally gelatinized starch, vessels, secretory cell, sclerenchyma cell, big and thin-walled cork cell; crystals of calcium oxalate and starch. Vessels are reticulate vessel fragments, scalariform vessel and spiral vessel, 15 – 40 μm in diameter. Secretory cell containing a mass of yellow glistened contents; rosette aggregate of calcium oxalate, 20 – 60 μm in diameter, and 1 – 5 μm in diameter, rarely up to 30 μm in diameter of its single crystal; sclerenchymatous cells and thin-walled cork cells. Starch grains are observed in simple grain and 2 to 6-compound grain, simple grain, 3 – 20 μm in diameter.

Identification To 2.0 g of Powdered Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (14:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Powdered Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 4.2%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract; not less than 14.0%.

Assay **(1)** Ginsenoside Rg_1—Weigh accurately about

1.0 g of Powdered Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg_1 RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rg_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rg_1\ (C_{42}H_{72}O_{14}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ginsenoside Rg_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of ginsenoside Rg_1 is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg_1 RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg_1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg_1 is not more than 1.5%.

(2) Ginsenoside Rb_1—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determined the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rb_1\ (C_{54}H_{92}O_{23}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of ginsenoside Rb_1 is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rb_1 RS and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb_1 and ginsenoside Rc are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

Containers and storage Containers—Tight containers.

Glehnia Root and Rhizome

Glehniae Radix cum Rhizoma

ハマボウフウ

Glehnia Root and Rhizome is the root and rhizome of *Glehnia littoralis* Fr. Schmidt ex Miquel (*Umbelliferae*).

Description Cylindrical to long conical root or rhizome, 10 – 20 cm in length, 0.5 – 1.5 cm in diameter; externally light yellow-brown to red-brown. Rhizome short, with fine ring nodes; roots having longitudinal wrinkes and numerous, dark red-brown, warty protrusions or transversely elongated protuberances. Brittle and easily breakable. A transverse section white and powdery, and under a magnifying glass, oil canals scattered as brown dots.

Odor, slight; taste, slightly sweet.

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Glehnia Root and Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Glehnia Root and Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Glycyrrhiza

Glycyrrhizae Radix

カンゾウ

Glycyrrhiza is the root and stolon, with (unpeeled) or without (peeled) the periderm, of *Glycyrrhiza uralensis* Fisher or *Glycyrrhiza glabra* Linné (*Leguminosae*).

It contains not less than 2.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Nearly cylindrical pieces, 0.5 – 3 cm in diameter, over 1 m in length. Glycyrrhiza is externally dark brown to red-brown, longitudinally wrinkled, and often has lenticels, small buds and scaly leaves; peeled Glycyrrhiza is externally light yellow and fibrous. The transverse section reveals a rather clear border between phloem and xylem, and a radial structure which often has radiating splits; a pith in Glycyrrhiza originated from stolon, but no pith from root.

Odor, slight; taste, sweet.

Under a microscope <*5.01*>, a transverse section reveals several layers of yellow-brown cork layers, and 1- to 3-cellular layer of cork cortex inside the cork layer; the cortex exhibiting medullary rays and obliterated sieve portions radiated alternately; the phloem exhibiting groups of phloem fibers with thick but incompletely lignified walls and surrounded by crystal cells; peeled Glycyrrhiza some times lacks periderm and a part of phloem; the xylem exhibiting large yellow vessels and medullary rays in 3 to 10 rows radiated alternately; the vessels accompanied with xylem fibers surrounded by crystal cells, and with xylem parenchyma cells; the parenchymatous pith only in Glycyrrhiza originated from stolon. The parenchyma cells contain starch grains and often solitary crystals of calcium oxalate.

Identification To 2 g of pulverized Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizic Acid RS or glycyrrhizic acid for thin-layer chromatography in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution and a spot from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of pulverized Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 15 minutes.

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak with the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Glycyrrhiza

Glycyrrhizae Radix Pulverata

カンゾウ末

Powdered Glycyrrhiza is the powder of Glycyrrhiza. It contains not less than 2.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Powdered Glycyrrhiza is light yellow-brown or light yellow to grayish yellow (powder of peeled Glycyrrhiza) in color. It has a slight odor and a sweet taste.

Under a microscope <*5.01*>, Powdered Glycyrrhiza reveals mainly yellow sclerenchymatous fiber bundles accompanied with crystal cell rows; vessels, 80 – 200 μm in diameter, with pitted, reticulate and scalariform pits, and with round perforations; parenchyma cells, containing starch grains and solitary crystals of calcium oxalate, their fragments, and cork tissues; but powder of peeled Glycyrrhiza shows no cork tissue; if any, a very few. Starch grains are simple grains, 2 – 20 μm in diameter; solitary crystals of calcium oxalate, 10 – 30 μm in a diameter.

Identification To 2 g of Powdered Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizic Acid RS or glycyrrhizic acid for thin-layer chromatography in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution and a spot from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Glycyrrhiza shows no stone cells.

(4) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of Powdered Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 15 minutes.

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak with the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Glycyrrhiza Extract

カンゾウエキス

Glycyrrhiza Extract contains not less than 4.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Method of preparation To 1 kg of fine cuttings of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza add 5 L of Water, Purified Water or Purified Water in Containers, and macerate for 2 days. Filter the macerated solution through a cloth filter. Add 3 L of Water, Purified Water or Purified Water in Containers to the residue, macerate again for 12 hours, and filter through a cloth filter. Evaporate the combined filtrates until the whole volume becomes 3 L. After cooling, add 1 L of Ethanol, and allow to stand in a cold place for 2 days. Filter, and evaporate the filtrate to a viscous extract.

Description Glycyrrhiza Extract is a brown to blackish brown, viscous extract, and has a characteristic odor and a

sweet taste.

It dissolves in water, forming a clear solution, or with a slight turbidity.

Identification To 0.8 g of Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 2 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Glycyrrhiza Extract as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Insoluble matter—Dissolve 2.0 g of Glycyrrhiza Extract in 18 mL of water, and filter. To 10 mL of the filtrate add 5 mL of ethanol (95): a clear solution results.

Assay Weigh accurately about 0.15 g of Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg of propyl parahydroxybenzoate for resolution check in 20 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, glycyrrhizic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Glycyrrhiza Extract contains not less than 6.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Method of preparation Boil coarse powder of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza with Water, Purified Water or Purified Water in Containers, filter the solution under pressure, and evaporate the filtrate.

Description Crude Glycyrrhiza Extract occurs as lustrous, dark yellow-red to blackish brown plates, rods or masses. It is comparatively brittle when cold, and the fractured surface is dark yellow-red, shell-like, and lustrous. It softens when warmed.

It has a characteristic odor and a sweet taste.

It dissolves in water with turbidity.

Identification To 0.6 g of Crude Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), dissolve by warming if necessary, cool, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Crude Glycyrrhiza Extract as directed in the Extracts (4) under General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Water-insoluble substances—Boil 5.0 g of pulverized Crude Glycyrrhiza Extract with 100 mL of water. After cooling, filter the mixture through tared filter paper, wash with water, and dry the residue at 105°C for 5 hours: the mass of the residue is not more than 1.25 g.

(3) Foreign matter—The filtrate obtained in (2) does not have a strong bitter taste.

(4) Starch—To about 1 g of pulverized Crude Glycyrrhiza Extract add water to make 20 mL, shake the mixture thoroughly, and filter. Examine the insoluble substance on the filter paper under a microscope: the residue contains no starch grains.

Total ash <*5.01*> Not more than 12.0% (1 g).

Assay Weigh accurately about 0.15 g of Crude Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg of propyl parahydroxybenzoate for resolution check in 20 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, glycyrrhizic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Goshajinkigan Extract

牛車腎気丸エキス

Goshajinkigan Extract contains not less than 4 mg and not more than 16 mg of loganin, not less than 6 mg and not more than 18 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.2 mg (for preparation prescribed Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Rehmannia Root	5 g	5 g
Cornus Fruit	3 g	3 g
Dioscorea Rhizome	3 g	3 g
Alisma Tuber	3 g	3 g
Poria Sclerotium	3 g	3 g
Moutan Bark	3 g	3 g
Cinnamon Bark	1 g	1 g
Powdered Processed Aconite Root (Powdered Processed Aconite Root 1)	1 g	—
Powdered Processed Aconite Root (Powdered Processed Aconite Root 2)	—	1 g
Achyranthes Root	3 g	3 g
Plantago Seed	3 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Goshajinkigan Extract occurs as a brown to dark brown powder or blackish brown viscous extract. It has slightly a characteristic odor and an acid taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at an *R*f value of about 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Cornus Fruit).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Alisma Tuber).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300 mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Powdered Processed Aconite Root).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.3 g of pulverized plantago seed for thin-layer chromatography, add 1 mL of methanol, warm on a water bath for 3 minutes, centrifuge after cooling, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value (around 0.3) with the deep blue spot from the standard solution (Plantago Seed).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 2 g of achyranthes root for thin-layer chromatography, add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value (around 0.4) with the dark red spot from the standard solution (Achyranthes Root).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5 respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ The dry extract: Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash ⟨*5.01*⟩ Not more than 9.0%, calculated on the dried basis.

Assay (1) Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately 10 mg of loganin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of loganin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine,

A_{TM} and A_{SM}, A_{TH} and A_{SH}, as well as A_{TA} and A_{SA}, in each solution, respectively.

Amount (mg) of benzoylmesaconine hydrochloride
$= C_{SM} \times A_{TM}/A_{SM} \times 10$

Amount (mg) of benzoylhypaconine hydrochloride
$= C_{SH} \times A_{TH}/A_{SH} \times 10$

Amount (mg) of 14-anisoylaconine hydrochloride
$= C_{SA} \times A_{TA}/A_{SA} \times 10$

C_{SM}: Concentration (mg/mL) of benzoylmesaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay
C_{SH}: Concentration (mg/mL) of benzoylhypaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay
C_{SA}: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Gypsum

Gypsum Fibrosum

セッコウ

Gypsum is natural hydrous calcium sulfate. It possibly corresponds to the formula $CaSO_4.2H_2O$.

Description Gypsum occurs as lustrous, white, heavy, fibrous, crystalline masses, which easily split into needles or very fine crystalline powder.

It is odorless and tasteless.

It is slightly soluble in water.

Identification To 1 g of pulverized Gypsum add 20 mL of water, allow to stand with occasional shaking for 30 minutes, and filter: the filtrate responds to the Qualitative Tests <*1.09*> (2) and (3) for calcium salt and to the Qualitative Tests <*1.09*> for sulfate.

Purity **(1)** Heavy metals <*1.07*>—Boil 4.0 g of pulverized Gypsum with 4 mL of acetic acid (100) and 96 mL of water for 10 minutes, cool, add water to make exactly 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 4.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Gypsum according to Method 2, and perform the test (not more than 5 ppm).

Containers and storage Containers—Well-closed containers.

Exsiccated Gypsum

Gypsum Exsiccatum

焼セッコウ

Exsiccated Gypsum possibly corresponds to the formula $CaSO_4.\frac{1}{2}H_2O$.

Description Exsiccated Gypsum occurs as a white to grayish white powder. It is odorless and tasteless.

It is slightly soluble in water, and practically insoluble in ethanol (95).

It absorbs moisture slowly on standing in air to lose its solidifying property.

When it is heated to yield an anhydrous compound at a temperature above 200°C, it loses its solidifying property.

Identification Shake 1 g of Exsiccated Gypsum with 20 mL of water for 5 minutes, and filter: the filtrate responds to the Qualitative Tests <*1.09*> (2) and (3) for calcium salt and to the Qualitative Tests <*1.09*> for sulfate.

Purity Alkalinity—Take 3.0 g of Exsiccated Gypsum in a glass-stoppered test tube, add 10 mL of water and 1 drop of phenolphthalein TS, and shake vigorously: no red color develops.

Solidification To 10.0 g of Exsiccated Gypsum add 10 mL of water, stir immediately for 3 minutes, and allow to stand: the period until water no longer separates, when the material is pressed with a finger, is not more than 10 minutes from the time when the water was added.

Containers and storage Containers—Tight containers.

Hachimijiogan Extract

八味地黄丸エキス

Hachimijiogan Extract contains not less than 4 mg and not more than 16 mg of loganin, not less than 6 mg and not more than 18 mg (for preparation prescribed 3 g of Moutan Bark) or not less than 5 mg and not more than 15 mg (for preparation prescribed 2.5 g of Moutan Bark) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.7 mg (for preparation prescribed 1 g of Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride), or not less than 0.2

mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), or not less than 0.1 mg (for preparation prescribed 0.5 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Rehmannia Root	5 g	5 g	5 g	6 g
Cornus Fruit	3 g	3 g	3 g	3 g
Dioscorea Rhizome	3 g	3 g	3 g	3 g
Alisma Tuber	3 g	3 g	3 g	3 g
Poria Sclerotium	3 g	3 g	3 g	3 g
Moutan Bark	3 g	3 g	3 g	2.5 g
Cinnamon Bark	1 g	1 g	1 g	1 g
Processed Aconite Root (Processed Aconite Root 1)	1 g	—	—	—
Powdered Processed Aconite Root (Powdered Processed Aconite Root 1)	—	1 g	—	0.5 g
Powdered Processed Aconite Root (Powdered Processed Aconite Root 2)	—	—	1 g	—

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Hachimijiogan Extract occurs as a grayish brown to brown powder or blackish brown viscous extract. It has a characteristic odor and a slightly bitter and acid taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at an *R*f value of about 0.6 (Rehmannia Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Cornus Fruit).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Alisma Tuber).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300 mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

(ii) To 2.0 g of dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of

methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet exactly 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5 %.

Loss on drying <*2.41*> The dry extract: Not more than 8.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Loganin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately 10 mg of loganin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of loganin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating con-

ditions, the number of theoretical plates and symmetry factor of the peak of loganin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of loganin is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <2.48> by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM}, A_{TH} and A_{SH}, as well as A_{TA} and A_{SA}, in each solution, respectively.

$$\text{Amount (mg) of benzoylmesaconine hydrochloride} = C_{SM} \times A_{TM}/A_{SM} \times 10$$

$$\text{Amount (mg) of benzoylhypaconine hydrochloride} = C_{SH} \times A_{TH}/A_{SH} \times 10$$

$$\text{Amount (mg) of 14-anisoylaconine hydrochloride} = C_{SA} \times A_{TA}/A_{SA} \times 10$$

C_{SM}: Concentration (mg/mL) of benzoylmesaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

C_{SH}: Concentration (mg/mL) of benzoylhypaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

C_{SA}: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Hangekobokuto Extract

半夏厚朴湯エキス

Hangekobokuto Extract contains not less than 2 mg and not more than 6 mg of magnolol, not less than 4 mg (for preparation prescribed 2 g of Perilla Herb) or not less than 6 mg (for preparation prescribed 3 g of Perilla Herb) of rosmarinic acid, and not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.8 mg and not more than 3.2 mg (for preparation prescribed 1.3 g of Ginger) or not less than 0.9 mg and not more than

3.6 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Pinellia Tuber	6 g	6 g	6 g	6 g
Poria Sclerotium	5 g	5 g	5 g	5 g
Magnolia bark	3 g	3 g	3 g	3 g
Perilla Herb	2 g	3 g	2 g	2 g
Ginger	1 g	1 g	1.3 g	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Hangekobokuto Extract is a light brown to dark brown powder or blackish brown viscous extract. It has a characteristic odor and has a bitter and astringent taste first then pungent later.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Magnolia Bark).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (60:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Perilla Herb).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 11.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 14.0%, calculated on the dried basis.

Assay **(1)** Magnolol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S \times 1/8$$

M_S: Amount (mg) of magnolol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: 1.0 mL per minute (the retention time of magnolol is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

(2) Rosmarinic acid—Conduct this procedure using

light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of rosmarinic acid for assay, dissolve in diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of rosmarinic acid in each solution.

Amount (mg) of rosmarinic acid = $M_S \times A_T/A_S \times 1/4$

M_S: Amount (mg) of rosmarinic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 330 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).

Flow rate: 1.0 mL per minute (the retention time of rosmarinic acid is about 11 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rosmarinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rosmarinic acid is not more than 1.5%.

(3) [6]-Gingerol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

Amount (mg) of [6]-gingerol = $M_S \times A_T/A_S \times 1/20$

M_S: Amount (mg) of [6]-gingerol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Hangeshashinto Extract

半夏瀉心湯エキス

Hangeshashinto Extract contains not less than 70 mg and not more than 210 mg (for preparation prescribed 2.5 g of Scutellaria Root) or not less than 80 mg and not more than 240 mg (for preparation prescribed 3 g of Scutellaria Root) of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 22 mg and not more than 66 mg (for preparation prescribed 2.5 g of Glycyrrhiza) or not less than 25 mg and not more than 75 mg (for preparation prescribed 3 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 7 mg and not more than 21 mg of berberine [expressed as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Pinellia Tuber	5 g	6 g	5 g
Scutellaria Root	2.5 g	3 g	2.5 g
Processed Ginger	2.5 g	3 g	—
Ginger	—	—	2.5 g
Ginseng	2.5 g	3 g	2.5 g
Glycyrrhiza	2.5 g	3 g	2.5 g
Jujube	2.5 g	3 g	2.5 g
Coptis Rhizome	1 g	1 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1), 2) or 3), using the crude drugs shown above.

Description Hangeshashinto Extract is a light yellow to yellow-brown powder or blackish brown viscous extract. It has a slightly odor and a hotter, bitter and slightly sweet taste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several

spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Scutellaria Root).

(2) For preparation prescribed Processed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 1 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Processed Ginger).

(3) For preparation prescribed Ginger—Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Ginger).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rg_1 RS or ginsenoside Rg_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(6) Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of coptisine chloride for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (15:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Coptis Rhizome).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract—Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract—Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diame-

ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

(3) Berberine—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.2 g of the dried substance), add exactly 50 mL of the mobile phase, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water <*2.48*> in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of berberine in each solution.

Amount (mg) of berberine chloride ($C_{20}H_{18}ClNO_4$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: 1.0 mL per minute (the retention time of berberine is about 8 minutes).

System suitability—

System performance: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatine and berberine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Hedysarum Root

Hedysari Radix

シンギ

Hedysarum Root is the root of *Hedysarum polybotrys* Handel-Mazzetti (*Leguminosae*).

Description Hedysarum Root is nearly cylindrical, 20 – 100 cm in length, 0.5 – 2.5 cm in diameter; outer surface yellowish brown to reddish brown, with irregular longitudinal wrinkles; often horizontal lenticels and scars of lateral roots; periderm peeled easily, internally light yellowish brown to light reddish brown; soft in texture, flexible and difficult to break; fractured surface fibrous, powdery; in transverse section nearly white in cortex, brownish around cambium, light yellowish brown in xylem; ray obvious.

Odor, slightly characteristic; taste, slightly sweet.

Under a microscope <*5.01*>, a transverse section reveals cork layer 6 – 8 cells layered, 2 – 4 cells layered parenchyma cells with sparingly thick wall inside the cork layer; ray obvious in secondary cortex and often appearing cracked tissue in outer portion of secondary cortex; phloem fiber bungles arranged stepwise in phloem; ray obvious in xylem, reticulate, scalariform, pitted, and spiral vessels; xylem tissues around vessels; thin walled cells containing solitary crystals of calcium oxalate in peripheral region of phloem fibers and

xylem fibers and appearing as crystal cell rows in a longitudinal section; solitary crystals of calcium oxalate 7 – 20 μm in diameter, starch grains simple or 2- to 8-compound grains in parenchyma.

Identification To 1.0 g of pulverized Hedysarum Root add 10 mL of methanol, shake for 10 minutes, and filter. Evaporate the solvent of the filtrate under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, 2-butanone and formic acid (60:40:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot at an *R*f value of about 0.4 is observed.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Hedysarum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Hedysarum Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 16.0% (6 hours).

Total ash <*5.01*> Not more than 5.5%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Hemp Fruit

Cannabis Fructus

マシニン

Hemp Fruit is the fruit of *Cannabis sativa* Linné (*Moraceae*).

Description Hemp Fruit is a slightly compressed void fruit, 4 – 5 mm in length, 3 – 4 mm in diameter; externally grayish green to grayish brown; pointed at one end, a scar of gynophore at the other end, and crest lines on both sides; outer surface lustrous with white mesh-like pattern; slightly hard pericarp; seed, slightly green in color and internally has grayish white albumen; 100 fruits weigh 1.6 – 2.7 g.

Practically odorless, aromatic on chewing; taste, mild and oily.

Under a microscope <*5.01*>, a transverse section reveals the exocarp to be a single-layered epidermis; mesocarp composed of parenchyma, a pigment cell layer and rows of short, small cells; endocarp made up of a layer of radially elongated stone cells; seed coat comprises a tubular cell layer and spongy tissue. Inside of the seed; exosperm consists of one layer of parenchymatous cells, endosperm of one to several layers of parenchymatous cells; most of the embryo composed of parenchyma, vascular bundles occurring in the center of hypocotyls and cotyledons; embryo parenchyma contains aleurone grains and oil drops.

Identification To 0.3 g of pulverized Hemp Fruit add 3 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (9:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a dark blue-purple spot appears at an *R*f value of about 0.6.

Purity Bract—When perform the test of foreign matter <*5.01*>, Hemp Fruit does not contain bract.

Loss on drying <*5.01*> Not more than 9.0% (6 hours).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Hochuekkito Extract

補中益気湯エキス

Hochuekkito Extract contains not less than 16 mg and not more than 64 mg of hesperidin, not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1 g of Bupleurum Root) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 2 g of Bupleurum Root) of saikosaponin b_2, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)	6)
Ginseng	4 g	4 g	4 g	4 g	4 g	4 g
Atractylodes Rhizome	4 g	—	4 g	—	4 g	4 g
Atractylodes Lancea Rhizom	—	4 g	—	4 g	—	—
Astragalus Root	4 g	4 g	4 g	4 g	3 g	4 g
Japanese Angelica Root	3 g	3 g	3 g	3 g	3 g	3 g
Citrus Unshiu Peel	2 g	2 g	2 g	2 g	2 g	2 g
Jujube	2 g	2 g	2 g	2 g	2 g	2 g
Bupleurum Root	2 g	2 g	1 g	1 g	2 g	1 g
Glycyrrhiza	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Ginger	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	—
Processed Ginger	—	—	—	—	—	0.5 g
Cimicifuga Rhizome	1 g	1 g	0.5 g	0.5 g	1 g	0.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 6), using the crude drugs shown above.

Description Hochuekkito Extract occurs as a light brown to brown powder or blackish brown viscous extract. It has a slight odor, and a sweet and bitter taste.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and shake. Take the 1-butanol layer, evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample

solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, shake, and take the hexane layer. To the hexane layer add anhydrous sodium sulfate to dry, filter, evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot appears an *R*f value of about 0.4, which shows a greenish brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allowing to cool (Atractylodes Lancea Rhizome).

(4) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 40 mL of a solution of potassium hydroxide in methanol (1 in 50), shake for 15 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 30 mL of water to the residue, then add 20 mL of diethyl ether, shake, and take the water layer. To the water layer add 20 mL of 1-butanol, shake, and take the 1-butanol layer. To the 1-butanol layer add 20 mL of water, shake, take the 1-butanol layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water, 1-butanol and acetic acid (100) (60:30:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution (Astragalus Root).

(5) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Japanese Angelica Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL of the sample solution and 20 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to ammonia vapor: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution (Citrus Unshiu Peel).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous

extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(9) For preparation prescribed Ginger—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

(10) For preparation prescribed Processed Ginger—Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for about 1 hour, separate the hexane layer, and use this as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 60 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Processed Ginger).

(11) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use (*E*)-isoferulic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the light yellowish white fluorescent spot obtained from the standard solution (Cimicifuga Rhizome).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 11.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Hesperidin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of hesperidin in each solution.

$$\text{Amount (mg) of hesperidin} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of hesperidin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hespiridin is not more than 1.5%.

(2) Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Honey

Mel

ハチミツ

Honey is the saccharine substances obtained from the honeycomb of *Apis mellifera* Linné or *Apis cerana* Fabricius (*Apidae*).

Description Honey is a light yellow to light yellow-brown, syrupy liquid. Usually it is transparent, but often opaque with separated crystals.

It has a characteristic odor and a sweet taste.

Specific gravity <2.56> Mix 50.0 g of Honey with 100 mL of water: the specific gravity of the solution is not less than d_{20}^{20}: 1.111.

Purity **(1)** Acidity—Mix 10 g of Honey with 50 mL of water, and titrate <2.50> with 1 mol/L potassium hydroxide VS (indicator: 2 drops of phenolphthalein TS): not more than 0.5 mL is required.

(2) Sulfate—Mix 1.0 g of Honey with 2.0 mL of water, and filter. To the filtrate add 2 drops of barium chloride TS: the solution does not show any change immediately.

(3) Ammonia-coloring substances—Mix 1.0 g of Honey with 2.0 mL of water, and filter. To the filtrate add 2 mL of ammonia TS: the solution does not show any change immediately.

(4) Resorcinol-coloring substances—Mix well 5 g of Honey with 15 mL of diethyl ether, filter, and evaporate the diethyl ether solution at ordinary temperature. To the residue add 1 to 2 drops of resorcinol TS: a yellow-red color may develop in the solution of resorcinol and in the residue, and a red to red-purple color which does not persist more than 1 hour.

(5) Starch or dextrin—(i) Shake 7.5 g of Honey with 15 mL of water, warm the mixture on a water bath, and add 0.5 mL of tannic acid TS. After cooling, filter, and to 1.0 mL of the filtrate add 1.0 mL of ethanol (99.5) containing 2 drops of hydrochloric acid: no turbidity is produced.

(ii) To 2.0 g of Honey add 10 mL of water, warm in a water bath, mix, and allow to cool. Shake 1.0 mL of the mixture with 1 drop of iodine TS: no blue, green or red-brown color develops.

(6) Foreign matter—Mix 1.0 g of Honey with 2.0 mL of water, centrifuge the mixture, and examine the precipitate

microscopically <*5.01*>: no foreign substance except pollen grains is observable.

Total ash <*5.01*> Not more than 0.4%.

Containers and storage Containers—Tight containers.

Houttuynia Herb

Houttuyniae Herba

ジュウヤク

Houttuynia Herb is the terrestrial part of *Houttuynia cordata* Thunberg (*Saururaceae*), collected during the flowering season.

Description Stem with alternate leaves and spikes; stem light brown, with longitudinal furrows and protruded nodes; when soaked in water and smoothed out, leaves wide ovate and cordate, 3 – 8 cm in length, 3 – 6 cm in width; light green-brown; margin entire, apex acuminate; petiole long, and membranous stipule at the base; spike, 1 – 3 cm in length, with numerous light yellow-brown achlamydeous florets, and the base enclosed by 4 long ovate, light yellow to light yellow-brown involucres.

Odor, slight; tasteless.

Identification Boil 2 g of pulverized Houttuynia Herb with 20 mL of ethyl acetate under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the filtrate to dryness, add 10 mL of water to the residue, warm the mixture on a water bath for 2 minutes, and, after cooling, filter. Shake well the filtrate with 20 mL of ethyl acetate in a separator, take 15 mL of ethyl acetate solution, and evaporate the solution on a water bath to dryness. Dissolve the residue in 5 mL of methanol, add 0.1 g of magnesium ribbon and 1 mL of hydrochloric acid, and allow the mixture to stand: a light red to red color develops.

Purity Foreign matter <*5.01*>—The amount of the rhizome, roots and other foreign matter contained in Houttuynia Herb is not more than 2.0%.

Total ash <*5.01*> Not more than 14.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 10.0%.

Containers and storage Containers—Well-closed containers.

Immature Orange

Aurantii Fructus Immaturus

キジツ

Immature Orange is the immature fruit or the fruit cut crosswise of *Citrus aurantium* Linné var. *daidai* Makino, *Citrus aurantium* Linné or *Citrus natsudaidai* Hayata (*Rutaceae*).

Description Nearly spherical fruit, 1 – 2 cm in diameter, or semispherical, 1.5 – 4.5 cm in diameter; external surface, deep green-brown to brown, and without luster, with numerous small dents associated with oil sacs; the outer portion of transverse section exhibits pericarp and mesocarp about 0.4 cm in thickness, yellow-brown in color in the region contacting epidermis, and light grayish brown color in the other parts; the central portion is radially divided into 8 to 16 small loculi; each loculus is brown and indented, often containing immature seeds.

Odor, characteristc; taste, bitter.

Identification To 0.5 g of pulverized Immature Orange add 10 mL of methanol, boil gently for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium ribbon and 1 mL of hydrochloric acid, and allow to stand: a red-purple color develops.

Total ash <*5.01*> Not more than 7.0%.

Containers and storage Containers—Well-closed containers.

Imperata Rhizome

Imperatae Rhizoma

ボウコン

Imperata Rhizome is the rhizome of *Imperata cylindrica* Beauvois (*Gramineae*), from which rootlets and scale leaves have been removed.

Description Long and thin cylindrical rhizome, 0.3 – 0.5 cm in diameter; sometimes branched; externally yellowish white, with slight longitudinal wrinkles, and with nodes at 2 – 3 cm intervals; difficult to break; fractured surface fibrous. Cross section irregularly round; thickness of cortex is slightly smaller than the diameter of the stele; pith often forms a hollow. Under a magnifying glass, a transverse section reveals cortex, yellowish white, and with scattered brown spots; stele, yellow-brown in color.

Odorless, and tasteless at first, but later slightly sweet.

Identification To 1 g of pulverized Imperata Rhizome add 20 mL of hexane, allow the mixture to stand for 30 minutes with occasional shaking, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 5 mL of acetic anhydride, place 0.5 mL of this solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to blue-purple color.

Purity **(1)** Rootlet and scaly leaf—When perform the test of foreign matter <*5.01*>, the amount of the rootlets and scaly leaves contained in Imperata Rhizome is not more than 3.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Imperata Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Imperata Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than rootlets and scaly leaves is not more than 1.0%.

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Containers and storage Containers—Well-closed contain-

ers.

Ipecac

Ipecacuanhae Radix

トコン

Ipecac is the root and rhizome of *Cephaelis ipecacuanha* A. Richard or *Cephaelis acuminata* Karsten (*Rubiaceae*).

It contains not less than 2.0% of the total alkaloids (emetine and cephaeline), calculated on the basis of dried material.

Description Slender, curved, cylindrical root, 3 – 15 cm in length, 0.3 – 0.9 cm in diameter; mostly twisted, and sometimes branched; outer surface gray, dark grayish brown, red-brown in color and irregularly annulated; when root fractured, cortex easily separable from the xylem; the cortex on the fractured surface is grayish brown, and the xylem is light brown in color: thickness of cortex up to about two-thirds of radius in thickened portion. Scales in rhizome opposite.

Odor, slight; powder irritates the mucous membrane of the nose; taste, slightly bitter and unpleasant.

Under a microscope <*5.01*>, the transverse section of Ipecac reveals a cork layer, consisting of brown thin-walled cork cells; in the cortex, sclerenchyma cells are absent; in the xylem, vessels and tracheids arranged alternately; parenchyma cells filled with starch grains and sometimes with raphides of calcium oxalate.

Identification To 0.5 g of pulverized Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small pieces of chlorinated lime: circumference of it turns red.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Ipecac according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Ipecac according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of pulverized Ipecac, in a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{TE} and A_{TC}, of emetine and cephaeline obtained with the sample solution, and the peak area, A_{SE}, of emetine obtained with the standard solution.

$$\text{Amount (mg) of total alkaloids (emetine and cephaeline)} = M_S \times \{A_{TE} + (A_{TC} \times 0.971)\}/A_{SE} \times 0.868$$

M_S: Amount (mg) of emetine hydrochloride for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust so that the retention time of emetine is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of emetine hydrochloride for assay and cephaeline hydrobromide in 0.01 mol/L hydrochloric acid TS to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cephaeline and emetine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of emetine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Ipecac

Ipecacuanhae Radix Pulverata

トコン末

Powdered Ipecac is the powder of Ipecac or its powder diluted with Potato Starch.

It contains not less than 2.0% and not more than 2.6% of the total alkaloids (emetine and cephaeline), calculated on the basis of dried material.

Description Powdered Ipecac occurs as a light grayish yellow to light brown powder. It has a slight odor, which is irritating to the nasal mucosa, and has a somewhat bitter and unpleasant taste.

Under a microscope <*5.01*>, Powdered Ipecac reveals starch grains and needle crystals of calcium oxalate; fragments of parenchyma cells containing starch grains or the needle crystals; substitute fibers,thin-walled cork tissue; vessels and tracheids with simple or bordered pits; a few wood fibers and wood parenchyma. Starch grains inherent in Ipecac, mainly 2 – 8-compound grains, rarely simple grains 4 – 22 μm in diameter; and needle crystals of calcium oxalate 25 – 60 μm in length.

Identification To 0.5 g of Powdered Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small pieces of chlorinated lime: circumfer-

ence of it turns red.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Ipecac according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Ipecac according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, groups of stone cells and sclerenchymatous fibers are not observed.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of Powdered Ipecac, transfer into a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{TE} and A_{TC}, of emetine and cephaeline obtained with the sample solution, and the peak area, A_{SE}, of emetine obtained with the standard solution.

$$\text{Amount (mg) of total alkaloids (emetine and cephaeline)} = M_S \times \{A_{TE} + (A_{TC} \times 0.971)\}/A_{SE} \times 0.868$$

M_S: Amount (mg) of emetine hydrochloride for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust so that the retention time of emetine is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of emetine hydrochloride for assay and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. When the procedure is run with 10 μL of this solution under the above operating conditions, cephaeline and emetine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of emetine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Ipecac Syrup

トコンシロップ

Ipecac Syrup is a syrup containing not less than 0.12 g and not more than 0.15 g of the total alkaloids (emetine and cephaeline) per 100 mL.

Method of preparation Take coarse powder of Ipecac, prepare the fluidextract as directed under Fluidextracts using a mixture of Ethanol and Purified Water or Purified Water in Containers (3:1), and evaporate the mixture under reduced pressure or add a suitable amount of Ethanol or Purified Water or Purified Water in Containers if necessary to get a solution containing 1.7 to 2.1 g of the total alkaloids (emetine and cephaeline) per 100 mL. To 70 mL of this solution add 100 mL of Glycerin and Simple Syrup to make 1000 mL, as directed under Syrups.

Description Ipecac Syrup is a yellow-brown, viscous liquid. It has a sweet taste and a bitter aftertaste.

Identification Take 2 mL of Ipecac Syrup into an evaporating dish, mix with 1 mL of hydrochloric acid, and add small pieces of chlorinated lime: circumference of it turns orange.

Purity Ethanol—Take exactly 5 mL of Ipecac Syrup, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, pipet 5 mL of ethanol (99.5), and add water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the rate of peak height of ethanol to that of the internal standard, Q_T and Q_S: Q_T is not larger than Q_S.

Internal standard solution—A solution of acetonitrile (1 in 20).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass-column about 3 mm in inside diameter and about 1.5 m in length, packed with ethylvinylbenzene-divinylbenzene porous co-polymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of between 105°C and 115°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethanol is 5 to 10 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions. Use a column giving elution of ethanol and the internal standard in this order, and clearly separating each peak.

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^3 CFU/mL and 10^2 CFU/mL, respectively. *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.

Assay Take exactly 5 mL of Ipecac Syrup, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use the solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the

standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{TE} and A_{TC}, of emetine and cephaeline with the sample solution, and the peak area, A_{SE}, of emetine with the standard solution.

$$\text{Amount (mg) of total alkaloids (emetine and cephaeline)} = M_S \times \{A_{TE} + (A_{TC} \times 0.971)\}/A_{SE} \times 1/2 \times 0.868$$

M_S: Amount (mg) of emetine hydrochloride for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium l-heptane sulfonate in 500 mL of water, adjust the pH to 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust so that the retention time of emetine is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of emetine hydrochloride for assay and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. When the procedure is run with 10 μL of this solution under the above operating conditions, cephaeline and emetine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of emetine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Japanese Angelica Root

Angelicae Acutilobae Radix

トウキ

Japanese Angelica Root is the root of *Angelica acutiloba* Kitagawa or *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino (*Umbelliferae*), usually after being passed through hot water.

Description Thick and short main root, with numerous branched roots, nearly fusiform; 10 – 25 cm in length; externally dark brown to red-brown, with longitudinal wrinkles and horizontal protrusions composed of numerous scars of fine rootlets; fractured surface is dark brown to yellow-brown in color, and smooth; and with a little remains of leaf sheath at the crown.

Odor, characteristic; taste, slightly sweet, followed by slight pungency.

Under a microscope <5.01>, a transverse section reveals 4 to 10 layers of cork, with several layers of collenchyma inside of the layer; the cortex exhibits many oil canals surrounded by secretory cells and often large hollows appear; boundary of phloem and xylem is distinct; in the xylem, numerous vessels radiate alternately with medullary rays; vessels in the outer part of the xylem are singly or in several groups, and disposed rather densely in a cuneiform pattern, but vessels in the region of the center are scattered very sparsely; starch grains are simple grains, not more than 20 μm in diameter, and rarely 2- to 5-compound grains, some times up to 25 μm in diameter; starch grains often gelatinized.

Purity **(1)** Leaf sheath—When perform the test of foreign matter <5.01>, the amount of leaf sheath contained in Japanese Angelica Root does not exceed 3.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Japanese Angelica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Japanese Angelica Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01>—The amount of foreign matter other than leaf sheath contained in Japanese Angelica Root does not exceed 1.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Well-closed containers.

Powdered Japanese Angelica Root

Angelicae Acutilobae Radix Pulverata

トウキ末

Powdered Japanese Angelica Root is the powder of Japanese Angelica Root.

Description Powdered Japanese Angelica Root occurs as a light grayish brown powder. It has a characteristic odor and a slight, sweet taste with a slightly pungent aftertaste.

Under a microscope <5.01>, Powdered Japanese Angelica Root reveals starch grains or masses of gelatinized starch, and fragments of parenchyma containing them; fragments of light yellow-brown cork tissue; fragments of rather thick-walled collenchyma and phloem tissue; fragments of oil canal surrounded by secretory cells; fragments, 20 – 60 μm in diameter, of scalariform and reticulate vessels with simple perforation; starch grains composed of simple grains not more than 20 μm in diameter, and rarely 2- to 5-compound grains, sometimes comes up to 25 μm.

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of Powdered Japanese Angelica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Japanese Angelica Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Japanese Angelica Root does not show remarkably lignified sclerenchymatous cells.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Japanese Gentian

Gentianae Scabrae Radix

リュウタン

Japanese Gentian is the root and rhizome of *Gentiana scabra* Bunge, *Gentiana manshurica* Kitagawa or *Gentiana triflora* Pallas (*Gentianaceae*).

Description Irregular, cylindrical, short rhizome with numerous, slender roots around, and externally yellow-brown to grayish yellow-brown. The root is 10 – 15 cm in length, about 0.3 cm in diameter, and has longitudinal, coarse wrinkles on the outer surface; flexible; fractured surface, smooth and yellow-brown in color. The rhizome is about 2 cm in length, about 0.7 cm in diameter, and has buds or short remains of stems at the top.

Odor, slight; taste, extremely bitter and lasting.

Under a microscope <*5.01*>, a transverse section of the young root reveals epidermis, exodermis and a few layers of primary cortex; usually, the outermost layer is endodermis consisting of characteristic cells divided into a few daughter cells, often with collenchyma of 1 to 2 layers contacting the inner side; secondary cortex having rents here and there, and irregularly scattered sieve tubes; vessels arranged rather radially in xylem, sieve tubes existing in xylem; the rhizome has a large pith, rarely with sieve tubes; parenchyma cells contain needle, plate or sand crystals of calcium oxalate and oil drops; starch grains usually absent.

Identification To 0.5 g of pulverized Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and the same *R*f value with the dark purple spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Japanese Gentian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Japanese Gentian according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Powdered Japanese Gentian

Gentianae Scabrae Radix Pulverata

リュウタン末

Powdered Japanese Gentian is the powder of Japanese Gentian.

Description Powdered Japanese Gentian occurs as a grayish yellow-brown powder. It has a slight odor and a lasting, extremely bitter taste.

Under a microscope <*5.01*>, Powdered Japanese Gentian reveals fragments of parenchyma cells containing oil droplets and fine crystals, fragments of endodermis and exodermis divided into daughter cells with suberized membrane, and fragments of vessels. Vessels mainly consist of reticulate vessels and scalariform vessels, 20 – 30 μm in diameter.

Identification To 0.5 g of Powdered Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots obtained from the sample solution and a dark purple spot obtained from the standard solution show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Japanese Gentian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Japanese Gentian according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Japanese Gentian usually reveals no stone cells and fibers. No starch grains; if any, very few.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Japanese Valerian

Valerianae Fauriei Radix

カノコソウ

Japanese Valerian is the root and rhizome of *Valeriana fauriei* Briquet (*Valerianaceae*).

Description Obovoid, short rhizome with numerous, fine and long roots; externally dark brown to grayish brown. The root, 10 – 15 cm in length, 0.1 – 0.3 cm in diameter; externally, with fine longitudinal wrinkles; brittle. The rhizome,

1 – 2 cm in length, 1 – 2 cm in diameter, with buds and remains of stem at the crown; hard in texture and difficult to break; flank of rhizome sometimes accompanied with stolons having thick and short or thin, long and extremely small, scaly leaves. Under a magnifying glass, the transverse section reveals a thick, light grayish brown cortical layer, and a grayish brown stele.

Odor, strong and characteristic; taste, slightly bitter.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Japanese Valerian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Japanese Valerian according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 5.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Japanese Valerian provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.3 mL.

Containers and storage Containers—Tight containers.

Powdered Japanese Valerian

Valerianae Fauriei Radix Pulverata

カノコソウ末

Powdered Japanese Valerian is the powder of Japanese Valerian.

Description Powdered Japanese Valerian occurs as a dark grayish brown powder. It is somewhat moist to the touch. It has a strong, characteristic odor and a slightly bitter taste.

Under a microscope <*5.01*>, Powdered Japanese Valerian reveals starch grains and fragments of parenchyma cells containing them; fragments of pitted vessels, reticulate vessels, ring vessels, and spiral vessels; fragments of exodermis containing oil droplets and composed of cells suberized and divided into daughter cells; fragments of yellow stone cells from the rhizome and the stolon; and very rarely, some fragments of epidermis and phloem fibers. Starch grains, simple grains 10 – 20 μm in diameter and 2- to 4-compound grains; oil droplets stained red with sudan III TS.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Japanese Valerian according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Japanese Valerian according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 5.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of Powdered Japanese Valerian provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage Containers—Tight containers.

Japanese Zanthoxylum Peel

Zanthoxyli Piperiti Pericarpium

サンショウ

Japanese Zanthoxylum Peel is the pericarps of the ripe fruit of *Zanthoxylum piperitum* De Candolle (*Rutaceae*), from which the seeds separated from the pericarps have been mostly removed.

Description Capsules of 2 or 3 flattened spheroidal mericarps, which are dehiscent in 2 pieces about 5 mm in diameter; the outer surface of pericarp, dark yellow-red to dark red-brown, with numerous dented spots originated from oil sacs; the inner surface, light yellowish white.

Odor, characteristically aromatic; taste, acrid, which gives numbing sensation to the tongue.

Under a microscope <*5.01*>, transverse section of Japanese Zanthoxylum Peel reveals the external epidermis and the adjoined unicellular layer containing red-brown tannin; the pericarp holds oil sacs being up to approximately 500 μm in diameter and sporadically vascular bundles consisting mainly of spiral vessels; the endocarp consists of stone cell layers; inner epidermal cells very small.

Identification To 2 g of pulverized Japanese Zanthoxylum Peel add 10 mL of water, shake for 5 minutes, add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and acetic acid (100) (20:20:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with an *R*f value of about 0.3 is observed.

Purity **(1)** Seed—When perform the test of foreign matter <*5.01*>, the amount of the seeds contained in Japanese Zanthoxylum Peel does not exceed 20.0%.

(2) Peduncle and twig—The amount of the peduncles and twigs contained in Japanese Zanthoxylum Peel does not exceed 5.0%.

(3) Foreign matter <*5.01*>—The amount of foreign matter other than peduncles and twigs contained in Japanese Zanthoxylum Peel does not exceed 1.0%.

Total ash <*5.01*> Not more than 8.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Essential oil content <*5.01*> Perform the test with 30.0 g of pulverized Japanese Zanthoxylum Peel: the volume of essential oil is not less than 1.0 mL.

Containers and storage Containers—Well-closed containers.

Powdered Japanese Zanthoxylum Peel

Zanthoxyli Piperiti Pericarpium Pulveratum

サンショウ末

Powdered Japanese Zanthoxylum Peel is the powder of Japanese Zanthoxylum Peel.

Description Powdered Japanese Zanthoxylum Peel occurs as a dark yellow-brown powder. It has a strong, characteristic aroma and an acrid taste leaving a sensation of numbness on the tongue.

Under a microscope <*5.01*>, Powdered Japanese Zanthoxylum Peel reveals fragments of inner tissue of pericarp consisting of stone cells with cell walls about 2.5 μm in thickness; fragments of spiral and ring vessels 10 – 15 μm in diameter; fragments of oil sacs containing essential oil or resin; fragments of epidermal cells, polygonal in surface view, containing tannin; numerous oil drops; masses of tannin, colored red by adding vanillin-hydrochloric acid TS.

Identification To 2 g of Powdered Japanese Zanthoxylum Peel add 10 mL of water, shake for 5 minutes, add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and acetic acid (100) (20:20:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with an *R*f value of about 0.3 is observed.

Total ash <*5.01*> Not more than 8.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Essential oil content <*5.01*> Perform the test with 30.0 g of Powdered Japanese Zanthoxylum Peel: the volume of essential oil is not less than 0.8 mL.

Containers and storage Containers—Tight containers.

Jujube

Zizyphi Fructus

タイソウ

Jujube is the fruit of *Zizyphus jujuba* Miller var. *inermis* Rehder (*Rhamnaceae*).

Description Ellipsoidal or broad ovoid fruit, 2 – 3 cm in length, 1 – 2 cm in diameter; externally reddish brown with coarse wrinkles, or dark grayish red with fine wrinkles, and both lustrous; both ends slightly dented, with a scar of style on one end and a scar of peduncle on the other; epicarp thin and leather; mesocarp thick, dark grayish brown in color, spongy, soft and adhesive; endocarp extremely hard, fusiform, and divided into two loculi; seeds flat and ovoid.

Odor, slight and characteristic; taste, sweet.

Purity **(1)** Rancidity—Jujube has no unpleasant, rancid odor and taste.

(2) Total BHC's and total DDT's <*5.01*> Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Jujube Seed

Zizyphi Semen

サンソウニン

Jujube Seed is the seed of *Zizyphus jujuba* Miller var. *spinosa* Hu ex H. F. Chou (*Rhamnaceae*).

Description Jujube Seed is a compressed ovate to orbicular, lenticular seed, 5 – 9 mm in lengh, 4 – 6 mm in width, 2 – 3 mm in thickness, externally brown to dark red-brown, glossy; hilum at one end, charaza at the other end; seed coat sightly flexible, covering, milky white endosperm and light yellow embryo. 100 seeds weigh 3.0 – 4.5 g.

Odor, slightly oily; taste, mild and slightly oily.

Under a microscope <*5.01*>, transverse section reveals seed coat composed of an upper epidermis, parenchyma and lower epidermis; upper epidermal cells sclerified and elongated in radial direction; lower epidermis covered with cuticle; endosperm composed of parenchyma, containing aggregated crystals of calcium oxalate, aleurone grains and starch grains; cotyledons composed of parenchyma that contains aleurone grains, starch grains and oil drops.

Identification To 2 g of pulverized Jujube Seed add 10 mL of methanol, and heat under a reflux condenser for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots appear at the *R*f value of about 0.3 and about 0.4, and these spots exhibit a fluorescence when examined under ultraviolet light (main wavelength: 365 nm) after spraying evenly dilute sulfuric acid on the plate and heating at 105°C for 5 minutes.

Purity Foreign matter <*5.01*>—Jujube Seed contains not more than 1.0% of the endocarp and other foreign matters.

Loss on drying <*5.01*> Not more than 11.0% (6 hours).

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.5%.

Containers and storage Containers—Well-closed containers.

Juzentaihoto Extract

十全大補湯エキス

Juzentaihoto Extract contains not less than 1.5 mg (for preparation prescribed 2.5 g of Ginseng) or not less than 1.8 mg (for preparation prescribed 3 g of Ginseng) of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), not less than 26 mg and not more than 78 mg of paeonifrolin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 8 mg and not more than 24 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Ginseng	3 g	3 g	2.5 g	3 g
Astragalus Root	3 g	3 g	2.5 g	3 g
Atractylodes Rhizome	3 g	—	3.5 g	3 g
Atractylodes Lancea Rhizome	—	3 g	—	—
Poria Sclerotium	3 g	3 g	3.5 g	3 g
Japanese Angelica Root	3 g	3 g	3.5 g	3 g
Peony Root	3 g	3 g	3 g	3 g
Rehmannia Root	3 g	3 g	3.5 g	3 g
Cnidium Rhizome	3 g	3 g	3 g	3 g
Cinnamon Bark	3 g	3 g	3 g	3 g
Glycyrrhiza	1.5 g	1.5 g	1 g	1g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Juzentaihoto Extract is a light brown to brown powder or blackish brown viscous extract. It has a slight odor and a sweet and bitter taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 15 mL of sodium hydroxide TS, centrifuge, and take the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and take the 1-butanol layer. To the 1-butanol layer add 10 mL of water, shake, centrifuge, and take the 1-butanol layer. Evaporate the layer under reduced pressure, to the residue add 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the dark brown spot from the standard solution (Ginseng).

(2) Use the sample solution obtained in (1) as the sample solution. Separately, dissolve 1 mg of astragaloside IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution (Astragalus Root).

(3) (For preparation prescribed Atractylodes Rhizome) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 5 mL of diethyl ether, shake, and centrifuge. Use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(4) (For preparation prescribed Atractylodes Lancea Rhizome) Shake 5.0 g of the dry extract (or 15.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Take the hexane layer, evaporate the hexane under reduced pressure, dissolve the residue in 2 mL of hexane, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.4, and this spot shows a green-brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allow to cool (Atractylodes Lancea Rhizome).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, then add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Cnidium Rhizome; Japanese Angelica Root).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sam-

ple solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Peony Root).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: a dark green spot is observed at an *R*f value of about 0.6 (Rehmannia Root).

(8) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination to the flask, and heat to boil under a reflux condenser. The graduated tube of the apparatus is previously filled with water to the standard line and added 2 mL of hexane. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution.

(9) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Ginsenoside Rb_1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to about 2 g of the dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine the supernatant liquids, add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column (about 10 mm in inside diameter and packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), washed just before use with methanol and then with diluted methanol (3 in 10)), and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rb_1\ (C_{54}H_{92}O_{23}) = M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl groups bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (400:100:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb_1 is about 16 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb_1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kakkonto Extract

葛根湯エキス

Kakkonto Extract contains not less than 9 mg and not more than 27 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 12 mg and not more than 36 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 14 mg and not more than 56 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 84 mg (for preparation prescribed 3 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 19 mg and not more than 57 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Pueraria Root	8 g	4 g	4 g	4 g
Ephedra Herb	4 g	4 g	3 g	3 g
Jujube	4 g	3 g	3 g	3 g
Cinnamon Bark	3 g	2 g	2 g	2 g
Peony Root	3 g	2 g	2 g	2 g
Glycyrrhiza	2 g	2 g	2 g	2 g
Ginger	1 g	1 g	1 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Kakkonto Extract occurs as a light brown to brown powder or blackish brown viscous extract. It has a characteristic odor, and a sweet first, then hot, and slightly bitter taste.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin RS or puerarin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Pueraria Root).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

(3) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution (Cinnamon Bark).

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Peony Root).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash ⟨*5.01*⟩ Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained with the sample solution, and the peak area, A_S, of ephedrine obtained with the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with water to make exactly 20 mL of eluate, and use this as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000

and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kakkontokasenkyushin'i Extract

葛根湯加川芎辛夷エキス

Kakkontokasenkyushin'i Extract contains not less than 9.5 mg and not more than 28.5 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 13 mg and not more than 39 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 17 mg and not more than 51 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 18 mg and not more than 54 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 1.5 mg and not more than 6 mg (for preparation prescribed 2 g of Magnolia Flower) or not less than 2 mg and not more than 8 mg (for preparation prescribed 3 g of Magnolia Flower) of magnoflorine [magnoflorine iodide ($C_{20}H_{24}INO_4$: 469.31)], per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Pueraria Root	4 g	4 g
Ephedra Herb	4 g	3 g
Jujube	3 g	3 g
Cinnamon Bark	2 g	2 g
Peony Root	2 g	2 g
Glycyrrhiza	2 g	2 g
Ginger	1 g	1 g
Cnidium Rhizome	3 g	2 g
Magnolia Flower	3 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Kakkontokasenkyushin'i Extract occurs as a light brown to brown powder or blackish brown viscous extract, having a characteristic order, and a sweet first, then a bitter and hot taste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin RS or puerarin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Pueraria Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

(3) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzoaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution

has the same color tone and *R*f value with the red-purple to purple spot obtained from the standard solution (Peony Root).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution (Ginger).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid TS, and then shake with 25 mL of diethyl ether. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Cnidium Rhizome).

(8) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, to 1 g of powdered magnolia flower add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the brown spot (*R*f value: about 0.4) obtained from the standard solution (Magnolia Flower).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine with the sample solution, and peak area, A_S, of ephedrine with standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, add 1 mL of acetic acid (100) to the effluent, then add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <2.48> by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 5/8$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

(4) Magnoflorine—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, add 3.0 mL of diluted sodium hydroxide TS (1 in 10), shake for 10 minutes, centrifuge, and remove the upper layer. Add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 3.0 mL of 0.1 mol/L hydrochloric acid TS and 20 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2) shake for 15 minutes, centrifuge, and separate the supernatant liquid. Combine the previous supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of magnoflorine iodide for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of magnoflorine in each solution.

$$\text{Amount (mg) of magnoflorine [as magnoflorine iodide } (C_{20}H_{24}INO_4)] = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of magnoflorine iodide for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 303 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of magnoflorine is about 20 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of magnoflorine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnoflorine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kamikihito Extract

加味帰脾湯エキス

Kamikihito Extract contains not less than 0.8 mg and not more than 3.2 mg of saikosaponin b_2, not less than 27 mg and not more than 81 mg of geniposide, and not less than 8 mg and not more than 24 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Ginseng	3 g	3 g	3 g	3 g
Atractylodes Rhizome	3 g			3 g
Atractylodes Lancea Rhizome		3 g	3 g	
Poria Sclerotium	3 g	3 g	3 g	3 g
Jujube Seed	3 g	3 g	3 g	3 g
Longan Aril	3 g	3 g	3 g	3 g
Astragalus Root	2 g	3 g	2 g	3 g
Japanese Angelica Root	2 g	2 g	2 g	2 g
Polygala Root	1.5 g	2 g	1 g	2 g
Bupleurum Root	3 g	3 g	3 g	3 g
Gardenia Fruit	2 g	2 g	2 g	2 g
Glycyrrhiza	1 g	1 g	1 g	1 g
Saussurea Root	1 g	1 g	1 g	1 g
Jujube	1.5 g	2 g	1 g	2 g
Ginger	0.5 g	1 g	1 g	0.5g
Moutan Bark				2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the preparation 1) to 4), using the crude drugs shown above. Or, prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive prepared as directed under Extracts, according to the preparation 2, using the crude drugs shown above.

Description Kamikihito Extract is a light yellow-brown to brown powder or blackish brown viscous extract. It has a slightly odor, and a slightly sweet, hot and bitter taste.

Identification **(1)** To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, and centrifuge. To the supernatant liquid add 10 mL of 1-butanol, shake, centrifuge, and separate the 1-butanol layer. To this layer add 10 mL of water, shake, centrifuge, and separate the 1-butanol layer. Evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of methanol, and use the solution as the sample solution. Separately, dissolve 1 mg of ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot obtained from the standard solution (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-purple spot obtained from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under reduced pressure, then dissolve the residue in 2 mL of hexane, and use the solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark violet spot is observed at an *R*f value of about 0.5, and this spot exhibits greenish brown when the plate is sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, and centri-

fuge. To the supernatant liquid add 10 mL of 1-butanol, shake, centrifuge, and separate 1-butanol layer. To the 1-butanol layer add 10 mL of water, shake, centrifuge, separate the 1-butanol layer, and evaporate the solvent under reduced pressure. Dissolve the residue in 2 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and ammonia solution (28) (9:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to blue-purple spot obtained from the standard solution (Astragalus Root).

(5) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, then dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [*Z*]-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Japanese Angelica Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of 1 mol/L hydrochloric acid TS, and heat for 10 minutes. After cooling, to 10 mL of this solution add 10 mL of ethyl acetate, shake, centrifuge, separate the ethyl acetate layer, and use this layer as the sample solution. Separately, to 2.0 g of a powder of polygala root add 30 mL of 1 mol/L hydrochloric acid TS, and heat for 10 minutes. After cooling, to 10 mL of this solution add 10 mL of ethyl acetate, shake, centrifuge, separate the ethyl acetate layer, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and acetic acid (100) (7:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfric acid TS on the plate, heat at 105°C for 1 minute, and observe while hot: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purplish red spot (at an *R*f value of about 0.5) obtained from the standard solution (Polygala Root).

(7) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of sodium hydroxide TS, shake, and centrifuge. To the supernatant liquid add 10 mL of 1-butanol, shake, centrifuge, and separate the 1-butanol layer. To this layer add 10 mL of water, shake, centrifuge, and separate the 1-butanol layer. Evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(8) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minute: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution (Gardenia Fruit).

(9) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot obtained from the standard solution (Glycyrrhiza).

(10) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, to 1.0 g of a powder of saussurea root add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, heat at 105°C for 2 minutes, and allow to cool: one of the spot among the several spots obtained

from the sample solution has the same color tone and *R*f value with the blue spot obtained from the standard solution (Saussurea Root).

(11) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, allow to cool, and spray water: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution (Ginger).

(12) For preparation prescribed Moutan Bark—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 15 mL of water, shake, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot obtained from the standard solution (Moutan Bark).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not less than 8.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 9.0% and 18.0%.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. Centrifuge, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as above, and remove the upper layer. To the residue add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Geniposide—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of geniposide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute (retention time of geniposide is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. Centrifuge, remove the supernatant liquid, then add 20 mL of diethyl ether, proceed in the same manner as above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kamishoyosan Extract

加味逍遙散エキス

Kamishoyosan Extract contains not less than 28 mg and not more than 84 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 25 mg and not more than 75 mg of geniposide, and not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 16 mg and not more than 48 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)	6)
Japanese Angelica Root	3 g	3 g	3 g	3 g	3 g	3 g
Peony Root	3 g	3 g	3 g	3 g	3 g	3 g
Atractylodes Rhizome	3 g	—	3 g	—	3 g	3 g
Atractylodes Lancea Rhizome	—	3 g	—	3 g	—	—
Poria Sclerotium	3 g	3 g	3 g	3 g	3 g	3 g
Bupleurum Root	3 g	3 g	3 g	3 g	3 g	3 g
Moutan Bark	2 g	2 g	2 g	2 g	2 g	2 g
Gardenia Fruit	2 g	2 g	2 g	2 g	2 g	2 g
Glycyrrhiza	2 g	2 g	1.5 g	1.5 g	1.5 g	1.5 g
Ginger	1 g	1 g	1 g	1 g	1.5 g	0.5 g
Mentha Herb	1 g	1 g	1 g	1 g	1 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 6), using the crude drugs shown above.

Description Kamishoyosan Extract occurs as a yellow-brown to brown powder or blackish brown viscous extract. It has slightly a characteristic odor, and a sweet, slightly hot, then bitter taste.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Japanese Angelica Root).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of

silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the orange fluorescent spot from the standard solution (Peony Root).

(3) For preparation prescribed Atractylodes Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 15 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Gardenia Fruit).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(9) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

(10) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution. Sepa-

rately, shake 0.2 g of powdered mentha herb with 10 mL of diluted phosphoric acid (1 in 30), add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (10:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-purple spot (around *R*f 0.6) from the standard solution (Mentha Herb).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(2) Geniposide—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of geniposide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute (the retention time of geniposide is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diame-

ter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Keishibukuryogan Extract

桂枝茯苓丸エキス

Keishibukuryogan Extract contains not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 3 g of Cinnamon Bark) or not less than 0.8 mg and not more than 3.2 mg (for preparation prescribed 4 g of Cinnamon Bark) of (*E*)-cinnamic acid, not less than 30 mg and not more than 90 mg (for preparation prescribed 3 g each of Moutan Bark and Peony Root) or not less than 40 mg and not more than 120 mg (for preparation prescribed 4 g each of Moutan Bark and Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 21 mg and not more than 63 mg (for preparation prescribed 3 g of Peach Kernel) or not less than 28 mg and not more than 84 mg (for preparation prescribed 4 g of Peach Kernel) of amygdalin, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Cinnamon Bark	4 g	3 g
Poria Sclerotium	4 g	3 g
Moutan Bark	4 g	3 g
Peach Kernel	4 g	3 g
Peony Root	4 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) using the crude drugs shown above, or prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive, prepared as directed under Extracts, according to the prescription 2), using the crude drugs shown above.

Description Keishibukuryogan Extract is a light brown to brown powder or blackish brown viscous extract. It has a characteristic odor and has a taste slightly sweet first then bitter later.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the green-brown spot from the standard solution (Peach Kernel).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia water (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the orange fluorescent spot from the standard solution (Peony Root).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 9.0% and 18.0%.

Assay **(1)** (*E*)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-cinnamic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-cinnamic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of (*E*)-cinnamic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute (the retention time of (*E*)-cinnamic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-cinnamic acid is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Amygdalin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and

not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Koi

Koi

コウイ

Koi is a saccharized substance obtained by hydrolysis of the starch of *Zea mays* Linné (*Gramineae*), *Manihot esculenta* Crantz (*Euphorbiaceae*), *Solanum tuberosum* Linné (*Solanaceae*), *Ipomoea batatas* Poiret (*Convolvulaceae*) or *Oryza sativa* Linné (*Gramineae*), or the seed of *Oryza sativa* Linné from which the seed coat is removed.

Koi is prepared by the following processes 1 or 2, and contains mainly maltose, sometimes glucose and maltotriose also.

Process 1. Saccharize starch with hydrochloric acid, oxalic acid, amylase or wort, then concentrate to dryness, and powder.

Process 2. To starch or a paste of starch prepared by adding water and heating, add hydrochloric acid, oxalic acid, amylase or wort to saccharize, and dry or concentrate.

Koi prepared by Process 1 is termed ''Koi 1'' and by Process 2 is termed ''Koi 2''. The label states the process.

Description

Koi 1: A white crystalline powder. It is odorless and has a sweet taste.

Koi 2: Colorless or brown, clear or semi-translucent, masses or viscous liquid. It is odorless and has a sweet taste.

Identification Dissolve exactly 0.50 g of Koi in a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 20.0 mg of maltose hydrate in a mixture of water and methanol (1:1) to make exactly 5 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography in equal size of circular spot each other. Develop the plate with a mixture of 2-butanone, water and acetic acid (100) (3:1:1) to a distance of about 7 cm, and dry at 105°C for 10 minutes the plate. Spray evenly 2,3,5-triphenyl-2*H*-tetrazolium chloride-methanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot obtained from the standard solution, and it is larger and more intense than the spot obtained from the standard solution.

Purity **(1)** Clarity of solution—A solution obtained by dissolving 2.0 g of Koi in 20 mL of hot water is practically clear.

(2) Heavy metals ⟨*1.07*⟩

Koi 1: Proceed with 1.0 g of Koi 1 according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

Koi 2: Proceed with 1.0 g of Koi 2 according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Koi according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying ⟨*5.01*⟩

Koi 1: Not more than 3.0% (1 g, 80°C, 4 hours).

Koi 2: Not more than 15.0% (1 g, 80°C, 4 hours). In the case where the sample is in masses, crush the masses, weigh accurately the mass, and put in a desiccator. In the case in viscous liquid, put in a weighing bottle to spread about 1 mm thick, weigh accurately the mass, and put the bottle in a desiccator.

Total ash ⟨*5.01*⟩ Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Hydrous Lanolin

加水ラノリン

Hydrous Lanolin is Purified Lanolin to which water is added. It contains not less than 70% and not more than 75% of Purified Lanolin (as determined by the test for Residue on evaporation).

Description Hydrous Lanolin is a yellowish white, ointment-like substance, and has a slight, characteristic odor, which is not rancid.

It is soluble in diethyl ether and in cyclohexane, with the separation of water.

When melted by heating on a water bath, it separates into a clear oily layer and a clear water layer.

Melting point: about 39°C.

Identification Dissolve 1 g of Hydrous Lanolin in 50 mL of cyclohexane, and remove the separated water. Superimpose carefully 1 mL of the cyclohexane solution on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and sulfuric acid layer shows a green fluorescence.

Acid value ⟨*1.13*⟩ Not more than 1.0.

Iodine value 18 – 36 Heat a suitable amount of Hydrous Lanolin on a water bath to remove its almost moisture, then weigh accurately about 0.8 g of the treated Hydrous Lanolin in a glass-stoppered 500-mL flask, and add 10 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus's TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in a light-resistant, well-closed container while occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\text{Iodine value} = (a - b) \times 1.269/M$$

M: amount (g) of Hydrous Lanolin taken
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration

Purity **(1)** Acidity or alkalinity—To 5 g of Hydrous Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride <*1.03*>—To 2.0 g of Hydrous Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of the dried residue obtained in the Residue on evaporation in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. Add dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observed in the same level with the spot of standard solution. For this test use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Residue on evaporation Weigh accurately about 12.5 g of Hydrous Lanolin, dissolve in 50 mL of diethyl ether, place it in a separator, transfer the separated aqueous layer to another separator, add 10 mL of diethyl ether, shake, and combine the diethyl ether layer and diethyl ether in the first separator. Shake the diethyl ether layer with 3 g of anhydrous sodium sulfate, and filter through dry filter paper. Wash the separator and the filter paper with two 20-mL portions of diethyl ether, combine the washings with the filtrate, evaporate on a water bath until the odor of diethyl ether is no longer perceptible, and dry in a desiccator (in vacuum, silica gel) for 24 hours: the content is not less than 70% and not more than 75%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Purified Lanolin

Adeps Lanae Purificatus

精製ラノリン

Purified Lanolin is the purified product of the fat-like substance obtained from the wool of *Ovis aries* Linné (*Bovidae*).

Description Purified Lanolin is a light yellow to yellowish brown, viscous, ointment-like substance, and has a faint, characteristic but not rancid odor.

It is very soluble in diethyl ether and in cyclohexane, freely soluble in tetrahydrofuran and in toluene, and very slightly soluble in ethanol (95). It is practically insoluble in water, but miscible without separation with about twice its mass of water, retaining ointment-like viscosity.

Melting point: 37 – 43°C

Identification Superimpose carefully 1 mL of a solution of Purified Lanolin in cyclohexane (1 in 50) on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and the sulfuric acid layer shows a green fluorescence.

Acid value <*1.13*> Not more than 1.0.

Iodine value 18 – 36 Weigh accurately about 0.8 g of Purified Lanolin in a glass-stoppered 500-mL flask, add 20 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus' TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in light-resistant, well-closed containers, with occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = (a - b) \times 1.269/M$$

M: amount (g) of Purified Lanolin taken
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the blank determination
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the titration of the sample

Purity **(1)** Acid or alkali—To 5 g of Purified Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride <*1.03*>—To 2.0 g of Purified Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of Purified Lanolin in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. And dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observable same level of the spot of standard solution. Use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Total ash ⟨*5.01*⟩ Not more than 0.1%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Lard

Adeps Suillus

豚脂

Lard is the fat obtained from *Sus scrofa* Linné var. *domesticus* Gray (*Suidae*).

Description Lard occurs as a white, soft, unctuous mass, and has a faint, characteristic odor and a bland taste.

It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: 36 – 42°C

Congealing point of the fatty acids: 36 – 42°C

Acid value ⟨*1.13*⟩ Not more than 2.0.

Saponification value ⟨*1.13*⟩ 195 – 203

Iodine value ⟨*1.13*⟩ 46 – 70

Purity **(1)** Moisture and coloration—Melt 5 g of Lard by heating on a water bath: it forms a clear liquid, from which no water separates. Observe the liquid in a layer 10 mm thick: the liquid is colorless to slightly yellow.

(2) Alkalinity—To 2.0 g of Lard add 10 mL of water, melt by warming on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: the layer is colorless.

(3) Chloride ⟨*1.03*⟩—To 1.5 g of Lard add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the opalescence of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, and add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

(4) Beef tallow—Dissolve 5 g of Lard in 20 mL of diethyl ether, stopper lightly with absorbent cotton, and allow to stand at 20°C for 18 hours. Collect the separated crystals, moisten them with ethanol (95), and examine under a microscope of 200 magnifications: the crystals are in the form of rhomboidal plates grouped irregularly, and do not contain prisms or needles grouped in fan-shaped clusters.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Leonurus Herb

Leonuri Herba

ヤクモソウ

Leonurus Herb is the aerial part of *Leonurus japonicus* Houttuyn or *Leonurus sibiricus* Linné (*Labiatae*), collected during the flowering season.

Description Stem, leaves, and flowers usually cross sectioned, stems squre, 0.2 – 3 cm in diameter, yellow-green to green-brown in color, covered densely with white short hairs; the pith white, a great parts of central of sections. Light in texture. Leaves opposite, petiolated, 3-dissected to 3-incised, each lobes split pinnately, and end lobes reveals linear-lanceolate, acute or acuminate, the upper surface light green, the lower surface bristle with white short hairs, grayish green. Flower, verticillate; sepal, tubular, and the upper end acerate with five lobes; light green to light green-brown in color, corolla labiate, light red-purple to light brown.

Odor, slightly; taste, slightly bitter, astringent.

Under a microscope ⟨*5.01*⟩, a transverse section of stem reveals four ridge, a parts of the ridge of *Leonurus sibiricus* Linné protruding knobby. Epidermis, observed non-glandular hairs from 1 to 3 cells, glandular hairs with head of 1 to 4 celled or glandular scale with 8 cells. Each ridge parts, beneath epidermis, collenchyma developed, development of xylem fibres remarkably. Cortex composed of several layers parenchymatous cells. Collateral vascular bundle arranged in a circle. Phloem fibres observed at the outer portion of phloem. Parenchymatous cells of cortex and pith observe needle crystals or plate-like crystals of calcium oxalate.

Identification To 1 g of pulverized Leonurus Herb add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of water and methanol (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS followed by immediate spraying of sodium nitrite TS on the plate: a grayish brown spot appears at an *R*f value of about 0.5, which color fades soon and then disappears after air-drying the plate.

Loss on drying ⟨*5.01*⟩ Not more than 12.0%.

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 2.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 12.0%.

Containers and storage Containers—Well-closed containers.

Lilium Bulb

Lilii Bulbus

ビャクゴウ

Lilium Bulb is the scaly leaves of *Lilium lancifolium* Thunberg, *Lilium brownii* F.E.Brown var. *colchesteri* Wilson, *Lilium brownii* F.E.Brown or *Lilium pumilum* De Candolle (*Liliaceae*), usually with the application of steaming.

Description Lilium Bulb reveals oblong with narrowed apex, lanceolate, or narrowly triangular boat-shaped, translucent, 1.3 – 6 cm in length, 0.5 – 2.0 cm in diameter, externally milky white to light yellow-brown occasionally purplish in color, nearly smooth; central portion somewhat thickend, circumferential portion thin, slightly waved, occasionally rolled inside; usually several lines of vascular bundles longitudinally in parallel are seen through parenchyma; hard in texture, easy to break; fractured surface horny and flat.

Odorless; taste, slightly acid and bitter.

Under a microscope <*5.01*>, the surface reveals epidermal cells rectangular to almost square, stomata nearly circular, the cells adjacent to stomata mostly 4 in number. Under a microscope <*5.01*>, a transverse section reveals the outermost layer composed of epidermal cells covered with smooth cuticle; beneath epidermis circular to quadrangular parenchymatous cells distributed evenly, palisade tissue not observed; in parenchyma of mesophyll collateral vascular bundles extended from adaxial side to abaxial side of scaly leaves are arranged almost in a transverse line; starch grains contained in parenchymatous cells, usually gelatinized.

Identification To 3 g of pulverized Lilium Bulb add 10 mL of 1-butanol, shake, add 10 mL of water, shake for 30 minutes, and centrifuge. Evaporate the supernatant liquid under reduced pressure, add 1 mL of methanol to the residue, shake gently, and use the supernatant liquid so obtained as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots appear at an *R*f value of about 0.3. When examine these spots under ultraviolet light (main wavelength: 365 nm) after spraying with sodium carbonate TS, they appear as blue-purple fluorescent spots.

Loss on drying <*5.01*> Not more than 16.0%.

Total ash <*5.01*> Not more than 4.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Lindera Root

Linderae Radix

ウヤク

Lindera Root is the root of *Lindera strychnifolia* Fernandez-Villar (*Lauraceae*).

Description Fusiform or rosary-like root, 10 – 15 cm in length, 10 – 25 mm in diameter; externally yellow-brown to brown, with a few scars of rootlets; a transverse section reveals cortex brown, xylem light yellow-brown, concentric circles and radially arranged lines brown; dense and hard in texture.

Odor, camphor-like; taste, bitter.

Under a microscope <*5.01*>, a transverse section of the root with periderm reveals a cork layer several cells thick, partially consisting of cork stone cells; cortex parenchyma sometimes contains oil cells and fibers; in xylem, vessels-xylem fibers and rays are arranged alternately; parenchyatous cells of cortex and xylem contain sandy and columnar crystals of calcium oxalate, simple starch grains 1 – 15 μm in diameter, and 2- to 4- compound starch grains.

Identification To 3 g of pulverized Lindera Root add 40 mL of hexane, and warm under a reflux condenser on a water bath for 30 minutes. After cooling, filter, to the residue add 10 mL of ammonia TS and 30 mL of a mixture of ethyl acetate and diethyl ether (1:1), shake vigorously for 20 minutes, and centrifuge. Separate the supernatant liquid, add 10 g of anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate, dissolve the residue with 0.5 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia water (28) (10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a yellow-brown spot appears at an *R*f value of about 0.4.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Lindera Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Lindera Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 2.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 6.0%.

Containers and storage Containers—Well-closed containers.

Lithospermum Root

Lithospermi Radix

シコン

Lithospermum Root is the root of *Lithospermum erythrorhizon* Siebold et Zuccarini (*Boraginaceae*).

Description Rather slender conical root, often branched, 6 – 10 cm in length, 0.5 – 1.5 cm in diameter; externally dark purple, coarse in texture, thin and easily peeled; mostly with twisted and deep longitudinal furrows, which sometimes reach to xylem; sometimes remains of stem at the crown; easily broken; fractured surface granular and with many clefts. Under a magnifying glass, a transverse section reveals a dark purple color at the outer portion of cortex, and light brown inner portion making irregular wave; xylem yellowish in color; the center of the crown is often cracked, and the surrounding part red-purple.

Odor, slight; taste, slightly sweet.

Identification **(1)** Heat 0.5 g of pulverized Lithospermum Root in a test tube: red vapor evolves, which condenses on the wall of the upper part of the tube into red-brown oil drops.

(2) Shake 0.5 g of pieces or powder of Lithospermum Root with 1 mL of ethanol (95), and to the red solution thereby obtained add 1 drop of sodium hydroxide TS: the red color changes to blue-purple. To this solution add 1 to 2 drops of dilute hydrochloric acid: the color turns red again.

(3) To 0.5 g of pulverized Lithospermum Root add 5 mL of ethanol (95), shake for 30 minutes, filter, and evaporate the filtrate at a temperature not higher than 40°C under reduced pressure. Add 1 mL of ethanol (95) to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (95) (3:1) to a distance of about 7 cm, and air-dry the plate: a red-purple spot appears at an *R*f value of about 0.75.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Lithospermum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Lithospermum Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 11.0%.

Acid-insoluble ash <*5.01*> Not more than 3.5%.

Containers and storage Containers—Well-closed containers.

Longan Aril

Longan Arillus

リュウガンニク

Longan Aril is the aril of *Euphoria longana* Lamarck (*Sapindaceae*).

Description Depressed ellipsoidal aril, 1 – 2 cm in length, about 1 cm in width; yellowish red-brown to blackish brown; soft in texture and mucous; when immersed in water, bell-shaped, with the tip split in several parts.

Odor, characteristic; taste, sweet.

Under a microscope <*5.01*>, a transverse section reveals the outmost layer composed of a single-layered epidermis, beneath this observed parenchyma consisting of depressed parenchyma cells; the innermost layer composed of slightly thick-walled epidermis; parenchyma contains red-brown to brown contents as well as solitary crystals, amorphous crystals and sand crystals of calcium oxalate.

Identification To 1 g of coarse cuttings of Longan Aril, add 10 mL of water, shake thoroughly, and filter. To 3 mL of the filtrate, add 3 mL of Fehling solution, and heat on a water bath: a red precipitate is produced.

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: Not less than 75.0%.

Containers and storage Containers—Well-closed containers.

Longgu

Fossilia Ossis Mastodi

リュウコツ

Longgu is the ossified bone of large mammal, and is mainly composed of calcium carbonate.

For Longgu used only for extracts, infusions and decoctions, the label states the restricted utilization forms.

Description Irregular masses or fragments, occasionally cylindrical masses; externally light grayish white, sometimes with grayish black or yellow-brown spots here and there; the outer part consists of a layer 2 – 10 mm in thickness, and is minute in texture, surrounding the light brown, porous portion; heavy and hard, but somewhat fragile in texture; when crushed, it changes into pieces and powder.

Odorless, tasteless, and strongly adhesive to the tongue on licking.

Identification **(1)** Dissolve 0.5 g of pulverized Longgu in 10 mL of dilute hydrochloric acid: it evolves a gas, and forms a slightly brownish and turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(2) The turbid solution obtained in (1) has a characteristic odor. Filter this solution and neutralize filtrate with ammonia TS: this solution responds to the Qualitative Tests <*1.09*> (1), (2) and (3) for calcium salt.

(3) Dissolve 0.1 g of pulverized Longgu in 5 mL of nitric

acid by warming, and add hexaammonium heptamolybdate TS: a yellow precipitate is produced.

Purity **(1)** Heavy metals <*1.07*>—To 2.0 g of pulverized Longgu, add 5 mL of water, shake, add gradually 6 mL of hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate, add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL, and use this solution as the control solution (not more than 20 ppm).

When being shown as extracts, infusions and decoctions on the label, the procedure and the limit are as follows.

To 20.0 g of pulverized Longgu, add 80 mL of water, shake occasionally in a water bath, heat to make about 40 mL, allow to cool, and filter. Proceed with this solution according to Method 3, and perform the test. To the control solution, add 1.0 mL of Standard Lead Solution (not more than 0.5 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.20 g of pulverized Longgu according to Method 2, and perform the test (not more than 10 ppm).

When being shown the restricted utilization forms as ''extracts, infusions and decoctions only'', the procedure and the limit are as follows.

Put 4.0 g of pulverized Longgu in a centrifuge tube, add 30 mL of water, and heat in a water bath with occasional shaking to make about 15 mL. After cooling, centrifuge, and perform the test using the supernatant liquid as the test solution (not more than 0.5 ppm).

Containers and storage Containers—Well-closed containers.

Powdered Longgu

Fossilia Ossis Mastodi Pulveratum

リュウコツ末

Powdered Longgu is the powder of Longgu.

Description Powdered Longgu occurs as a light grayish-white to light grayish brown. It is odorless and tasteless.

Identification **(1)** Dissolve 0.1 g of Powdered Longgu in 5 mL of nitric acid by warming, and add hexaammonium heptamolybdate TS: a yellow precipitate is produced.

(2) Dissolve 0.5 g of Powdered Longgu in 10 mL of dilute hydrochloric acid: it evolves a gas, and forms a slightly brownish and turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(3) The turbid solution obtained in (2) has a characteristic odor. Filter this solution, and neutralize filtrate with ammonia TS: the solution responds to the Qualitative test <*1.09*> (1), (2) and (3) for calcium salt.

Purity **(1)** Heavy metals <*1.07*>—To 2.0 g of Powdered Longgu add 5 mL of water, shake to mix, add gradually 6 mL of hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.20 g of Powdered Longgu according to Method 2, and perform the test (not more than 10 ppm).

Containers and storage Containers—Well-closed containers.

Lonicera Leaf and Stem

Lonicerae Folium Cum Caulis

ニンドウ

Lonicera Leaf and Stem is the leaves and stems of *Lonicera japonica* Thunberg (*Caprifoliaceae*).

Description Leaves and opposite leaves on short stem; leaf, ovate and entire, with short petiole, 3 – 7 cm in length, 1 – 3 cm in width; upper surface greenish brown, lower surface light grayish green; under a magnifying glass, both surfaces pubescent. Stem, 1 – 4 mm in diameter; externally grayish yellow-brown to purplish brown, a transverse section of stem, round and hollow.

Almost odorless; taste, slightly astringent, followed by a litter bitterness.

Under a microscope <*5.01*>, a transverse section of leaf reveals the outermost layer of upper and lower surfaces to be composed of a single-layered epidermis, uni-cellular non-glandular hairs and multi-cellular glandular hairs on epidermis; in midvein, several-layered collenchyma present beneath the epidermis and vascular bundles in the center; in mesophyll, palisade layer adjacent to upper epidermis, spongy tissue adjacent to lower epidermis; glandular hairs contain brown secretion, parenchymatous cells contain aggregate crystals of calcium oxalate, and occasionally starch grains.

Identification To 1 g of pulverized Lonicera Leaf and Stem add 5 mL of methanol, shake for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (1). Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution (2).

Purity Stem—Lonicera Leaf and Stem does not contains the stems larger than 5 mm in diameter.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 9.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 12.0%.

Containers and storage Containers—Well-closed containers.

Loquat Leaf

Eriobotryae Folium

ビワヨウ

Loquat Leaf is the leaf of *Eriobotrya japonica* Lindley (*Rosaceae*).

Description Loquat Leaf is an oblong to wide lanceolate leaf, 12 – 30 cm in length, 4 – 9 cm in width; pointed at the apex and wedge-shaped at the base; roughly serrate leaf with short petiole; occasionally being cut into strips 5 – 10 mm in shorter diameter and several cm in longer diameter; upper surface green to green-brown in color, lower surface light green-brown with light brown woolly hairs; vein, light yellow-brown in color, raised out on the lower surface of the leaf.

Odor, slight; practically tasteless.

Under a microscope ⟨*5.01*⟩, a transverse section of Loquat Leaf reveals thick cuticle on both surfaces; palisade tissue, mostly 4 to 5 layers with several large cells without chloroplast; at main vein, ring of collateral bundle partly cut by intruding fundamental tissue at xylem side, and group of fiber attaching to phloem; solitary and clustered crystals of calcium oxalate in mesophyll; woolly hair, unicellular and curved, about 25 μm in thickness, and up to 1.5 mm in length.

Identification To 0.3 g of pulverized Loquat Leaf add 10 mL of methanol, warm on a water bath for 5 minutes with occasional shaking, cool, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water and acetonitrile (3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 10 minutes: a red-purple principal spot appears at an *R*f value of about 0.5.

Purity Total BHC's and total DDT's ⟨*5.01*⟩—Not more than 0.2 ppm, respectively.

Loss on drying ⟨*5.01*⟩ Not more than 15.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 16.0%.

Containers and storage Containers—Well-closed containers.

Lycium Bark

Lycii Cortex

ジコッピ

Lycium Bark is the root bark of *Lycium chinense* Miller or *Lycium barbarum* Linné (*Solanaceae*).

Description Tubular to semitubular bark, 1 – 6 mm in thickness; externally light brown to light yellow-brown, periderm peeled easily as scale; internally grayish brown, longitudinally striate; brittle in texture; fractured surface, grayish white, not fibrous.

Odor, weak and characteristic; taste, slightly sweet at first.

Under a microscope ⟨*5.01*⟩, a transverse section reveals periderm composed of a cork layer of several layers of thin walled cork cells; in cortex parenchyma cells containing sandy crystals of calcium oxalate sparsely distributed, occasionally a few fibers observed; parenchyma cells contain starch grains, 1 – 10 μm in diameter; stone cells very rare.

Identification To 1.0 g of pulverized Lycium Bark add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, ammonium acetate solution (1 in 20) and acetic acid (100) (2:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, heat at 105°C for 2 minutes, then spray evenly sodium nitrite TS, and allow to stand for 5 minutes: a dark brown principal spot appears at an *R*f value of about 0.4.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Lycium Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Lycium Bark according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying ⟨*5.01*⟩ Not more than 11.5% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 20.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 3.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 10.0%.

Containers and storage Containers—Well-closed containers.

Lycium Fruit

Lycii Fructus

クコシ

Lycium Fruit is the fruit of *Lycium chinense* Miller or *Lycium barbarum* Linné (*Solanaceae*).

Description Fusiform fruit with acute apex, 6 – 20 mm in length, 3 – 8 mm in diameter, pericarp red to dark red, externally roughly wrinkled; under a magnifying glass, a

transverse section of fruit reveals two locules containing numerous seeds; seed light brown to light yellow-brown, about 2 mm in a diameter, compressed reniform.

Odor, characteristic; taste, sweet, later slightly bitter.

Identification To 1.0 g of pulverized Lycium Fruit add 5 mL of ethyl acetate, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (10:1) to a distance of about 7 cm, and air-dry the plate: a yellow principal spot appears at an *R*f value of about 0.6.

Purity Foreign matter <*5.01*>—It contains not more than 2.0% of foreign matter such as peduncle or others.

Total ash <*5.01*> Not more than 8.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Well-closed containers.

Magnolia Bark

Magnoliae Cortex

コウボク

Magnolia Bark is the bark of the trunk of *Magnolia obovata* Thunberg (*Magnolia hypoleuca* Siebold et Zuccarini), *Magnolia officinalis* Rehder et Wilson or *Magnolia officinalis* Rehder et Wilson var. *biloba* Rehder et Wilson (*Magnoliaceae*).

It contains not less than 0.8% of magnolol.

Description Plate-like or semi-tubular bark, 2 – 7 mm in thickness; externally grayish white to grayish brown, and rough, sometimes cork layer removed, and externally red-brown; internally light brown to dark purplish brown; cut surface extremely fibrous, and light red-brown to purplish brown.

Odor, slight; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals a thick cork layer or several thin cork layers, and internally adjoining the circular tissue of stone cells of approximately equal in diameter; primary cortex thin; fiber groups scattered in the pericycle; groups of phloem fibers lined alternately with the other tissue of phloem between medullary rays in the secondary cortex, and then these tissues show a latticework; oil cells scattered in the primary and secondary cortex, but sometimes observed in the narrow medullary rays.

Identification To 1.0 g of pulverized Magnolia Bark add 10 mL of methanol, stir for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: a yellow spot appears at an *R*f value of about 0.3.

Total ash <*5.01*> Not more than 6.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 11.0%.

Assay Weigh accurately about 0.5 g of pulverized Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of magnolol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust so that the retention time of magnolol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Magnolia Bark

Magnoliae Cortex Pulveratus

コウボク末

Powdered Magnolia Bark is the powder of Magnolia Bark.

It contains not less than 0.8% of magnolol.

Description Powdered Magnolia Bark occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope <*5.01*>, Powdered Magnolia Bark reveals starch grains and parenchyma cells containing them; stone cells of various sizes or its groups; fibers 12 to 25 μm in diameter; yellowish red-brown cork tissue; oil cells containing a yellow-brown to red-brown substance. Simple starch

grains about 10 μm in diameter and 2- to 4-compound starch grains.

Identification To 1.0 g of Powdered Magnolia Bark add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: a yellow spot appears at an *R*f value of about 0.3.

Total ash <*5.01*> Not more than 6.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 11.0%.

Assay Weigh accurately about 0.5 g of Powdered Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of magnolol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust so that the retention time of magnolol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Magnolia Flower

Magnoliae Flos

シンイ

Magnolia Flower is the flower bud of *Magnolia salicifolia* Maximowicz, *Magnolia kobus* De Candolle, *Magnolia biondii* Pampanini, *Magnolia sprengeri* Pampanini or *Magnolia heptapeta* Dandy (*Magnolia denudata* Desrousseaux) (*Magnoliaceae*).

Description Magnolia Flower is a fusiform flower bud, 15 – 45 mm in length, 6 – 20 mm in diameter at central part; often having ligneous peduncles on base; usually 3 bracts, externally with sparse hairs, brown to dark brown, or with dense hairs, grayish white to light yellow-brown, and the inner surface of 3 bracts smooth and dark brown in color; interior perianth of 9 pieces or 12 pieces, same size or outer three pieces are smaller; 50 – 100 stamens and numerous pistils. Brittle in texture.

Odor, characteristic; taste, acrid and slightly bitter.

Identification To 1 g of pulverized Magnolia Flower add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetone, water and formic acid (5:3:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a yellow-red spot appears at an *R*f value of about 0.3.

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 5.5%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-extract: not less than 13.0%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Magnolia Flower: the volume of essential oil is not less than 0.5 mL.

Containers and storage Containers—Well-closed containers.

Mallotus Bark

Malloti Cortex

アカメガシワ

Mallotus Bark is the bark of *Mallotus japonica* Mueller Argoviensis (*Euphorbiaceae*).

Description Mallotus Bark is flat or semitubular pieces of bark, 1 – 3 mm in thickness; externally greenish gray to brownish gray brow in color, with a vertically striped shape gathering numerous lenticels; internal surface light yellow-brown to grayish brown in color, and smooth with numerous fine striped lines; easy to break; slightly fibrous at fracture surface.

Mallotus Bark has a slight odor, a bitter taste and slightly astringent.

Identification To 0.5 g pulverized Mallotus Bark add 10 mL of methanol, warm on a water bath for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of bergenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (100:17:13) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark blue spot obtained from the standard solution.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 12.0%.

Acid-insoluble ash <*5.01*> Not more than 2.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 11.0%.

Containers and storage Containers—Well-closed containers.

Malt

Fructus Hordei Germinatus

バクガ

Malt is the dried ripe cariopsis of *Hordeum vulgare* Linné (*Gramineae*), after being germinated.

Description Oval caryopsis, 10 mm in length, 3 – 4 mm in width, furrowed on one surface; externally light yellow, sometimes with plumule at one end, with hairs and sometimes with roots at the other end; cross section of caryopsis white and powdery; easily broken and light in texture.

Odor, slight; taste, slightly sweet.

Under a microscope <*5.01*>, a transverse section of the caryopsis reveals glume, pericarp, seed coat and endosperm in this order from the outside; 2 – 4 layered aleurone layers on the circumference of endosperm; endosperm filled with starch grains; starch grains as spheroidal or ellipsoidal, large grains about 20 μm and small grains about 2 μm in diameter mixed together.

Identification To 3.0 g of pulverized Malt add 5 mL of methanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Liquid Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (8:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly a solution of 0.1 g of 2,3-indolinedione in 50 mL of acetone on the plate, and heat at 105°C for 5 minutes: a blue-purple spot appears at an *R*f value of about 0.4.

Loss on drying <*5.01*> Not more than 11.0%.

Total ash <*5.01*> Not more than 2.6%.

Acid-insoluble ash <*5.01*> Not more than 0.8%.

Extract content <*5.01*> Dilute ethanol-soluble extract: Not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Maoto Extract

麻黄湯エキス

Maoto Extract contains not less than 15 mg and not more than 45 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 48 mg and not more than 192 mg of amygdalin, and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)
Ephedra Herb	5 g
Apricot Kernel	5 g
Cinnamon Bark	4 g
Glycyrrhiza	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1), using the crude drugs shown above, or prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive prepared as directed under Extracts, according to the prescription 1), using the crude drugs shown above.

Description Maoto Extract occurs as a light brown powder or blackish brown viscous extract, having a slightly order, and a sweet and bitter, then a slightly astringent taste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green-brown spot obtained from the standard solution (Apricot Kernel).

(3) Perform the test according to the following (i) or (ii)

(Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity (1) Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 13.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 10.0% and 22.0%.

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine all the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained from the sample solution, and peak area, A_S, of ephedrine from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Amygdalin—Weigh accurately about 0.5 g of the dry

extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, then elute with water to make exactly 20 mL, and use this effluent as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizinic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Mentha Herb

Menthae Herba

ハッカ

Mentha Herb is the terrestrial part of *Mentha arvensis* Linné var. *piperascens* Malinvaud (*Labiatae*).

Description Stem with opposite leaves; stem, square, light brown to red-purple in color, and with fine hairs; when smoothed by immersing in water, leaf, ovate to oblong, with acute apex and base, 2 – 8 cm in length, 1 – 2.5 cm in width, margin irregularly serrated; the upper surface, light brown-yellow to light green-yellow, and the lower surface, light green to light green-yellow in color; petiole 0.3 – 1 cm in length. Under a magnifying glass, leaf reveals hairs, glandular hairs and scales.

It has a characteristic aroma and gives a cool feeling on keeping in the mouth.

Identification To 1.0 g of pulverized Mentha Herb add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of menthol in 1 mL of diethyl ether, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity Foreign matter <*5.01*>—The amount of roots and other foreign matter contained in Mentha Herb does not exceed 2.0%.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 12.0%.

Acid-insoluble ash <*5.01*> Not more than 2.5%.

Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Mentha Herb after adding 1 mL of silicone resin to the sample in the flask: the volume of essential oil is not less than 0.4 mL.

Containers and storage Containers—Well-closed containers.

Mentha Oil

Oleum Menthae Japonicae

ハッカ油

Mentha Oil is the essential oil which is distilled with steam from the terrestrial parts of *Mentha arvensis* Linné var. *piperascens* Malinvaud (*Labiatae*), and from which solids are removed after cooling.

It contains not less than 30.0% of menthol ($C_{10}H_{20}O$: 156.27).

Description Mentha Oil is a colorless or pale yellow, clear liquid. It has a characteristic, pleasant aroma and has a pungent taste, followed by a cool aftertaste.

It is miscible with ethanol (95), with ethanol (99.5), with warm ethanol (95), and with diethyl ether.

It is practically insoluble in water.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.455 – 1.467

Optical rotation ⟨*2.49*⟩ α_D^{20}: −17.0 – −36.0° (100 mm).

Specific gravity ⟨*1.13*⟩ d_{25}^{25}: 0.885 – 0.910

Acid value ⟨*1.13*⟩ Not more than 1.0.

Purity **(1)** Clarity and color of solution—To 1.0 mL of Mentha Oil add 3.5 mL of diluted ethanol (7 in 10), and shake: Mentha Oil dissolves clearly. To the solution add 10 mL of ethanol (95): the solution is clear or has no more turbidity, if any, than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 mL of Mentha Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Weigh accurately about 5 g of Mentha Oil, and dissolve in ethanol (95) to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 10 g of *l*-menthol for assay, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak area of menthol to that of the internal standard.

$$\text{Amount (mg) of menthol } (C_{10}H_{20}O) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of *l*-menthol for assay taken

Internal standard solution—A solution of *n*-ethyl caprylate in ethanol (95) (1 in 25).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 25% of polyethylene glycol 6000 for gas chromatography supported on acid-washed 180 – 250 μm siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 150°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of the internal standard is about 10 minutes.

Selection of column: Proceed with 1 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and *l*-menthol in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Mentha Water

ハッカ水

Method of preparation

Mentha Oil	2 mL
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Aromatic Waters, with the above ingredients.

Description Mentha Water is a clear, colorless liquid, having the odor of mentha oil.

Containers and storage Containers—Tight containers.

Moutan Bark

Moutan Cortex

ボタンピ

Moutan Bark is the root bark of *Paeonia suffruticosa* Andrews (*Paeonia moutan* Sims) (*Paeoniaceae*).

It contains not less than 1.0% of paeonol.

Description Tubular to semi-tubular bark, about 0.5 cm in thickness, 5 – 8 cm in length, 0.8 – 1.5 cm in diameter; externally dark brown to purplish brown, with small and transversely elongated ellipsoidal scars of lateral roots, and with longitudinal wrinkles; internally, light grayish brown to purplish brown and smooth; fractured surface coarse; white crystals often attached on the internal and fractured surfaces.

Odor, characteristic; taste, slightly pungent and bitter.

Identification To 2.0 g of pulverized Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of hexane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultravi-

olet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity **(1)** Xylem—When perform the test of foreign matter <*5.01*>, the amount of the xylem contained in Moutan Bark is not more than 5.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Moutan Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Moutan Bark according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than xylem contained in Moutan Bark is not exceed 1.0%.

(5) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Assay Weigh accurately about 0.3 g of pulverized Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of paeonol for assay, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeonol in each solution.

$$\text{Amount (mg) of paeonol} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of paeonol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust so that the retention time of paeonol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg of paeonol for assay and 5 mg of butyl parahydroxybenzoate for resolution check in methanol to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, paeonol and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeonol is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Moutan Bark

Moutan Cortex Pulveratus

ボタンピ末

Powdered Moutan Bark is the powder of Moutan Bark.

It contains not less than 0.7% of paeonol.

Description Powdered Moutan Bark occurs as a light grayish yellow-brown powder. It has a characteristic odor and a slight, pungent and bitter taste.

Under a microscope <*5.01*>, Powdered Moutan Bark reveals starch grains and fragments of parenchyma containing them; fragments of cork tissue containing tannin; fragments of somewhat thick-walled collenchyma, medullary rays, and phloem parenchyma; rosette aggregates of calcium oxalate and also fragments of parenchyma cells containing them. Starch grains are simple or 2- to 10-compound grains, 10 – 25 μm in diameter; rosette aggregates are 20 – 30 μm in diameter.

Identification **(1)** To 2.0 g of Powdered Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of hexane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

(2) Evaporate to dryness 1 mL of the sample solution obtained in (1), dissolve the residue in 50 mL of ethanol (95), and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima at around 228 nm, 274 nm and 313 nm.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Moutan Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Moutan Bark according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, usually vessels and other sclerenchymatous cells are not observable.

(4) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of Powdered Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly

100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of paeonol for assay, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeonol in each solution.

$$\text{Amount (mg) of paeonol} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of paeonol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust so that the retention time of paeonol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg of paeonol for assay and 5 mg of butyl parahydroxybenzoate for resolution check in methanol to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, paeonol and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeonol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Mukoi-Daikenchuto Extract

無コウイ大建中湯エキス

Mukoi-Daikenchuto Extract contains not less than 1.8 mg of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), and not less than 1.4 mg and not more than 4.2 mg of [6]-shogaol, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)
Japanese Zanthoxylum Peel	2 g
Ginseng	3 g
Processed Ginger	5 g

Prepare a dry extract as directed under Extracts, according to the prescription 1), using crude drugs shown above.

Description Mukoi-Daikenchuto Extract is a light brown powder. It has a slight odor, and has a pungent taste.

Identification (1) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, then shake with 10 mL of diethyl ether, centrifuge, and use the supernatant liquid as the sample solution. Separately, powder japanese zanthoxylum peel, shake 2.0 g with 10 mL of water, then shake with 5 mL of diethyl ether, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and acetic acid (100) (20:20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot (*R*f value: about 0.3) from the standard solution (Japanese Zanthoxylum Peel).

(2) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(3) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Processed ginger).

Purity (1) Heavy metals <*1.07*>—Prepare the test solution with 2.0 g of Mukoi-Daikenchuto Extract as directed under Extracts (4), and perform the test (not more than 15 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Mukoi-Daikenchuto Extract according to Method 3, and perform the test (not more than 1 ppm).

Loss on drying <*2.41*> Not more than 5.9% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%.

Assay (1) Ginsenoside Rb_1—Weigh accurately about 2 g of Mukoi-Daikenchuto Extract, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), and repeat the same procedure. Combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL

of sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column [10 mm in inside diameter, packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), and washed just before using with methanol and then diluted methanol (3 in 10)], and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rb_1\ (C_{54}H_{92}O_{23}) = M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl group bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (400:100:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb_1 is about 16 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb_1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

(2) [6]-Shogaol—Weigh accurately about 0.5 g of Mukoi-Daikenchuto Extract, add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of [6]-shogaol for assay, dissolve in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (3 in 4) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-shogaol in each solution.

$$\text{Amount (mg) of [6]-shogaol} = M_S \times A_T/A_S \times 1/10$$

M_S: Amount (mg) of [6]-shogaol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 0.1 g of oxalic acid dihydrate in 600 mL of water, and add 400 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of [6]-shogaol is about 30 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Mulberry Bark

Mori Cortex

ソウハクヒ

Mulberry Bark is the root bark of *Morus alba* Linné (*Moraceae*).

Description Tubular, semi-tubular or cord-like bark, 1 – 6 mm thick, often in fine lateral cuttings; externally, white to yellow-brown; in the case of the bark with periderm, its periderm is yellow-brown in color, easy to peel, with numerous longitudinal, fine wrinkles and numerous red-purple lenticels laterally elongated; inner surface, dark yellow-brown in color and flat; cross section, white to light brown in color, and fibrous.

Odor, slight; taste, slight.

Under a microscope <*5.01*>, a transverse section of bark with periderm reveals 5 to 12 layers of cork cells in the outer portion; phloem fibers or their bundles scattered in the cortex, arranged alternately and stepwise with phloem parenchyma; lactiferous tubes; solitary crystals of calcium oxalate; starch grains as spheroidal or ellipsoidal, simple or compound grains, simple grain 1 – 7 μm in diameter.

Identification Heat 1 g of pulverized Mulberry Bark with 20 mL of hexane under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 10 mL of acetic anhydride, place 0.5 mL of the solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Mulberry Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Mulberry Bark according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of the root

xylem and other foreign matter is not more than 1.0%.

Total ash ⟨*5.01*⟩ Not more than 11.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nelumbo Seed

Nelumbis Semen

レンニク

Nelumbo Seed is the seed of *Nelumbo nucifera* Gaertner (*Nymphaeaceae*), usually with the endocarp, sometime being removed the embryo.

Description Ovoid to ellipsoidal seed, at the base a papillate protuberance surrounded with shallow depression, 1.0 – 1.7 cm in length, 0.5 – 1.2 cm in width; externally light reddish brown to light yellowish brown; projection part dark reddish brown; endocarp not lustrous and hardly peeled off; endosperm yellowish white, a green embryo in the center.

Almost odorless; taste, slightly sweet and oily, embryo is extremely bitter.

Under a microscope ⟨*5.01*⟩, a transverse section of the seed at central portion reveals endocarp composed of parenchyma or endocarp occasionally left out; seed coat composed of epidermis and parenchyma of compressed cells; vascular bundles scattered in parenchyma; endosperm composed of epidermis and parenchyma; aggregate crystals of calcium oxalate and tannin-like substances contained in endocarp remained; parenchymatous cells of seed coat contain tannin-like substances; parenchyma of endosperm contain starch grains.

Identification To 0.5 g of pulverized Nelumbo Seed add 5 mL of water, shake for 5 minutes, and centrifuge. To 0.5 mL of the supernatant liquid add 1 drop of a solution of 1-naphthol in ethanol (99.5) (1 in 5), mix, then add gently 1 mL of sulfuric acid: the solution shows a purple color.

Loss on drying ⟨*5.01*⟩ Not more than 14.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 5.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 14.5%.

Containers and storage Containers—Well-closed containers.

Notopterygium

Notopterygii Rhizoma

キョウカツ

Notopterygium is the rhizome and root of *Notopterygium incisum* Ting ex H. T. Chang or *Notopterygium forbesii* Boissieu (*Umbelliferae*).

Description Notopterygium is slightly curved, cylindrical to conical, 3 – 10 cm in length, 5 – 20 mm in diameter; rhizome occasionally branched; externally yellow-brown to dark brown. The rhizome with nearly orbicular, hollowed stem scars at the apex, sometimes having short residue of stem; externally node rising, internode short; root scars in warty processes on the node; externally root has coarse longitudinal wrinkles and lateral root scars in warty processes; light and slightly brittle in texture, easy to break. The transverse section of the rhizome reveals numerous radial cracks; cortex yellow-brown to brown; xylem light yellow to light grayish yellow; pith grayish white to light brown. Under a magnifying glass, the rhizome reveals brown, fine points of resin canals in the cortex and pith.

Odor, characteristic; taste, slightly acid at first, followed by a slightly pungent and slightly numbing aftertaste.

Under a microscope ⟨*5.01*⟩, transverse section shows the outermost layer to be composed of a cork layer several to a dozen or so cells thick; collenchyma just inside of the cork layer; oil canals scattered in cortex, large ones more than 300 μm in diameter; intercellular space occurring in radial direction in cortex; oil canals scattered in pith, large ones more than 500 μm in diameter; parenchymatous cells contain simple and 2- to 3-compound starch grains.

Identification To 0.3 g of pulverized Notopterygium add 3 mL of hexane in a glass-stoppered centrifuge tube, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of methanol and water (9:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at an *R*f value of about 0.5, which shows a dark purple color under ultraviolet light (main wavelength: 254 nm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Notopterygium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Notopterygium according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying ⟨*5.01*⟩ Not more than 13.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 6.5%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 20.0%.

Containers and storage Containers—Well-closed containers.

Nuphar Rhizome

Nupharis Rhizoma

センコツ

Nuphar Rhizome is the longitudinally split rhizome of *Nuphar japonicum* De Candolle (*Nymphaeaceae*).

Description Usually, longitudinally split irregular column, twisted, bent or somewhat pressed, 20 – 30 cm in length, about 2 cm in width; the outer surface, dark brown, and the cross section, white to grayish white in color; one side shows nearly round to blunt triangular scars of petiole about 1 cm in diameter, and the other side numerous scars of roots less

than 0.3 cm in diameter; light, spongy in texture, and easily broken; fractured surface flat and powdery. Under a magnifying glass, a transverse section reveals a black outer portion, and porous tissue with scattered vascular bundles in the inner portion.

Odor, slight; taste, slightly bitter and unpleasant.

Identification Boil 1 g of pulverized Nuphar Rhizome with 20 mL of methanol under a reflux condenser on a water bath for 15 minutes, cool, and filter. Evaporate the filtrate to dryness, warm the residue with 5 mL of dilute acetic acid on a water bath for 1 minute, cool, and filter. Spot 1 drop of the filtrate on a piece of filter paper, air-dry the paper, spray Dragendorff's TS for spraying on it, and allow it to stand: a yellow-red color appears.

Purity **(1)** Petiole—When perform the test of foreign matter <*5.01*>, the amount of the petioles contained in Nuphar Rhizome does not exceed 3.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Nuphar Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Nuphar Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than petioles is not more than 1.0%.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 10.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nutmeg

Myristicae Semen

ニクズク

Nutmeg is the seed of *Myristica fragrans* Houttuyn (*Myristicaceae*), usually from which the seed coat has been removed.

Description Ovoid-globose to ellipsoidal seeds, 1.5 – 3.0 cm in length, 1.3 – 2.0 cm in diameter; externally grayish brown, with wide and shallow longitudinal furrows and fine wrinkles; usually, grayish white to grayish yellow and slightly protruding hilum at one end, grayish brown to dark brown and slightly concave chalaza at the other end; cross section has a marble-like appearance with the dark brown thin perisperm extending irregularly into the light yellowish white to light brown endosperm.

Odor, characteristic and strong; taste, acrid and slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals perisperm composed of outer and inner layers, the outer layer composed of parenchyma containing dark red-brown contents and the inner layer composed of parenchyma containing red-brown contents with a number of large oil cells and scattered vascular bundles; in parenchyma cells of endosperm, simple or compound starch grains and aleurone grains observed.

Identification To 1 g of pulverized Nutmeg add 5 mL of methanol, allow to stand for 10 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Loss on drying <*5.01*> Not more than 16.0% (6 hours).

Total ash <*5.01*> Not more than 2.5%.

Essential oil content <*5.01*> When the test is performed with 10.0 g of pulverized Nutmeg, the essential oil content is not less than 0.5 mL.

Containers and storage Containers—Well-closed containers.

Nux Vomica

Strychni Semen

ホミカ

Nux Vomica is the seed of *Strychnos nux-vomica* Linné (*Loganiaceae*).

When dried, it contains not less than 1.07% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Description Disk, often slightly bent, 1 – 3 cm in diameter, 0.3 – 0.5 cm in thickness; externally light grayish yellow-green to light grayish brown, covered densely with lustrous appressed hairs radiating from the center to the circumference; on both sides, the margin and the central part bulged a little; the dot-like micropyle situated at one point on the margin, and from which usually a raised line runs to the center on one side; extremely hard in texture; when cracked upon soaking in water, the seed coat thin, the interior consisting of two horny, light grayish yellow endosperms, and leaving a central narrow cavity at the center; a white embryo, about 0.7 cm in length, situated at one end between the inner surfaces of the endosperms.

Odorless; taste, very bitter and persisting.

Identification **(1)** To 3 g of pulverized Nux Vomica add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer

of sulfuric acid shows a purple color which turns immediately red to red-brown.

Total ash ⟨*5.01*⟩ Not more than 3.0%.

Assay Weigh accurately about 1 g of pulverized Nux Vomica, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 1 mL of ammonia solution (28). To this solution add 20 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure three times with the residue using 20-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Calculate the ratio, Q_T and Q_S, of the peak area of strychnine to that of the internal standard.

$$\text{Amount (mg) of strychnine } (C_{21}H_{22}N_2O_2) = M_S \times Q_T/Q_S \times 1/5 \times 0.841$$

M_S: Amount (mg) of strychnine nitrate for assay taken, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in water to make 1000 mL, and mix with acetonitrile and triethylamine (45:5:1), and adjust the mixture with phosphoric acid to pH 3.0.

Flow rate: Adjust so that the retention time of Strychnine is about 17 minutes.

Selection of column: Proceed with 5 μL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly separating each peak.

Containers and storage Containers—Well-closed containers.

Nux Vomica Extract

ホミカエキス

Nux Vomica Extract contains not less than 6.15% and not more than 6.81% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation After defatting 1000 g of coarse powder of Nux Vomica with hexane, extract with the percolation method, using a mixture of 750 mL of Ethanol, 10 mL of Acetic Acid and 240 mL of Purified Water or Purified Water in Containers as the first solvent, and 70 vol% ethanol as the second solvent. Combine the extracts, and prepare the dry extract as directed under Extracts. Where, an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used instead of 70 vol% ethanol.

Description Nux Vomica Extract occurs as yellow-brown to brown powder. It has a slight characteristic odor, and an extremely bitter taste.

Identification Extract 0.5 g of Nux Vomica Extract with 0.5 mL of ammonia TS and 10 mL of chloroform with occasional shaking. Filter the chloroform extract, evaporate the filtrate on a water bath until most of the chloroform is removed, and proceed as directed in the Identification under Nux Vomica.

Purity Heavy metals ⟨*1.07*⟩—Prepare the test solution with 1.0 g of Nux Vomica Extract as directed in the Extracts (4), and perform the test (not more than 30 ppm).

Assay Weigh accurately about 0.2 g of Nux Vomica Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to disperse the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 100 mL. Then, proceed as directed in the Assay under Nux Vomica.

$$\text{Amount (mg) of strychnine } (C_{21}H_{22}N_2O_2) = M_S \times Q_T/Q_S \times 1/5 \times 0.841$$

M_S: Amount (mg) of strychnine nitrate for assay taken, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Nux Vomica Extract Powder

ホミカエキス散

Nux Vomica Extract Powder contains not less than 0.61% and not more than 0.68% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation

Nux Vomica Extract	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

To Nux Vomica Extract add 100 mL of Purified Water or Purified Water in Containers, then warm, and soften with stirring. Cool, add 800 g of Starch, Lactose Hydrate or their mixture little by little, and mix well. Dry, preferably at a low temperature, and dilute with a sufficient additional quantity of Starch, Lactose or their mixture to make 1000 g of the homogeneous powder.

Description Nux Vomica Extract Powder occurs as a yellow-brown to grayish brown powder. It has a slight, characteristic odor and a bitter taste.

Identification **(1)** To 3 g of Nux Vomica Extract Powder add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer of sulfuric acid shows a purple color which turns immediately red to red-brown.

Assay Weigh accurately about 2.0 g of Nux Vomica Extract Powder, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to separate the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 5 mL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratio, Q_T and Q_S, of the peak area of strychnine to that of the internal standard.

$$\text{Amount (mg) of strychnine } (C_{21}H_{22}N_2O_2) = M_S \times Q_T/Q_S \times 1/5 \times 0.841$$

M_S: Amount (mg) of strychnine nitrate for assay taken, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (6.8 in 1000), acetonitrile and triethylamine (45:5:1), adjusted the pH to 3.0 with phosphoric acid.

Flow rate: Adjust so that the retention time of strychnine is about 17 minutes.

Selection of column: Proceed with 5 μL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nux Vomica Tincture

ホミカチンキ

Nux Vomica Tincture contains not less than 0.097 w/v% and not more than 0.116 w/v% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation

Nux Vomica, in coarse powder	100 g
70 vol% Ethanol	a sufficient quantity
	To make 1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers.

Description Nux Vomica Tincture is a yellow-brown liquid. It has an extremely bitter taste.

Specific gravity d_{20}^{20}: about 0.90

Identification Heat 20 mL of Nux Vomica Tincture on a water bath to remove ethanol, cool, transfer to a separator, add 2 mL of ammonia TS and 20 mL of chloroform, and shake well for 2 to 3 minutes. Filter the chloroform layer through a pledget of absorbent cotton, warm the filtrate on a water bath to remove most of chloroform, and proceed as directed in the Identification under Nux Vomica.

Alcohol number <*1.01*> Not less than 6.7 (Method 2).

Assay Pipet 3 mL of Nux Vomica Tincture into a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the

diethyl ether on a water bath. Dissolve the residue with 10 mL of the mobile phase, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.8-μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (separately determine the loss on drying), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and the standard solution as directed in the Assay under Nux Vomica.

$$\text{Amount (mg) of strychnine } (C_{21}H_{22}N_2O_2) = M_S \times Q_T/Q_S \times 1/20 \times 0.841$$

M_S: Amount (mg) of strychnine nitrate for assay taken, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Olive Oil

Oleum Olivae

オリブ油

Olive Oil is the fixed oil obtained by expression from the ripe fruit of *Olea europaea* Linné (*Oleaceae*).

Description Olive Oil is a light yellow oil. It has a faint odor, which is not rancid, and has a bland taste.
It is miscible with diethyl ether, with petroleum ether.
It is slightly soluble in ethanol (95).
The whole or a part of it congeals between 0°C and 6°C.
Congealing point of the fatty acids: 17 – 26°C

Specific gravity ⟨*1.13*⟩ d_{25}^{25}: 0.908 – 0.914

Acid value ⟨*1.13*⟩ Not more than 1.0.

Saponification value ⟨*1.13*⟩ 186 – 194

Unsaponifiable matters ⟨*1.13*⟩ Not more than 1.5%.

Iodine value ⟨*1.13*⟩ 79 – 88

Purity **(1)** Drying oil—Mix 2 mL of Olive Oil with 10 mL of diluted nitric acid (1 in 4), add 1 g of powdered sodium nitrite little by little with thorough shaking, and allow to stand in a cold place for 4 to 10 hours: the mixture congeals to a white solid.

(2) Peanut oil—Weigh exactly 1.0 g of Olive Oil, dissolve in 60 mL of sulfuric acid-hexane-methanol TS, boil for 2.5 hours on a water bath under a reflux condenser, cool, transfer to a separator, and add 100 mL of water. Wash the flask with 50 mL of petroleum ether, add the washing to the separator, shake, allow to stand, and separate the petroleum ether layer. Extract the water layer with another 50 mL of petroleum ether, and combine the petroleum ether layer with the former petroleum ether solution. Wash the petroleum ether solution repeatedly with 20-mL portions of water until the washings show no more acidity to methyl orange TS. Then add 5 g of anhydrous sodium sulfate, shake, filter, wash anhydrous sodium sulfate with two 10-mL portions of petroleum ether, filter the washings using the former separator, combine the filtrates, distil the petroleum ether on a water bath, passing nitrogen. Dissolve the residue in acetone to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 0.067 g of methyl behenate in acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Measure the peak heights, H_T and H_S, of methyl behenate of respective solutions: H_T is not higher than H_S.

Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter), coated with polyethylene glycol 20 mol/L in a ratio of 5%.
Column temperature: A constant temperature of about 220°C.
Carrier gas: Nitrogen.
Flow rate: Adjust so that the retention time of methyl behenate is about 18 minutes.
Detection sensitivity: Adjust so that the peak height of methyl behenate obtained from 2 μL of the standard solution is 5 to 10 mm.

Containers and storage Containers—Tight containers.

Ophiopogon Root

Ophiopogonis Radix

バクモンドウ

Ophiopogon Root is the enlarged part of the root of *Ophiopogon japonicus* Ker-Gawler (*Liliaceae*).

Description Fusiform root, 1 – 2.5 cm in length, 0.3 – 0.5 cm in diameter, somewhat sharp at one end, and somewhat rounded at the other; externally light yellow to light yellow-brown, with longitudinal wrinkles of various sizes; when fractured, cortex flexible and friable, stele strong; fractured surface of cortex light yellow-brown in color, slightly translucent and viscous.
Odor, slight; taste, slightly sweet and mucous.
Under a microscope ⟨*5.01*⟩, a transverse section reveals brown, 4- to 5-layer velamen internally adjoining the epidermis; a single-layer exodermis inside the velamen, and cortex of parenchyma cells inside the exodermis; endodermis is distinct; about 20 protoxylems in actionstele; cortex parenchyma contains columnar crystals and needle raphides of calcium oxalate; oil drops in the exodermis.

Purity **(1)** Rootlets—When perform the test of foreign matter ⟨*5.01*⟩, the amount of the rootlets contained in Ophiopogon Root is not exceed 1.0%.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Ophiopogon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Ophiopogon Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 3.0%.

Containers and storage Containers—Well-closed containers.

Powdered Opium

Opium Pulveratum

アヘン末

Powdered Opium is a homogeneous powder of opium obtained from *Papaver somniferum* Linné (*Papaveraceae*). Starch or Lactose Hydrate may be added.

Powdered Opium contains not less than 9.5% and not more than 10.5% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Description Powdered Opium occurs as a yellow-brown to dark brown powder.

Identification **(1)** To 0.1 g of Powdered Opium add 5 mL of diluted ethanol (7 in 10), dissolve by treating with ultrasonic waves for 10 minutes, and add diluted ethanol (7 in 10) to make 10 mL. Filter this solution, and use the filtrate as the sample solution. Separately, dissolve 25 mg of Morphine Hydrochloride Hydrate, 12 mg of Codeine Phosphate Hydrate, 2 mg of Papaverine Hydrochloride, and 12 mg of Noscapine Hydrochloride Hydrate separately in 25 mL of diluted ethanol (7 in 10), and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: each spot from the sample solution shows the same color tone and *R*f value of each spot obtained from the standard solution (1), the standard solution (2), the standard solution (3), and the standard solution (4) (morphine, codeine, papaverine and noscapine), respectively.

(2) To 0.1 g of Powdered Opium add 5 mL of water, and shake the mixture for 5 minutes. Filter, to the filtrate add 1 mL of a solution of hydroxylammonium chloride (3 in 10) and 1 drop of iron (III) chloride TS, and shake: a red-brown color is produced. To this solution add immediately 5 mL of diethyl ether, and shake: the diethyl ether layer has no red-purple color (meconic acid).

Loss on drying ⟨*2.41*⟩ Not more than 8.0% (1 g, 105°C, 5 hours).

Assay Place about 5 g of Powdered Opium, accurately weighed, in a mortar, and triturate it with exactly 10 mL of water. Add 2 g of calcium hydroxide and exactly 40 mL of water, and stir the mixture for 20 minutes. Filter, and shake 30 mL of the filtrate with 0.1 g of magnesium sulfate heptahydrate for 1 minute. To the mixture add 0.3 g of calcium hydroxide, shake for 1 minute, and allow to stand for 1 hour. Filter, place 20 mL of the filtrate, exactly measured, in a glass-stoppered flask, and add 10 mL of diethyl ether and 0.3 g of ammonium chloride. Shake vigorously with caution. When crystals begin to separate out, shake for 30 minutes with a mechanical shaker, and set aside overnight at a temperature of 5°C to 10°C. Decant the diethyl ether layer and filter first, and then the water layer through filter paper 7 cm in diameter. Wash the adhering crystals in the flask with three 5-mL portions of water saturated with diethyl ether, and wash the crystals on the filter paper with each of these washings. Wash the top of the glass-stoppered flask and the upper part of the filter paper with final 5 mL of water saturated with diethyl ether. Transfer the crystals and the filter paper to a beaker. Dissolve the crystals remaining in the glass-stoppered flask with the aid of 15 mL of 0.05 mol/L sulfuric acid VS, accurately measured, and pour the solution into the beaker. Wash the glass-stoppered flask with four 5-mL portions of water, and add the washings to the solution in the beaker. Titrate ⟨*2.50*⟩ the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of methyl red-methylene blue TS).

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.

Diluted Opium Powder

アヘン散

Diluted Opium Powder contains not less than 0.90% and not more than 1.10% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Powdered Opium	100 g
Starch or a suitable diluent	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

Description Diluted Opium Powder occurs as a light brown powder.

Identification **(1)** Proceed with 1 g of Diluted Opium Powder as directed in the Identification (1) under Powdered Opium.

(2) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (2) under Powdered Opium.

Assay Place about 50 g of Diluted Opium Powder, accurately weighed, in a glass-stoppered flask, and stir with 250 mL of dilute ethanol in a water bath at 40°C for 1 hour. Filter the mixture through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, and add 50 mL of dilute ethanol. Stir the mixture in a water bath at 40°C for 10 minutes, and filter through the same glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Evaporate the combined filtrate in a mortar to dryness on a water bath. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, and, after cooling, triturate it with exactly 10 mL of water. Proceed with this solution as directed in Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.

Opium Tincture

アヘンチンキ

Opium Tincture contains not less than 0.93 w/v% and not more than 1.07 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Powdered Opium	100 g
35 vol% Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers in place of 35 vol% Ethanol.

Description Opium Tincture is a dark red-brown liquid. It is affected by light.

Identification **(1)** To 1 mL of Opium Tincure add diluted ethanol (7 in 10) to make 10 mL, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (1) under Powdered Opium.

(2) Evaporate 1 mL of Opium Tincture to dryness on a water bath, and proceed with the residue as directed in the Identification (2) under Powdered Opium.

Alcohol number <*1.01*> Not less than 3.5 (Method 1).

Assay Evaporate 50 mL of Opium Tincture, accurately measured, on a water bath to dryness. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, cool, and triturate with exactly 10 mL of water. Proceed with this solution as directed in the Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Opium Ipecac Powder

アヘン·トコン散

Opium Ipecac Powder contains not less than 0.90% and not more than 1.10% of morphine ($C_{17}H_{19}NO_3$: 285.34).

Method of preparation

Powdered Opium	100 g
Powdered Ipecac	100 g
Starch or a suitable ingredient	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

Description Opium Ipecac Powder occurs as a light brown powder.

Identification **(1)** Proceed with 1 g of Opium Ipecac Powder as directed in the Identification (1) under Powdered Opium.

(2) Proceed with 1 g of Opium Ipecac Powder as directed in the Identification (2) under Powdered Opium.

(3) Shake frequently a mixture of 3 g of Opium Ipecac Powder and 5 mL of hydrochloric acid, and allow to stand for 1 hour. Filter the solution into an evaporating dish. Add 5 mg of chlorinated lime to the filtrate: an orange color is produced at the circumference of the chlorinated lime (emetine).

Assay Weigh accurately about 50 g of Opium Ipecac Powder in a glass stoppered flask, add 250 mL of dilute ethanol, warm in a water bath at 40°C for 1 hour with stirring, and filter through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, add 50 mL of dilute ethanol, warm in a water bath at 40°C for 10 minutes with stirring, and filter through the glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Combine all the filtrates in a mortar, evaporate on a water bath to dryness, add 10 mL of ethanol (99.5) to the residue, and evaporate again. After cooling, triturate the residue with an exactly measured 10 mL of water, add 2 g of calcium hydroxide and an exactly measured 40 mL of water, stir the mixture for 20 minutes, and filter. To 30 mL of the filtrate add 0.1 g of magnesium sulfate heptahydrate, shake for 1 minute, then add 0.3 g of calcium hydroxide, shake for 1 minute, allow to stand for 1 hour, and filter. To an exactly measured 20 mL of the filtrate add 5 mL of sodium hydroxide TS, and adjust the pH to between 9.0 and 9.2 with ammonium chloride. Extract the solution successively with 60 mL, 40 mL and 30 mL of a mixture of chloroform and ethanol (95) (3:1). Combine all the extracts, distil, then evaporate off the solvent on a water bath. Dissolve the residue in 20 mL of dilute sodium hydroxide TS and 10 mL of diethyl ether with shaking, add 0.5 g of ammonium chloride, shake vigorously with caution, and proceed as directed in the Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.

Orange Oil

Oleum Aurantii

オレンジ油

Orange Oil is the essential oil obtained by expression from the peel of the edible fruit of *Citrus* species (*Rutaceae*).

Description Orange Oil is a yellow to yellow-brown liquid. It has a characteristic, aromatic odor, and a slightly bitter taste.

It is miscible with an equal volume of ethanol (95) with turbidity.

Refractive index <*2.45*> n_D^{20}: 1.472 – 1.474

Optical rotation <*2.49*> α_D^{20}: +43 – +50° (50 mm).

Specific gravity <*1.13*> d_{20}^{20}: 0.842 – 0.848

Purity Heavy metals <*1.07*>—Proceed with 1.0 mL of Orange Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Orange Peel Syrup

トウヒシロップ

Method of preparation

Orange Peel Tincture	200 mL
Simple Syrup	a sufficient quantity
To make	1000 mL

Prepare as directed under Syrups, with the above ingredients. An appropriate quantity of Sucrose and Purified Water or Purified Water in Containers may be used in place of Simple Syrup.

Description Orange Peel Syrup is a brownish yellow to reddish brown liquid. It has a characteristic odor, a sweet taste and a bitter aftertaste.

Specific gravity d_{20}^{20}: about 1.25

Identification To 25 mL of Orange Peel Syrup add 50 mL of ethyl acetate, shake for 5 minutes, allow to stand until clear ethyl acetate layer separate, and take the ethyl acetate layer, and evaporate on a water bath to dryness. Dissolve the residue in 10 mL of ethanol (95), filter if necessary, and use this solution as the sample solution. Separately, dissolve 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: one of the spot among the several spots from the sample solution and a grayish green spot from the standard solution show the same color tone and the same *R*f value.

Containers and storage Containers—Tight containers.

Orange Peel Tincture

トウヒチンキ

Method of preparation

Bitter Orange Peel, in coarse powder	200 g
70 vol% Ethanol	a sufficient quantity
To make	1000 mL

Prepare as directed under Tinctures, with the above ingredients. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 70 vol% Ethanol.

Description Orange Peel Tincture is a yellowish brown liquid. It has a characteristic odor, and a bitter taste.

Specific gravity d_{20}^{20}: about 0.90

Identification To 5.0 mL of Orange Peel Tincture add 5 mL of ethanol (95), filter if necessary, and use the filtrate as the sample solution. Proceed as directed in the Identification under Bitter Orange Peel.

Alcohol number <*1.01*> Not less than 6.6 (Method 2).

Containers and storage Containers—Tight containers.

Orengedokuto Extract

黄連解毒湯エキス

Orengedokuto Extract contains not less than 20 mg and not more than 80 mg of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 30 mg and not more than 90 mg (for preparation prescribed 2 g of Gardenia Fruit) or not less than 45 mg and not more than 135 mg (for preparation prescribed 3 g of Gardenia Fruit) of geniposide, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Coptis Rhizome	1.5 g	1.5 g	2 g	2 g
Phellodendron Bark	1.5 g	3 g	2 g	1.5 g
Scutellaria Root	3 g	3 g	3 g	3 g
Gardenia Fruit	2 g	3 g	2 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Orengedokuto Extract occurs as a yellow-brown to red-brown powder or blackish brown viscous extract. It has a characteristic odor and a very bitter taste.

Identification **(1)** Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of coptisine chloride for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (15:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Coptis Rhizome).

(2) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 5 mL of water, then add 25 mL of ethyl acetate, and shake. Separate the ethyl acetate layer, evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of limonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot

obtained from the standard solution (Phellodendron Bark).

(3) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Scutellaria Root).

(4) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution (Gardenia Fruit).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Lead—Take 5.0 g of the dry extract (or an amount of the viscous extract, equivalent to 5.0 g of the dried substance) in a platinum, quartz or porcelain crucible, heat gently, and then incinerate by ignition at 450 to 550°C. After cooling, add a small amount of 2 mol/L nitric acid TS, filter if necessary, and wash the crucible and filter several times with small portions of 2 mol/L nitric acid TS. Combine the washings and the filtrate, add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, to 2.5 mL of Standard Lead Solution add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 5 ppm).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: A lead hollow-cathode lamp.
Wavelength: 283.3 nm.

(3) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 7.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 12.0%, calculated on the dried basis.

Assay **(1)** Berberine—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.2 g of dried substance), add exactly 50 mL of the mobile phase, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water <*2.48*> in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of berberine in each solution.

$$\text{Amount (mg) of berberine chloride } (C_{20}H_{18}ClNO_4) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: 1.0 mL per minute (the retention time of berberine is about 8 minutes).

System suitability—

System performance: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatine and berberine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Geniposide—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.2 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of geniposide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute (the retention time of geniposide is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Containers and storage Containers—Tight containers.

Oriental Bezoar

Bezoar Bovis

ゴオウ

Oriental Bezoar is a stone formed in the gall sac of *Bos taurus* Linné var. *domesticus* Gmelin (*Bovidae*).

Description Spherical or massive stone, 1 – 4 cm in diameter; externally yellow-brown to red-brown; light, fragile and easily broken. Fractured surface shows yellow-brown to red-brown annular rings, often containing white granular substances or thin layers in these annular rings.

Odor, slight; taste, slightly bitter, followed by slight sweetness.

Identification **(1)** Shake 0.1 g of pulverized Oriental Bezoar with 10 mL of petroleum ether for 30 minutes, filter, and wash the residue with 10 mL of petroleum ether. Shake 0.01 g of the residue with 3 mL of acetic anhydride for 1 to 2 minutes, add a mixture of 0.5 mL of acetic anhydride and 2 drops of sulfuric acid, and shake: a yellow-red to deep red color develops, and changes through dark red-purple to dark red-brown.

(2) Shake well 0.01 g of Oriental Bezoar with 1 mL of hydrochloric acid and 10 mL of chloroform, separate the chloroform layer when it acquires a yellow-brown color, and shake with 5 mL of barium hydroxide TS: a yellow-brown precipitate is produced.

Purity **(1)** Synthetic dye—To 2 mg of pulverized Oriental Bezoar add 1 mL dilute hydrochloric acid: no violet color develops.

(2) Starch—To 5 mg of pulverized Oriental Bezoar add 2 mL of water, and heat on a water bath for 5 minutes. Cool and add 2 to 3 drops of iodine TS: no blue-purple color develops.

(3) Sucrose—To 0.02 g of pulverized Oriental Bezoar add 10 mL of water, shake for 15 minutes, and filter. To 1 mL of the filtrate add 2 mL of anthrone TS, and shake: no deep blue-green to dark green color develops.

Total ash <*5.01*> Not more than 10.0%.

Content of the active principle Weigh accurately about 0.5 g of pulverized Oriental Bezoar in a flask, add 50 mL of petroleum ether, warm under a reflux condenser on a water bath for 2 hours, and filter. Place the residue along with the filter paper in the flask, add 2 mL of hydrochloric acid and 40 mL of chloroform, warm under a reflux condenser on a water bath for 1 hour, and filter into a tared flask. Wash the filter paper with a small quantity of chloroform, combine the washings with the filtrate, and distil off the chloroform. Dry the residue in a desiccator (silica gel) for 24 hours, and weigh: the mass of the residue is not less than 12.0%.

Containers and storage Containers—Well-closed containers.

Otsujito Extract

乙字湯エキス

Otsujito Extract contains not less than 1.2 mg and not more than 4.8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 17 mg and not more than 51 mg (for preparation prescribed 2 g of Glycyrrhiza) or not less than 25 mg and not more than 75 mg (for preparation prescribed 3 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 0.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 1.5 mg of rhein (for preparation prescribed 0.5 g of Rhubarb) or not less than 1 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 3 mg of rhein (for preparation prescribed 1 g of Rhubarb) per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Japanese Angelica Root	6 g	6 g	6 g
Bupleurum Root	5 g	5 g	5 g
Scutellaria Root	3 g	3 g	3 g
Glycyrrhiza	2 g	2 g	3 g
Cimicifuga Rhizome	1.5 g	1 g	1 g
Rhubarb	1 g	0.5 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above.

Description Otsujito Extract occurs as light brown to brown powder or blackish brown viscous extract, having a slightly order, and a hot and slight sweet taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, separate the diethyl ether layer, and use this layer as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Japanese Angelica Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot obtained from the standard solution (Scutellaria Root).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Use (*E*)-isoferulic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the light yellowish white fluorescent spot obtained from the standard solution (Cimicifuga Rhizome).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as

directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.5%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the

standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

(4) Sennoside A—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, pour it into a column about 10 mm in inside diameter (previously prepared by packing 0.36 g of strongly basic ion-exchange resin for column chromatography, and washing with 10 mL of methanol and 10 mL of diluted methanol (1 in 2)) to flow out, wash out the column with 10 mL of diluted methanol (1 in 2), then flow out with a mixture of water, methanol and formic acid (25:25:1) to obtain exactly 5 mL of the outflow liquid, and use this liquid as the sample solution. Separately, weigh accurately about 5 mg of Sennoside A RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/8$$

M_S: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (2460:540:1).

Flow rate: 1.0 mL per minute (the retention time of sennoside A is about 14 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sennoside A are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

(5) Rhein—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 80 mL of water, shake, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 20 mL of iron (III) chloride TS, heat in a water bath under a reflux condenser for 30 minutes, add 3 mL of hydrochloric acid, and heat in addition under a reflux condenser for 30 minutes. After cooling, extract three times with 25 mL each of diethyl ether, combine all the diethyl ether layers, evaporate the solvent under reduced pressure, dissolve the residue in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhein for assay, and dissolve in acetone to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of rhein in each solution.

$$\text{Amount (mg) of rhein} = M_S \times A_T/A_S \times 2/5$$

M_S: Amount (mg) of rhein for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL per minute (the retention time of rhein is about 17 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rhein are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rhein is not more than 1.5%.

Containers and storage Containers—Tight containers.

Oyster Shell

Ostreae Testa

ボレイ

Oyster Shell is the shell of *Ostrea gigas* Thunberg (*Ostreidae*).

Description Irregularly curved, foliaceous or lamellated broken pieces. The unbroken oyster shell forms a bivalve 6 – 10 cm in length and 2 – 5 cm in width. The upper valve is flat and the lower one is somewhat hollow. Both the upper and lower edges of the valve are irregularly curved and bite with each other. The surface of the valve is externally light greenish gray-brown and internally milky in color.

Almost odorless and tasteless.

Identification **(1)** Dissolve 1 g of sample pieces of Oyster Shell in 10 mL of dilute hydrochloric acid by heating: it evolves a gas, and forms a very slightly red, turbid solution in which a transparent, thin suspended matter remains. Pass the evolved gas through calcium hydroxide TS: a white precipitate is produced.

(2) The solution obtained in (1) has a slight, characteristic odor. Filter this solution and neutralize with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for calcium salt.

(3) Ignite 1 g of pulverized Oyster Shell: it turns blackish brown in color at first, and evolves a characteristic odor. Ignite it further: it becomes almost white.

Purity Barium—Dissolve 1 g of pulverized Oyster Shell in 10 mL of dilute hydrochloric acid: the solution does not respond to the Qualitative Tests (1) <*1.09*> for barium salt.

Containers and storage Containers—Well-closed containers.

Powdered Oyster Shell

Ostreae Testa Pulverata

ボレイ末

Powdered Oyster Shell is the powder of Oyster Shell.

Description Powdered Oyster Shell occurs as a grayish white powder. It is almost odorless and tasteless.

Identification **(1)** Dissolve 1 g of Powdered Oyster Shell in 10 mL of dilute hydrochloric acid by heating: it evolves a gas, and forms a very slightly red, turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(2) The solution obtained in (1) has a slight, characteristic odor. Filter this solution, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for calcium salt.

(3) Ignite 1 g of Powdered Oyster Shell: it turns blackish brown in color at first evolving a characteristic odor. Ignite it further: it becomes almost white.

Purity **(1)** Water-soluble substances—Shake 3.0 g of Powdered Oyster Shell with 50 mL of freshly boiled and cooled water for 5 minutes, filter, and evaporate 25 mL of the filtrate to dryness. Dry the residue at 105°C for 1 hour, cool, and weigh: the mass of the residue does not exceed 15 mg.

(2) Acid-insoluble substances—To 5.0 g of Powdered Oyster Shell add 100 mL of water, and add hydrochloric acid in small portions with stirring until the solution becomes acid. Boil the acidic mixture with additional 1 mL of hydrochloric acid. After cooling, collect the insoluble substance by filtration, and wash it with hot water until the last washing no longer gives any reaction in Qualitative Tests <*1.09*> (2) for chloride. Ignite the residue and weigh: the mass of the residue does not exceed 25 mg.

(3) Barium—Dissolve 1 g of Powdered Oyster Shell in 10 mL of dilute hydrochloric acid: the solution does not responds to the Qualitative Tests <*1.09*> (1) for barium salt.

Loss on drying <*2.41*> Not more than 4.0% (1 g, 180°C, 4 hours).

Containers and storage Containers—Tight containers.

Panax Japonicus Rhizome

Panacis Japonici Rhizoma

チクセツニンジン

Panax Japonicus Rhizome is the rhizome of *Panax japonicus* C. A. Meyer (*Araliaceae*), usually after being treated with hot water.

Description Irregularly cylidrical rhizome with distinct nodes, 3 – 20 cm in length, 1 – 1.5 cm in diameter, internode 1 – 2 cm; externally light yellow-brown, with fine longitudinal wrinkles; stem scars, hollowed at the center, protruding on the upper surface, and root scars protruding as knobs on internodes; easily broken; fractured surface approximately flat, and light yellow-brown in color; horny in texture.

Odor, slight; taste, slightly bitter.

Identification Shake 0.5 g of pulverized Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of the spot among the several spots obtained from the sample solution shows the same color tone and *R*f value with the purple-red spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Panax Japonicus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Panax Japonicus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Powdered Panax Japonicus Rhizome

Panacis Japonici Rhizoma Pulveratum

チクセツニンジン末

Powdered Panax Japonicus Rhizome is the powder of Panax Japonicus Rhizome.

Description Powdered Panax Japonicus Rhizome occurs as a light grayish yellow-brown powder, and has a slight odor and a slightly bitter taste.

Under a microscope <*5.01*>, Powdered Panax Japonicus Rhizome reveals mainly starch grains or gelatinized starch masses, and fragments of parenchyma cells containing them; also fragments of cork tissue, somewhat thick-walled collenchyma, phloem tissue, and reticulate vessels; rarely fragments of scalariform vessels with a simple perforation, fibers and fiber bundles, rosette aggregates of calcium oxalate, and parenchyma cells containing them; yellow to orange-yellow resin; starch grains consisting of simple grains or 2- to 4-compound grains, simple grains, 3 – 18 μm in diameter; rosette aggregates of calcium oxalate are 20 – 60 μm in diameter.

Identification Shake 0.5 g of Powdered Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of the spot among the several spots obtained from the sample solution shows the same color tone and *R*f value with the purple-red spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Panax Japonicus Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Panax Japonicus Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 5.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Peach Kernel

Persicae Semen

トウニン

Peach Kernel is the seed of *Prunus persica* Batsch or *Prunus persica* Batsch var. *davidiana* Maximowicz (*Rosaceae*).

It contains not less than 1.2% of amygdalin, calculated on the basis of dried material.

Description Flattened, asymmetric ovoid seed, 1.2 – 2.0 cm in length, 0.6 – 1.2 cm in width, and 0.3 – 0.7 cm in thickness; somewhat sharp at one end, and round at the other end with chalaza; seed coat red-brown to light brown; externally, its surface being powdery by easily detachable stone cells of epidermis; numerous vascular bundles running and rarely branching from chalaza through the seed coat, and, appearing as dented longitudinal wrinkles; when soaked in boiling water and softened, the seed coat and thin, translucent, white albumen easily separated from the cotyledone; cotyledone white in color.

Almost odorless; taste, slightly bitter and oily.

Under a microscope <*5.01*>, the outer surface of seed coat reveals polygonal, long polygonal, or obtuse triangular stone cells on the protrusion from vascular bundles, shape of which considerably different according to the position, and their cell walls almost equally thickened; in lateral view, appearing as a square, rectangle or obtuse triangle.

Identification To 1.0 g of ground Peach Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution.

Purity **(1)** Rancidity—Grind Peach Kernel with boiling water: no odor of rancid oil is perceptible.

(2) Foreign matter <*5.01*>—When perform the test with not less than 250 g of Peach Kernel, it contains not more than 0.10% of broken pieces of endocarp.

Loss on drying <*5.01*> Not more than 8.0% (6 hours).

Assay Weigh accurately 0.5 g of ground Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under

Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Peach Kernel

Persicae Semen Pulveratum

トウニン末

Powdered Peach Kernel is the powder of the Peach Kernel.

It contains not less than 1.2% of amygdalin, calculated on the basis of dried material.

Description Powdered Peach Kernel occurs as a reddish-light brown to light brown powder. It is almost odorless and is oily and has slightly a bitter taste.

Under a microscope <5.01>, Powdered Peach Kernel fragments of outer seed coat epidermis; elliptical to ovoid, containing yellow-brown compound 50 to 80 μm in diameter and stone cell; cap-like shape to ovoid, yellow-brown in color. The stone cell is element of epidermis, 50 to 80 μm in diameter and 70 to 80 μm in height, cell wall of the top, 12 to 25 μm thickness, the base 4 μm in thickness, with obvious and numerous pits. Inner seed coat, yellow-brown, irregular and somewhat long polygon, 15 to 30 μm in diameter; and fragments of cotyledon and albumen containing aleurone grains and fatted oil, Aleurone grains are almost spherical grains, 5 to 10 μm in diameter.

Identification To 1.0 g of Powdered Peach Kernel add 10 mL of methanol, and immediately heat under a reflux condenser on a water bath for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly thymol-sulfuric acid-methanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution.

Loss on drying <5.01> Not more than 8.5% (6 hours).

Total ash <5.01> Not more than 3.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Assay Weigh accurately 0.5 g of Powdered Peach Kernel, add 40 mL of diluted methanol (9 in 10), heat immediately under a reflux condenser on a water bath for 30 minutes, and cool. Filter the mixture, add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test exactly with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of amygdalin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Peanut Oil

Oleum Arachidis

ラッカセイ油

Peanut Oil is the fixed oil obtained from the seeds of *Arachis hypogaea* Linné (*Leguminosae*).

Description Peanut Oil is a pale yellow, clear oil. It is odorless or has a slight odor. It has a mild taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95).

Specific gravity d_{25}^{25}: 0.909 – 0.916

Congealing point of the fatty acids: 22 – 33°C

Identification Saponify 5 g of Peanut Oil by boiling with 2.5 mL of sodium hydroxide solution (3 in 10) and 12.5 mL of ethanol (95). Evaporate the ethanol, dissolve the residue in 50 mL of hot water, and add dilute hydrochloric acid in excess until the free fatty acids separate as an oily layer. Cool the mixture, remove the separated fatty acids, and dissolve them in 75 mL of diethyl ether. To the diethyl ether solution add a solution of 4 g of lead (II) acetate trihydrate in 40 mL of ethanol (95), and allow the mixture to stand for 18 hours. Filter the supernatant liquid, transfer the precipitate to the filter with the aid of diethyl ether, and filter by suction. Place the precipitate in a beaker, heat it with 40 mL of dilute hydrochloric acid and 20 mL of water until the oily layer is entirely clear, cool, and decant the water layer. Boil the fatty acids with 50 mL of diluted hydrochloric acid (1 in 100). When the solution prepared by dissolving 0.1 g of the fatty acids in 10 mL of ethanol (95) is not darkened by the addition of 2 drops of sodium sulfide TS, allow the fatty acids to solidify, and press them between dry filter papers to exclude moisture. Dissolve the solid fatty acid in 25 mL of diluted ethanol (9 in 10) with the aid of gentle heat, and then cool to 15°C to crystallize the fatty acids. Recrystallize them from diluted ethanol (9 in 10) and dry in a desiccator (phosphorus (V) oxide, in vacuum) for 4 hours: the melting point <*1.13*> of the dried crystals is between 73°C and 76°C.

Acid value <*1.13*> Not more than 0.2.

Saponification value <*1.13*> 188 – 196

Unsaponifiable matters <*1.13*> Not more than 1.5%.

Iodine value <*1.13*> 84 – 103

Containers and storage Containers—Tight containers.

Peony Root

Paeoniae Radix

シャクヤク

Peony Root is the root of *Paeonia lactiflora* Pallas (*Paeoniaceae*).

It contains not less than 2.0% of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), calculated on the basis of dried material.

Description Cylindrical root, 7 – 20 cm in length, 1 – 2.5 cm in diameter; externally brown to light grayish brown, with distinct longitudinal wrinkles, with warty scars of lateral roots, and with laterally elongated lenticels; fractured surface dense in texture, light grayish brown, and with light brown radial lines in xylem.

Odor, characteristic; taste, slightly sweet at first, followed by an astringency and a slight bitterness.

Identification **(1)** Shake 0.5 g of pulverized Peony Root with 30 mL of ethanol (95) for 15 minutes, and filter. Shake 3 mL of the filtrate with 1 drop of iron (III) chloride TS: a blue-purple to blue-green color is produced, and it changes to dark blue-purple to dark green.

(2) To 2 g of pulverized Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Peony Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Peony Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Assay Weigh accurately about 0.5 g of pulverized Peony Root, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust so that the retention time of paeoniflorin is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Peony Root

Paeoniae Radix Pulverata

シャクヤク末

Powdered Peony Root is the powder of Peony Root.

It contains not less than 2.0% of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), calculated on the basis of dried material.

Description Powdered Peony Root occurs as a light grayish brown powder, and has a characteristic odor and a slightly sweet taste at first, followed by an astringency and a slight bitterness.

Under a microscope <*5.01*>, Powdered Peony Root reveals starch grains and fragments of parenchyma cells containing them; fragments of cork cells, vessels, tracheids and xylem fibers; rosette aggregates of calcium oxalate, and fragments of rows of crystal cells containing them. Starch grains consist of simple grains, 5 – 25 μm in diameter, occasionaly 2- to 3-compound grains.

Identification **(1)** Shake 0.5 g of Powdered Peony Root with 30 mL of ethanol (95) for 15 minutes, and filter. To 3 mL of the filtrate add 1 drop of iron (III) chloride TS, and mix: a blue-purple to blue-green color is produced, and thereafter it changes to dark blue-purple to dark green.

(2) To 2 g of Powdered Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Peony Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Peony Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Peony Root does not show groups of light yellow stone cells and fibers.

Loss on drying <*5.01*> Not less than 14.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Assay Weigh accurately about 0.5 g of Powdered Peony Root, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust so that the retention time of paeoniflorin is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Perilla Herb

Perillae Herba

ソヨウ

Perilla Herb is the leaves and the tips of branches of *Perilla frutescens* Britton var. *crispa* W. Deane (*Labiatae*).

It contains not less than 0.08% of perillaldehyde, calculated on the basis of dried material.

Description Usually, contracted and wrinkled leaves, often with thin stems. Both surfaces of the leaf are brownish purple, or the upper surface is grayish green to brownish green, and the lower surface is brownish purple in color. When smoothed by immersion in water, the lamina is ovate to obcordate, 5 – 12 cm in length, 5 – 8 cm in width; the apex, acuminate; the margin, serrate; the base, broadly cuneate; petiole, 3 – 5 cm in length; cross sections of stem and petiole, square. Under a magnifying glass, hairs are observed on both surfaces of the leaf, but abundantly on the vein and sparsely on other parts; small glandular hairs are observed on the lower surface.

Odor, characteristic; taste slightly bitter.

Identification To 0.6 g of pulverized Perilla Herb, add 10 mL of diethyl ether, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS for spray on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple spot obtained from the standard solution.

Purity **(1)** Stem—When perform the test of foreign matter <*5.01*>, Perilla Herb does not contain its stems equal to or greater than 3 mm in diameter.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than the stems contained in Perilla Herb does not exceed 1.0%.

(3) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 16.0%.

Acid-insoluble ash <*5.01*> Not more than 2.5%.

Assay Weigh accurately about 0.2 g of freshly prepared pulverized Perilla Herb, put in a glass-stoppered centrifuge tube, add 20 mL of methanol, shake for 10 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 20 mL of methanol, and proceed in the same manner. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of perillaldehyde for assay, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of perillaldehyde in each solution.

Amount (mg) of perillaldehyde = $M_S \times A_T/A_S \times 1/20$

M_S: Amount (mg) of perillaldehyde for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 1 mg of (*E*)-asarone in the standard solution to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, perillaldehyde and (*E*)-asarone are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of perillaldehyde is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Peucedanum Root

Peucedani Radix

ゼンコ

Peucedanum Root is the root of **1)** *Peucedanum praeruptorum* Dunn (Peucedanum Praeruptorum Root) or **2)** *Angelica decursiva* Franchet et Savatier (*Peucedanum decursivum* Maximowicz) (*Umbelliferae*) (Angelica Decursiva Root).

Description **1)** Peucedanum Praeruptorum Root—Slender obconical to cylindrical root, occasionally dichotomized at the lower part, 3 – 15 cm in length, 0.8 – 1.8 cm in diameter at the crown; externally light brown to dark brown; ring-node-like wrinkles numerous at the crown, sometimes with hair-like remains of petioles; the root having somewhat deep longitudinal wrinkles and scars of cutting off of lateral roots; transverse section surface light brown to whitish in color; brittle in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer composed of a cork layer, inner tangential walls of some cork cells thickened; collenchyma just inside of the cork layer; in cortex numerous oil canals scattered and intercellular air spaces observed; occasionally phloem fibers observed at the terminal portion of phloem; vessels and scattered oil canals in xylem; starch grains in parenchyma, 2 to 10 several-compound grains.

2) Angelica Decursiva Root—Similar to 1), but without hair-like remains of petioles at the crown.

Odor, characteristic; taste, slightly bitter.

Under a microscope ⟨*5.01*⟩, a transverse section reveals, similar to 1), but cell wall of cork cells not thickened, phloem fibers not observed at the terminal portion of phloem, nor oil canals observed in xylem.

Identification **(1)** Peucedanum Praeruptorum Root—To 1 g of pulverized Peucedanum Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (±)-praeruptorin A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the fluorescent spot obtained from the standard solution.

(2) Angelica Decursiva Root—To 1 g of pulverized Peucedanum Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of nodakenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the fluorescent spot obtained from the standard solution.

Purity Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of pulverized Peucedanum Root according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying ⟨*5.01*⟩ Not more than 13.0%.

Total ash ⟨*5.01*⟩ Not more than 7.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 2.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 20.0%.

Containers and storage Containers—Well-closed containers.

Pharbitis Seed

Pharbitidis Semen

ケンゴシ

Pharbitis Seed is the seed of *Pharbitis nil* Choisy (*Convolvulaceae*).

Description Longitudinally quartered or sexpartite globe, 4 – 6 mm in length, 3 – 5 mm in width; externally black to grayish red-brown or grayish white, smooth, but slightly shrunken and coarsely wrinkled. The transverse section almost fan-shaped, light yellow-brown to light grayish brown, and dense in texture. Under a magnifying glass, the surface of the seed coat reveals dense, short hairs; dented hilum at the bottom of the ridge. Seed coat thin, the outer layer dark gray, and the inner layer light gray; two irregularly folded cotyledons in the transverse section at one end; two thin membranes from the center of the dorsal side to the ridge separating cotyledons but unrecognizable in the transverse section of the other end having hilum; dark gray secretory pits in the section of the cotyledon. 100 seeds weighing about 3.5 g.

When cracked, odor, slight; taste, oily and slightly pungent.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Containers and storage Containers—Well-closed containers.

Phellodendron Bark

Phellodendri Cortex

オウバク

Phellodendron Bark is the bark of *Phellodendron amurense* Ruprecht or *Phellodendron chinense* Schneider (*Rutaceae*), from which the periderm has been removed.

It contains not less than 1.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

Description Flat or rolled semi-tubular pieces of bark, 2 – 4 mm in thickness; externally grayish yellow-brown to grayish brown, with numerous traces of lenticels; the internal surface yellow to dark yellow-brown in color, with fine vertical lines, and smooth; fractured surface fibrous and bright yellow.

Odor, slight; taste, extremely bitter; mucilaginous; it colors the saliva yellow on chewing.

Under a microscope ⟨*5.01*⟩, a transverse section reveals primary ray expands outward and looks fan shaped in secondary cortex, and sometimes ray differentiated later converges outward; groups of stone cells yellow and scattered in primary ray; groups of phloem fibers light yellow to yellow, lined alternately with the other tissue of phloem between rays, and then these tissues show obviously latticework; solitary crystals of calcium oxalate, single and compound starch grains observed in parenchyma.

Identification **(1)** To 1 g of pulverized Phellodendron Bark add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter to remove the diethyl ether. Collect the powder on the filter paper, add 10 mL of ethanol (95), allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid, add 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS or berberine chloride hydrate for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a

distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value.

(3) Stir up pulverized Phellodendron Bark with water: the solution becomes gelatinous owing to mucilage.

Loss on drying ⟨*5.01*⟩ Not more than 11.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 7.5%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Assay Weigh accurately about 0.5 g of pulverized Phellodendron Bark, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of berberine in each solution.

Amount (mg) of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$)]
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 mm in particle diameter).

Column temperature: A constant temperature of about 40°C

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Perform the test with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly separating each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions the relative deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Phellodendron Bark

Phellodendri Cortex Pulveratus

オウバク末

Powdered Phellodendron Bark is the powder of Phellodendron Bark.

It contains not less than 1.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

Description Powdered Phellodendron Bark occurs as a bright yellow to yellow powder. It has a slight odor and an extremely bitter taste, is mucilaginous, and colors the saliva yellow on chewing.

Under a microscope ⟨*5.01*⟩, Powdered Phellodendron Bark reveals fragments of yellow, thick-walled fiber bundles or fibers, and fibers often accompanied by crystal cell rows; fewer groups of stone cells together with idioblasts; fragments of parenchyma cells containing starch grains and oil droplets; fragments of medullary ray and phloem; mucilage cells and mucilage masses. Numerous solitary crystals of calcium oxalate, 7 – 20 μm in diameter; starch grains, simple grains and 2- to 4-compound grains, simple grain, 2 – 6 μm in diameter; oil droplets, stained red with sudan III TS.

Identification **(1)** To 1 g of Powdered Phellodendron Bark add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter to remove the diethyl ether. Collect the powder on the filter paper, add 10 mL of ethanol (95), allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid, add 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS or berberine chloride hydrate for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value.

(3) Stir up Powdered Phellodendron Bark with water: the solution becomes gelatinous owing to mucilage.

Purity Curcuma—Place Powdered Phellodendron Bark on filter paper, drop diethyl ether on it, and allow to stand. Take the powder off the filter paper, and drip 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope ⟨*5.01*⟩, Powdered Phellodendron Bark does not contain gelatinized starch or secretory cells containing yellow-red resin.

Loss on drying ⟨*5.01*⟩ Not more than 11.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 7.5%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Assay Weigh accurately about 0.5 g of Powdered Phellodendron Bark, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux con-

denser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To obtained residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of berberine in each solution.

Amount (mg) of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$)]
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When repeat the test 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Compound Phellodendron Powder for Cataplasm

パップ用複方オウバク散

Method of preparation

Powdered Phellodendron Bark	660 g
Powdered Gardenia Fruit	325 g
d- or *dl*-Camphor	10 g
dl- or *l*-Menthol	5 g
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Phellodendron Powder for Cataplasm occurs as a yellow-brown powder, having a characteristic odor.

Identification Shake thoroughly 0.2 g of Compound Phellodendron Powder for Cataplasm with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS or berberine chloride hydrate for thin-layer chromatography in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow to yellow-green fluorescent spot from the standard solution (phellodendron bark).

Containers and storage Containers—Tight containers.

Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder

オウバク·タンナルビン·ビスマス散

Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder contains not less than 12.9% and not more than 16.3% of bismuth (Bi: 208.98).

Method of preparation

Powdered Phellodendron Bark	300 g
Albumin Tannate	300 g
Bismuth Subnitrate	200 g
Scopolia Extract	10 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients. Scopolia Extract Powder may be used in place of Scopolia Extract.

Description Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder is brownish yellow in color, and has a bitter taste.

Identification **(1)** Shake thoroughly 0.1 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride RS or berberine chloride hydrate for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value (phellodendron bark).

(2) To 0.3 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 20 mL of ethanol (95), heat in a water bath for 3 minutes with shaking, cool, and filter.

To 10 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced. Allow to stand: a bluish black precipitate is produced (albumin tannate).

(3) To 0.3 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 10 mL of diluted pyridine (1 in 5), warm in a water bath for 3 minutes with shaking, cool, and filter. Add 1 mL of ninhydrin-ascorbic acid TS to the filtrate, and heat in a water bath: a blue color is produced (albumin tannate).

(4) To 0.5 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 5 mL of dilute hydrochloric acid and 10 mL of water, warm, shake thoroughly, and filter. The filtrate responds to the Qualitative Tests <*1.09*> for bismuth salt.

Assay Weigh accurately about 0.7 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder, shake well with 10 mL of water and 20 mL of diluted nitric acid (1 in 3), add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.23 g of bismuth nitrate pentahydrate, add 20 mL of diluted nitric acid (1 in 3) and water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions. On the other hand, determine the absorbance A_0 of the solution prepared in the same manner using 20 mL of diluted nitric acid (1 in 3) instead of the standard solution.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: A bismuth hollow-cathode lamp.
Wavelength: 223.1 nm.

$$\text{Amount (mg) of bismuth (Bi)} = M \times (A_T - A_0)/(A_S - A_0) \times 0.431$$

M: Amount (mg) of bismuth nitrate pentahydrate taken

Containers and storage Containers—Well-closed containers.

Picrasma Wood

Picrasmae Lignum

ニガキ

Picrasma Wood is the wood of *Picrasma quassioides* Bennet (*Simaroubaceae*).

Description Light yellow chips, slices or short pieces of wood; a transverse section reveals distinct annual rings and thin medullary rays; tissue dense in texture.

Odorless; taste, extremely bitter and lasting.

Under a microscope <*5.01*>, it reveals medullary rays consisting of 1 – 5 cells wide for transverse section, and 5 – 50 cells high for longitudinal section; vessels of spring wood up to about 150 μm in diameter, but those of autumn wood only one-fifth as wide; vessels, single or in groups, scattered in the xylem parenchyma; wall of wood fibers extremely thickened; medullary rays and xylem parenchyma cells contain rosette aggregates of calcium oxalate and starch grains. Vivid yellow or red-brown, resinous substance often present in the vessels.

Purity Foreign matter <*5.01*>—The amount of foreign matter contained in Picrasma Wood does not exceed 1.0%.

Total ash <*5.01*> Not more than 4.0%.

Containers and storage Containers—Well-closed containers.

Powdered Picrasma Wood

Picrasmae Lignum Pulveratum

ニガキ末

Powdered Picrasma Wood is the powder of Picrasma Wood.

Description Powdered Picrasma occurs as a grayish white to light yellow powder. It is odorless, and has an extremely bitter and lasting taste.

Under a microscope <*5.01*>, Powdered Picrasma Wood reveals fragments of vessels of various sizes, xylem fibers and xylem parenchyma cells; fragments of medullary rays containing starch grains; all tissues lignified; a few crystals of calcium oxalate observed. Starch grains are 5 to 15 μm in diameter.

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Pinellia Tuber

Pinelliae Tuber

ハンゲ

Pinellia Tuber is the tuber of *Pinellia ternata* Breitenbach (*Araceae*), from which the cork layer has been removed.

Description Slightly flattened spherical to irregular-shaped tuber; 0.7 – 2.5 cm in diameter and 0.7 – 1.5 cm in height; externally white to grayish white-yellow; the upper end dented, where the stem has been removed, with root scars dented as numerous small spots on the circumference; dense in texture; cross section white and powdery.

Almost odorless; tasteless at first, slightly mucous, but leaving a strong acrid taste.

Under a microscope <*5.01*>, a transverse section reveals mainly tissue of parenchyma filled with starch grains, and scattered with a few mucilage cells containing raphides of calcium oxalate. Starch grains mostly 2- to 3-compound grains, usually 10 – 15 μm in diameter, and simple grains, usually 3 – 7 μm in diameter; raphides of calcium oxalate 25 – 150 μm in length.

Purity **(1)** Rhizome of Arisaema species and others—Under a microscope <*5.01*>, no mucilage canal is revealed on the outer layer of cortex.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulver-

ized Pinellia Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Pinellia Tuber according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 3.5%.

Containers and storage Containers—Well-closed containers.

Plantago Herb

Plantaginis Herba

シャゼンソウ

Plantago Herb is the entire plant of *Plantago asiatica* Linné (*Plantaginaceae*), collected during the flowering season.

Description Usually wrinkled and contracted leaf and spike, grayish green to dark yellow-green in color; when soaked in water and smoothed out, the lamina is ovate to orbicular-ovate, 4 – 15 cm in length, 3 – 8 cm in width; apex acute, and base sharply narrowed; margin slightly wavy, with distinct parallel veins; glabrous or nearly glabrous; petiole is rather longer than the lamina, and its base is slightly expanded with thin-walled leaf-sheath; scape is 10 – 50 cm in length, one-third to one-half of the upper part forming the spike, with dense florets; the lower part of inflorescence often shows pyxidia; roots usually removed, but, if any, fine roots are closely packed.

Odor, slight; tasteless.

Identification To 2.0 g of pulverized Plantago Herb add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: a dark blue spot appears at an *R*f value of about 0.55.

Total ash <*5.01*> Not more than 15.0%.

Acid-insoluble ash <*5.01*> Not more than 4.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 14.0%.

Containers and storage Containers—Well-closed containers.

Plantago Seed

Plantaginis Semen

シャゼンシ

Plantago Seed is the seed of *Plantago asiatica* Linné (*Plantaginaceae*).

Description Flattened ellipsoidal seed, 2 – 2.5 mm in length, 0.7 – 1 mm in width, 0.3 – 0.5 mm in thickness; externally brown to yellow-brown and lustrous. Under a magnifying glass, the surface of the seed is practically smooth, with the dorsal side protruding like a bow, and with the ventral side somewhat dented; micropyle and raphe not observable. 100 seeds weigh about 0.05 g.

Odorless; taste, slightly bitter and mucous.

Under a microscope <*5.01*>, a transverse section reveals a seed coat consisting of three layers of epidermis composed of cells containing mucilage, a vegetative layer, and a pigment layer of approximately equidiameter cells; in the interior, endosperm thicker than seed coat, enclosing two cotyledons.

Identification **(1)** To 1 g of Plantago Seed add 2 mL of warm water, and allow the mixture to stand for 10 minutes: the seed coat swells to discharge mucilage.

(2) To 1.0 g of pulverized Plantago Seed add 5 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.2 g of powdered plantago seed for thin-layer chromatography add 1 mL of methanol, and warm on a water bath for 3 minutes. After cooling, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: the spot appeared at an *R*f value of about 0.25 obtained from the sample solution has the same color tone with the dark blue spot appeared at an *R*f value of about 0.25 obtained from the standard solution.

Purity Foreign matter <*5.01*>—The amount of foreign matter contained in Plantago Seed does not exceed 2.0%.

Total ash <*5.01*> Not more than 5.5%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Platycodon Root

Platycodi Radix

キキョウ

Platycodon Root is the root of *Platycodon grandiflorum* A. De Candolle (*Campanulaceae*).

Description Irregular, somewhat thin and long fusiform to conical root, often branched; externally grayish brown, light brown or white; main root 10 – 15 cm in length, 1 – 3 cm in

diameter; the upper end, with dented scars of removed stems; the neighborhood, with fine lateral wrinkles and longitudinal furrows and also slightly constricted; the greater part of the root, except the crown, covered with coarse longitudinal wrinkles, lateral furrows and lenticel-like lateral lines; hard in texture, but brittle; fractured surface not fibrous, often with cracks. Under a magnifying glass, a transverse section reveals cambium and its neighborhood often brown in color; cortex slightly thinner than xylem, almost white and with scattered cracks; xylem white to light brown in color, and the tissue slightly denser than cortex.

Odor, slight; tasteless at first, later acrid and bitter.

Identification **(1)** Boil 0.5 g of pulverized Platycodon Root with 10 mL of water for a while, allow to cool, and shake the mixture vigorously: a lasting fine foam is produced.

(2) Warm 0.2 g of pulverized Platycodon Root with 2 mL of acetic anhydride on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red to red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to green color.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Platycodon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Platycodon Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 4.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Powdered Platycodon Root

Platycodi Radix Pulverata

キキョウ末

Powdered Platycodon Root is the powder of Platycodon Root.

Description Powdered Platycodon Root occurs as a light grayish yellow to light grayish brown powder. It has a slight odor, and is tasteless at first, later acrid and bitter.

Under a microscope <*5.01*>, Powdered Platycodon Root reveals numerous fragments of colorless parenchyma cells; fragments of reticulate vessels and scalariform vessels; fragments of sieve tubes and lactiferous tubes; fragments of cork layer are sometimes observed. Usually, starch grains are not observed, but very rarely simple grain.

Identification **(1)** Boil 0.5 g of Powdered Platycodon Root with 10 mL of water for a while, allow to cool, and shake the mixture vigorously: a lasting fine foam is produced.

(2) Warm 0.2 g of Powdered Platycodon Root with 2 mL of acetic anhydride on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red to red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to green color.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Platycodon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Platycodon Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Platycodon Root does not show fibers, stone cells or other foreign matter.

Total ash <*5.01*> Not more than 4.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Containers and storage Containers—Well-closed containers.

Platycodon Fluidextract

キキョウ流エキス

Method of preparation Take coarse powder of Platycodon Root, and prepare the fluidextract as directed under Fluidextracts using 25 vol% ethanol. An appropriate quantity of Ethanol and Purified Water or Purified Water in Containers may be used in place of 25 vol% ethanol.

Description Platycodon Fluidextract is a red-brown liquid. It is miscible with water, producing slight turbidity. It has a mild taste at first, followed by an acrid and bitter taste.

Identification **(1)** Shake vigorously 0.5 mL of Platycodon Fluidextract with 10 mL of water: a lasting fine foam is produced.

(2) Dissolve 1 drop of Platycodon Fluidextract in 2 mL of acetic anhydride, and add gently 0.5 mL of sulfuric acid: a red to red-brown color develops at the zone of contact.

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Platycodon Fluidextract as directed in the Fluidextracts (4), and perform the test (not more than 30 ppm).

(2) Starch—Mix 1 mL of Platycodon Fluidextract with 4 mL of water, and add 1 drop of dilute iodine TS: no purple or blue color develops.

Content of the active principle Transfer exactly 5 mL of Platycodon Fluidextract to a tared beaker, evaporate to dryness on a water bath, and dry at 105°C for 5 hours: the mass of the residue is not less than 0.50 g.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Pogostemon Herb

Pogostemoni Herba

カッコウ

Pogostemon Herb is the terrestrial part of *Pogostemon cablin* Bentham (*Labiatae*).

Description Stems with opposite leaves, leaves wrinkled and shriveled. When smoothed by immersion in water, leaves are obovate to ovate-oblong, 2.5 – 10 cm in length, 2.5 – 7 cm in width, with obtusely serrate margins and petioles at the cuneate bases; the upper surface of leaves dark brown, the lower surface grayish brown, both sides covered densely with hairs. Stems are square, solid, grayish green, covered with grayish to yellowish white hairs; the pith broad, whitish, spongy. Under a magnifying glass, leaf reveales hairs, glandular hairs and glandular scales.

Odor, distinct; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of petiole reveals central portion of the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center divided into two groups. Under a microscope <*5.01*>, a transverse section of the midvein of lamina reveals the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center arranged in fan-shape. Under a microscope <*5.01*>, a transverse section of stem reveals several-cells-layered collenchyma beneath epidermis, occasionally with cork layer developed; beneath cortex, collateral vascular bundles arranged in a circle, phloem fibers in groups observed at the outer portion of phloem; oil droplets observed in parenchymat cells of cortex, needle, solitary or columnar crystals of calcium oxalate in parenchyma cells of pith.

Identification To 0.5 g of pulverized Pogostemon Herb, add 5 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes; a blue-purple spot appears at an *R*f value of about 0.4.

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 13.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Essential oil content <*5.01*> When the test is performed with 50.0 g of pulverized Pogostemon Herb in a flask with 1 mL of silicon resin added, the essential oil content is not less than 0.3 mL.

Containers and storage Containers—Well-closed containers.

Polygala Root

Polygalae Radix

オンジ

Polygala Root is the root or the root bark of *Polygala tenuifolia* Willdenow (*Polygalaceae*).

Description Thin, long and bent, cylindrical or tubular root; main root, 10 – 20 cm in length, 0.2 – 1 cm in diameter, sometimes with one to several lateral roots; externally light grayish brown, with coarse longitudinal wrinkles, and with deep lateral furrows cracked to some degree here and there; brittle, and fractured surface not fibrous; margin of the transverse section irregularly undulate; cortex, comparatively thick, with large cracks here and there; xylem usually round to elliptical, light brown in color, and often tears in a wedge-like shape.

Odor, slight; taste, slightly acrid.

Identification **(1)** Shake vigorously 0.5 g of pulverized Polygala Root with 10 mL of water: a lasting fine foam is produced.

(2) To 0.5 g of pulverized Polygala Root add 2 mL of acetic anhydride. After shaking well, allow to stand for 2 minutes, and filter. To the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a light blue-green to brown color.

Purity **(1)** Stem—When perform the test of foreign matter <*5.01*>, the amount of the stems contained in Polygala Root does not exceed 10.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Polygala Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Polygala Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than the stems is not more than 1.0%.

(5) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 6.0%.

Containers and storage Containers—Well-closed containers.

Powdered Polygala Root

Polygalae Radix Pulverata

オンジ末

Powdered Polygala Root is the powder of Polygala Root.

Description Powdered Polygala Root occurs as a light grayish yellow-brown powder. It has a slight odor and a slightly acrid taste.

Under a microscope <*5.01*>, Powdered Polygala Root reveals fragments of cork layers, pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers and xylem parenchyma cells with a small number of simple pits; frag-

ments of parenchyma cells containing substances such as oil droplets, rosette aggregates and solitary crystals of calcium oxalate. Oil drop-like contents stained red with sudan III TS.

Identification **(1)** Shake vigorously 0.5 g of Powdered Polygala Root with 10 mL of water: a lasting fine foam is produced.

(2) To 0.5 g of Powdered Polygala Root add 2 mL of acetic anhydride. After shaking well, allow to stand for 2 minutes, and filter. To the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a light blue-green to brown color.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Polygala Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Polygala Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Polygala Root does not show stone cells or starch grains.

(4) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Total ash <*5.01*> Not more than 6.0%.

Containers and storage Containers—Well-closed containers.

Polygonatum Rhizome

Polygonati Rhizoma

オウセイ

Polygonatum Rhizome is the rhizome of *Polygonatum falcatum* A. Gray, *Polygonatum sibiricum* Redouté, *Polygonatum kingianum* Collett et Hemsley or *Polygonatum cyrtonema* Hua (*Liliaceae*), usually after being steamed.

Description Irregularly cylindrical rhizome, 3 – 10 cm in length, 0.5 – 3 cm in diameter; or irregular massive rhizome, 5 – 10 cm in length, 2 – 6 cm in diameter, occasionally branched; both rhizomes with many cyclic nodes and longitudinally striate; externally yellow-brown to blackish brown; stem scars, round, concave at their center, and protuberant on the upper surface; root scars on the lower surface; cut surface flat and horny.

Odor, slight; taste, slightly sweet.

Under a microscope <*5.01*>, a transverse section of the rhizome reveals epidermis coated with cuticle; inside of epidermis parenchyma lie; numerous vascular bundles and mucilage cells scattered in parenchyma; vascular bundles collateral or amphivasal concentric; mucilage cells contain raphides of calcium oxalate.

Identification **(1)** To 0.5 g of fine cutted Polygonatum Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtate add gently 0.5 mL of sulfuric acid: a red-brown color appears at the zone of contact.

(2) To 1.0 g of fine cutted Polygonatum Rhizome add 10 mL of dilute hydrochloric acid, boil gently for 2 minutes, and filter. Neutralize the filtrate with sodium hydroxide TS. To 3 mL of this solution add 1 mL of Fehling's TS, and warm: red precipitates appear.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Polygonutum Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Polygonutum Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Polygonum Root

Polygoni Multiflori Radix

カシュウ

Polygonum Root is the root of *Polygonum multiflorum* Thunberg (*Polygonaceae*), often being cut into round slices.

Description Polygonum Root is nearly fusiform, 10 – 15 cm in length, 2 – 5 cm in diameter; externally red-brown to dark brown; roughly wrinkled; a cross section light red-brown or light grayish brown, with numerous abnormal vascular bundles scattering irregularly around the large vascular bundles near center; heavy and hard in texture.

Odor, slight and characteristic; taste, astringent and slightly bitter.

Under a microscope <*5.01*>, transverse section reveals the outermost layer to be several cells thick and composed of cork; cork cells contain brown substances; cortex composed of parenchyma; abnormal vascular bundles, exhibiting a ring of cambium; xylem lies inside of the cambium, and phloem outside; fibers lie outside the phloem; central portion of root lignified; parenchymatous cells contain aggregated crystals of calcium oxalate, and both simple and 2- to 8-compound starch grains; navel of starch grain obvious.

Identification To 1 g of pulverized Polygonum Root add 10 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 2 mL of methanol, and use this as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water, methanol and acetic acid (100) (200:10:10:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a fluorescent bluish white spot appears at an *R*f value of about 0.3.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Polygonum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Polygonum Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 14.0% (6 hours).

Total ash <*5.01*> Not more than 5.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 17.0%.

Containers and storage Containers—Well-closed containers.

Polyporus Sclerotium

Polyporus

チョレイ

Polyporus Sclerotium is the sclerotium of *Polyporus umbellatus* Fries (*Polyporaceae*).

Description Irregularly shaped mass, usually 5 – 15 cm in length; externally blackish brown to grayish brown, with numerous dents and coarse wrinkles; breakable; fractured surface rather soft and cork-like, and almost white to light brown in color, and a white speckled pattern on the inner region; light in texture.

Odorless and tasteless.

Identification Warm, while shaking, 0.5 g of pulverized Polyporus Sclerotium with 5 mL of acetone on a water bath for 2 minutes, filter, and evaporate the filtrate to dryness. Dissolve the residue in 5 drops of acetic anhydride, and add 1 drop of sulfuric acid: a red-purple color develops, and immediately changes to dark green.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Polyporus Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Polyporus Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 16.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 4.0%.

Containers and storage Containers—Well-closed containers.

Powdered Polyporus Sclerotium

Polyporus Pulveratus

チョレイ末

Powdered Polyporus Sclerotium is the powder of the Polyporus Sclerotium.

Description Powdered Polyporus Sclerotium occurs as a light grayish brown to light brown powder. It is almost odorless, has a slightly bitter taste, and is gritty between the teeth on chewing.

Under a microscope ⟨*5.01*⟩, Powdered Polyporus Sclerotium reveals hypha, 1 to 2 μm, rarely up to 13 μm in diameter, and colorless transparent; granule strongly refracting light; and a few mucilage plates; sometimes fragments of false tissue consisting of them; somewhat brown false tissues; and solitary crystal of calcium oxalate. Solitary crystal is 10 to 40 μm in diameter, sometimes 100 μm in diameter.

Identification Warm, while shaking, 0.5 g of Powdered Polyporus Sclerotium with 5 mL of acetone on a water bath for 2 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 5 drops of acetic anhydride, and add 1 drop of sulfuric acid: a red-purple color develops, and immediately changes to dark green.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of Powdered Polyporus Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Powdered Polyporus Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 16.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 4.0%.

Containers and storage Containers—Tight containers.

Poria Sclerotium

Poria

ブクリョウ

Poria Sclerotium is the sclerotium of *Wolfiporia cocos* Ryvarden et Gilbertson (*Poria cocos* Wolf) (*Polyporaceae*), from which usually the external layer has been mostly removed.

Description Mass, about 10 – 30 cm in diameter, up to 0.1 – 2 kg in mass; usually it appears as broken or chipped pieces; white or slightly reddish white; sclerotium with remaining outer layer is dark brown to dark red-brown in color, coarse, which fissures; hard in texture, but brittle.

Almost odorless, tasteless, and slightly mucous.

Identification **(1)** Warm 1 g of pulverized Poria Sclerotium with 5 mL of acetone on a water bath for 2 minutes with shaking, and filter. Evaporate the filtrate to dryness, dissolve the residue in 0.5 mL of acetic anhydride, and add 1 drop of sulfuric acid: a light red color develops, which changes immediately to dark green.

(2) To a section or powder of Poria Sclerotium add 1 drop of iodine TS: a deep red-brown color is produced.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Poria Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Poria Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Powdered Poria Sclerotium

Poria Pulveratum

ブクリョウ末

Powdered Poria Sclerotium is the powder of Poria Sclerotium.

Description Powdered Poria Sclerotium occurs as a white to grayish white powder. It is almost odorless and tasteless, but is slightly mucous.

Under a microscope <*5.01*>, Powdered Poria Sclerotium reveals colorless and transparent hyphae strongly refracting light, and fragments of false tissue consisting of granules and mucilage plates. Thin hyphae, 2 – 4 μm in diameter; thick ones, usually 10 – 20 μm, up to 30 μm.

Identification **(1)** Warm 1 g of Powdered Poria Sclerotium with 5 mL of acetone on a water bath for 2 minutes with shaking, and filter. Evaporate the filtrate to dryness, dissolve the residue in 0.5 mL of acetic anhydride, and add 1 drop of sulfuric acid: a light red color develops, which changes immediately to dark green.

(2) To Powdered Poria Sclerotium add 1 drop of iodine TS: a deep red-brown color is produced.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Poria Sclerotium according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Poria Sclerotium according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Poria Sclerotium does not show starch grains.

Total ash <*5.01*> Not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Prepared Glycyrrhiza

Glycyrrhizae Radix Praeparata

シャカンゾウ

Prepared Glycyrrhiza is prepared by roasting Glycyrrhiza.

It contains not less than 2.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Usually cut; external surface dark brown to dark red-brown and with longitudinal wrinkles; cut surface brown to light yellow-brown; in case periderm fallen off, external surface brown to light yellow-brown and fibrous; on transversely cut surface cortex and xylem almost distinctly defined, and exhibits radial structure; sometimes radial cleft observed.

Odor, fragrant; taste sweet, followed by slight bitterness.

Identification To 2.0 g of pulverized Prepared Glycyrrhiza add 10 mL of ethyl acetate, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Shake the residue with 5 mL of ethyl acetate and 5 mL of 0.1 mol/L hydrochloric acid TS for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 3 minutes, and allow to cool: a red-purple spot is observed at an *R*f value of about 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Prepared Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Prepared Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 8.0% (6 hours).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of pulverized Prepared Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 15 minutes.

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak with the relative retention time of about 0.9

to glycyrrhizic acid, and the peak of glycyrrhizic acid is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Processed Aconite Root

Aconiti Radix Processa

ブシ

Processed Aconite Root is the tuberous root of *Aconitum carmichaeli* Debeaux or *Aconitum japonicum* Thunberg (*Ranunculaceae*) prepared by the following processes.

Process 1: Autoclaving. [Processed Aconite Root 1]

Process 2: Heating or autoclaving after rinsing in salt or rock salt solution. [Processed Aconite Root 2]

Process 3: Treating with calcium hydroxide after rinsing in salt solution. [Processed Aconite Root 3]

Processed Aconite Root 1, Processed Aconite Root 2 and Processed Aconite Root 3 contain the total alkaloid [as benzoyl aconin ($C_{32}H_{45}NO_{10}$: 603.70)] of not less than 0.7% and not more than 1.5%, not less than 0.1% and not more than 0.6%, and not less than 0.5% and not more than 0.9%, calculated on the dried bases, respectively.

The label indicates the treating process.

Description **1)** Processed Aconite Root 1: Cut pieces irregularly polygonal, less than 10 mm in diameter; externally dark grayish brown to blackish brown; hard in texture; cut surface flat, light brown to dark brown, usually horny and lustrous.

Odor, weak and characteristic.

Under a microscope <*5.01*>, transverse and longitudinal sections reveal pitted, scaraliform, reticulate and spiral vessels; starch grains in parenchymatous cells usually gelatinized but sometimes not gelatinized; starch grains, simple, spherical or ellipsoid, 2 – 25 μm in diameter, or 2- to a dozen or so- compound, hilum of starch grain distinct.

2) Processed Aconite Root 2: Nearly obconical root, 15 – 30 mm in length, 12 – 16 mm in diameter, slices cut longitudinally or transversely, 20 – 60 mm in length, 15 – 40 mm in width, and 200 – 700 μm in thickness, or cut pieces irregularly polygonal, less than 12 mm in diameter; externally light brown to dark brown or yellow-brown; hard in texture, usually without wrinkles; cut surface flat, light brown to dark brown or yellowish white to light yellow-brown, usually horny, semi-transparent and lustrous.

Odor, weak and characteristic.

Under a microscope <*5.01*>, transverse and longitudinal sections reveal metaderm, primary cortex, endodermis, secondary cortex, cambium, and xylem; primary cortex contains oblong to oblong-square sclerenchymatous cells, 30 – 75 μm in short axis, 60 – 150 μm in long axis; endodermis single layered, endodermal cells elongated in tangential direction; cambium, star shaped or irregular polygons to orbicular; a group of vessel in xylem v-shaped; sometimes isolated ring of cambium appears in secondary cortex or in pith; vessels, pitted, scaraliform, reticulate and spiral; starch grains in parenchymatous cells gelatinized.

3) Processed Aconite Root 3: Cut pieces irregularly polygonal, less than 5 mm in diameter; externally grayish brown; hard in texture; cut surface flat, light grayish brown to grayish white, not lustrous.

Odor, weak and characteristic.

Under a microscope <*5.01*>, transverse and longitudinal sections reveal pitted, scaraliform, reticulate and spiral vessels; starch grains, simple, spherical or ellipsoid, 2 – 25 μm in diameter, or 2- to a dozen or so- compound, hilum of starch grain distinct.

Identification To 3 g of pulverized Processed Aconite Root in a glass-stoppered centrifuge tube add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the diethyl ether layer. Evaporate the layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28) (40:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Processed Aconite Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Processed Aconite Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and aconitum diester alkaloids standard solution for purity as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the heights of the peaks corresponding to aconitine, H_{TA} and H_{SA}, jesaconitine, H_{TJ} and H_{SJ}, hypaconitine, H_{TH} and H_{SH}, and mesaconitine, H_{TM} and H_{SM}, respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 60 μg, 60 μg, 280 μg and 140 μg, respectively, and the total amount of them is not more than 450 μg.

Amount (μg) of aconitine ($C_{34}H_{47}NO_{11}$)
$= C_{SA}/M \times H_{TA}/H_{SA} \times 10$

Amount (μg) of jesaconitine ($C_{35}H_{49}NO_{12}$)
$= C_{SJ}/M \times H_{TJ}/H_{SJ} \times 10$

Amount (μg) of hypaconitine ($C_{33}H_{45}NO_{10}$)
$= C_{SH}/M \times H_{TH}/H_{SH} \times 10$

Amount (μg) of mesaconitine ($C_{33}H_{45}NO_{11}$)
$= C_{SM}/M \times H_{TM}/H_{SM} \times 10$

C_{SA}: Concentration (μg/mL) of aconitine for purity in aconitum diester alkaloids standard solution for purity
C_{SJ}: Concentration (μg/mL) of jesaconitine for purity in aconitum diester alkaloids standard solution for purity
C_{SH}: Concentration (μg/mL) of hypaconitine for purity in aconitum diester alkaloids standard solution for purity
C_{SM}: Concentration (μg/mL) of mesaconitine for purity in aconitum diester alkaloids standard solution for purity
M: Amount (g) of Processed Aconite Root taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust so that the retention time of mesaconitine is about 31 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make 10 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying ⟨*5.01*⟩ Not more than 15.0% (6 hours).

Total ash ⟨*5.01*⟩

Processed Aconite Root 1: Not more than 4.0%.
Processed Aconite Root 2: Not more than 12.0%.
Processed Aconite Root 3: Not more than 19.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.9%.

Assay Weigh accurately about 2 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, and add 1.6 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 0.8 mL of ammonia TS and 20 mL of diethyl ether, and proceed as above. Repeat this process more three times. Combine all extracts, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL of ethanol (99.5), add 30 mL of freshly boiled and cooled water, and titrate ⟨*2.50*⟩ with 0.01 mol/L hydrochloric acid VS until the color of the solution changes from green to gray-blue through blue-green (indicator: 3 drops of methyl red-methylene blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L hydrochloric acid VS
= 6.037 mg of total alkaloid [as benzoylaconine ($C_{32}H_{45}NO_{10}$)]

Containers and storage Containers—Well-closed containers.

Powdered Processed Aconite Root

Aconiti Radix Processa et Pulverata

ブシ末

Powdered Processed Aconite Root is the powder of Processed Aconite Root prepared by the process 1 or process 2, the powder of Processed Aconite Root prepared by process 1, or the powder of Processed Aconite Root prepared by the process 1 to which Corn Starch or Lactose Hydrate is added.

Process 1: Autoclaving. [Powdered Processed Aconite Root 1]
Process 2: Heating or autoclaving after rinsing in salt or rock salt solution. [Powdered Processed Aconite Root 2]

Powdered Processed Aconite Root 1 and Powdered Processed Aconite Root 2 contain the total alkaloid [as benzoyl aconin ($C_{32}H_{45}NO_{10}$: 603.70)] of not less than 0.4% and not more than 1.2%, and not less than 0.1% and not more than 0.3%, calculated on the dried bases, respectively.

The label indicates the treating process.

Description **1)** Powdered Processed Aconite Root 1: Powdered Processed Aconite Root 1 occurs as a light grayish brown powder. It has a characteristic odor.

Under a microscope ⟨*5.01*⟩, Powered Processed Aconite Root 1 reveals gelatinized starch masses or starch grains and parenchymatous cells containing them, fragments of red-brown metaderm, fragments of pitted, scaraliform, reticulate and spiral vessels; also square to oblong-square sclerenchymatous cells, 30 – 150 μm in diameter, 100 – 250 μm in length, cell wall of sclerenchymatous cells, 6 – 12 μm in thickness; starch grains of *Aconitum carmichaeli Debeaux* or *Aconitum japonicum* Thunberg (*Ranunculaceae*) origin, simple, spherical or ellipsoid, 2 – 25 μm in diameter, or 2- to a dozen or so- compound, hilum of starch grain distinct.

2) Powdered Processed Aconite Root 2: Powdered Processed Aconite Root 2 occurs as a light yellowish white powder. It has a characteristic odor.

Under a microscope ⟨*5.01*⟩, Powered Processed Aconite Root 2 reveals gelatinized starch masses and parenchymatous cells containing them, fragments of red-brown metaderm, fragments of pitted, scaraliform, reticulate and spiral vessels; also square to oblong-square sclerenchymatous cells, 30 – 150 μm in diameter, 100 – 250 μm in length, cell wall of sclerenchymatous cells, 6 – 12 μm in thickness.

Identification To 3 g of Powdered Processed Aconite Root in a glass-stoppered centrifuge tube add 20 mL of diethyl

ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the diethyl ether layer. Evaporate the layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28) (40:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Processed Aconite Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Processed Aconite Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and aconitum diester alkaloids standard solution for purity as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the heights of the peaks corresponding to aconitine, H_{TA} and H_{SA}, jesaconitine, H_{TJ} and H_{SJ}, hypaconitine, H_{TH} and H_{SH}, and mesaconitine, H_{TM} and H_{SM}, respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 55 μg, 40 μg, 55 μg and 120 μg, respectively, and the total amount of them is not more than 230 μg.

Amount (μg) of aconitine ($C_{34}H_{47}NO_{11}$)
$= C_{SA}/M \times H_{TA}/H_{SA} \times 10$

Amount (μg) of jesaconitine ($C_{35}H_{49}NO_{12}$)
$= C_{SJ}/M \times H_{TJ}/H_{SJ} \times 10$

Amount (μg) of hypaconitine ($C_{33}H_{45}NO_{10}$)
$= C_{SH}/M \times H_{TH}/H_{SH} \times 10$

Amount (μg) of mesaconitine ($C_{33}H_{45}NO_{11}$)
$= C_{SM}/M \times H_{TM}/H_{SM} \times 10$

C_{SA}: Concentration (μg/mL) of aconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SJ}: Concentration (μg/mL) of jesaconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SH}: Concentration (μg/mL) of hypaconitine for purity in aconitum diester alkaloids standard solution for purity

C_{SM}: Concentration (μg/mL) of mesaconitine for purity in aconitum diester alkaloids standard solution for purity

M: Amount (g) of Powdered Processed Aconitine Root taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust so that the retention time of mesaconitine is about 31 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make 10 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying <*5.01*> Not more than 11.0% (6 hours).

Total ash <*5.01*>

Powdered Processed Aconite Root 1: Not more than 4.0%.

Powdered Processed Aconite Root 2: Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 0.7%.

Assay Weigh accurately about 2 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, and add 1.6 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 0.8 mL of ammonia TS and 20 mL of diethyl ether, and proceed as above. Repeat this process more three times. Combine all extracts, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL of ethanol (99.5), add 30 mL of freshly boiled and cooled water, and titrate <*2.50*> with 0.01 mol/L hydrochloric acid VS until the color of the solution changes from green to gray-blue through blue-green (indicator: 3 drops of methyl red-methylene blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L hydrochloric acid VS
= 6.037 mg of total alkaloid [as benzoylaconine ($C_{32}H_{45}NO_{10}$)]

Containers and storage Containers—Well-closed containers.

Processed Ginger

Zingiberis Rhizoma Processum

カンキョウ

Processed Ginger is the rhizome of *Zingiber officinale* Roscoe (*Zingiberaceae*), after being passed through hot water or being steamed.

It contains not less than 0.10% of [6]-shogaol ($C_{17}H_{24}O_3$: 276.37), calculated on the basis of dried material.

Description Irregularly compressed and often branched massive rhizome; branched parts slightly curved ovoid or oblong- ovoid, 2 – 4 cm in length, and 1 – 2 cm in diameter; external surface grayish yellow to grayish yellow-brown, with wrinkles and ring node; fractured surface brown to dark brown, transparent and horny; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles scattered throughout the surface.

Odor, characteristic; taste, extremely pungent.

Under a microscope <*5.01*>, a transverse section reveals cork layer, cortex and stele in this order from the outside; cortex and stele, divided by a single-layered endodermis, composed of parenchyma; vascular bundles surrounded by fibers scattered in cortex and stele; oil cells contain yellow oily substances, scattered in parenchyma; parenchyma cells contain solitary crystals of calcium oxalate, and gelatinized starch.

Identification To 2 g of pulverized Processed Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution (1). To the residue add 5 mL of methanol, proceed in the same manner as above, and use so obtained solution as the sample solution (2). Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of sucrose in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution (1) and standard solution (1) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution (1) has the same color tone and *R*f value with the spot from the standard solution (1). Spot 10 μL each of the sample solution (2) and standard solution (2) on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (8:5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution (2) has the same color tone and *R*f value with the spot from the standard solution (2).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Processed Ginger according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Processed Ginger according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 15.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.0%.

Assay Weigh accurately about 1 g of pulverized Processed Ginger, place in a centrifuge tube, add 30 mL of the mobile phase, shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of the mobile phase, and repeat the extraction twice more. To the combined all extracts add the mobile phase to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-shogaol for assay, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-shogaol in each solution.

$$\text{Amount (mg) of [6]-shogaol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-shogaol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and water (3:2).

Flow rate: Adjust so that the retenton time of [6]-shogaol is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-shogaol is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Prunella Spike

Prunellae Spica

カゴソウ

Prunella Spike is the spike of *Prunella vulgaris* Linné var. *lilacina* Nakai (*Labiatae*).

Description Spikes in nearly cylindrical and wheat ear-like shape, 3 – 6 cm in length, 1 – 1.5 cm in diameter, externally grayish brown; spikes composed of a floral axis having numerous bracts and calyxes; corollas often remaining on the upper part; a calyx usually enclosing four mericarps; bract, cordate to eccentric, and exhibiting white hairs on the vein, as on the calyx; light in texture.

Almost odorless and tasteless.

Purity **(1)** Stem—When perform the test of foreign matter ⟨*5.01*⟩, the amount of the stems contained in Prunella Spike does not exceed 5.0%.

(2) Foreign matter ⟨*5.01*⟩—The amount of foreign matter other than the stems contained in Prunella Spike does not exceed 1.0%.

Total ash ⟨*5.01*⟩ Not more than 13.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 5.0%.

Containers and storage Containers—Well-closed containers.

Pueraria Root

Puerariae Radix

カッコン

Pueraria Root is the root of *Pueraria lobata* Ohwi (*Leguminosae*), from which periderm has been removed.

It contains not less than 2.0% of puerarin ($C_{21}H_{20}O_9$: 416.38), calculated on the basis of dried material.

Description Usually cut into small pieces of irregular hexagons of about 0.5 cm cube, or cut into longitudinally plate-like pieces 20 – 30 cm in length, 5 – 10 cm in width, and about 1 cm in thickness; externally light grayish yellow to grayish white; transverse section showing concentric annulate ring or part of it formed by abnormal growth of cambium. Under a magnifying glass, phloem light grayish yellow in color; in xylem, numerous vessels appearing as small dots; medullary rays slightly dented; vertical section showing longitudinal patterns formed alternately by fibrous xylem and parenchyma; easily breakable lengthwise, and its section extremely fibrous.

Odorless; taste, at first slightly sweet, followed by a slight bitterness.

Under a microscope ⟨*5.01*⟩, a transverse section reveals fiber bundles accompanied by crystal cells in phloem; distinct vessels and xylem fibers in xylem; starch grains numerous in parenchyma, mainly composed of polygonal simple grains, rarely 2- to 3-compound grains, 2 – 18 μm, mostly 8 – 12 μm, in size, with hilum or cleft in the center, and also with striae.

Identification To 2 g of pulverized Pueraria Root add 10 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Puerarin RS or puerarin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Pueraria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Pueraria Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying ⟨*5.01*⟩ Not less than 13.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Assay Weigh accurately about 0.3 g of pulverized Pueraria Root, add 50 mL of diluted methanol (1 in 2), and heat under a reflex condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and perform as the same as above. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Puerarin RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of puerarin in each solution.

$$\text{Amount (mg) of puerarin } (C_{21}H_{20}O_9) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Puerarin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (9:1).

Flow rate: Adjust so that the retention time of puerarin is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry coefficient of the peak of puerarin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of puerarin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Quercus Bark

Quercus Cortex

ボクソク

Quercus Bark is the bark of *Quercus acutissima* Carruthers, *Quercus serrata* Murray, *Quercus mongolica* Fischer ex Ledebour var. *crispula* Ohashi or *Quercus variabilis* Blume (*Fagaceae*).

Description Plate-like or semi-tubular pieces of bark, 5 – 15 mm in thickness; externally grayish brown to dark brown, with thick periderm and longitudinal coarse splits; internally

brown to light brown, with longitudinal ridges, the transverse section brown to light brown, white small spots composed of stone cells in groups observed sporadically.

Almost odorless, tasteless.

Under a microscope <*5.01*>, a transverse section reveals a cork layer with scattered cork stone cells; in secondary cortex fiber bundles lined almost stepwide, large groups of stone cells arranged irregularly; in parenchyma aggregated crystals of calcium oxalate scattered; adjacent to stone cells and fiber cells, cells containing solitary crystals of calcium oxalate observed, and these cells form crystal cell rows in a longitudinal section.

Identification To 2 g of pulverized Quercus Bark, add 10 mL of ethyl acetate, shake for 10 minutes, and centrifuge to remove ethyl acetate. Add 10 mL of acetone to the residue, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): Two consecutive fluorescent spots in different colors are observed at an *R*f value of about 0.4. Then, spray evenly diluted sulfuric acid on the plate, heat at 105°C. Examine under ultraviolet light (main wavelength: 365 nm): one of these spots produces fluorescence.

Loss on drying <*5.01*> Not more than 11.0% (6 hours).

Total ash <*5.01*> Not more than 8.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Rape Seed Oil

Oleum Rapae

ナタネ油

Rape Seed Oil is the fixed oil obtained from the seed of *Brassica campestris* Linné subsp. *napus* Hooker filius et Anderson var. *nippo-oleifera* Makino (*Cruciferae*).

Description Rape Seed Oil is a clear, pale yellow, slightly viscous oil. It is odorless or has a slight odor and a mild taste.

It is miscible with diethyl ether and with petroleum diethyl ether. It is slightly soluble in ethanol (95).

Specific gravity d_{25}^{25}: 0.906 – 0.920

Acid value <*1.13*> Not more than 0.2.

Saponification value <*1.13*> 169 – 195

Unsaponifiable matters <*1.13*> Not more than 1.5%.

Iodine value <*1.13*> 95 – 127

Containers and storage Containers—Tight containers.

Red Ginseng

Ginseng Radix Rubra

コウジン

Red Ginseng is the root of *Panax ginseng* C. A. Meyer (*Panax schinseng* Nees) (*Araliaceae*), after being steamed.

It contains not less than 0.10% of ginsenoside Rg_1 ($C_{42}H_{72}O_{14}$: 801.01) and not less than 0.20% of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), calculated on the basis of dried material.

Description Thin and long cylindrical to fusiform root, often branching out into 2 to 5 lateral roots from the middle; 5 – 25 cm in length, main root 0.5 – 3 cm in diameter; externally light yellow-brown to red-brown, and translucent and with longitudinal wrinkles; crown somewhat constricted, and sometimes with short remains of stem; fractured surface flat; horny and hard in texture.

Odor, characteristic; taste, at first slightly sweet, followed by a slight bitterness.

Identification **(1)** To 0.2 g of pulverized Red Ginseng add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

(2) To 2.0 g of pulverized Red Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (14:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Red Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of pulverized Red Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Foreign matter <*5.01*>—The amount of stems and other foreign matter contained in Red Ginseng does not exceed 2.0%.

(4) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 15.5% (6 hours).

Total ash <*5.01*> Not more than 4.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 18.0%.

Assay **(1)** Ginsenoside Rg_1—Weigh accurately about 1 g of pulverized Red Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for

15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg_1 RS (separately determine the water <2.48> by coulometric titration, using 10 mg) dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rg_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rg_1\ (C_{42}H_{72}O_{14}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ginsenoside Rg_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of ginsenoside Rg_1 is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg_1 RS and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg_1 and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg_1 is not more than 1.5%.

(2) Ginsenoside Rb_1—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rb_1\ (C_{54}H_{92}O_{23}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust so that the retention time of ginsenoside Rb_1 is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rb_1 RS and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb_1 and ginsenoside Rc are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Rehmannia Root

Rehmanniae Radix

ジオウ

Rehmannia Root is the root of *Rehmannia glutinosa* Liboschitz var. *purpurea* Makino or *Rehmannia glutinosa* Liboschitz (*Scrophulariaceae*), with the application of steaming (prepared one: Juku-jio) or without it (non-prepared one: Kan-jio).

Description 1) Kan-jio—Massive or fusiform root, narrow at one or both ends, 5 – 10 cm in length, 0.5 – 3.0 cm in diameter, sometimes broken or markedly deformed in shape; externally yellow-brown, blackish brown or black, with deep, longitudinal wrinkles and constrictions; soft in texture; transversely cut surface yellow-brown, blackish brown, or black and peripheral portion darker.

Odor, characteristic; taste, slightly sweet at first, followed by a slight bitterness.

Under a microscope <5.01>, a transverse section reveals 7 – 15 layers of cork; cortex composed entirely of parenchyma; cells containing brown secretes scattered in cortex; xylem practically filled with parenchyma; vessels radially lined, mainly reticulate vessels.

2) Juku-jio—Irregularly massive root, or massive or fusiform root, narrow at one or both ends, 5 – 10 cm in length, 0.5 – 3.0 cm in diameter; externally black, usually lustrous, with deep, longitudinal wrinkles and constrictions; soft in texture and mucous; transversely cut surface black.

Odor, characteristic; taste, sweet at first, followed by a slight bitterness.

Under a microscope <5.01>, a transverse section reveals 7 – 15 layers of cork; cortex composed entirely of parenchyma; cells containing brown secretes scattered in cortex; xylem practically filled with parenchyma, often parenchyma partially broken and gaps observed; vessels radially lined, mainly reticulate vessels.

Identification 1) Kan-jio—Sake 0.5 g of the fine cutting of Rehmannia Root with 5 mL of water, add 20 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of stachyose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions

as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution. When further heat for more than 5 minutes, a blue spot is not observed at just lower than the spot mentioned above, or even appears it is only few.

2) Juku-jio—Sake 0.5 g of the fine cutting of Rehmannia Root with 5 mL of water, add 20 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of fructose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution (1). Separately, dissolve 3 mg of manninotriose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat at 105°C for 10 minutes: the principal spot obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution (1). Furthermore, one of the spot from the several spots obtained from the sample solution has the same color tone and *R*f value with the blue spot obtained from the standard solution (2).

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Rehmannia Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Rehmannia Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Containers and storage Containers—Well-closed containers.

Rhubarb

Rhei Rhizoma

ダイオウ

Rhubarb is usually the rhizome of *Rheum palmatum* Linné, *Rheum tanguticum* Maximowicz, *Rheum officinale* Baillon, *Rheum coreanum* Nakai or their interspecific hybrids (*Polygonaceae*).

It contains not less than 0.25% of sennosides A ($C_{42}H_{38}O_{20}$: 862.74), calculated on the basis of dried material.

Description Ovoid, oblong-ovoid or cylindrical rhizome, often cut crosswise or longitudinally, 4 – 10 cm in diameter, 5 – 15 cm in length. In the case of Rhubarb without most part of cortex, the outer surface is flat and smooth, yellow-brown to light brown in color, and sometimes exhibiting white, fine reticulations; thick and hard in texture. In the case of Rhubarb with cork layer, externally dark brown or reddish black, and with coarse wrinkles; rough and brittle in texture. The fractured surface of Rhubarb is not fibrous; transverse section grayish brown, light grayish brown or brown in color, having patterns of blackish brown tissue complicated with white and light brown tissues; near the cambium, the patterns often radiate, and in pith, consist of whirls of tissues radiated from the center of a small brown circle 1 – 3 mm in diameter and arranged in a ring or scattered irregularly.

Odor, characteristic; taste, slightly astringent and bitter; when chewed, gritty between the teeth, and coloring the saliva yellow.

Under a microscope <5.01>, the transverse section reveals mostly parenchyma cells; small abnormal cambium-rings scattered here and there in the pith; the cambium-rings produce phloem inside and xylem outside, accompanied with 2 to 4 rows of medullary rays containing brown-colored substances, and the rays run radiately from the center of the ring towards the outside forming whirls of tissues; parenchyma cells contain starch grains, brown-colored substances or crystal druses of calcium oxalate.

Identification To 1.0 g of pulverized Rhubarb add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography<2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow spot obtained from the standard solution, and the spot develops a red color on spraying sodium carbonate TS.

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of pulverized Rhubarb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Rhubarb according to Method 4, and perform the test (not more than 5 ppm).

(3) Raponticin—To 0.1 g of pulverized Rhubarb add exactly 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of raponticin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, 2-butanon, water and formic acid (10:7:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the chromatogram obtained with the sample solution shows no spot having the same color tone and *R*f value with the blue fluorescent spot obtained with the standard solution.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 30.0%.

Assay Weigh accurately about 0.5 g of pulverized Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS, (separately determine the water <2.48> by coulometric titration, using 10 mg) dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4 – 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A RS and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Rhubarb

Rhei Rhizoma Pulveratum

ダイオウ末

Powdered Rhubarb is the powder of Rhubarb.

It contains not less than 0.25% of sennoside A ($C_{42}H_{38}O_{20}$: 862.74), calculated on the basis of dried materials.

Description Powdered Rhubarb occurs as a brown powder. It has a characteristic odor and a slightly astringent and bitter taste; is gritty between the teeth and colors the saliva yellow on chewing.

Under a microscope <5.01>, Powdered Rhubarb reveals starch grains, dark brown substances or druses of calcium oxalate, fragments of parenchyma cells containing them, and reticulate vessels. The starch grains are spherical, simple, or 2- to 4-compound grains. Simple grain, 3 – 18 μm in diameter, rarely 30 μm; crystal druses of calcium oxalate, 30 – 60 μm in diameter, sometimes exceeding 100 μm.

Identification To 1.0 g of Powdered Rhubarb add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow spot obtained from the standard solution, and the spot develops a red color on spraying sodium carbonate TS.

Purity **(1)** Heavy metals <1.07>—Proceed with 3.0 g of Powdered Rhubarb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Rhubarb according to Method 4, and perform the test (not more than 5 ppm).

(3) Raponticin—To 0.1 g of Powdered Rhubarb add exactly 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of raponticin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, 2-butanon, water and formic acid (10:7:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the chromatogram obtained with the sample solution shows no spot having the same color tone and *R*f value with the blue fluorescent spot obtained with the standard solution.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Assay Weigh accurately about 0.5 g of Powdered Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS, (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of senno-

side A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column about 4 – 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A RS and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Compound Rhubarb and Senna Powder

複方ダイオウ·センナ散

Method of preparation

Powdered Senna Leaves	110 g
Powdered Rhubarb	110 g
Sulfur	555 g
Magnesium Oxide	225 g
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Rhubarb and Senna Powder occurs as a yellow-brown powder, having a characteristic odor and a bitter taste.

Identification To 2 g of Compound Rhubarb and Senna Powder add 50 mL of water, warm on a water bath for 30 minutes, and filter. Add 2 drops of dilute hydrochloric acid to the filtrate, shake with two 20-mL portions of diethyl ether, and remove the diethyl ether layer. Add 5 mL of hydrochloric acid to the aqueous layer, and heat it on a water bath for 30 minutes. Cool, shake with 20 mL of diethyl ether, take the diethyl ether layer, add 10 mL of sodium hydrogen carbonate TS, and shake: the aqueous layer is red in color.

Containers and storage Containers—Well-closed containers.

Rikkunshito Extract

六君子湯エキス

Rikkunshito Extract contains not less than 2.4 mg of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), not less than 16 mg and not more than 48 mg of hesperidin, and not less than 8 mg and not more than 24 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Ginseng	4 g	4 g
Atractylodes Rhizome	4 g	—
Atractylodes Lancea Rhizome	—	4 g
Poria Sclerotium	4 g	4 g
Pinellia Tuber	4 g	4 g
Citrus Unshiu Peel	2 g	2 g
Jujube	2 g	2 g
Glycyrrhiza	1 g	1 g
Ginger	0.5 g	0.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Rikkunshito Extract is a light brown to brown powder or blackish brown viscous extract. It has an odor and a sweet and bitter taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome— Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate.

Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Take the hexane layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of hexane, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value about 0.4, and this spot shows green-brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allow to cool (Atractylodes Lancea Rhizome).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution (Citrus Unshiu Peel).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 30 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0 % (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Ginsenoside Rb_1—Weigh accurately about 2 g of the dry extract (or an amount of the viscous extract, equivalent to about 2 g of the dried substance), add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 15 mL of diluted methanol (3 in 5), repeat the same procedure. Combine the supernatant liquids, add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of sodium hydroxide TS, allow to stand for 30 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 20 mL. Apply exactly 5 mL of this solution to a column (about 10 mm in inside diameter and packed with 0.36 g of octadecylsilanized silica gel for pre-treatment (55 – 105 μm in particle size), washed just before use with methanol and then with diluted methanol (3 in 10)), and wash the column in sequence with 2 mL of diluted methanol (3 in 10), 1 mL of sodium carbonate TS and 10 mL of diluted methanol (3 in 10). Finally, elute with methanol to collect exactly 5 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb_1 RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb_1 in each solution.

$$\text{Amount (mg) of ginsenoside } Rb_1\ (C_{54}H_{92}O_{23}) = W_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of Ginsenoside Rb_1 RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with carbamoyl groups bound silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

60°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (400:100:1).

Flow rate: 1.0 mL per minute (the retention time of ginsenoside Rb_1 is about 16 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ginsenoside Rb_1 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb_1 is not more than 1.5%.

(2) Hesperidin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of hesperidin in each solution.

$$\text{Amount (mg) of hesperidin} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of hesperidin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hesperidin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Rose Fruit

Rosae Fructus

エイジツ

Rose Fruit is the pseudocarp of fruit of *Rosa multiflora* Thunberg (*Rosaceae*).

Description The pseudocarp, spherical, ellipsoidal or spheroidal, 5 – 9.5 mm in length, 3.5 – 8 mm in diameter; the external surface red to dark brown in color, smooth and lustrous; often with peduncle about 10 mm in length at one end, and with pentagonal remains of calyx without sepal at the other end; internal wall of receptacle covered densely with silvery hairs; the interior containing 5 – 10 mature nuts; the nut, irregularly angular ovoid, about 4 mm in length, about 2 mm in diameter; external surface, light yellow-brown; obtuse at one end, and slightly acute at the other.

Odor, slight; taste of receptacle, sweet and acid, and of nut, mucilaginous at first, later astringent, bitter and irritative.

Identification Boil gently 1 g of pulverized Rose Fruit with 20 mL of methanol for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 0.5 mL of hydrochloric acid, and allow the mixture to stand: a light red to red color develops.

Purity Foreign matter <*5.01*>—The amount of the peduncle and other foreign matter contained in Rose Fruit is not more than 1.0%.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Containers and storage Containers—Well-closed containers.

Powdered Rose Fruit

Rosae Fructus Pulveratus

エイジツ末

Powdered Rose Fruit is the powder of Rose Fruit.

Description Powdered Rose Fruit occurs as a grayish yellow-brown powder. It has a slight odor, and has a slightly mucilaginous, astringent, bitter, and slightly acid taste.

Under a microscope ⟨*5.01*⟩, Powdered Rose Fruit reveals fragments of extremely thick-walled hairs 35 – 70 μm in diameter, fragments of epidermis and hypodermis containing brown tannin masses, fragments of thin-walled fundamental tissue containing grayish brown substances, fragments of fine vessels, and solitary or twin crystals or rosette agregates of calcium oxalate (components of receptacle); fragments of sclerenchyma, fiber groups, fine vessels, and fragments of epidermis containing brown tannin and mucilage (components of pericarp); fragments of endosperm composed of polygonal cells containing aleuron grains and fatty oil, fragments of outer epidermis composed of polygonal cells containing tannin, and fragments of inner epidermis composed of elongated cells having wavy lateral walls (components of seed).

Identification Boil gently 1 g of Powdered Rose Fruit with 20 mL of methanol for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 0.5 mL of hydrochloric acid, and allow the mixture of stand: a light red to red color develops.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Containers and storage Containers—Well-closed containers.

Rosin

Resina Pini

ロジン

Rosin is the resin obtained from the exudation of plants of *Pinus* species (*Pinaceae*) from which essential oil has been removed.

Description Rosin occurs as a light yellow to light brown, glassily transparent, brittle mass, the surfaces of which are often covered with a yellow powder. The fractured surface is shell-like and lustrous.

It has a slight odor.

It melts easily, and burns with a yellow-brown flame.

It is freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether.

A solution of Rosin in ethanol (95) is acidic.

Acid value ⟨*1.13*⟩ 150 – 177

Total ash ⟨*5.01*⟩ Not more than 0.1%.

Containers and storage Containers—Well-closed containers.

Royal Jelly

Apilac

ローヤルゼリー

Royal Jelly is the viscous liquid or its dried substance secreted by the secreting gland on the head of *Apis mellifera* Linné or *Apis cerana* Fabricius (*Apidae*).

It contains not less than 4.0% and not more than 8.0% of 10-hydroxy-2-(*E*)-decenoic acid, calculated on the basis of dried material.

Description Slightly viscous liquid or powder, milky white to light yellow in color. Odor, characteristic; taste, astringent and acid.

Identification To a portion of Royal Jelly, equivalent to 0.2 g of dried substance, add 5 mL of water, 1 mL of dilute hydrochloric acid and 10 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 2 mg of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of 1-propanol and ammonia solution (28) (7:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with a portion of Royal Jelly, equivalent to 1.0 g of the dried substance, according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with an amount of Royal Jelly, equivalent to 0.40 g of the dried substance according to Method 3, and perform the test (not more than 5 ppm).

Loss on drying ⟨*5.01*⟩ The slightly viscous liquid: Not less than 57.0% and not more than 77.0% (6 hours).

The powder: Not less than 7.0% and not more than 13.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 4.0%, calculated on the dried basis.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%, calculated on the dried basis.

Assay Weigh accurately a portion of Royal Jelly, equivalent to 0.2 g of the dried substance, add 20 mL of methanol, treat with ultrasonic waves for 30 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 10-

hydroxy-2-(*E*)-decenoic acid for assay, dissolve in methanol to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 10-hydroxy-2-(*E*)-decenoic acid to that of the internal standard.

Amount (mg) of 10-hydroxy-2-(*E*)-decenoic acid
$= M_S \times Q_T/Q_S \times 3/4$

M_S: Amount (mg) of 10-hydroxy-2-(*E*)-decenoic acid for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, methanol for liquid chromatography and phosphoric acid (550:450:1).

Flow rate: Adjust so that the retention time of 10-hydroxy-2-(*E*)-decenoic acid is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 10-hydroxy-2-(*E*)-decenoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 10-hydroxy-2-(*E*)-decenoic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At not exceeding 10°C.

Ryokeijutsukanto Extract

苓桂朮甘湯エキス

Ryokeijutsukanto Extract contains not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, and not less than 21 mg and not more than 63 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Poria Sclerotium	6 g	6 g
Cinnamon Bark	4 g	4 g
Atractylodes Rhizome	3 g	—
Atractylodes Lancea Rhizome	—	3 g
Glycyrrhiza	2 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Ryokeijutsukanto Extract occurs as a brown powder or blackish brown viscous extract. It has an odor, and a sweet first then bitter taste.

Identification **(1)** To 1.0 g of dry extract (or 3.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of dry extract (or 3.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plat, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of dry extract (or 6.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of dry extract (or 3.0 g of the viscous extract) of Ryokeijutsukanto Extract add 10 mL of water,

shake, then add 10 mL of 1-butanol, and shake. Centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) of Ryokeijutsukanto Extract as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) of Ryokeijutsukanto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 8.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g 105°C, 5 hours).

Total ash <*5.01*> Not more than 8.0%, calculated on the dried basis.

Assay **(1)** (*E*)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance) of Ryokeijutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-cinnamic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-cinnamic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of (*E*)-cinnamic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1).

Flow rate: 1.0 mL per minute (the retention time of (*E*)-cinnamic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-cinnamic acid is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance) of Ryokeijutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Safflower

Carthami Flos

コウカ

Safflower is the tubulous flower of *Carthamus tinctorius* Linné (*Compositae*) without any treatment or with most of the yellow pigment removed, and sometimes with pressed into a flat slab.

Description Red to red-brown corolla, yellow style and sta-

men, rarely mixed with immature ovary; total length about 1 cm; corolla, tubular and with 5 lobes; 5 stamens surrounding long pistil; pollen grains yellow and approximately spherical, about 50 μm in diameter, with fine protrusions on the surface. The pressed slab, about 0.5 cm in thickness, consists of a collection of numerous corollas.

Odor, characteristic; taste, slightly bitter.

Identification Boil 0.2 g of Safflower with 10 mL of dilute ethanol under a reflux condenser for 15 minutes, and after cooling, filter. Place 3 mL of the filtrate in a small glass vessel about 3 cm in both internal diameter and height, hang a piece of filter paper, 20 mm by 300 mm, so that one end of the filter paper reaches the bottom of the vessel, and allow the paper to soak up the liquid for 1 hour. Transfer and immediately hang the paper in another glass vessel of the same type, containing 3 mL of water, and allow the paper to soak up the water for 1 hour: most of the upper part of the paper is colored light yellow, and the lower portion, light red.

Purity Foreign matter <*5.01*>—The amount of ovaries, stems, leaves and other foreign matter contained in Safflower does not exceed 2.0%.

Total ash <*5.01*> Not more than 18.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Saffron

Crocus

サフラン

Saffron is the stigma of *Crocus sativus* Linné (*Iridaceae*).

Description Thin cord-like stigma, externally dark yellow-red to red-brown, 1.5 – 3.5 cm in length, tripartite or separate; the end of partite part widened and the other end narrowed gradually.

Odor, strong and characteristic; taste, bitter; colors the saliva yellow on chewing.

Under a microscope <*5.01*>, when softened by immersion in water, the upper end has numerous tubular protrusions about 150 μm in length, with a small number of pollen grains.

Identification Add 1 drop of sulfuric acid to Saffron: the color changes to dark blue which gradually turns red-brown through purple.

Purity **(1)** Aniline dyes—Shake 0.05 g of Saffron with 10 mL of chloroform: the solution is colorless, or only slightly yellow.

(2) Glycerol, sugar or honey—Saffron has no sweet taste. Press it between two pieces of paper: no spot is left on the paper.

(3) Yellow style—When perform the test of foreign matter <*5.01*>, the yellow style in Saffron does not exceed 10.0%.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 7.5%.

Content of the active principle Crocin—Dry Saffron in a desiccator (silica gel) for 24 hours, and powder. To exactly 0.100 g of the powder add 150 mL of warm water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, cool, and filter. Pipet 1 mL of the filtrate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve exactly 98 mg of carbazochrome sodium sulfonate trihydrate in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 438 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance of the sample solution is larger than that of the standard solution.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Saibokuto Extract

柴朴湯エキス

Saibokuto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 90 mg and not more than 270 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Bupleurum Root	7 g	7 g
Pinellia Tuber	6 g	5 g
Poria Sclerotium	5 g	5 g
Scutellaria Root	3 g	3 g
Magnolia Bark	3 g	3 g
Jujube	3 g	3 g
Ginseng	3 g	3 g
Glycyrrhiza	2 g	2 g
Perilla Herb	2 g	2 g
Ginger	1 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Saibokuto Extract is a light brown powder or blackish brown viscous extract, having a slightly odor and a slight sweet first, then a bitter taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the

spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the diethyl ether of the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Scutellaria Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the diethyl ether of the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution (Magnolia Bark).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the diethyl ether of the layer under reduced pressure, add to the residue 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (60:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution (Perilla Herb).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the diethyl ether of the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Ginger).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution.

Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <2.48> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Saikokeishito Extract

柴胡桂枝湯エキス

Saikokeishito Extract contains not less than 1.5 mg and not more than 6 mg of saikosaponin b_2, not less than 60 mg and not more than 180 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 17 mg and not more than 51 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 63 mg (for preparation prescribed 2.5 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 13 mg and not more than 39 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 17 mg

and not more than 51 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Bupleurum Root	5 g	5 g	5 g	5 g
Pinellia Tuber	4 g	4 g	4 g	4 g
Scutellaria Root	2 g	2 g	2 g	2 g
Peony Root	2 g	2.5 g	2 g	2 g
Jujube	2 g	2 g	2 g	2 g
Ginseng	2 g	2 g	2 g	2 g
Cinnamon Bark	2.5 g	2.5 g	2.5 g	2 g
Glycyrrhiza	1.5 g	1.5 g	1.5 g	2 g
Ginger	0.5 g	1 g	1 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Saikokeishito Extract is a yellow-brown powder or blackish brown viscous extract, having a slightly odor and a slight sweet first, then a bitter and slightly pungent taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Scutellaria Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105 °C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Peony Root).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(5) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for about 1 hour, separate the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydradine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several

spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add to the residue 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Ginger).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Lead—Take 5.0 g of the dry extract (or an amount of the viscous extract, equivalent to 5.0 g of the dried substance) in a platinum, quartz or porcelain crucible, heat gently, and then incinerate by ignition at 450 to 550°C. After cooling, add a small amount of 2 mol/L nitric acid TS to the residue, filter if necessary, and wash the crucible several times with small portions of 2 mol/L nitric acid TS. Combine the washings and the filtrate, add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, to 2.5 mL of Standard Lead Solution add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 5 ppm).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: A lead hollow-cathode lamp.
Wavelength: 283.3 nm.

(3) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(4) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Saireito Extract

柴苓湯エキス

Saireito Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.37), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Bupleurum Root	7 g	7 g
Pinellia Tuber	5 g	5 g
Ginger	1 g	1 g
Scutellaria Root	3 g	3 g
Jujube	3 g	3 g
Ginseng	3 g	3 g
Glycyrrhiza	2 g	2 g
Alisma Tuber	6 g	5 g
Polyporus Sclerotium	4.5 g	3 g
Poria Sclerotium	4.5 g	3 g
Atractylodes Rhizome	4.5 g	—
Atractylodes Lancea Rhizome	—	3 g
Cinnamon Bark	3 g	2 g

Prepare a dry extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Saireito Extract occurs as a light yellow-brown powder. It has slightly a characteristic odor, and a sweet, then bitter taste.

Identification **(1)** To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(2) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 15 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

(3) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, air-dry the plate, and spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Scutellaria Root).

(4) To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Ginseng).

(5) To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Glycyrrhiza).

(6) To 2.0 g of Saireito Extract add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Alisma Tuber).

(7) For preparation prescribed Atractylodes Rhizome—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atracty-

lenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plat, heat at 105°C for 5 minutes, examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(8) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(9) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Cinnamon Bark).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Saireito Extract as directed under Extract (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Saireito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> Not more than 10.0% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of Saireito Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and the standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute. (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Salvia Miltiorrhiza Root

Salviae Miltiorrhizae Radix

タンジン

Salvia Miltiorrhiza Root is the root of *Salvia miltiorrhiza* Bunge (*Labiatae*).

Description Salvia Miltiorrhiza Root is nearly cylindrical, 5 – 25 cm in length, 0.3 – 1.5 cm in diameter; slightly curved, often with lateral roots; outer surface reddish brown, dark reddish brown or blackish brown; with irregular rough wrinkles; hard in texture, but easily broken; fracture surface fine or rough with clefts; cortex grayish yellow white or reddish brown, xylem light yellowish white or blackish brown.

Odor, slight; taste, sweet at first and followed by slight bitterness and astringency.

Under a microscope <*5.01*>, a transverse section reveals usually cork layer at the outermost portion, rarely parenchyma or endodermis outside the cork layer; several sclerenchyma cells observed or not in secondary cortex and phloem; cambium obvious; vessels radially arranged in secondary xylem, sometimes radial lines of vessels unite in the center of root; xylem fibers surrounding vessels; primary xylem divided into 2 – 3; vessels of secondary xylem mainly pitted vessels and reticulate vessels in a longitudinal section.

Identification To 1.0 g of pulverized Salvia Miltiorrhiza Root add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter. Evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethyl acetate, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 10 cm, and air-dry the plate: a red-brown spot at an *R*f value of about 0.4 is observed.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Salvia Miltiorrhiza Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Salvia Miltiorrhiza Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 16.0% (6 hours).

Total ash <*5.01*> Not more than 7.5%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 42.0%.

Containers and storage Containers—Well-closed containers.

Saposhnikovia Root and Rhizome

Saposhnikoviae Radix

ボウフウ

Saposhnikovia Root and Rhizome is the root and rhizome of *Saposhnikovia divaricata* Schischkin (*Umbelliferae*).

Description Long and narrow, conical rhizome and root, 15 – 20 cm in length, 0.7 – 1.5 cm in diameter; externally light brown; rhizome reveals dense crosswise wrinkles like ring nodes, and sometimes reveals brown and hair-like remains of leaf sheath; the root reveals many longitudinal wrinkles and scars of rootlets; in a transverse section, cortex is grayish brown in color and reveals many lacunae, and xylem is yellow in color.

Odor, slight; taste, slightly sweet.

Identification To 1 g of pulverized Saposhnikovia Root and Rhizome, add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glucosyl-5-*O*-methylvisamminol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (10:2:1) to a dis-

tance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Saposhnikovia Root and Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Saposhnikovia Root and Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter <*5.01*>—The amount of stems and other foreign matter is not more than 2.0%.

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 1.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 20.0%.

Containers and storage Containers—Well-closed containers.

Sappan Wood

Sappan Lignum

ソボク

Sappan Wood is the duramen of *Caesalpinia sappan* Linné (*Leguminosae*).

Description Chips, slices or short pieces of wood; yellowish red to grayish yellow-brown, sometimes with light brown to grayish white splint woods; hard in texture; a transverse section shows a pattern like annual ring.

Almost odorless; almost tasteless.

Under a microscope <*5.01*>, a transverse section reveals ray composed of 1 – 2 rows of slender and long cells; the area between rays filled with fiber cells, and large and oblong vessels scattered there; solitary crystals of calcium oxalate in parenchymatous cells of the innermost of xylem.

Identification To 0.5 g of pulverized Sappan Wood add 10 mL of dilute ethanol, shake, and filter. To 5 mL of the filtrate add 2 to 3 drops of sodium hydroxide TS: a dark red color develops.

Purity Put a small piece of Sappan Wood in calcium hydroxide TS: no purple-blue color develops.

Loss on drying <*5.01*> Not more than 11.5% (6 hours).

Total ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 7.0%.

Containers and storage Containers—Well-closed containers.

Saussurea Root

Saussureae Radix

モッコウ

Saussurea Root is the root of *Saussurea lappa* Clarke (*Compositae*).

Description Nearly cylindrical roots, 5 – 20 cm in length, 1 – 6 cm in diameter; some of them slightly bent, and sometimes longitudinally cut; scar of stem dented on the top of the root with crown; externally yellow-brown to grayish brown, with coarse longitudinal wrinkles and fine reticulate furrows, and also with remains of lateral roots; sometimes root from which periderm has been removed; hard and dense in texture, and difficult to break. A transverse section is yellow-brown to dark brown, and cambium part has a dark color. Under a magnifying glass, medullary rays distinct, here and there, large clefts, and brown oil sacs scattered; in old root, pith existing in the center, and often forming a hollow.

Odor, characteristic; taste, bitter.

Identification To 1.0 g of pulverized Saussurea Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and cool: a red-purple spot at an *R*f value of about 0.5 and a grayish blue to grayish brown spot just below it are observed.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Saussurea Root according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Saussurea Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Add iodine TS dropwise to a transverse section: no blue-purple color develops.

Total ash <*5.01*> Not more than 4.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 17.0%.

Containers and storage Containers—Well-closed containers.

Schisandra Fruit

Schisandrae Fructus

ゴミシ

Schisandra Fruit is the fruit of *Schisandra chinensis* Baillon (*Schisandraceae*).

Description Sap fruit of irregular sphere or spheroid, about 6 mm in diameter; externally dark red to blackish brown in color, with wrinkles, and occasionally with white powder; seeds, kidney-shaped, externally yellow-brown to dark red-brown, lustrous, with distinct raphe on the dorsal side; external seed coat easily peeled but internal seed coat adhering closely to the albumen.

Odor, slight; taste, acid, later astringent and bitter.

Identification To 1.0 g of pulverized Schisandra Fruit add 10 mL of methanol, warm on a water bath for 3 minutes with shaking, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-violet spot obtained from the standard solution.

Purity Foreign matter <*5.01*>—The amount of receptacle, peduncle and other foreign matter contained in Schisandra Fruit is not more than 1.0%.

Total ash <*5.01*> Not more than 5.0%.

Containers and storage Containers—Well-closed containers.

Schizonepeta Spike

Schizonepetae Spica

ケイガイ

Schizonepeta Spike is the spike of *Schizonepeta tenuifolia* Briquet (*Labiatae*).

Description Oblong spike, 5 – 10 cm in length, 0.5 – 0.8 cm in diameter, purplish green-brown to green-brown in color. Spike, with calyx-tubes containing small labiate flower or often fruits; sometimes leaves under spike; leaf, linear or small lanceolate; stem, prismatic, purple-brown in color. Under a magnifying glass, it reveals short hairs.

It has a characteristic aroma and slightly cool feeling on keeping in the mouth.

Identification To 1 g of pulverized Schizonepeta Spike add 10 mL of ethyl acetate, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes. After cooling for more than 10 minutes under an adequate humidity, examine under ultraviolet light (main wavelength: 365 nm): two spots, one is a bluish fluorescent spot with an *R*f value of about 0.5 and the another is a yellowish fluorescent spot with an *R*f value of about 0.1, are observed.

Total ash <*5.05*> Not more than 11.0%.

Acid-insoluble ash <*5.05*> Not more than 3.0%.

Extract content <*5.05*> Dilute ethanol-soluble extract: not less than 8.0%.

Containers and storage Containers—Well-closed containers.

Scopolia Rhizome

Scopoliae Rhizoma

ロートコン

Scopolia Rhizome is the rhizome with root of *Scopolia japonica* Maximowicz, *Scopolia carniolica* Jacquin or *Scopolia parviflora* Nakai (*Solanaceae*).

When dried, it contains not less than 0.29% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

Description Chiefly irregularly branched, slightly curved rhizome, about 15 cm in length, about 3 cm in diameter, occasionally longitudinally cut; externally grayish brown, with wrinkles; constrictions make the rhizome appear nodular; rarely, stem base at one end; stem scars at upper side of each node; roots or root scars on both sides and lower surface of rhizome; fractured surface granular, grayish white to light brown in color, with lighter colored cortex.

Odor characteristic; taste sweet, later slightly bitter.

Under a microscope <*5.01*>, xylem reveals groups of vessels arranged stepwise, and accompanied with xylem sieve tubes in medullary rays; parenchyma cells contain starch grains, and sometimes sand crystals of calcium oxalate.

Identification **(1)** To 1 g of pulverized Scopolia Rhizome add 10 mL of diethyl ether and 0.5 mL of ammonia TS, shake for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, transfer the filtrate and the washing to a separator, add 20 mL of diluted sulfuric acid (1 in 50), shake well, and drain off the acid extract into another separator. Render the solution slightly alkaline with ammonia TS, add 10 mL of diethyl ether, shake well, transfer the diethyl ether layer to a porcelain dish, and evaporate the diethyl ether on a water bath. To the residue add 5 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Cool, dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple to purple color develops.

(2) Place 2.0 g of pulverized Scopolia Rhizome in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl

acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate RS or atropine sulfate hydrate for thin-layer chromatography and 1 mg of Scopolamine Hydrobromide RS or scopolamine hydrobromide hydrate for thin-layer chromatography in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution and each yellow-red spot from the standard solutions show the same color tone and the same *R*f value.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Scopolia Rhizome according to Method 3, and perform the test. Prepare the control solution with 4.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Scopolia Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 7.0%.

Assay Weigh accurately about 0.7 g of pulverized Scopolia Rhizome, previously dried at 60°C for 8 hours, in a glass-stoppered, centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 25 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Scopolamine Hydrobromide Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of standard stock solution A and 1 mL of standard stock solution B, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Calculate the ratios, Q_{TA} and Q_{SA}, of the peak area of hyoscyamine (atropine), and the ratios, Q_{TS} and Q_{SS}, of the peak area of scopolamine to that of the internal standard in each solution, calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

Amount (mg) of hyoscyamine ($C_{17}H_{23}NO_3$)
$= M_{SA} \times Q_{TA}/Q_{SA} \times 1/5 \times 0.855$

Amount (mg) of scopolamine ($C_{17}H_{21}NO_4$)
$= M_{SS} \times Q_{TS}/Q_{SS} \times 1/25 \times 0.789$

M_{SA}: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

M_{SS}: amount (mg) of Scopolamine Hydrobromide RS taken, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadesilcylanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to pH 3.5, and add water to make 1000 mL. To 9 parts of this solution add 1 part of acetonitrile.

Flow rate: Adjust so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and with the resolution between the peaks of atropine and the internal standard being not less than 4.

Containers and storage Containers—Well-closed containers.

Scopolia Extract

ロートエキス

Scopolia Extract contains not less than 0.90% and not more than 1.09% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

Method of preparation Extract the coarse powder of Scopolia Rhizome with 35 vol% ethanol, Water, Purified Water or Purified Water in Containers, and prepare the viscous extract as directed under Extracts.

Description Scopolia Extract is brown to dark brown in color. It has a characteristic odor, and a bitter taste.

It dissolves in water with a slight turbidity.

Identification **(1)** Dissolve 4 g of Scopolia Extract in 10 mL of water, add 8 mL of ammonia TS and 80 mL of diethyl ether, stopper tightly, shake for 1 hour, add 2.5 g of powdered tragacanth, shake vigorously, allow to stand for 5 minutes, and separate the diethyl ether layer into a porcelain dish. Evaporate the diethyl ether on a water bath, add 5 drops of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple to purple color develops.

(2) Mix 0.5 g of Scopolia Extract with 30 mL of ammonia TS in a flask, and transfer the mixture to a separator. Add 40 mL of ethyl acetate to the separator, and shake the mixture. After drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in Identification (2) under Scopolia Rhizome.

Purity Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Scopolia Extract as directed in the Extracts (4), and perform the test (not more than 30 ppm).

Assay Weigh accurately about 0.4 g of Scopolia Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Proceed as directed under Scopolia Rhizome.

$$\text{Amount (mg) of hyoscyamine } (C_{17}H_{23}NO_3) = M_{SA} \times Q_{TA}/Q_{SA} \times 1/5 \times 0.855$$

$$\text{Amount (mg) of scopolamine } (C_{17}H_{21}NO_4) = M_{SS} \times Q_{TS}/Q_{SS} \times 1/25 \times 0.789$$

M_{SA}: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

M_{SS}: Amount (mg) of Scopolamine Hydrobromide RS taken, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Scopolia Extract Powder

ロートエキス散

Scopolia Extract Powder contains not less than 0.085% and not more than 0.110% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

Method of preparation

Scopolia Extract	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make	1000 g

To Scopolia Extract add 100 mL of Purified Water or Purified Water in Containers, then warm and soften the mixture with stirring. Cool, add 800 g of starch, Lactose Hydrate or their mixture little by little, and mix well. Dry preferably at a low temperature, and dilute with a sufficient additional quantity of starch, Lactose Hydrate or their mixture to make 1000 g of homogeneous powder.

Description Scopolia Extract Powder is a brownish yellow to grayish yellow-brown powder. It has a faint, characteristic odor and a slightly bitter taste.

Identification (1) To 20 g of Scopolia Extract Powder add 15 mL of water and 8 mL of ammonia TS, mix homogeneously, add 100 mL of diethyl ether and 7 g of sodium chloride, stopper tightly, shake for 1 hour, add 5 g of powdered tragacanth, and shake vigorously. Allow to stand for 5 minutes, take the clearly separated diethyl ether layer, and filter. Proceed with the filtrate as directed in the Identification (1) under Scopolia Extract.

(2) Place 5.0 g of Scopolia Extract Powder in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in the Identification (2) under Scopolia Rhizome.

Assay Weigh accurately about 4 g of Scopolia Extract Powder, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to take the diethyl ether layer. Repeat this procedure three times with the water layer, using 25-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (separately determine the loss on drying <*2.41*> under the same manner as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 25 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying <*2.41*> under the same manner as Scopolamine Hydrobromide Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of the standard stock solution A and 1 mL of the standard stock solution B, add exactly 3 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_{TA} and Q_{SA}, of the peak area of hyoscyamine (atropine), and ratios, Q_{TS} and Q_{SS}, of the peak area of scopolamine to that of the internal standard in each solution, calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

$$\text{Amount (mg) of hyoscyamine } (C_{17}H_{23}NO_3) = M_{SA} \times Q_{TA}/Q_{SA} \times 1/5 \times 0.855$$

$$\text{Amount (mg) of scopolamine } (C_{17}H_{21}NO_4) = M_{SS} \times Q_{TS}/Q_{SS} \times 1/25 \times 0.789$$

M_{SA}: Amount (mg) of Atropine Sulfate RS taken, calculated on the dried basis

M_{SS}: Amount (mg) of Scopolamine Hydrobromide RS taken, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate

in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of a solution obtained by dissolving 6.8 g of potassium dihydrogenphosphate in 900 mL of water, adding 10 mL of triethylamine, adjusting the pH to 3.5 with phosphoric acid, and adding water to make 1000 mL, and acetonitrile (9:1).

Flow rate: Adjust so that the retention time of scopolamine is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and determine the resolution. Use a column giving elution of scopolamine, atropine and the internal standard in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and the resolution between the peaks of atropine and the internal standard being not less than 4.

Containers and storage Containers—Tight containers.

Scopolia Extract and Carbon Powder

ロートエキス·カーボン散

Method of preparation

Scopolia Extract	5 g
Medicinal Carbon	550 g
Natural Aluminum Silicate	345 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare before use as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract and Carbon Powder is easily dustable and black in color. It is tasteless.

Containers and storage Containers—Well-closed containers.

Compound Scopolia Extract and Diastase Powder

複方ロートエキス·ジアスターゼ散

Method of preparation

Scopolia Extract	8 g
Diastase	200 g
Precipitate Calcium Carbonate	300 g
Sodium Bicarbonate	250 g
Magnesium Oxide	100 g
Powdered Gentian	50 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare before use as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Compound Scopolia Extract and Diastase Powder is light yellow in color. It has a bitter taste.

Containers and storage Containers—Well-closed containers.

Scopolia Extract and Ethyl Aminobenzoate Powder

ロートエキス·アネスタミン散

Scopolia Extract and Ethyl Aminobenzoate Powder contains not less than 22.5% and not more than 27.5% of ethyl aminobenzoate ($C_9H_{11}NO_2$: 165.19).

Method of preparation

Scopolia Extract	10 g
Ethyl Aminobenzoate	250 g
Magnesium Oxide	150 g
Sodium Bicarbonate	500 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract and Ethyl Aminobenzoate Powder is slightly brownish white in color. It has a slightly bitter taste, leaving a sensation of numbness on the tongue.

Identification (1) To 2 g of Scopolia Extract and Ethyl Aminobenzoate Powder add 20 mL of diethyl ether, shake, and filter through a glass filter (G4). Wash the residue with three 10-mL portions of diethyl ether, combine the filtrate and the washings, evaporate to dryness, and perform the following test with the residue (ethyl aminobenzoate).

(i) Dissolve 0.01 g of the residue in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(ii) Dissolve 0.1 g of the residue in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add

iodine TS dropwise: a brown precipitate is produced.

(iii) Warm 0.05 g of the residue with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

(2) To the diethyl ether-insoluble residue obtained in (1) add 30 mL of water, shake gently, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for sodium salt and for bicarbonate.

(3) To the water-insoluble residue obtained in (2) add 10 mL of dilute hydrochloric acid, shake, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for magnesium salt.

(4) Place 30 g of Scopolia Extract and Ethyl Aminobenzoate Powder in a glass-stoppered conical flask, add 100 mL of water, shake for 30 minutes, and filter immediately by suction through a glass filter (G3). Transfer the residue in the flask to the same glass filter with the filtrate, and filter the residue by suction while pressing vigorously the residue on the same glass filter. Place 75 mL of the filtrate in a 300-mL beaker, and add cautiously 10 mL of diluted sulfuric acid (1 in 3). Add 0.2 mL of bromocresol green TS to this solution, and add dilute sulfuric acid dropwise while shaking thoroughly, until the color of the solution changes from green to yellow-green. After cooling, place this solution in a separator, wash with two 25-mL portions of a mixture of hexane and diethyl ether (1:1) by shaking well, and place the water layer in another separator. Make slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, separate the diethyl ether layer, add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate RS or atropine sulfate hydrate for thin-layer chromatography and 1 mg of Scopolamine Hydrobromide RS or scopolamine hydrobromide hydrate for thin-layer chromatography in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution, standard solution (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia solution (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution show the same color tone and the same *R*f value with each yellow-red spot from the standard solutions, respectively.

Assay Weigh accurately about 0.3 g of Scopolia Extract and Ethyl Aminobenzoate Powder, transfer to a Soxhlet extractor, extract with 100 mL of diethyl ether for 1 hour, and evaporate the diethyl ether on a water bath. Dissolve the residue in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the sample solution. Weigh accurately about 75 mg of Ethyl Aminobenzoate RS, previously dried in a desiccator (silica gel) for 3 hours, dissolve in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, to each add 10 mL of 1 mol/L hydrochloric acid TS, then add 1 mL of a solution of sodium nitrite (1 in 200), prepared before use, and allow to stand for 5 minutes with occasional shaking. Add 5 mL of ammonium amidosulfate TS, shake well, and allow to stand for 10 minutes. Add 2 mL of *N*-*N*-diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS, mix immediately, and add water to make exactly 50 mL. Allow to stand for 2 hours, determine the absorbances, A_T and A_S, of these solutions at 550 nm, as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a blank prepared in the same manner with 5 mL of water in place of the sample solution.

$$\text{Amount (mg) of ethyl aminobenzoate } (C_9H_{11}NO_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ethyl Aminobenzoate RS taken

Containers and storage Containers—Well-closed containers.

Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder

ロートエキス·パパベリン·アネスタミン散

Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder contains not less than 10.8% and not more than 13.2% of ethyl aminobenzoate ($C_9H_{11}NO_2$: 165.19).

Method of preparation

Scopolia Extract	15 g
Papaverine Hydrochloride	15 g
Ethyl Aminobenzoate	120 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder is brownish yellow to grayish yellow-brown in color. It has a slightly bitter taste, leaving a sensation of numbness on the tongue.

Identification (1) To 4 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder add 20 mL of diethyl ether, shake, and filter through a glass filter (G4). Wash the residue with three 10-mL portions of diethyl ether, combine the filtrate and the washings, evaporate to dryness, and perform the following test with the residue (ethyl aminobenzoate):

(i) Dissolve 0.01 g of the residue in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution respounds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(ii) Dissolve 0.1 g of the residue in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(iii) Warm 0.05 g of the residue with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

(2) To the diethyl ether-insoluble residue obtained in (1) add 20 mL of chloroform, shake well, filter, and further wash the residue with 10 mL of chloroform. Combine the filtrate and the washing, transfer this solution to a separator, and add 10 mL of 0.1 mol/L hydrochloric acid TS. After shaking, separate the chloroform layer, add 2 g of anhy-

drous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dry the residue at 105°C for 3 hours, and perform the following tests (papaverine hydrochloride):

(i) To 1 mg of the residue add 1 drop of formaldehyde solution-sulfuric acid TS: a colorless or light yellow-green color, changing to red-purple, is produced.

(ii) Dissolve 1 mg of the residue in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and view under ultraviolet light: the solution shows a yellow-green fluorescence.

(3) Place 20 g of Scopolia Extract, Paraverine and Ethyl Aminobenzoate Powder in a glass-stopperd conical flask, add 80 mL of water, shake for 15 minutes, and filter by suction through a glass filter (G3). Transfer 60 mL of the filtrate to a separator, add 0.5 mL of 1 mol/L hydrochloric acid TS, and extract with three 20-mL portions of chloroform by shaking. Make the aqueous layer slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, and separate the diethyl ether layer. Add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol (95), and use the solution as the sample solution. Dissolve 20 mg of atropine sulfate hydrate for thin-layer chromatography, 10 mg of scopolamine hydrobromide hydrate and 20 mg of papaverine hydrochloride in 10 mL each of ethanol (95), and use these solutions as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution, standard solution (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and dry the plate at 80°C for 20 minutes. After cooling, spray Dragendorff's TS for spraying upon the plate evenly: three yellow-red principal spots obtained from the sample solution and the corresponding spots obtained from standard solutions (1), (2) and (3) show the same *R*f values.

Assay Weigh accurately about 0.6 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder, transfer to a Soxhlet extractor, and extract with 100 mL of diethyl ether for 1 hour, and evaporate the diethyl ether on a water bath. Dissolve the residue in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of Ethyl Aminobenzoate RS, previously dried in a desiccator (silica gel) for 3 hours, dissolve in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add 10 mL of 1 mol/L hydrochloric acid TS to each, then add 1 mL of a solution of sodium nitrite (1 in 200) prepared before use, and allow to stand for 5 minutes with occasional shaking. Add 5 mL of ammonium amidosulfate TS, shake well, and allow to stand for 10 minutes. Add 2 mL of *N*-*N*-diethyl-*N*′-1-naphthylethylenediamine oxalate-acetone TS, mix immediately, and add water to make exactly 50 mL. Allow to stand for 2 hours, and determine the absorbances, A_T and A_S, of these solutions at 550 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a blank prepared in the same manner with 5 mL of water in place of the sample solution.

$$\text{Amount (mg) of ethyl aminobenzoate } (C_9H_{11}NO_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ethyl Aminobenzoate RS taken

Containers and storage Containers—Well-closed containers.

Scopolia Extract and Tannic Acid Suppositories

ロートエキス·タンニン坐剤

Method of preparation

Scopolia Extract	0.5 g
Tannic Acid	1 g
Cacao Butter or a suitable base	a sufficient quantity

Prepare 10 suppositories as directed under Suppositories, with the above ingredients.

Description Scopolia Extract and Tannic Acid Suppositories are light brown in color.

Identification **(1)** To 2 Scopolia Extract and Tannic Acid Suppositories add 20 mL of diethyl ether, and dissolve the base of suppositories with shaking for 10 minutes. Shake thoroughly the mixture with 15 mL of water, separate the water layer, and filter. To the filtrate add 10 mL of chloroform, shake well, and separate the chloroform layer. Take 5 mL of the chloroform solution, add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

(2) To 1 mL of the aqueous layer obtained in (1) after extraction with diethyl ether, add 2 drops of iron (III) chloride TS: a bluish-black color develops. Allow to stand: a bluish-black precipitate is formed (tannic acid).

Containers and storage Containers—Well-closed containers.

Scutellaria Root

Scutellariae Radix

オウゴン

Scutellaria Root is the root of *Scutellaria baicalensis* Georgi (*Labiatae*), from which the periderm has been removed.

It contains not less than 10.0% of baicalin ($C_{21}H_{18}O_{11}$: 446.36), calculated on the basis of dried material.

Description Cone-shaped, cylindrical, semitubular or flattened root, 5 – 20 cm in length, 0.5 – 3 cm in diameter; externally yellow-brown, with coarse and marked longitudinal wrinkles, and with scattered scars of lateral root and remains of brown periderm; scars of stem or remains of stem at the crown; sometimes central portion of xylem rotted, often forming a hollow; hard in texture and easily broken; fractured surface fibrous and yellow in color.

Almost odorless; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals 6 – 20 layered cork remaining, cortex composed of parenchyma,

sclerencyma cells scattered in cortex; xylem composed of parenchyma, vessels and small amount of xylem fibers observed in xylem; vessels usually in groups and arranged in tangential direction, radial direction or in irregular form; in case where central portion of xylem rotted, cork layer observed around hollow; parenchyma cells of cortex and xylem contain simple and compound starch grains.

Identification **(1)** Boil gently 0.5 g of pulverized Scutellaria Root with 20 mL of diethyl ether under a reflux condenser on a water bath for 5 minutes, cool, and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol (95), and to 3 mL of the solution add 1 to 2 drops of dilute iron (III) chloride TS: a grayish green color develops, and it changes to purple-brown.

(2) To 1 g of pulverized Scutellaria Root add 25 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin RS or baicalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the dark green spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Scutellaria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Scutellaria Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Assay Weigh accurately about 0.5 g of pulverized Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 5$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust so that the retention time of baicalin is about 6 minutes.

System suitability—

System performance: Dissolve 1 mg of Baicalin RS and 2 mg of methyl parahydroxybenzoate for resolution check in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Scutellaria Root

Scutellariae Radix Pulverata

オウゴン末

Powdered Scutellaria Root is the powder of Scutellaria Root.

It contains not less than 10.0% of baicalin ($C_{21}H_{18}O_{11}$: 446.36), calculated on the basis of dried material.

Description Powdered Scutellaria Root occurs as a yellow-brown powder. It is almost odorless, and has a slight, bitter taste.

Under a microscope <*5.01*>, Powdered Scutellaria Root reveals fragments of parenchyma cells containing small amount of simple and compound starch grains, fragments of short reticulate vessel elements and fusiform, stick-like and ellipsoidal to spherical sclerenchyma cells; also a few fragments of spiral vessels and xylem fibers are observed.

Identification **(1)** Boil gently 0.5 g of Powdered Scutellaria Root with 20 mL of diethyl ether under a reflux condenser on a water bath for 5 minutes, cool, and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol (95), and to 3 mL of the solution add 1 to 2 drops of dilute iron (III) chloride TS: a grayish green color develops, and it changes to purple-brown later.

(2) To 1 g of Powdered Scutellaria Root add 25 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin RS or baicalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solu-

tion and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the dark green spot from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Scutellaria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Scutellaria Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Scutellaria Root does not show crystals of calcium oxalate.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 6.0%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of Powdered Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 5$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust so that the retention time of baicalin is about 6 minutes.

System suitability—

System performance: Dissolve 1 mg of Baicalin RS and 2 mg of methyl parahydroxybenzoate for resolution check in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Senega

Senegae Radix

セネガ

Senega is the root of *Polygala senega* Linné or *Polygala senega* Linné var. *latifolia* Torrey et Gray (*Polygalaceae*).

Description Slender, conical root often branched, 3 – 10 cm in length; main root 0.5 – 1.5 cm in diameter; externally light grayish brown to grayish brown; with many longitudinal wrinkles and sometimes with twisted protruding lines; tuberously enlarged crown, with remains of stems and red buds; branched rootlets twisted; a transverse section reveals grayish brown cortex and yellowish white xylem; usually round, and sometimes cuneate to semicircular; cortex on the opposite side is thickened.

Odor, characteristic, resembling the aroma of methyl salicylate; taste, sweet at first but leaving an acrid taste.

Under a microscope <*5.01*>, a transverse section of the main root reveals a cork layer consisting of several rows of light brown cork cells; secondary cortex composed of parenchyma cells and sieve tubes, traversed by medullary rays, 1 to 3 cells wide; medullary rays on zylem not distinct. Its parenchyma cells contain oil droplets, but starch grains and calcium oxalate crystals are absent.

Identification **(1)** Shake vigorously 0.5 g of pulverized Senega with 10 mL of water: a lasting fine foam is produced.

(2) Shake 0.5 g of pulverized Senega with 30 mL of water for 15 minutes, and filter. Take 1 mL of the filtrate, mix with 50 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum at about 317 nm.

Purity **(1)** Stem—When perform the test of foreign matter <*5.01*>, the amount of the stems contained in Senega does not exceed 2.0%.

(2) Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Senega according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Senega according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <*5.01*>—The amount of foreign matter other than the stems is not more than 1.0%.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Powdered Senega

Senegae Radix Pulverata

セネガ末

Powdered Senega is the powder of Senega.

Description Powdered Senega occurs as a light brown powder, and has a characteristic odor resembling the aroma of methyl salicylate; taste, sweet at first, but later acrid.

Under a microscope ⟨*5.01*⟩, Powdered Senega reveals fragments of pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers with oblique pits; fragments of xylem parenchyma cells with simple pits; fragments of phloem parenchyma containing oily droplets; fragments of exodermis often composed of cells suberized and divided into daughter cells; oily droplets stained red by sudan III TS. The parenchyma cells of Powdered Senega do not contain starch grains and crystals of calcium oxalate.

Identification **(1)** Shake vigorously 0.5 g of Powdered Senega with 10 mL of water: a lasting fine foam is produced.

(2) Shake 0.5 g of Powdered Senega with 30 mL of water for 15 minutes, and filter. Take 1 mL of the filtrate, mix with 50 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum at about 317 nm.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of Powdered Senega according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Powdered Senega according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope ⟨*5.01*⟩, stone cells, starch grains or crystals of calcium oxalate are not observed.

Loss on drying ⟨*5.01*⟩ Not more than 13.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 5.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 2.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 30.0%.

Containers and storage Containers—Well-closed containers.

Senega Syrup

セネガシロップ

Method of preparation

Senega, in moderately fine cutting	40 g
Sucrose	780 g
10 vol% Ethanol	a sufficient quantity
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Add 400 mL of 10 vol% ethanol to Senega, and macerate for one or two days. Filter the extract, wash the residue with a small amount of 10 vol% Ethanol, filter, and combine the filtrate of the extracts and washings until total volume measures about 500 mL. Dissolve Sucrose in the mixture, by warming if necessary, and dilute to 1000 mL with Purified Water or Purified Water in Containers. May be prepared with an appropriate quantity of Ethanol and Purified Water or Purified Water in Containers in place of 10 vol% Ethanol.

Description Senega Syrup is a yellow-brown, viscous liquid. It has a characteristic odor resembling methyl salicylate and a sweet taste.

Identification Add 5 mL of water to 1 mL of Senega Syrup, and shake: lasting small bubbles are produced.

Containers and storage Containers—Tight containers.

Senna Leaf

Sennae Folium

センナ

Senna Leaf is the leaflets of *Cassia angustifolia* Vahl or *Cassia acutifolia* Delile (*Leguminosae*).

It contains not less than 1.0% of total sennosides [sennoside A ($C_{42}H_{38}O_{20}$: 862.74) and sennoside B ($C_{42}H_{38}O_{20}$: 862.74)], calculated on the basis of dried material.

Description Lanceolate to narrow lanceolate leaflets, 1.5 – 5 cm in length, 0.5 – 1.5 cm in width, light grayish yellow to light grayish yellow-green in color; margin entire, apex acute, base asymmetric, petiole short; under a magnifying glass, vein marked, primary lateral veins running toward the apex along the margin and joining the lateral vein above; lower surface having slight hairs.

Odor slight; taste, bitter.

Under a microscope ⟨*5.01*⟩, a transverse section of Senna Leaf reveals epidermis with thick cuticle, with numerous stomata, and with thick-walled, warty unicellular hairs; epidermal cells are often separated into two loculi by a septum which is in parallel with the surface of the leaf, and contain mucilage in the inner loculus; palisade of a single layer under each epidermis; spongy tissue, consisting of 3 to 4 layers, and containing clustered or solitary crystals of calcium oxalate; cells adjacent to vascular bundle, forming crystal cell rows.

Identification **(1)** Macerate 0.5 g of pulverized Senna Leaf in 10 mL of diethyl ether for 2 minutes, and filter. Add

5 mL of ammonia TS to the filtrate: a yellow-red color is produced in the water layer. To the residue of maceration add 10 mL of water, and macerate for 2 minutes. Filter, and add 5 mL of ammonia TS: a yellow-red color is produced in the water layer.

(2) To 2 g of pulverized Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the aqueous layer with undissolved sodium chloride, and adjust to pH 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use the separated tetrahydrofuran layer as the sample solution. Separately, dissolve 1 mg of Sennoside A RS or sennoside A for thin-layer chromatography in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red fluorescent spot obtained from the standard solution.

Purity **(1)** Rachis and fruit—When perform the test of foreign matter <*5.01*>, the amount of rachis and fruits contained in Senna Leaf does not exceed 5.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than rachis and fruits contained in Senna Leaf does not exceed 1.0%.

(3) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 12.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of pulverized Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of diluted methanol (7 in 10), shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure once more, combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Sa}, of sennoside A, and the peak areas, A_{Tb} and A_{Sb}, of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennosides.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_{Sa} \times A_{Ta}/A_{Sa} \times 1/4$$

$$\text{Amount (mg) of sennoside B } (C_{42}H_{38}O_{20}) = M_{Sb} \times A_{Tb}/A_{Sb} \times 1/2$$

M_{Sa}: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

M_{Sb}: Amount (mg) of Sennoside B RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet aborption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0) (1 in 10) and acetonitrile (17:8).

Flow rate: Adjust so that the retention time of sennoside A is about 26 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Senna Leaf

Sennae Folium Pulveratum

センナ末

Powdered Senna Leaf is the powder of Senna Leaf. It contains not less than 1.0% of total sennosides [sennoside A ($C_{42}H_{38}O_{20}$: 862.74) and sennoside B ($C_{42}H_{38}O_{20}$: 862.74)], calculated on the basis of dried material.

Description Powdered Senna Leaf occurs as a light yellow to light grayish yellow-green powder. It has a slight odor and a bitter taste.

Under a microscope <*5.01*>, Powdered Senna Leaf reveals fragments of vessels and vein tissue accompanied with crystal cell rows; fragments of thick-walled, bent, unicellular hairs; fragments of palisade and spongy tissue; clustered and solitary crystals of calcium oxalate, 10 to 20 μm in diameter.

Identification **(1)** Macerate 0.5 g of Powdered Senna Leaf in 10 mL of diethyl ether for 2 minutes, and filter. Add

5 mL of ammonia TS to the filtrate: a yellow-red color is produced in the water layer. To the residue of maceration add 10 mL of water, and macerate for 2 minutes. Filter, and add 5 mL of ammonia TS: a yellow-red color is produced in the water layer.

(2) To 2 g of Powdered Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the aqueous layer with undissolved sodium chloride, and adjust to pH 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use the separated tetrahydrofuran layer as the sample solution. Separately, dissolve 1 mg of Sennoside A RS or sennoside A for thin-layer chromatography in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red fluorescent spot obtained from the standard solution.

Purity **(1)** Foreign matter <*5.01*>—Under a microscope, stone cells and thick fibers are not observable.

(2) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 12.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of Powdered Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of diluted methanol (7 in 10), shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure once more, combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Sa}, of sennoside A, and the peak areas, A_{Tb} and A_{Sb}, of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennoside.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_{Sa} \times A_{Ta}/A_{Sa} \times 1/4$$

$$\text{Amount (mg) of sennoside B } (C_{42}H_{38}O_{20}) = M_{Sb} \times A_{Tb}/A_{Sb} \times 1/2$$

M_{Sa}: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

M_{Sb}: Amount (mg) of Sennoside B RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution (pH 5.0) (1 in 10) and acetonitrile (17:8).

Flow rate: Adjust so that the retention time of sennoside A is about 26 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Sesame

Sesami Semen

ゴマ

Sesame is the seed of *Sesamum indicum* Linné (*Pedaliaceae*).

Description Ovate to spatulate seed, 3 – 4 mm in length, about 2 mm in width, about 1 mm in thickness; externally dark brown to black, rarely light brown to brown. Under a magnifying glass, thin ridges are observed on edges. 100 seeds weigh about 0.2 – 0.3 g.

Odorless; taste, slightly sweet and oily.

Under a microscope <*5.01*>, transverse section reveals a seed coat consisting of palisade epidermis and flattened parenchyma; in the interior, endosperm and cotyledon; epidermal cells contain orbicular crystals of calcium oxalate and black pigment; parenchymatous cells of endosperm and cotyledon contain oil drops and aleurone grains.

Identification Grind a suitable amount of Sesame. To 1.0 g of the ground add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of sesamin for thin-layer chromatography in 5 mL of methanol, and use this solution

as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (10:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the brown spot obtained from the standard solution.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Sesame Oil

Oleum Sesami

ゴマ油

Sesame Oil is the fixed oil obtained from the seeds of *Sesamum indicum* Linné (*Pedaliaceae*).

Description Sesame Oil is a clear, pale yellow oil. It is odorless or has a faint, characteristic odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether.
It is slightly soluble in ethanol (95).
It congeals between 0°C and −5°C.
Congealing point of the fatty acids: 20 – 25°C

Identification To 1 mL of Sesame Oil add 0.1 g of sucrose and 10 mL of hydrochloric acid, and shake for 30 seconds: the acid layer becomes light red and changes to red on standing.

Specific gravity ⟨*1.13*⟩ d_{25}^{25}: 0.914 – 0.921

Acid value ⟨*1.13*⟩ Not more than 0.2.

Saponification value ⟨*1.13*⟩ 187 – 194

Unsaponifiable matters ⟨*1.13*⟩ Not more than 2.0%.

Iodine value ⟨*1.13*⟩ 103 – 118

Containers and storage Containers—Tight containers.

Shakuyakukanzoto Extract

芍薬甘草湯エキス

Shakuyakukanzoto Extract contains not less than 50 mg and not more than 150 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 50 mg and not more than 150 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Peony Root	6 g	5 g
Glycyrrhiza	6 g	5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Shakuyakukanzoto Extract occurs as a light brown powder or brown viscous extract. It has slightly an odor, and a sweet taste.

Identification **(1)** Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Peony Root).

(2) Shake 0.5 g of dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying ⟨*2.41*⟩ The dry extract: Not more than 8.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash ⟨*5.01*⟩ Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Paeoniflorin—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to 0.2 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and

standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to 0.2 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Shimbuto Extract

真武湯エキス

Shimbuto Extract contains not less than 26 mg and not more than 78 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 0.8 g of Ginger) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 1 g of Ginger) or not less than 0.9 mg and not more than 3.6 mg (for preparation prescribed 1.5 g of Ginger) of [6]-gingerol, and not less than 0.7 mg (for preparation prescribed 1 g of Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride) or not less than 0.2 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride) or not less than 0.1 mg (for preparation prescribed 1 g of Powdered Processed Aconite Root 2) of total alkaloids (as benzoylmesaconine hydrochloride and 14-benzoylhypacomine hydrochloride) or not less than 0.1 mg (for preparation prescribed 0.5 g of Powdered Processed Aconite Root 1) of total alkaloids (as benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or as benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Poria Sclerotium	5 g	5 g	5 g	4 g
Peony Root	3 g	3 g	3 g	3 g
Atractylodes Rhizome	3 g	—	3 g	—
Atractylodes Lancea Rhizome	—	3 g	—	3 g
Ginger	1 g	1 g	0.8 g	1.5 g
Processed Aconite Root (Processed Aconite Root 1)	1 g	—	—	—
Powdered Processed Aconite Root (Powdered Processed Aconite Root 1)	—	1 g	—	0.5 g
Powdered Processed Aconite Root (Powdered Processed Aconite Root 2)	—	—	1 g	—

Prepare a dry extract as directed under Extracts, according to the prescription 1) to 4), using the crude drugs shown above.

Description Shimbuto Extract occurs as light yellow-brown to brown powder. It has a characteristic odor and a hot and bitter taste.

Identification **(1)** To 2.0 g of Shimbuto Extract, add 10

mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybezaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Peony Root).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.4. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heated at 105°C for 5 minutes and allowed to cool: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-green spot from the standard solution (Ginger).

(5) To 3.0 g of Shimbuto Extract, add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the supernatant liquid under reduced pressure, add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellow-brown spot from the standard solution (Processed Aconite Root or Powdered Processed Aconite Root).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Shimbuto Extract as directed in the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Shimbuto Extract according to Method 3, and perform the test (not more than 3 ppm).

(3) Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately 1.0 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of aconitum diester alkaloids standard solution for purity, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the heights of the peaks corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the sample solution are not higher than the respective heights corresponding to aconitine, jesaconitine, hypaconitine and mesaconitine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica

gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of mesaconitine is about 31 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 7.0% (1 g, 105°C, 5 hours).

Total ash ⟨*5.01*⟩ Not more than 10.0%.

Assay (1) Paeoniflorin—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water ⟨*2.48*⟩ by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(2) [6]-gingerol—Weigh accurately about 0.5 g of Shimbuto Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of [6]-gingerol for assay, dissolve in diluted methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of [6]-gingerol in each solution.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of [6]-gingerol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (620:380:1).

Flow rate: 1.0 mL per minute (the retention time of [6]-gingerol is about 15 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of [6]-gingerol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of [6]-gingerol is not more than 1.5%.

(3) Total alkaloids—Weigh accurately about 1 g of Shimbuto Extract, add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the upper layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the water layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the supernatant liquids, and evaporate to dryness under reduced pressure. Dissolve the residue with a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and the aconitum monoester alkaloids standard solution TS for assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_{TM} and A_{SM}, A_{TH} and A_{SH}, as well as A_{TA} and A_{SA}, in each solution, respectively.

$$\text{Amount (mg) of benzoylmesaconine hydrochloride} = C_{SM} \times A_{TM}/A_{SM} \times 10$$

$$\text{Amount (mg) of benzoylhypaconine hydrochloride} = C_{SH} \times A_{TH}/A_{SH} \times 10$$

$$\text{Amount (mg) of 14-anisoylaconine hydrochloride} = C_{SA} \times A_{TA}/A_{SA} \times 10$$

C_{SM}: Concentration (mg/mL) of benzoylmesaconine hydrochloride for assay in aconitum monoester

alkaloids standard solution TS for assay

C_{SH}: Concentration (mg/mL) of benzoylhypaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

C_{SA}: Concentration (mg/mL) of 14-anisoylaconine hydrochloride for assay in aconitum monoester alkaloids standard solution TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute (the retention time of benzoylmesaconine is about 15 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the aconitum monoester alkaloids standard solution TS for assay under the above operating conditions, the relative standard deviation of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Shosaikoto Extract

小柴胡湯エキス

Shosaikoto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Bupleurum Root	7 g	6 g
Pinellia Tuber	5 g	5 g
Ginger	1 g	1 g
Scutellaria Root	3 g	3 g
Jujube	3 g	3 g
Ginseng	3 g	3 g
Glycyrrhiza	2 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Shosaikoto Extract occurs as a light brown to grayish brown powder or black-grayish brown viscous extract. It has a slight odor, and a sweet first then slightly pungent and bitter taste.

Identification **(1)** Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the diethyl ether under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 15 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Ginger).

(3) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, air-dry the plate, and spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Scutellaria Root).

(4) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_1 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution

on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Ginseng).

(5) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to about 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatog-

raphy <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Shoseiryuto Extract

小青竜湯エキス

Shoseiryuto Extract contains not less than 10 mg and not more than 30 mg of the total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 26 mg and not more than 78 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Ephedra Herb	3 g	3 g
Peony Root	3 g	3 g
Processed Ginger	3 g	—
Ginger	—	3 g
Glycyrrhiza	3 g	3 g
Cinnamon Bark	3 g	3 g
Asiasarum Root	3 g	3 g
Schisandra Fruit	3 g	3 g
Pinellia Tuber	6 g	6 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Shoseiryuto Extract occurs as a light brown to brown powder or blackish brown viscous extract. It has a characteristic odor and a acid first then pungent taste.

Identification **(1)** Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid(100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

(2) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution (Peony Root).

(3) For preparation prescribed Processed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 1 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Processed Ginger).

(4) For preparation prescribed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the

spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green spot obtained from the standard solution (Ginger).

(5) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(6) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. Previously, add water up to the base point line of the graduated tube of the apparatus, and then add 2 mL of hexane. After heating under reflux for about 1 hour, take the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane and shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(7) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of asarinin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Asiasarum Root).

(8) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot obtained from the standard solution (Schisandra Fruit).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Cadmium—Take 5.0 g of the dry extract (or an amount of the viscous extract, equivalent to 5.0 g of the dried substance) in a platinum, quartz or porcelain crucible, heat weakly, then incinerate by ignition at 450°C. After cooling, add a small amount of 2 mol/L nitric acid TS to the residue, filter if necessary, wash the crucible several times with small portions of 2 mol/L nitric acid TS, combine the filtrate and washings, add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, to 5.0 mL of Standard Cadmium Solution add 2 mol/L nitric acid TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*>: the absorbance of the sample solution is not more than that of the standard solution (not more than 1 ppm).

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Cadmium hollow-cathode lamp.
Wavelength: 228.8 nm.

(3) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 12.0%, calculated on the dried basis.

Assay **(1)** Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g

of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained from the sample solution, and the peak area, A_S, of ephedrine obtained from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS, (separately determined the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS, (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of dilute acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Simple Ointment

単軟膏

Method of preparation

Yellow Beeswax	330 g
Fixed oil	a sufficient quantity
	To make 1000 g

Prepare as directed under Ointments, with the above ingredients.

Description Simple Ointment is yellow in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Sinomenium Stem and Rhizome

Sinomeni Caulis et Rhizoma

ボウイ

Sinomenium Stem and Rhizome is the climbing stem and rhizome of *Sinomenium acutum* Rehder et Wilson (*Menispermaceae*), usually cut transversely.

Description Round or elliptic sections, 0.2 – 0.4 cm in thickness, 1 – 4.5 cm in diameter; cortex on both fractured surfaces, light brown to dark brown; in xylem, grayish brown vessel portions and dark brown medullary rays lined alternately and radially; flank, dark gray, with longitudinal wrinkles and warty protrusions.

Almost odorless; taste, bitter.

Under a microscope ⟨*5.01*⟩, a transverse section reveals extremely thick-walled stone cells in primary cortex and pericycle; irregular-sized vessels lined nearly stepwise in the vessel portion; cells of medullary ray mostly not lignified, and extremely thick-walled and large stone cells scattered here and there; primary cortex containing needle crystals of calcium oxalate; medullary rays containing starch gains, mainly simple grain, 3 – 20 μm in diameter, and small needle crystals of calcium oxalate.

Identification To 0.5 g of pulverized Sinomenium Stem and Rhizome add 10 mL of dilute acetic acid, heat for 2 minutes on a water bath with frequent shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: an orange-yellow precipitate is immediately produced.

Total ash ⟨*5.01*⟩ Not more than 7.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Smilax Rhizome

Smilacis Rhizoma

サンキライ

Smilax Rhizome is the rhizome of *Smilax glabra* Roxburgh (*Liliaceae*).

Description Flattened and irregular cylindrical tuber, often with node-like branches; usually 5 – 15 cm in length, 2 – 5 cm in diameter; the outer surface grayish yellow-brown to yellow-brown, and the upper surface scattered with knotty remains of stem; transverse section irregular elliptical to obtuse triangular, consisting of extremely thin cortical layer and mostly of stele.

Odor, slight; almost tasteless.

Under a microscope ⟨*5.01*⟩, a transverse section reveals a 2- to 3-cell-wide cork layer, with extremely narrow cortical layer, usually consisting of a 2- to 4-cell-wide, thick-walled parenchyma cells, showing large mucilage cells here and there; mucilage cell containing raphides of calcium oxalate; stele consisting chiefly of parenchyma cells, and scattered with vascular bundles; parenchyma cells containing starch grains composed mostly of simple grains, 12 – 36 μm in diameter, and sometimes mixed with 2- to 4-compound grains.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Smilax Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Smilax Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 5.0%.

Containers and storage Containers—Well-closed containers.

Powdered Smilax Rhizome

Smilacis Rhizoma Pulveratum

サンキライ末

Powdered Smilax Rhizome is the powder of Smilax Rhizome.

Description Powdered Smilax Rhizome occurs as a light yellow-brown powder, and has a slight odor, and is practically tasteless.

Under a microscope ⟨*5.01*⟩, Powdered Smilax Rhizome reveals starch grains and fragments of parenchyma cells containing them; fragments of raphides of calcium oxalate contained in mucilage masses; fragments of lignified parenchyma cells of cortical layer; fragments of cork cells and scalariform vessels; starch grains composed mostly of simple grains, and mixed with a few 2- to 4-compound grains 12 – 36 μm in diameter.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of Powdered Smilax Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Powdered Smilax Rhizome according to Method 4, and

perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Smilax Rhizome does not show a large quantity of stone cells or thick-walled fibers.

Total ash <*5.01*> Not more than 5.0%.

Containers and storage Containers—Well-closed containers.

Sodium Bicarbonate and Bitter Tincture Mixture

苦味重曹水

Method of preparation

Sodium Bicarbonate	30 g
Bitter Tincture	20 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare before use, with the above ingredients.

Description Sodium Bicarbonate and Bitter Tincture Mixture is a clear, yellowish liquid, having a bitter taste.

Containers and storage Containers—Tight containers.

Anhydrous Sodium Sulfate

Sal Mirabilis Anhydricus

無水ボウショウ

Na_2SO_4: 142.04
[*7757-82-6*]

Anhydrous Sodium Sulfate is mainly sodium sulfate (Na_2SO_4) containing no water of crystallization.

It, when dried, contains not less than 99.0% of sodium sulfate (Na_2SO_4).

Description Anhydrous Sodium Sulfate occurs as white, crystals or powder. It is odorless and has a salty and slightly bitter taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** A solution of Anhydrous Sodium Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

(2) A solution of Anhydrous Sodium Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for sulfate.

Purity **(1)** Acidity or alkalinity—Dissolve 0.5 g of Anhydrous Sodium Sulfate in 5 mL of freshly boiled and cooled water: the solution is clear and colorless, and neutral.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of previously dried Anhydrous Sodium Sulfate. Prepare the control solution with 0.5 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of previously dried Anhydrous Sodium Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of previously dried Anhydrous Sodium Sulfate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 0.5% (4 g, 105°C, 4 hours).

Assay Weigh accurately about 0.4 g of previously dried Anhydrous Sodium Sulfate, dissolve in 200 mL of water, add 1 mL of hydrochloric acid, boil, and gradually add 8 mL of barium chloride TS. Heat the solution in a water bath for 1 hour. After cooling, filter through a filter paper for quantitative analysis (No.5C), wash the residue on the filter paper with water until the washings do not give the turbidity with silver nitrate TS. After drying the residue together with the paper, ignite at 500 – 800°C to constant mass, and weigh the mass of the residue as the amount of barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of sodium sulfate (Na_2SO_4)
= amount (mg) of barium sulfate ($BaSO_4$) × 0.609

Containers and storage Containers—Well-closed containers.

Sodium Sulfate Hydrate

Sal Mirabilis

ボウショウ

$Na_2SO_4.10H_2O$: 322.19
[*7727-73-3*]

Sodium Sulfate Hydrate is mainly decahydrate of sodium sulfate (Na_2SO_4).

It, when dried, contains not less than 99.0% of sodium sulfate (Na_2SO_4: 142.04).

Description Sodium Sulfate Hydrate occurs as colorless or white, crystals or crystalline powder. It is odorless and has a cooling and salty taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is quickly efflorescent in air, soluble in its own water of crystallization at about 33°C and lost the water at 100°C.

Identification **(1)** A solution of Sodium Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

(2) A solution of Sodium Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for sulfate.

Purity **(1)** Acidity or alkalinity—Dissolve 0.5 g of Sodium Sulfate Hydrate in 5 mL of freshly boiled and cooled water: the solution is clear and colorless, and neutral.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of previously dried Sodium Sulfate Hydrate. Prepare the control solution with 0.5 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of previously dried Sodium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of previously dried Sodium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <*2.41*> 51.0 – 57.0% (4 g, 105°C, 4 hours).

Assay Weigh accurately about 0.4 g of previously dried Sodium Sulfate Hydrate, dissolve in 200 mL of water, add 1 mL of hydrochloric acid, boil, and gradually add 8 mL of barium chloride TS. Heat the solution in a water bath for 1 hour. After cooling, filter through a filter paper for quantitative analysis (No.5C), wash the residue on the filter paper with water until the washings do not give the turbidity with silver nitrate TS. After drying the residue together with the paper, ignite at 500 – 800°C to constant mass, and weigh the mass of the residue as the amount of barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of sodium sulfate (Na_2SO_4)
= amount (mg) of barium sulfate ($BaSO_4$) × 0.609

Containers and storage Containers—Well-closed containers.

Sophora Root

Sophorae Radix

クジン

Sophora Root is the root of *Sophora flavescens* Aiton (*Leguminosae*) or often such root from which the periderm has been removed.

Description Cylindrical root, 5 – 20 cm in length, 2 – 3 cm in diameter; externally dark brown to yellow-brown, with distinct longitudinal wrinkles, and with laterally extended lenticels; root without periderm, externally yellowish white, with somewhat fibrous surface; the transversely cut surface, light yellow-brown; cortex, 0.1 – 0.2 cm in thickness, slightly tinged with dark color near cambium, forming a crack between xylem.

Odor, slight; taste, extremely bitter and lasting.

Identification To 0.5 g of pulverized Sophora Root add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes with occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: an orange-yellow precipitate is produced immediately.

Purity **(1)** Stem—When perform the test of foreign matter ⟨*5.01*⟩, the amount of its stems contained in Sophora Root does not exceed 10.0%.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Sophora Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Sophora Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter ⟨*5.01*⟩—The amount of foreign matter other than stems is not more than 1.0%.

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Sophora Root

Sophorae Radix Pulverata

クジン末

Powdered Sophora Root is the powder of Sophora Root.

Description Powdered Sophora Root occurs as a light brown powder. It has a slight odor, and an extremely bitter and lasting taste.

Under a miscroscope ⟨*5.01*⟩, Powdered Sophora Root reveals mainly starch grains and fragments of parenchyma cells containing them, fibers, bordered pitted vessels, reticulate vessels; a few fragments of corky tissue and solitary crystals of calcium oxalate. Starch grains usually composed of 2- to 4-compound grains 15 – 20 μm in diameter, and simple grains 2 – 5 μm in diameter.

Identification To 0.5 g of Powdered Sophora Root add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes while occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: an orange-yellow precipitate is produced immediately.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of Powdered Sophora Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Powdered Sophora Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 6.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Soybean Oil

Oleum Sojae

ダイズ油

Soybean Oil is the fixed oil obtained from the seeds of *Glycine max* Merrill (*Leguminosae*).

Description Soybean Oil is a clear, pale yellow oil. It is odorless or has a slight odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It congeals between −10°C and −17°C.

Congealing point of the fatty acids: 22 – 27°C

Specific gravity ⟨*1.13*⟩ d_{25}^{25}: 0.916 – 0.922

Acid value ⟨*1.13*⟩ Not more than 0.2.

Saponification value ⟨*1.13*⟩ 188 – 195

Unsaponifiable matter ⟨*1.13*⟩ Not more than 1.0%.

Iodine value ⟨*1.13*⟩ 126 – 140

Containers and storage Containers—Tight containers.

Sweet Hydrangea Leaf

Hydrangeae Dulcis Folium

アマチャ

Sweet Hydrangea Leaf is the leaf and twig of *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (*Saxifragaceae*), usually crumpled.

Description Usually wrinkled and contracted leaf, dark green to dark yellow-green in color. When soaked in water and smoothed out, it is lanceolate to acuminately ovate, 5 – 15 cm in length, 2 – 10 cm in width; margin serrated, base slightly wedged; coarse hair on both surfaces, especially on the veins; lateral veins not reaching the margin but curving upwards and connecting with each other; petiole short and less than one-fifth of the length of lamina.

Odor, slight; taste, characteristically sweet.

Identification To 1.0 g of pulverized Sweet Hydrangea Leaf add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of sweet hydrangea leaf dihydroisocoumarin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, hexane and formic acid (5:5:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two of the spots among the several spots obtained from the sample solution have the same color tone and *R*f value with the spots obtained from the standard solution.

Purity **(1)** Stem—When perform the test of foreign matter <*5.01*>, the amount of stems contained in Sweet Hydrangea Leaf does not exceed 3.0%.

(2) Foreign matter <*5.01*>—The amount of foreign matter other than stems contained in Sweet Hydrangea Leaf does not exceed 1.0%.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 12.0%.

Acid-insoluble ash <*5.01*> Not more than 2.5%.

Containers and storage Containers—Well-closed containers.

Powdered Sweet Hydrangea Leaf

Hydrangeae Dulcis Folium Pulveratum

アマチャ末

Powdered Sweet Hydrangea Leaf is the powder of Sweet Hydrangea Leaf.

Description Powdered Sweet Hydrangea Leaf occurs as a dark yellow-green powder, and has a faint odor and a characteristic, sweet taste.

Under a microscope <*5.01*>, Powdered Sweet Hydrangea Leaf reveals fragments of epidermis with wavy lateral cell wall; stomata with two subsidiary cells; unicellular and thin-walled hair with numerous protrusions of the surface, 150 – 300 μm in length; fragments of palisade tissue and spongy tissue; fragments of vascular bundle and mucilage cells containing raphides of calcium oxalate 50 – 70 μm in length.

Identification Mix 0.5 g of Powdered Sweet Hydrangea Leaf with 8 mL of a mixture of diethyl ether and petroleum ether (1:1), shake well, filter, and evaporate the filtrate to dryness. Dissolve the residue in 1 mL of dilute ethanol, and add 1 drop of dilute iron (III) chloride TS: a red-purple color develops, which disappears on the addition of 2 to 3 drops of dilute sulfuric acid.

Purity Foreign matter <*5.01*>—Under a microscope, Powdered Sweet Hydrangea Leaf does not show stone cells, a large quantity of fibers or starch grains.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 12.0%.

Acid-insoluble ash <*5.01*> Not more than 2.5%.

Containers and storage Containers—Well-closed containers.

Swertia Herb

Swertiae Herba

センブリ

Swertia Herb is the whole herb of *Swertia japonica* Makino (*Gentianaceae*) collected during the blooming season.

It contains not less than 2.0% of swertiamarin ($C_{16}H_{22}O_{10}$: 374.34), calculated on the basis of dried material.

Description Herb, 10 – 50 cm in length, having flowers, opposite leaves, stems, and, usually, with short, lignified roots; stems square, about 2 mm in diameter, often with branches; the leaves and stems dark green to dark purple or yellow-brown in color; the flowers white to whitish, and the roots yellowbrown. When smoothed by immersing in water, leaves, linear or narrow lanceolate, 1 – 4 cm in length, 0.1 – 0.5 cm in width, entire, and sessile; corolla split deeply as five lobes; the lobes narrow, elongated ellipse shape, and under a magnifying glass, with two elliptical nectaries juxtaposed at the base of the inner surface; the margin of lobe resembles eyelashes; the five stamens grow on the tube of the corolla and stand alternately in a row with corolla-lobes; peduncle distinct.

Odor, slight; taste, extremely bitter and persisting.

Identification To 1 g of pulverized Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin RS or swertiamarin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one of the spot among the several spots obtained from the sample solution

has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity Foreign matter <*5.01*>—The amount of straw and other foreign matters contained in Swertia Herb is not more than 1.0%.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 20.0%.

Assay Weigh accurately about 1 g of moderately fine powder of Swertia Herb in a glass-stoppered centrifuge tube, add 40 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Swertiamarin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in methanol to make exactly 20 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of swertiamarin in each solution.

$$\text{Amount (mg) of swertiamarin } (C_{16}H_{22}O_{10}) = M_S \times A_T/A_S \times 5$$

M_S: Amount (mg) of Swertiamarin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (91:9).

Flow rate : Adjust so that the retention time of swertiamarin is about 12 minutes.

System suitability—

System performance: Dissolve 1 mg each of Swertiamarin RS and theophylline in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, theophylline and swertiamarin are eluted in this order with the resolution of these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of swertiamarin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Swertia Herb

Swertiae Herba Pulverata

センブリ末

Powdered Swertia Herb is the powder of Swertia Herb.

It contains not less than 2.0% of swertiamarin ($C_{16}H_{22}O_{10}$: 374.34), calculated on the basis of dried material.

Description Powdered Swertia Herb occurs as a grayish yellow-green to yellow-brown powder. It has a slight odor, and extremely bitter, persistent taste.

Under a microscope <*5.01*>, Powdered Swertia Herb reveals xylem tissues with fibers (components of stems and roots); assimilation tissues (components of leaves and calyces); striated epidermis (components of stems and peduncles); tissues of corollas and filaments with spiral vessels; cells of anthers and their inner walls; spherical pollen grains with granular patterns (components of flowers), about 30 μm in diameter; starch grains are simple grain, about 6 μm in diameter, and very few.

Identification To 1 g of Powdered Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin RS or swertiamarin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity Foreign matter—Under a microscope <*5.01*>, crystals of calcium oxalate, a large quantity of starch grains and groups of stone cells are not observable.

Loss on drying <*5.01*> Not more than 12.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 20.0%.

Assay Weigh accurately about 1 g of Powdered Swertia Herb in a glass-stoppered centrifuge tube, add 40 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Swertiamarin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in methanol to make exactly 20 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard

solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of swertiamarin in each solution.

$$\text{Amount (mg) of swertiamarin } (C_{16}H_{22}O_{10}) = M_S \times A_T/A_S \times 5$$

M_S: Amount (mg) of Swertiamarin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (91:9).

Flow rate: Adjust so that the retention time of swertiamarin is about 12 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sweriamarin RS and theophylline in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, theophylline and swertiamarin are eluted in this order with the resolution of these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of swertiamarin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Swertia and Sodium Bicarbonate Powder

センブリ·重曹散

Method of preparation

Powdered Swertia Herb	30 g
Sodium Bicarbonate	700 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Swertia and Sodium Bicarbonate Powder occurs as a light grayish yellow powder, having a bitter taste.

Identification **(1)** To 10 g of Swertia and Sodium Bicarbonate Powder add 10 mL of ethanol (95), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Swertiamarin RS or swertiamarin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 30 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Proceed as directed in the Identification under Powdered Swertia Herb.

(2) To 0.5 g of Swertia and Sodium Bicarbonate Powder add 10 mL of water. After stirring, centrifuge the mixture with 500 revolutions per minute. Smear, using a small glass rod, the slide glass with a small amount of the precipitate, add 1 drop of a mixture of water and glycerin (1:1), and put a cover glass on it so that the tissue section spreads evenly without overlapping each other, taking precaution against inclusion of bubbles, and use this as the preparation for microscopic examination. If the precipitate separates into two layers, proceed with the upper layer in the same manner, and use as the preparation for microscopic examination. Heat the preparation for microscopic examination in a short time: the preparation reveals the yellow-green to yellow-brown, approximately spherical pollen grains with granular patterns under a microscope <*5.01*>. The pollen grains are 25 – 34 μm in diameter.

(3) The supernatant liquid obtained in (2) by centrifuging responds to the Qualitative Tests <*1.09*> (1) for bicarbonate.

Containers and storage Containers—Well-closed containers.

Toad Cake

Bufonis Crustum

センソ

Toad Cake is the parotoid secretion of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider (*Bufonidae*).

When dried, it contains not less than 5.8% of bufo steroid.

Description A round disk with slightly dented bottom and protuberant surface, about 8 cm in diameter, about 1.5 cm in thickness, the mass of one disk being about 80 to 90 g; or a round disk with almost flattened surfaces on both sides, about 3 cm in diameter, and about 0.5 cm in thickness, the mass of one disk being about 8 g; externally red-brown to blackish brown, somewhat lustrous, approximately uniform and horny, hard in texture, and difficult to break; fractured surface nearly flat, and edges of broken pieces red-brown and translucent.

Odorless; taste, bitter and irritating, followed a little later by a lasting sensation of numbness.

Identification To 0.3 g of pulverized Toad Cake add 3 mL of acetone, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of resibufogenin for thin-layer chromatography in 2 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane and acetone (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Total ash <*5.01*> Not more than 5.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of pulverized Toad

Cake, previously dried in a desiccator (silica gel) for 24 hours, add 50 mL of methanol, heat under a reflux condenser on a water bath for 1 hour, cool, and filter. Wash the residue with 30 mL of methanol, and combine the washing and filtrate. To this solution add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg, about 20 mg and about 20 mg of bufalin for assay, cinobufagin for assay and resibufogenin for assay, respectively, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Calculate the ratios, Q_{TB} and Q_{SB}, of the peak area of bufalin, Q_{TC} and Q_{SC}, of the peak area of cinobufagin, and Q_{TR} and Q_{SR}, of the peak area of resibufogenin, respectively, to that of the internal standard, and designate the total amount as an amount of bufosteroid.

Amount (mg) of bufalin = $M_{SB} \times Q_{TB}/Q_{SB}$

Amount (mg) of cinobufagin = $M_{SC} \times Q_{TC}/Q_{SC}$

Amount (mg) of resibufogenin = $M_{SR} \times Q_{TR}/Q_{SR}$

M_{SB}: Amount (mg) of bufalin for assay taken
M_{SC}: Amount (mg) of cinobufagin for assay taken
M_{SR}: Amount (mg) of resibufogenin for assay taken

Internal standard solution—A solution of indometacin in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 300 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (11:9).

Flow rate: Adjust so that the retention time of the internal standard is 16 to 19 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin, resibufogenin and the internal standard in this order, and clearly dividing each peak.

Containers and storage Containers—Well-closed containers.

Tokakujokito Extract

桃核承気湯エキス

Tokakujokito Extract contains not less than 38 mg and not more than 152 mg of amygdalin, not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, not less than 3 mg of sennosides A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 9 mg of rhein, and not less than 13 mg and not more than 39 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Peach Kernel	5 g	5 g	5 g
Cinnamon Bark	4 g	4 g	4 g
Rhubarb	3 g	3 g	3 g
Glycyrrhiza	1.5 g	1.5 g	1.5 g
Anhydrous Sodium Sulfate	1 g	0.9 g	
Sodium Sulfate			2 g

Prepare a dry extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above. Or, prepare a dry extract by adding Light Anhydrous Silicic Acid to an extractive, prepared as directed under Extracts, according to the prescription 2), using the crude drugs shown above.

Description Tokakujokito Extract occurs as a greenish yellow-brown to dark brown powder. It has characteristic odor and, salty, slightly astringent, and then slightly sweet taste.

Identification **(1)** To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green-brown spot obtained from the standard solution (Peach Kernel).

(2) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of Tokakujokito Extract in a 300-mL of hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of

the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution .

(ii) To 2.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(3) To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

(4) To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Tokakujokito Extract as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Tokakujokito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 8.0% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not less than 20.0% and more than 40.0%.

Assay **(1)** Amygdalin—Weigh accurately about 0.5 g of Tokakujokito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, elute through a column prepared previously with 2 g of polyamide for column chromatography using water to make exactly 20 mL of effluent , and use this solution as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of amygdalin for assay taken

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

(2) (*E*)-Cinnamic acid—Conduct this procedure using light-resistant vessels. Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of diethyl ether and 10 mL of water, shake for 10 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diethyl ether, proceed in the same manner as above, and repeat this procedure two more times. Combine all the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-cinnamic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-cinnamic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of (*E*)-cinnamic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (800:200:1).

Flow rate: 1.0 mL per minute (the retention time of (*E*)-cinnamic acid is about 22 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-cinnamic acid is not more than 1.5%.

(3) Sennoside A—Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, remove the upper layer, then add 20 mL of ethyl acetate, proceed in the same manner as above, and remove the upper layer. To the water layer obtained add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Sennoside A RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of sennoside A in each solution.

$$\text{Amount (mg) of sennoside A } (C_{42}H_{38}O_{20}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Sennoside A RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (840:160:1).

Flow rate: 1.0 mL per minute (the retention time of sennoside A is about 20 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sennoside A are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

(4) Rhein—Weigh accurately about 0.5 g of Tokakujokito Extract, add 80 mL of water, shake, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 20 mL of iron (III) chloride TS, heat in a water bath under a reflux condenser for 30 minutes, add 3 mL of hydrochloric acid, and heat in addition under a reflux condenser for 30 minutes. After cooling, extract three times with 25 mL each of diethyl ether, combine all the diethyl ether layers, evaporate the solvent under reduced pressure, dissolve the residue to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of rhein for assay, and dissolve in acetone to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of rhein in each solution.

$$\text{Amount (mg) of rhein} = M_S \times A_T/A_S \times 4/5$$

M_S: Amount (mg) of rhein for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL per minute (the retention time of rhein is about 17 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rhein are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rhein is not more than 1.5%.

(5) Glycyrrhizic acid—Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, remove the upper layer, then add 20 mL of ethyl acetate, proceed in the same manner as above, and remove the upper layer. To resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Tokishakuyakusan Extract

当帰芍薬散エキス

Tokishakuyakusan Extract contains not less than 0.6 mg and not more than 2.4 mg of (*E*)-ferulic acid, not less than 34 mg and not more than 102 mg (for preparation prescribed 4 g of Peony Root) or not less than 51 mg and not more than153 mg (for preparation prescribed 6 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.4 mg of atractylenolide III (for preparation prescribed Atractylodes Rhizome) or not less than 0.1 mg of atractylodin (for preparation prescribed Atractylodes Lancea Rhizome), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Japanese Angelica Root	3 g	3 g	3 g	3 g
Cnidium Rhizome	3 g	3 g	3 g	3 g
Peony Root	6 g	6 g	4 g	4 g
Poria Sclerotium	4 g	4 g	4 g	4 g
Atractylodes Rhizome	4 g	4 g	4 g	—
Atractylodes Lancea Rhizome	—	—	—	4 g
Alisma Tuber	4 g	5 g	4 g	4 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the preparation 1) to 4), using the crude drugs shown above.

Description Tokishakuyakusan Extract is a light brown to brown powder or blackish brown viscous extract. It has a characteristic odor, and a slight sweet taste at first and a bitter taste later.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid TS, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Peony Root).

(3) For preparation prescribed Atractylodes Rhizome—Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 0.5 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone

(7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot observed at an *R*f value of about 0.4. The spot shows greenish brown color after spraying evenly 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allowing to cool (Atractylodes Lancea Rhizome).

(5) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium carbonate TS, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the yellowish fluorescent spot from the standard solution (Alisma Tuber).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay **(1)** (*E*)-Ferulic acid—Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-ferulic acid for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-ferulic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-ferulic acid} = M_S \times A_T/A_S \times 1/50$$

M_S: Amount (mg) of (*E*)-ferulic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate in 1000 mL of water, and add 2 mL of phosphoric acid. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of (*E*)-ferulic acid is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-ferulic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-ferulic acid is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Paeoniflorin RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg of albiflorin in 10 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Atractylenolide III—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh

accurately about 10 mg of atractylenolide III for assay, previously dried in a desiccator (silica gel) for more than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of atractylenolide III in each solution.

$$\text{Amount (mg) of atractylenolide III} = M_S \times A_T/A_S \times 1/40$$

M_S: Amount (mg) of atractylenolide III for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (550:450:1).

Flow rate: 1.0 mL per minute (the retention time of atractylenolide III is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylenolide III are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylenolide III is not more than 1.5%.

(4) Atractylodin—Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with exactly 10 μL each of the sample solution and atractylodin TS for assay as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of atractylodin in each solution.

$$\text{Amount (mg) of atractylodin} = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of atractylodin in atractylodin TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 330 mL of a mixture of water and phosphoric acid (55:1) add 670 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of atractylodin is about 13 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of atractylodin TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylodin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of atractylodin TS for assay under the above operating conditions, the relative standard deviation of the peak area of atractylodin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Tragacanth

Tragacantha

トラガント

Tragacanth is the exudation obtained from the trunks of *Astragalus gummifer* Labillardiére or other species of the same genus (*Leguminosae*).

Description Tragacanth occurs as curved, flattened or lamellate fragments, 0.5 – 3 mm in thickness. It is white to light yellow in color, translucent, and horny in texture. It is easily broken, and swells in water.

Odorless; tasteless and mucilaginous.

Identification **(1)** To 1 g of pulverized Tragacanth add 50 mL of water: a nearly uniform, slightly turbid mucilage is formed.

(2) To pulverized Tragacanth add dilute iodine TS, and examine the mixture microscopically <*5.01*>: a few blue-colored starch grains are observable.

Purity Karaya gum—Boil 1 g of Tragacanth with 20 mL of water until a mucilage is formed, add 5 mL of hydrochloric acid, and again boil the mixture for 5 minutes: no light red to red color develops.

Total ash <*5.01*> Not more than 4.0%.

Containers and storage Containers—Well-closed containers.

Powdered Tragacanth

Tragacantha Pulverata

トラガント末

Powdered Tragacanth is the powder of Tragacanth.

Description Powdered Tragacanth occurs as a white to yellowish white powder. It is odorless, tasteless and mucilaginous.

Under a microscope <*5.01*>, it, immersed in olive oil or liquid paraffin, reveals numerous angular fragments with a small amount of the circular or irregular lamellae or of starch grains. Starch grains are spherical to elliptical, mostly simple and occasionally 2- to 4-compound grains, simple grain, 3 – 25 μm in diameter. The fragments are swollen and altered with water.

Identification **(1)** To 1 g of Powdered Tragacanth add 50 mL of water: a nearly uniform, slightly turbid mucilage is formed.

(2) To Powdered Tragacanth add dilute iodine TS, and examine the mixture microscopically <*5.01*>: a few blue-colored starch grains are observable.

Purity Karaya gum—Boil 1 g of Powdered Tragacanth with 20 mL of water until a mucilage is formed, add 5 mL of hydrochloric acid, and again boil the mixture for 5 minutes: no light red to red color develops.

Total ash ⟨*5.01*⟩ Not more than 4.0%.

Containers and storage Containers—Tight containers.

Tribulus Fruit

Tribuli Fructus

シツリシ

Tribulus Fruit is the fruit of *Tribulus terrestris* Linné (*Zygophyllaceae*).

Description Pentagonal star shaped fruit, composed of five mericarps, 7 – 12 mm in diameter, often each mericarp separated; externally grayish green to grayish brown; a pair of longer and shorter spines on surface of each mericarp, the longer spine 3 – 7 mm in length, the shorter one 2 – 5 mm in length, numerous small processes on midrib; pericarp hard in texture, cut surface light yellow; each mericarp contains 1 – 3 seeds.

Almost odorless; taste, mild at first, followed by bitterness.

Under a microscope ⟨*5.01*⟩, a transverse section reveals epicarp composed of a single-layered epidermis; mesocarp composed of parenchyma and sclerenchyma layer; endocarp composed of several-layered fiber cells; a single-layer of cell between mesocarp and endocarp contain solitary crystals of calcium oxalate; cotyledons of seed contain oil drops and aleurone grains, and occasionally starch grains.

Identification To 2 g of pulverized Tribulus Fruit add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and water (40:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at an *R*f value of about 0.4.

Purity **(1)** Peduncle—When perform the test of foreign matter ⟨*5.01*⟩, the amount of peduncle contained in Tribulus Fruit does not exceed 4.0%.

(2) Foreign matters ⟨*5.01*⟩—Not more than 1.0% of foreign matters other than peduncle.

Loss on drying ⟨*5.01*⟩ Not more than 11.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 13.0%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.5%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 8.5%.

Containers and storage Containers—Well-closed containers.

Trichosanthes Root

Trichosanthis Radix

カロコン

Trichosanthes Root is the root of *Trichosanthes kirilowii* Maximowicz, *Trichosanthes kirilowii* Maximowicz var. *Japonica* Kitamura or *Trichosanthes bracteata* Voigt (*Cucurbitaceae*), from which the cortical layer has been removed.

Description Irregular cylindrical root 5 – 10 cm in length, 3 – 5 cm in diameter, often cut lengthwise; externally light yellowish white, and with irregular pattern of vascular bundles appearing as brownish yellow lines; fractured surface somewhat fibrous and light yellow in color; under a magnifying glass, the transverse section reveals wide medullary rays and brownish yellow spots or small holes formed by vessels.

Odorless; taste, slightly bitter.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of pulverized Trichosanthes Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of pulverized Trichosanthes Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash ⟨*5.01*⟩ Not more than 4.0%.

Containers and storage Containers—Well-closed containers.

Turmeric

Curcumae Rhizoma

ウコン

Turmeric is the rhizome of *Curcuma longa* Linné (*Zingiberaceae*) with or without cork layers, usually with the application of blanching.

It contains not less than 1.0% and not more than 5.0% of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), calculated on the basis of dried material.

Description Turmeric is a main rhizome or a lateral rhizome; main rhizome, nearly ovoid, about 3 cm in diameter, about 4 cm in length; lateral rhizome, cylindrical, with round tips, curved, about 1 cm in diameter, 2 - 6 cm in length; both main and lateral rhizomes with cyclic nodes; rhizome with cork layer, yellow-brown, lustrous; rhizome without cork layer, dark yellow-red, with yellow-red powders on surface; hard in texture, not easily broken; transversely cut surface yellow-brown to red-brown, lustrous like wax.

Odor, characteristic; taste, slightly bitter and stimulant, it colors a saliva yellow on chewing.

Under a microscope ⟨*5.01*⟩, a transverse section reveals the outermost layer to be composed of a cork layer 4 – 10 cells thick; sometimes a cork layer partly remains; cortex and stele, divided by a single-layered endodermis, composed of parenchyma, vascular bundles scattered; oil cells scattered in

parenchyma; parenchymatous cells contain yellow substances, sandy and solitary crystals of calcium oxalate, and gelatinized starch.

Identification **(1)** To 0.5 g of pulverized Turmeric, add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (11:9:1) to a distance about 7 cm, and air-dry the plate: a yellow spot appears at an *R*f value of about 0.4.

(2) To 0.2 g of pulverized Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), centrifuge after shaking for 20 minutes. Perform the test with the supernatant liquid as directed in the Assay, and determine the peak areas of curcumin, demethoxycurcumin and bisdemethoxycurcumin: the peak area of curcumin is larger than the peak area of demethoxycurcumin and is larger than 0.69 times the peak area of bisdemethoxycurcumin.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Turmeric according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Turmeric according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 17.0% (6 hours).

Total ash <*5.01*> Not more than 7.5%.

Acid-insoluble ash <*5.01*> Not more than 1.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 9.0%.

Assay Weigh accurately about 0.2 g of pulverized Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), and proceed in the same manner as described above. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of curcumin for assay, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as described under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TC}, A_{TD} and A_{TD} of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area A_S of curcumin in the standard solution.

$$\text{Amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin)} = M_S \times (A_{TC} + A_{TD} + A_{TB} \times 0.69)/A_S \times 1/5$$

M_S: Amount (mg) of curcumin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (56:43:1).

Flow rate: 1.0 mL per minute (the retention time of curcumin is about 11 minutes).

System suitability—

System performance: Dissolve 1 mg each of curcumin for assay, demethoxycurcumin and bisdemethoxycurcumin in methanol to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, bisdemethoxycurcumin, demethoxycurcumin and curcumin are eluted in this order with the resolution among these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of curcumin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Powdered Turmeric

Curcumae Rhizoma Purveratum

ウコン末

Powdered Turmeric is the powder of Turmeric.

It contains not less than 1.0% and not more than 5.0% of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), calculated on the basis of dried material.

Description Powdered Turmeric occurs as a yellow-brown to dark yellow-brown powder. It has a characteristic odor and a bitter, stimulant taste, and colors the saliva yellow on chewing.

Under a microscope <*5.01*>, all elements are yellow in color; it reveals parenchymatous cells containing mainly masses of gelatinized starch or yellow substances, also fragments of scalariform vessels; fragments of cork layers, epidermis, thick-walled xylem parenchymatous cells, and non-glandular hairs are occasionally observed.

Identification **(1)** To 0.5 g of Powdered Turmeric, add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (11:9:1) to a distance about 7 cm, and air-dry the plate: a yellow spot appears at an *R*f value of about 0.4.

(2) To 0.2 g of Powdered Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), centrifuge after shaking for 20 minutes. Perform the test with the supernatant liquid as directed in the Assay, and determine the peak areas of curcumin, demethoxycurcumin and bisdemethoxycurcumin: the peak area of curcumin is larger than the peak area of demethoxycurcumin and is larger than 0.69 times the peak area of bisdemethoxycurcumin.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Turmeric according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g

of Powdered Turmeric according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying ⟨*5.01*⟩ Not more than 17.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 7.5%.

Acid-insoluble ash ⟨*5.01*⟩ Not more than 1.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-soluble extract: not less than 9.0%.

Assay Weigh accurately about 0.2 g of Powdered Turmeric, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue, add 25 mL of a mixture of methanol and acetic acid (100) (99:1), and proceed in the same manner as described above. Combine all the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of curcumin for assay, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as described under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{TC}, A_{TD} and A_{TB} of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the sample solution as well as the peak area A_S of curcumin in the standard solution.

$$\text{Amount (mg) of total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin)} = M_S \times (A_{TC} + A_{TD} + A_{TB} \times 0.69)/A_S \times 1/5$$

M_S: Amount (mg) of curcumin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilianized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (56:43:1).

Flow rate: 1.0 mL/per minute (the retention time of curcumin is about 11 minutes).

System suitability—

System performance: Dissolve 1 mg each of curcumin for assay, demethoxycurcumin and bisdemethoxycurcumin in methanol to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, bisdemethoxycurcumin, demethoxycurcumin and curcumin are eluted in this order with the resolution among these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of curcumin is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Turpentine Oil

Oleum Terebinthinae

テレビン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

Description Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.465 – 1.478

Specific gravity ⟨*1.13*⟩ d_{20}^{20}: 0.860 – 0.875

Purity **(1)** Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15°C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

Distilling range ⟨*2.57*⟩ 150 – 170°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Uncaria Hook

Uncariae Uncis Cum Ramulus

チョウトウコウ

Uncaria Hook is, hook or the hook-bearing stem of *Uncaria rhynchophylla* Miquel, *Uncaria sinensis* Haviland or *Uncaria macrophylla* Wallich (*Rubiaceae*), sometimes after being passed through hot water or steamed.

Uncaria Hook contains not less than 0.03% of total alkaloids (rhynchophylline and hirsutine), calculated on the basis of dried material.

Description Uncaria Hook is uncinate hook or short stem with opposite or single hook; the hook, 1 – 4 cm in length, curved and acuminate; externally red-brown to dark brown or grayish brown, some one with hairs, the transverse section oblong to elliptical, light brown; stem thin and prismatic square to cylindrical, 2 – 5 mm in diameter, externally, red-brown to dark brown or grayish brown; the transverse section, square to elliptical; the pith light brown, square to elliptical; hard in texture.

Odorless and practically tasteless.

Under a microscope ⟨*5.01*⟩, a transverse section of the hook reveals vascular bundles in the cortex, unevenly dis-

tributed and arranged in a ring. Parenchyma cells in the secondary cortex containing sand crystals of calcium oxalate.

Identification To 1 g of pulverized Uncaria Hook add 20 mL of methanol, boil under a reflux condenser on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, add 5 mL of dilute acetic acid to the residue, warm the mixture on a water bath for 1 minute, and filter after cooling. Spot 1 drop of the filtrate on a filter paper, air-dry, spray Dragendorff's TS for spraying on it, and allow to stand: a yellow-red color develops.

Loss on drying ⟨*5.01*⟩ Not more than 12.0% (6 hours).

Total ash ⟨*5.01*⟩ Not more than 4.0%.

Extract content ⟨*5.01*⟩ Dilute ethanol-souble extract: not less than 8.5%.

Assay Weigh accurately about 0.2 g of moderately fine powder of Uncaria Hook, transfer into a glass-stoppered centrifuge tube, add 30 mL of a mixture of methanol and dilute acetic acid (7:3), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add two 10-mL portions of a mixture of methanol and dilute acetic acid (7:3), proceed in the same manner, and combine all of the supernatant liquid. To the combined liquid add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately about 5 mg of rhynchophylline for assay, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 10 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_{Ta} and A_{Tb}, of rhynchophylline and hirsutine obtained from the sample solution, and the peak area, A_S, of rhynchophylline from the standard solution (1).

Amount (mg) of total alkaloids (rhynchophylline and hirsutine)
$= M_S \times (A_{Ta} + 1.405A_{Tb})/A_S \times 1/20$

M_S: Amount (mg) of rhynchophylline for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 200 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL, and add 350 mL of acetonitrile.

Flow rate: Adjust so that the retention time of rhynchophylline is about 17 minutes.

System suitability—

System performance: Dissolve 5 mg of rhynchophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and reflux for 10 minutes or warm at about 50°C for 2 hours. After cooling, to 1 mL of the solution so obtained add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline is appears in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak areas of rhynchophylline is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Uva Ursi Fluidextract

ウワウルシ流エキス

Uva Ursi Fluidextract contains not less than 3.0 w/v% of arbutin.

Method of preparation Prepare an infusion from Bearberry Leaf, in coarse powder, as directed under Fluidextracts, using hot Purified Water or hot Purified Water in Containers. Remove a part of the accompanying tannin, evaporate the mixture under reduced pressure, if necessary, and add Purified Water or Purified Water in Containers to adjust the percentage. It may contain an appropriate quantity of Ethanol.

Description Uva Ursi Fluidextract is a yellow-brown to dark red-brown liquid, and has a bitter and astringent taste.

It is miscible with water and with ethanol (95).

Identification To 1 mL of Uva Ursi Fluidextract add 30 mL of a mixture of ethanol (95) and water (7:3), shake, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (2) under Bearberry Leaf.

Purity Heavy metals ⟨*1.07*⟩—Prepare the test solution with 1.0 g of Uva Ursi Fluidextract as direct under the Fluidextracts (4), and perform the test (not more than 30 ppm).

Assay Pipet 1 mL of Uva Ursi Fluidextract, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Bearberry Leaf.

Amount (mg) of arbutin $= M_S \times A_T/A_S$

M_S: Amount (mg) of arbutin for assay taken

Containers and storage Containers—Tight containers.

Wood Creosote

Creosotum Ligni

木クレオソート

Wood Creosote is a mixture of phenols obtained from by using wood tar derived from dry distillation of stems and branches of various plants of genus *Pinus* (*Pinaceae*), genus *Cryptomeria* (*Taxodiaceae*), genus *Fagus* (*Fagaceae*), genus *Afzelia* (genus *Intsia*); (*Leguminosae*), genus *Shorea* (*Dipterocarpaceae*) or genus *Tectona* (*Verbenaceae*), followed by distillation and collection at 180 to 230°C, then further purification and then re-distillation.

Wood Creosote contains not less than 23% and not more than 35% of guaiacol ($C_7H_8O_2$: 124.14).

Description Wood Creosote is a colorless or pale yellow, clear liquid. It has a characteristic odor.

It is slightly soluble in water.

It is miscible with methanol and with ethanol (99.5).

Its saturated solution is acidic.

It is highly refractive.

It gradually changes in color by light or by air.

Identification Use the sample solution obtained in the Assay as the sample solution. Separately, dissolve 0.1 g of phenol, *p*-cresol, guaiacol, and 2-methoxy-4-methylphenol in methanol respectively, to make 100 mL. To 10 mL of each solution add methanol to make 50 mL, and use these solutions as standard solution (1), standard solution (2), standard solution (3) and standard solution (4). Perform the test with 10 μL each of the sample solution, standard solution (1), (2), (3) and (4) as directed under Liquid Chromatography <*2.01*> according to the following conditions: the main peaks obtained with the sample solution show the same retention times with those obtained with the standard solutions (1), (2), (3) and (4).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

Specific gravity <*2.56*> d_{20}^{20}: not less than 1.076.

Purity **(1)** Coal Creosote—Accurately measure 10 mL of Wood Creosote, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, to 1 mg each of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene add a small quantity of ethyl acetate, if necessary, and add methanol to make 100 mL. To 1 mL of this solution add methanol to make 100 mL, and use this soiution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions: No peaks are detected with the sample solution for the retention times corresponding to benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene of the standard solution. Change these conditions if any peak is detected for retention times that correspond to benzo[*a*]pyrene, benz[*a*]anthracene or dibenz[*a*,*h*]anthracene, to verify that such a peak does not belong to benzo[*a*]pyrene, benz[*a*]anthracene or dibenz[*a*,*h*]anthracene.

Operating conditions—

Detector: A mass spectrometer (EI).

Monitored ions:

Benz[*a*]anthracene: Molecular ion *m/z* 228, Fragment ion *m/z* 114	About 14 to 20 minutes
Benzo[*a*]pyrene: Molecular ion *m/z* 252, Fragment ion *m/z* 125	About 20 to 25 minutes
Dibenz[*a*,*h*]anthracene: Molecular ion *m/z* 278, Fragment ion *m/z* 139	About 25 to 30 minutes

Column: A quartz tube 0.25 mm in inside diameter and 30 m in length, with internal coating 0.25 – 0.5 μm in thickness made of 5% diphenyl-95% dimethyl polysiloxane for gas chromatography.

Column temperature: Inject sample at a constant temperature in vicinity of 45°C, then raise temperature to 240°C at the rate of 40°C per minute, maintain the temperature at 240°C for 5 minutes, then raise temperature to 300°C at the rate of 4°C per minute, then raise the temperature to 320°C at the rate of 10°C per minute, then maintain temperature at 320°C for 3 minutes.

Injection port temperature: A constant temperature in vicinity of 250°C.

Interface temperature: A constant temperature in vicinity of 300°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of benzo[*a*]pyrene is about 22 minutes.

Split ratio: Splitless.

System suitability—

Test for required detectability: Accurately measure 1 mL of standard solution and add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the test is performed with conditions described above with 1 μL of the solution for system suitability test, the SN ratio of each substance is not less than 3.

System performance: When the procedure is run with conditions described above with 1 μL of the solution for system suitability test, the elution takes place in order of benz[*a*]anthracene, benzo[*a*]pyrene and then dibenz[*a*,*h*]anthracene.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of benzo[*a*]pyrene, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene is respectively not more than 10%.

(2) Acenaphthene—To 0.12 g of Wood Creosote add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of acenaphthene in methanol to make 50 mL. To 5 mL of this solution add methanol to make 20 mL. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions: No peaks are detected with sample solution for the retention time corresponding to acenaphthene of the standard solution. Change these conditions if any peak is detected for the retention time corresponding to acenaphthene, to verify that such a peak does not belong to athenaphthene.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.25 mm inside diameter and 60 m in length, with internal coating 0.25 – 0.5 μm in thickness made of polymethylsiloxane for gas chromatography.

Column temperature: Perform injection at a constant temperature in vicinity of 45°C, then raise the temperature by 11.5°C per minute until reaching 160°C, then raise the temperature by 4°C per minute until reaching 180°C, then raise the temperature by 8°C until reaching 270°C, then maintain temperature at 270°C for 3 minutes.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of acenaphthene is about 18 minutes.

Split ratio: Splitless.

System suitability—

Test for required detectability: Accurately measure 1 mL of the standard solution, add methanol to make exactly 10 mL, and use this solution as the solution for system suitability test. When the procedure is run with conditions described above with 1 μL of solution for system suitability test, the SN ratio of acenaphthene is not less than 3.

System repeatability: When the test is repeated 6 times

with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acenaphthene is not more than 6.0%.

(3) Other impurities

Add 2 mL of petroleum benzin to 1.0 mL of Wood Creosote, then add 2 mL of barium hydroxide test solution, agitate to mix and allow to stand. No blue or muddy brown color develops in the upper layer of the mixture. Furthermore, no red color develops in the lower layer.

Distilling range ⟨*2.57*⟩ 200 – 220°C, not less than 85 vol%.

Assay To about 0.1 g of Wood Creosote, accurately weighed, add methanol to make exactly 50 mL. Pipet 10 mL of this solution add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, add methanol to about 30 mg of accurately measured guaiacol for assay to make exactly 50 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of guaiacol in each solution.

$$\text{Amount (mg) of guaiacol } (C_7H_8O_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of guaiacol for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Mixture of water and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of guaiacol is about 9 minutes.

System suitability—

System performance: Dissolve 2 mg each of guaiacol and phenol in methanol to make 10 mL. The procedure is run with conditions described above with 10 μL of this solution, the elution takes place in order of phenol then guaiacol, with the degree in separation of not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of guaiacol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Yokukansan Extract

抑肝散エキス

Yokukansan Extract contains not less than 0.15 mg of total alkaloids (rhyncophylline and hirsutine), not less than 0.6 mg and not more than 2.4 mg of saikosaponin b_2, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Japanese Angelica Root	3 g	3 g
Uncaria Hook	3 g	3 g
Cnidium Rhizome	3 g	3 g
Atractylodes Rhizome	4 g	—
Atractylodes Lancea Rhizome	—	4 g
Poria Sclerotium	4 g	4 g
Bupleurum Root	2 g	2 g
Glycyrrhiza	1.5 g	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Yokukansan Extract is a light brown to grayish brown powder or a blackish brown viscous extract. It has a slightly odor, and a slightly bitter and acid taste.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, separate the diethyl ether layer, and use this layer as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(2) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 20 mL of water and 2 mL of ammonia TS, shake, then add 20 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use the solution as the sample solution. Separately, dissolve 1 mg each of rhyncophyllin for thin-layer chromatography and hirsutine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): at least one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with one of the two dark violet spots obtained from the standard solution (Uncaria Hook).

(3) For preparation prescribed Atractylodes Rhizome— To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenoide III for thin-layer chromatography in 2 mL of methanol, and use this solution

as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red to purple-red spot obtained from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under reduced pressure, dissolve the residue in 2 mL of hexane, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark violet spot is observed at an *R*f value of about 0.4. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: the spot exhibits a greenish brown color (Atractylodes Lancea Rhizome).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

Purity **(1)** Heavy metals <1.07>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of the dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of the dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <5.01> Not less than 10.0%, calculated on the dried basis.

Assay **(1)** Total alkaloids (rhyncophylline and hirsutine)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3 mL of 1 mol/L hydrochloric acid TS and 7 mL of water, and shake for 10 minutes, centrifuge, and remove the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, and proceed in the same manner as above. To the resultant aqueous layer add 10 mL of sodium hydroxide TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diethyl ether, and repeat the process above two more times. Combine all the supernatant liquids, evaporate the solvent under reduced pressure at not more than 40°C, dissolve the residue in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg each of rhyncophylline for assay and hirsutine for assay, dissolve in a mixture of methanol and diluted acetic acid (7:3) to make exactly 100 mL. Pipet 10 mL of this solution, add the mixture of methanol and diluted acetic acid (7:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TR} and A_{TH}, and A_{SR} and A_{SH}, of rhyncophylline and hirsutine in each solution.

Amount (mg) of total alkaloids (rhyncophylline and hirsutine)
$$= (M_{SR} \times A_{TR}/A_{SR} + M_{SH} \times A_{TH}/A_{SH}) \times 1/50$$

M_{SR}: Amount (mg) of rhyncophylline for assay taken
M_{SH}: Amount (mg) of hirsutine for assay taken

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 1 g of sodium lauryl sulfate add 600 mL of methanol, shake, then add 400 mL of water and 1 mL of phosphoric acid.

Flow rate: 1.0 mL per minute (the retention times of rhyncophylline and hirsutine are about 17 minutes and about 47 minutes, respectively).

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peaks of rhyncophylline and hirsutine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rhyncophylline and hirsutine is not more than 1.5%, respectively.

(2) Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Use saikosaponin b_2 standard TS for assay as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of saikosaponin b_2 in saikosaponin b_2 standard TS for assay

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, calculated on the anhydrous basis

Operation conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 12 minutes).

Systemic suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Zedoary

Zedoariae Rhizoma

ガジュツ

Zedoary is the rhizome of *Curcuma zedoaria* Roscoe (*Zingiberaceae*), usually after being passed through hot water.

Description Nearly ovoid rhizome, 4 – 6 cm in length, 2.5 – 4 cm in diameter; externally grayish yellow-brown to grayish brown; nodes protruded as rings; internode of 0.5 – 0.8 cm, with thin, longitudinal wrinkles, scars of removed roots, and small protrusions of branched rhizomes; under a magnifying glass, external surface covered with coarse hairs; horny in texture and difficult to cut; transverse section grayish brown in color; cortex 2 – 5 mm in thickness, stele thick, a light grayish brown ring separating them.

Odor, characteristic; taste, pungent, bitter and cooling.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Zedoary according to Method 3, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Zedoary according to Method 4, and perform the test (not more than 5 ppm).

Total ash <*5.01*> Not more than 7.0%.

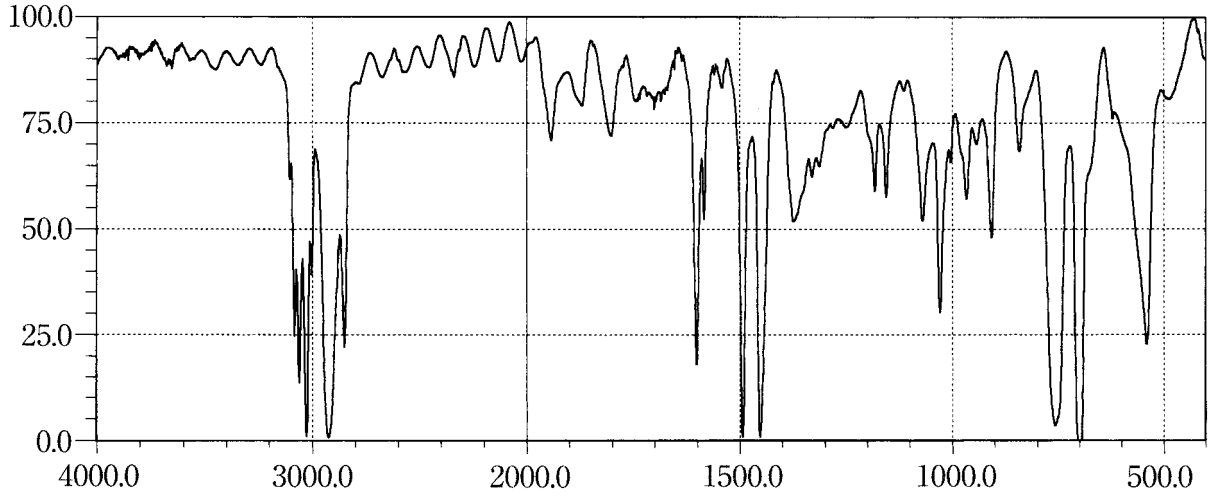
Essential oil content <*5.01*> Perform the test with 50.0 g of pulverized Zedoary, provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.5 mL.

Containers and storage Containers—Well-closed containers.

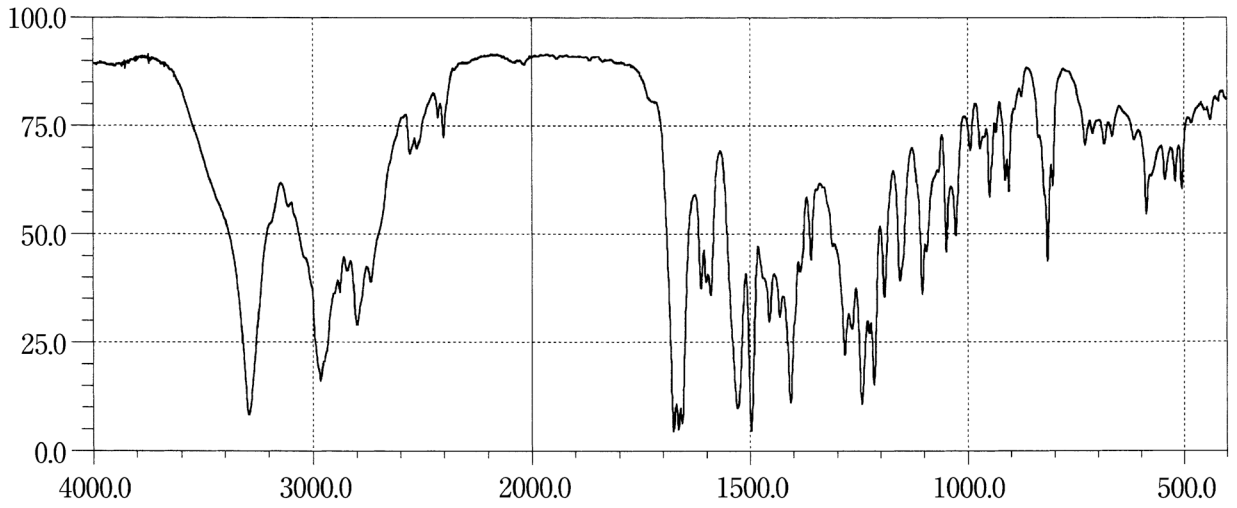
INFRARED REFERENCE SPECTRA

The infrared reference spectra presented here were obtained by the use of Fourier-transform infrared spectrophotometers under the conditions specified in the individual monographs. The horizontal axis indicates the wave numbers (cm^{-1}) and the vertical axis indicates the transmittance (%). A spectrum of polystyrene obtained in the same manner is also presented for reference.

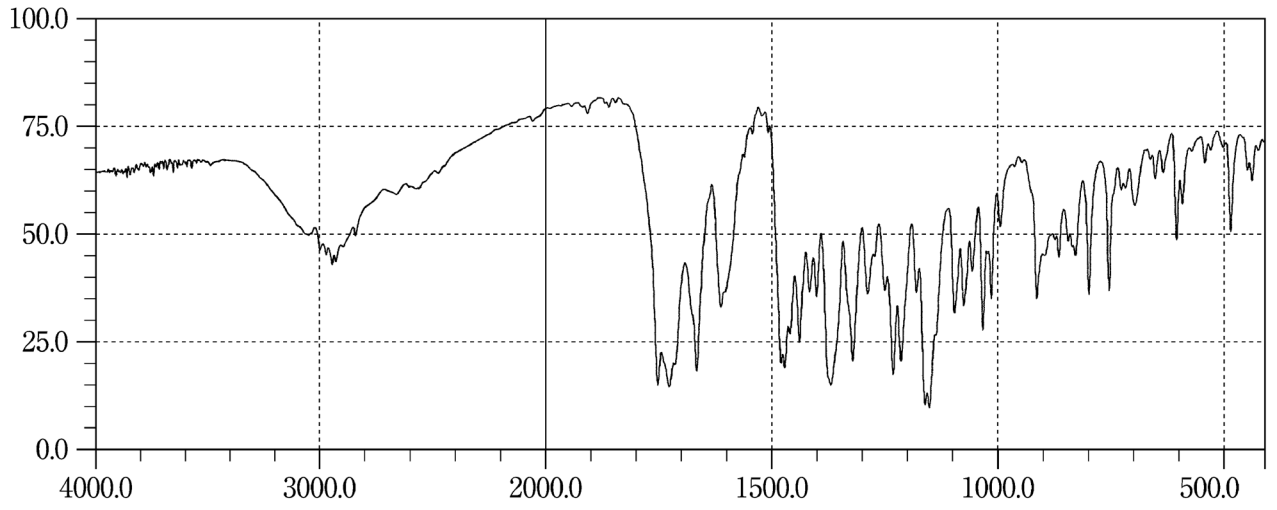
Polystyrene



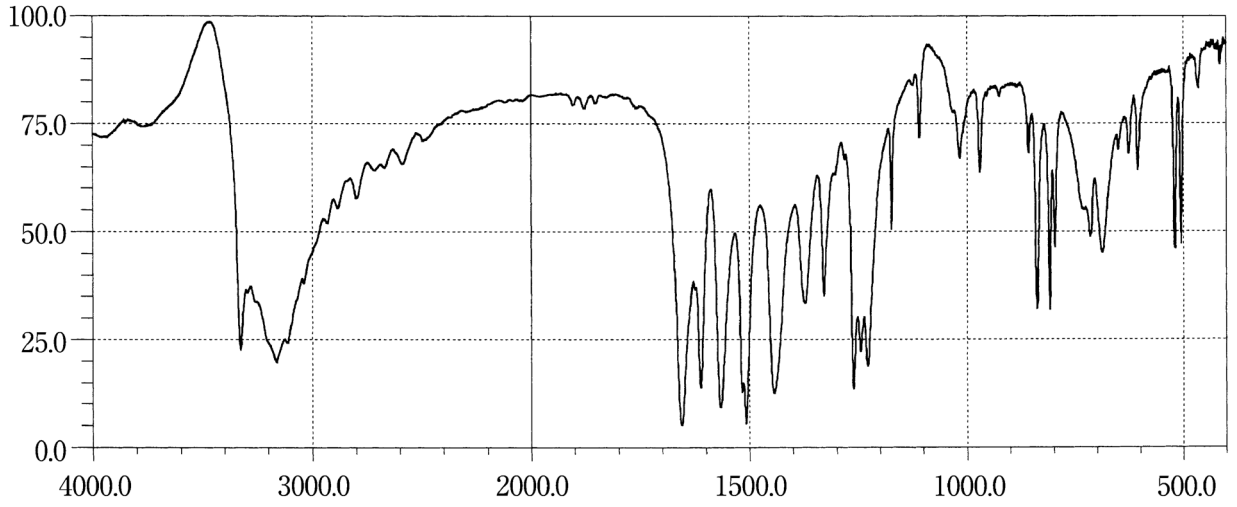
Acebutolol Hydrochloride



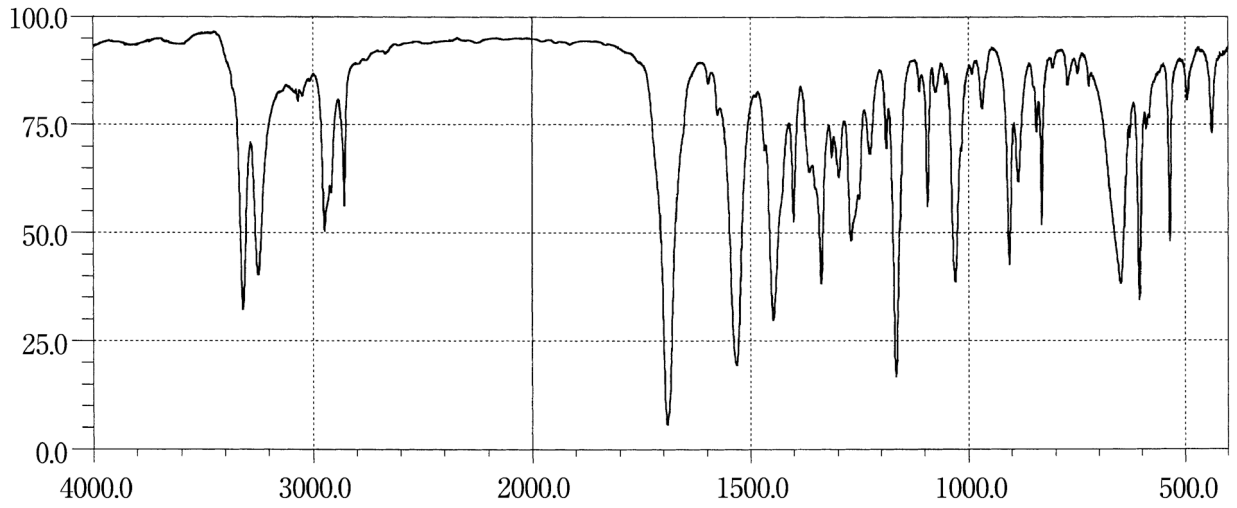
Acemetacin



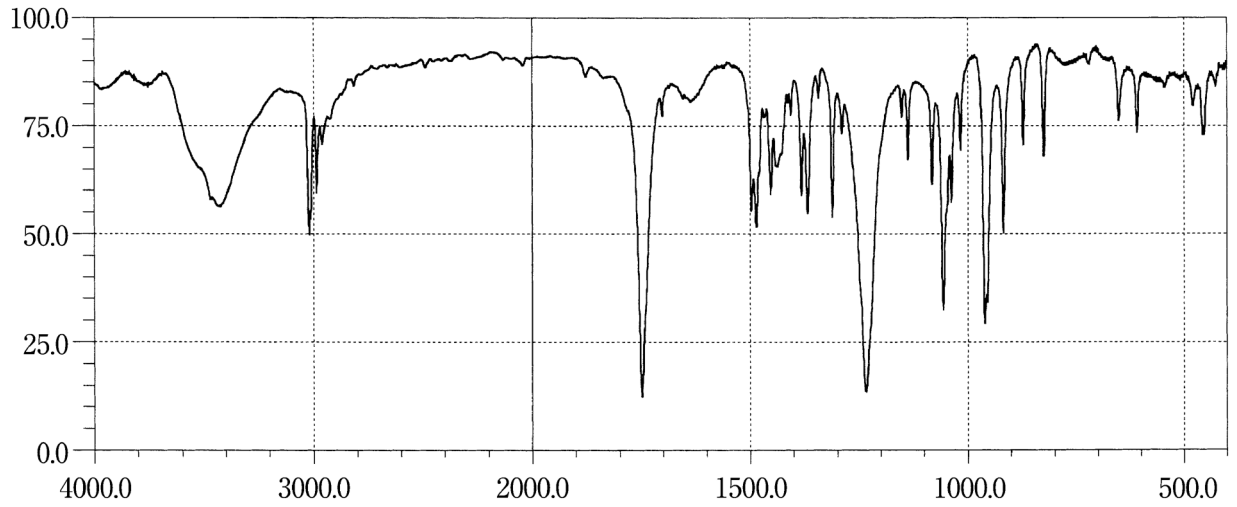
Acetaminophen



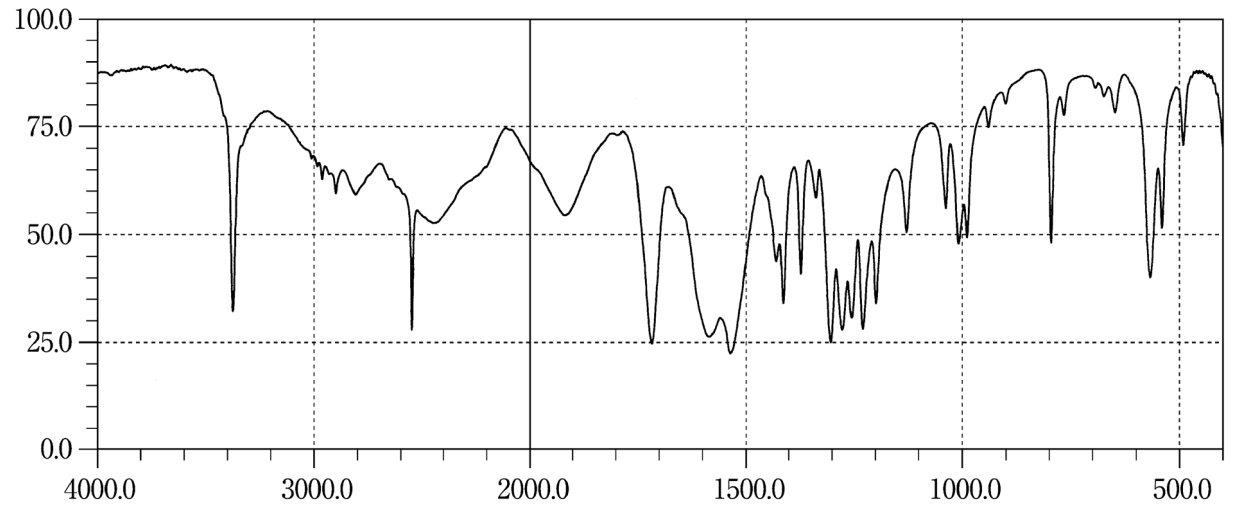
Acetohexamide



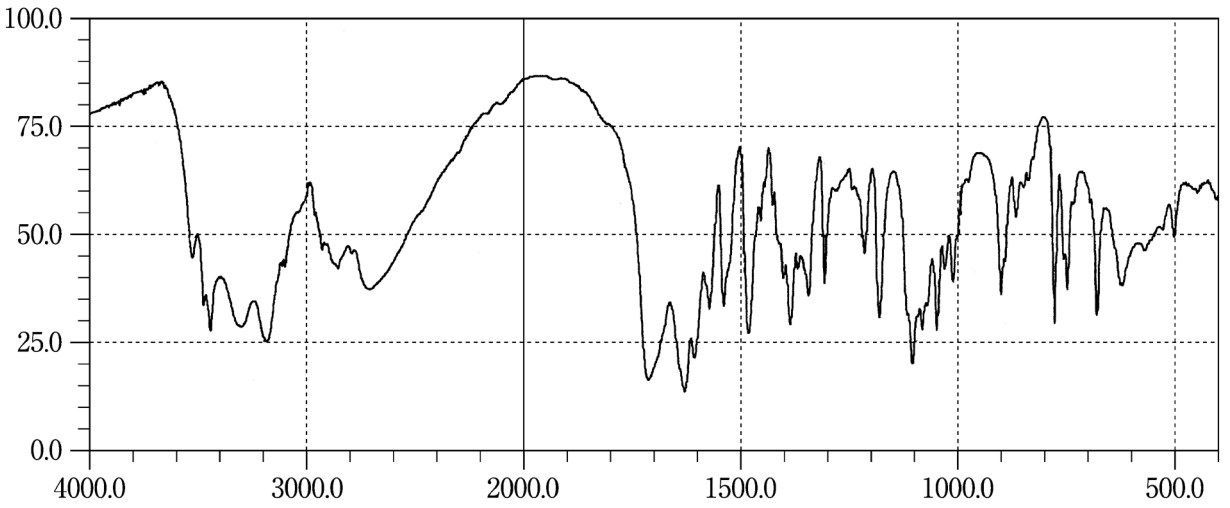
Acetylcholine Chloride for Injection



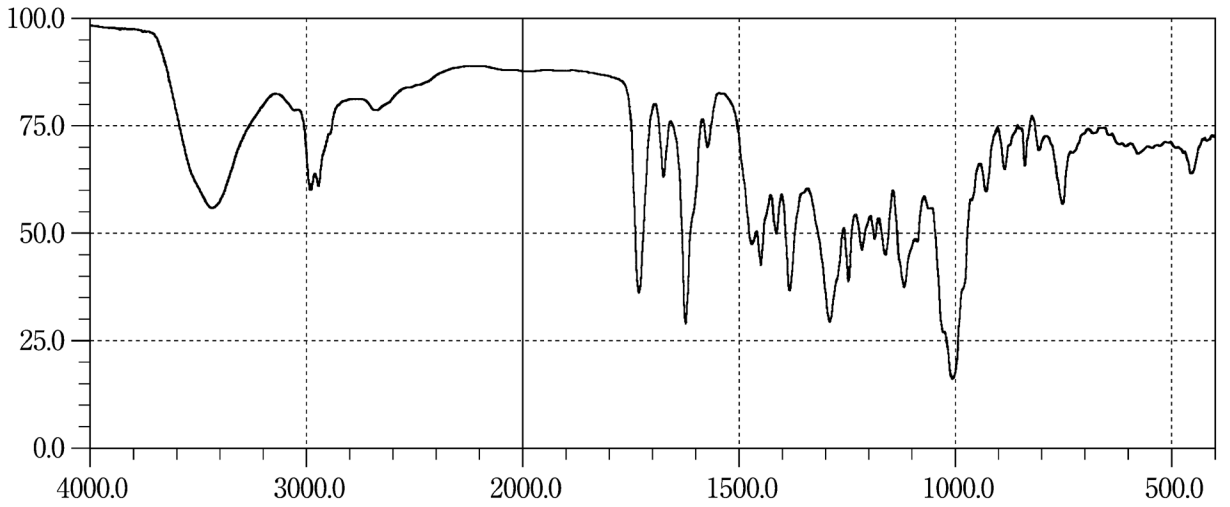
Acetylcysteine



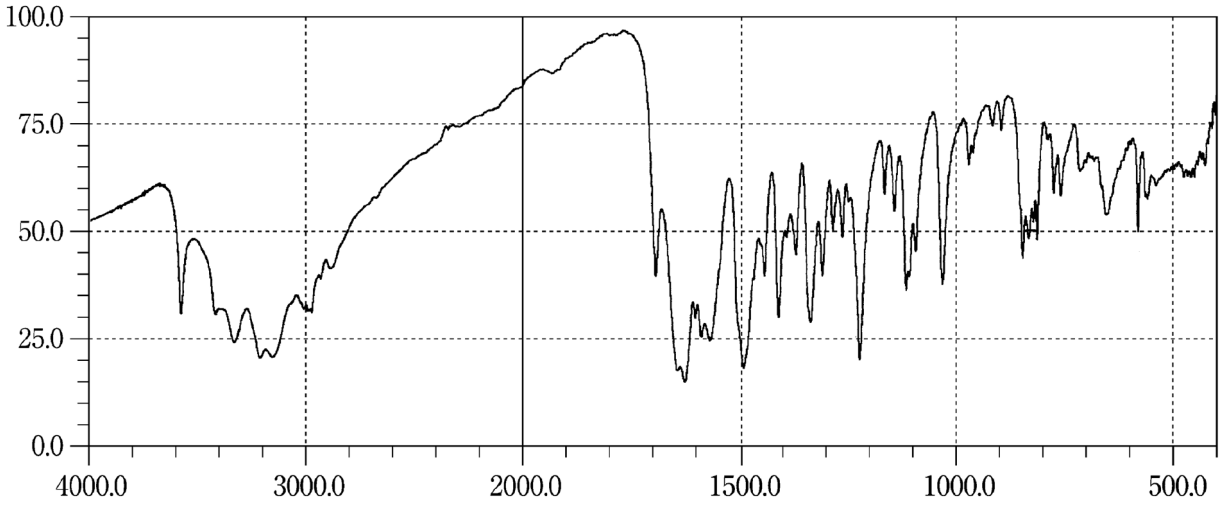
Aciclovir



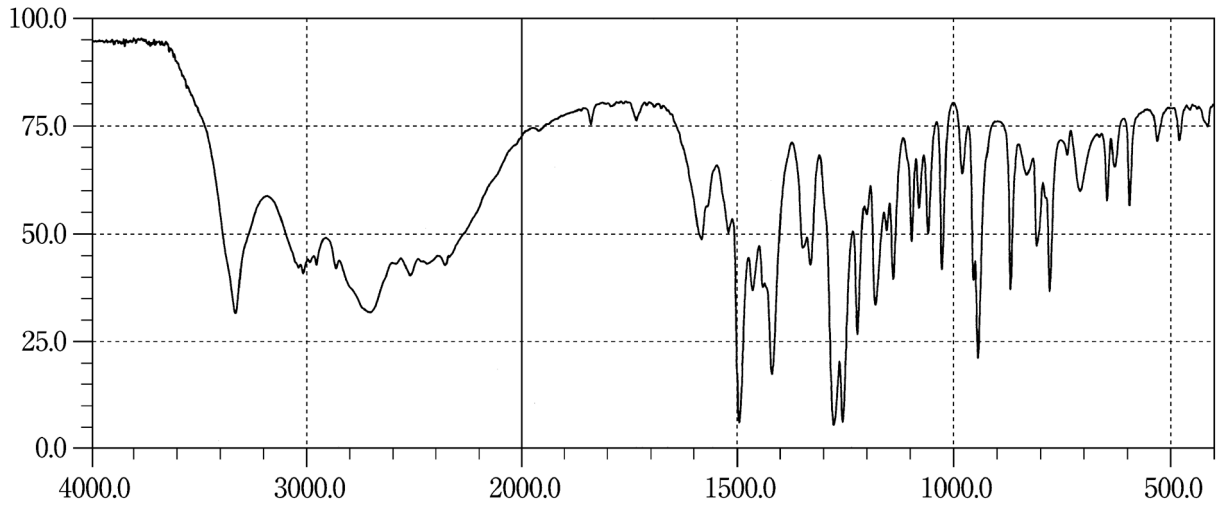
Aclarubicin Hydrochloride



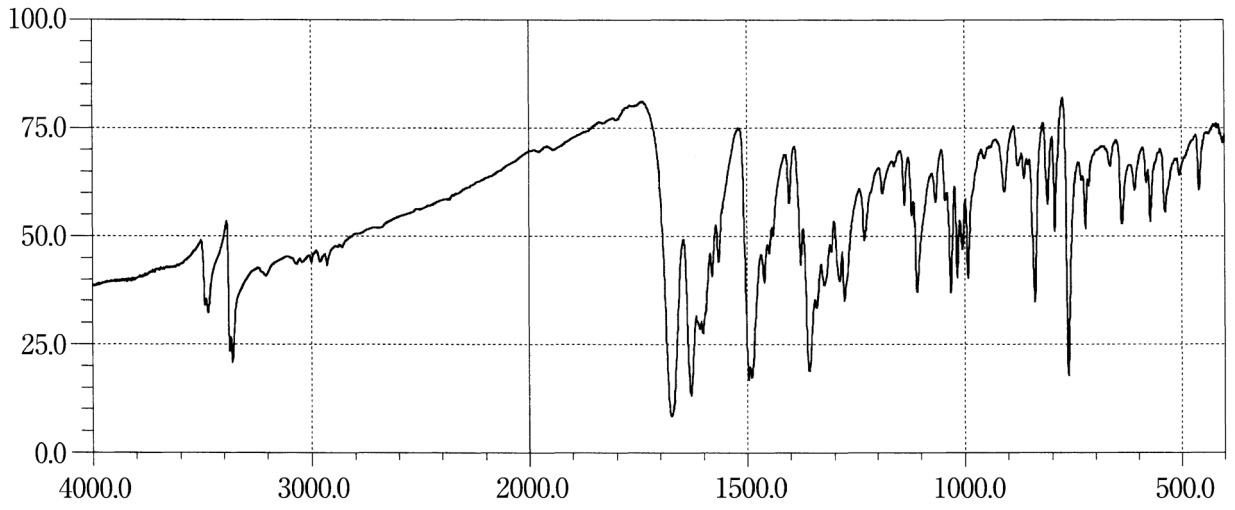
Acrinol Hydrate



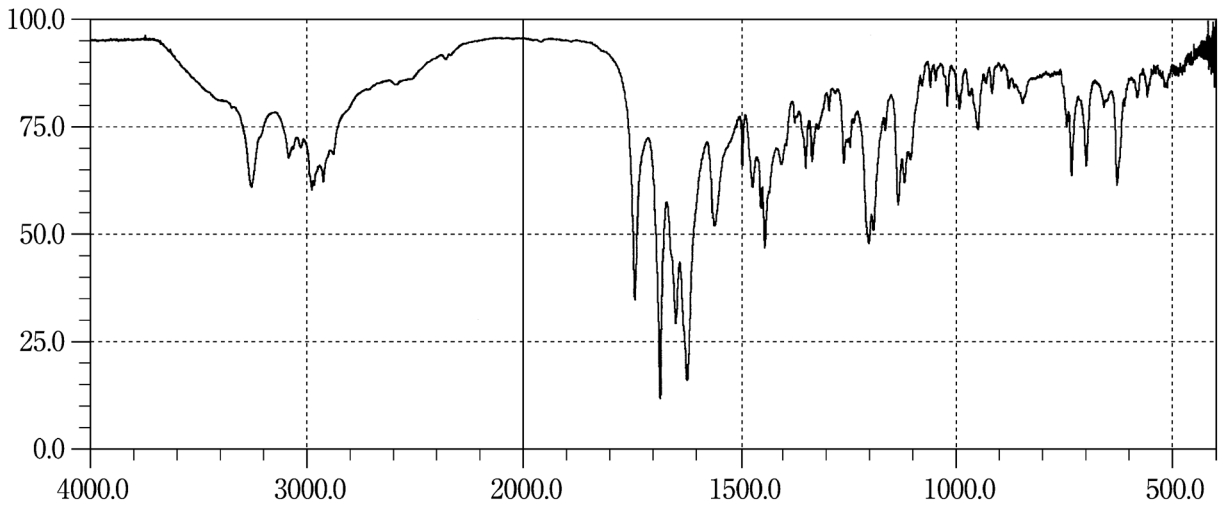
Adrenaline



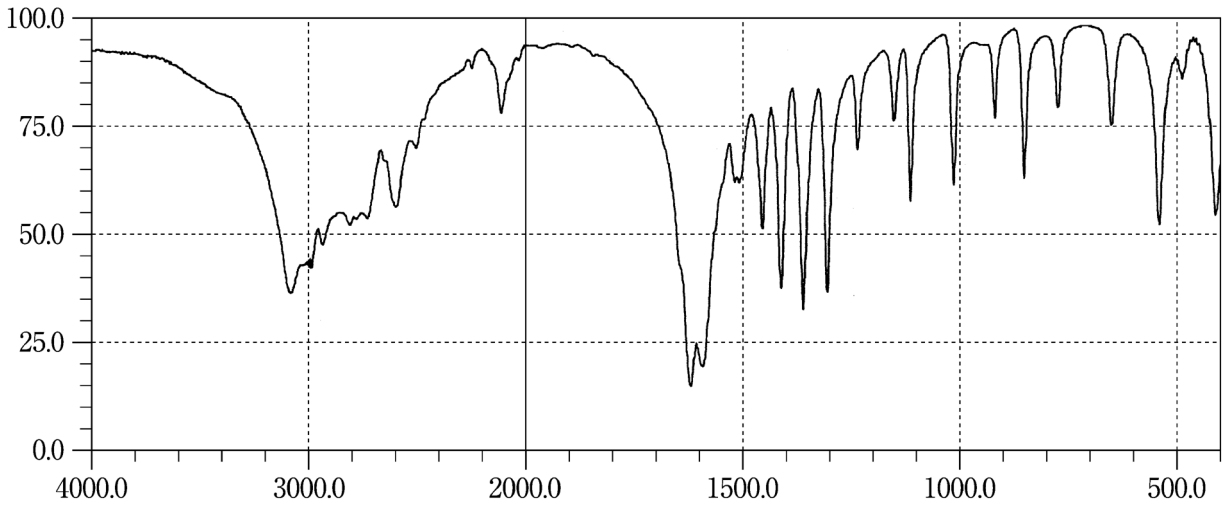
Afloqualone



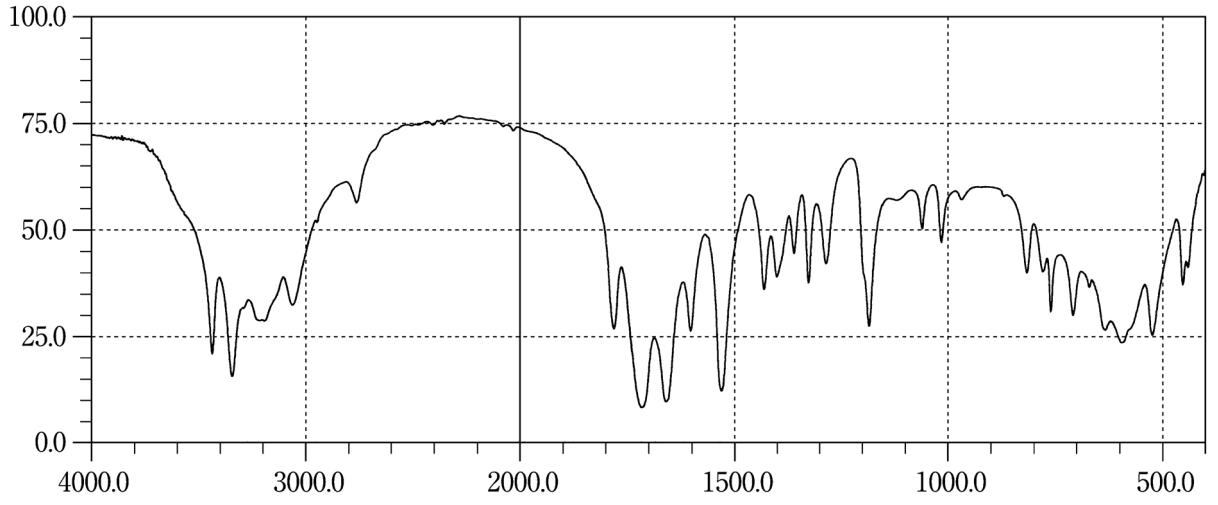
Alacepril



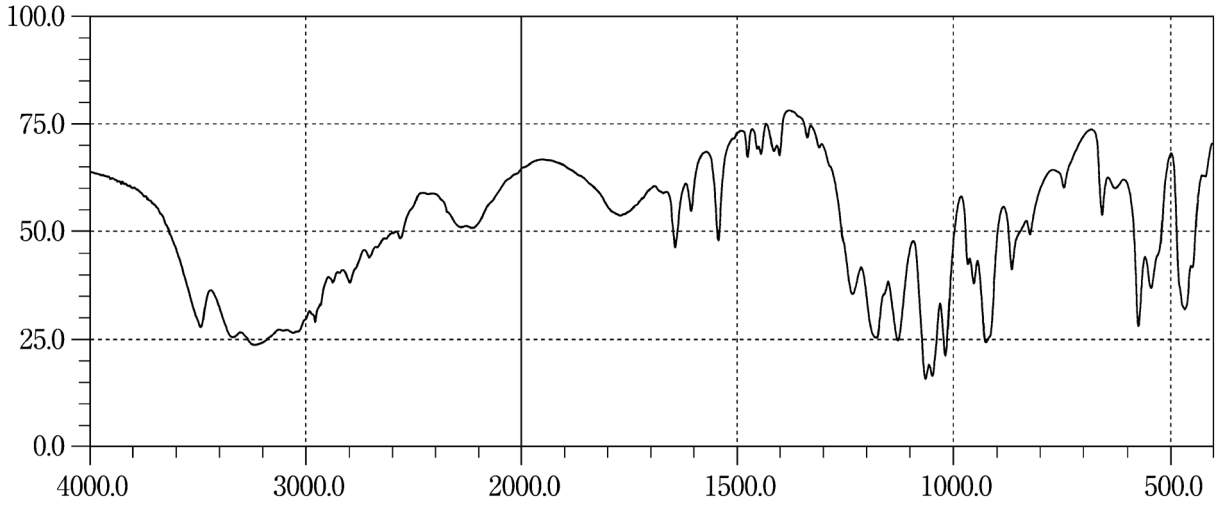
L-Alanine



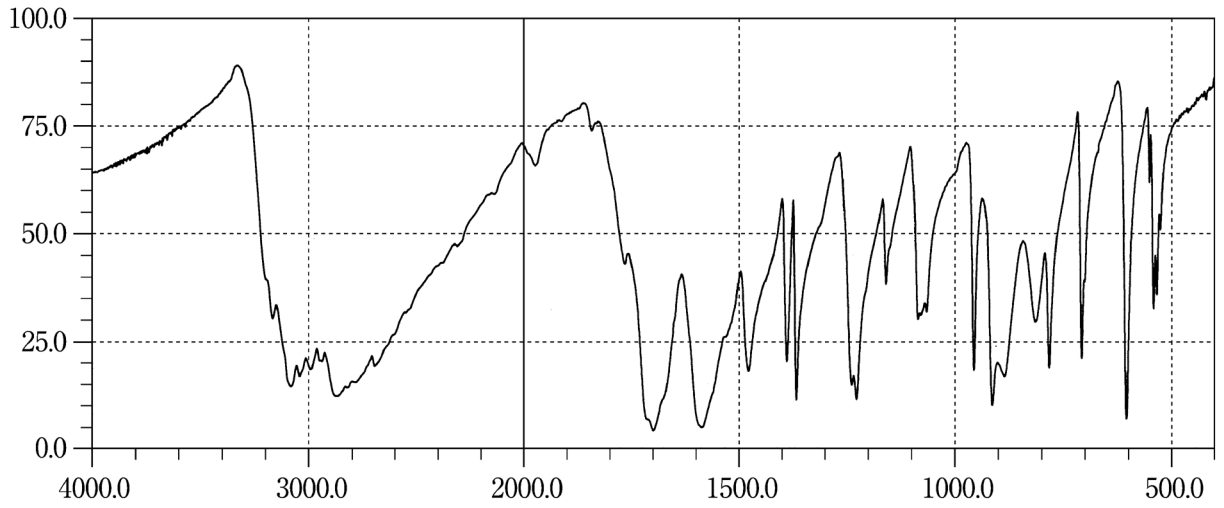
Aldioxa



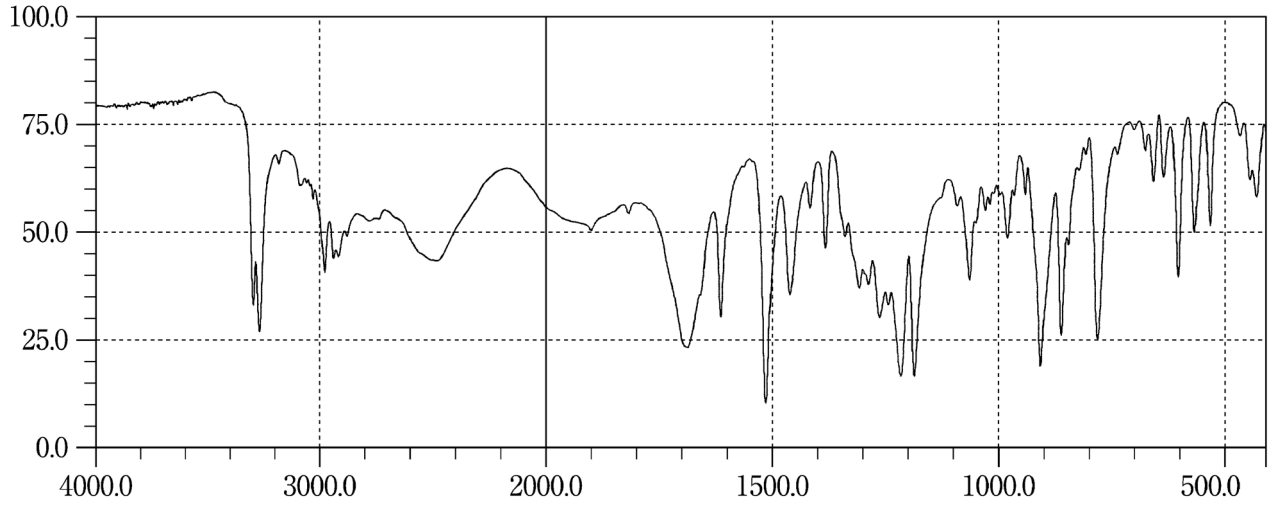
Alendronate Sodium Hydrate



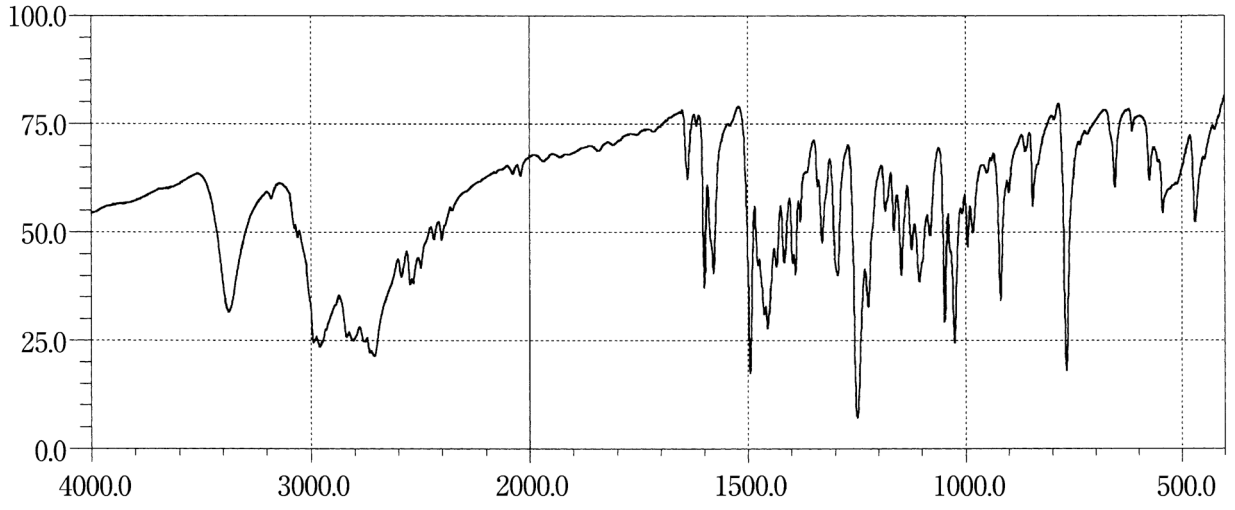
Allopurinol



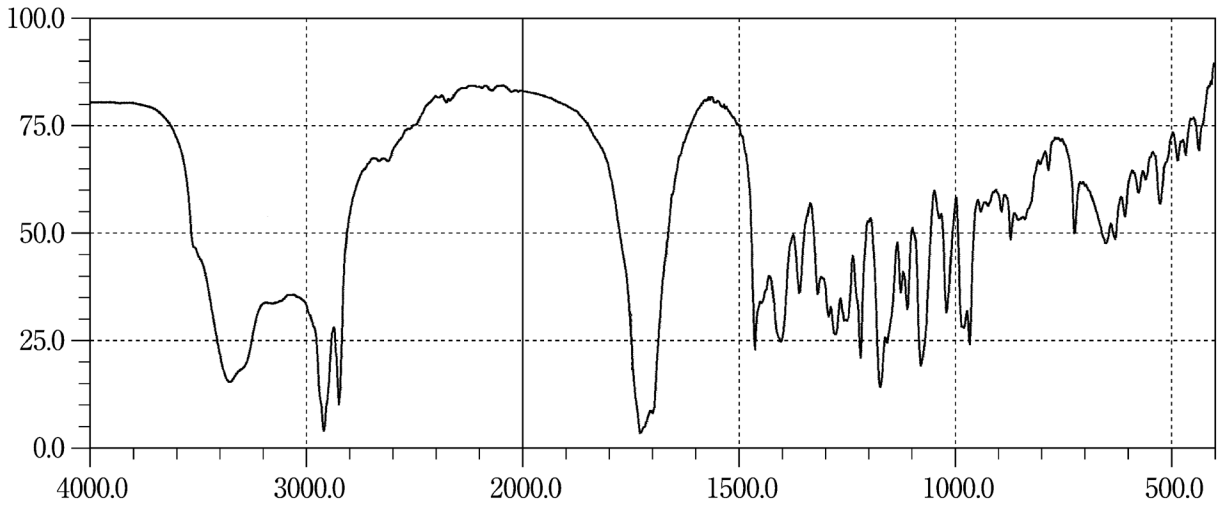
Alminoprofen



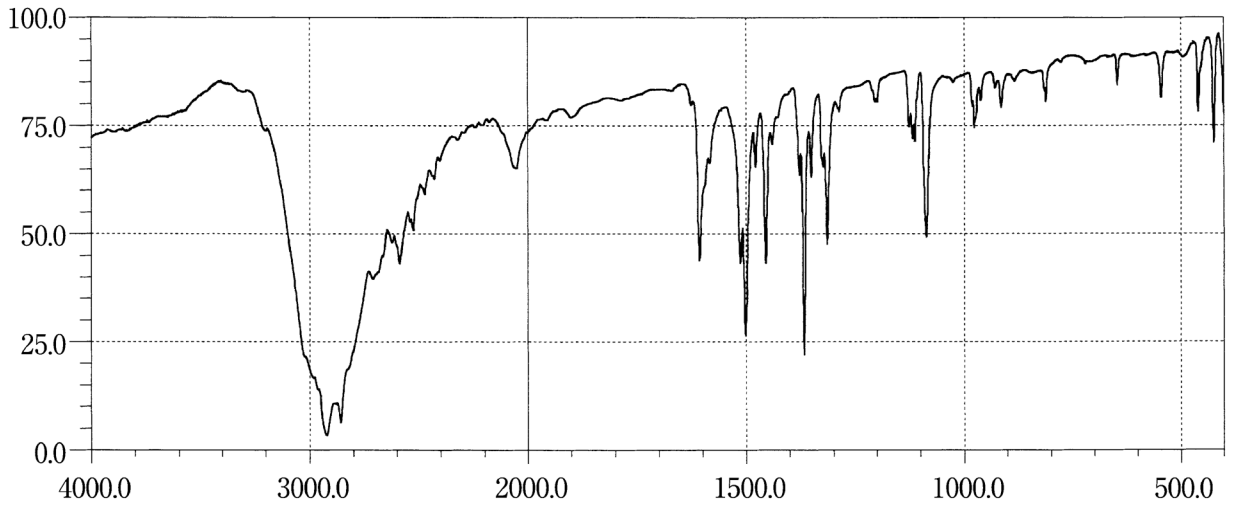
Alprenolol Hydrochloride



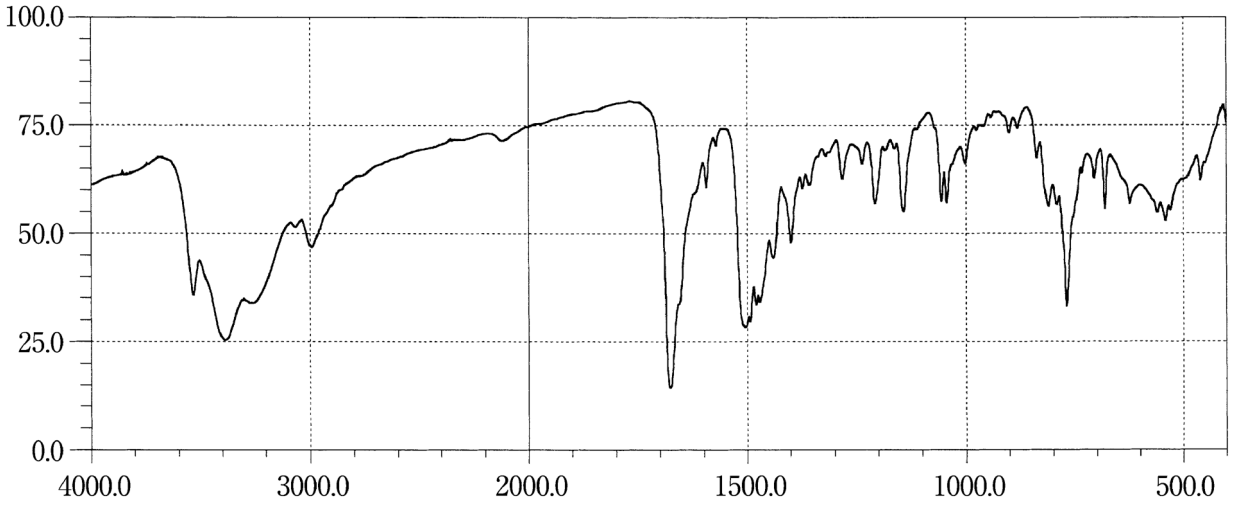
Alprostadil



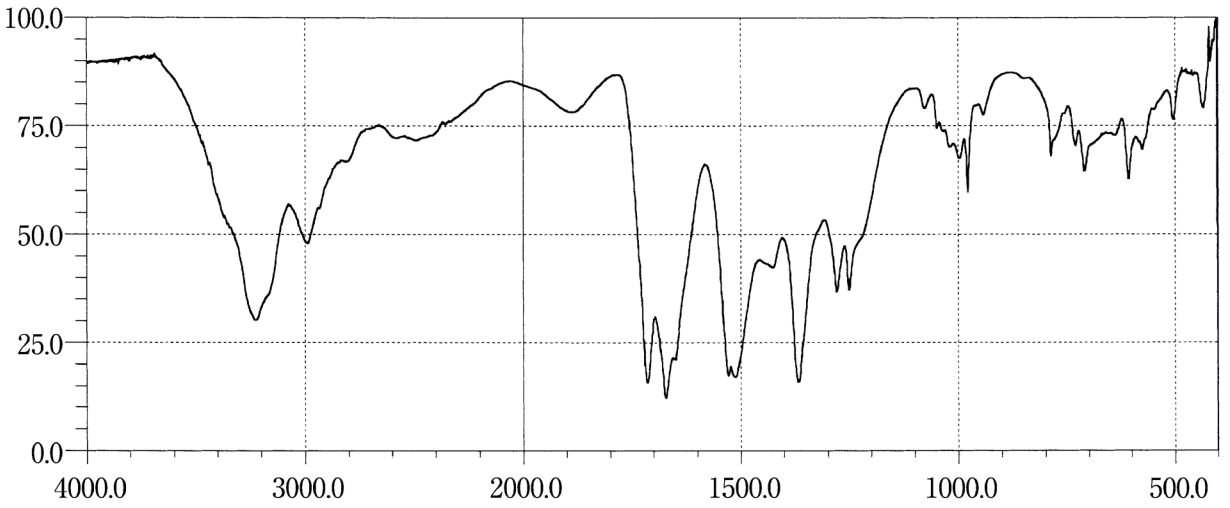
Amantadine Hydrochloride



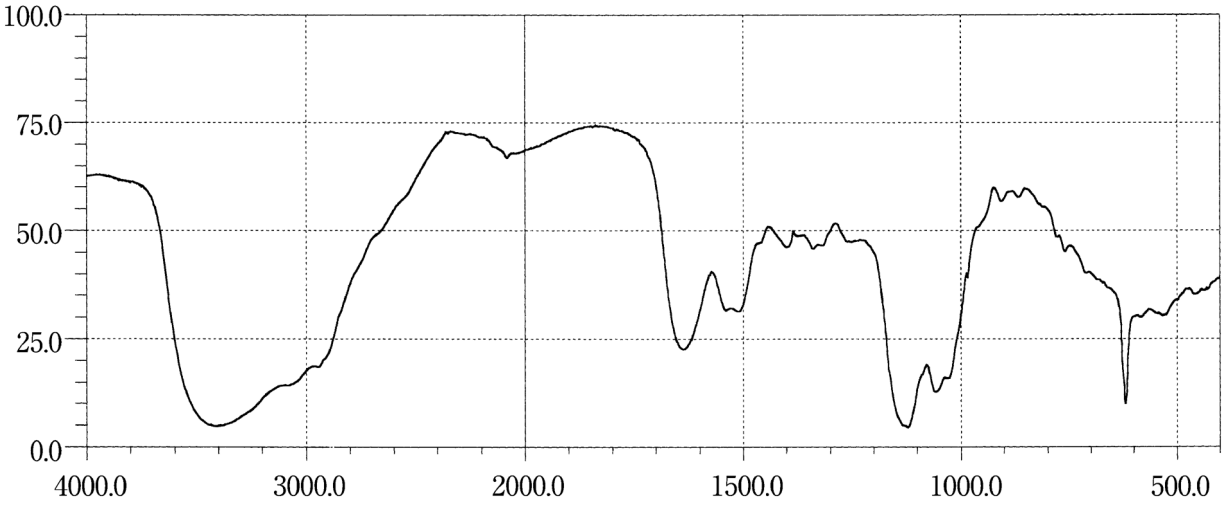
Ambenonium Chloride



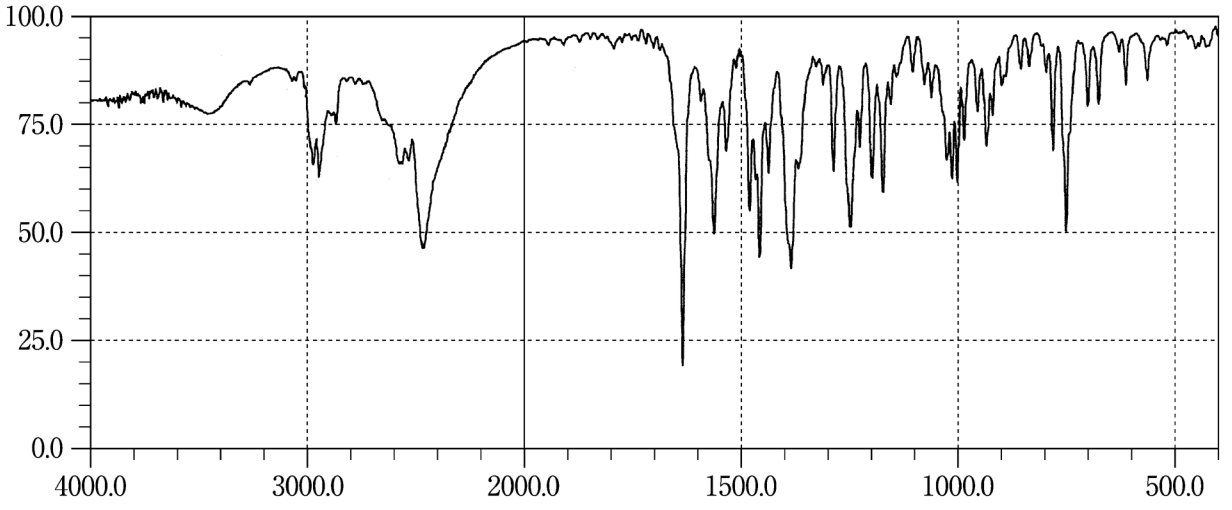
Amidotrizoic Acid



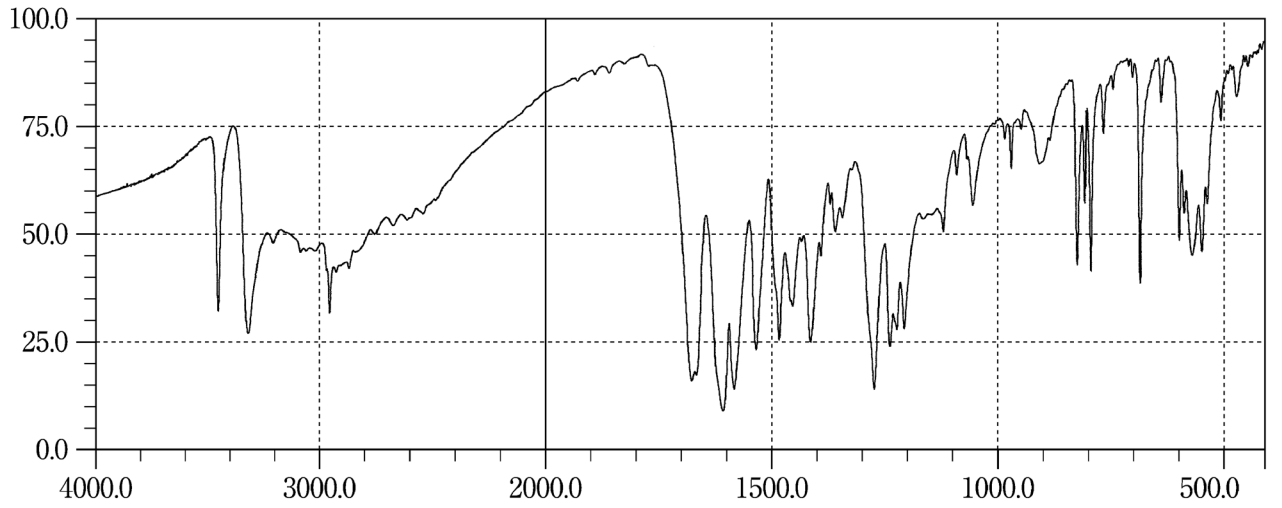
Amikacin Sulfate



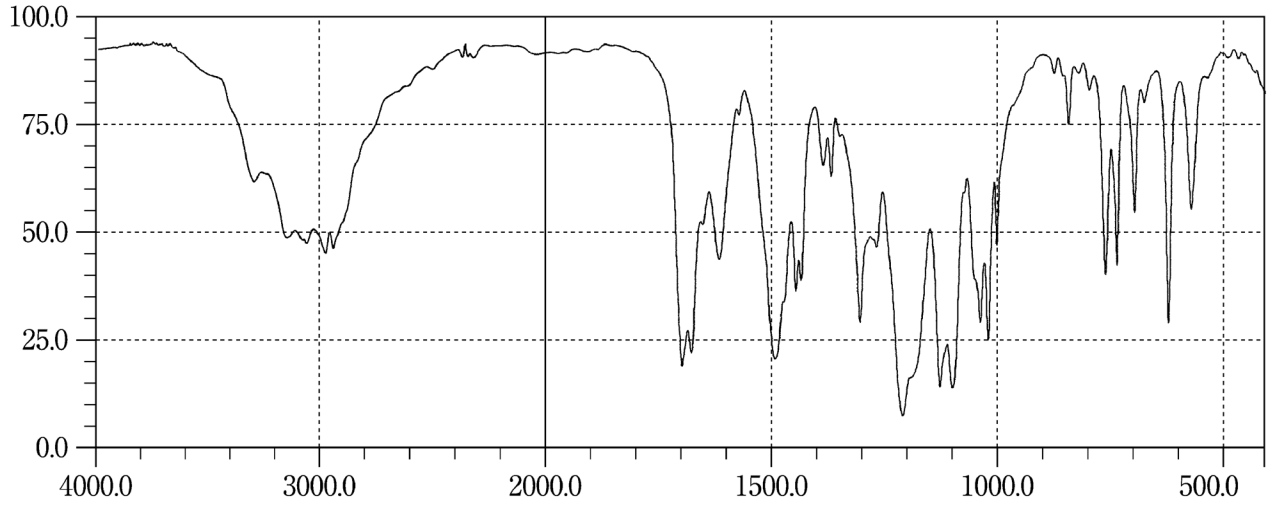
Amiodarone Hydrochloride



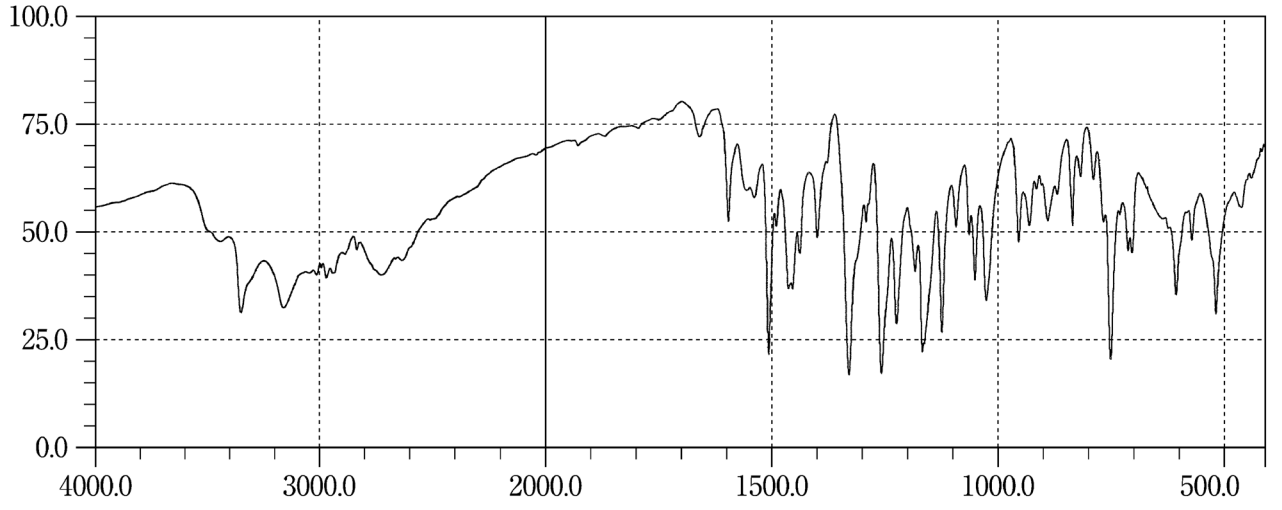
Amlexanox



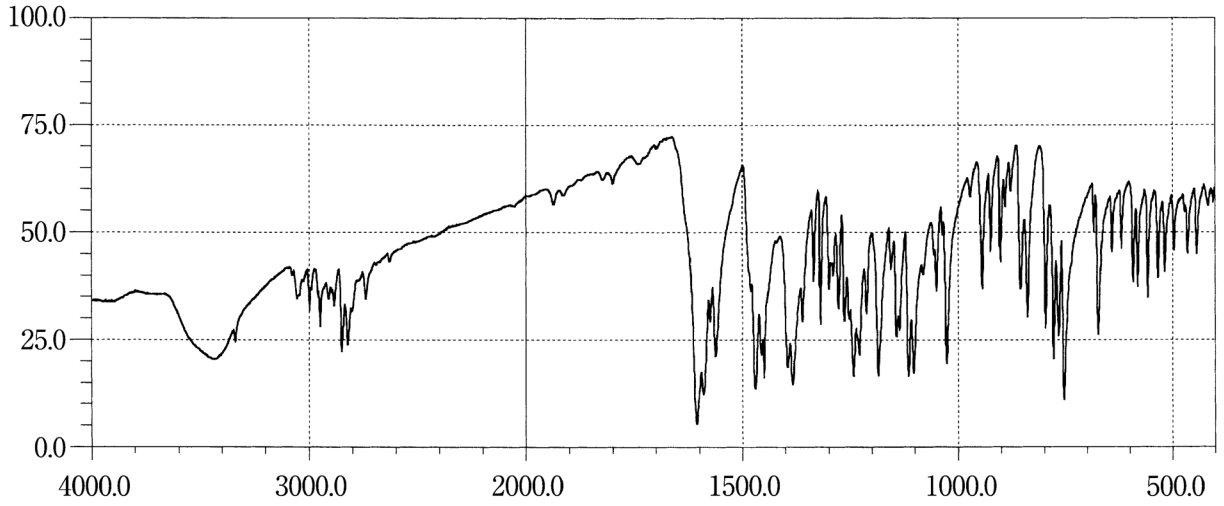
Amlodipine Besilate



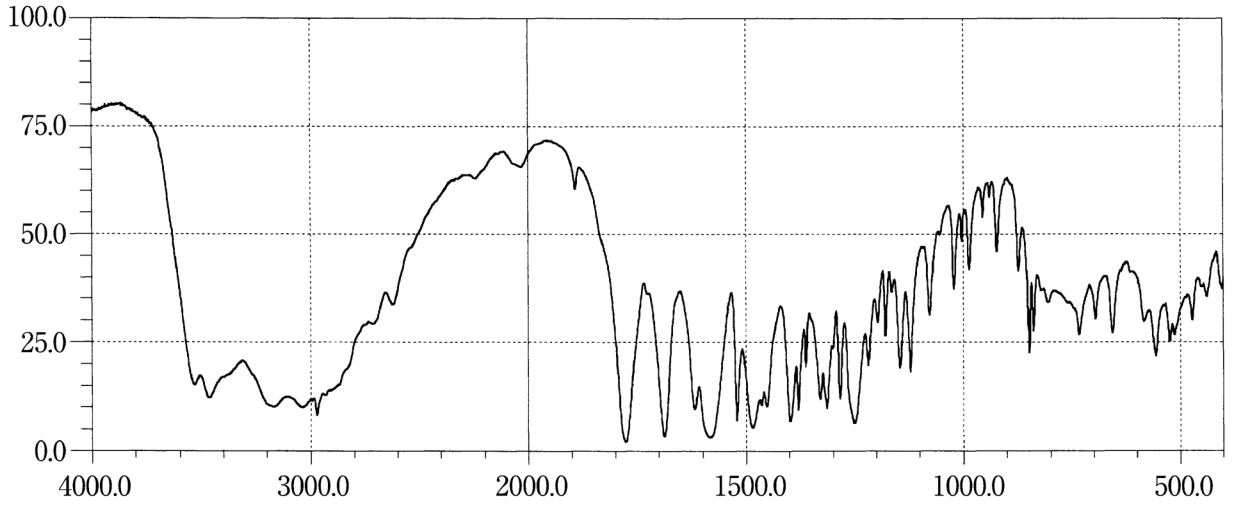
Amosulalol Hydrochloride



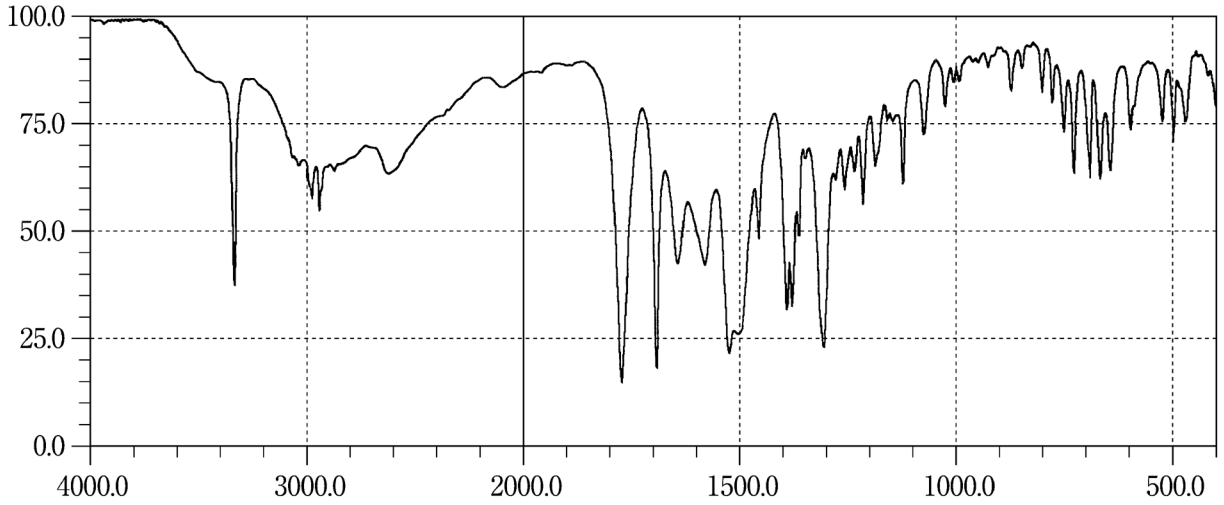
Amoxapine



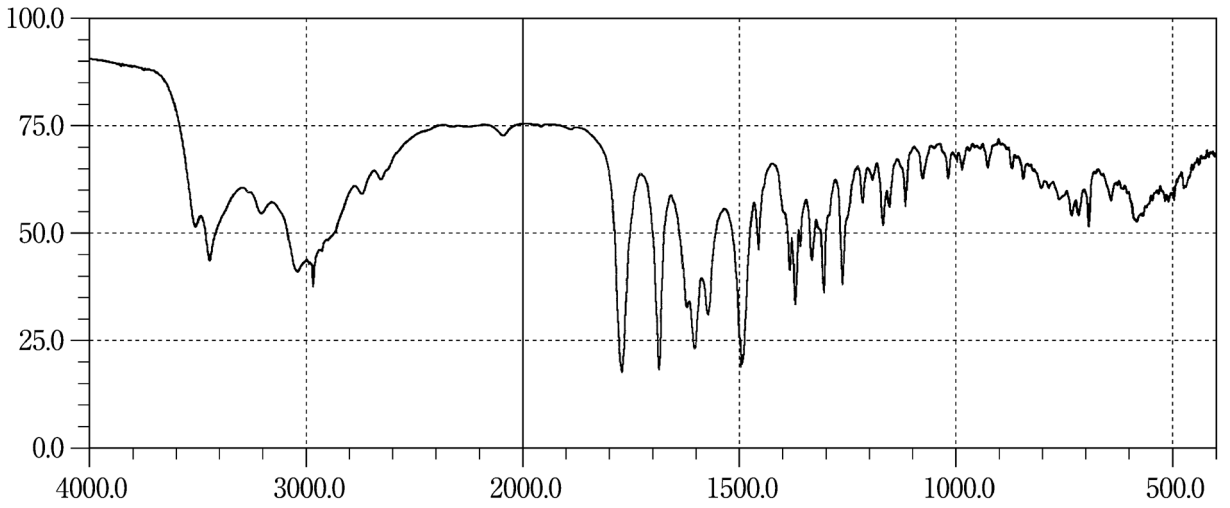
Amoxicillin Hydrate



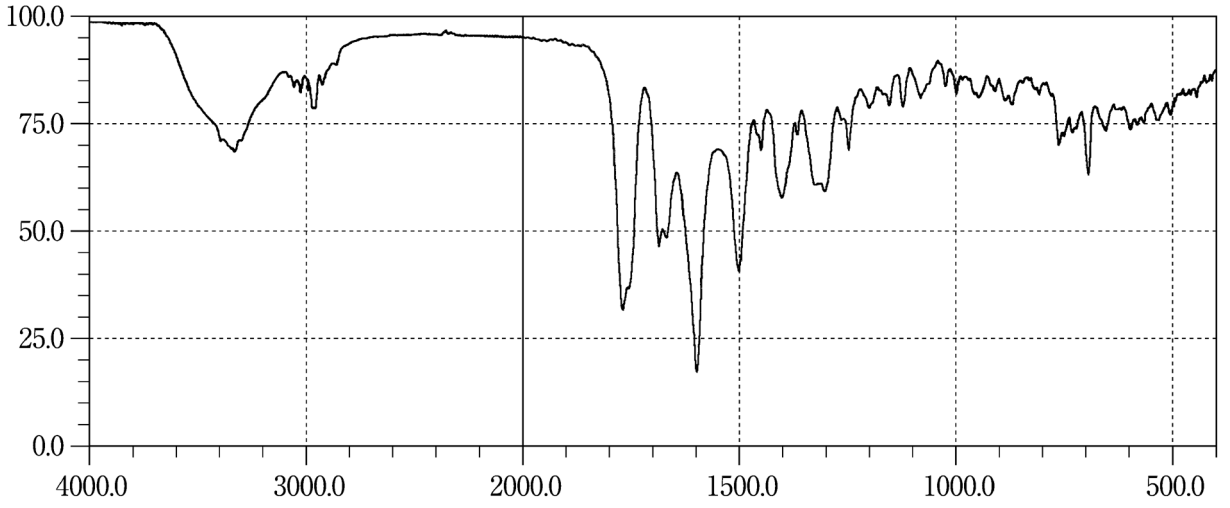
Anhydrous Ampicillin



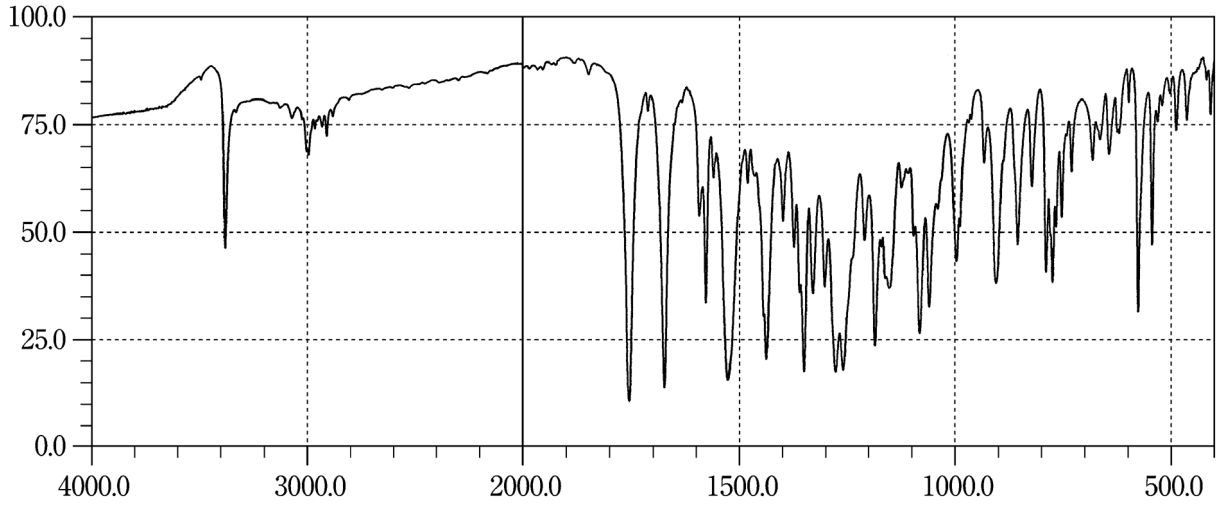
Ampicillin Hydrate



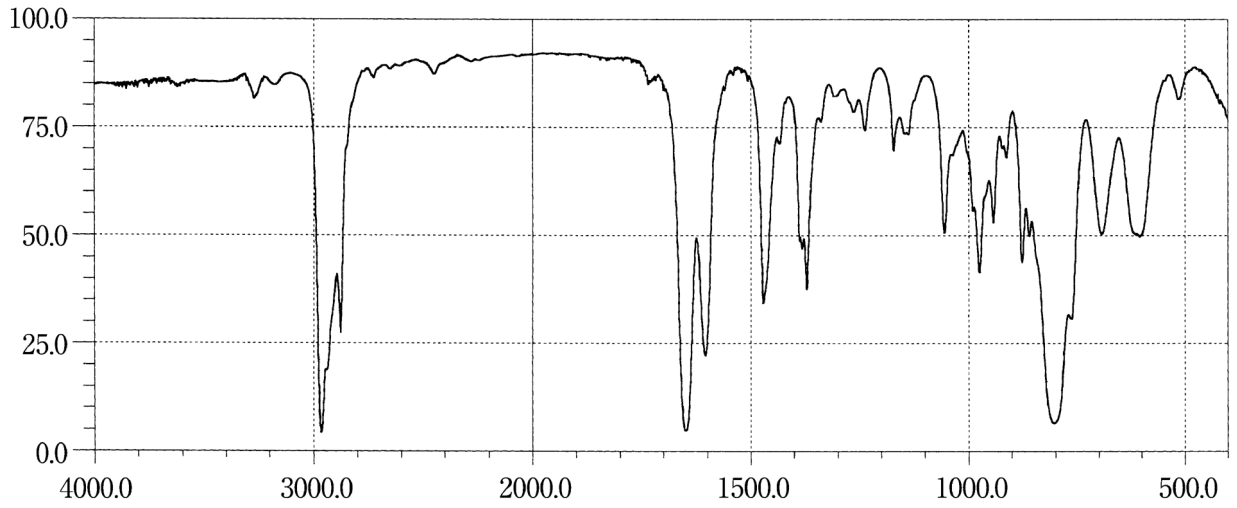
Ampicillin Sodium



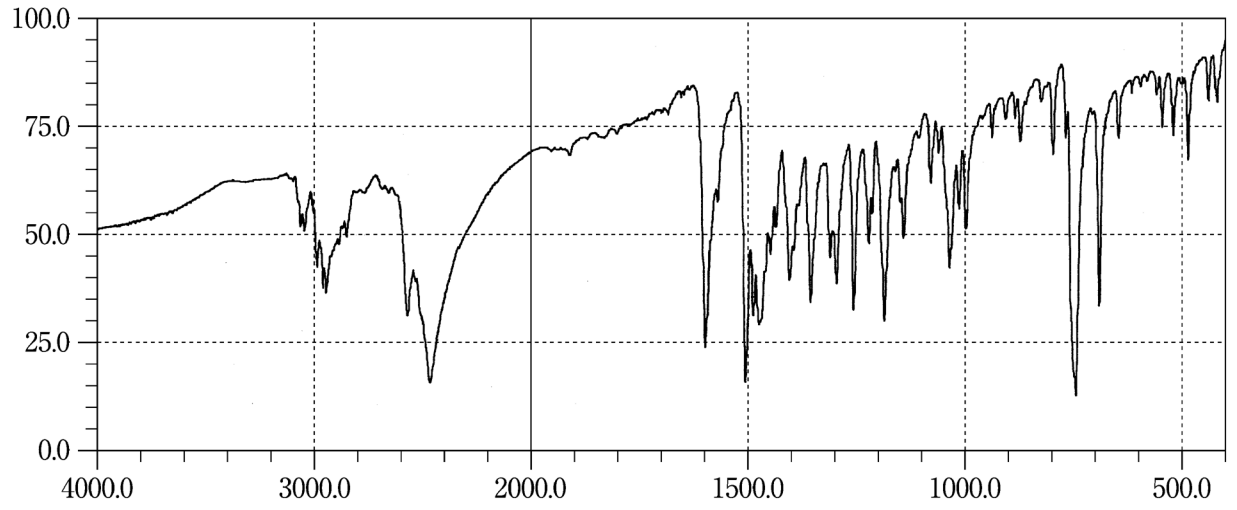
Ampiroxicam



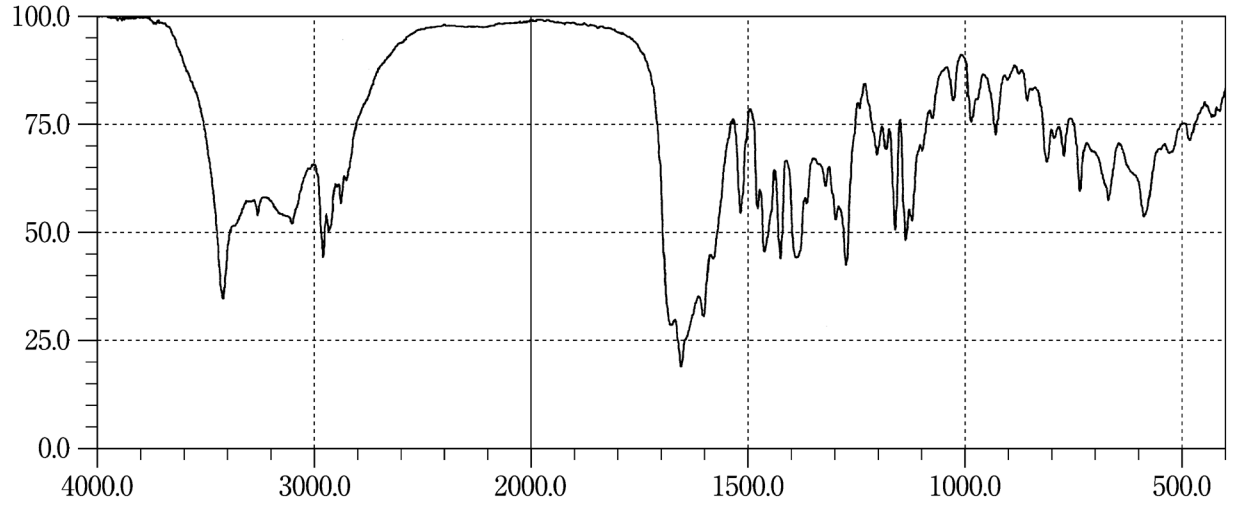
Amyl Nitrite



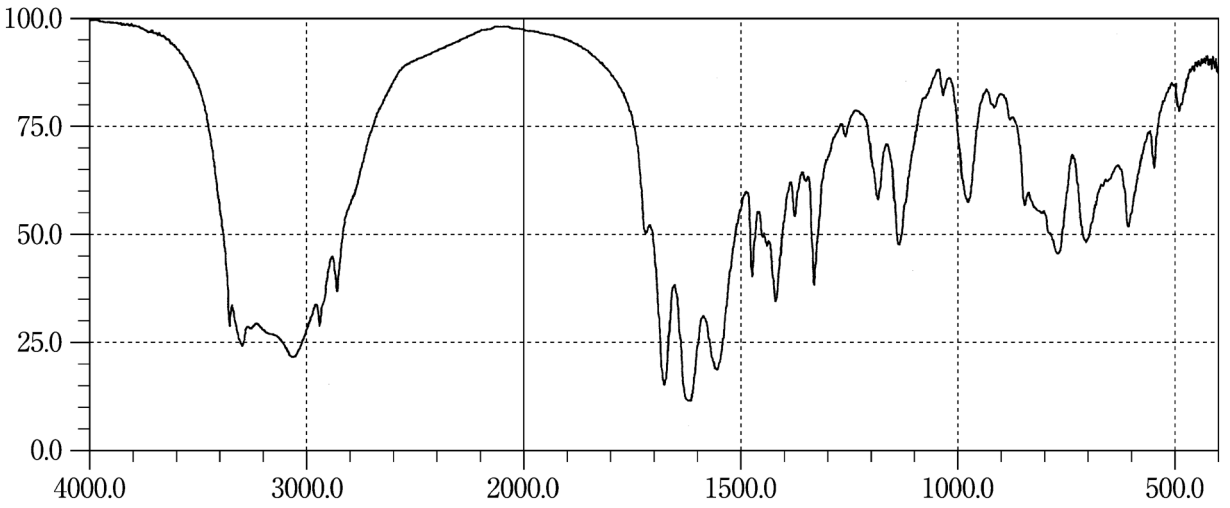
Aprindine Hydrochloride



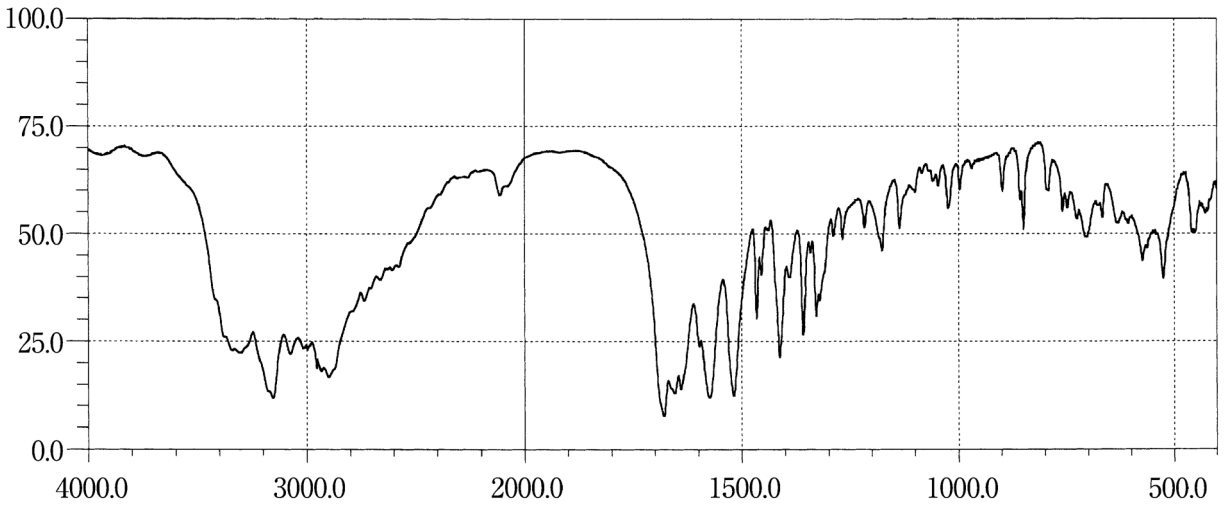
Argatroban Hydrate



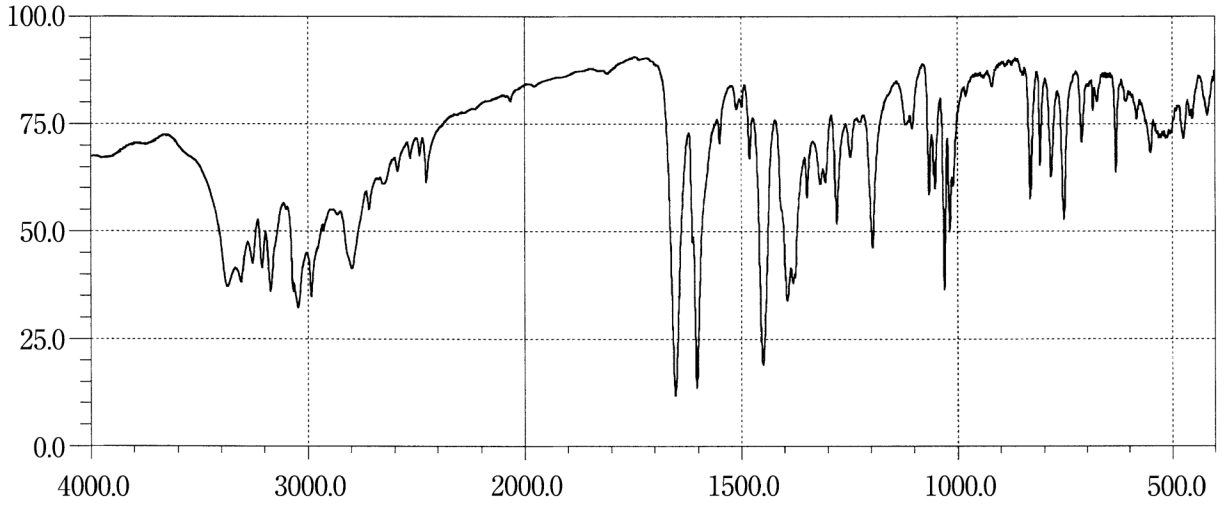
L-Arginine



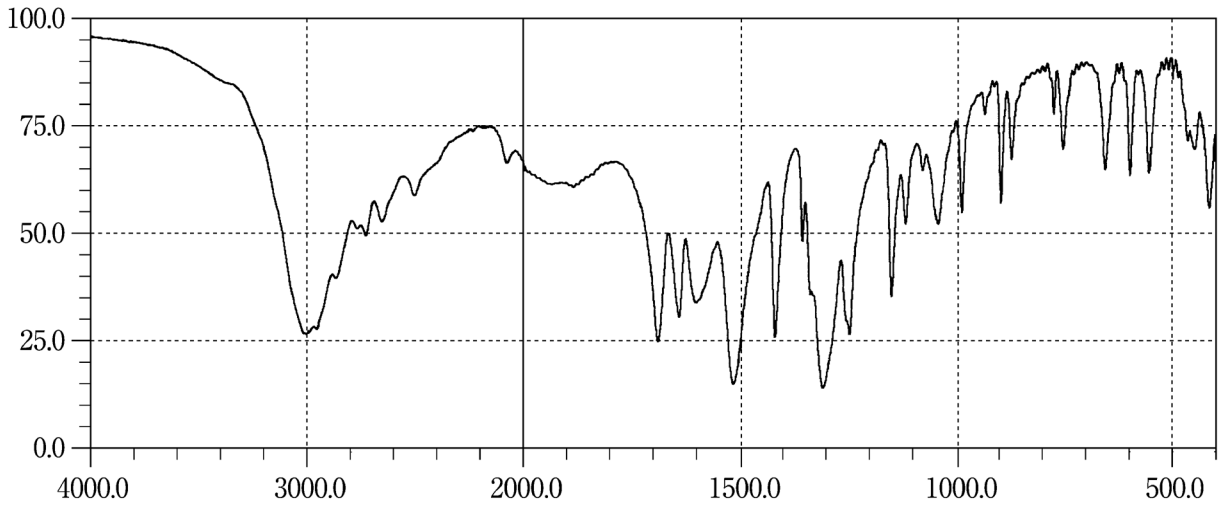
L-Arginine Hydrochloride



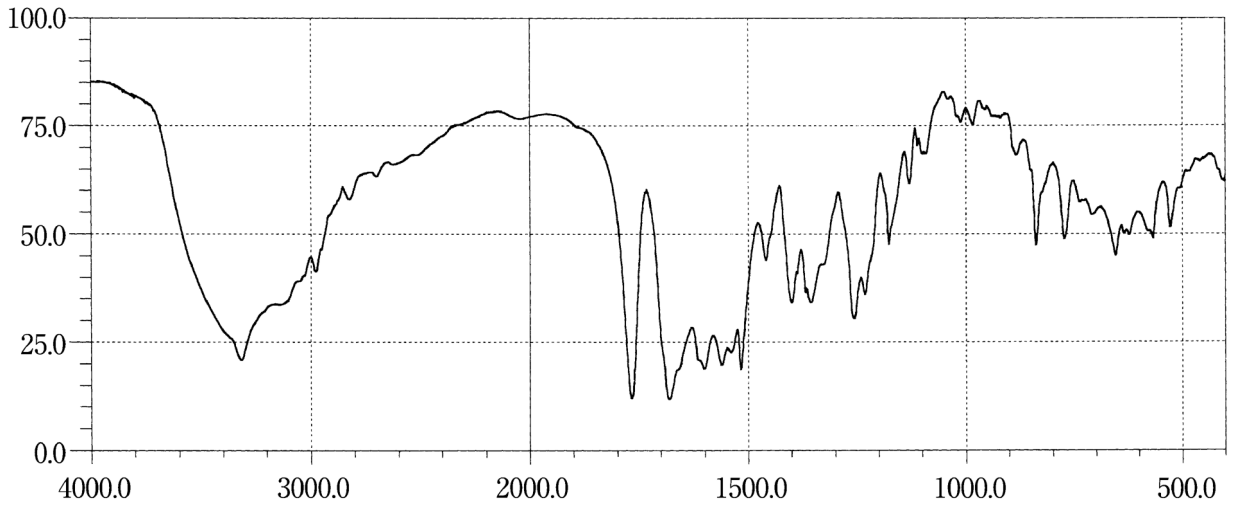
Arotinolol Hydrochloride



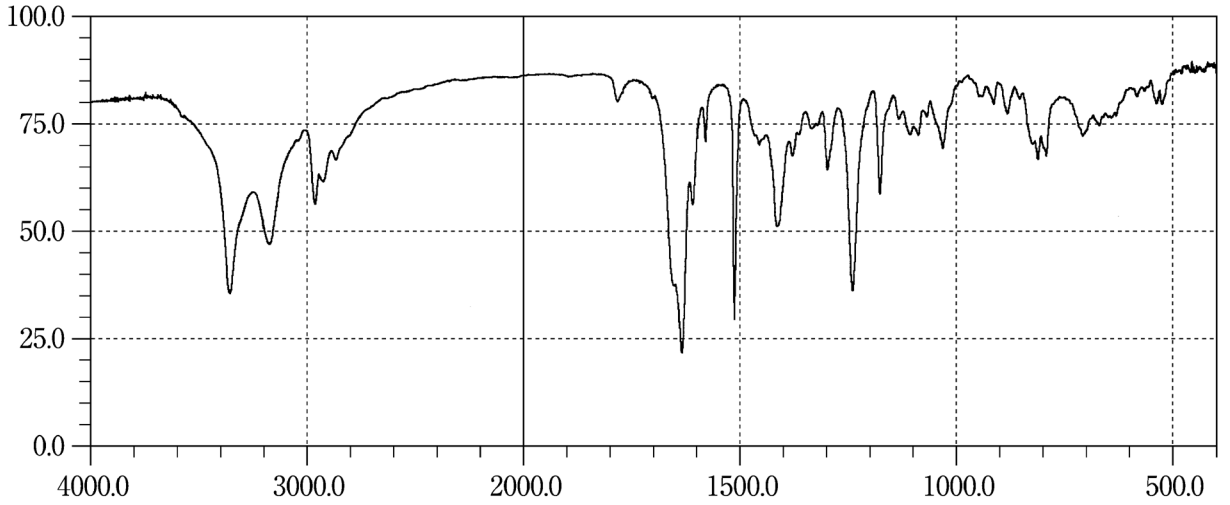
L-Aspartic Acid



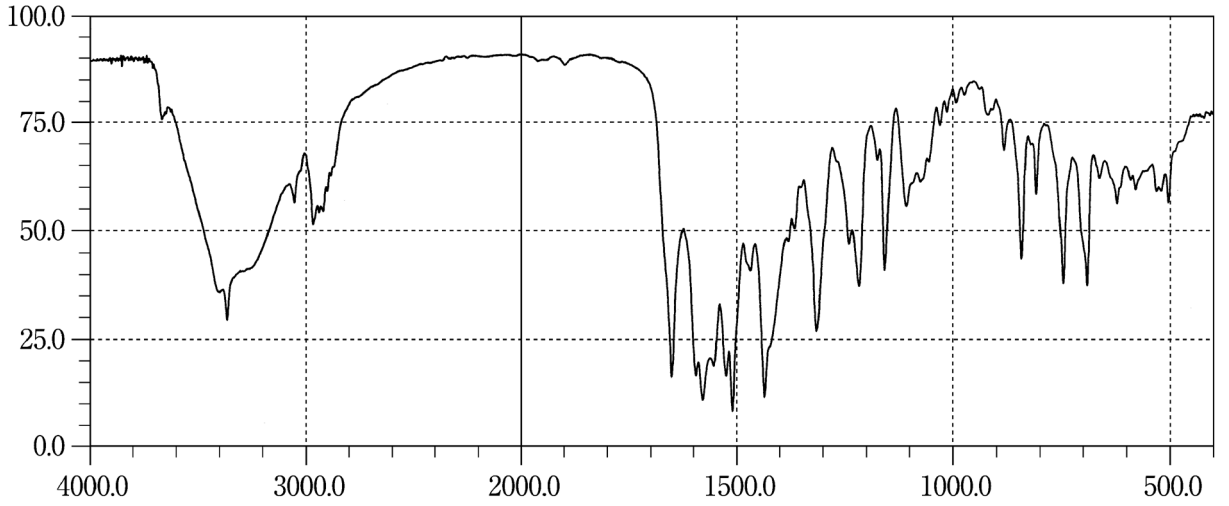
Aspoxicillin Hydrate



Atenolol



Atorvastatin Calcium Hydrate



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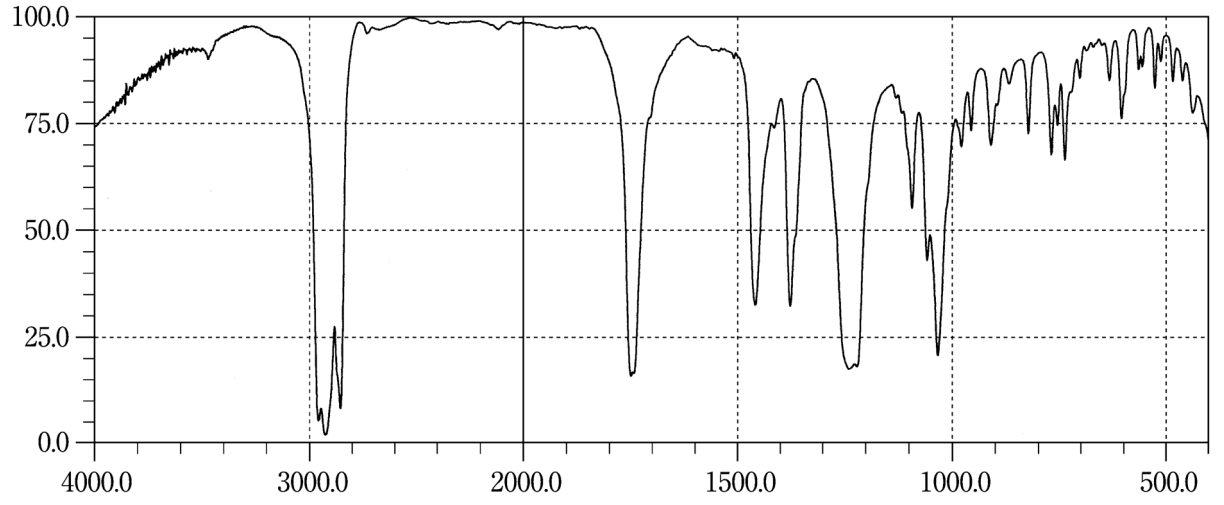
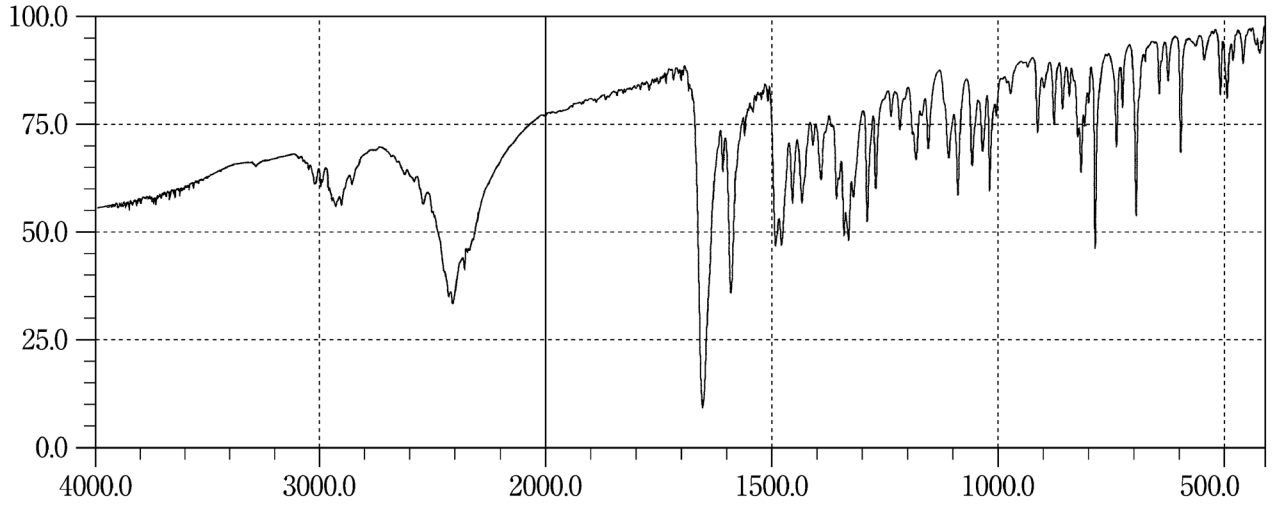
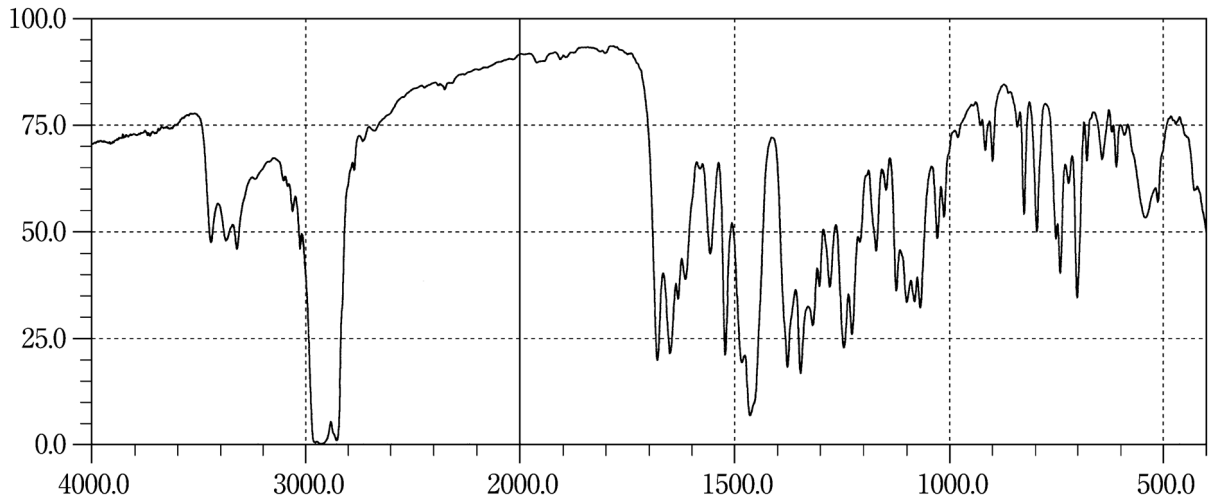


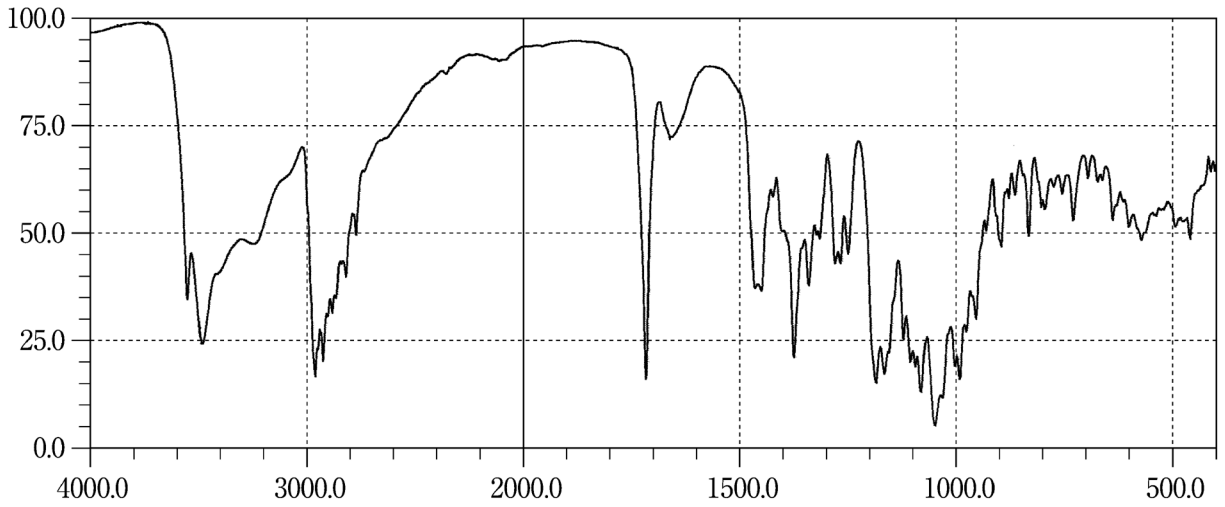
Azelastine Hydrochloride



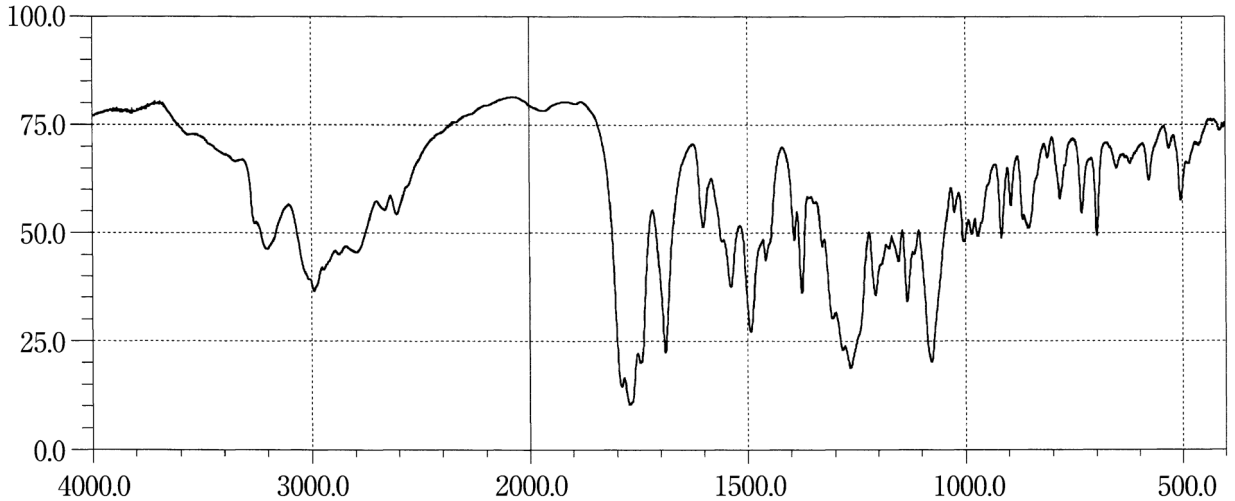
Azelnidipine



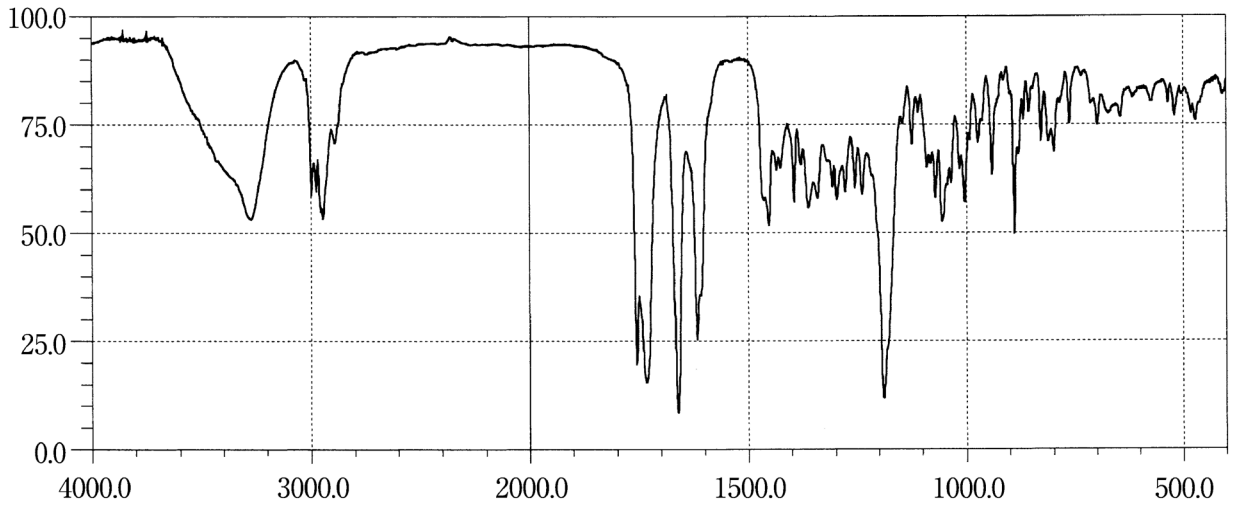
Azithromycin Hydrate



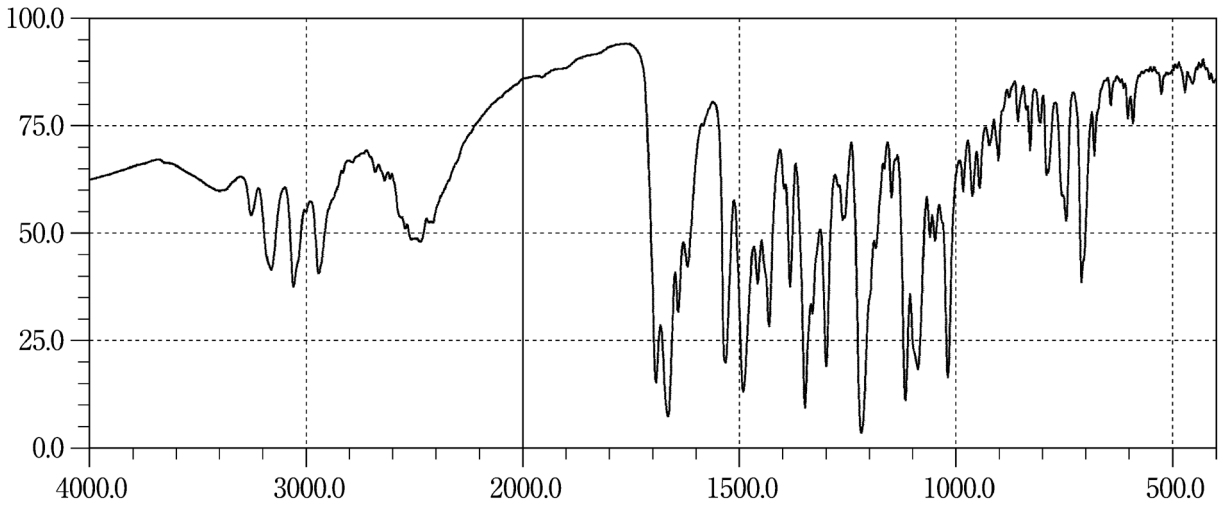
Bacampicillin Hydrochloride



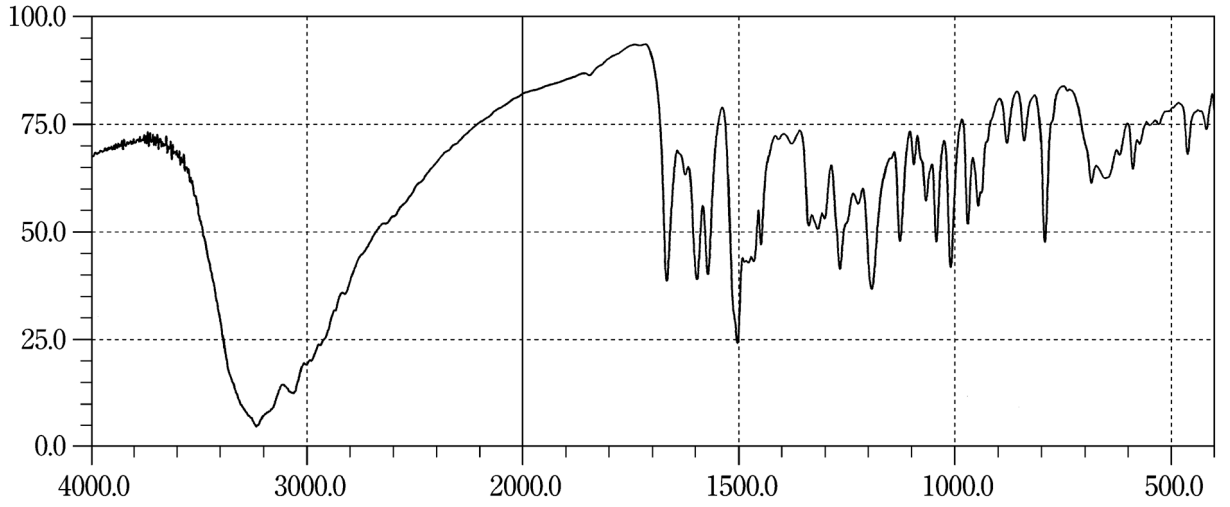
Beclometasone Dipropionate



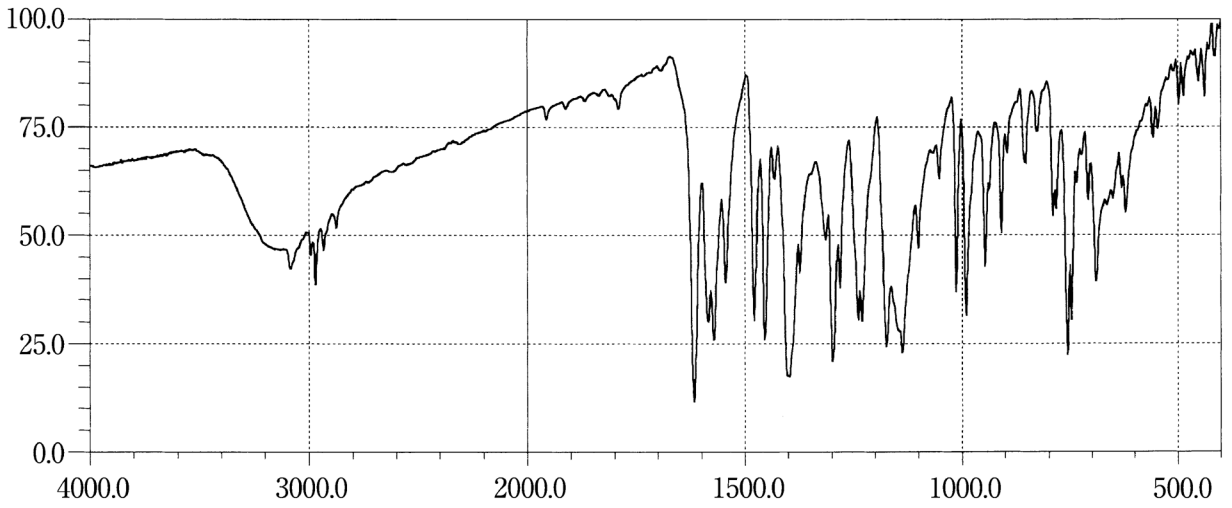
Benidipine Hydrochloride



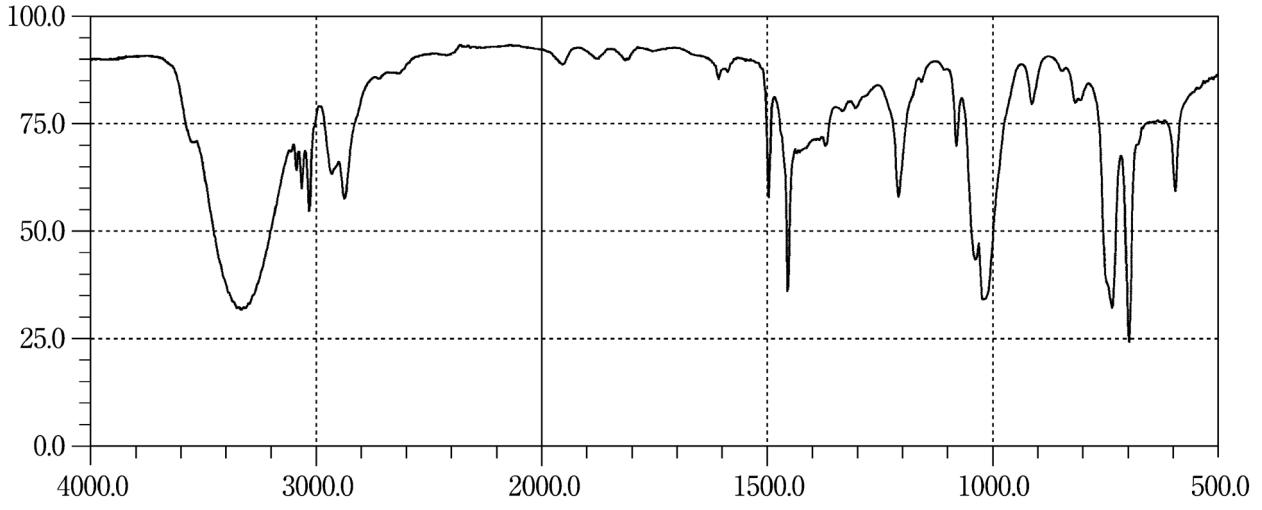
Benserazide Hydrochloride



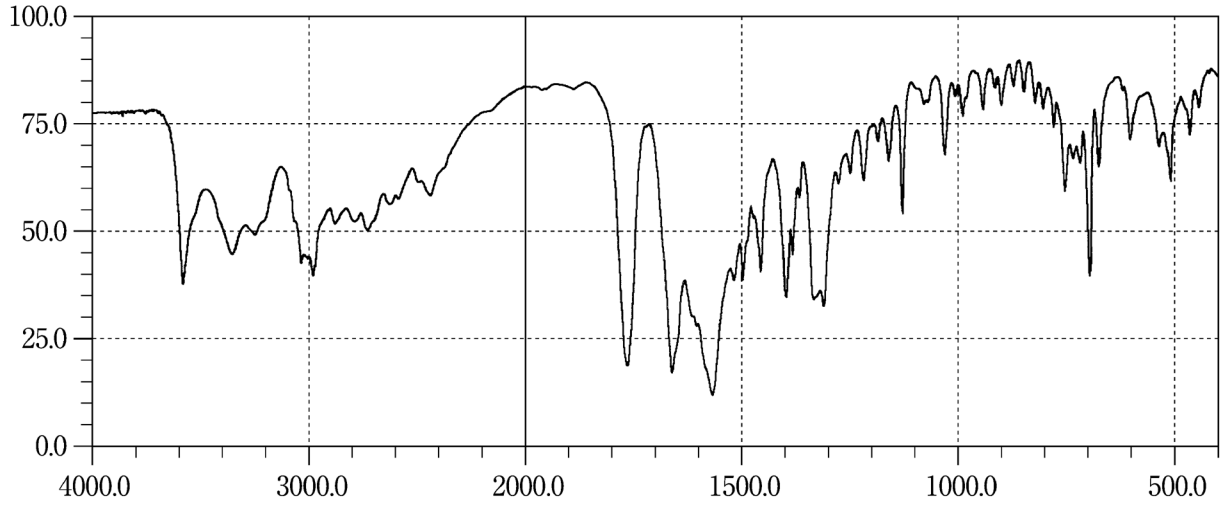
Benzbromarone



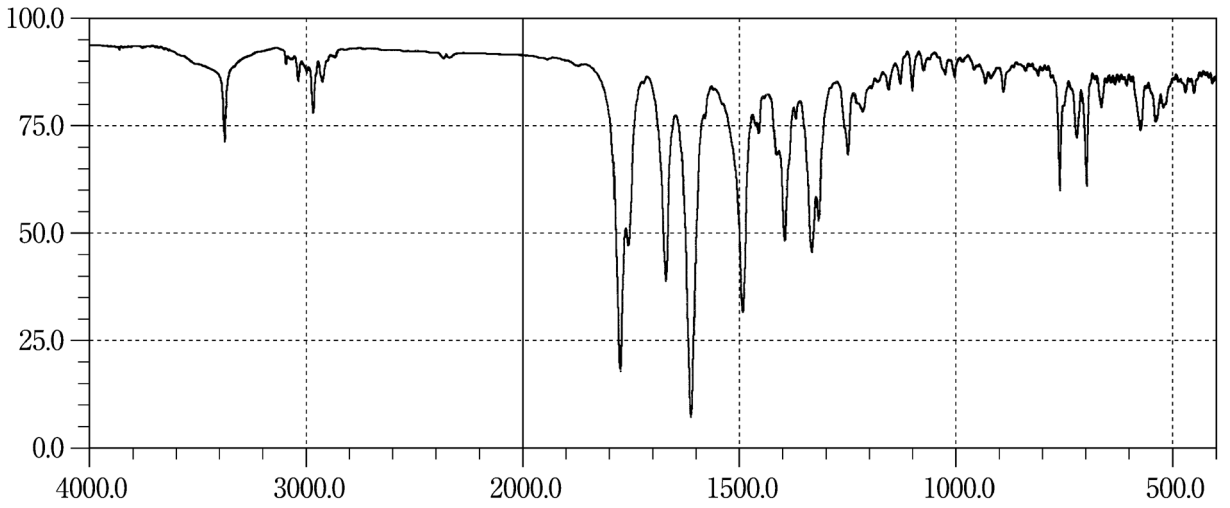
Benzyl Alcohol



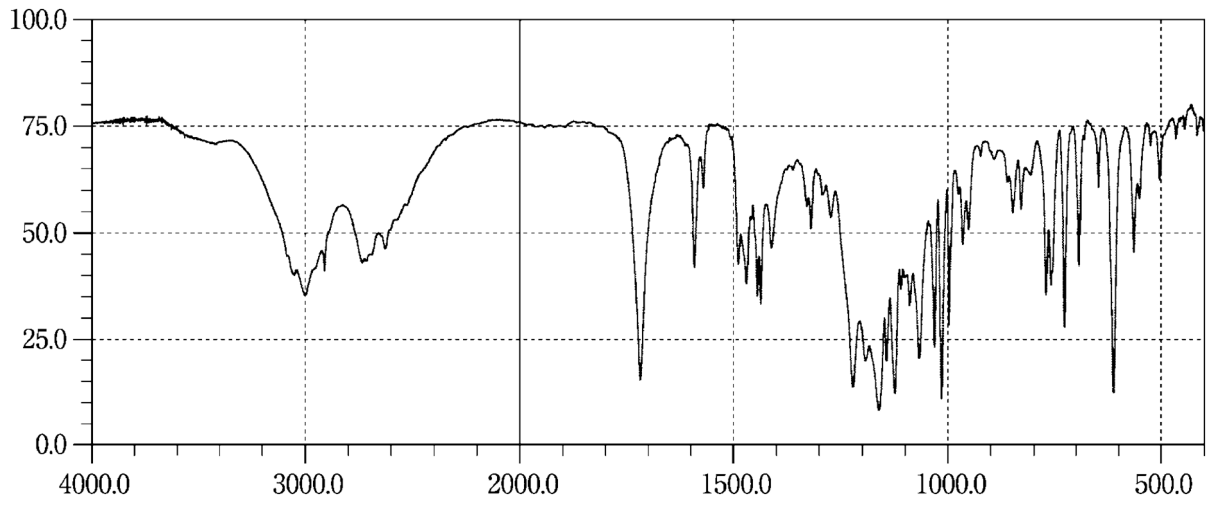
Benzylpenicillin Benzathine Hydrate



Benzylpenicillin Potassium



Bepotastine Besilate



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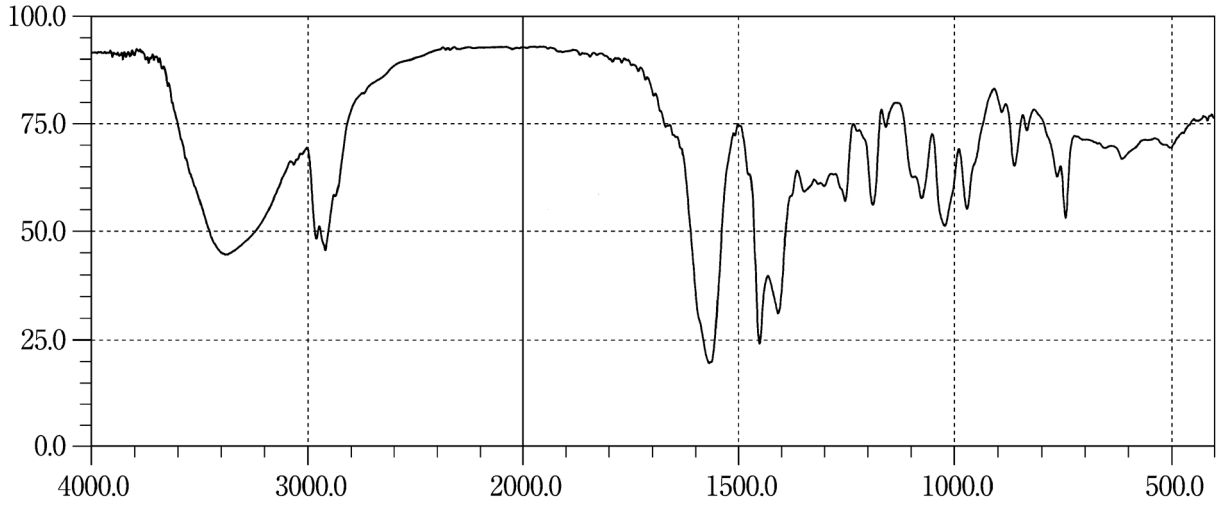
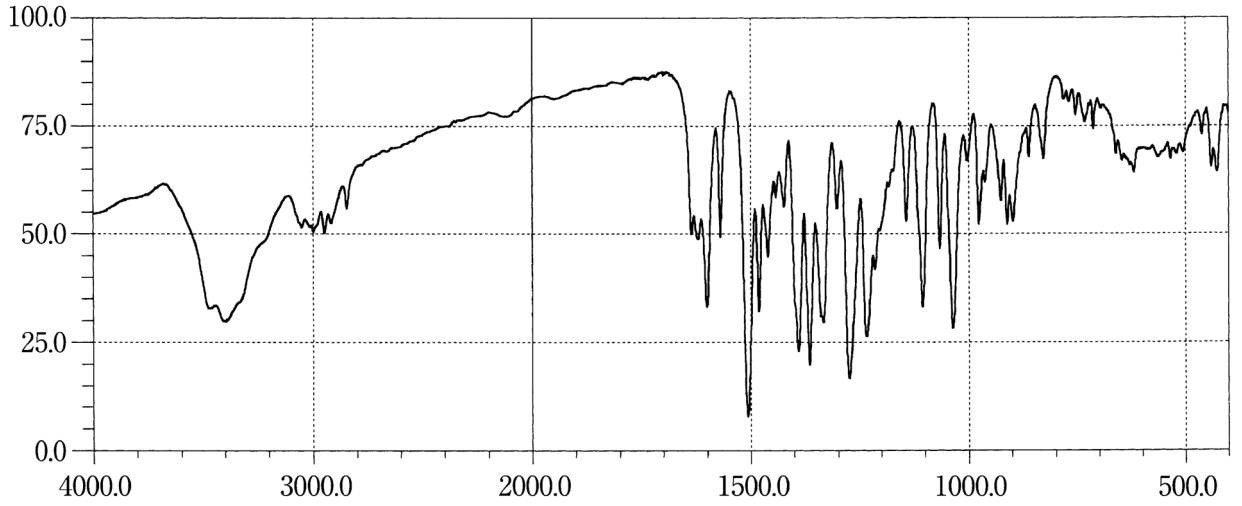
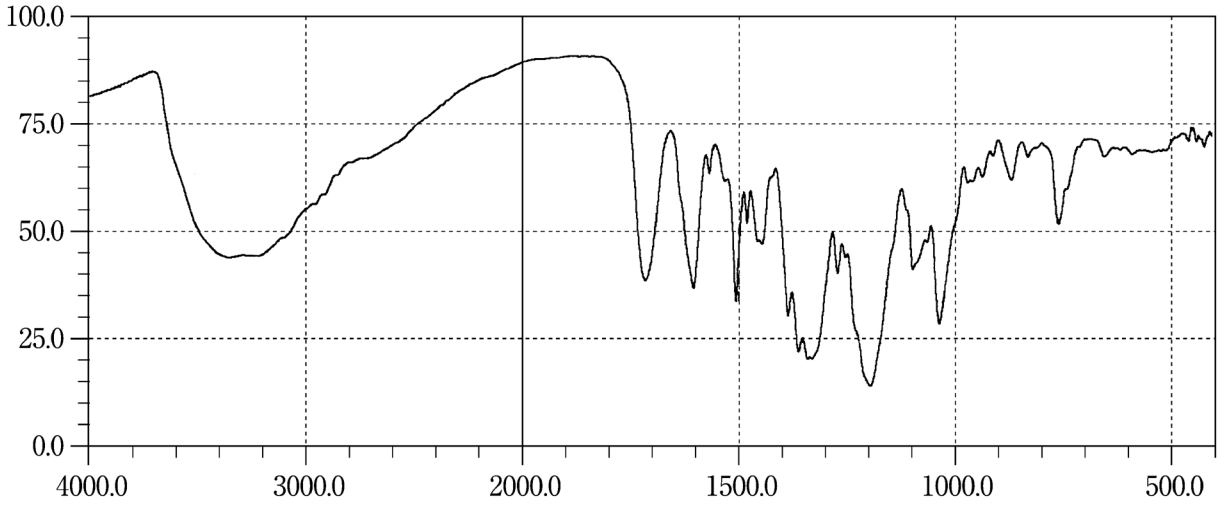


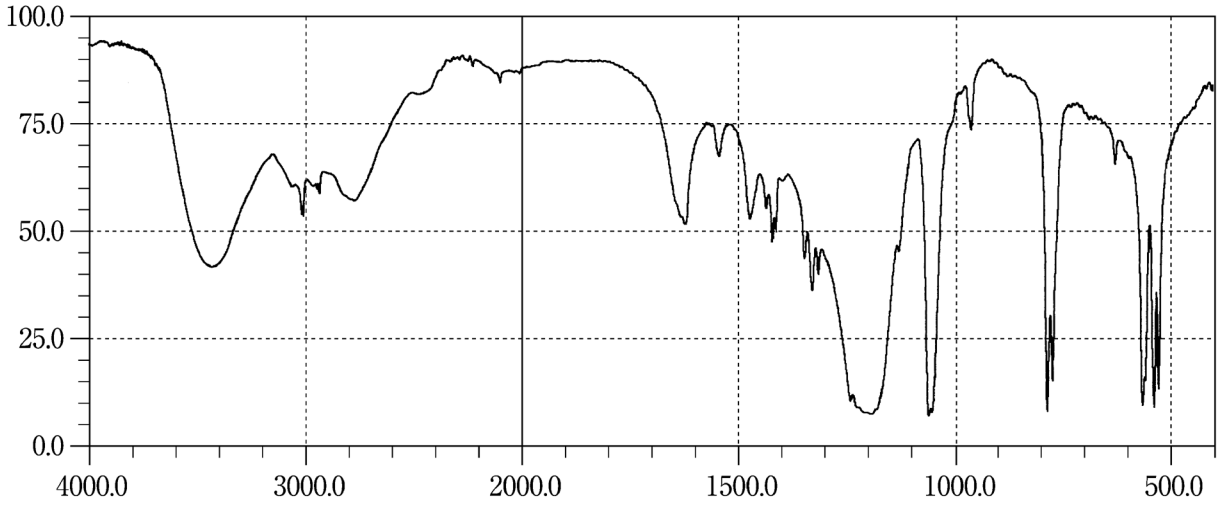
Berberine Chloride Hydrate



Berberine Tannate



Betahistine Mesilate



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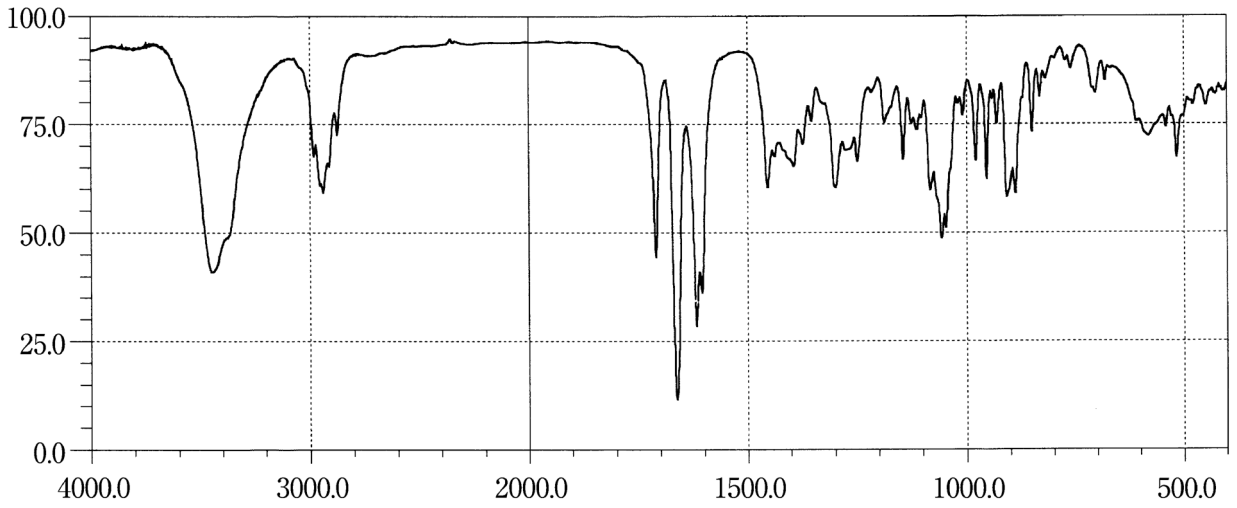
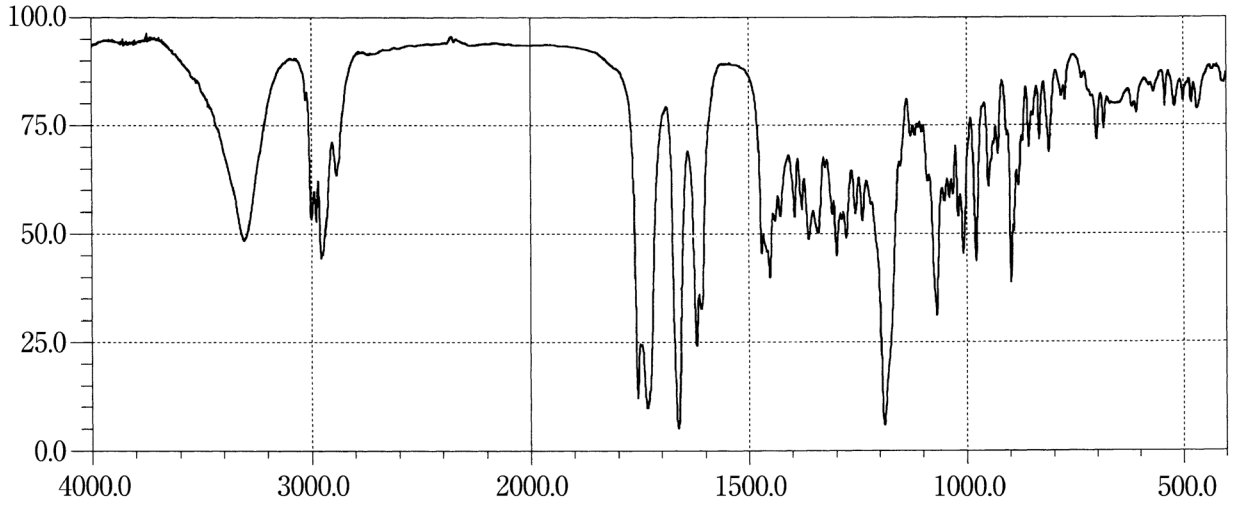
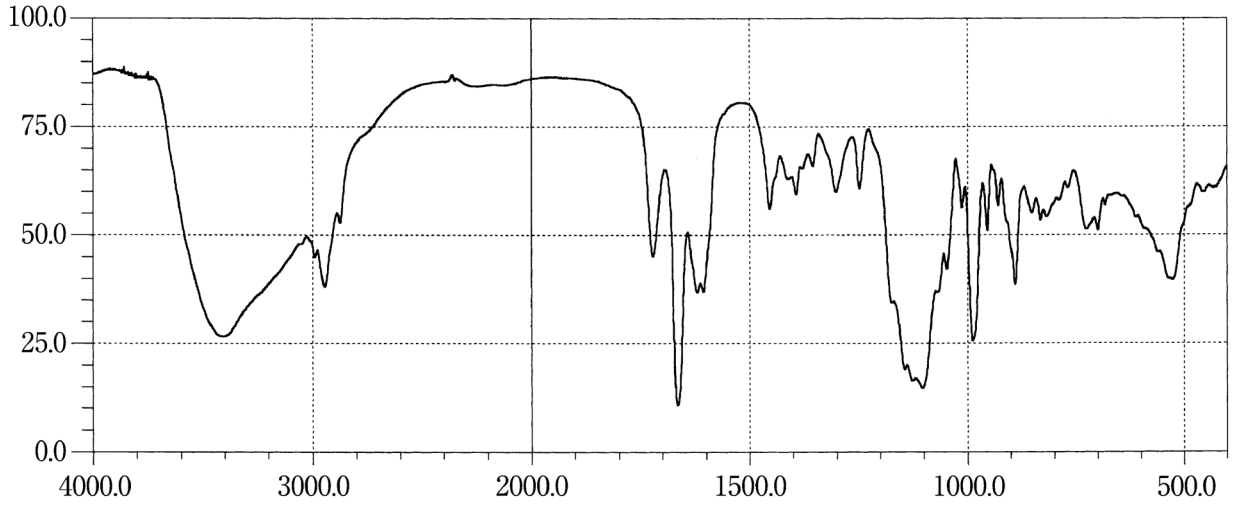


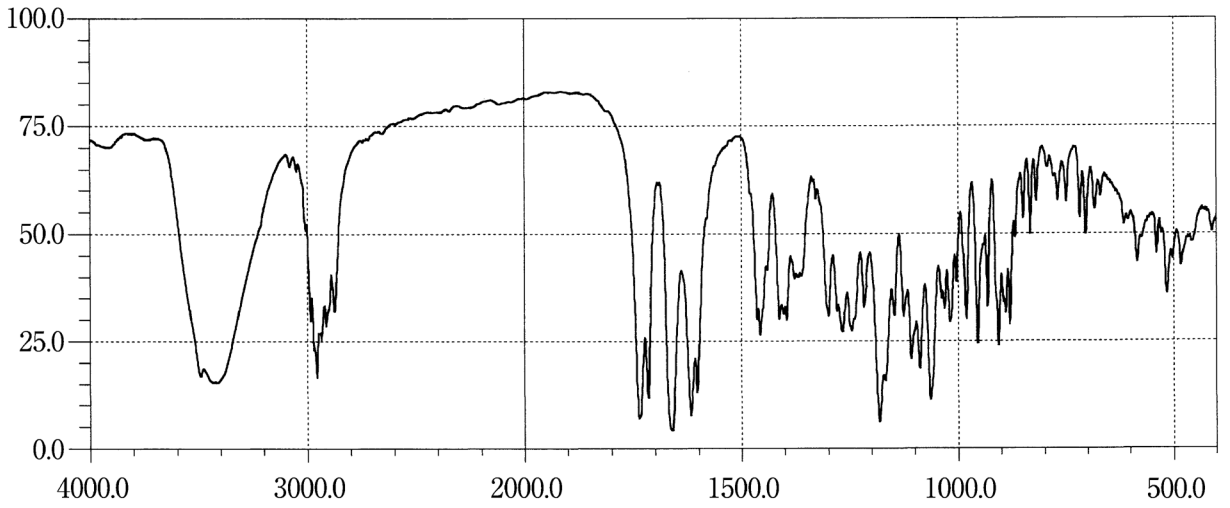
Betamethasone Dipropionate



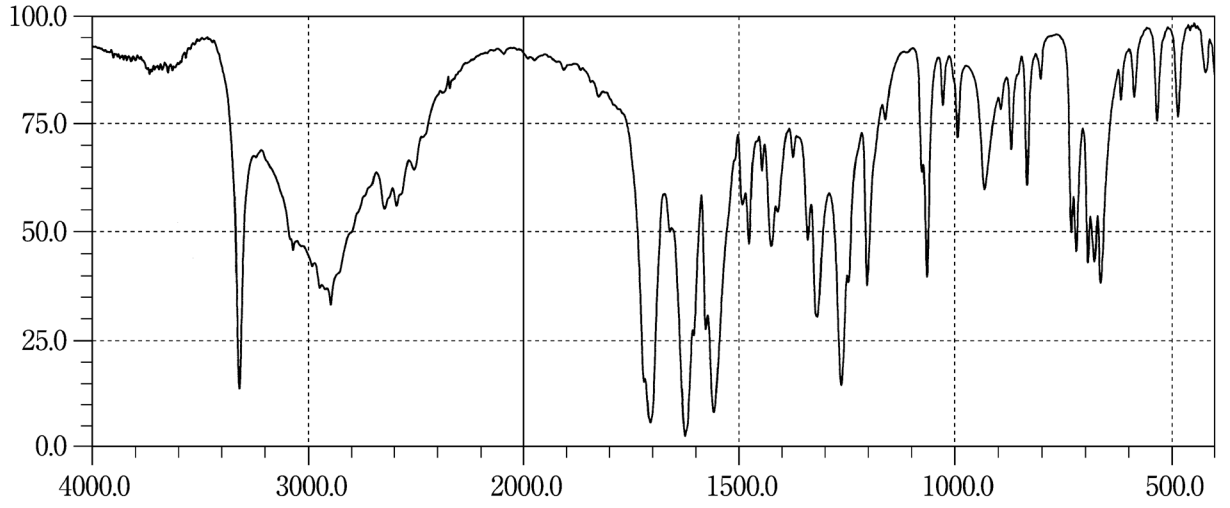
Betamethasone Sodium Phosphate



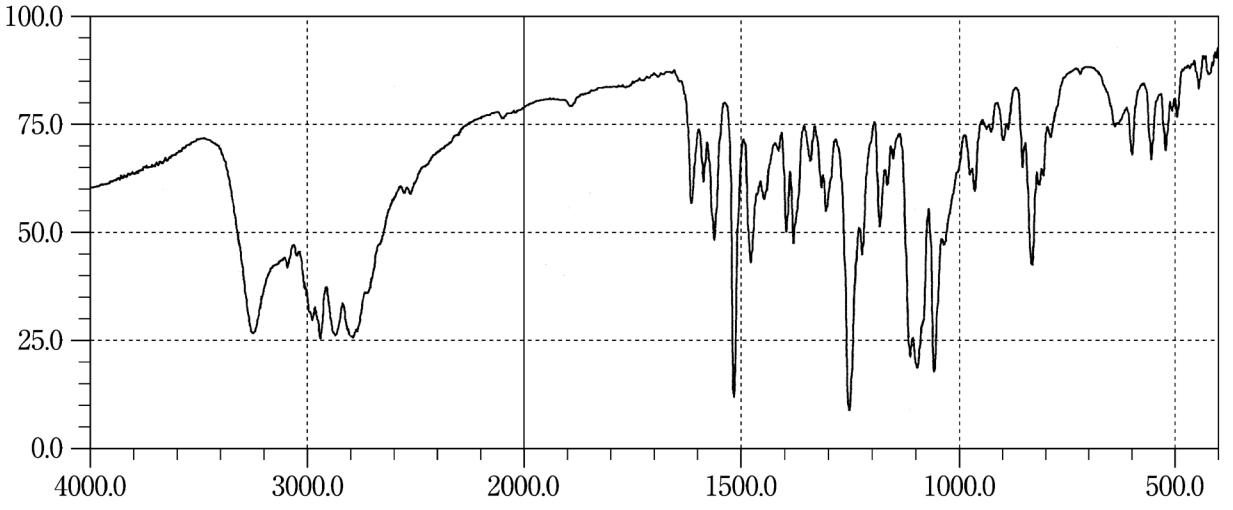
Betamethasone Valerate



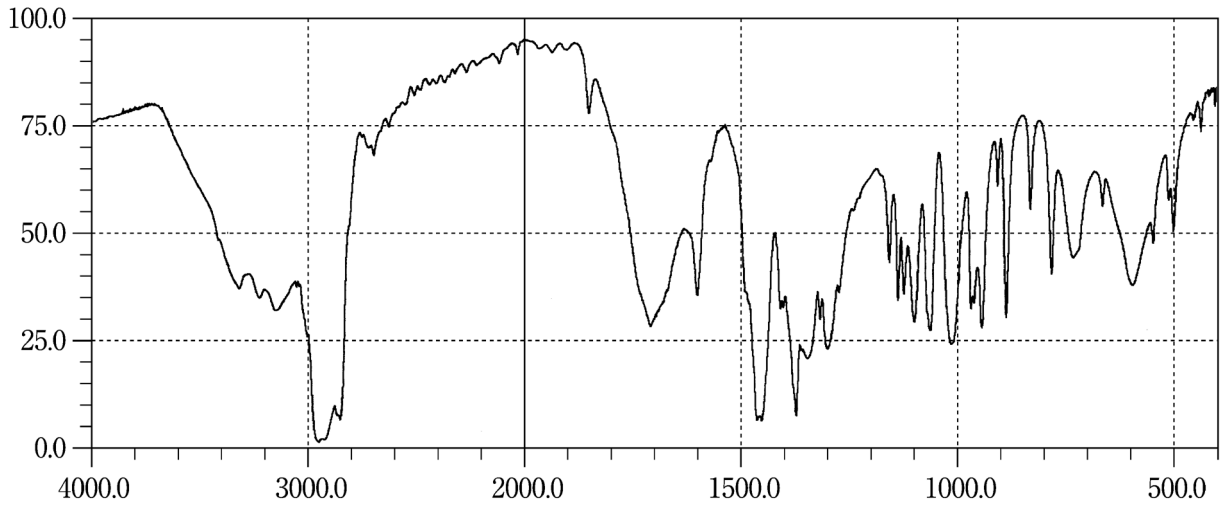
Betamipron



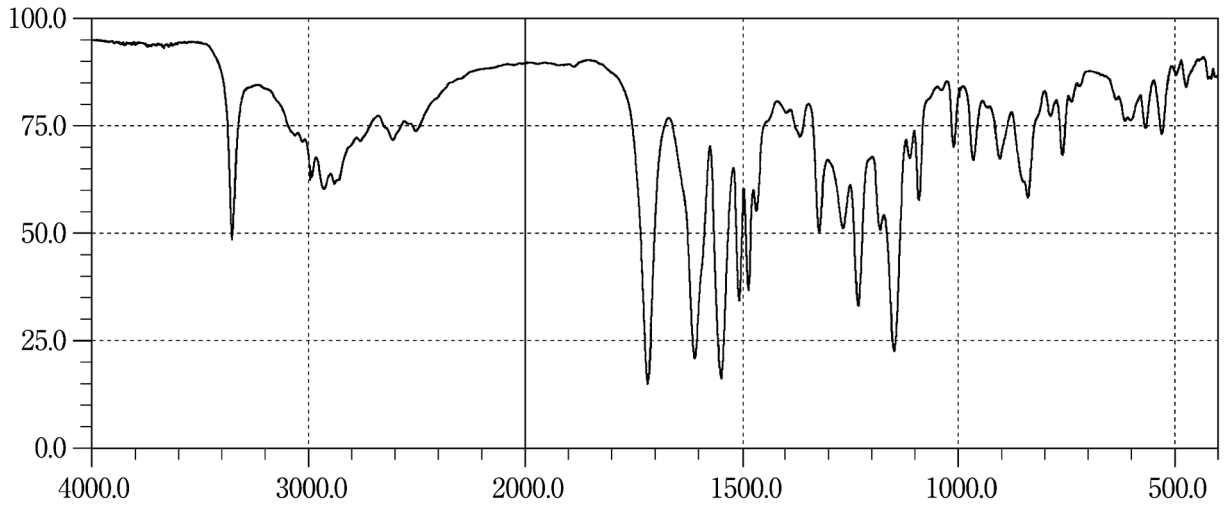
Betaxolol Hydrochloride



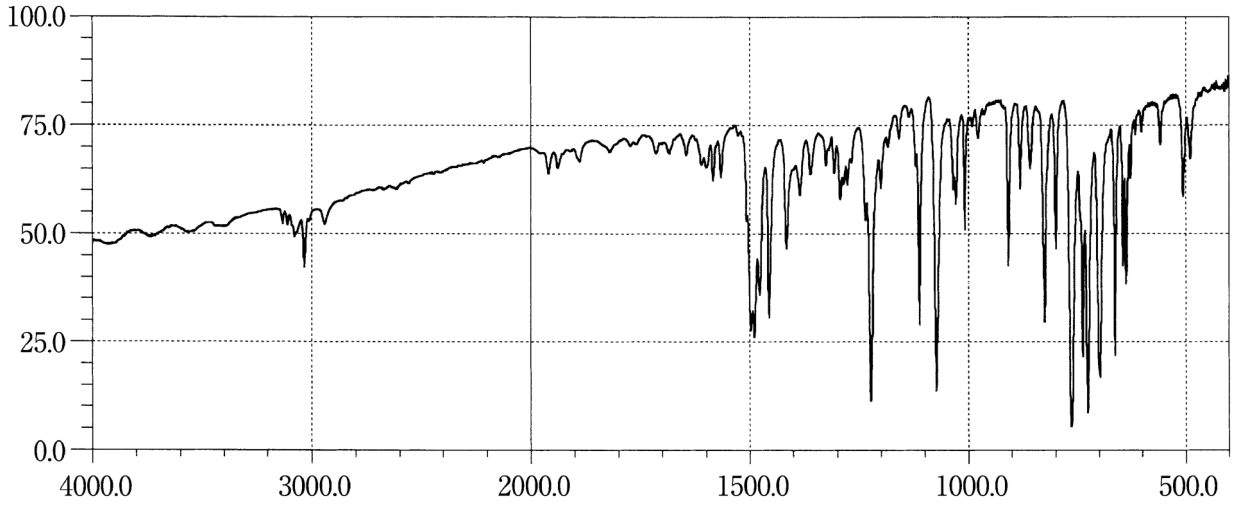
Bethanechol Chloride



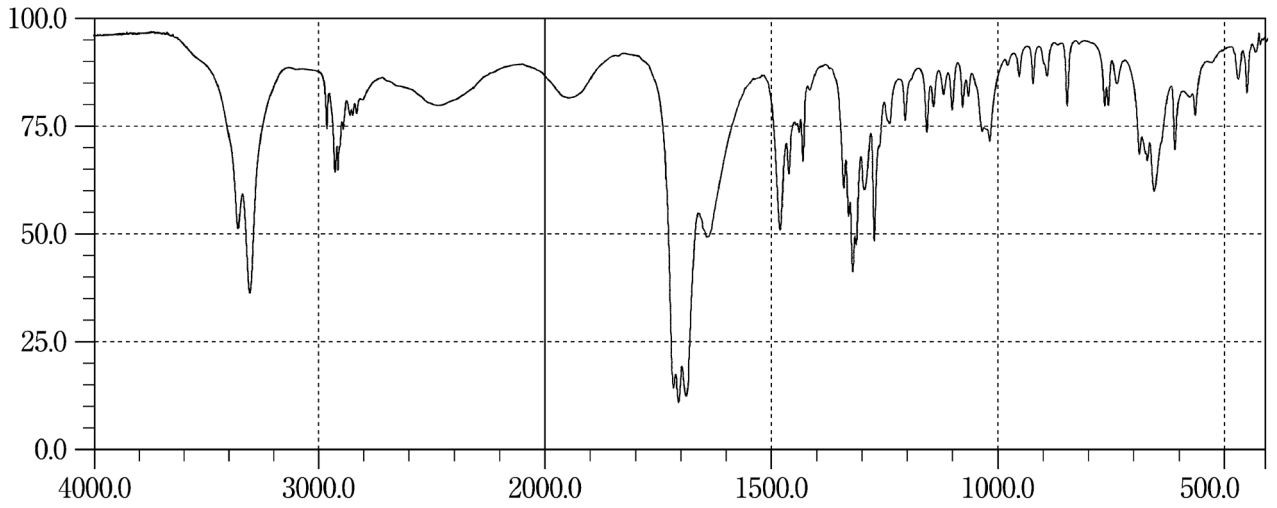
Bezafibrate



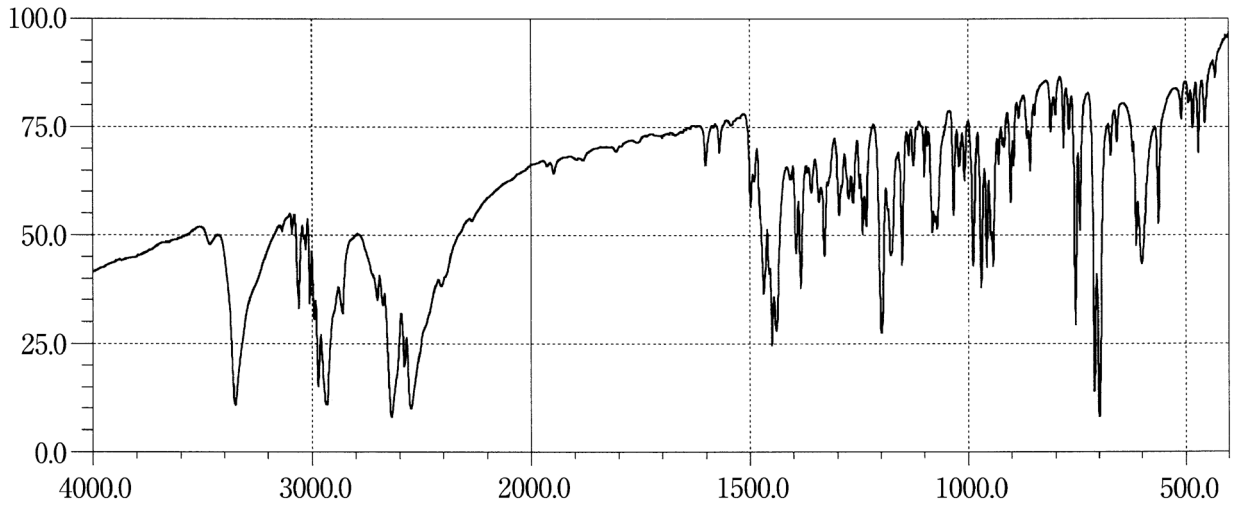
Bifonazole



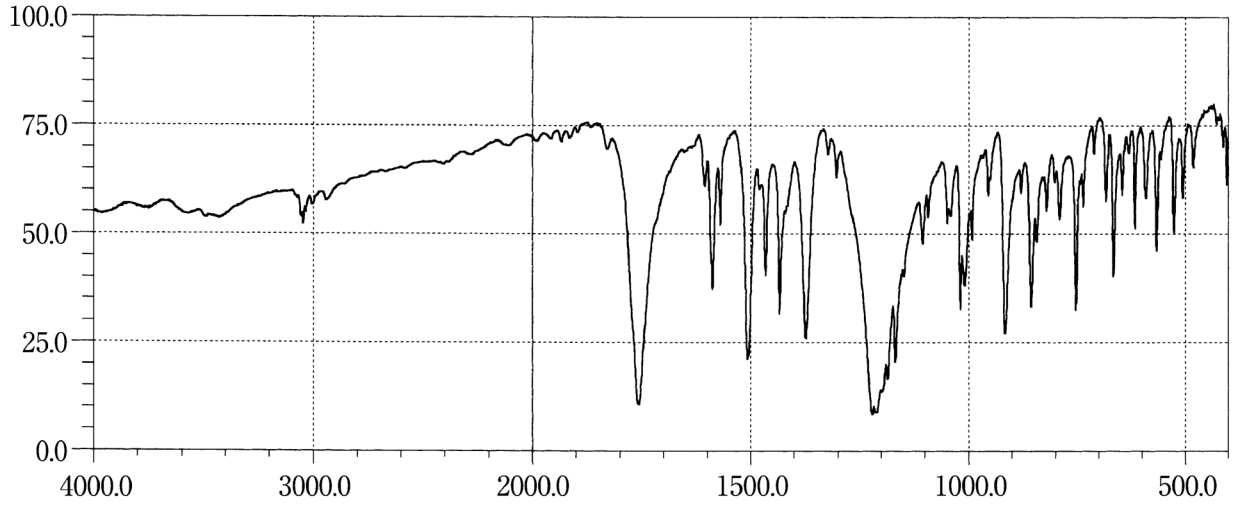
Biotin



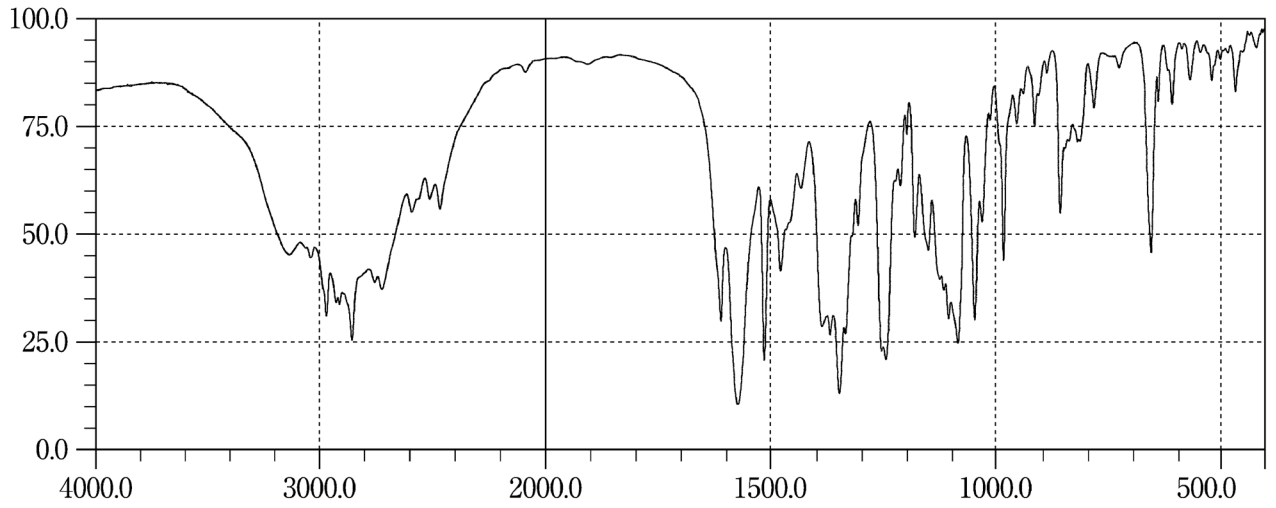
Biperiden Hydrochloride



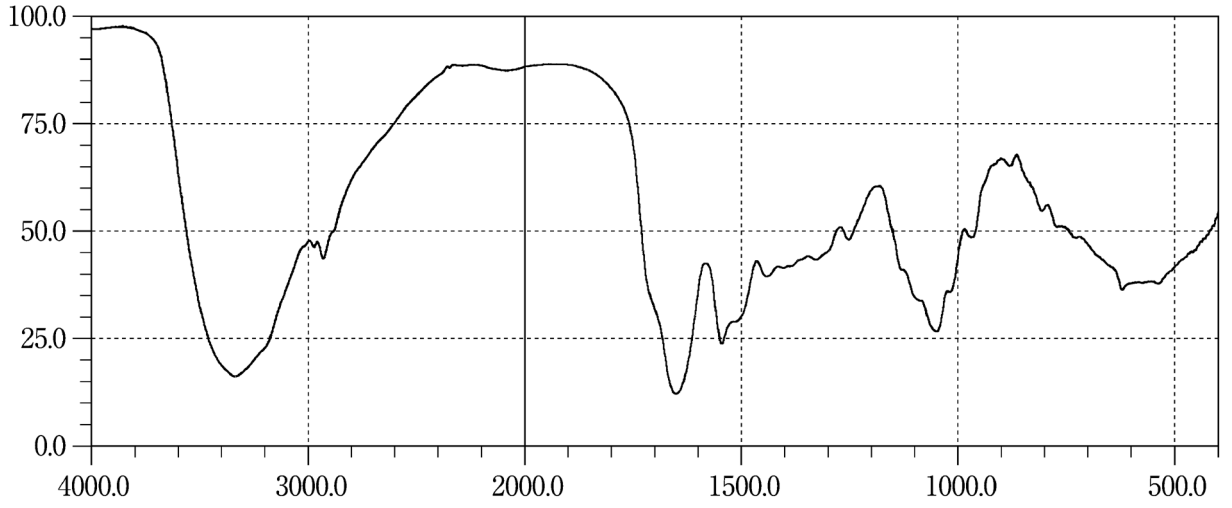
Bisacodyl



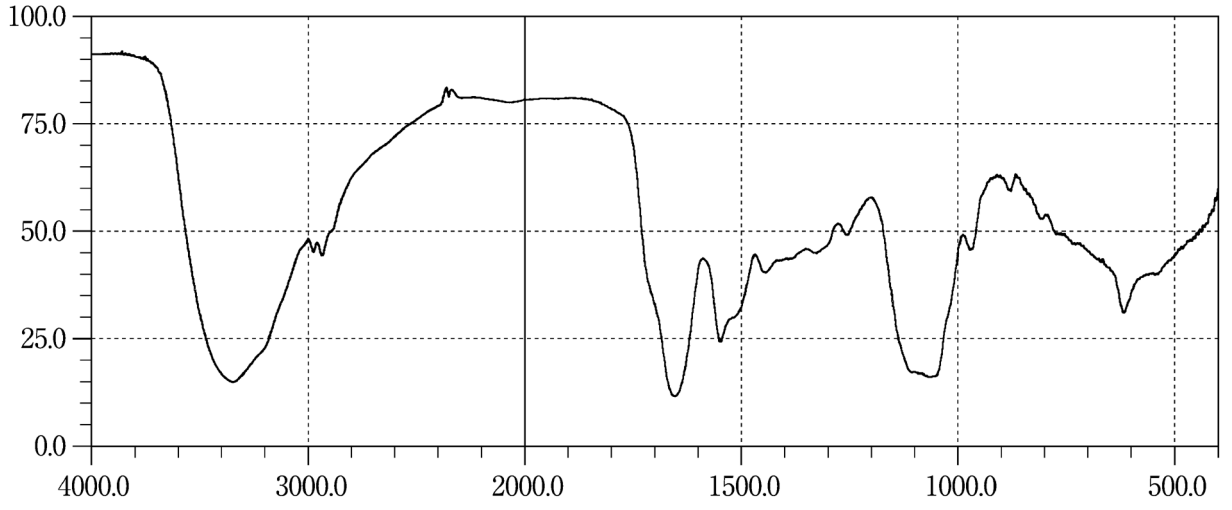
Bisoprolol Fumarate



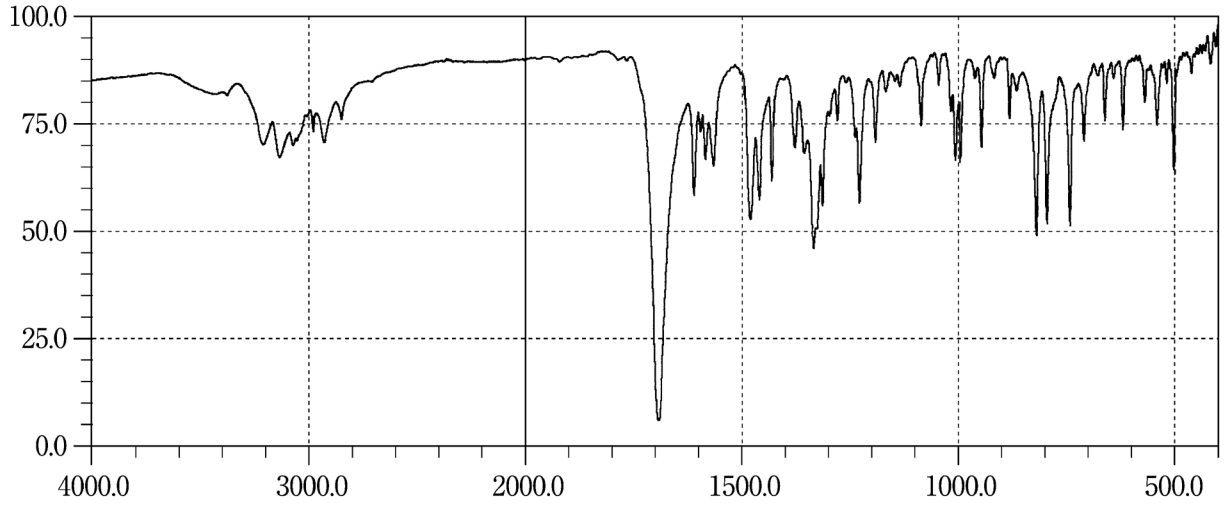
Bleomycin Hydrochloride



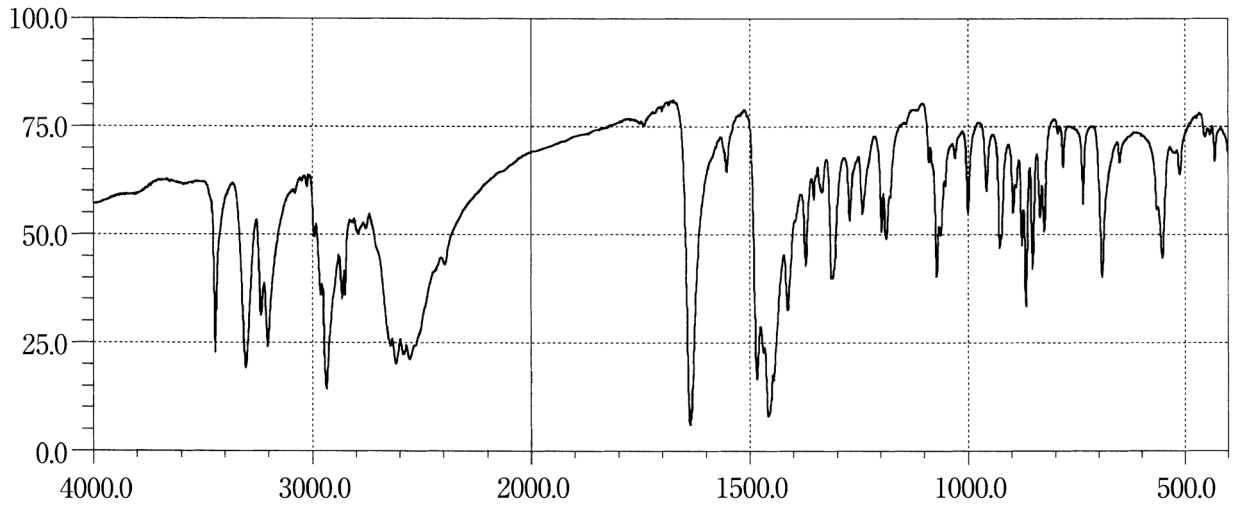
Bleomycin Sulfate



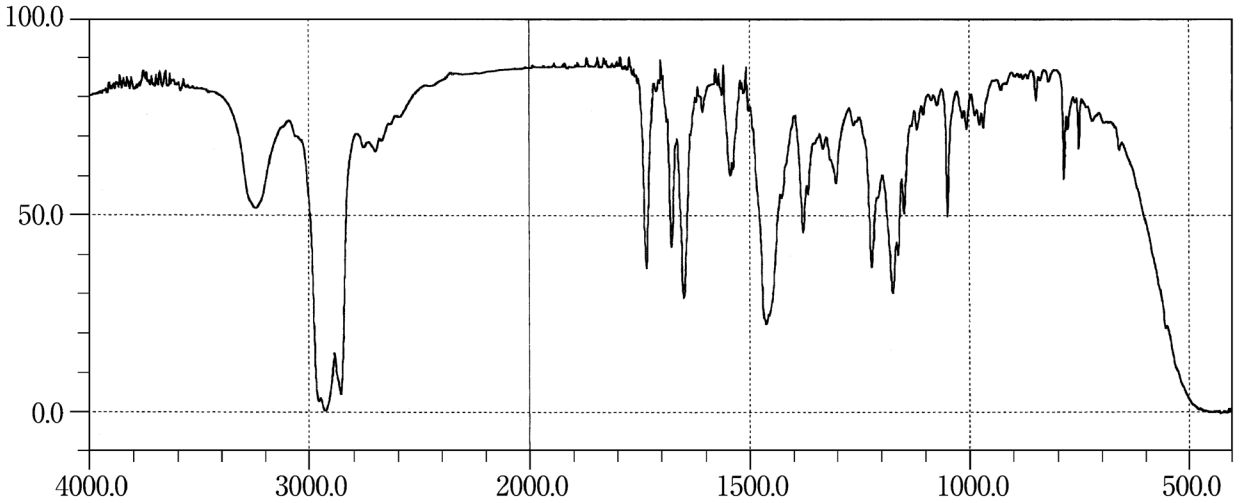
Bromazepam



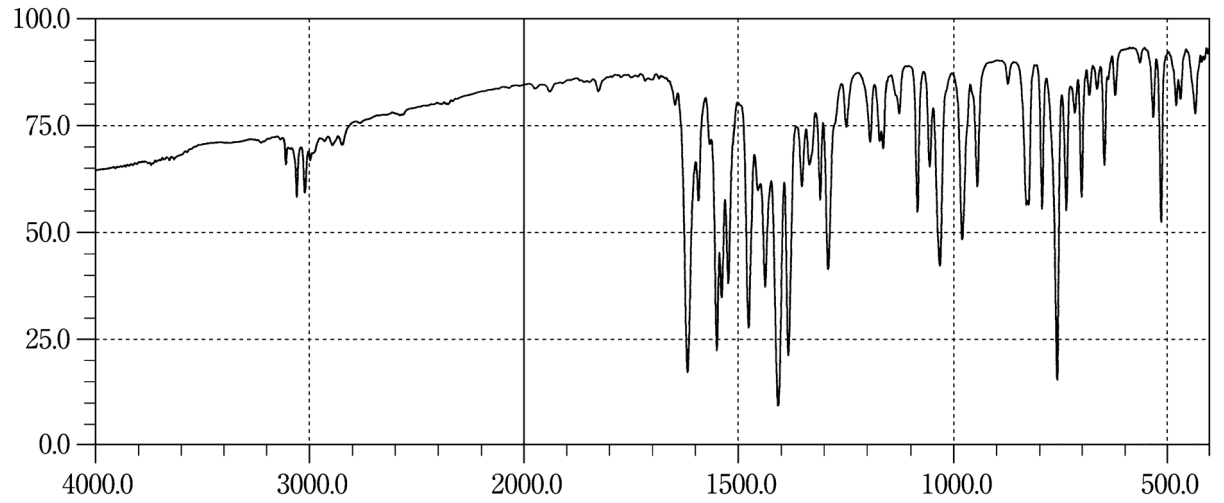
Bromhexine Hydrochloride



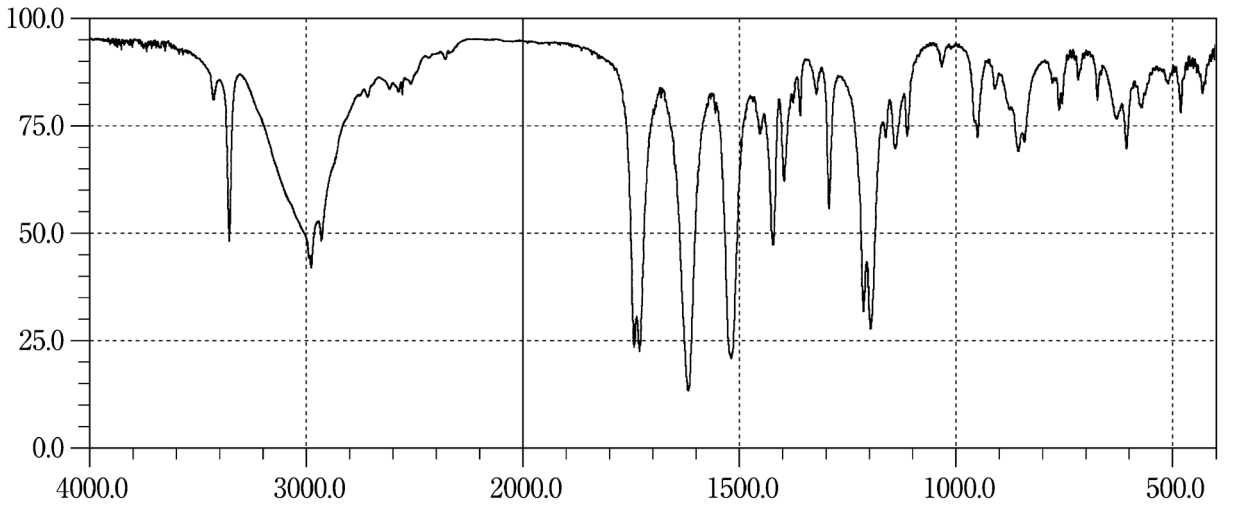
Bromocriptine Mesilate



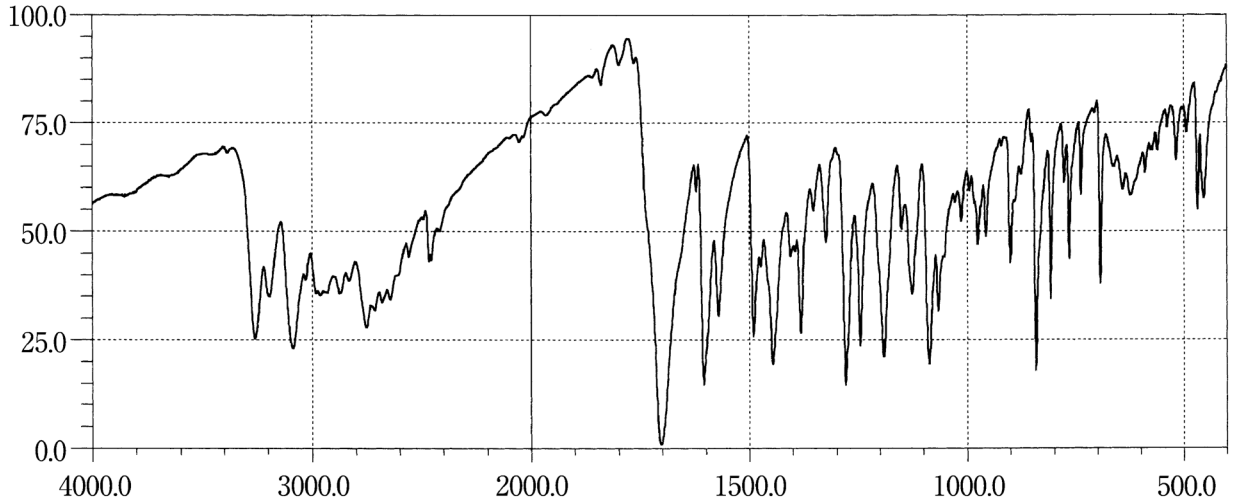
Brotizolam



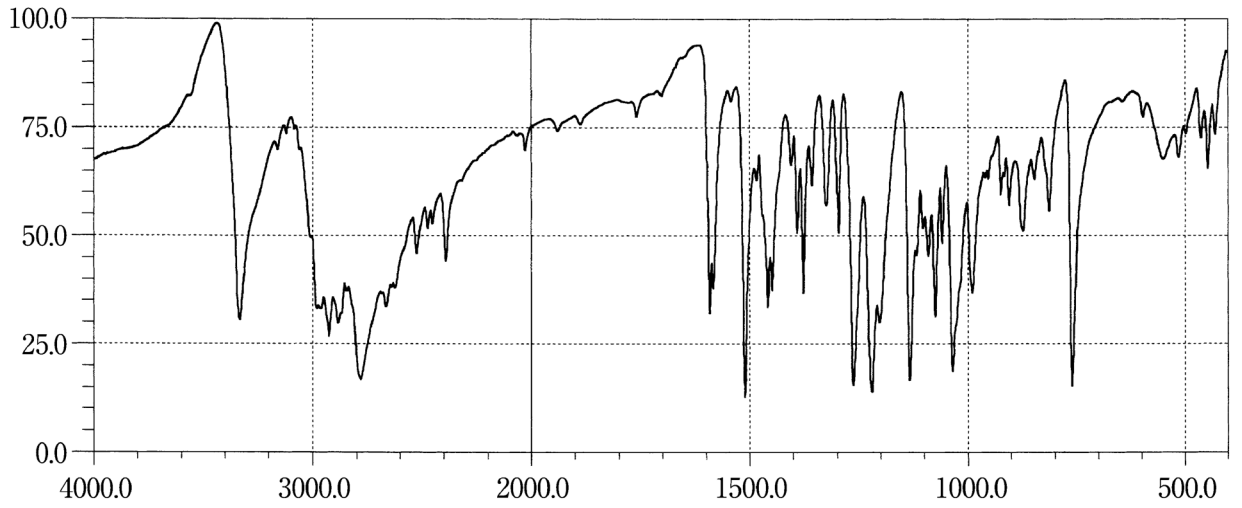
Bucillamine



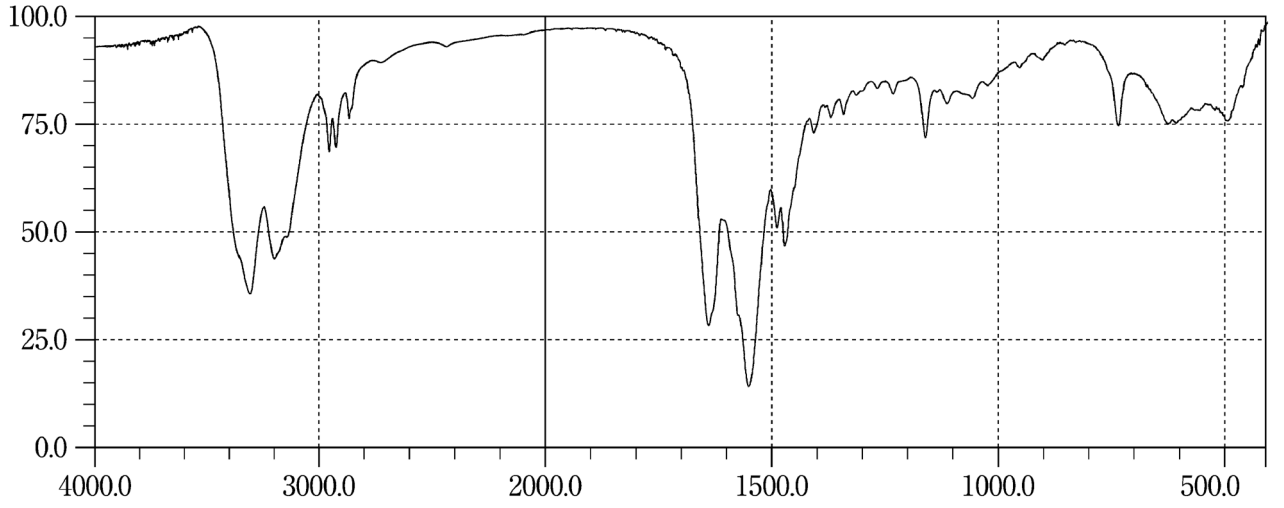
Bucumolol Hydrochloride



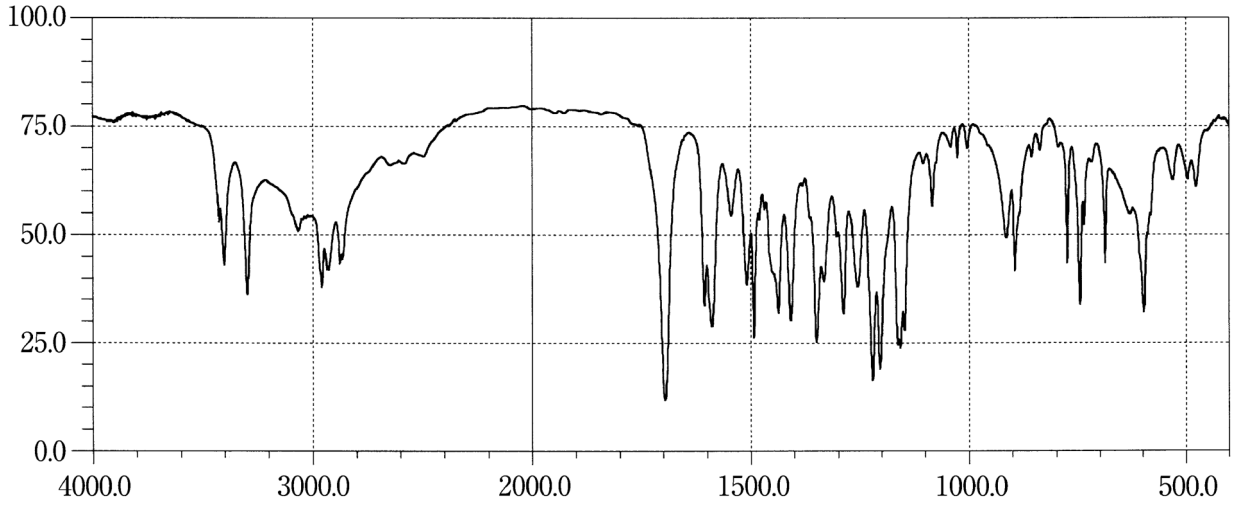
Bufetolol Hydrochloride



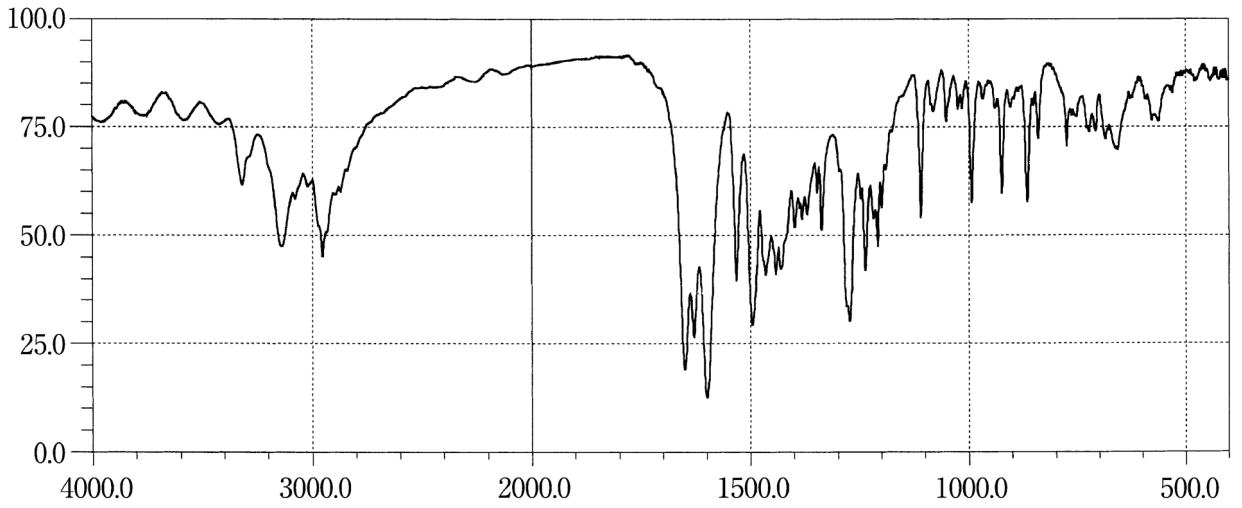
Buformin Hydrochloride



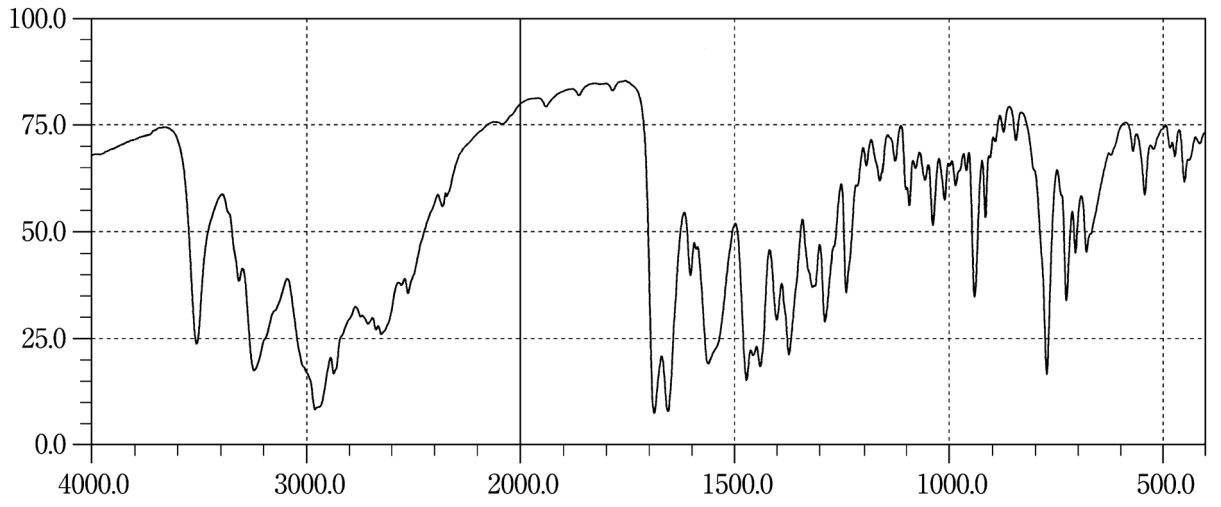
Bumetanide



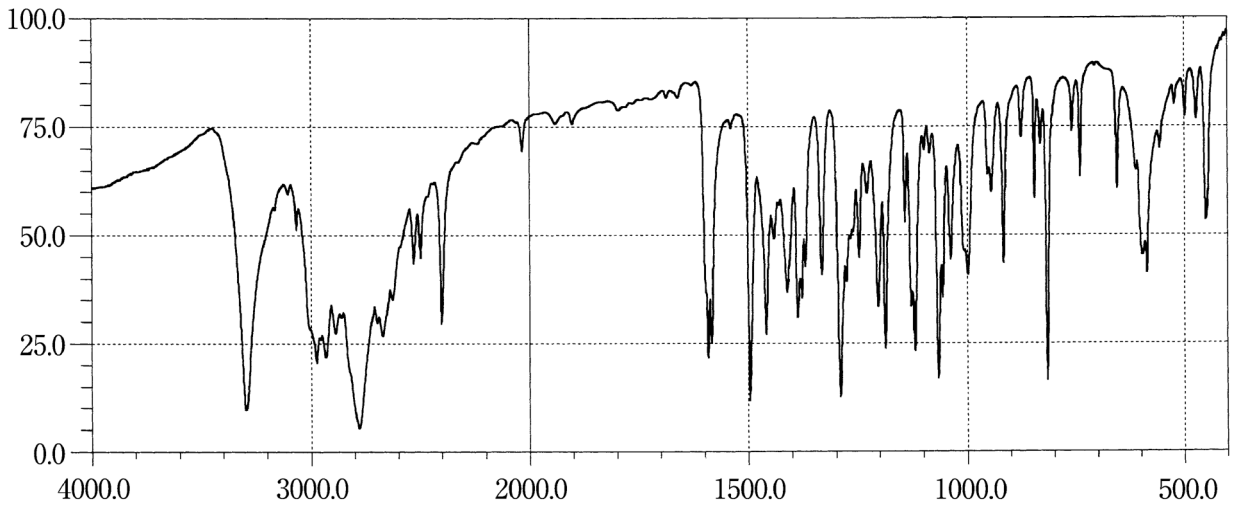
Bunazosin Hydrochloride



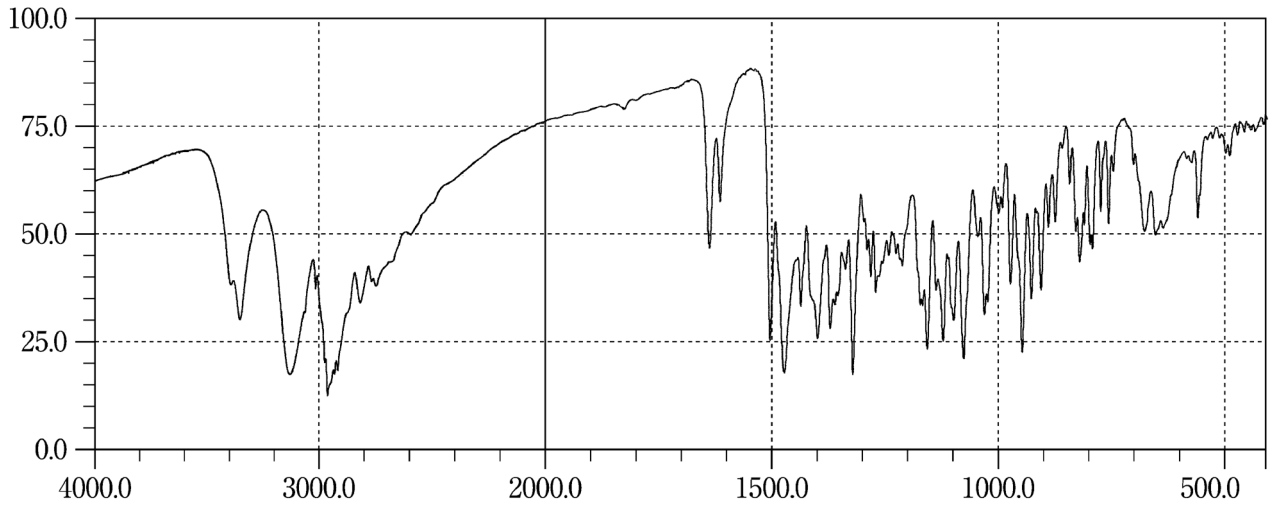
Bupivacaine Hydrochloride Hydrate



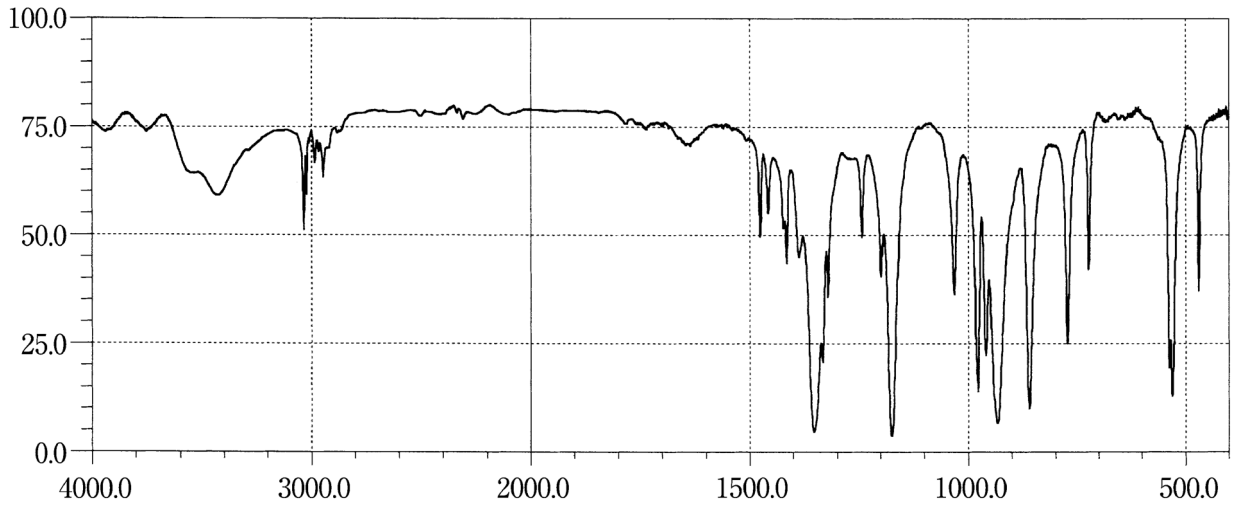
Bupranolol Hydrochloride



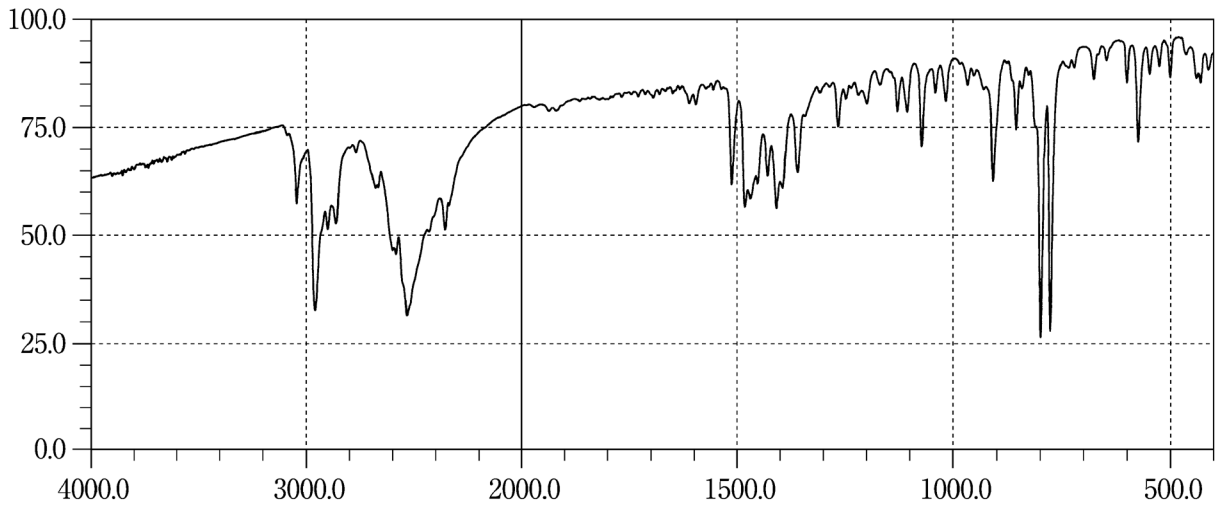
Buprenorphine Hydrochloride



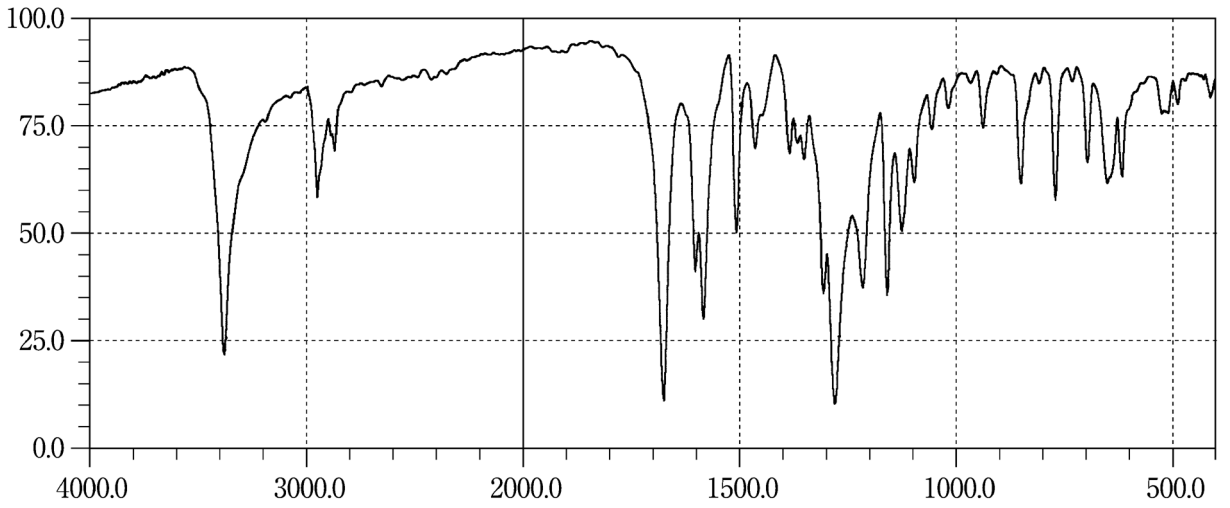
Busulfan



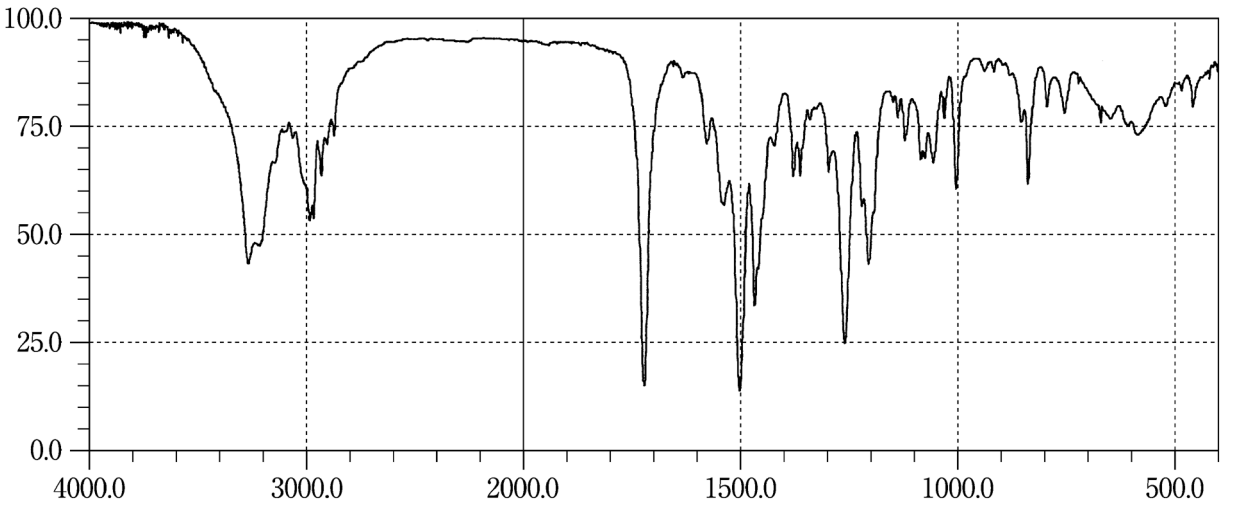
Butenafine Hydrochloride



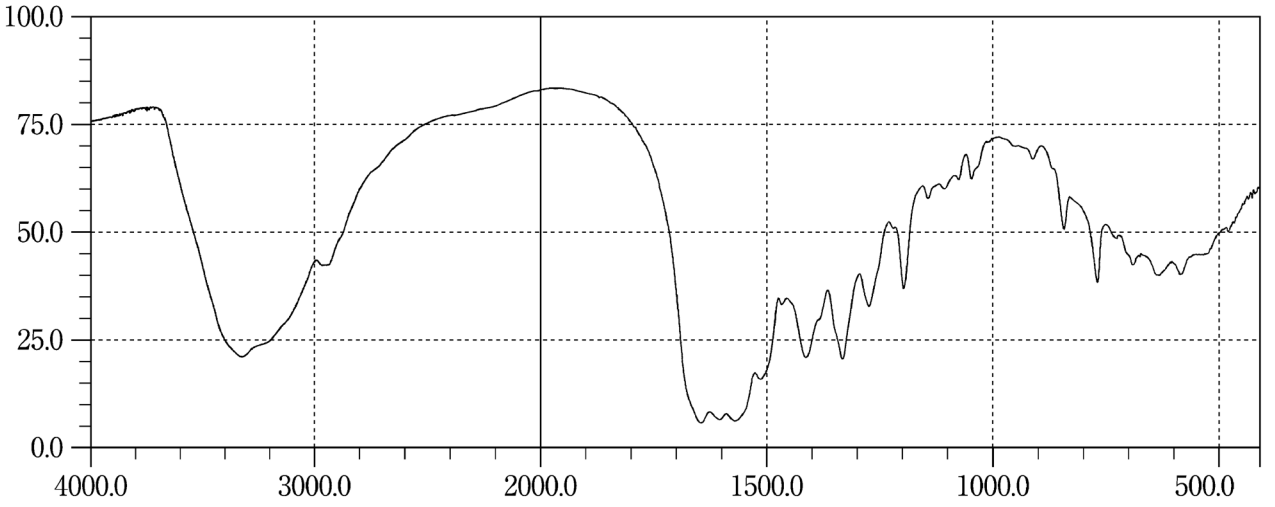
Butyl Parahydroxybenzoate



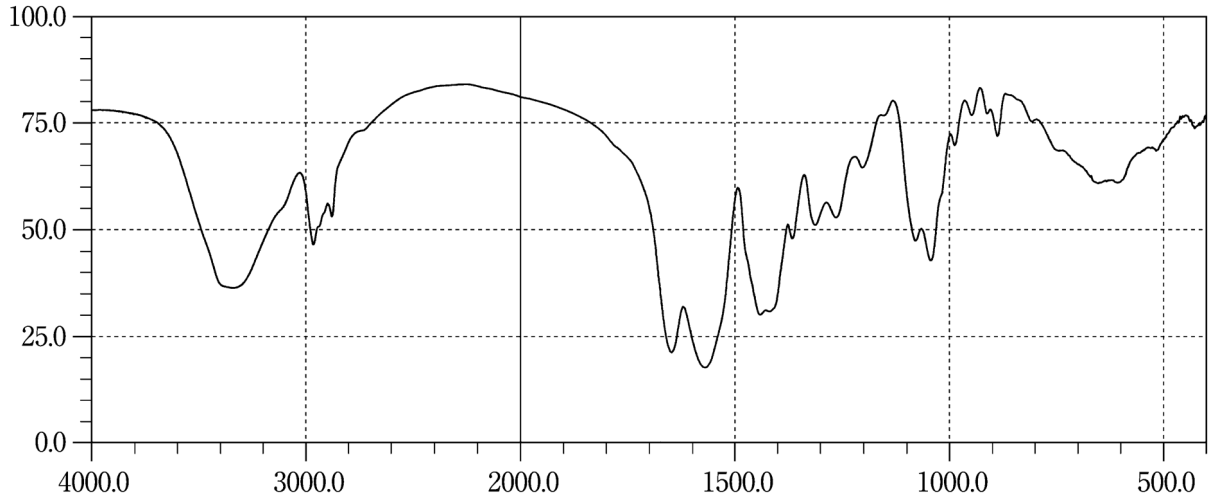
Cadralazine



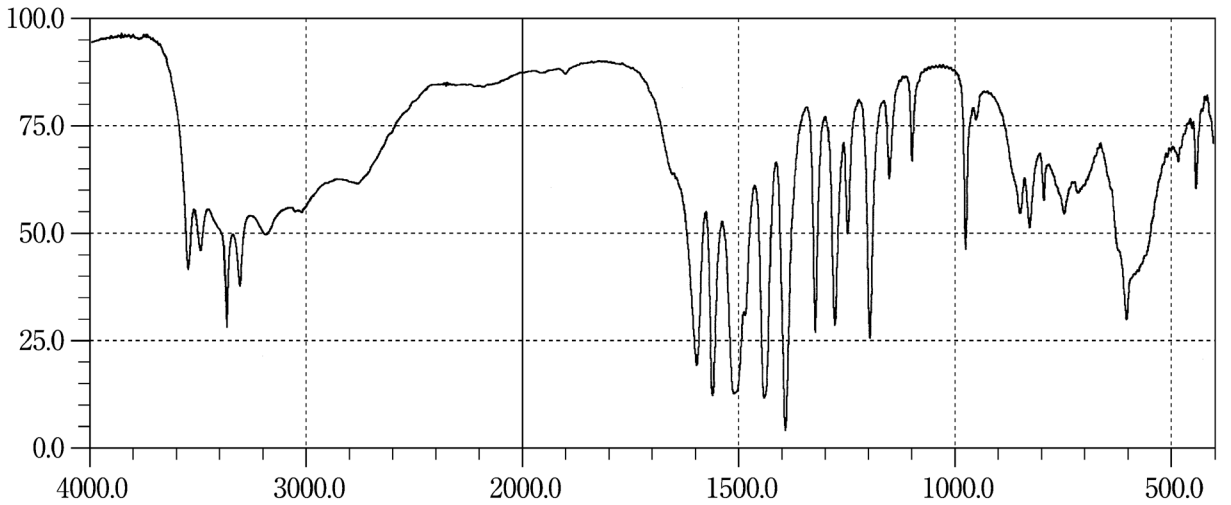
Calcium Folinate



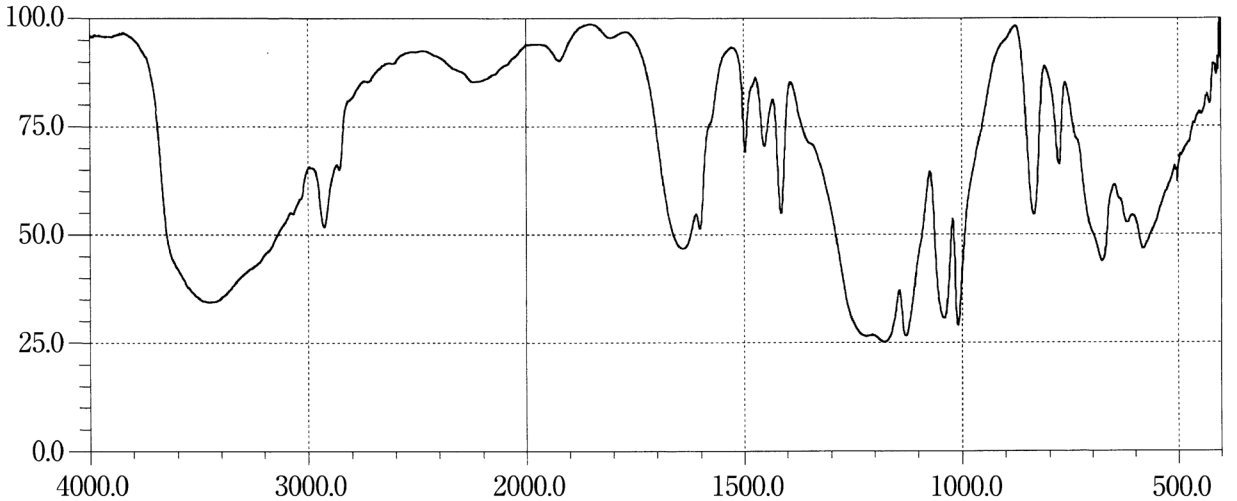
Calcium Pantothenate



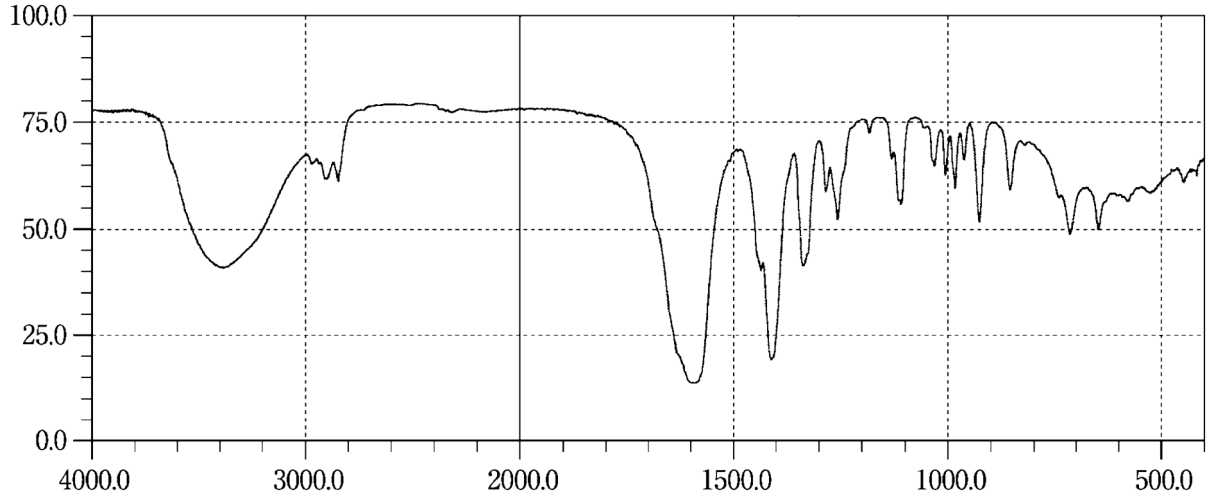
Calcium Paraaminosalicylate Hydrate



Calcium Polystyrene Sulfonate



Calcium Sodium Edetate Hydrate



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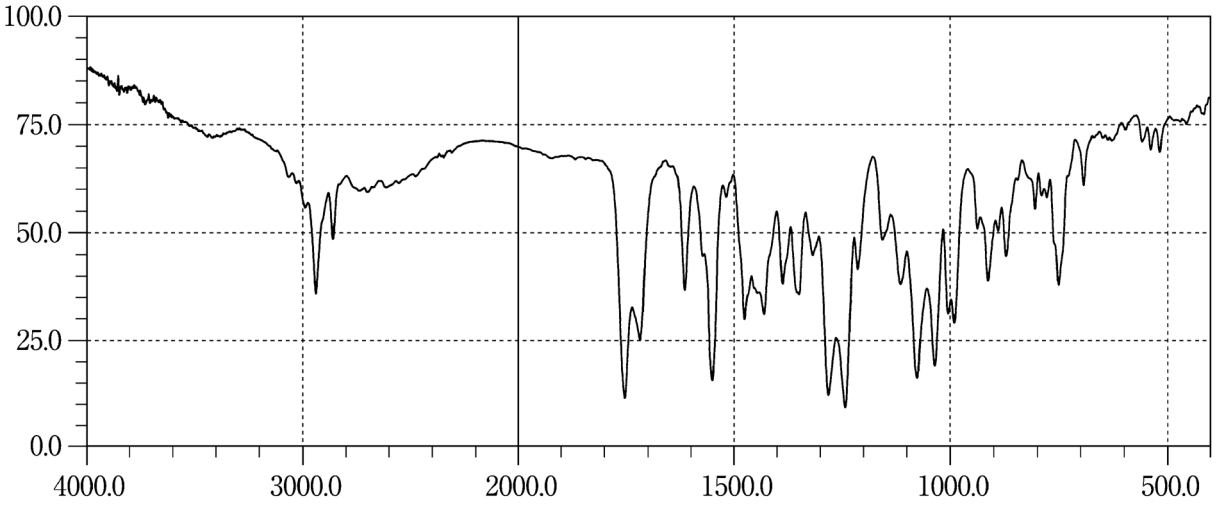
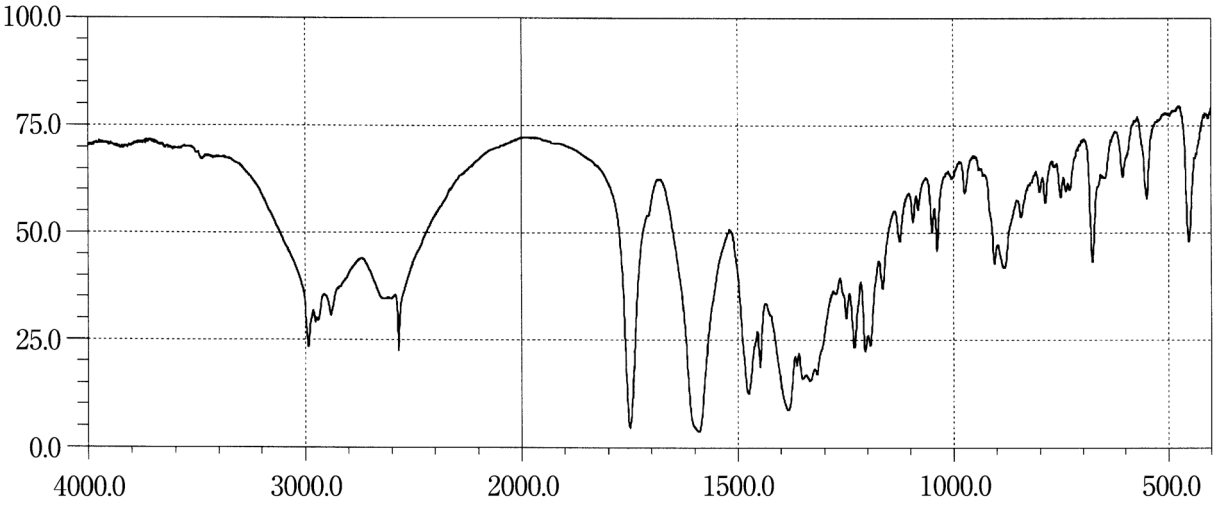
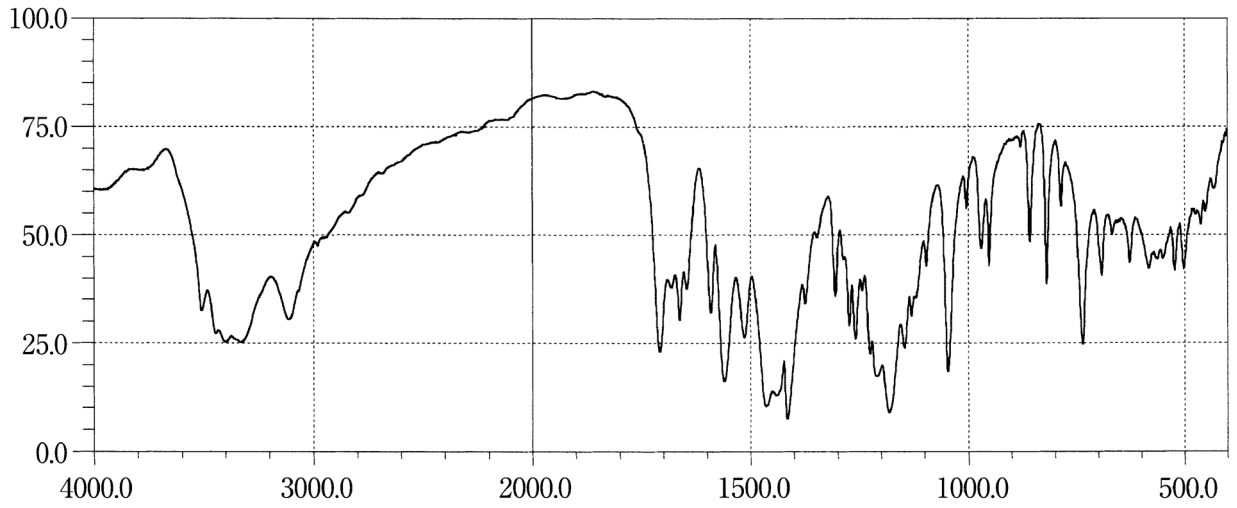


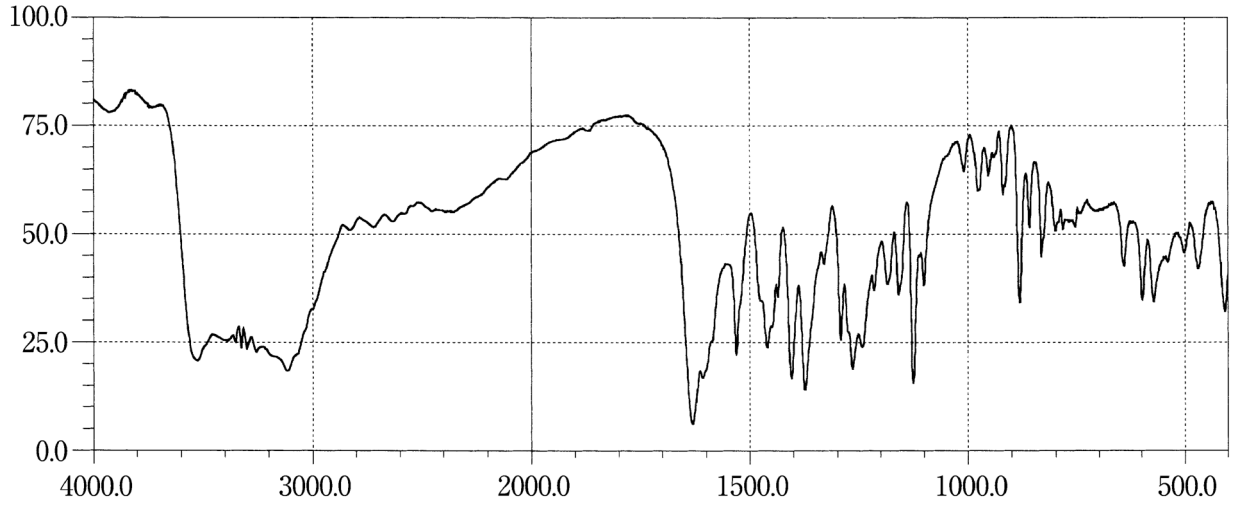
Captopril



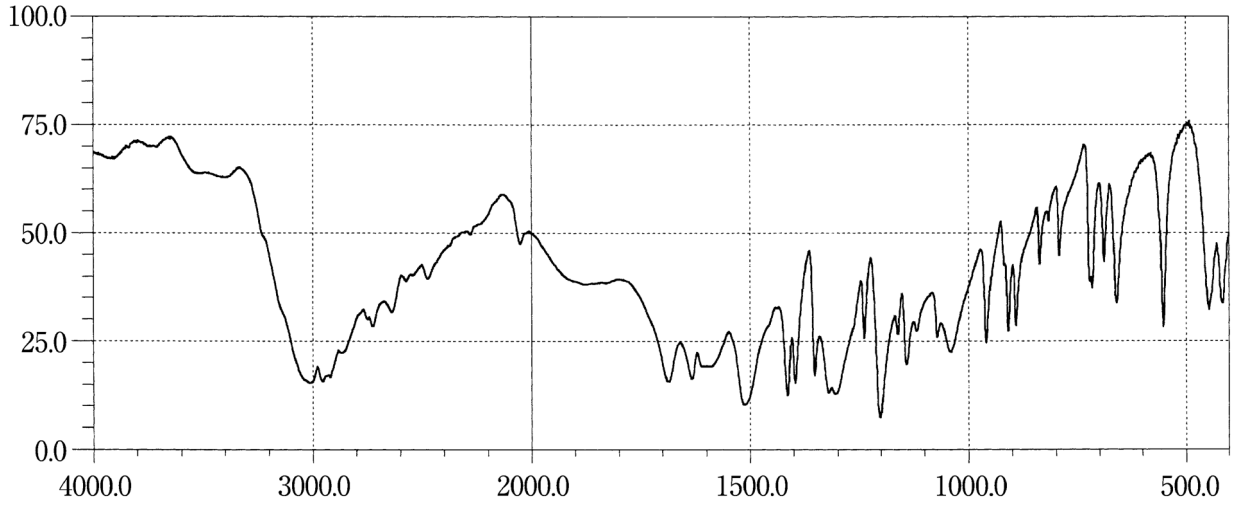
Carbazochrome Sodium Sulfonate Hydrate



Carbidopa Hydrate



L-Carbocisteine



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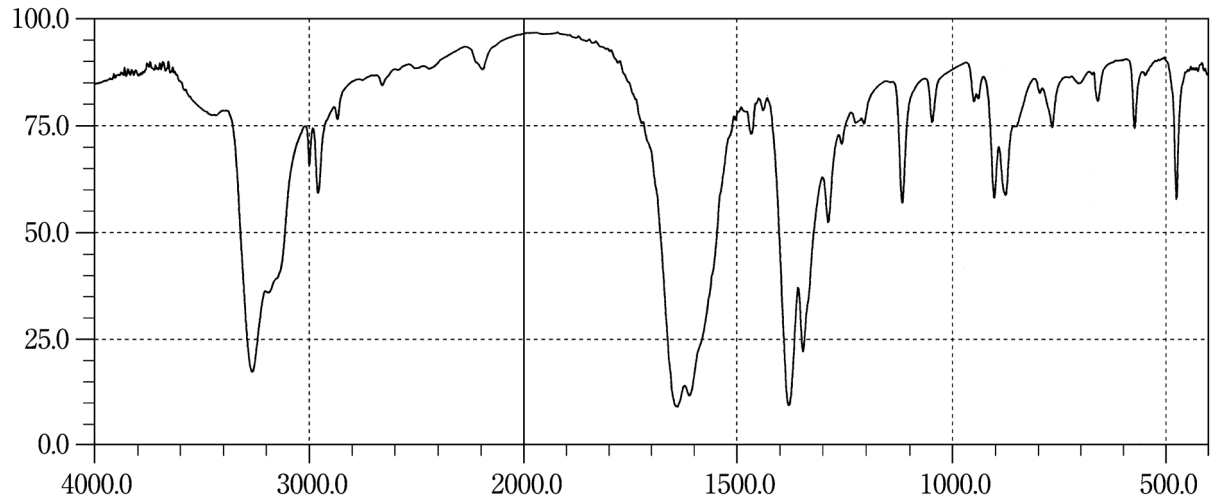

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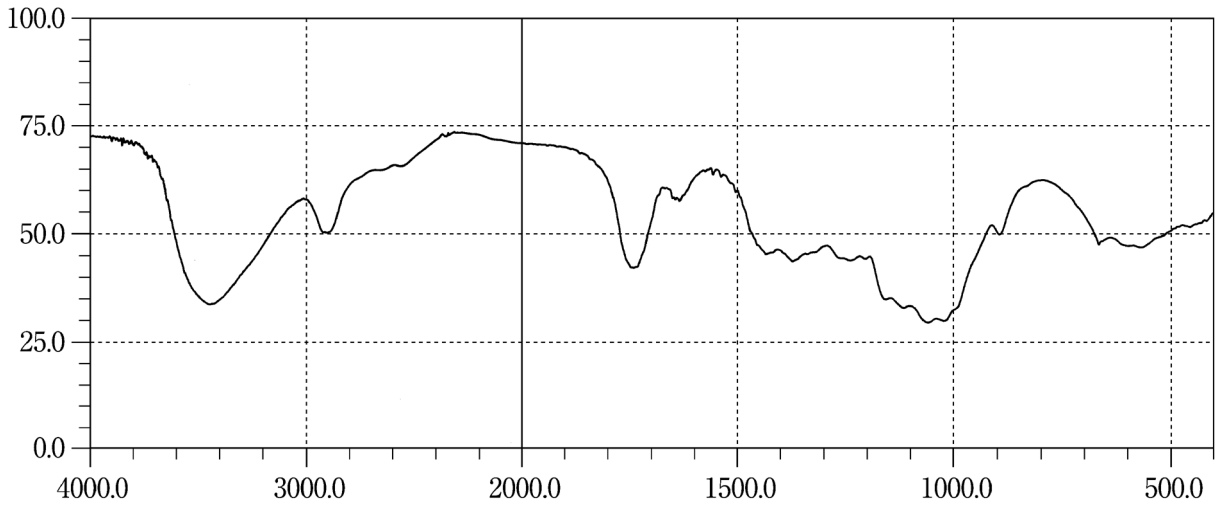

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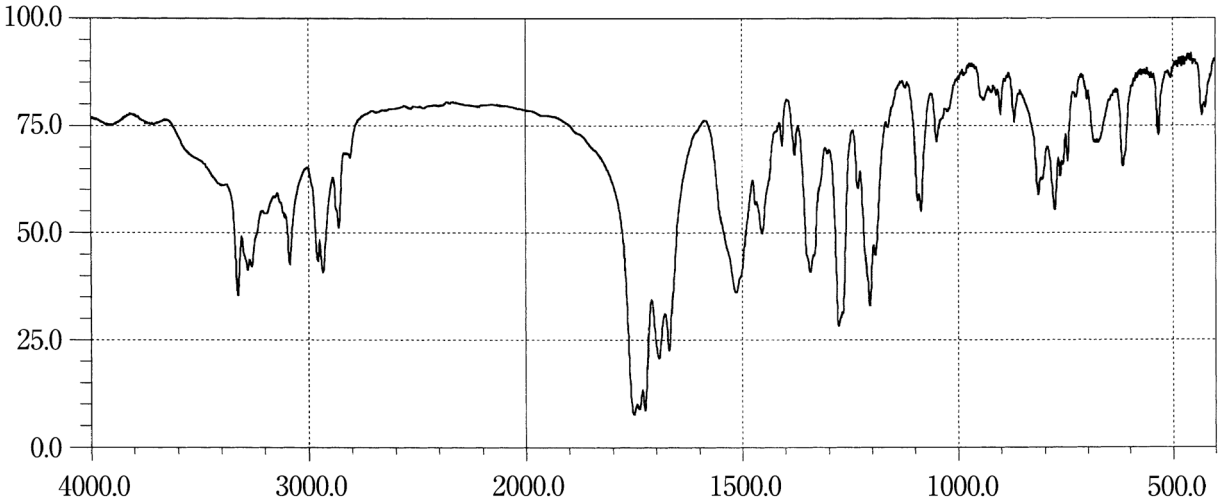
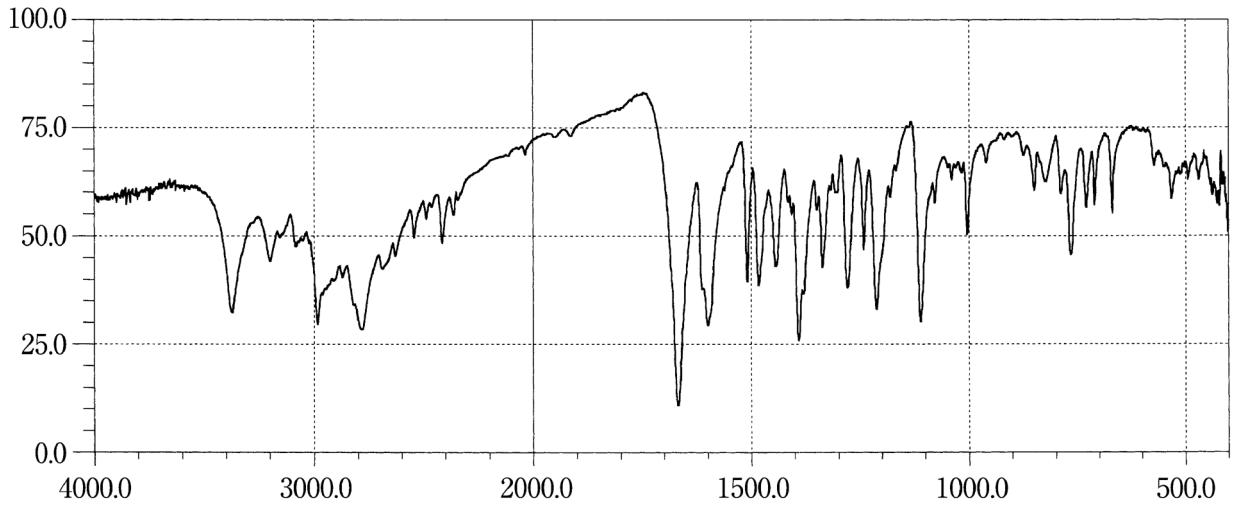
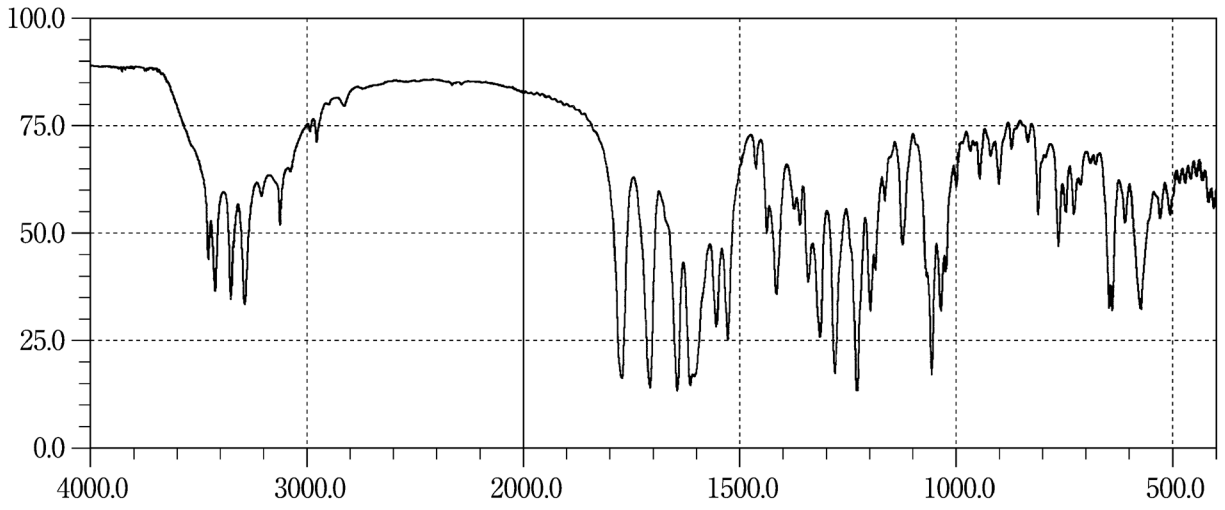


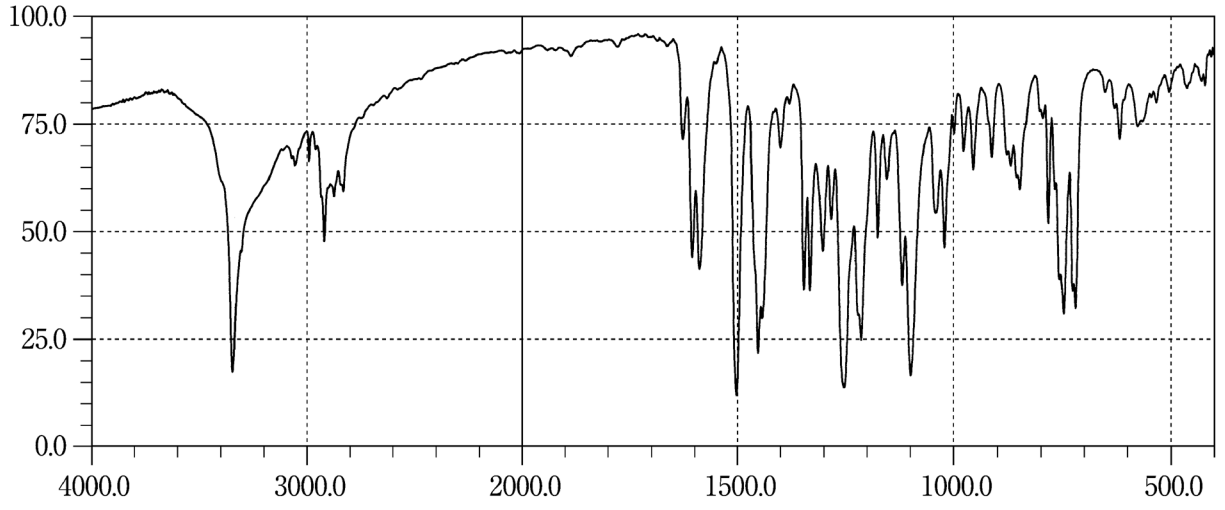
Carteolol Hydrochloride



Carumonam Sodium



Carvedilol



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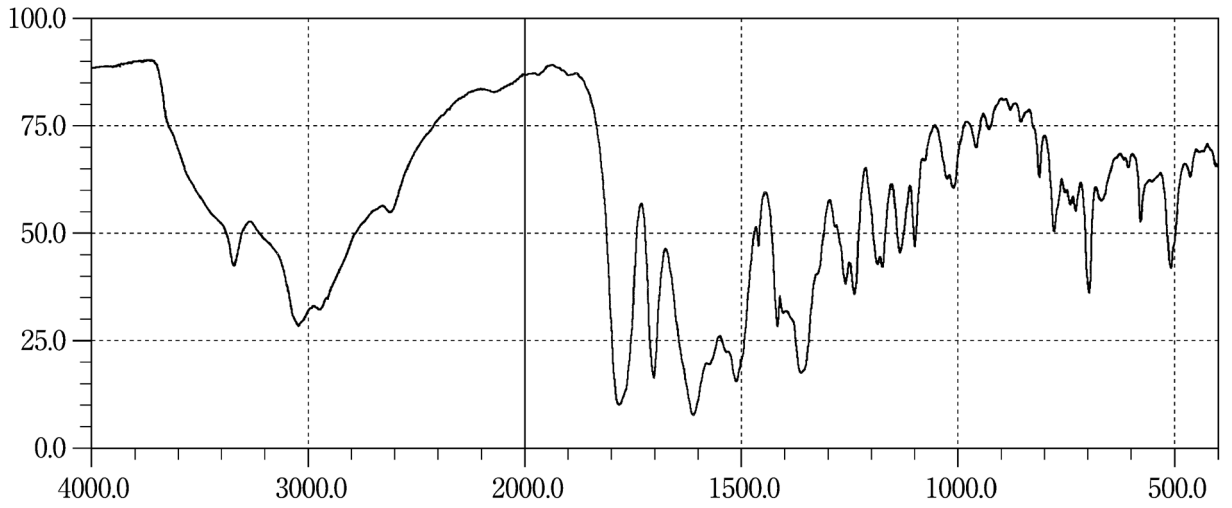

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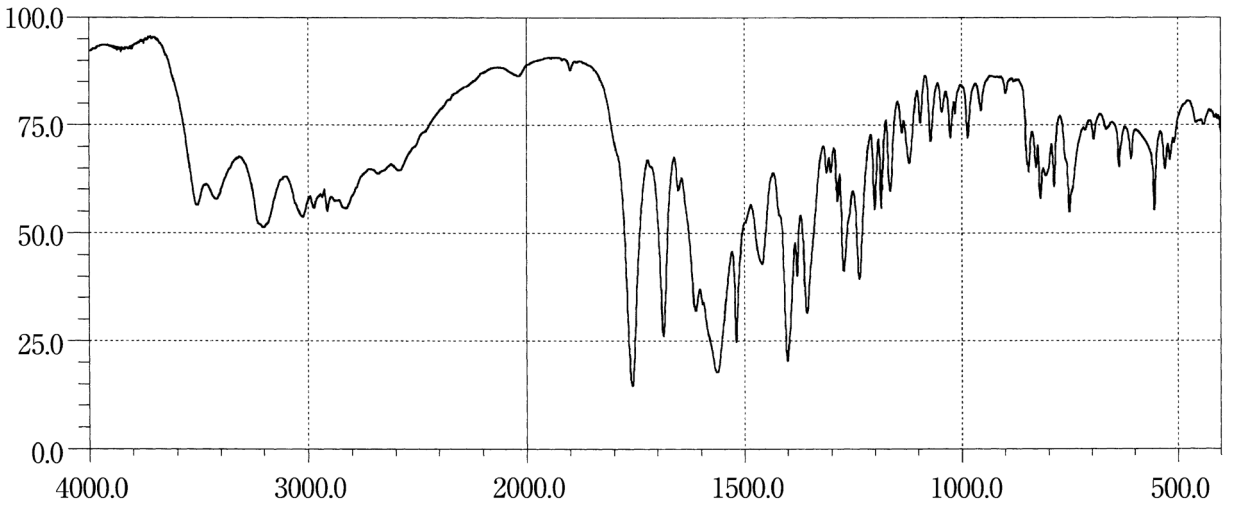

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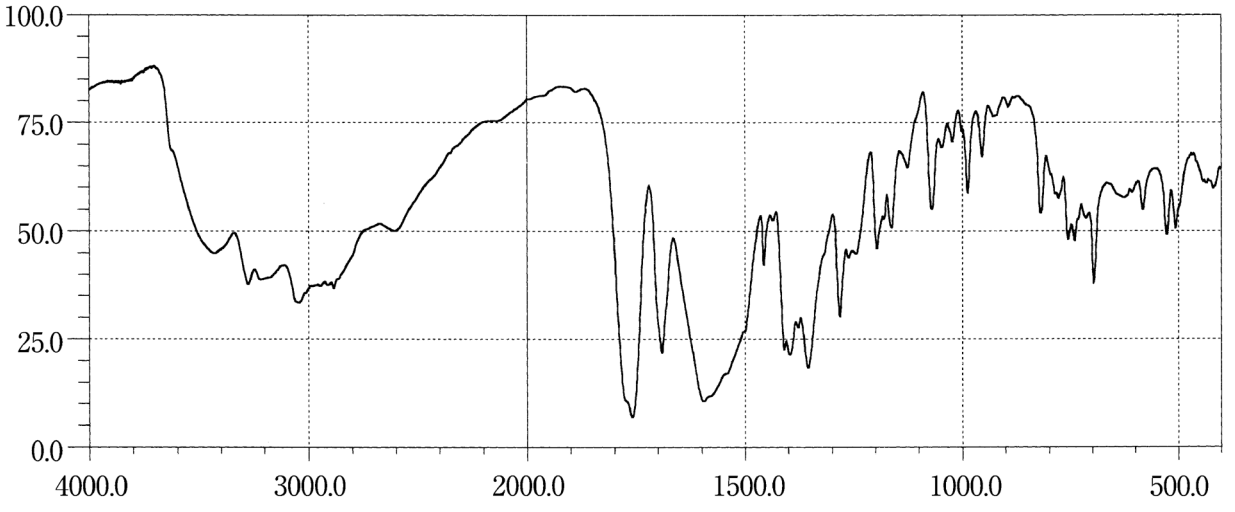
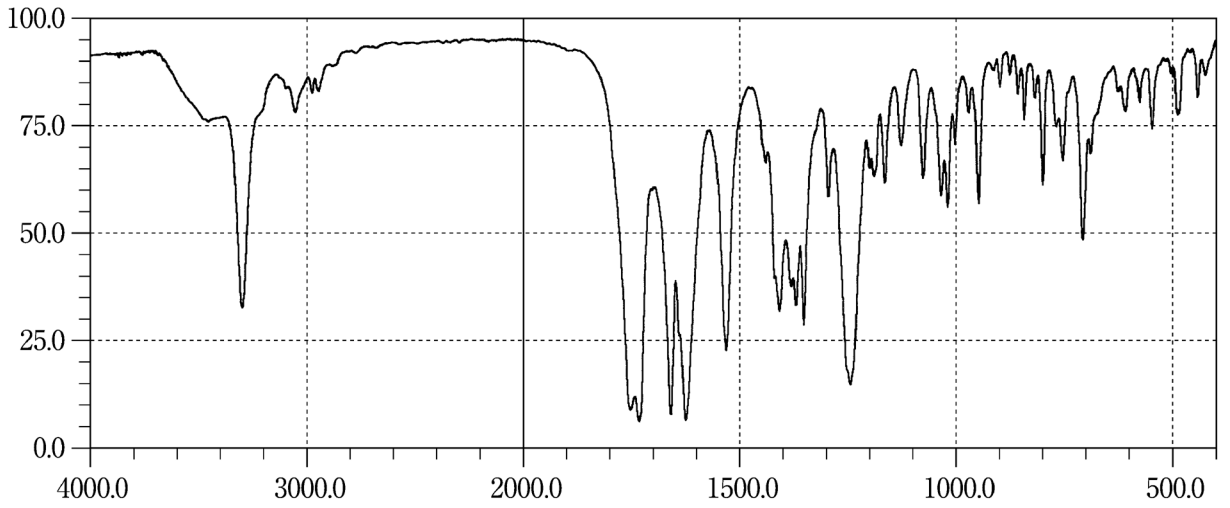
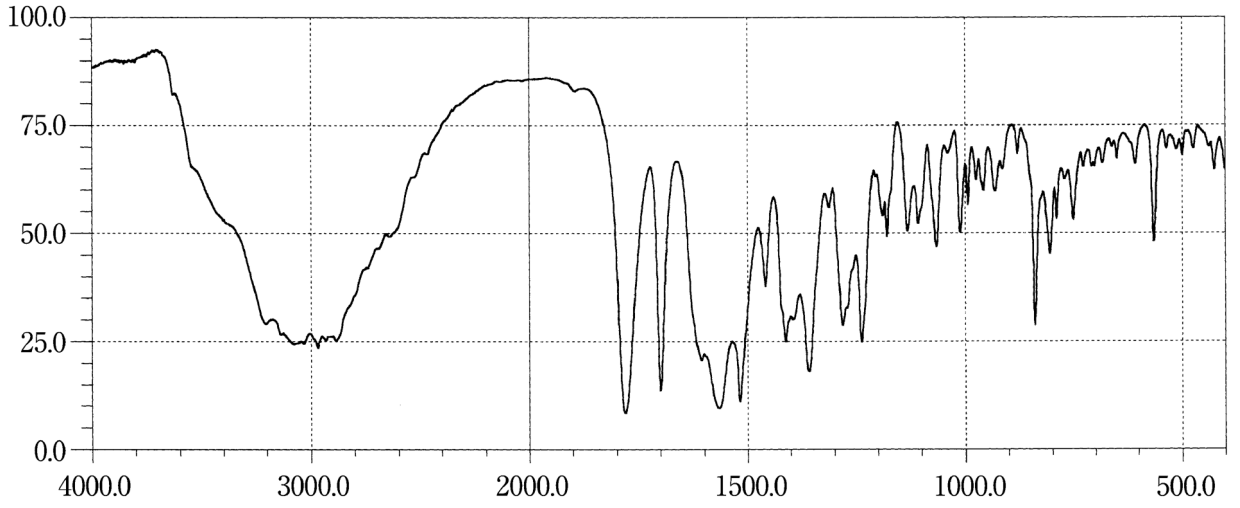


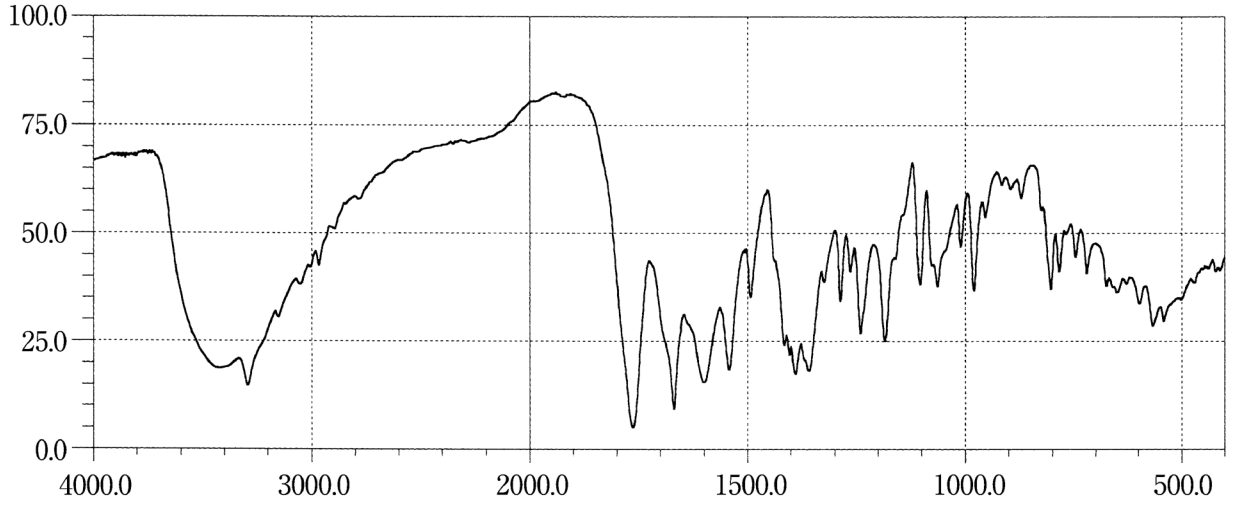
Cefalotin Sodium



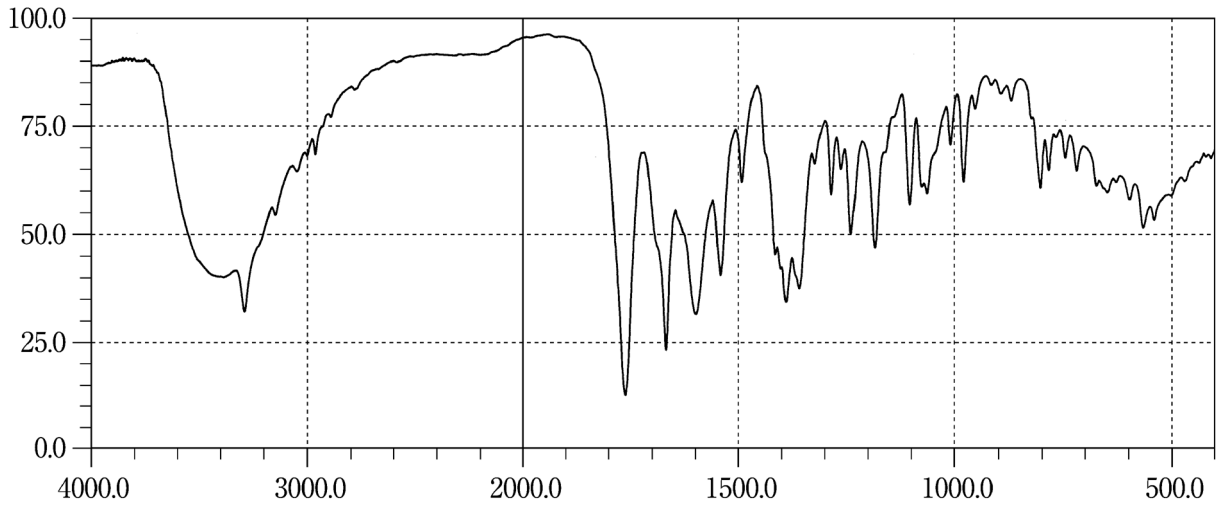
Cefatrizine Propylene Glycolate



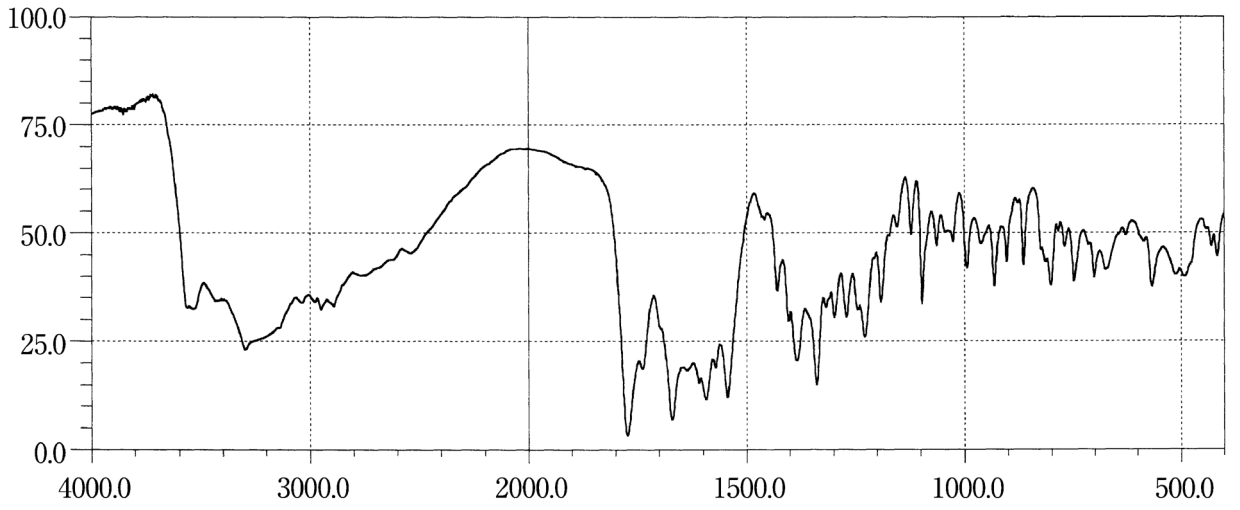
Cefazolin Sodium



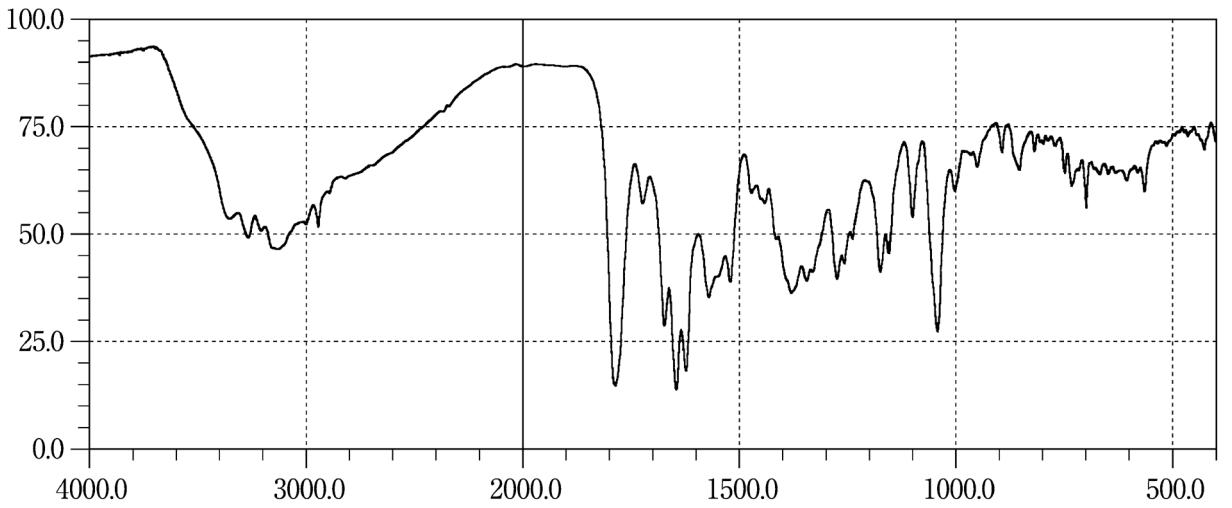
Cefazolin Sodium Hydrate



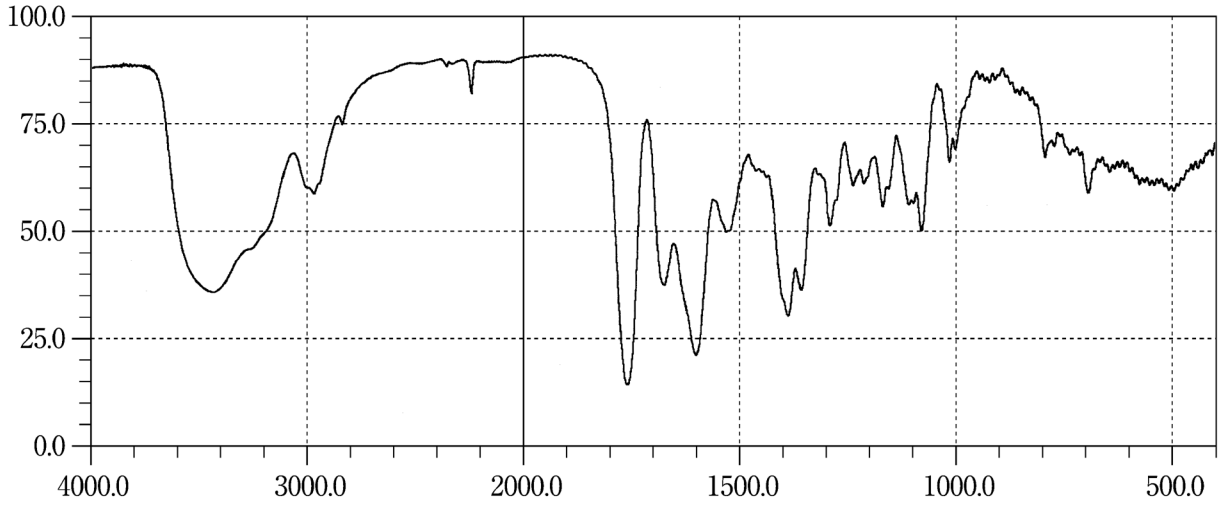
Cefixime Hydrate



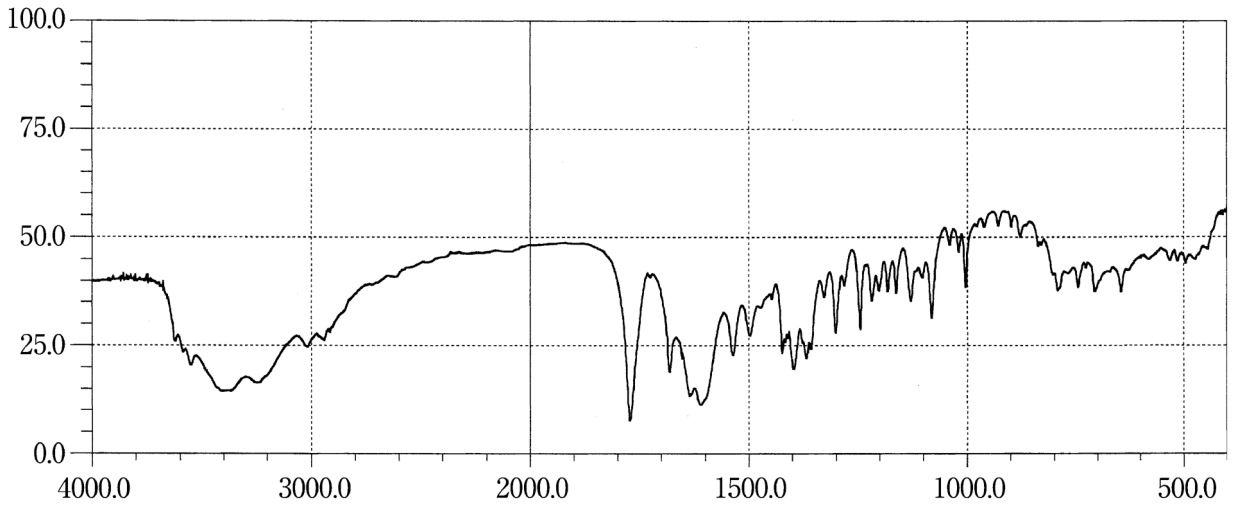
Cefmenoxime Hydrochloride



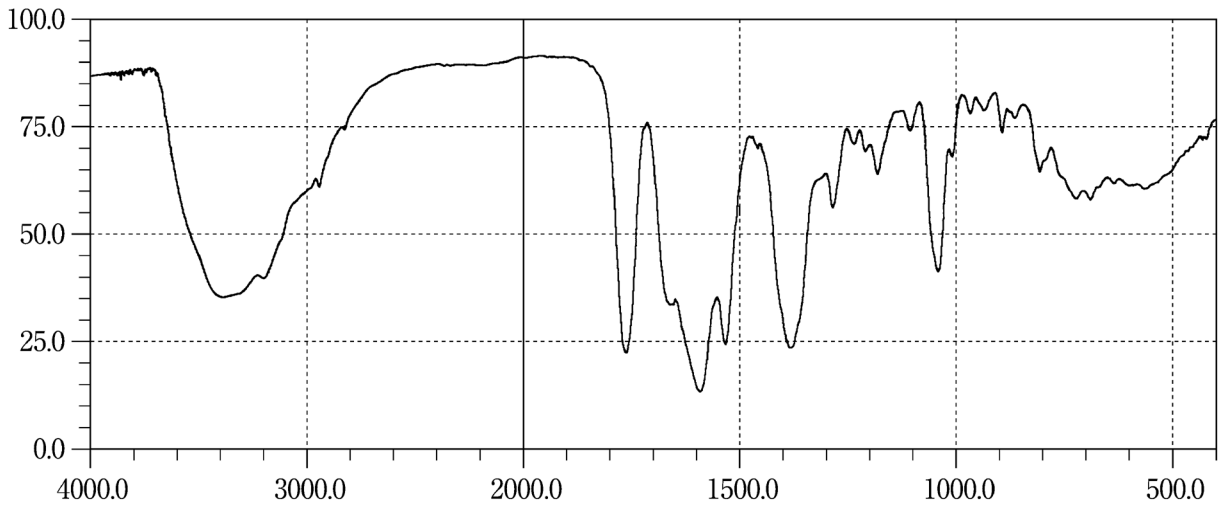
Cefmetazole Sodium



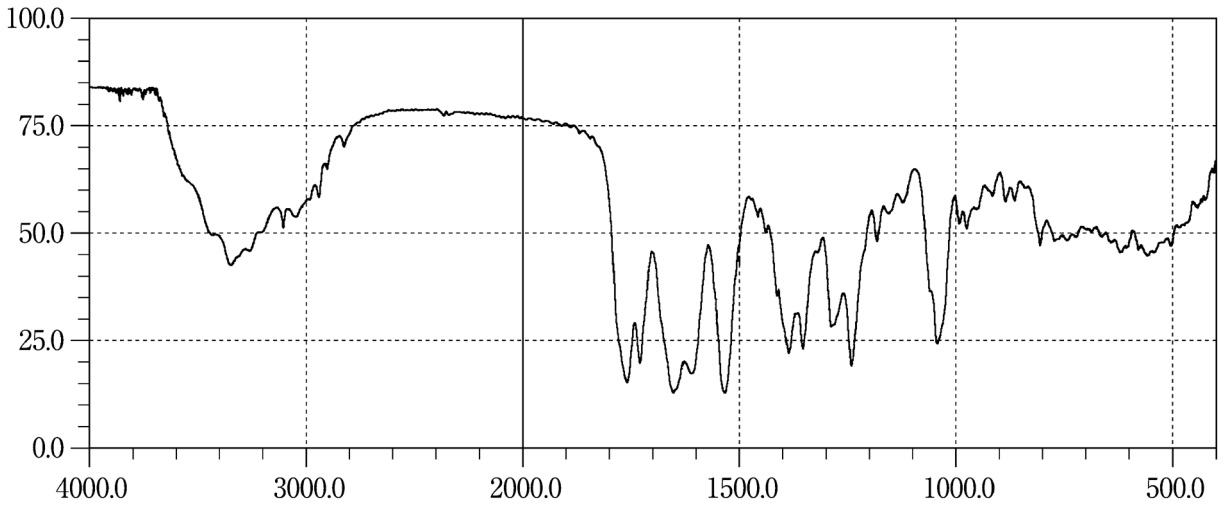
Cefminox Sodium Hydrate



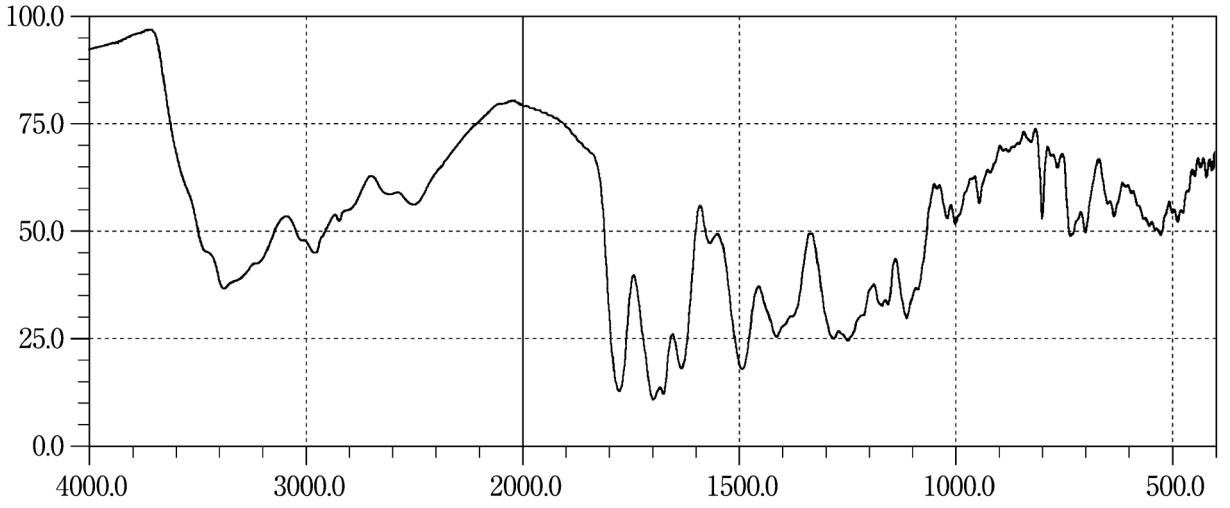
Cefodizime Sodium



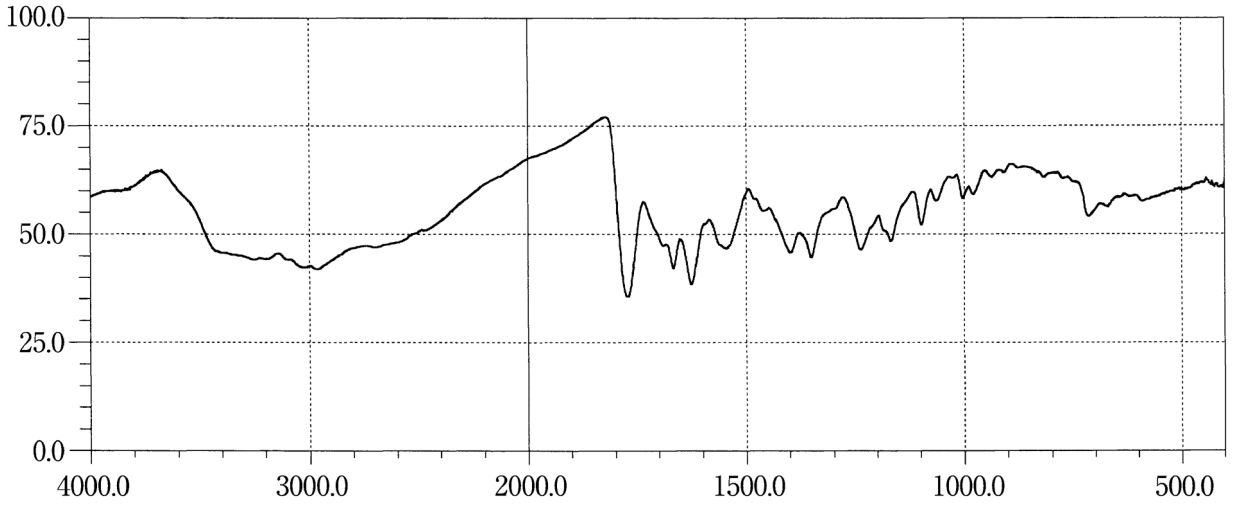
Cefotaxime Sodium



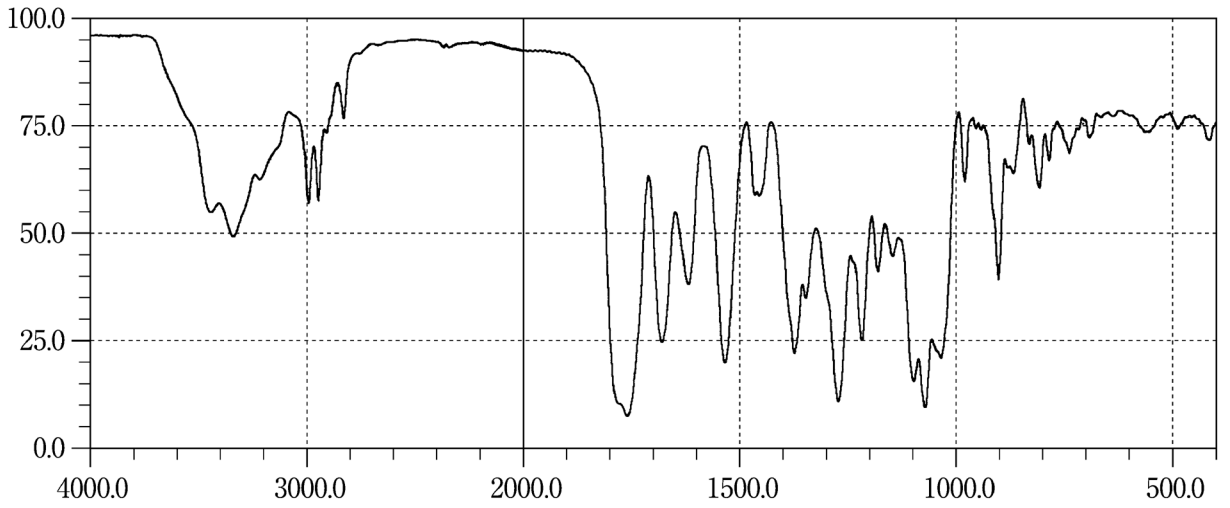
Cefotetan



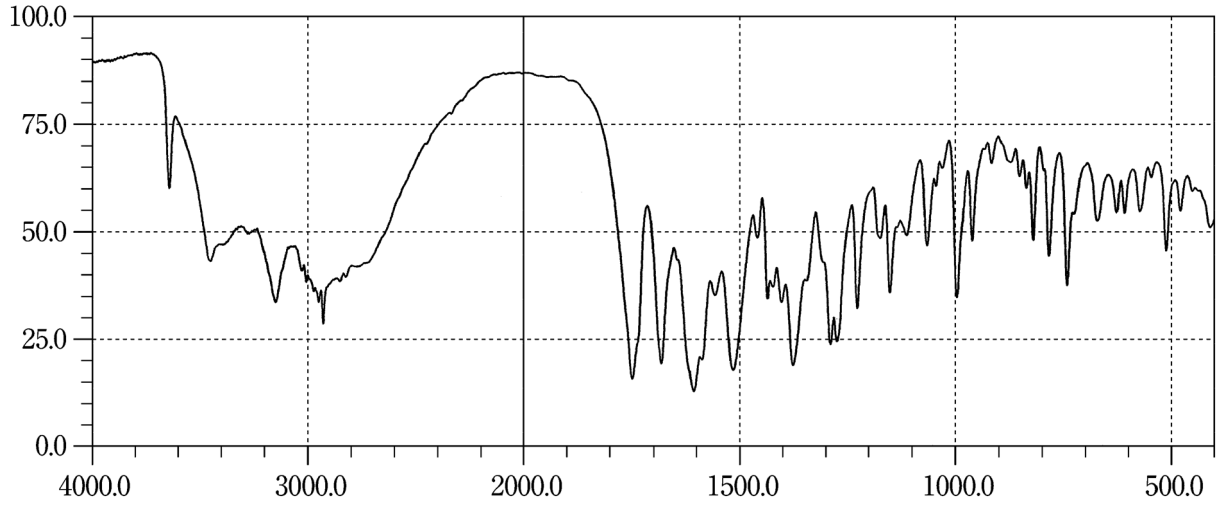
Cefotiam Hydrochloride



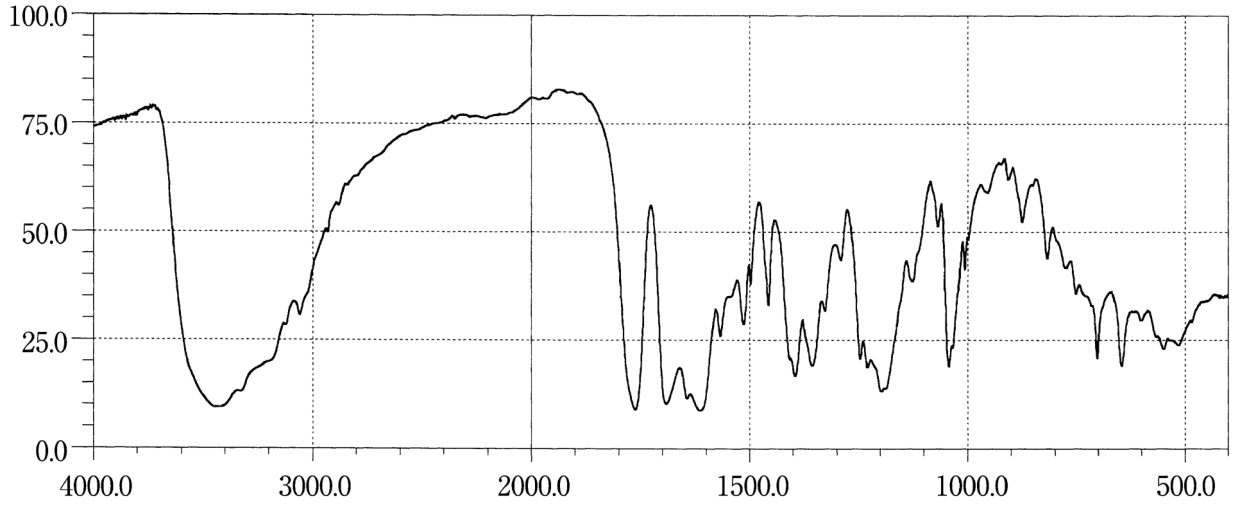
Cefpodoxime Proxetil



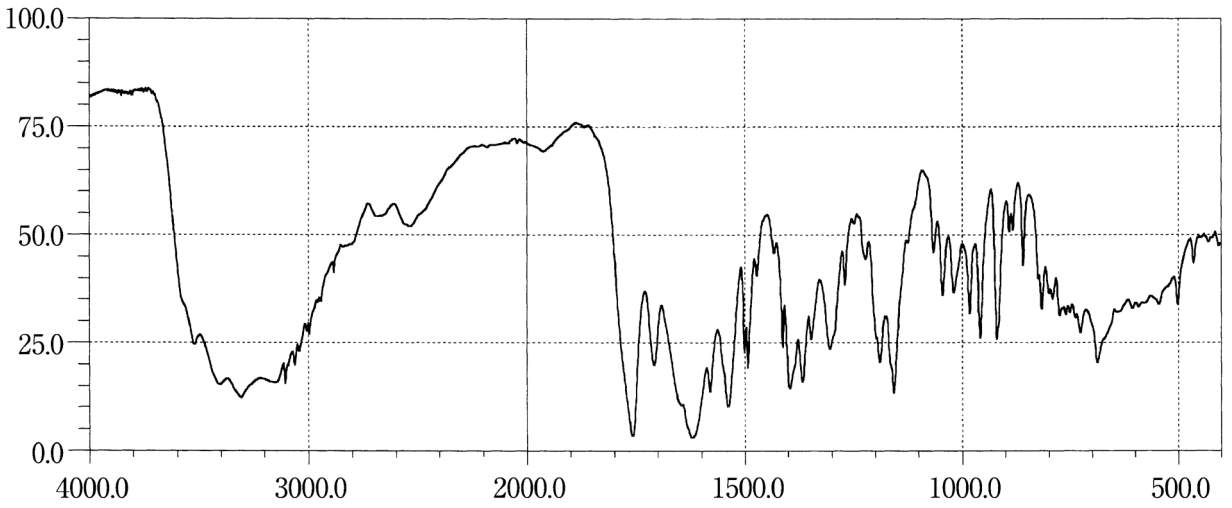
Cefroxadine Hydrate



Cefsulodin Sodium



Ceftazidime Hydrate



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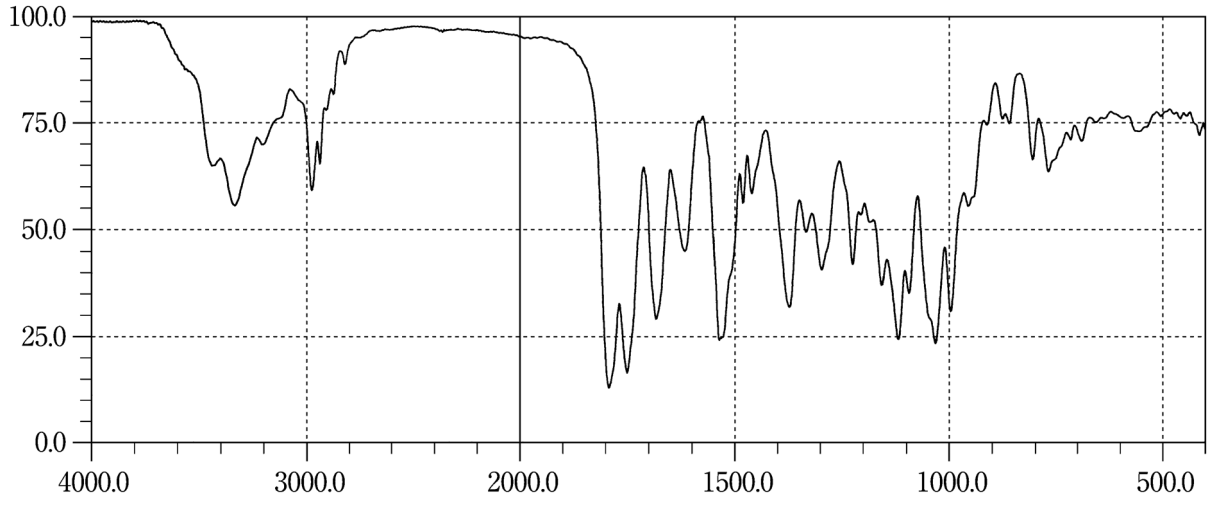

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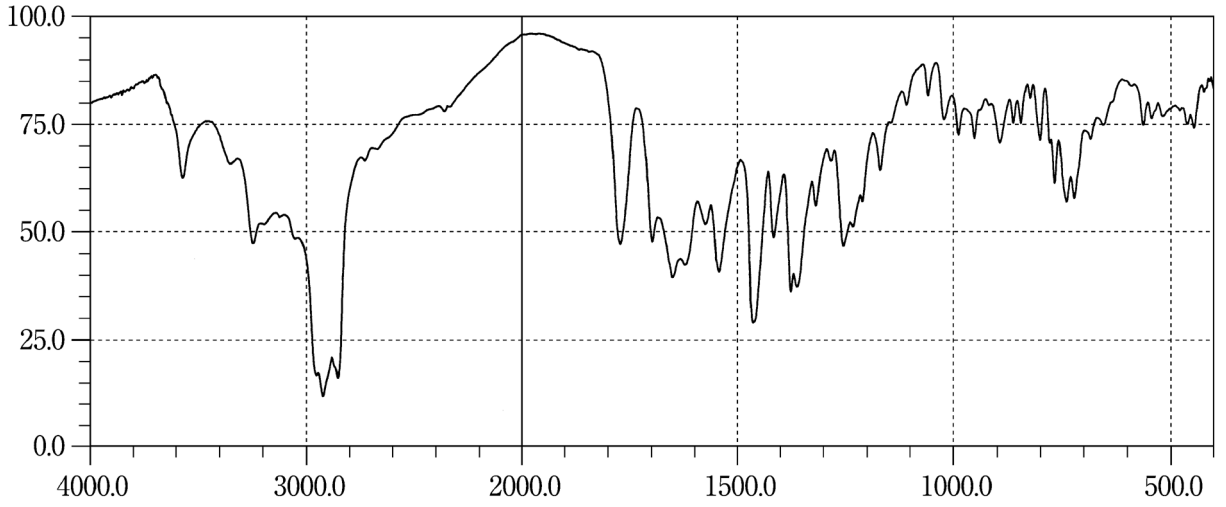
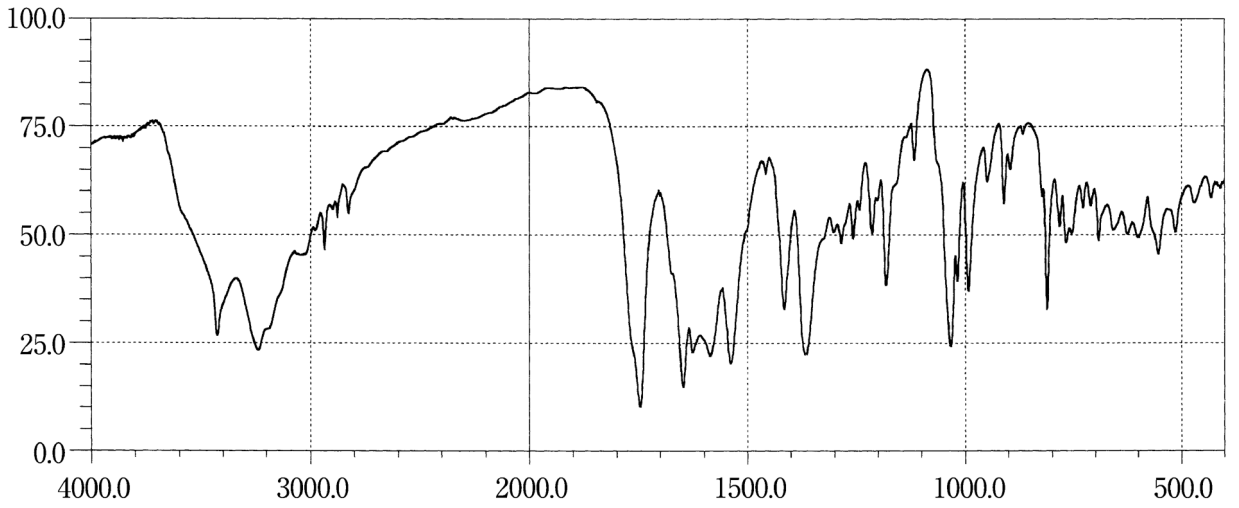
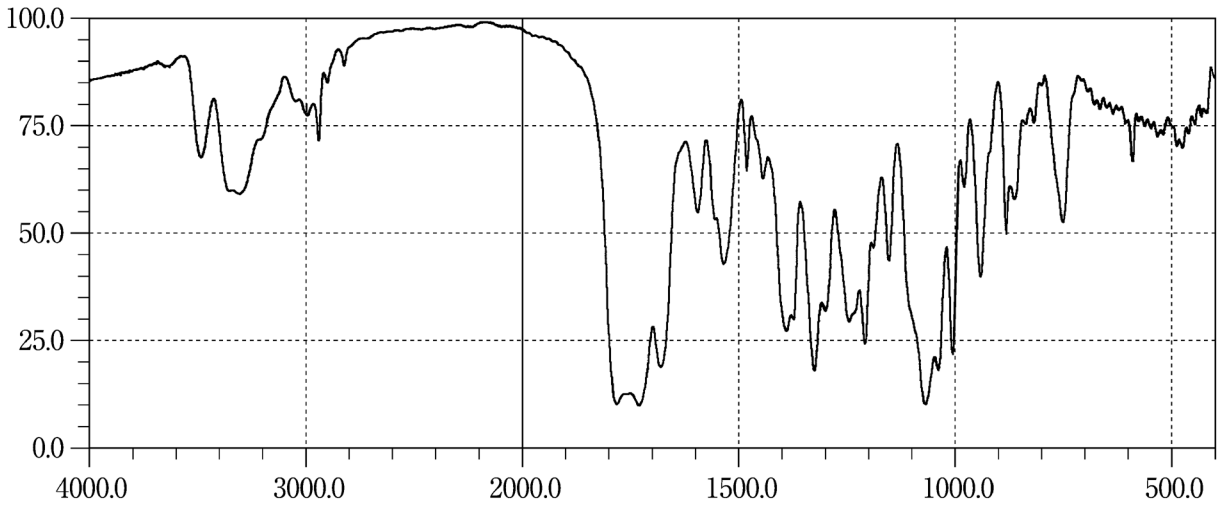


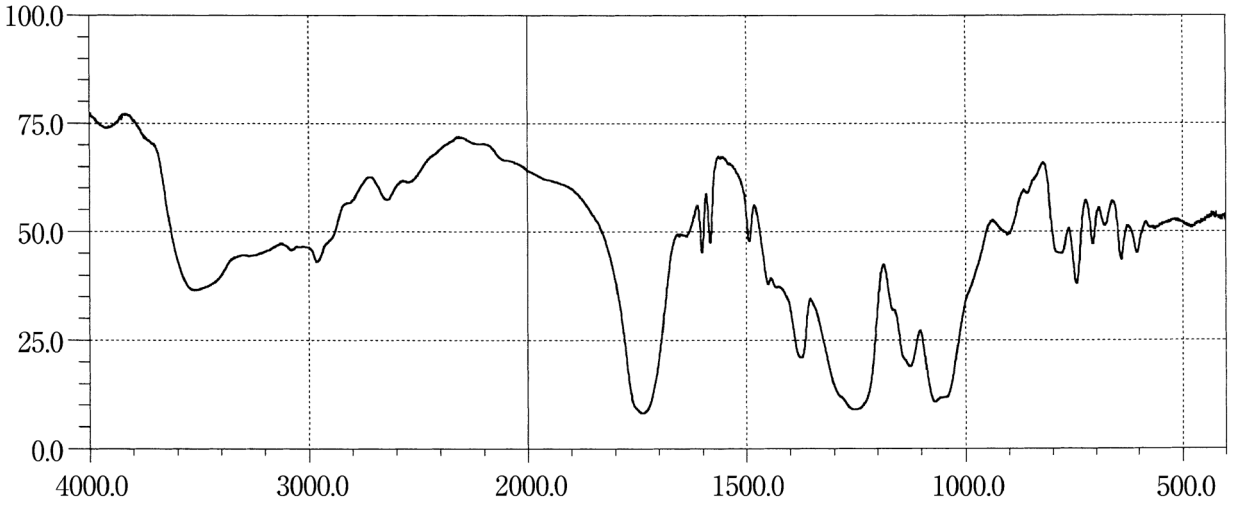
Ceftizoxime Sodium



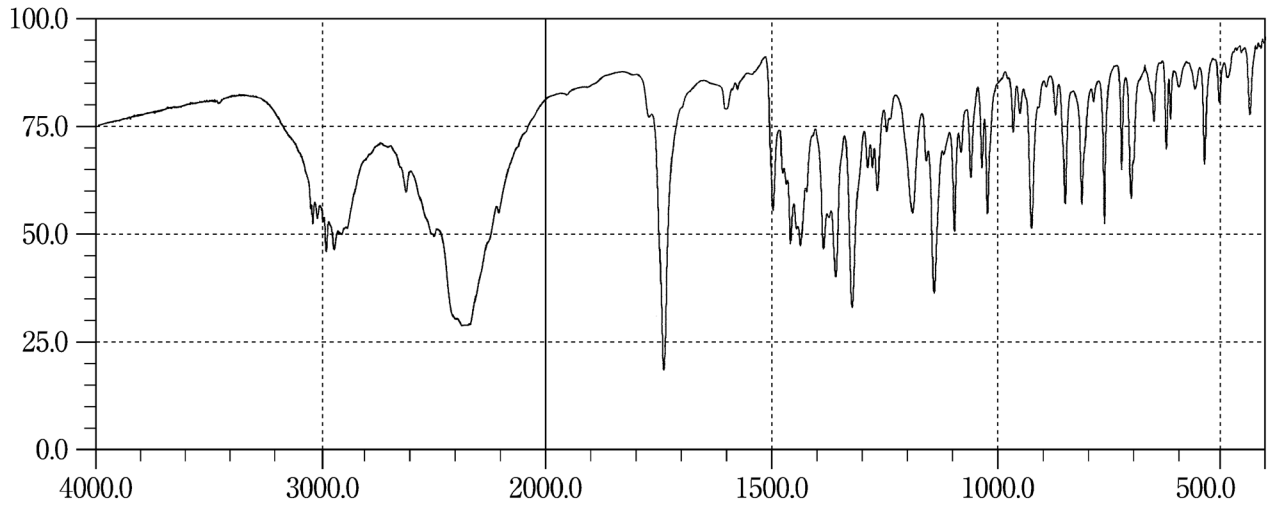
Cefuroxime Axetil



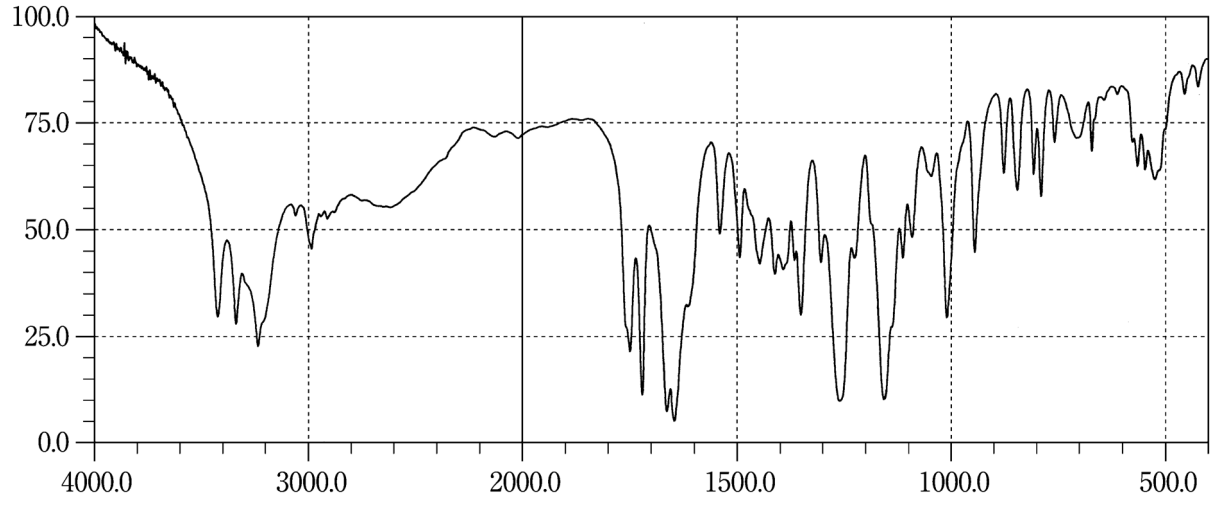
Cellacefate



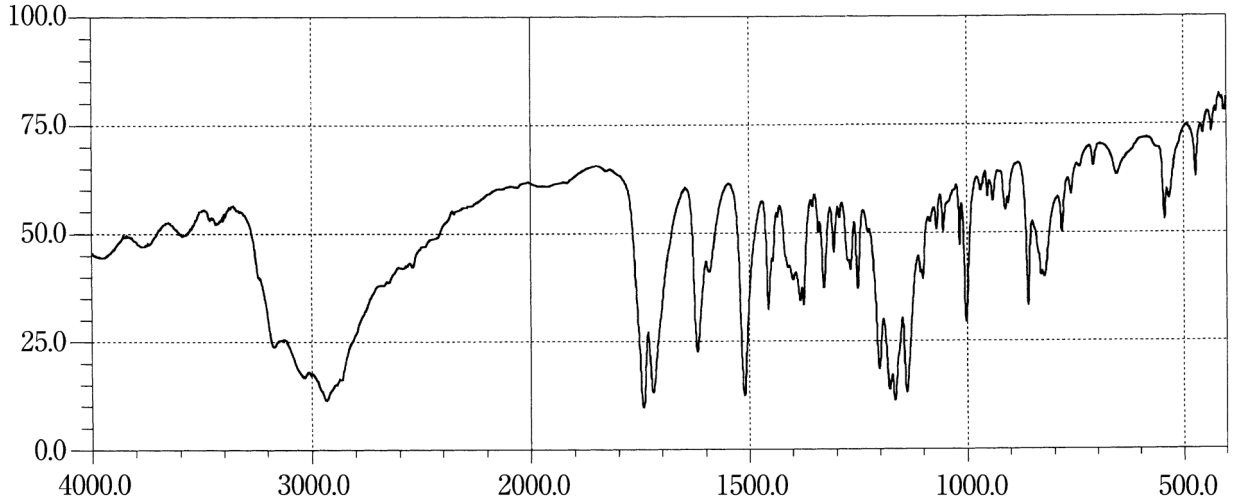
Cetirizine Hydrochloride



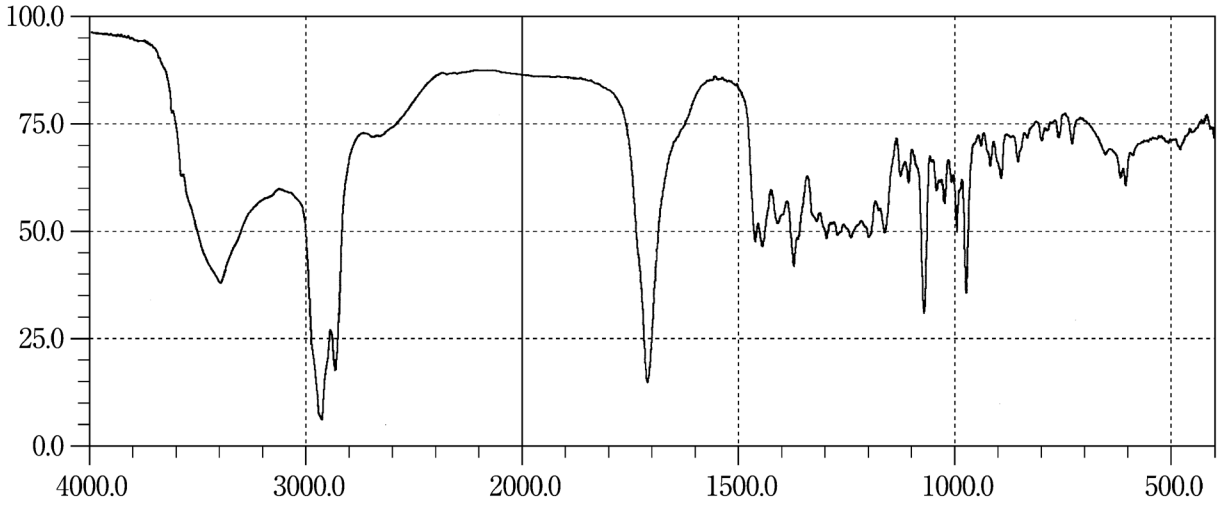
Cetotiamine Hydrochloride Hydrate



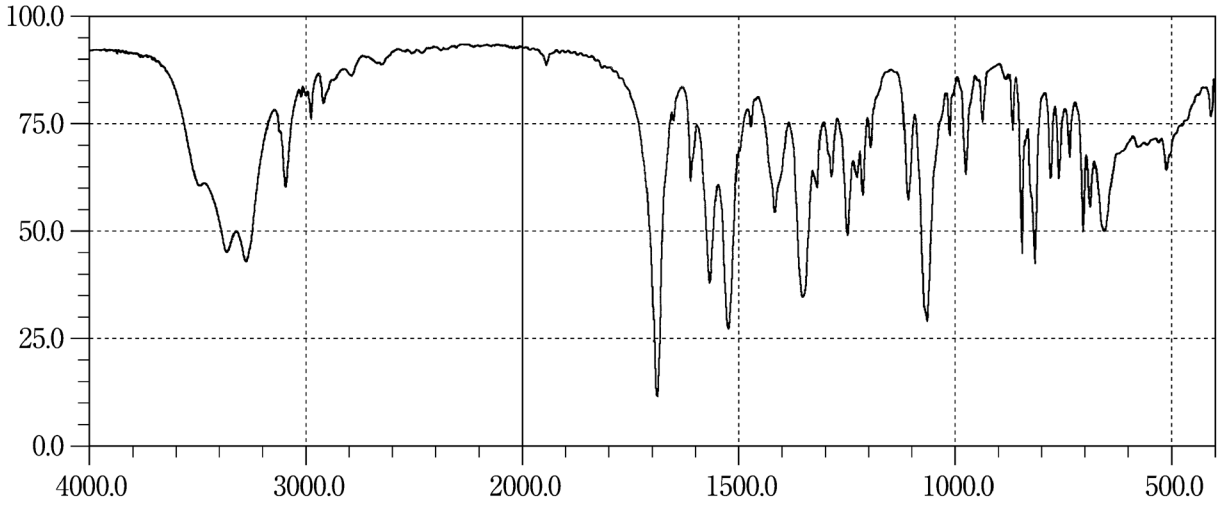
Cetraxate Hydrochloride



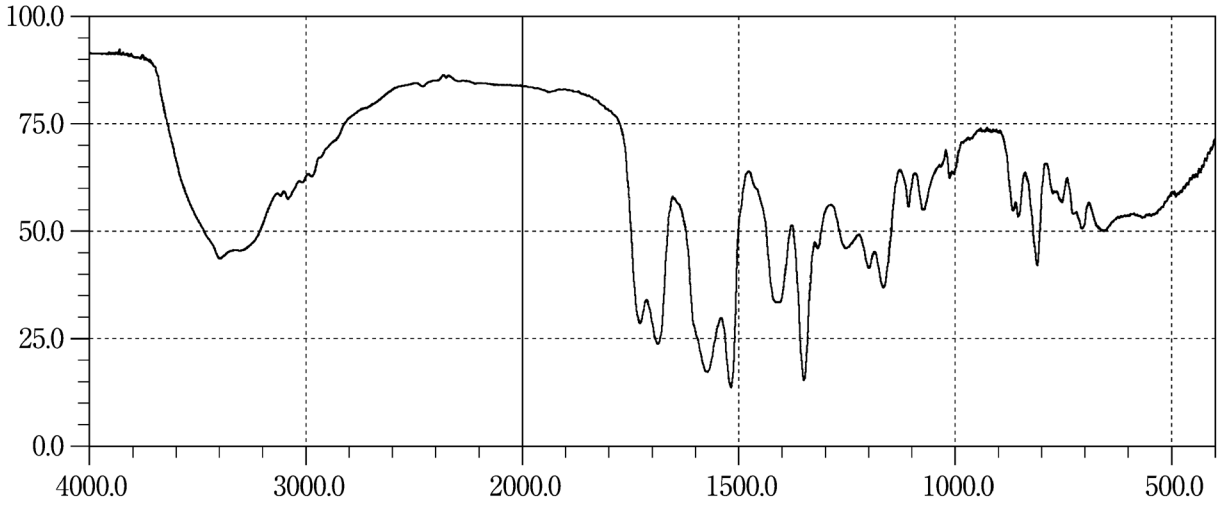
Chenodeoxycholic Acid



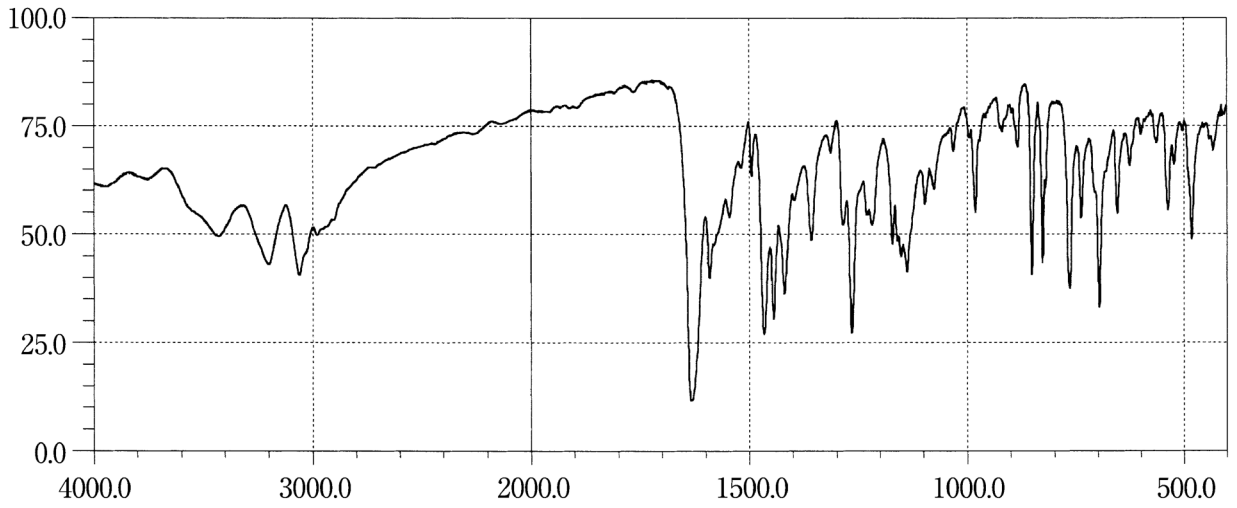
Chloramphenicol



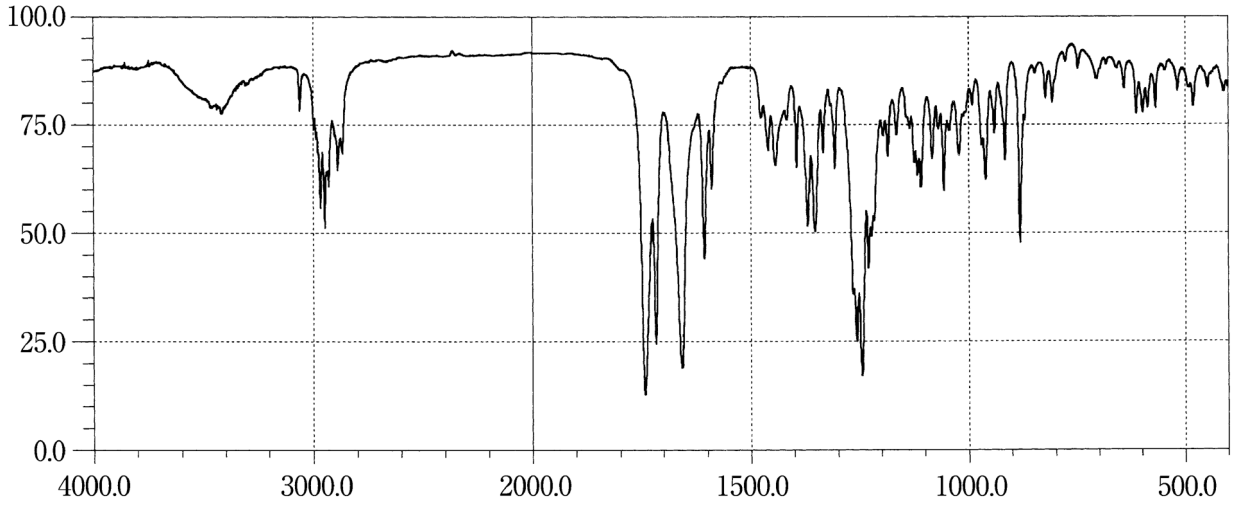
Chloramphenicol Sodium Succinate



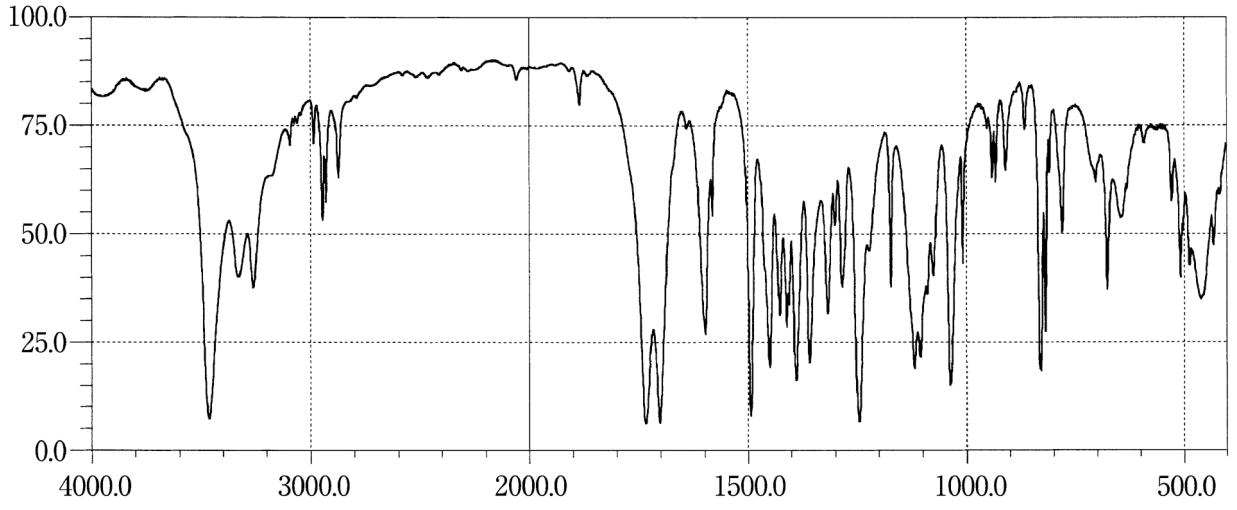
Chlordiazepoxide



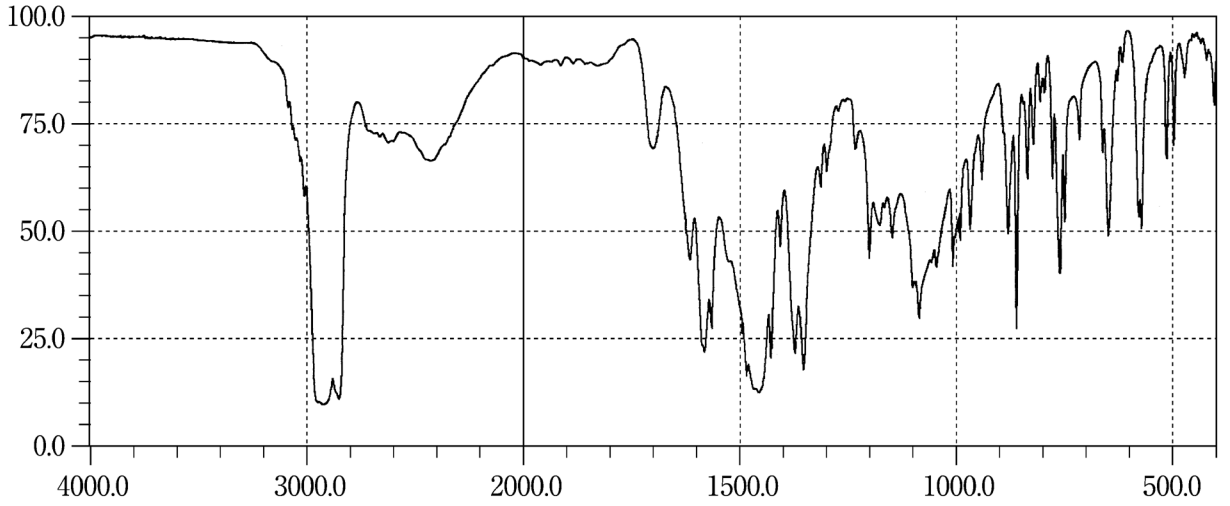
Chlormadinone Acetate



Chlorphenesin Carbamate



Chlorpheniramine Maleate



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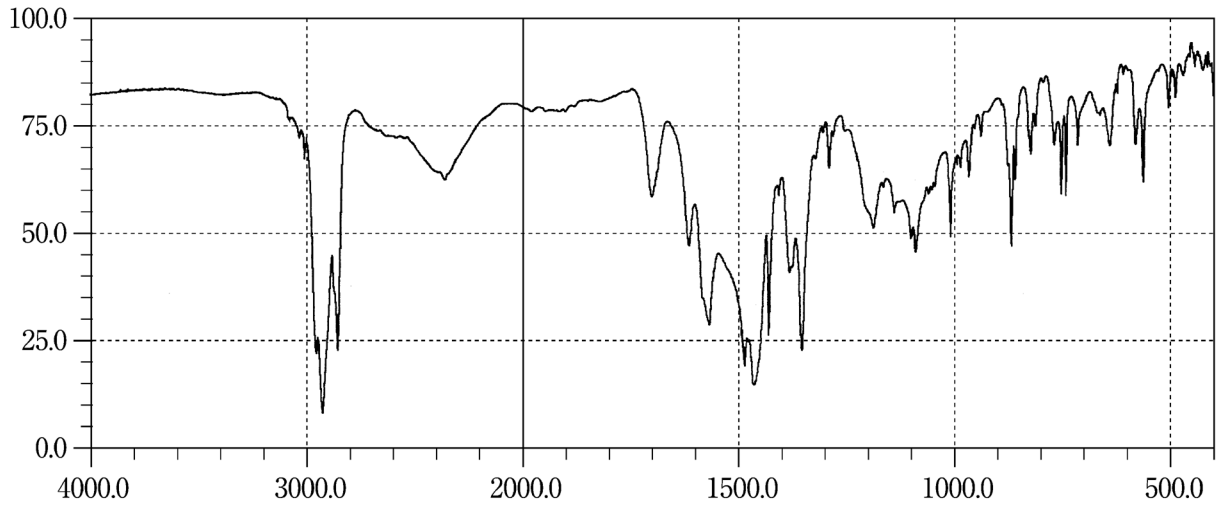
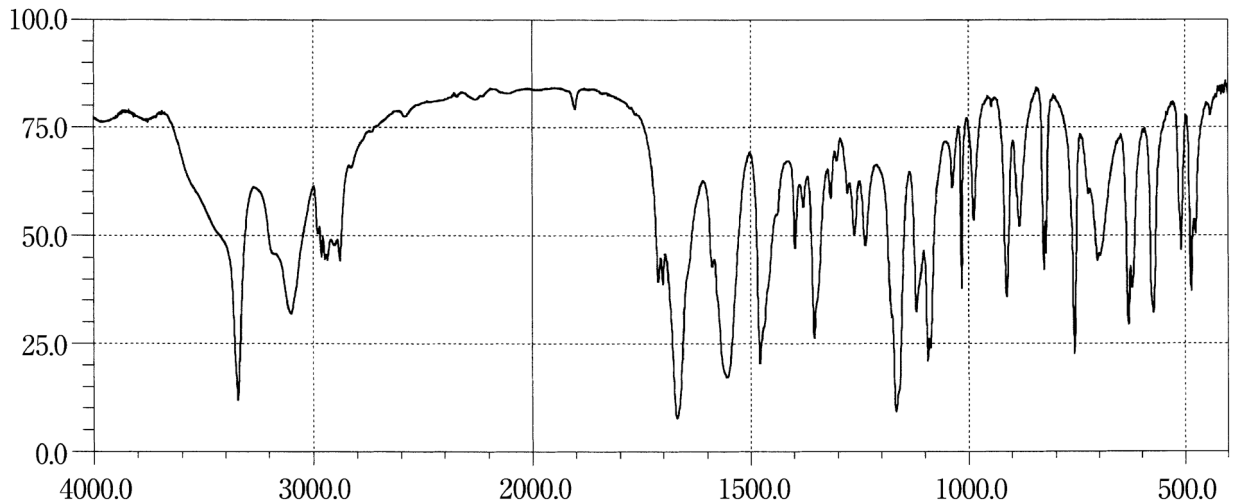
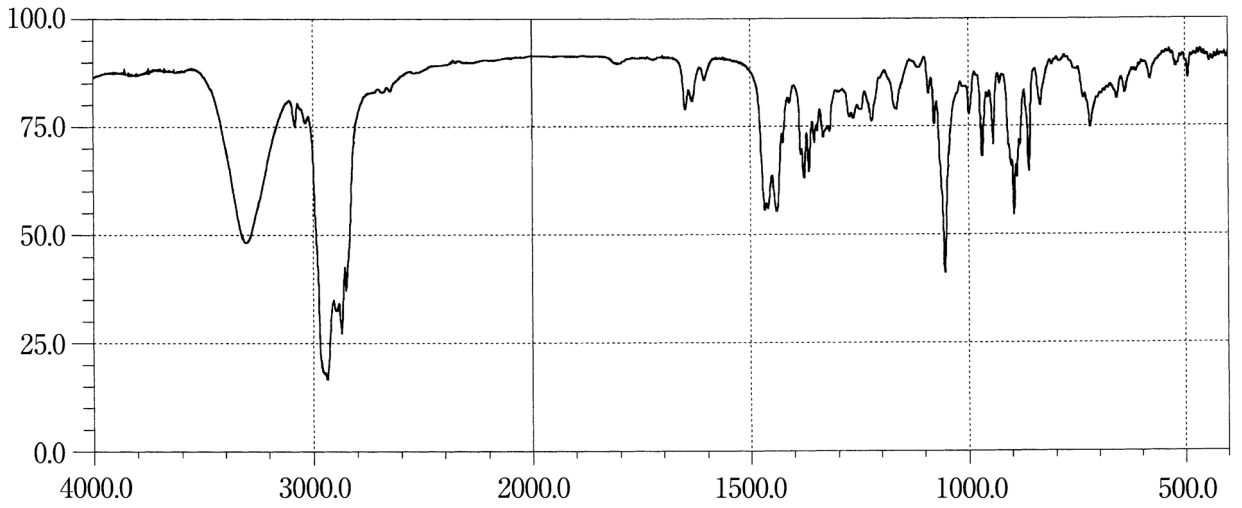


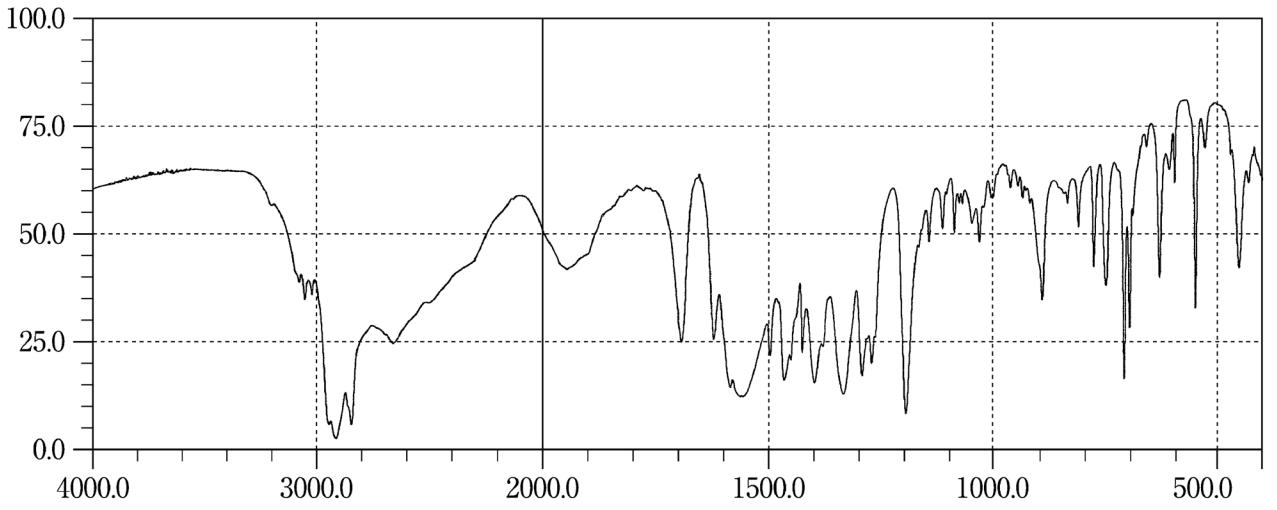
Chlorpropamide



Cholecalciferol



Cibenzoline Succinate



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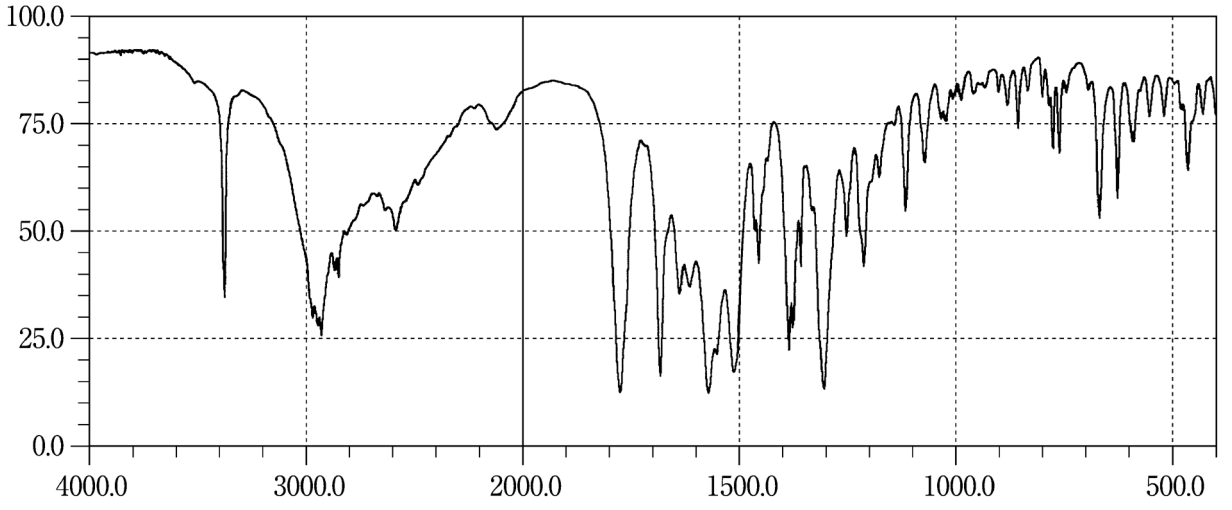
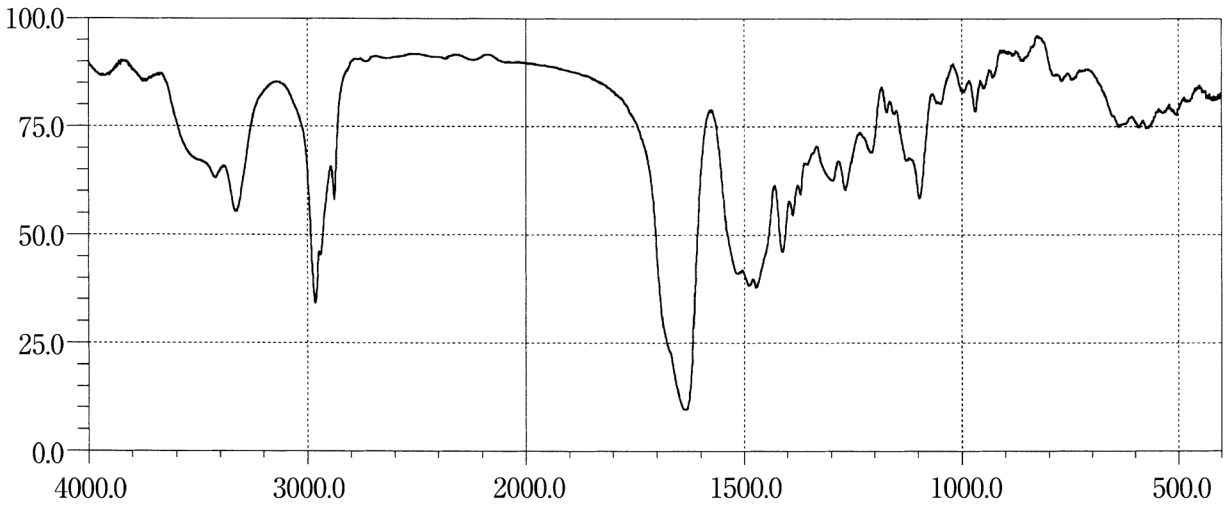
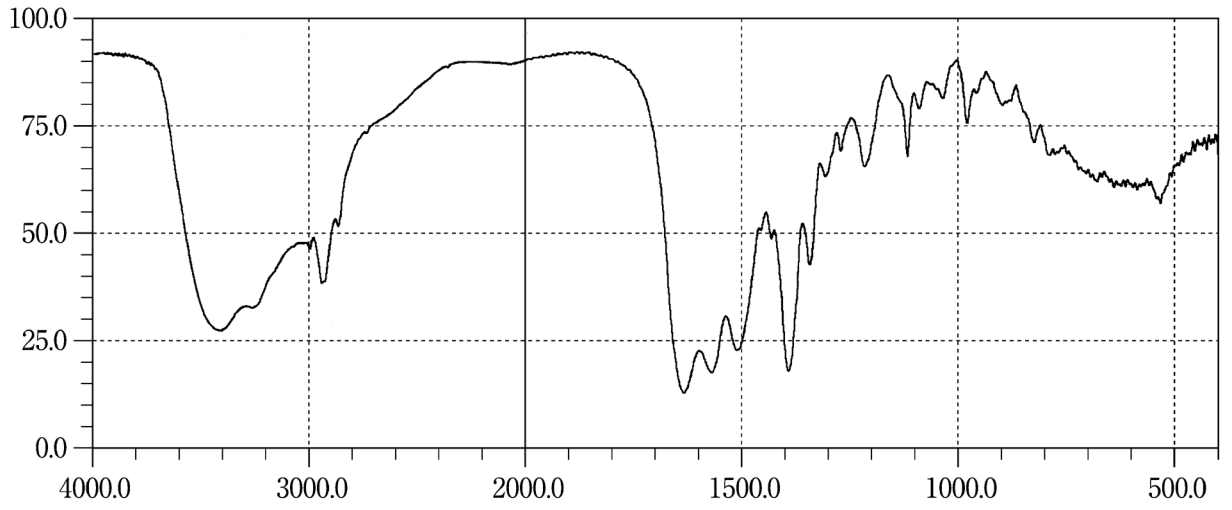


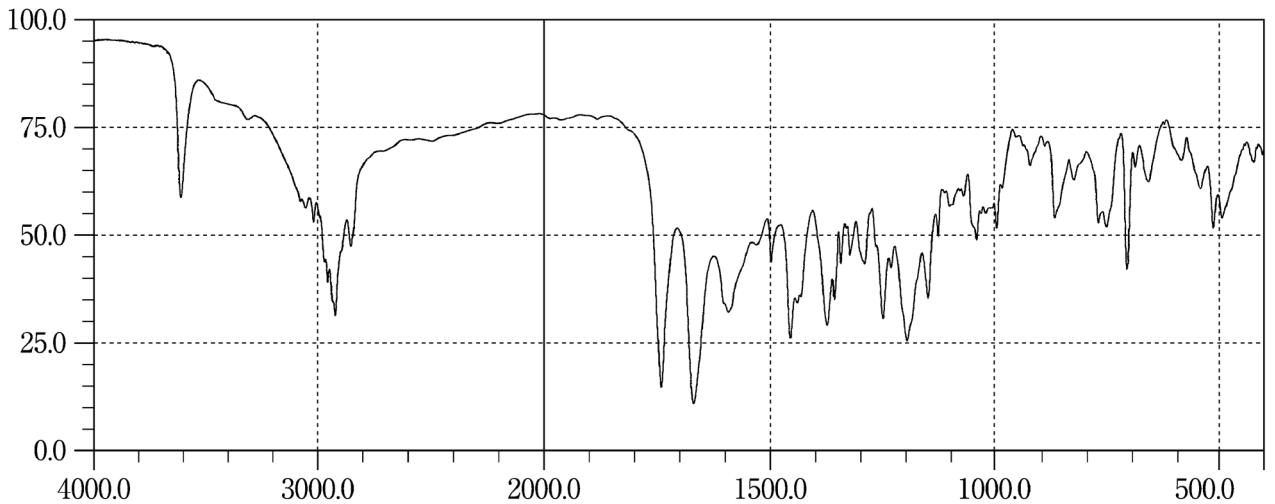
Ciclosporin



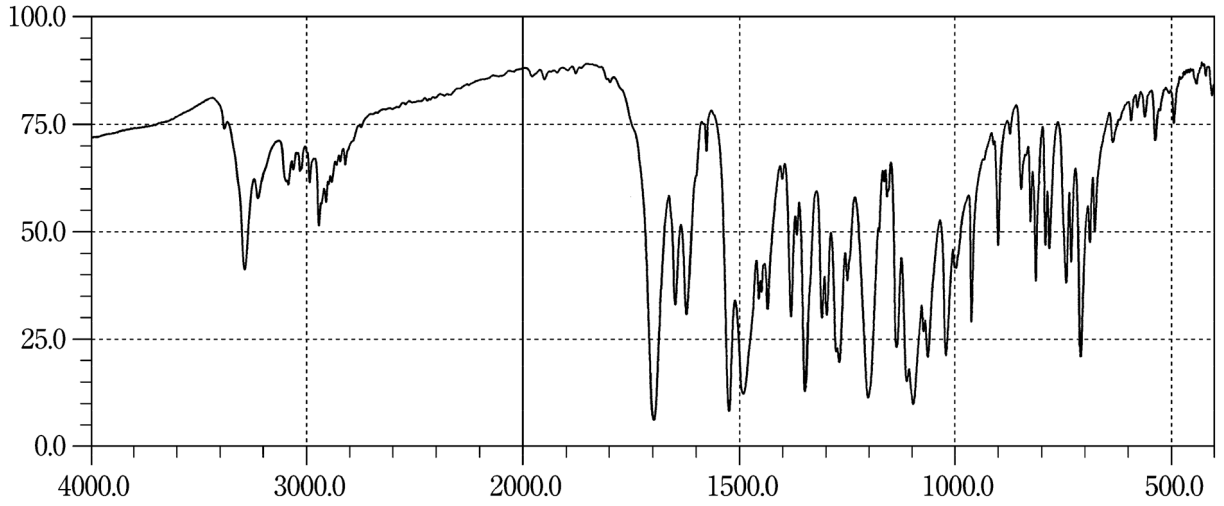
Cilastatin Sodium



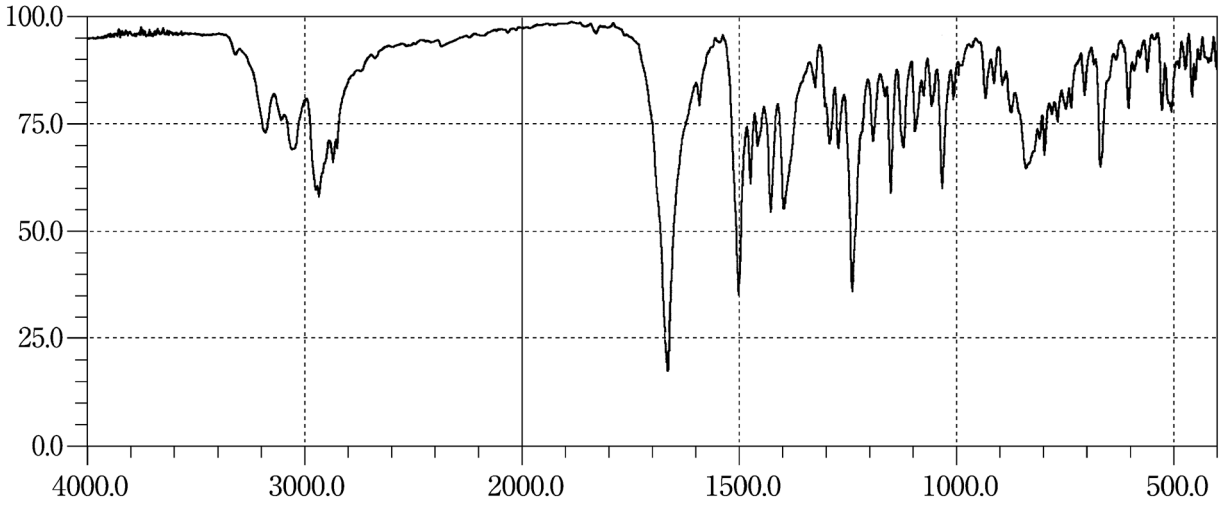
Cilazapril Hydrate



Cilnidipine



Cilostazol



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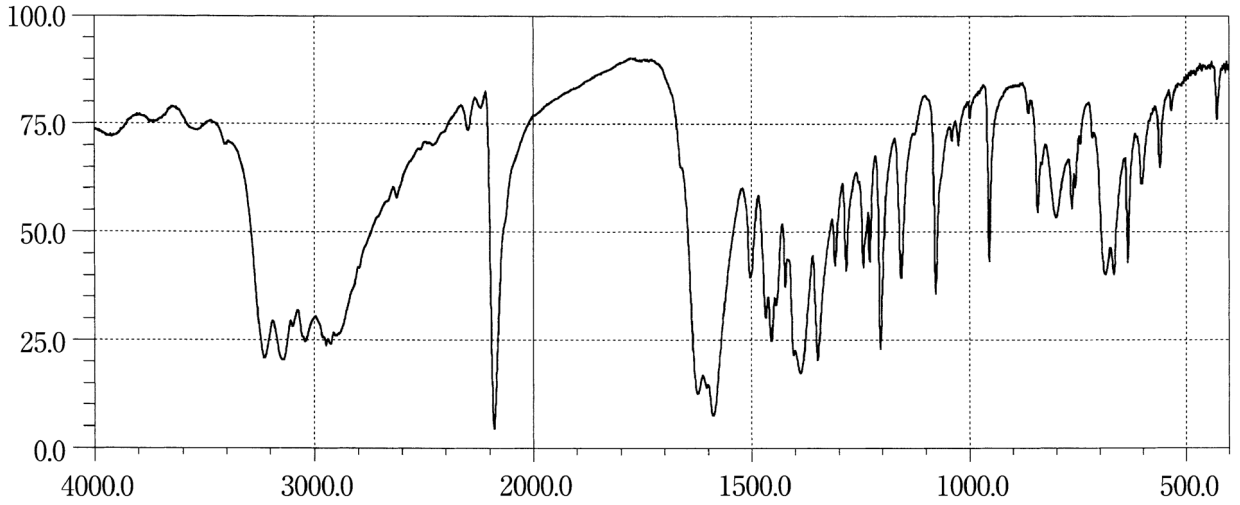
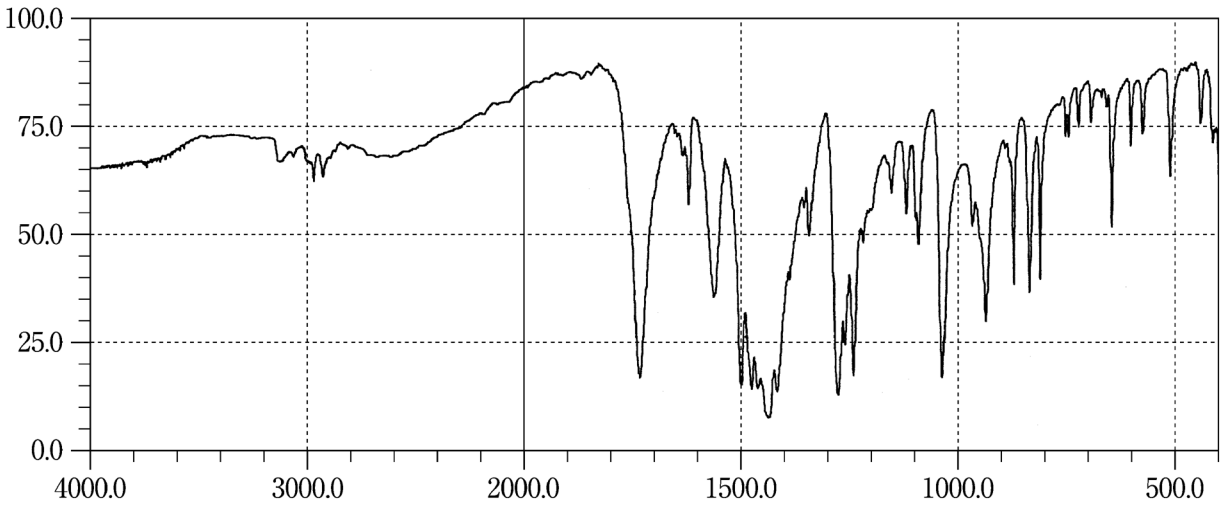
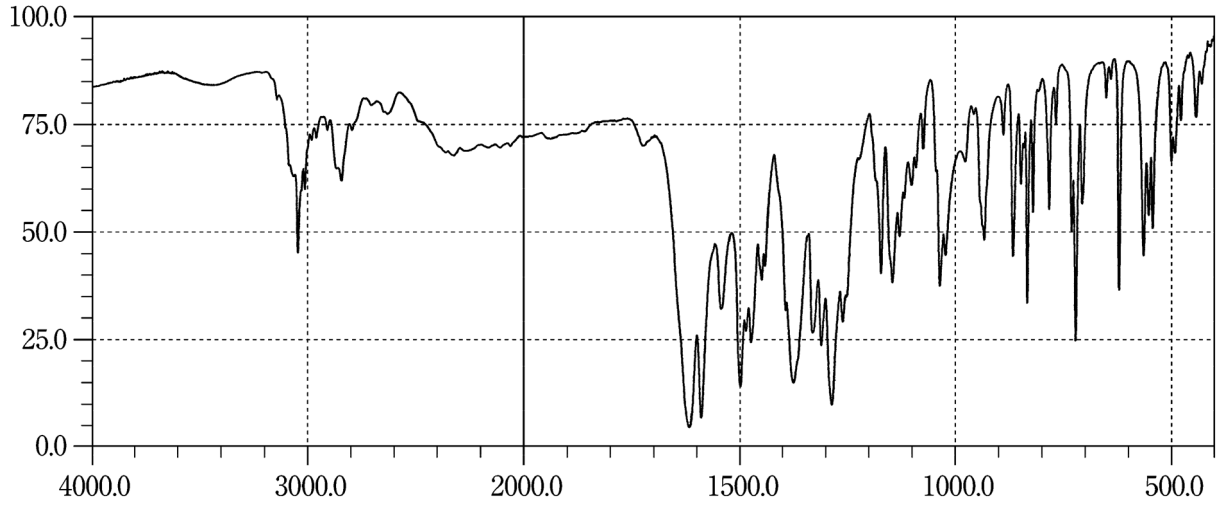


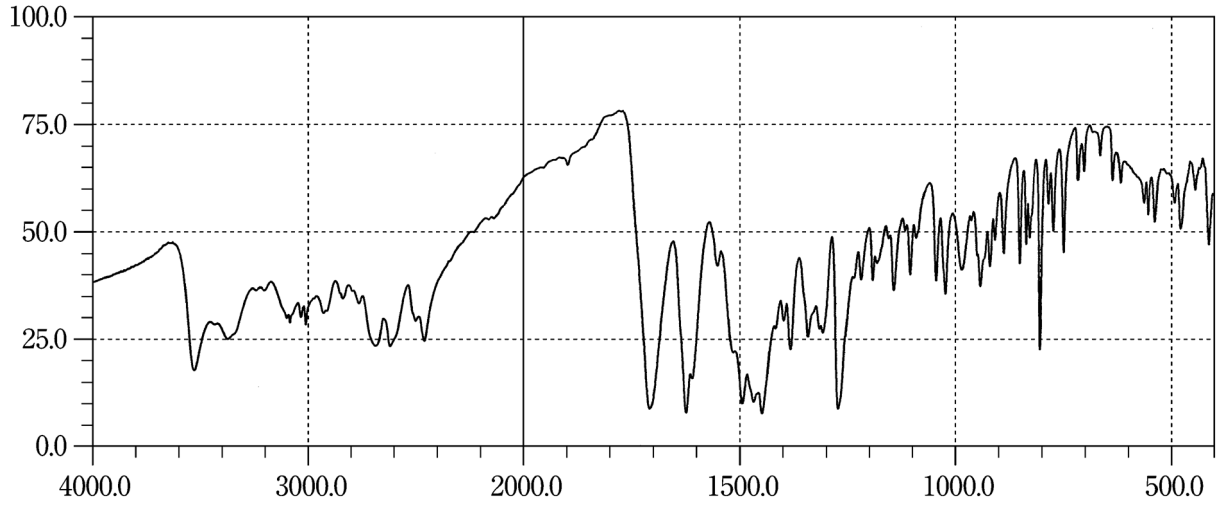
Cinoxacin



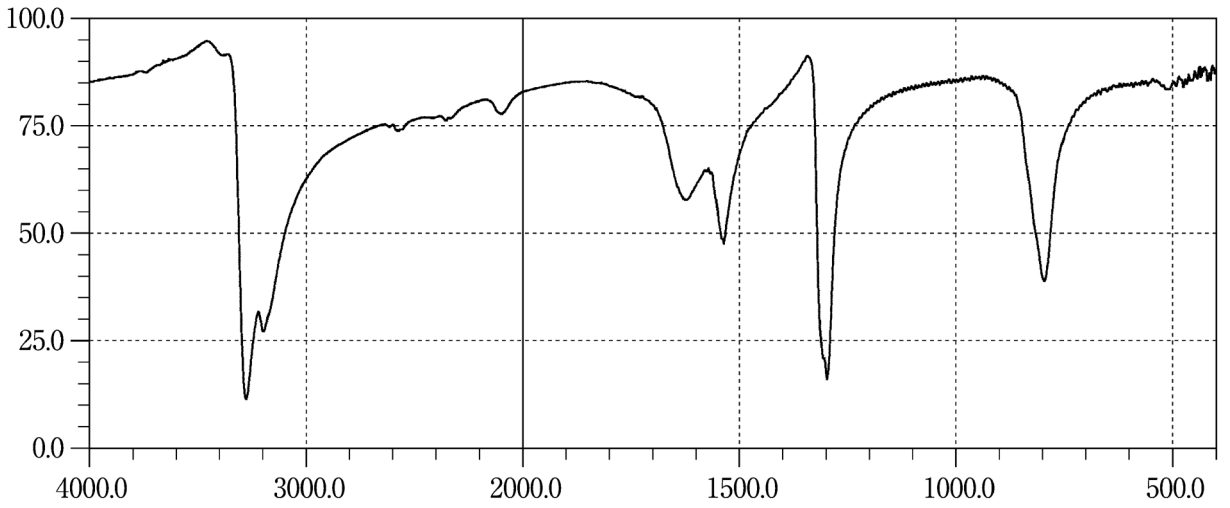
Ciprofloxacin



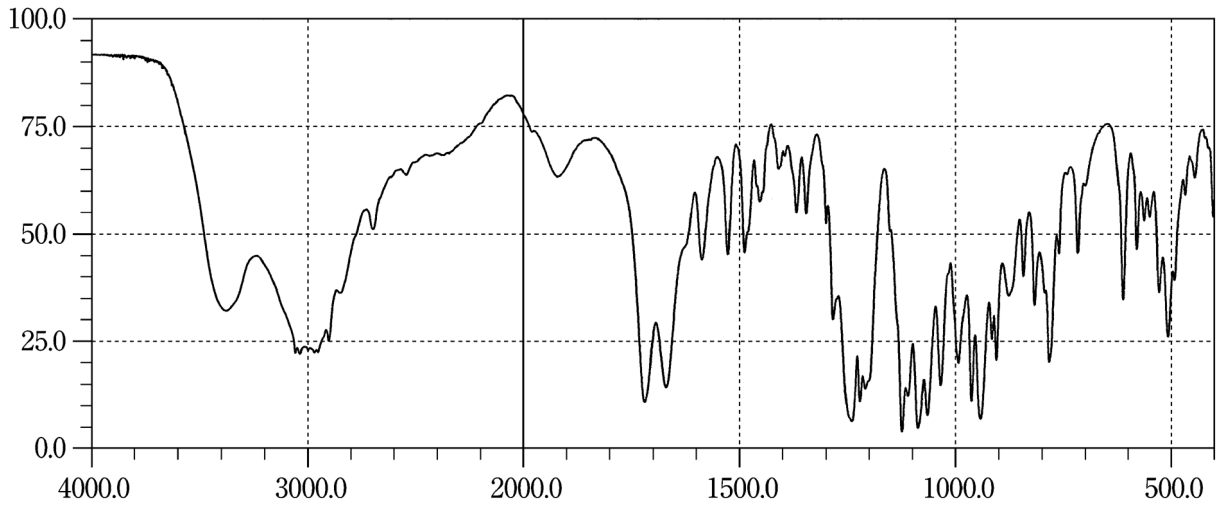
Ciprofloxacin Hydrochloride Hydrate



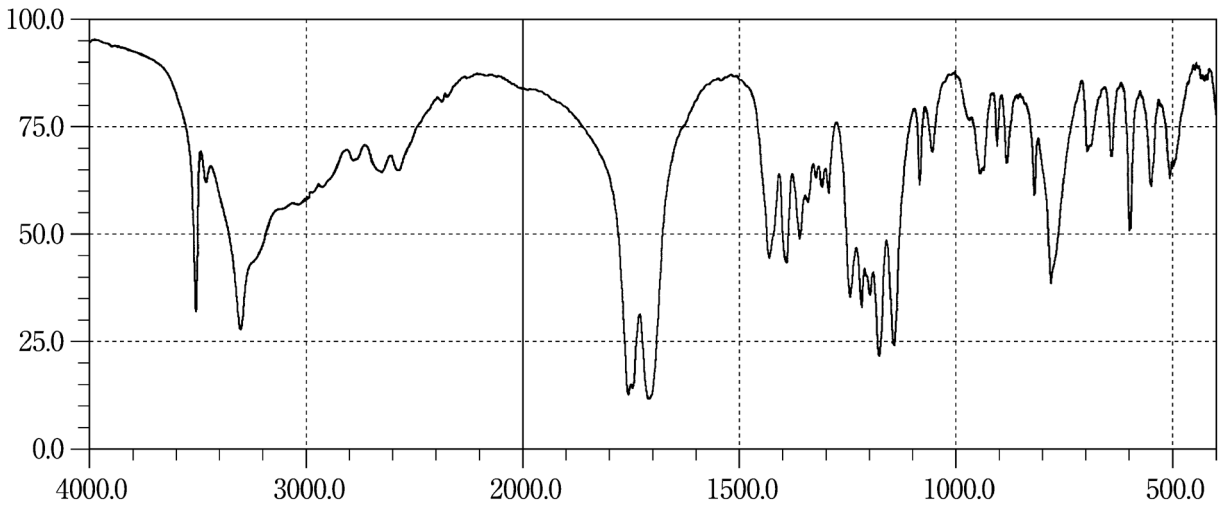
Cisplatin



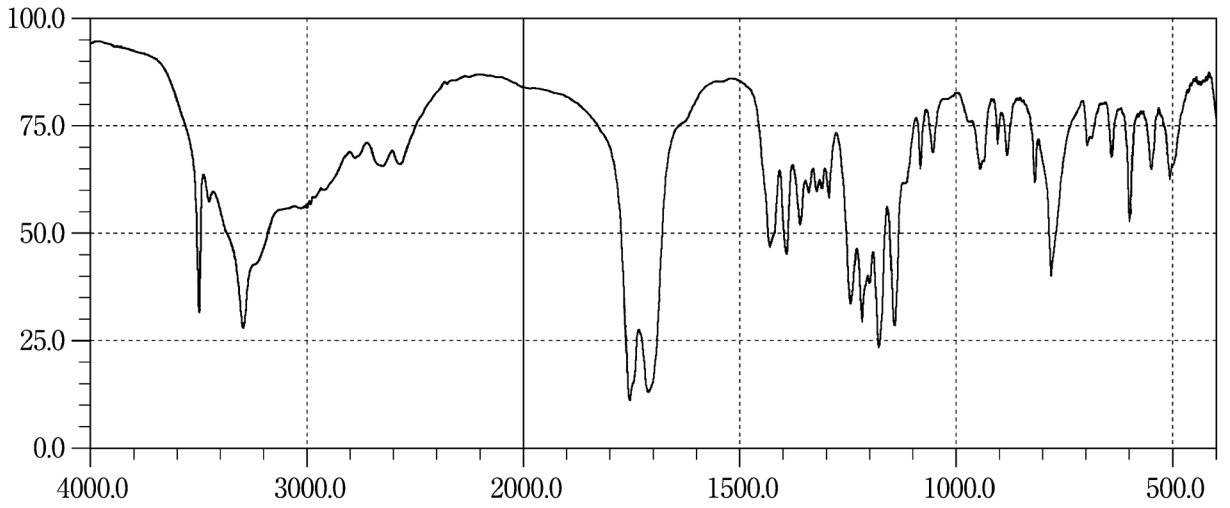
Citicoline



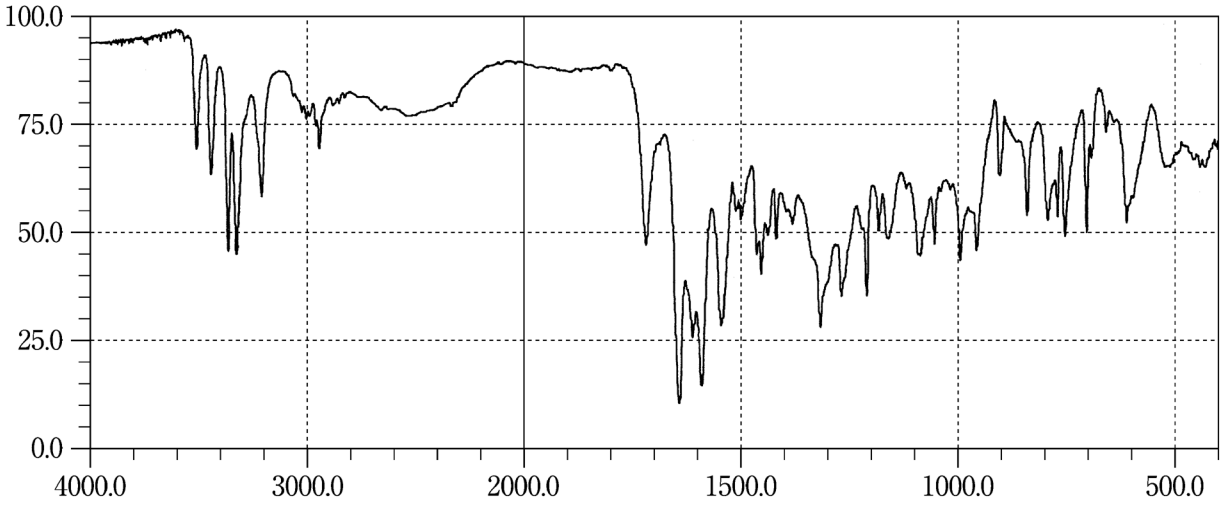
Anhydrous Citric Acid



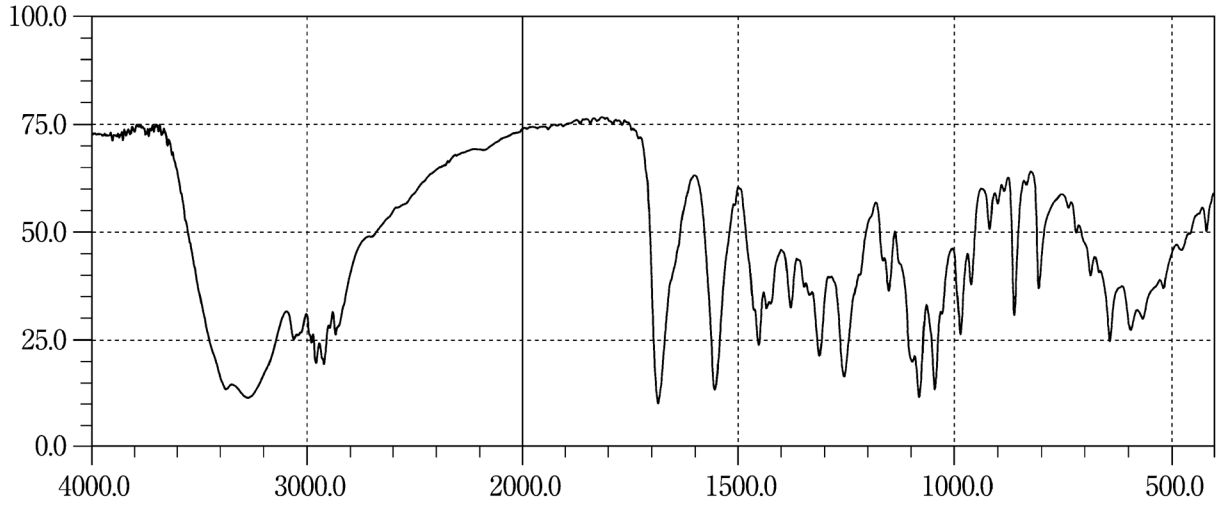
Citric Acid Hydrate



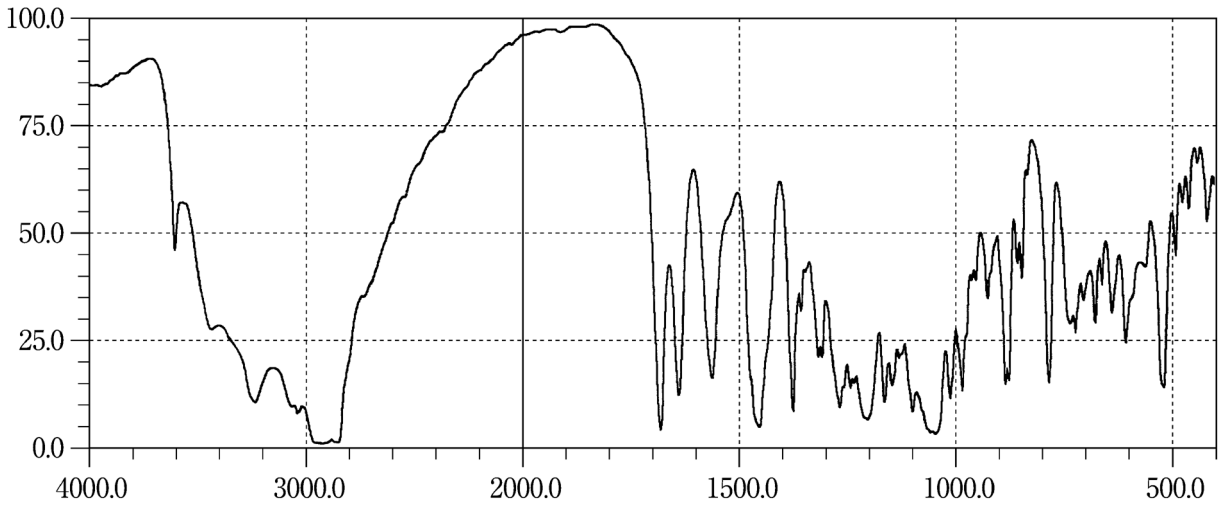
Clebopride Malate



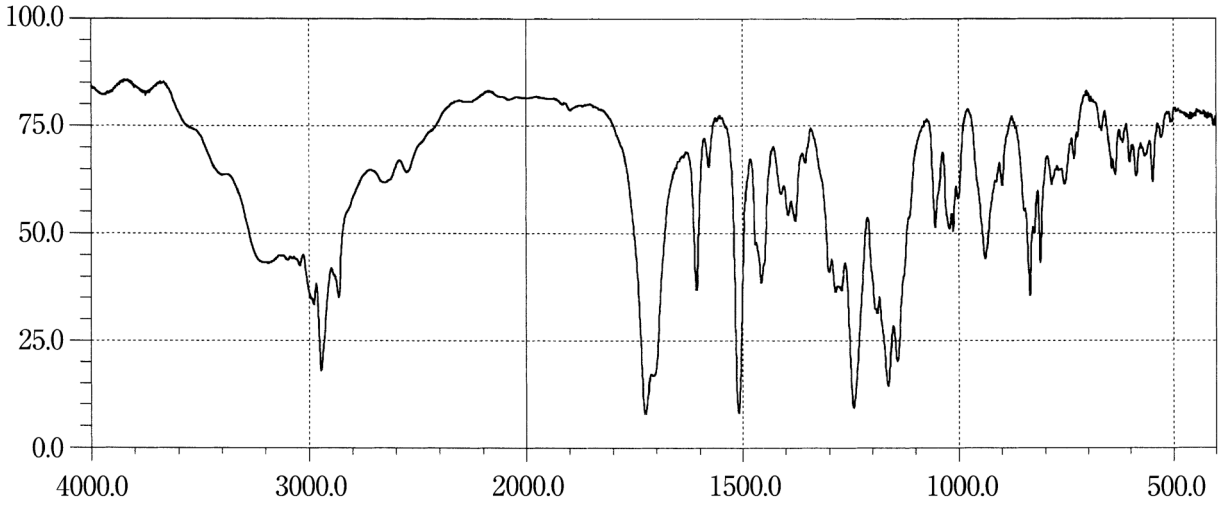
Clindamycin Hydrochloride



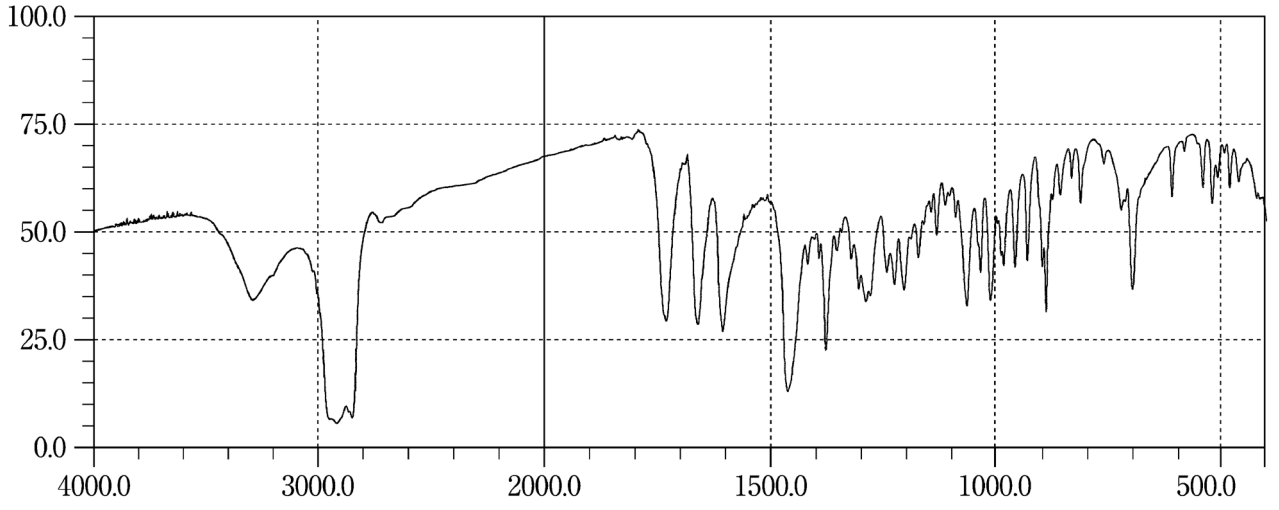
Clindamycin Phosphate



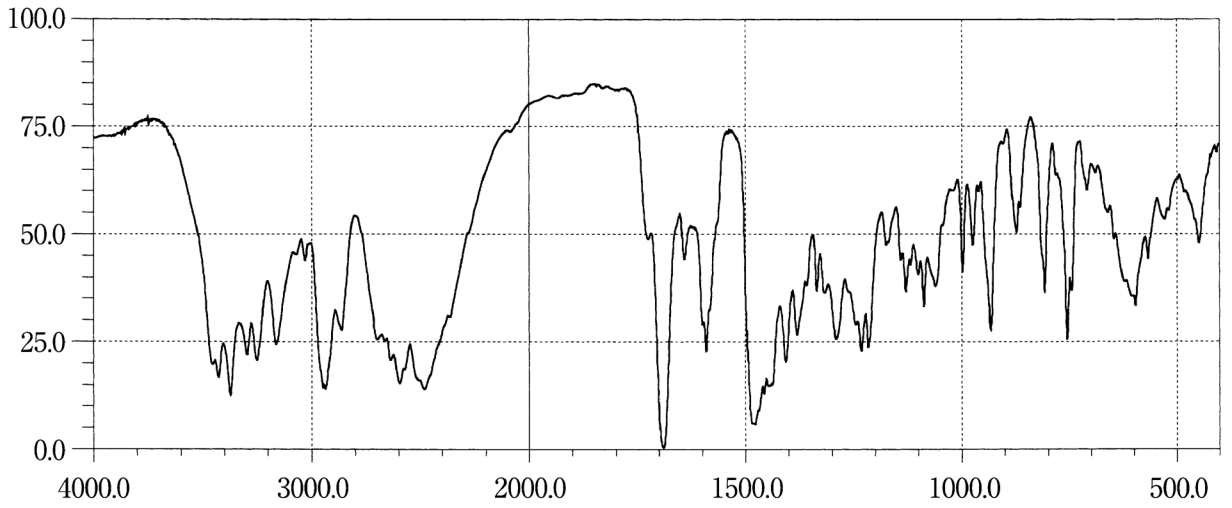
Clinofibrate



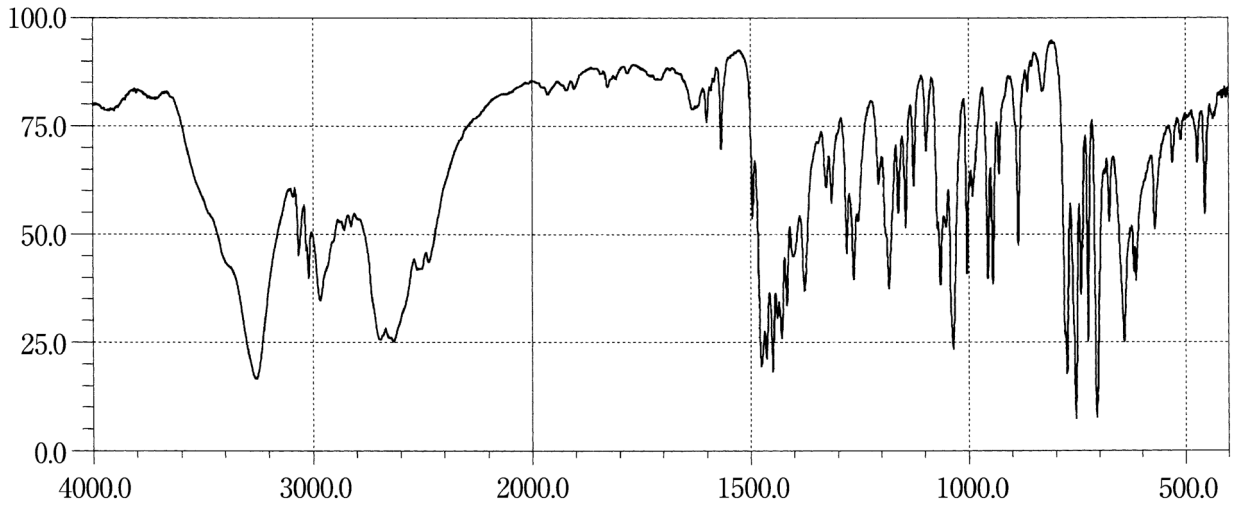
Clobetasol Propionate



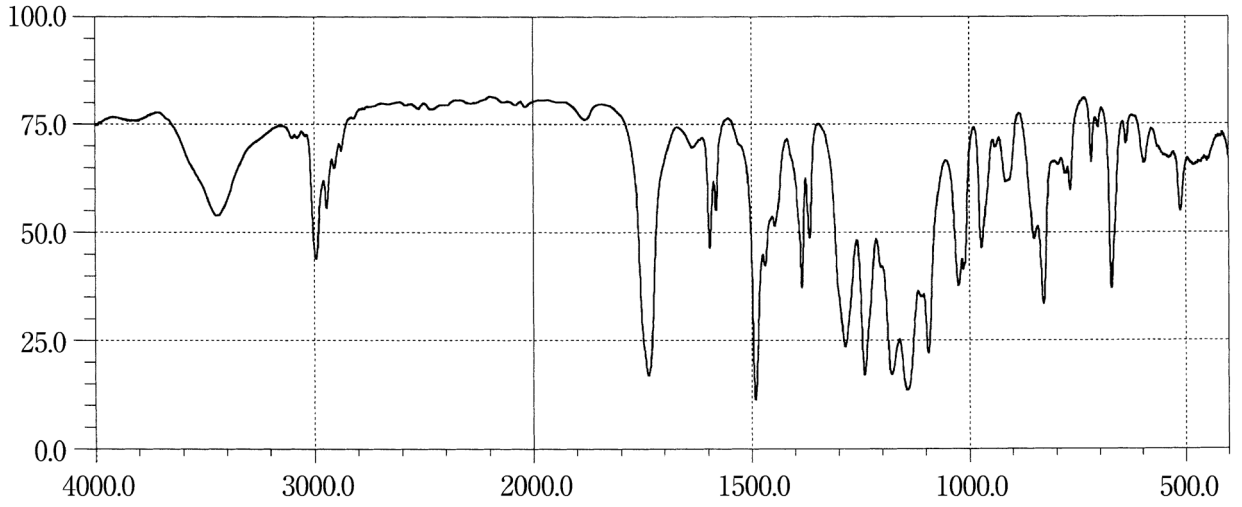
Clocapramine Hydrochloride Hydrate



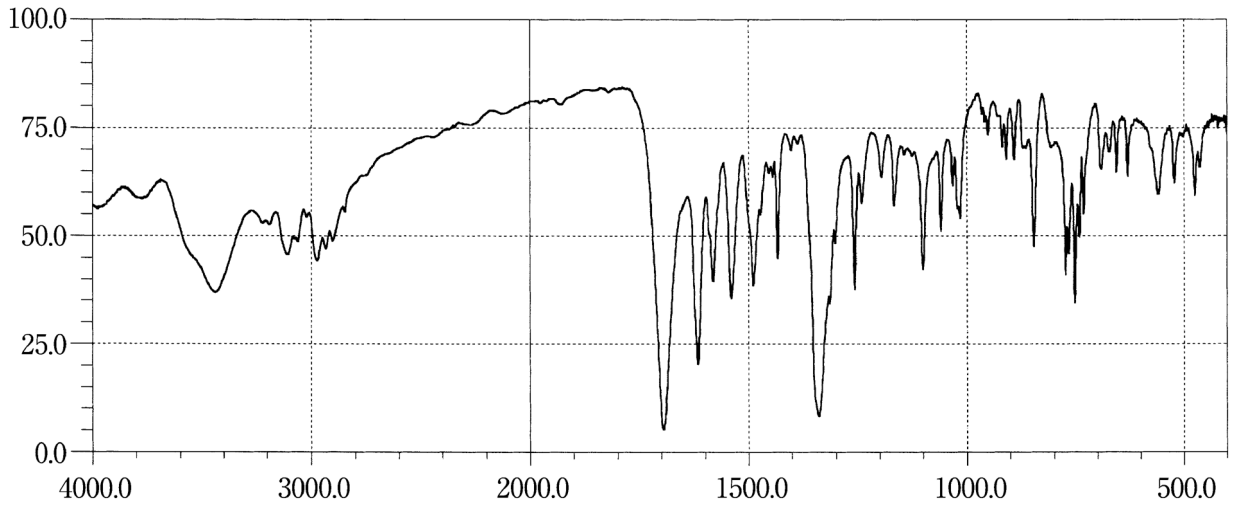
Clofedanol Hydrochloride



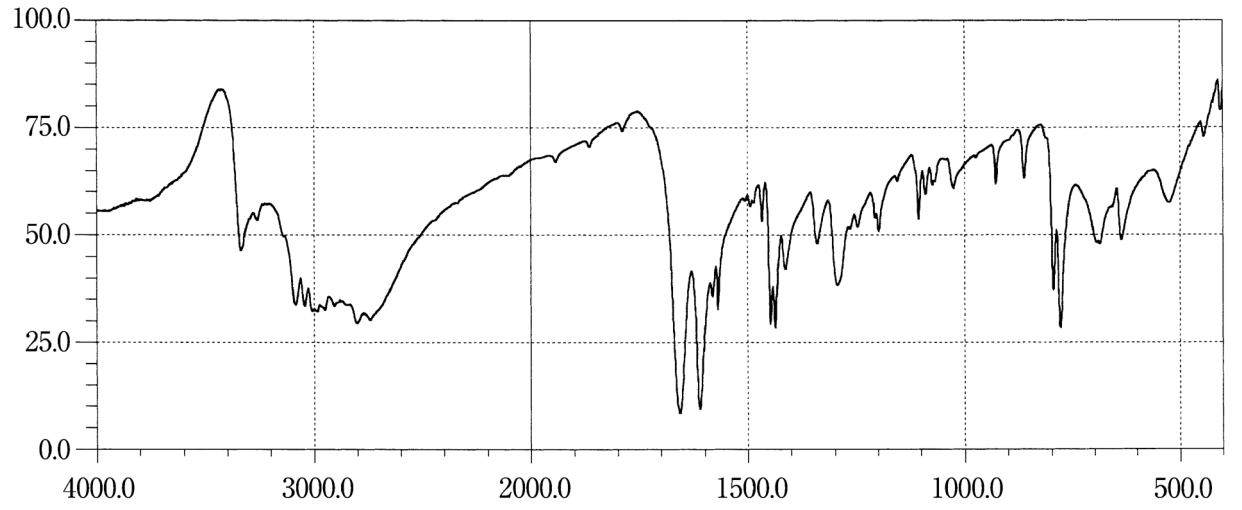
Clofibrate



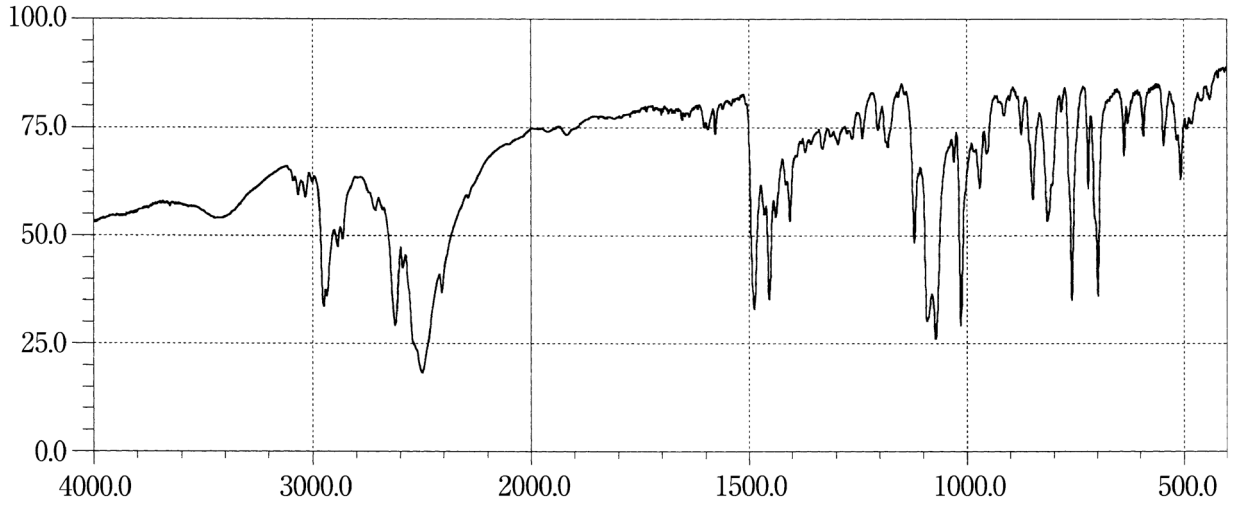
Clonazepam



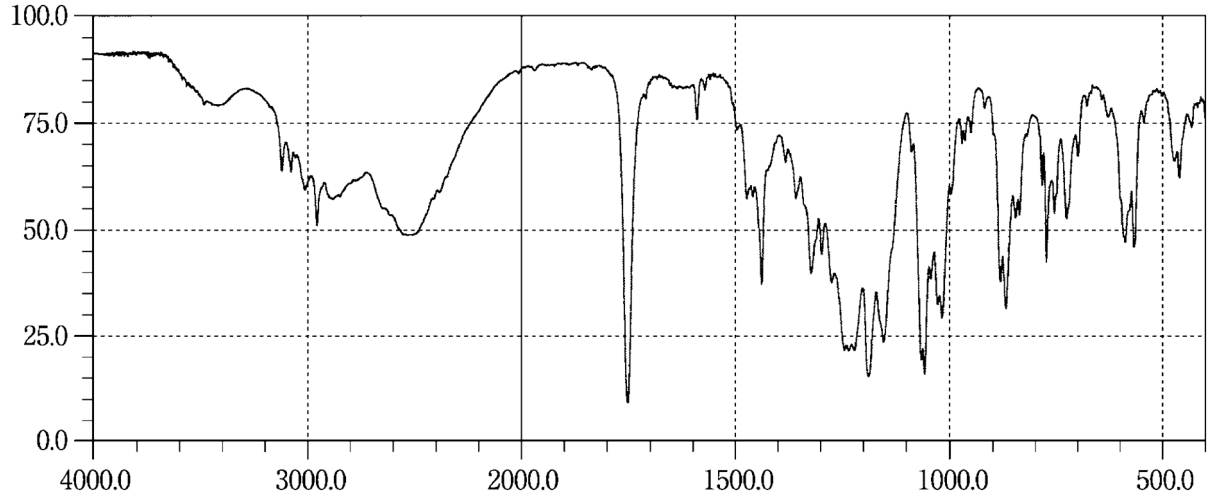
Clonidine Hydrochloride



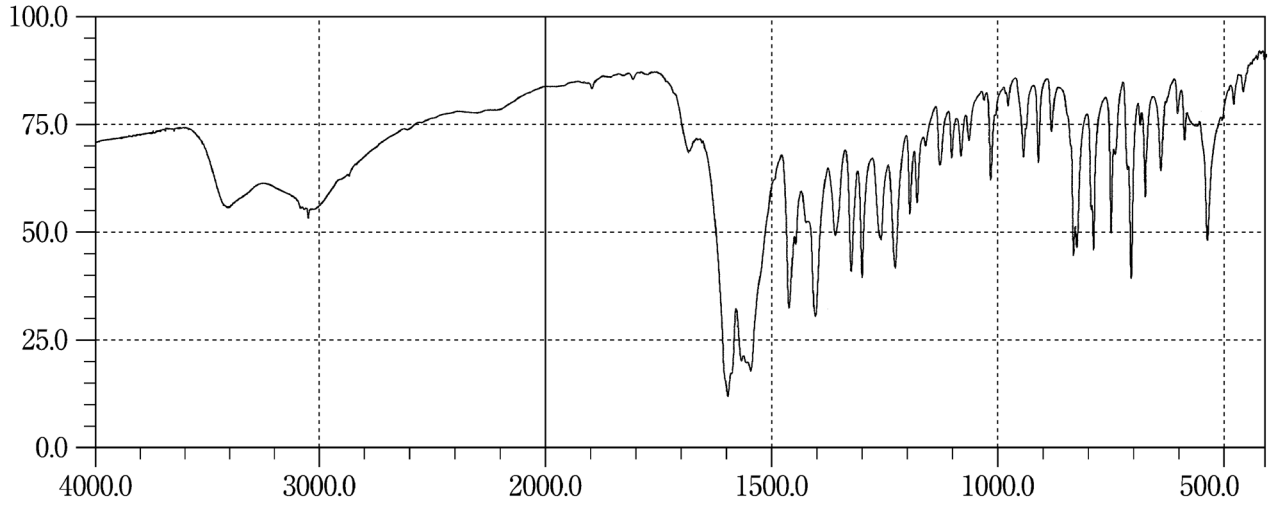
Cloperastine Hydrochloride



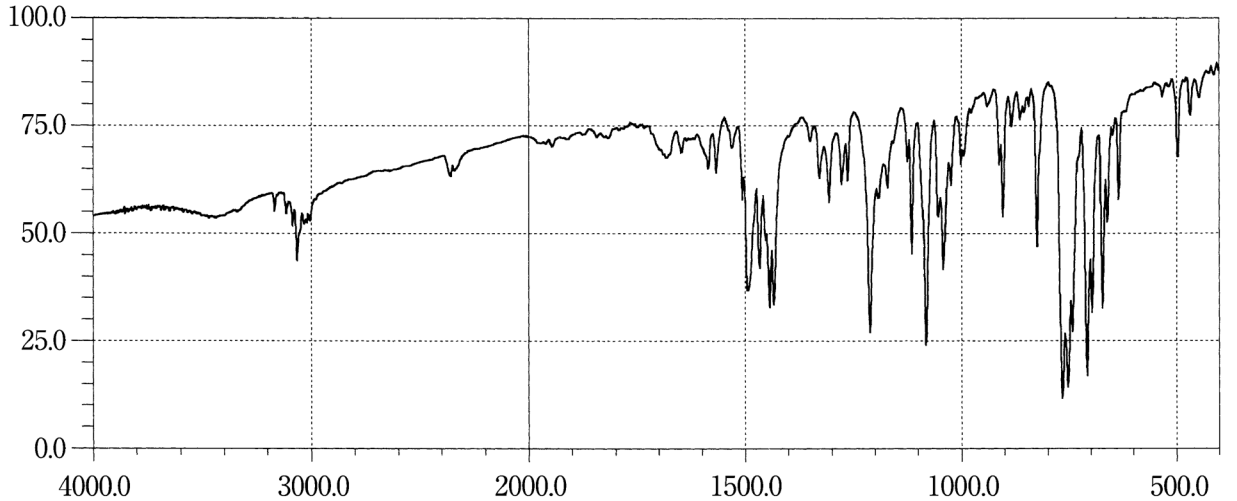
Clopidogrel Sulfate



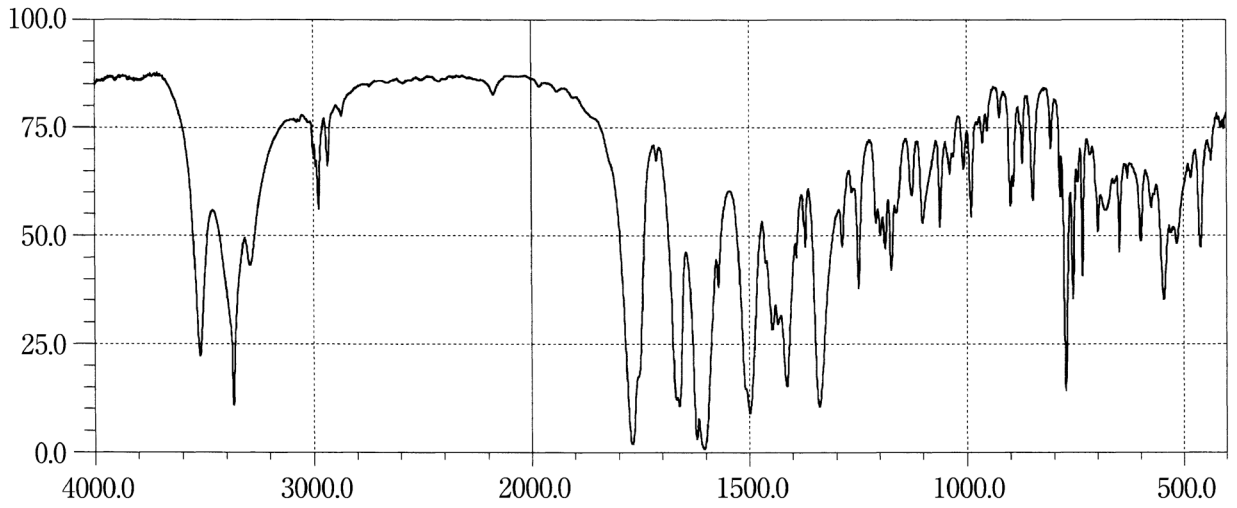
Clorazepate Dipotassium



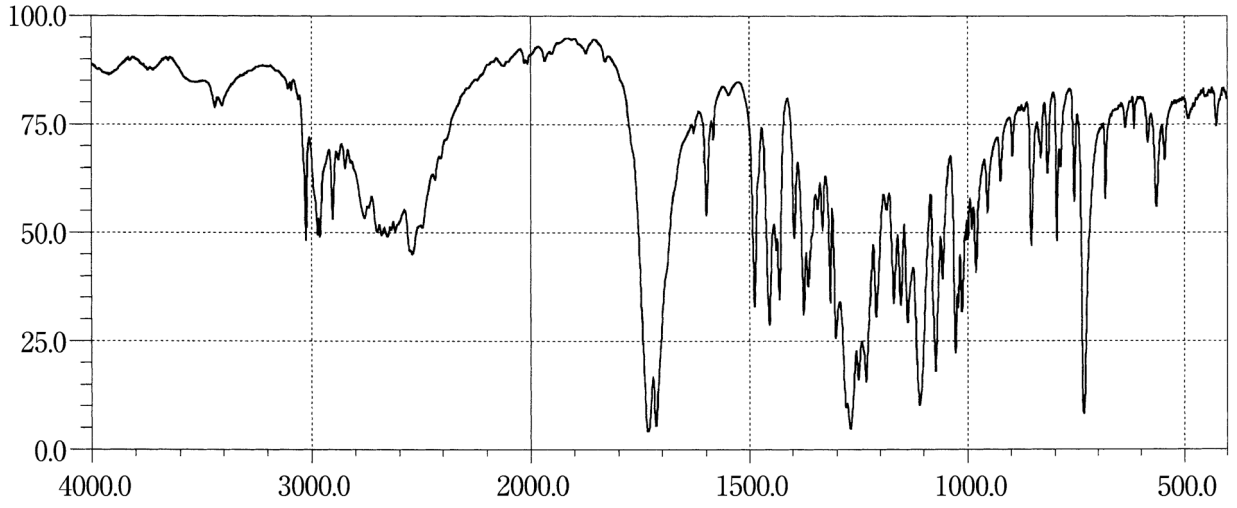
Clotrimazole



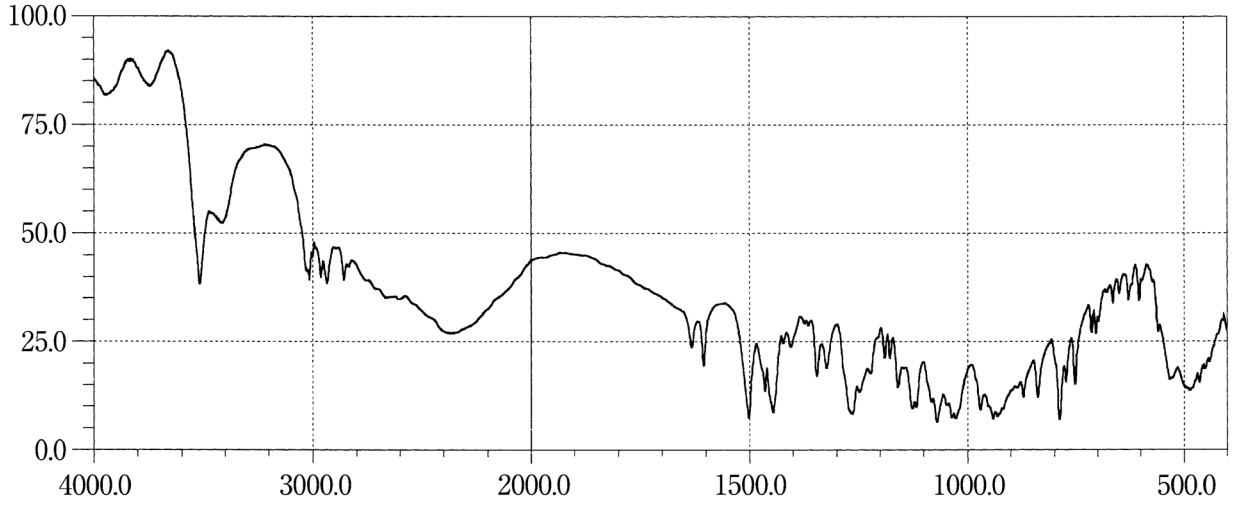
Cloxacillin Sodium Hydrate



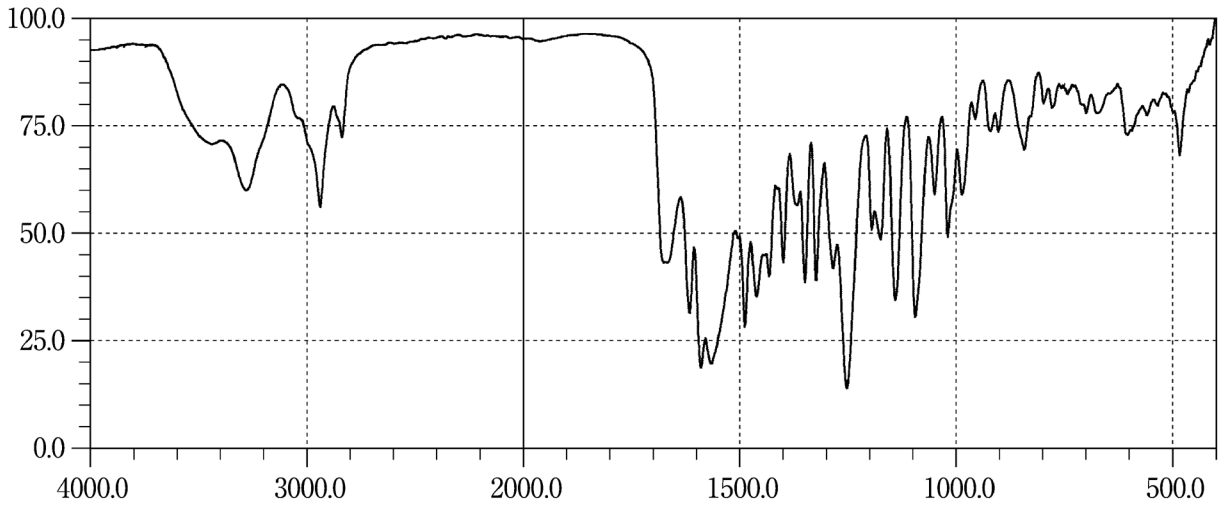
Cocaine Hydrochloride



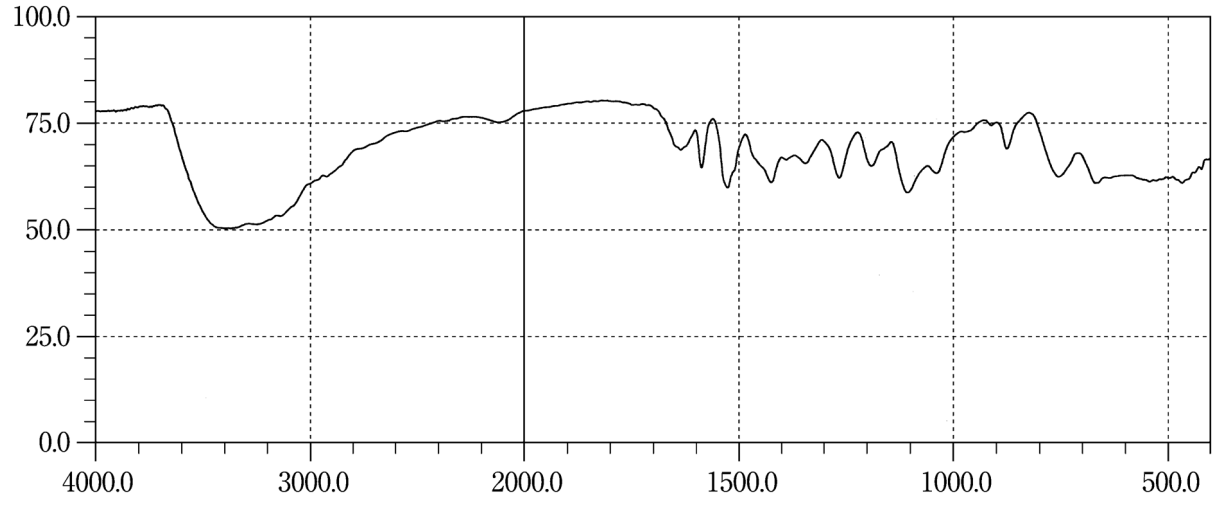
Codeine Phosphate Hydrate



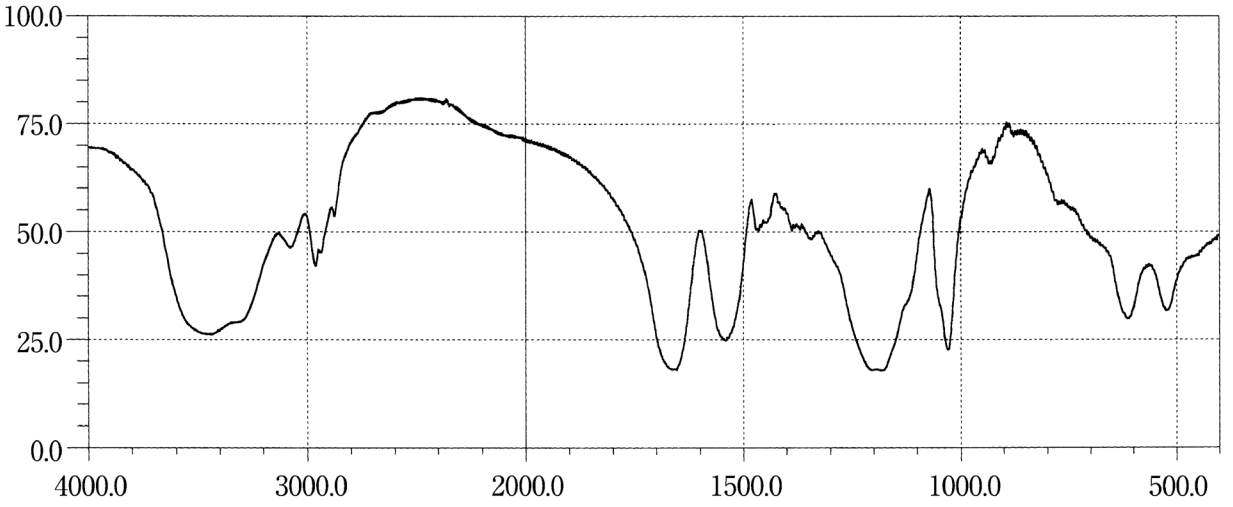
Colchicine



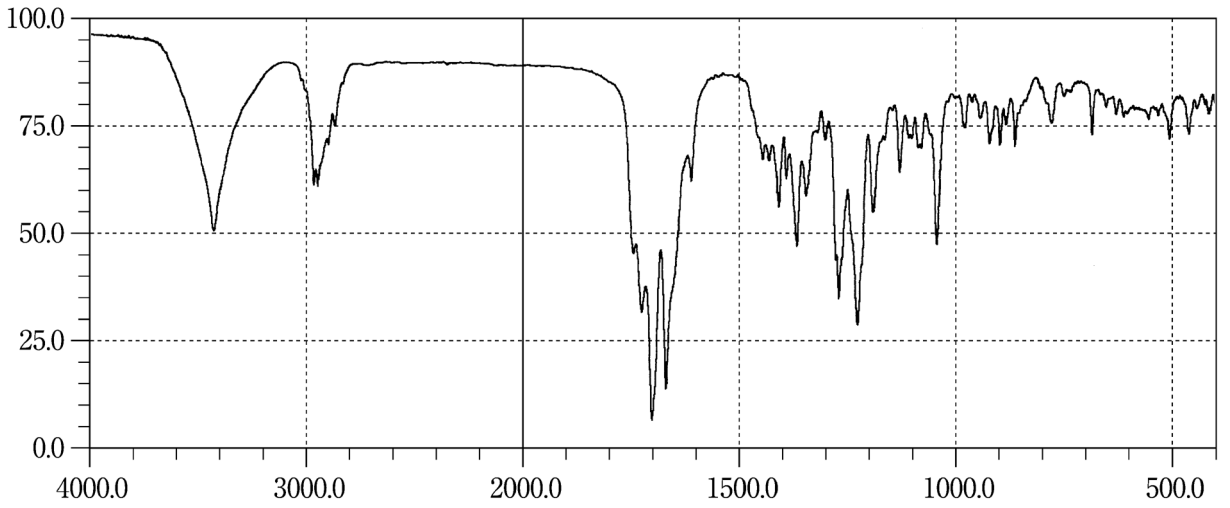
Colestimide



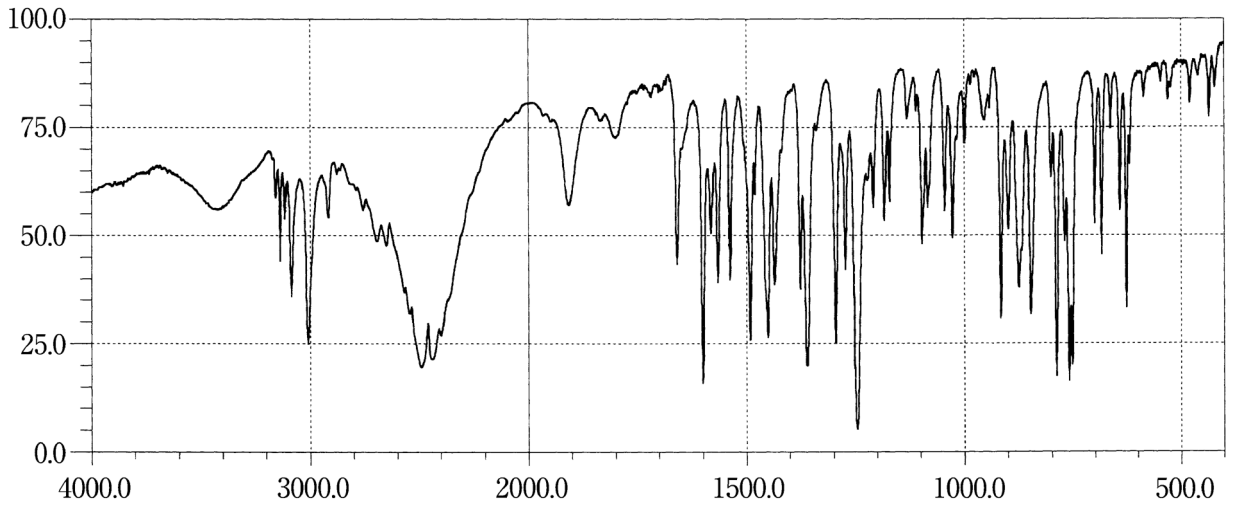
Colistin Sodium Methanesulfonate



Cortisone Acetate



Croconazole Hydrochloride



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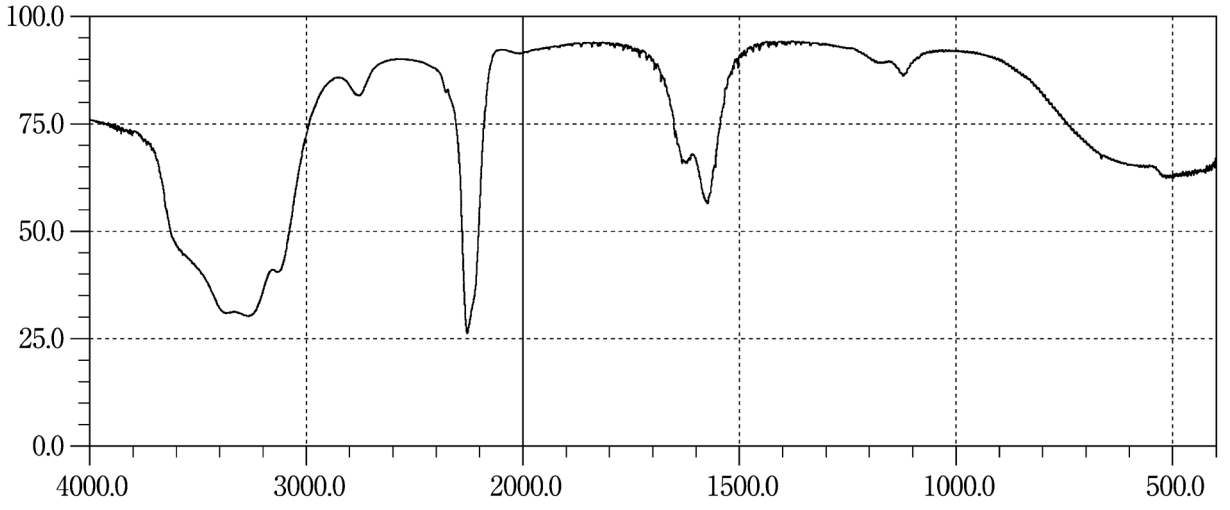
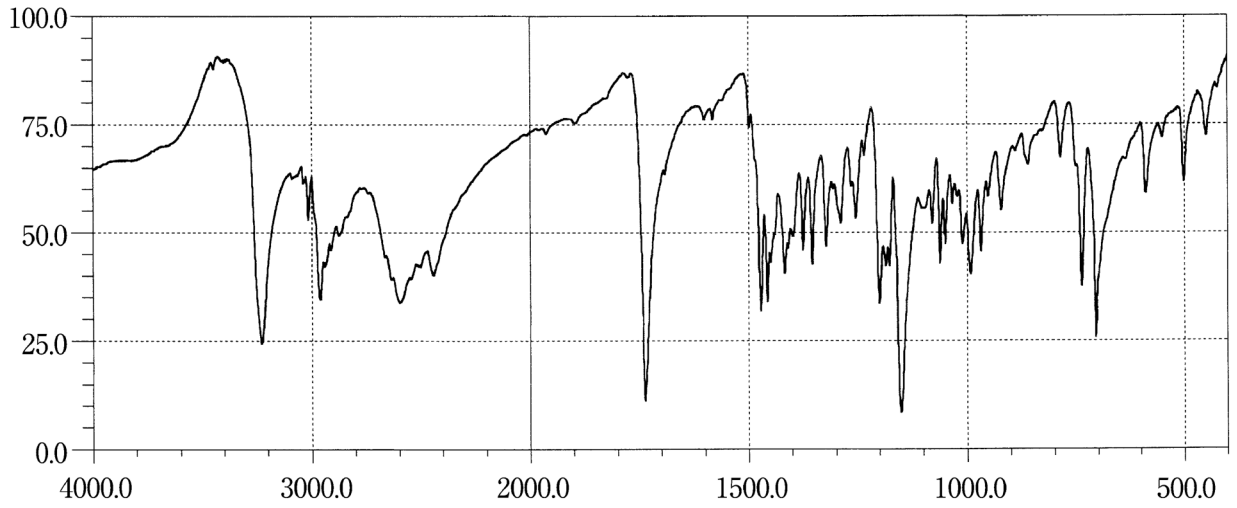
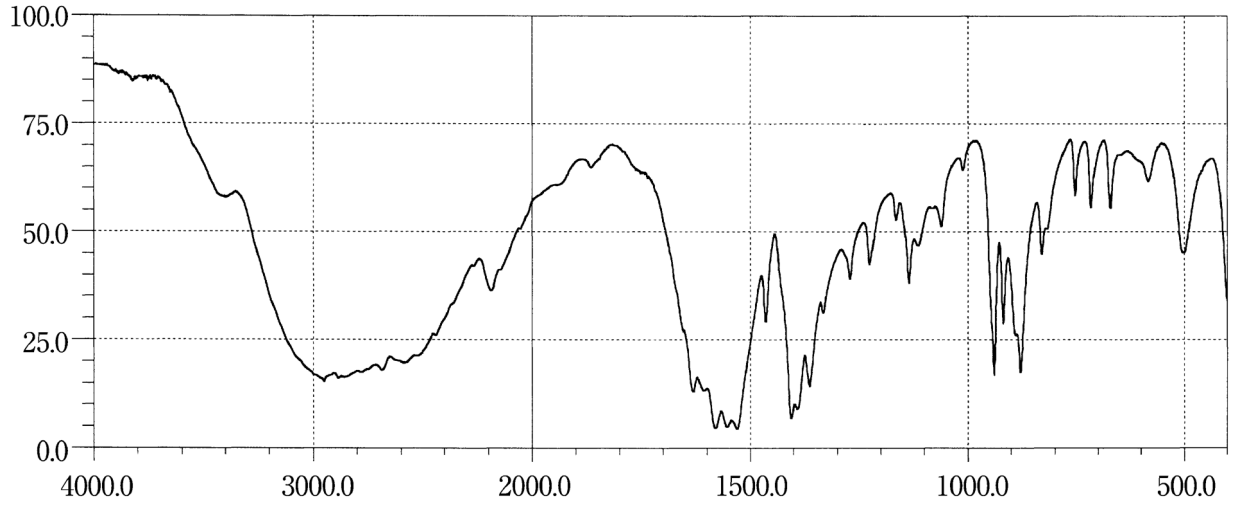


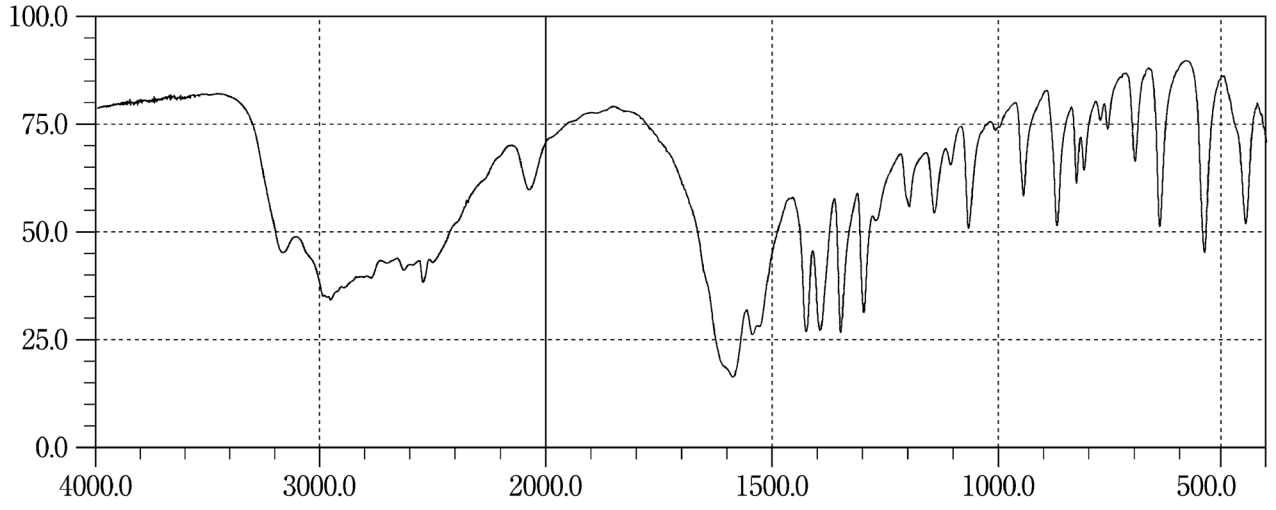
Cyclopentolate Hydrochloride



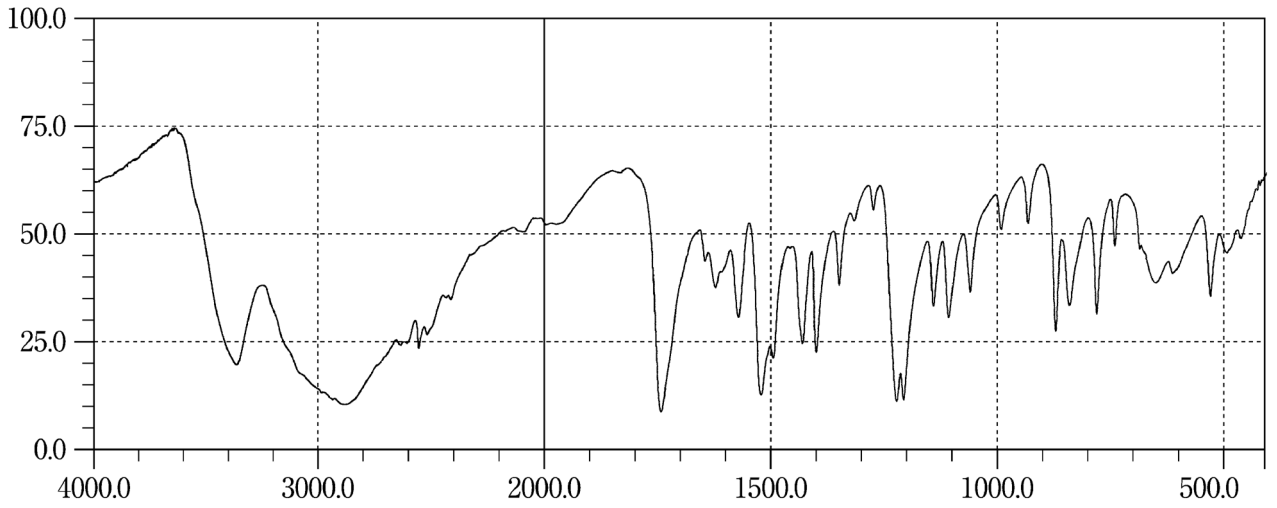
Cycloserine



L-Cysteine



L-Cysteine Hydrochloride Hydrate



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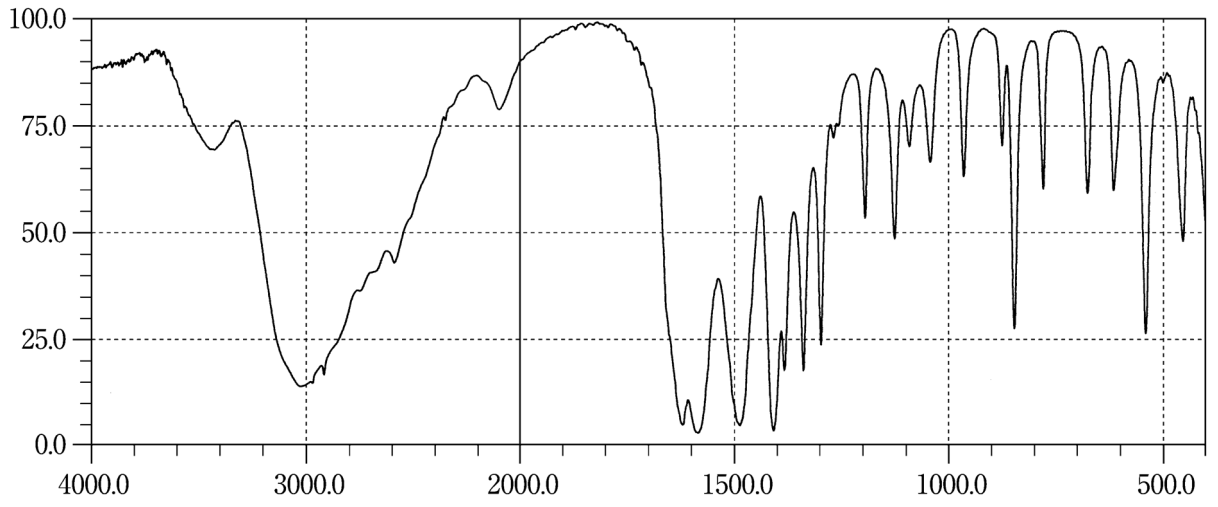
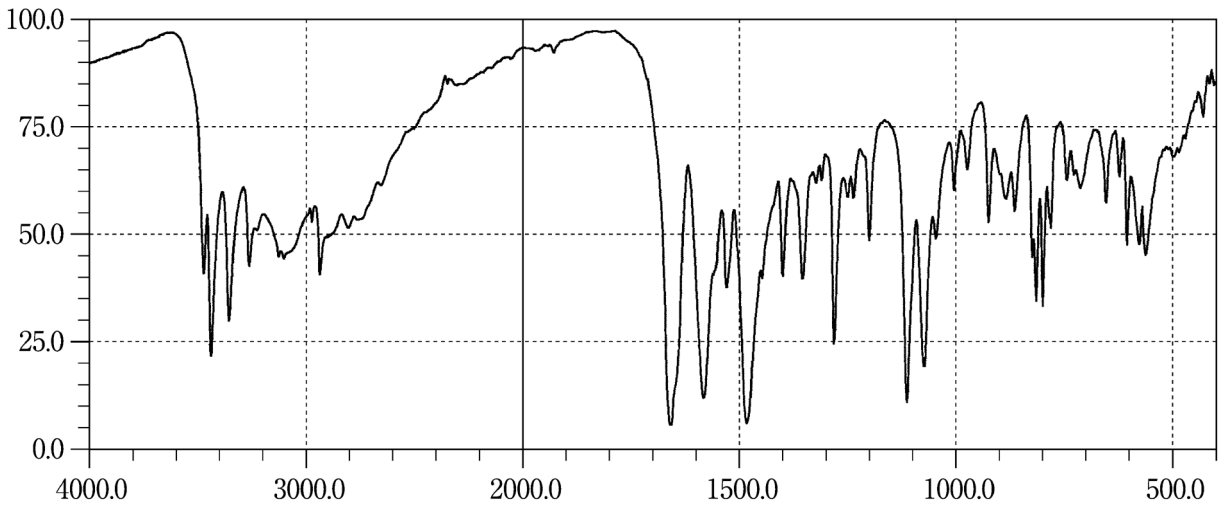
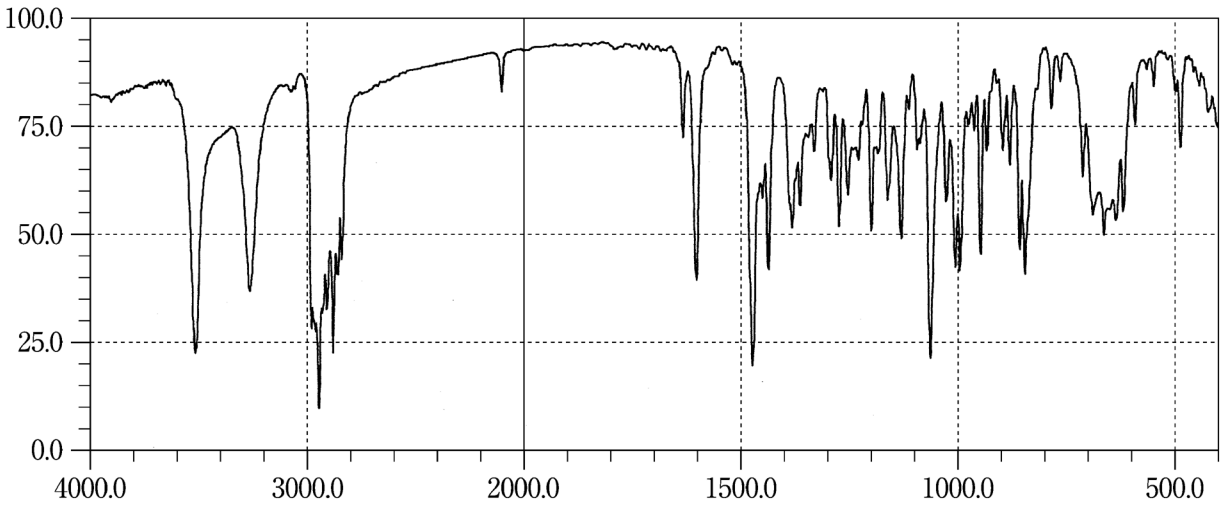


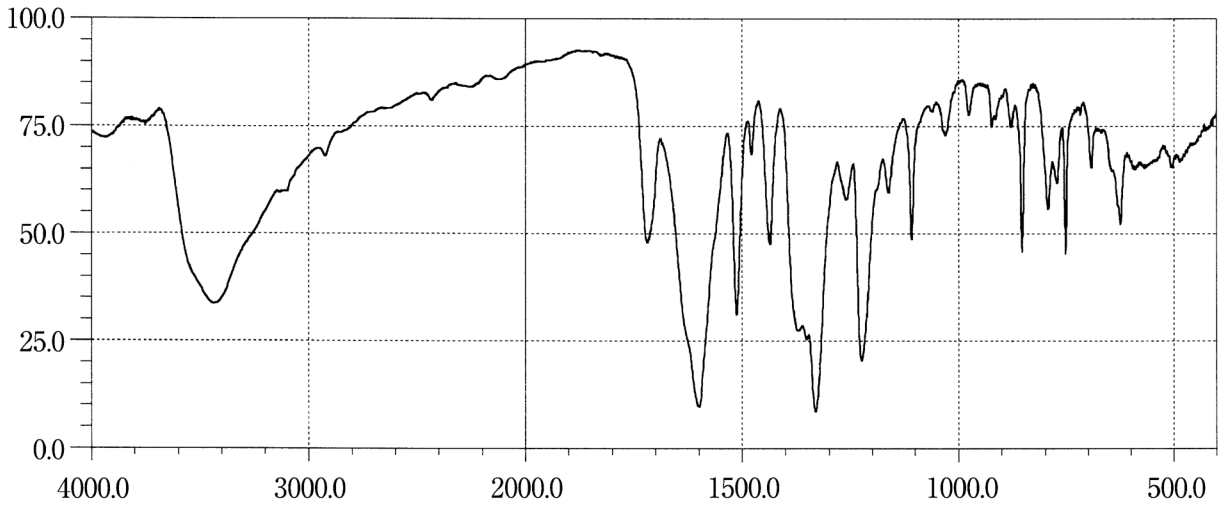
Cytarabine



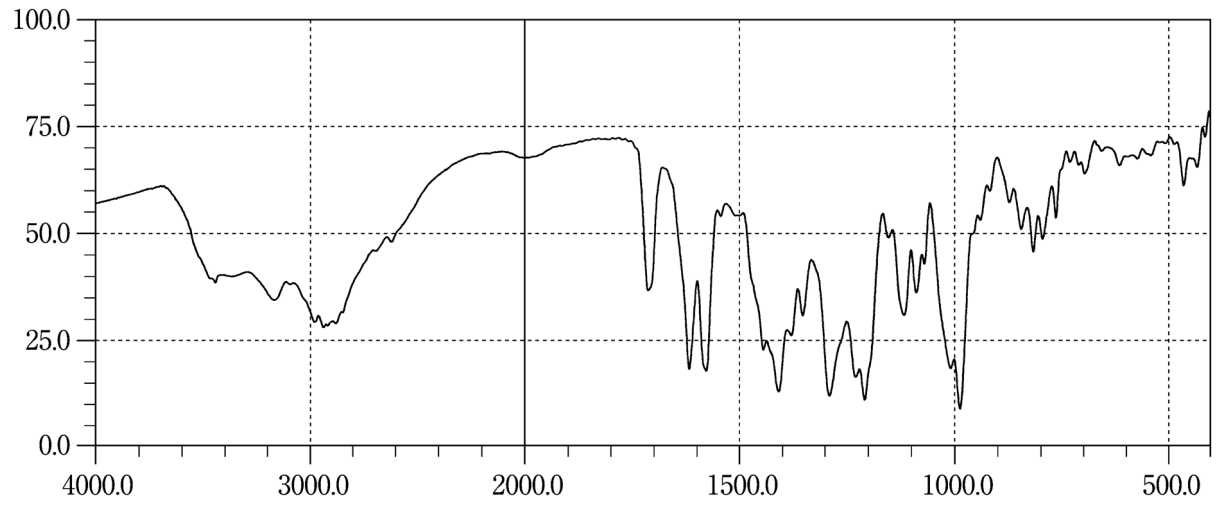
Danazol



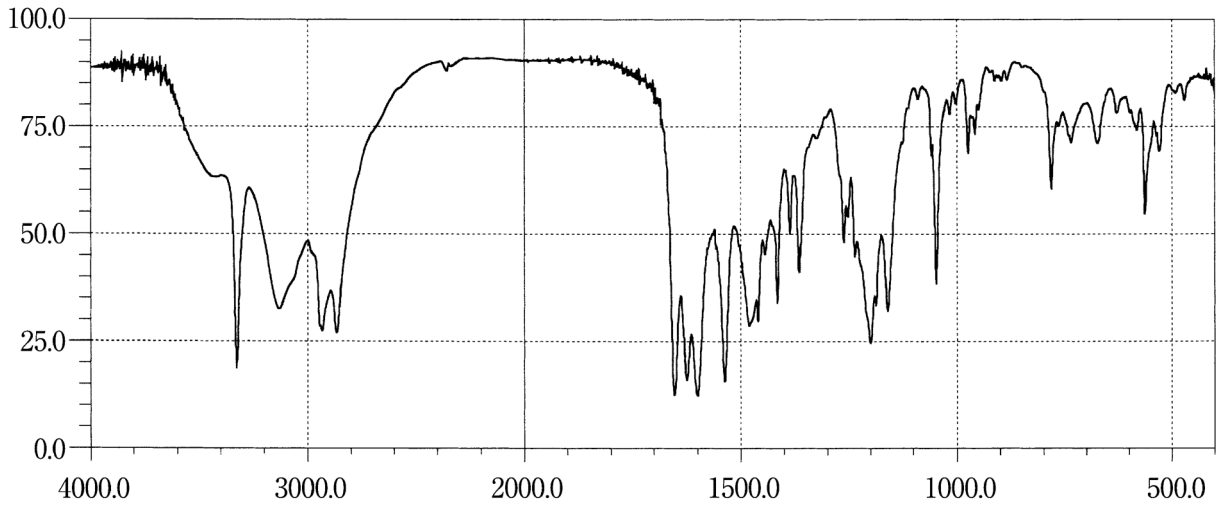
Dantrolene Sodium Hydrate



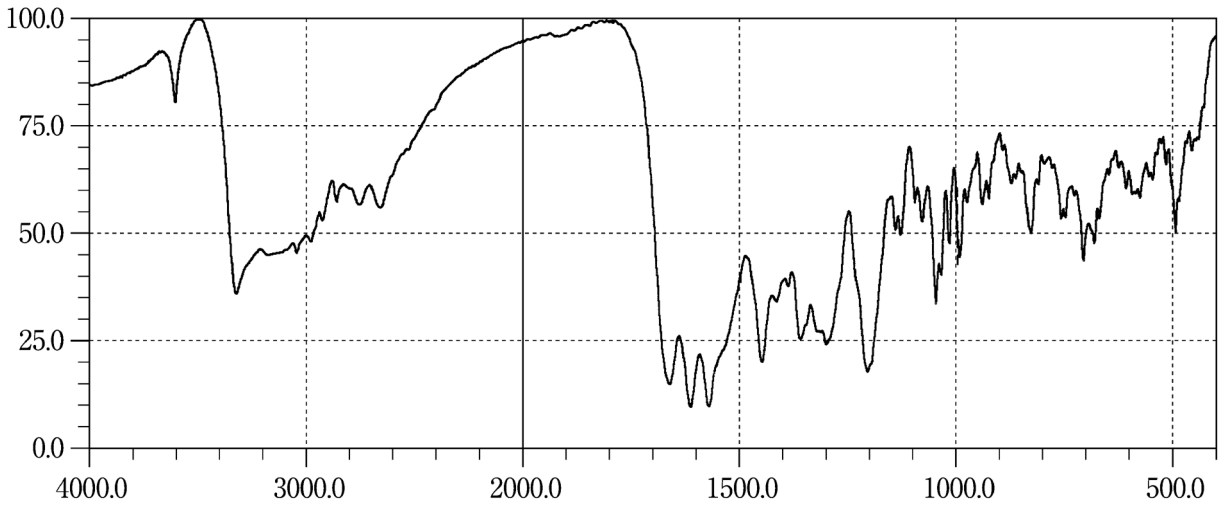
Daunorubicin Hydrochloride



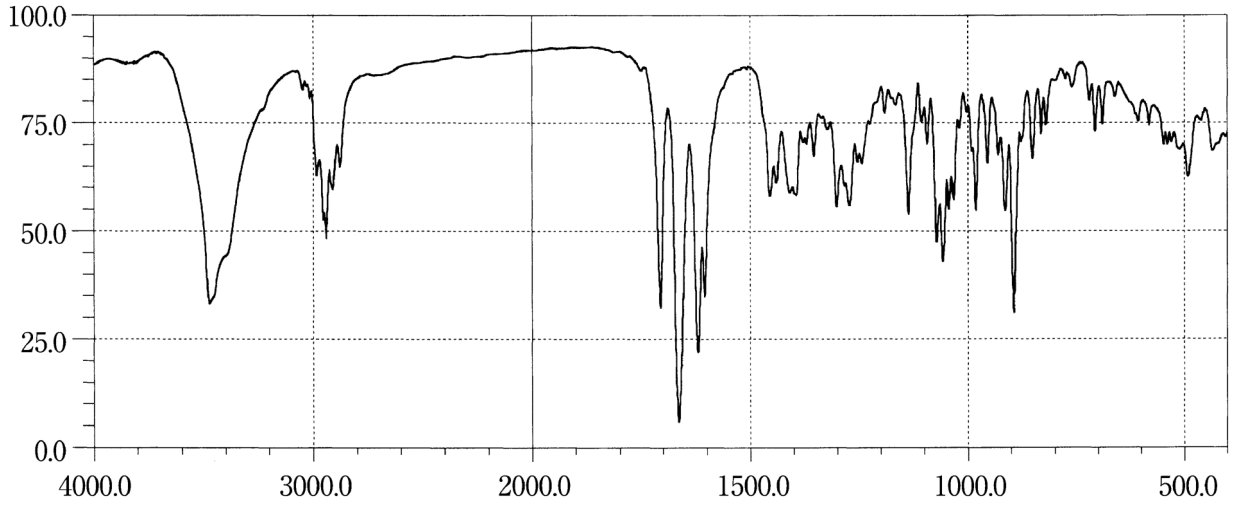
Deferoxamine Mesilate



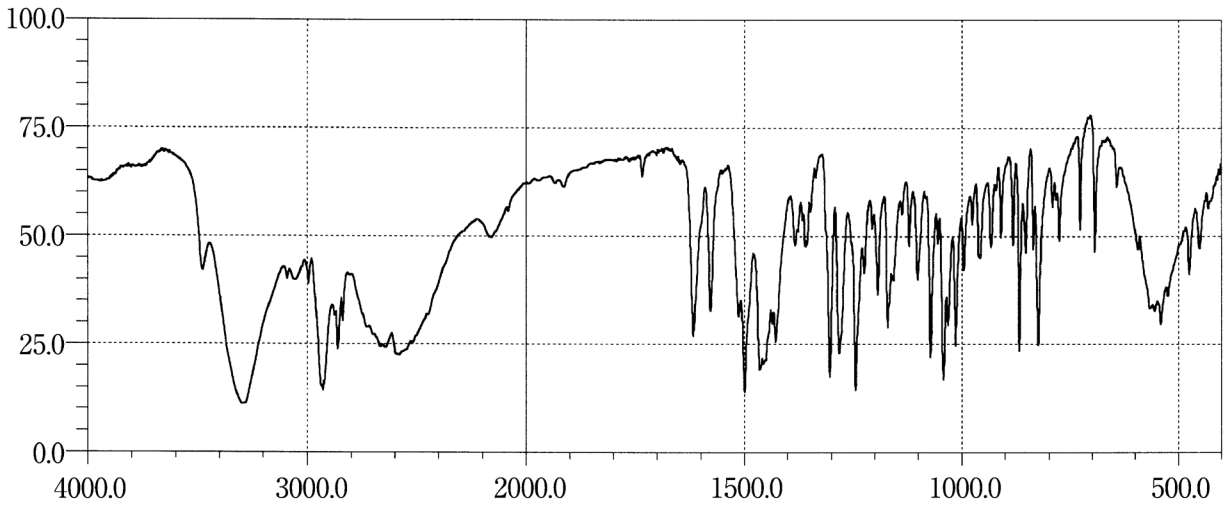
Demethylchlortetracycline Hydrochloride



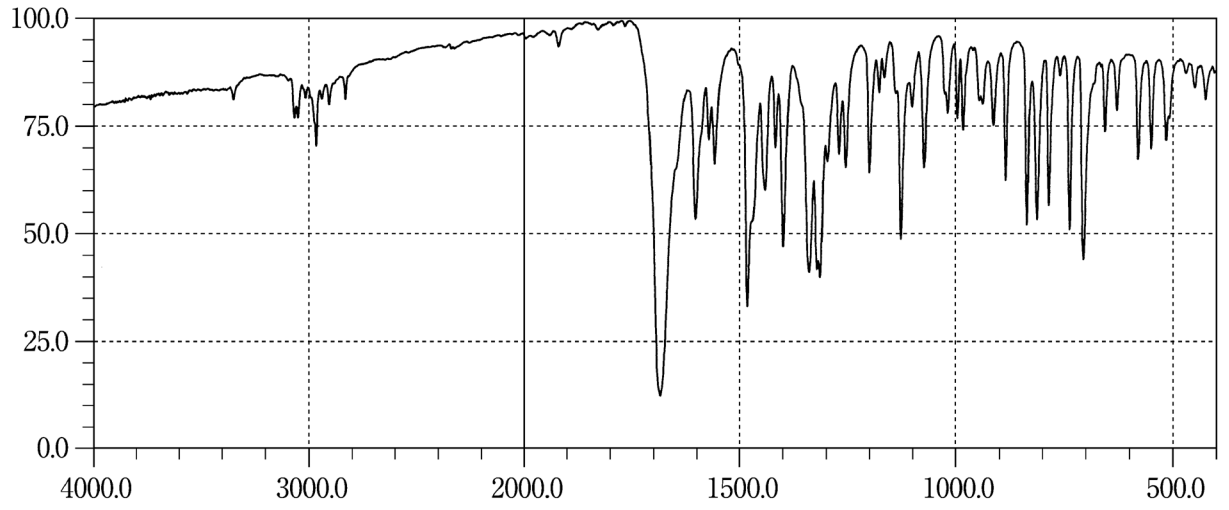
Dexamethasone



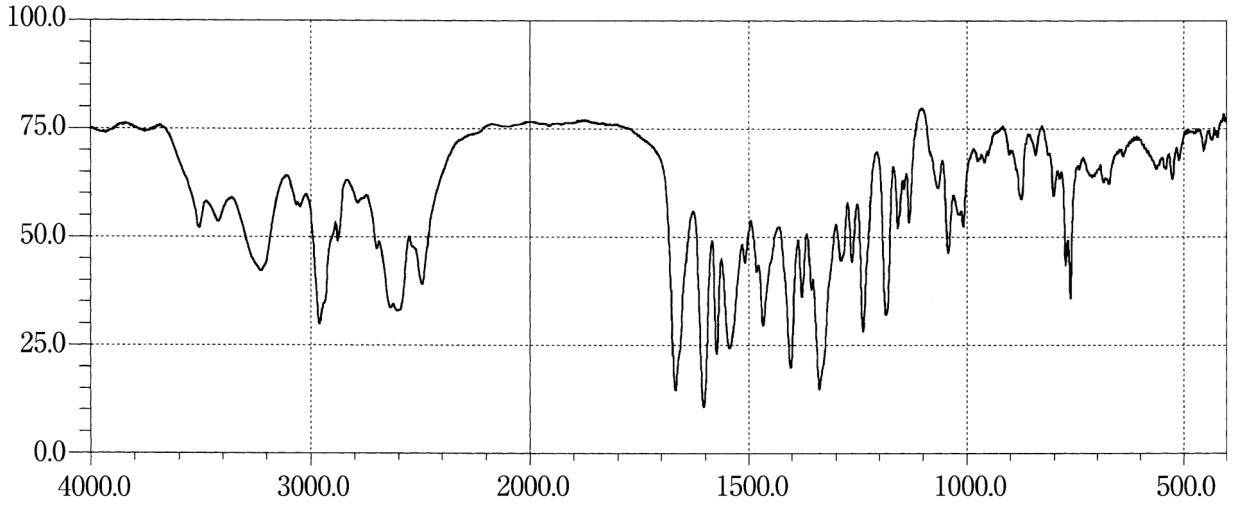
Dextromethorphan Hydrobromide Hydrate



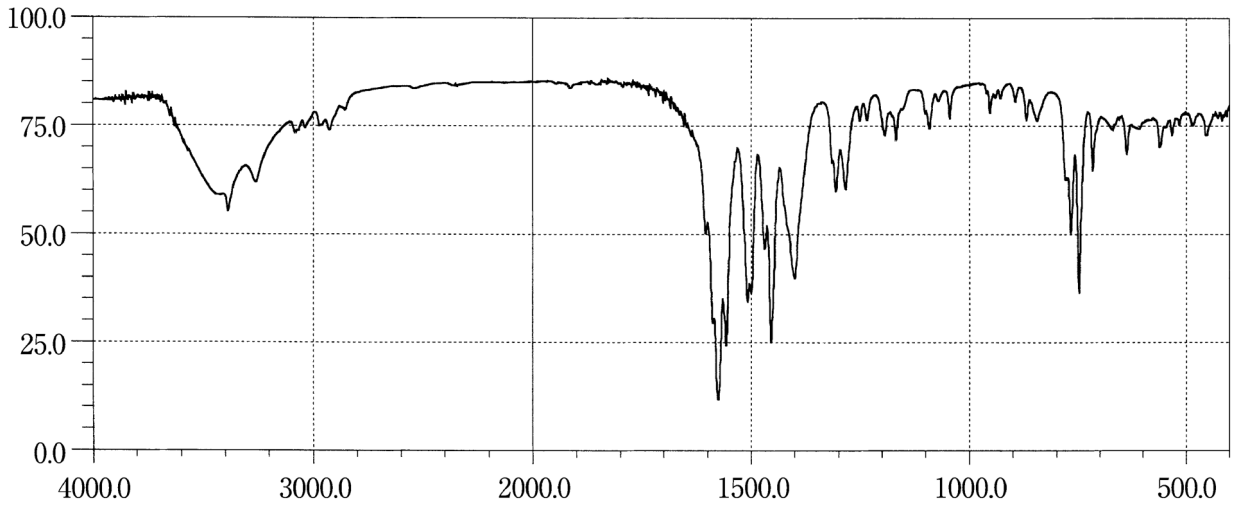
Diazepam



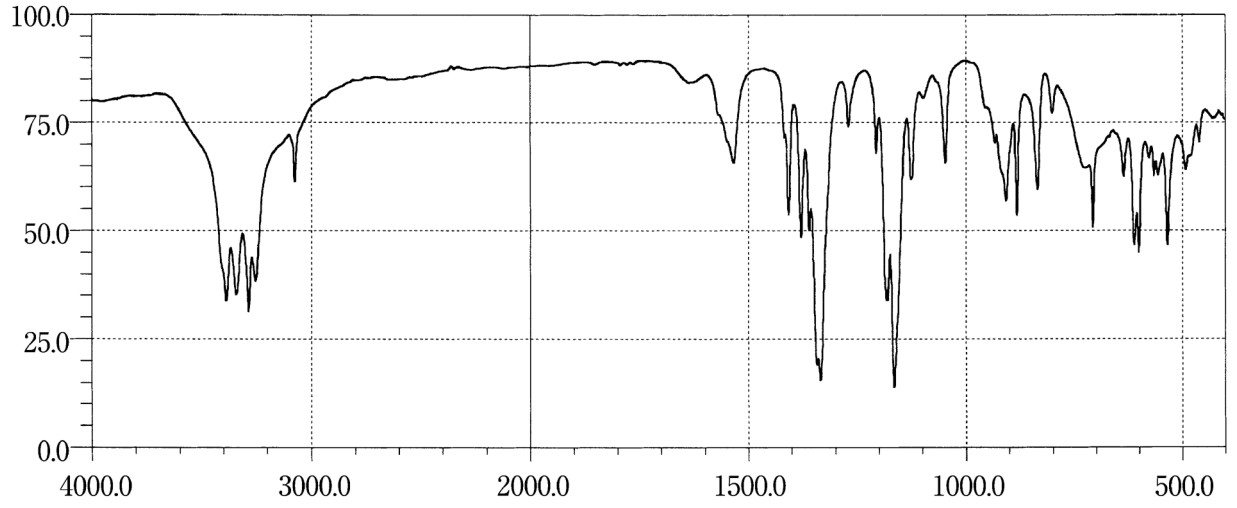
Dibucaine Hydrochloride



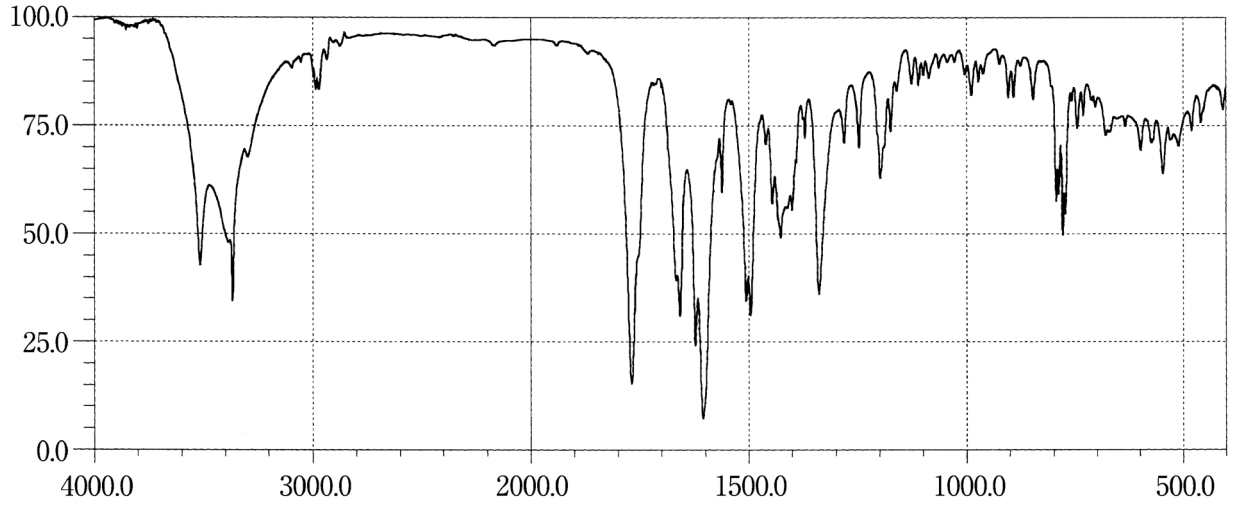
Diclofenac Sodium



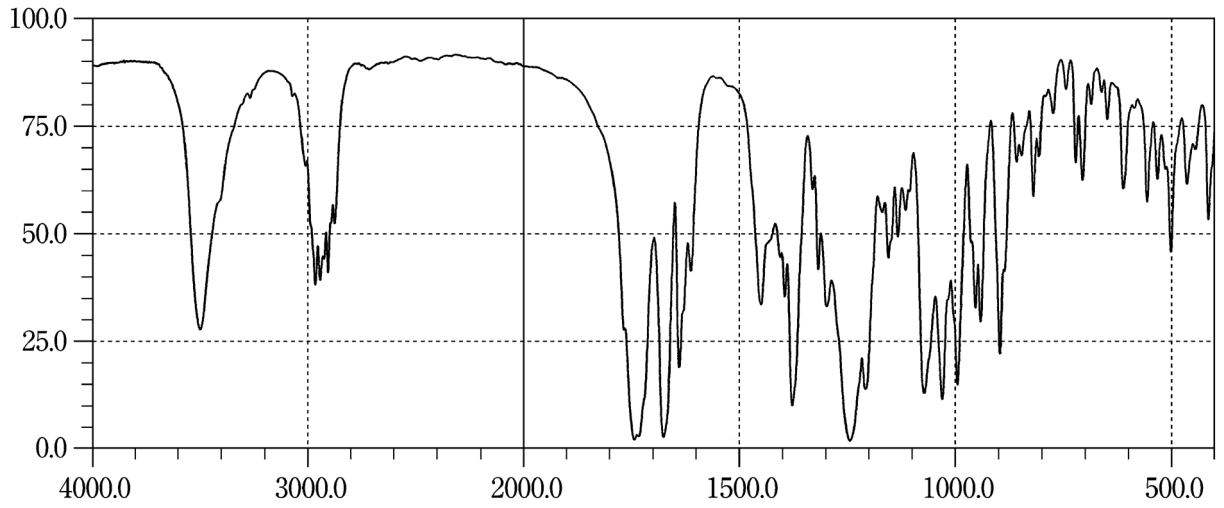
Diclofenamide



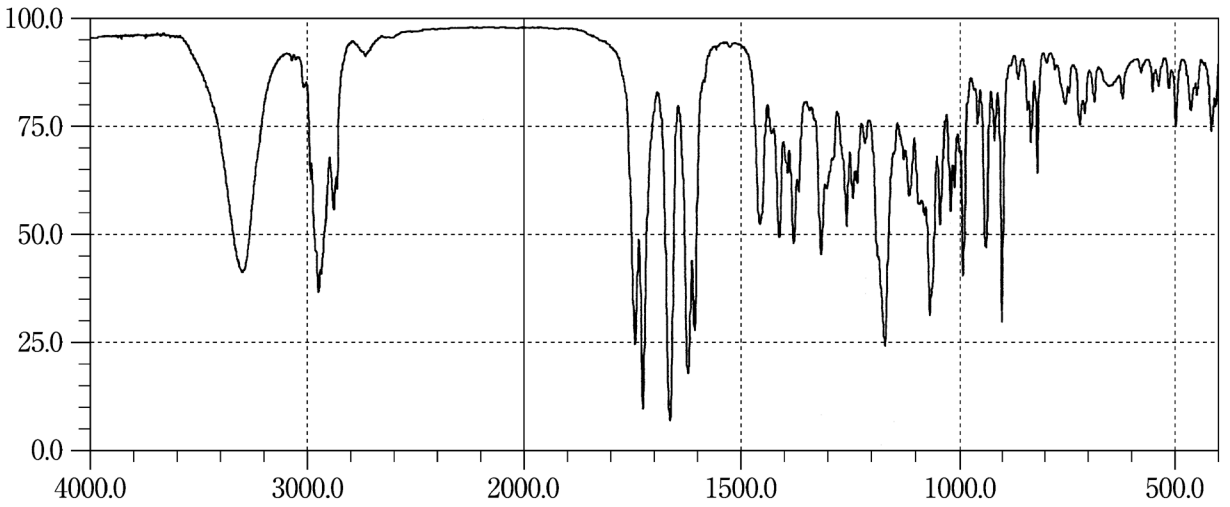
Dicloxacillin Sodium Hydrate



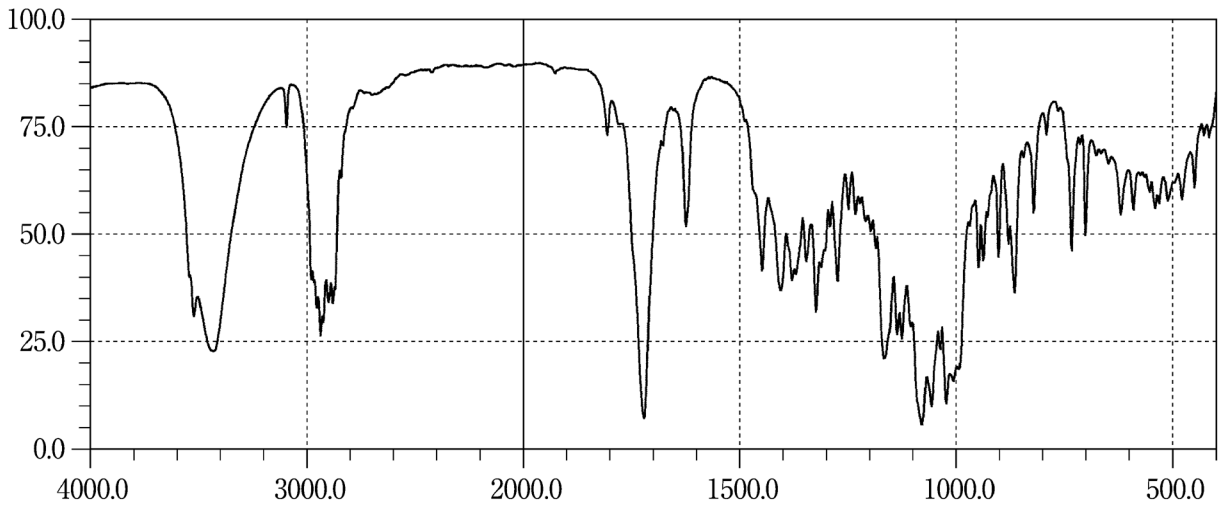
Diflorasone Diacetate



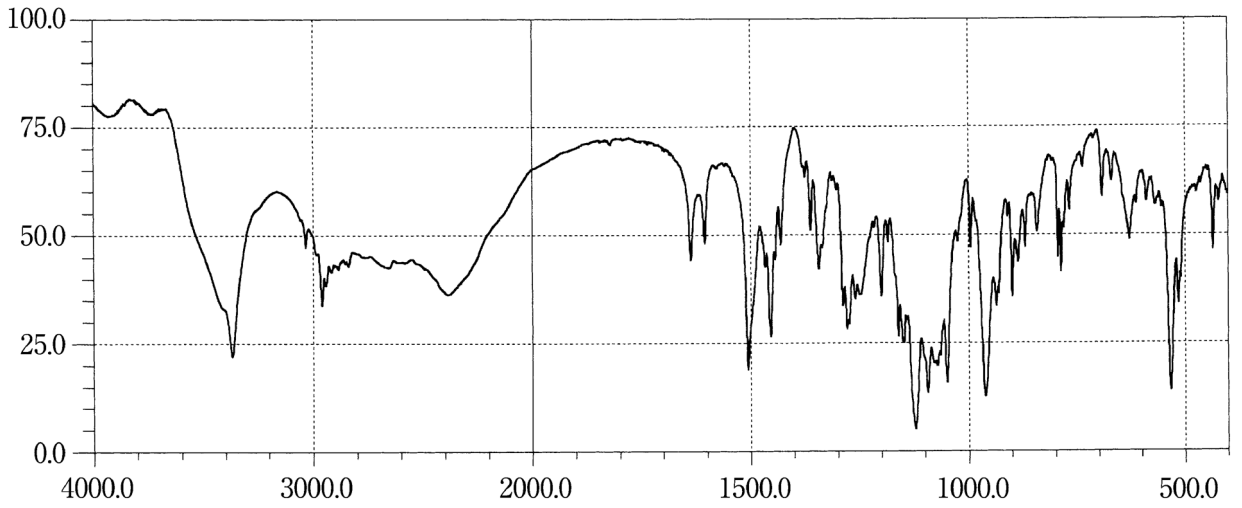
Diflucortolone Valerate



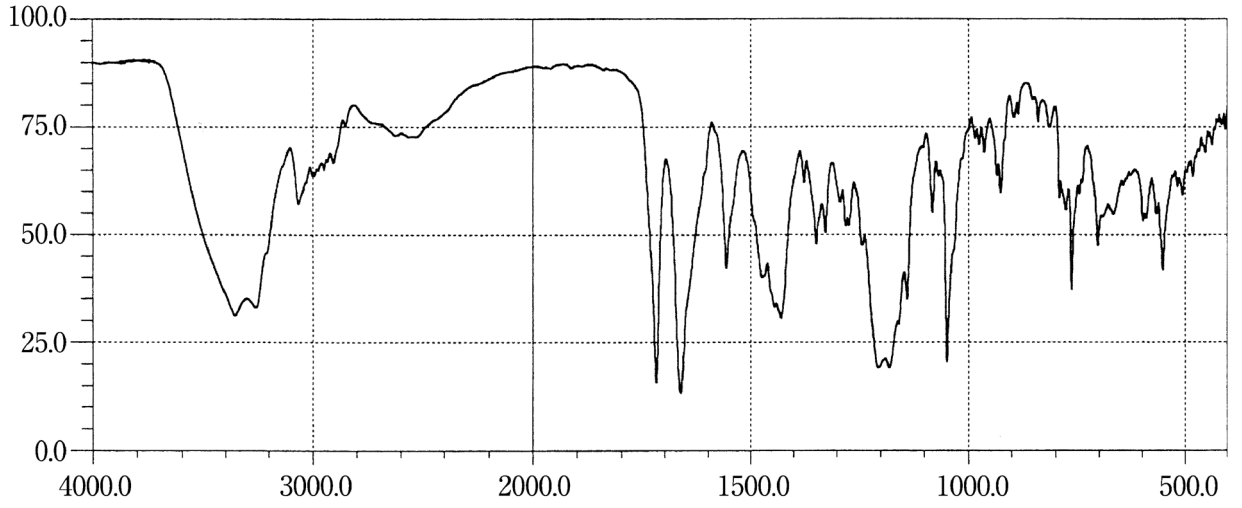
Digoxin



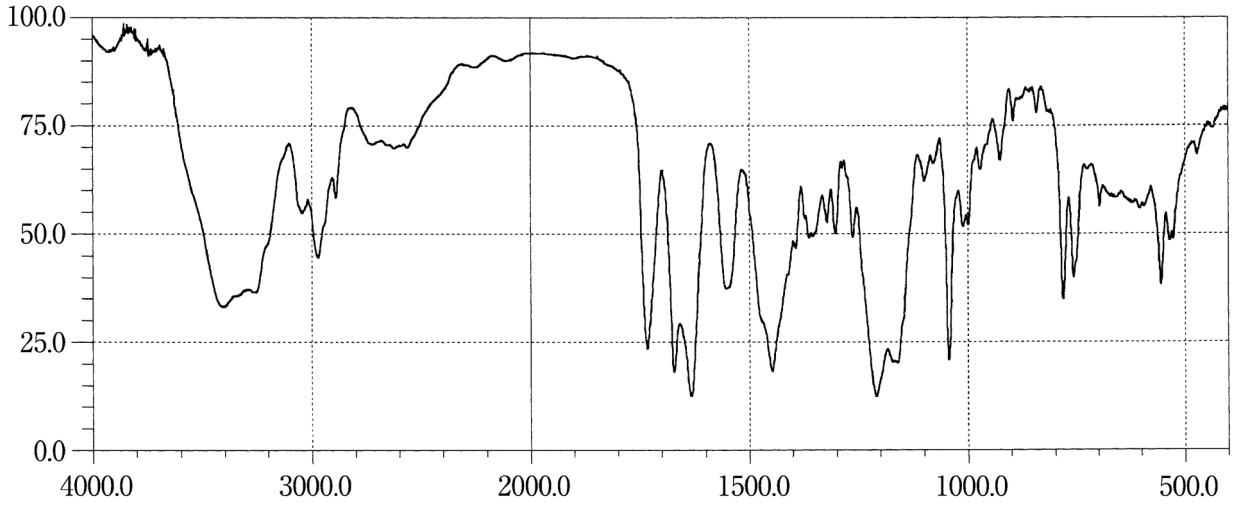
Dihydrocodeine Phosphate



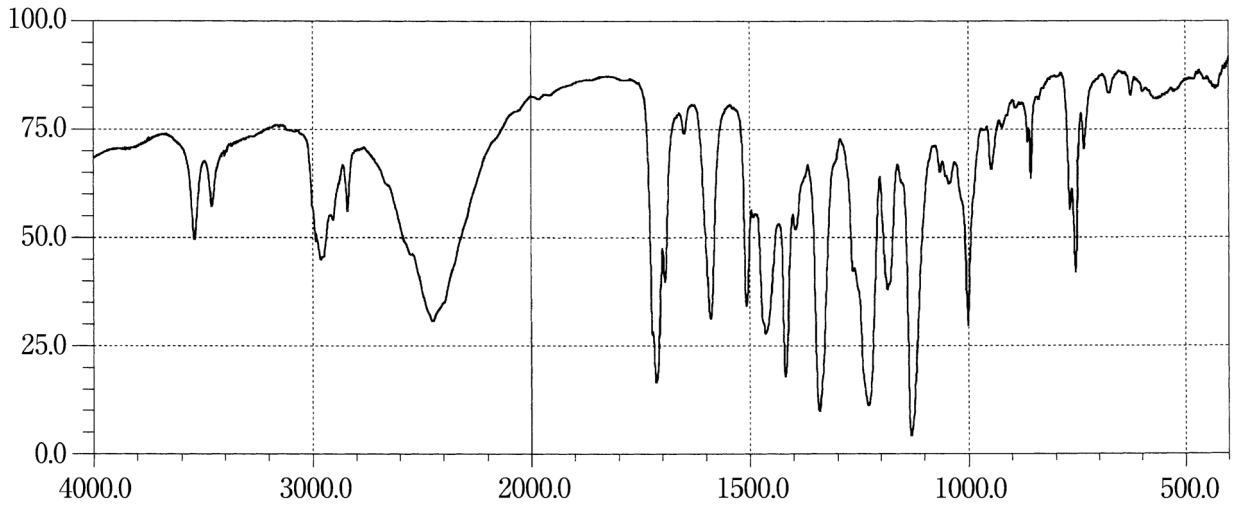
Dihydroergotamine Mesilate



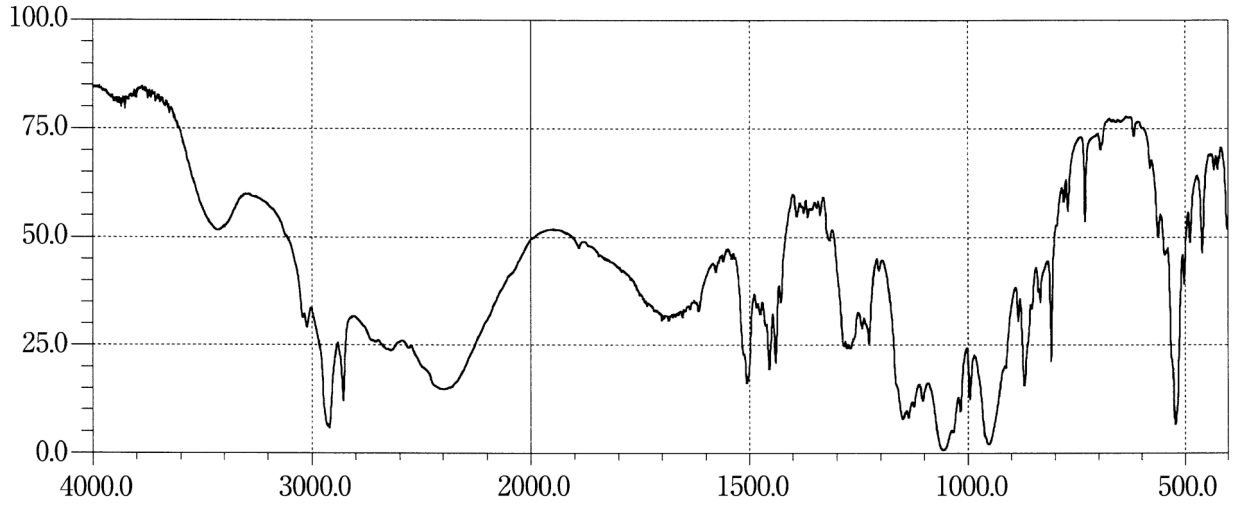
Dihydroergotoxine Mesilate



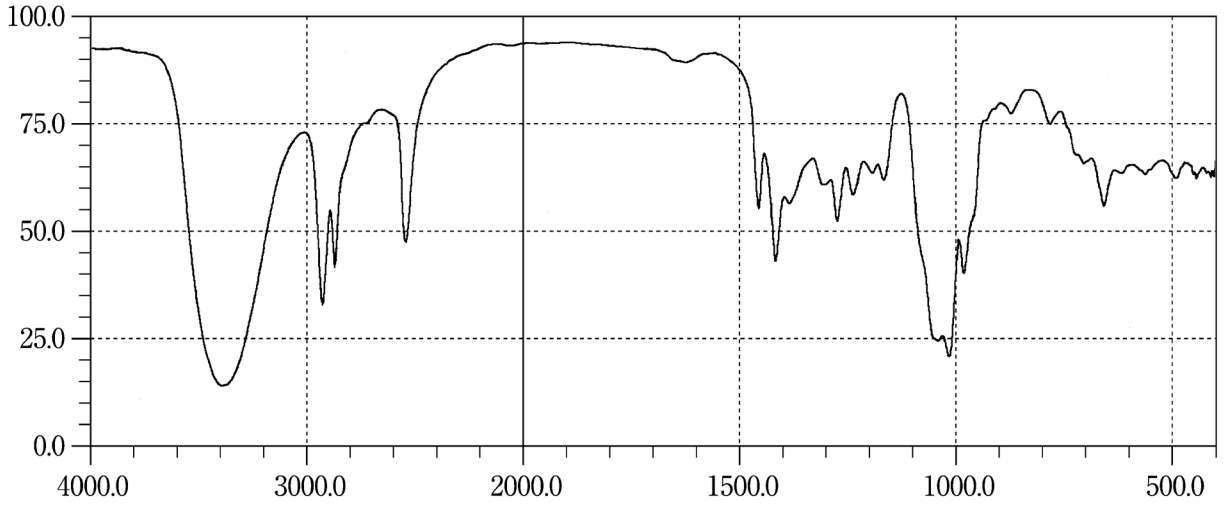
Dilazep Hydrochloride Hydrate



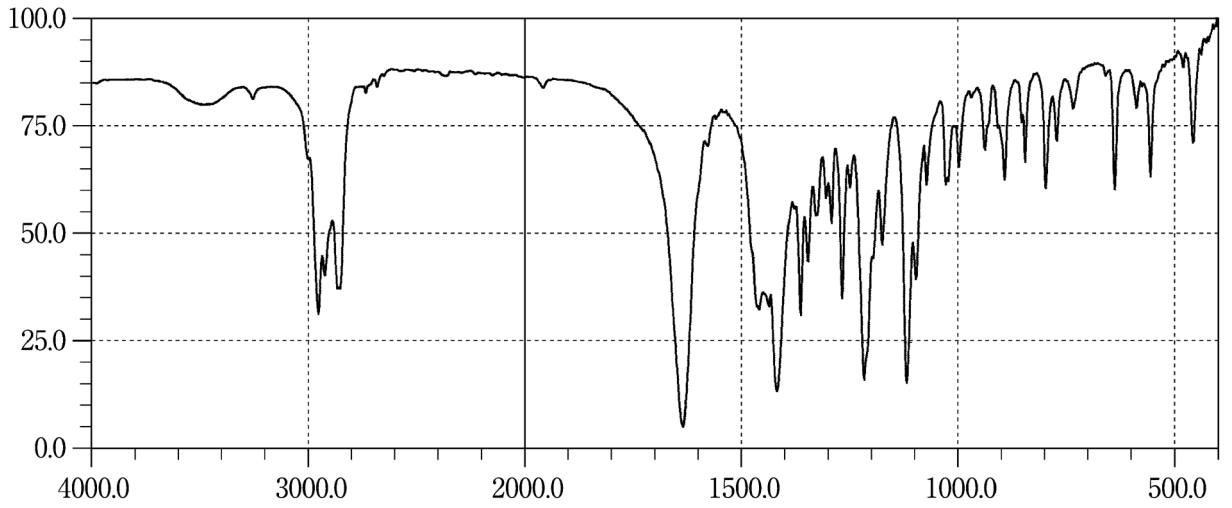
Dimemorfan Phosphate



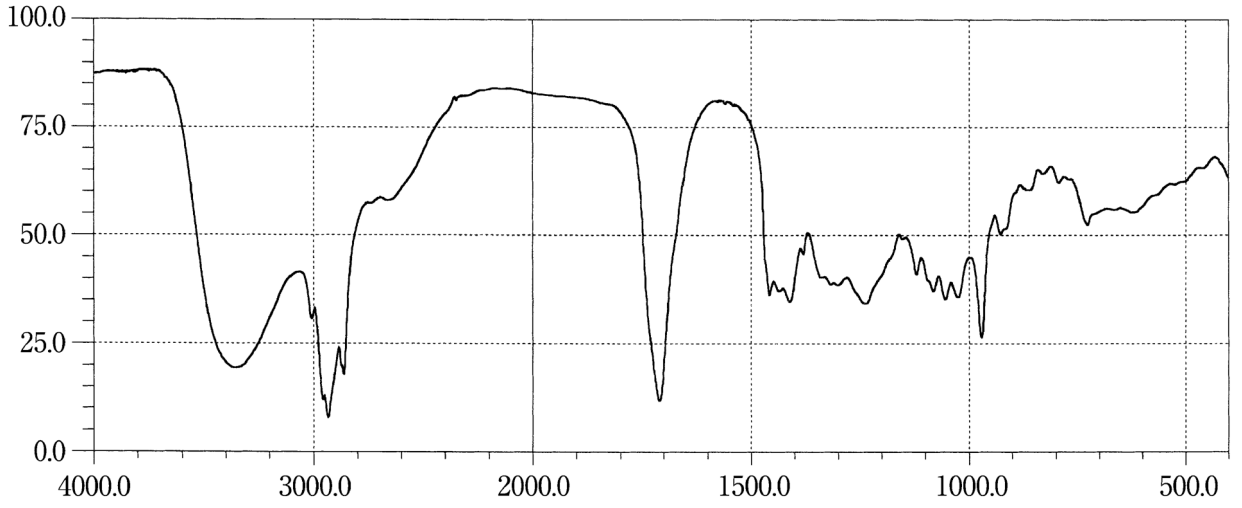
Dimercaprol



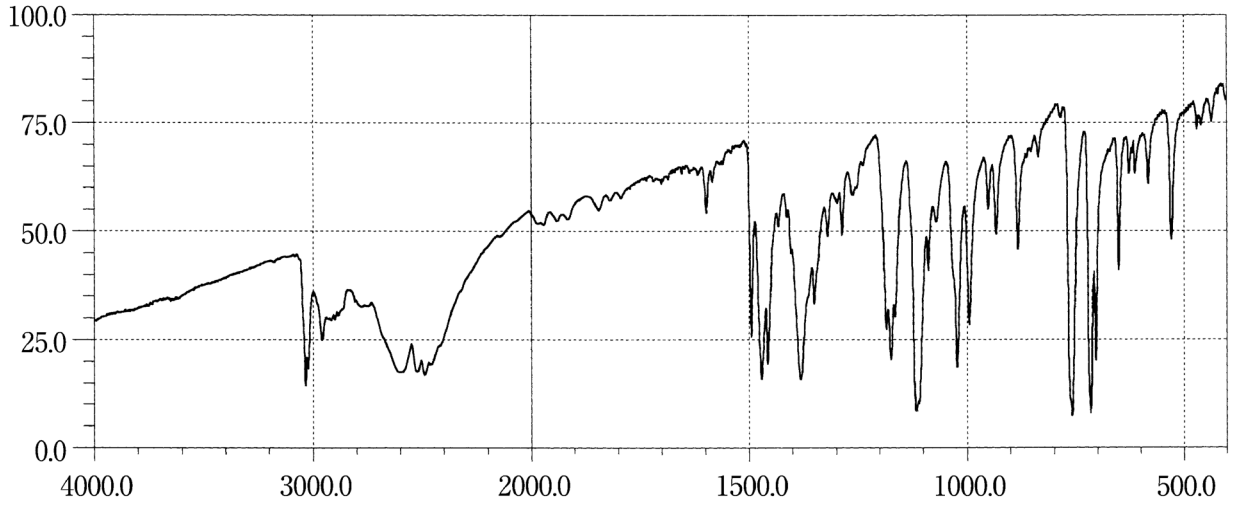
Dimorpholamine



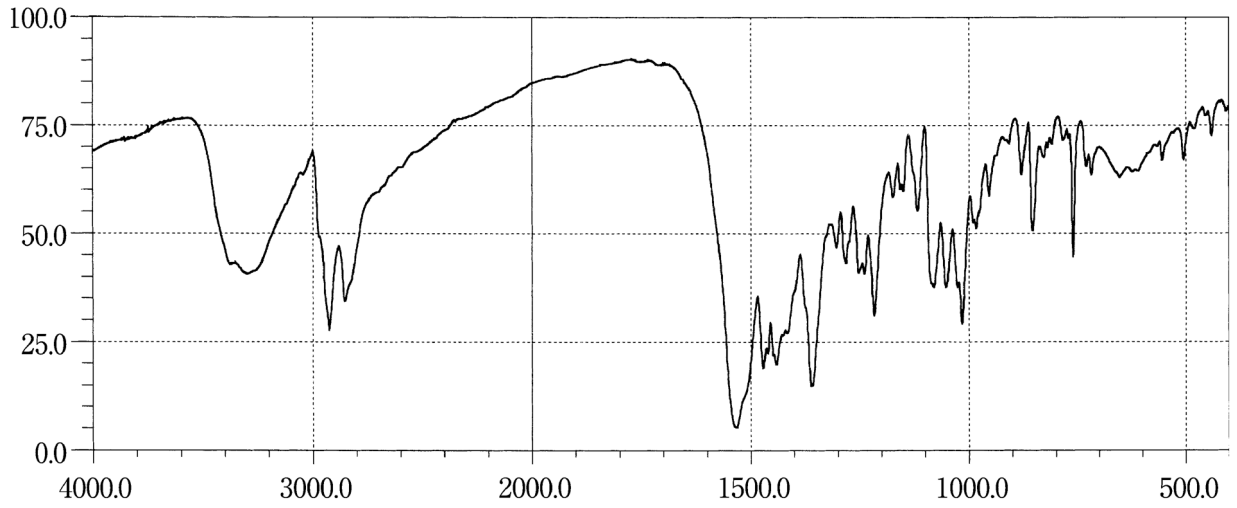
Dinoprost



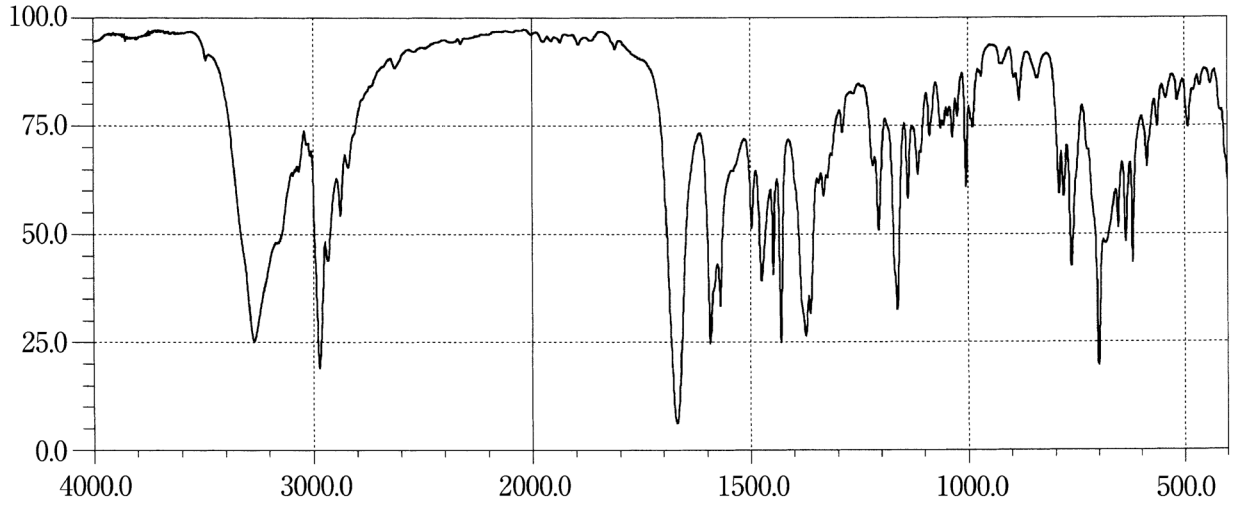
Diphenhydramine Hydrochloride



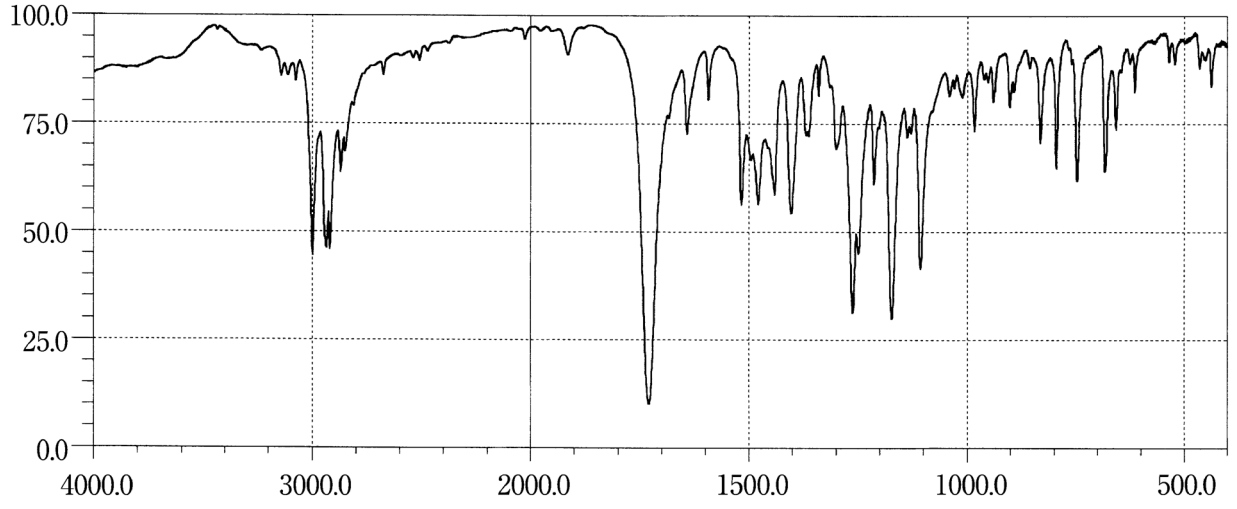
Dipyridamole



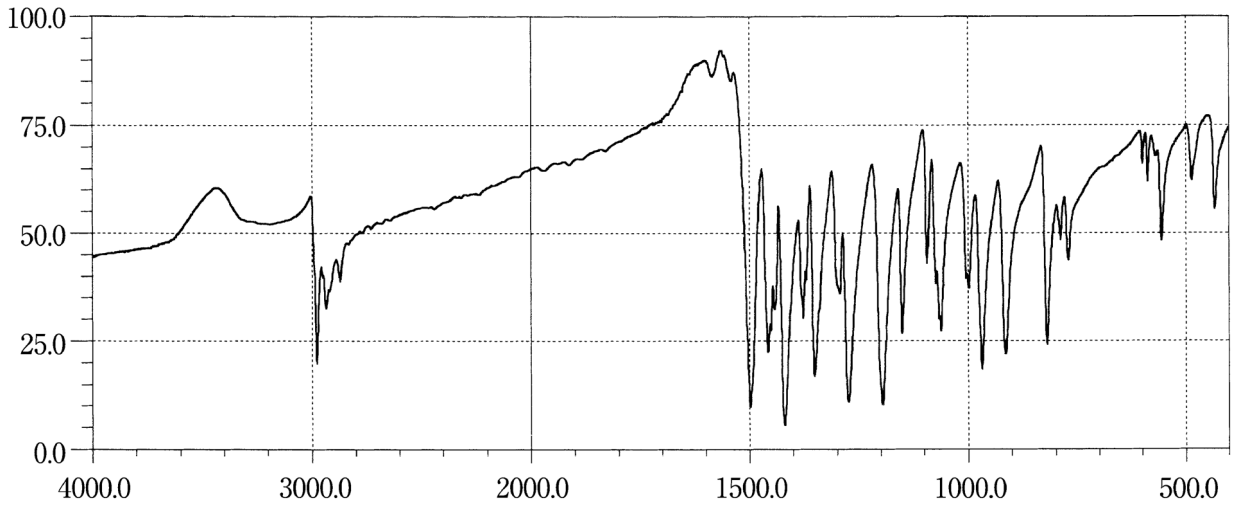
Disopyramide



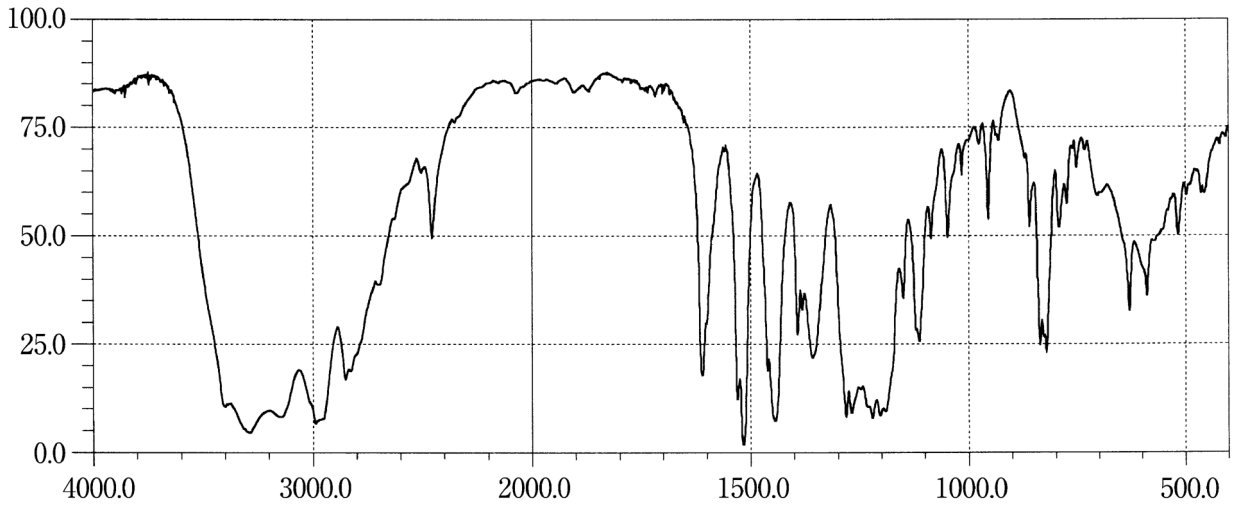
Distigmine Bromide



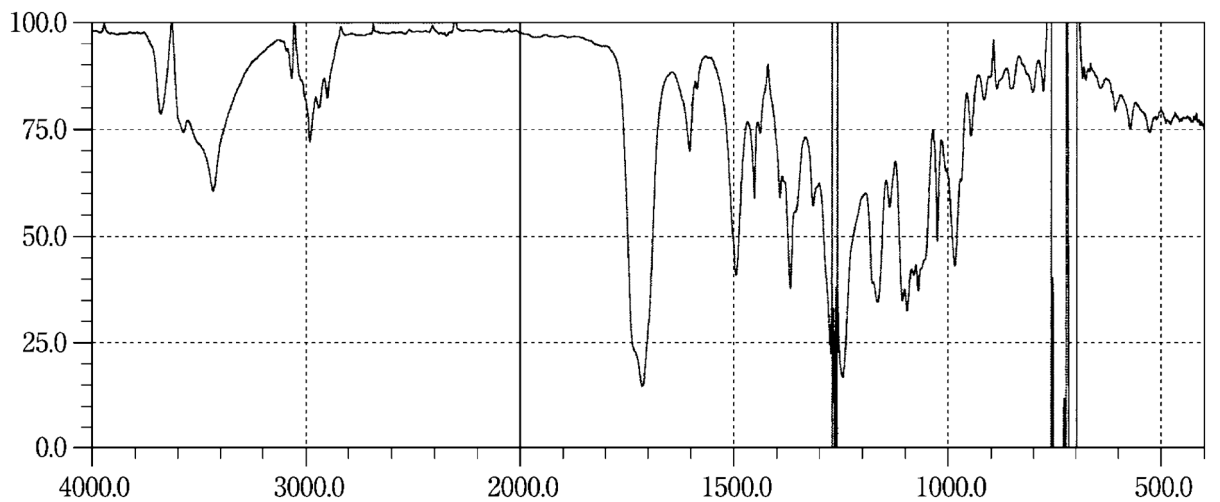
Disulfiram



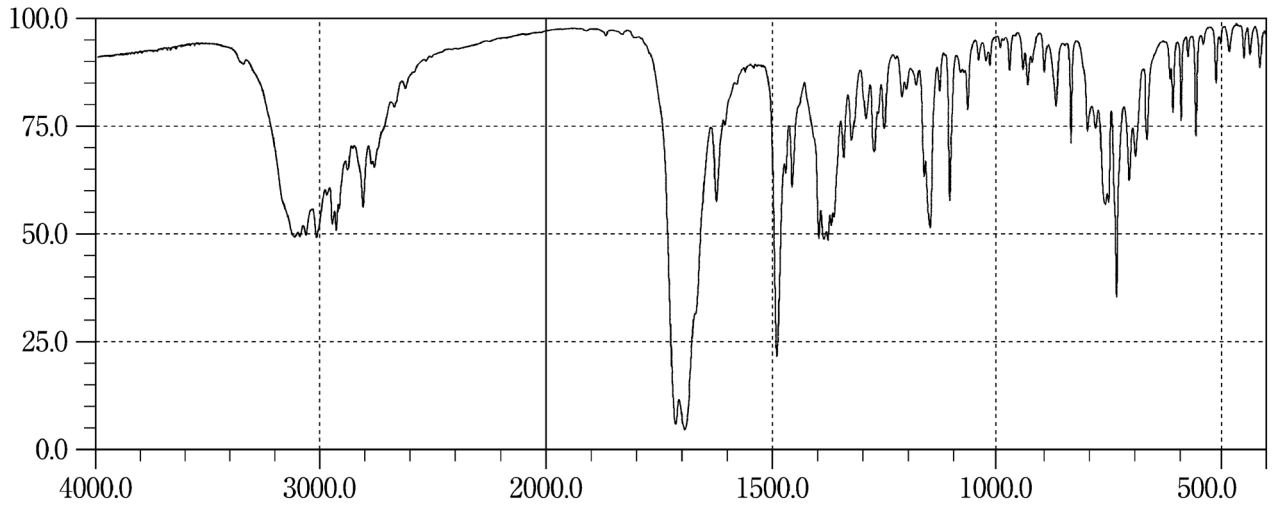
Dobutamine Hydrochloride



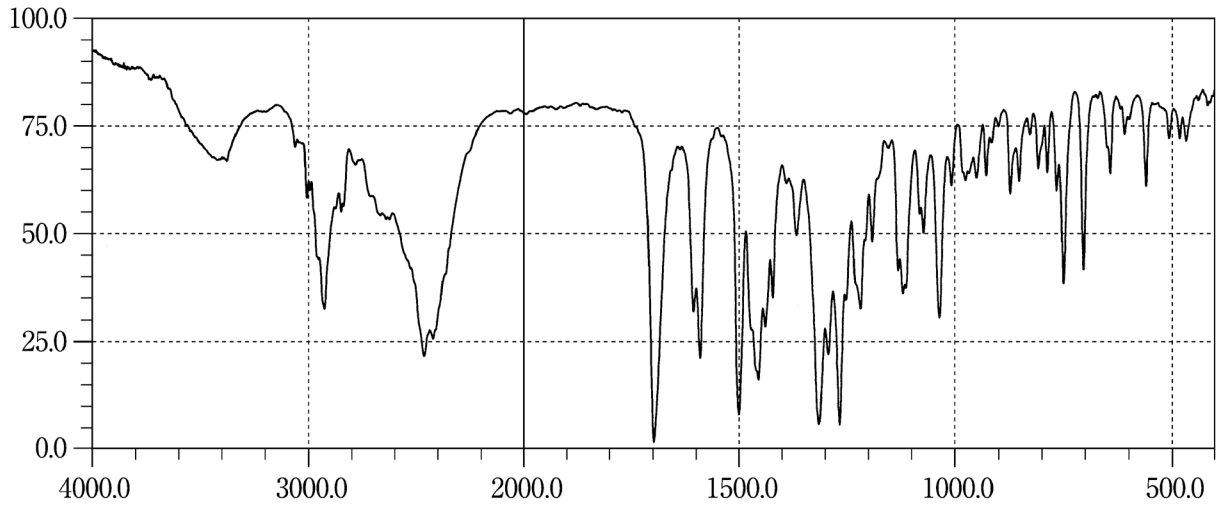
Docetaxel Hydrate



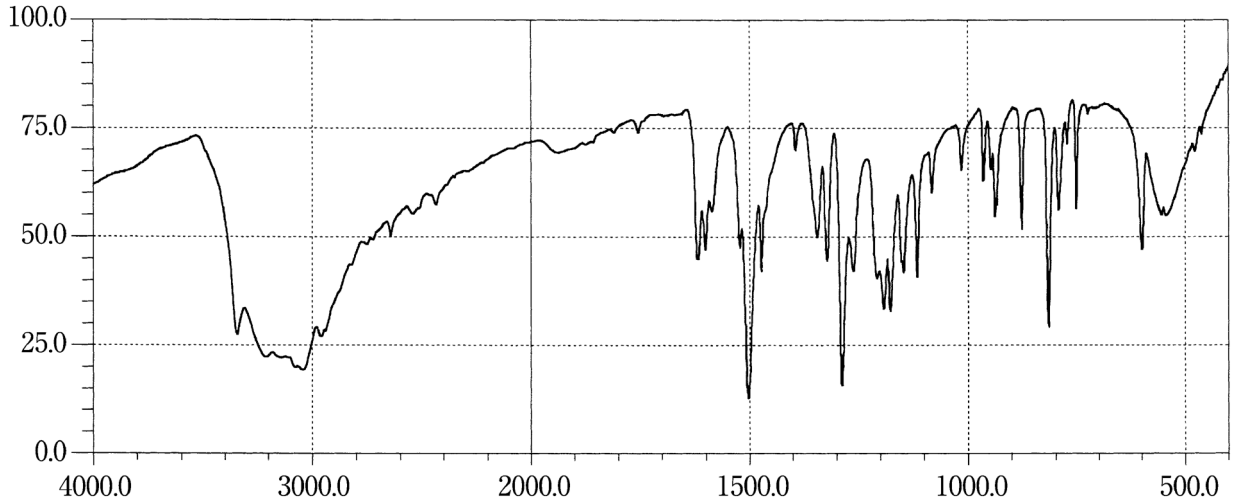
Domperidone



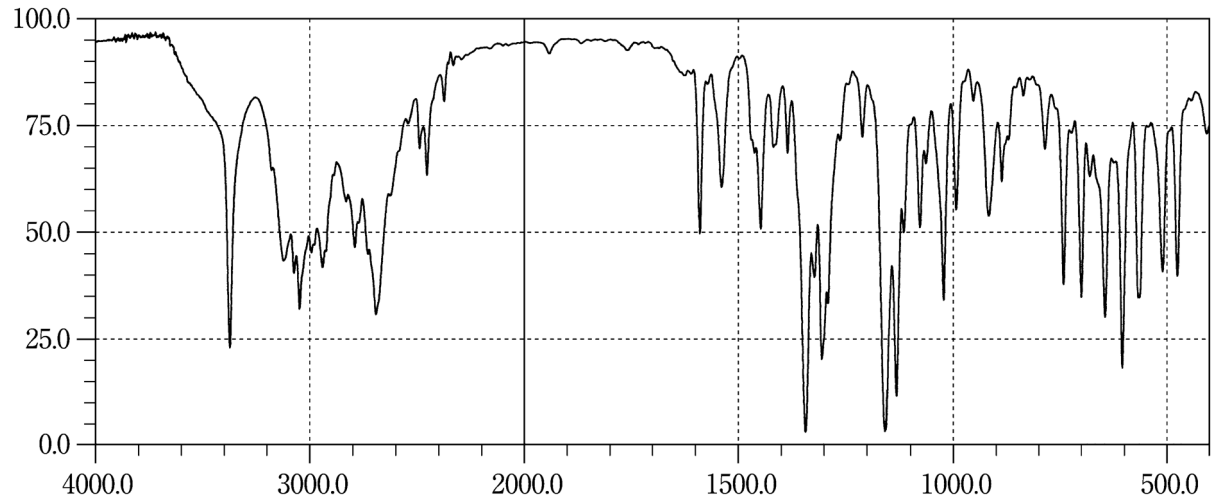
Donepezil Hydrochloride



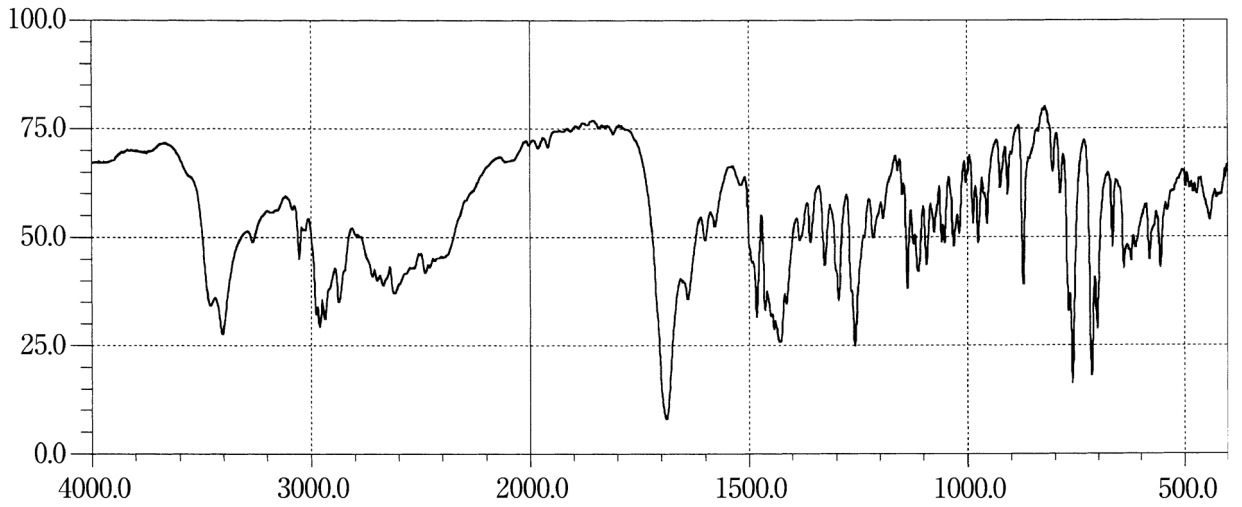
Dopamine Hydrochloride



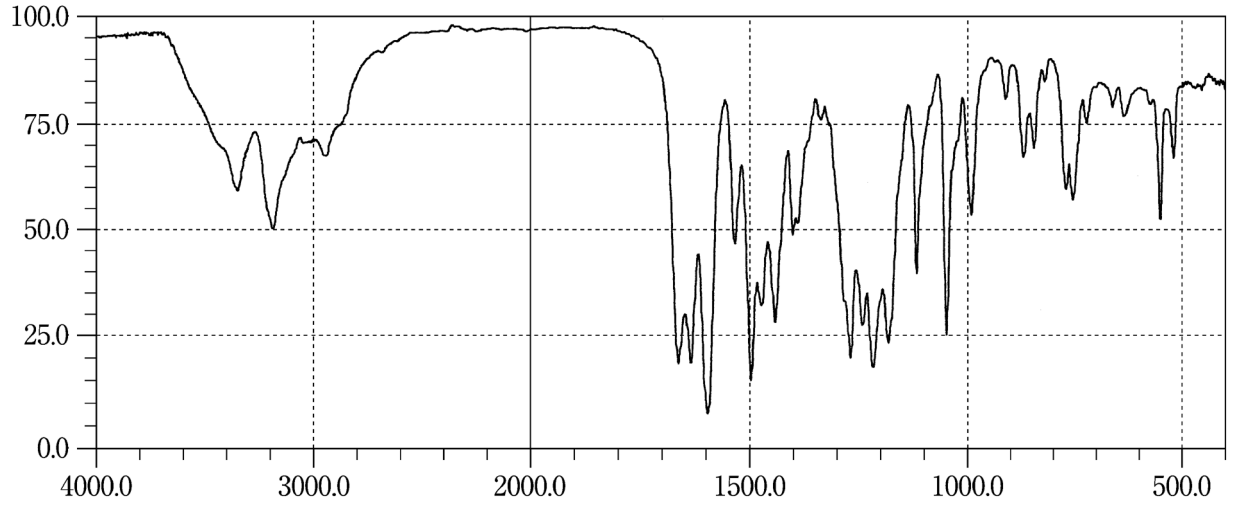
Dorzolamide Hydrochloride



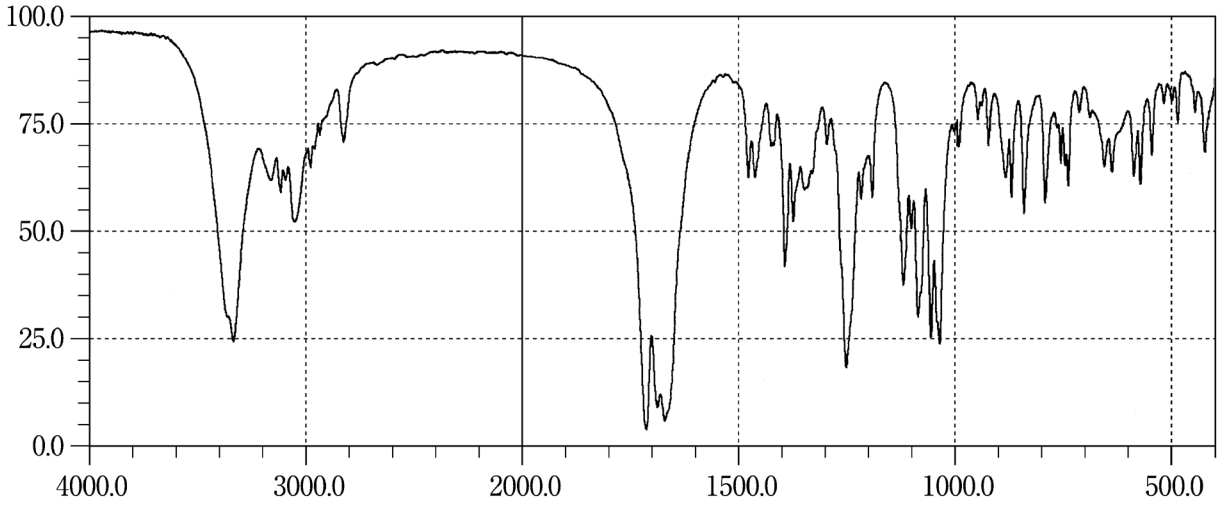
Doxapram Hydrochloride Hydrate



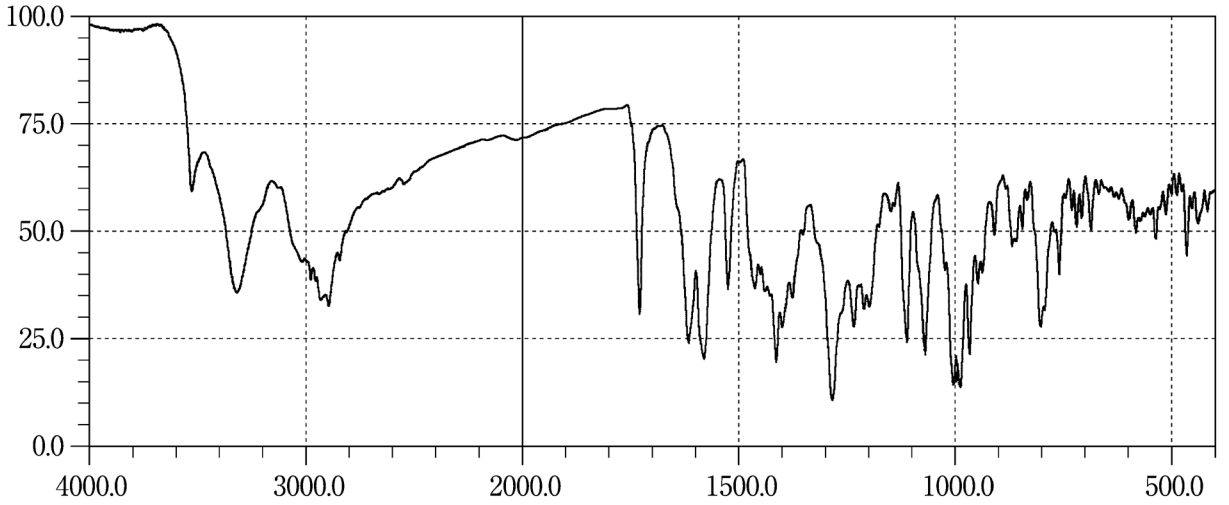
Doxazosin Mesilate



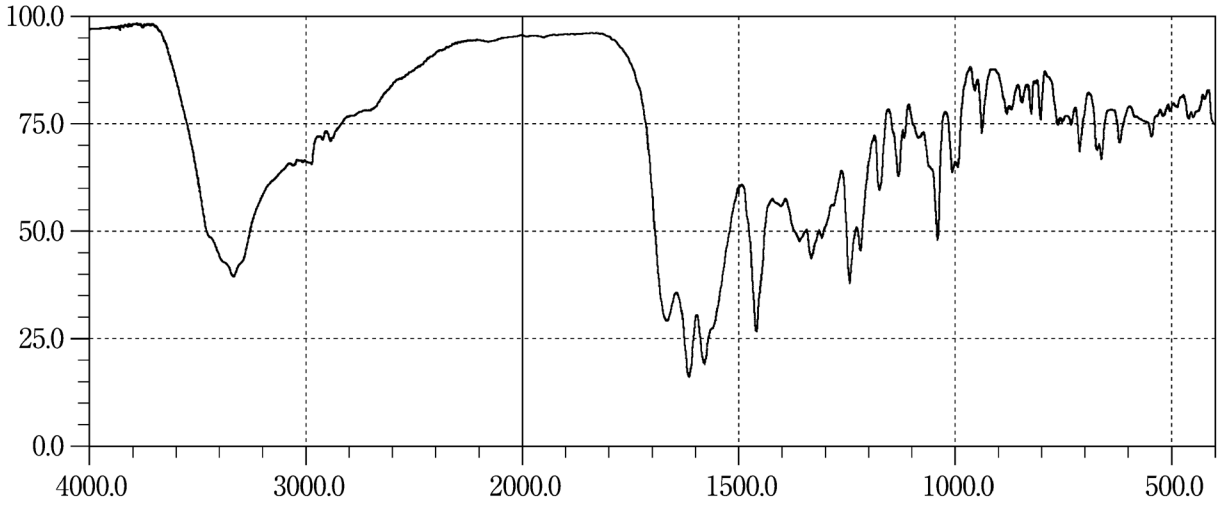
Doxifluridine



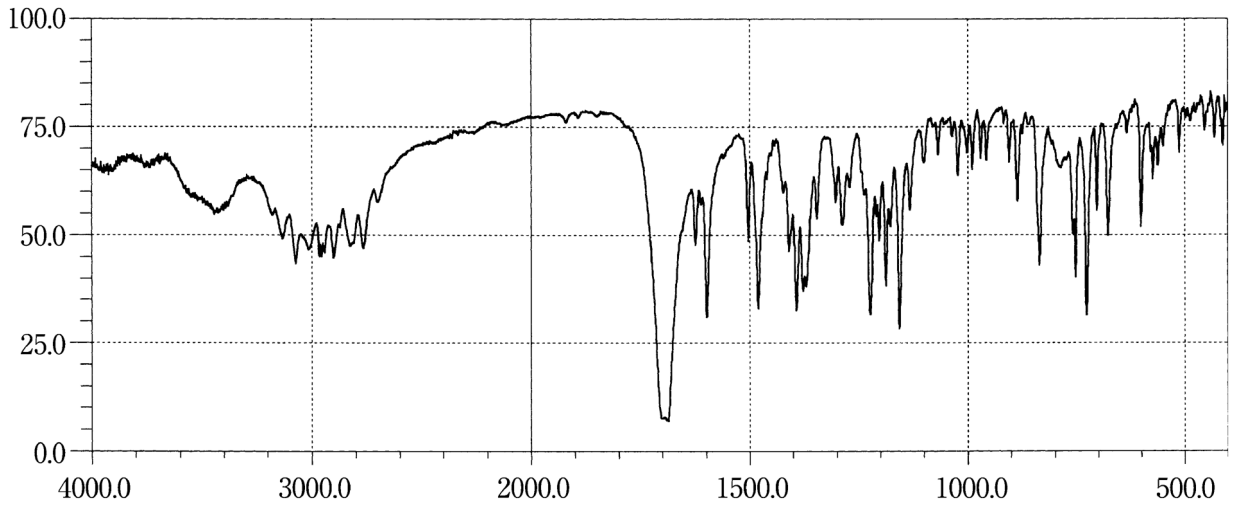
Doxorubicin Hydrochloride



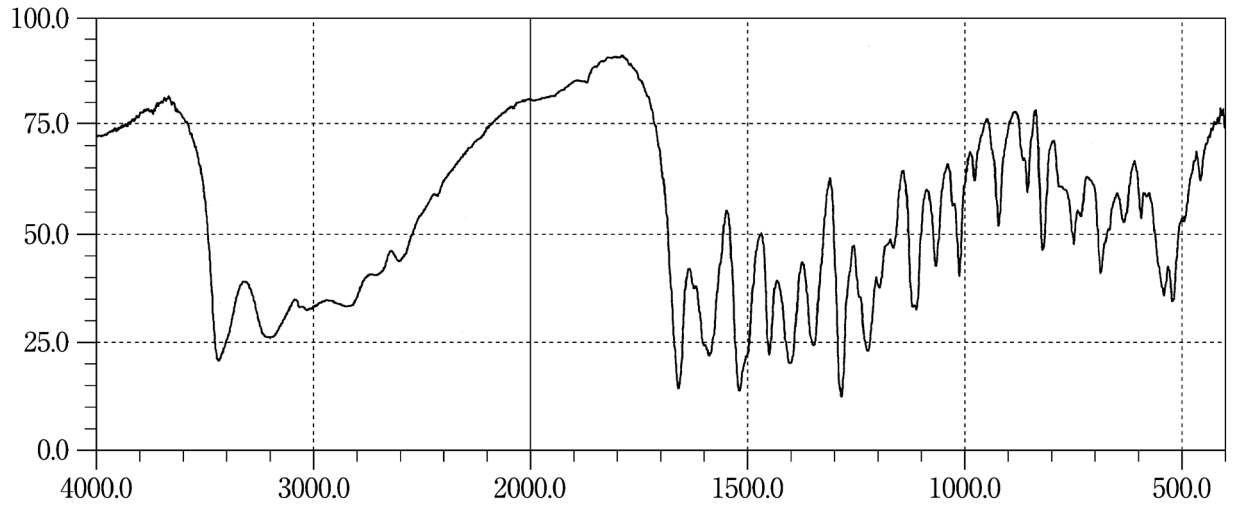
Doxycycline Hydrochloride Hydrate



Droperidol



Droxidopa



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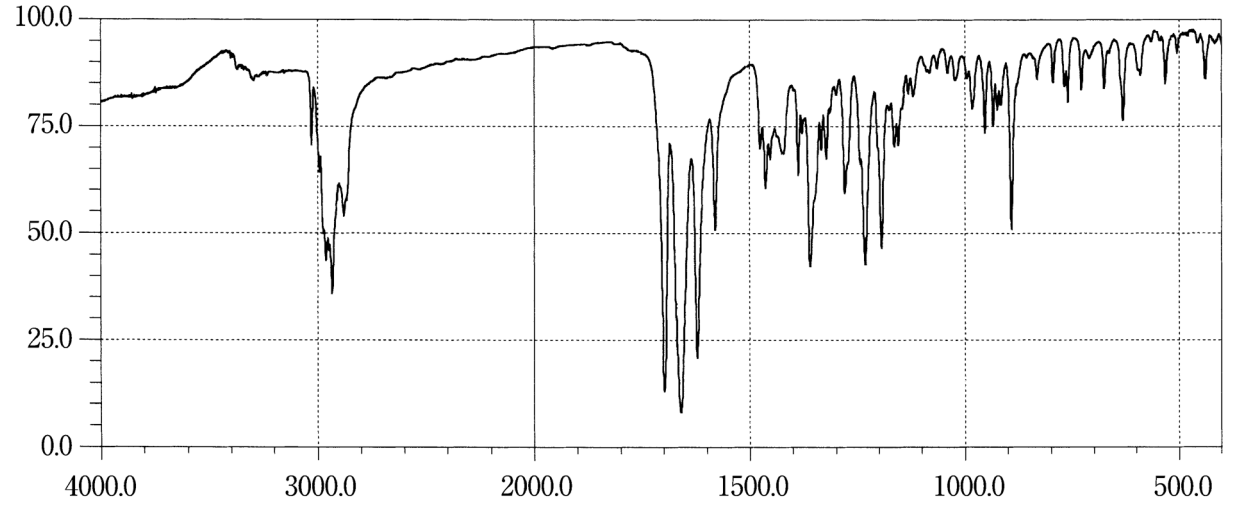

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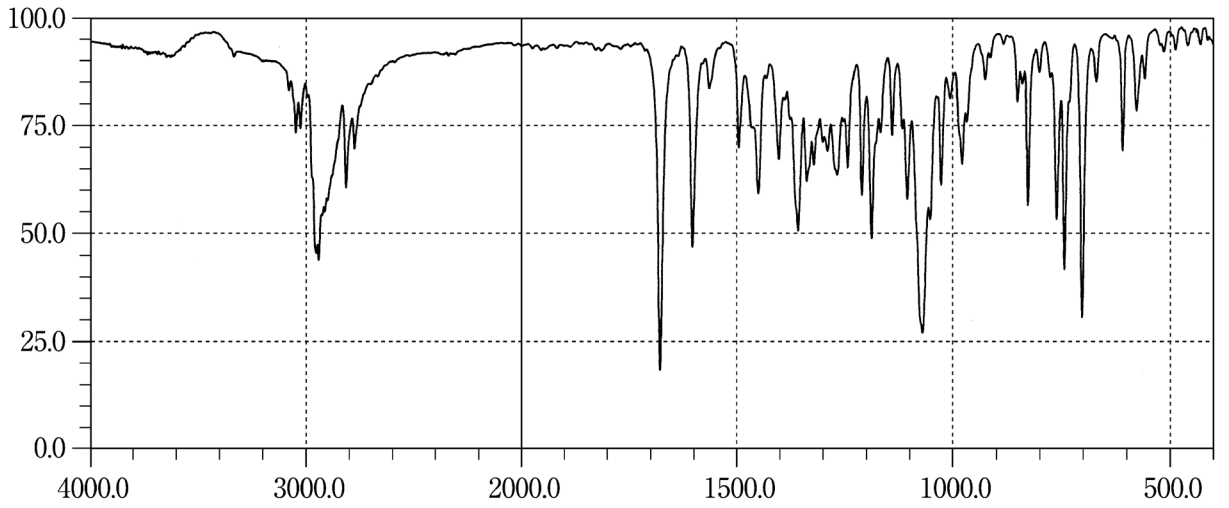

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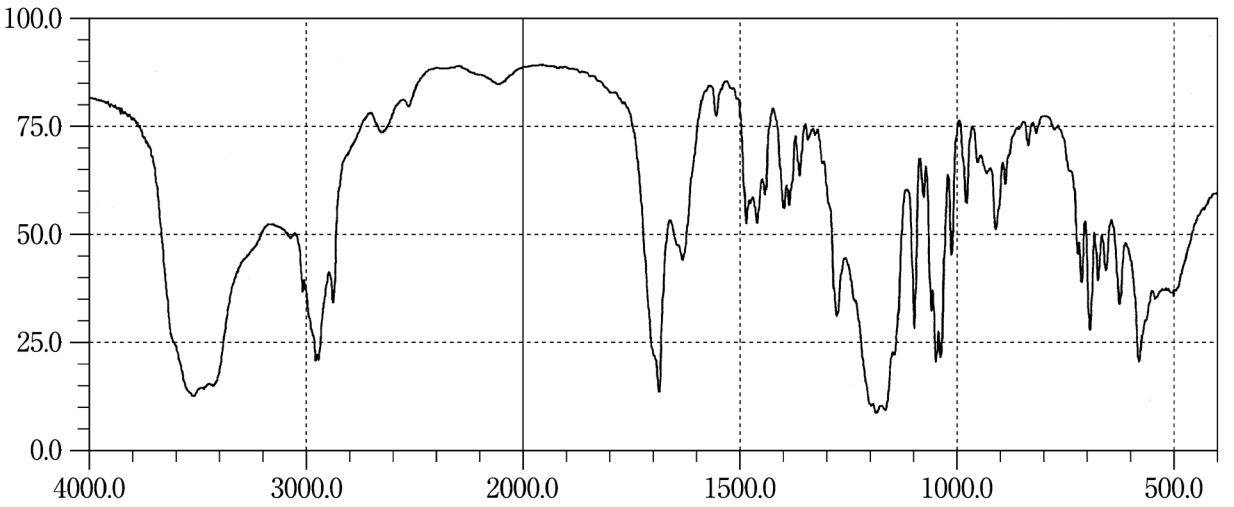

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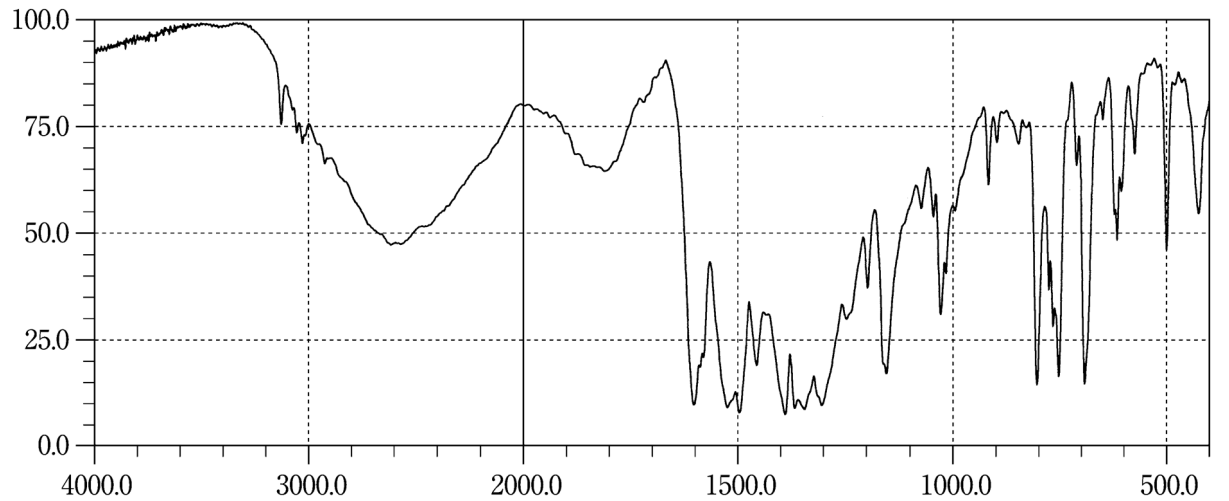

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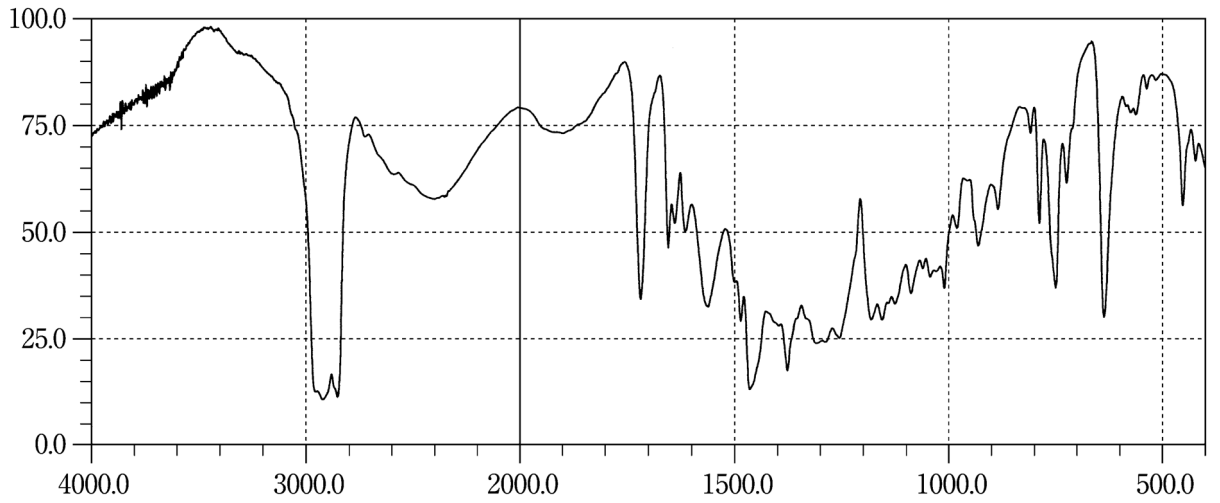

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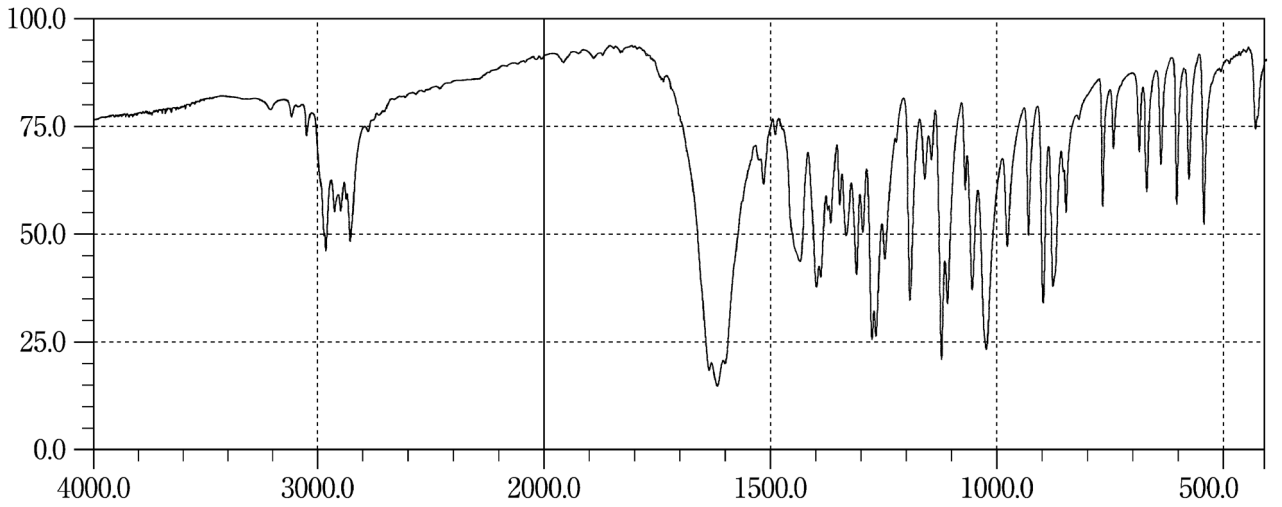

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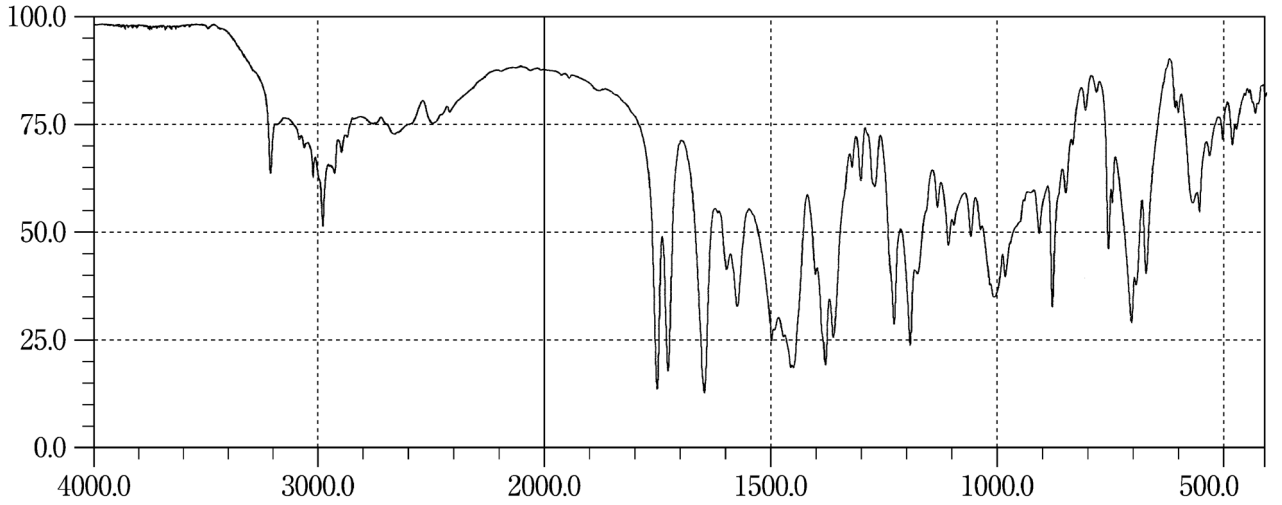

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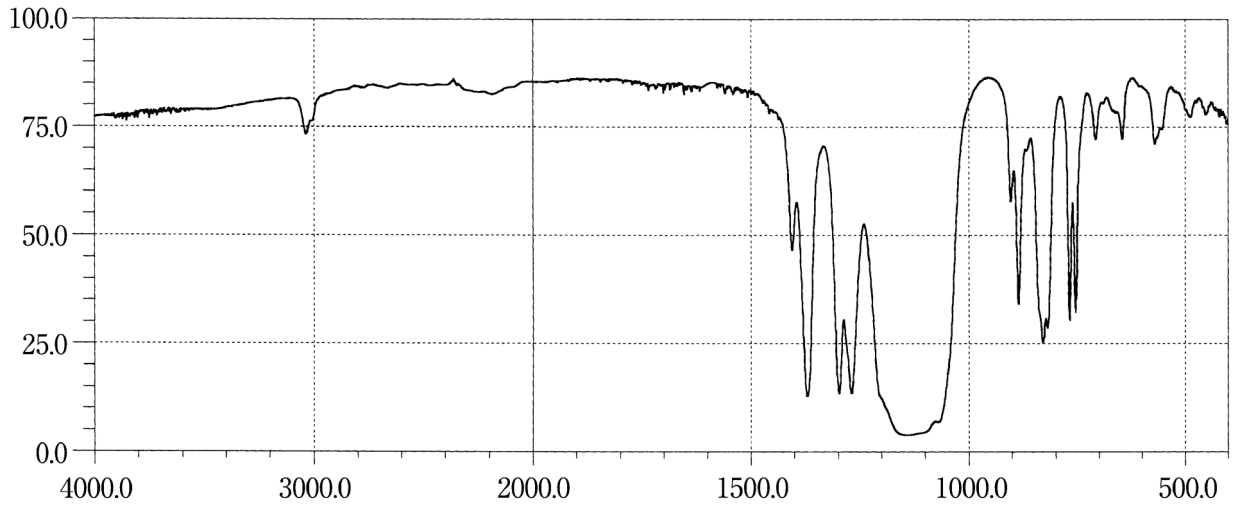

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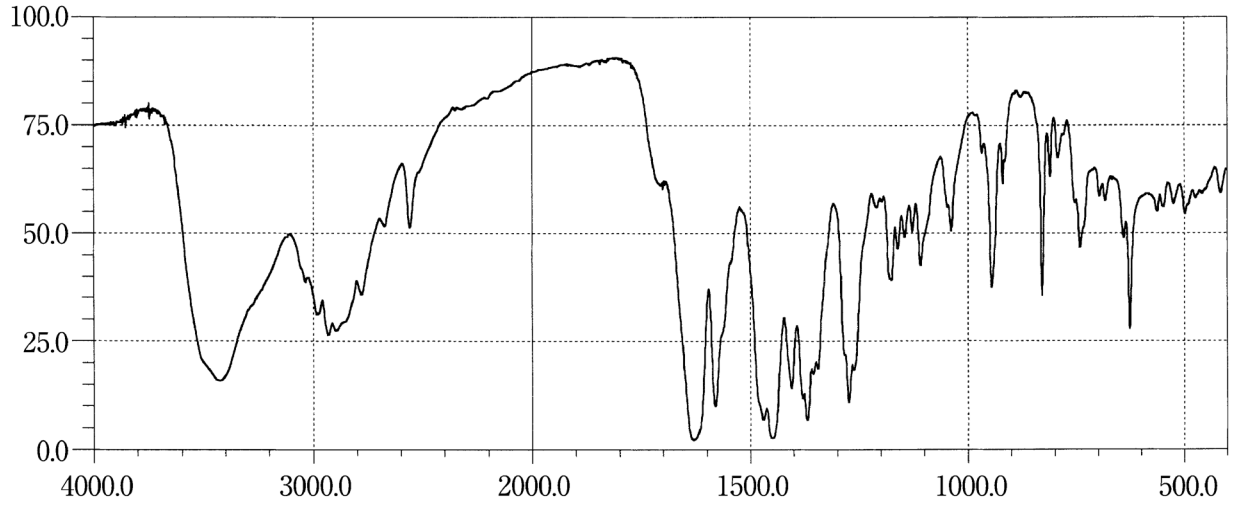

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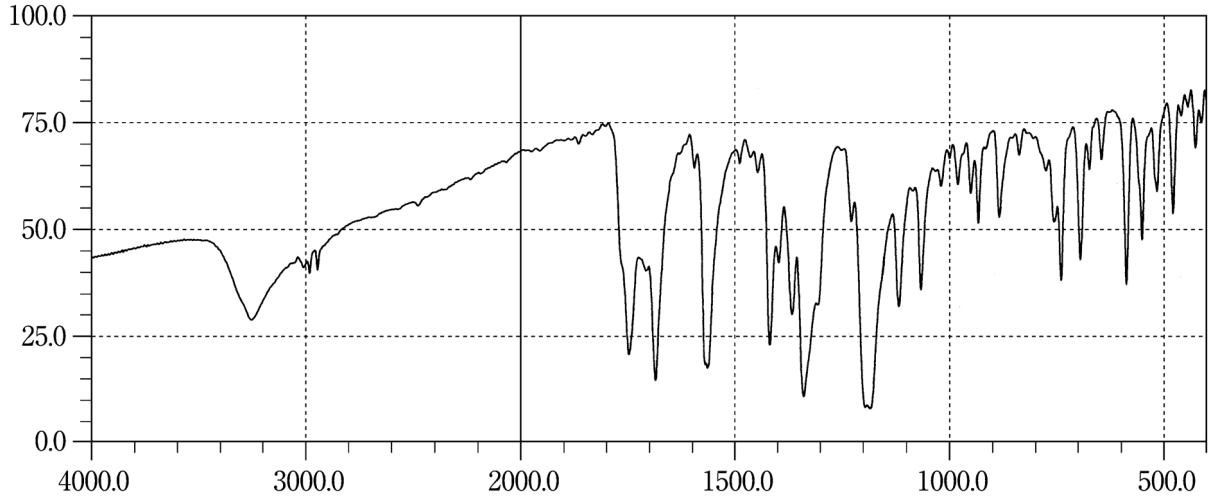
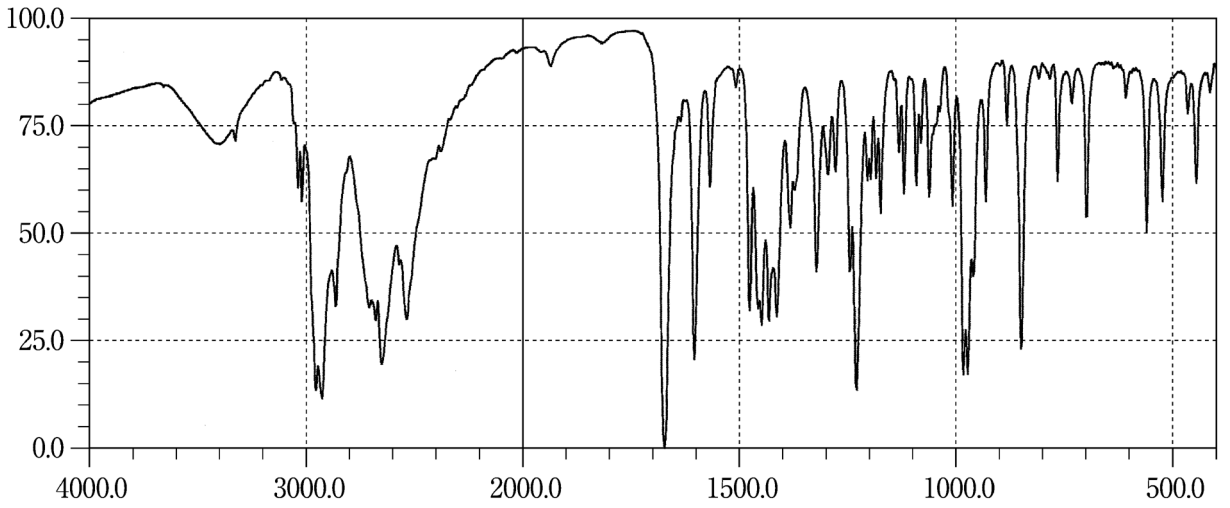


Eperisone Hydrochloride



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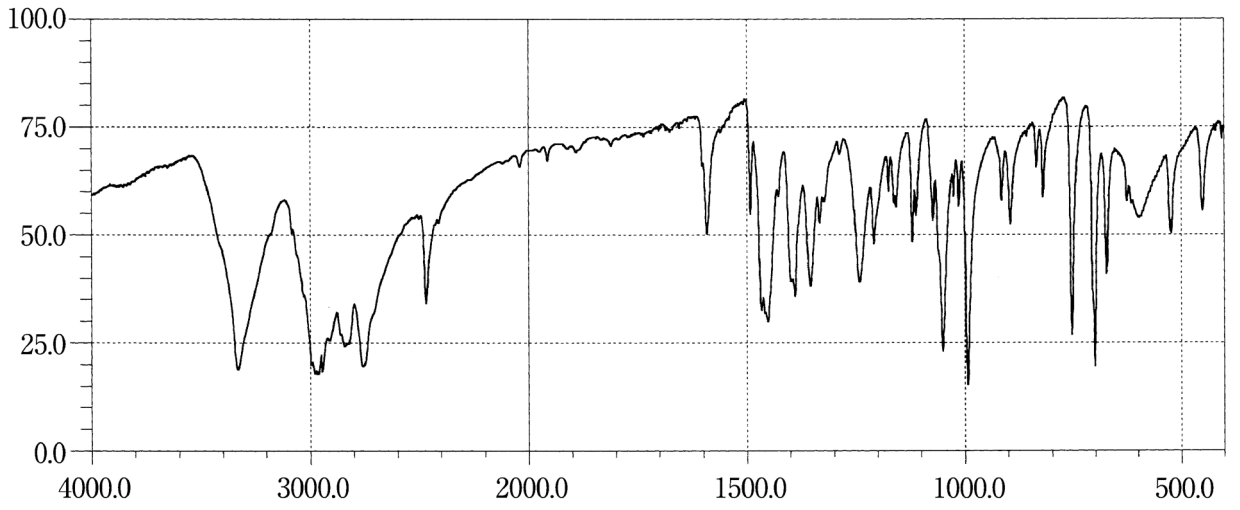

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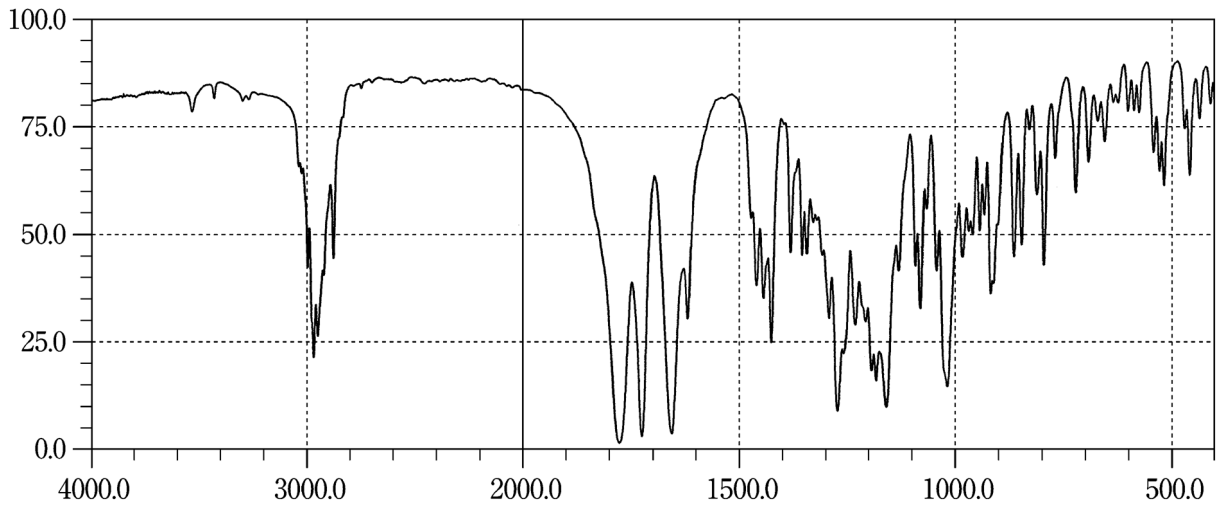

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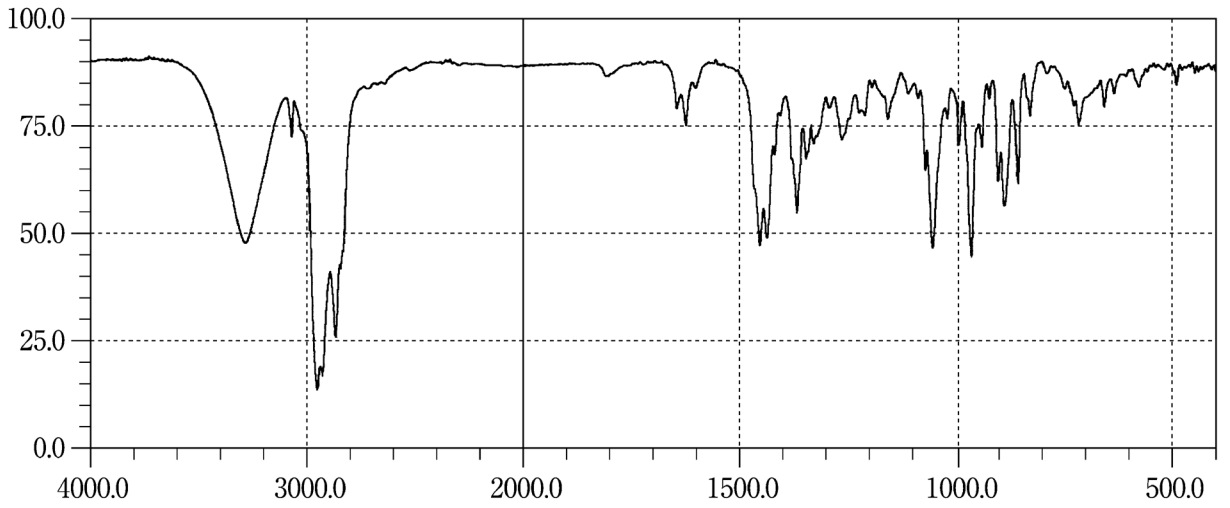
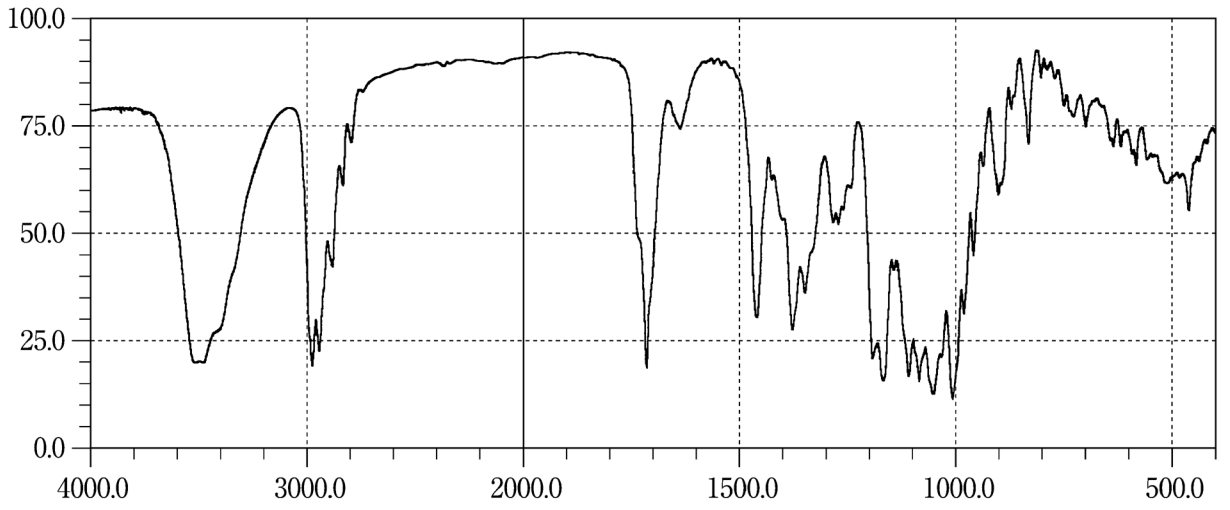


Erythromycin



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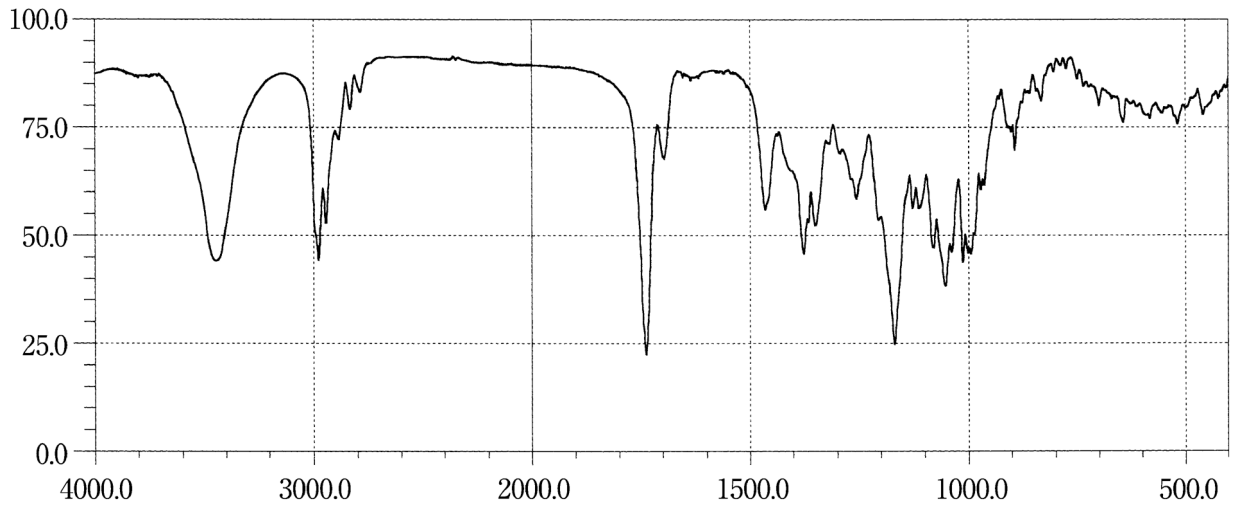

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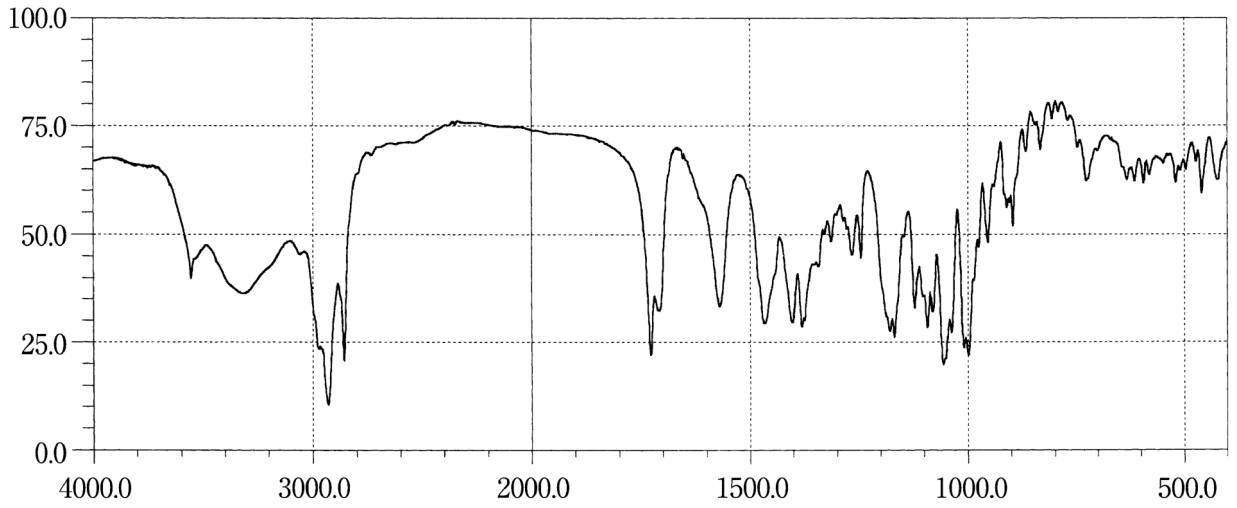

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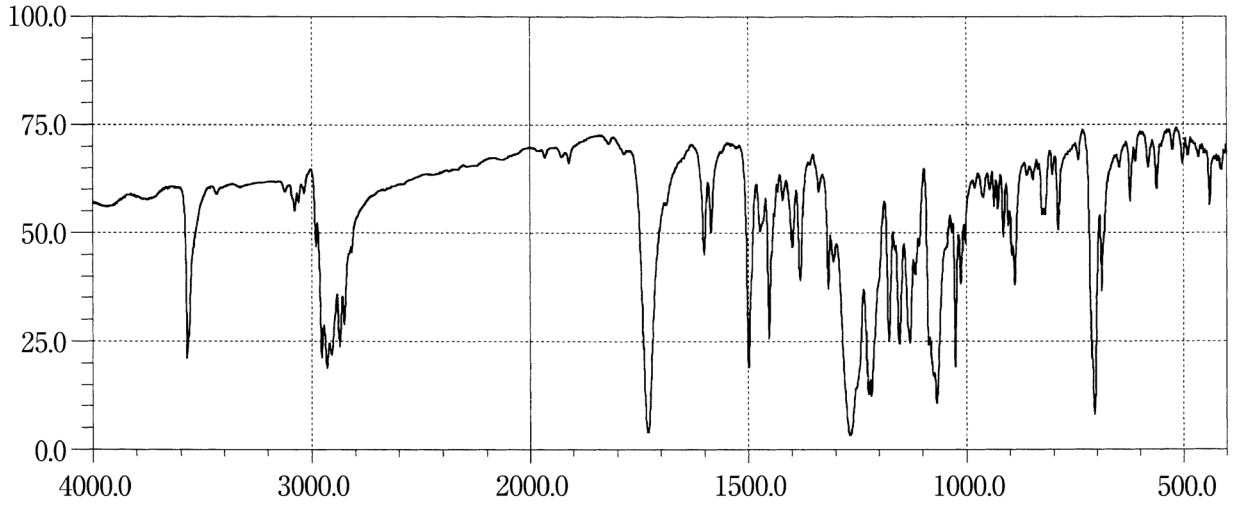

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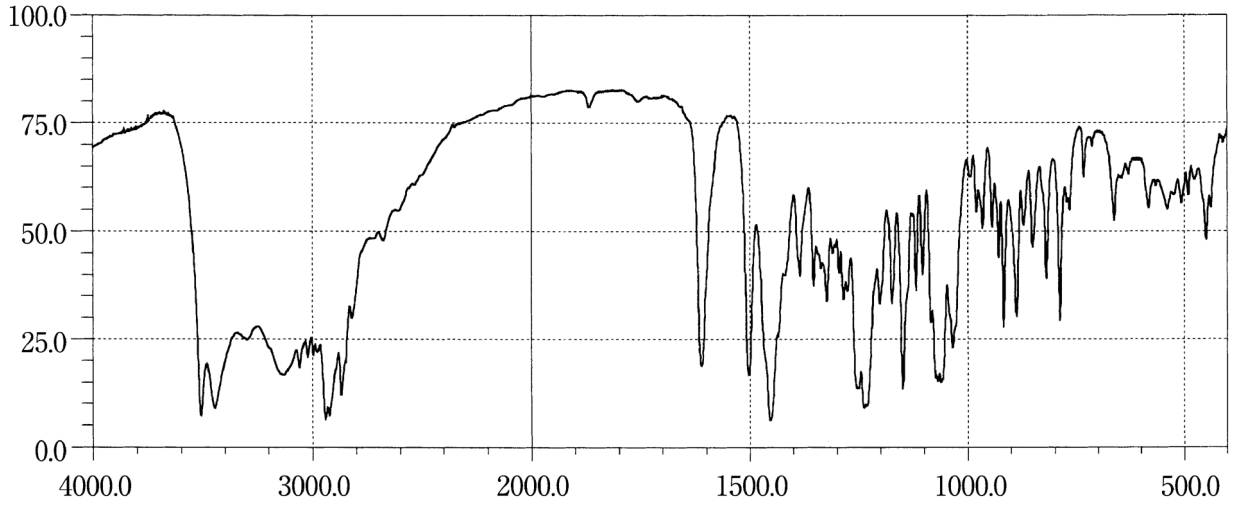

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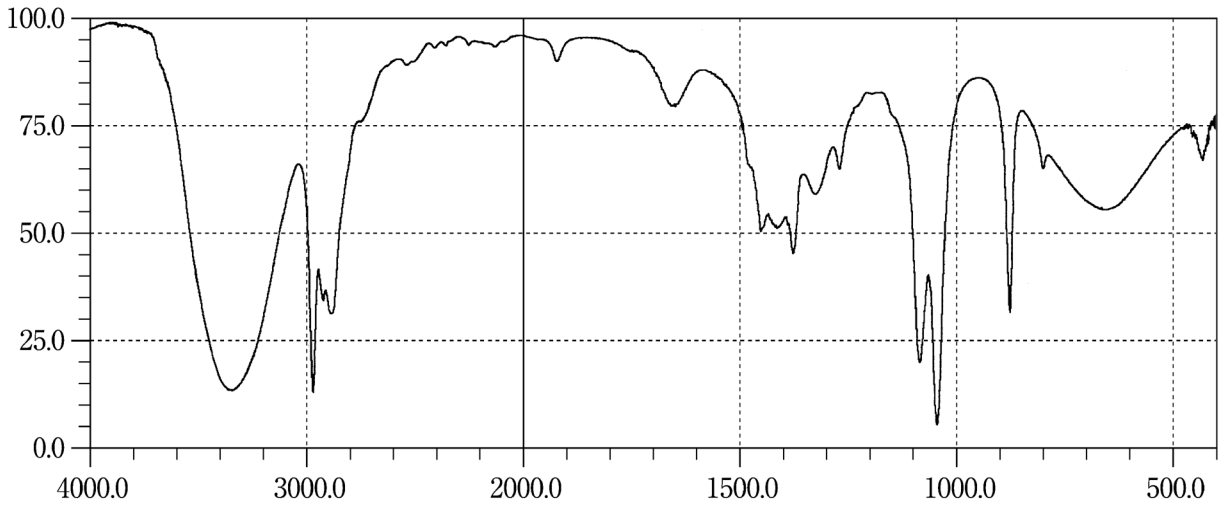

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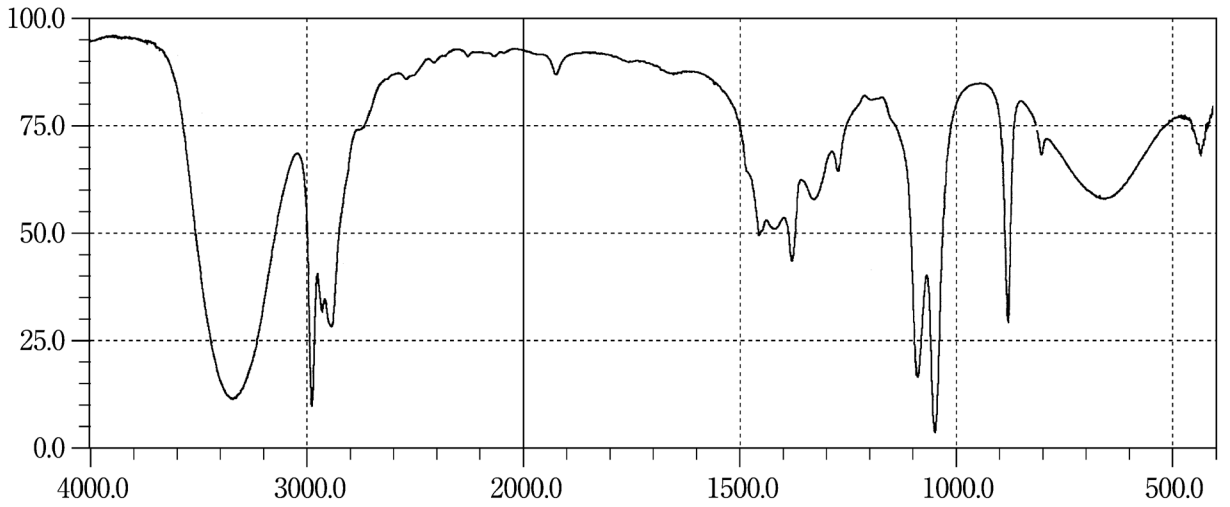

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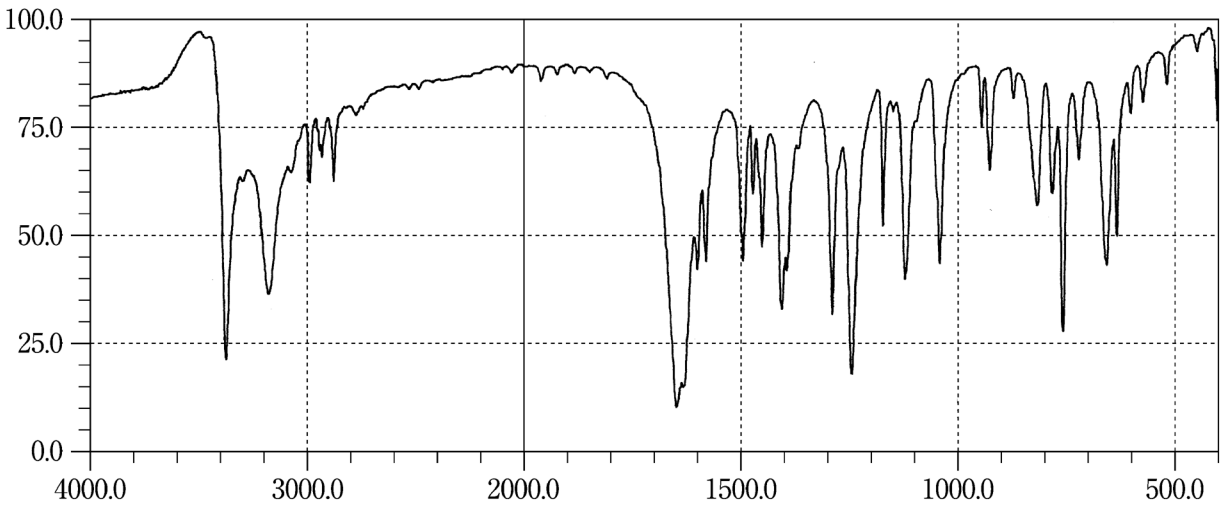

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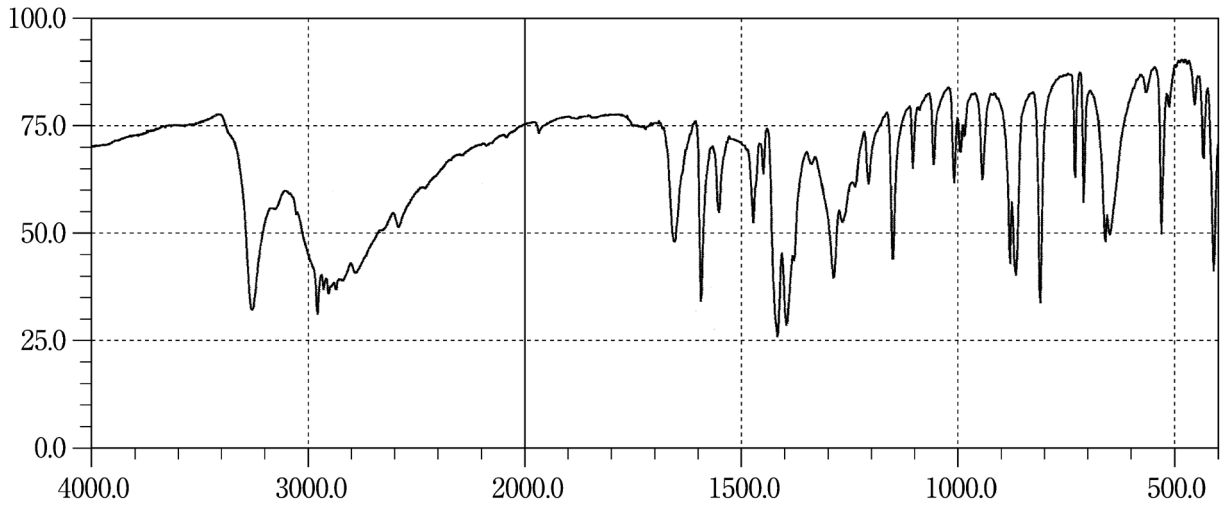

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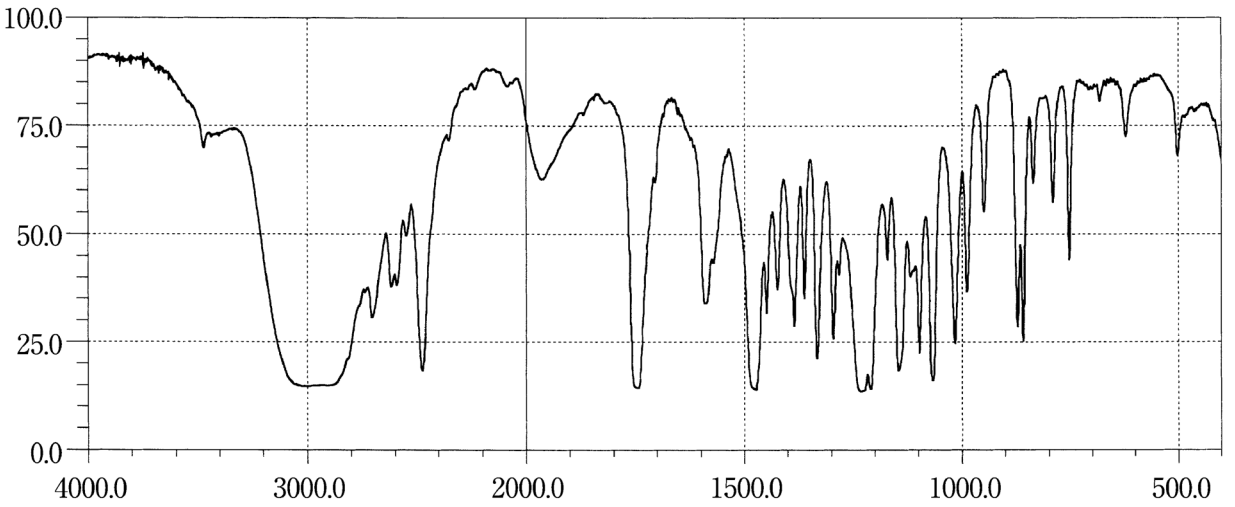

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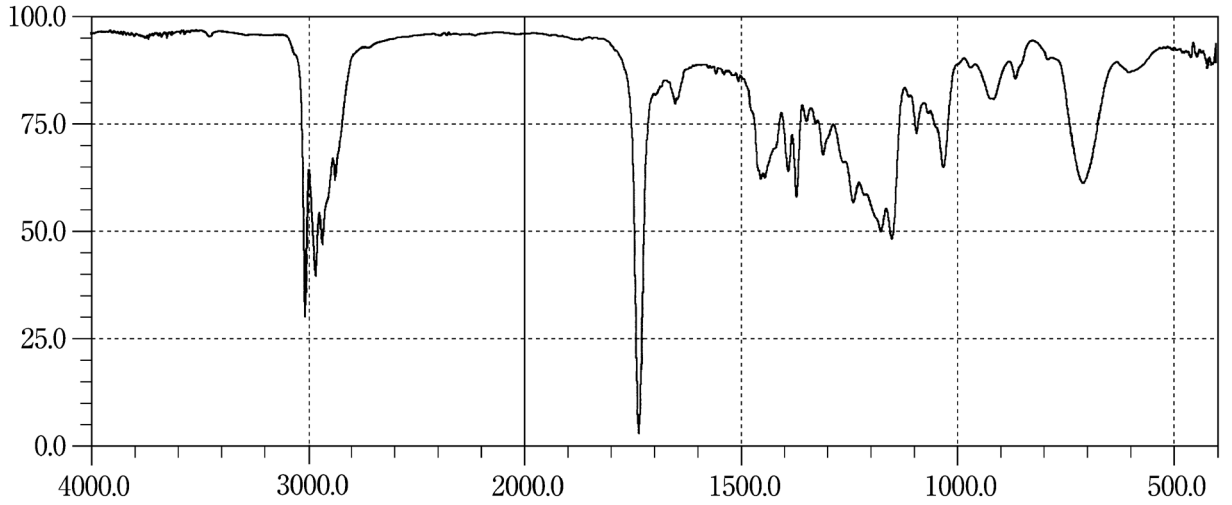
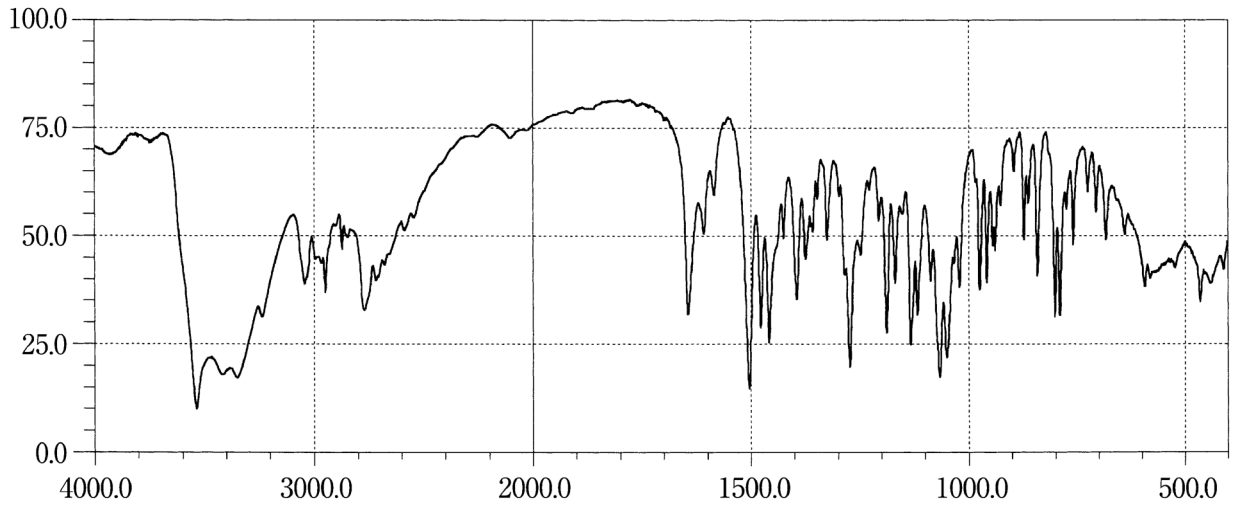
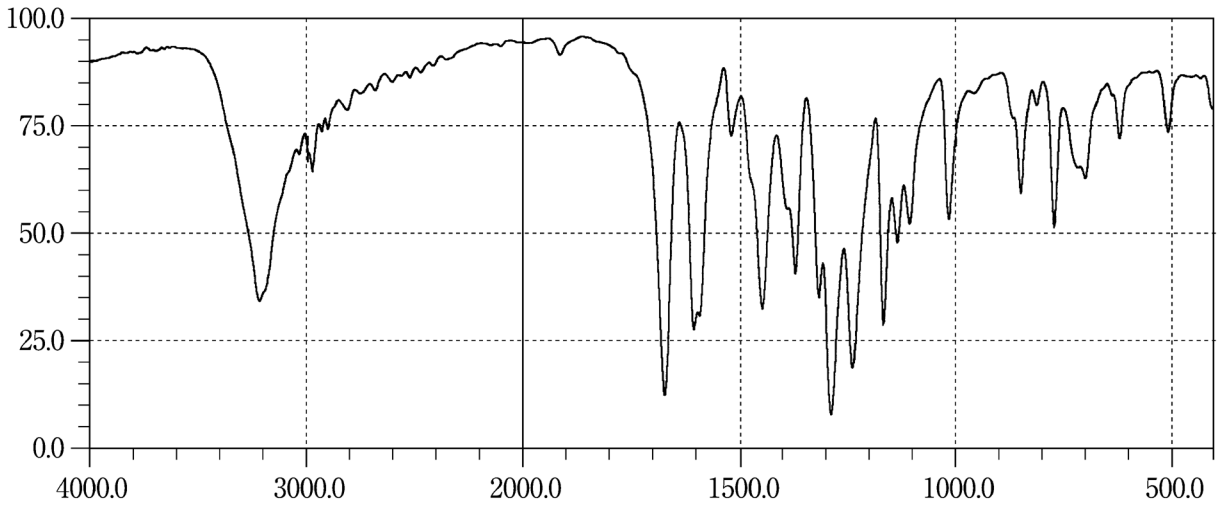


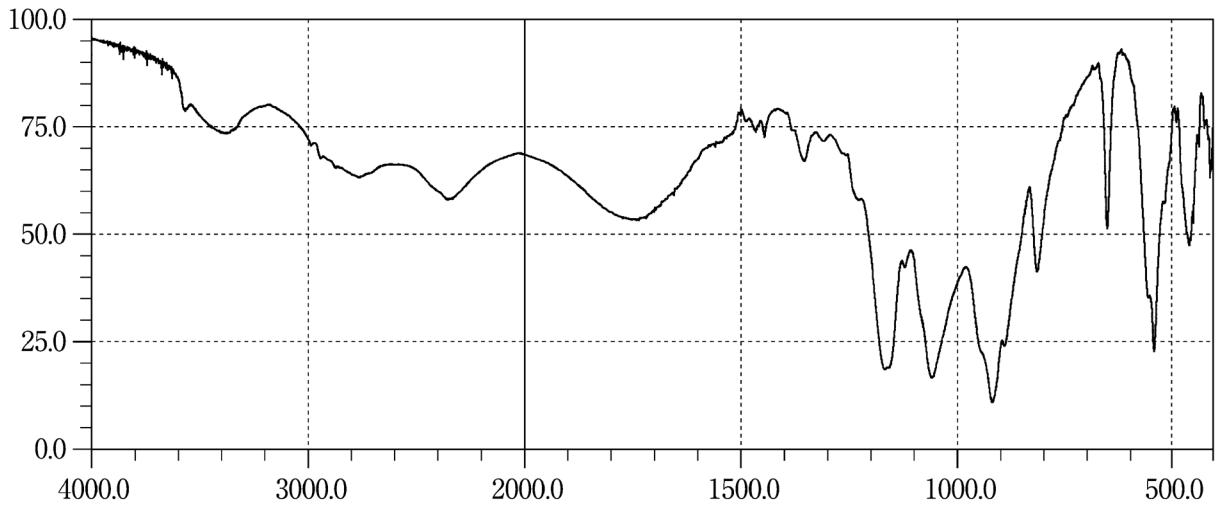
Ethylmorphine Hydrochloride Hydrate



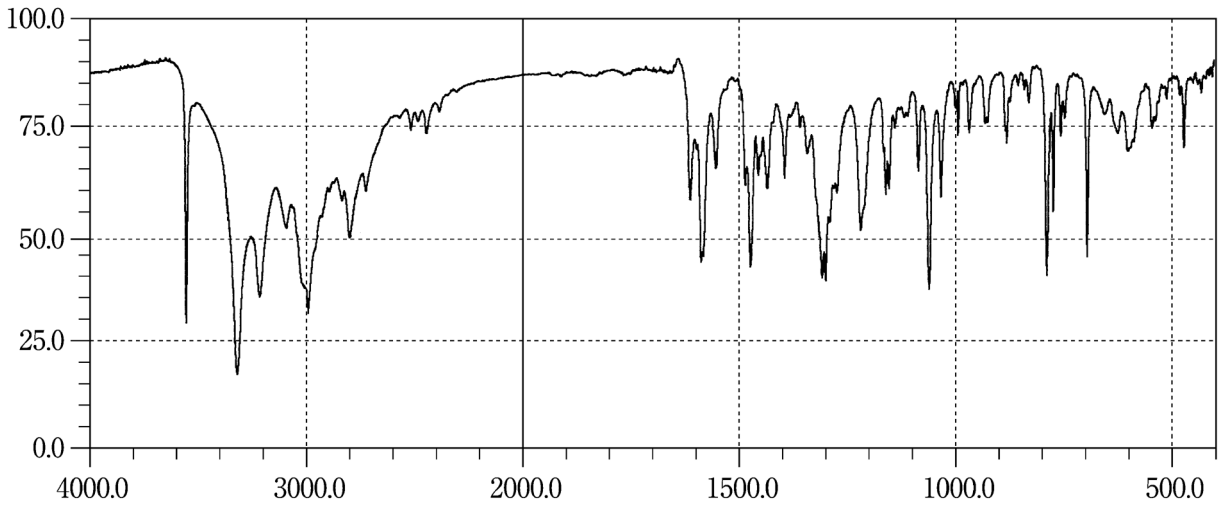
Ethyl Parahydroxybenzoate



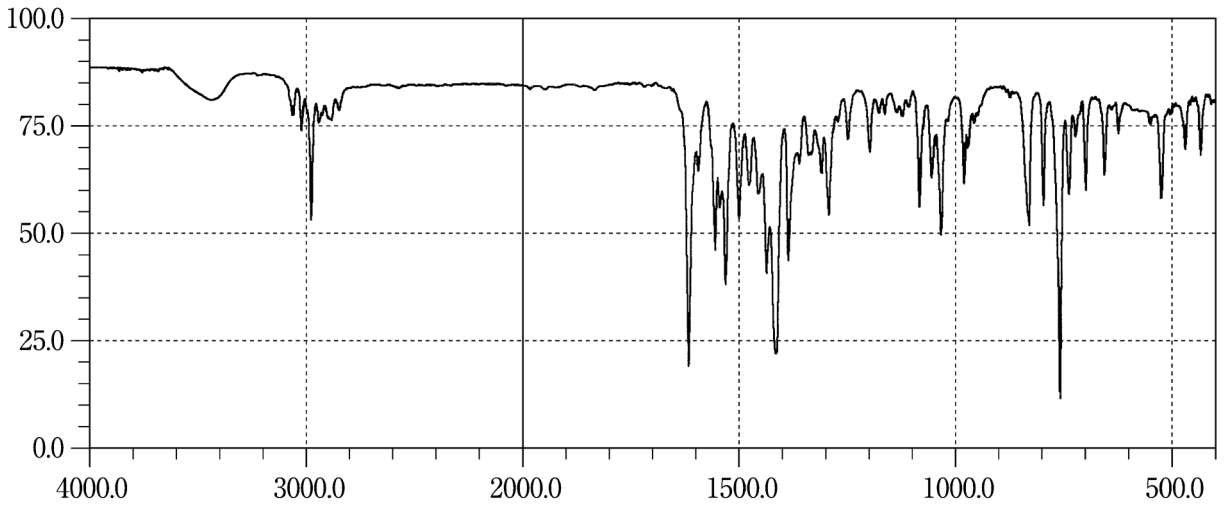
Etidronate Disodium



Etilefrine Hydrochloride



Etizolam



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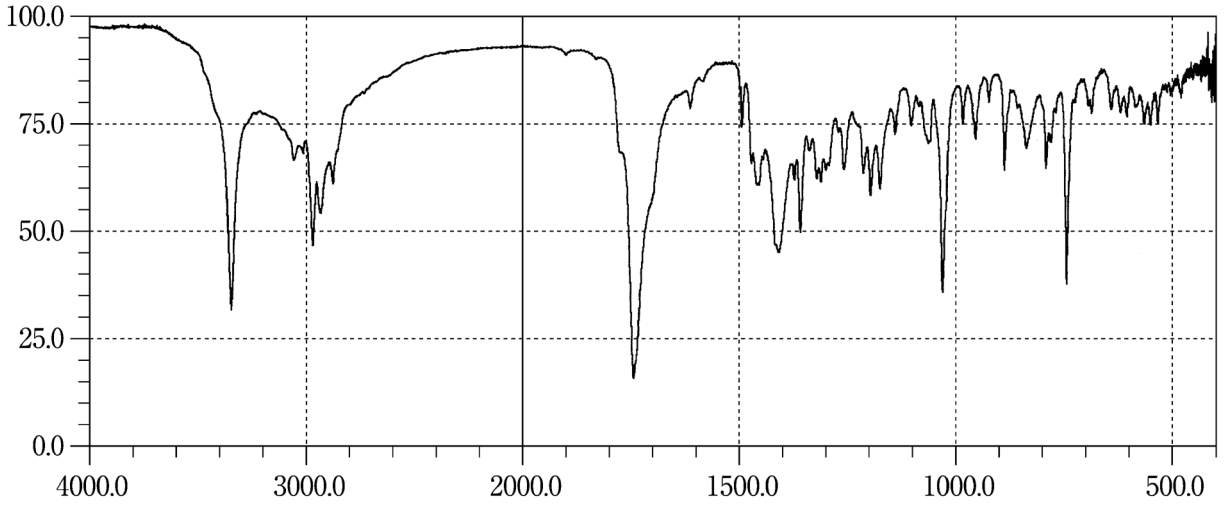

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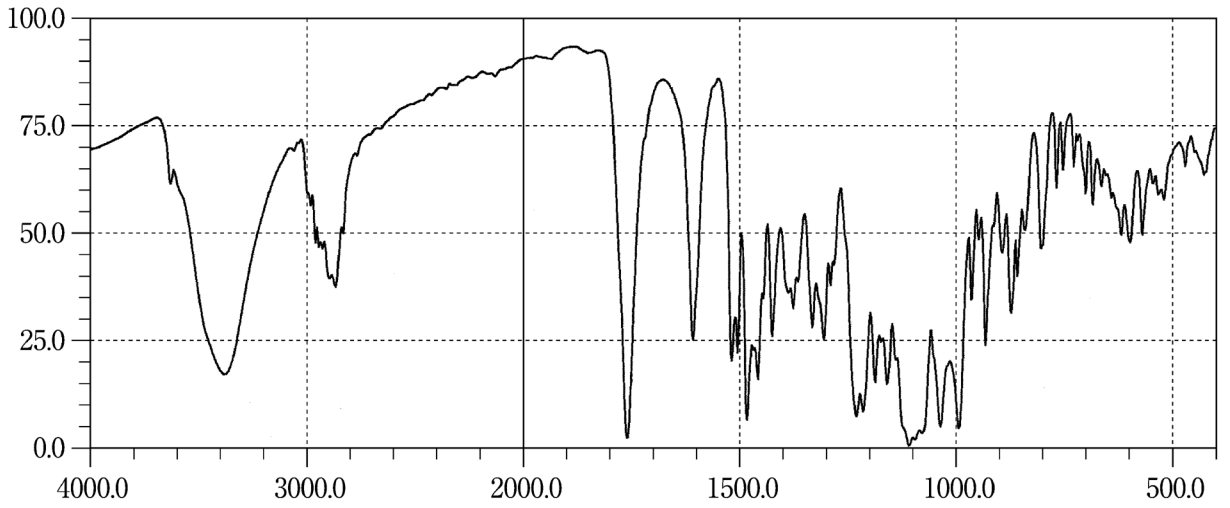

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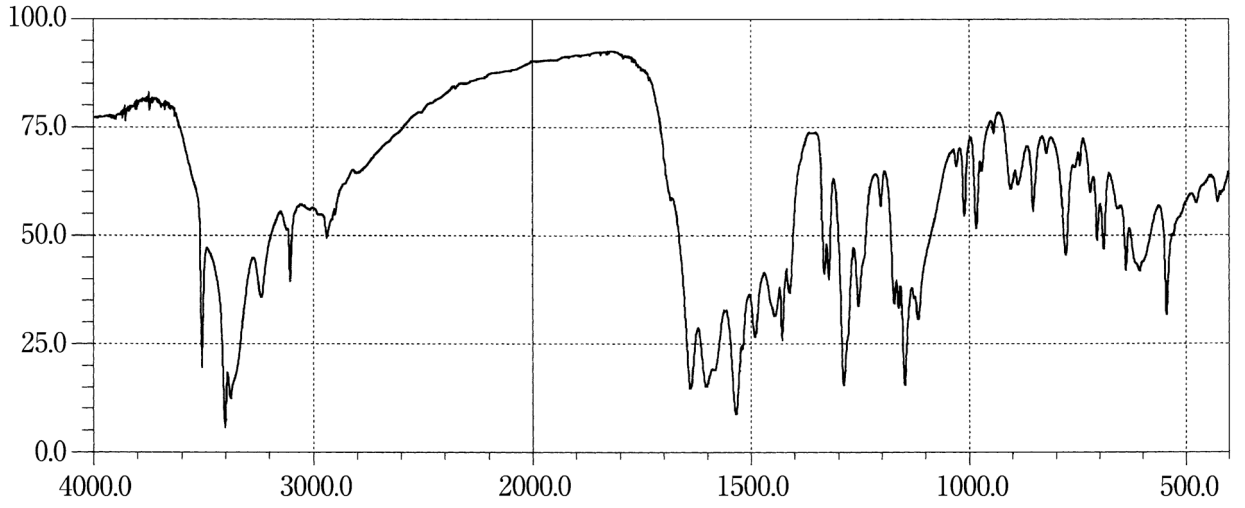

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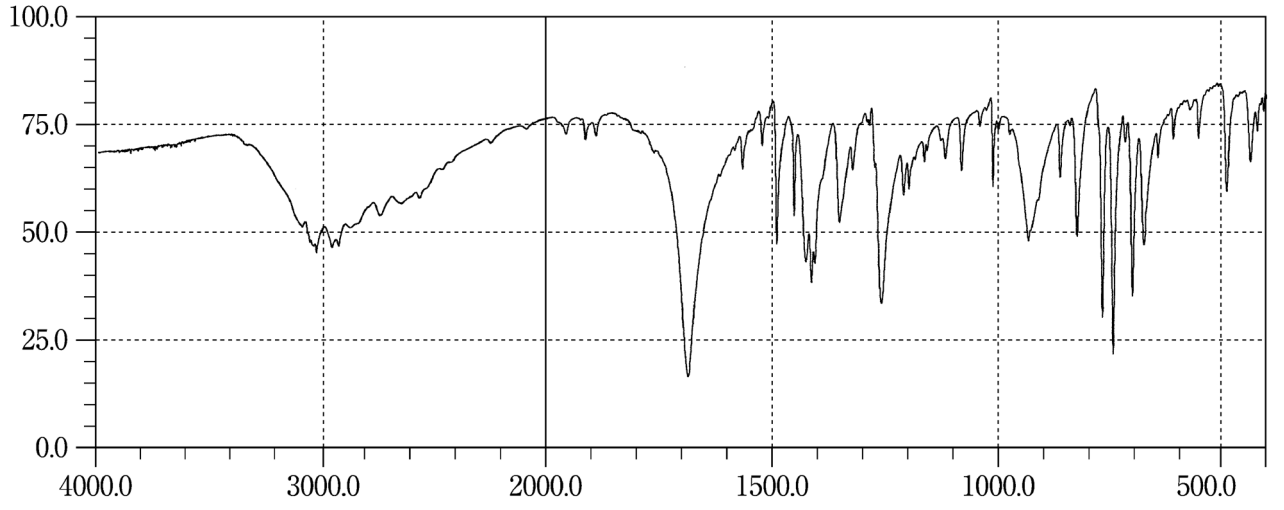

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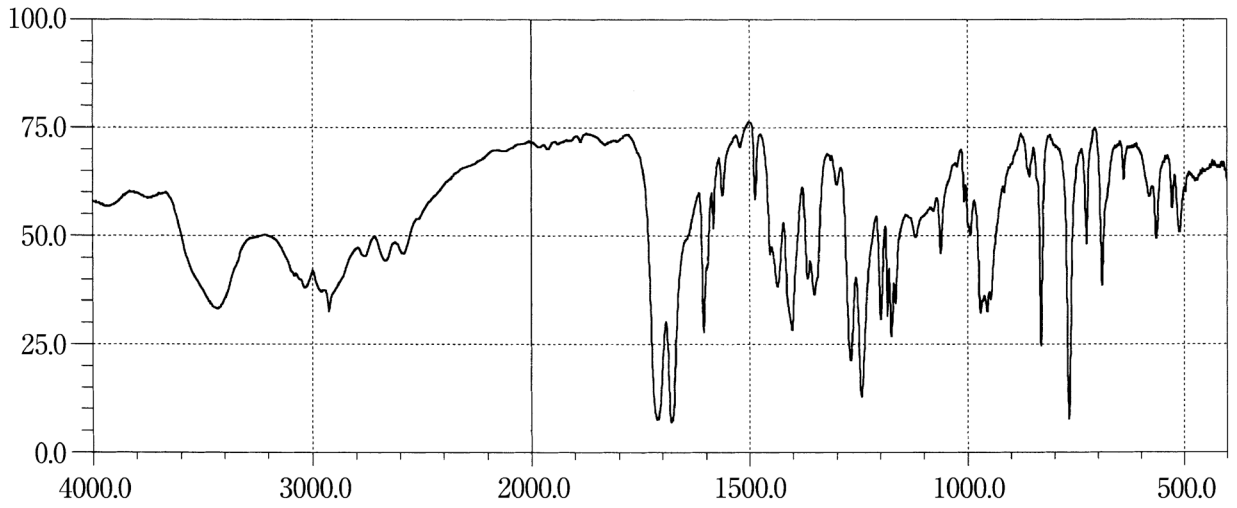
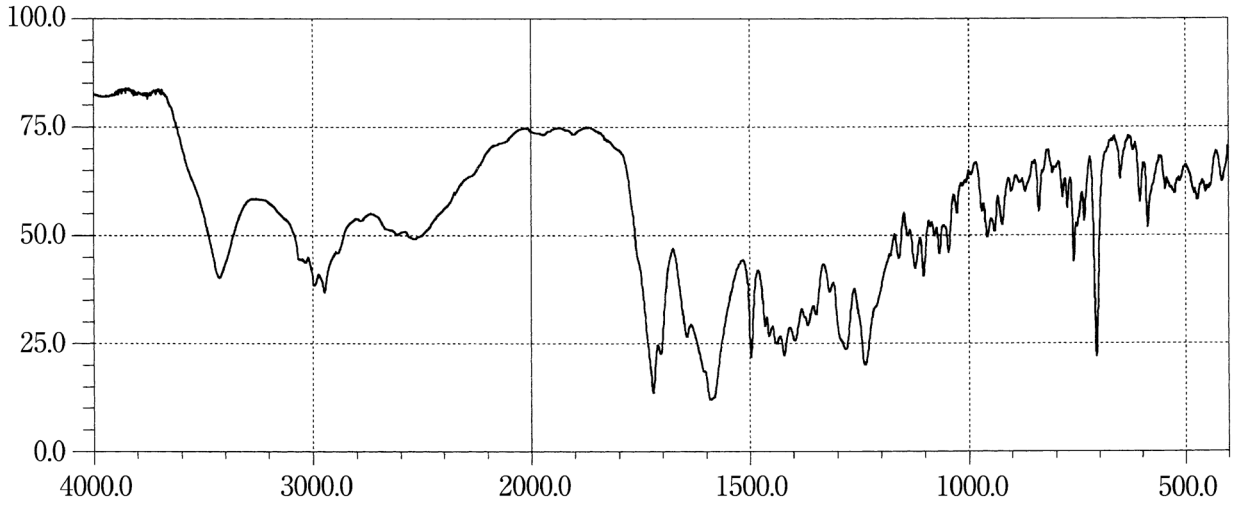
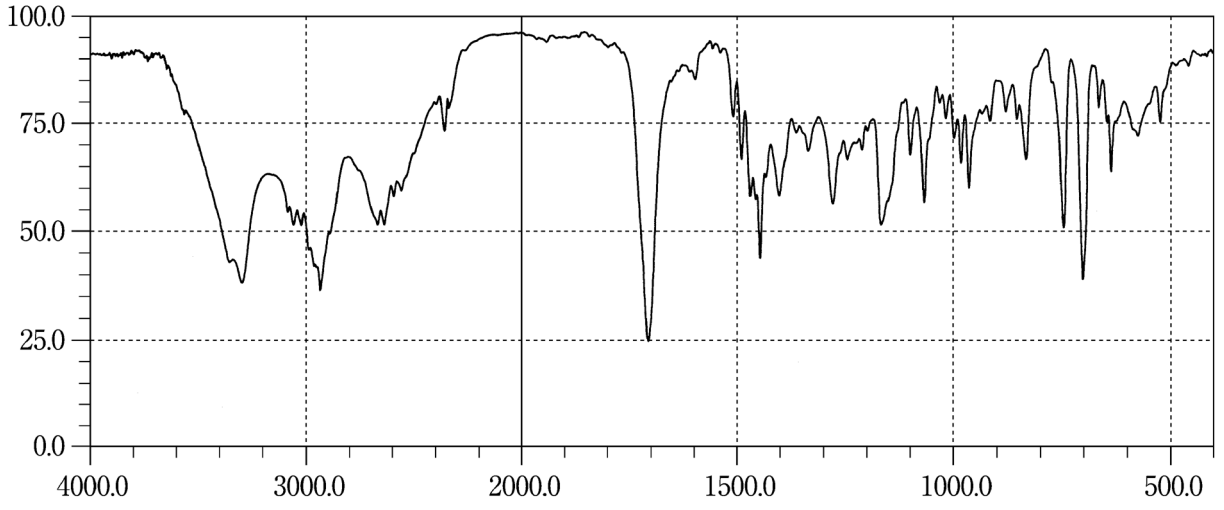


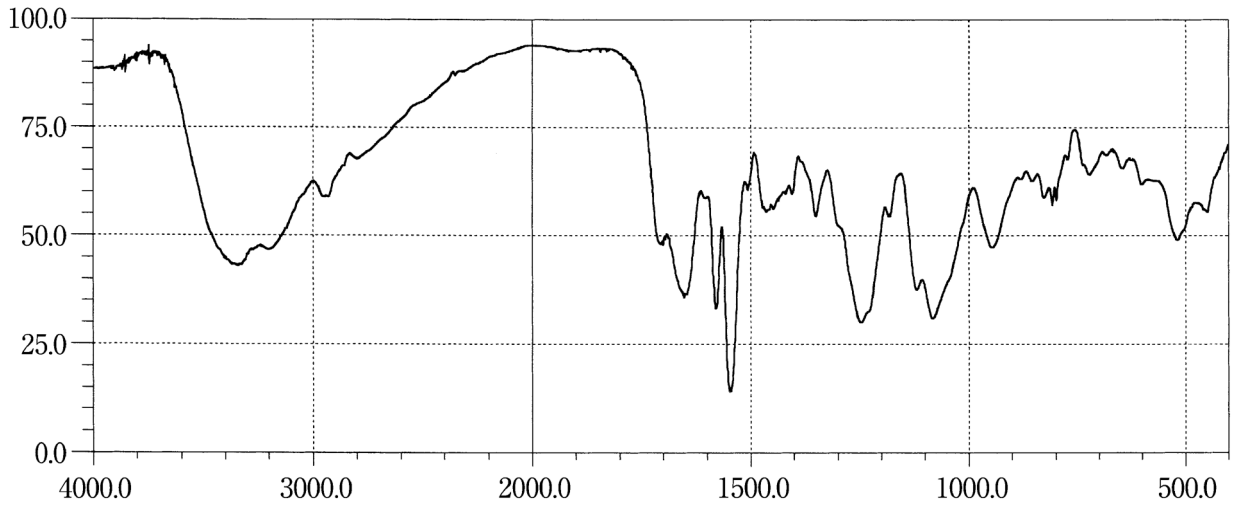
Fentanyl Citrate



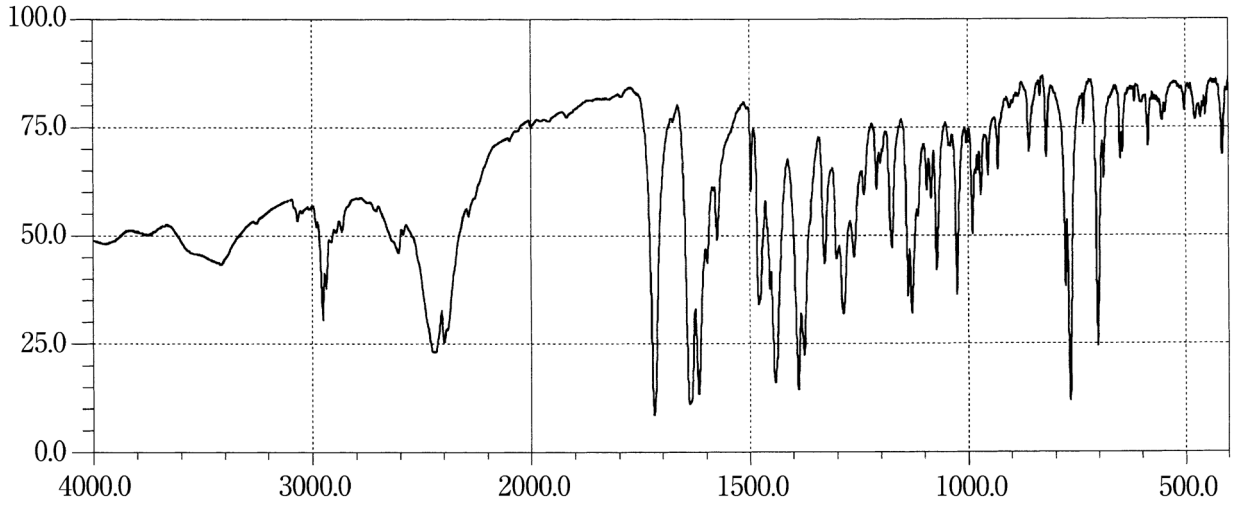
Fexofenadine Hydrochloride



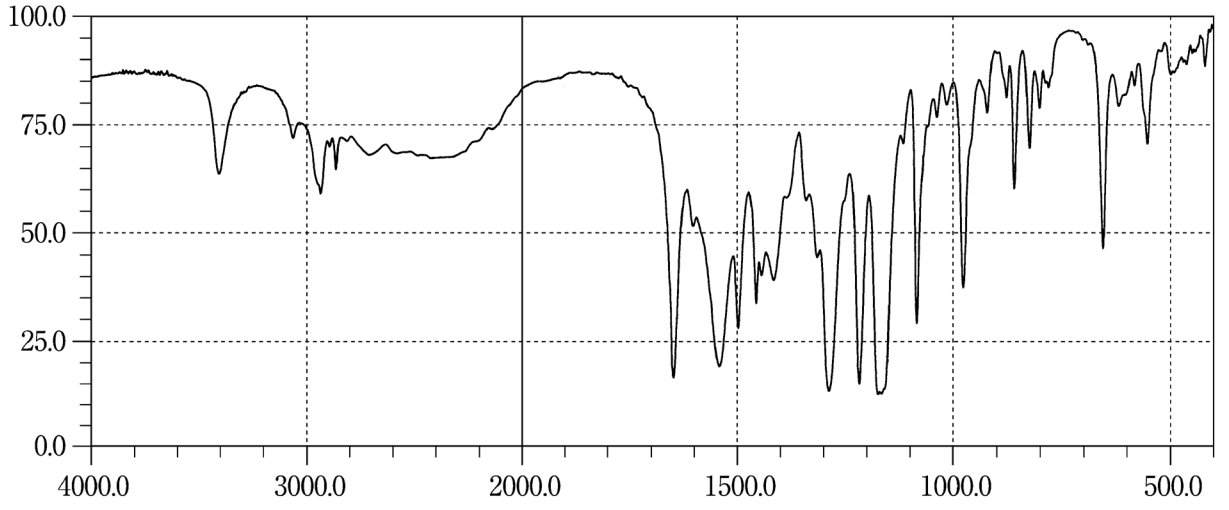
Flavin Adenine Dinucleotide Sodium



Flavoxate Hydrochloride



Flecainide Acetate



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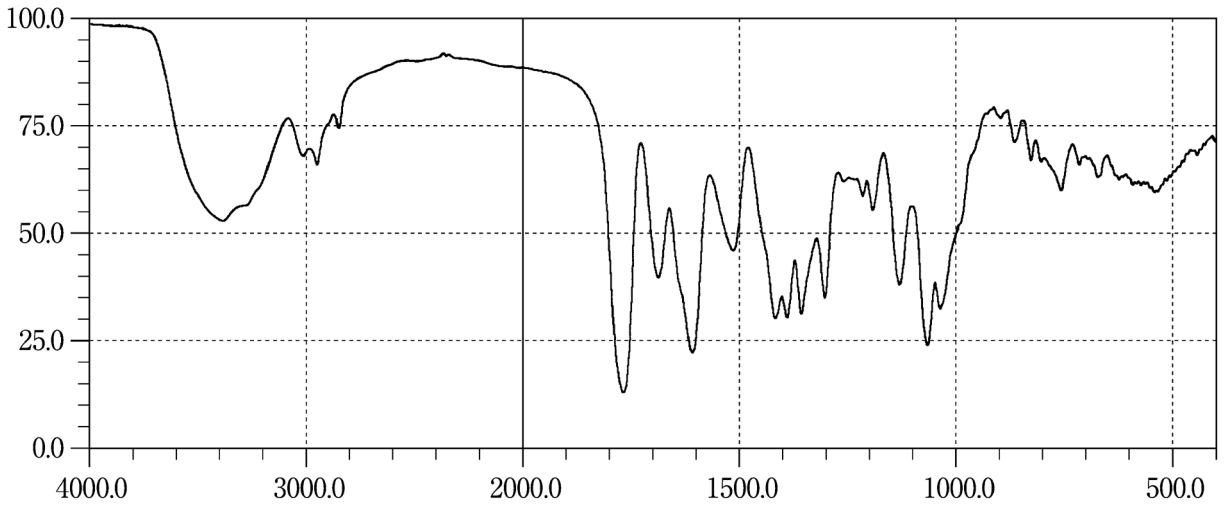

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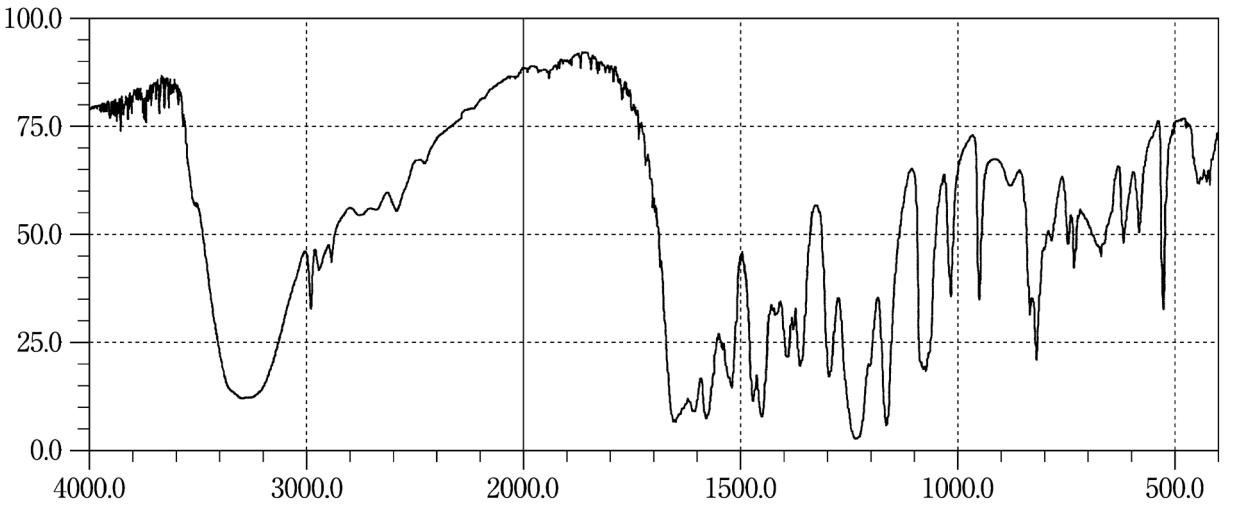
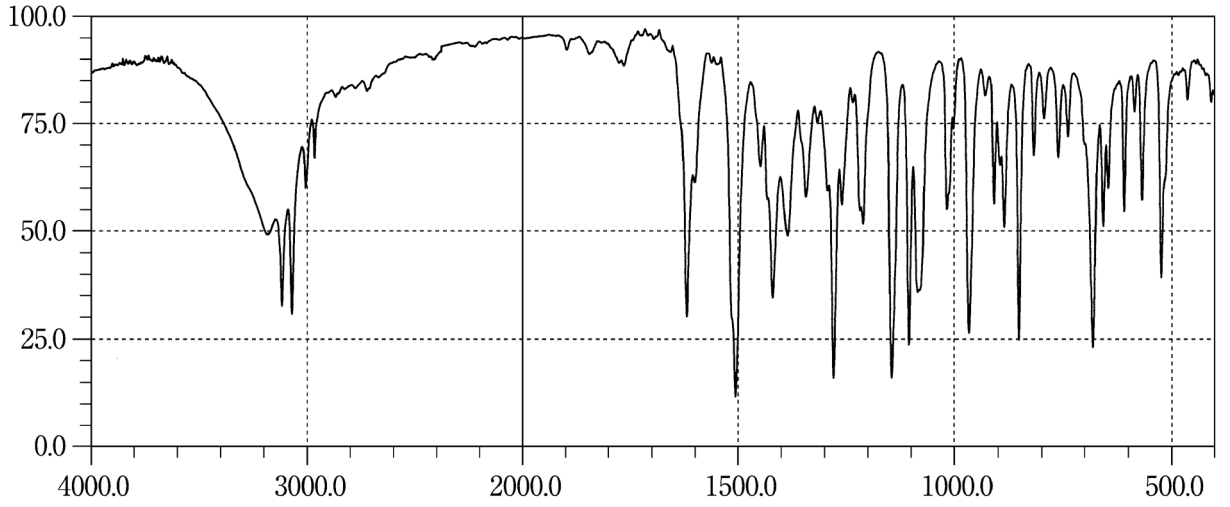
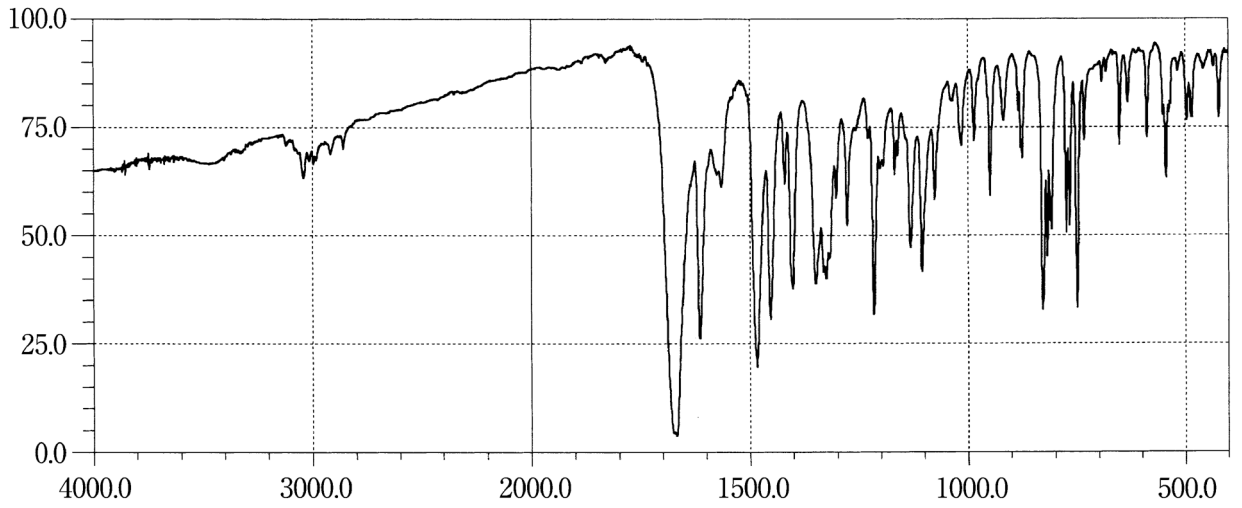


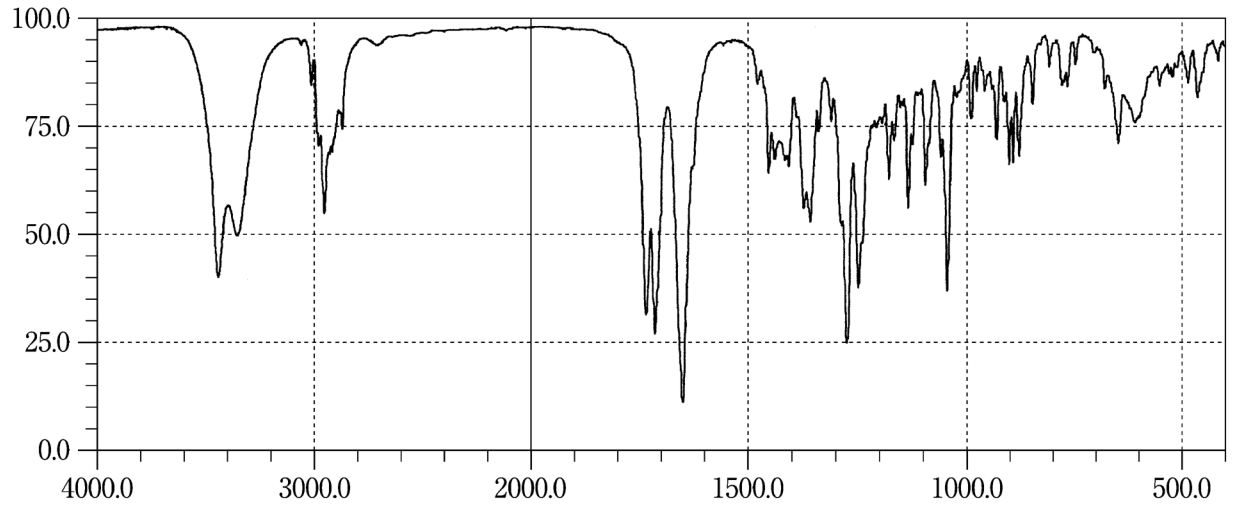
Fluconazole



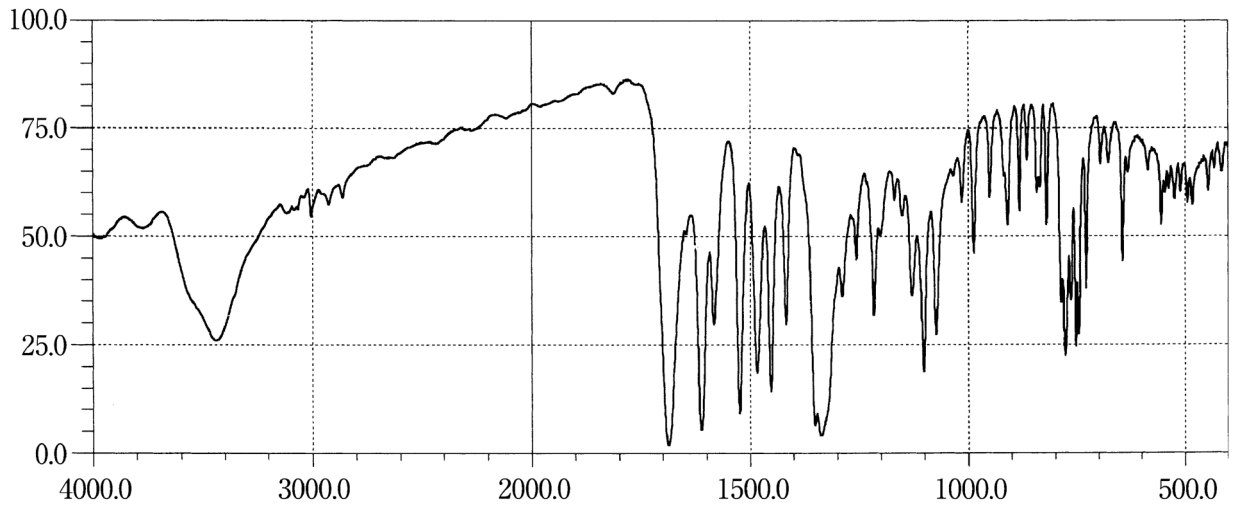
Fludiazepam



Fludrocortisone Acetate



Flunitrazepam



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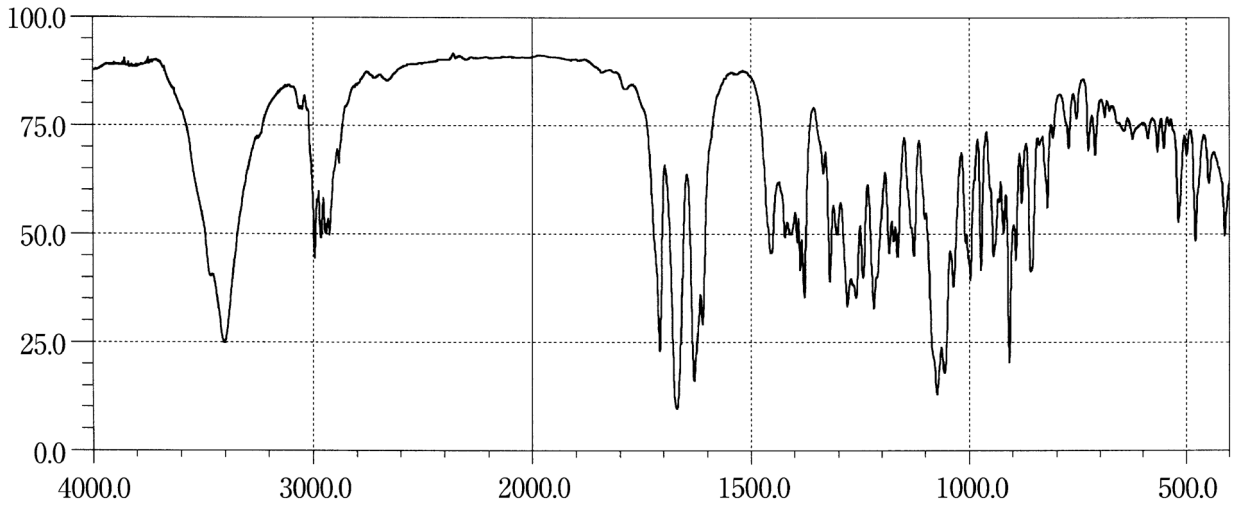

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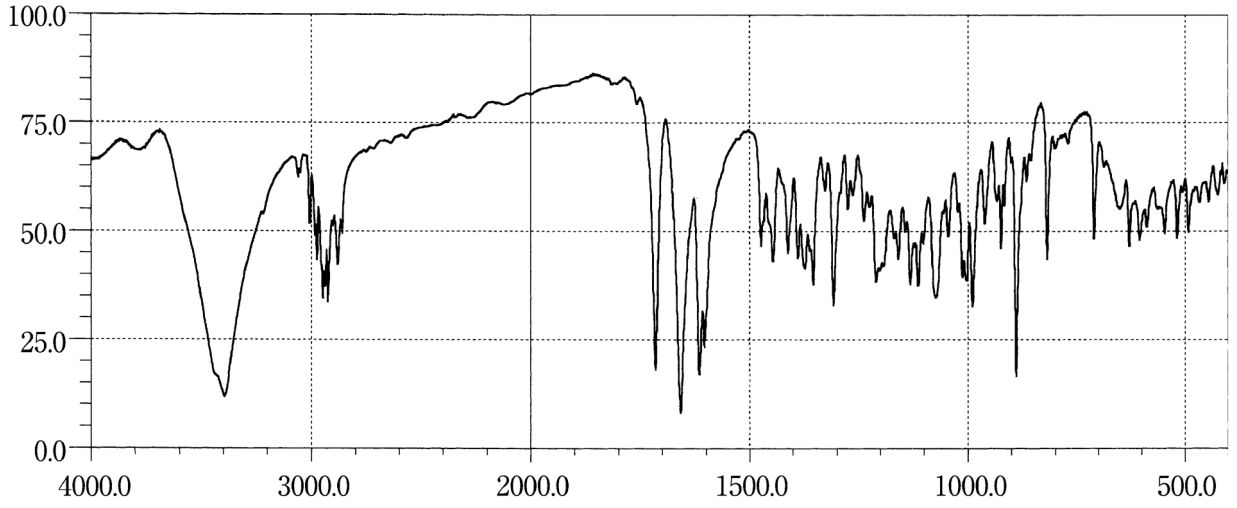
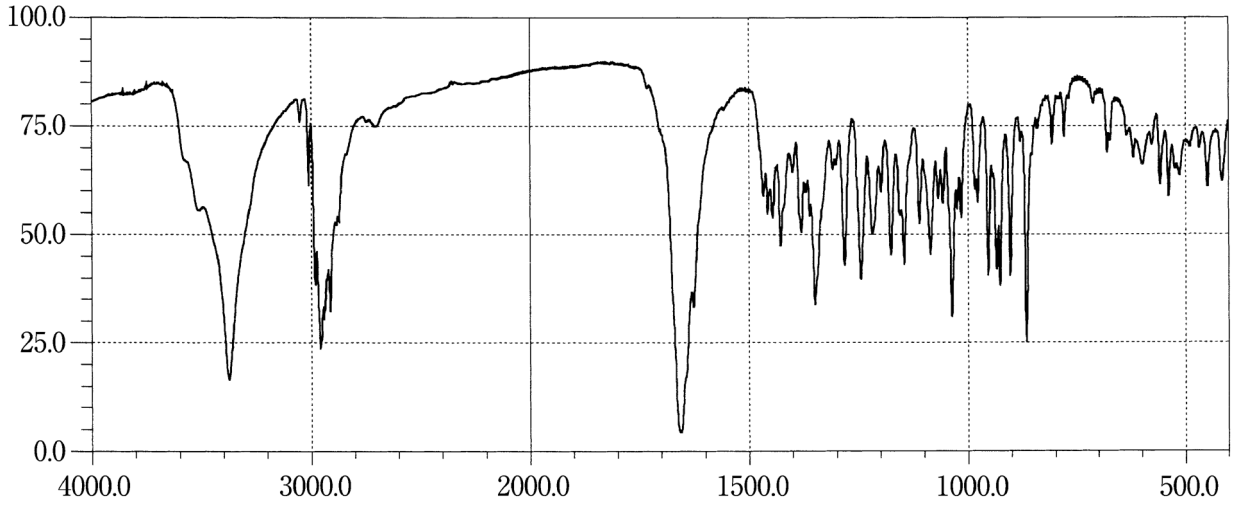
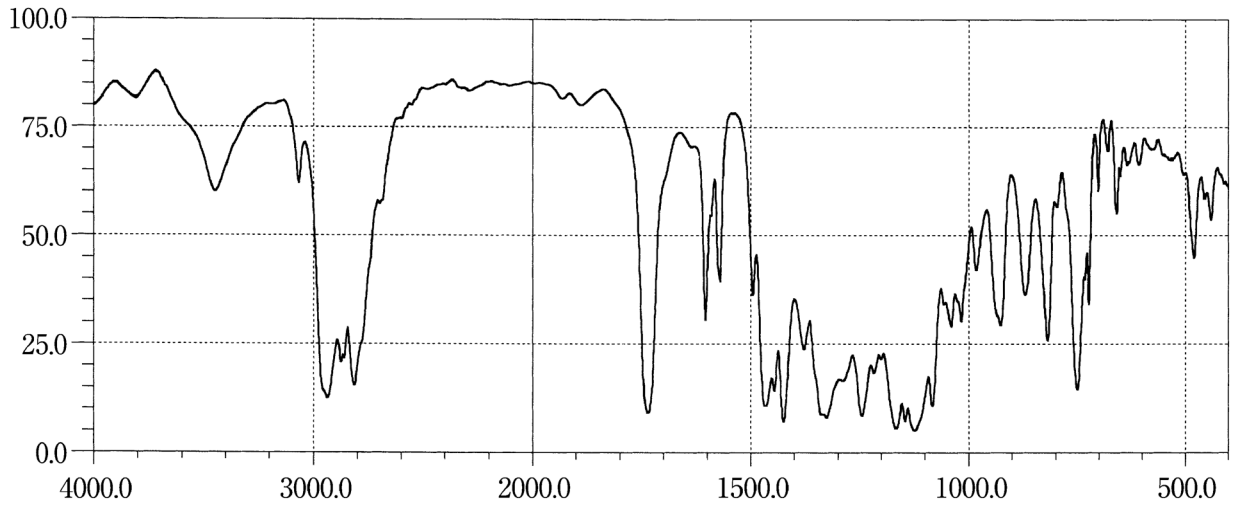


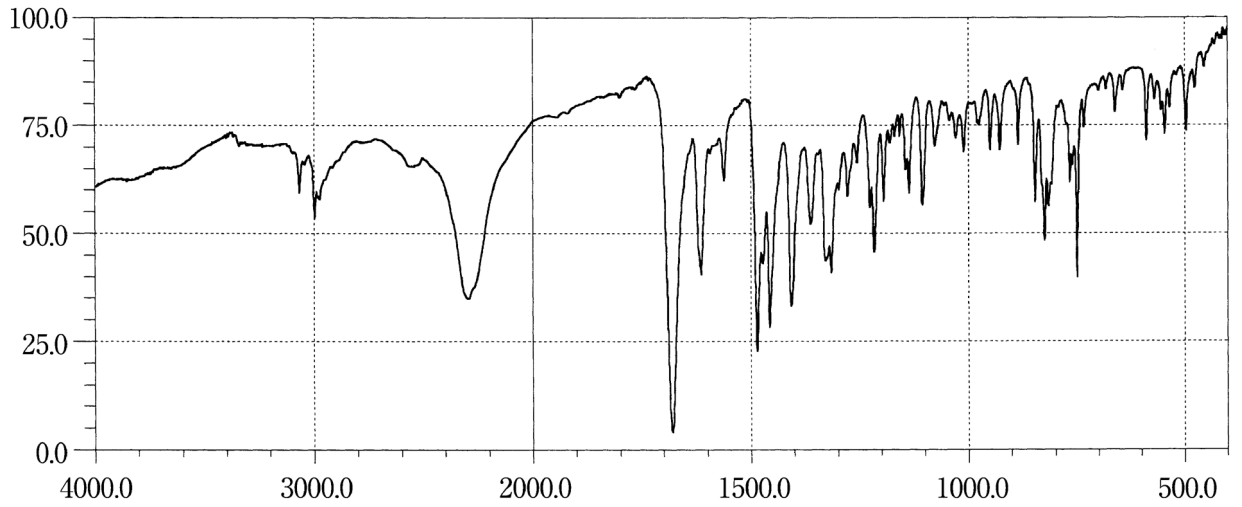
Fluoxymesterone



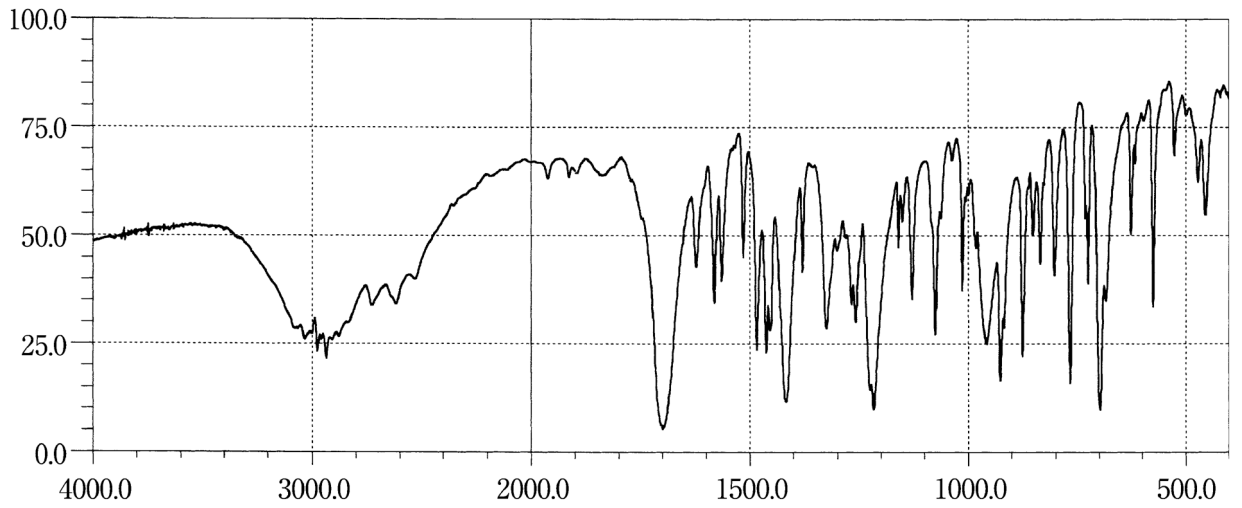
Fluphenazine Enanthate



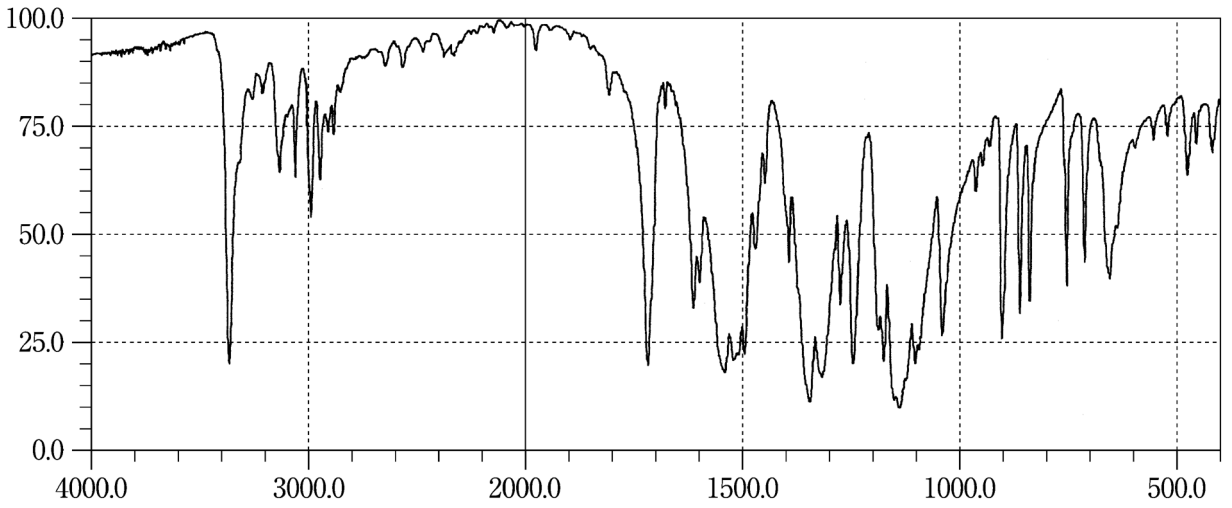
Flurazepam Hydrochloride



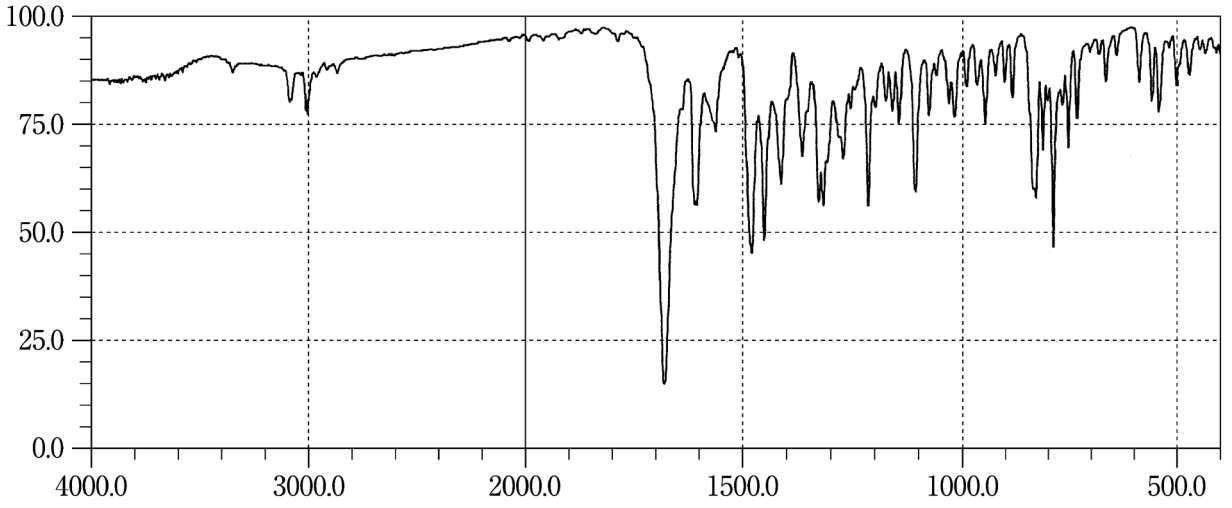
Flurbiprofen



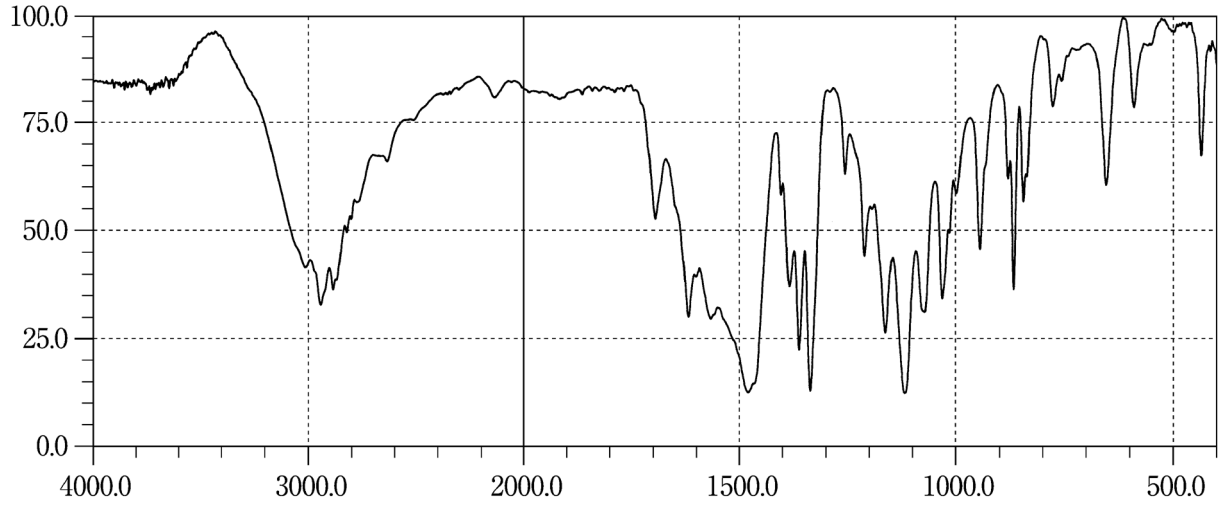
Flutamide



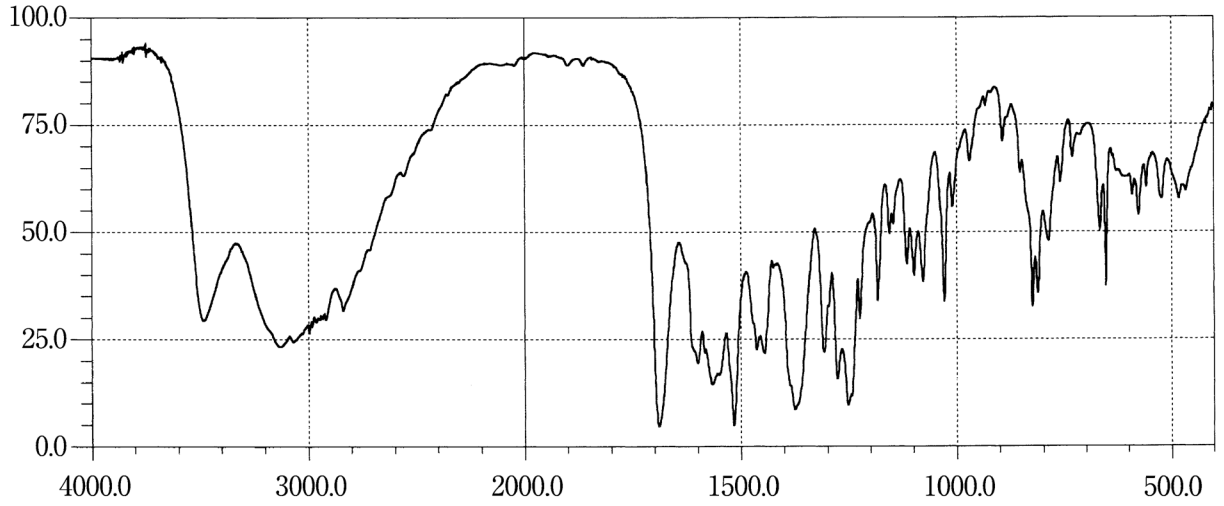
Flutoprazepam



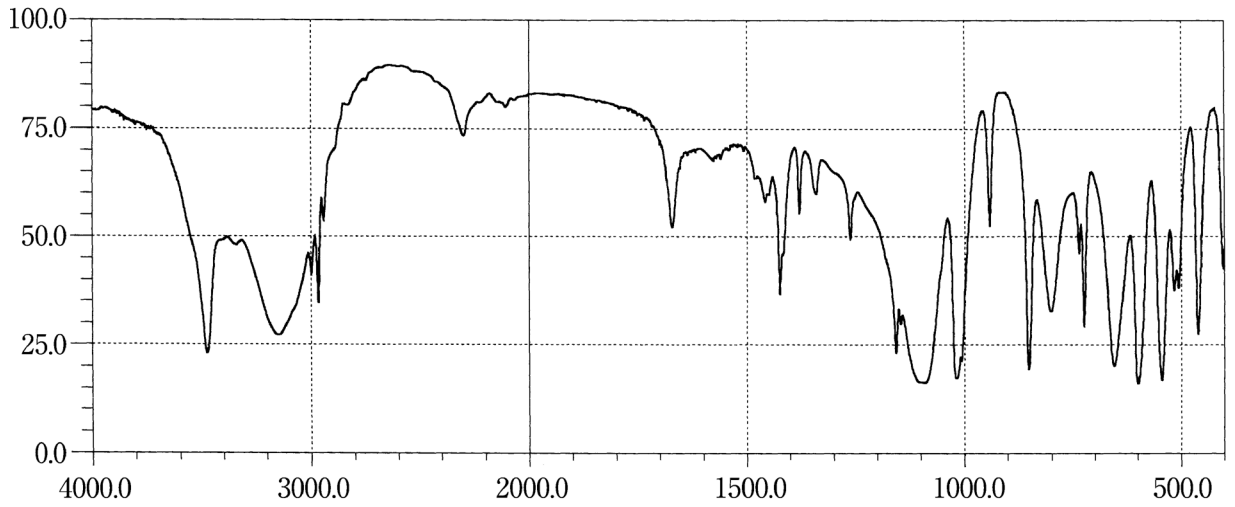
Fluvoxamine Maleate



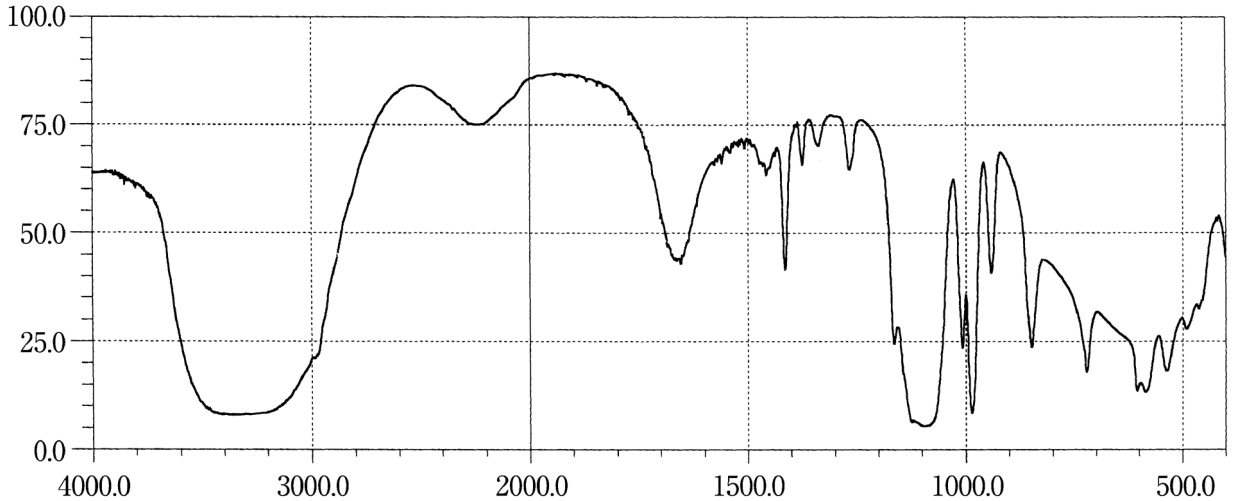
Formoterol Fumarate Hydrate



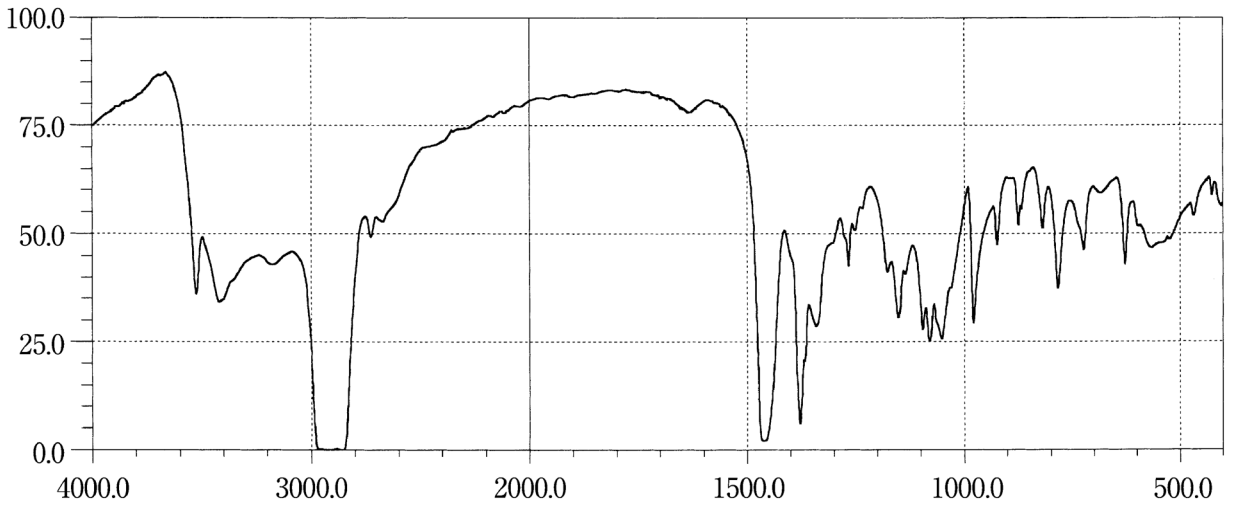
Fosfomycin Calcium Hydrate



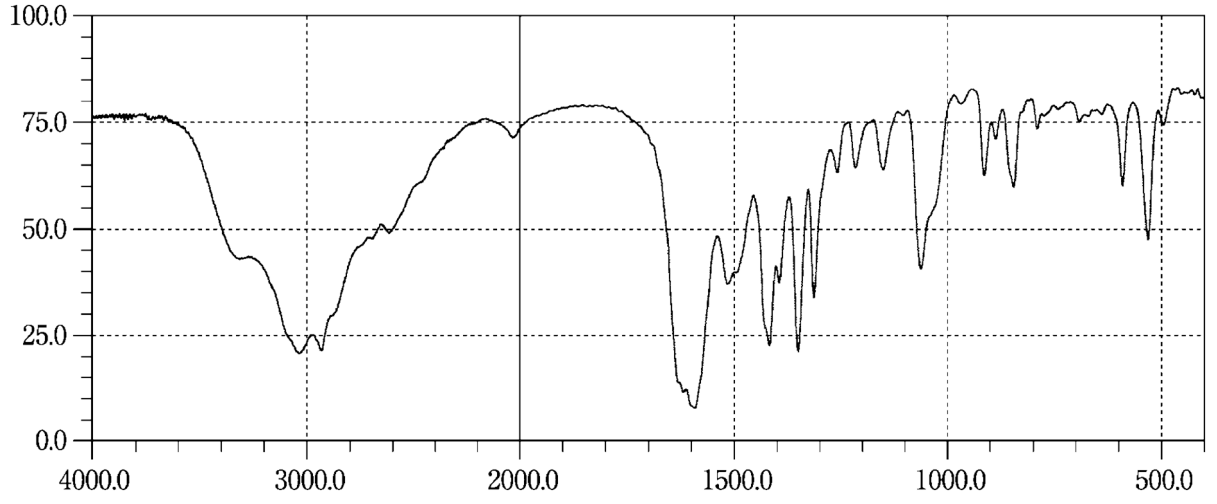
Fosfomycin Sodium



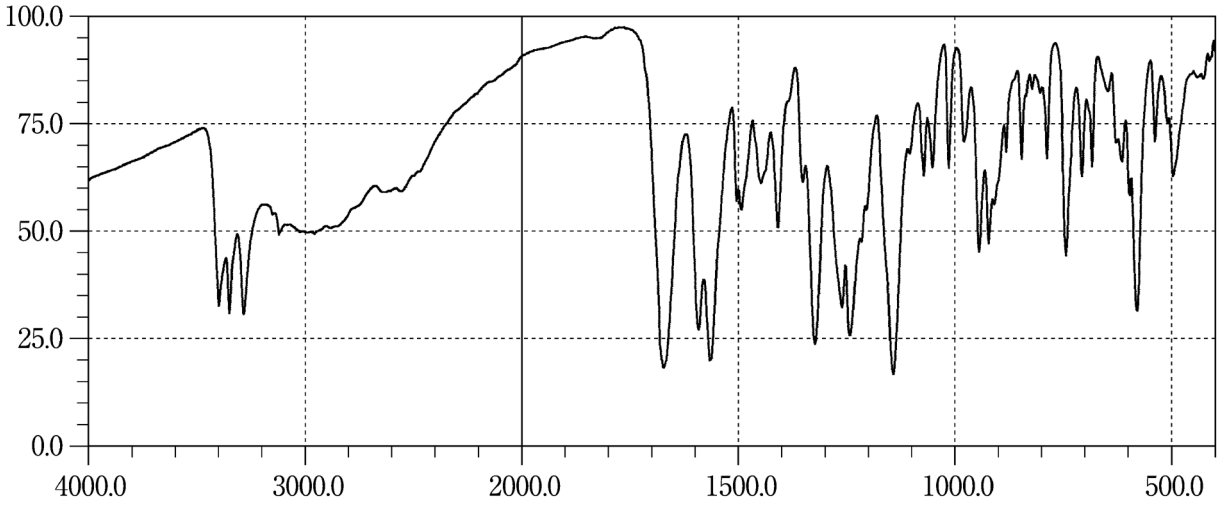
Fructose



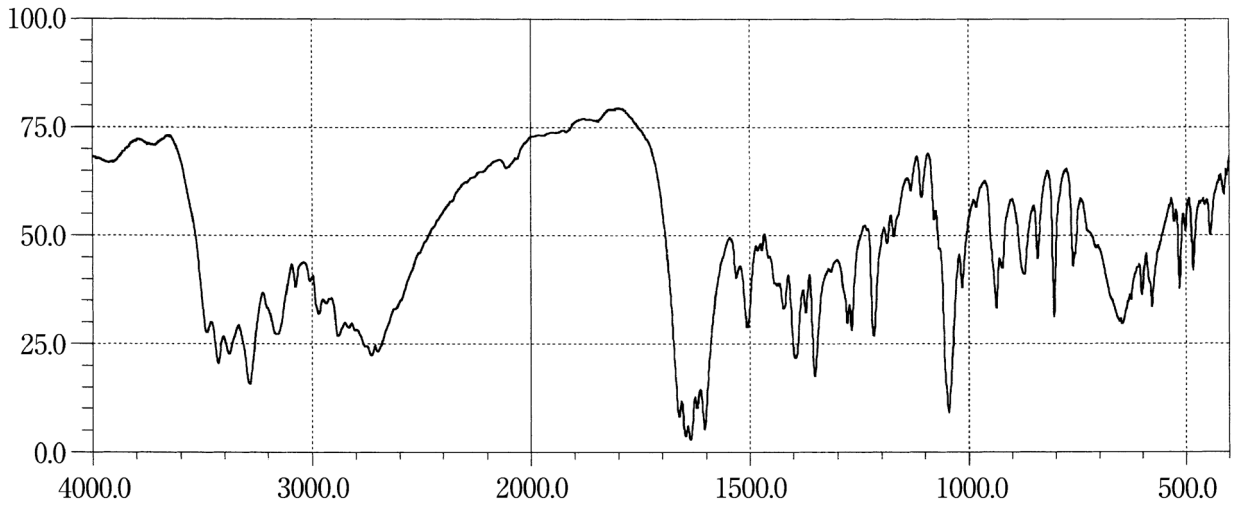
Fudosteine



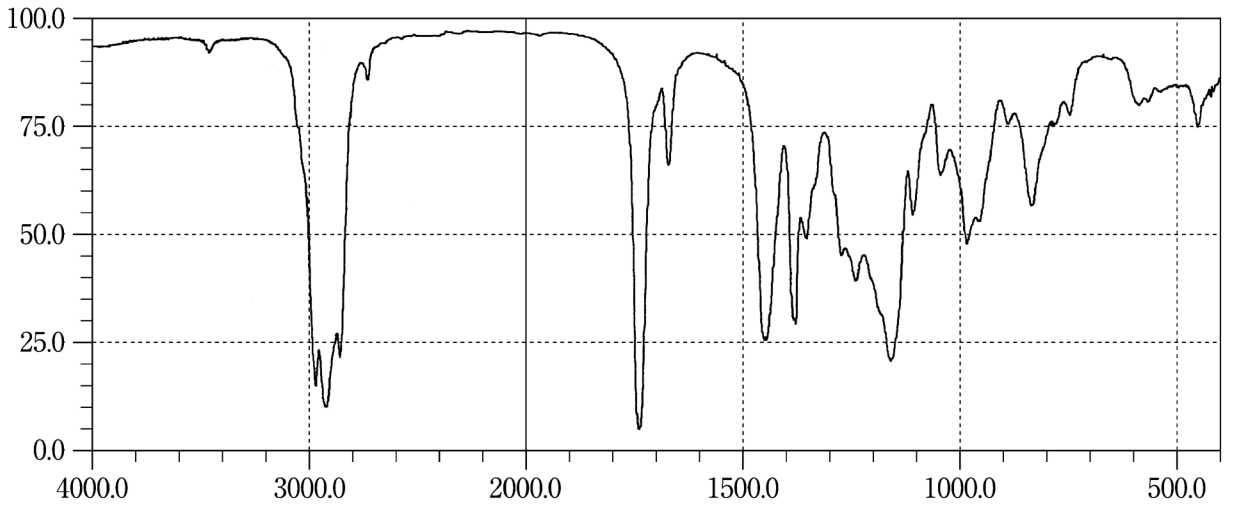
Furosemide



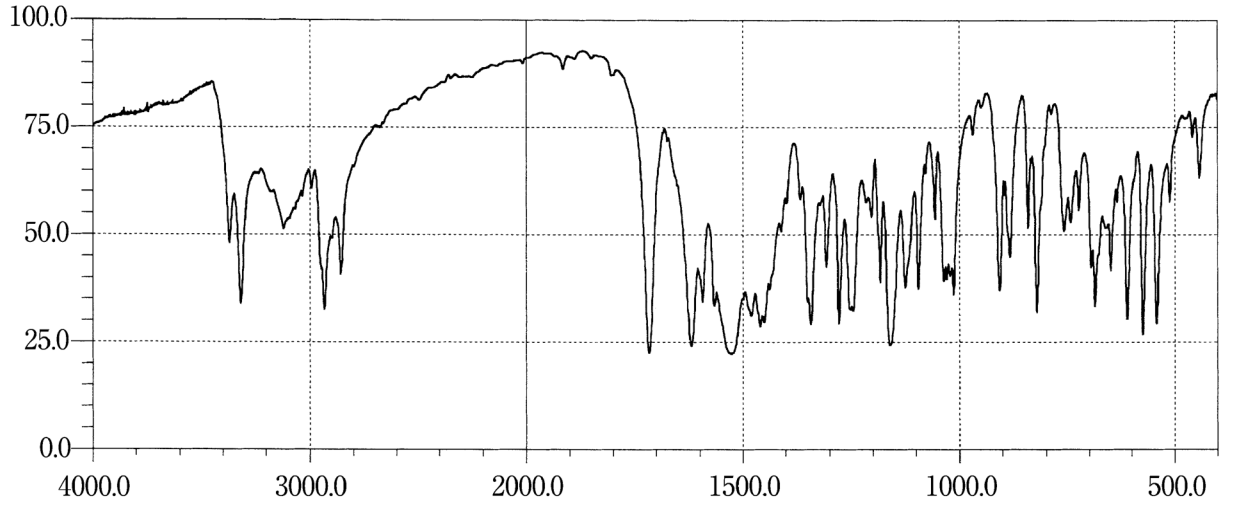
Fursultiamine Hydrochloride



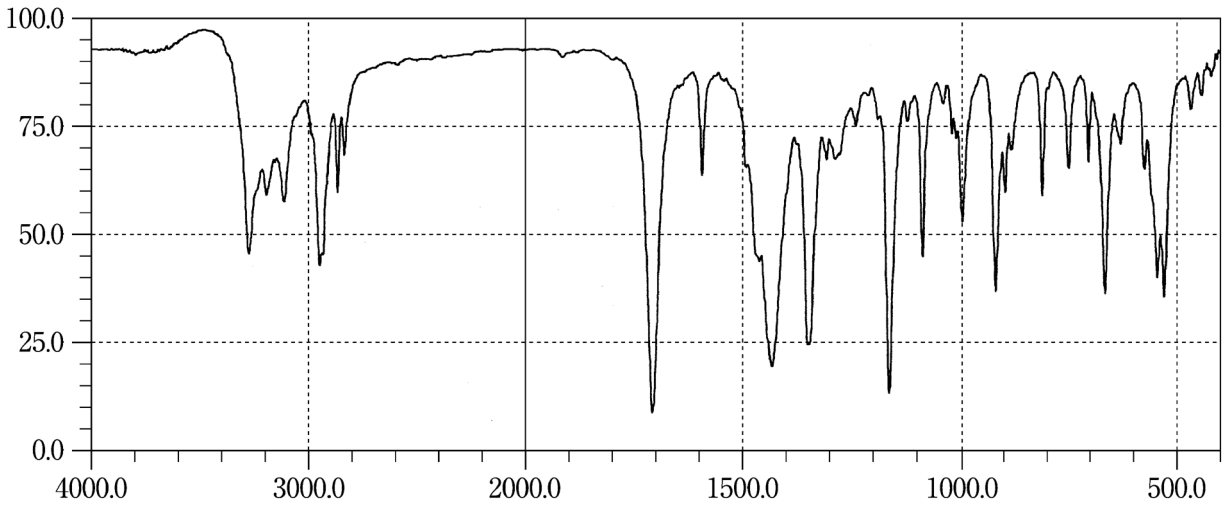
Gefarnate



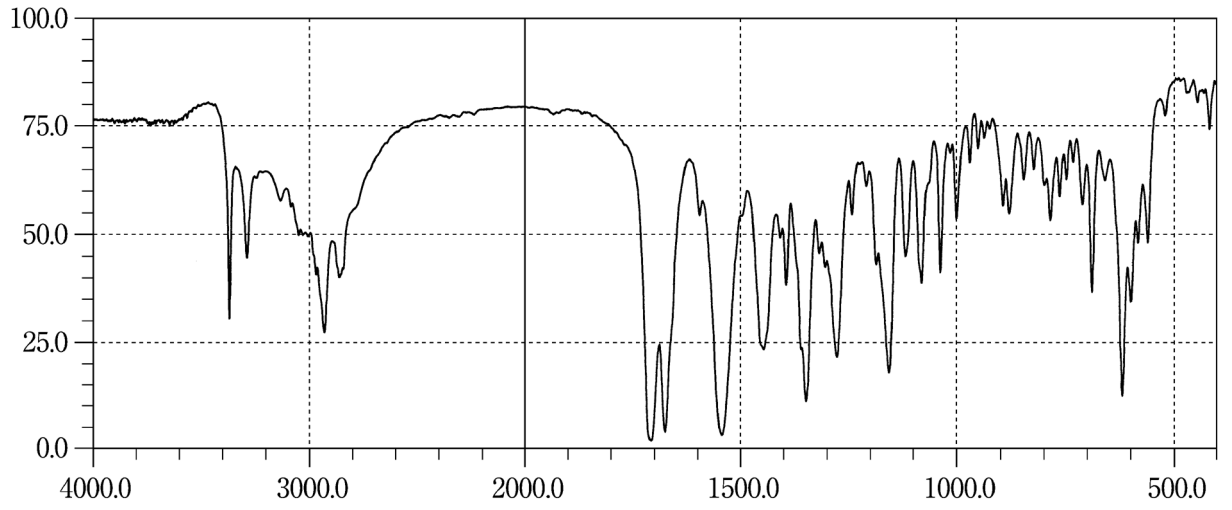
Glibenclamide



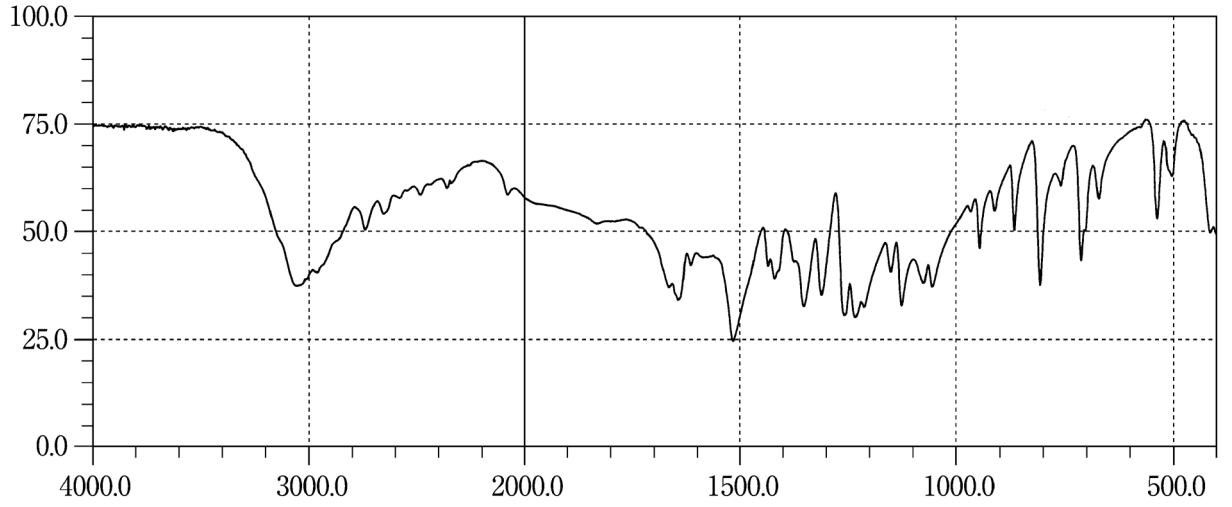
Gliclazide



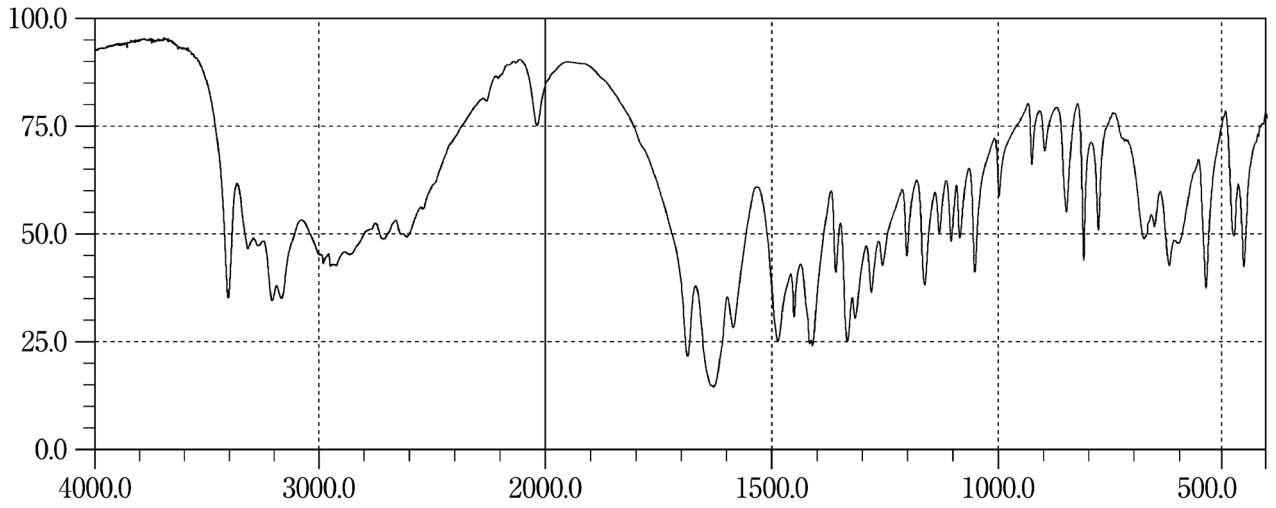
Glimepiride



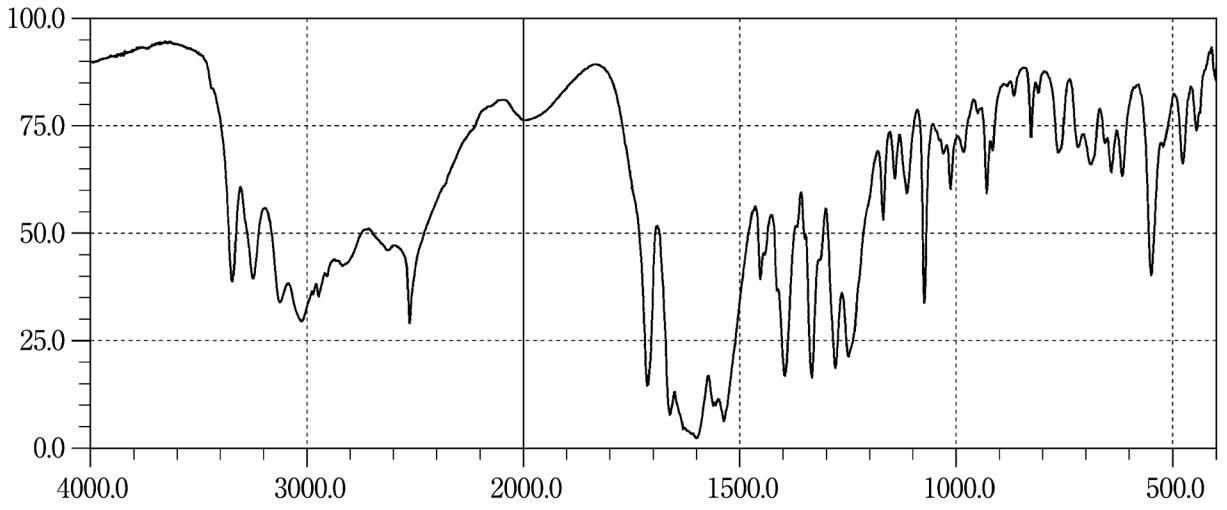
L-Glutamic Acid



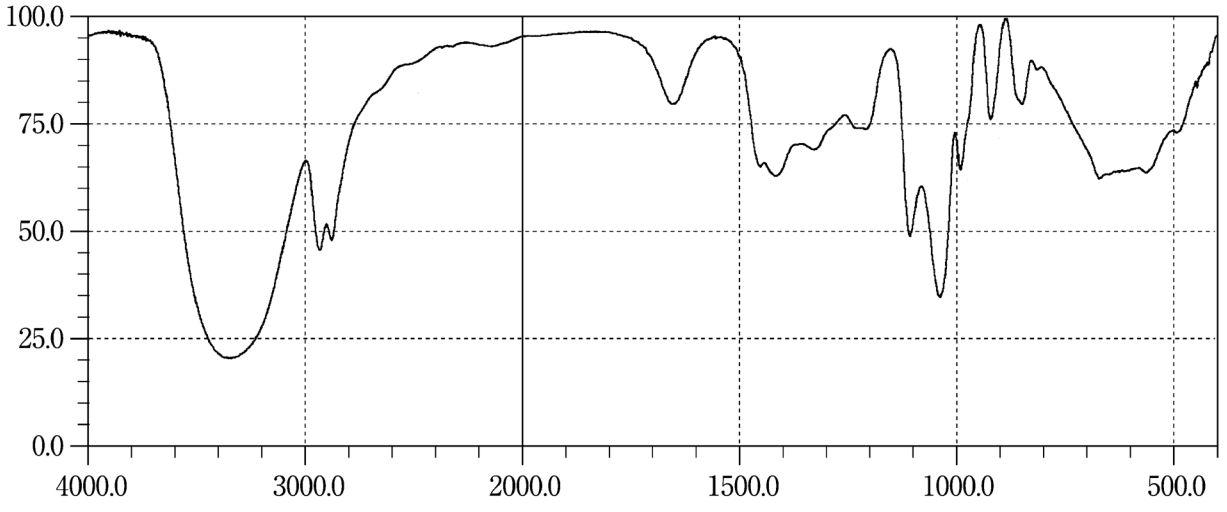
L-Glutamine



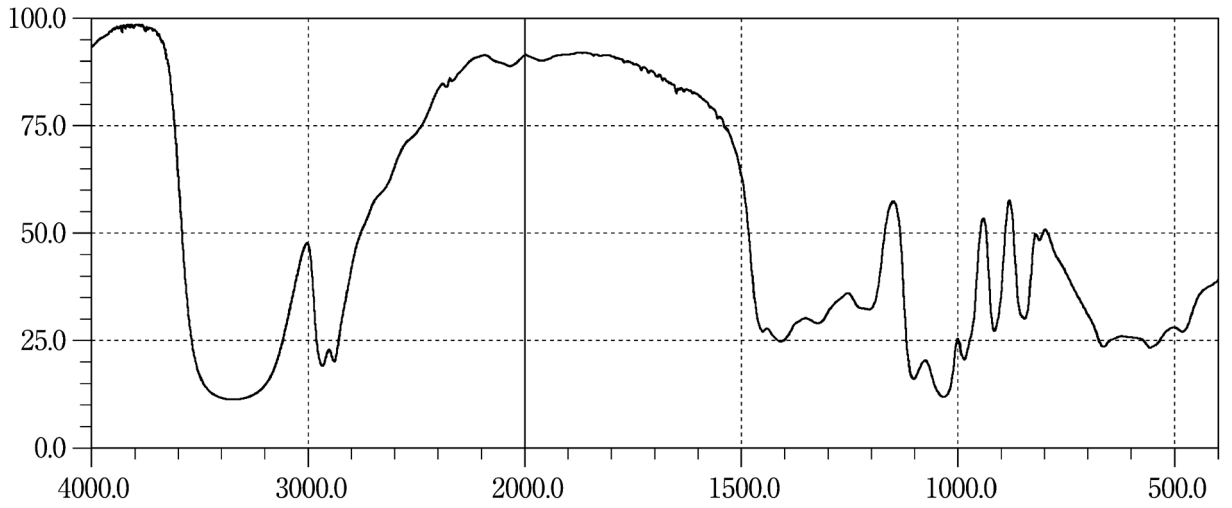
Glutathione



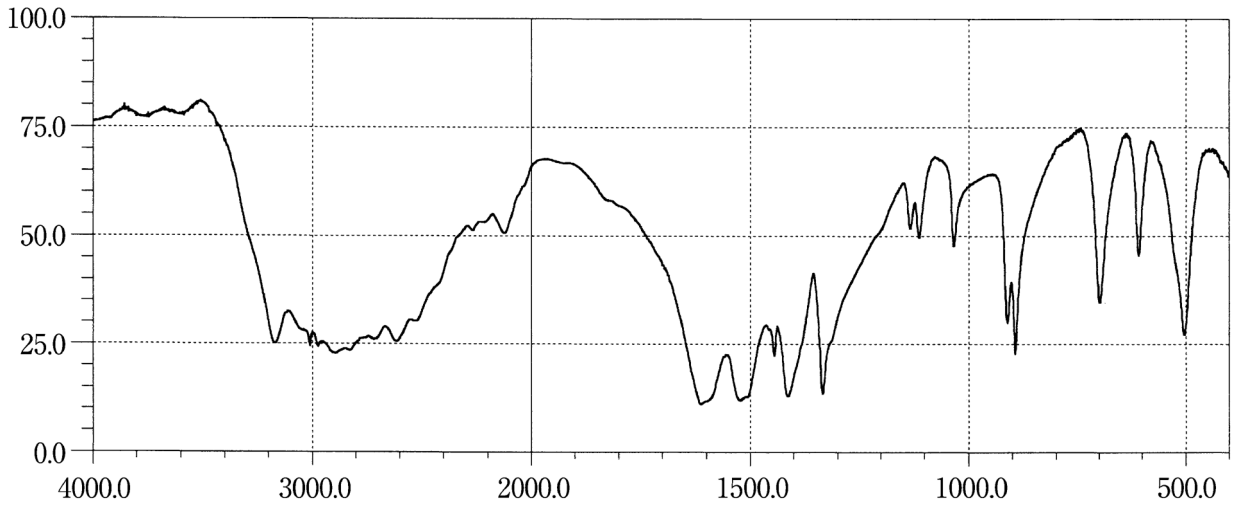
Glycerin



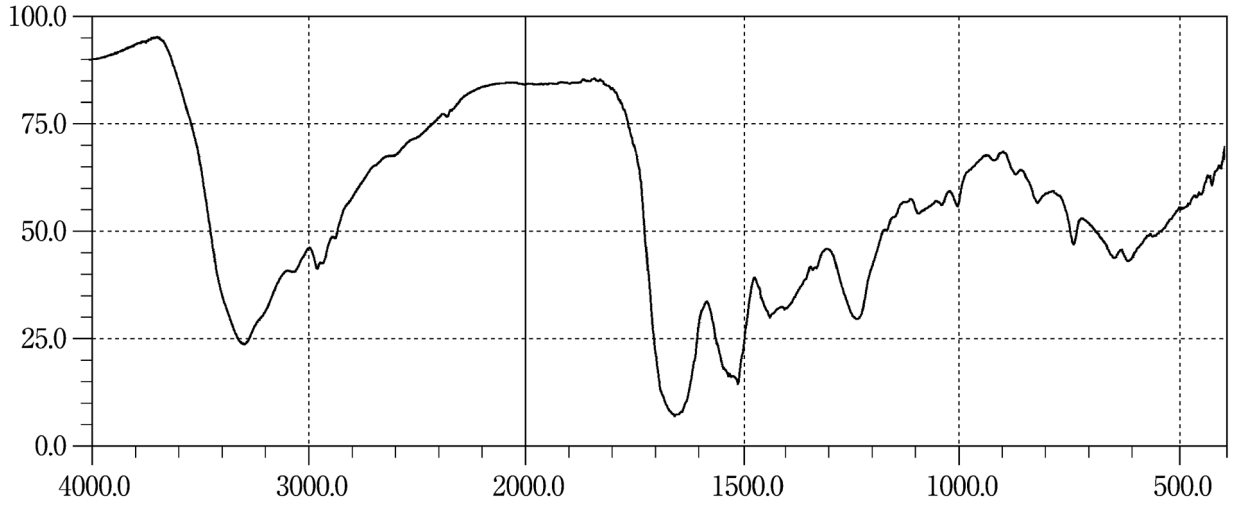
Concentrated Glycerin



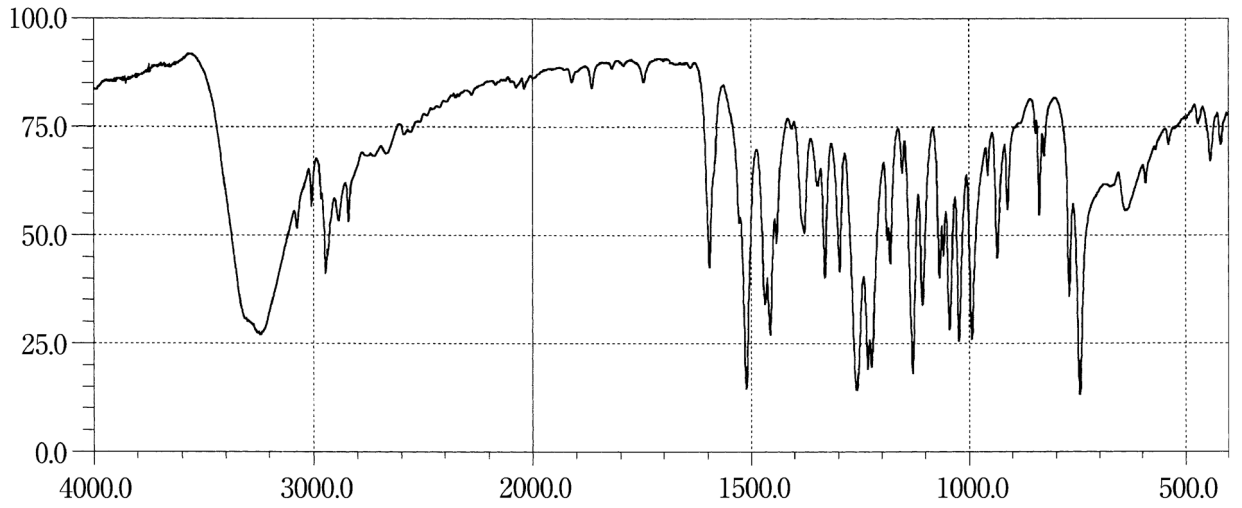
Glycine



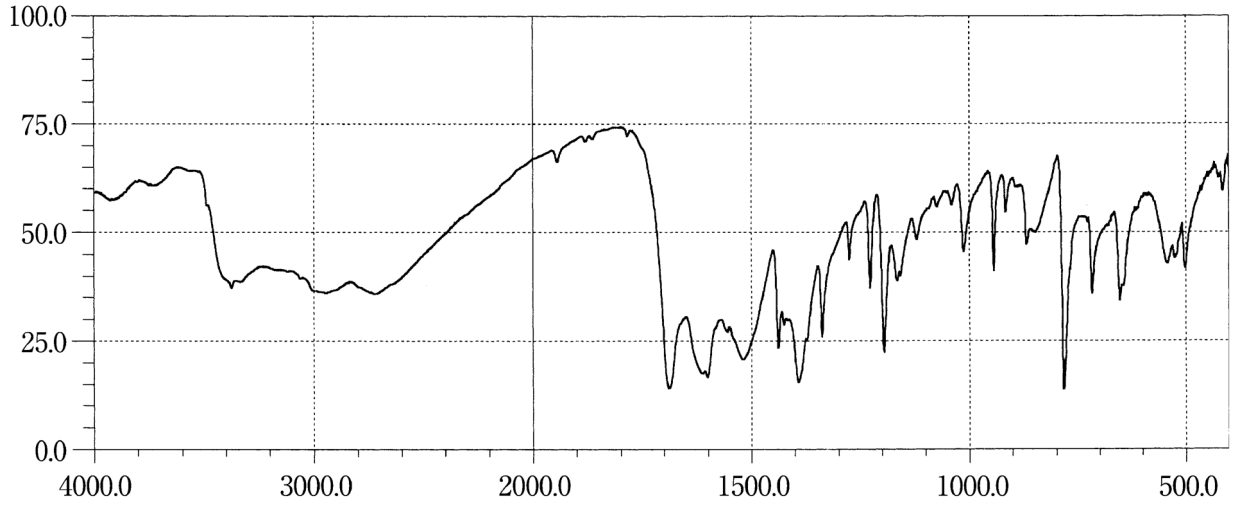
Gonadorelin Acetate



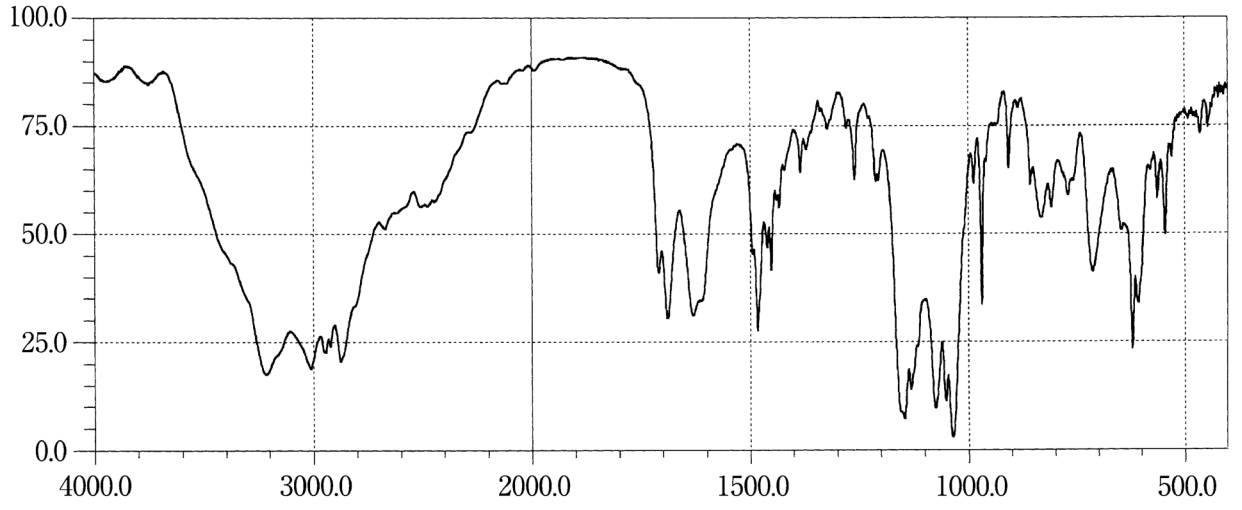
Guaifenesin



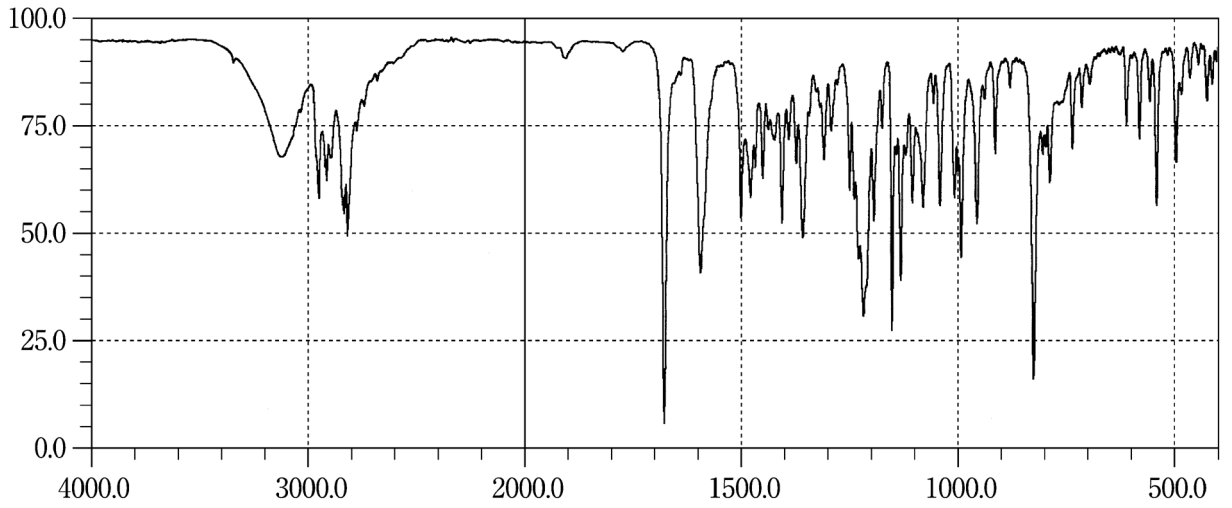
Guanabenz Acetate



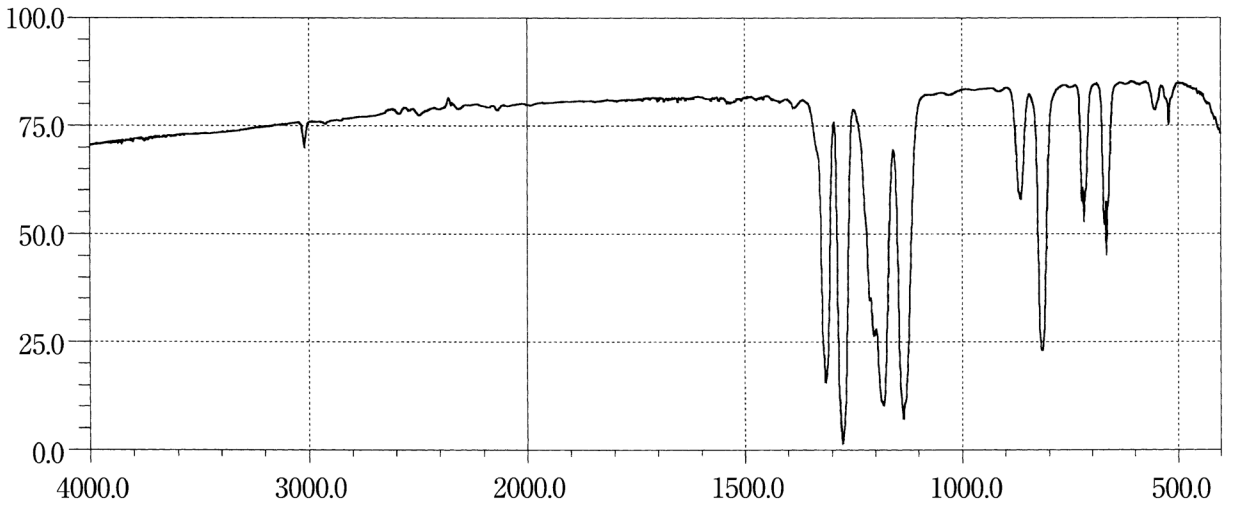
Guanethidine Sulfate



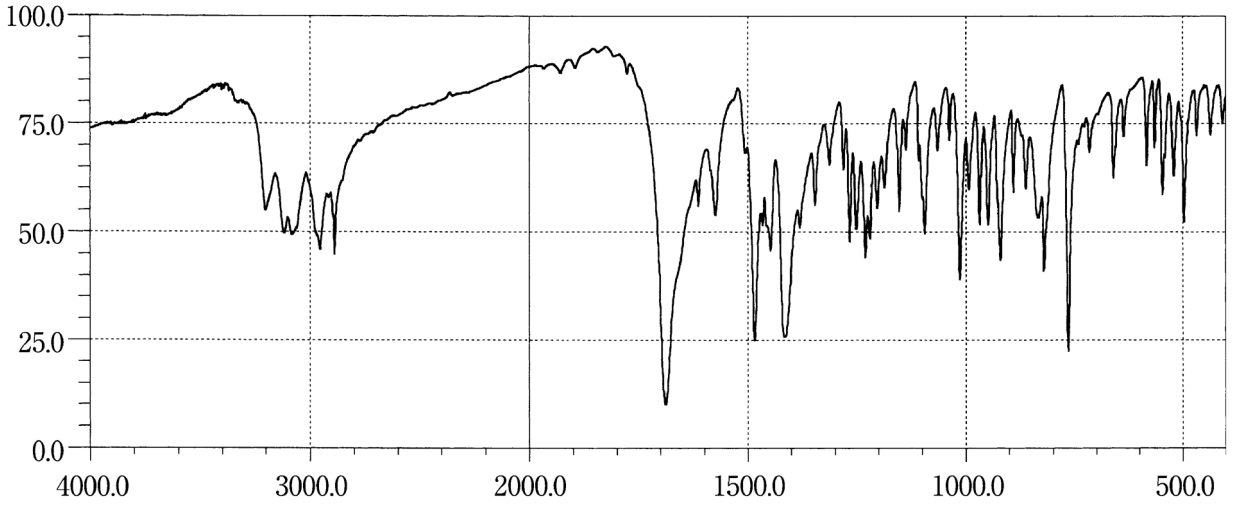
Haloperidol



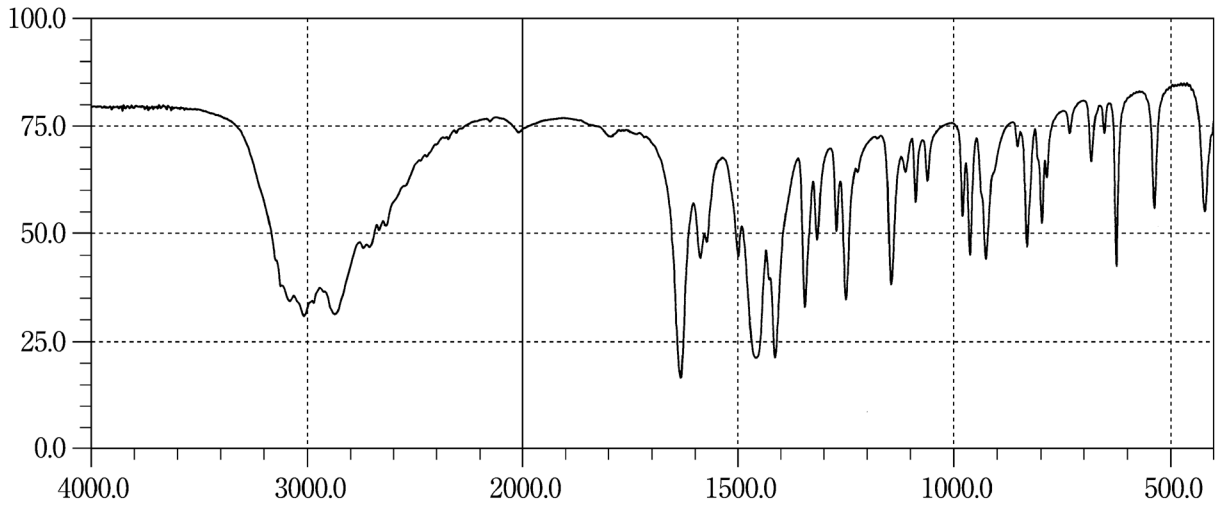
Halothane



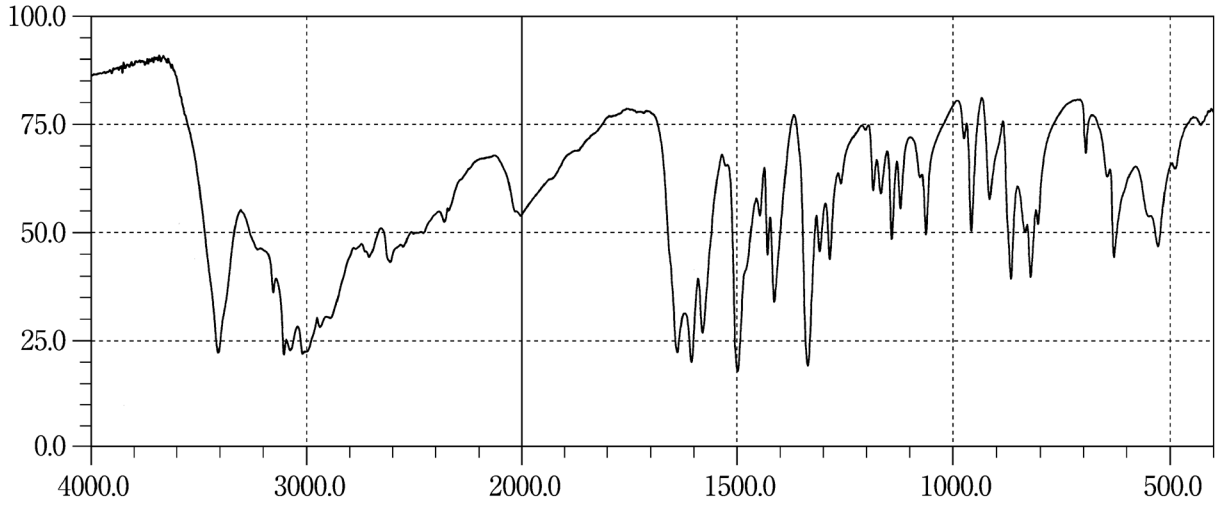
Haloxazolam



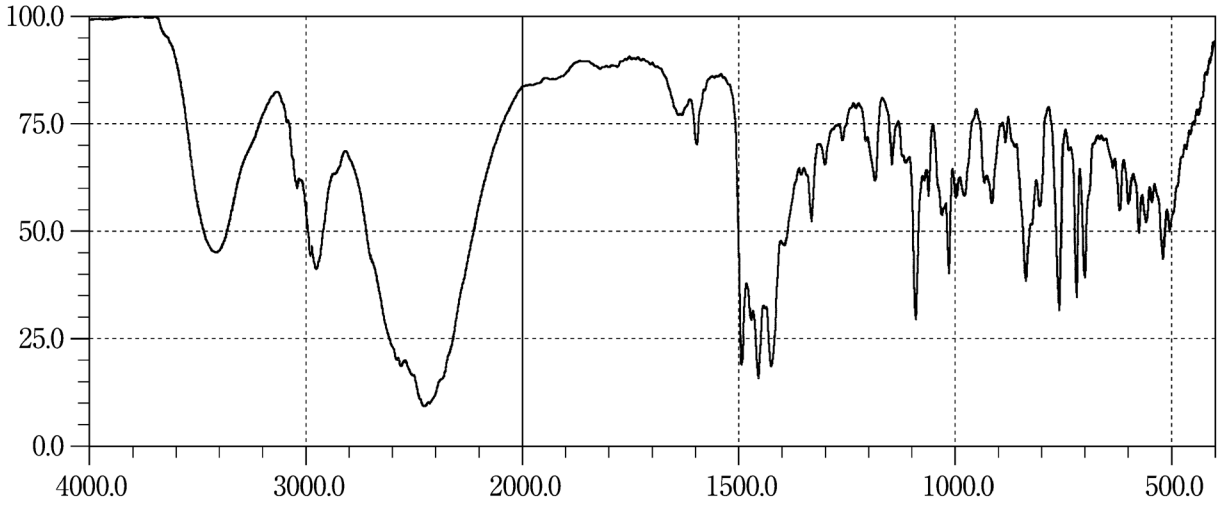
L-Histidine



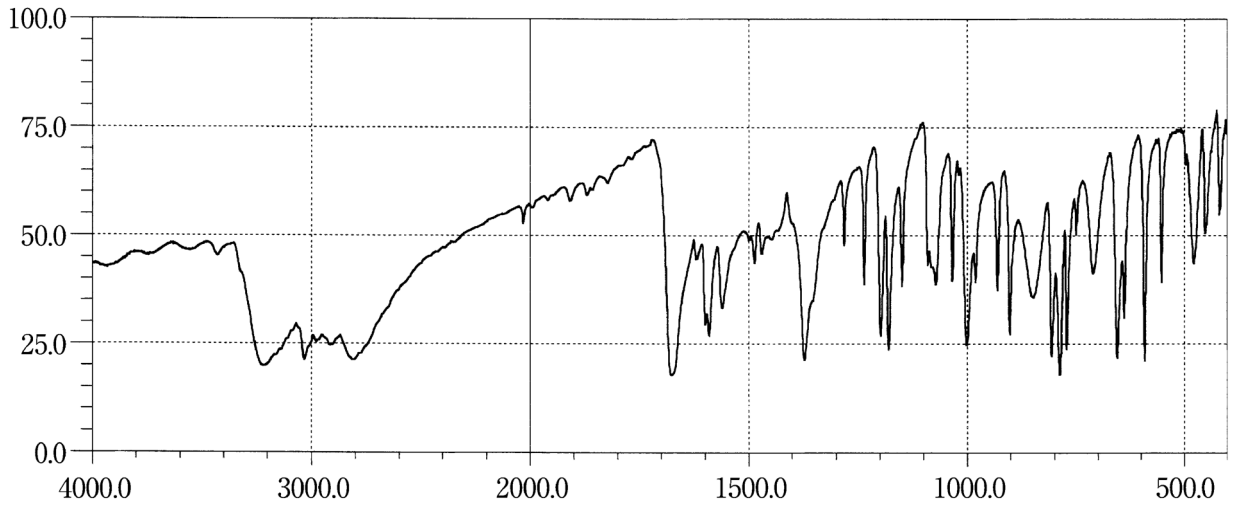
L-Histidine Hydrochloride Hydrate



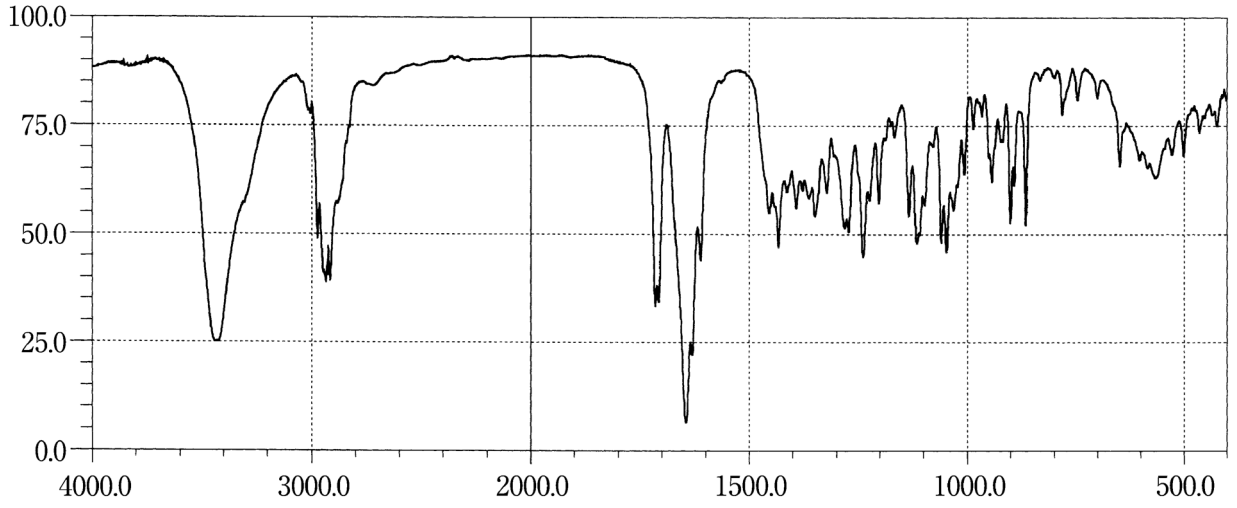
Homochlorcyclizine Hydrochloride



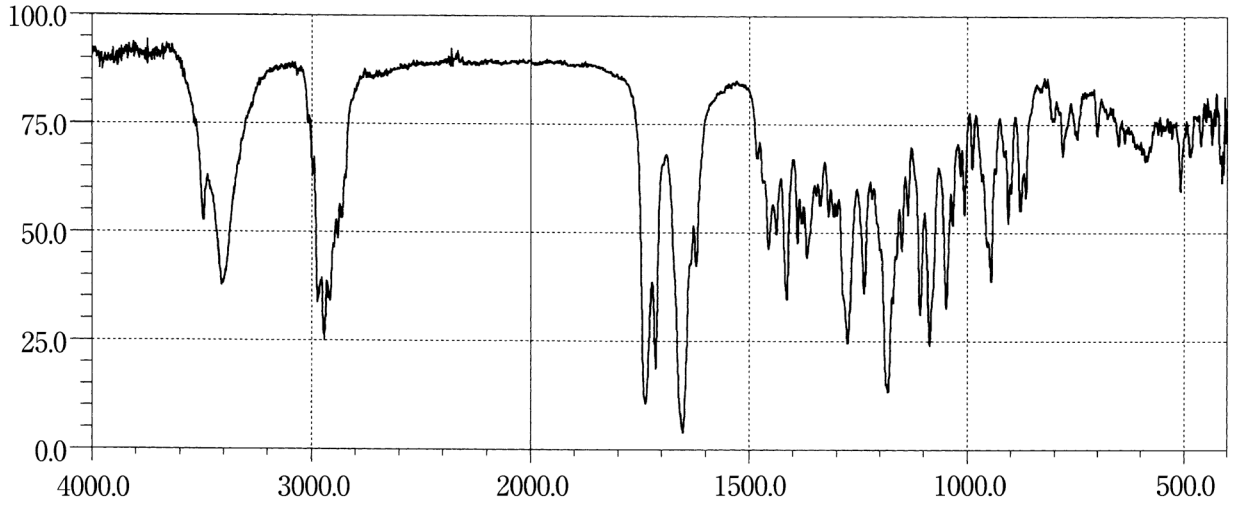
Hydralazine Hydrochloride



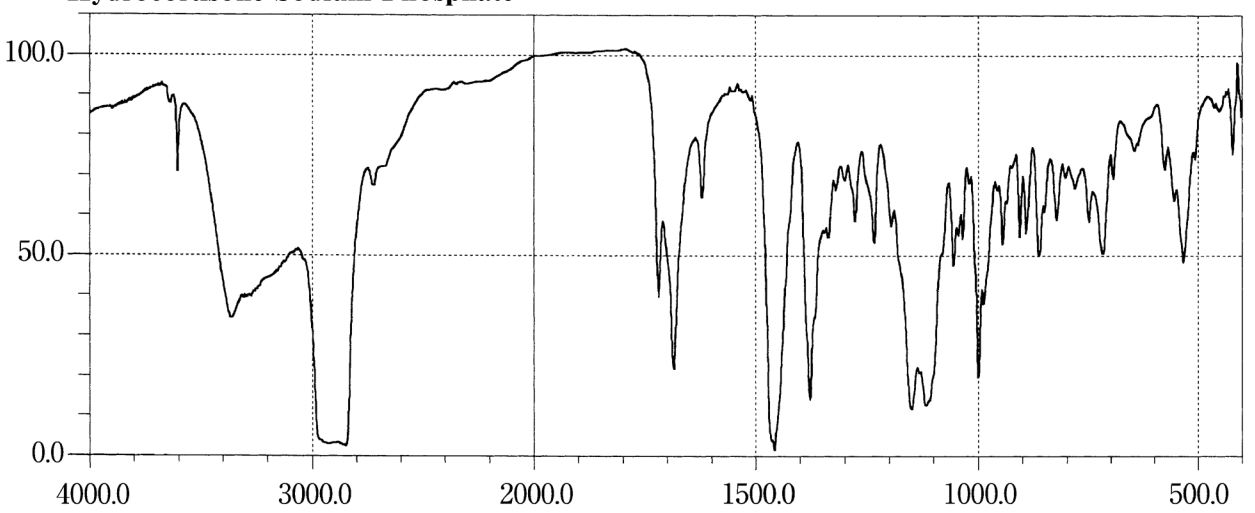
Hydrocortisone



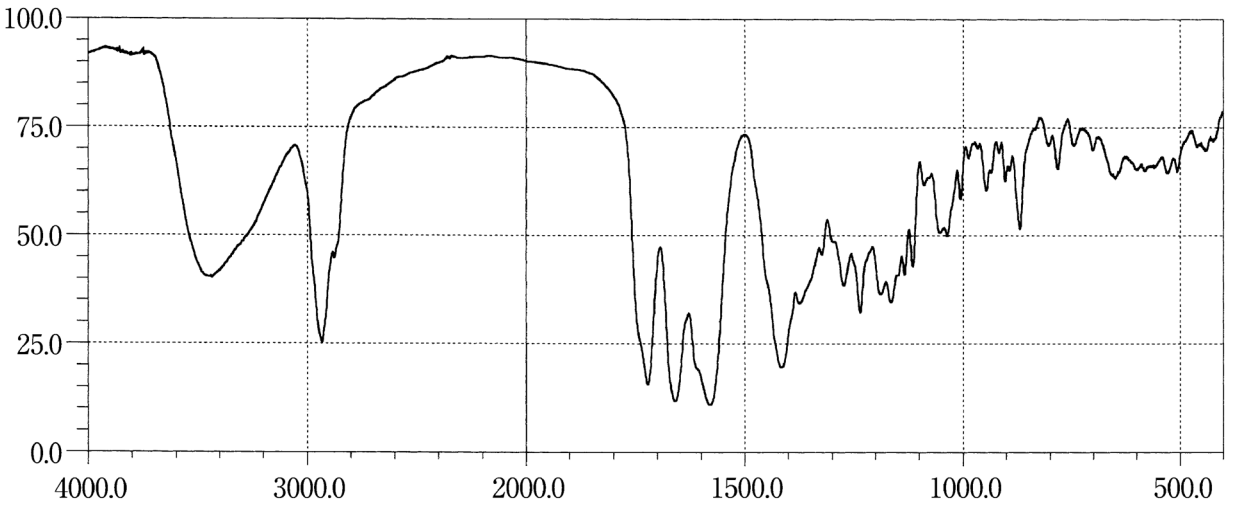
Hydrocortisone Butyrate



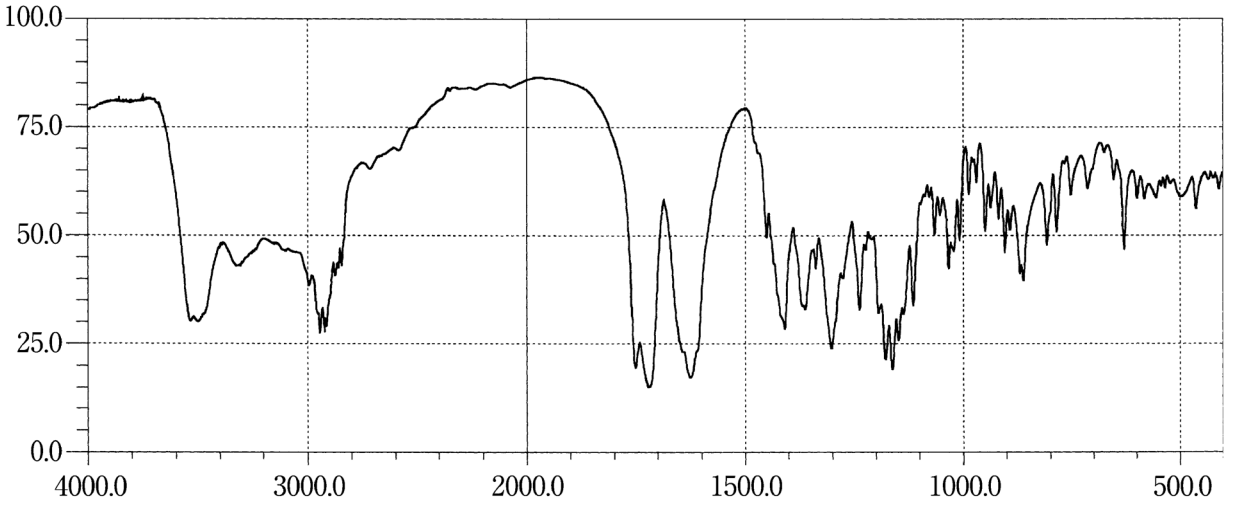
Hydrocortisone Sodium Phosphate



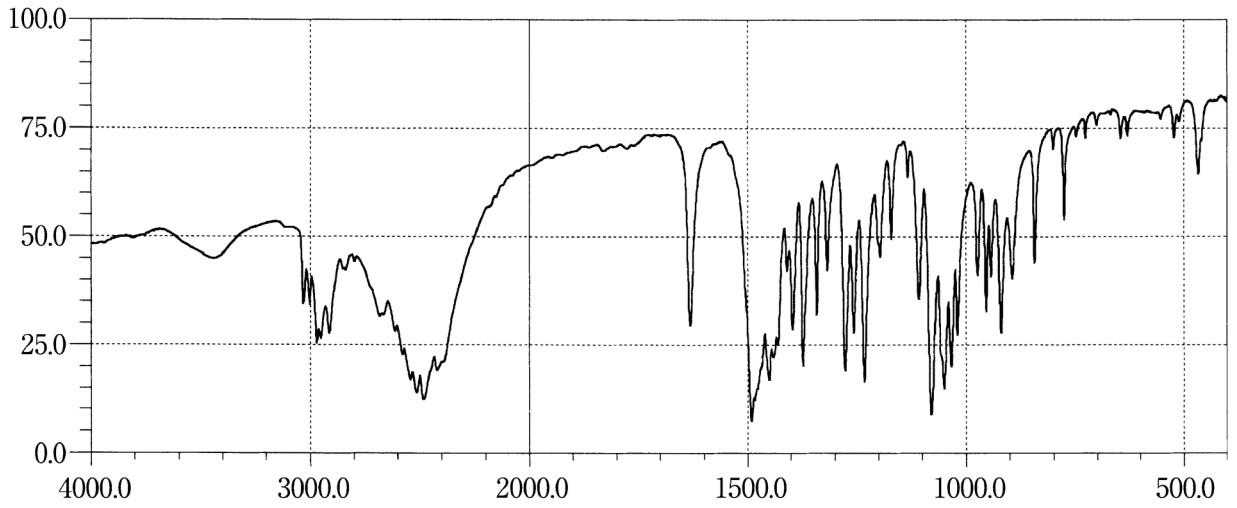
Hydrocortisone Sodium Succinate



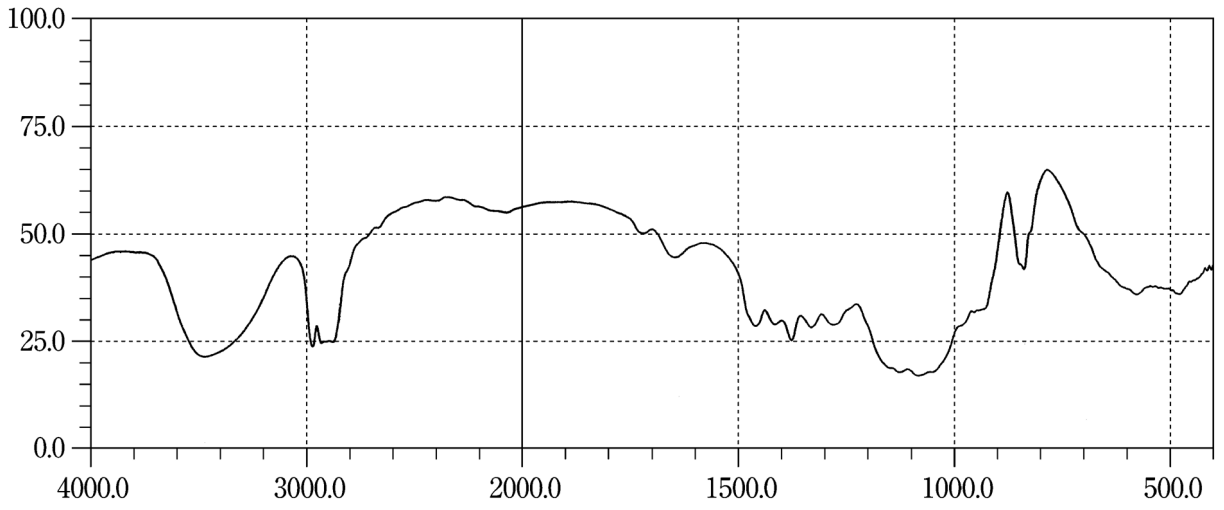
Hydrocortisone Succinate



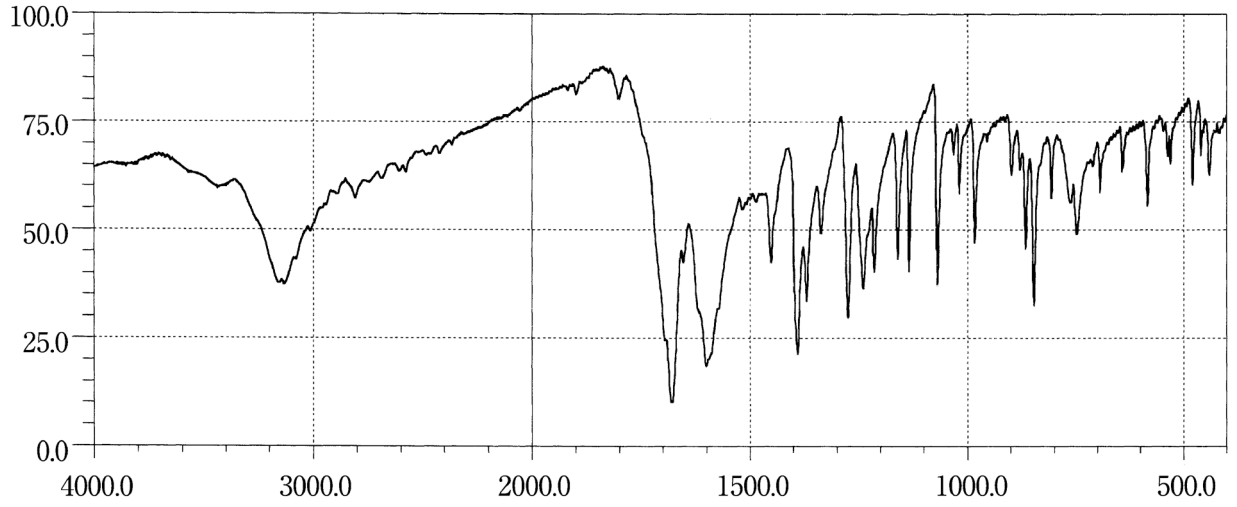
Hydrocotarnine Hydrochloride Hydrate



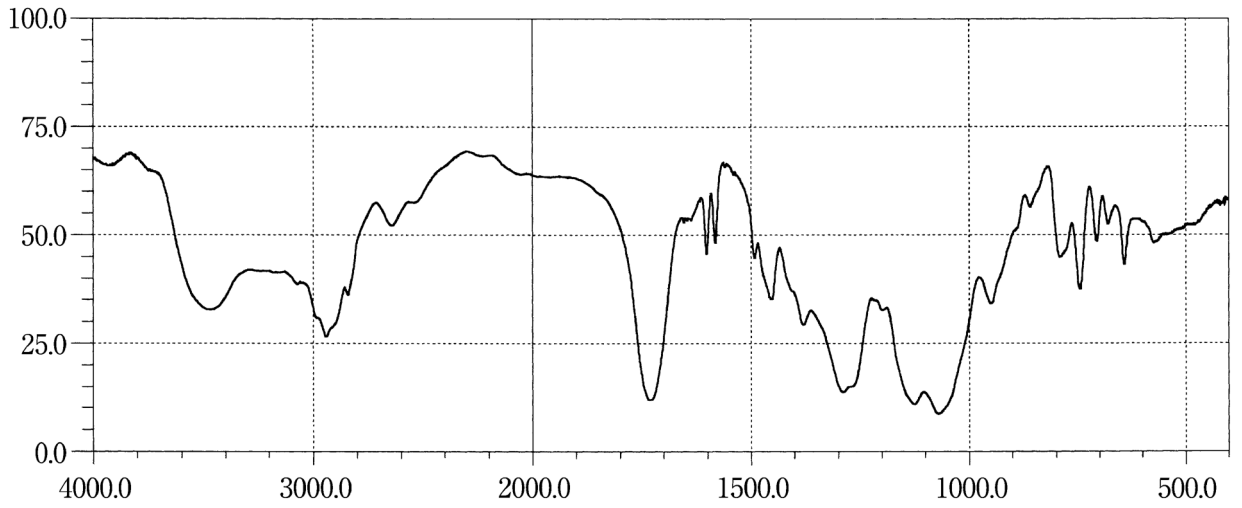
Hydroxypropylcellulose



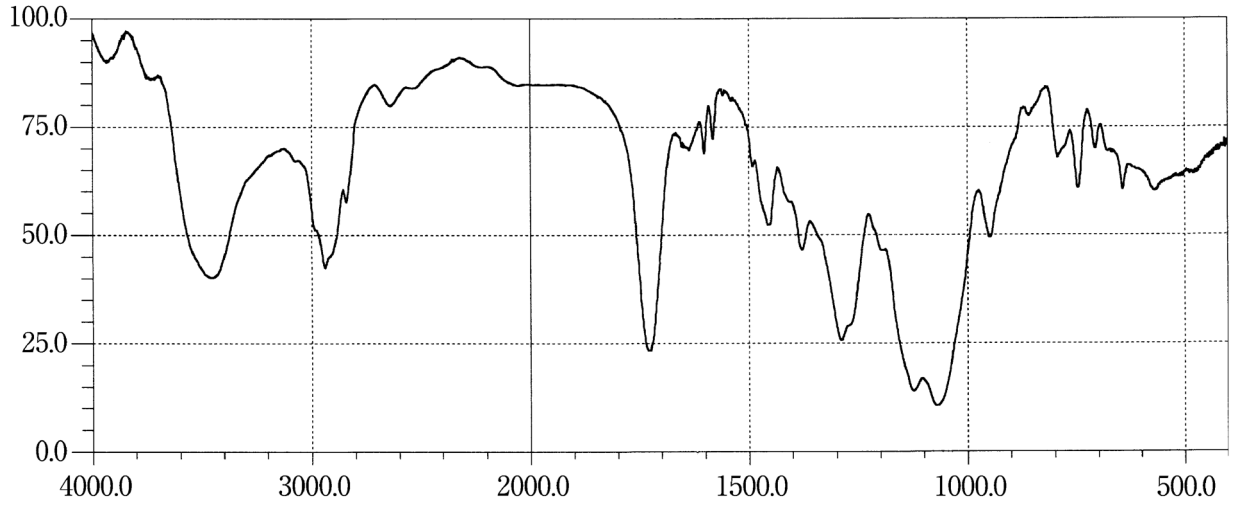
Hymecromone



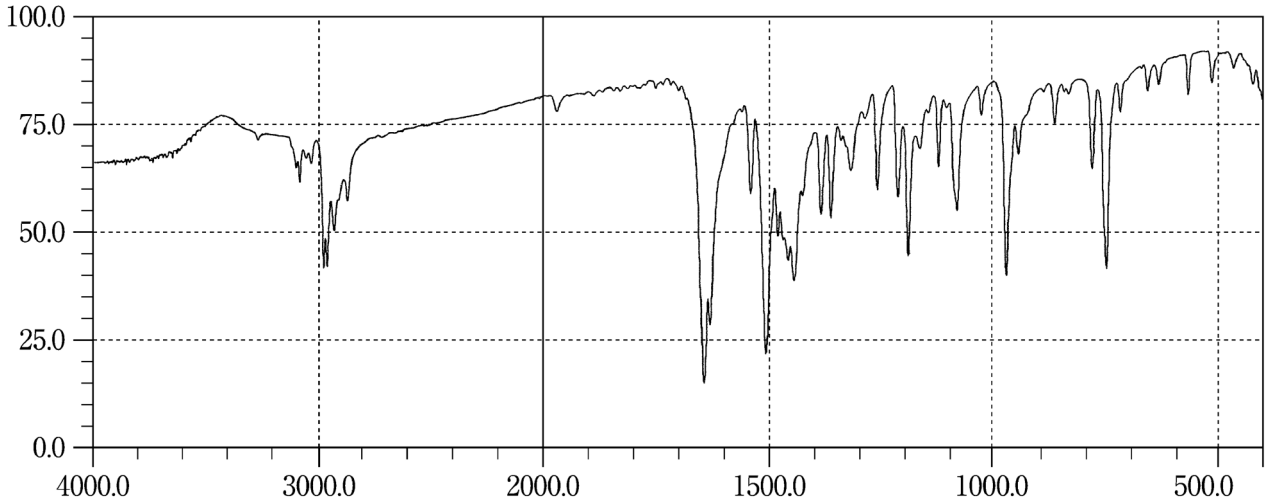
Hypromellose Phthalate (200731)



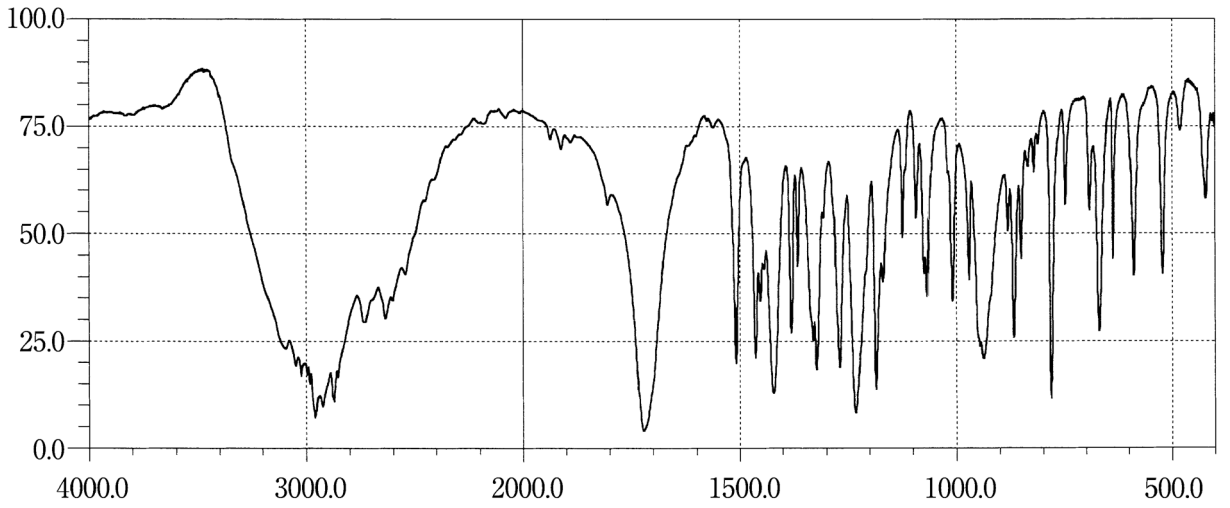
Hypromellose Phthalate (220824)



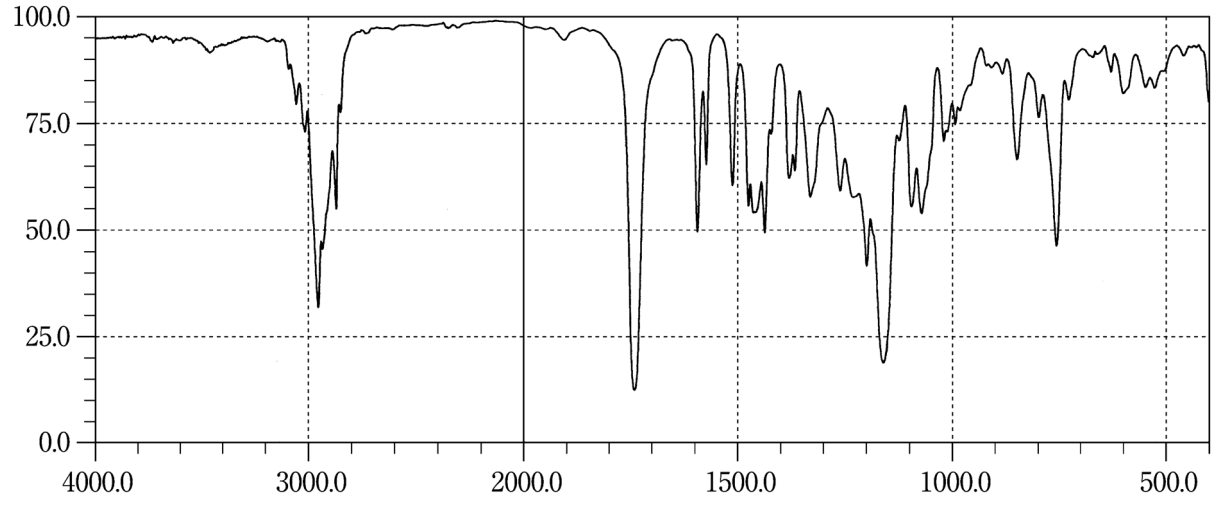
Ibudilast



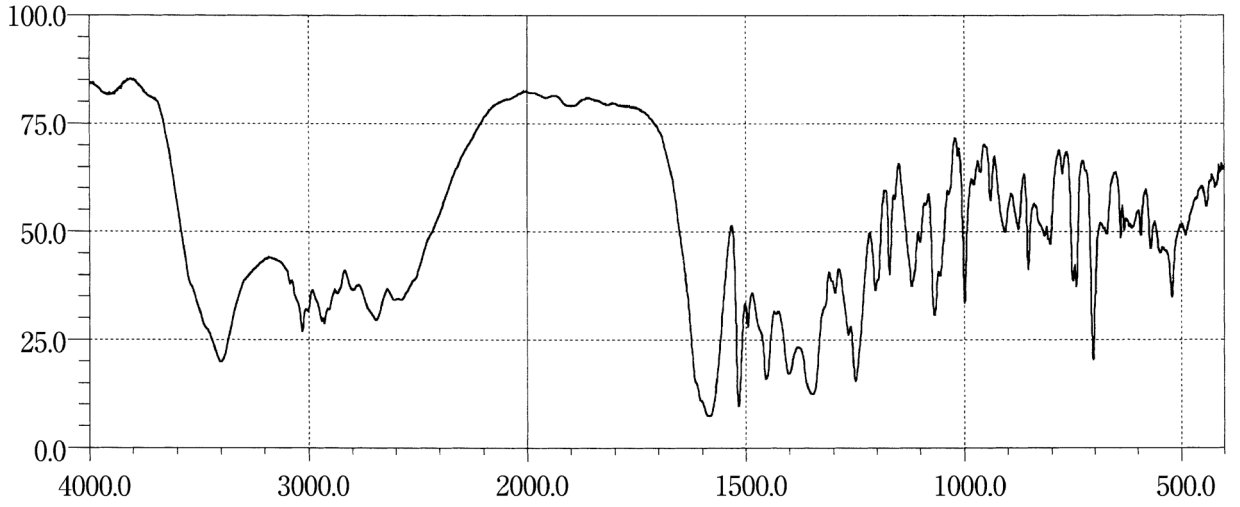
Ibuprofen



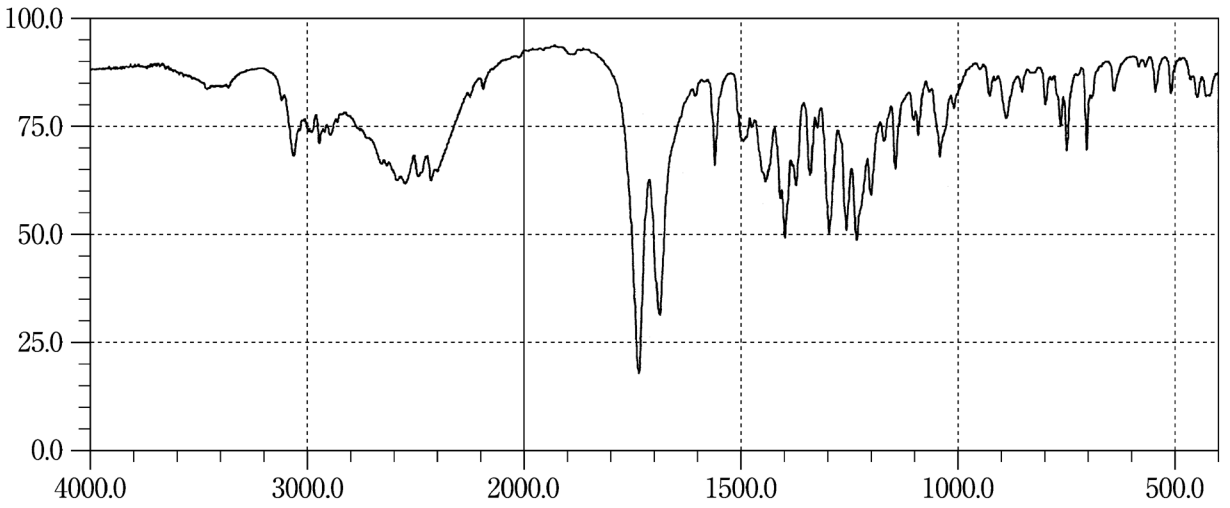
Ibuprofen Piconol



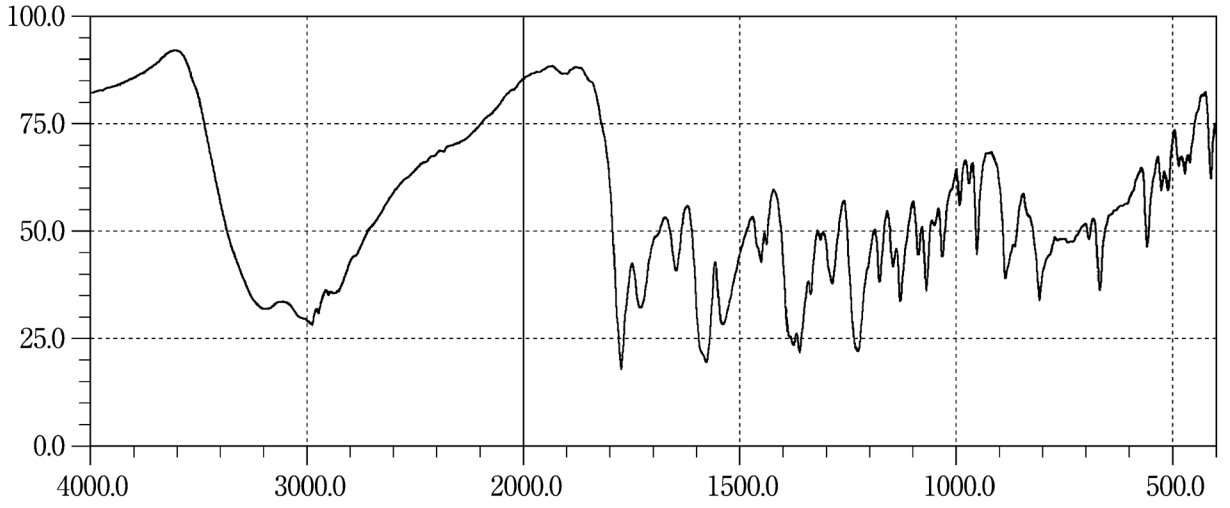
Ifenprodil Tartrate



Imidapril Hydrochloride



Imipenem Hydrate



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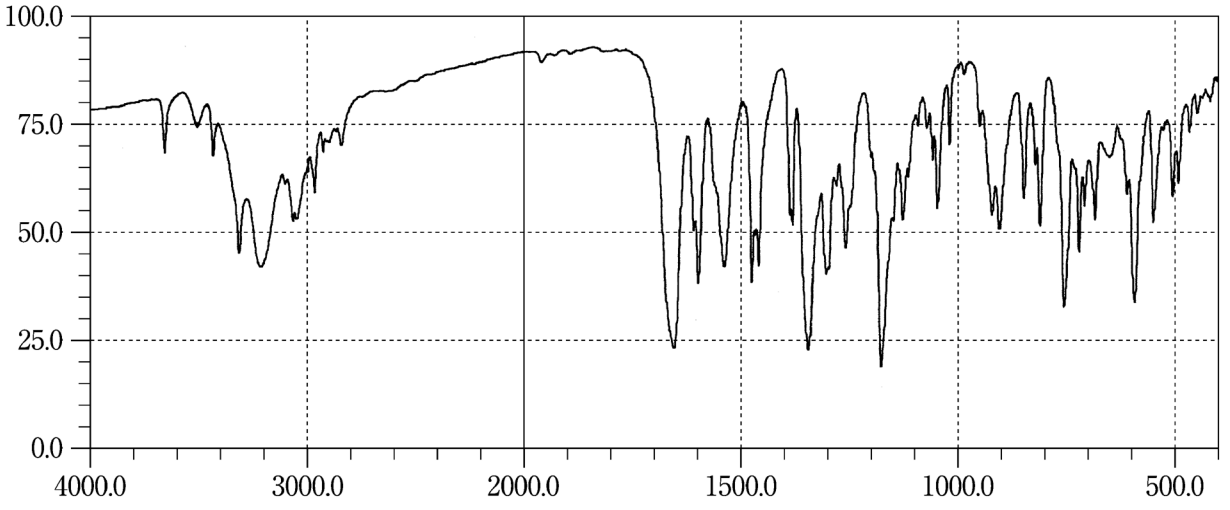
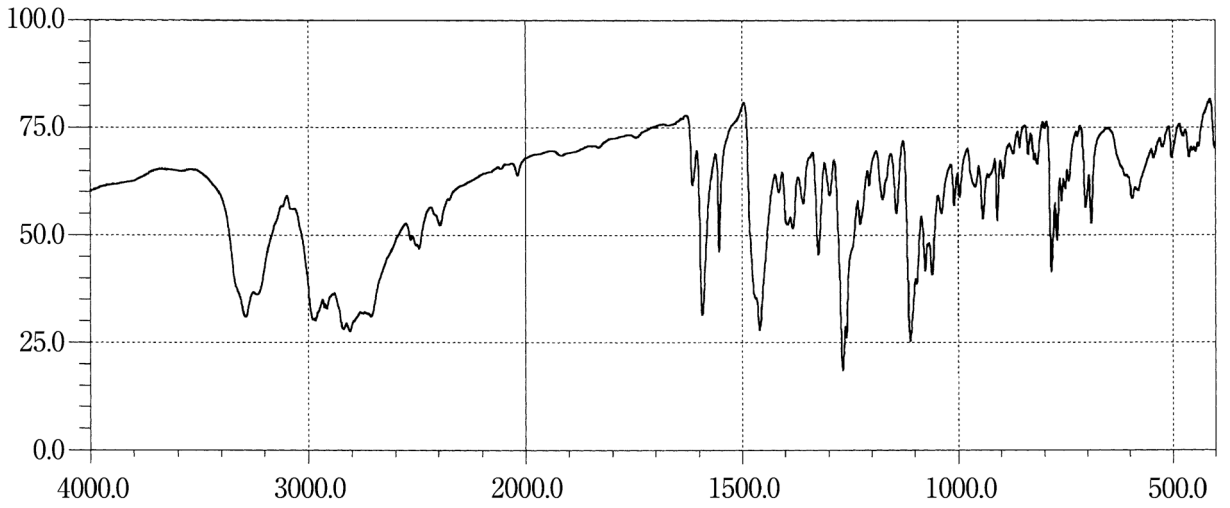
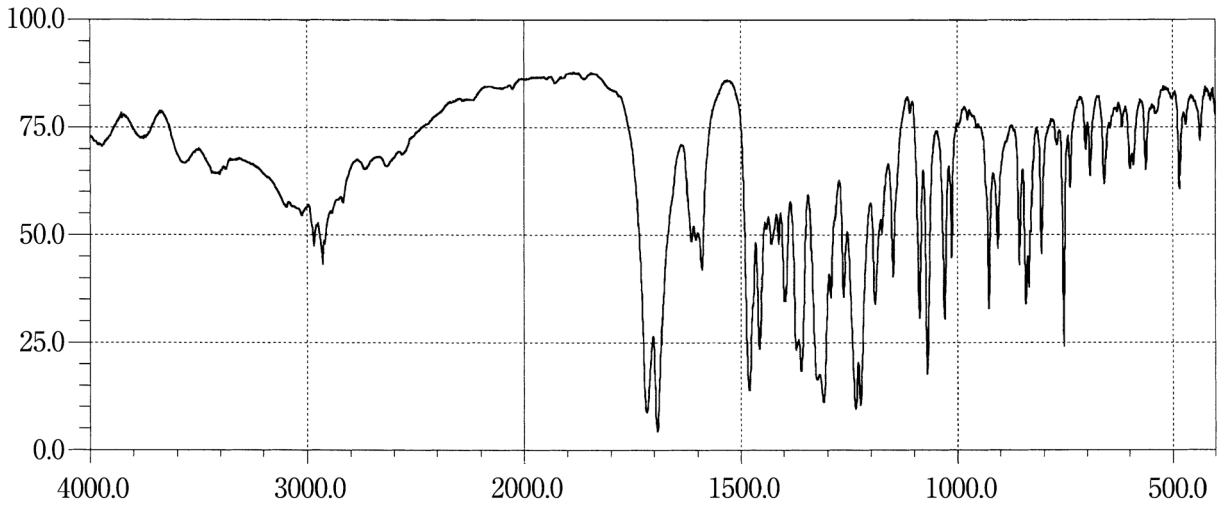


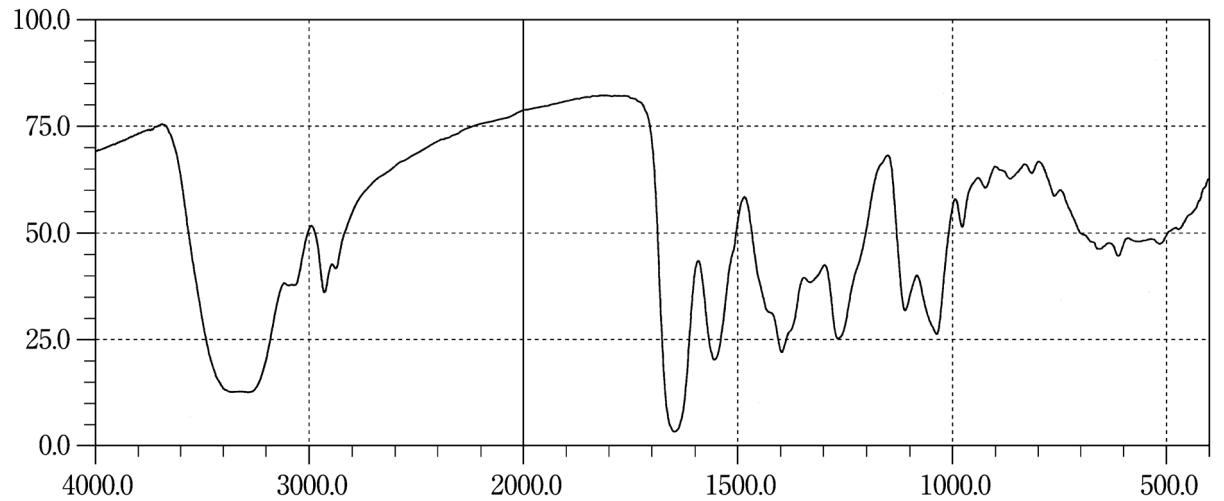
Indenolol Hydrochloride



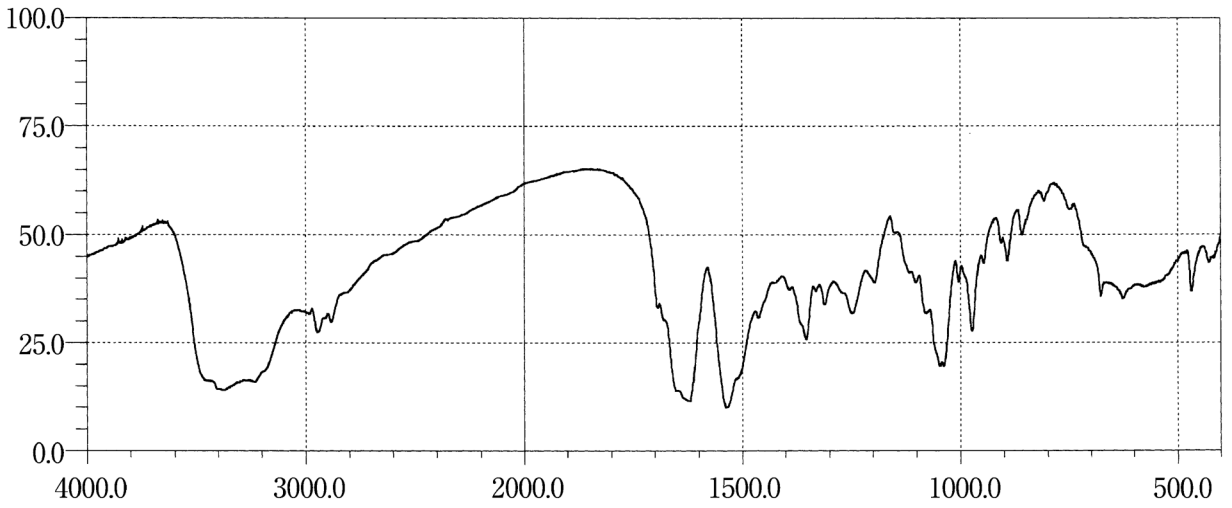
Indometacin



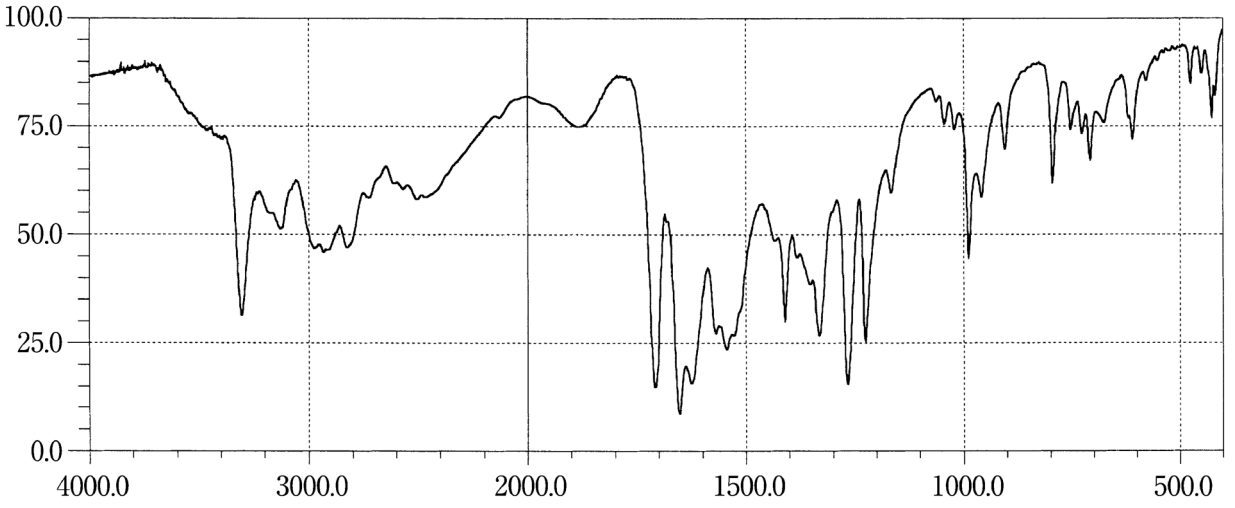
Iohexol



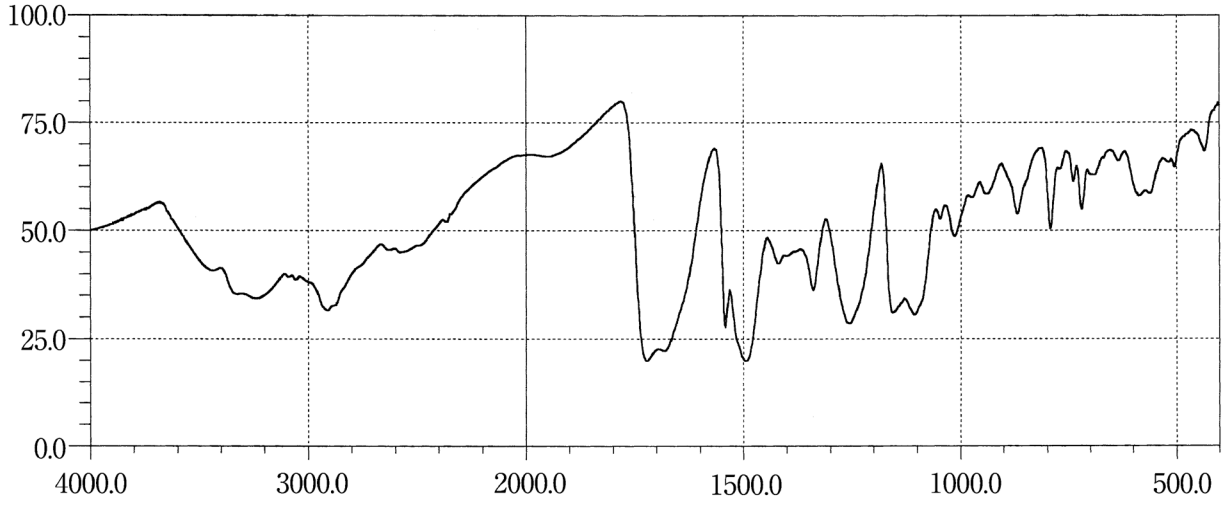
Iopamidol



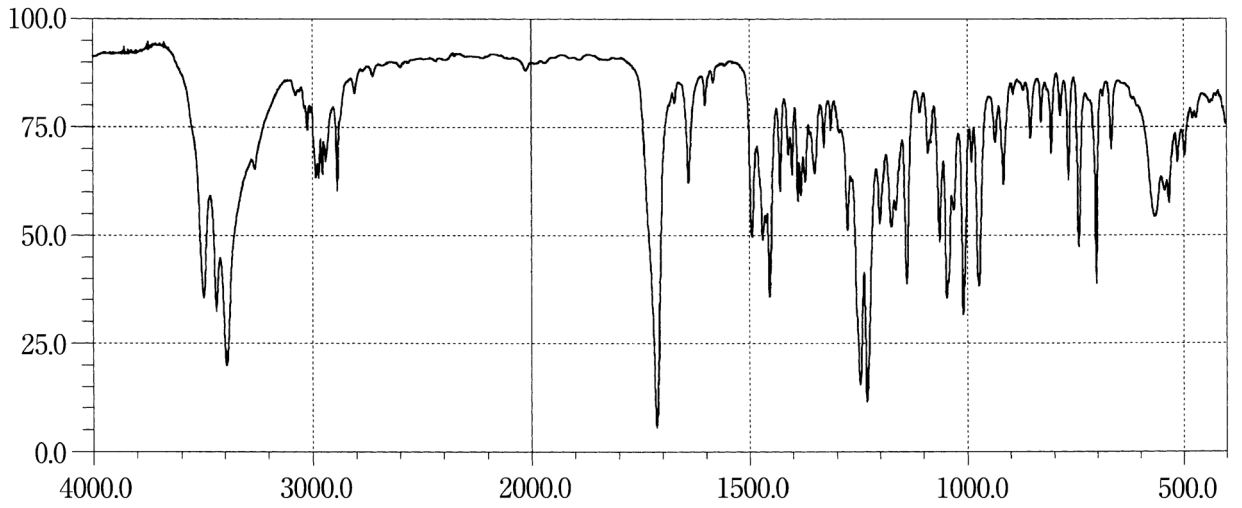
Iotalamic Acid



Iotroxic Acid



Ipratropium Bromide Hydrate



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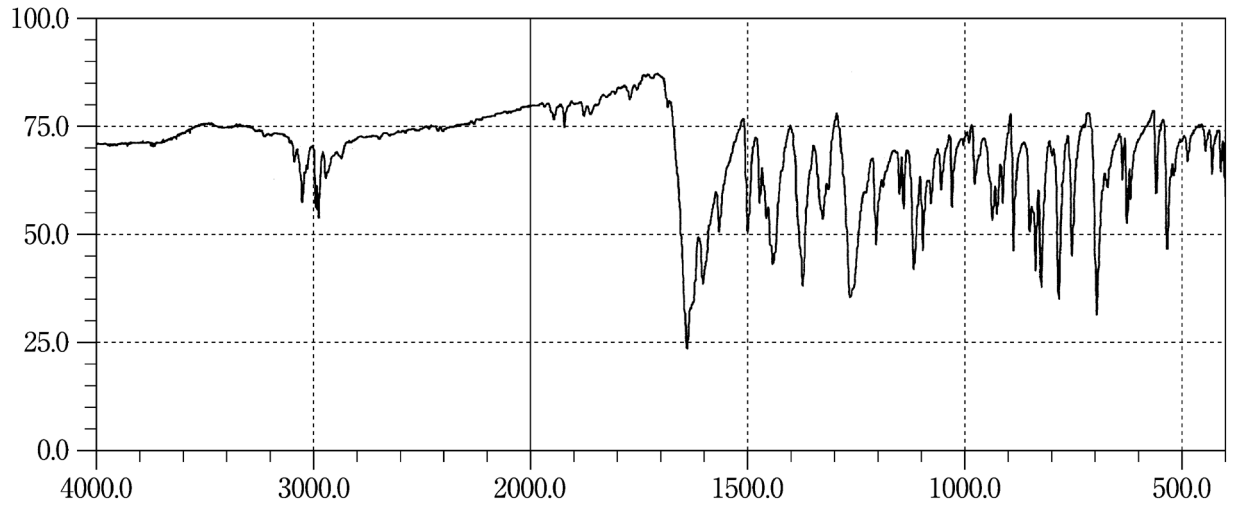

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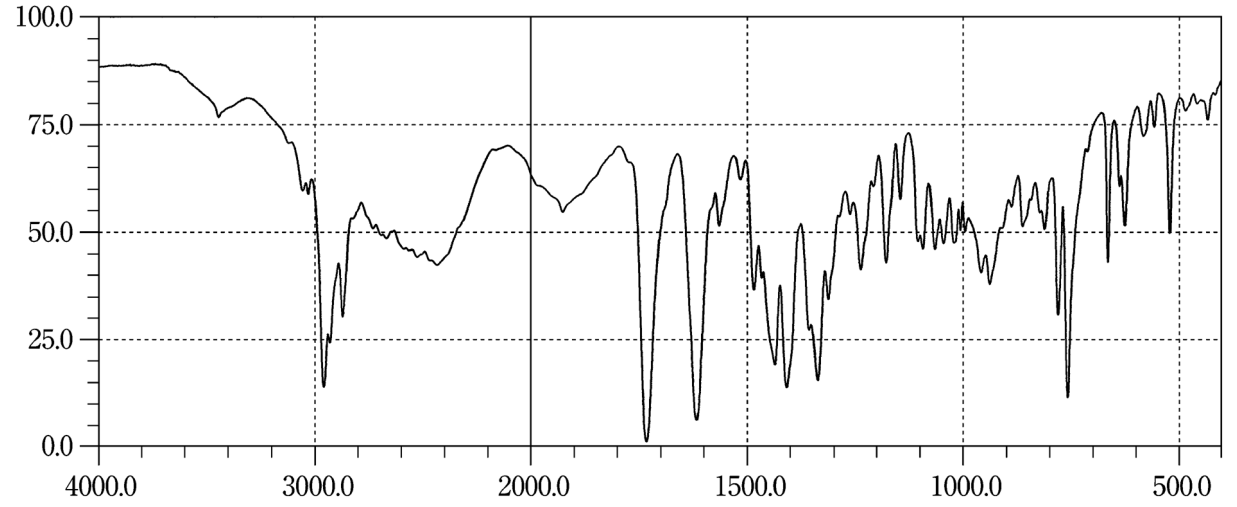
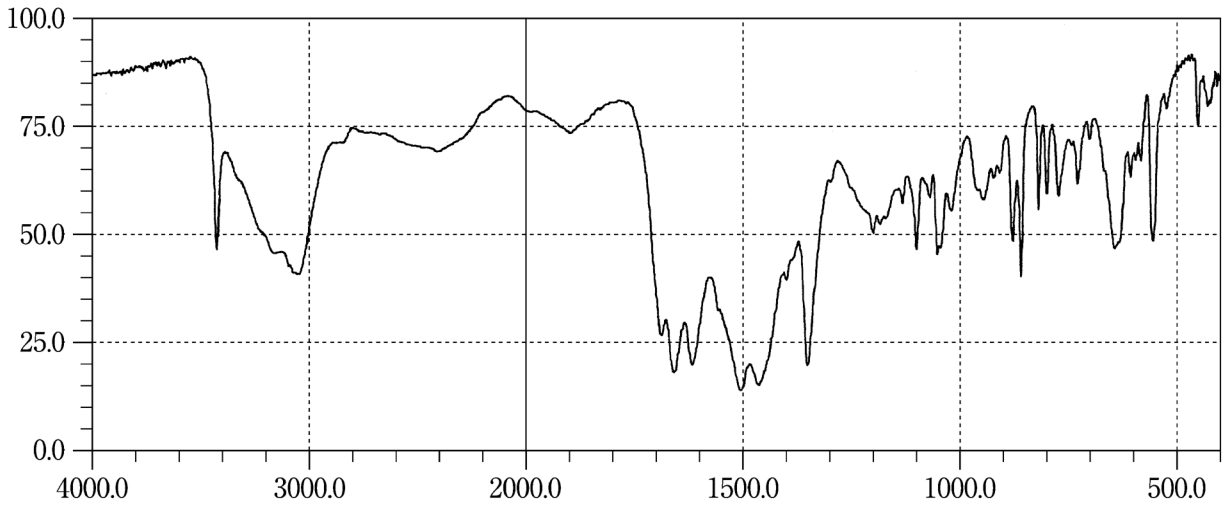
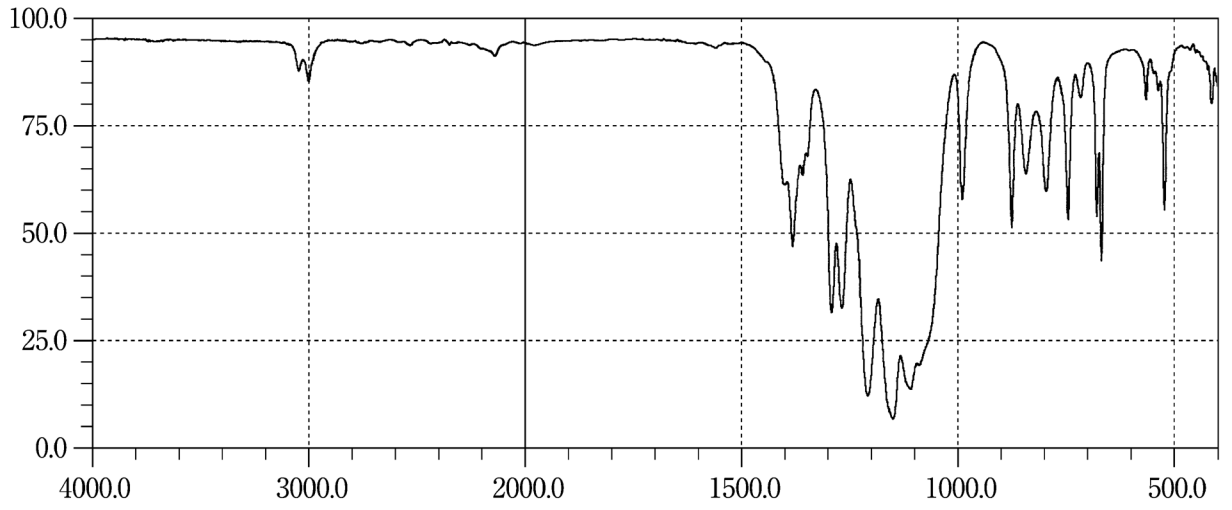


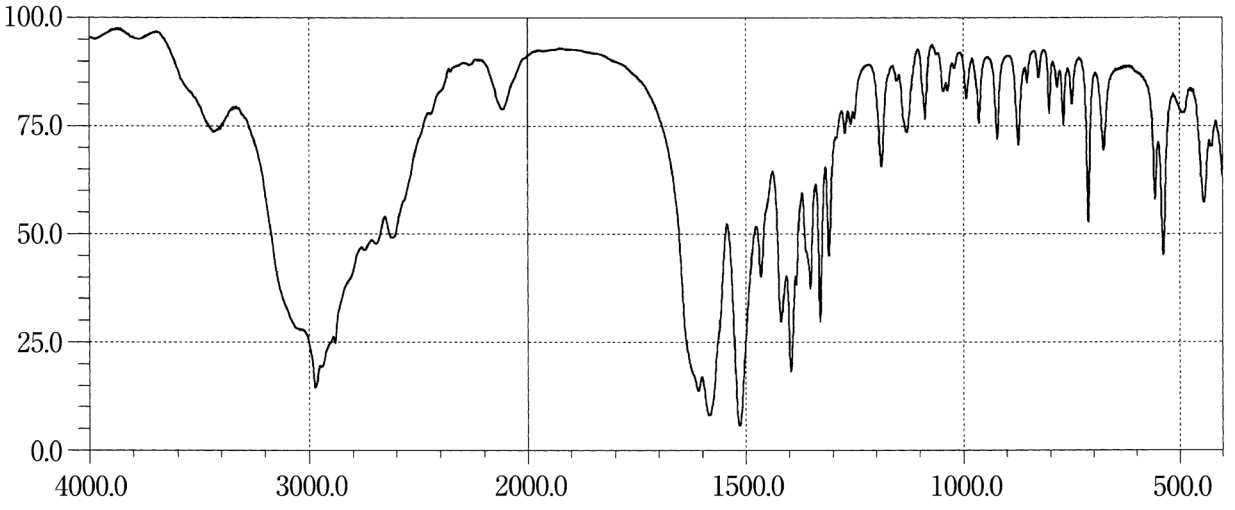
Irsogladine Maleate



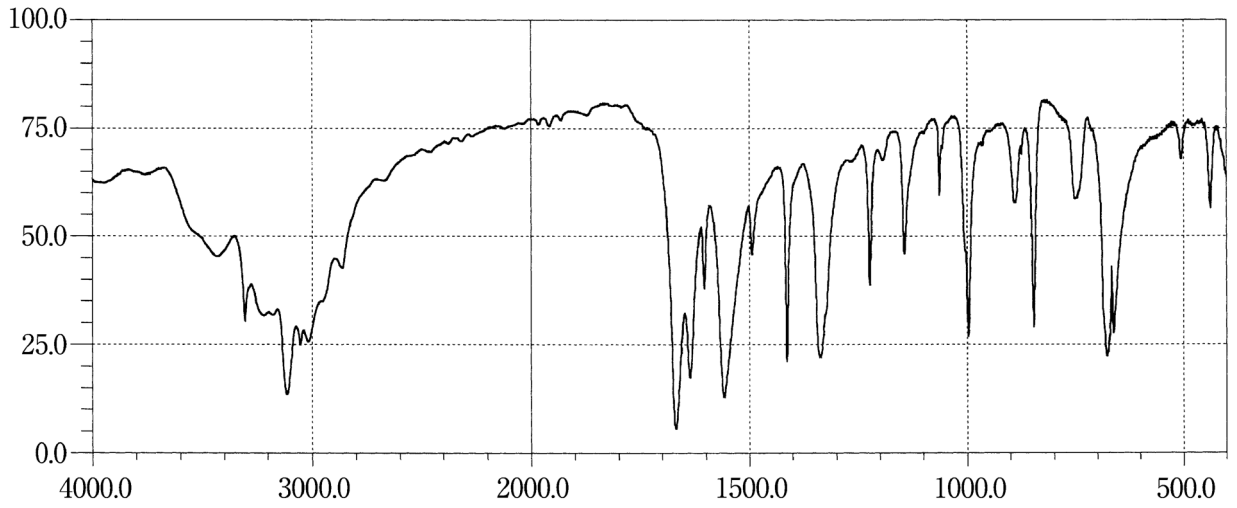
Isoflurane



L-Isoleucine



Isoniazid



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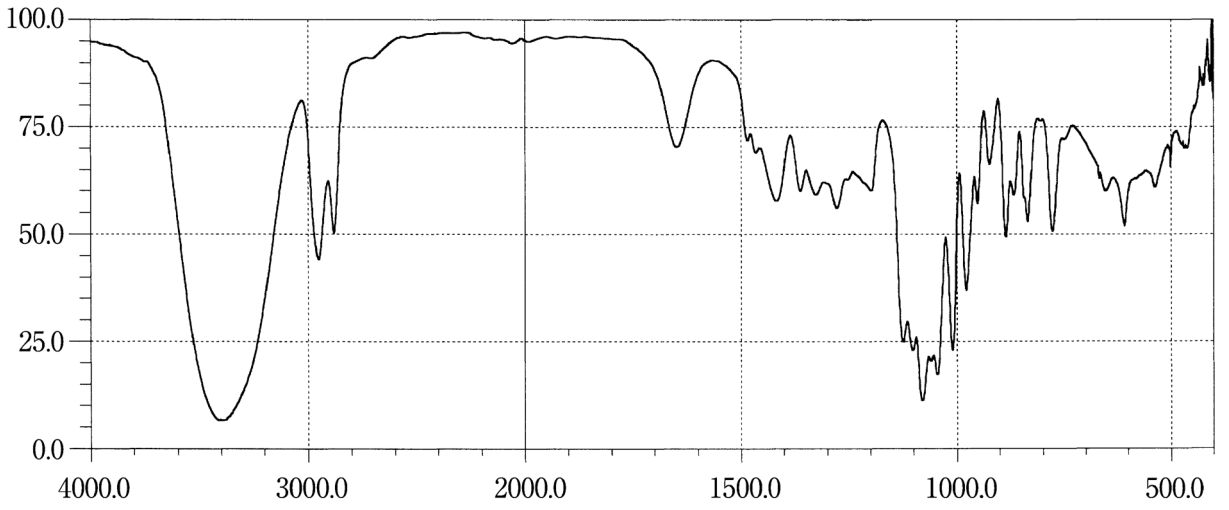

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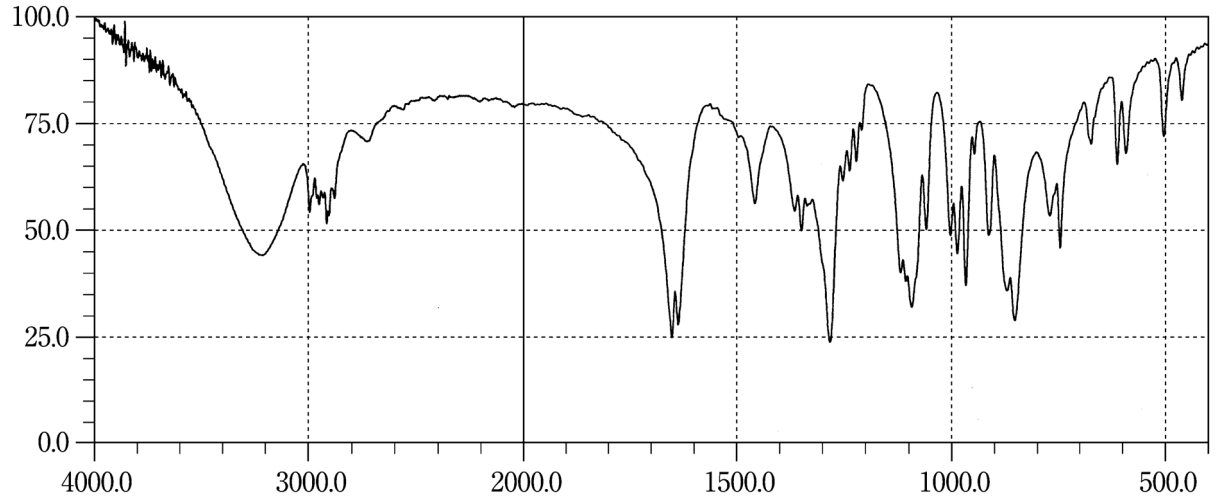
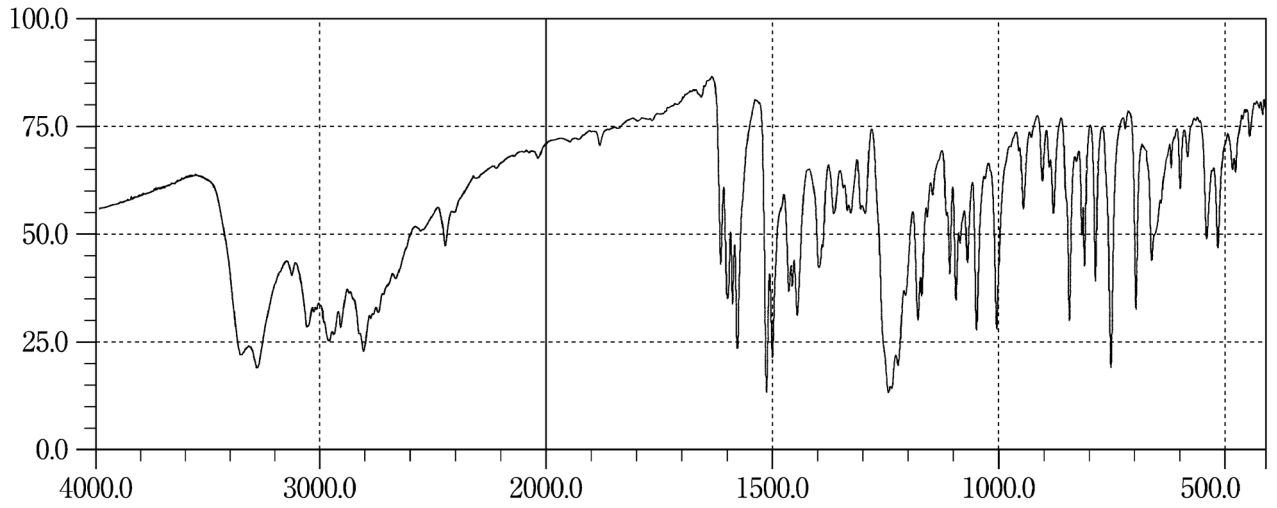
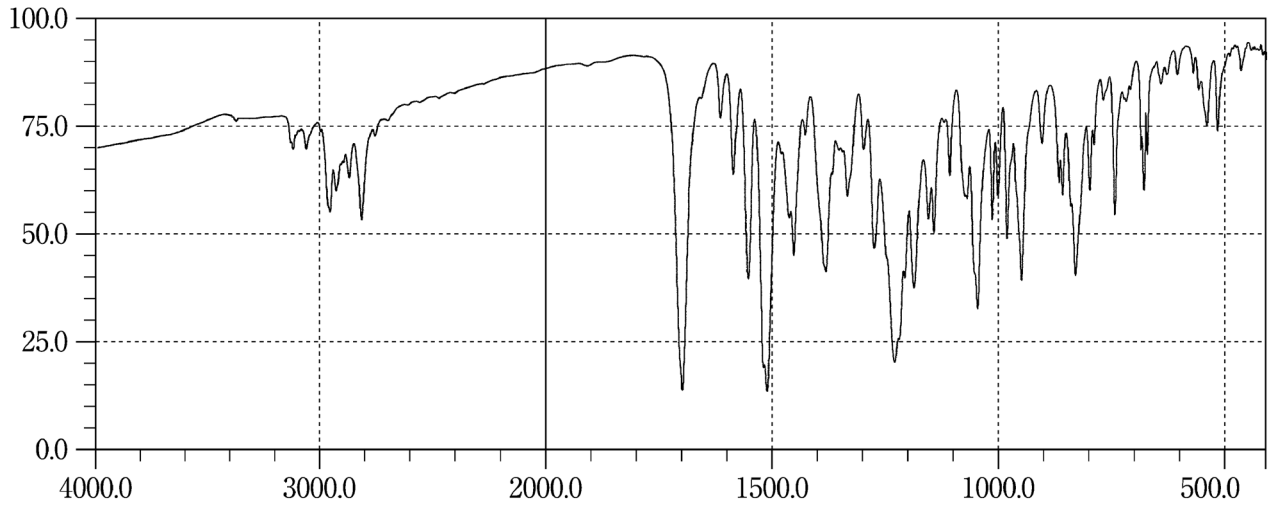


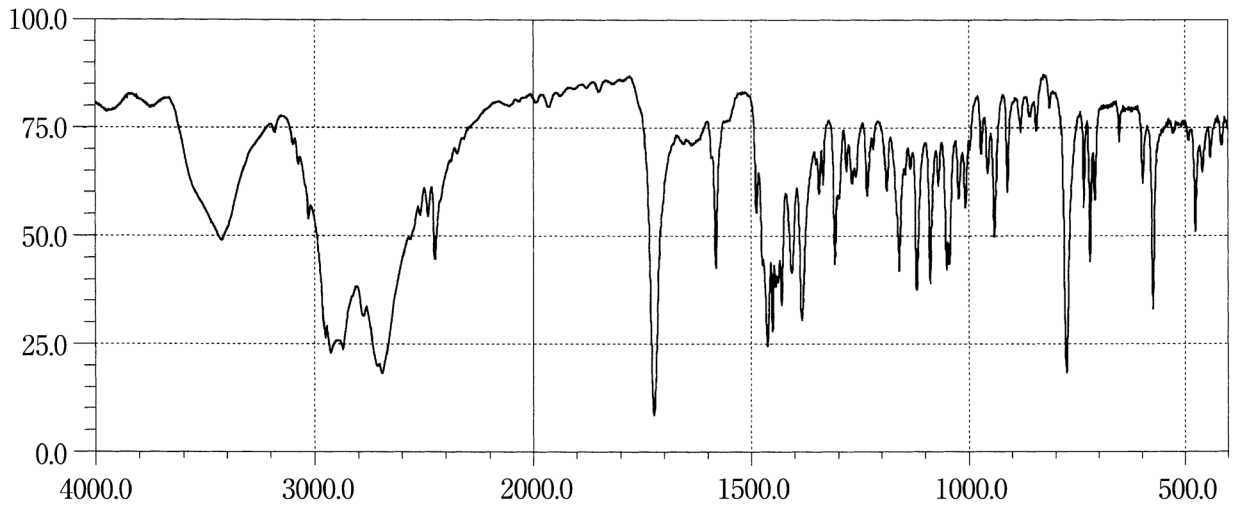
Isoxsuprine Hydrochloride



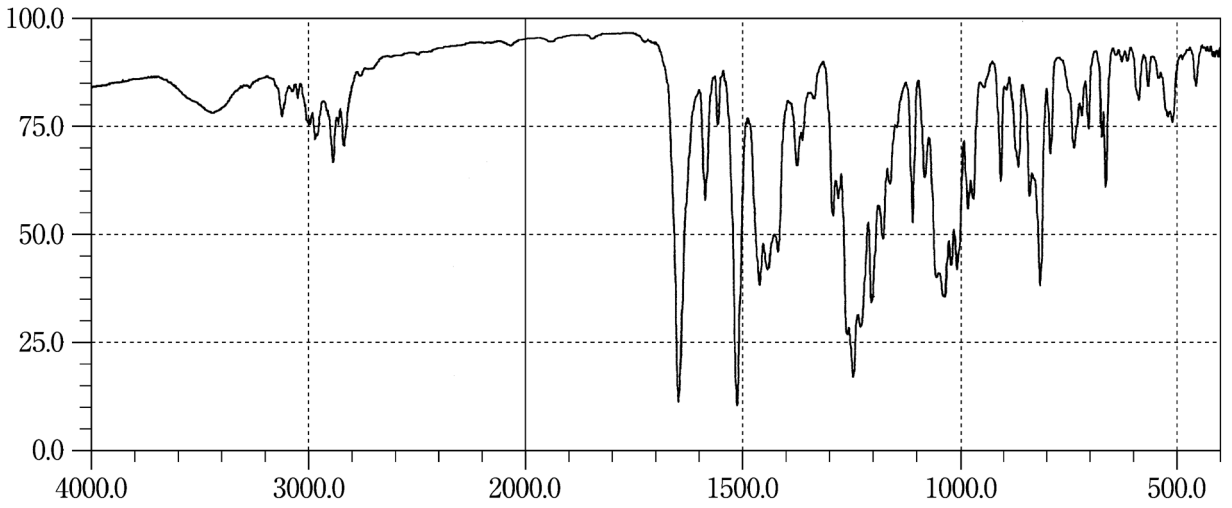
Itraconazole



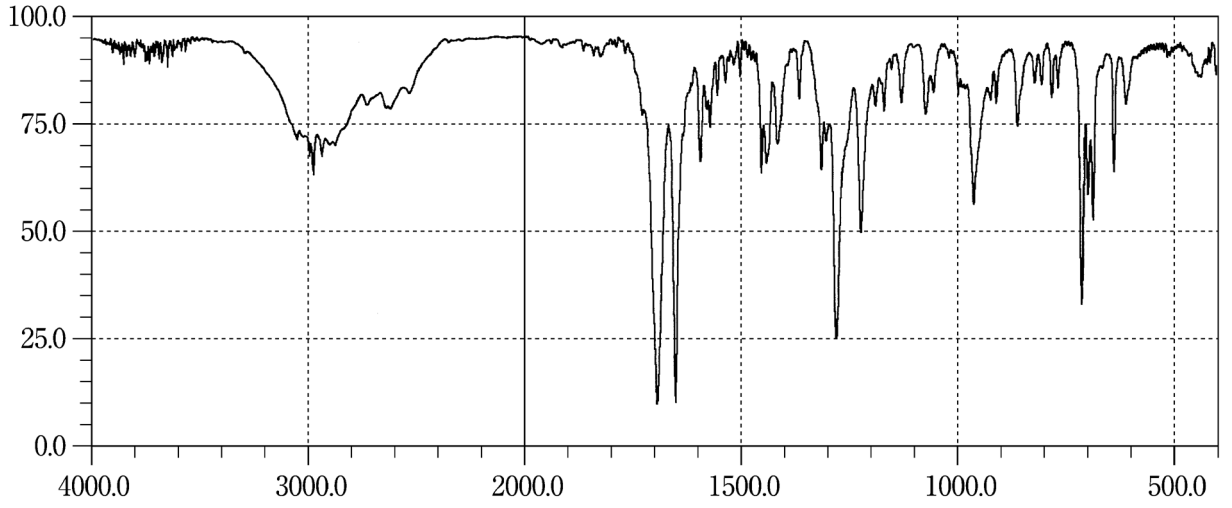
Ketamine Hydrochloride



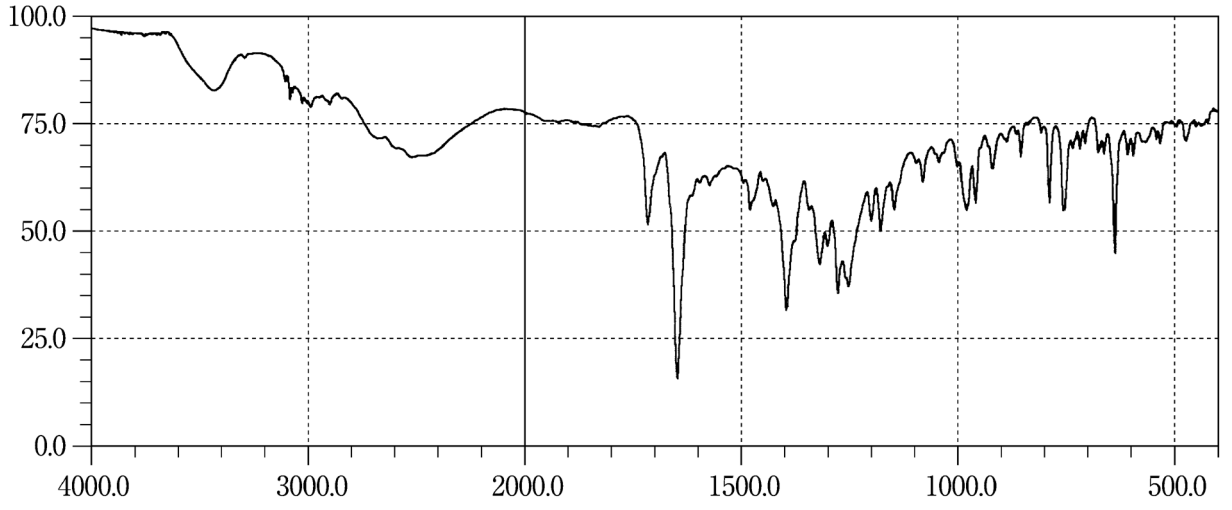
Ketoconazole



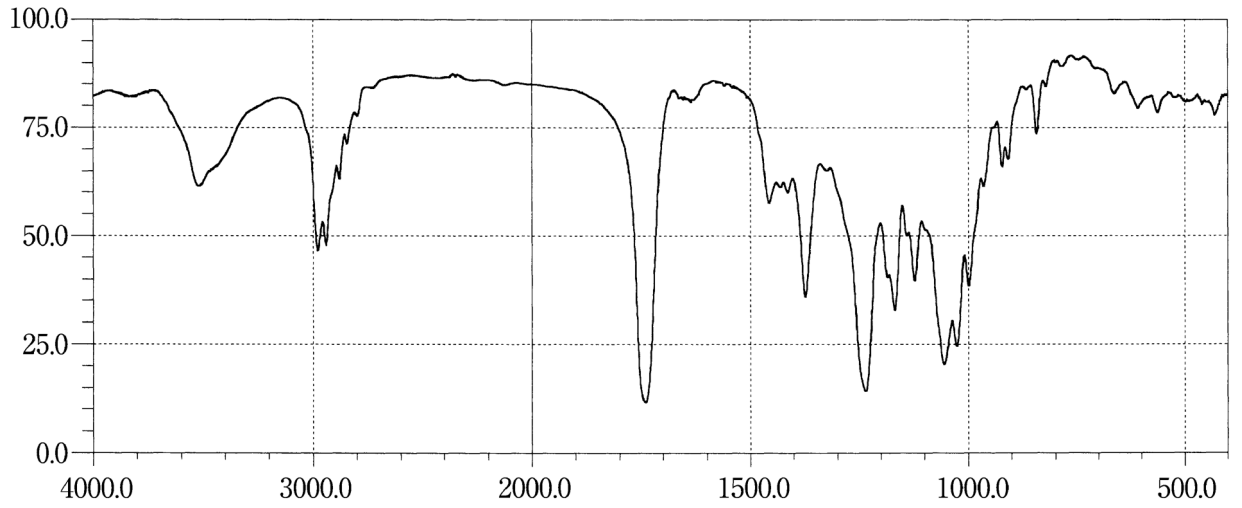
Ketoprofen



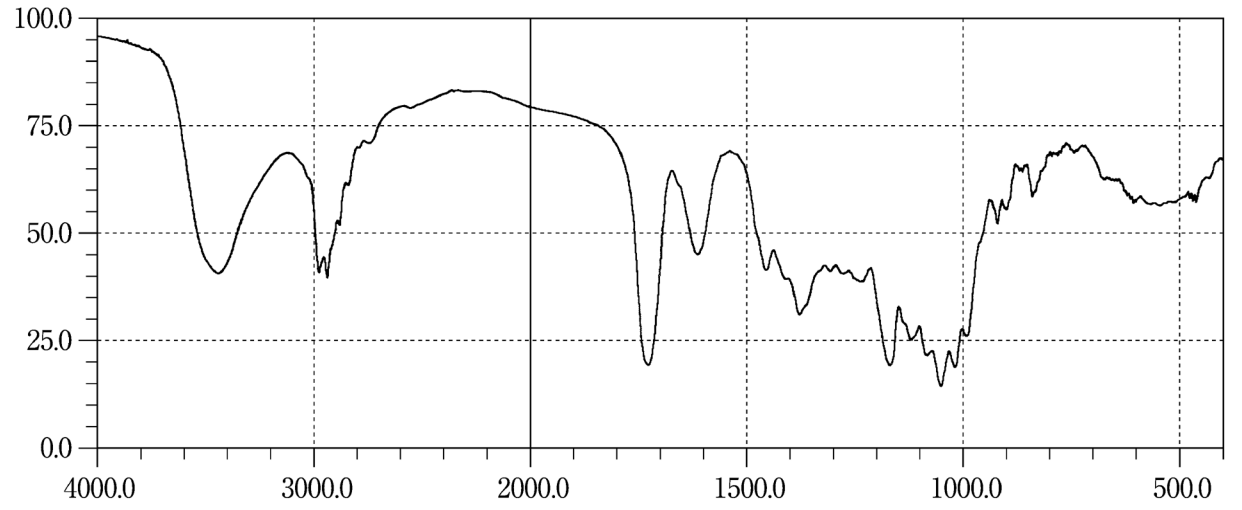
Ketotifen Fumarate



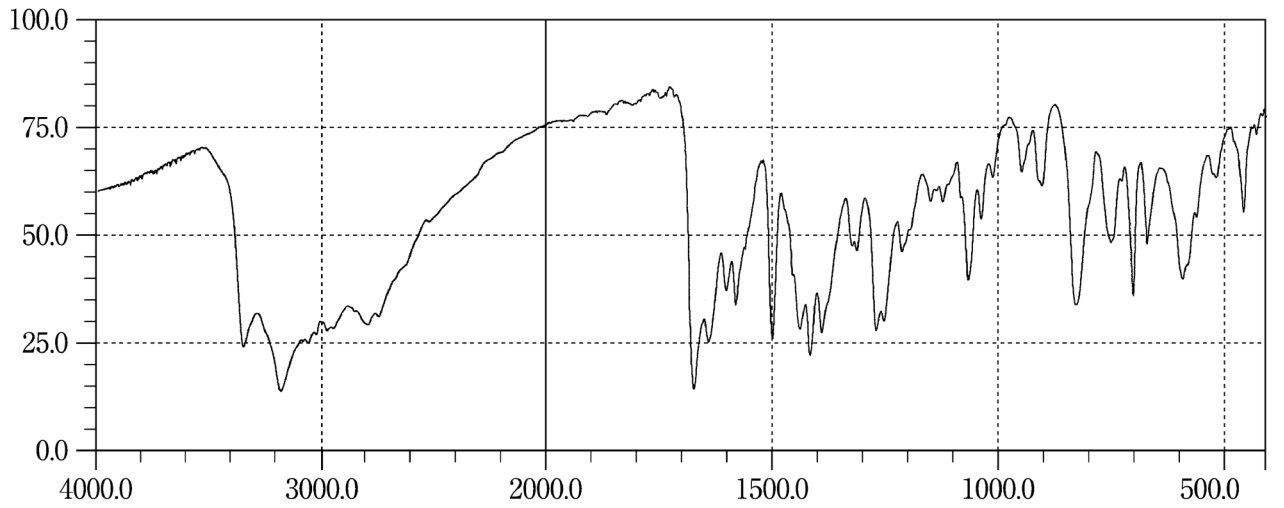
Kitasamycin Acetate



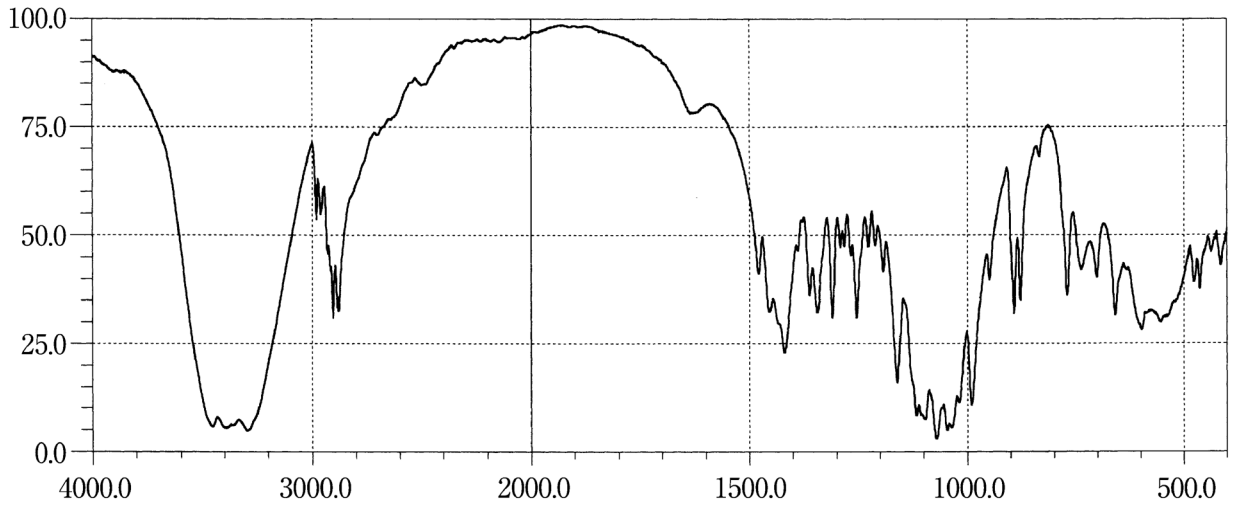
Kitasamycin Tartrate



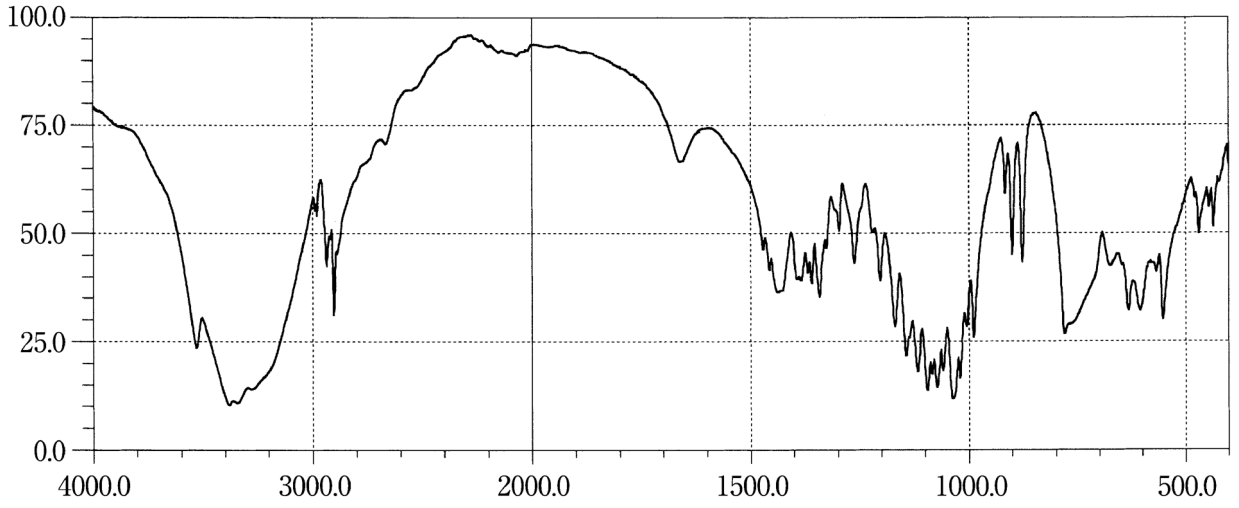
Labetalol Hydrochloride



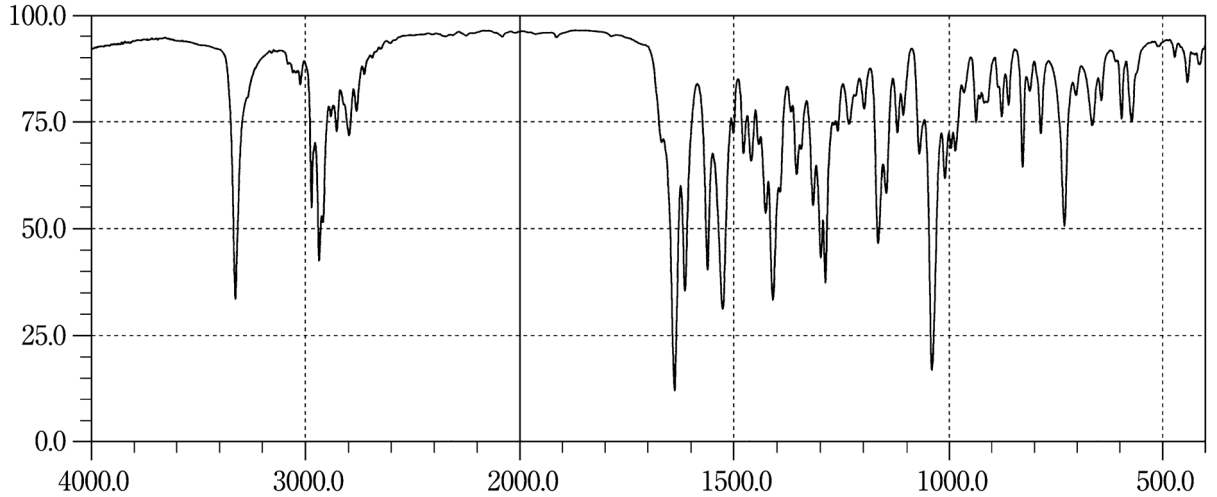
Anhydrous Lactose



Lactose Hydrate

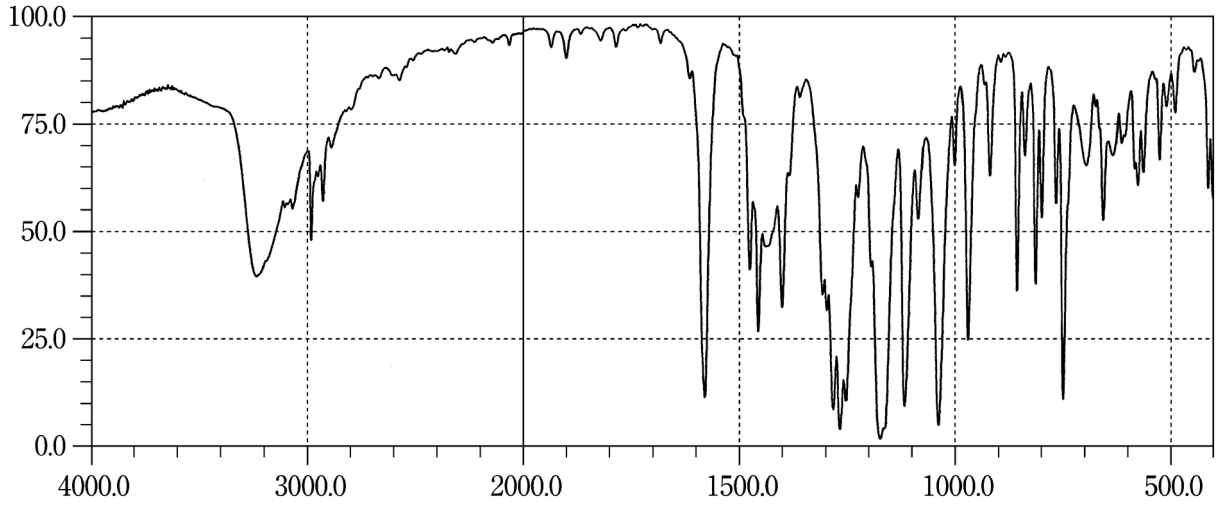


Lafutidine

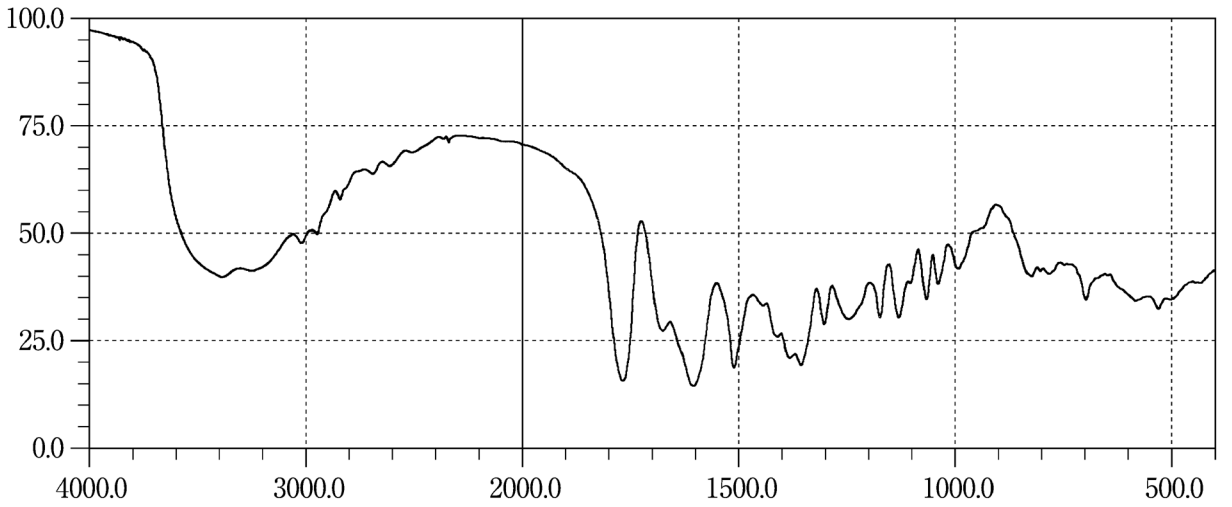


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

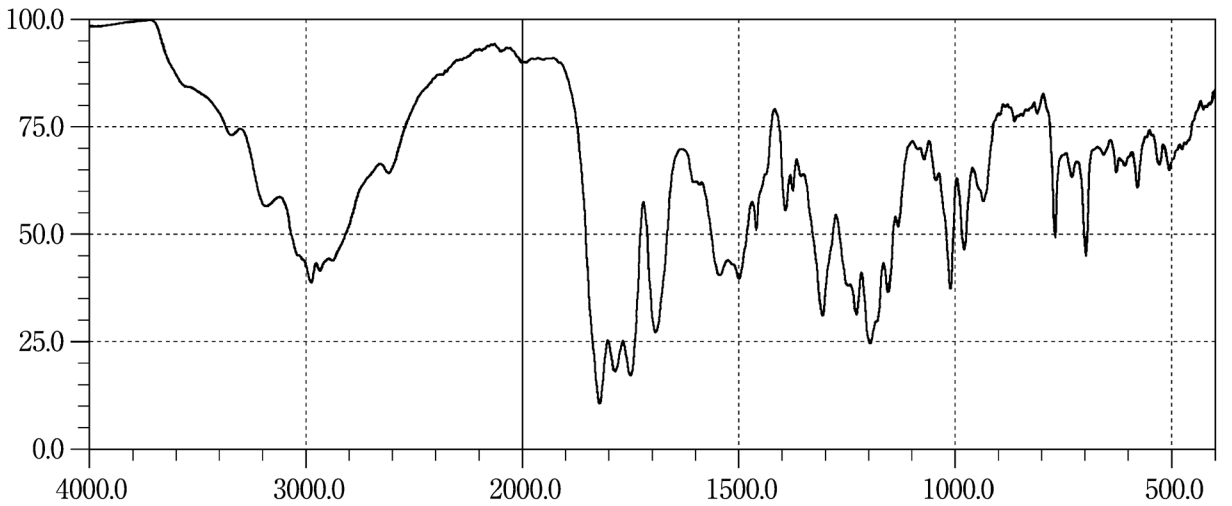
Lansoprazole



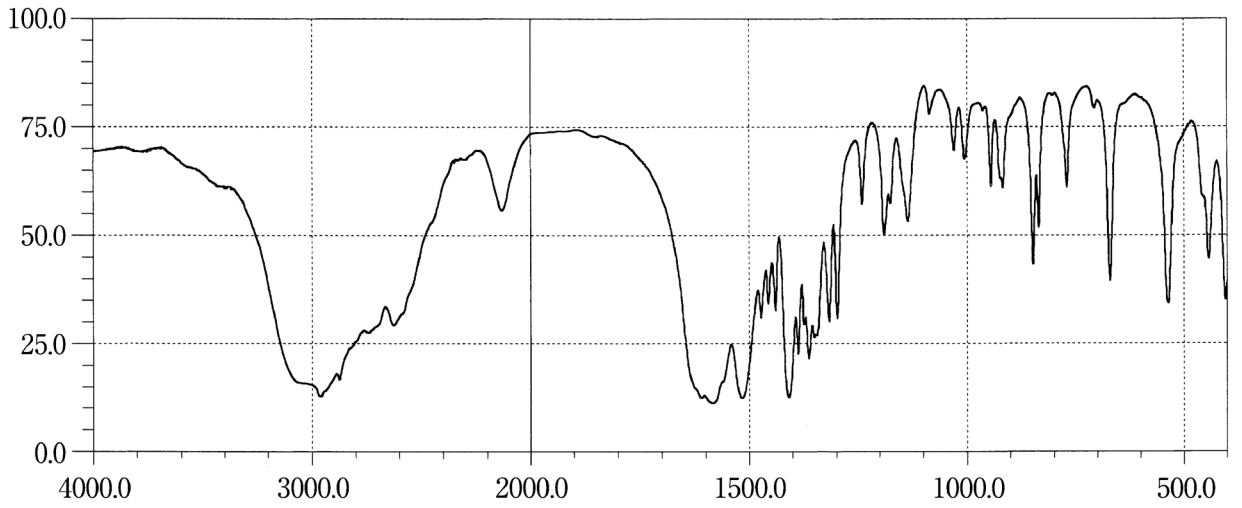
Latamoxef Sodium



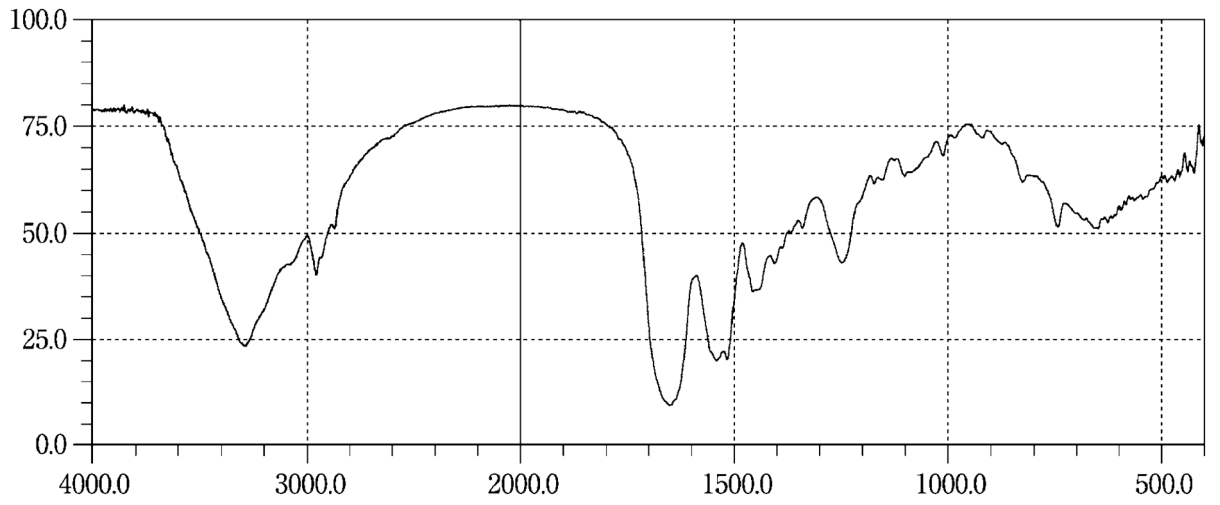
Lenampicillin Hydrochloride



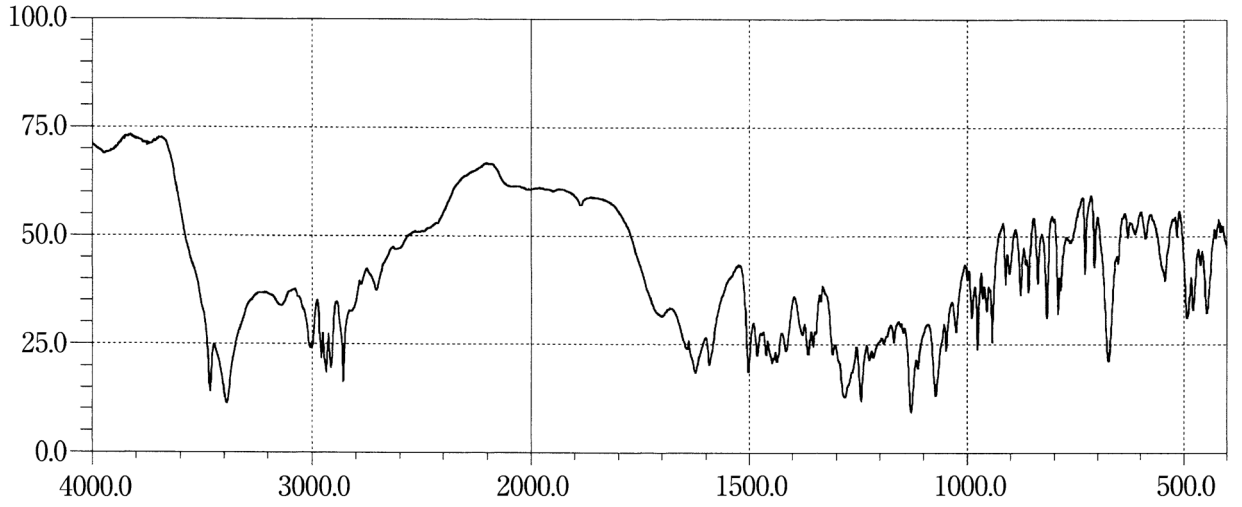
L-Leucine



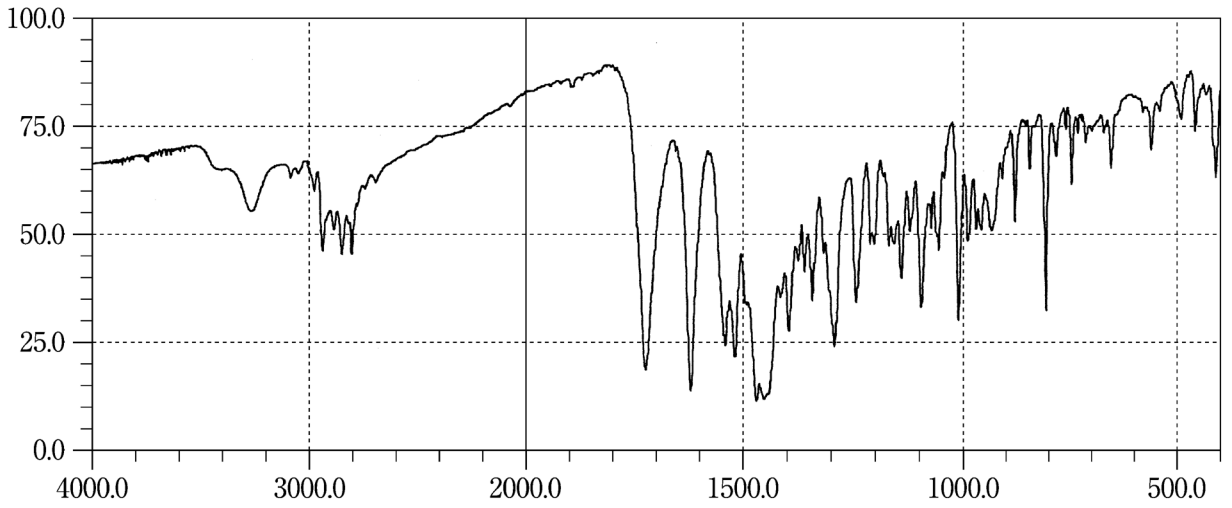
Leuprorelin Acetate



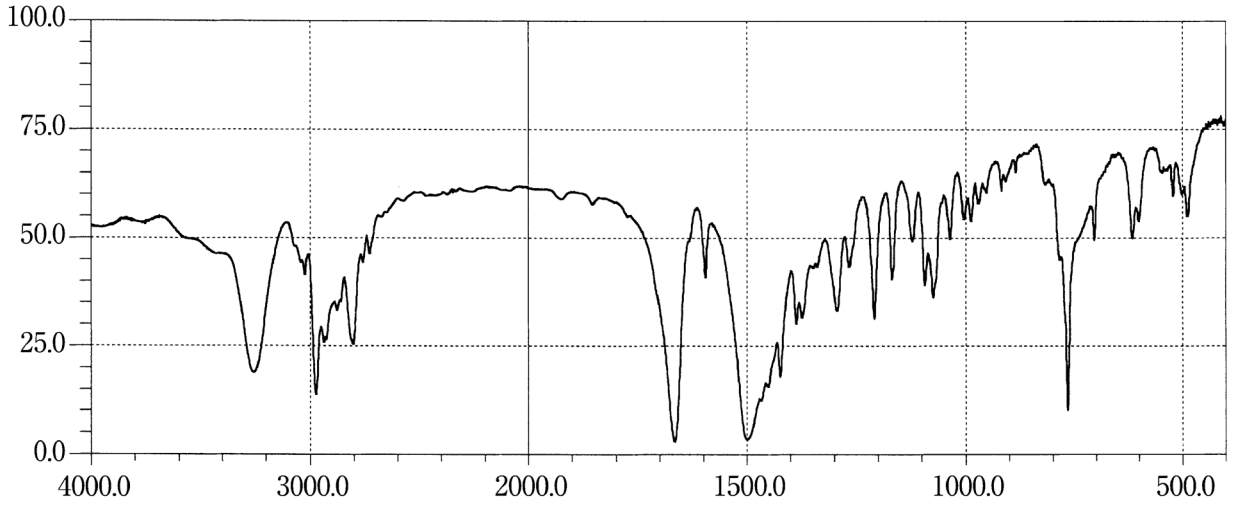
Levallorphan Tartrate



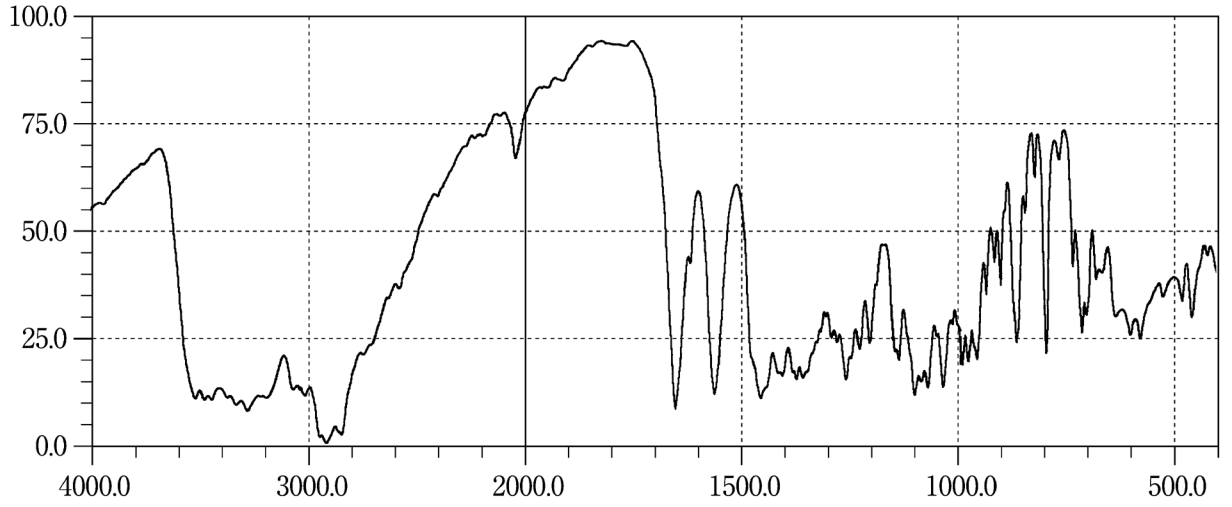
Levofloxacin Hydrate



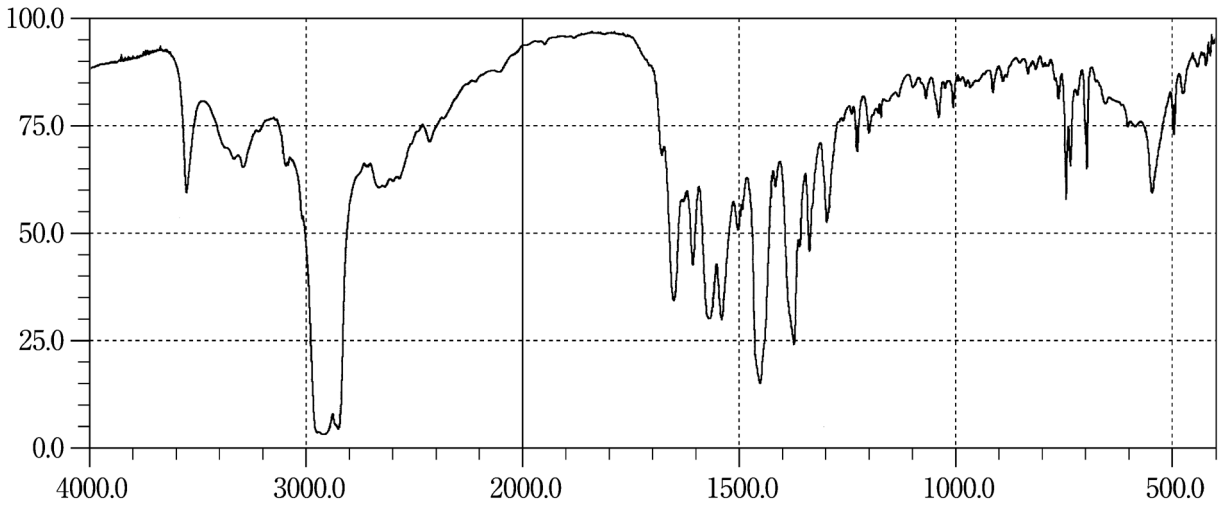
Lidocaine



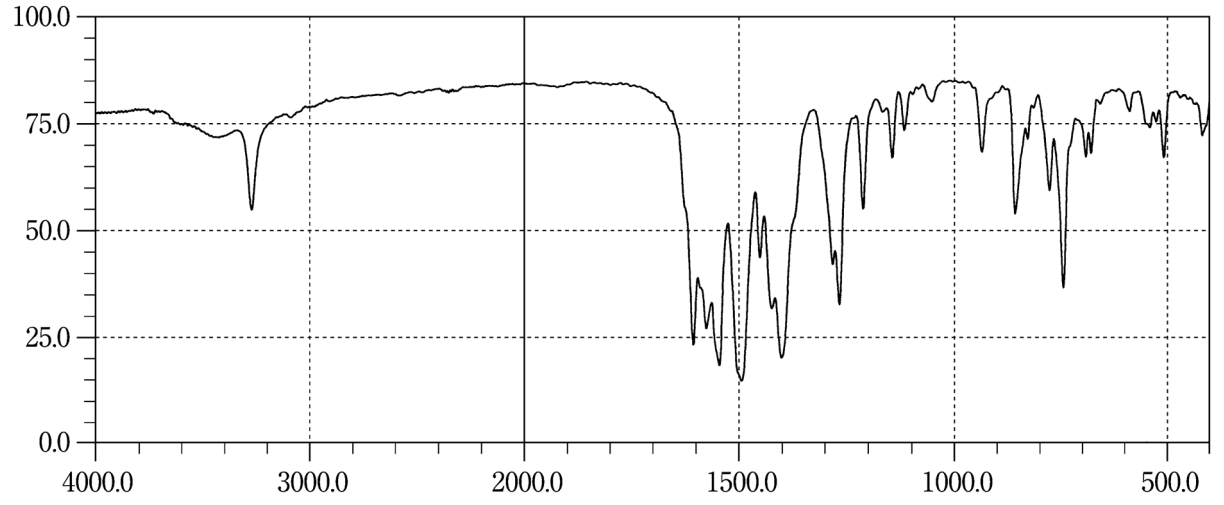
Lincomycin Hydrochloride Hydrate



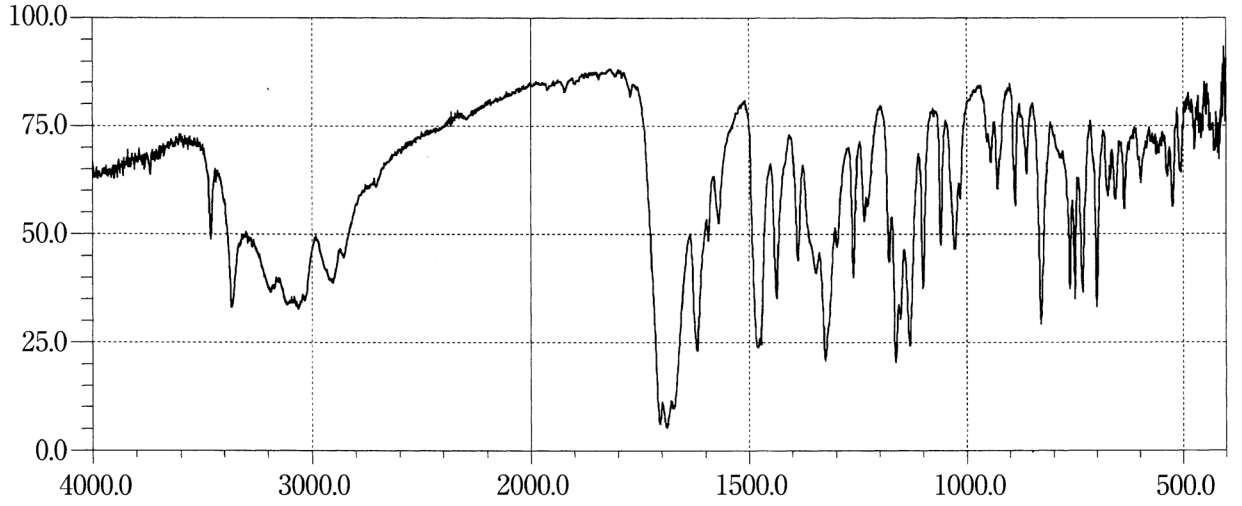
Lisinopril Hydrate



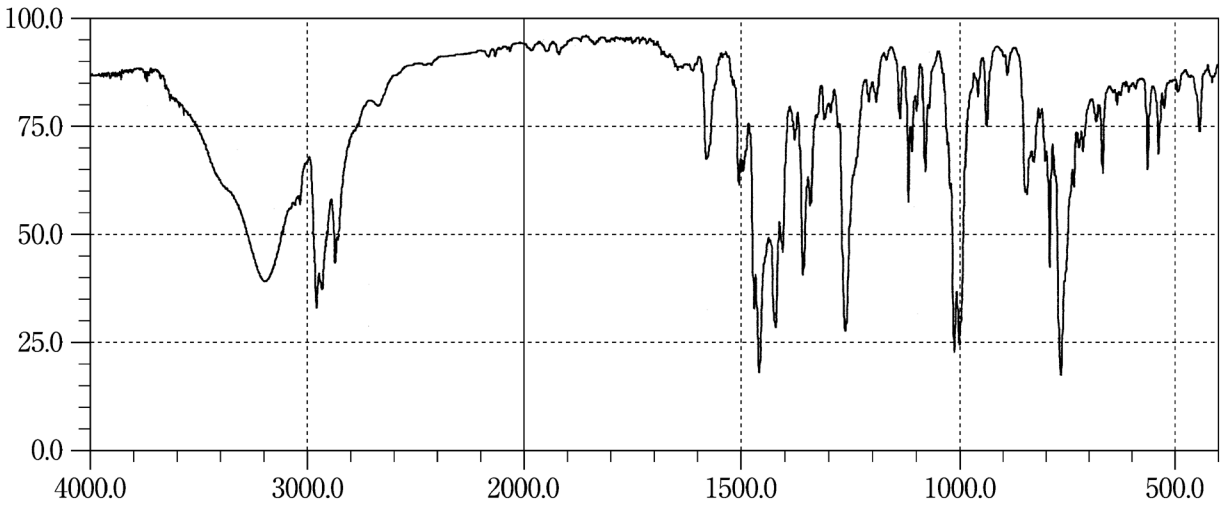
Lobenzarit Sodium



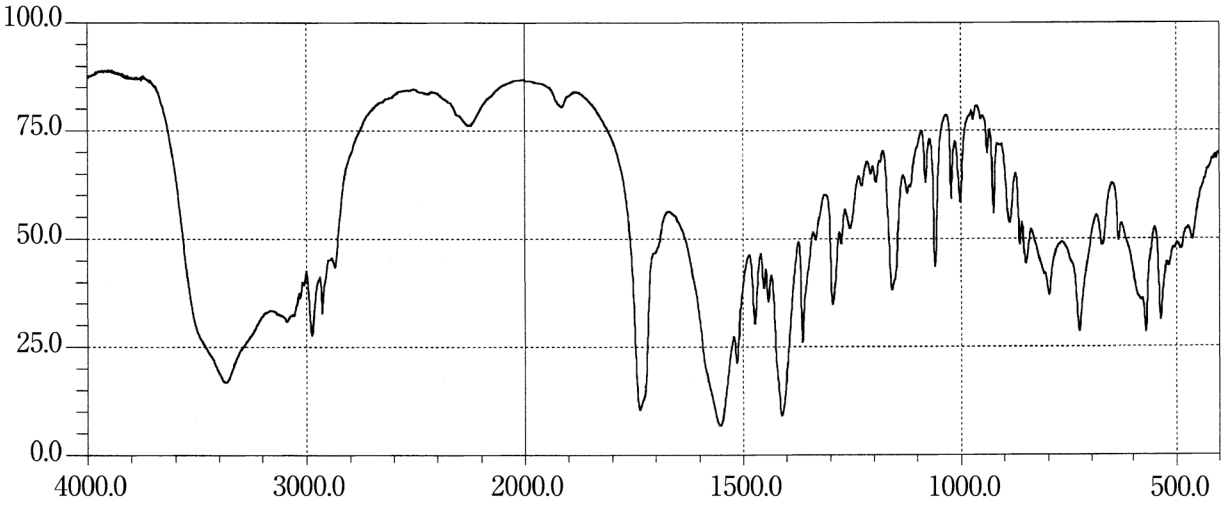
Lorazepam



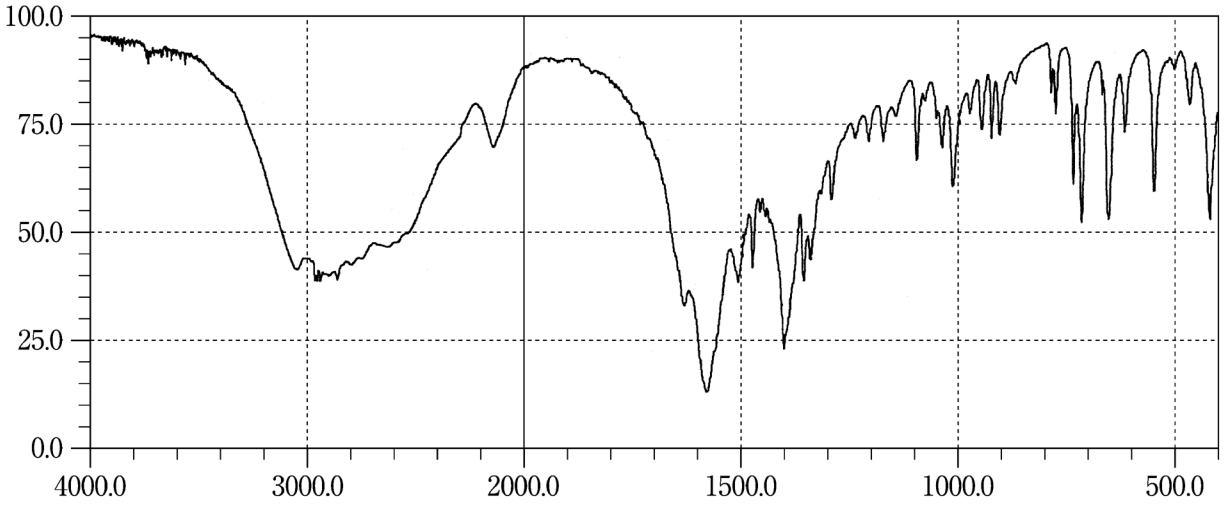
Losartan Potassium



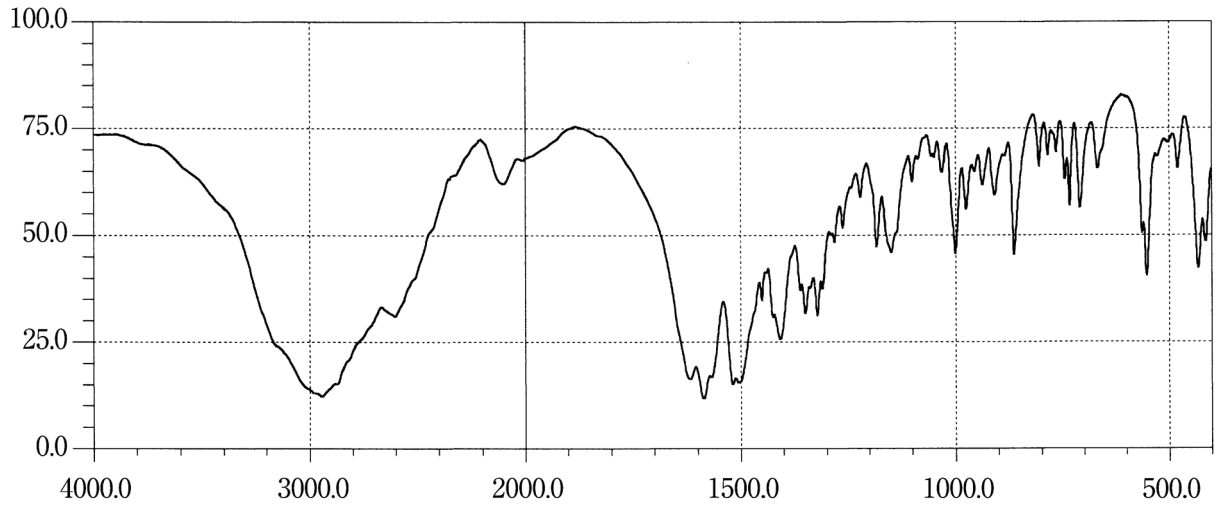
Loxoprofen Sodium Hydrate



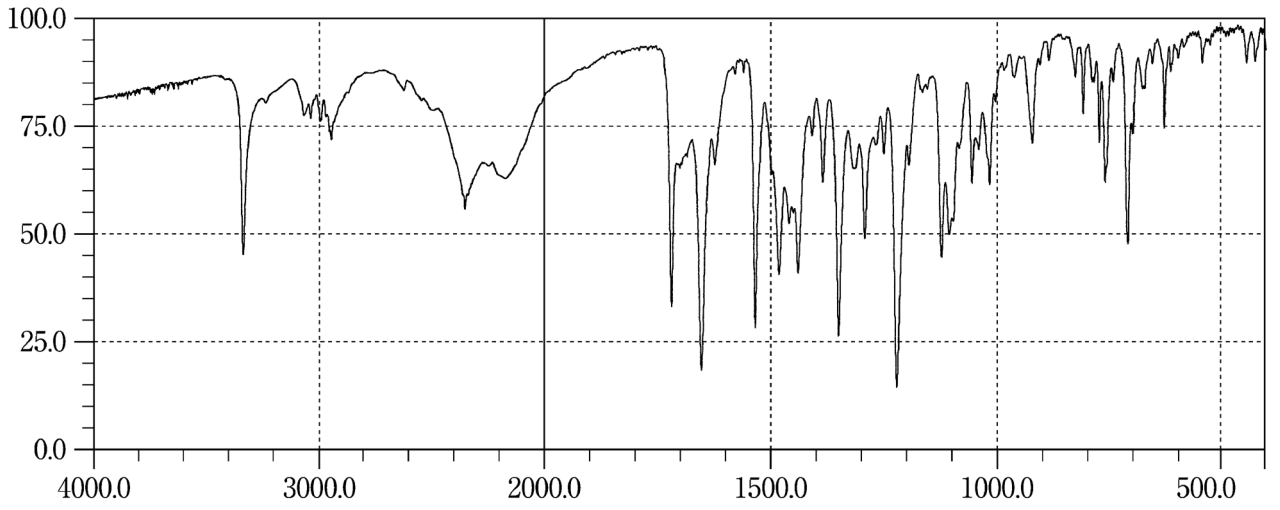
L-Lysine Acetate



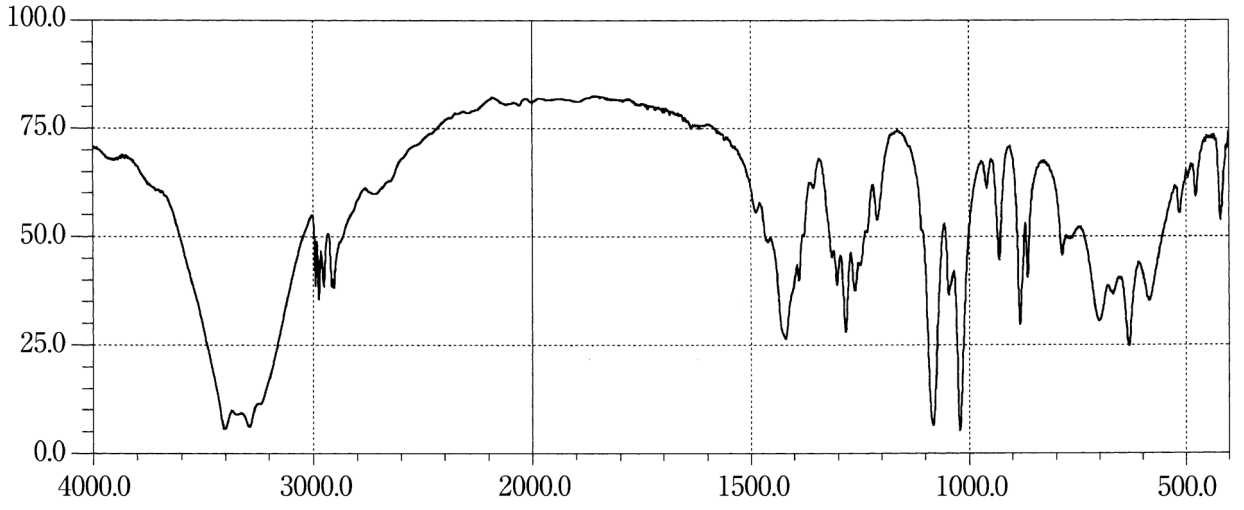
L-Lysine Hydrochloride



Manidipine Hydrochloride

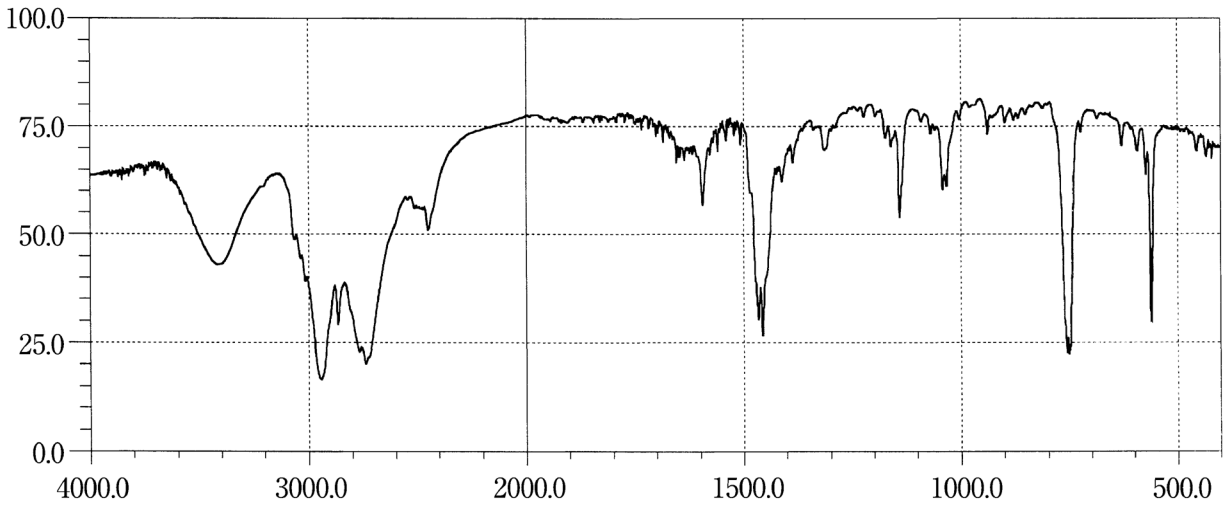


D-Mannitol

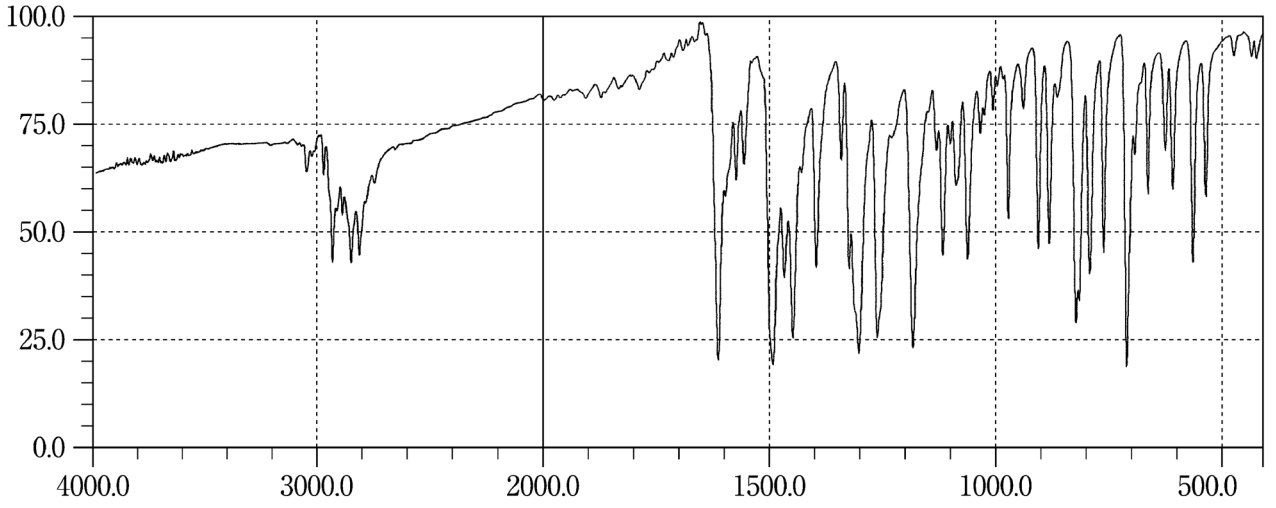


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

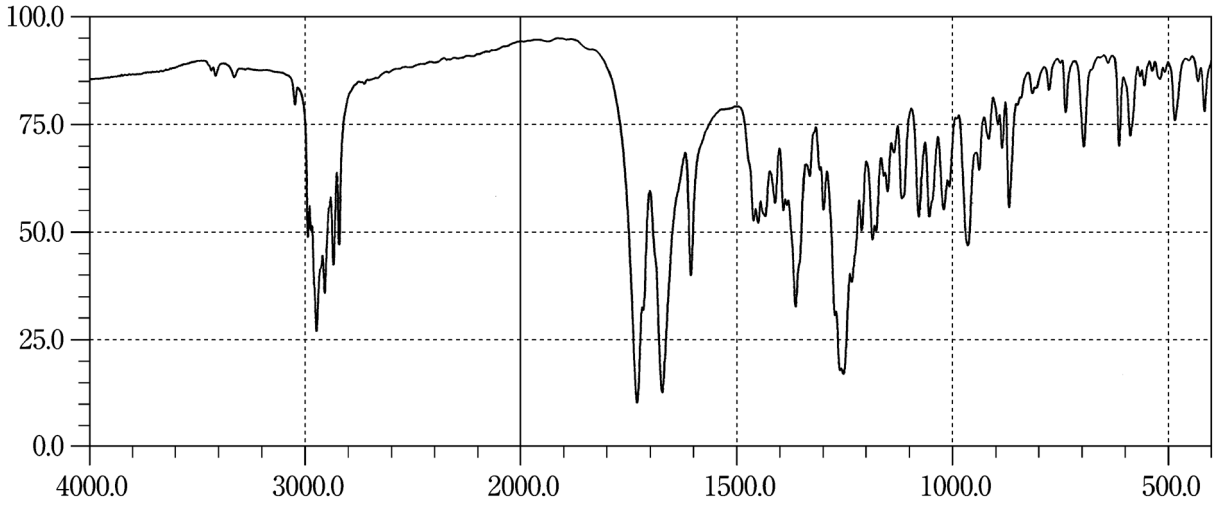
Maprotiline Hydrochloride



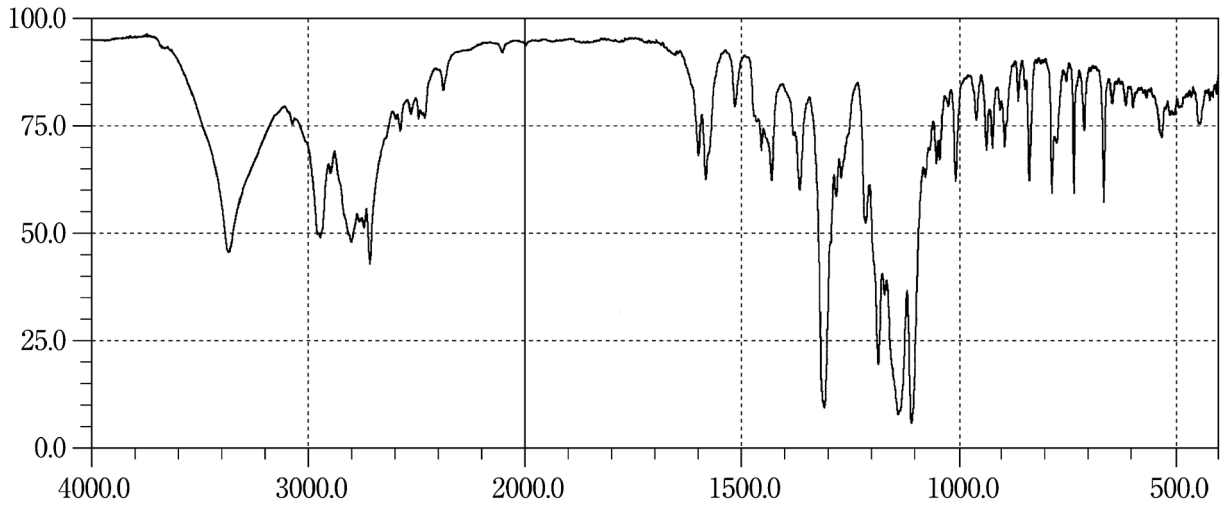
Medazepam



Medroxyprogesterone Acetate



Mefloquine Hydrochloride



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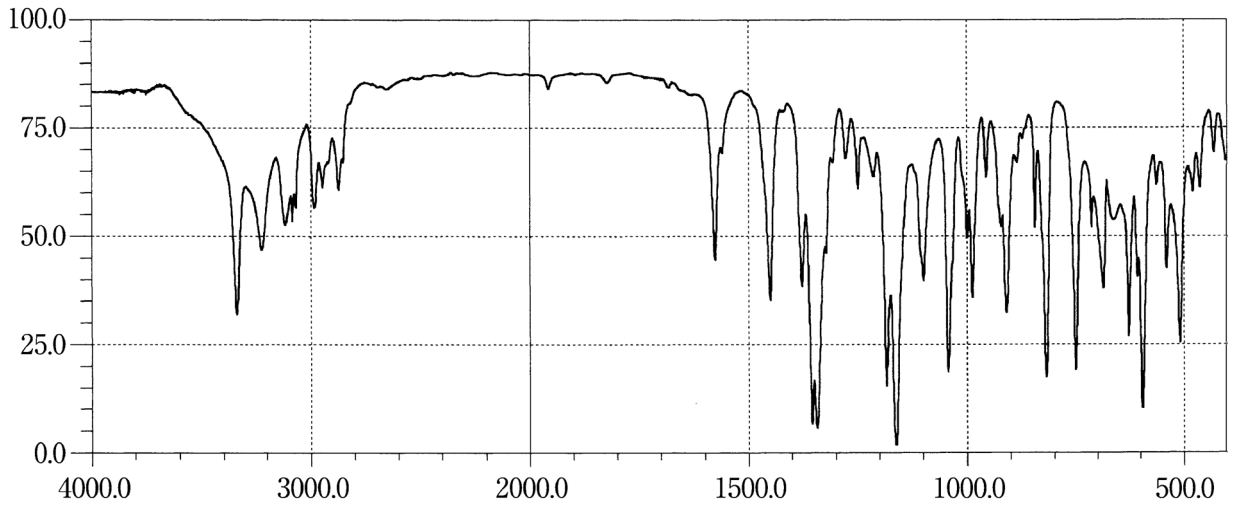

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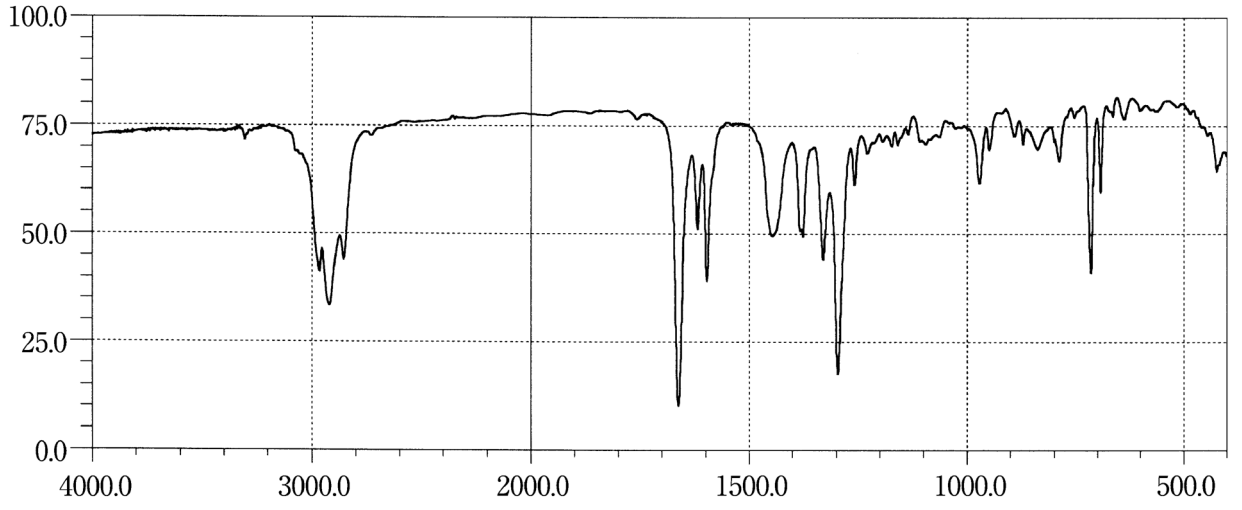
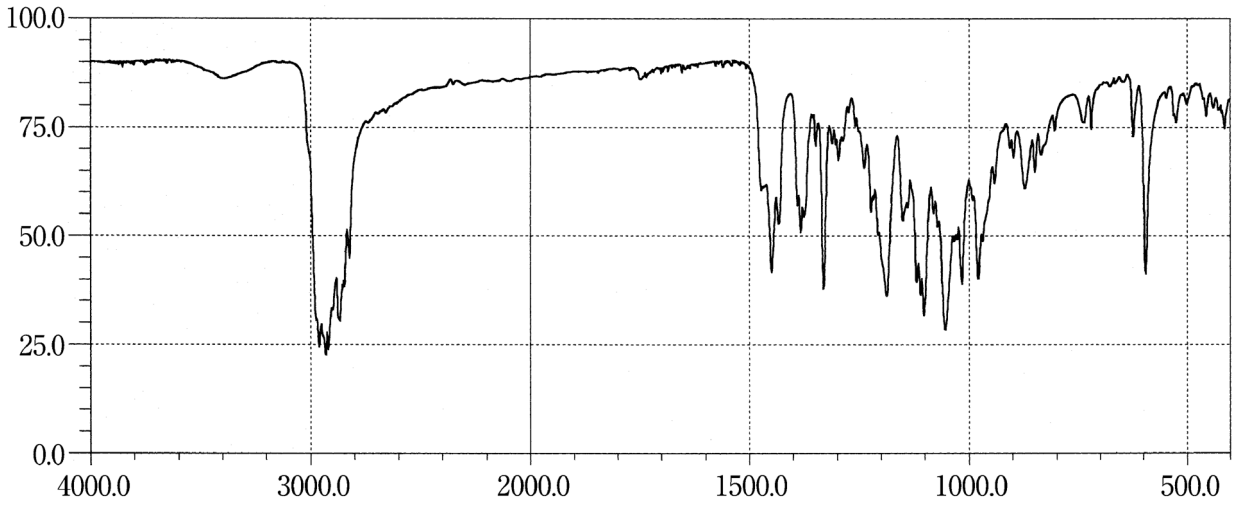


Mepitiostane



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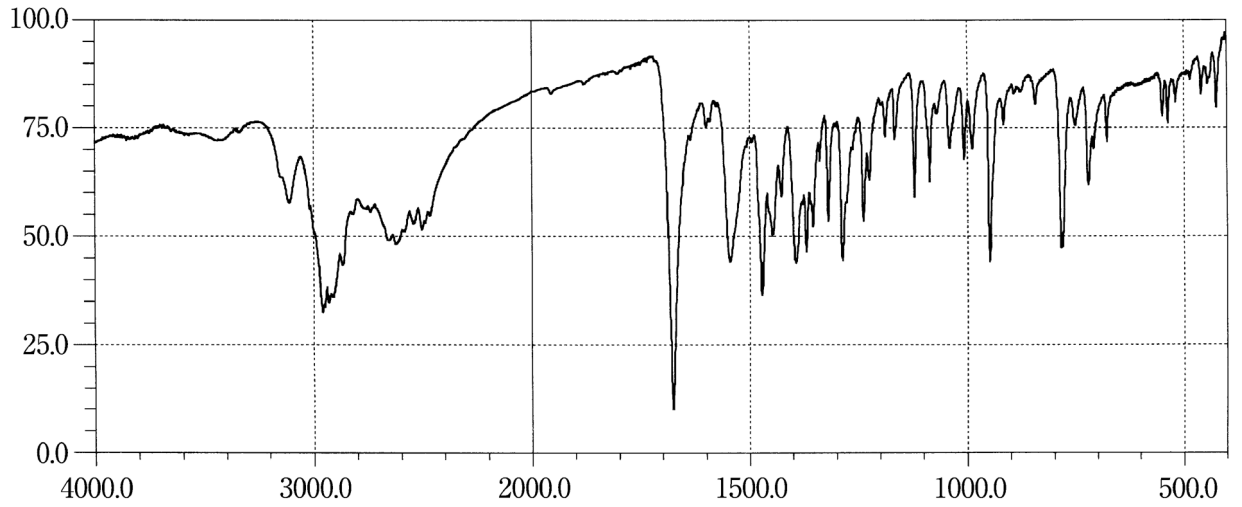

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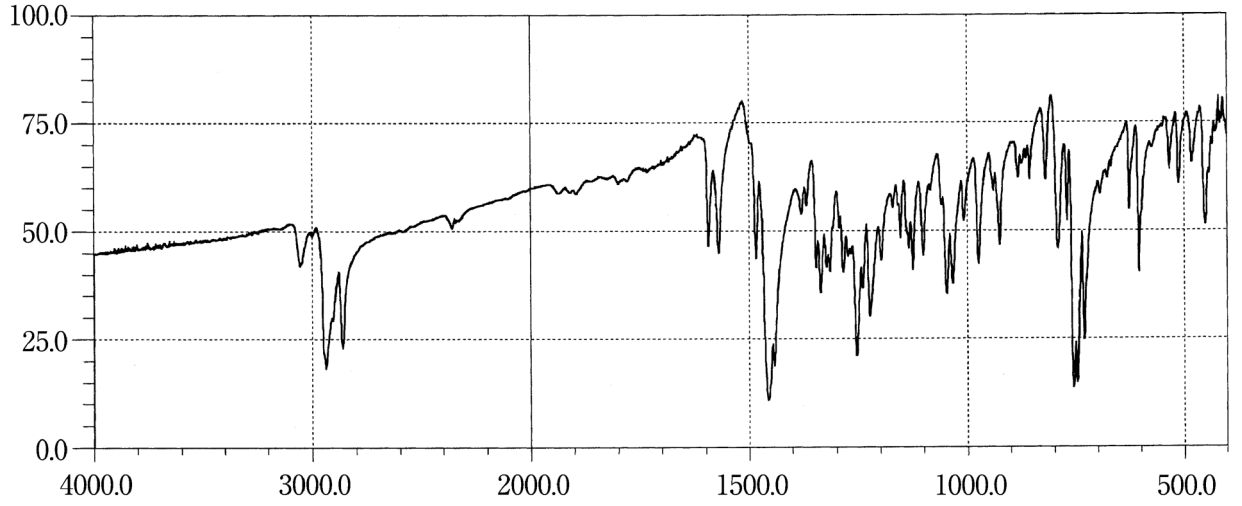

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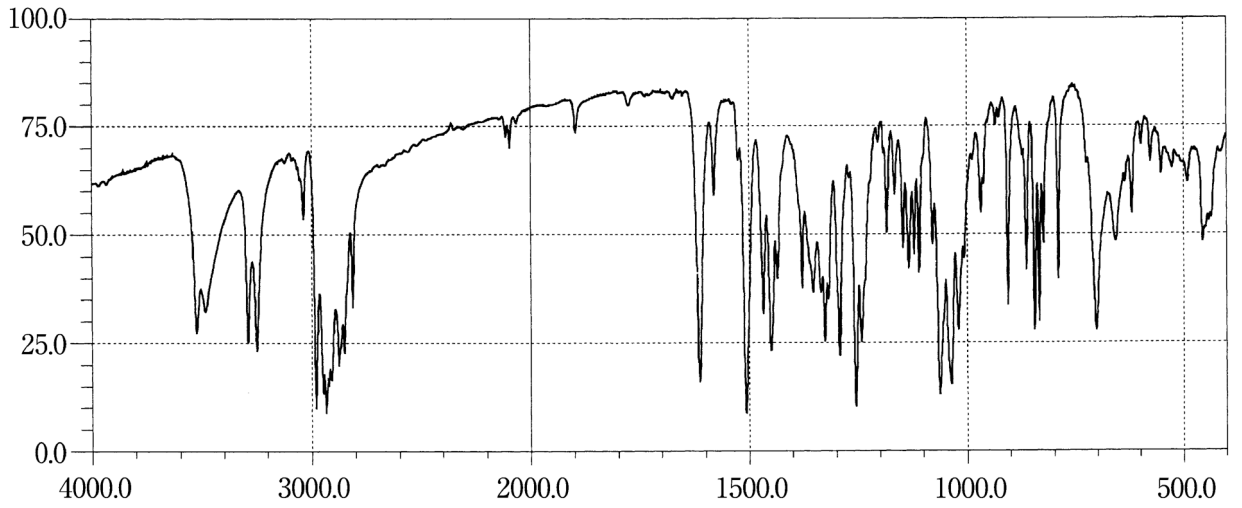
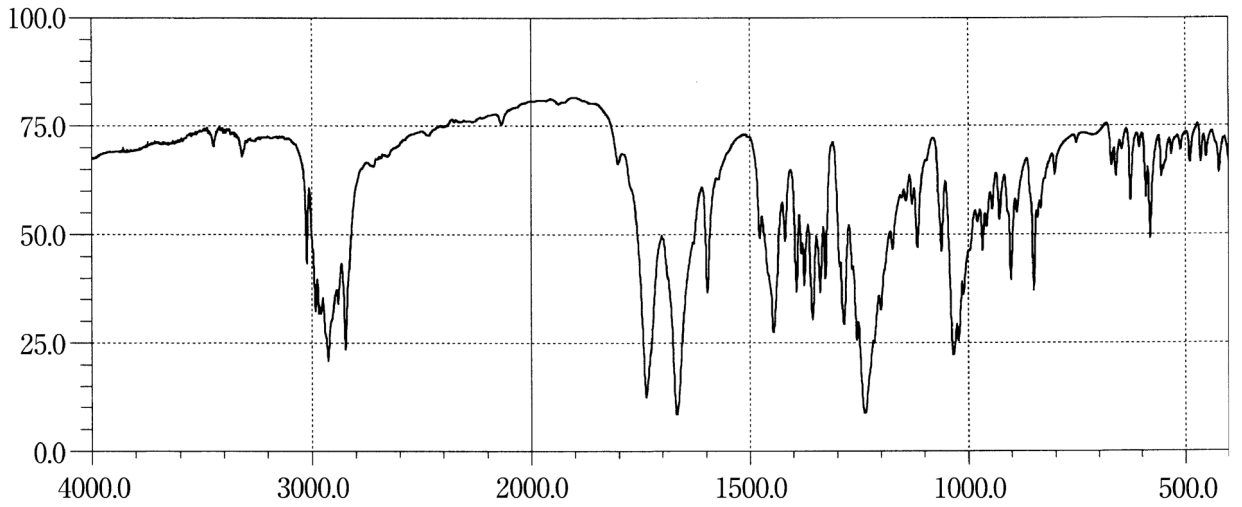
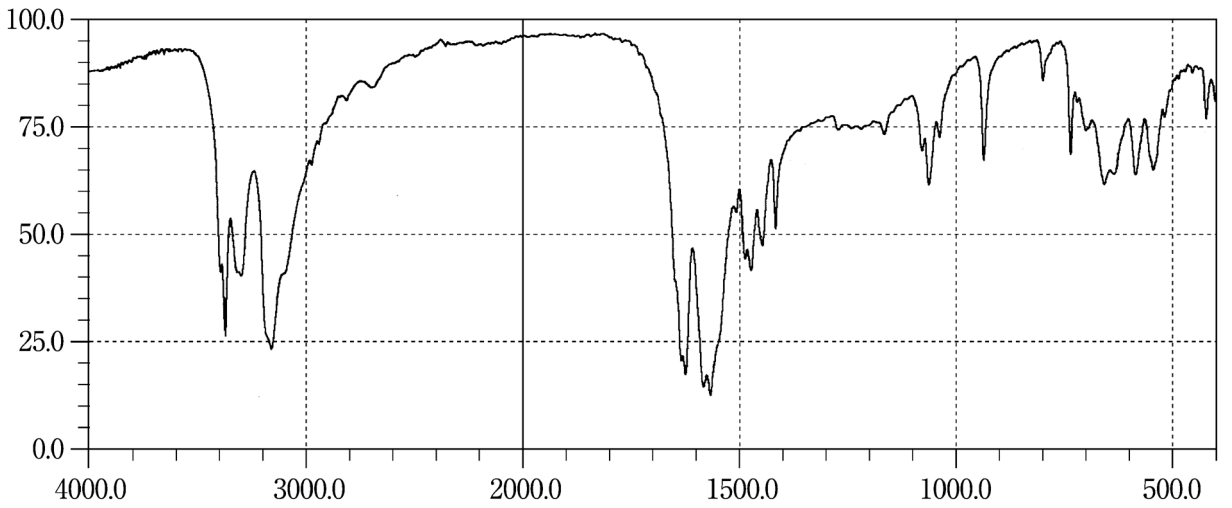


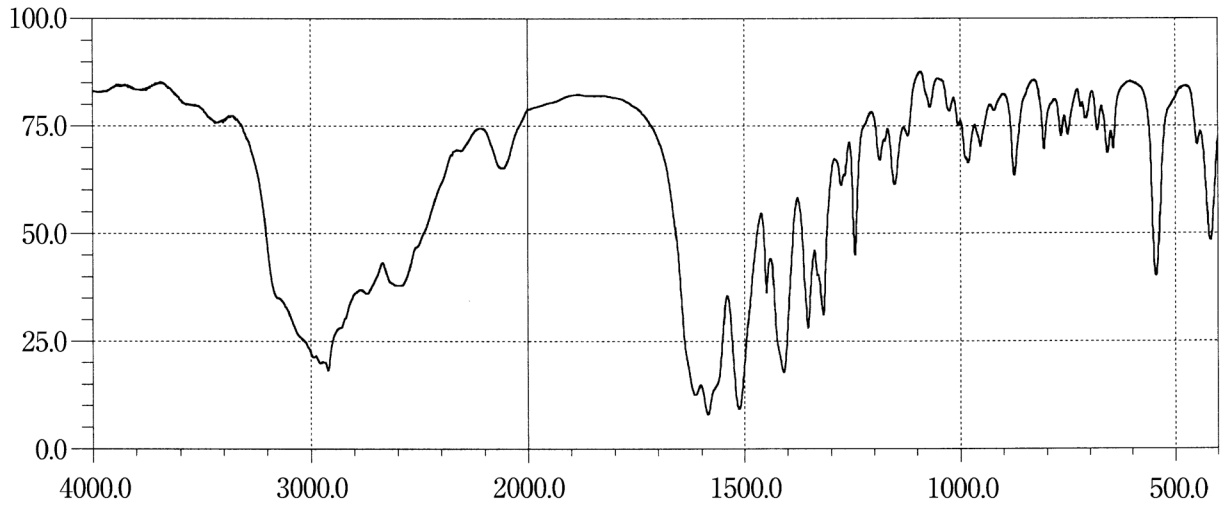
Metenolone Acetate



Metformin Hydrochloride



L-Methionine



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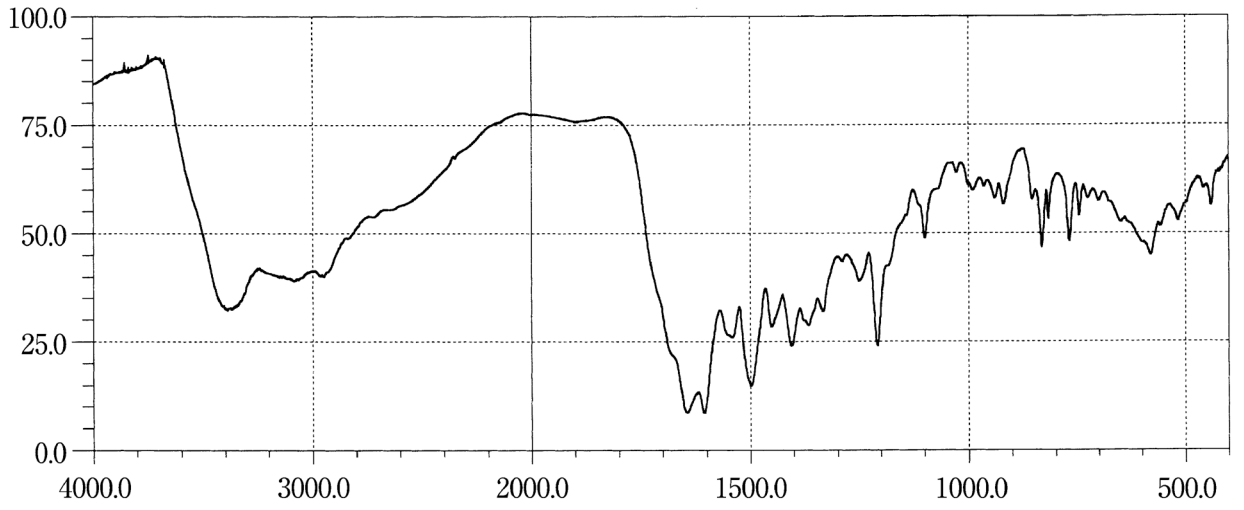
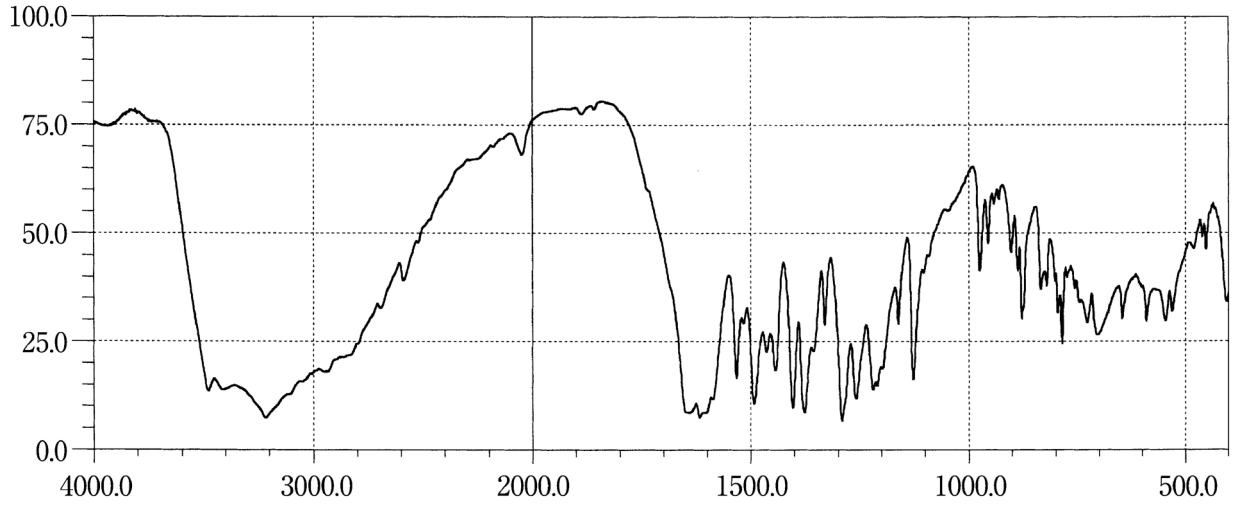
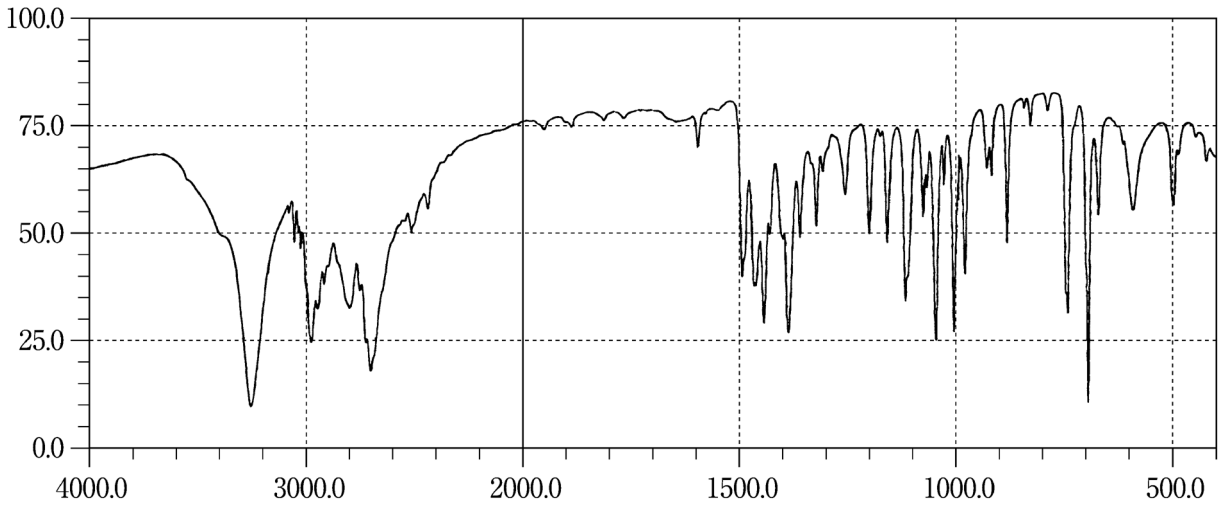


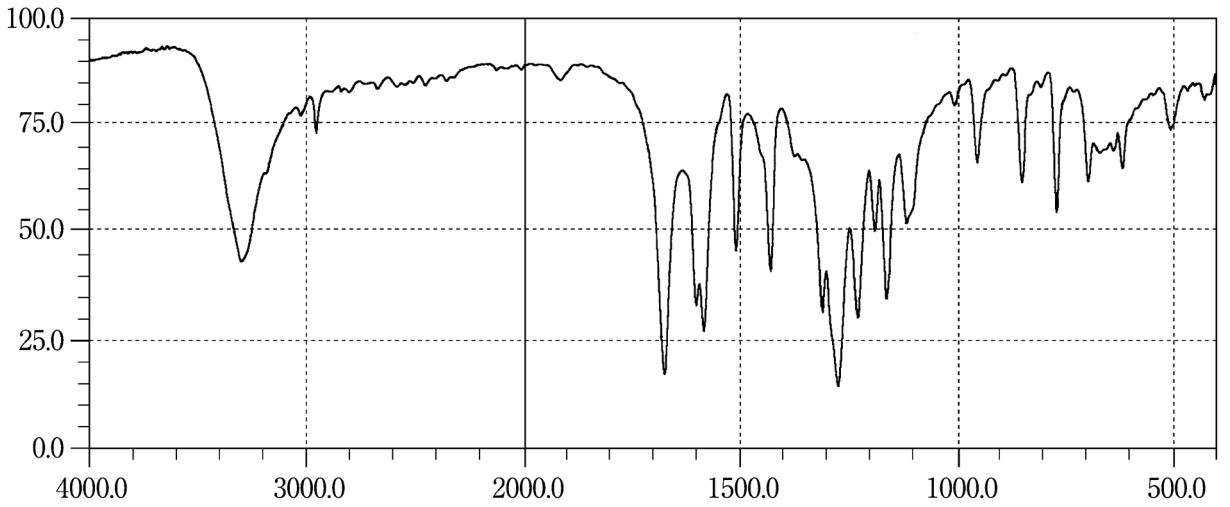
Methyldopa Hydrate



_dl_-Methylephedrine Hydrochloride



Methyl Parahydroxybenzoate



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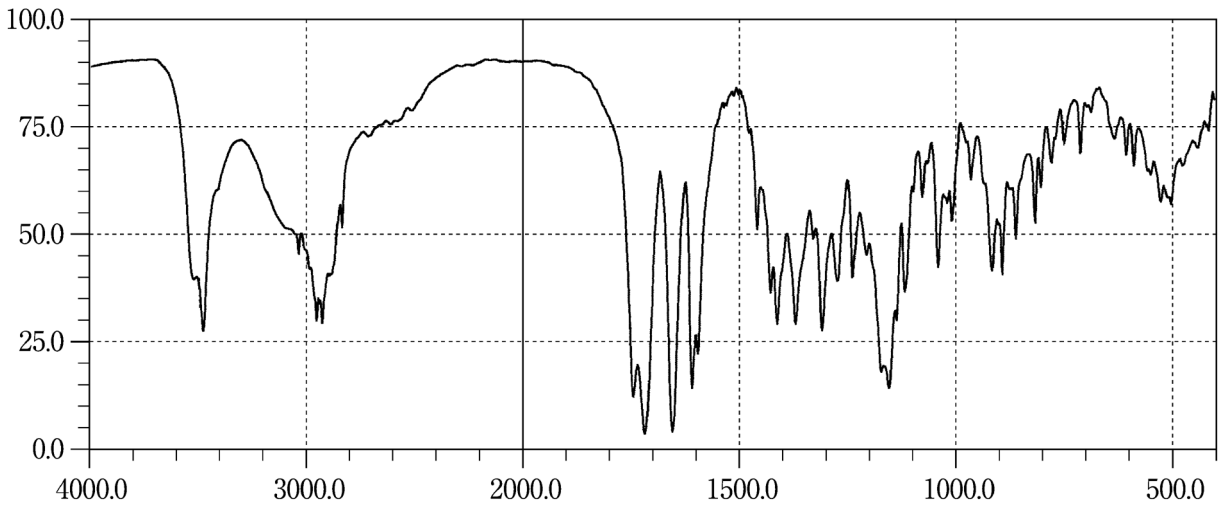

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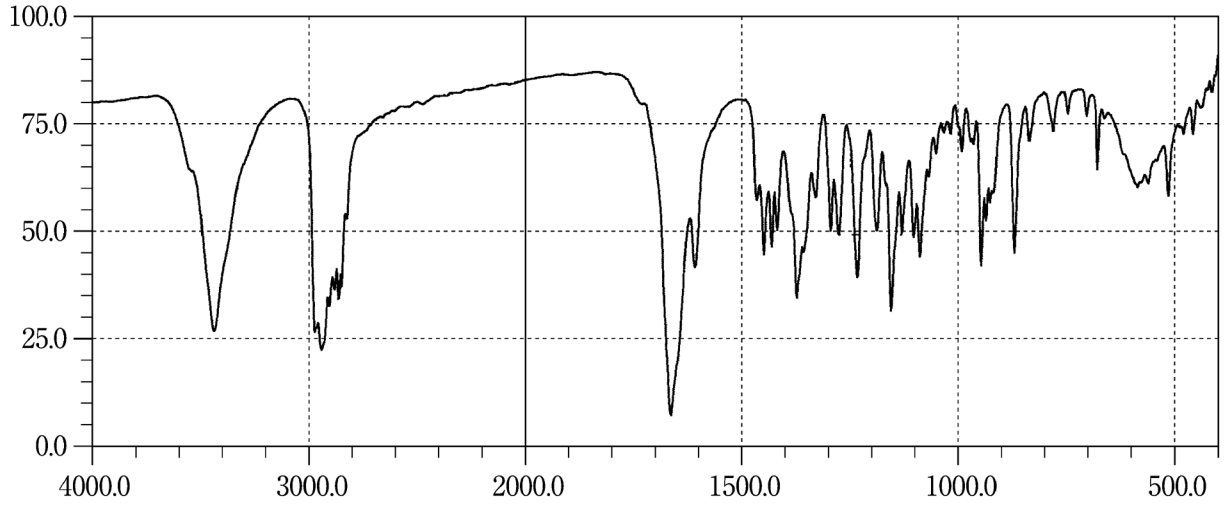

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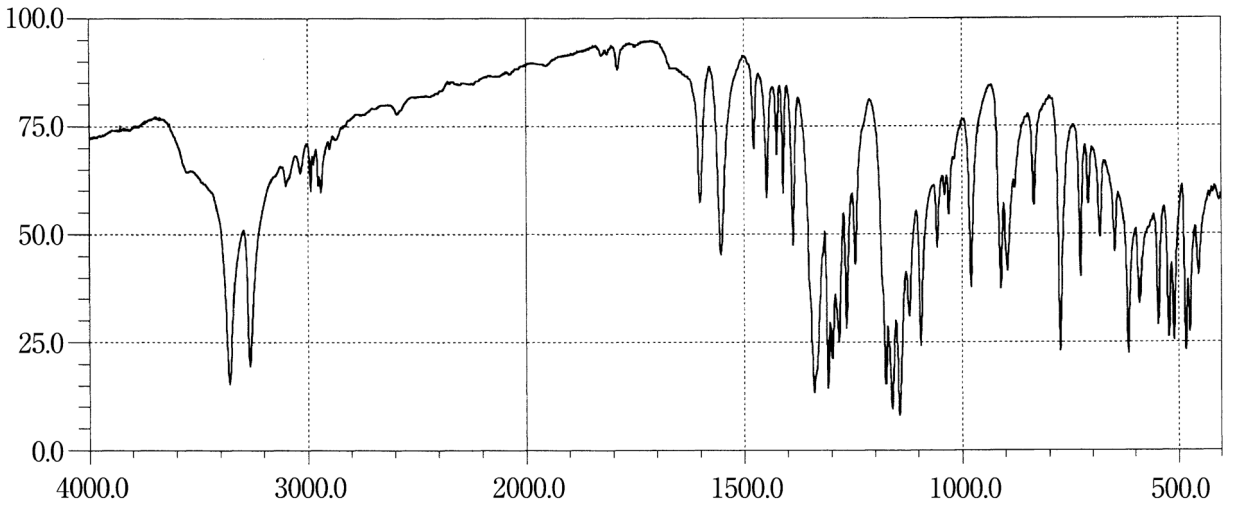

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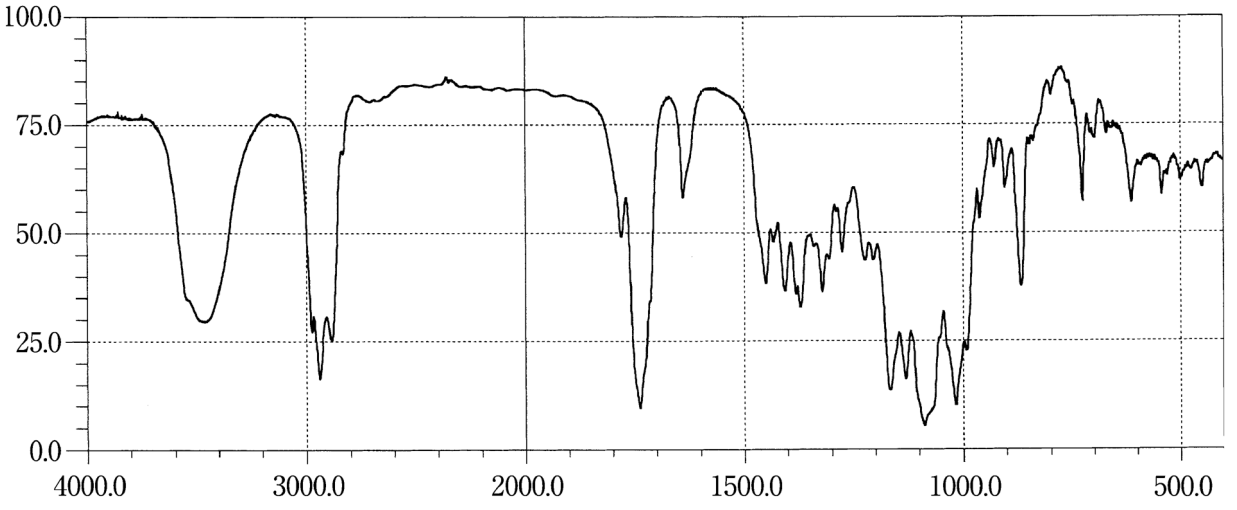

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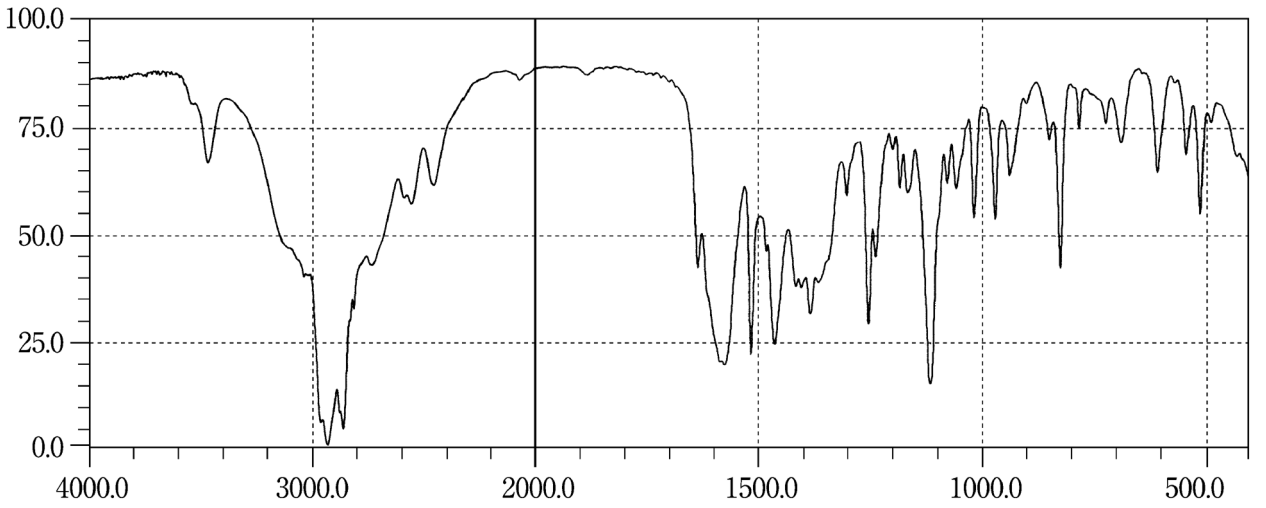

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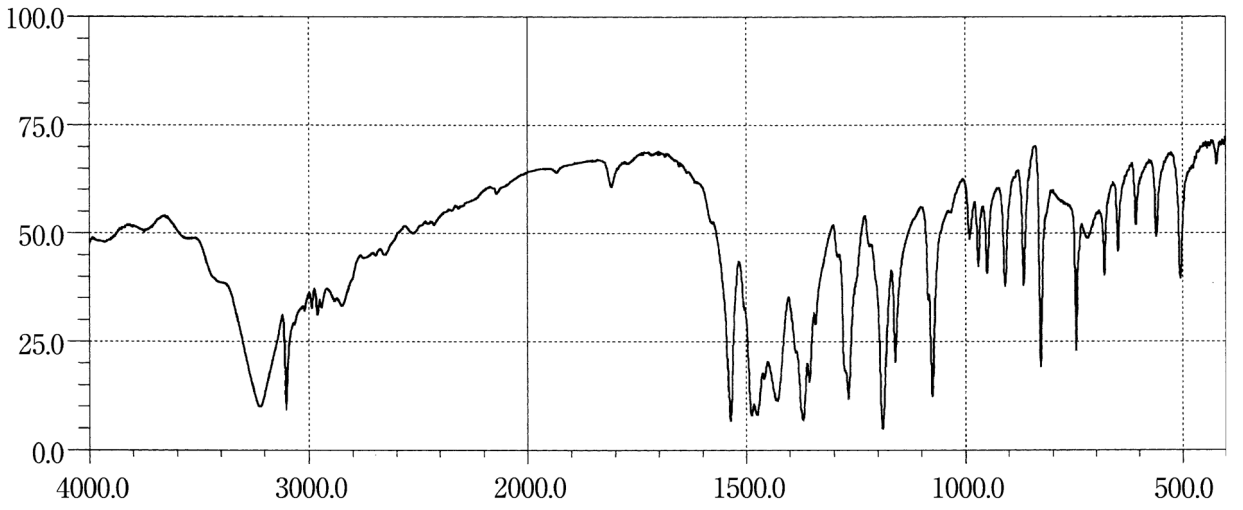
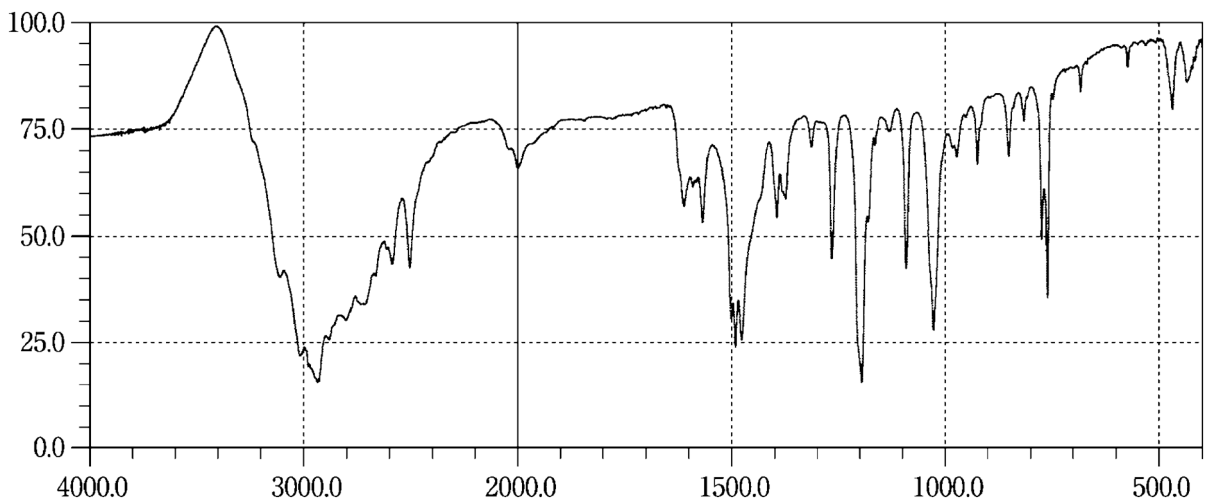


Mexiletine Hydrochloride



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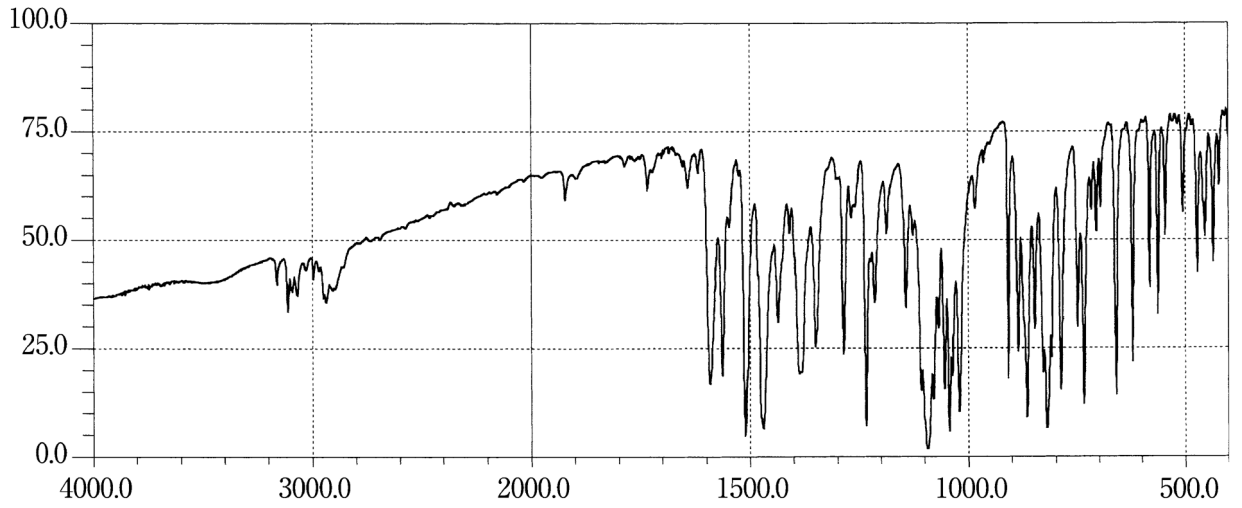
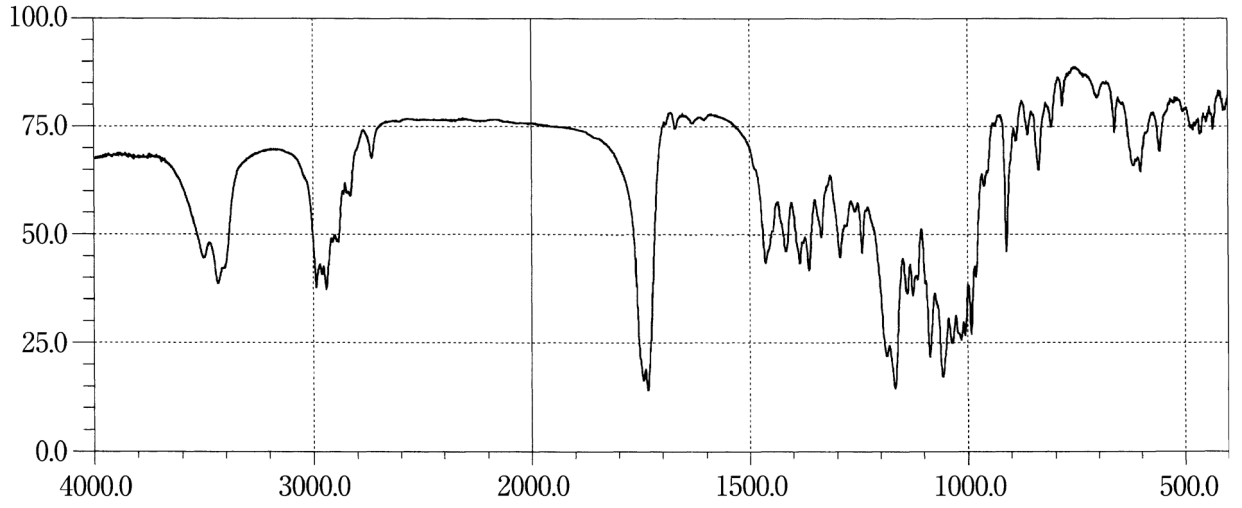
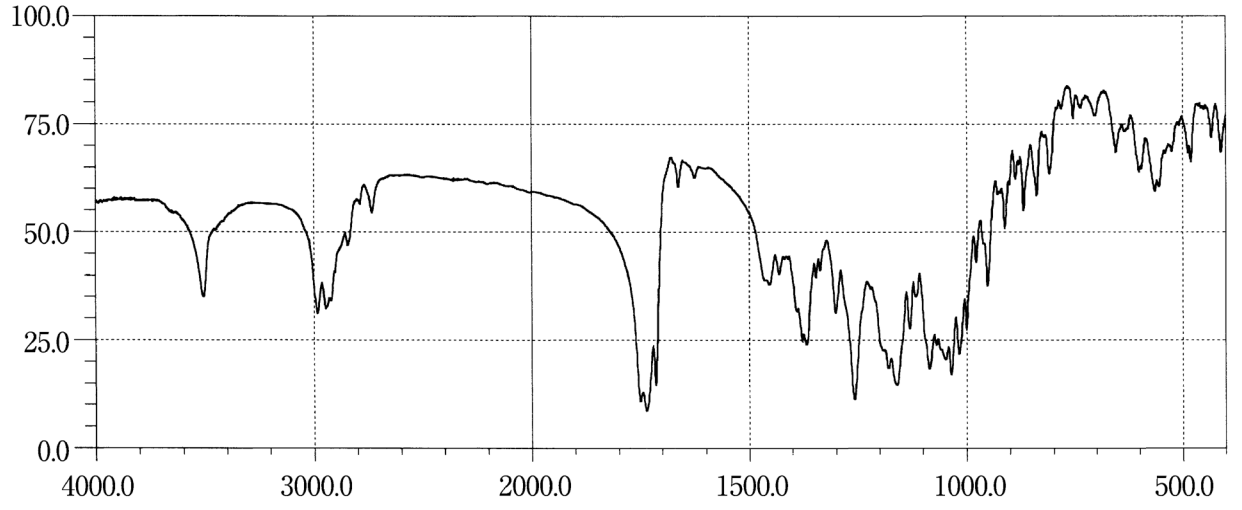


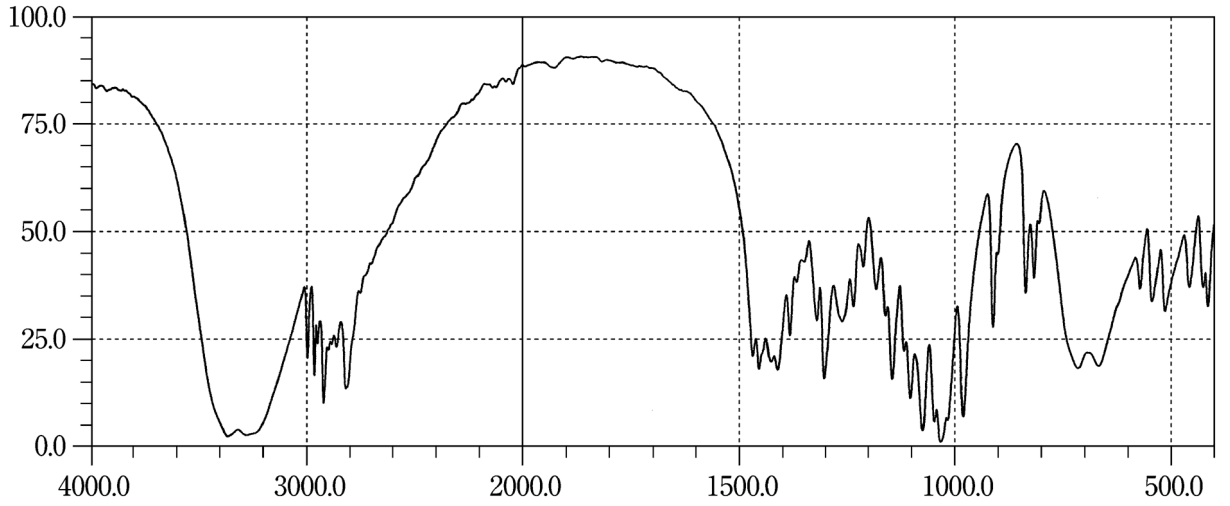
Midecamycin



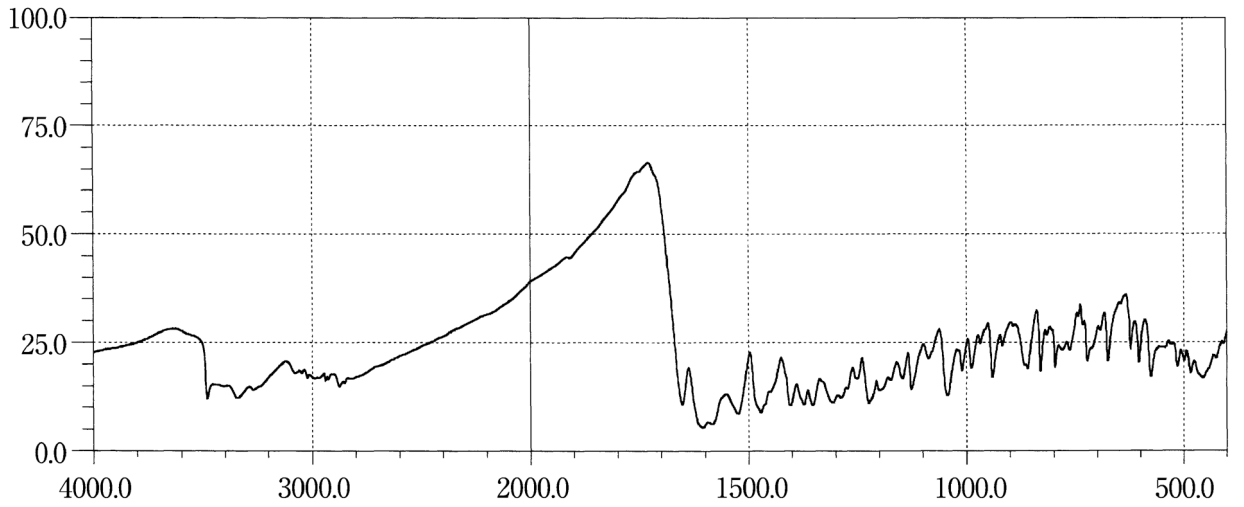
Midecamycin Acetate



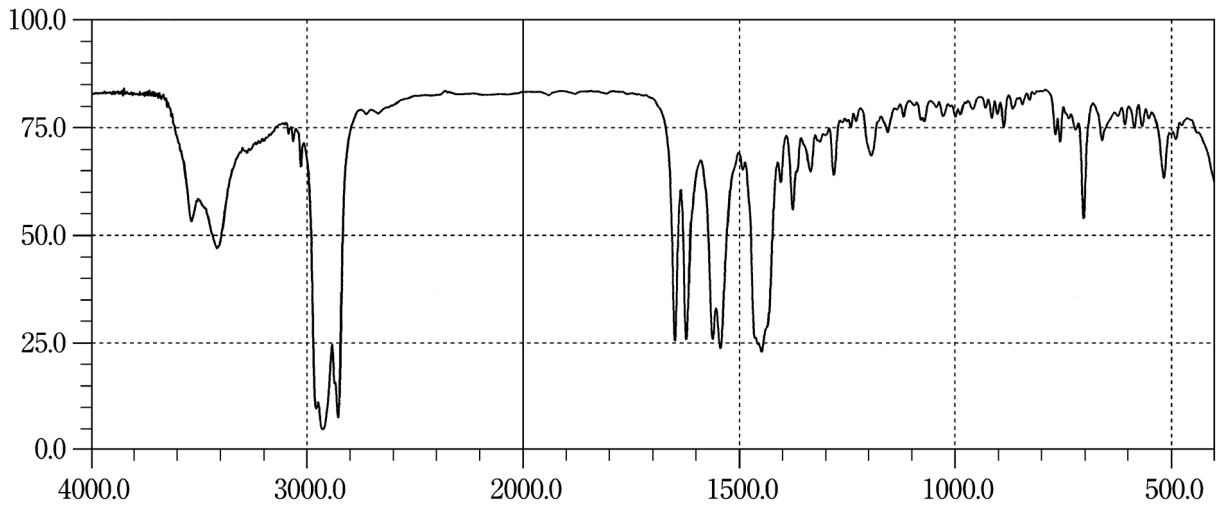
Miglitol



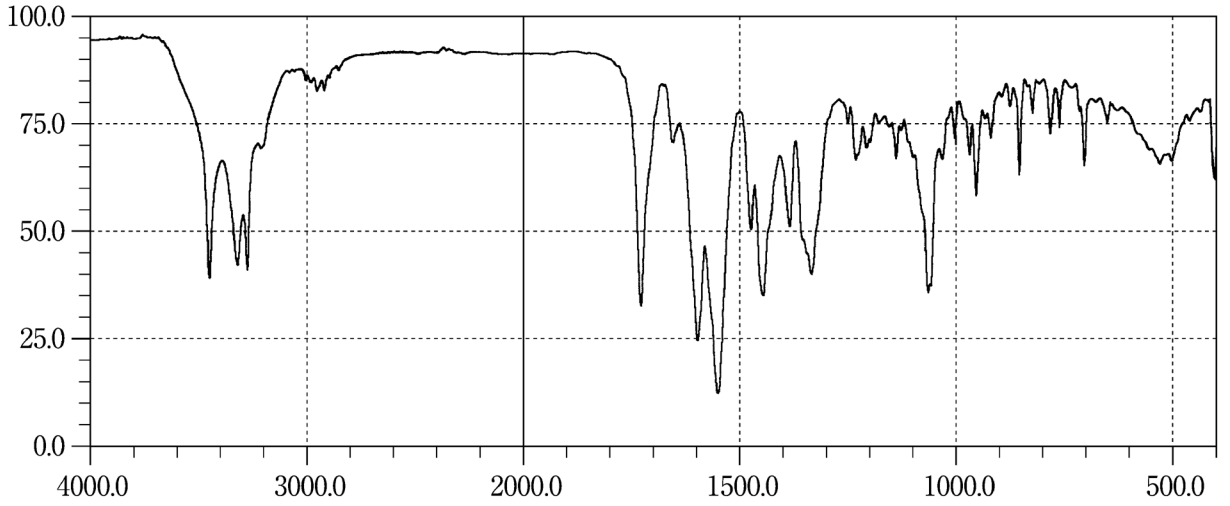
Minocycline Hydrochloride



Mitiglinide Calcium Hydrate



Mitomycin C



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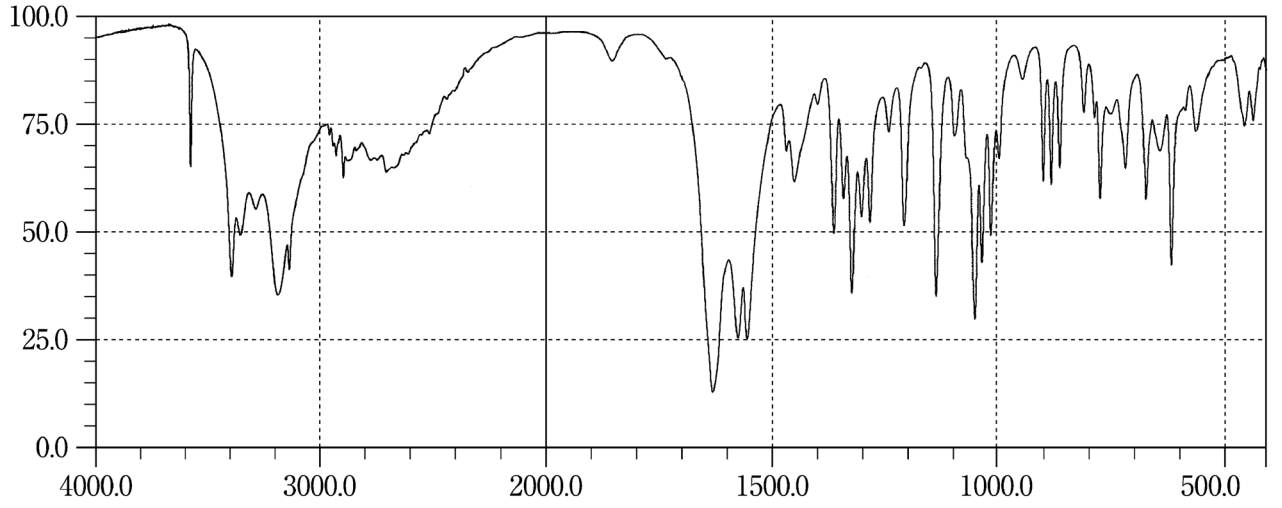

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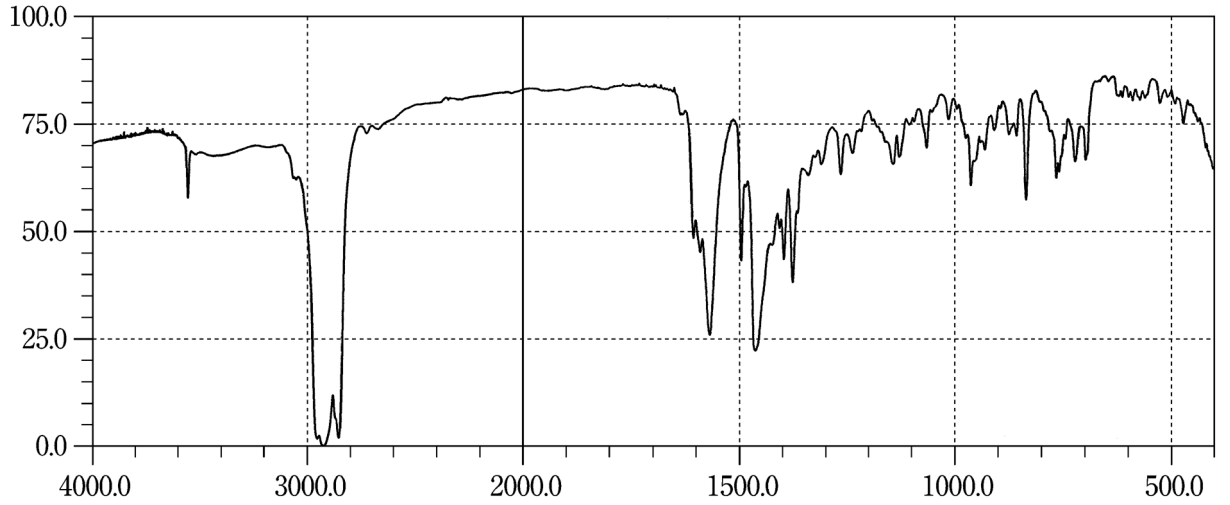

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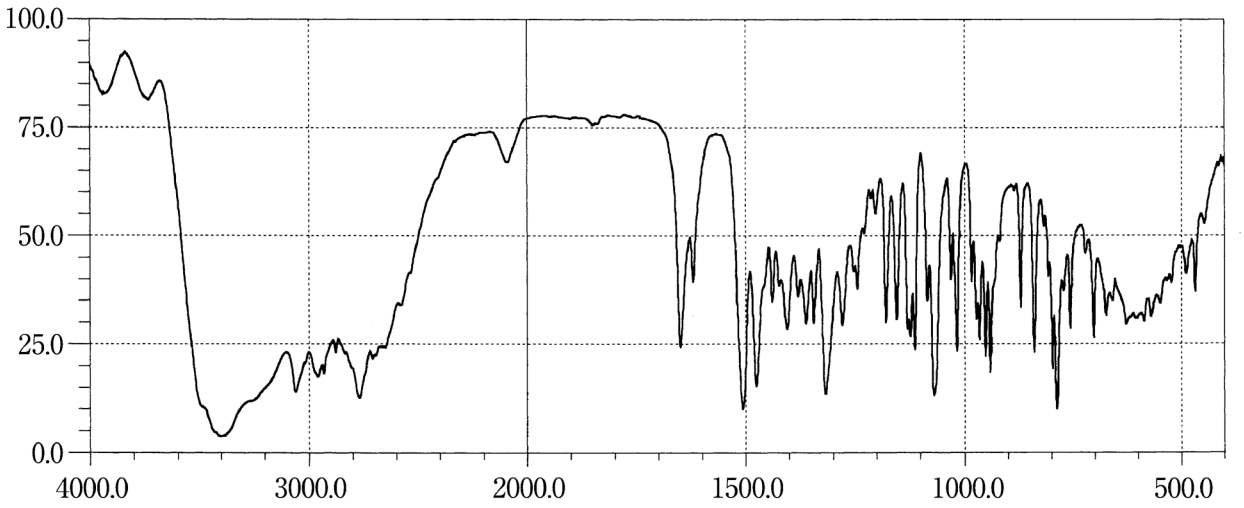
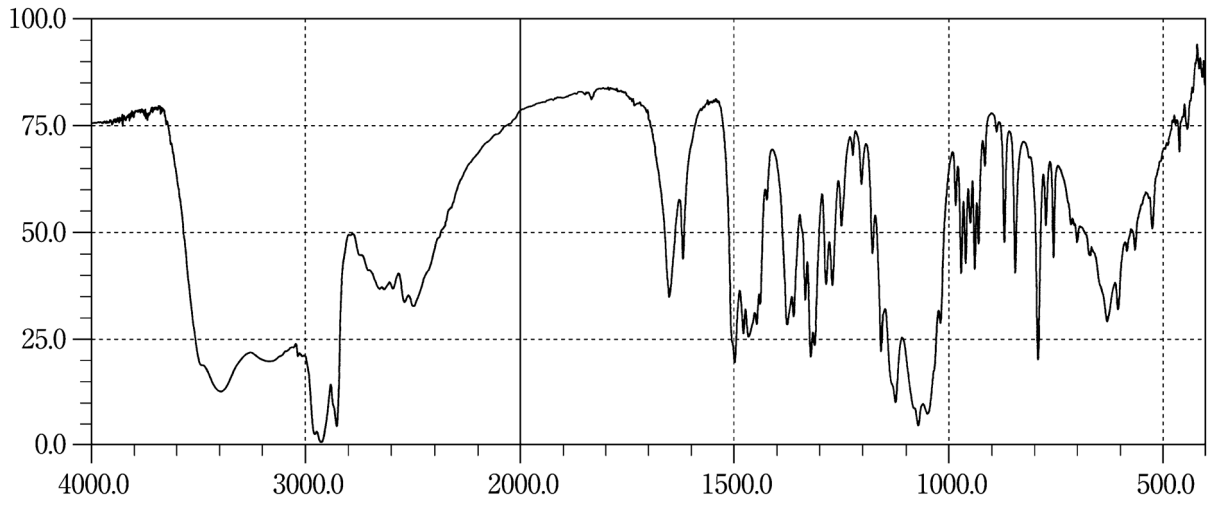
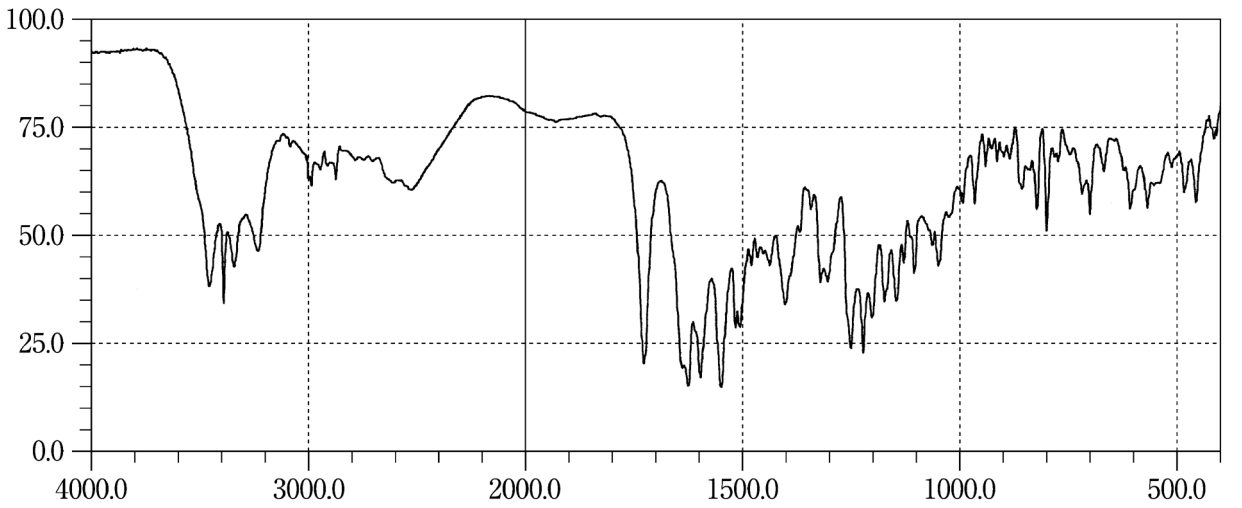


Morphine Sulfate Hydrate



Mosapride Citrate Hydrate



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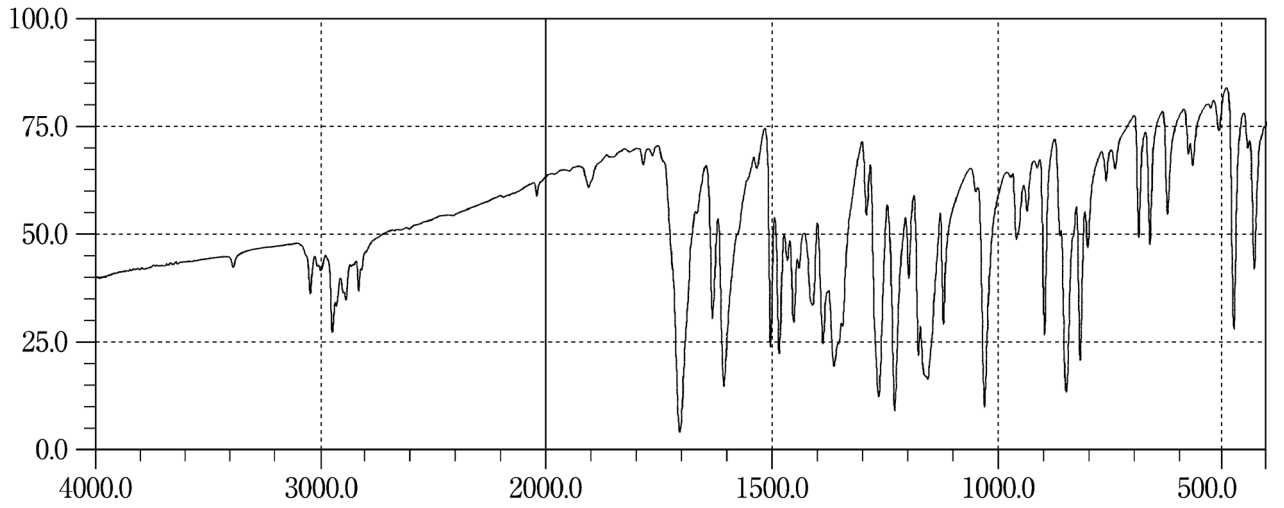
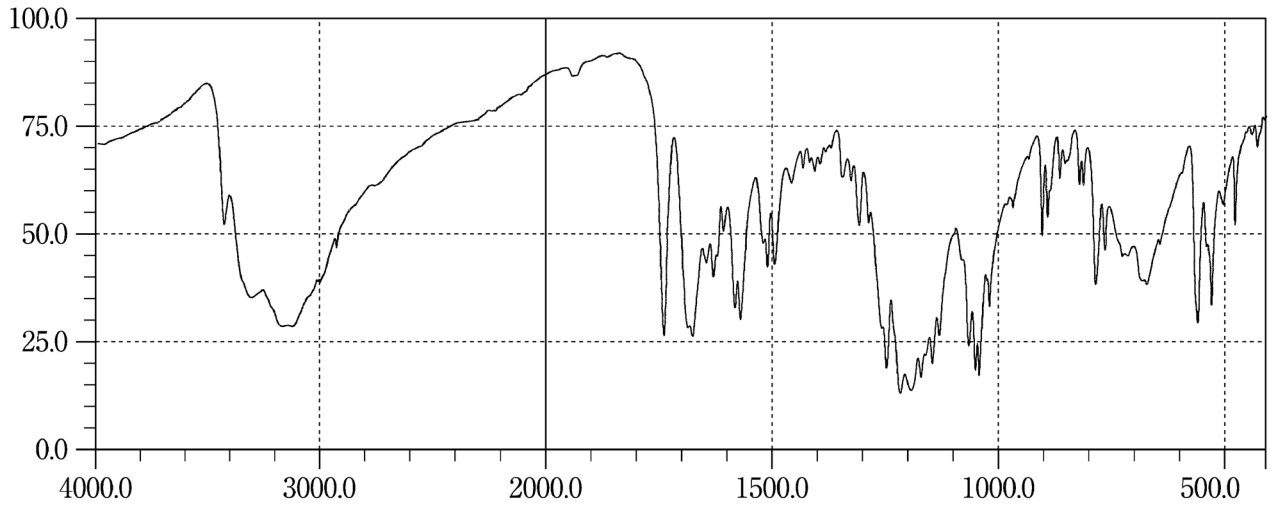


Nafamostat Mesilate



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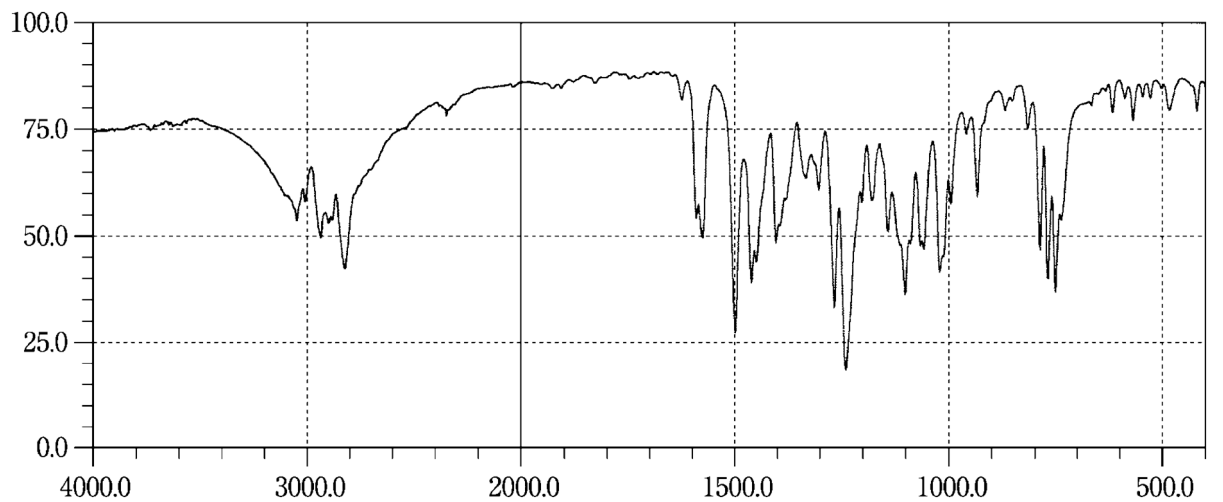
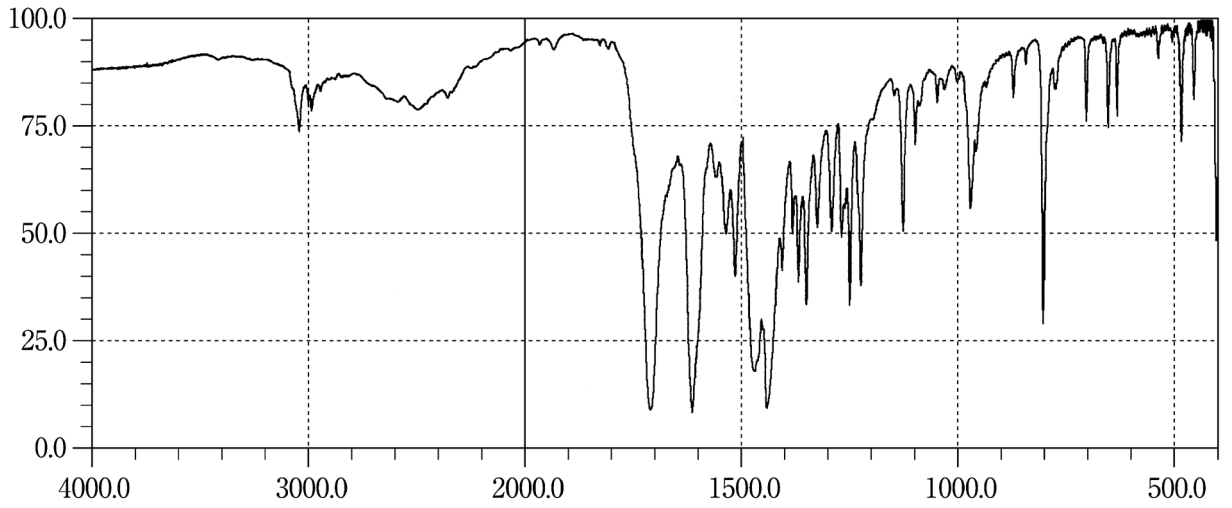
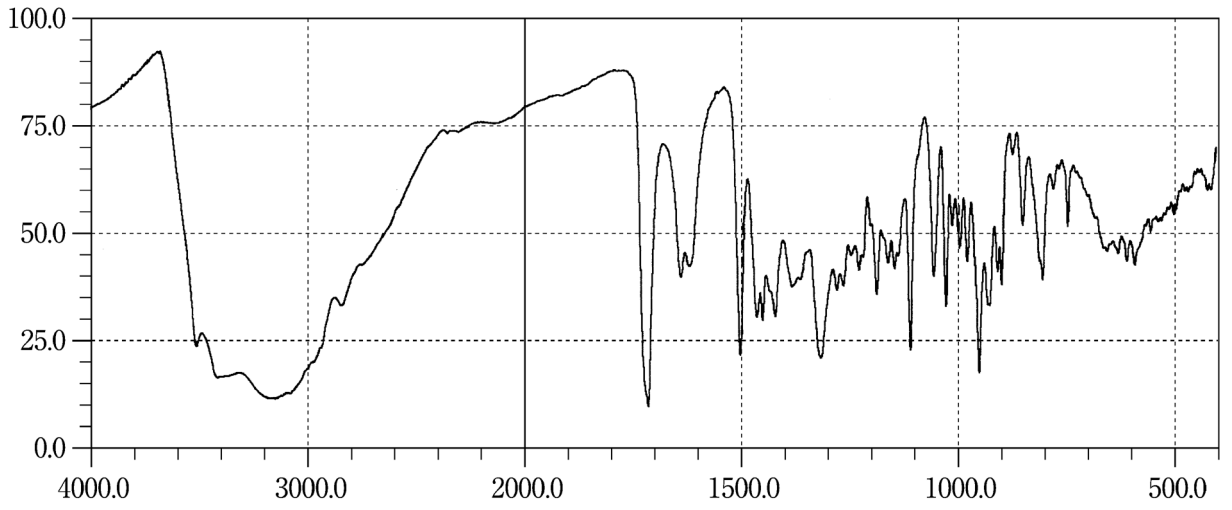


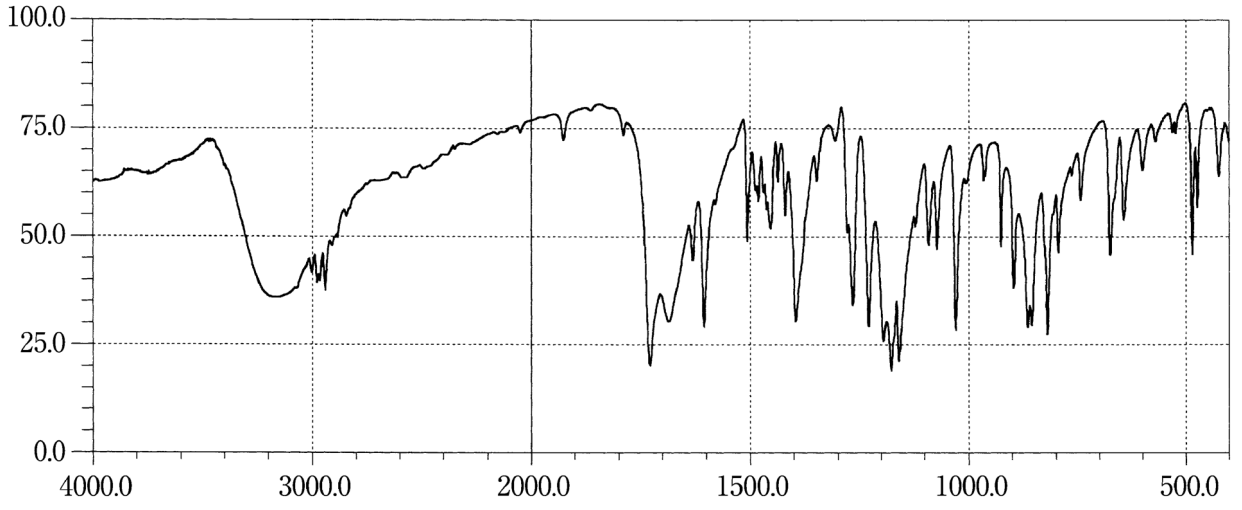
Nalidixic Acid



Naloxone Hydrochloride



Naproxen



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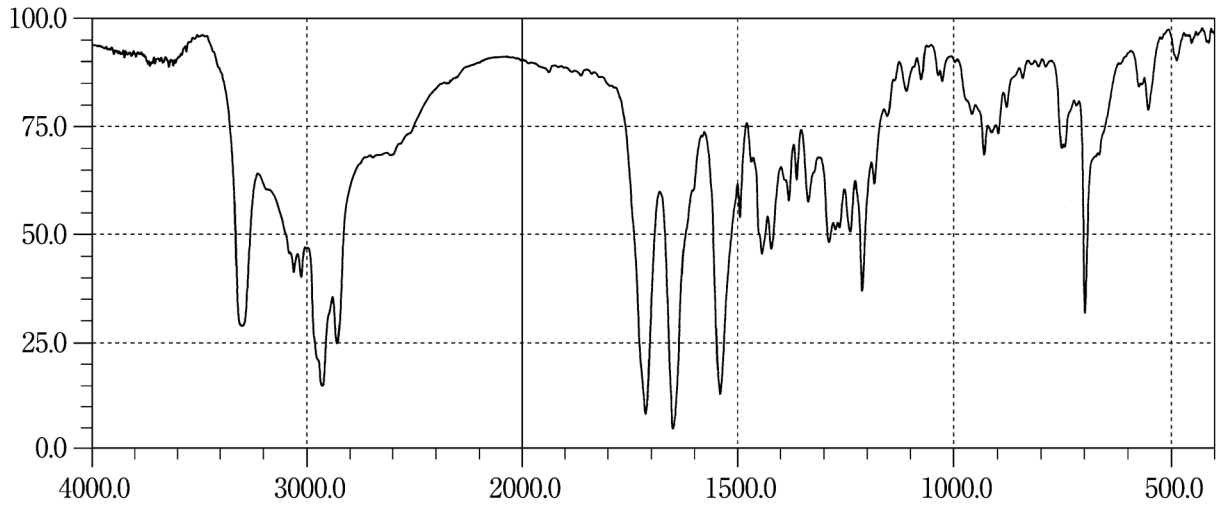
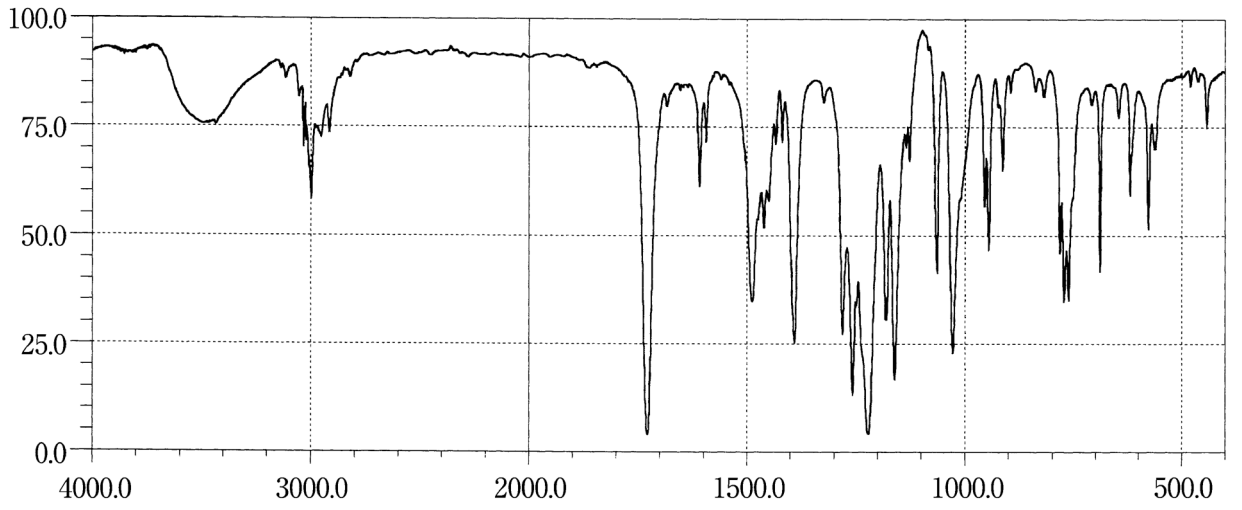
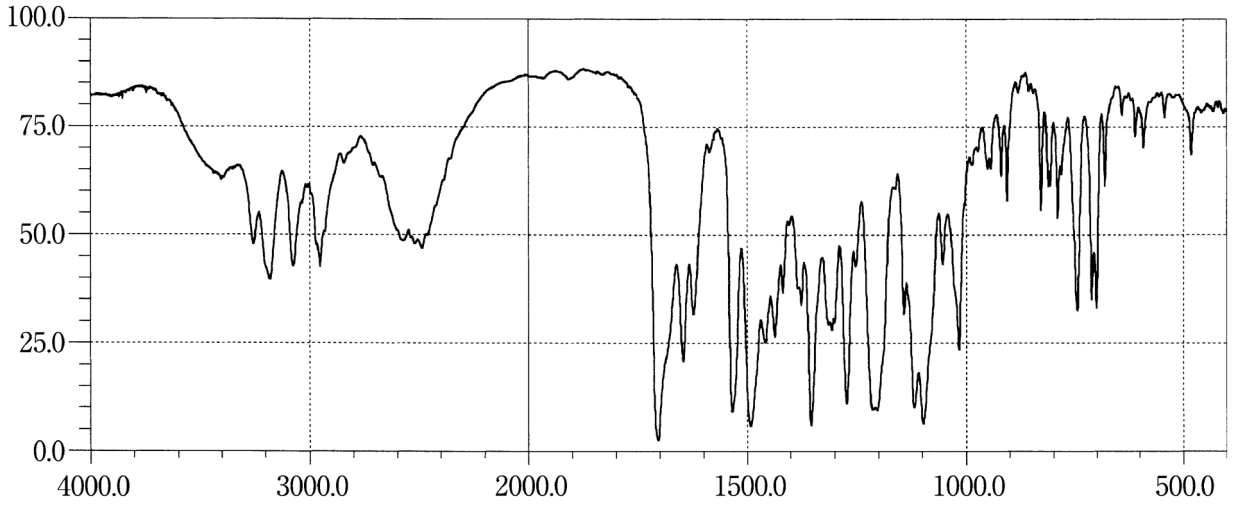


Neostigmine Methylsulfate



Nicardipine Hydrochloride



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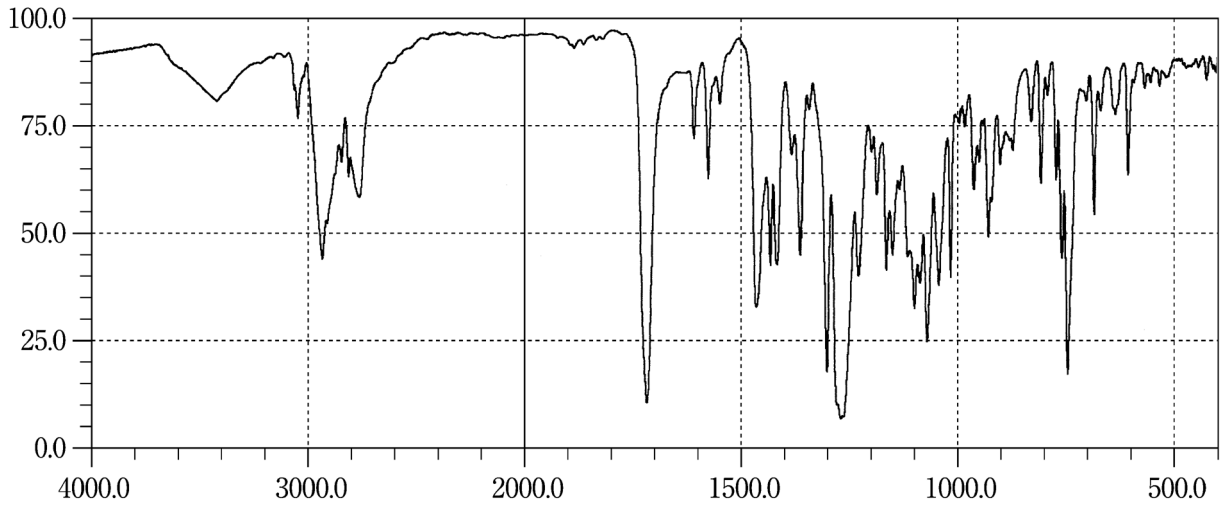
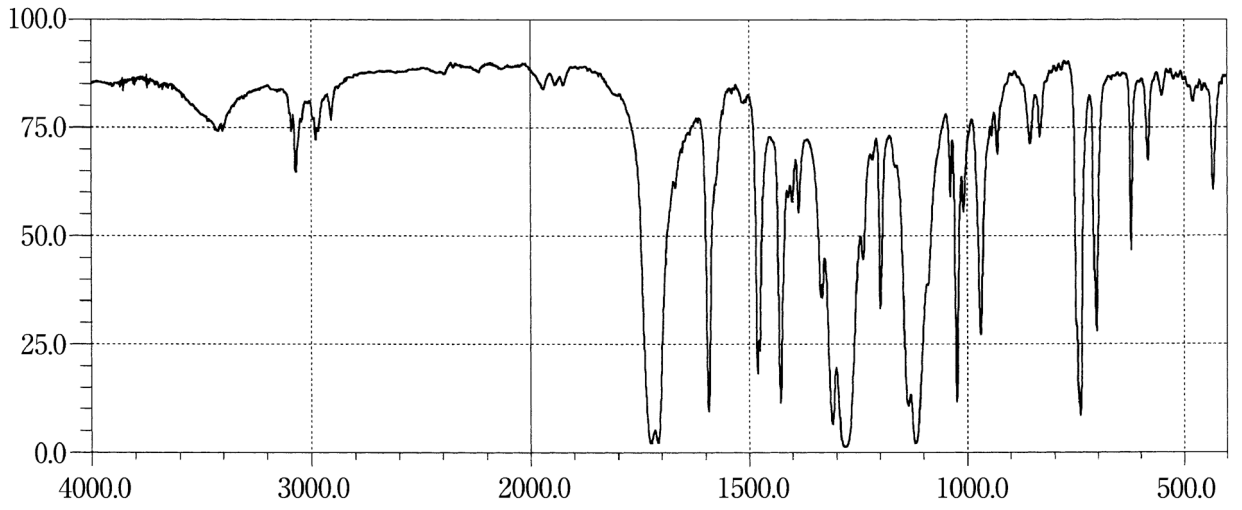
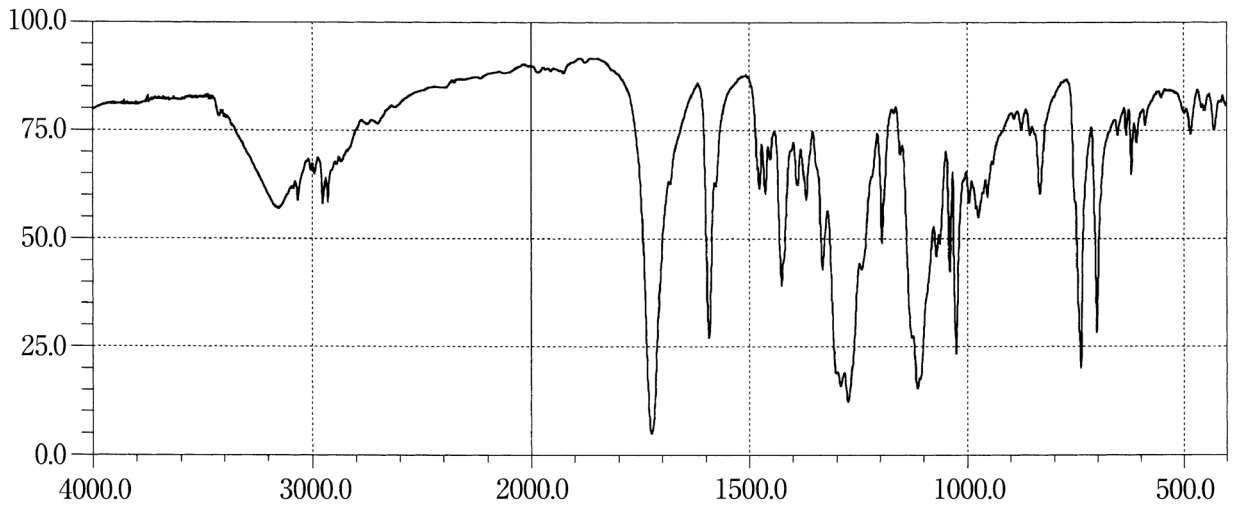


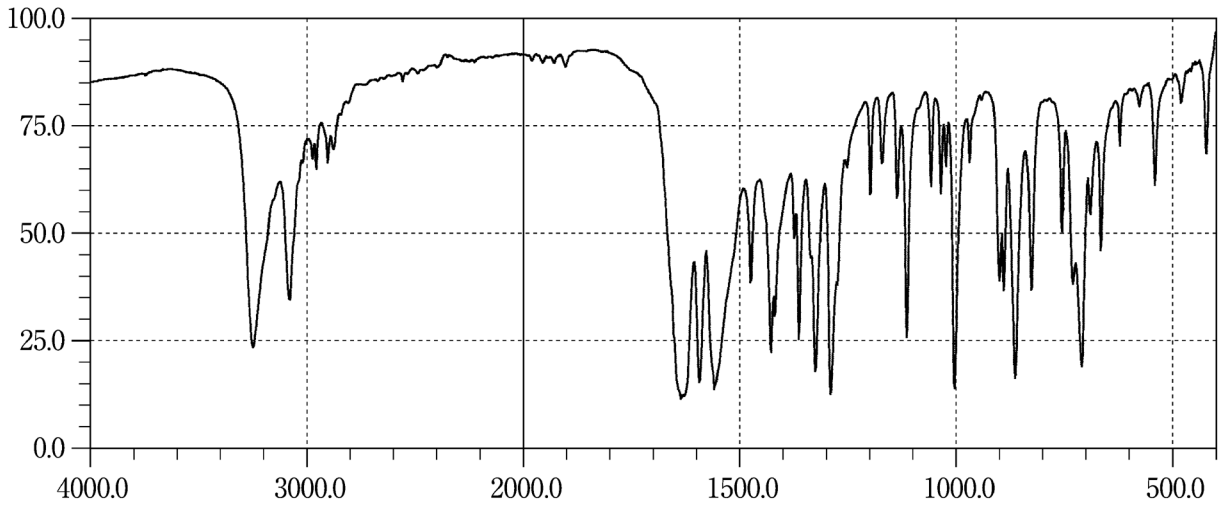
Niceritrol



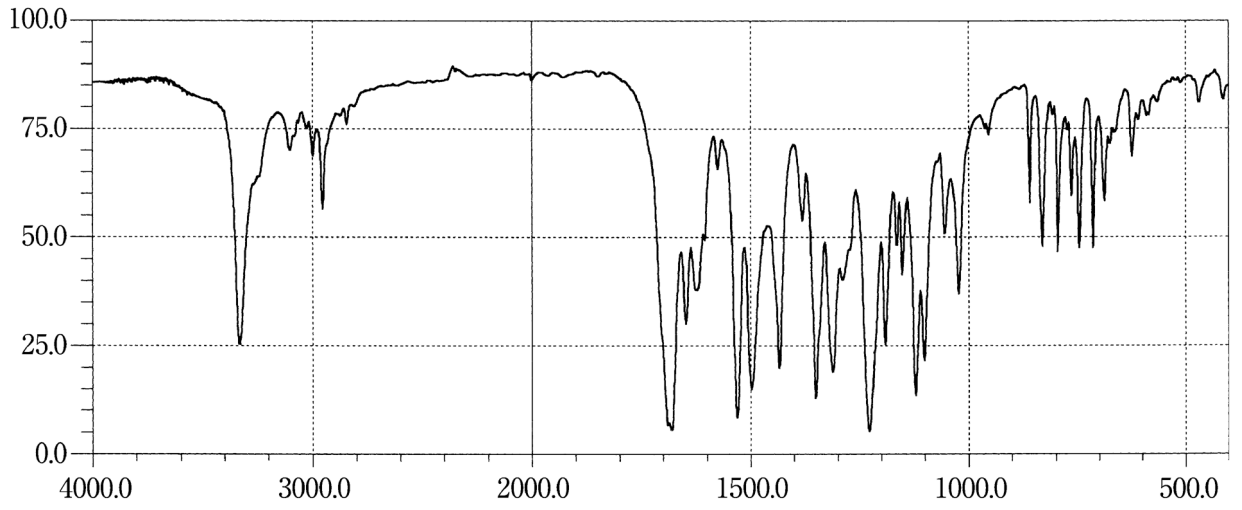
Nicomol



Nicorandil



Nifedipine



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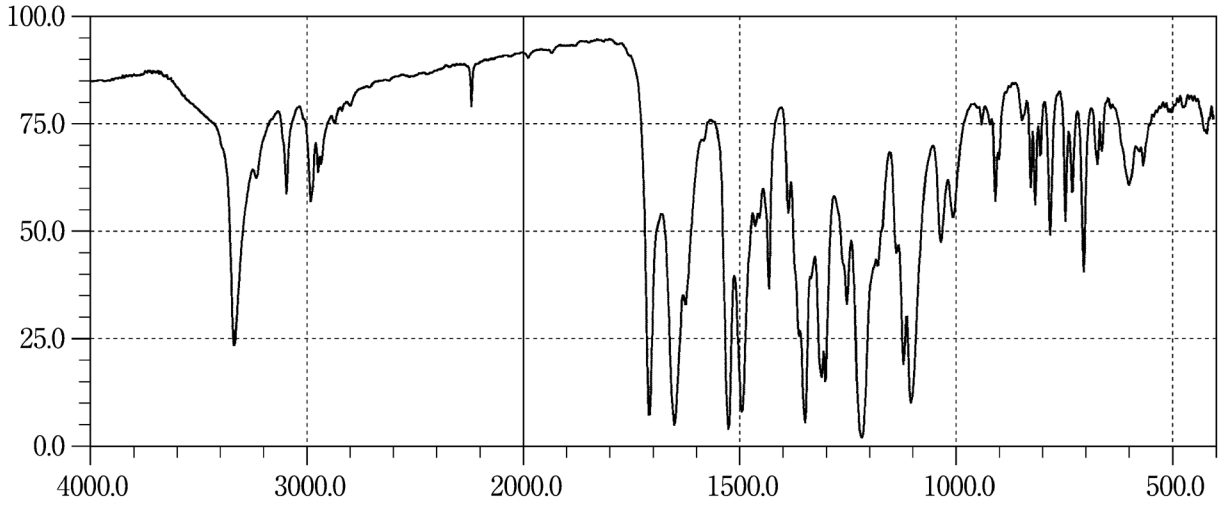

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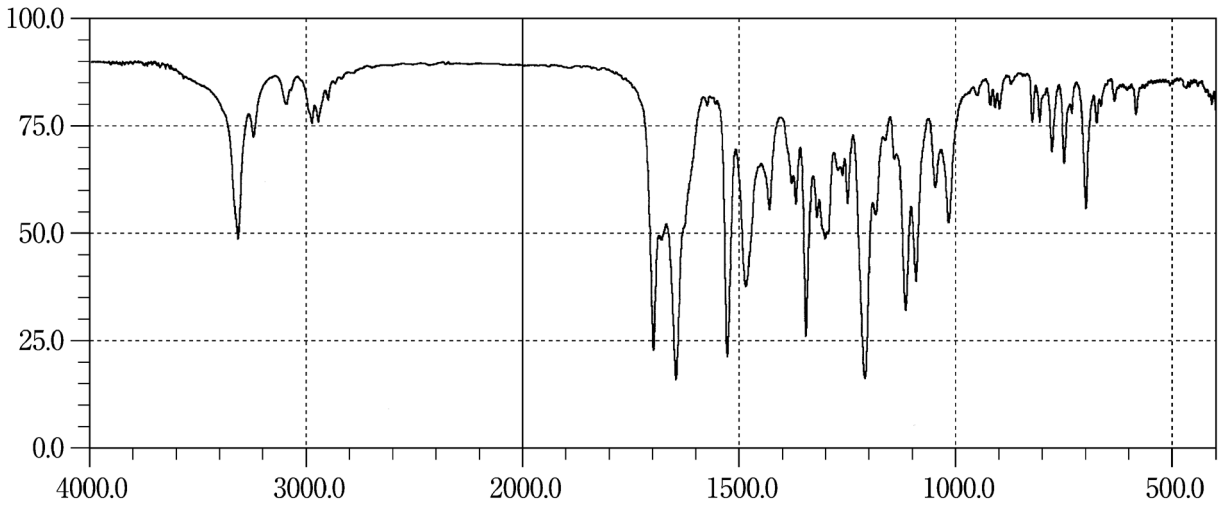

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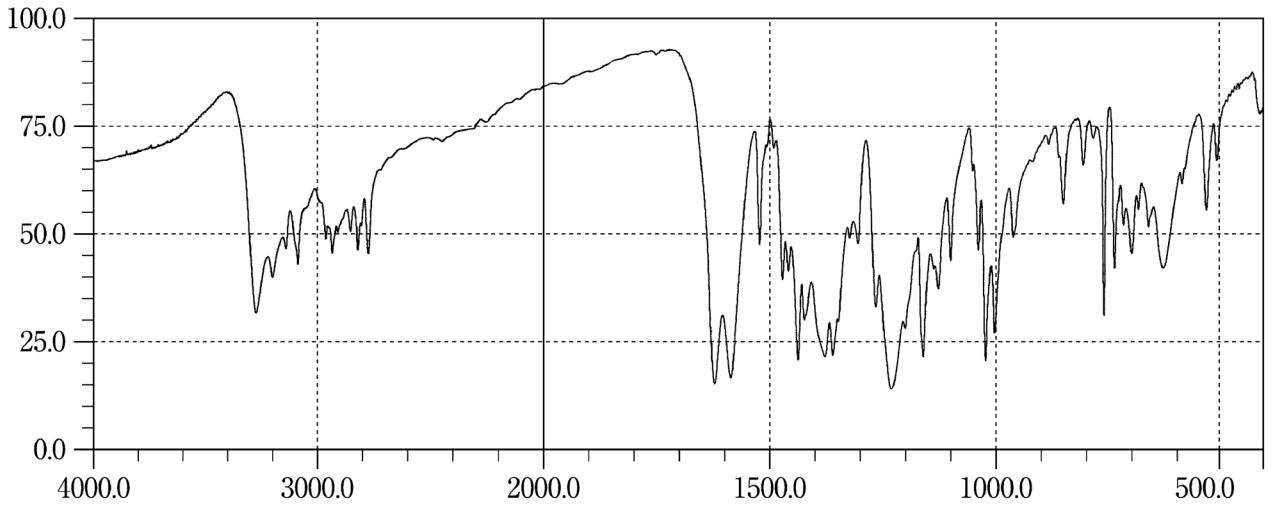

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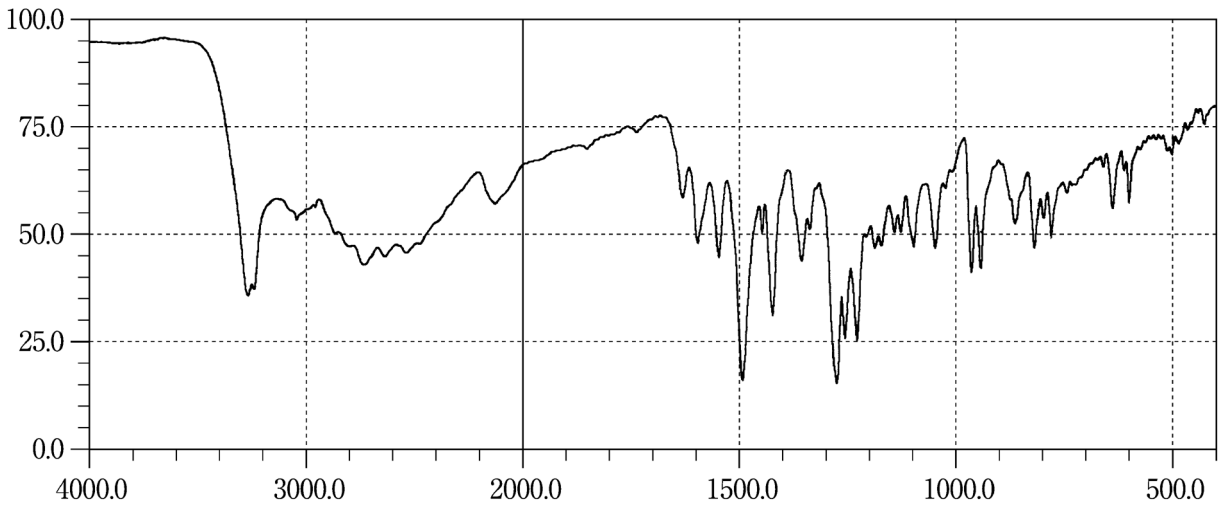

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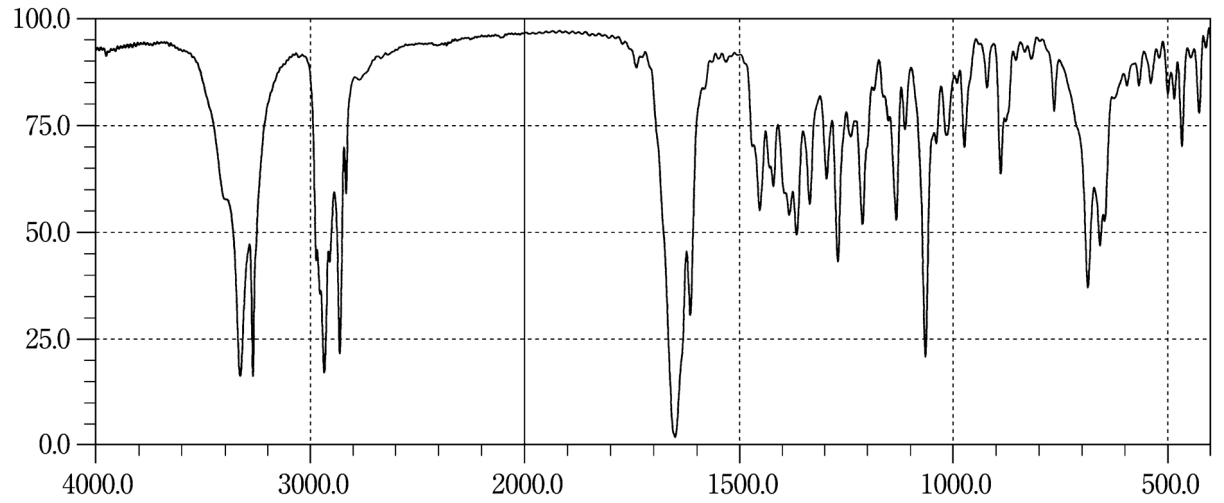
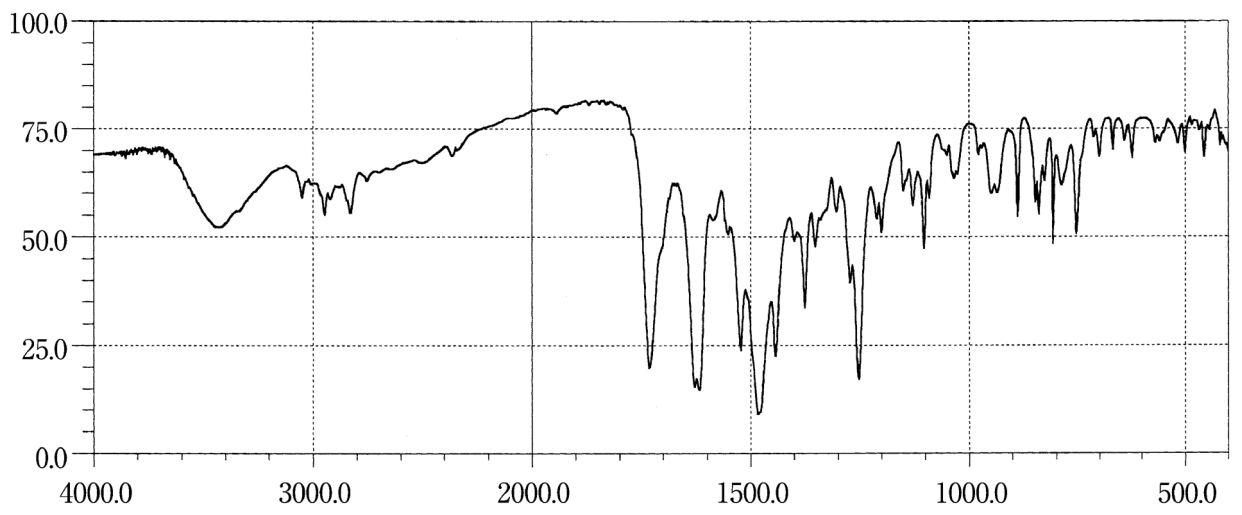
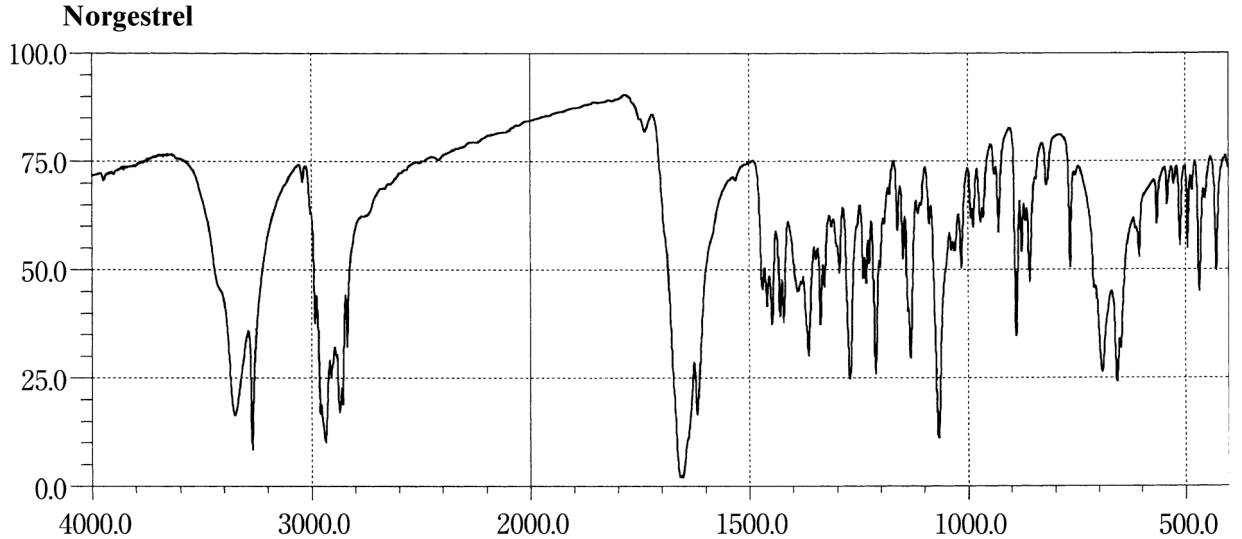


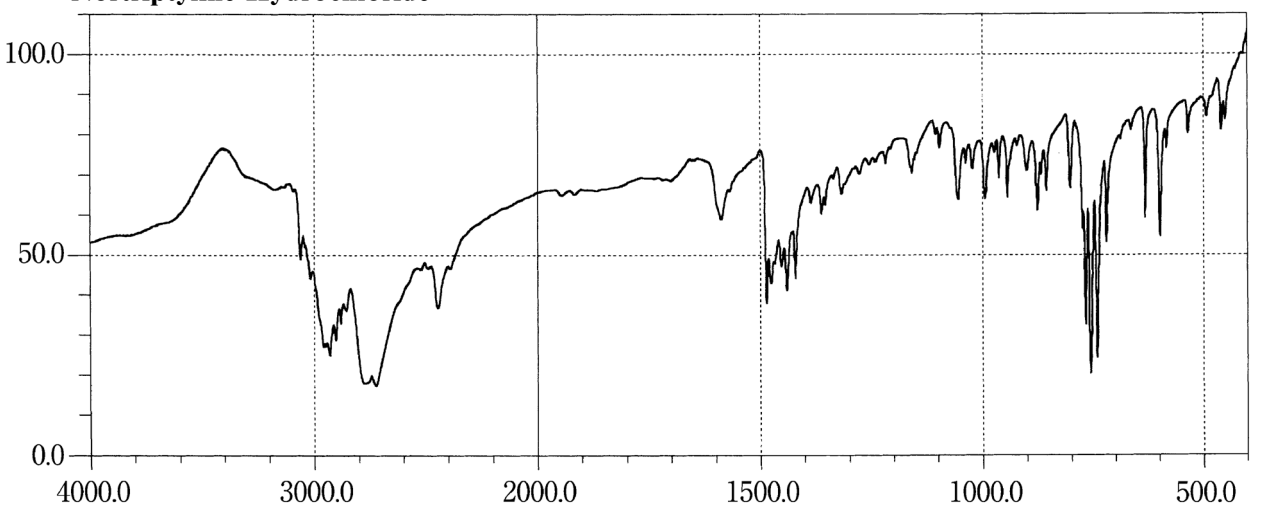
Norfloxacin



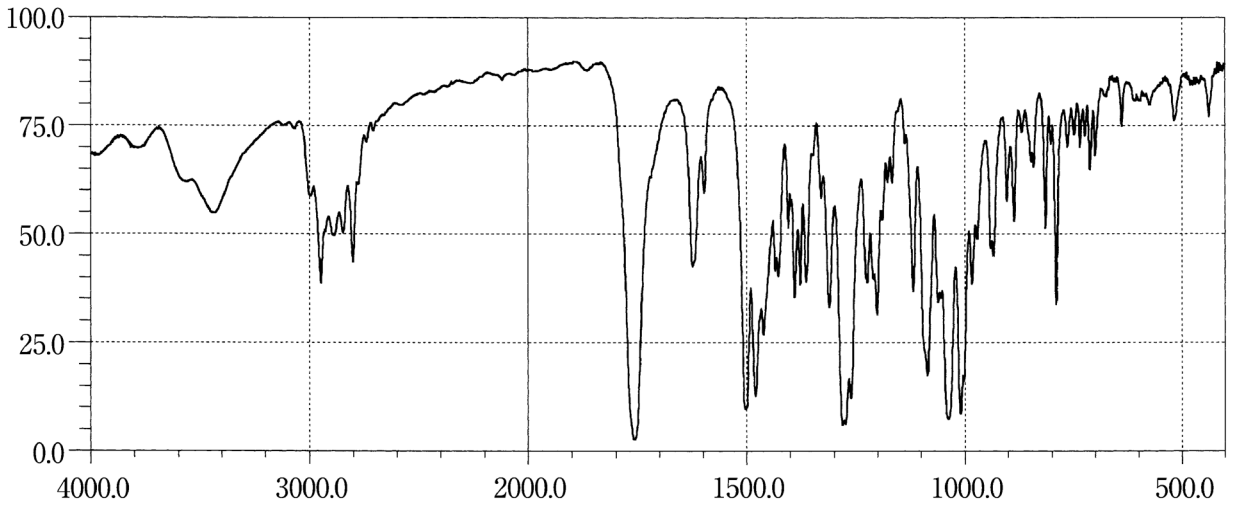
Norgestrel



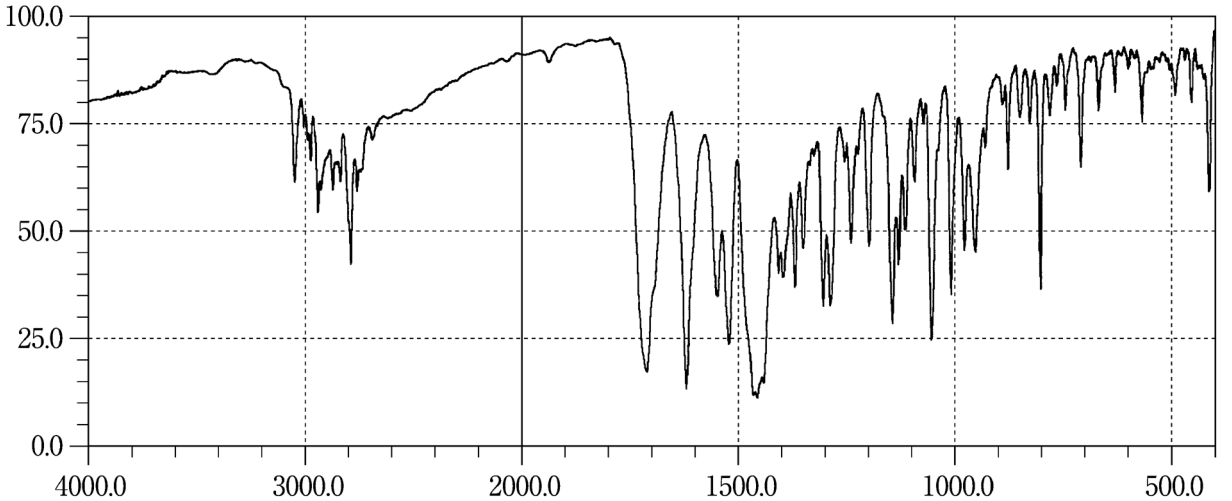
Nortriptyline Hydrochloride



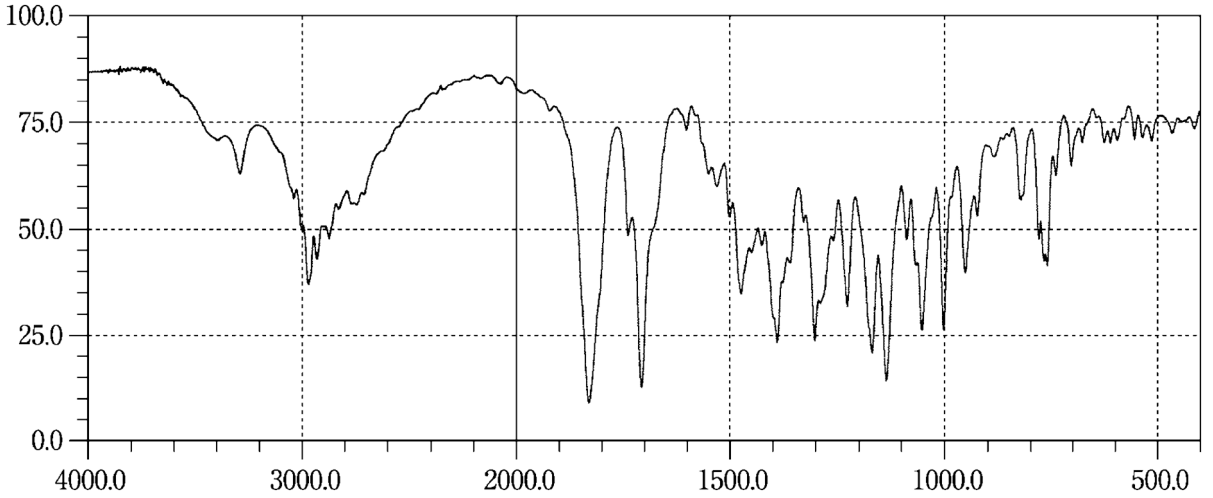
Noscapine



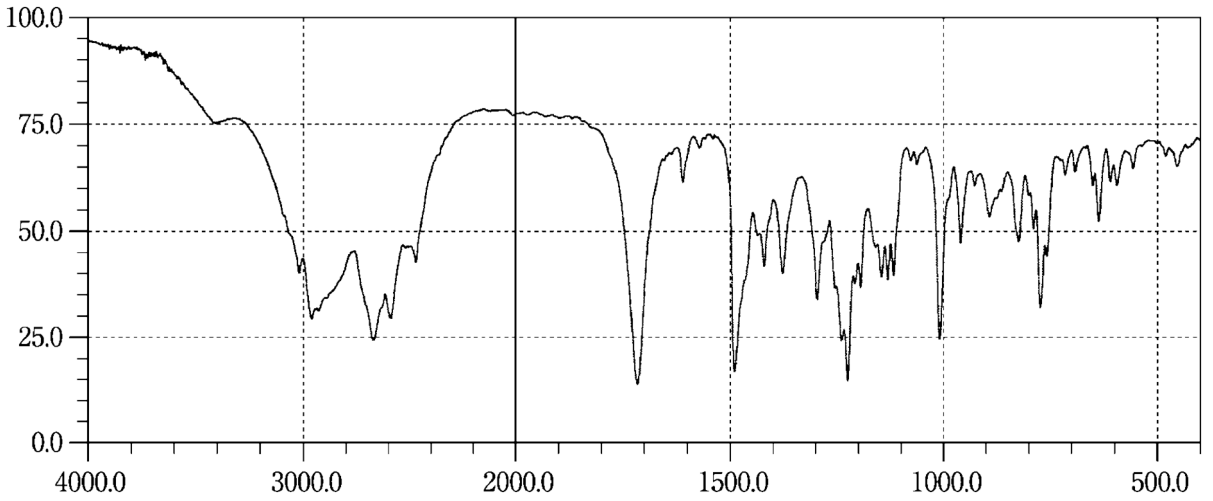
Ofloxacin



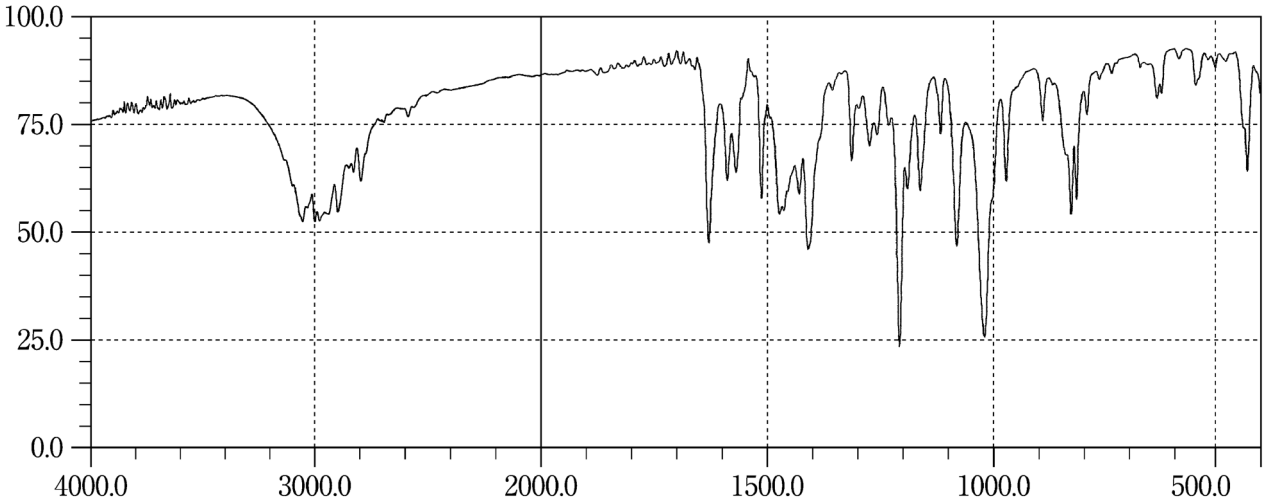
Olmesartan Medoxomil



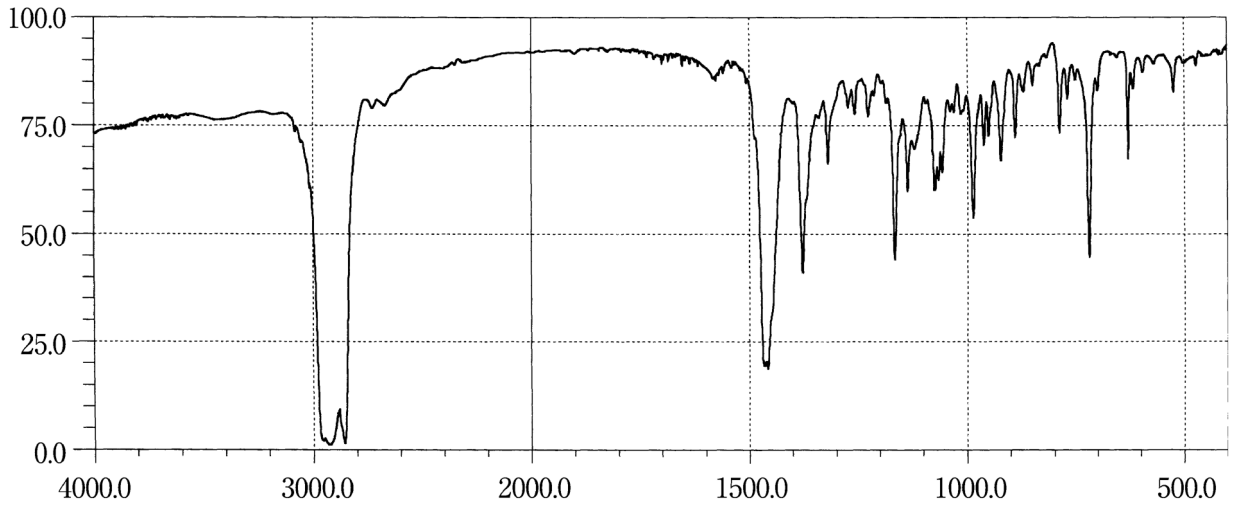
Olopatadine Hydrochloride



Omeprazole



Oxapium Iodide



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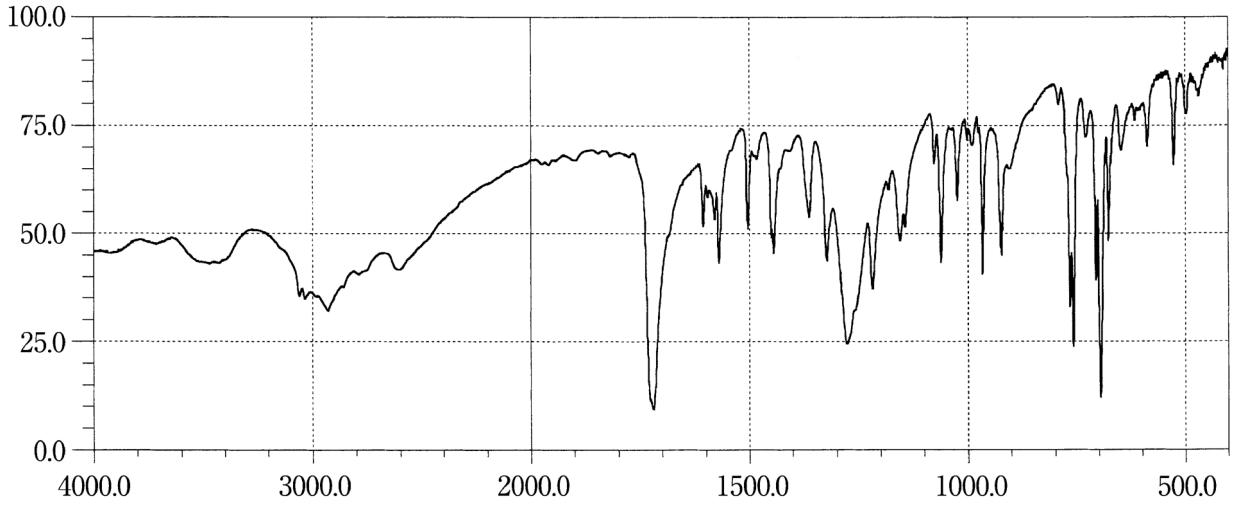
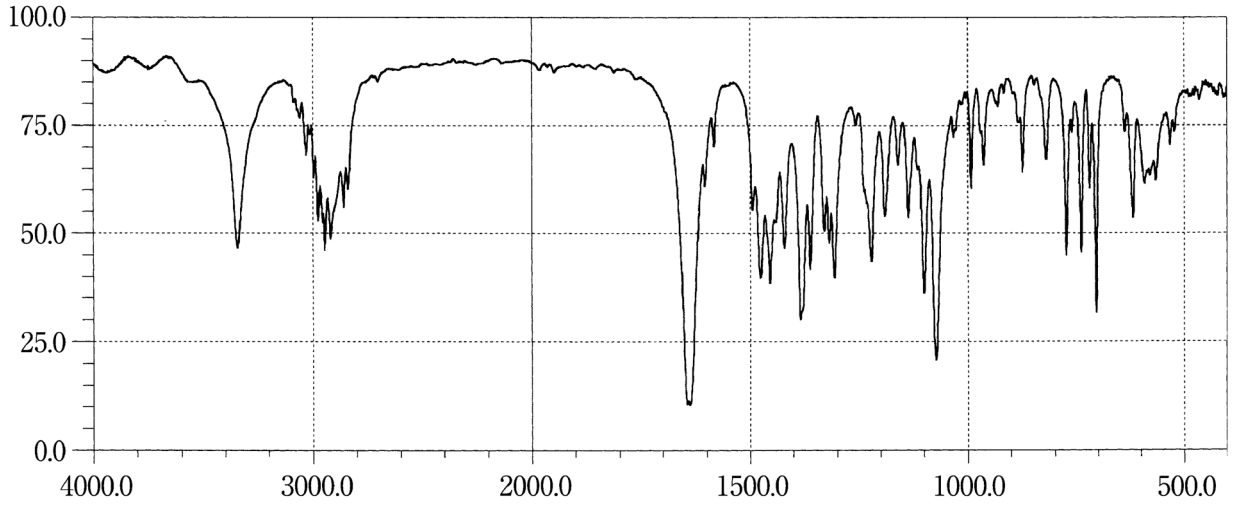
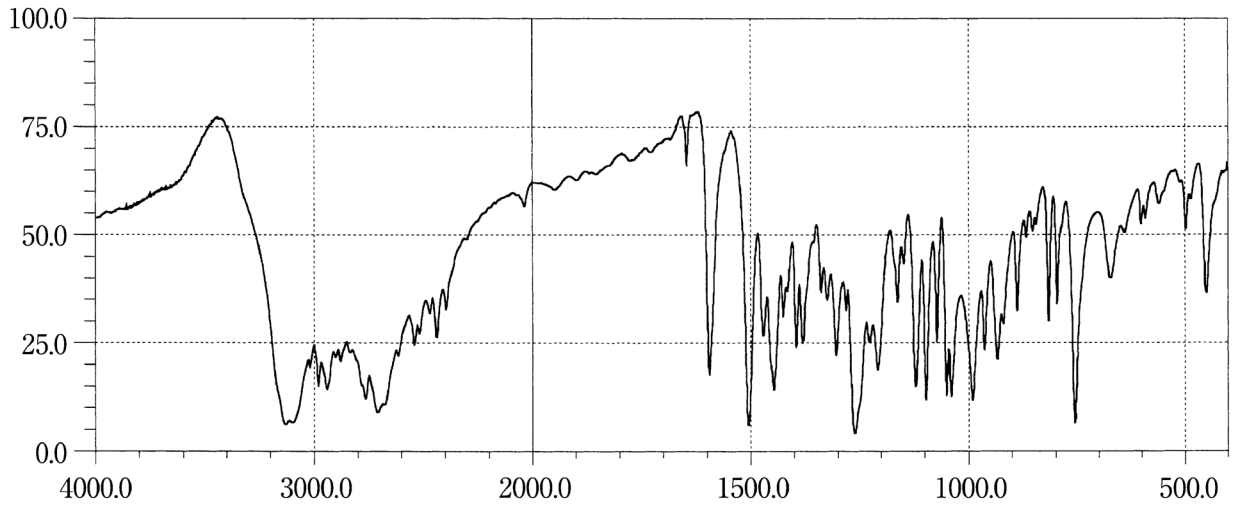


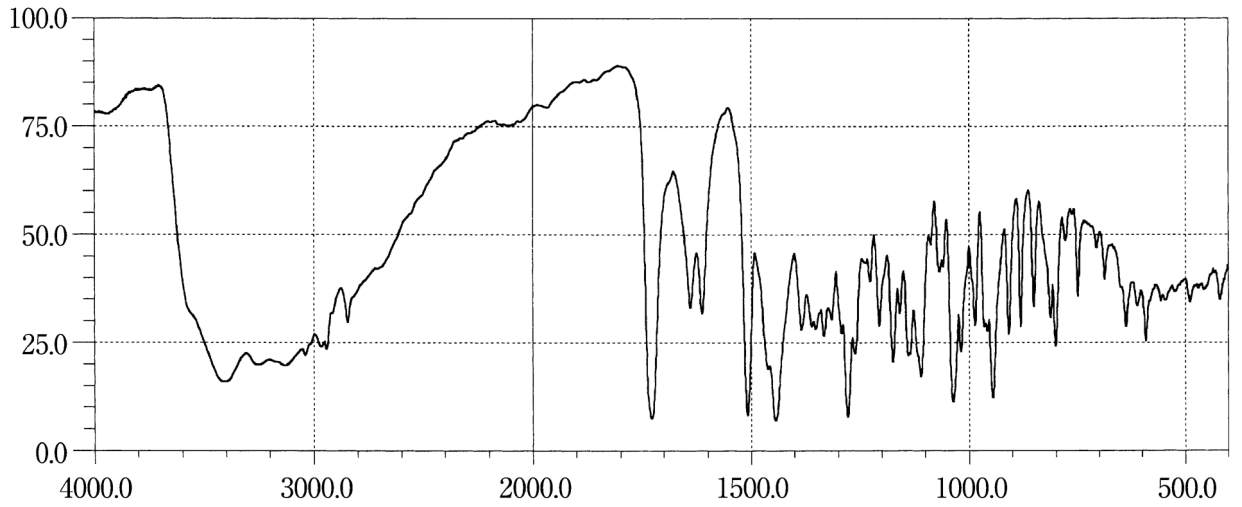
Oxethazaine



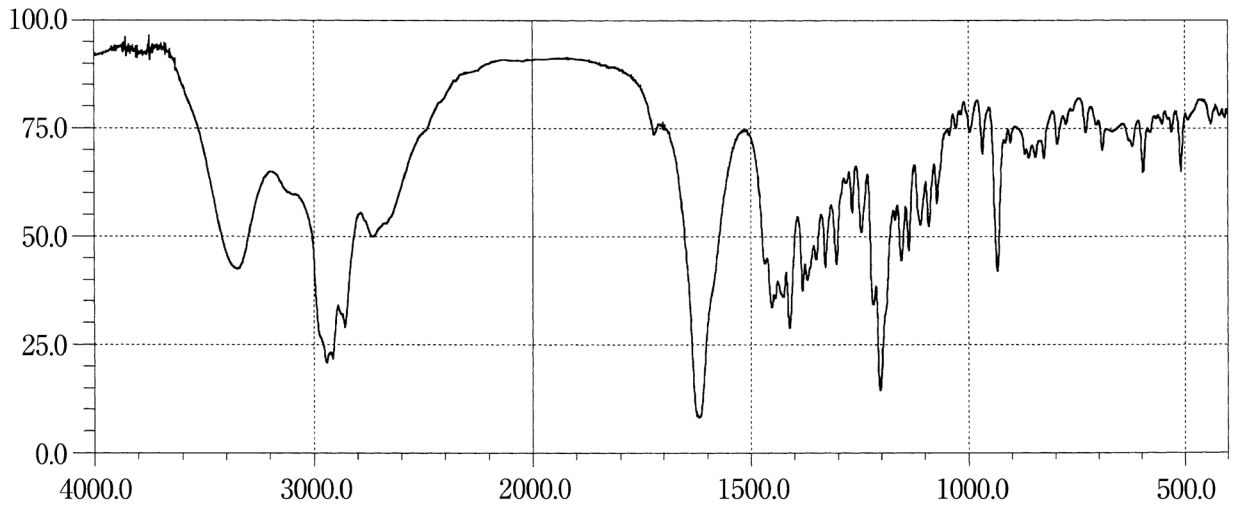
Oxprenolol Hydrochloride



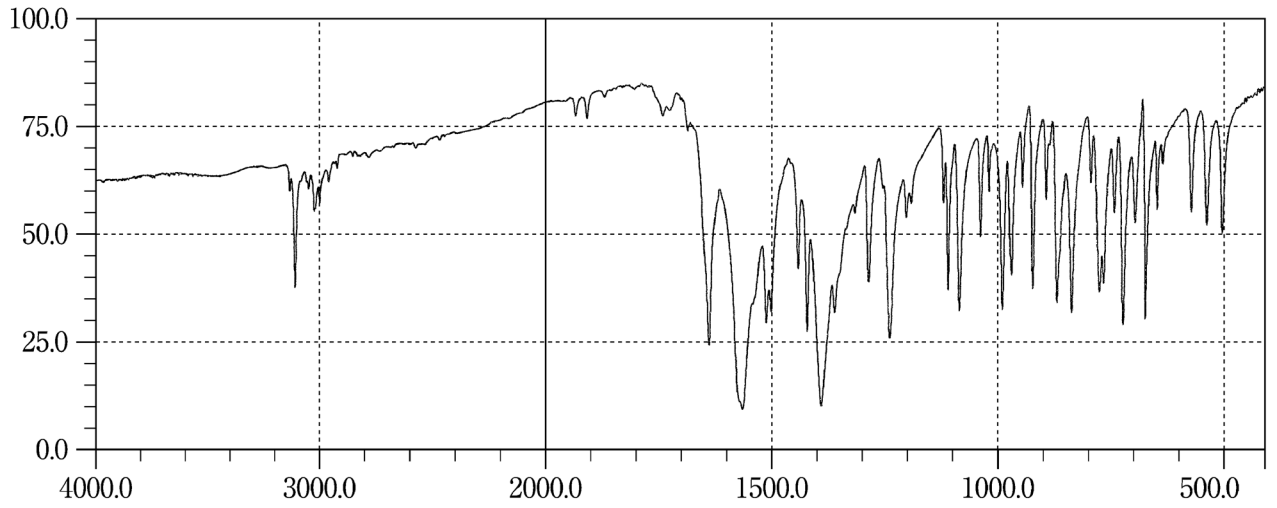
Oxycodone Hydrochloride Hydrate



Oxymetholone



Ozagrel Sodium



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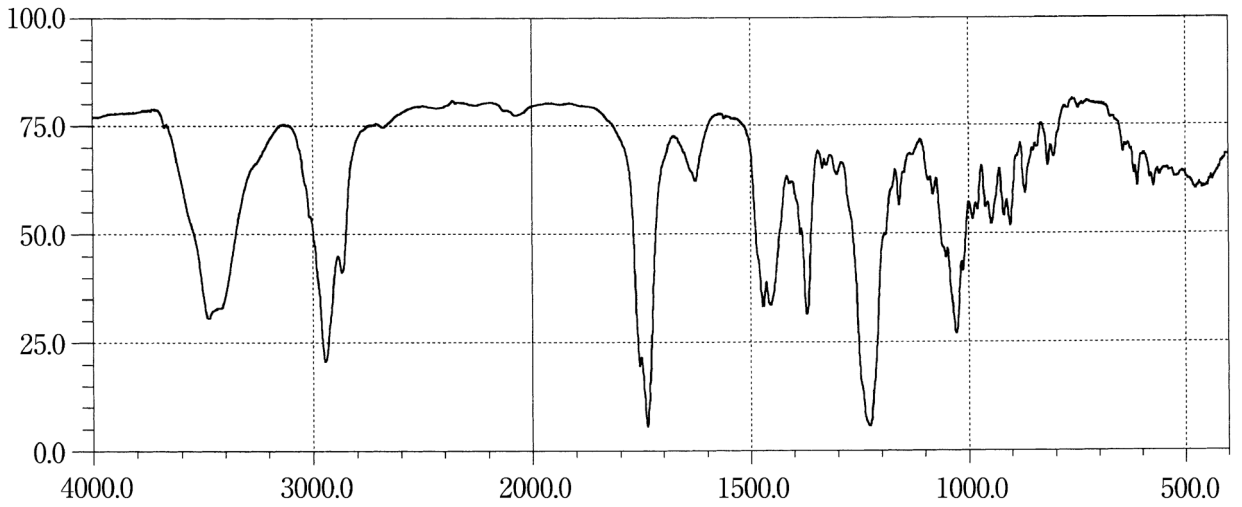

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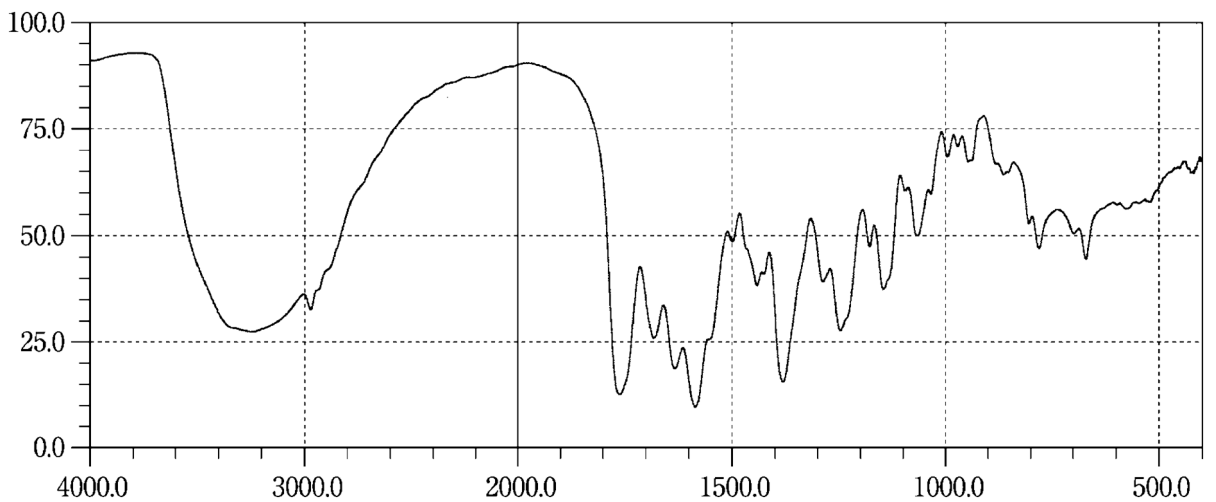
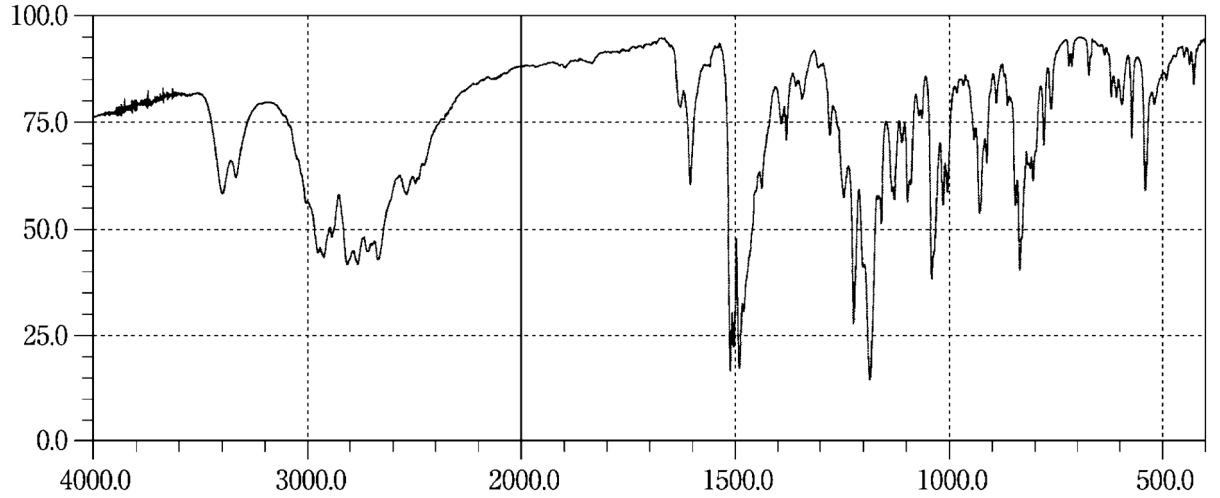
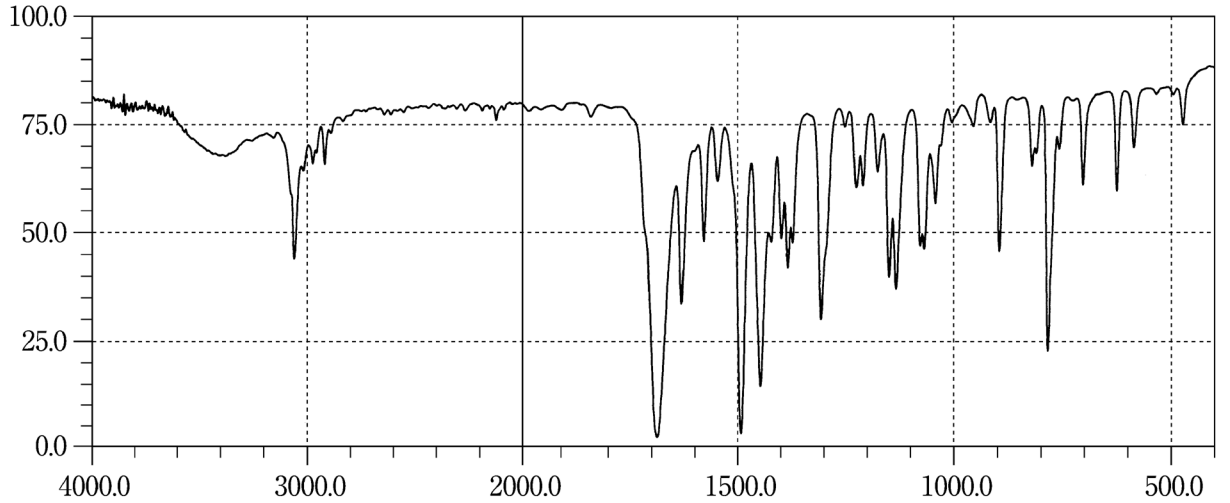


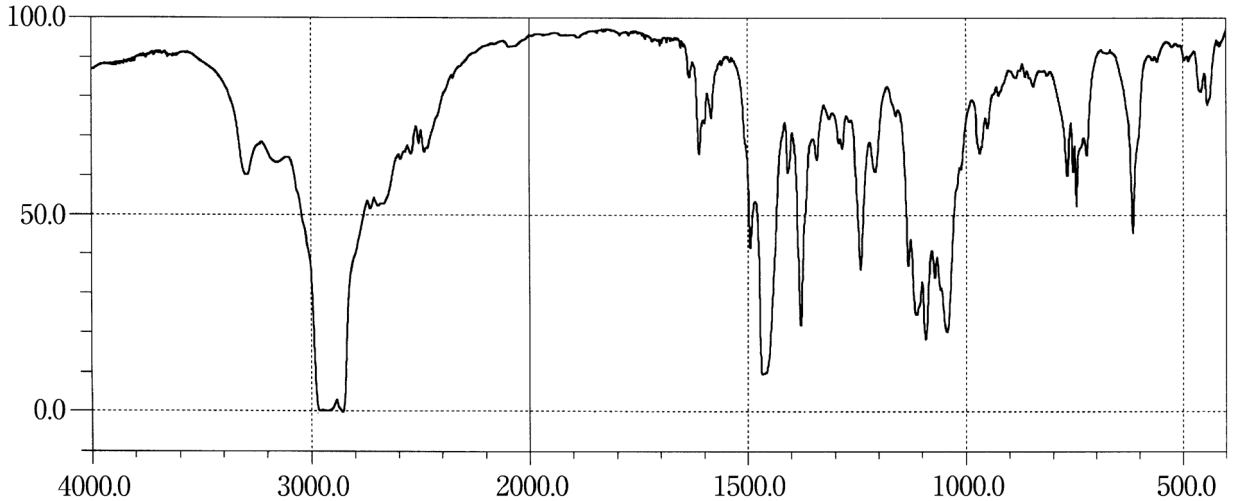
Paroxetine Hydrochloride Hydrate



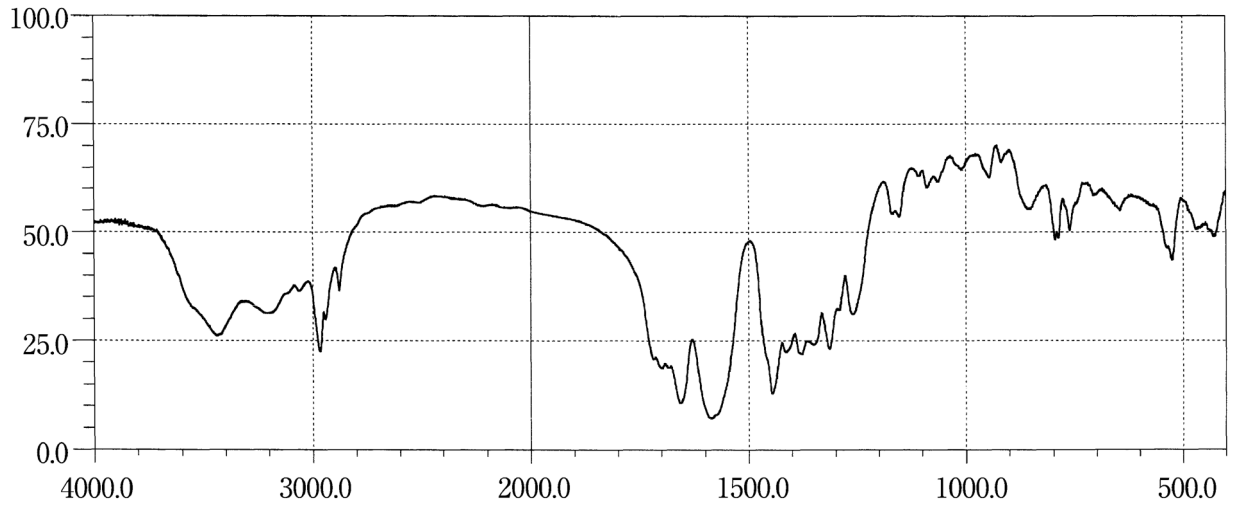
Pemirolast Potassium



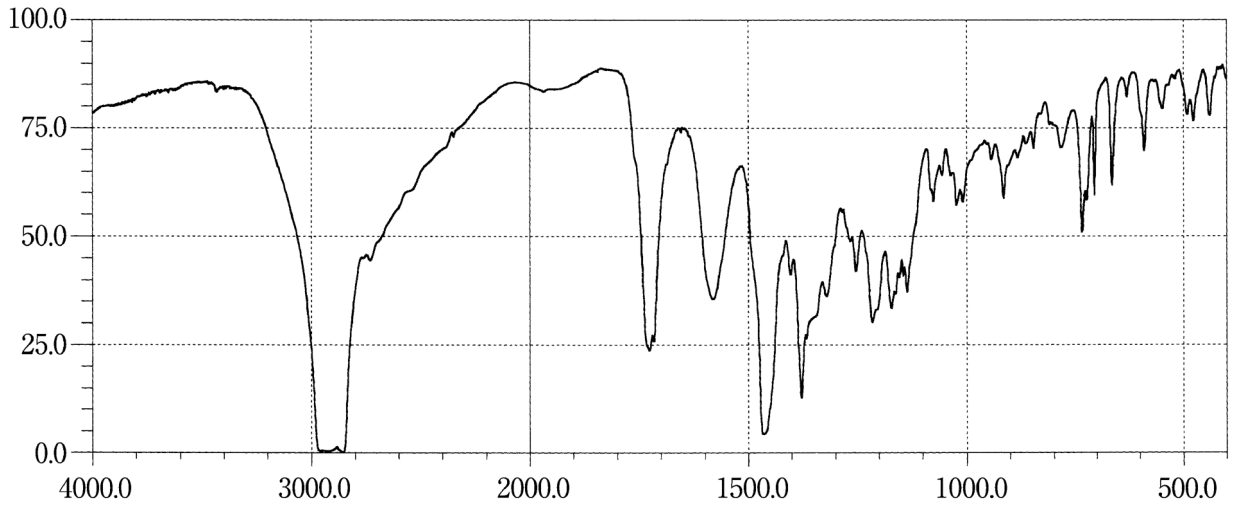
Penbutolol Sulfate



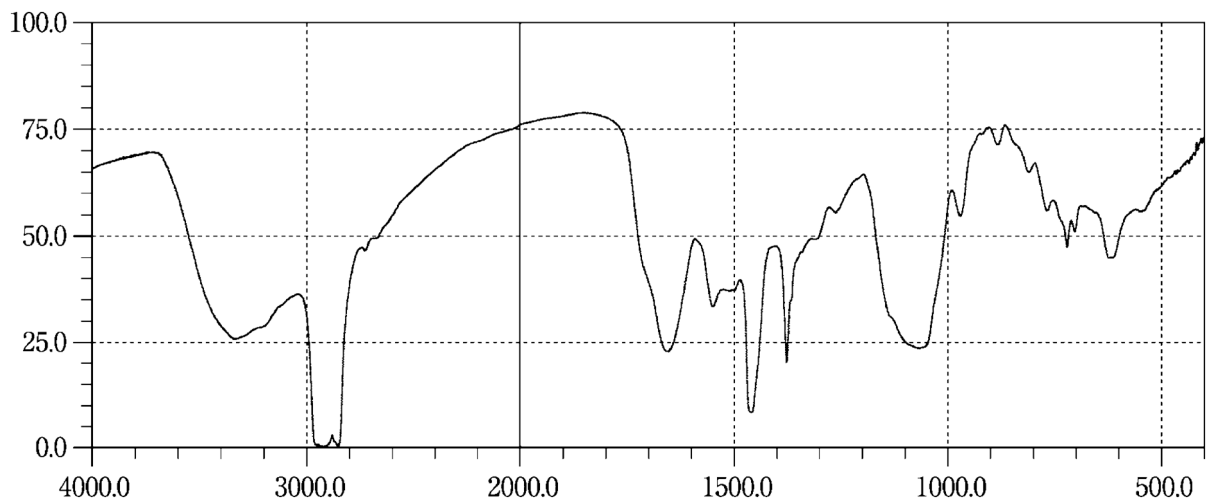
Pentobarbital Calcium



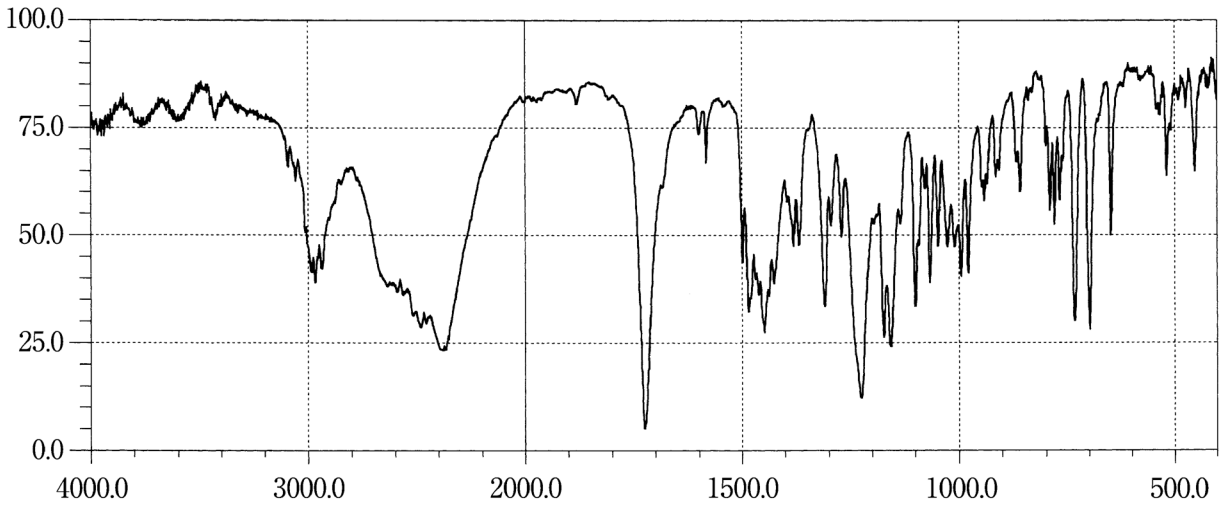
Pentoxyverine Citrate



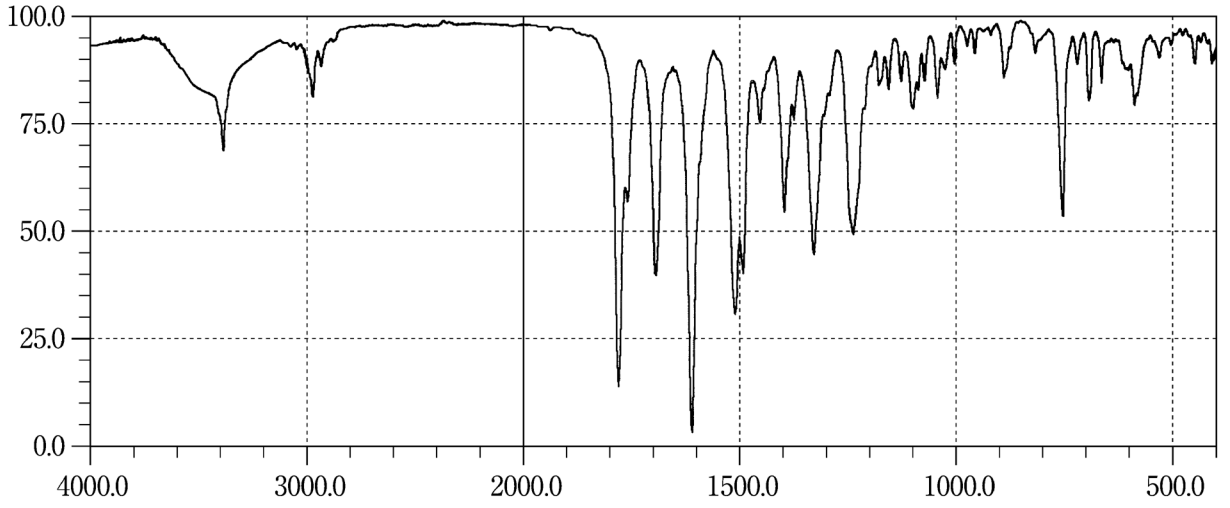
Peplomycin Sulfate



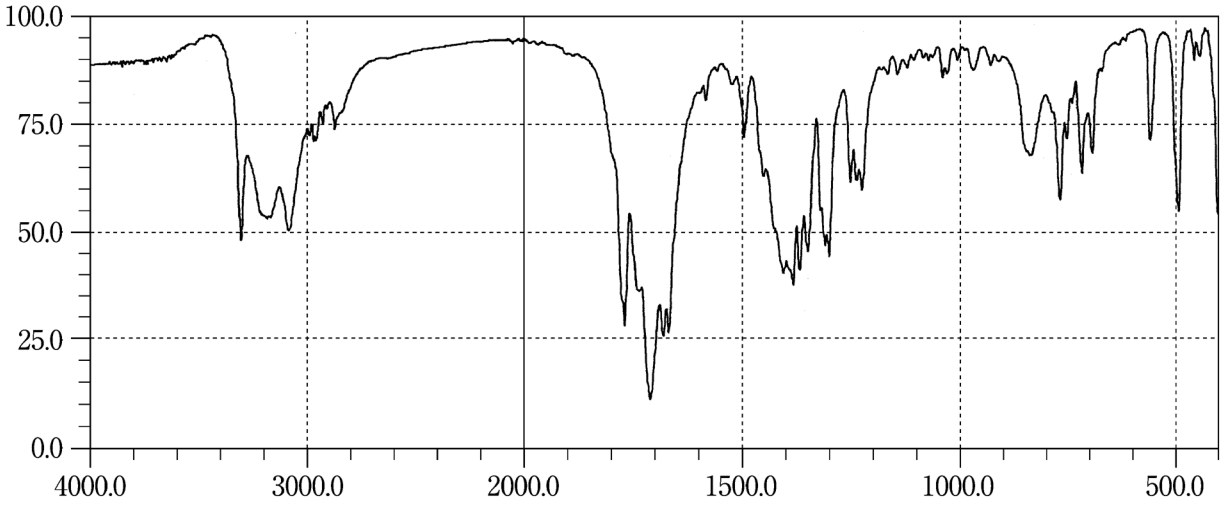
Pethidine Hydrochloride



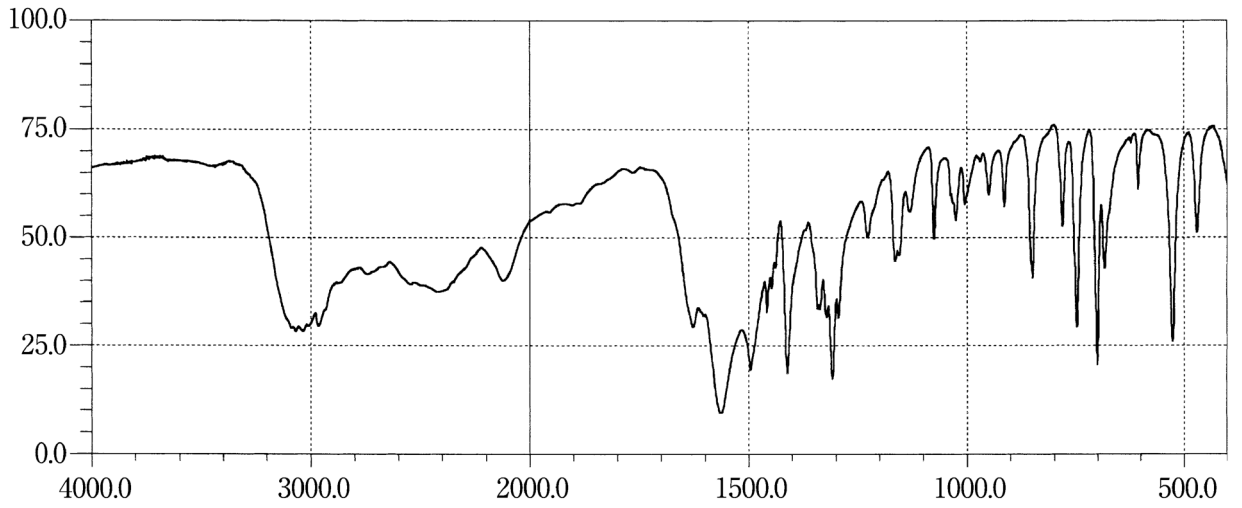
Phenethicillin Potassium



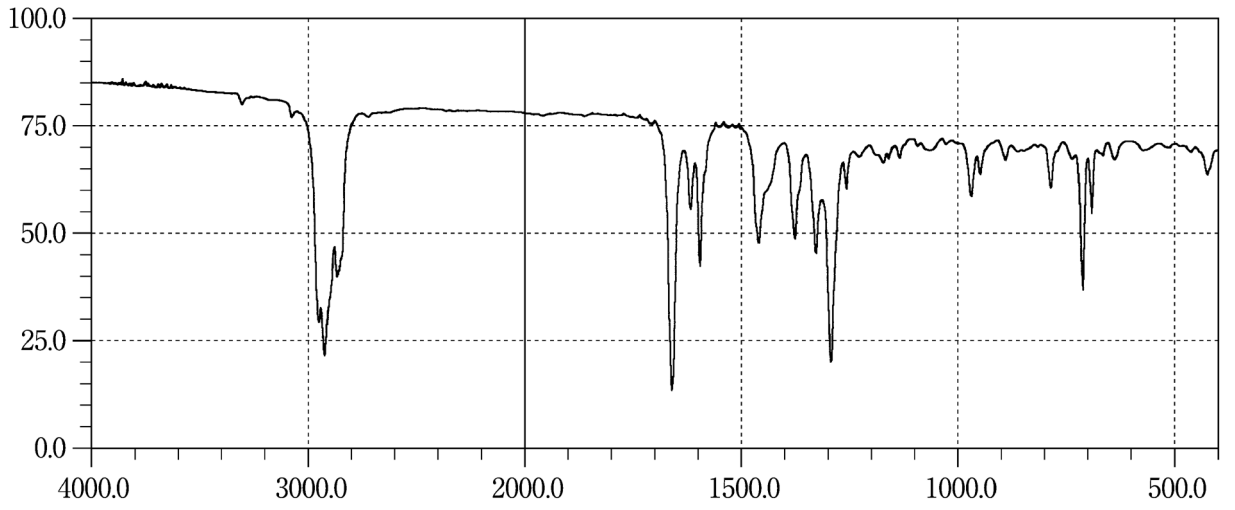
Phenobarbital



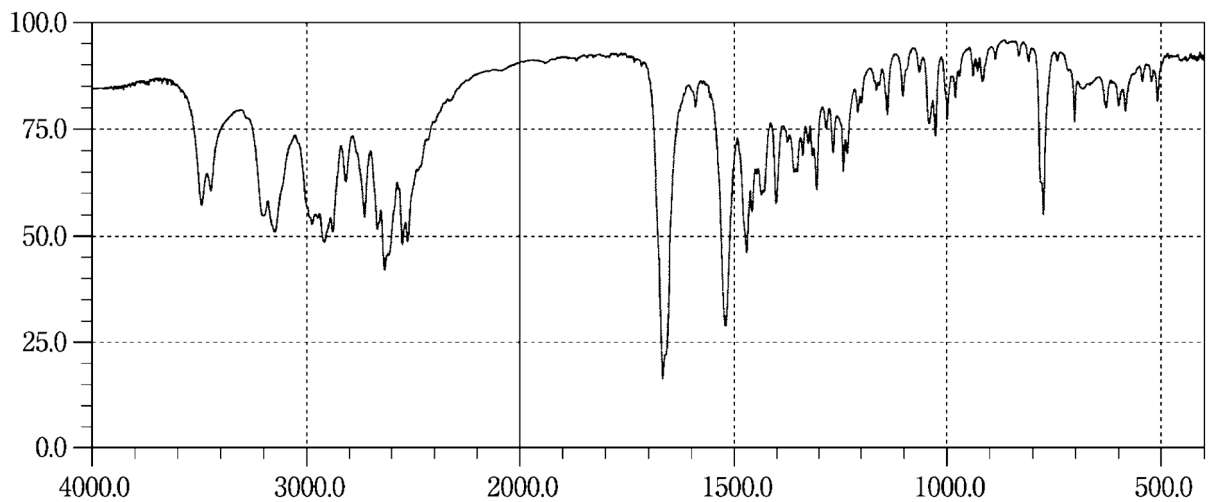
L-Phenylalanine



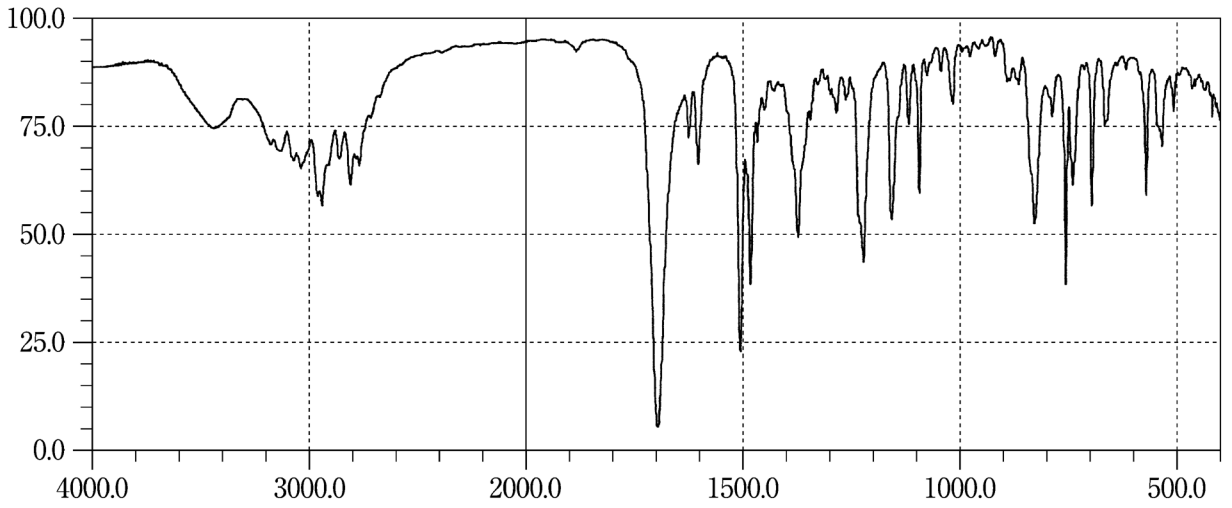
Phytonadione



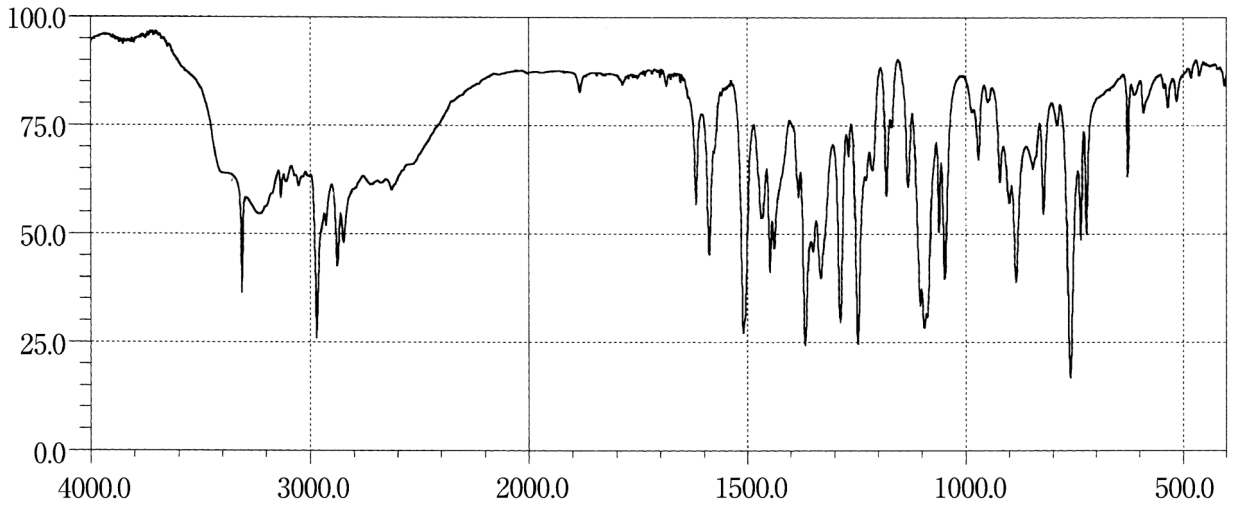
Pilsicainide Hydrochloride Hydrate



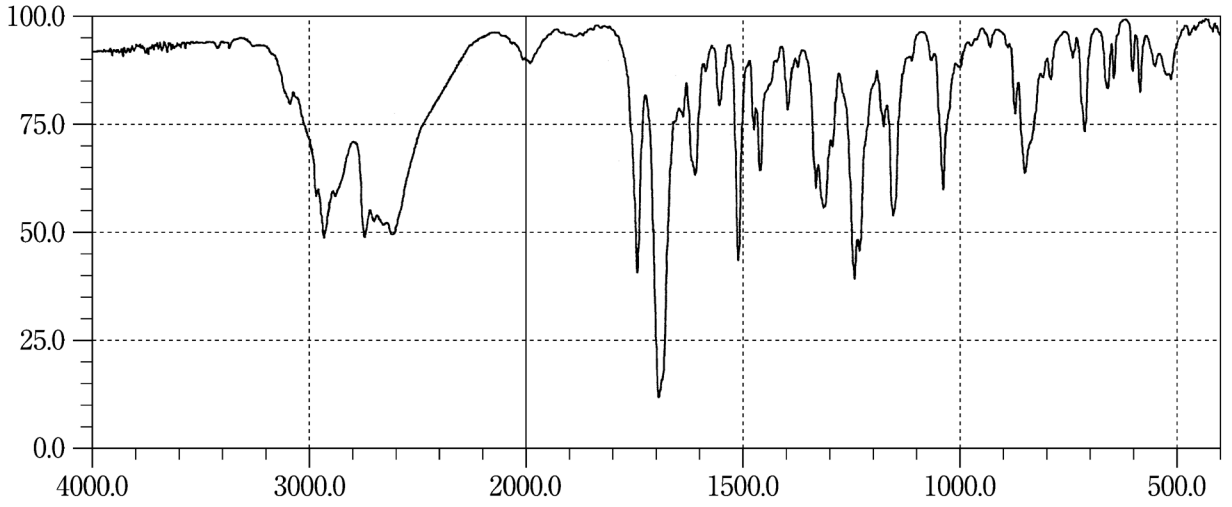
Pimozide



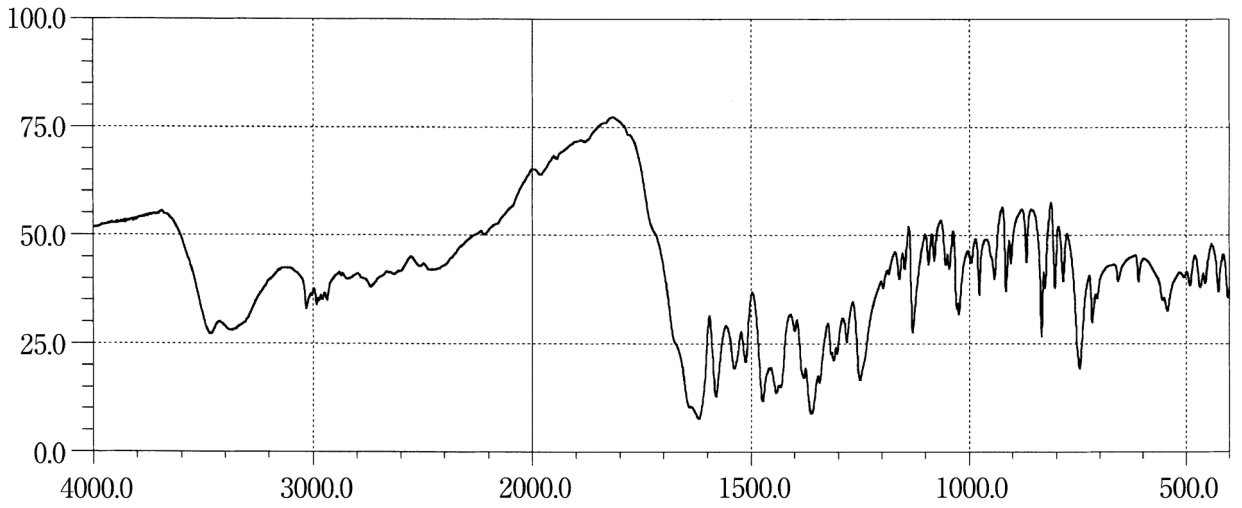
Pindolol



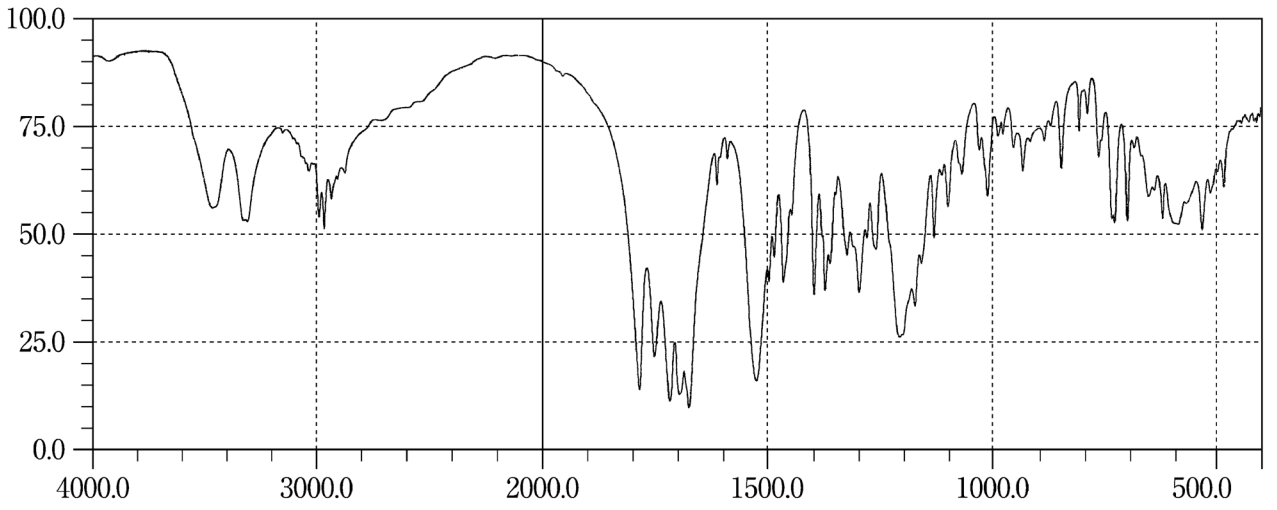
Pioglitazone Hydrochloride



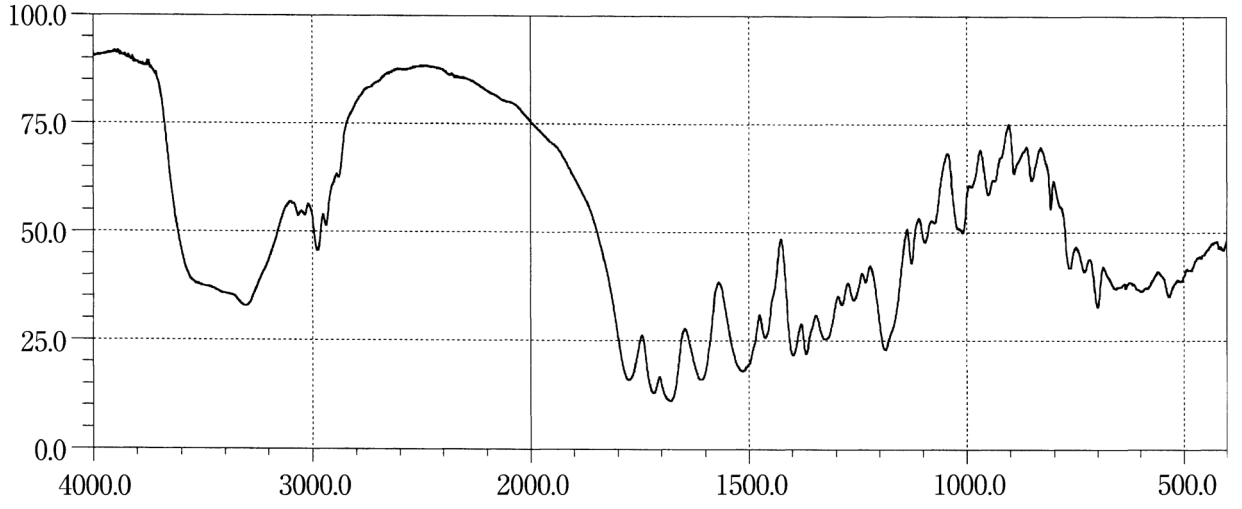
Pipemidic Acid Hydrate



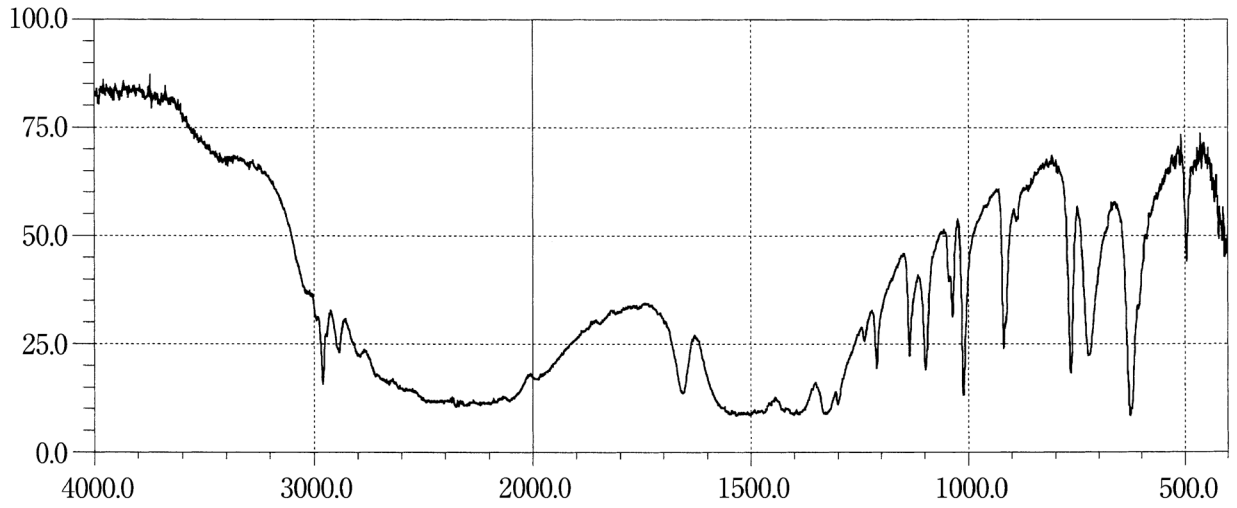
Piperacillin Hydrate



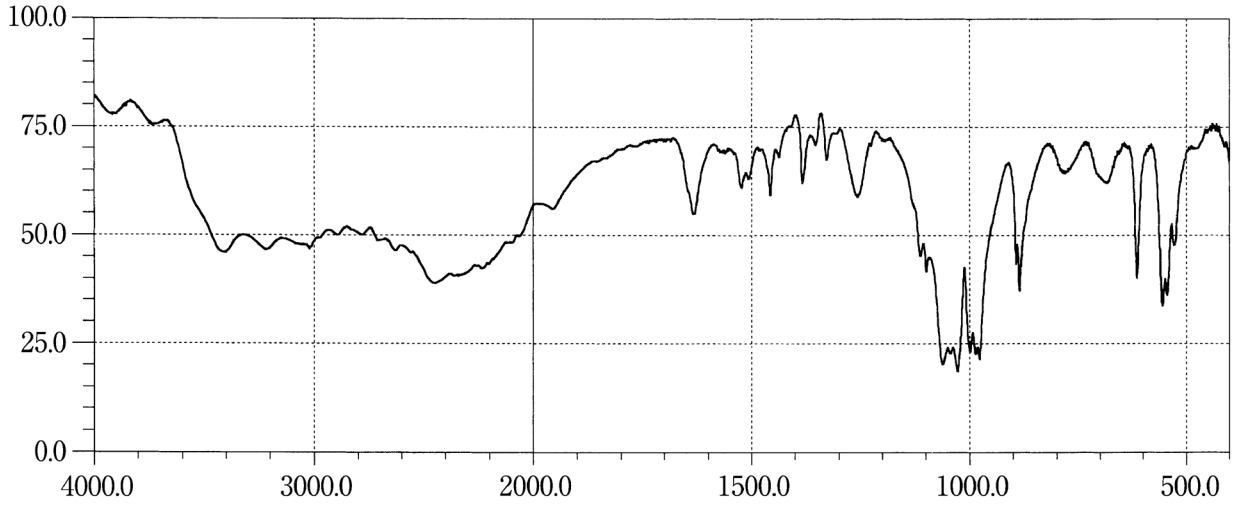
Piperacillin Sodium



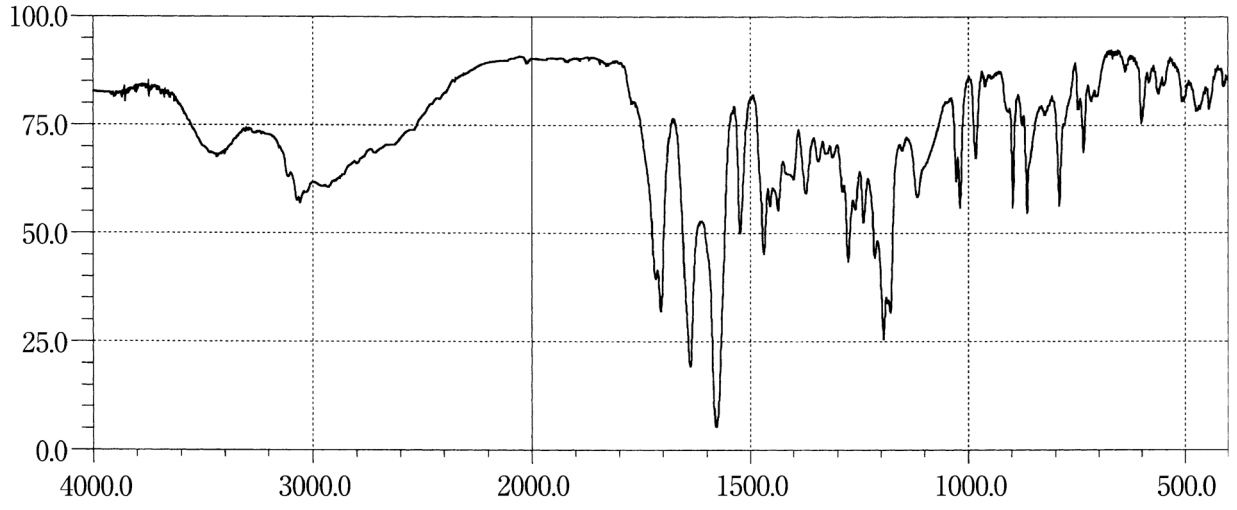
Piperazine Adipate



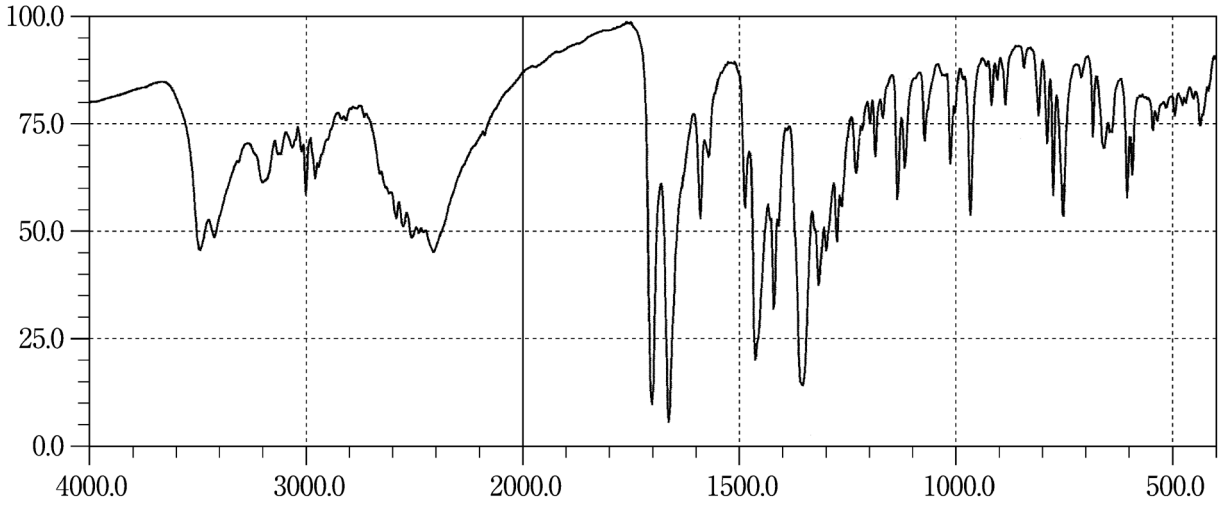
Piperazine Phosphate Hydrate



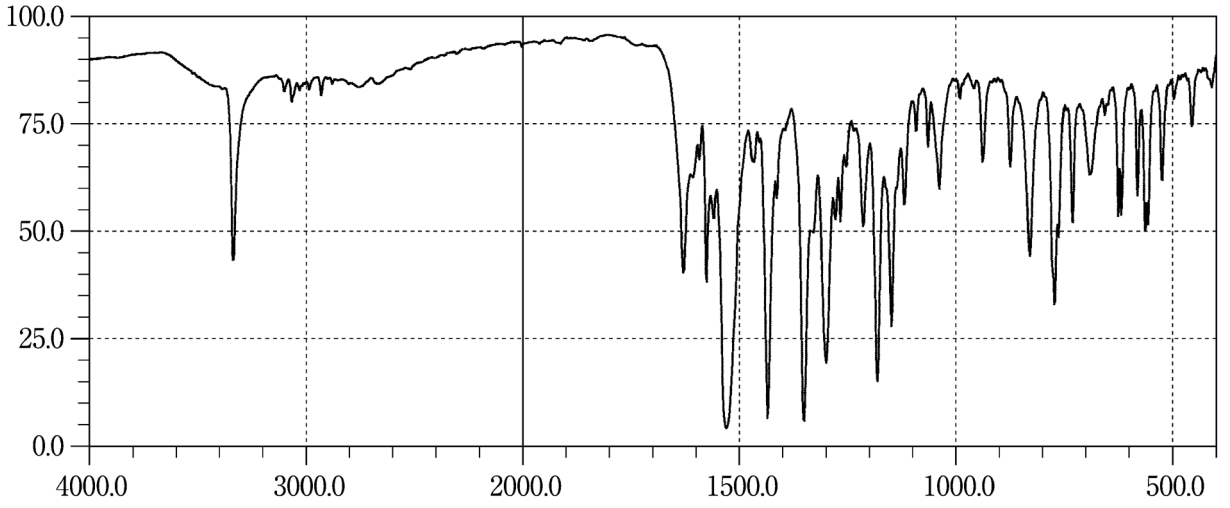
Pirenoxine



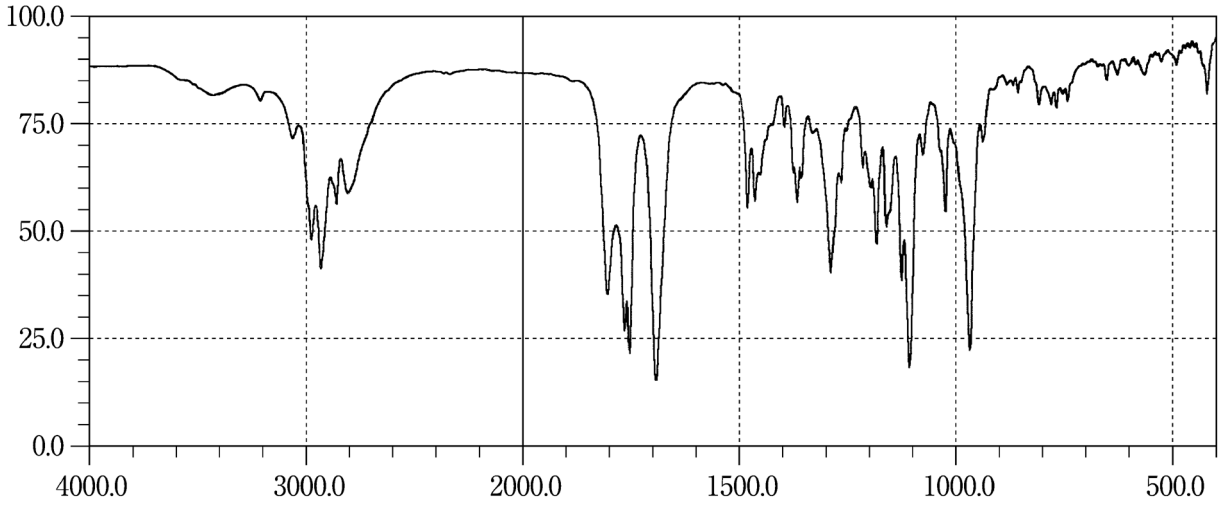
Pirenzepine Hydrochloride Hydrate



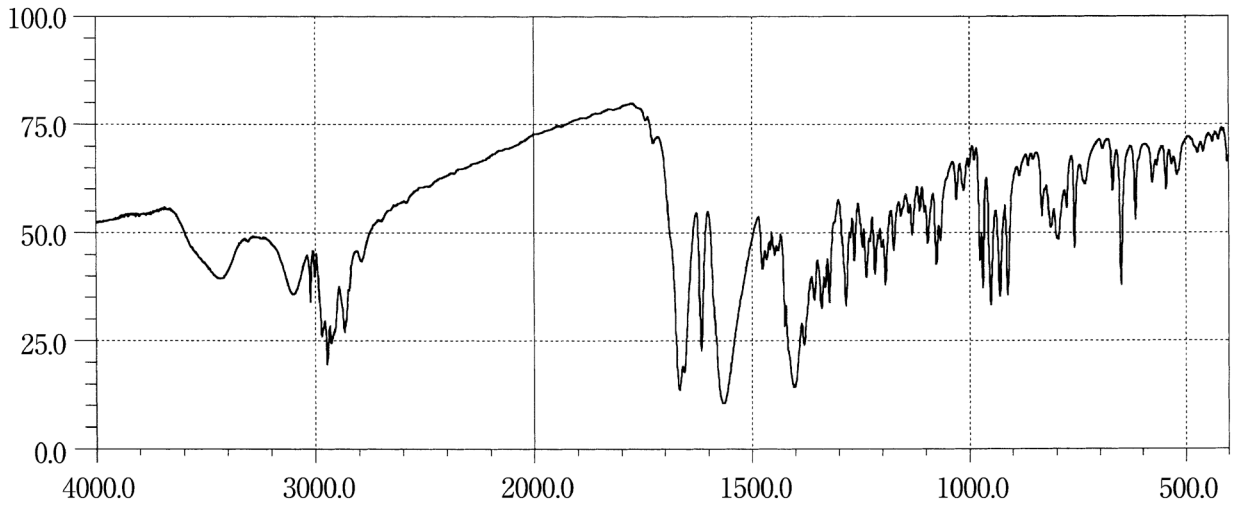
Piroxicam



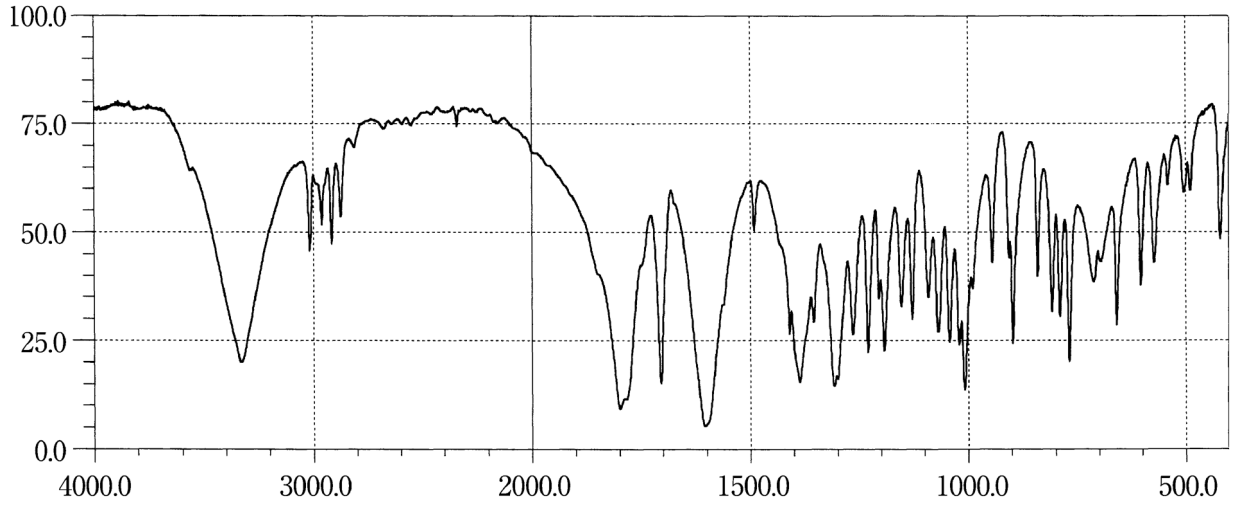
Pivmecillinam Hydrochloride



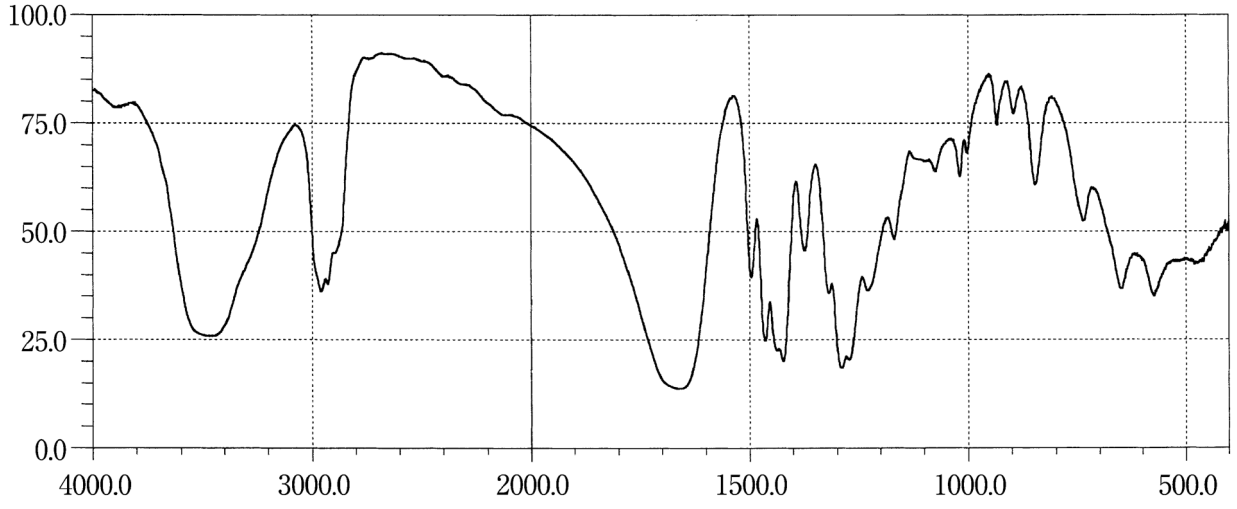
Potassium Canrenoate



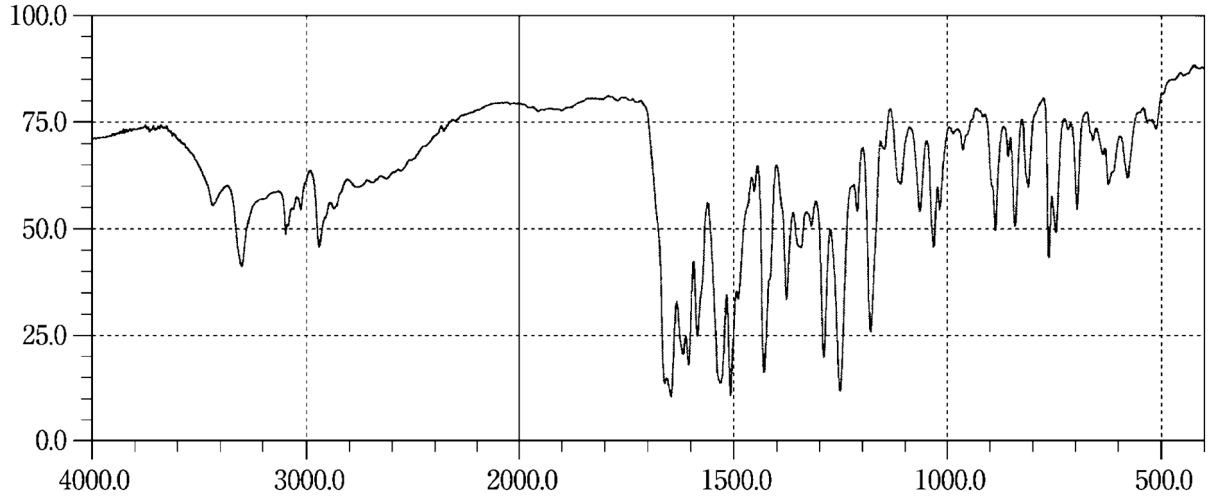
Potassium Clavulanate



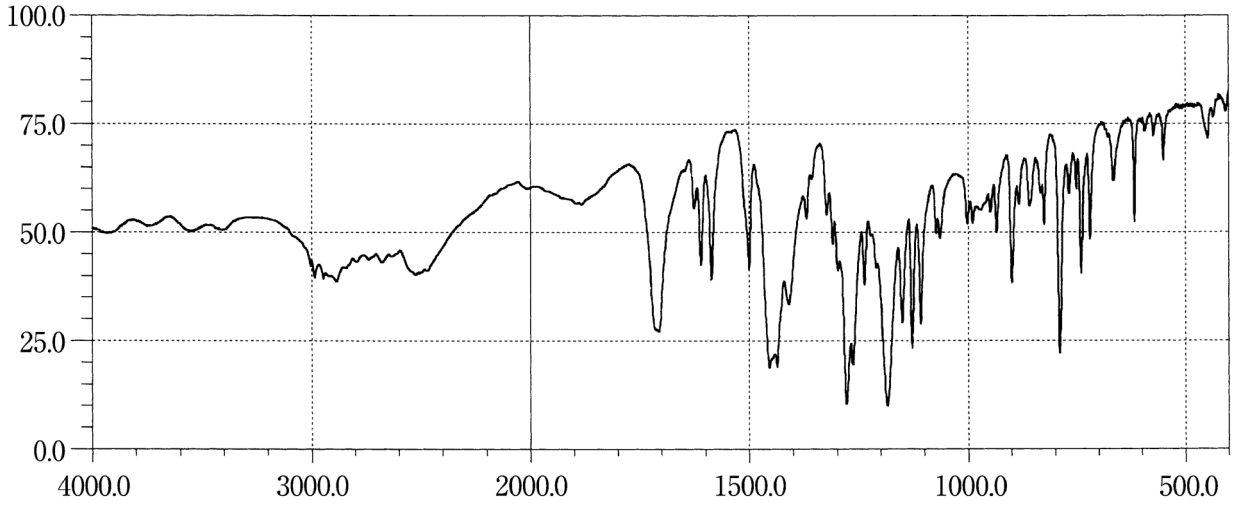
Povidone



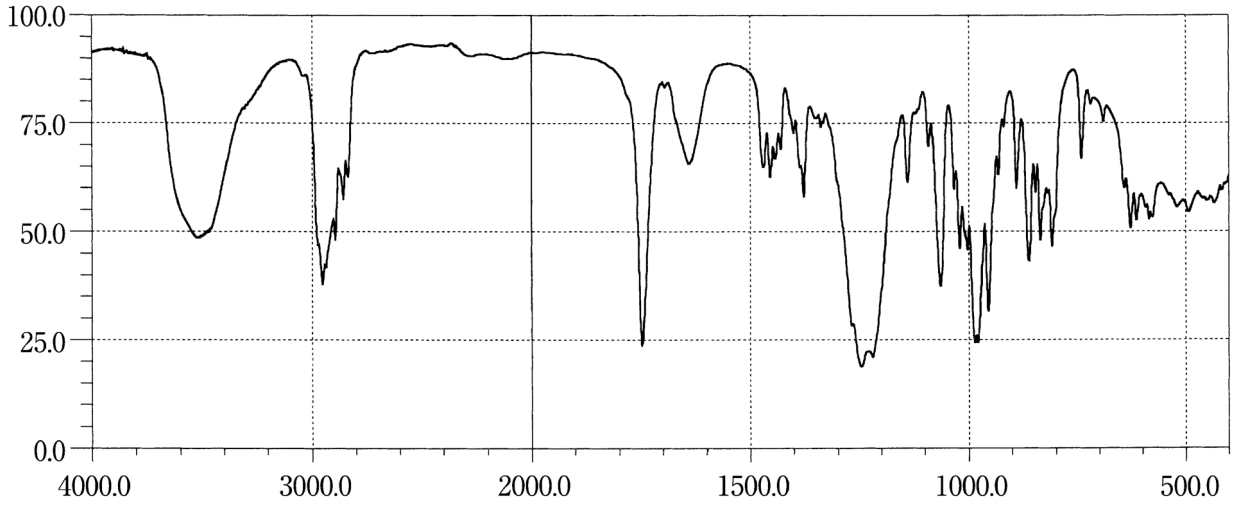
Pranlukast Hydrate



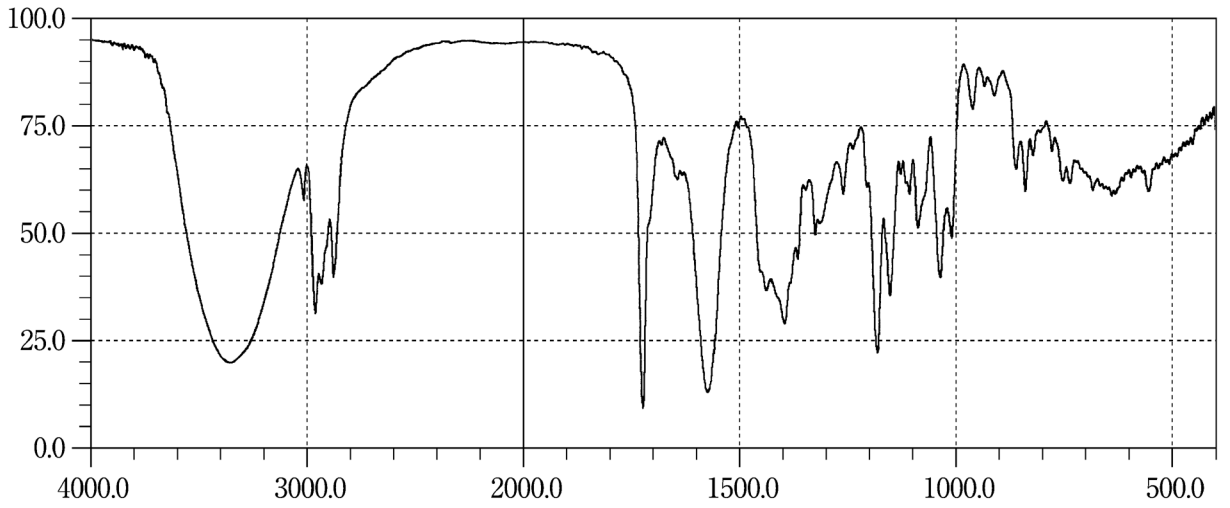
Pranoprofen



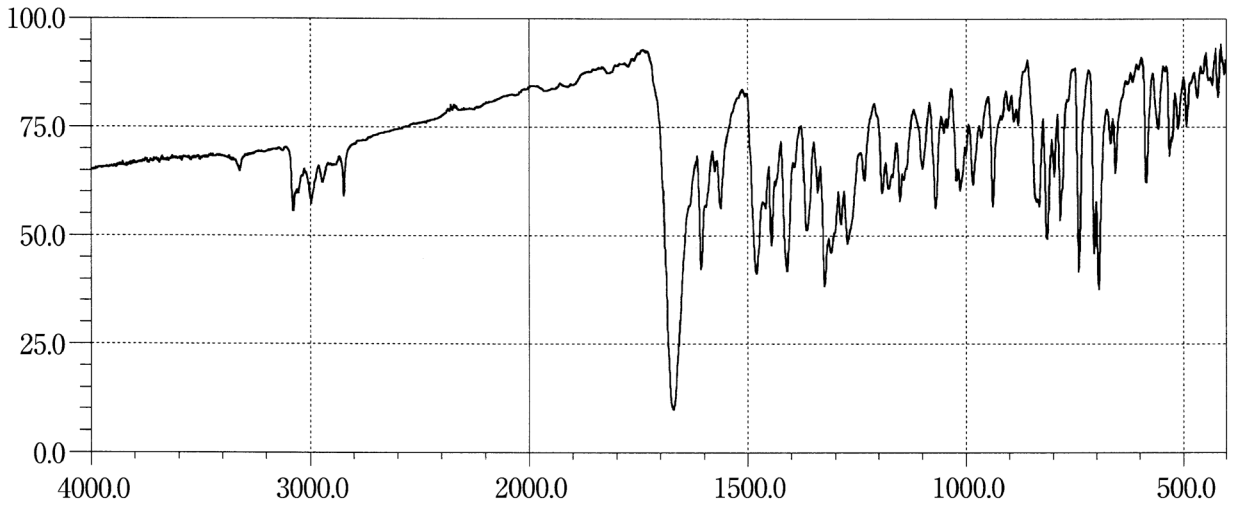
Prasterone Sodium Sulfate Hydrate



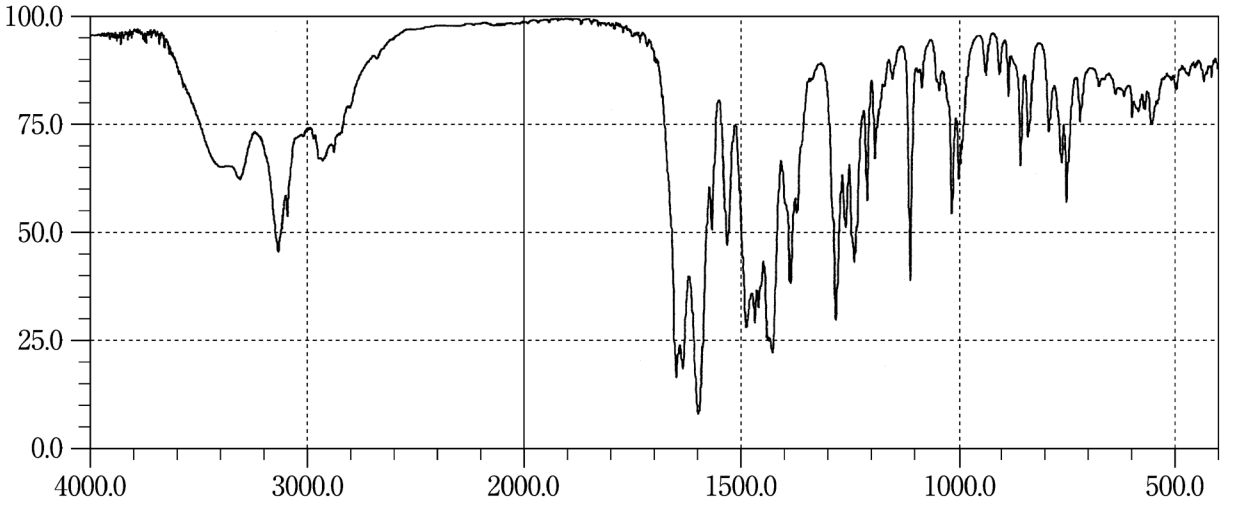
Pravastatin Sodium



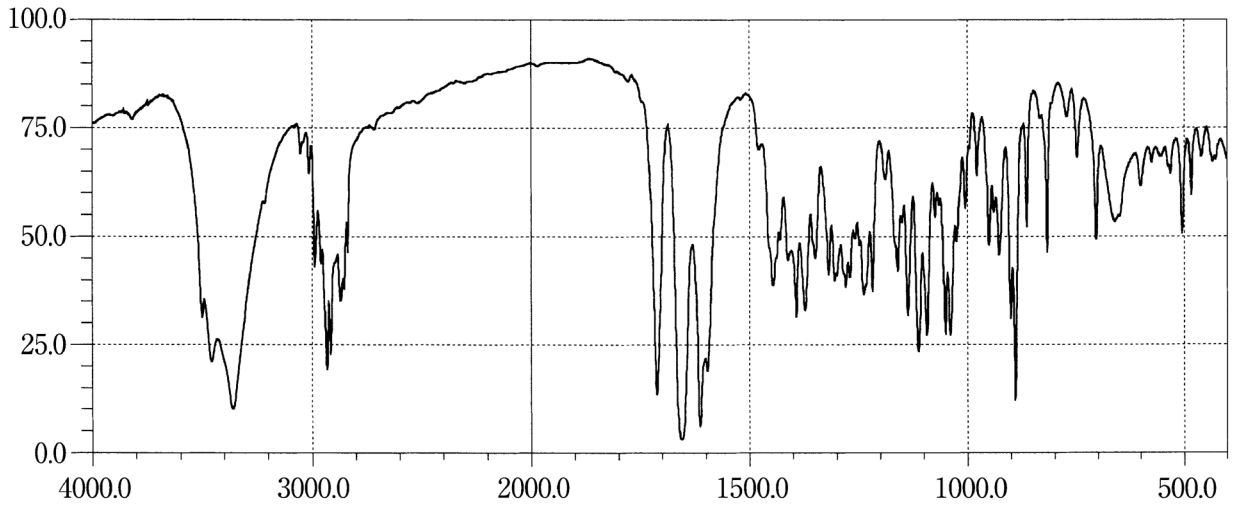
Prazepam



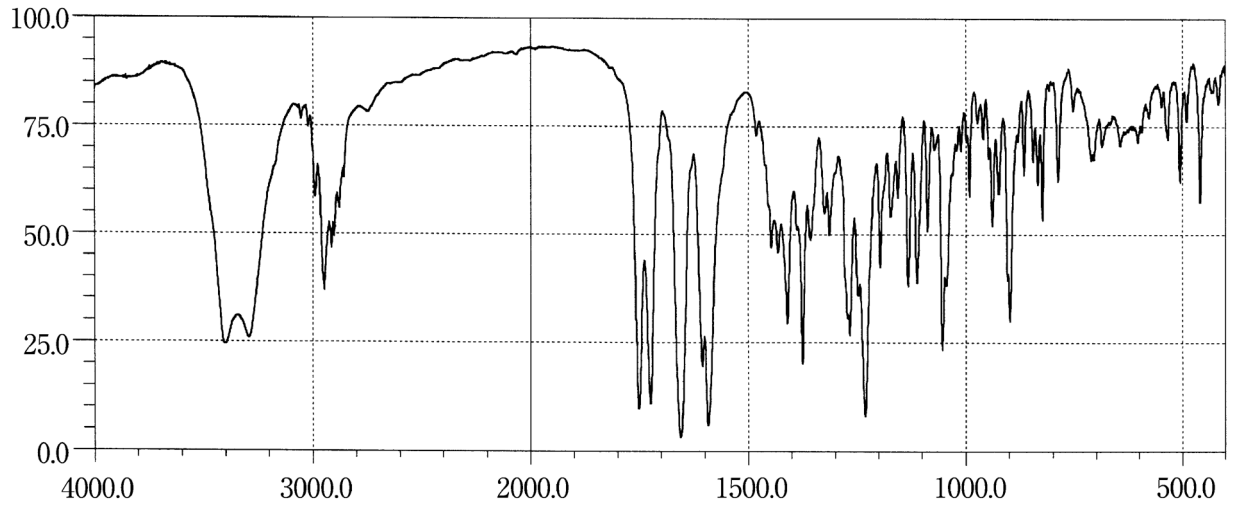
Prazosin Hydrochloride



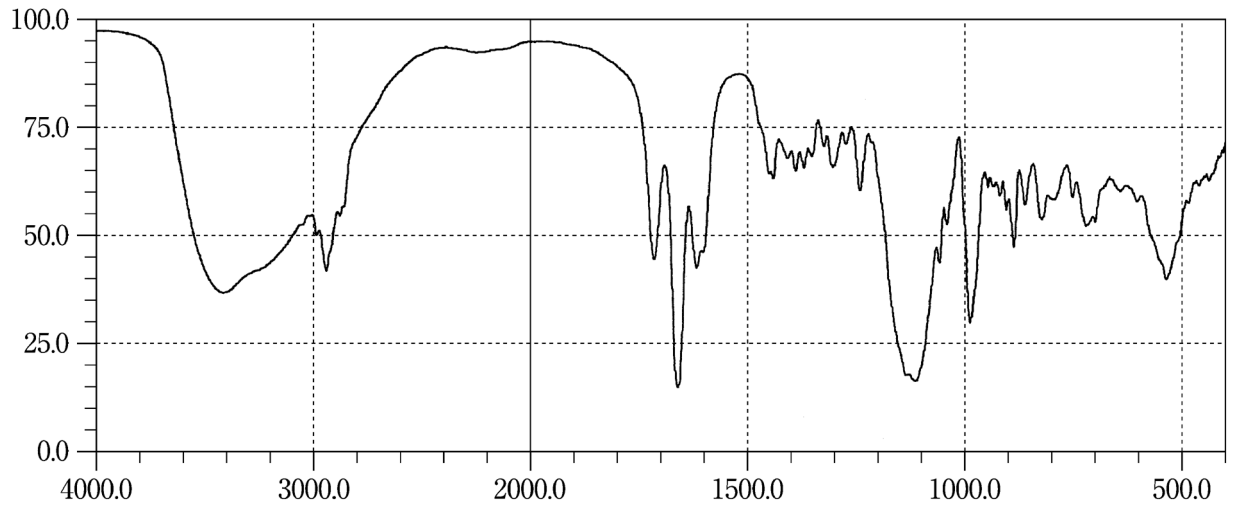
Prednisolone



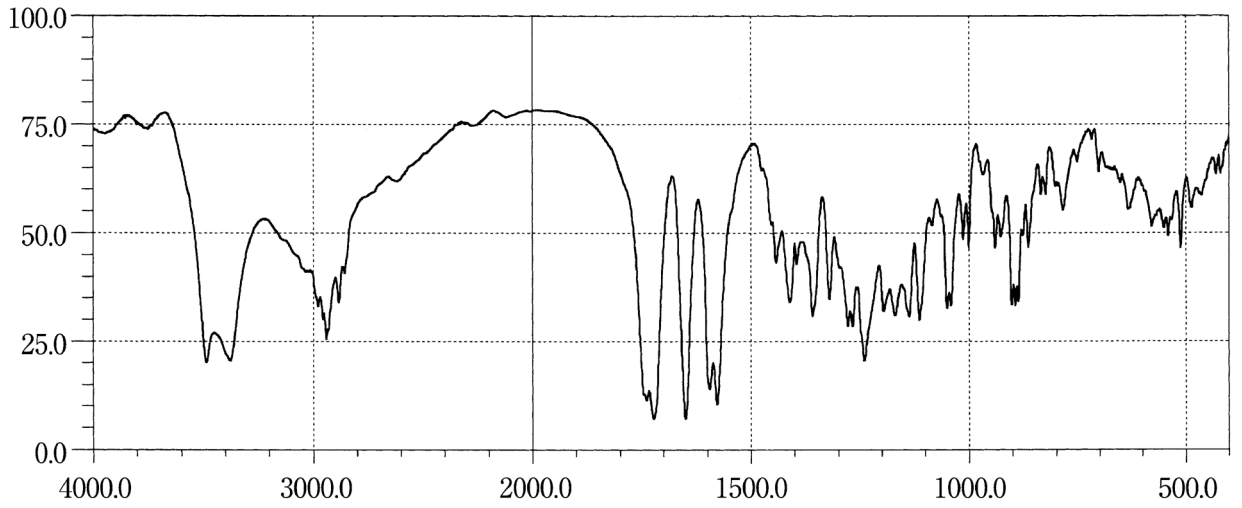
Prednisolone Acetate



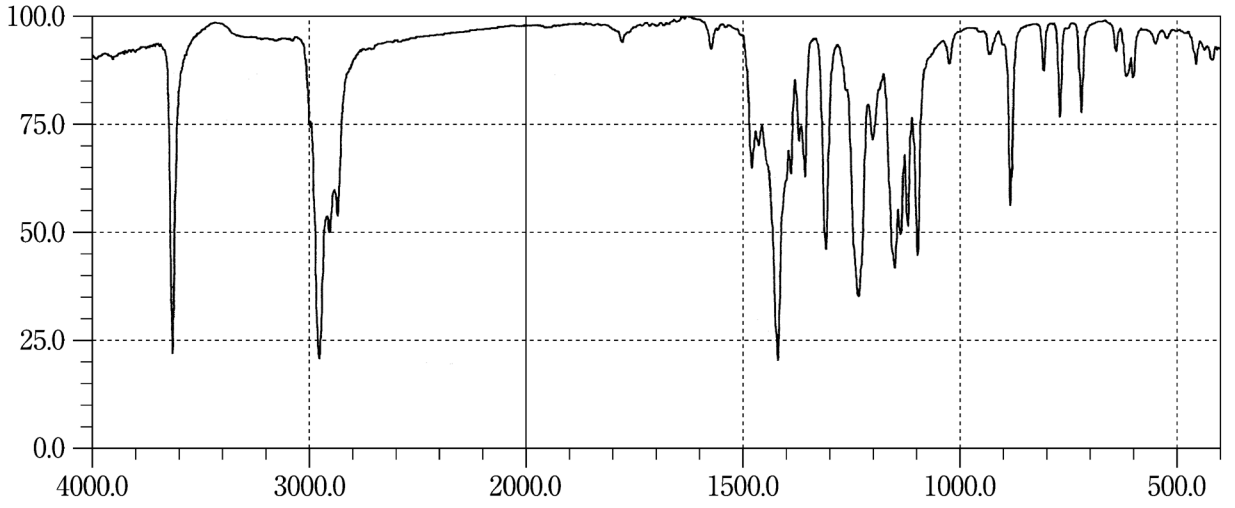
Prednisolone Sodium Phosphate



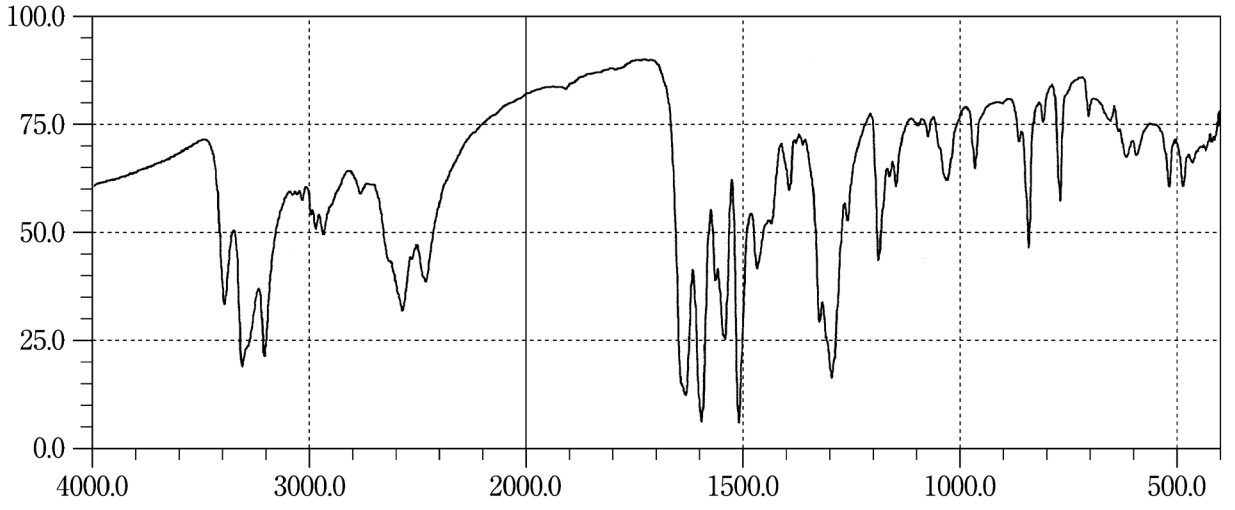
Prednisolone Succinate



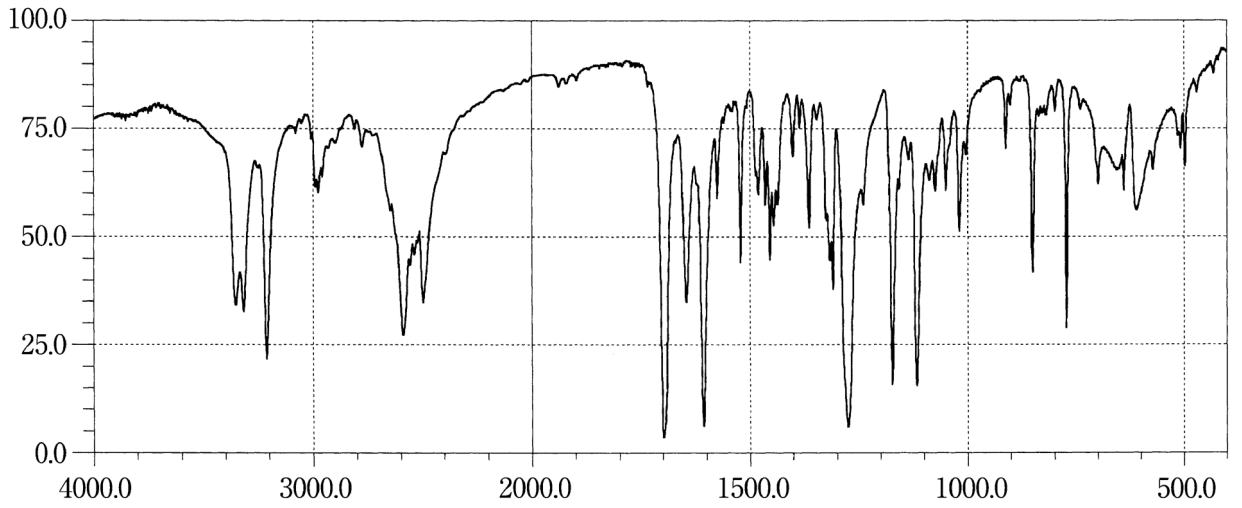
Probucol



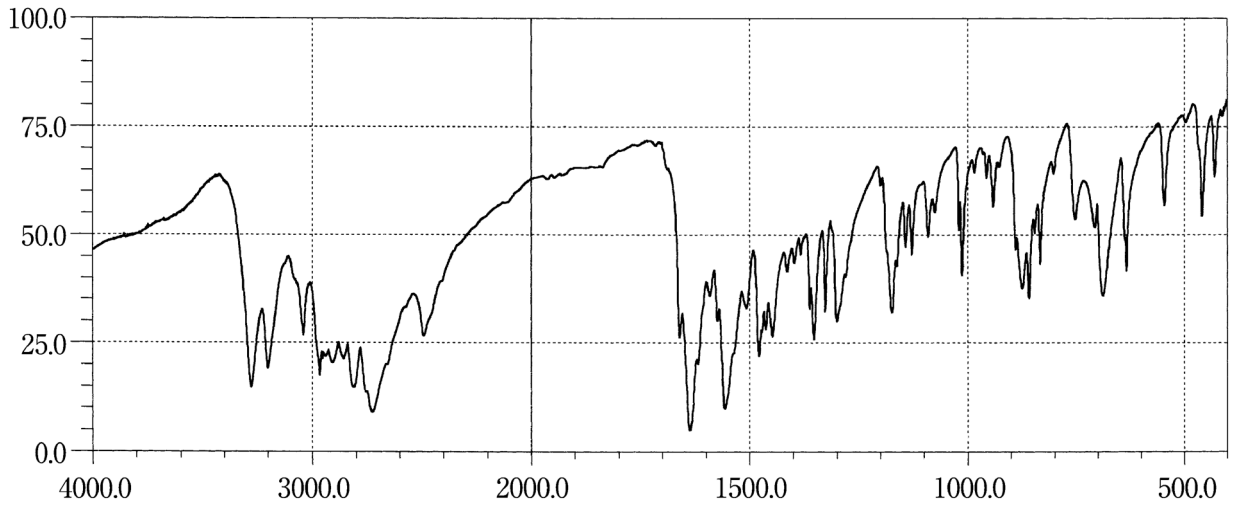
Procainamide Hydrochloride



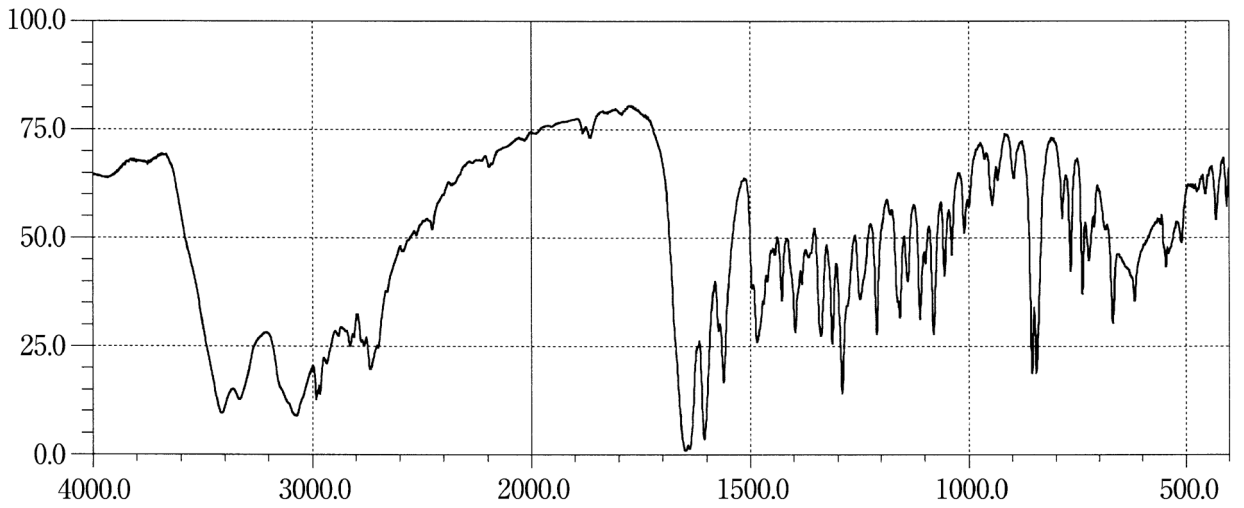
Procaine Hydrochloride



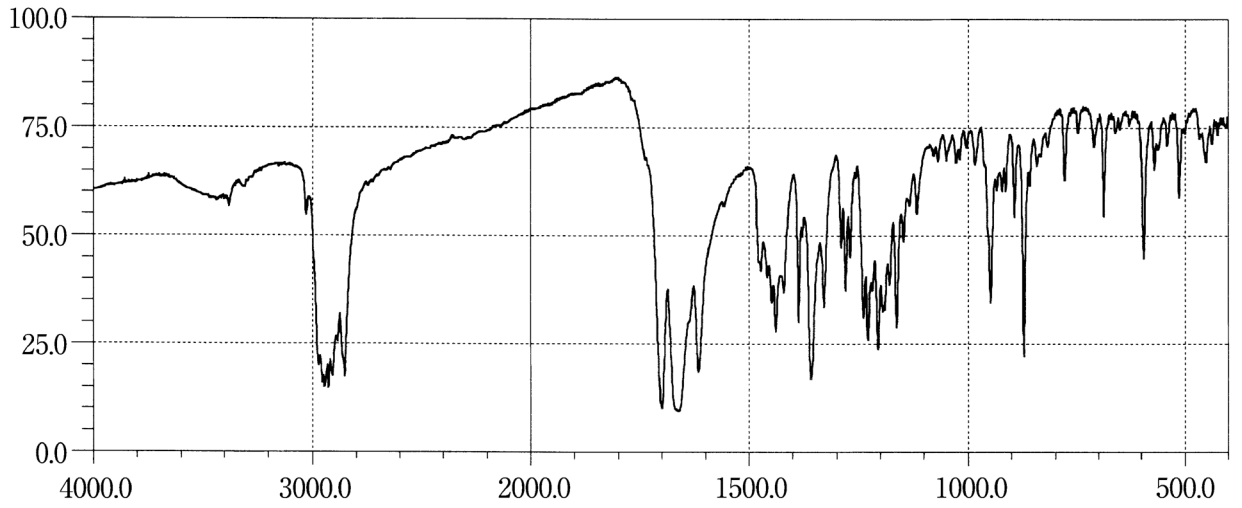
Procarbazine Hydrochloride



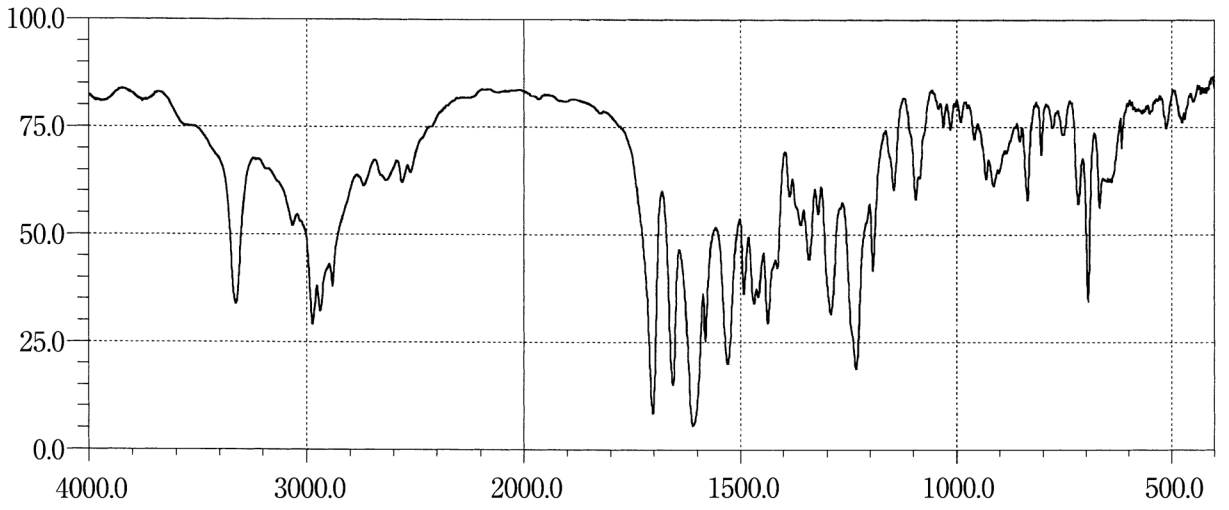
Procaterol Hydrochloride Hydrate



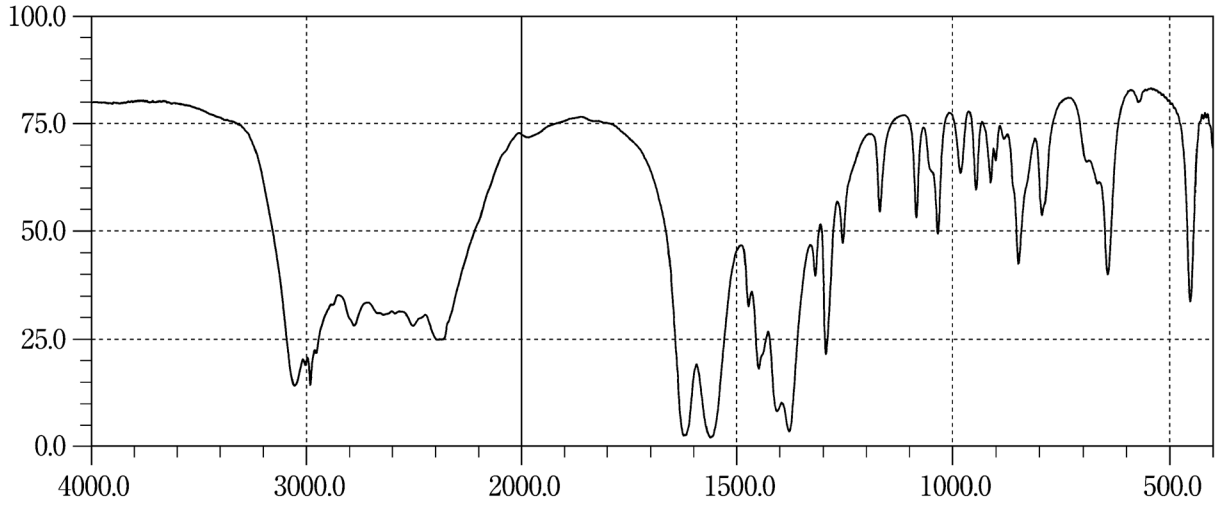
Progesterone



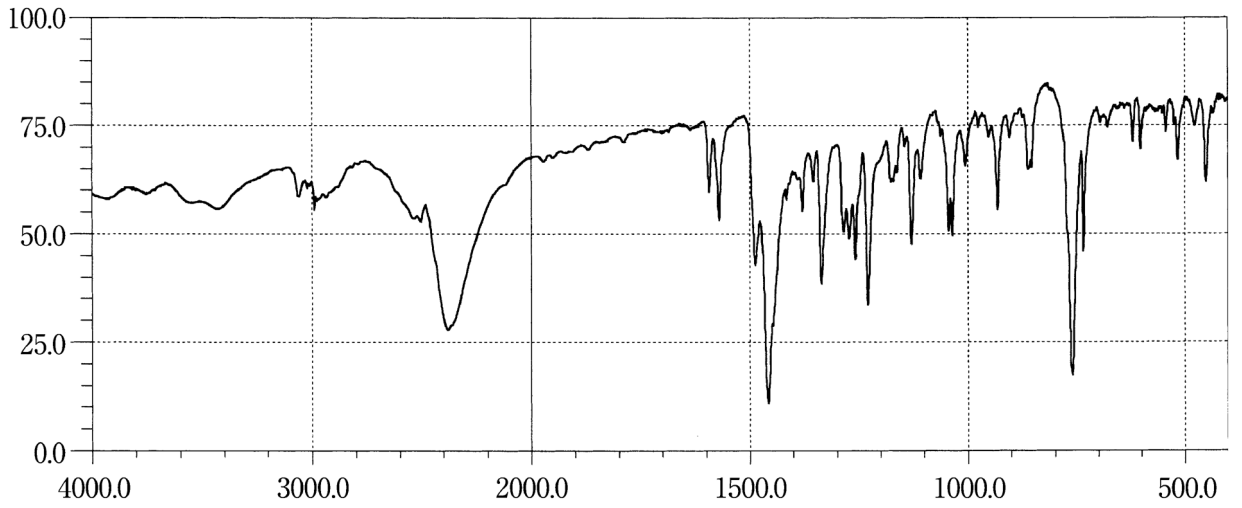
Proglumide



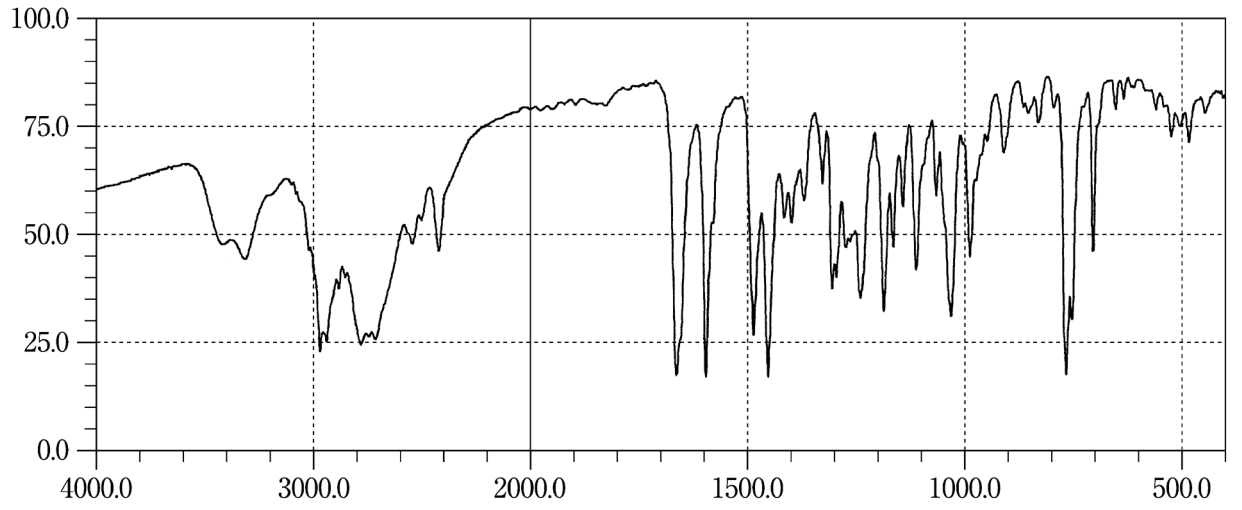
L-Proline



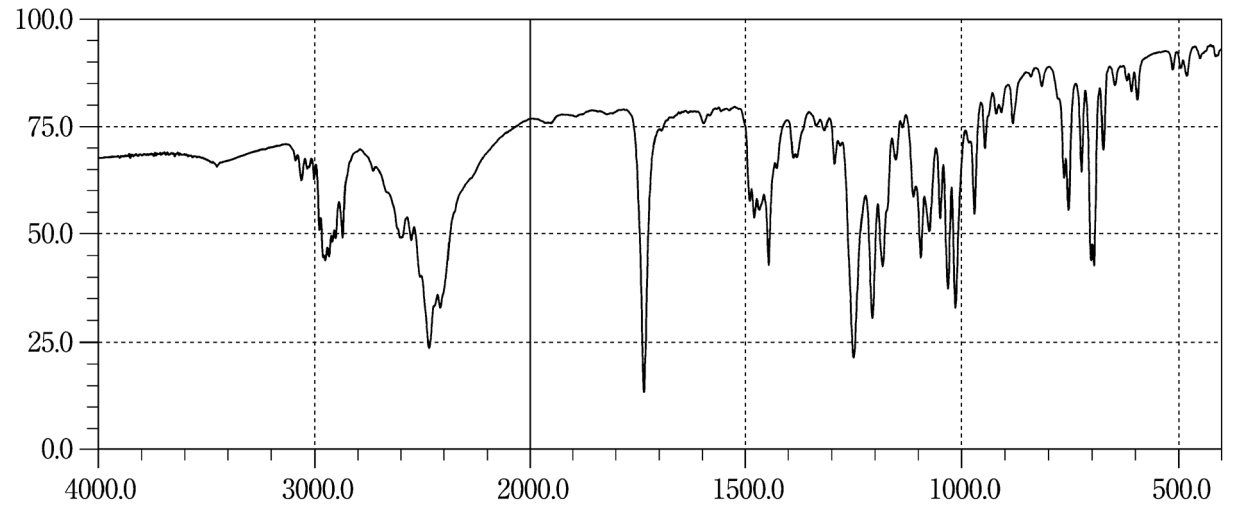
Promethazine Hydrochloride



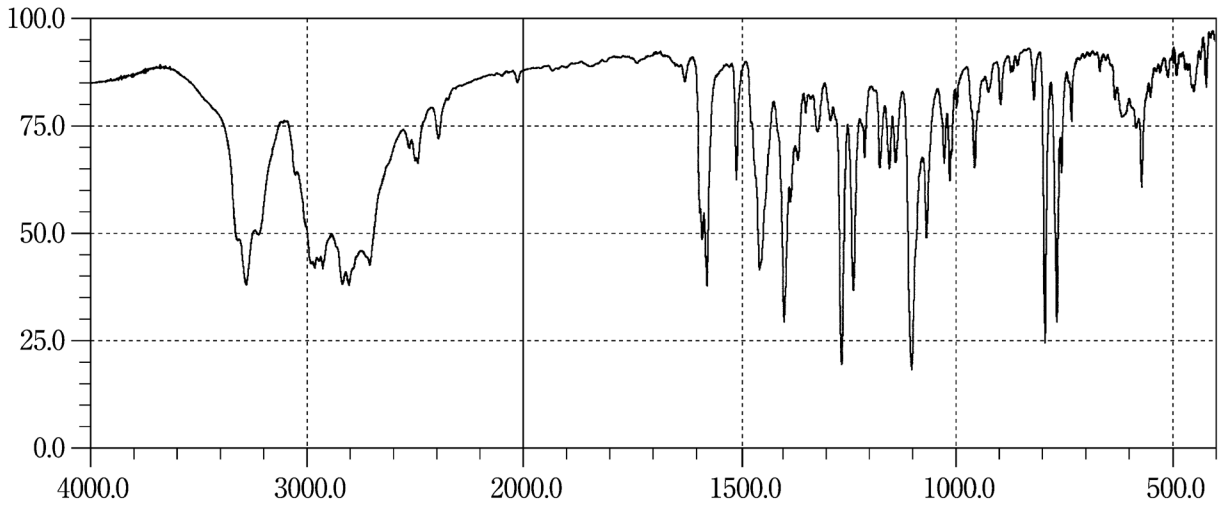
Propafenone Hydrochloride



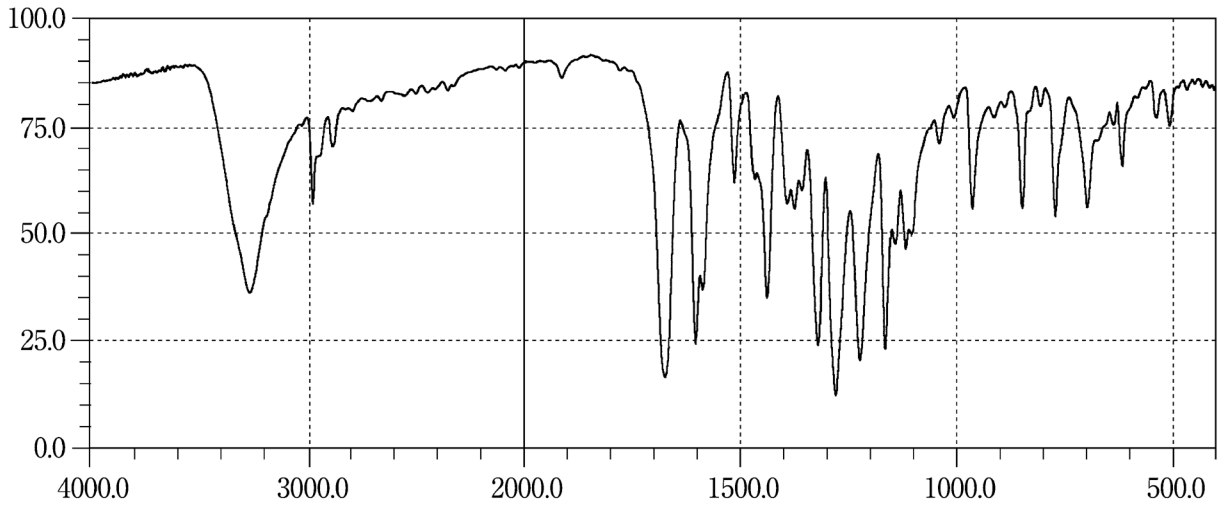
Propiverine Hydrochloride



Propranolol Hydrochloride



Propyl Parahydroxybenzoate



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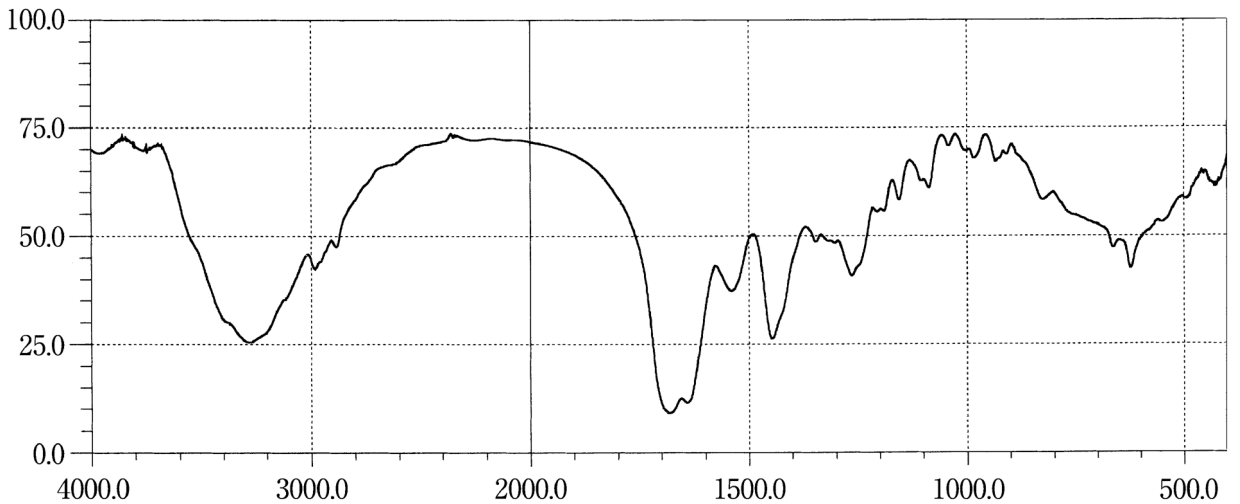
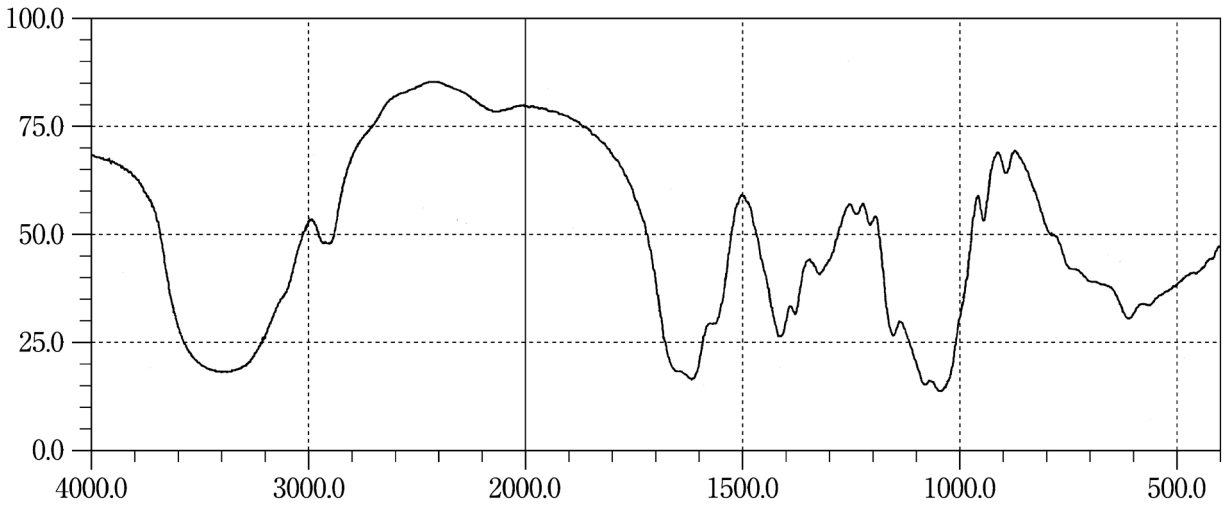
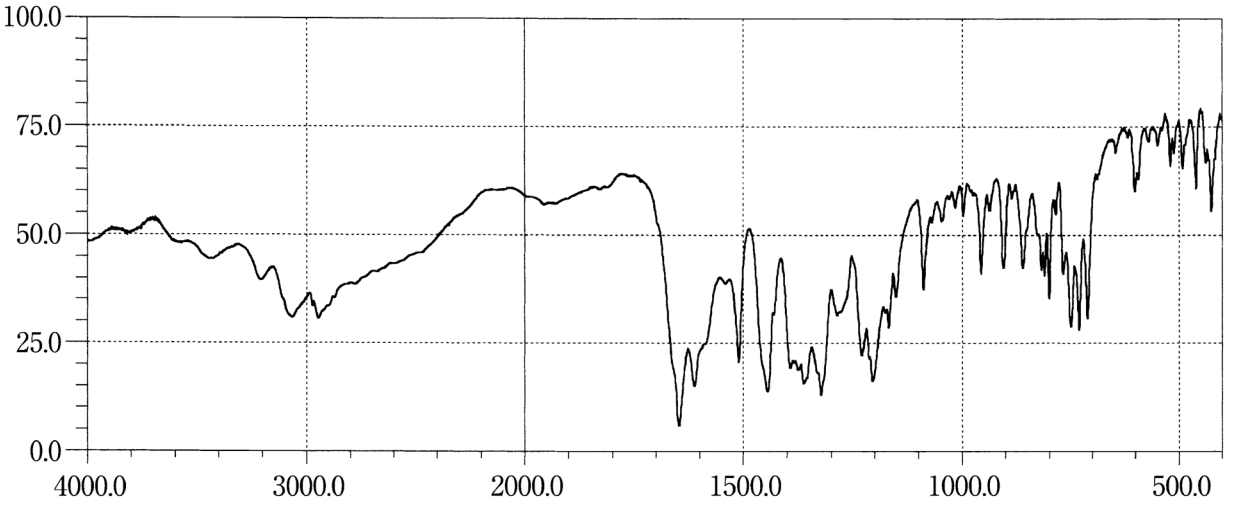


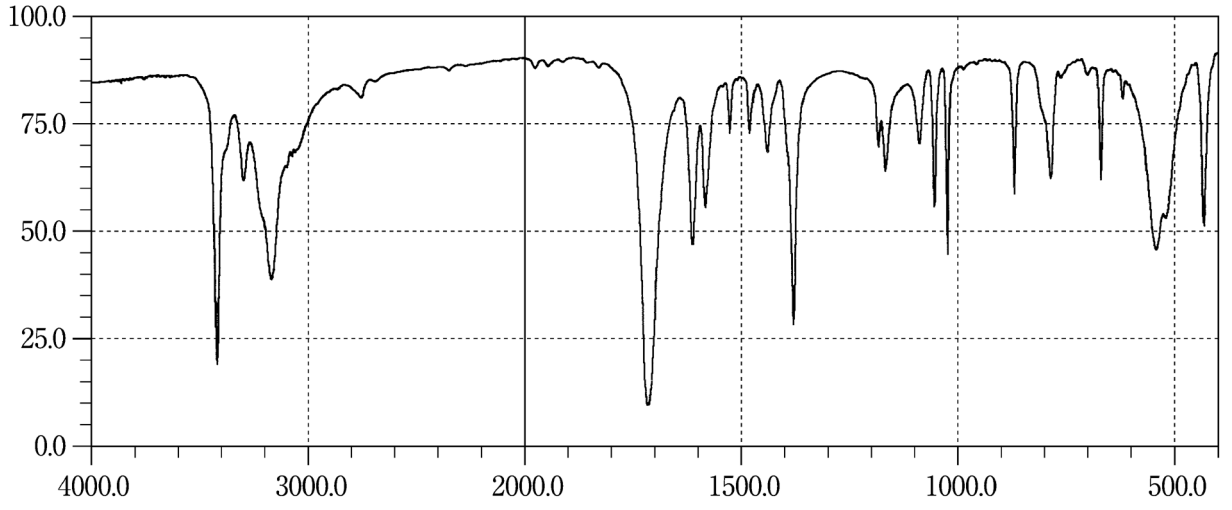
Purified Sodium Hyaluronate



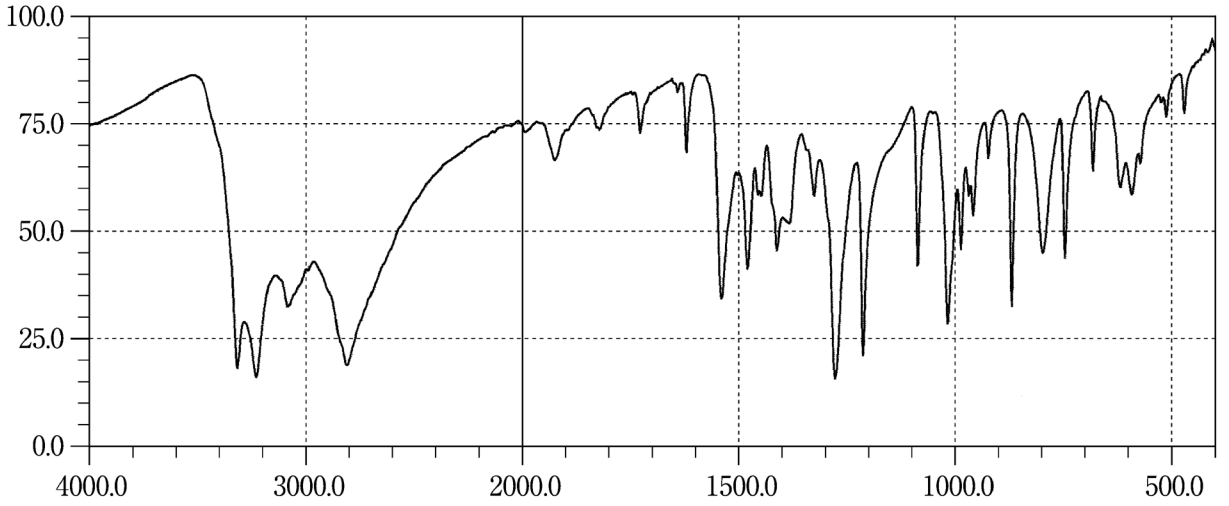
Pyrantel Pamoate



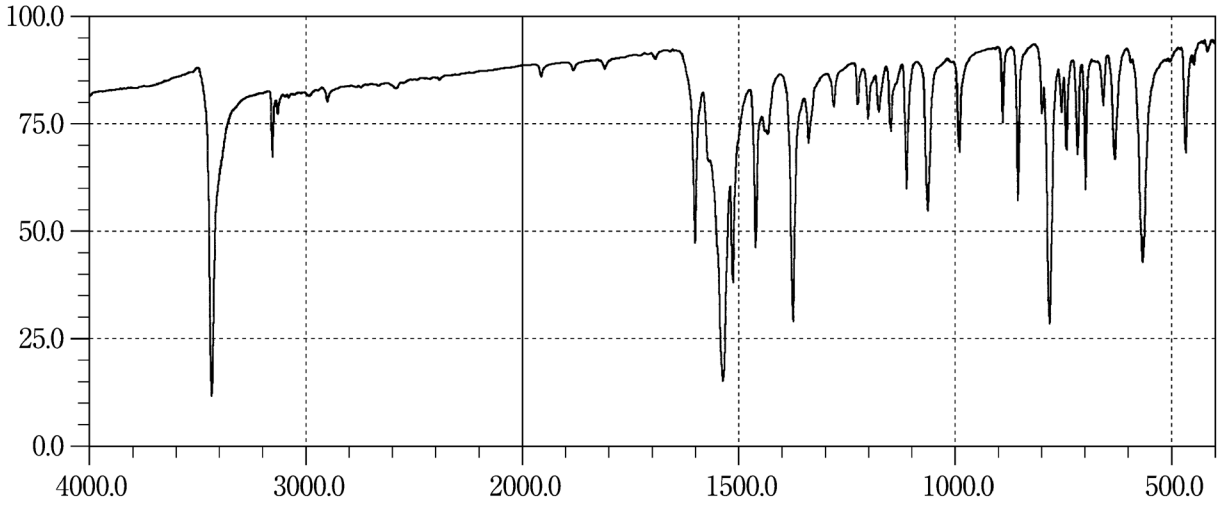
Pyrazinamide



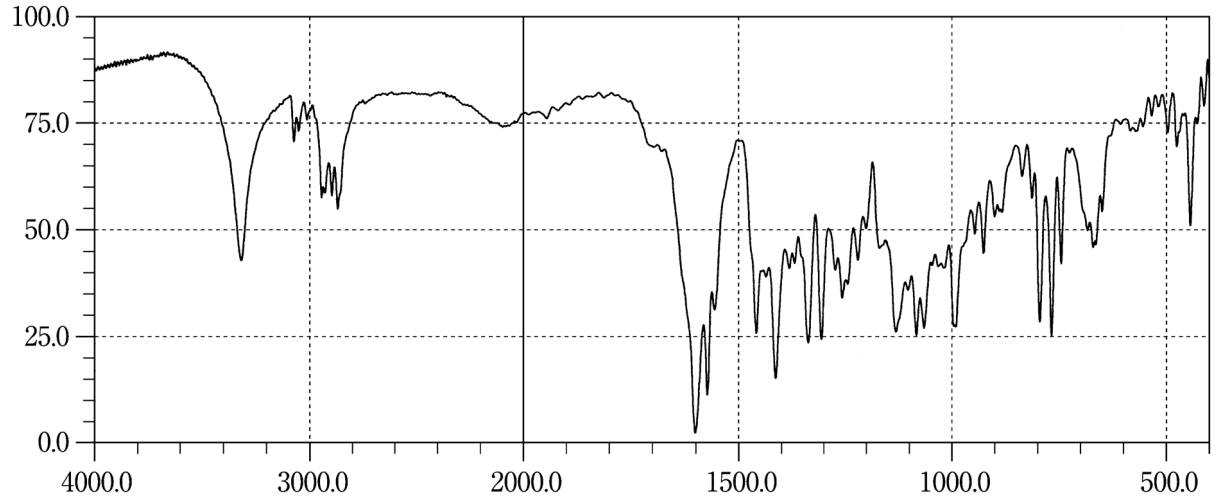
Pyridoxine Hydrochloride



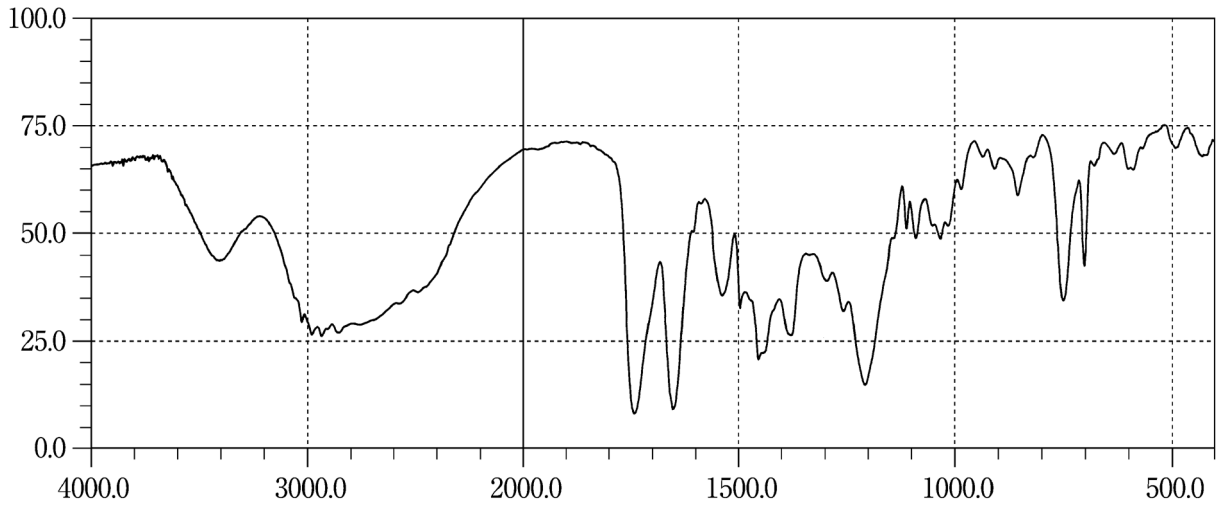
Pyrrolnitrin



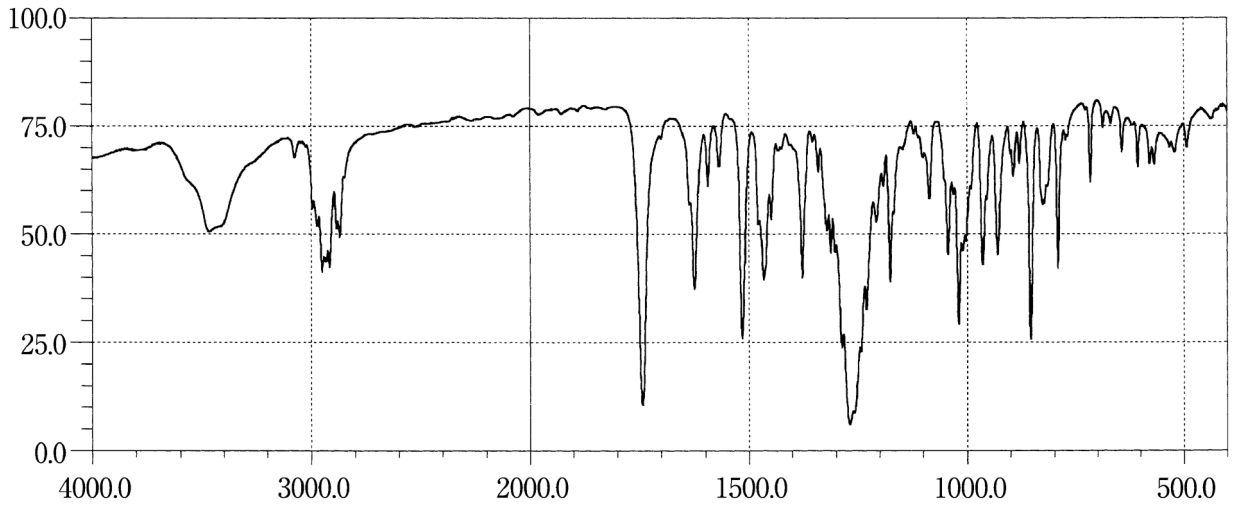
Quetiapine Fumarate



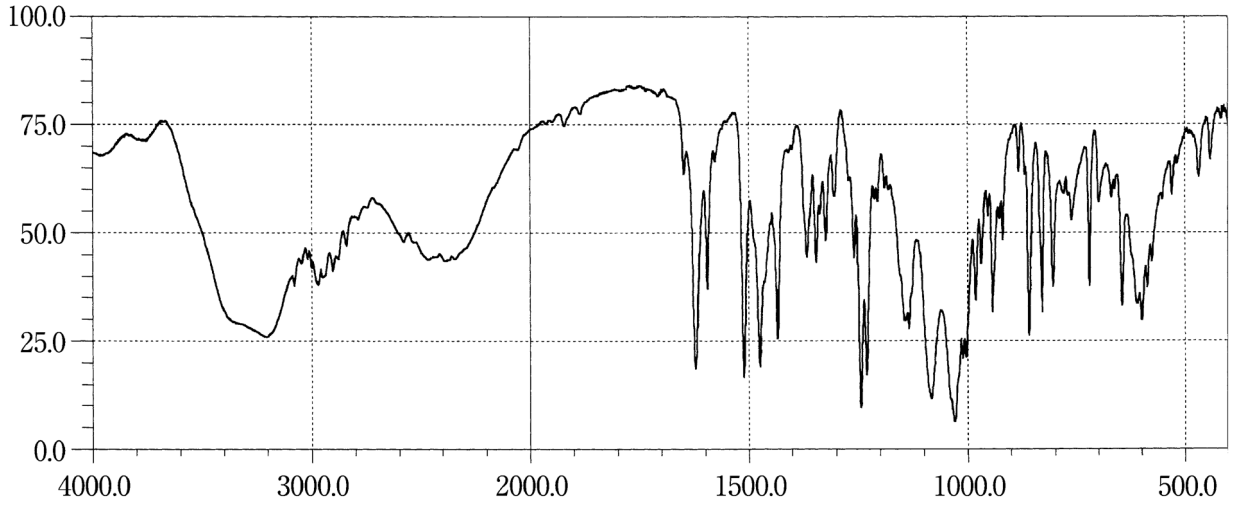
Quinapril Hydrochloride



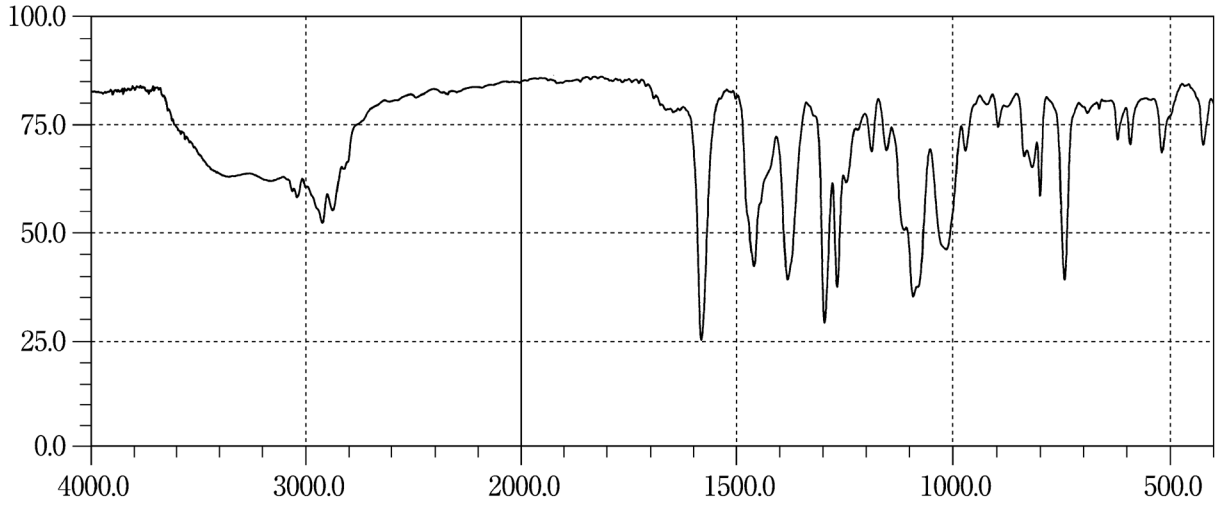
Quinine Ethyl Carbonate



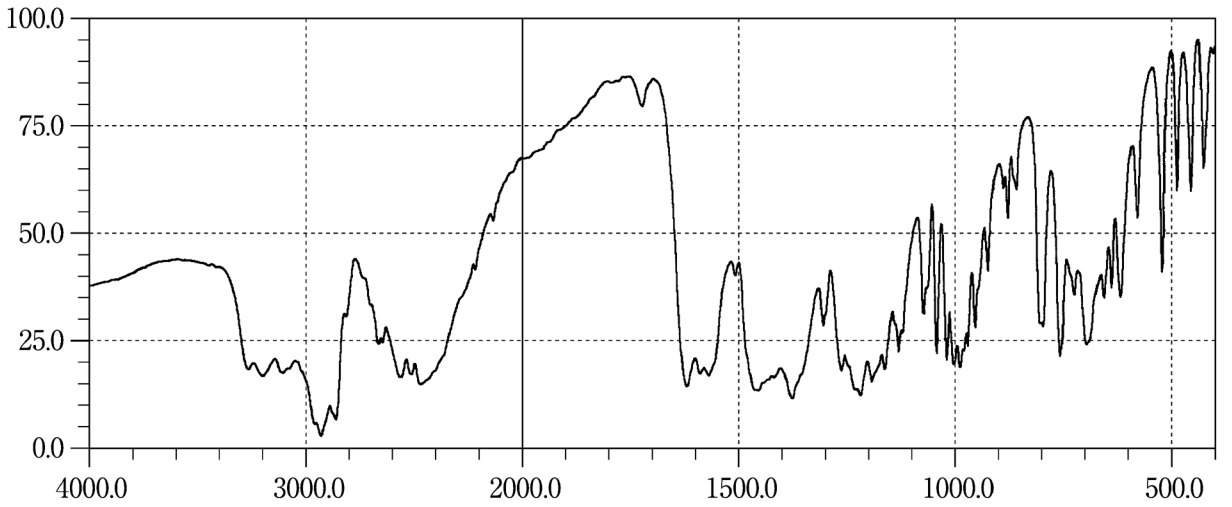
Quinine Sulfate Hydrate



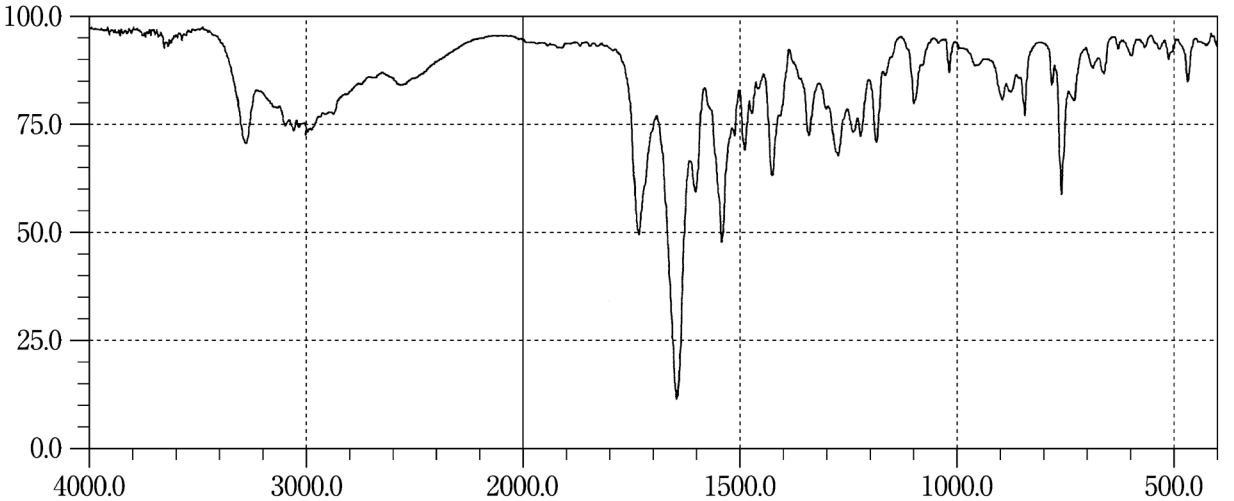
Rabeprazole Sodium



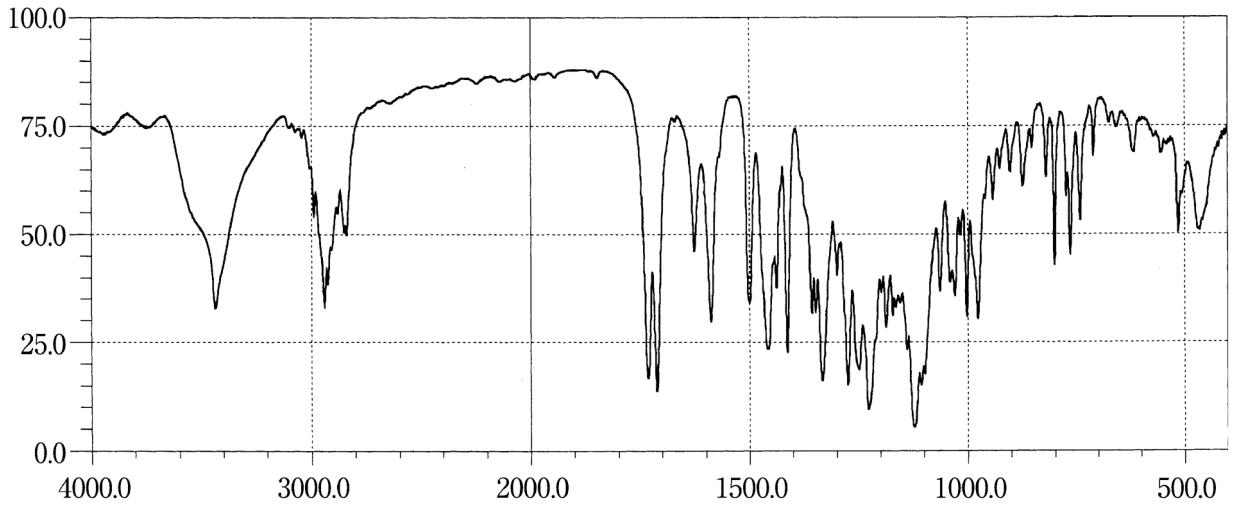
Ranitidine Hydrochloride



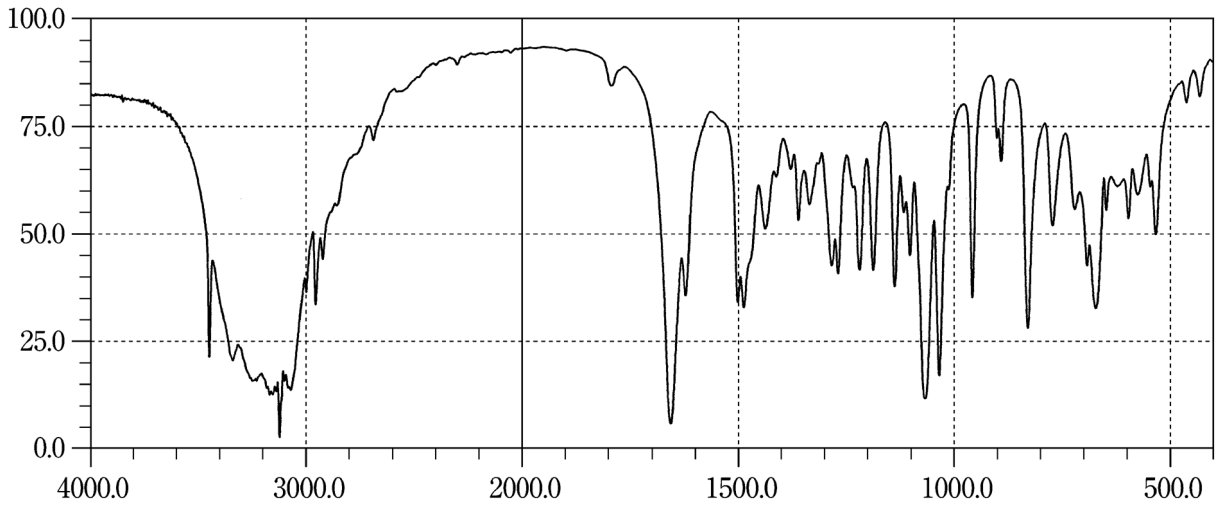
Rebamipide



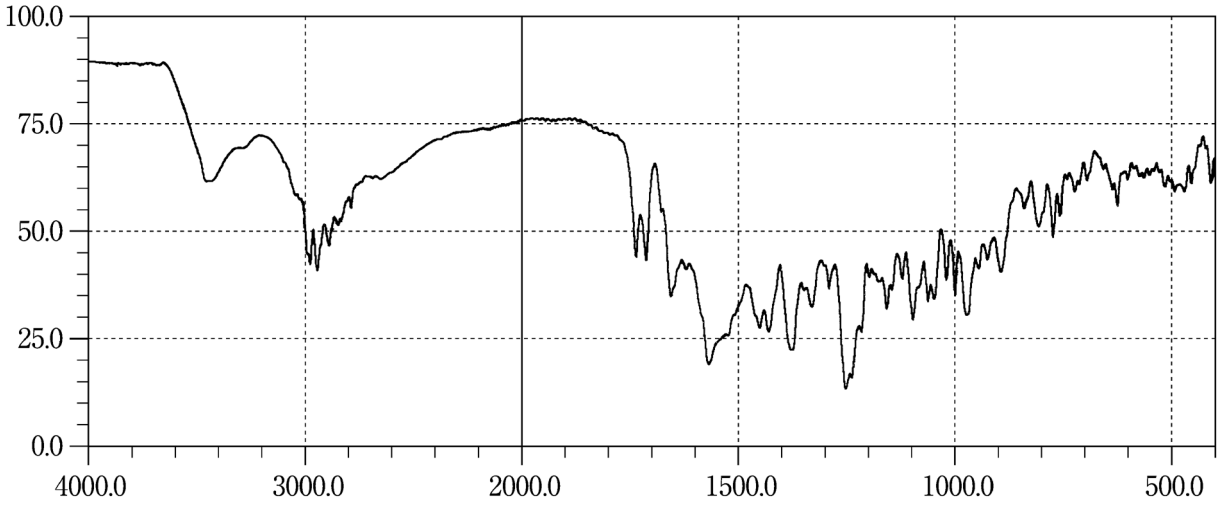
Reserpine



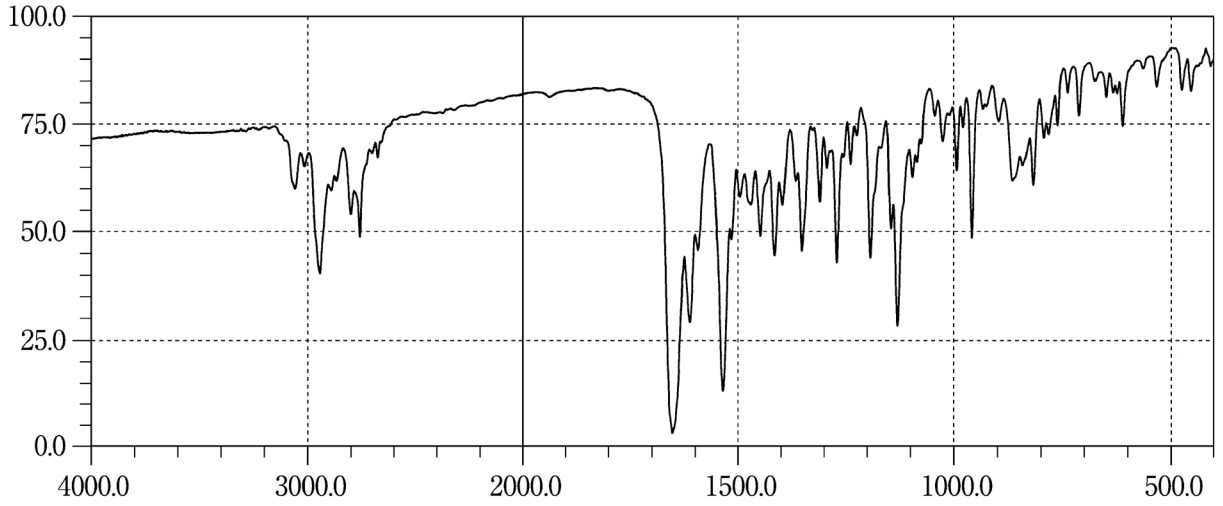
Ribavirin



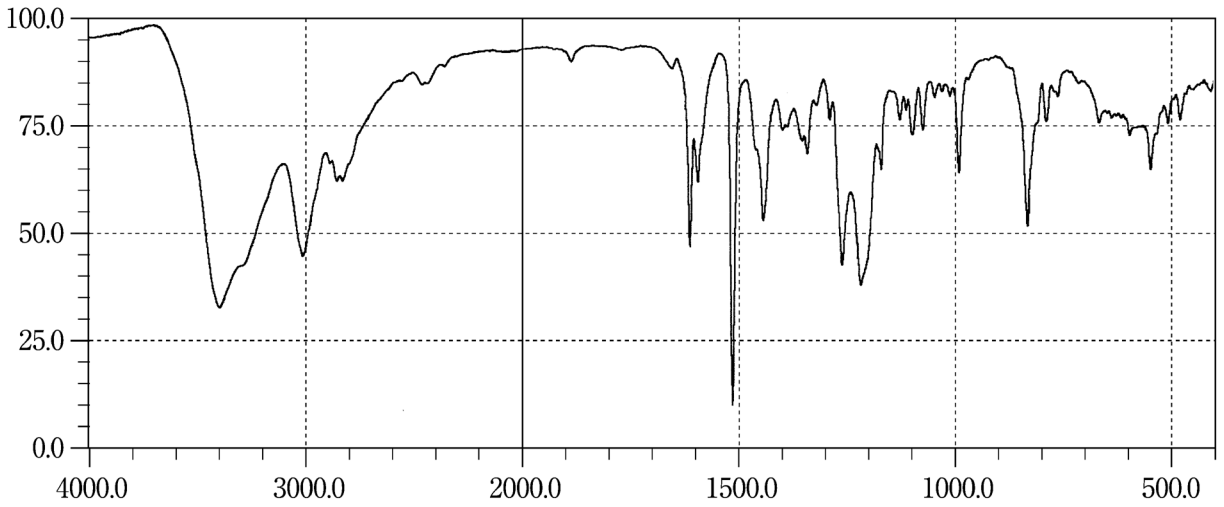
Rifampicin



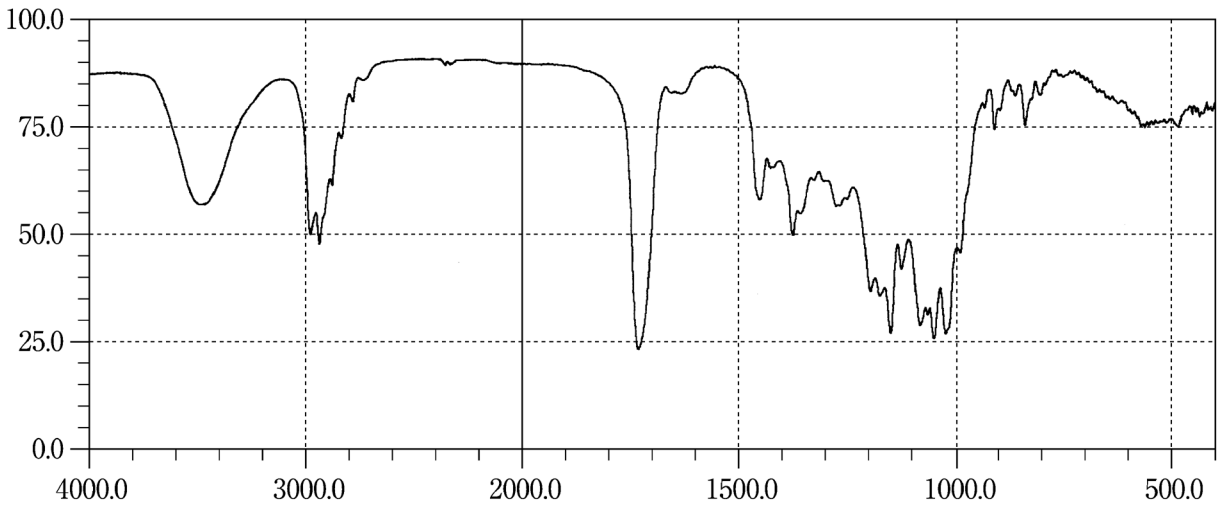
Risperidone



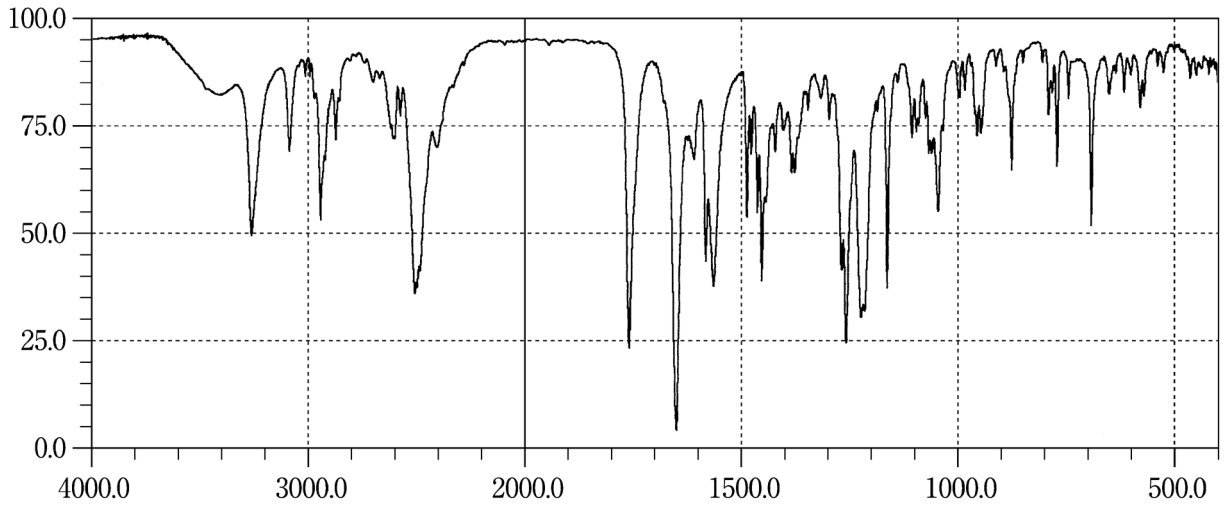
Ritodrine Hydrochloride



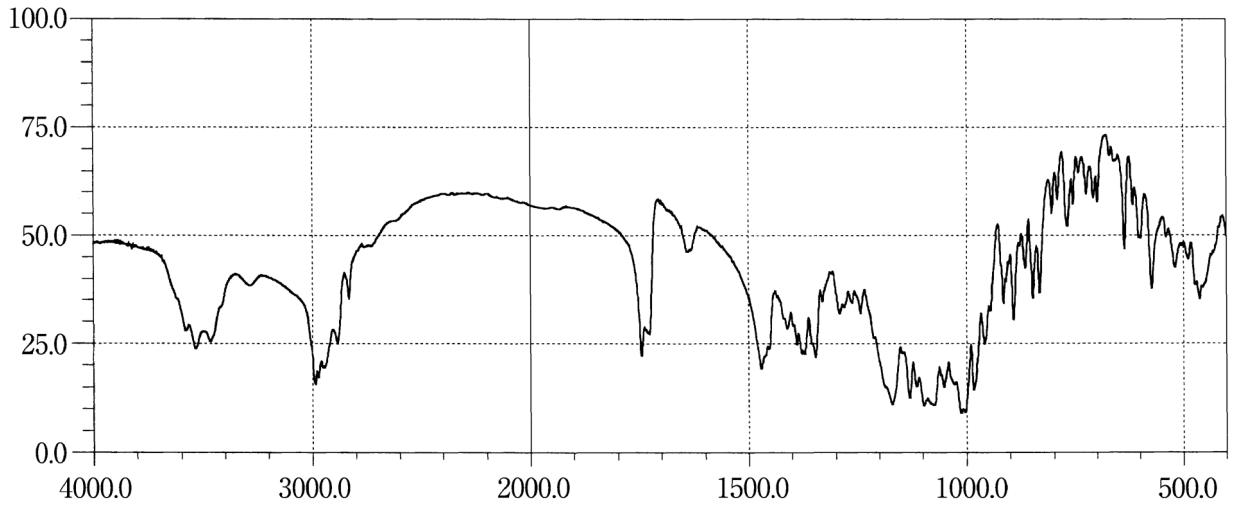
Rokitamycin



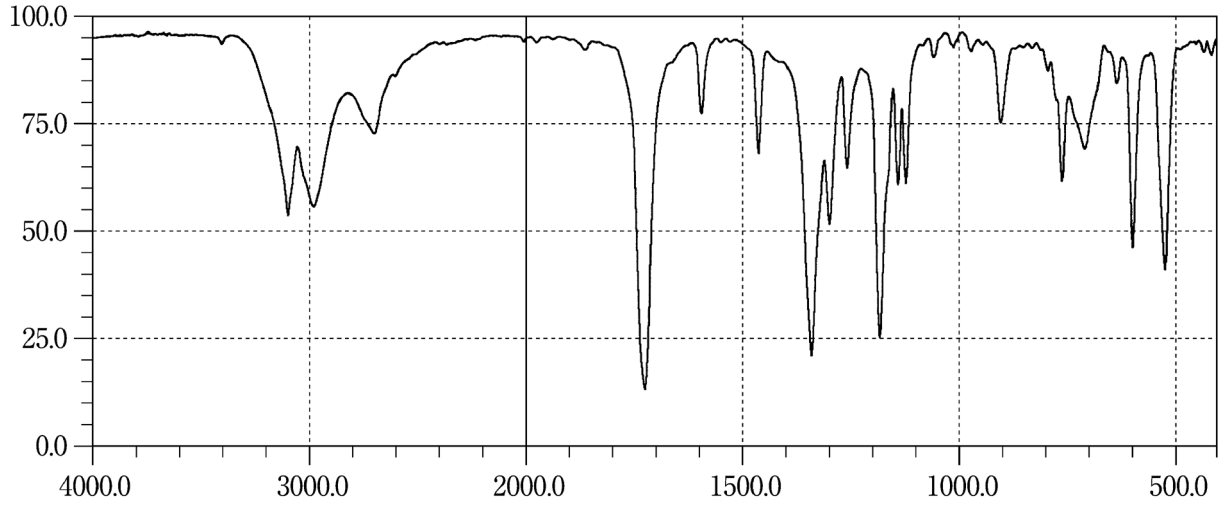
Roxatidine Acetate Hydrochloride



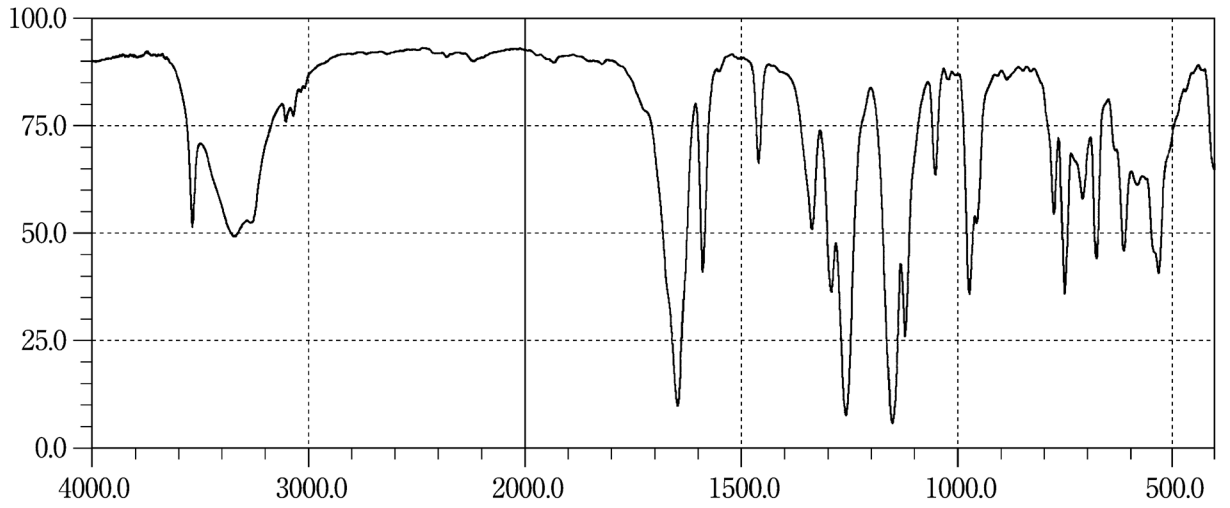
Roxithromycin



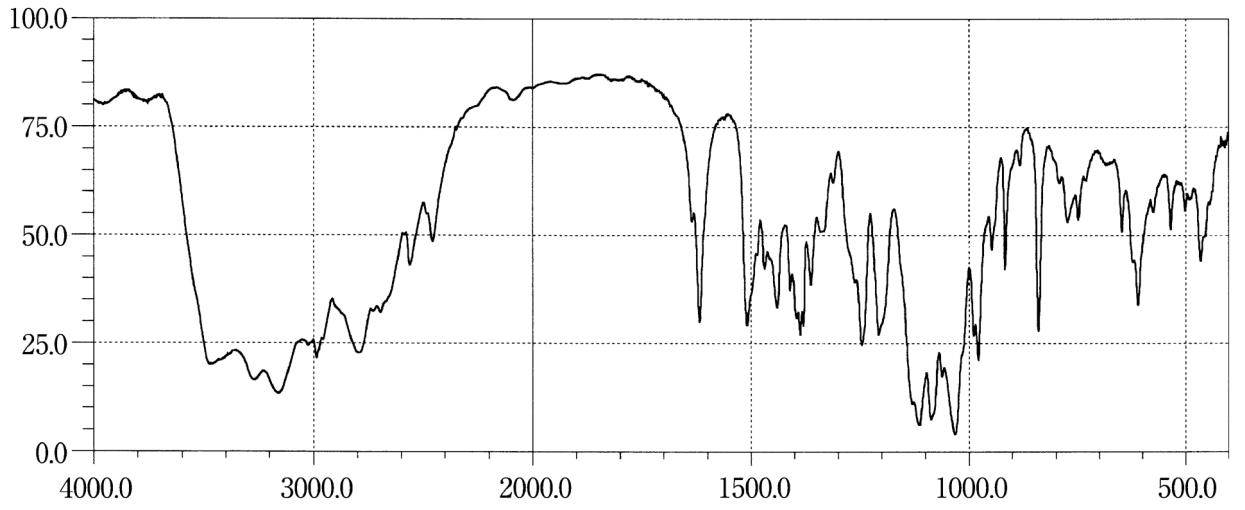
Saccharin



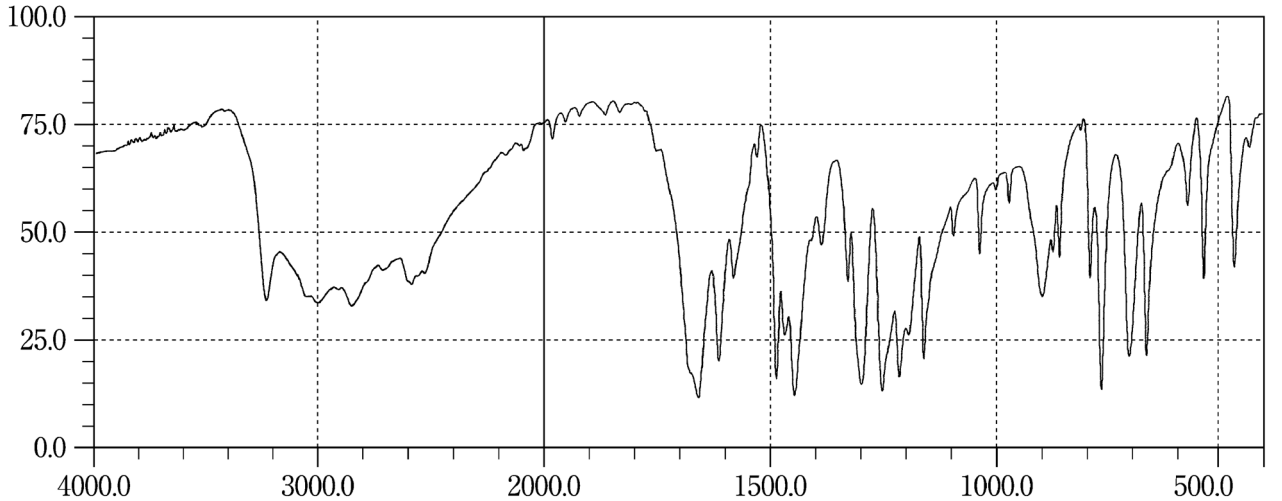
Saccharin Sodium Hydrate



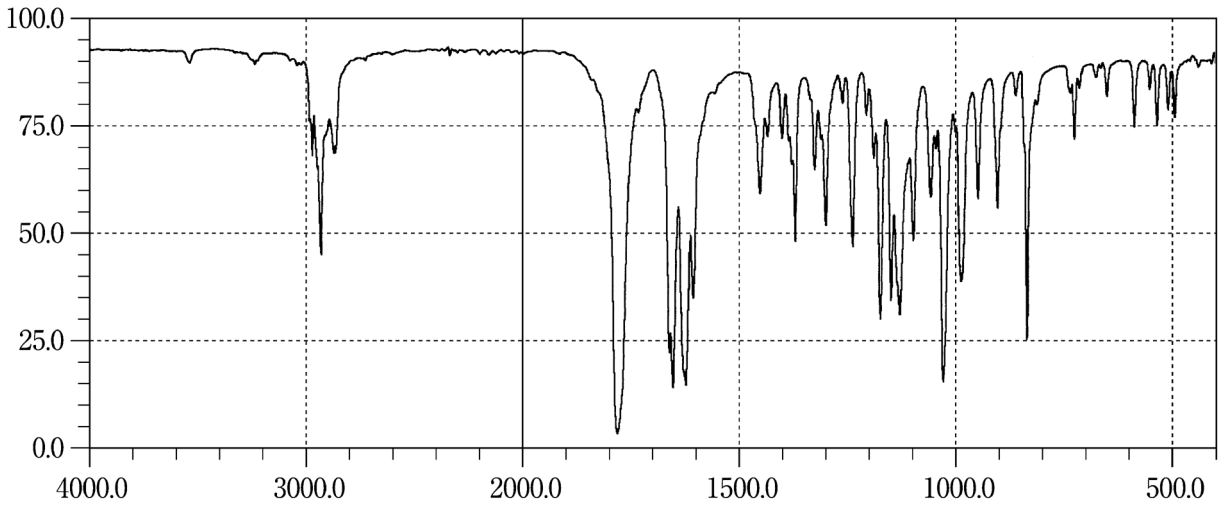
Salbutamol Sulfate



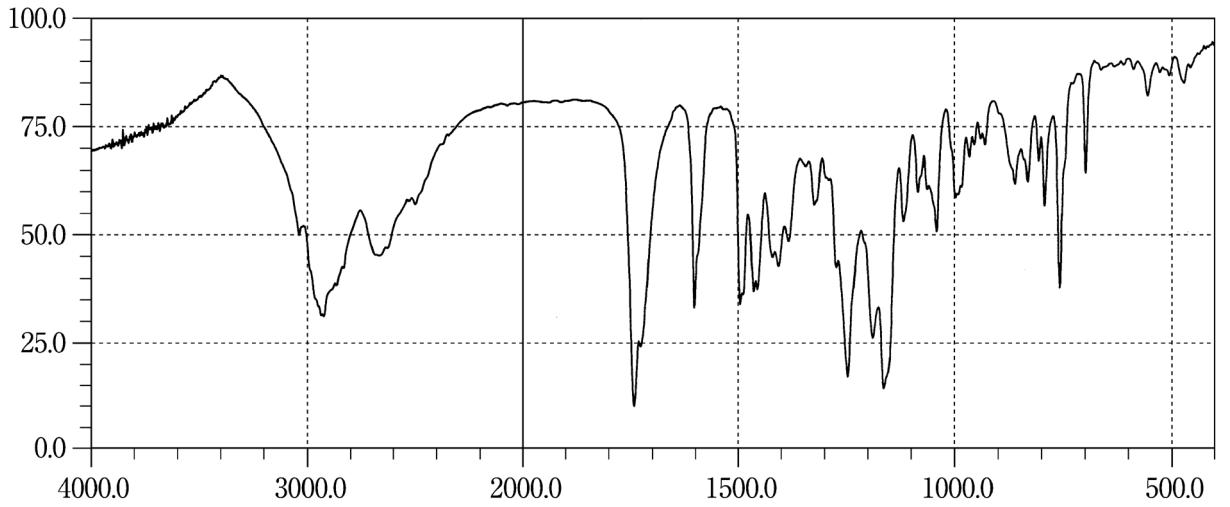
Salicylic Acid



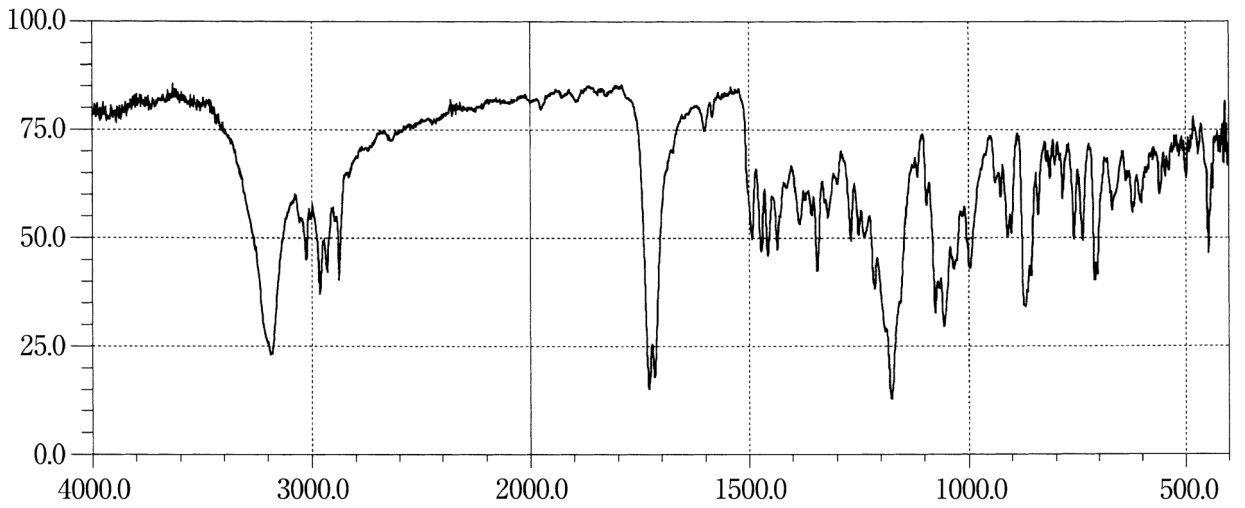
Santonin



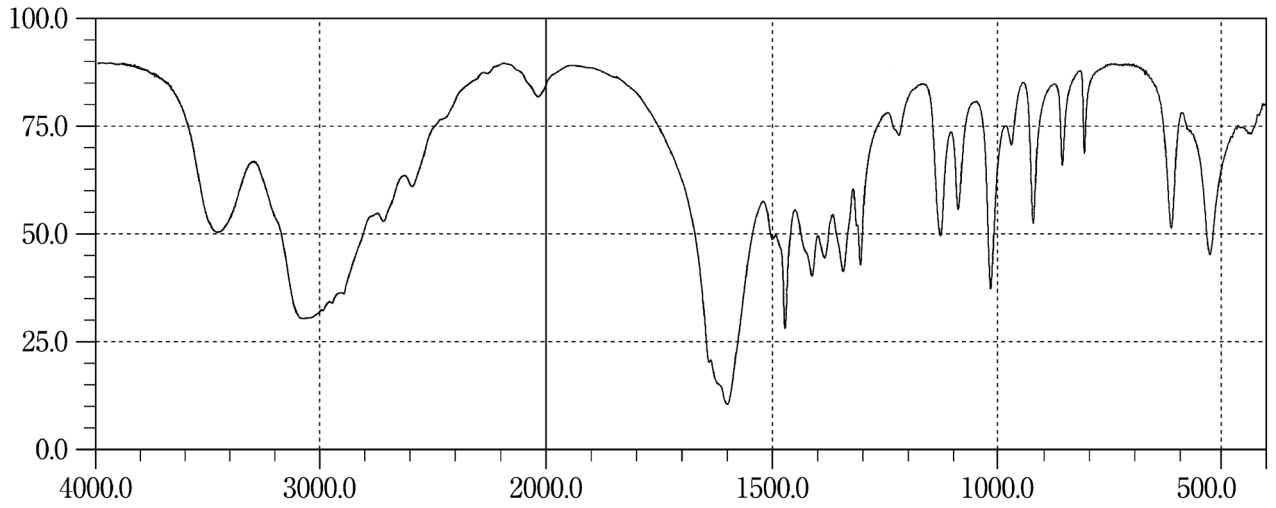
Sarpogrelate Hydrochloride



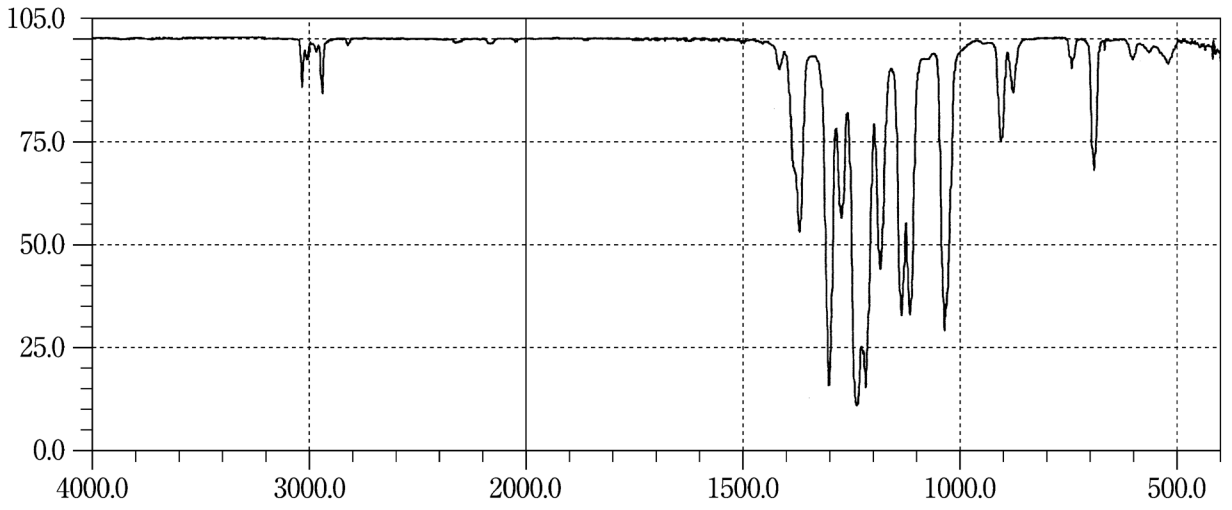
Scopolamine Butylbromide



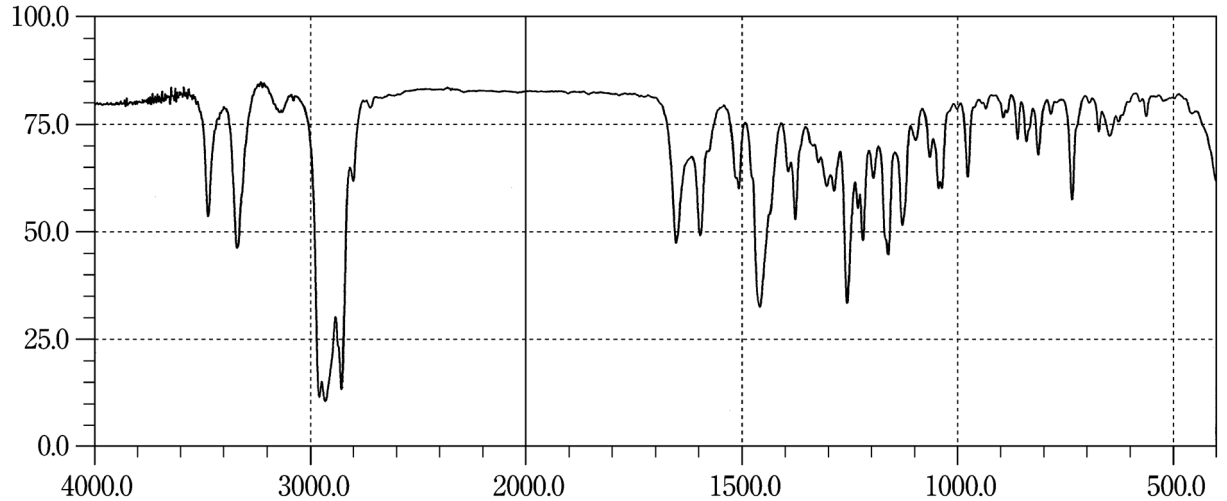
L-Serine



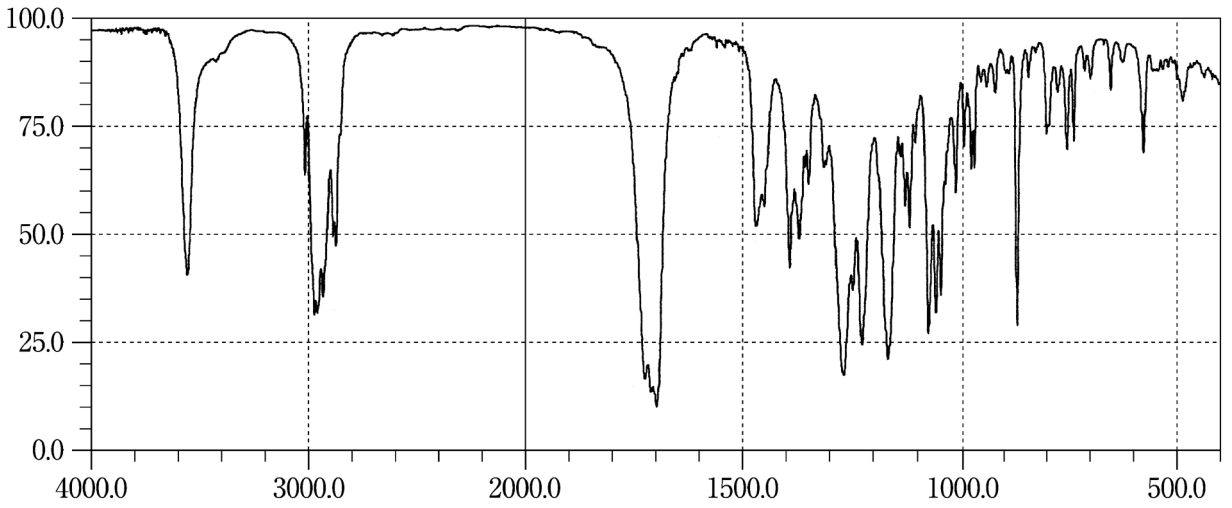
Sevoflurane



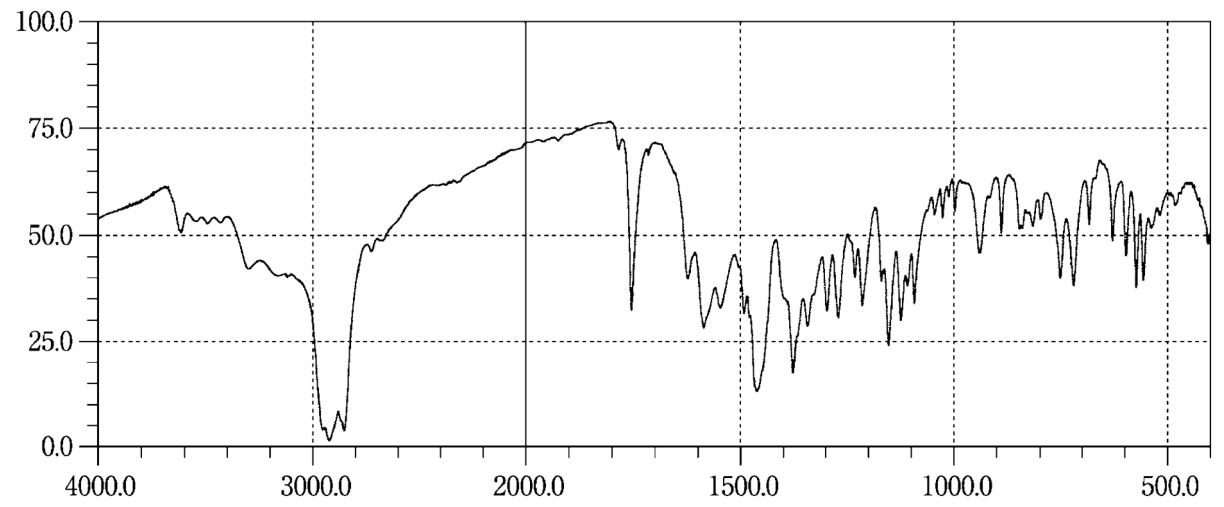
Silodosin



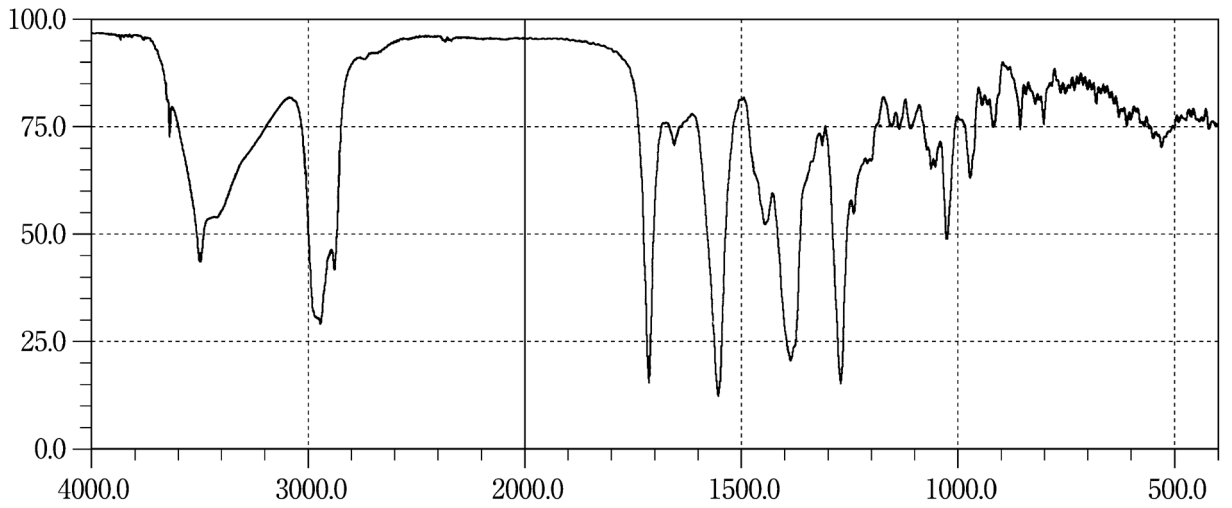
Simvastatin



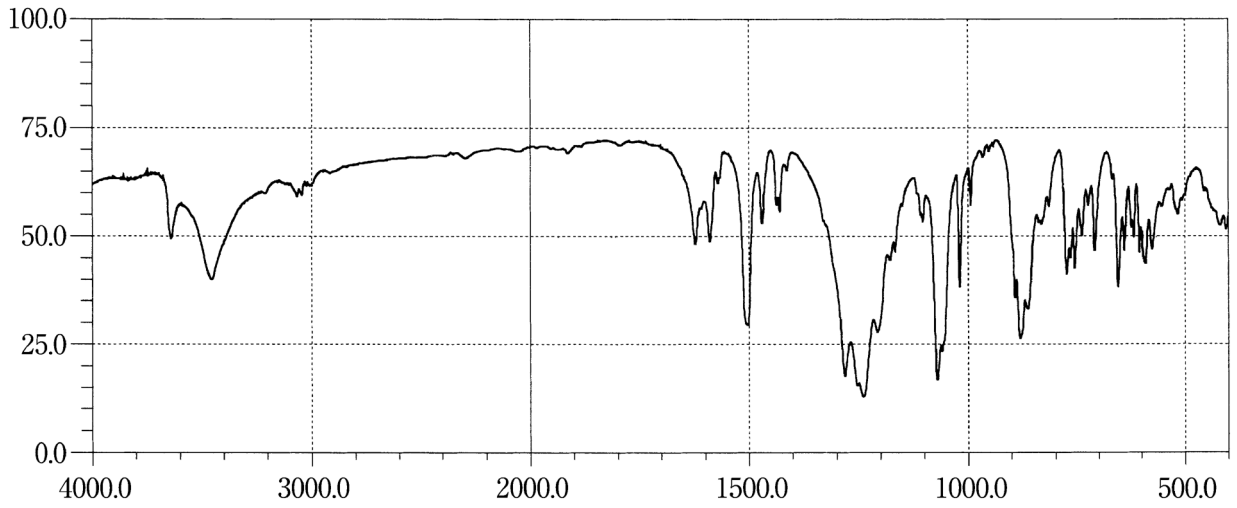
Sivelestat Sodium Hydrate



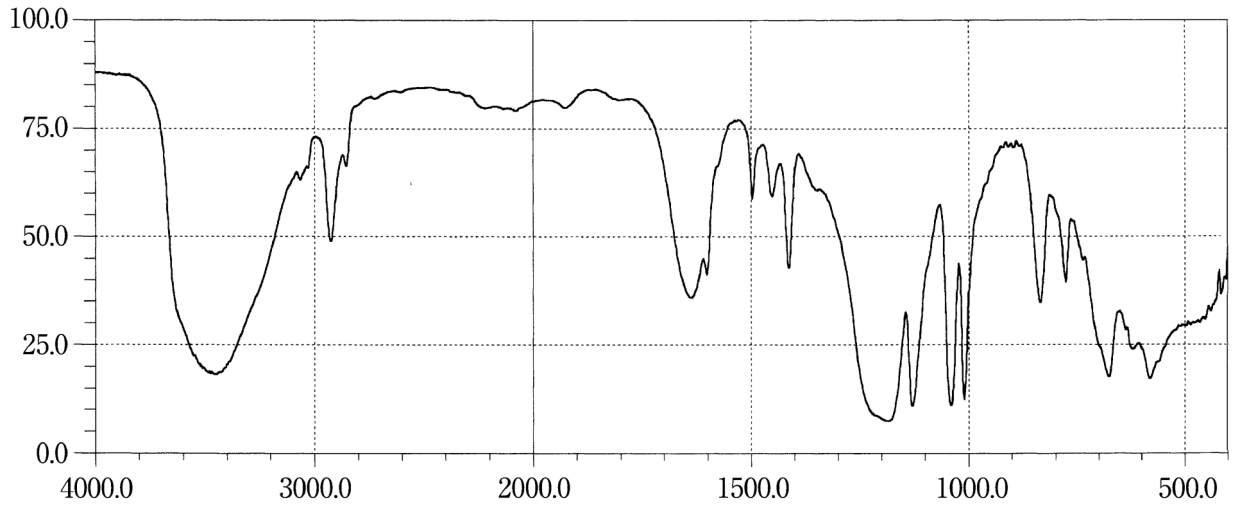
Sodium Fusidate



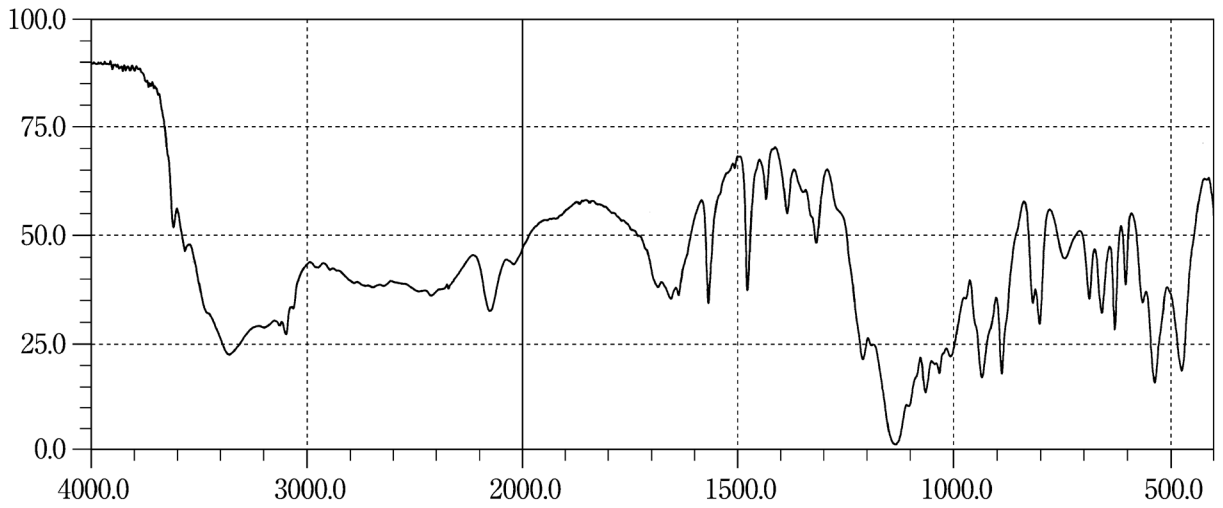
Sodium Picosulfate Hydrate



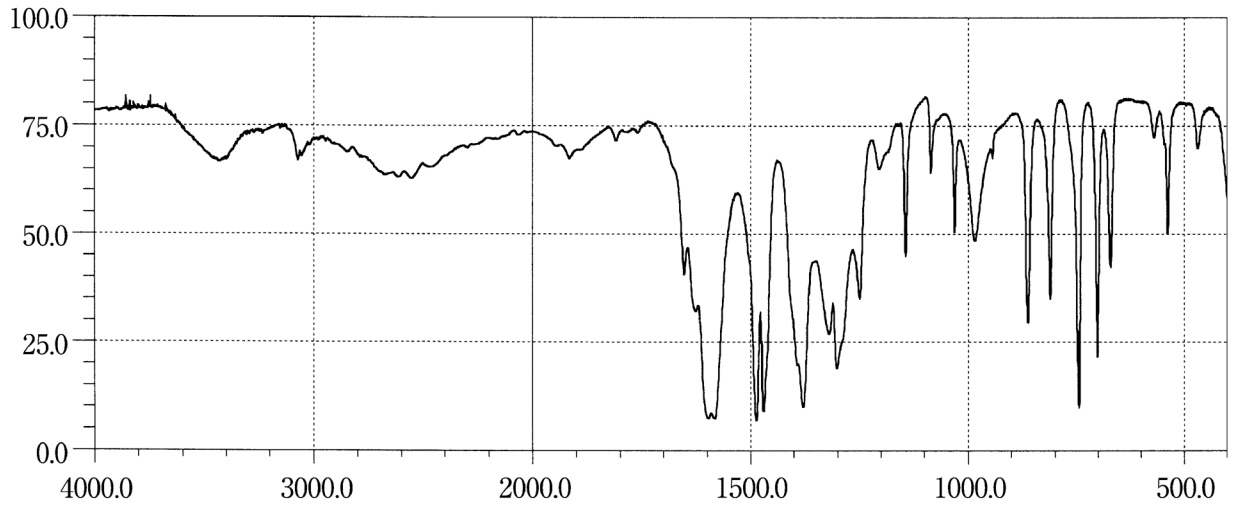
Sodium Polystyrene Sulfonate



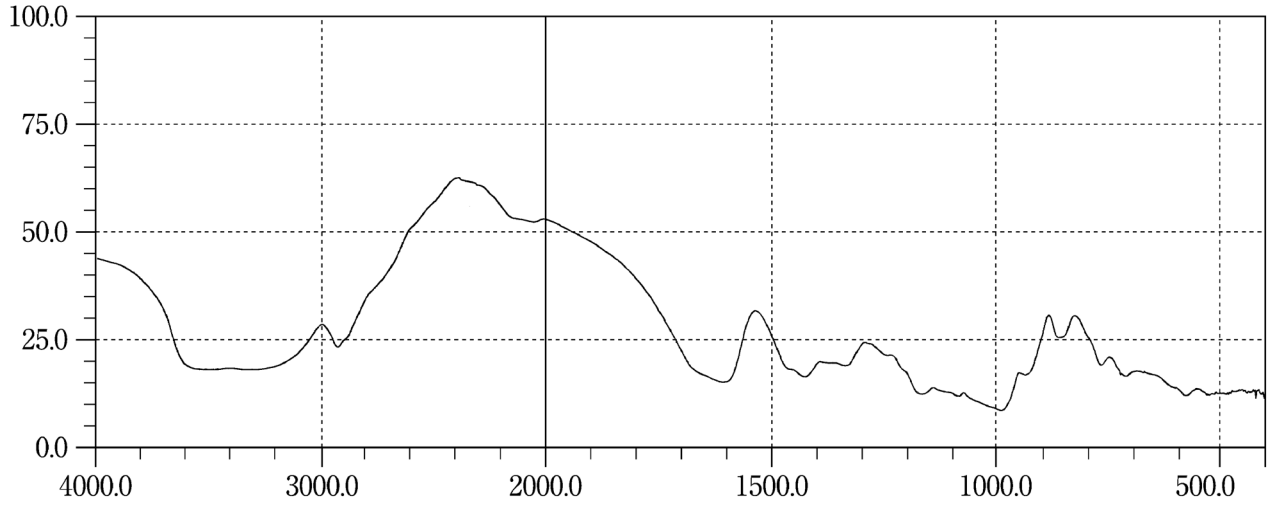
Sodium Risedronate Hydrate



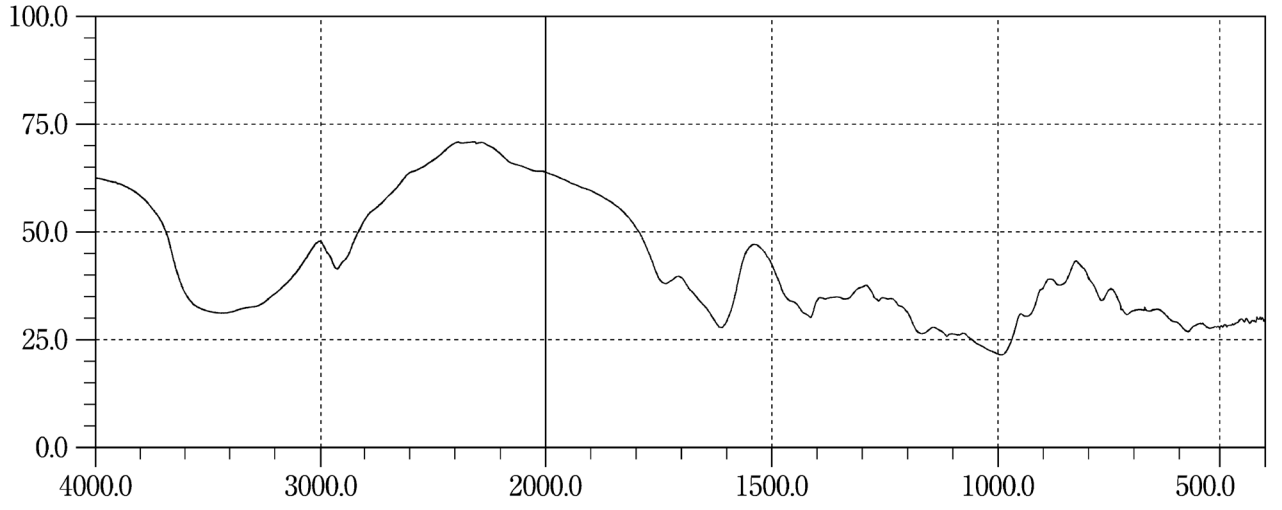
Sodium Salicylate



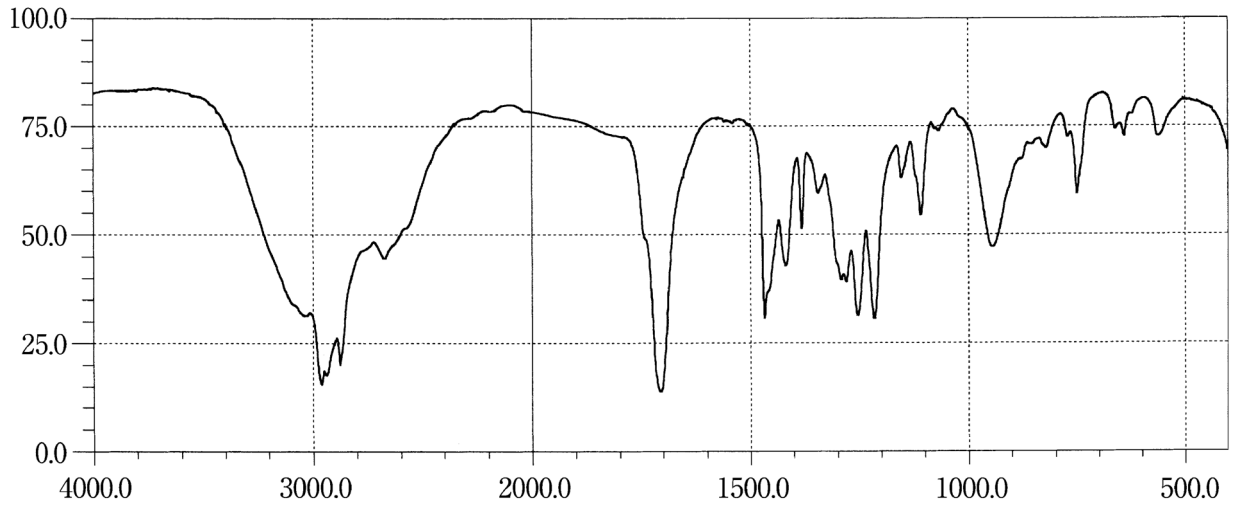
Sodium Starch Glycolate, Type A



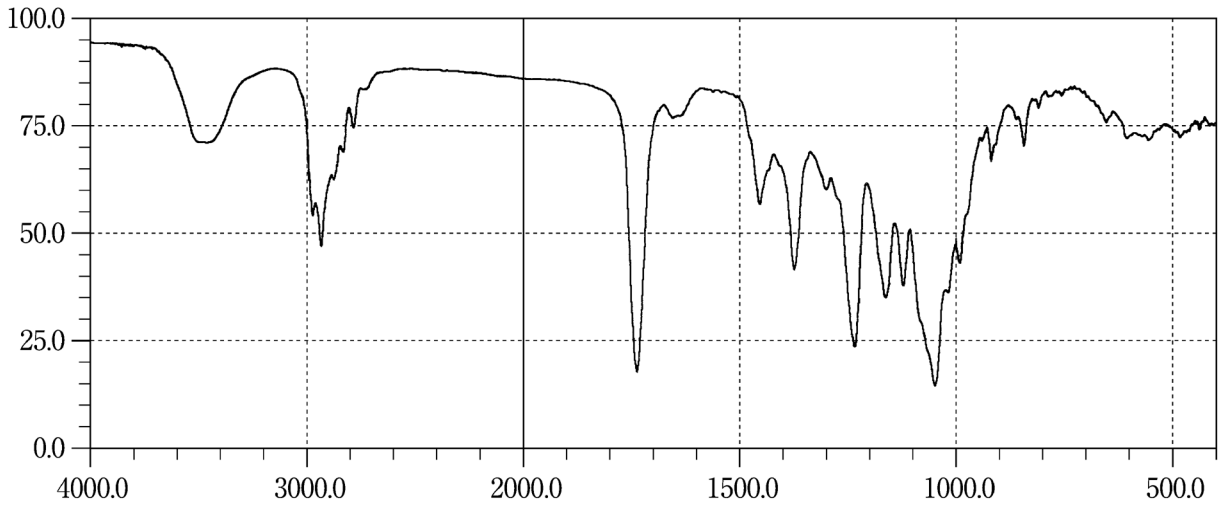
Sodium Starch Glycolate, Type B



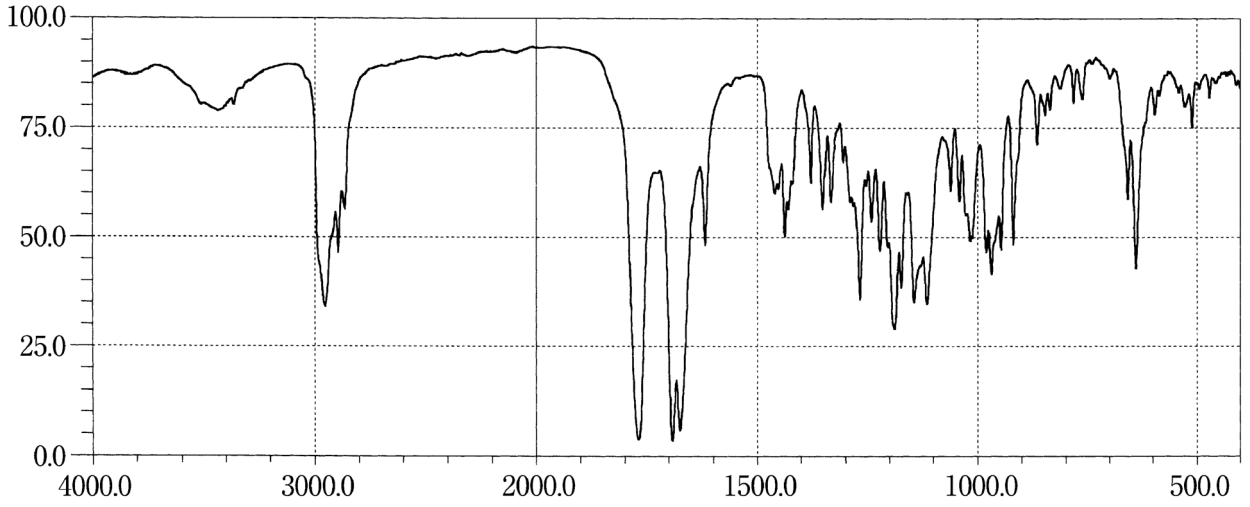
Sodium Valproate



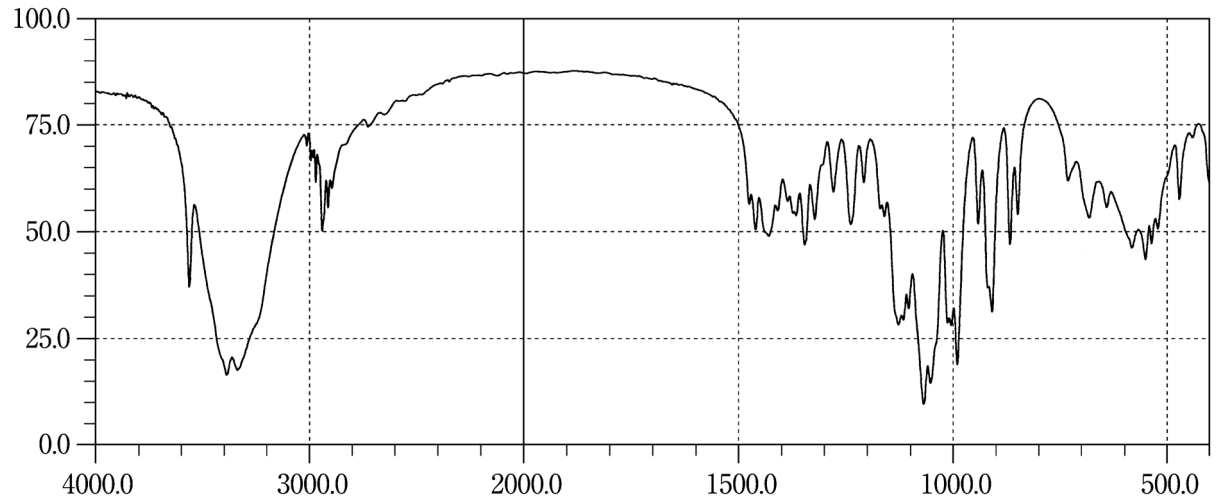
Spiramycin Acetate



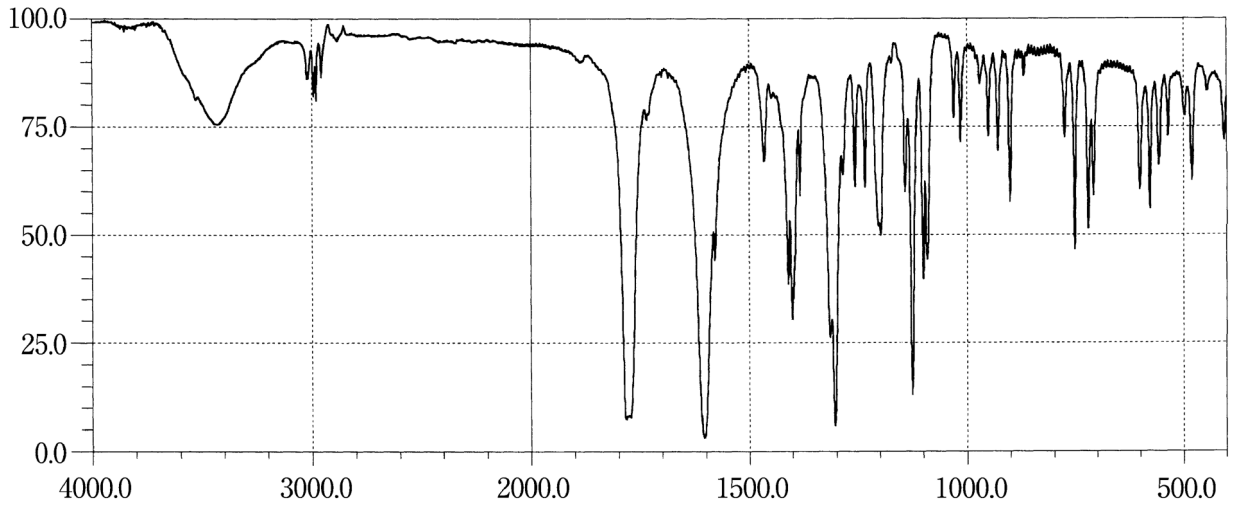
Spironolactone



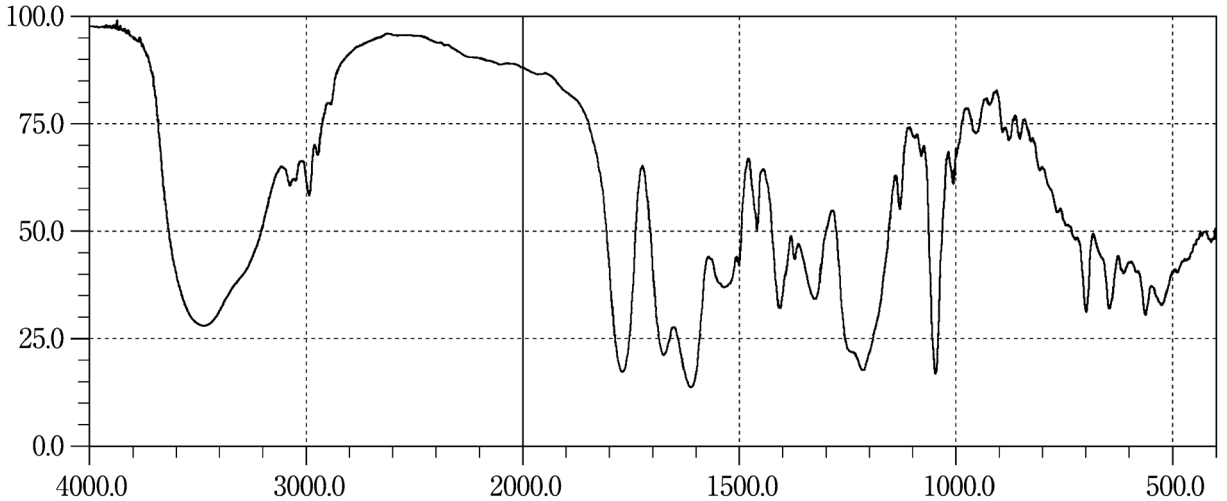
Sucrose



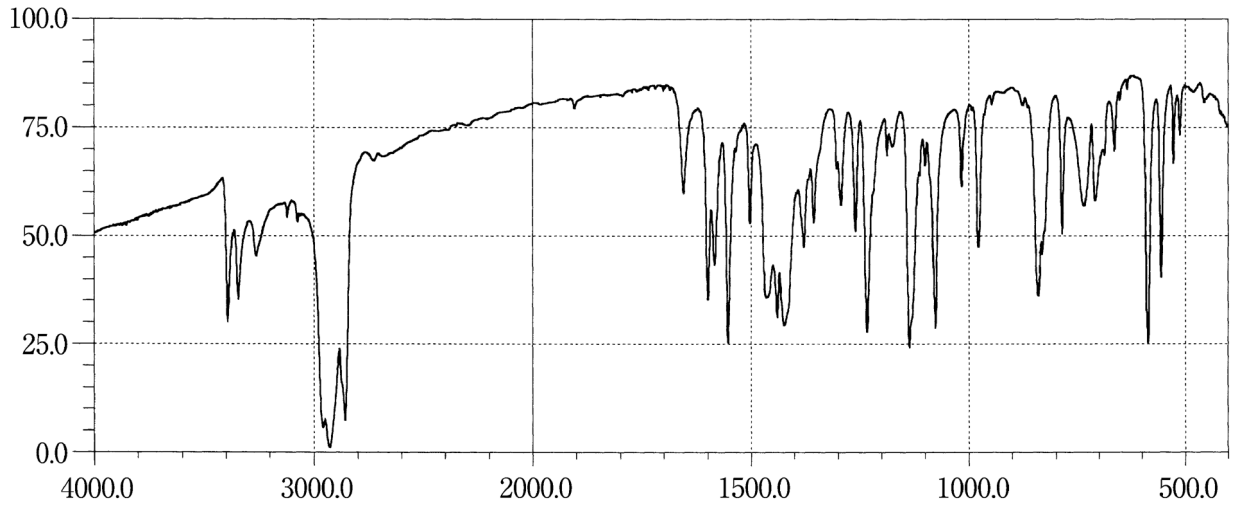
Sulbactam Sodium



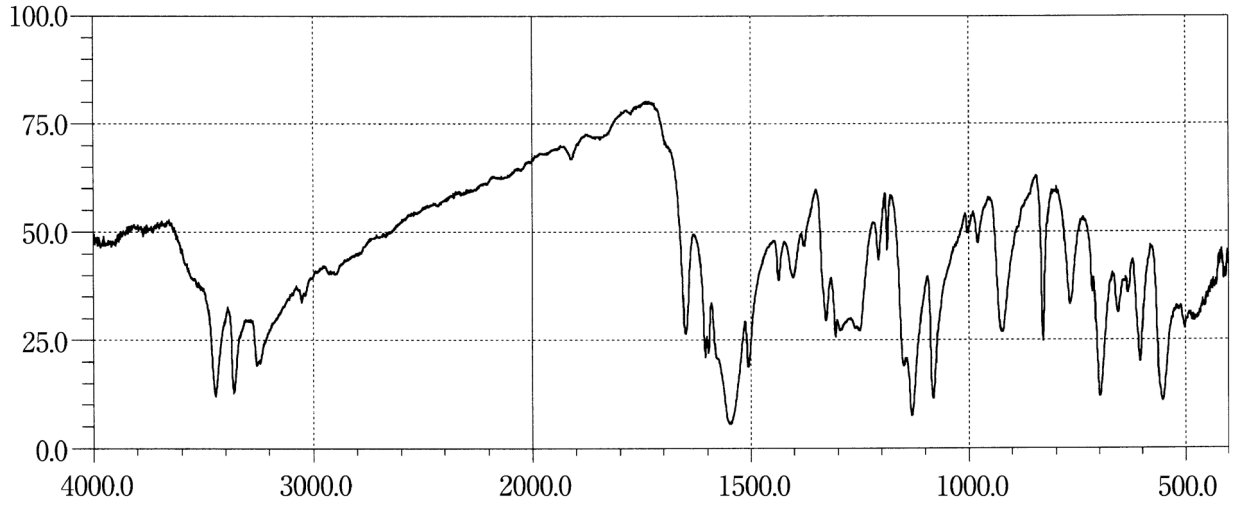
Sulbenicillin Sodium



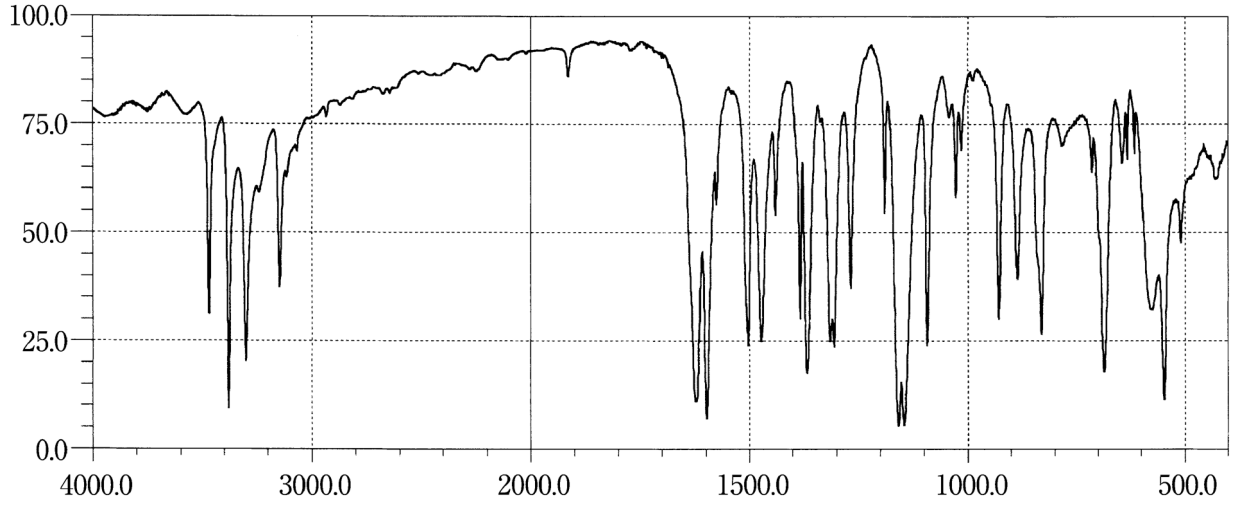
Sulfadiazine Silver



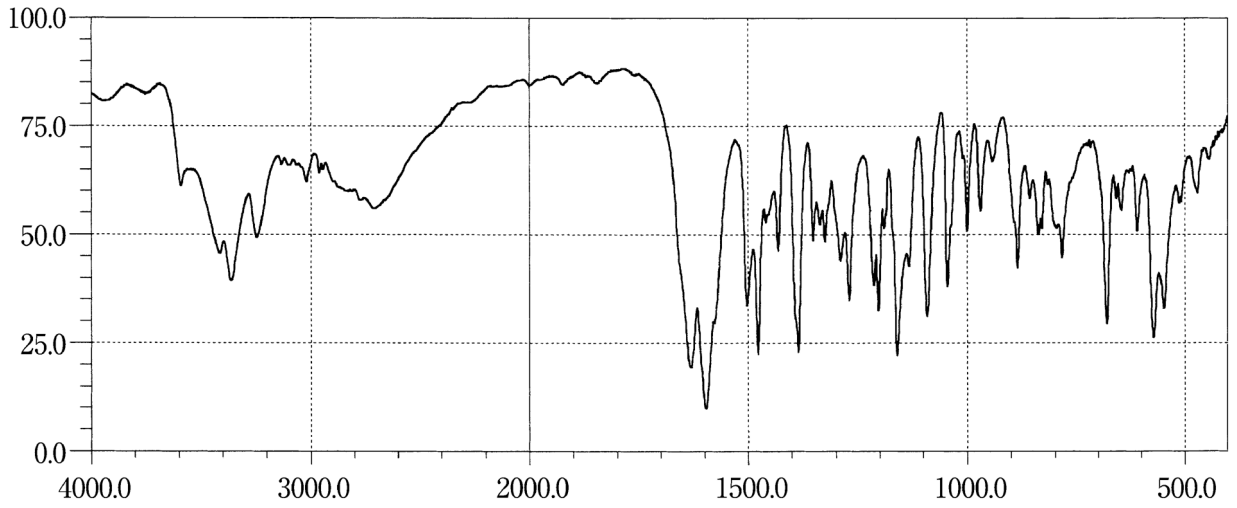
Sulfamethizole



Sulfamethoxazole



Sulfamonomethoxine Hydrate



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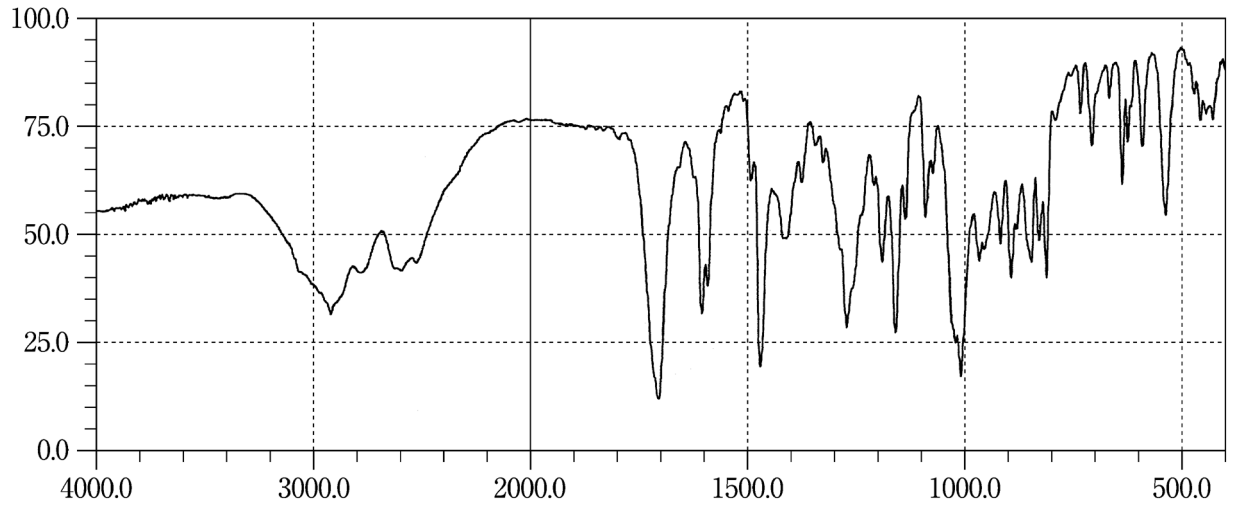
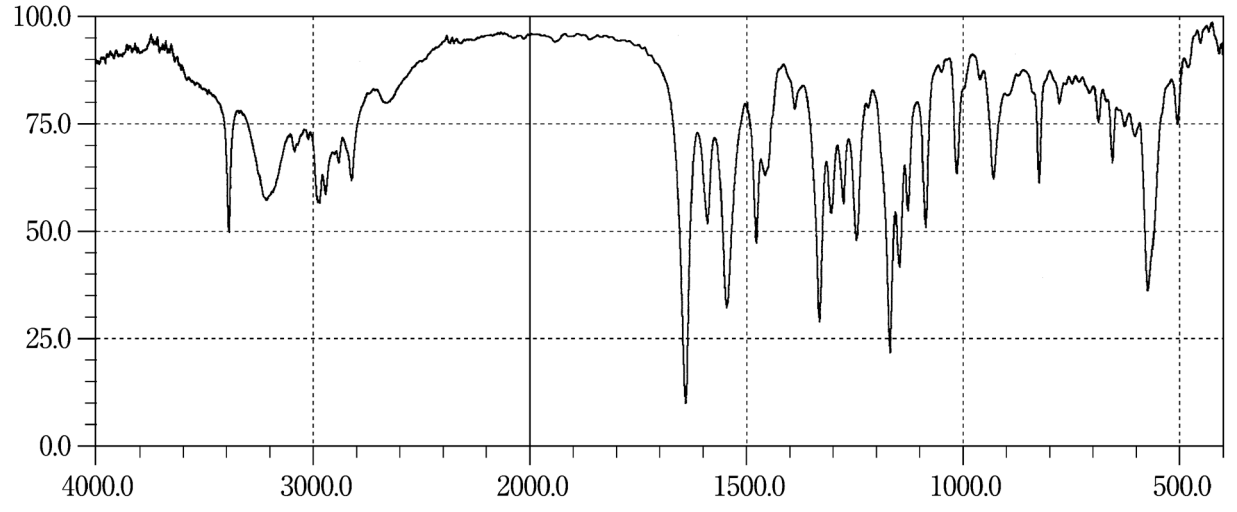
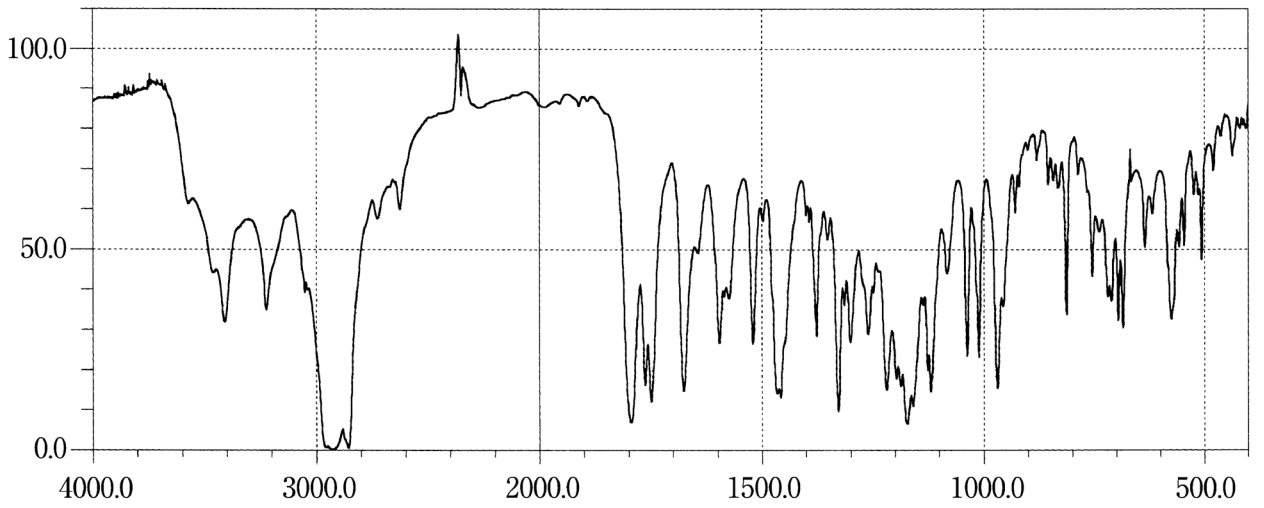


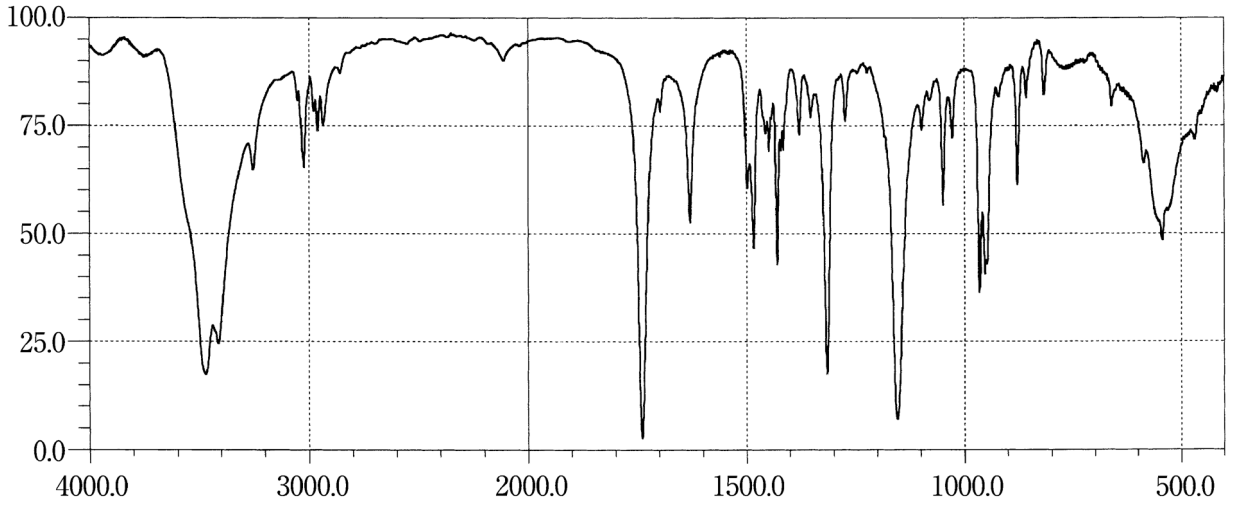
Sulpiride



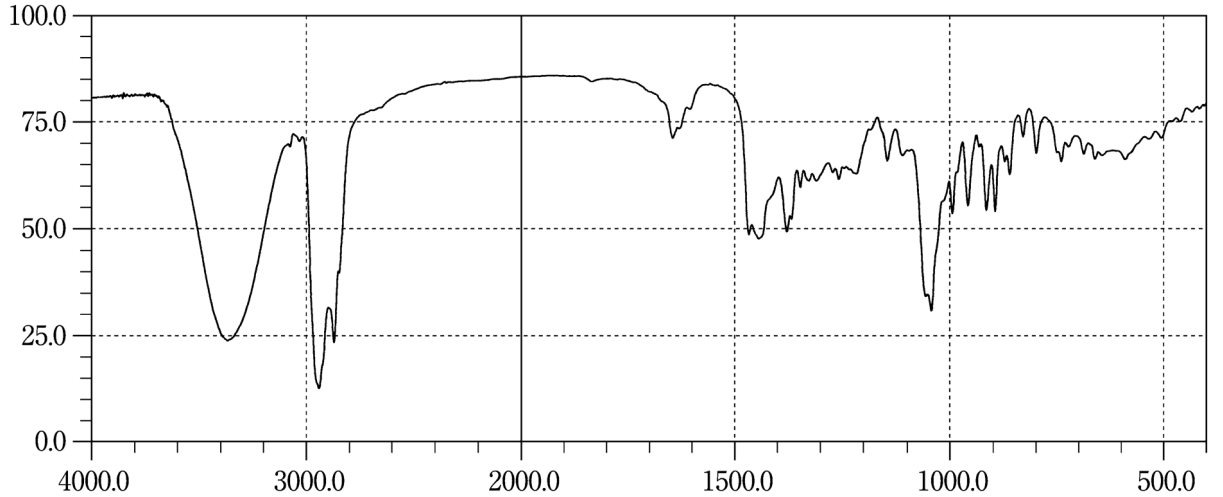
Sultamicillin Tosilate Hydrate



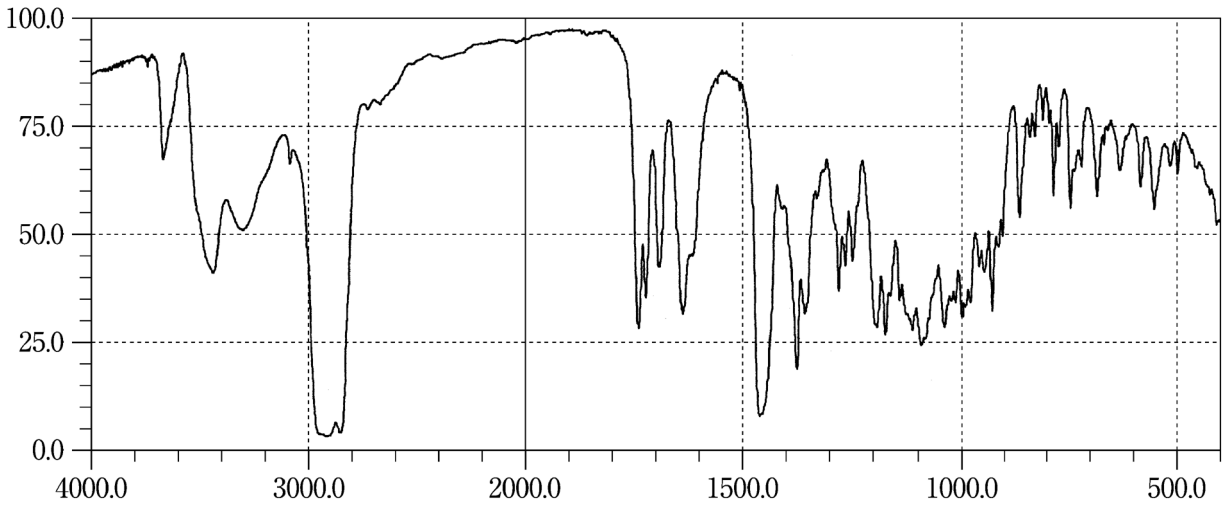
Suxamethonium Chloride Hydrate



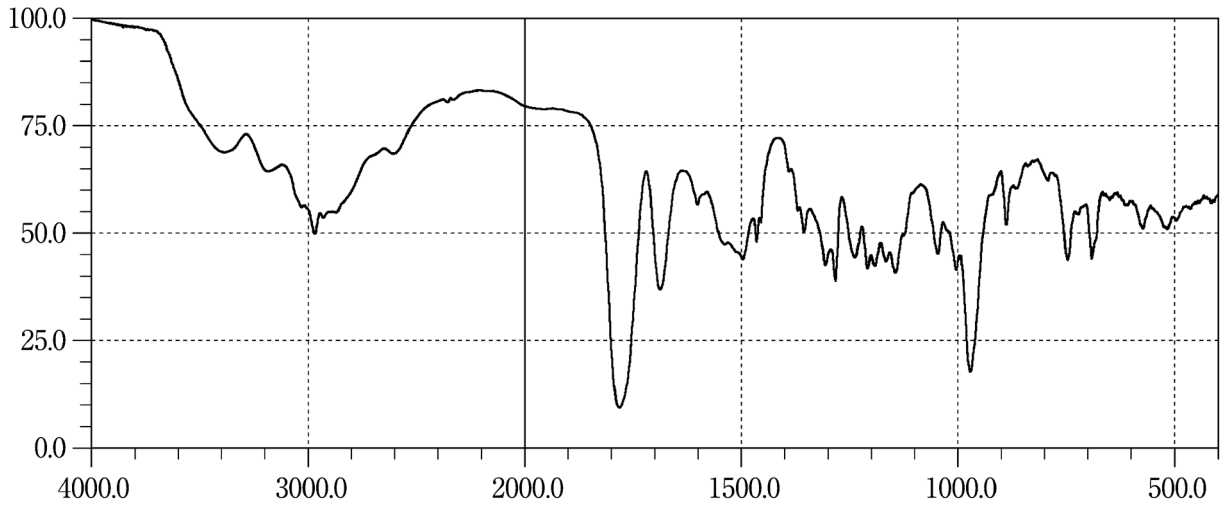
Tacalcitol Hydrate



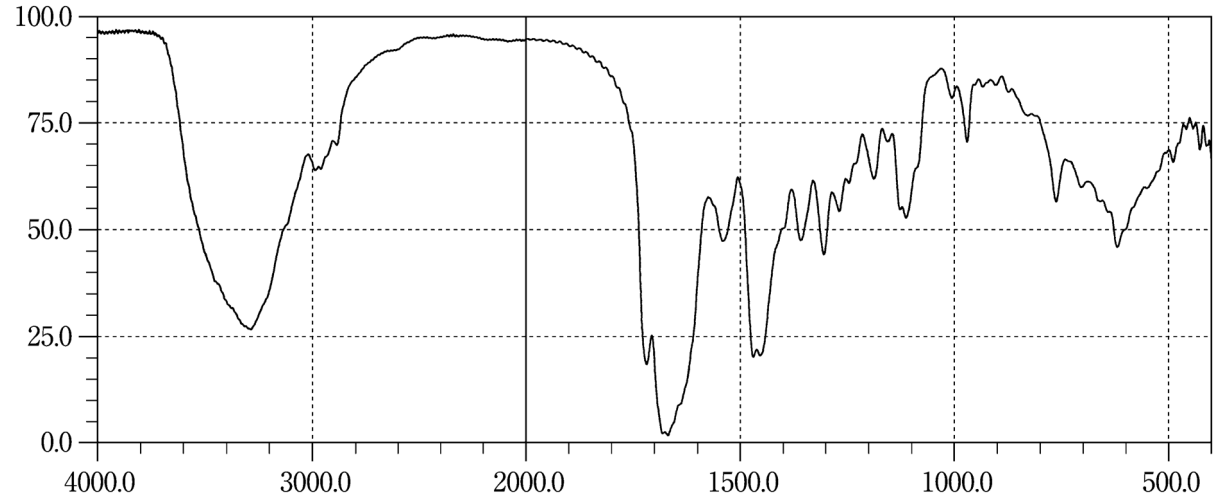
Tacrolimus Hydrate



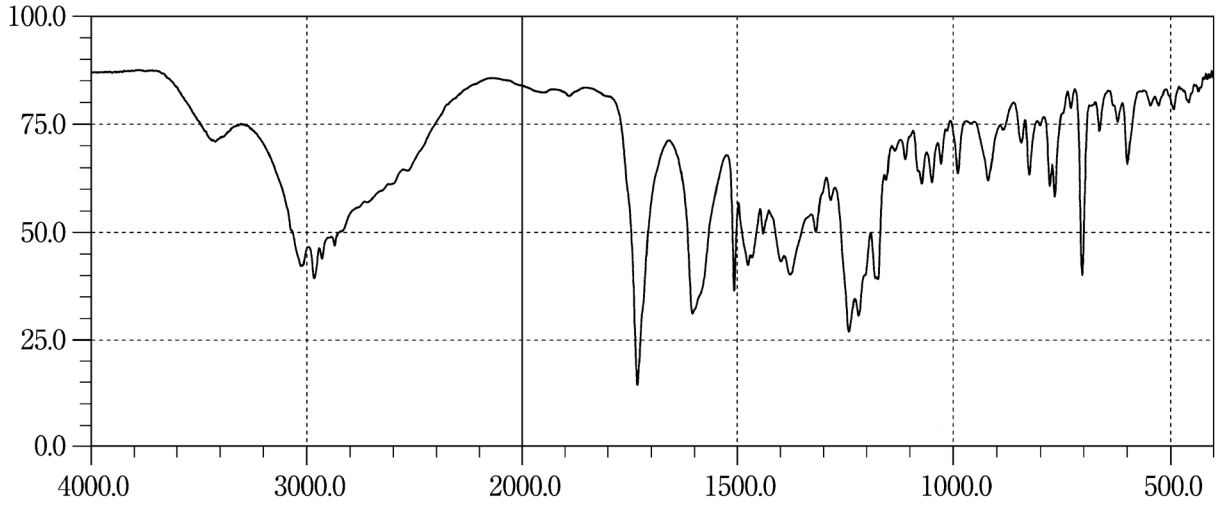
Talampicillin Hydrochloride



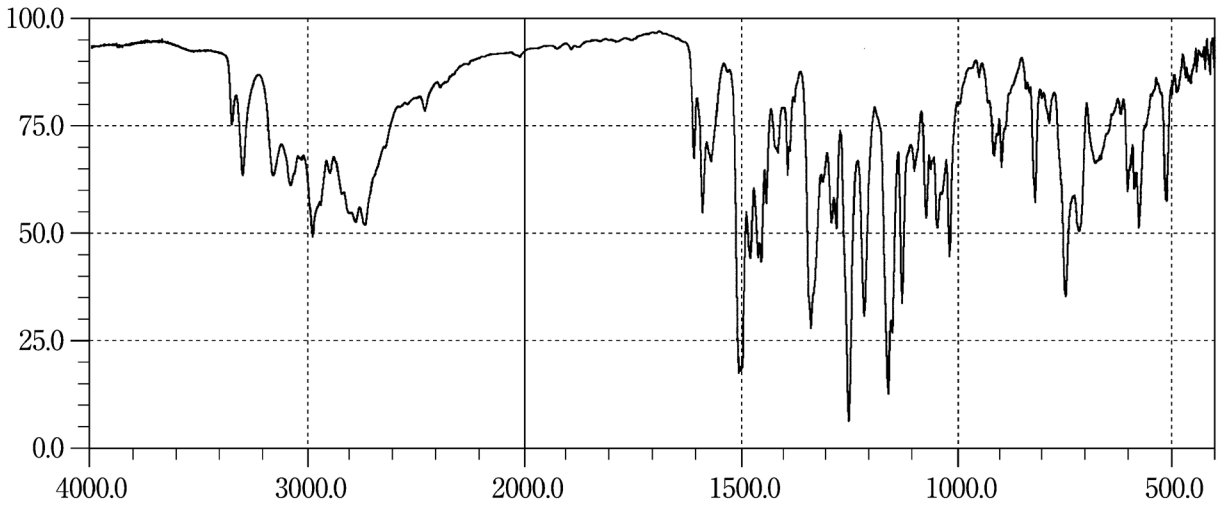
Taltirelin Hydrate



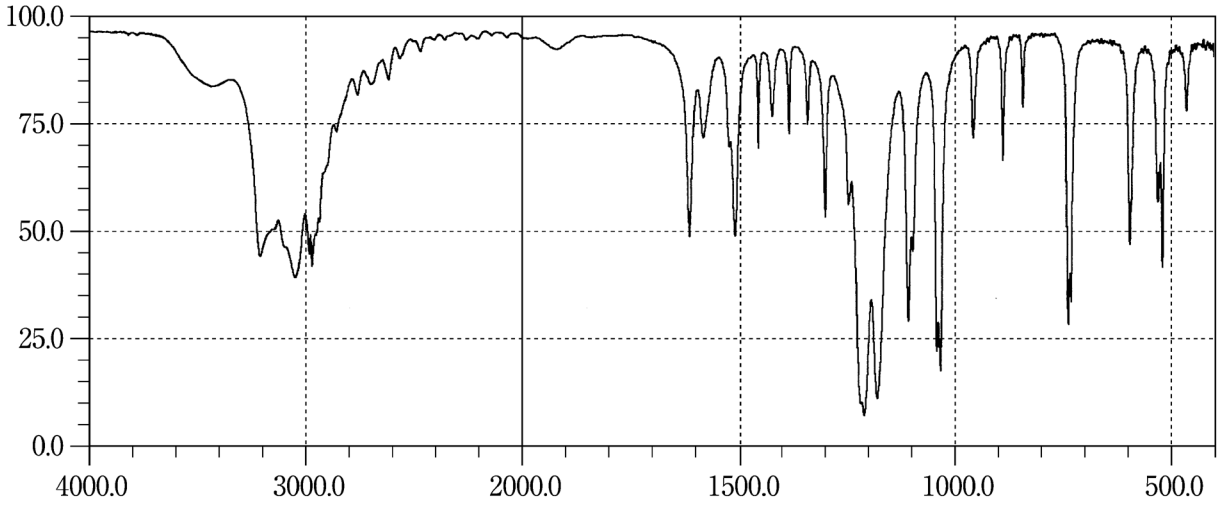
Tamoxifen Citrate



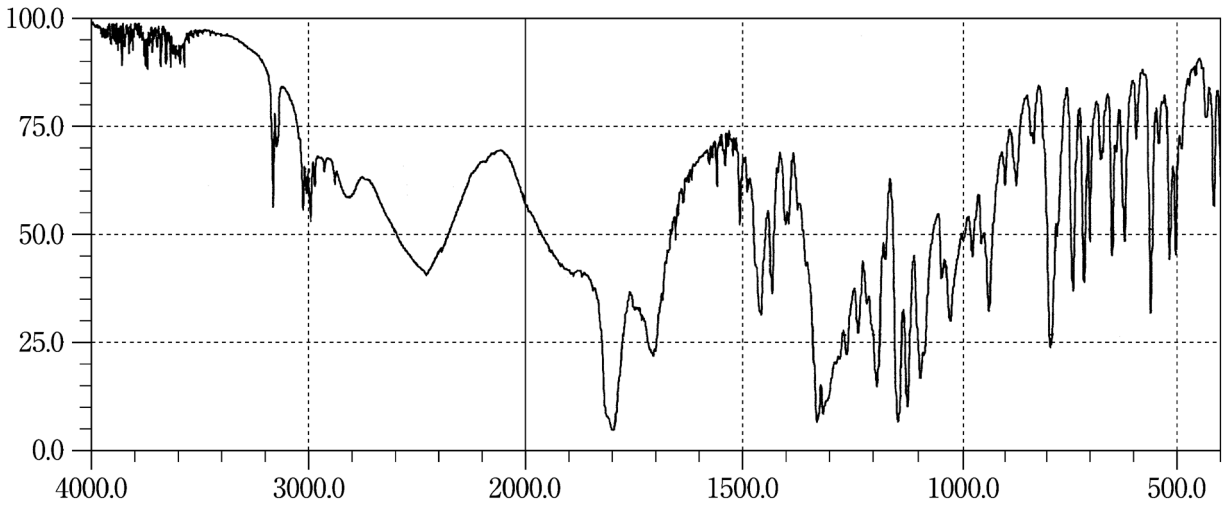
Tamsulosin Hydrochloride



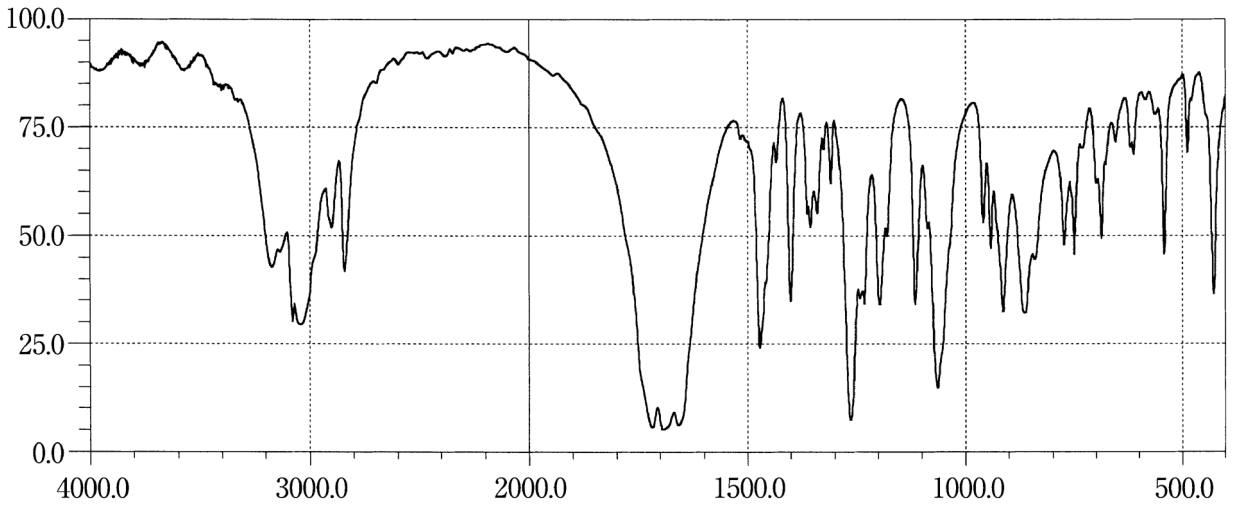
Taurine



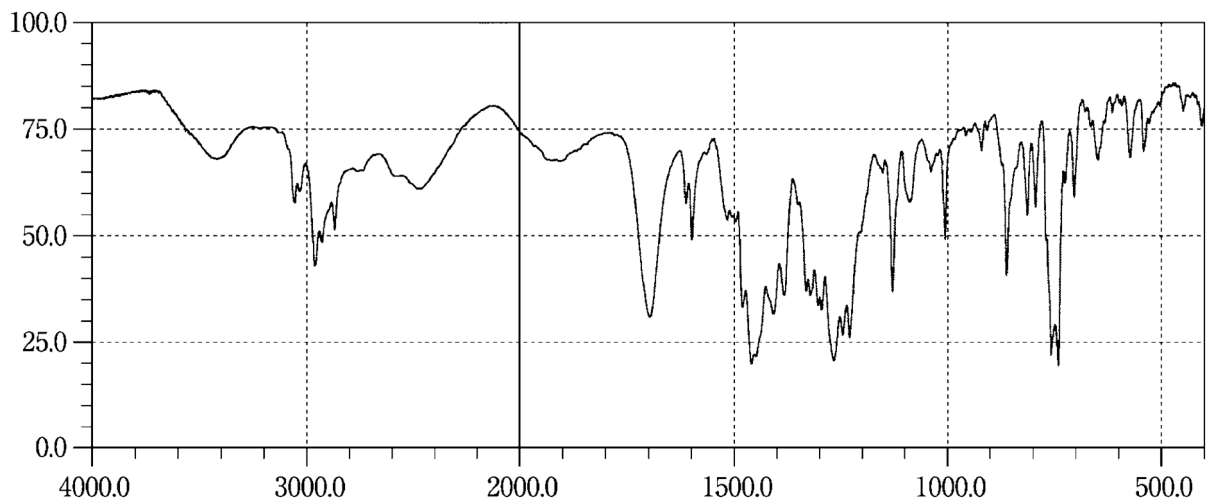
Tazobactam



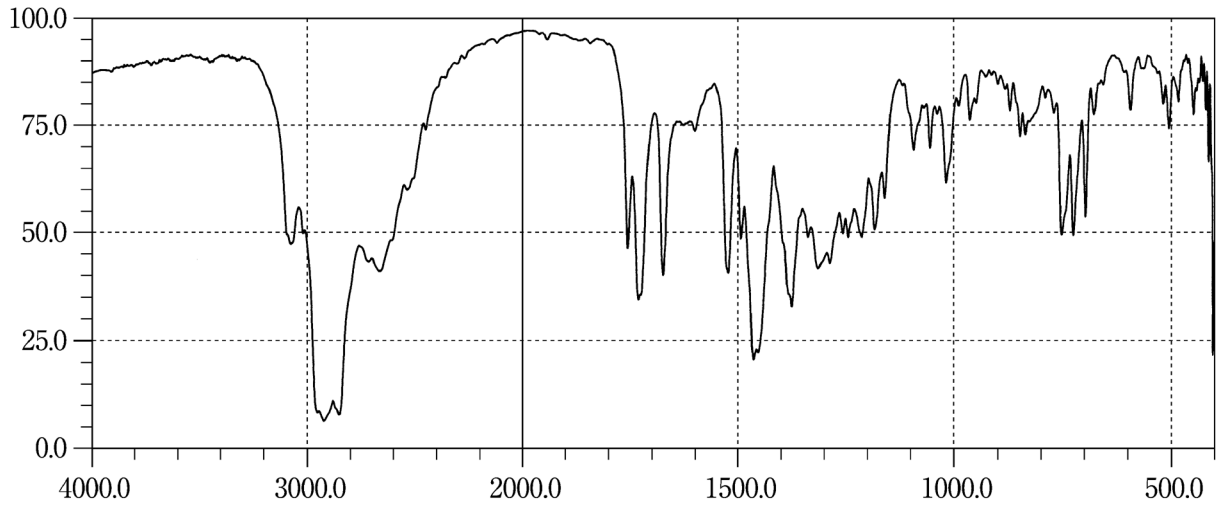
Tegafur



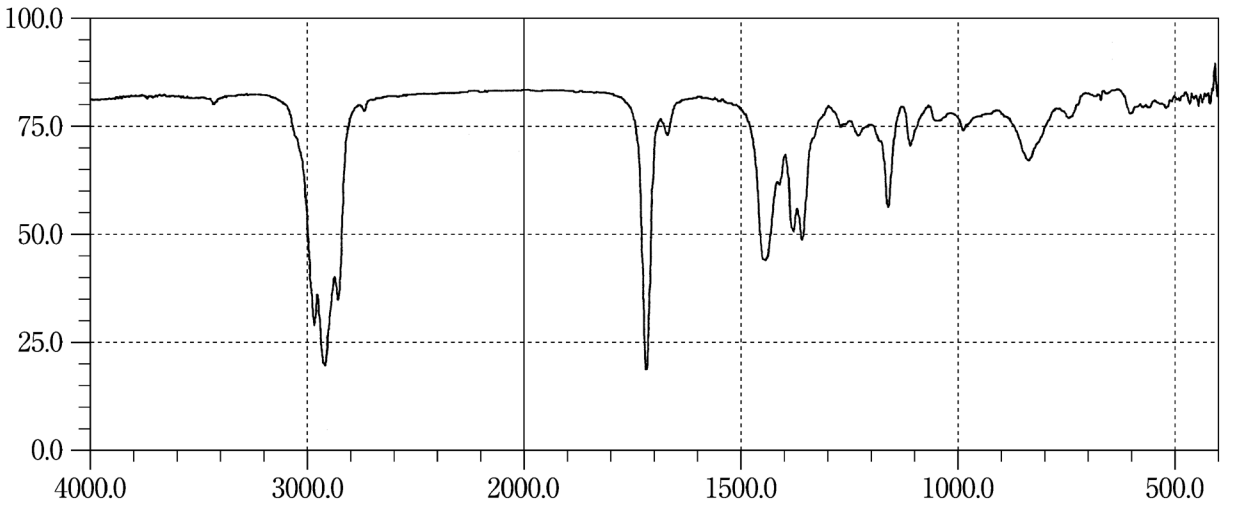
Telmisartan



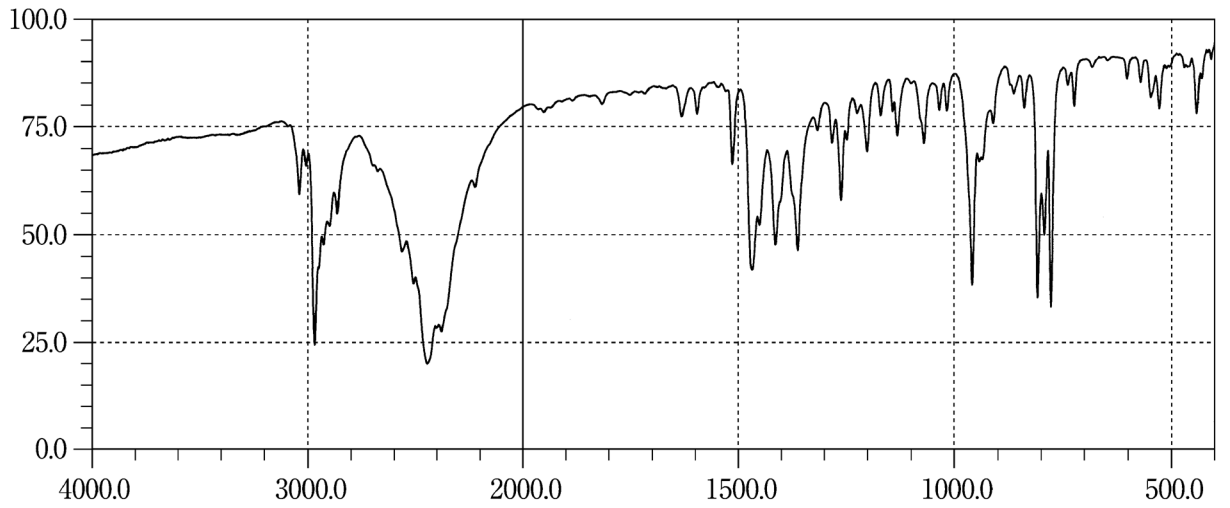
Temocapril Hydrochloride



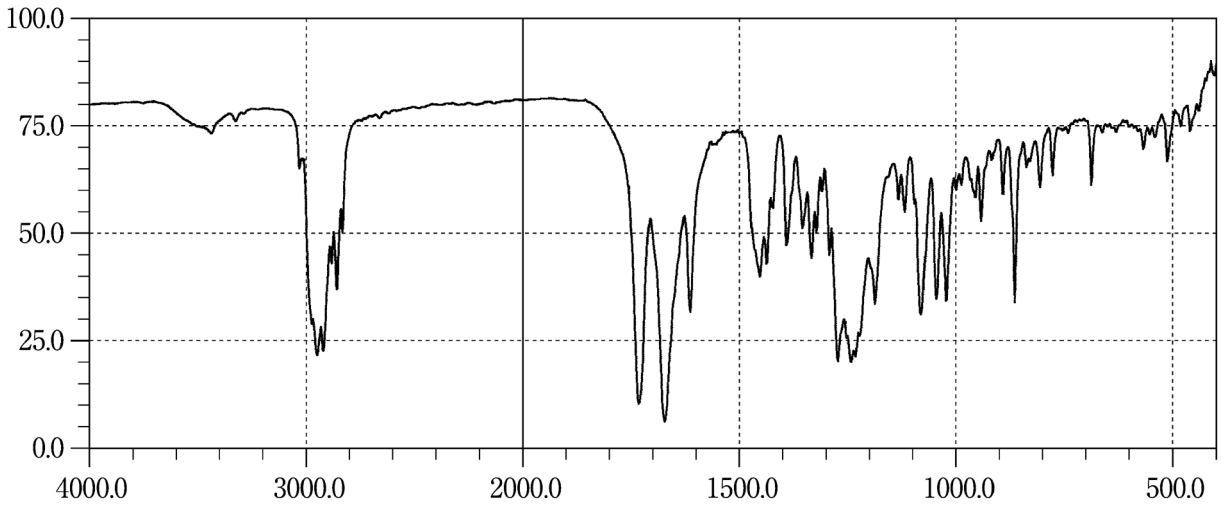
Teprenone



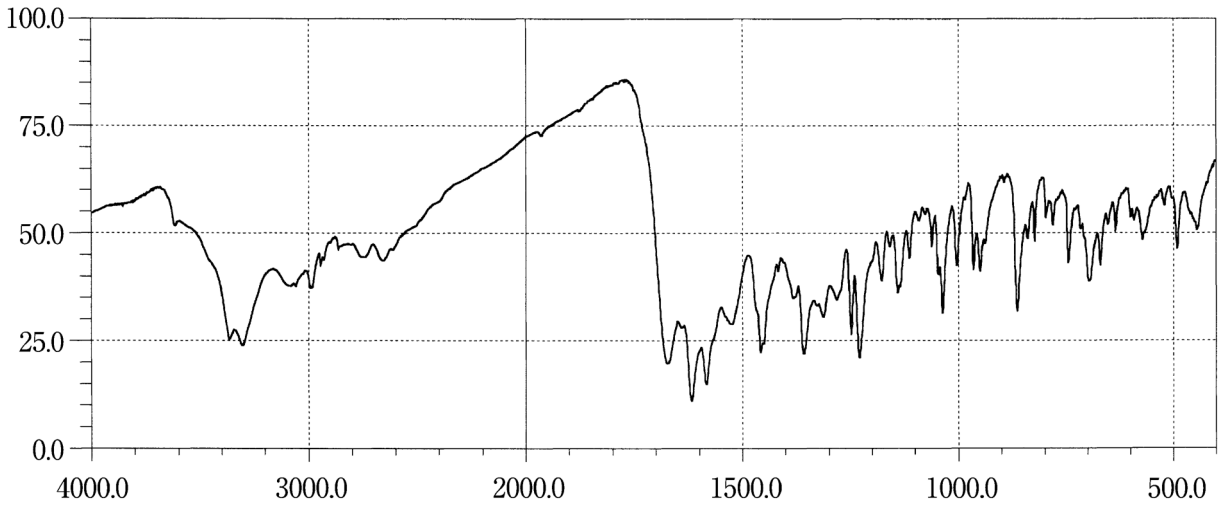
Terbinafine Hydrochloride



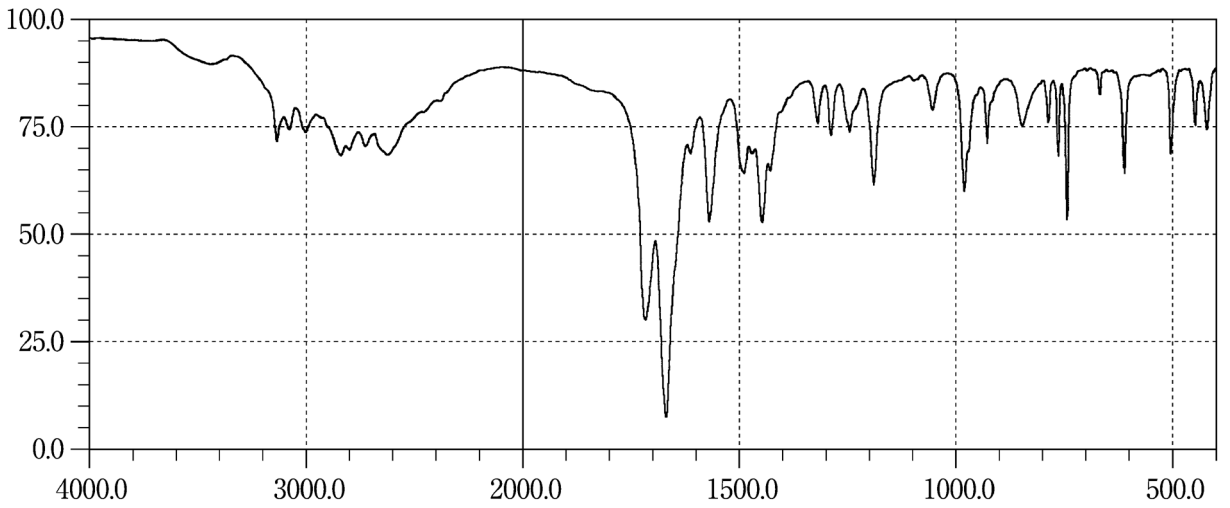
Testosterone Propionate



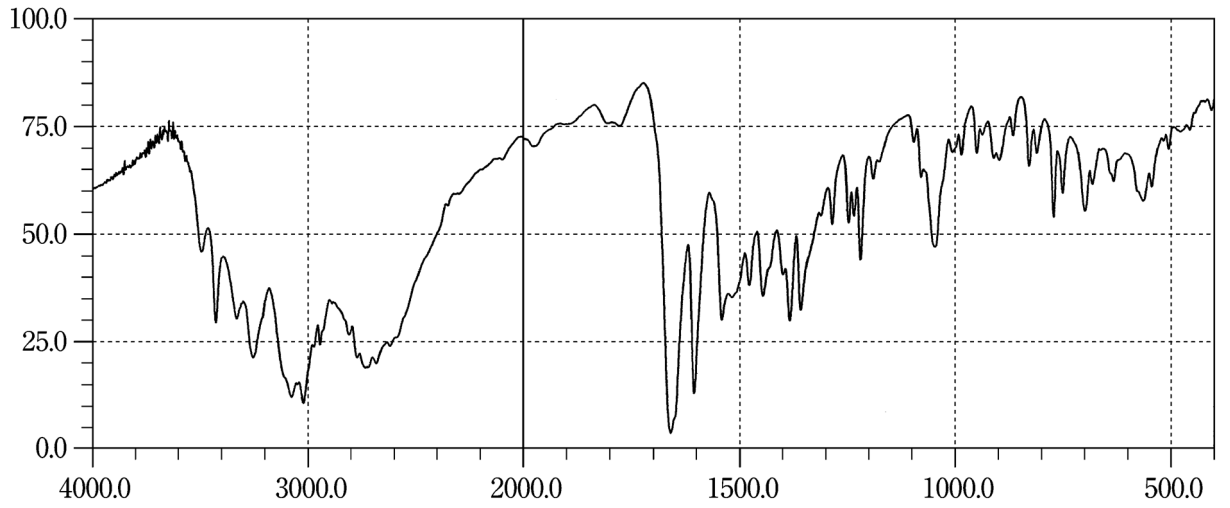
Tetracycline Hydrochloride



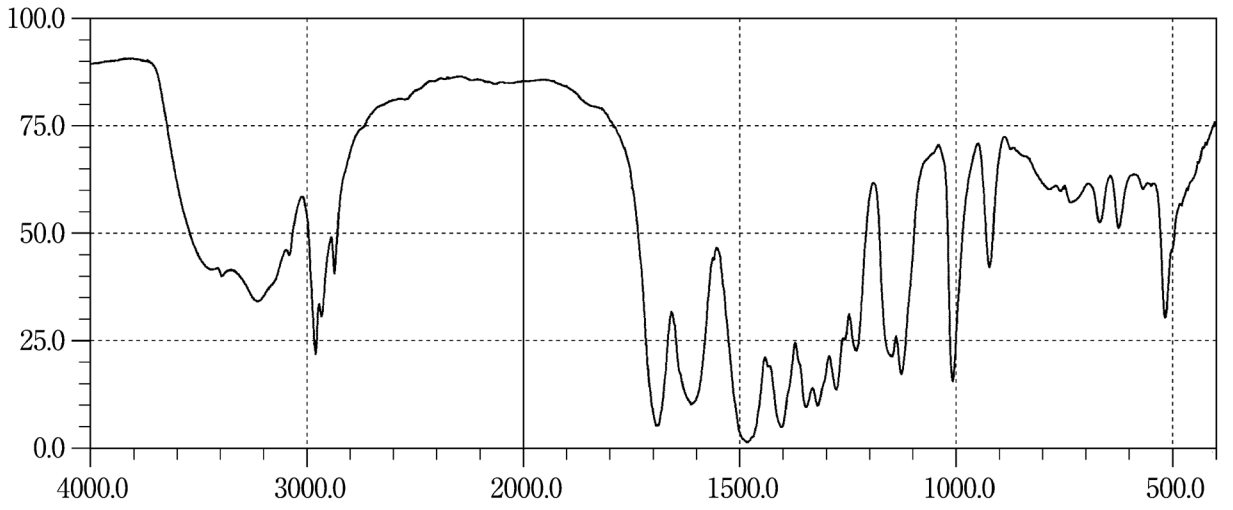
Theophylline



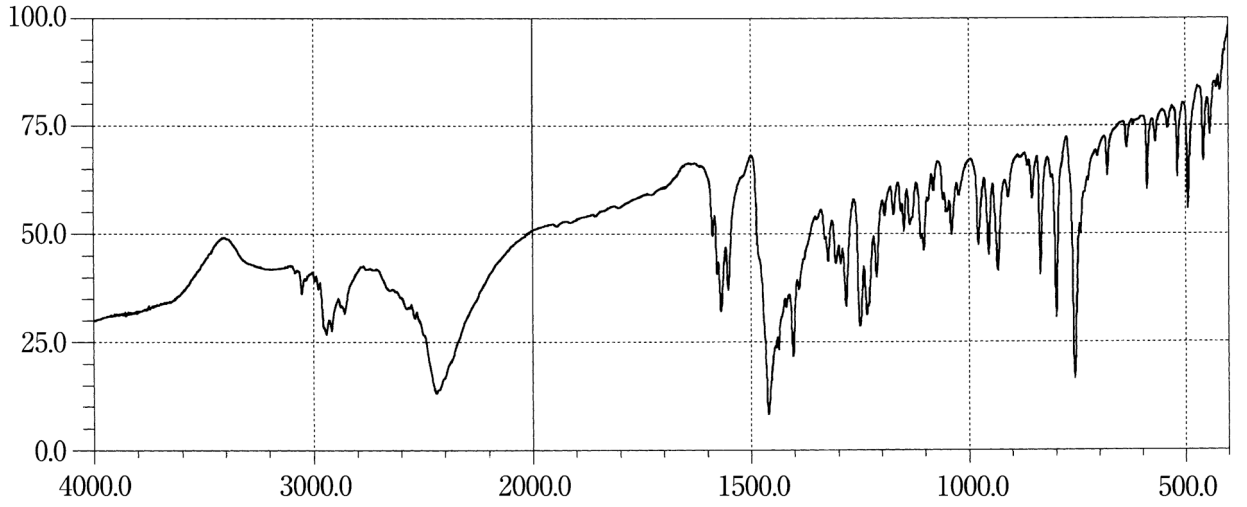
Thiamine Chloride Hydrochloride



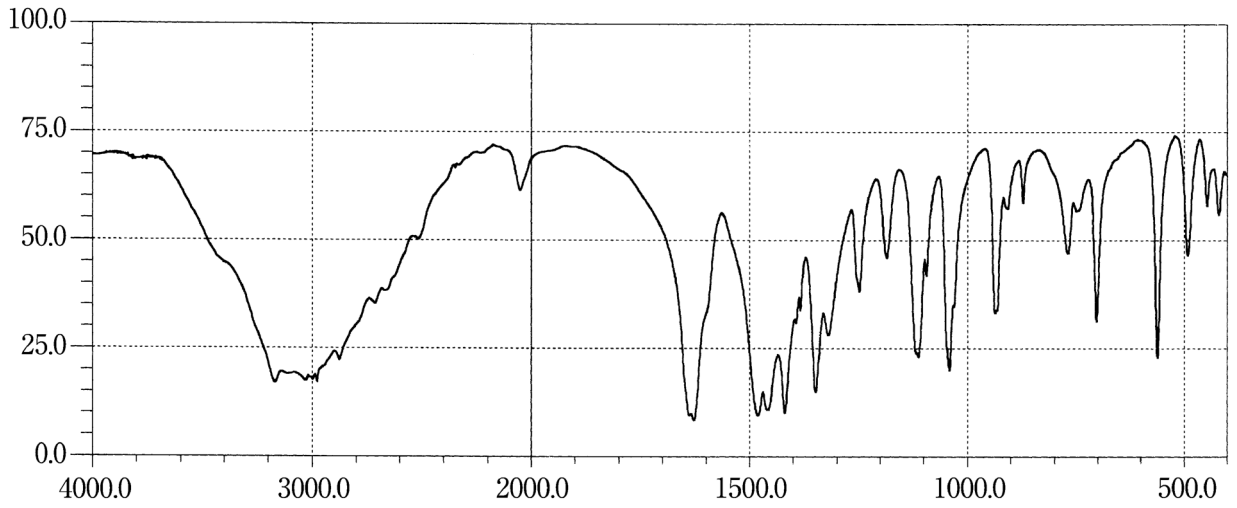
Thiamylal Sodium



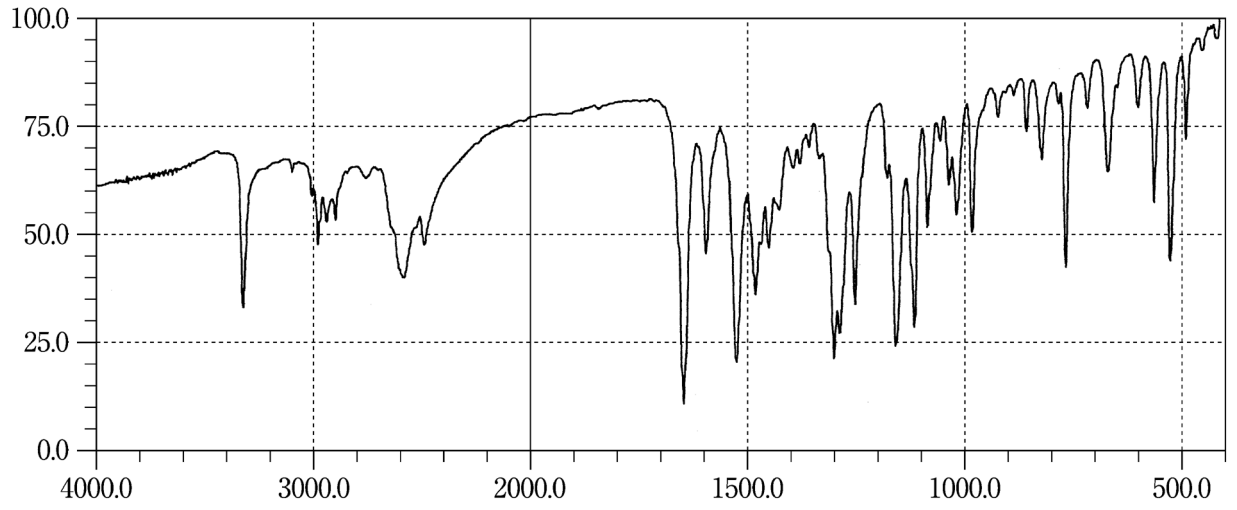
Thioridazine Hydrochloride



L-Threonine



Tiapride Hydrochloride



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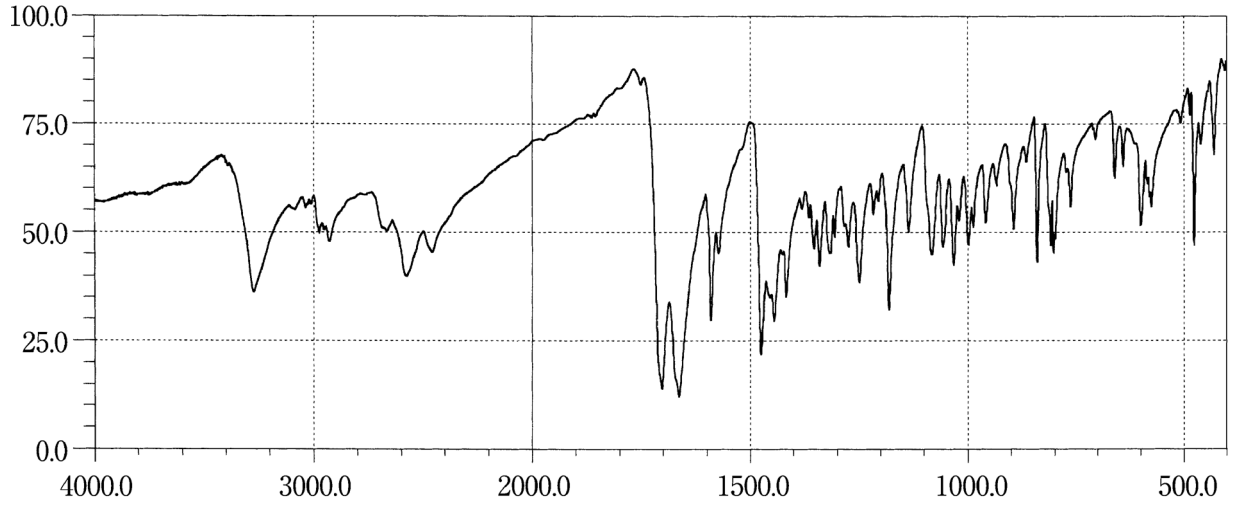
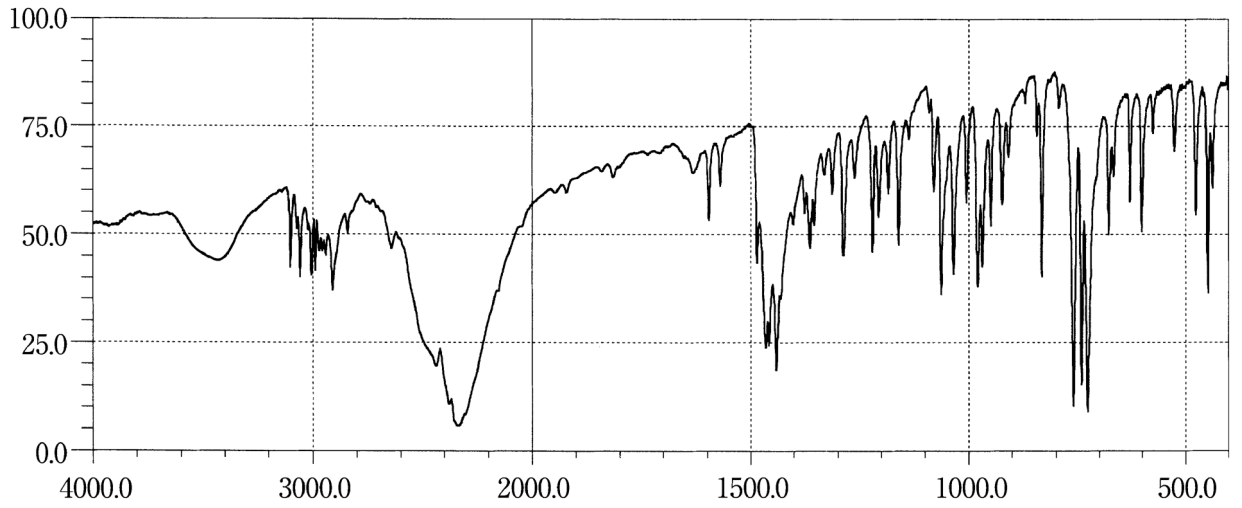
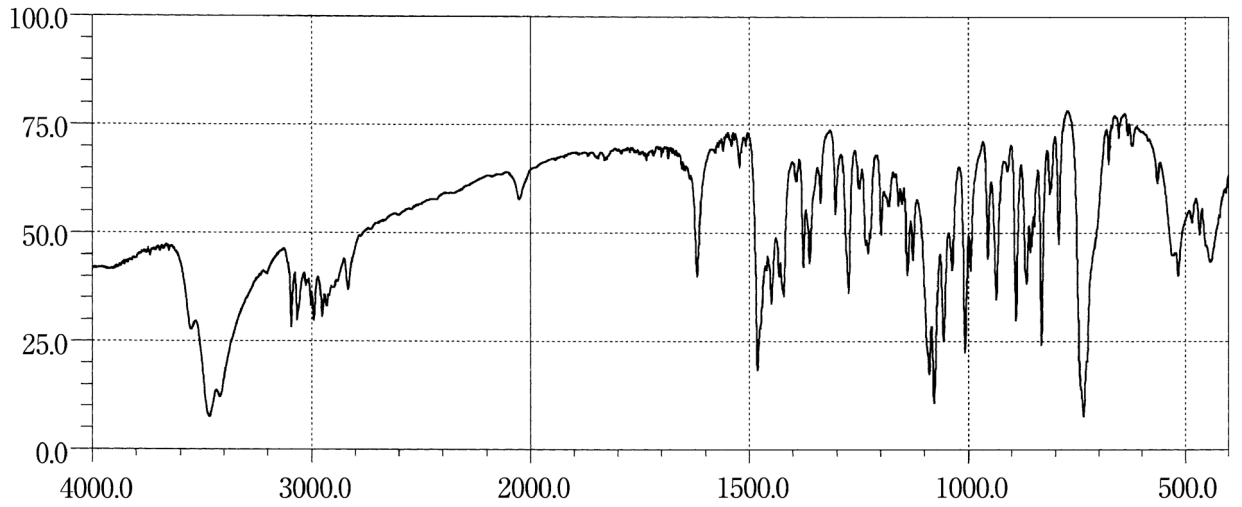


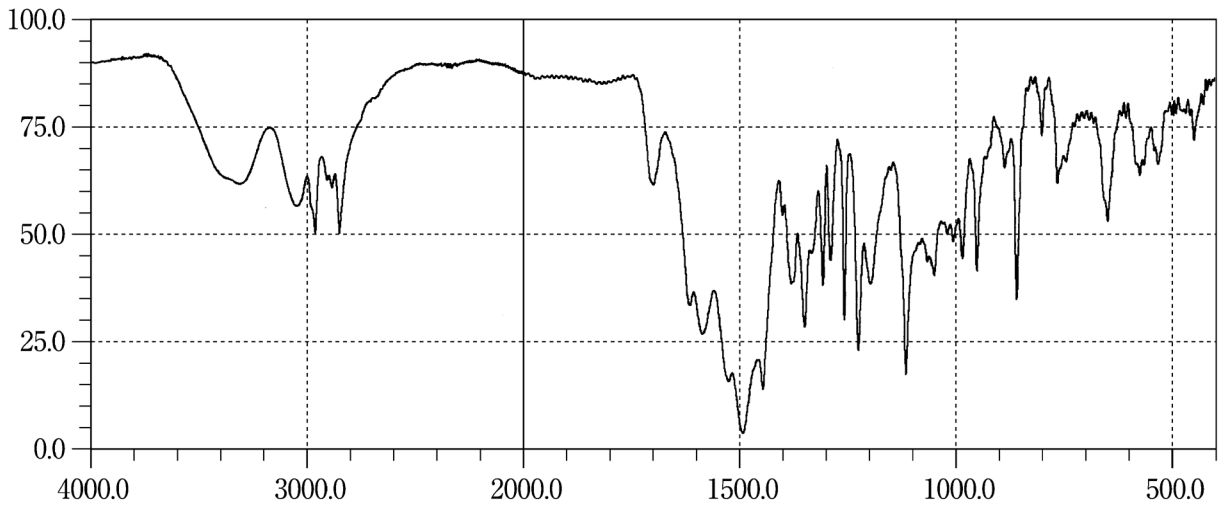
Ticlopidine Hydrochloride



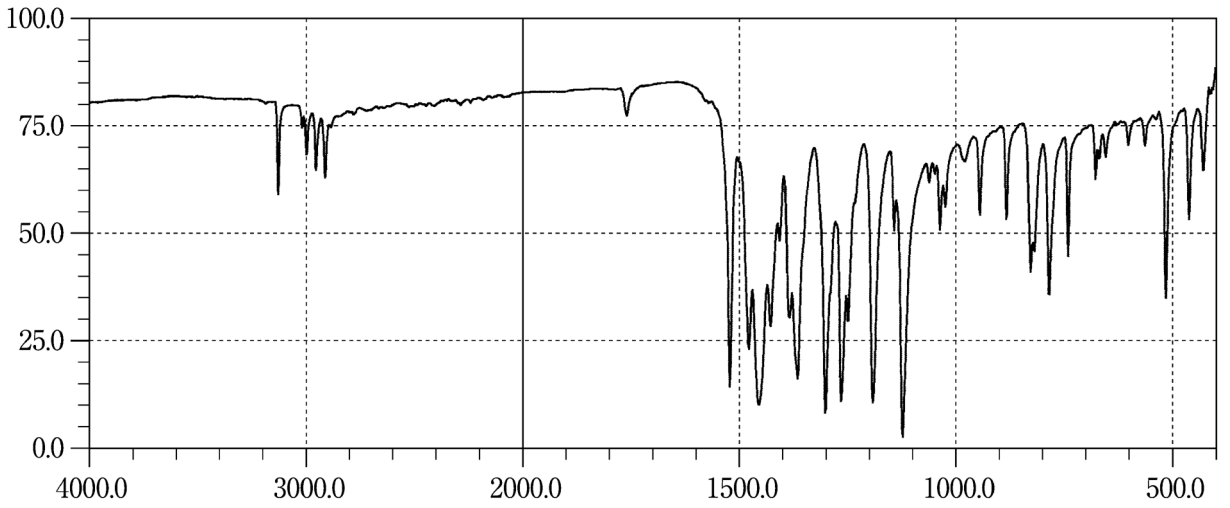
Timepidium Bromide Hydrate



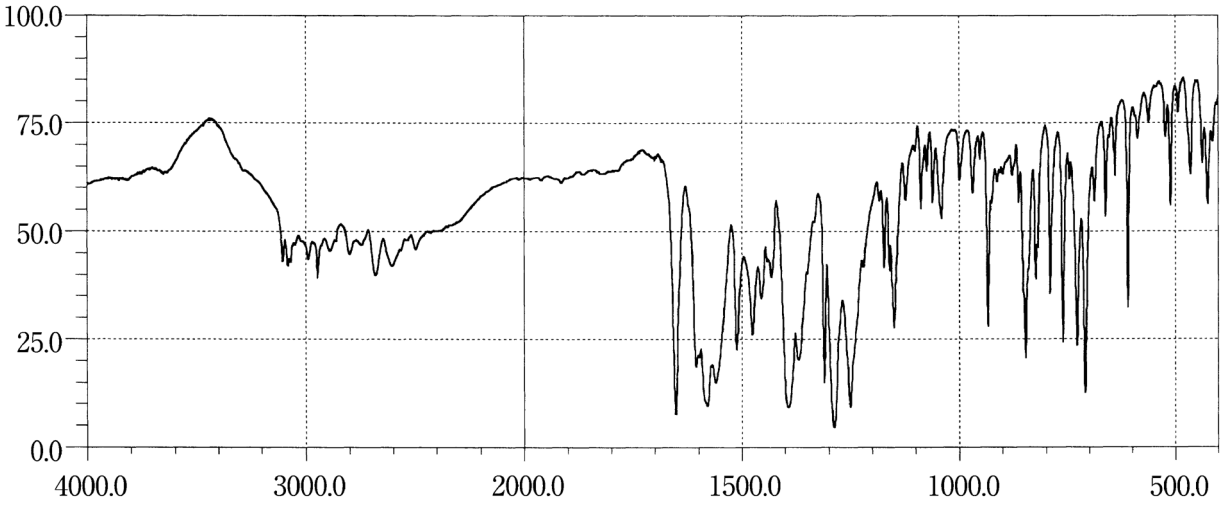
Timolol Maleate



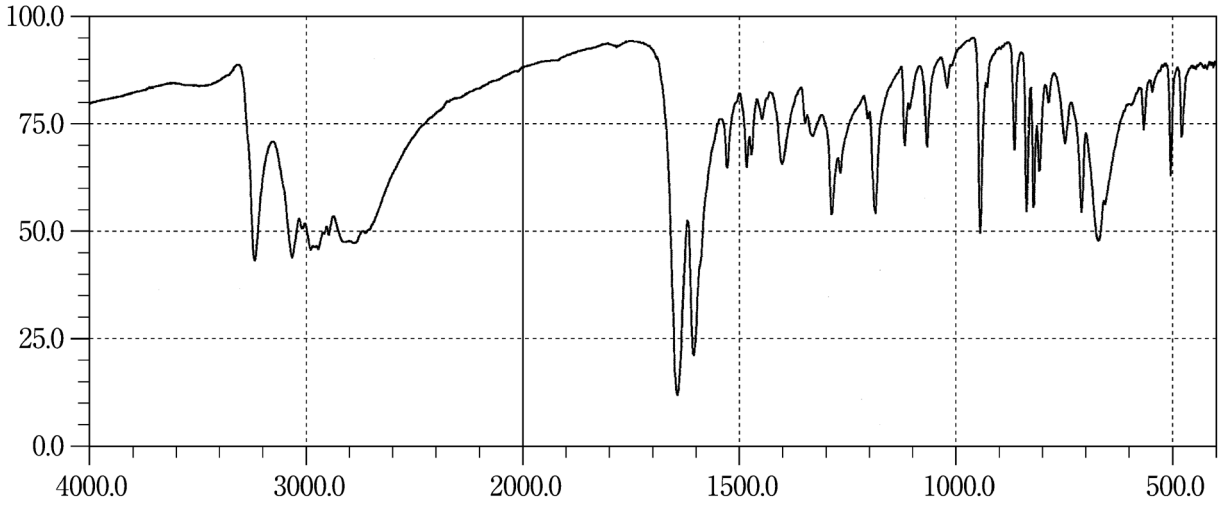
Tinidazole



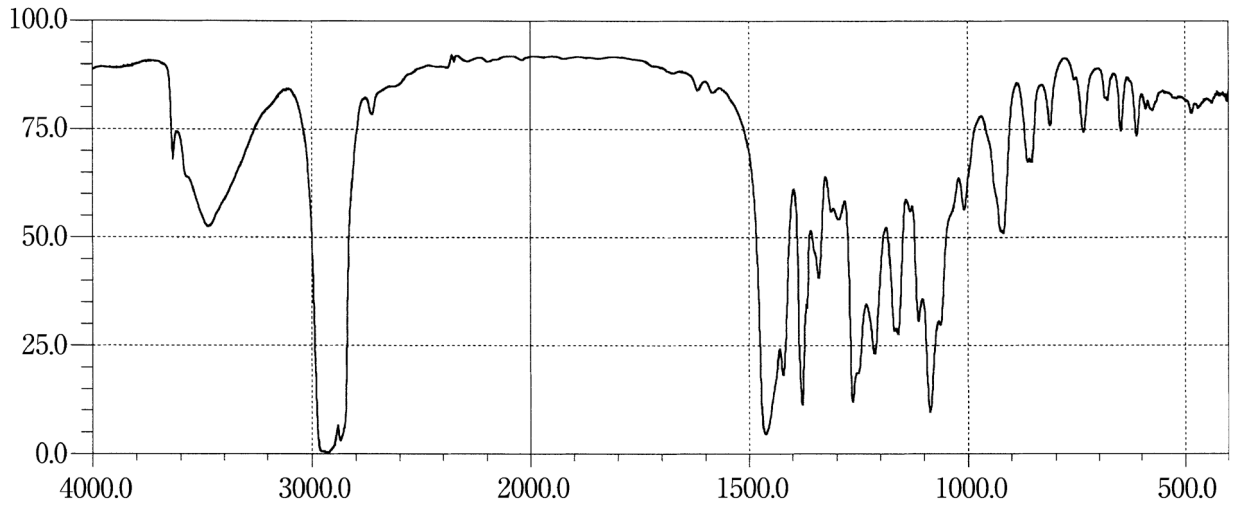
Tipepidine Hibenzate



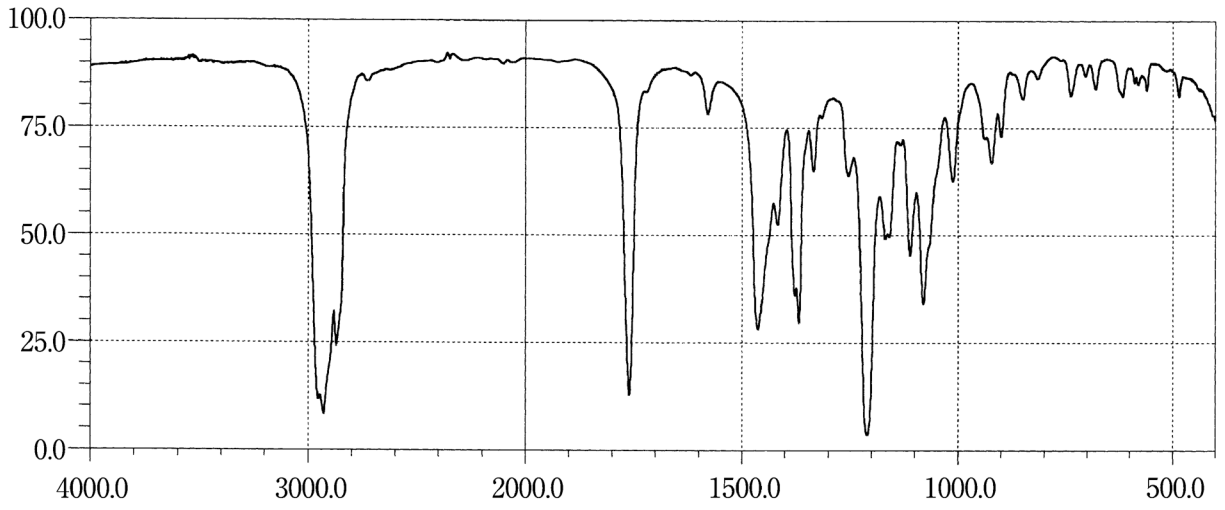
Tizanidine Hydrochloride



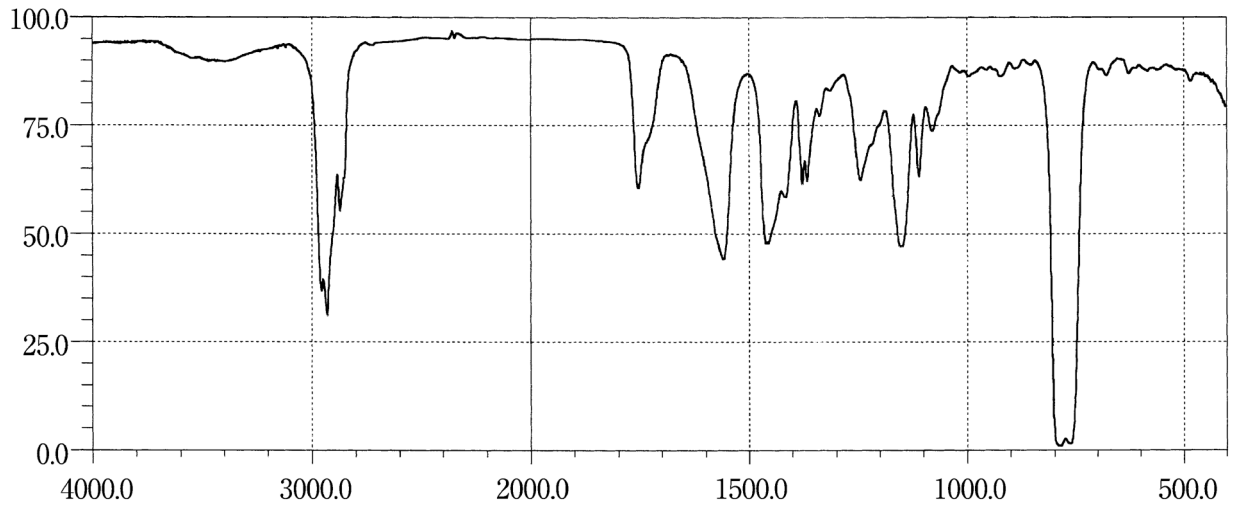
Tocopherol



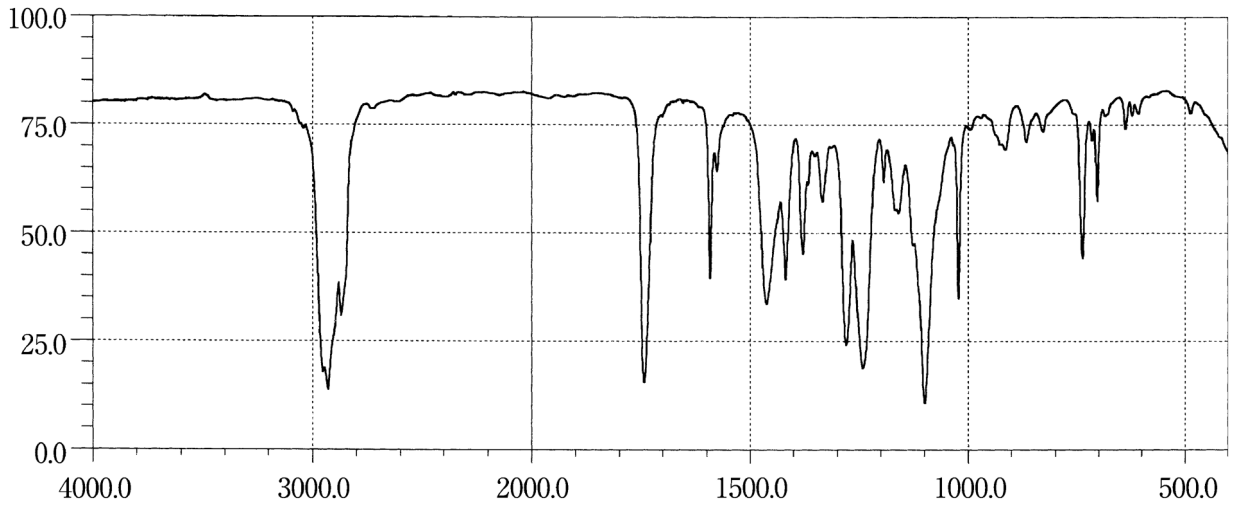
Tocopherol Acetate



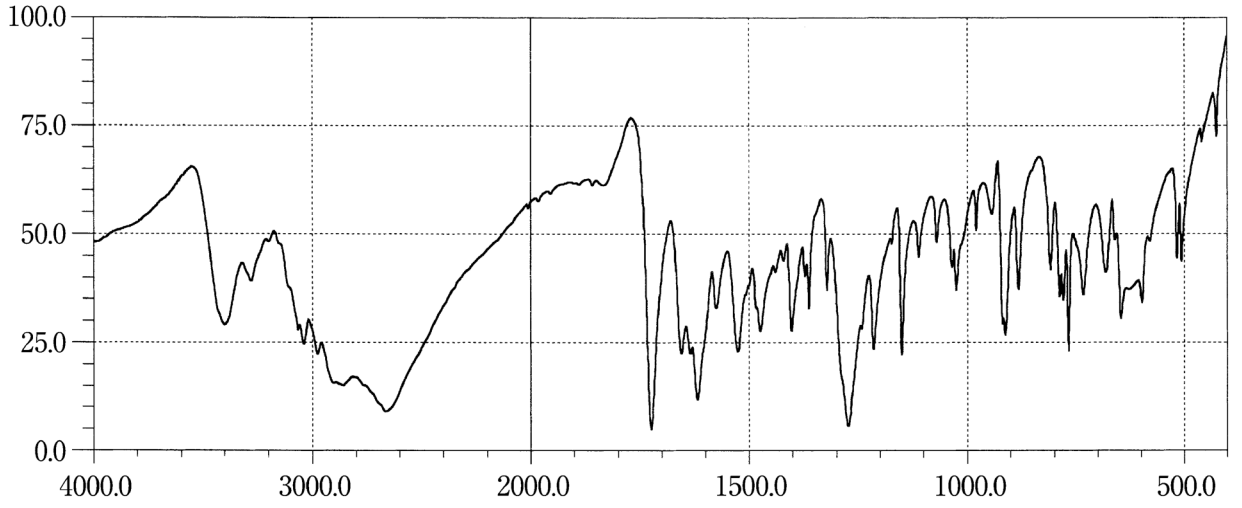
Tocopherol Calcium Succinate



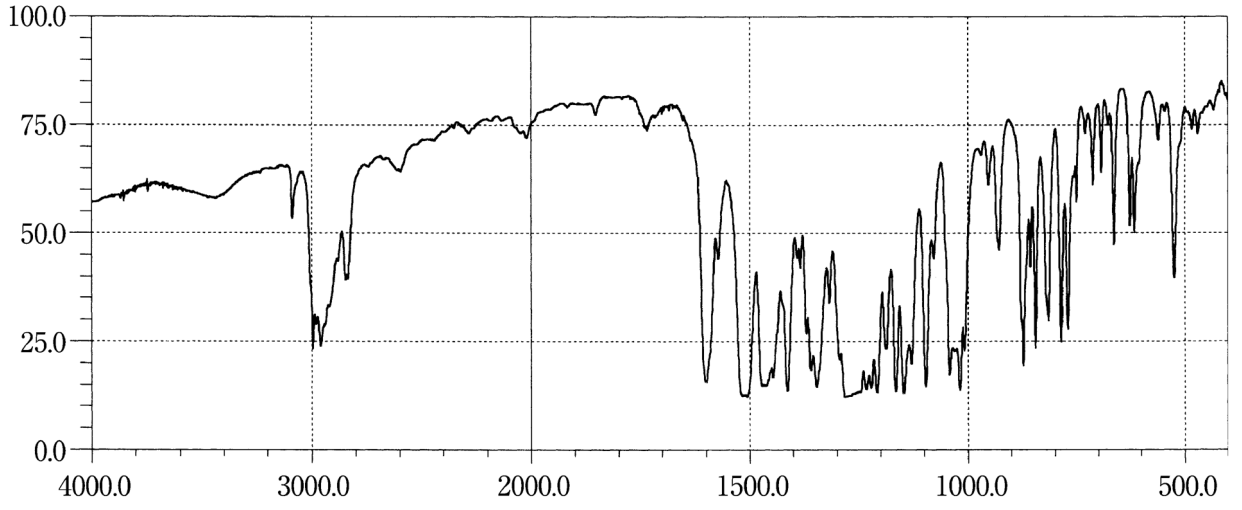
Tocopherol Nicotinate



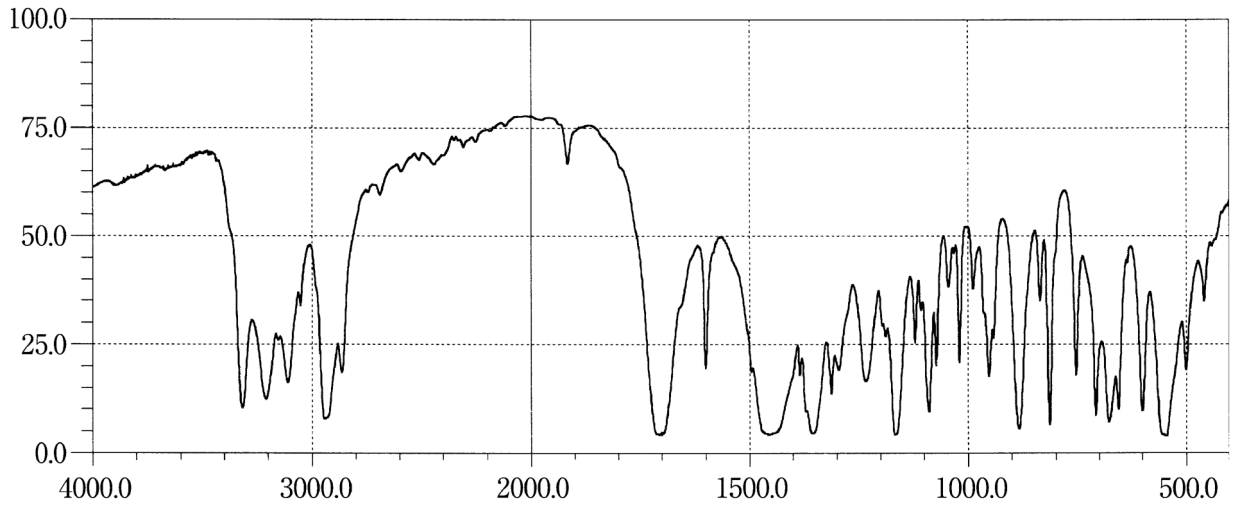
Todralazine Hydrochloride Hydrate



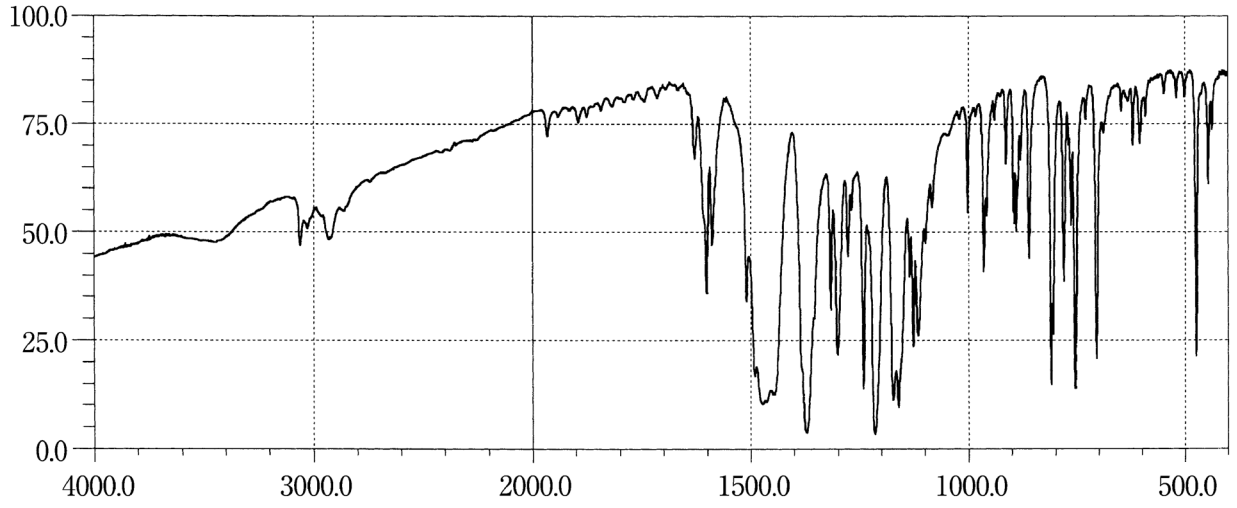
Tofisopam



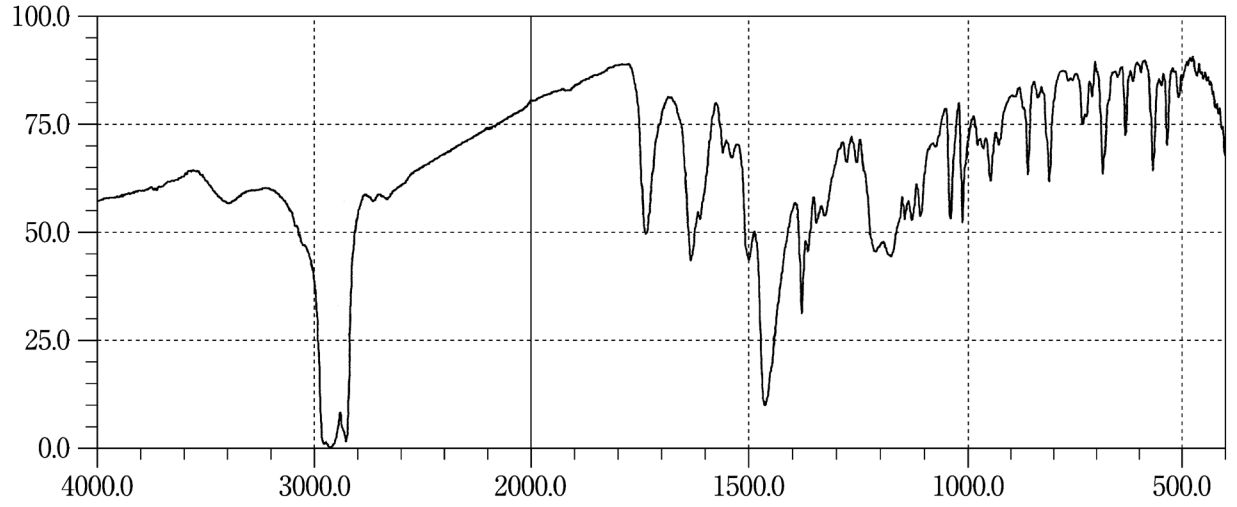
Tolazamide



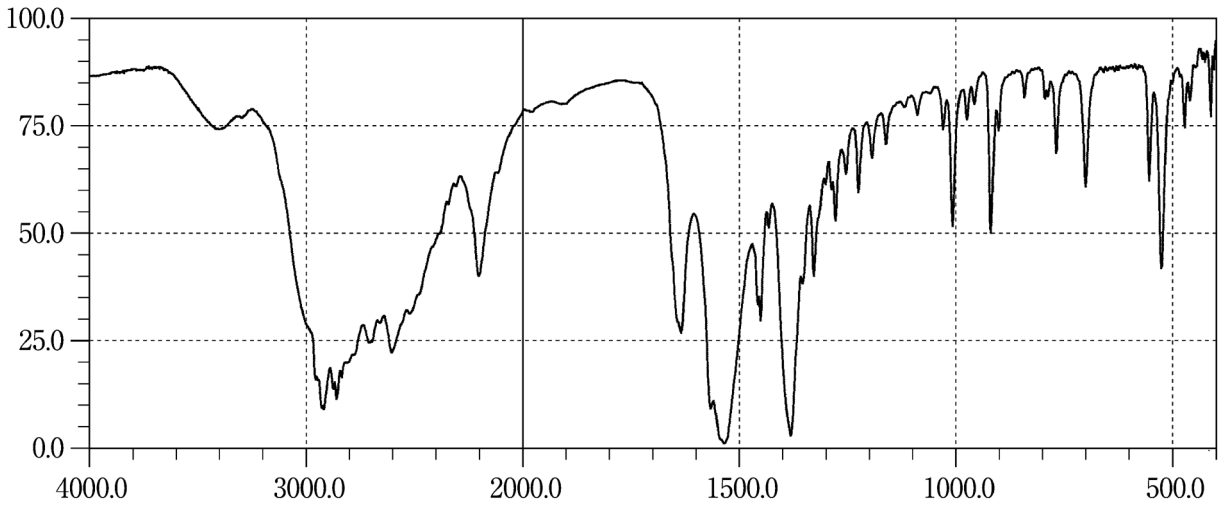
Tolnaftate



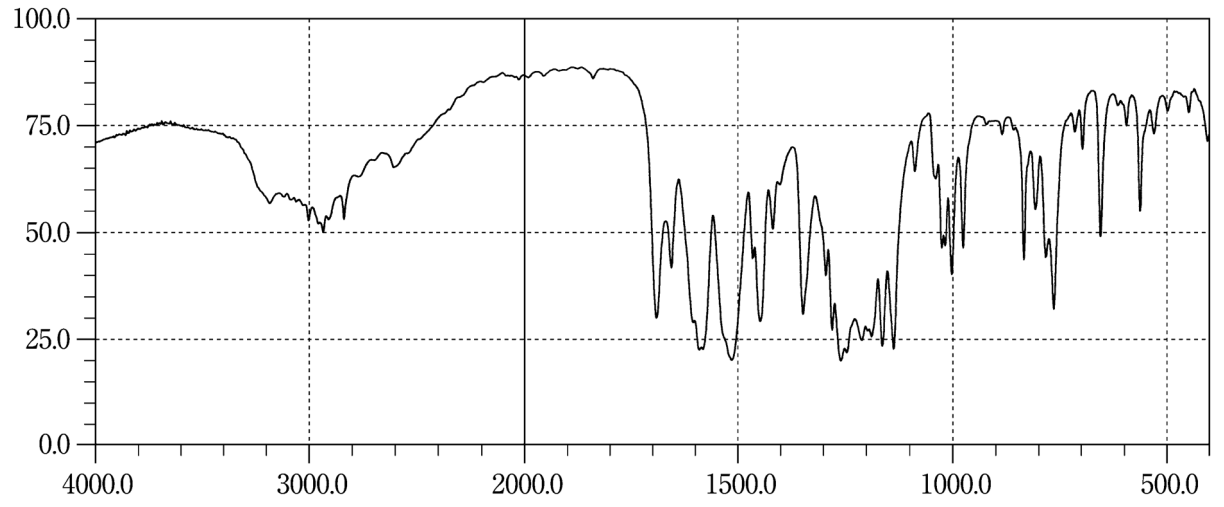
Tosufloxacin Tosilate Hydrate



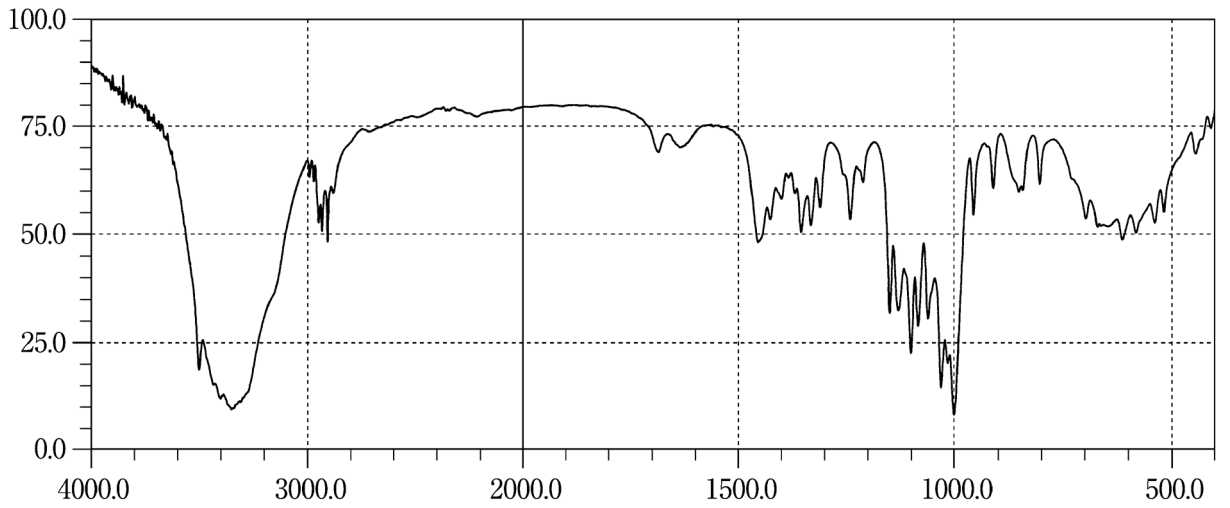
Tranexamic Acid



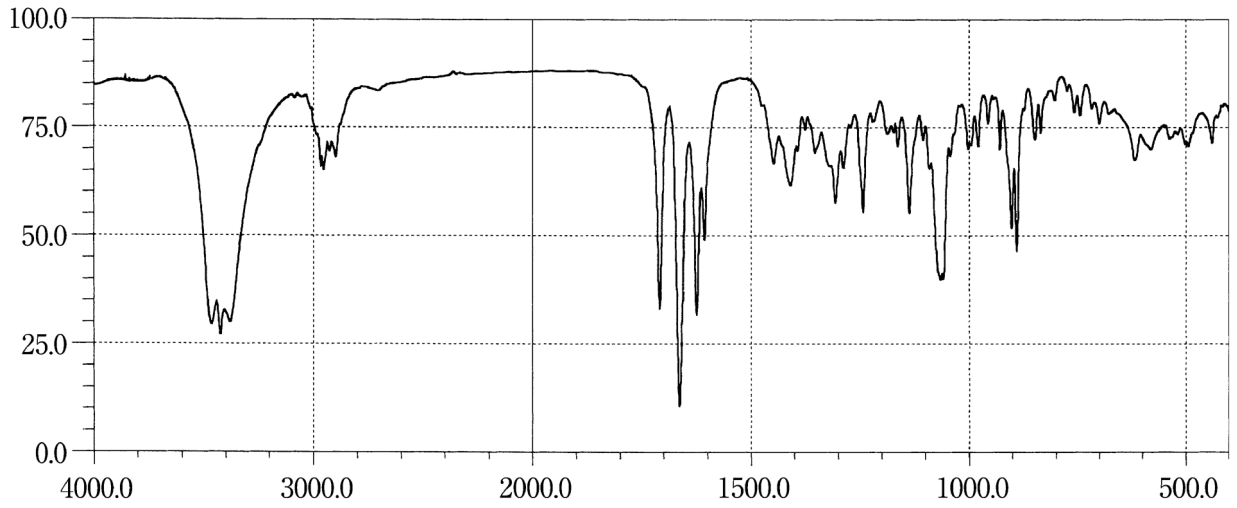
Tranilast



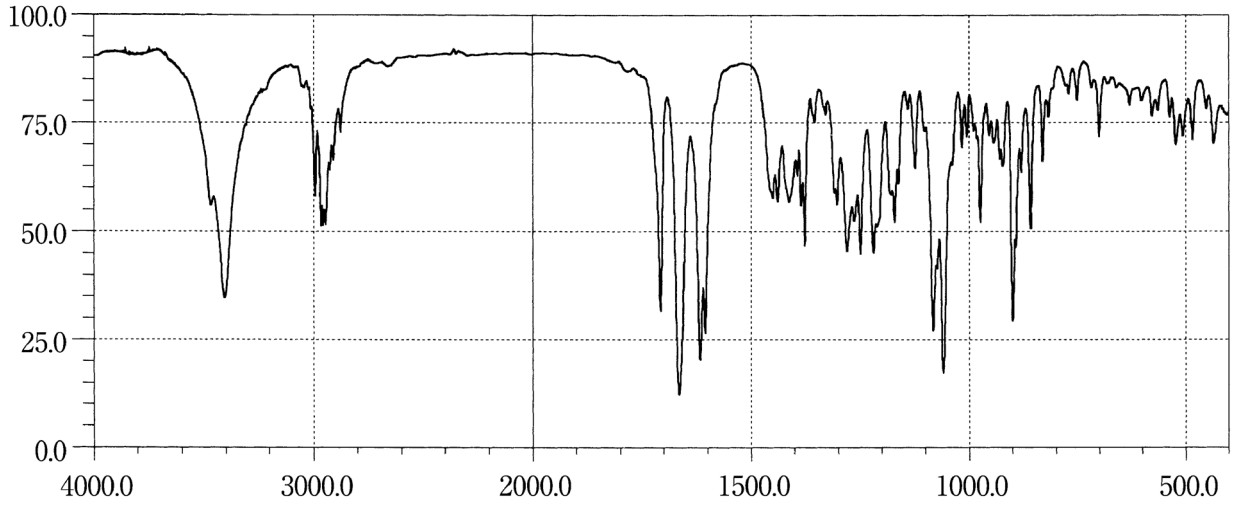
Trehalose Hydrate



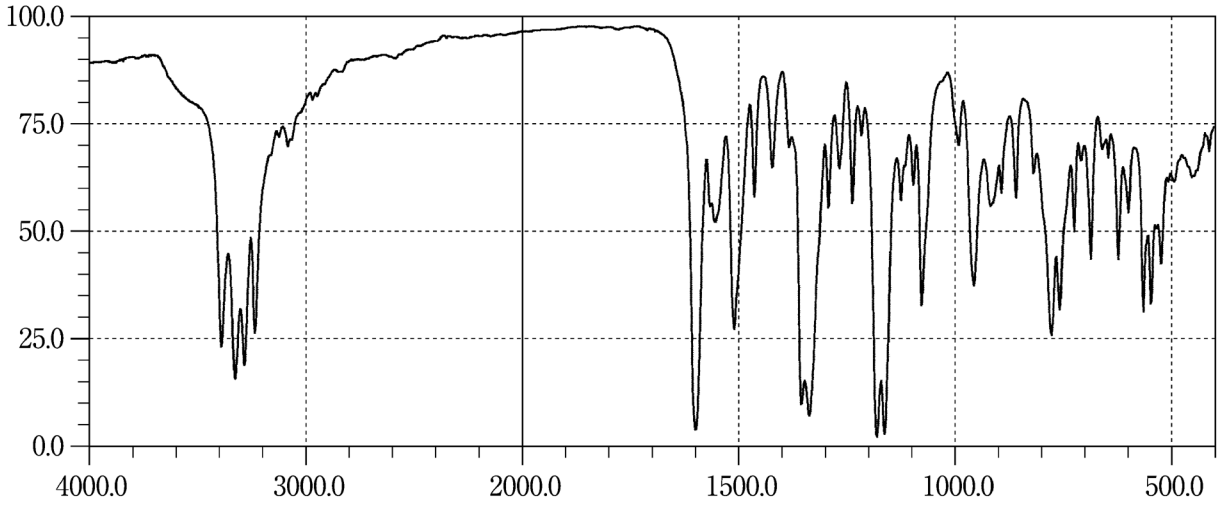
Triamcinolone



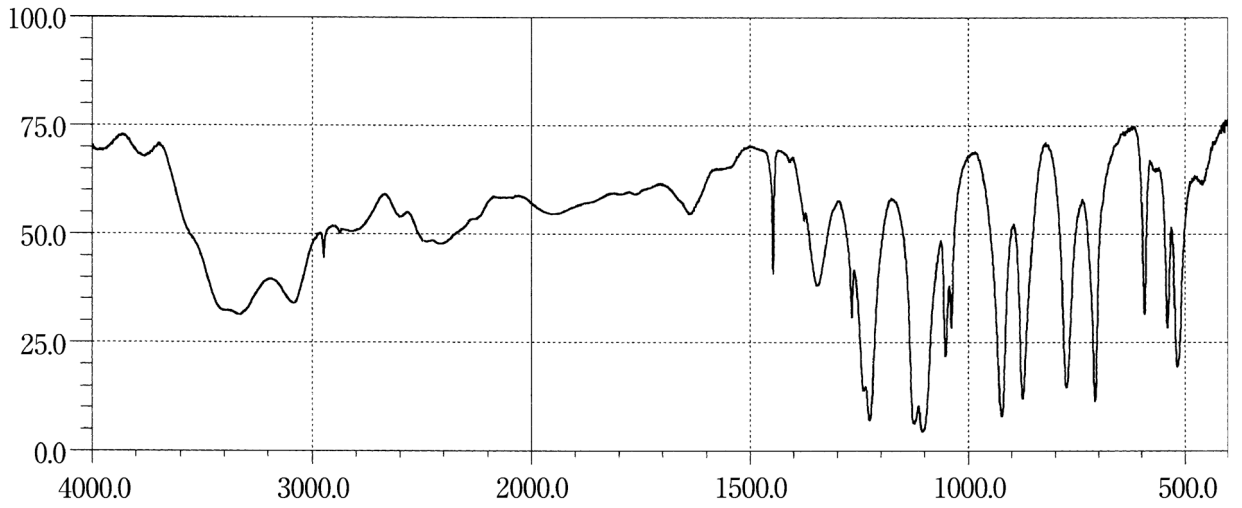
Triamcinolone Acetonide



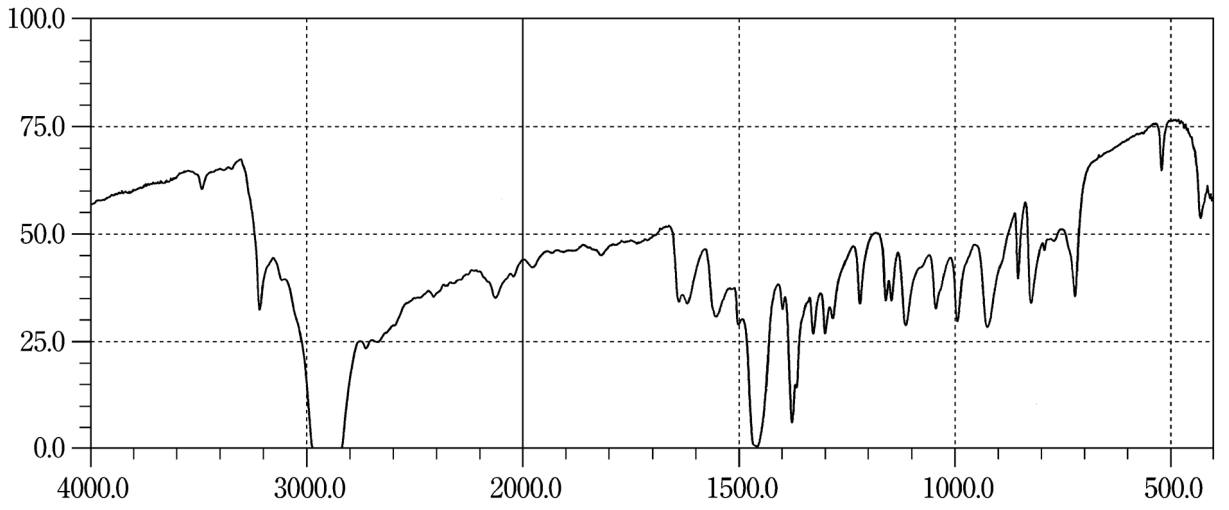
Trichlormethiazide



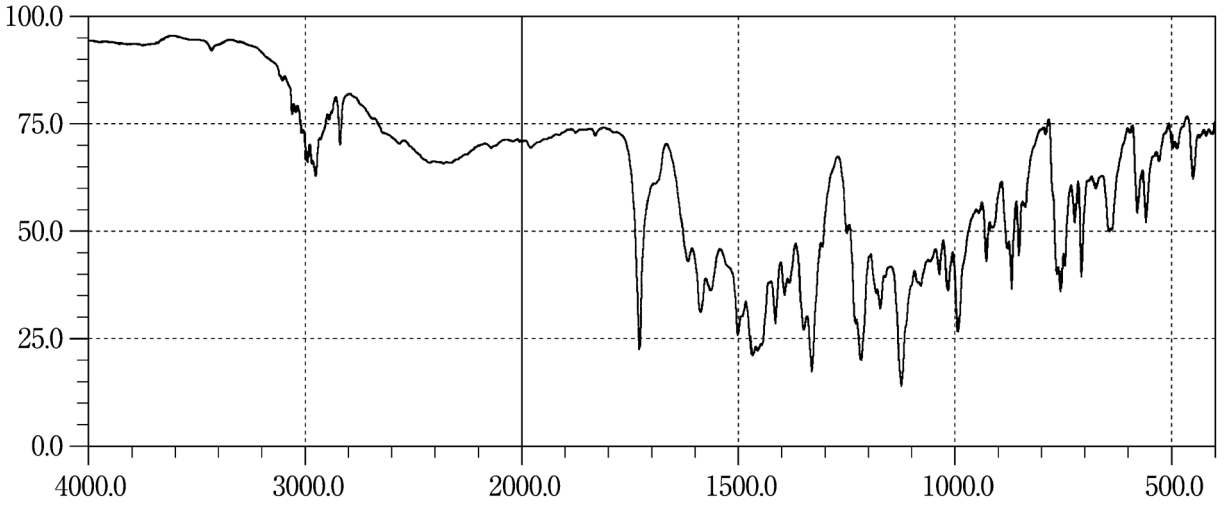
Triclofos Sodium



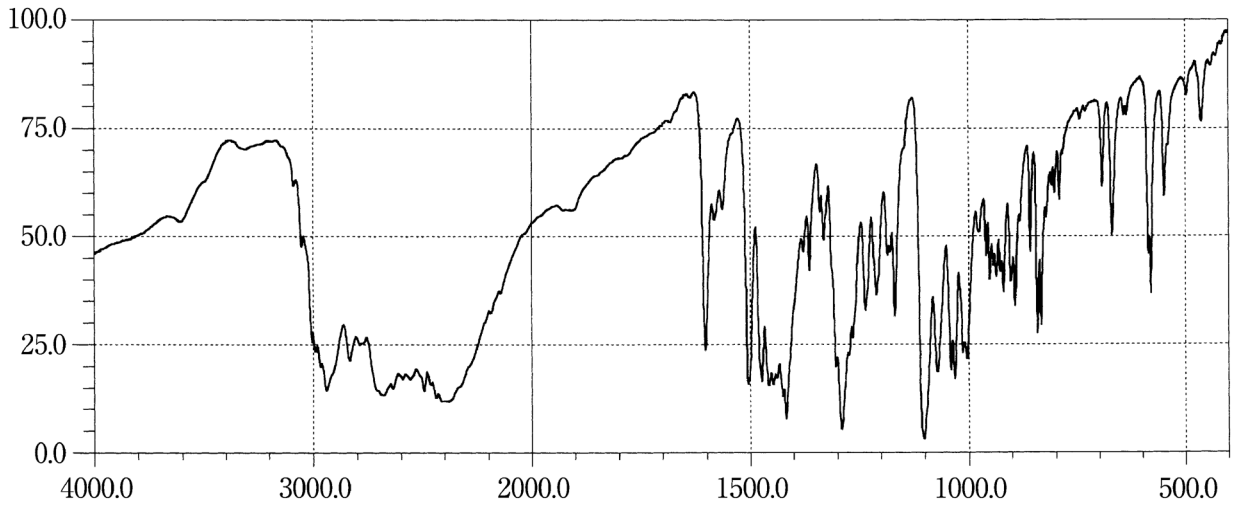
Trientine Hydrochloride



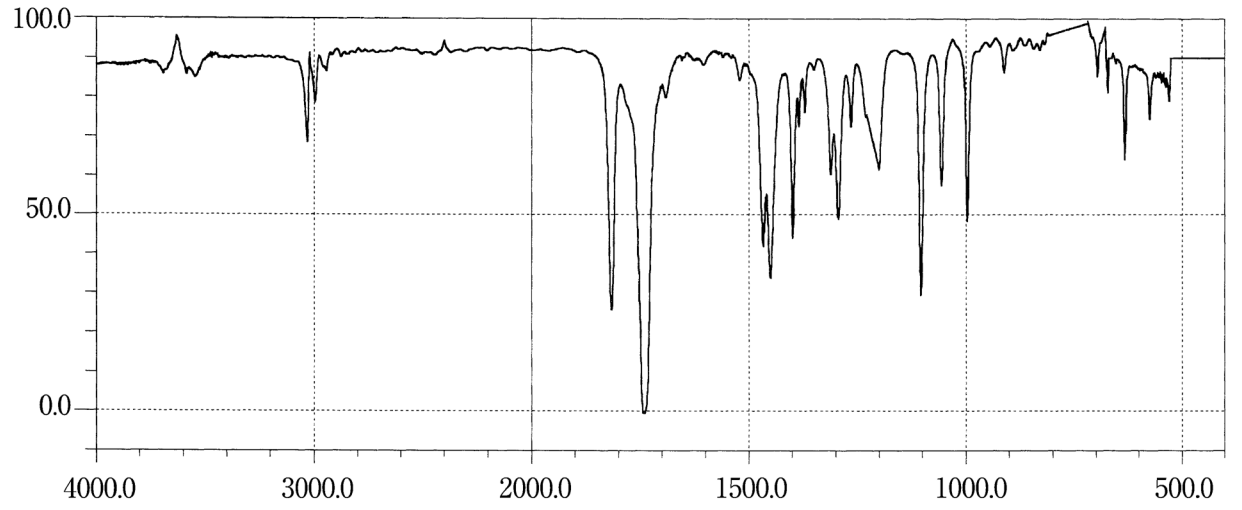
Trimebutine Maleate



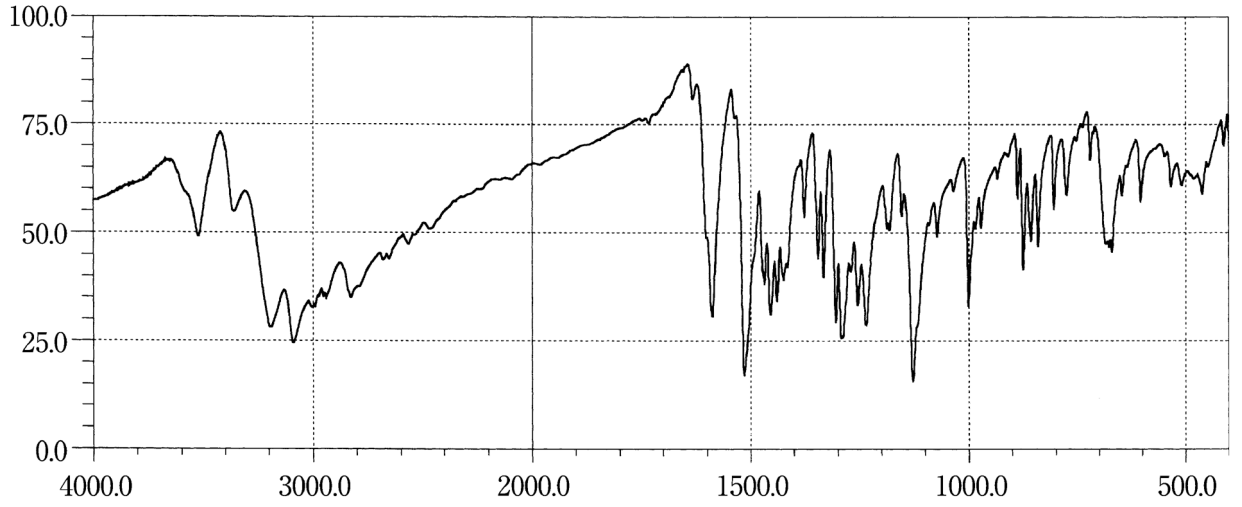
Trimetazidine Hydrochloride



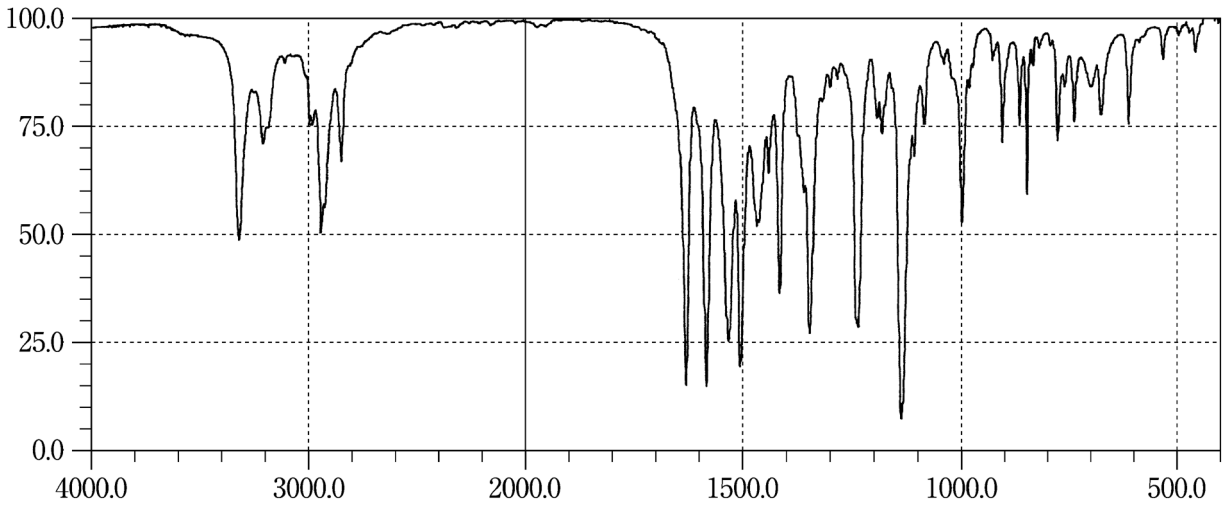
Trimethadione



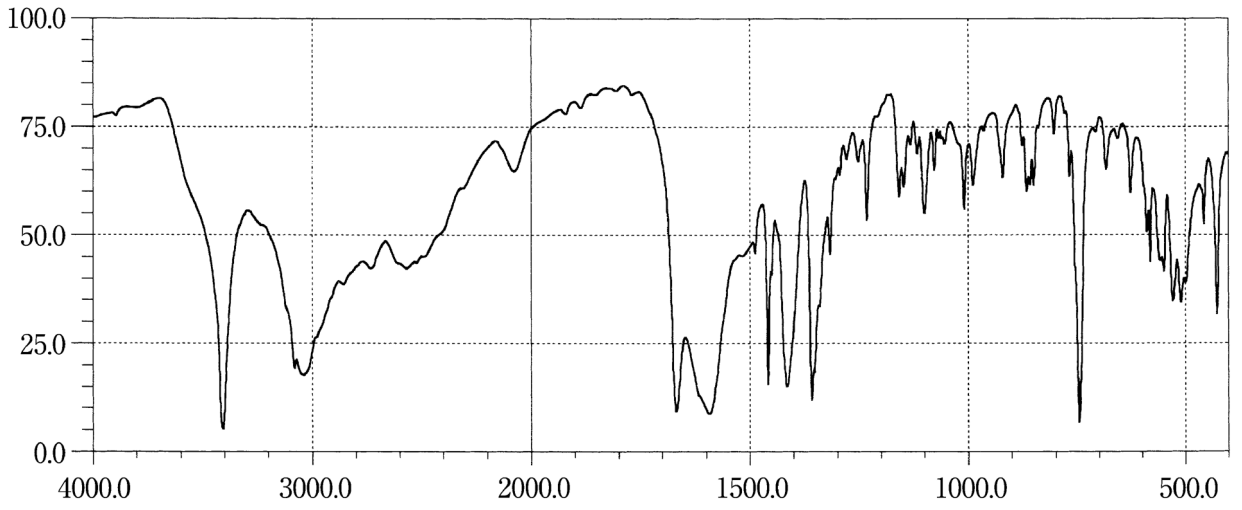
Trimetoquinol Hydrochloride Hydrate



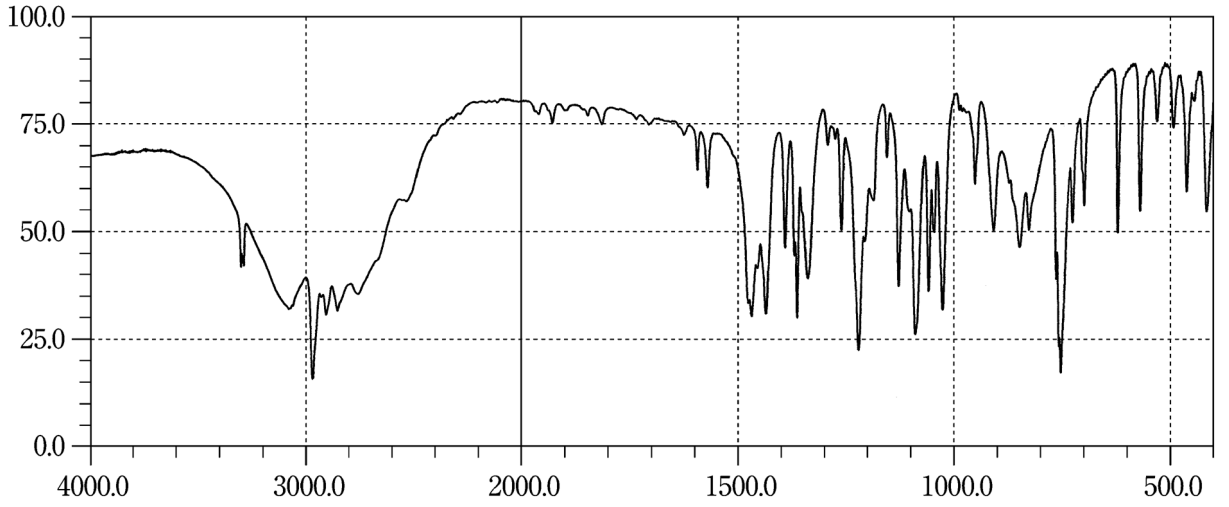
Troxipide



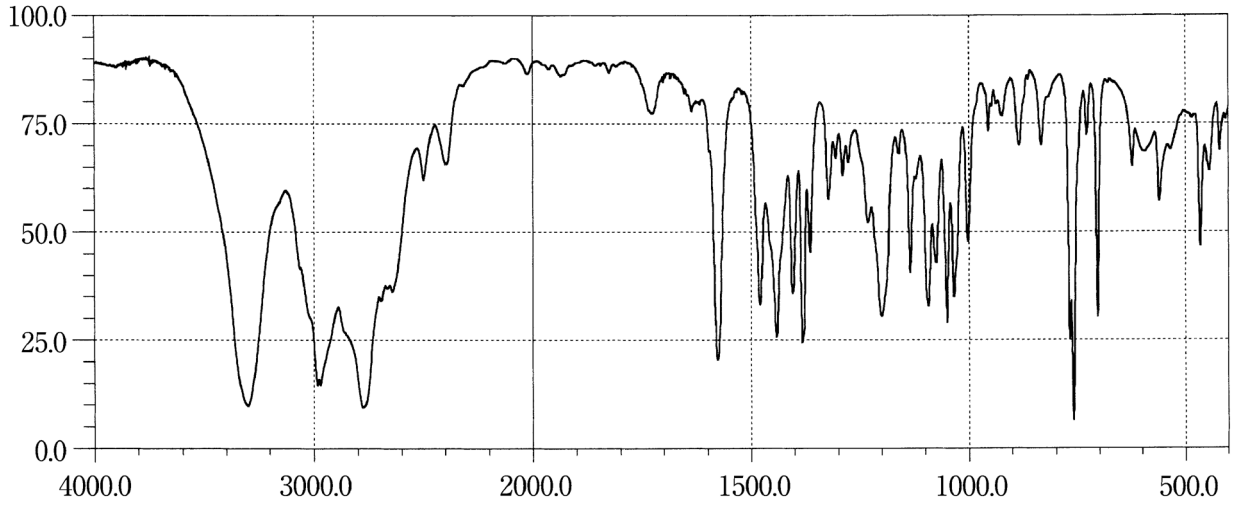
L-Tryptophan



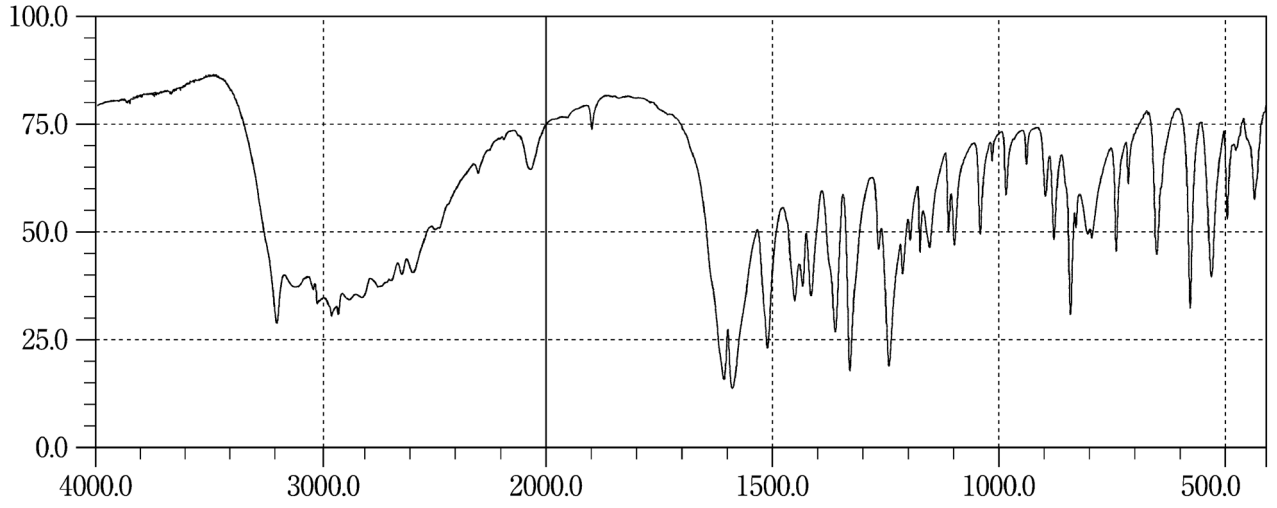
Tulobuterol



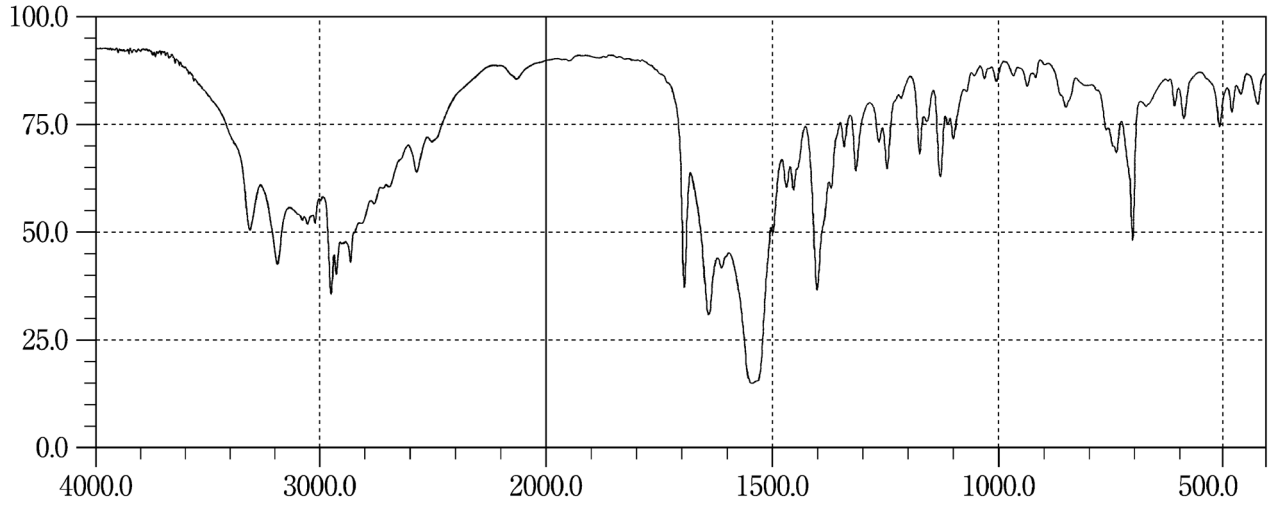
Tulobuterol Hydrochloride



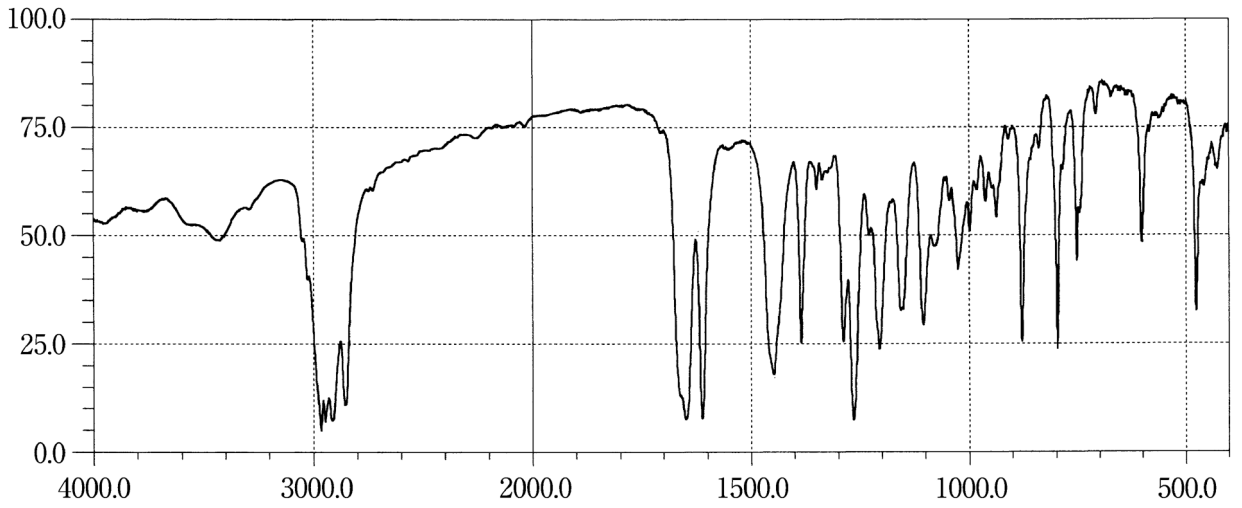
L-Tyrosine



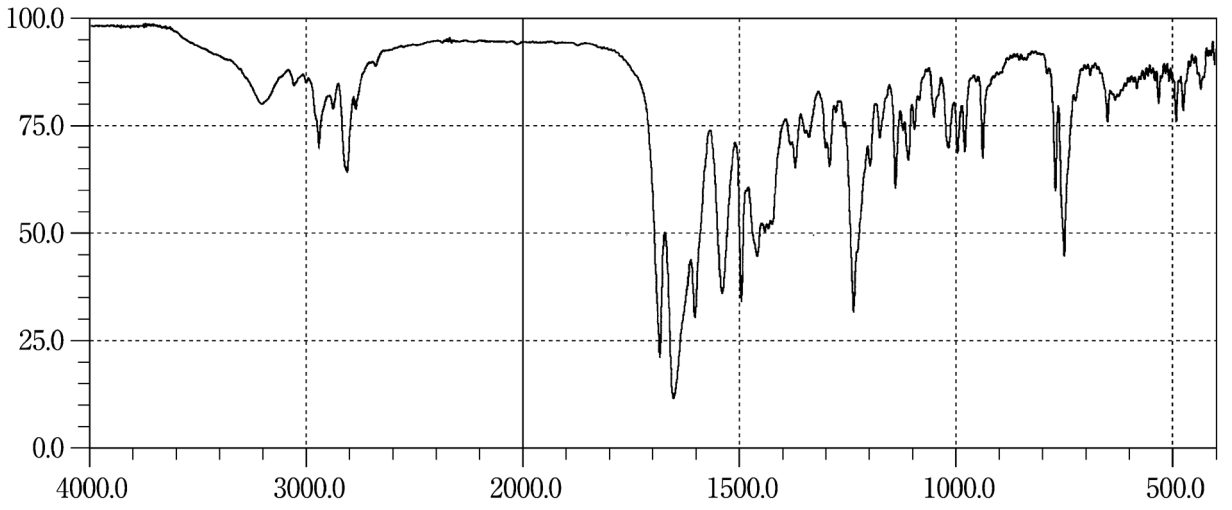
Ubenimex



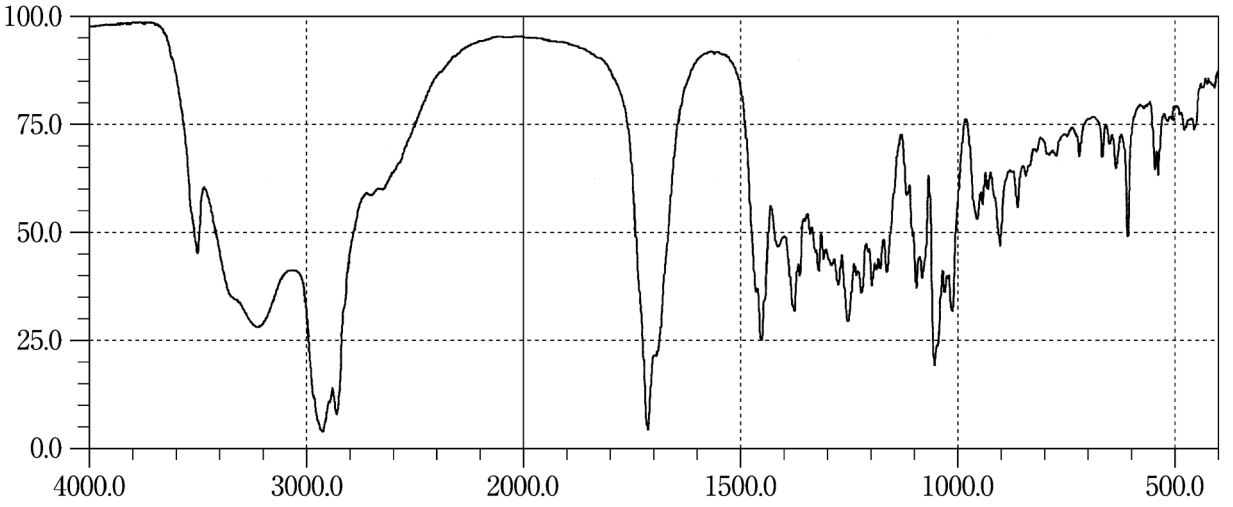
Ubidecarenone



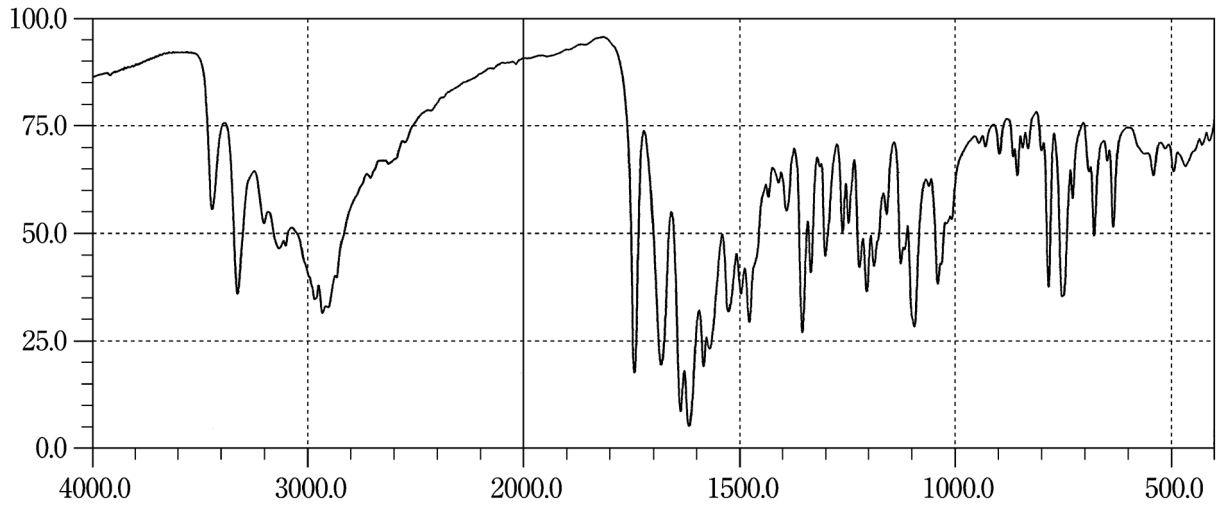
Urapidil



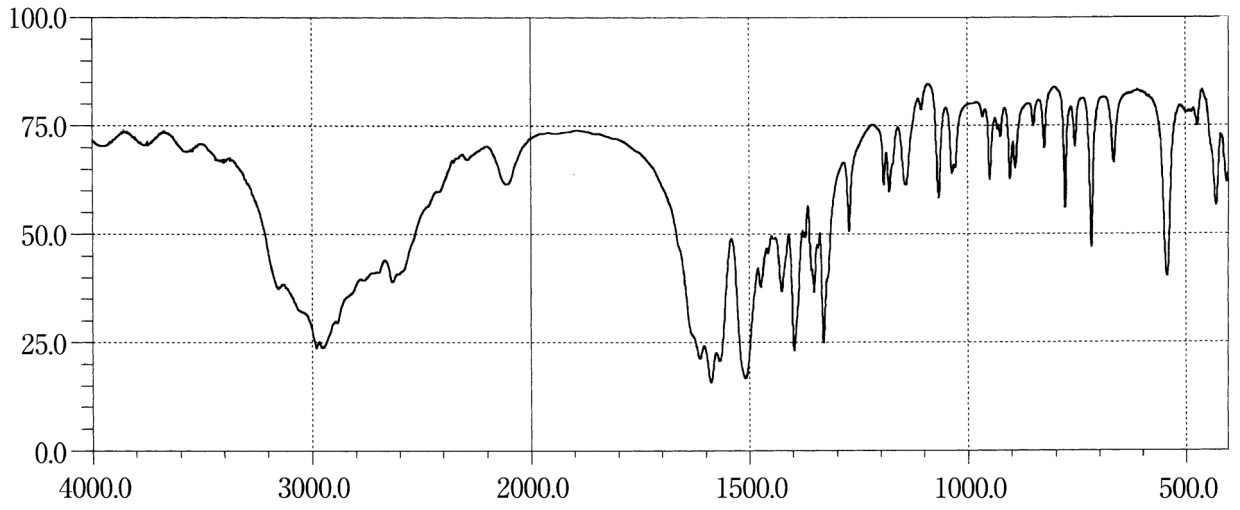
Ursodeoxycholic Acid



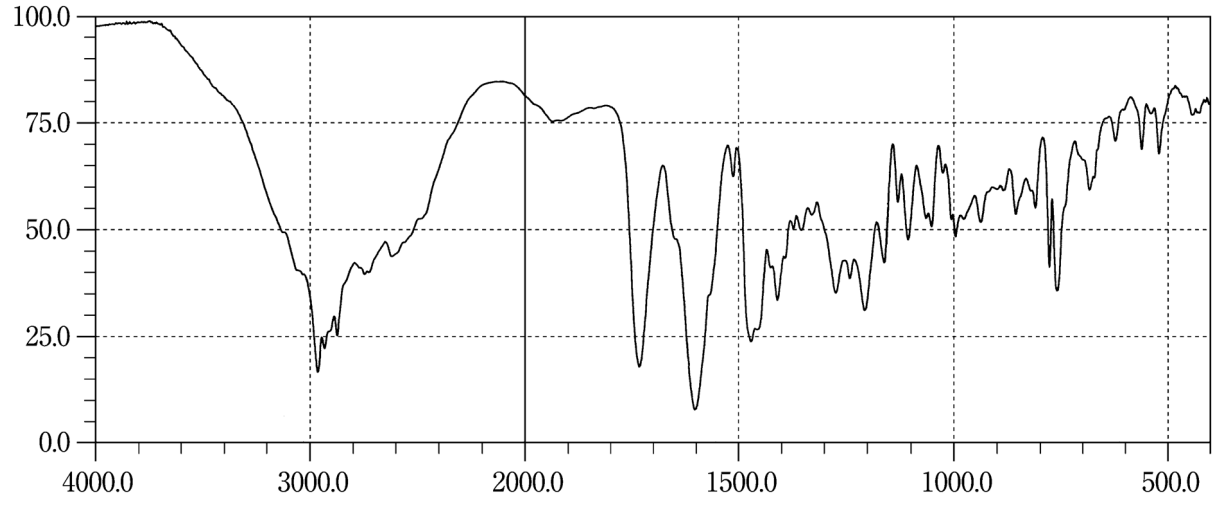
Valaciclovir Hydrochloride



L-Valine

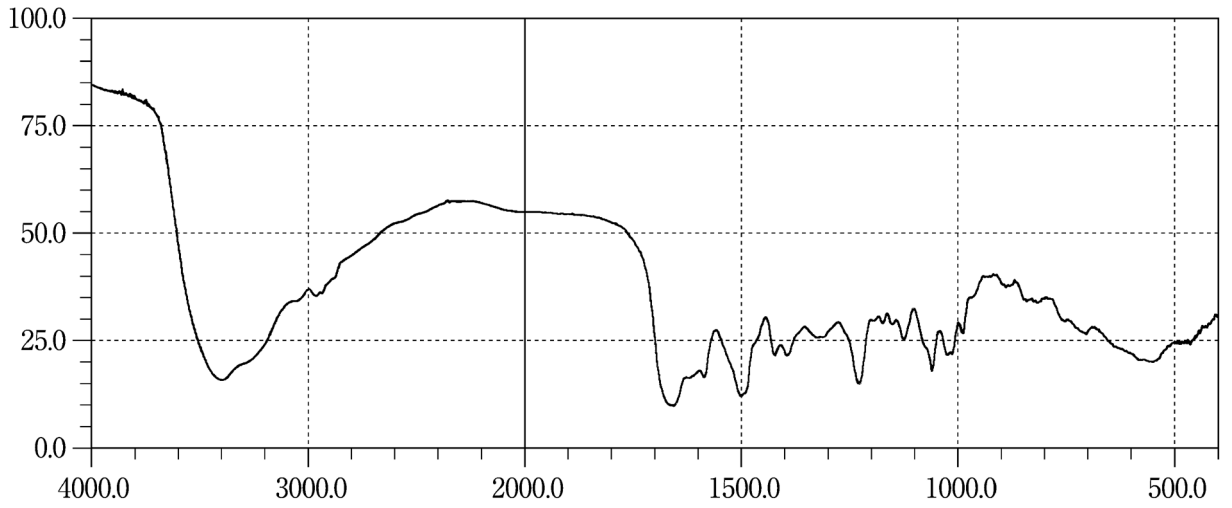


Valsartan

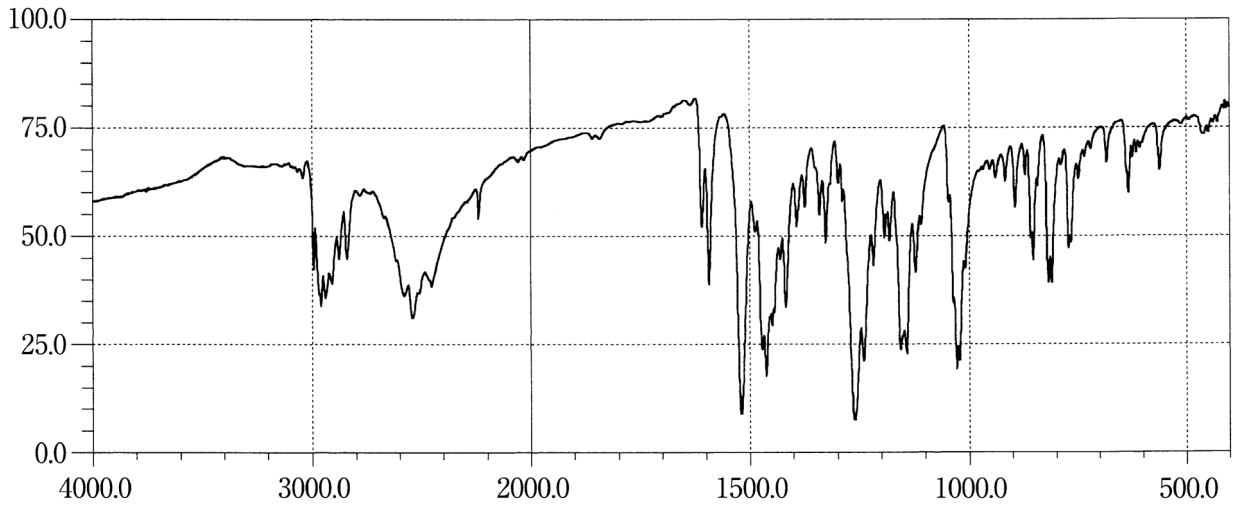


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

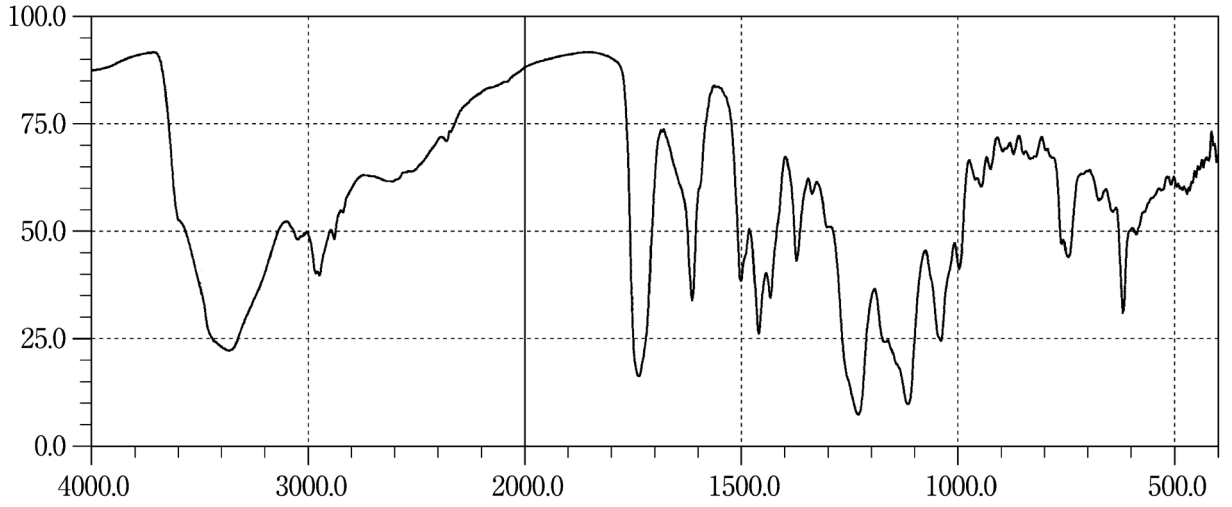
Vancomycin Hydrochloride



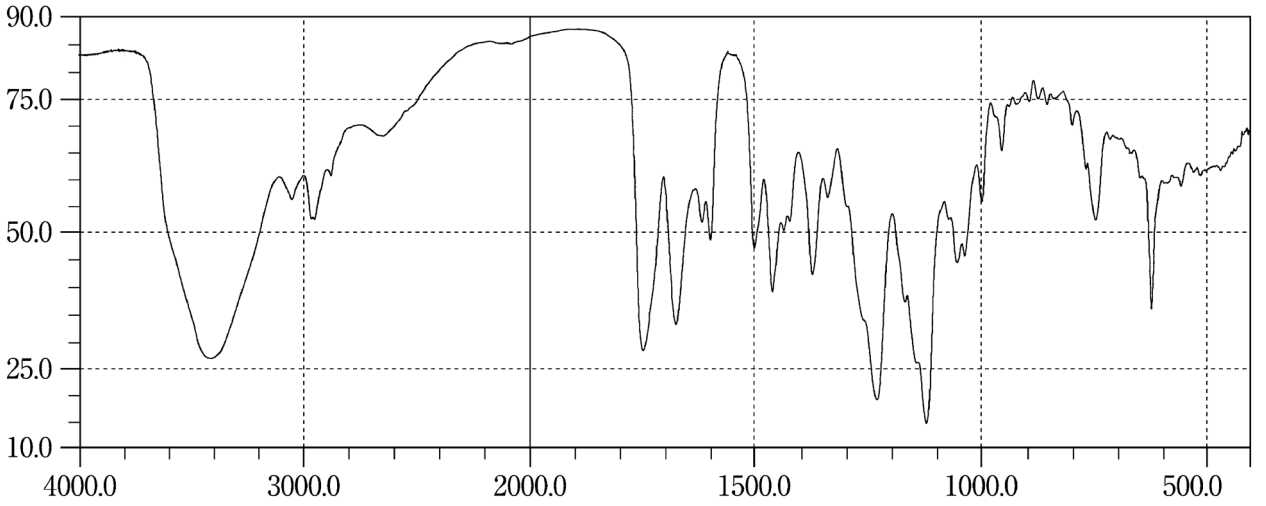
Verapamil Hydrochloride



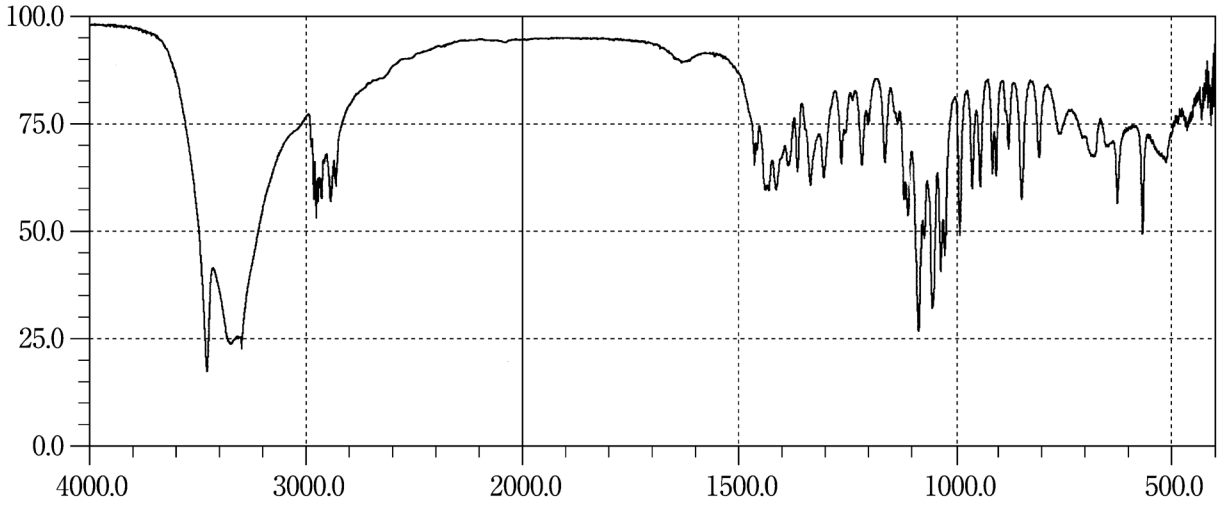
Vinblastine Sulfate



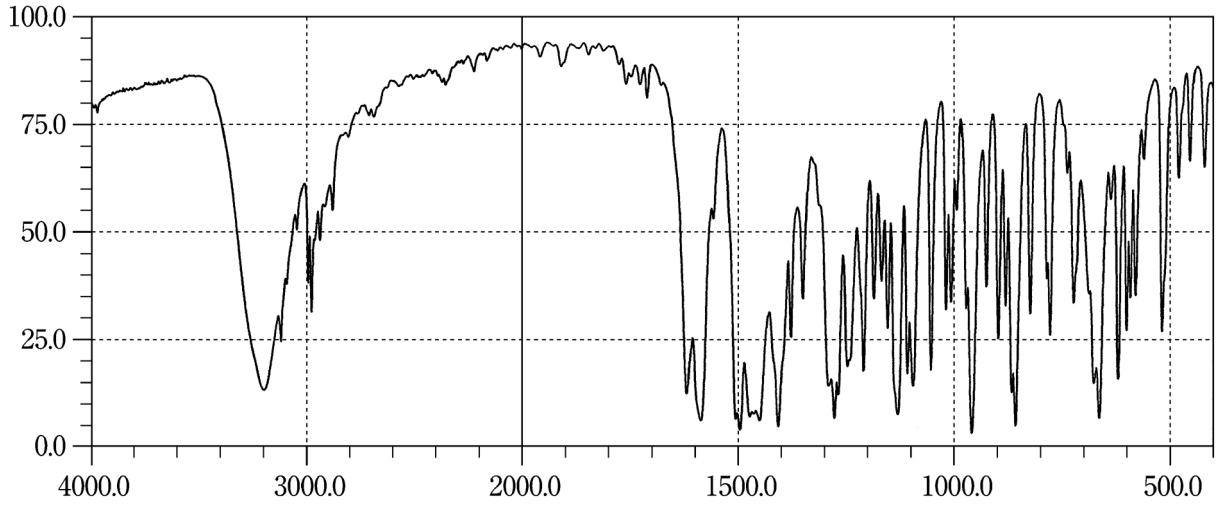
Vincristine Sulfate



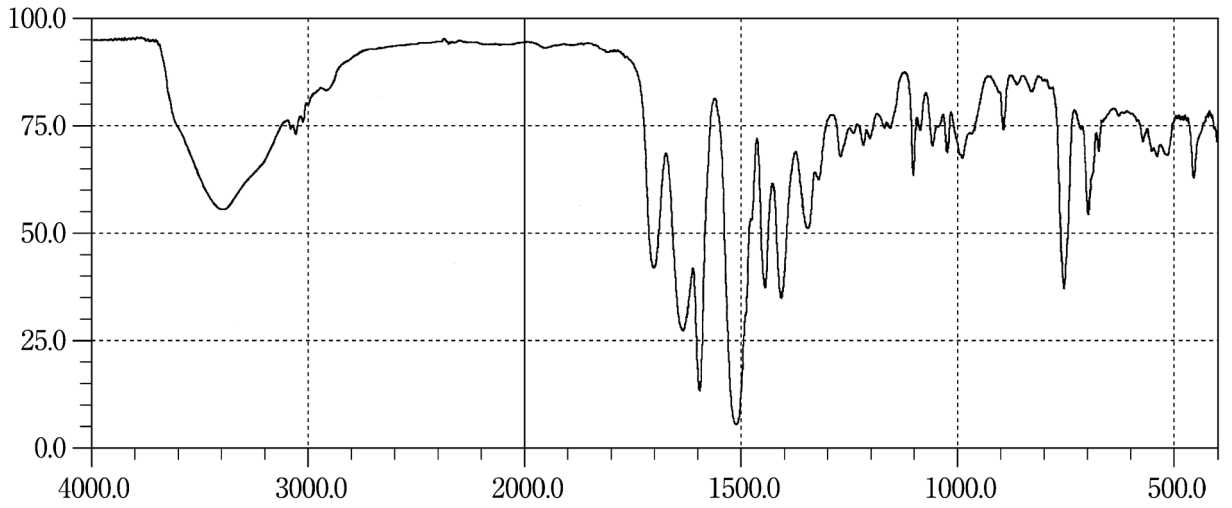
Voglibose



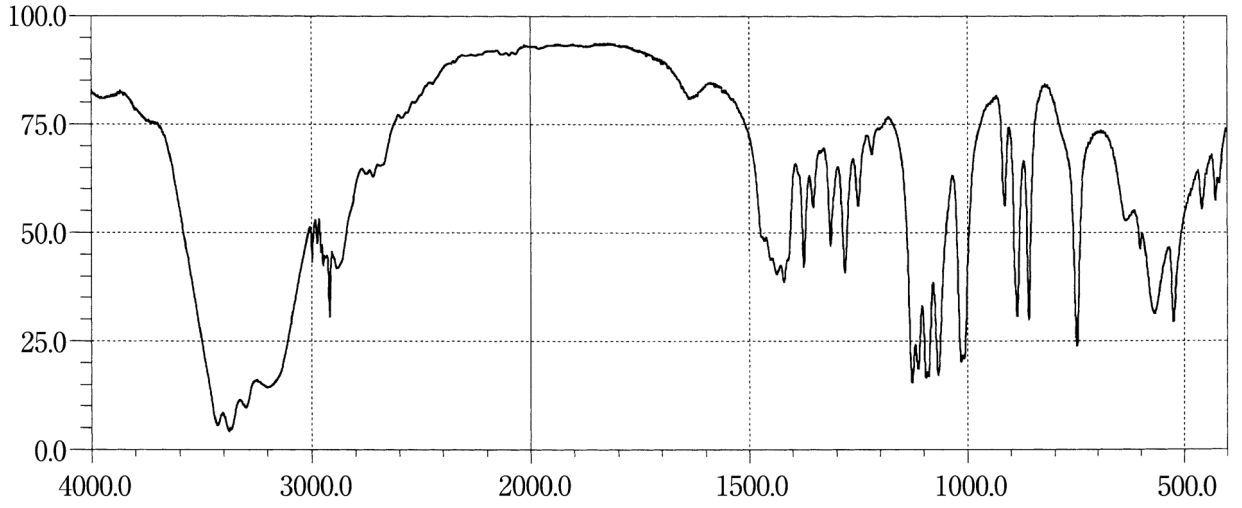
Voriconazole



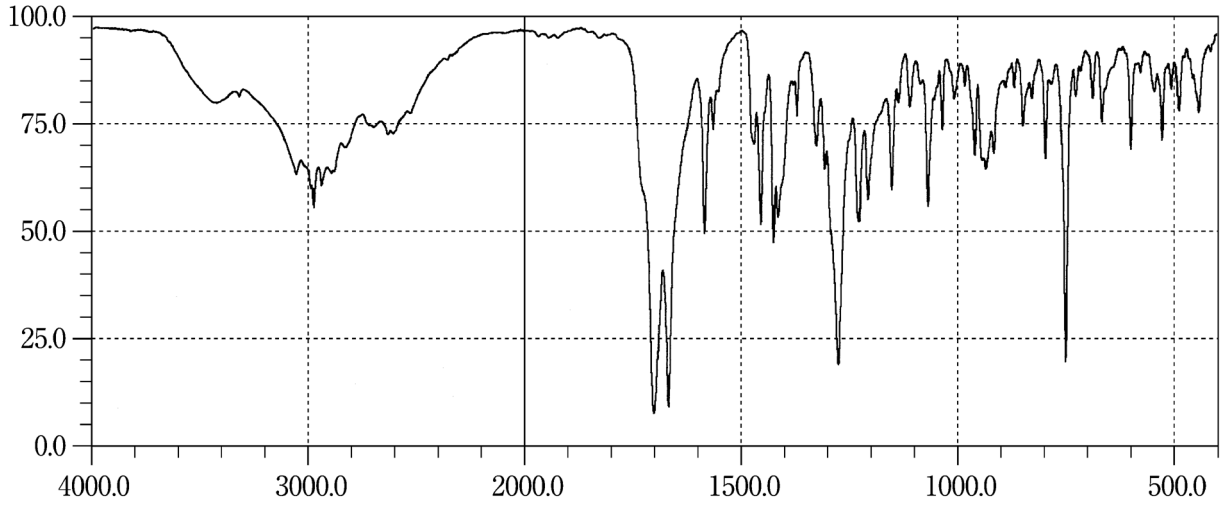
Warfarin Potassium



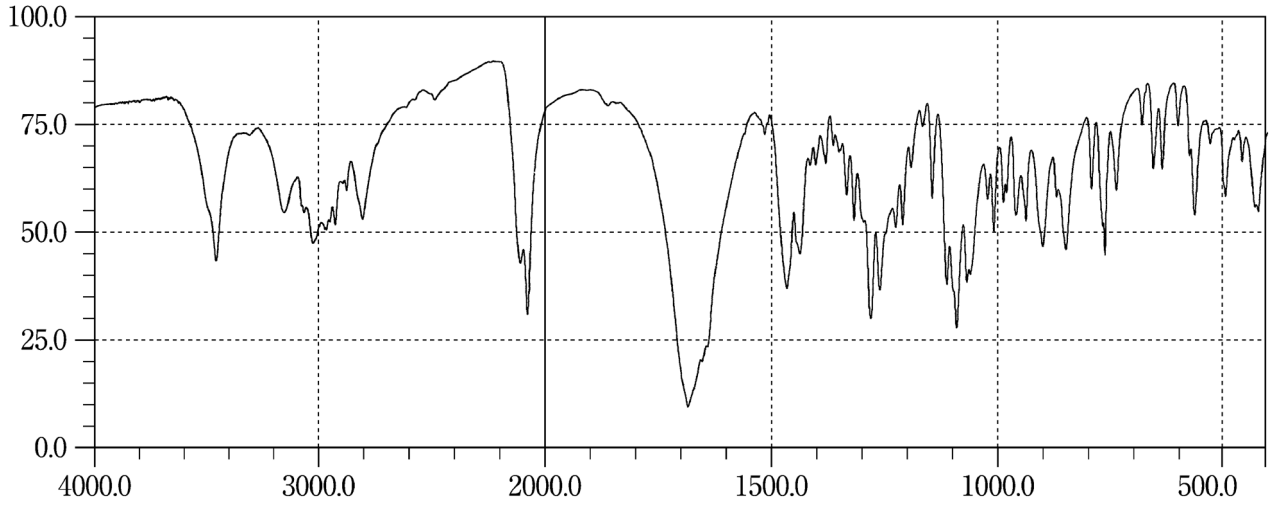
Xylitol



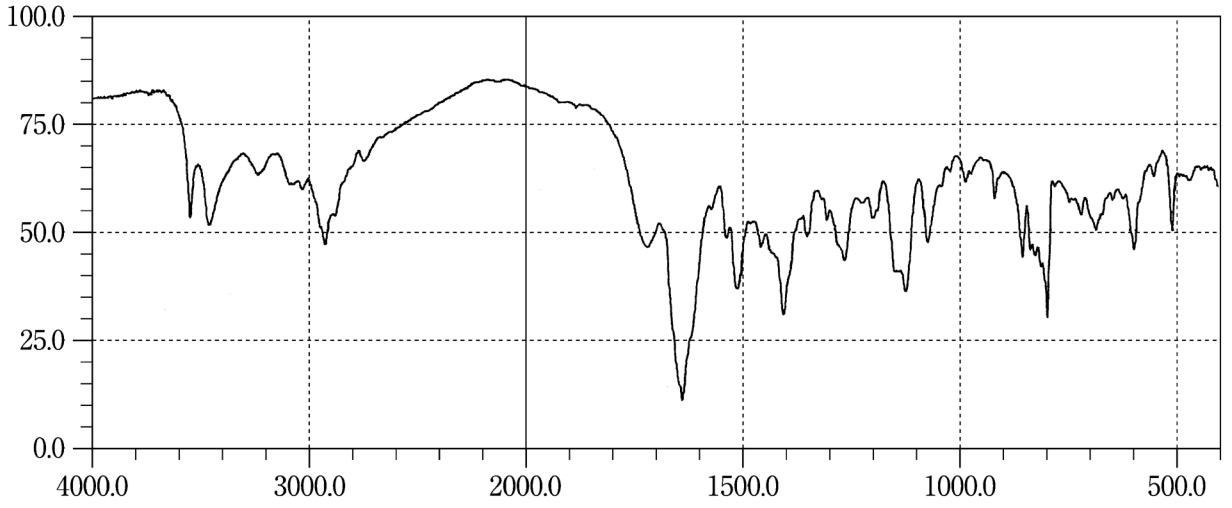
Zaltoprofen



Zidovudine



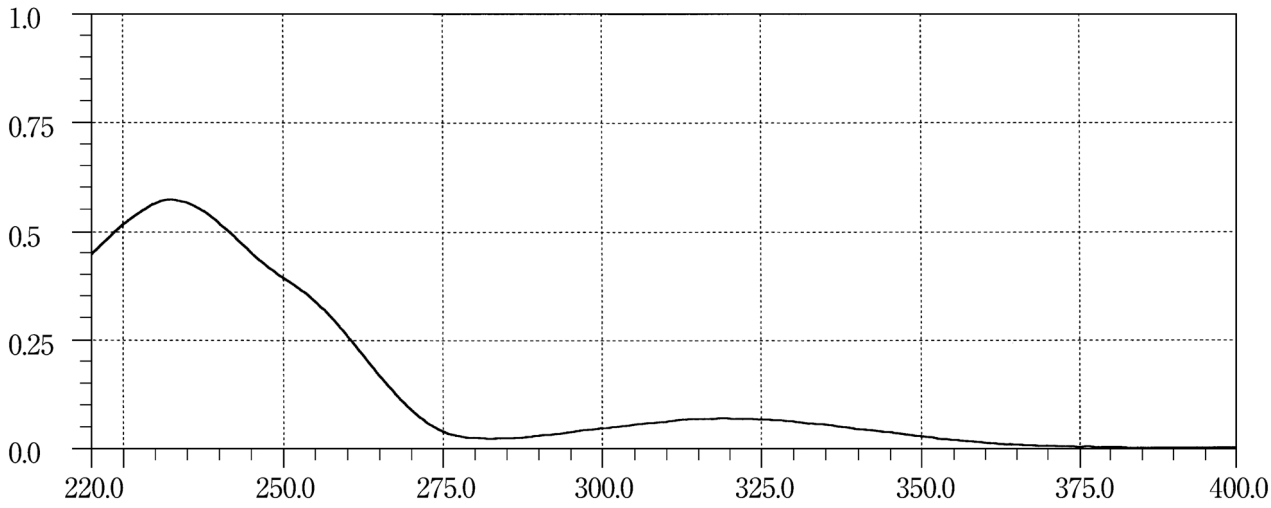
Zolpidem Tartrate



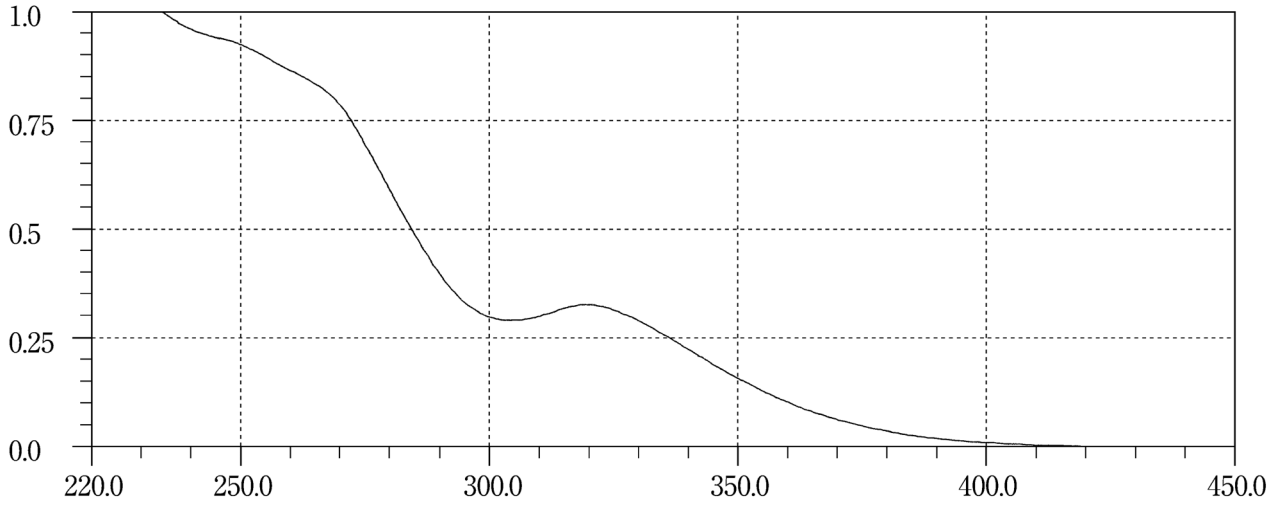
ULTRAVIOLET-VISIBLE REFERENCE SPECTRA

The ultraviolet-visible reference spectra presented here were obtained by the use of double-beam spectrophotometers with sample solutions prepared as specified in the individual monographs. The horizontal axis indicates the wavelength (nm) and the vertical axis indicates the absorbance.

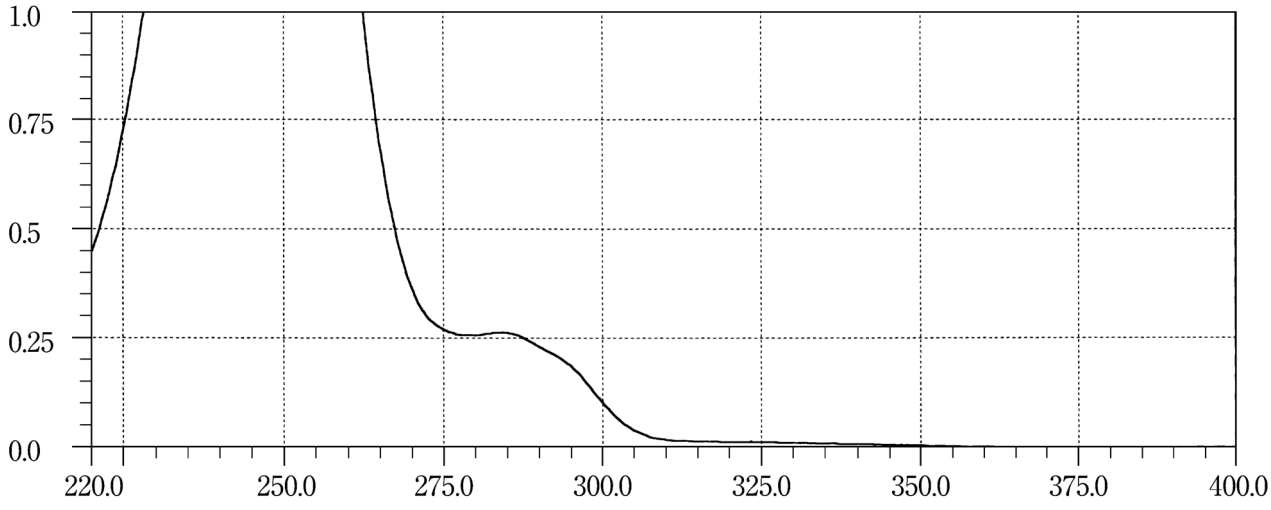
Acebutolol Hydrochloride



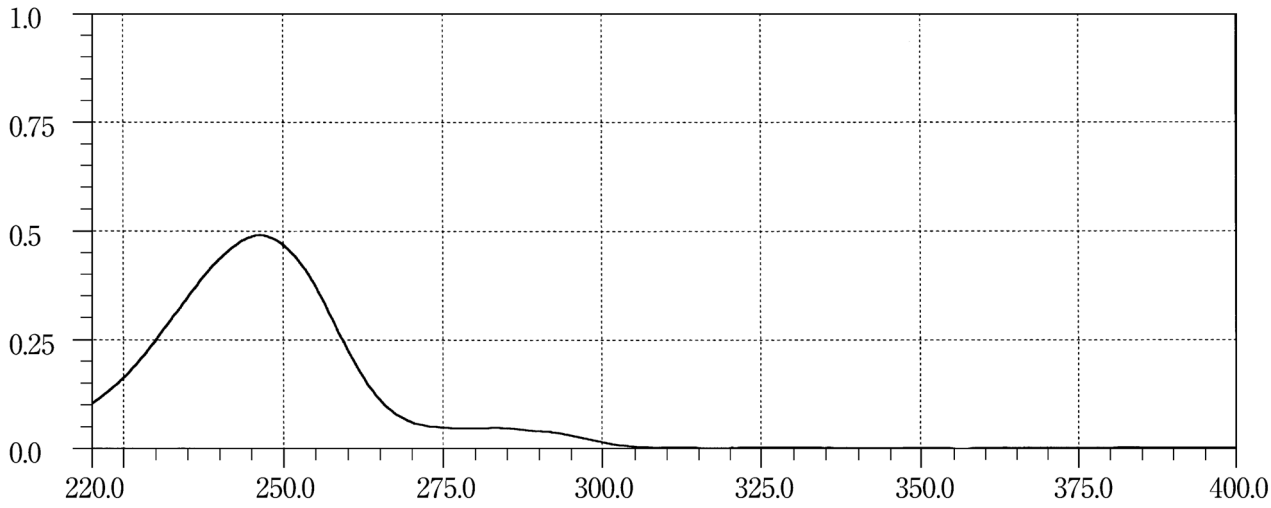
Acemetacin



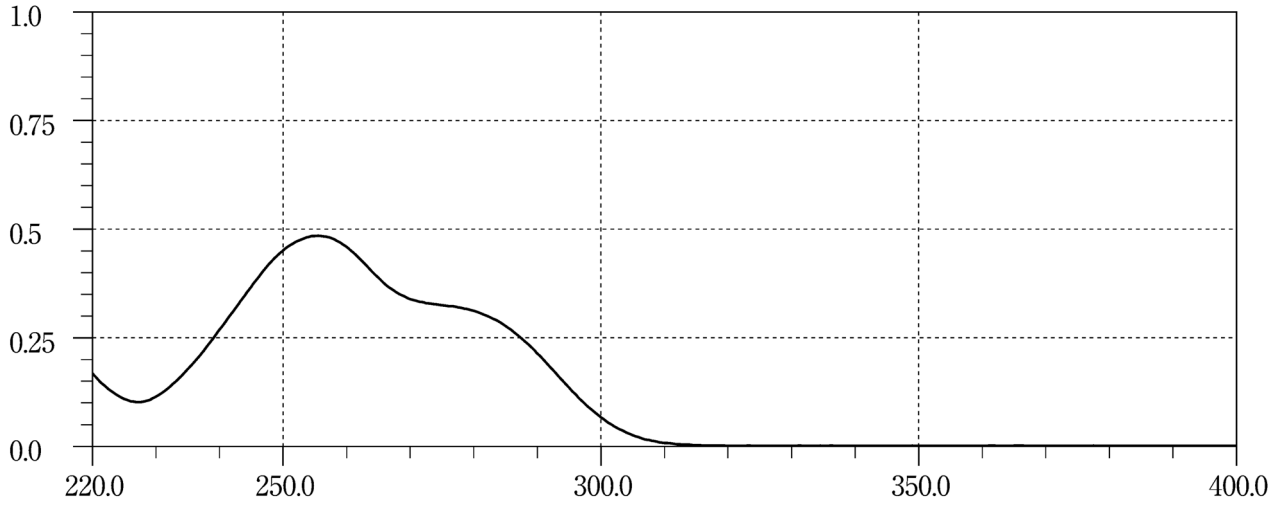
Acetohexamide 1



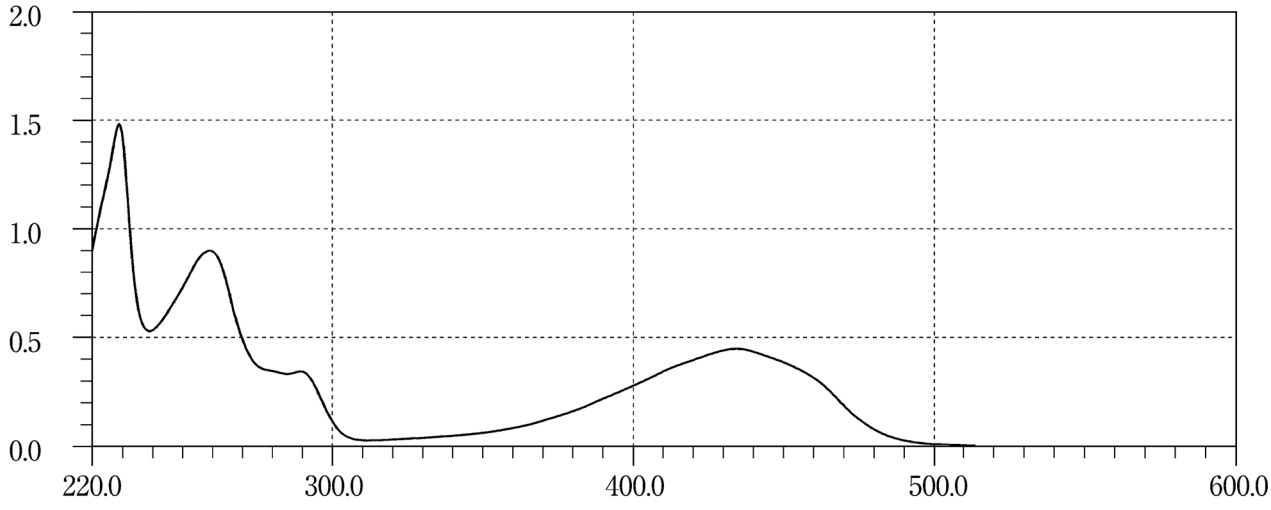
Acetohexamide 2



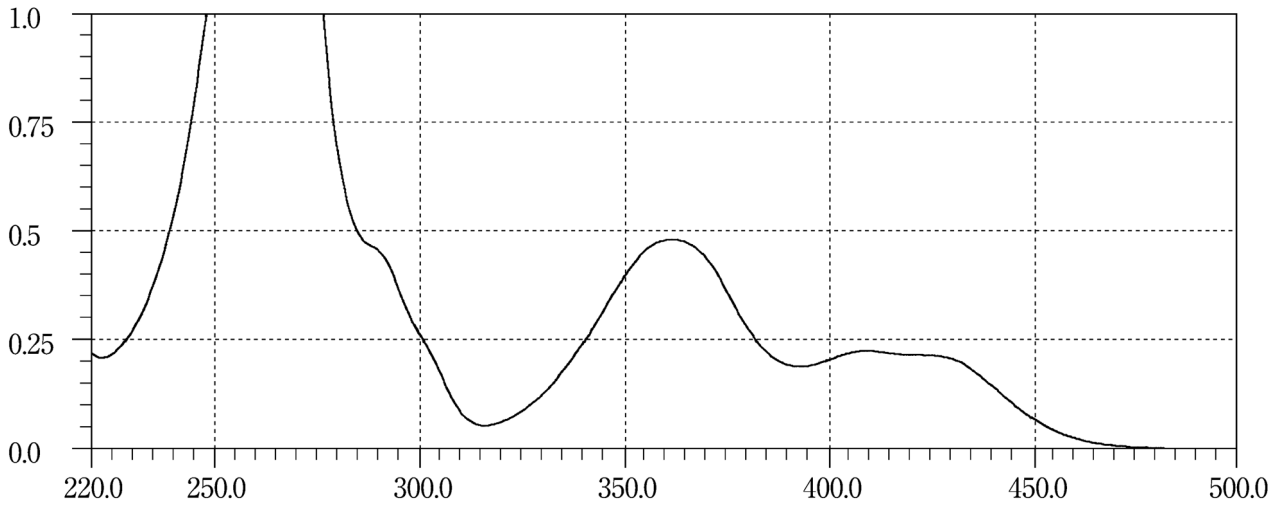
Aciclovir



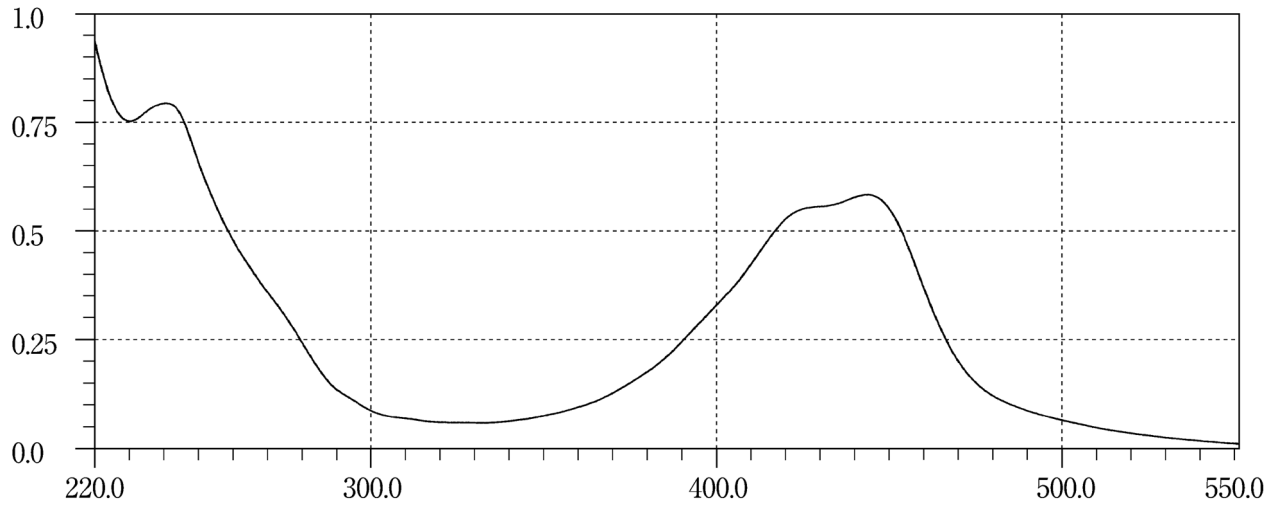
Aclarubicin Hydrochloride



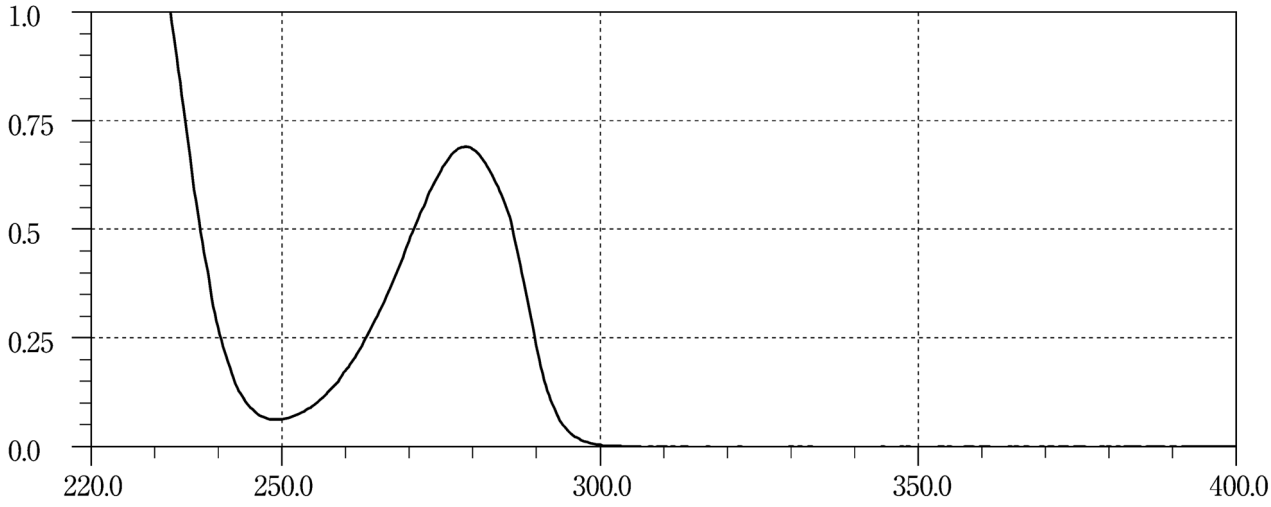
Acrinol Hydrate



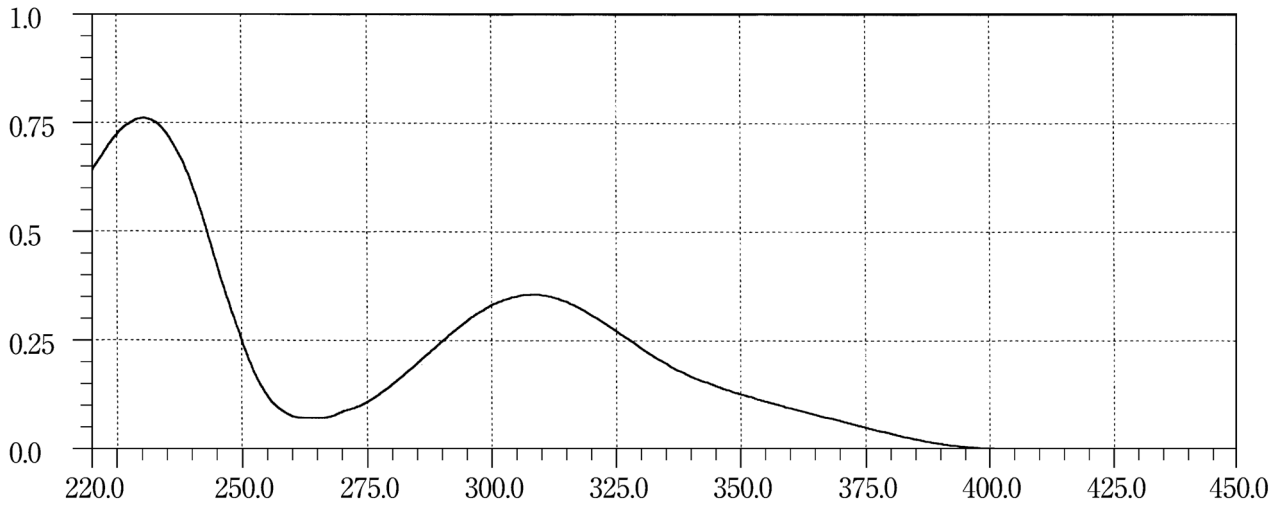
Actinomycin D



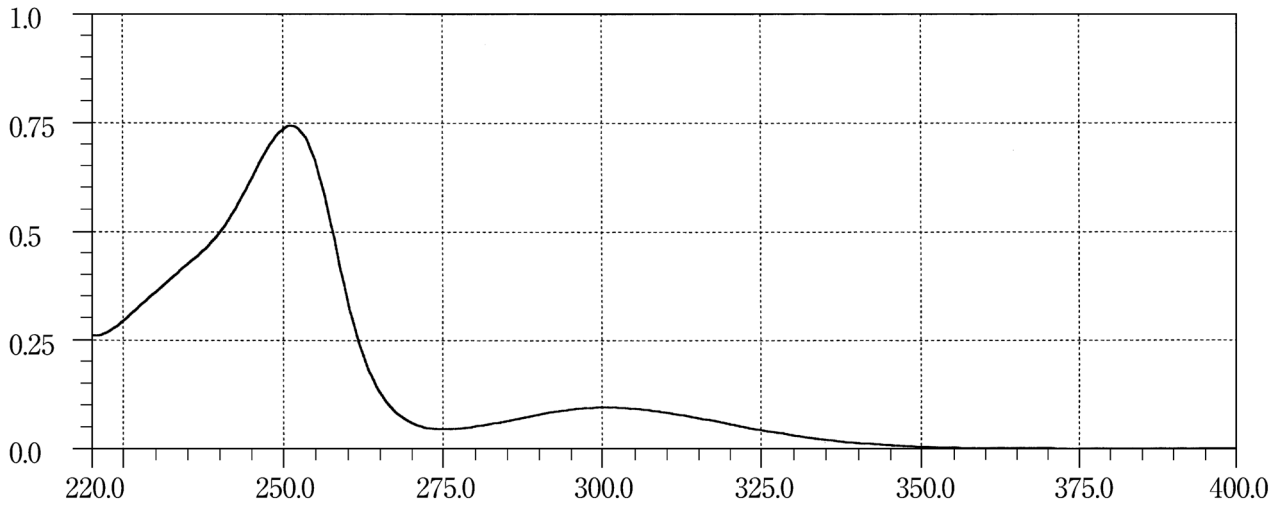
Adrenaline



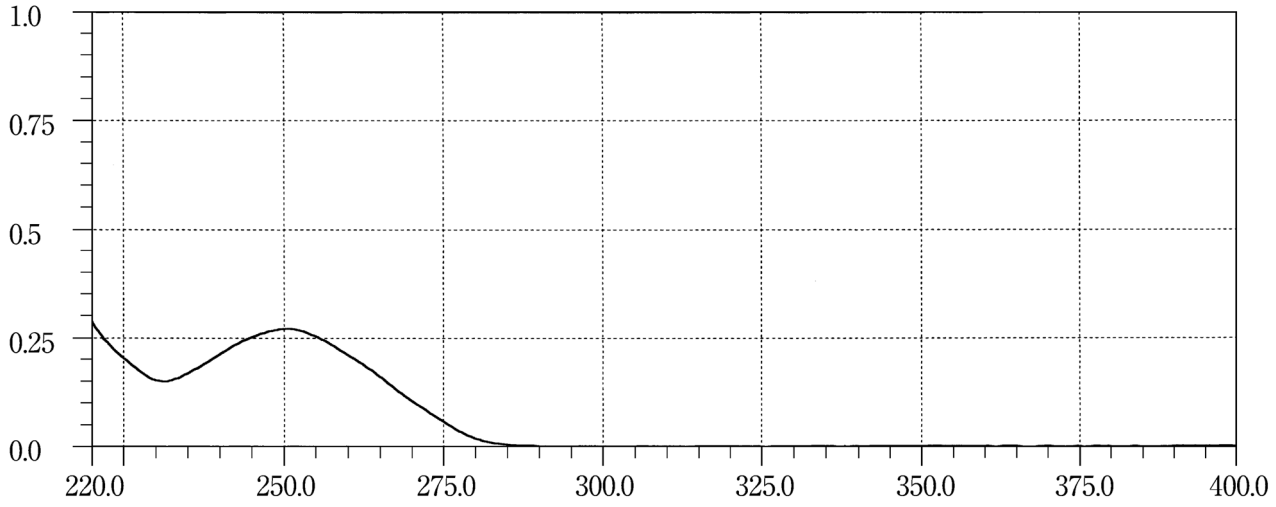
Afloqualone



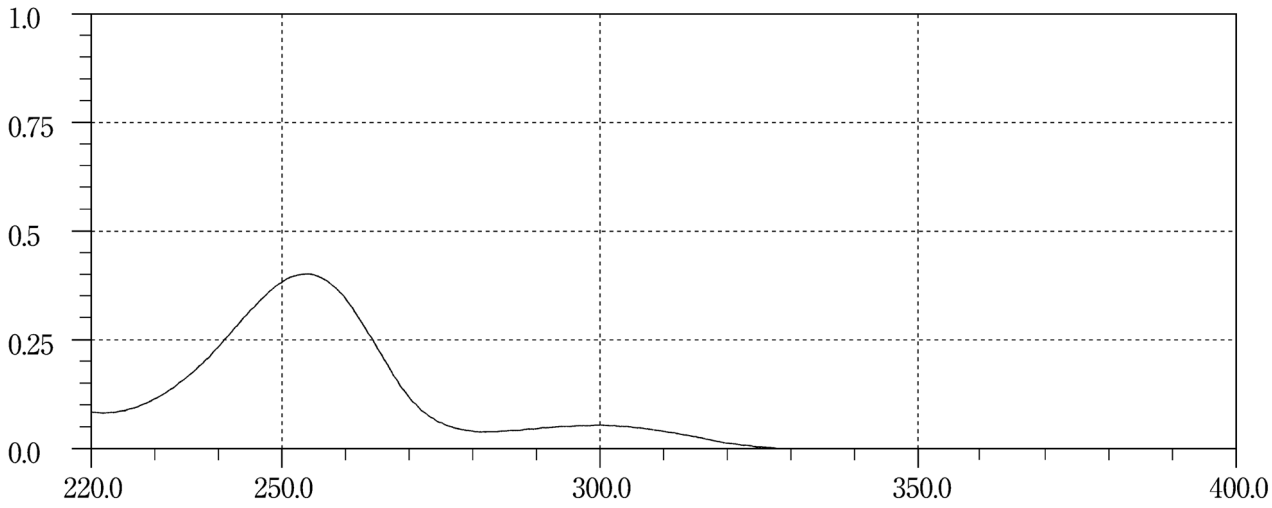
Alimemazine Tartrate



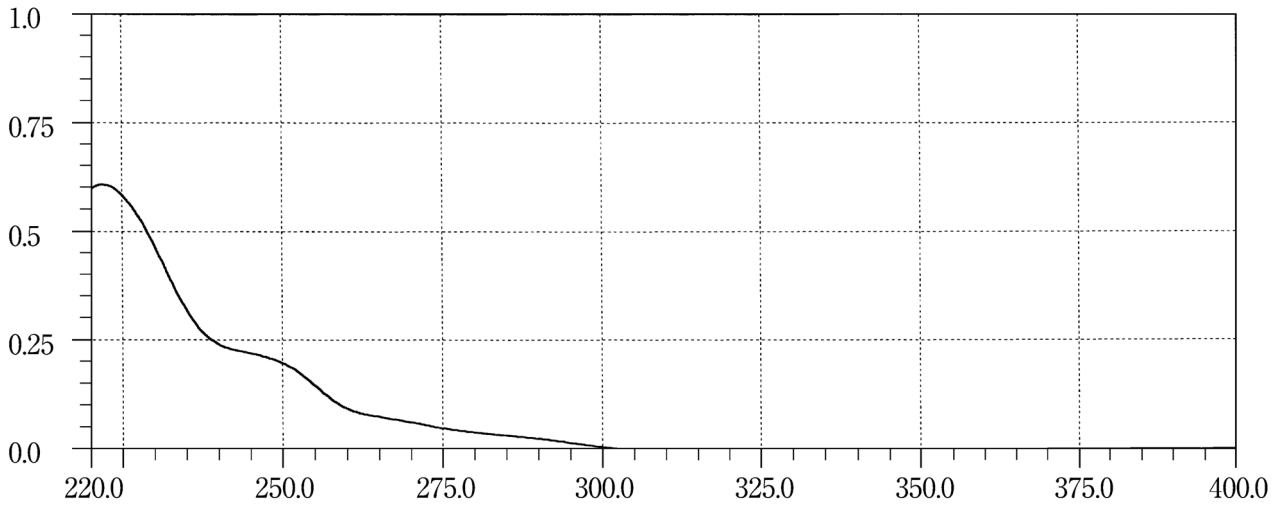
Allopurinol



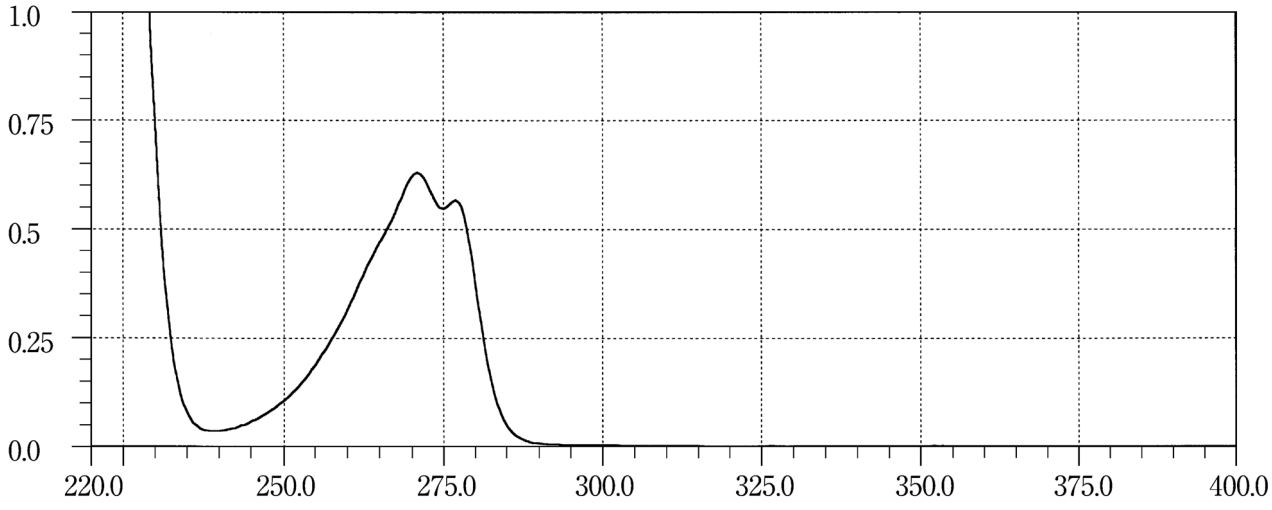
Alminoprofen



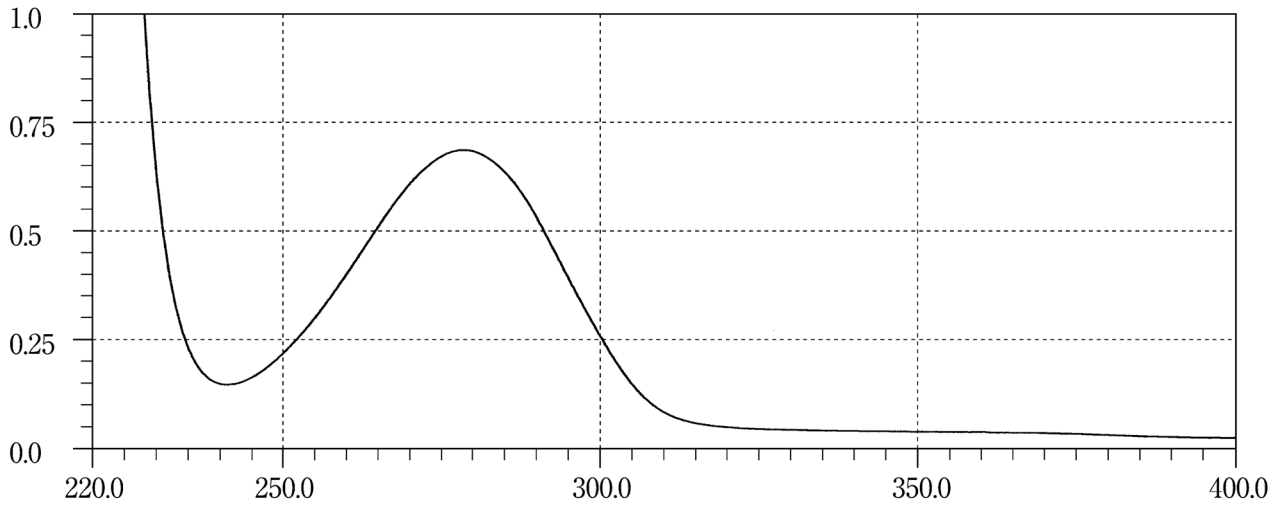
Alprazolam



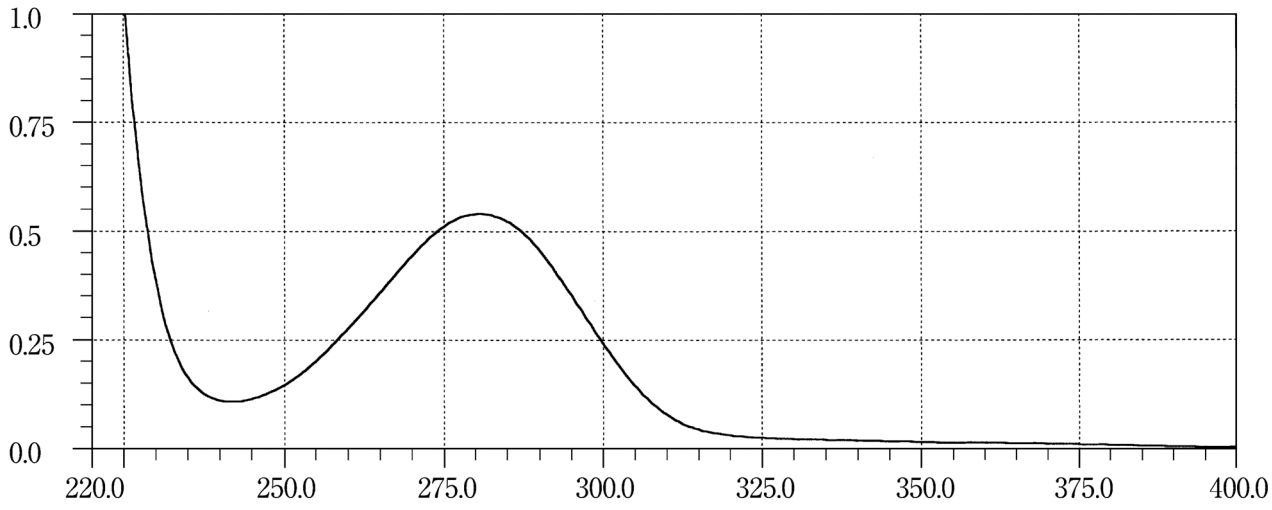
Alprenolol Hydrochloride



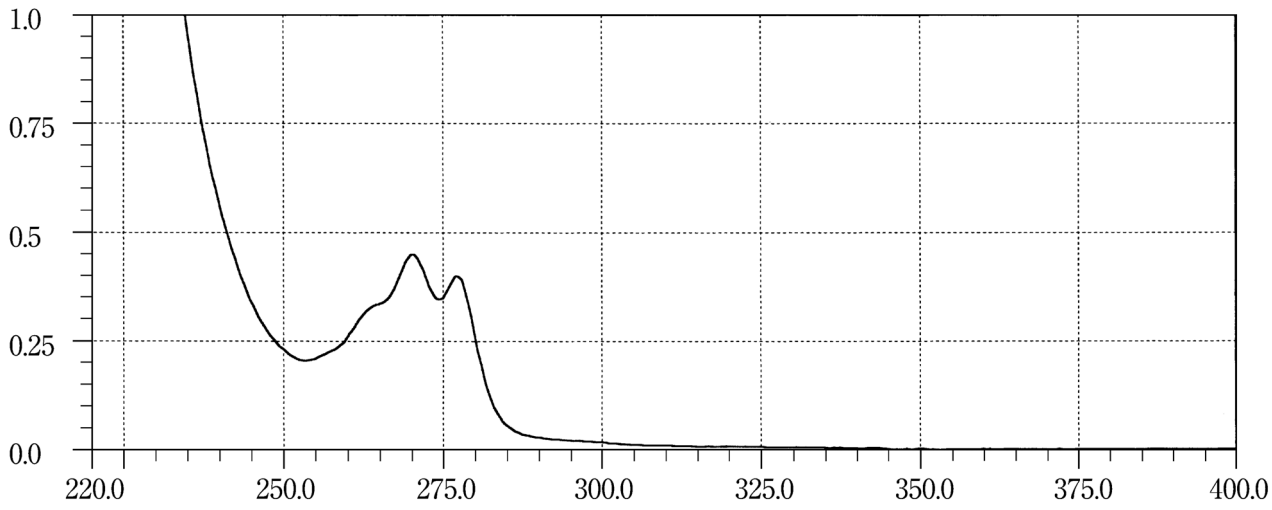
Alprostadil



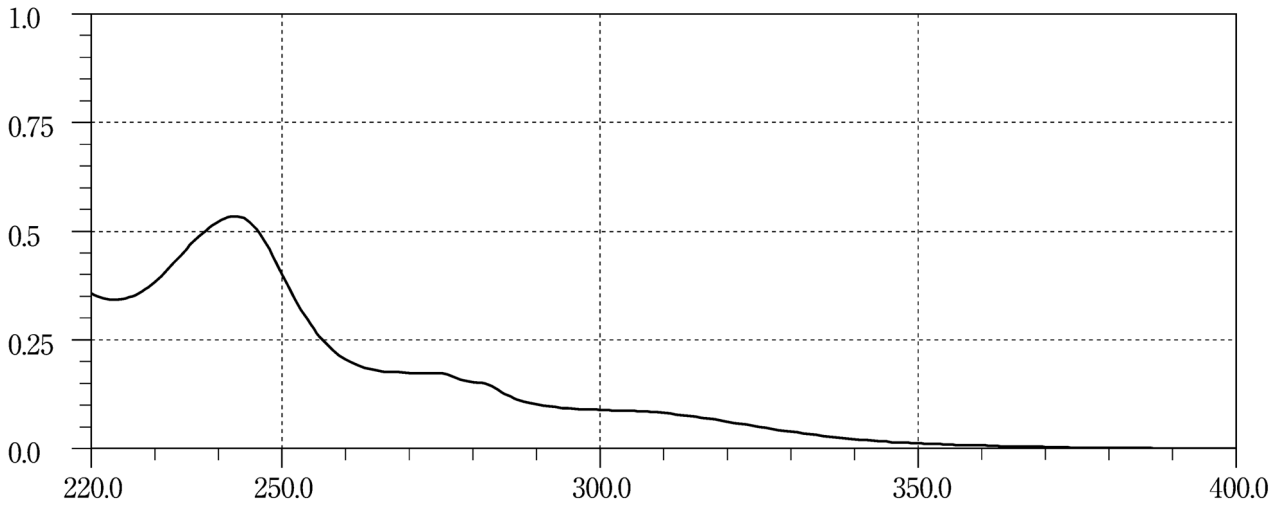
Alprostadil Alfadex



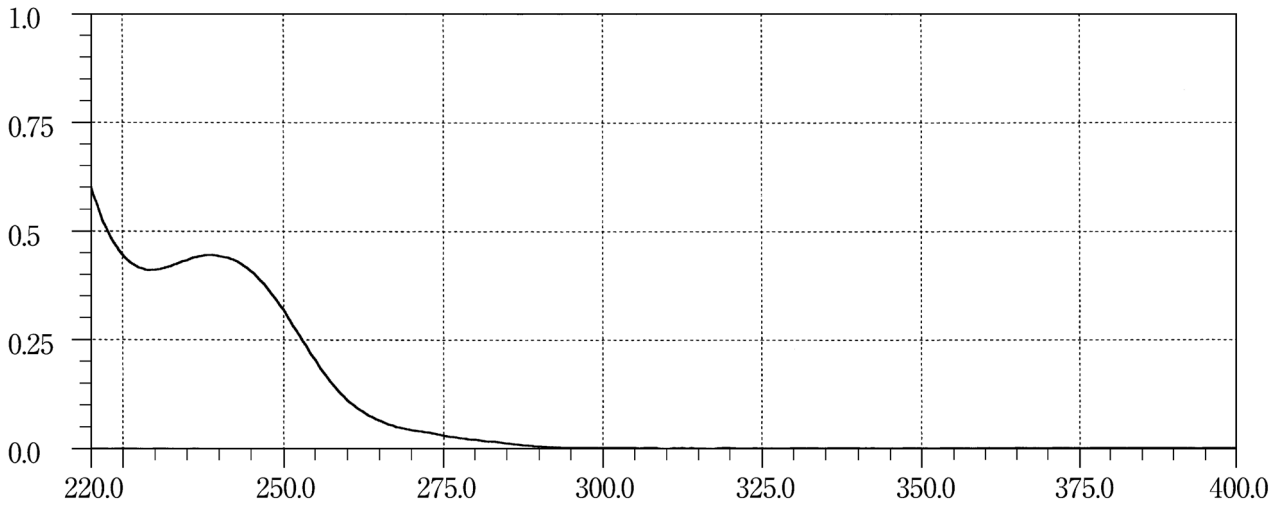
Ambenonium Chloride



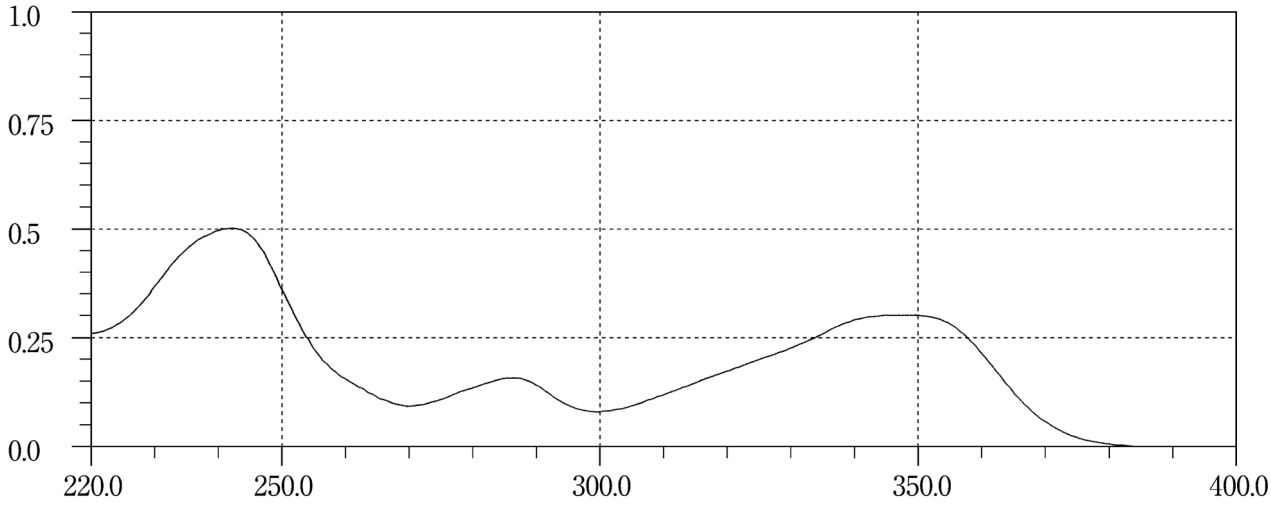
Amiodarone Hydrochloride



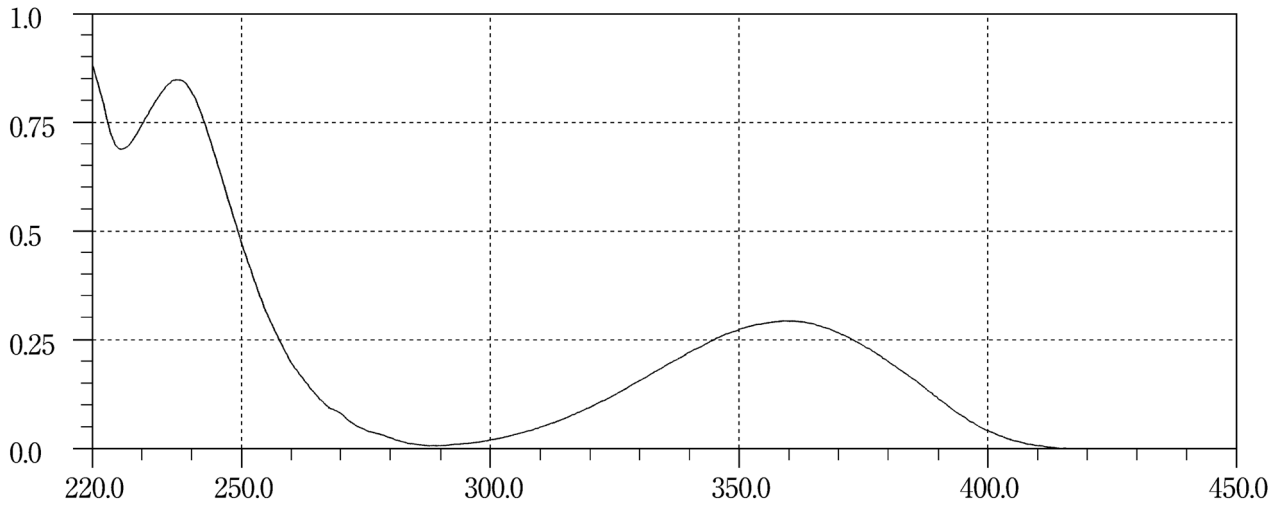
Amitriptyline Hydrochloride



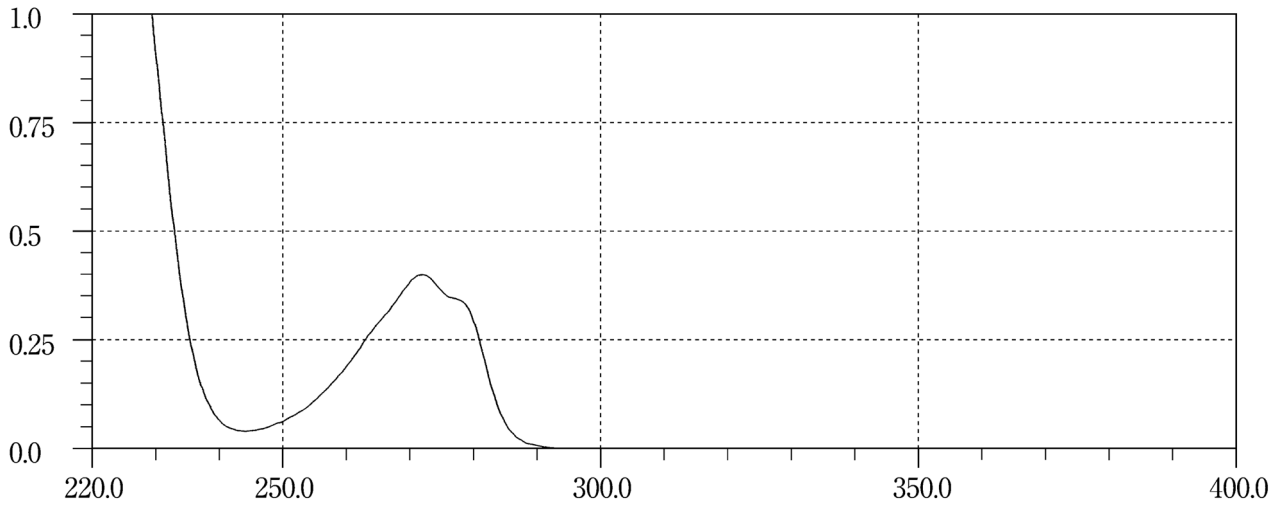
Amlexanox



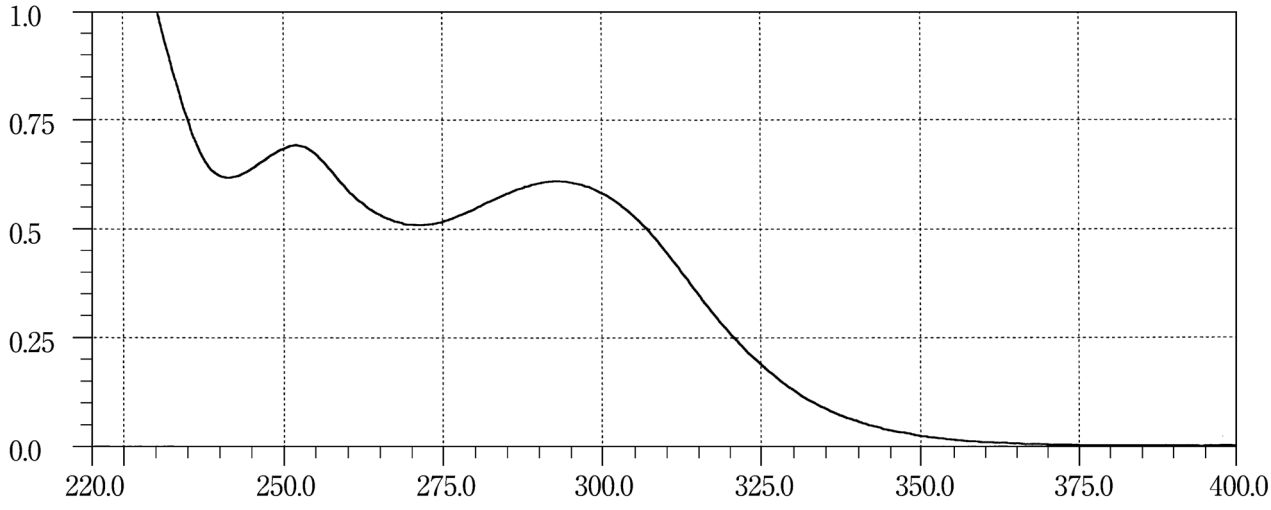
Amlodipine Besilate



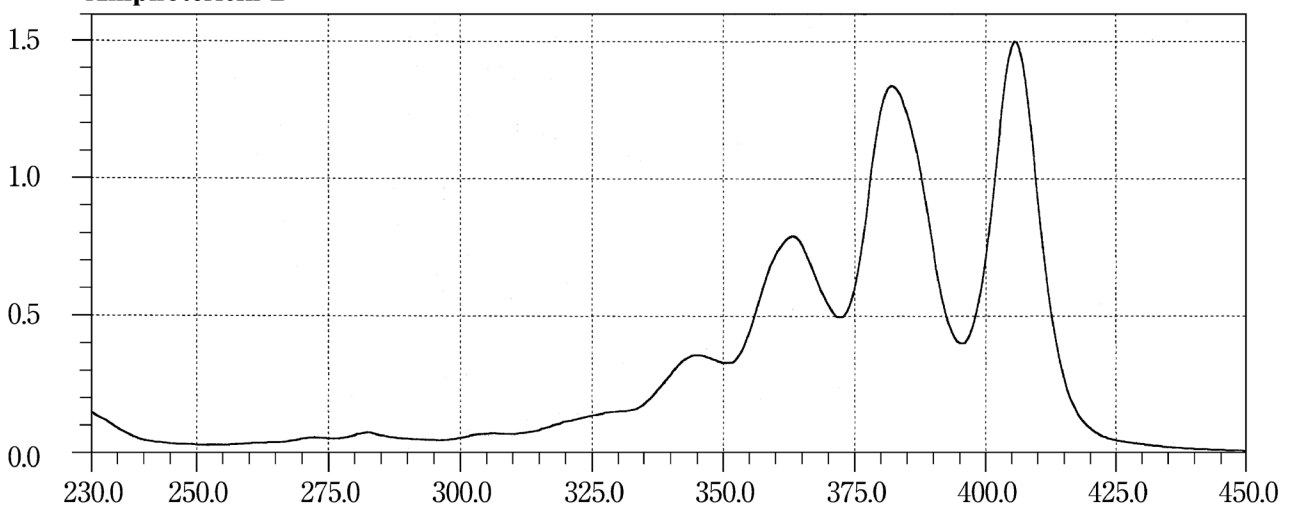
Amosulalol Hydrochloride



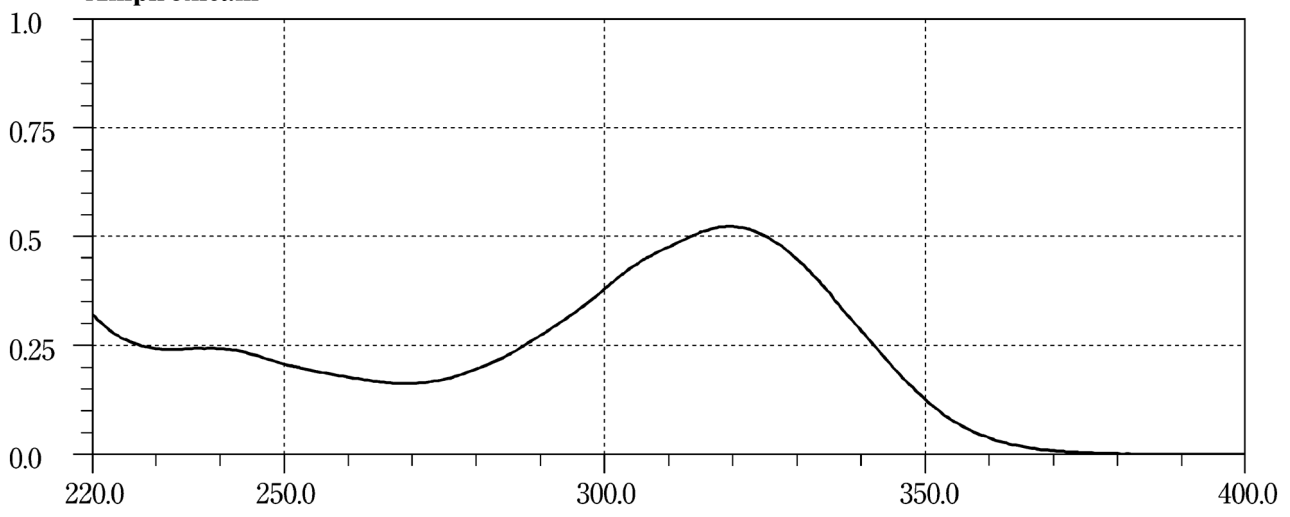
Amoxapine



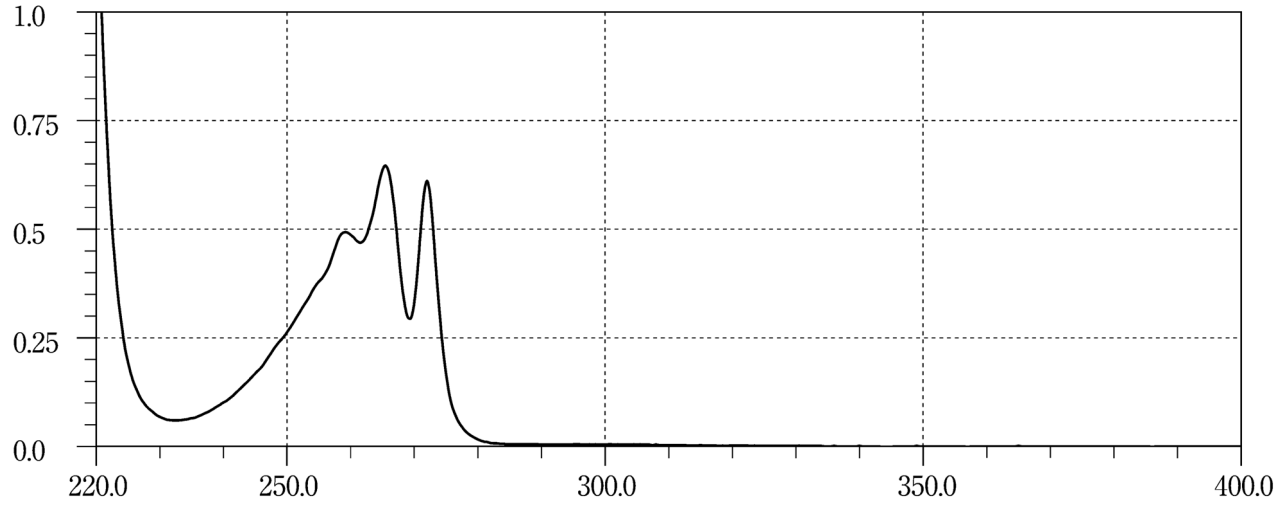
Amphotericin B



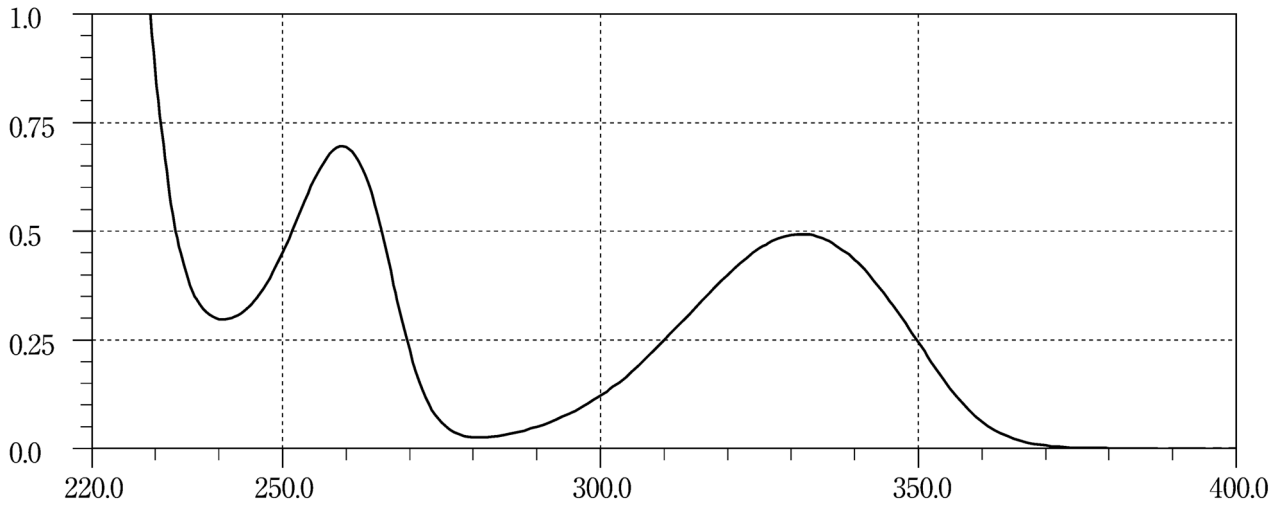
Ampiroxicam



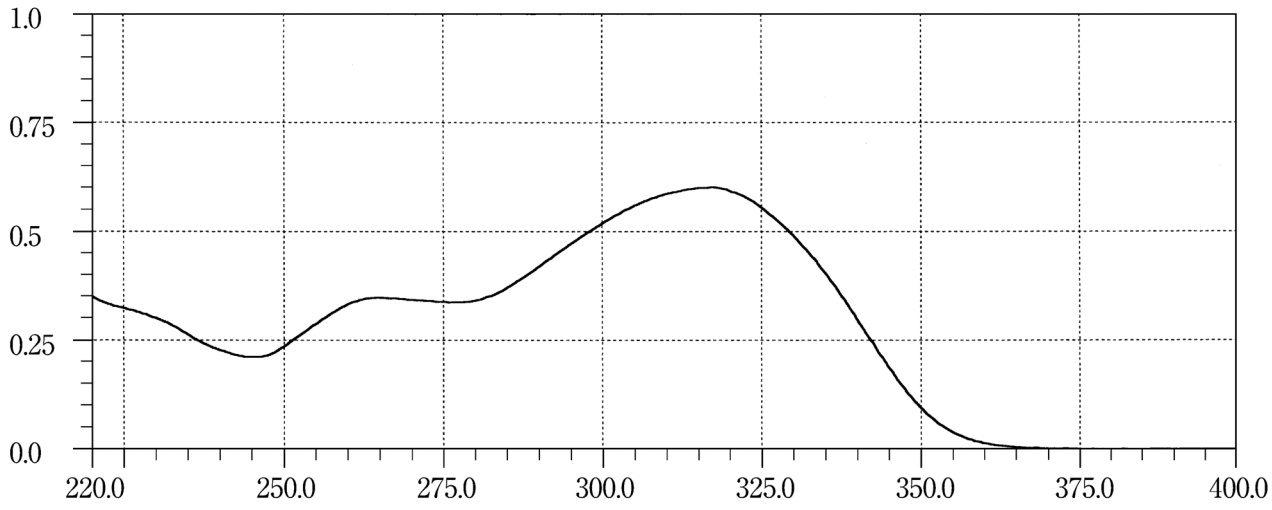
Aprindine Hydrochloride



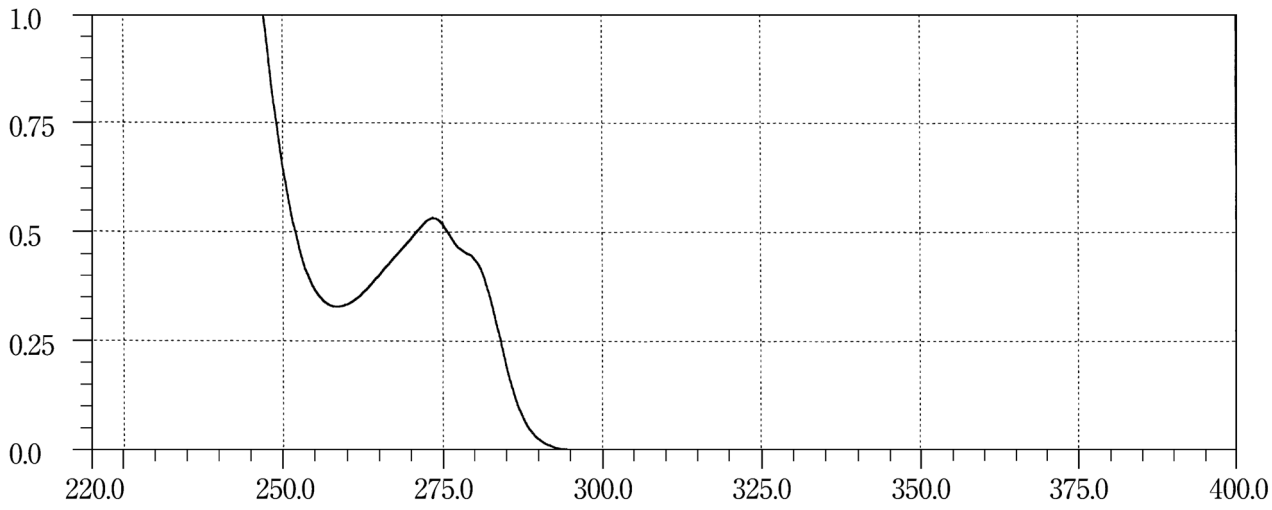
Argatroban Hydrate



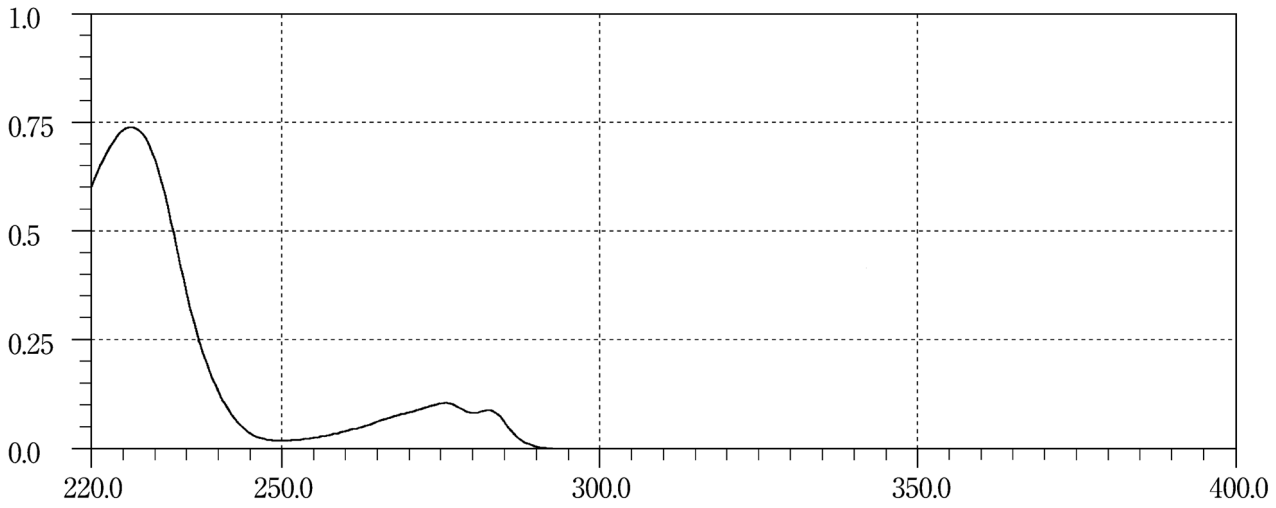
Arotinolol Hydrochloride



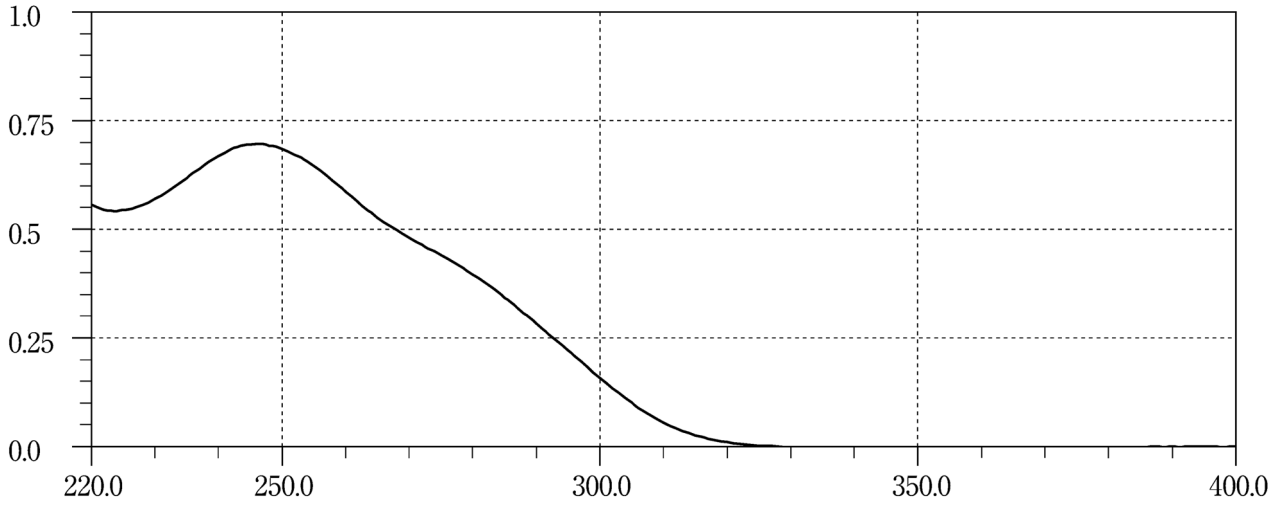
Aspoxicillin Hydrate



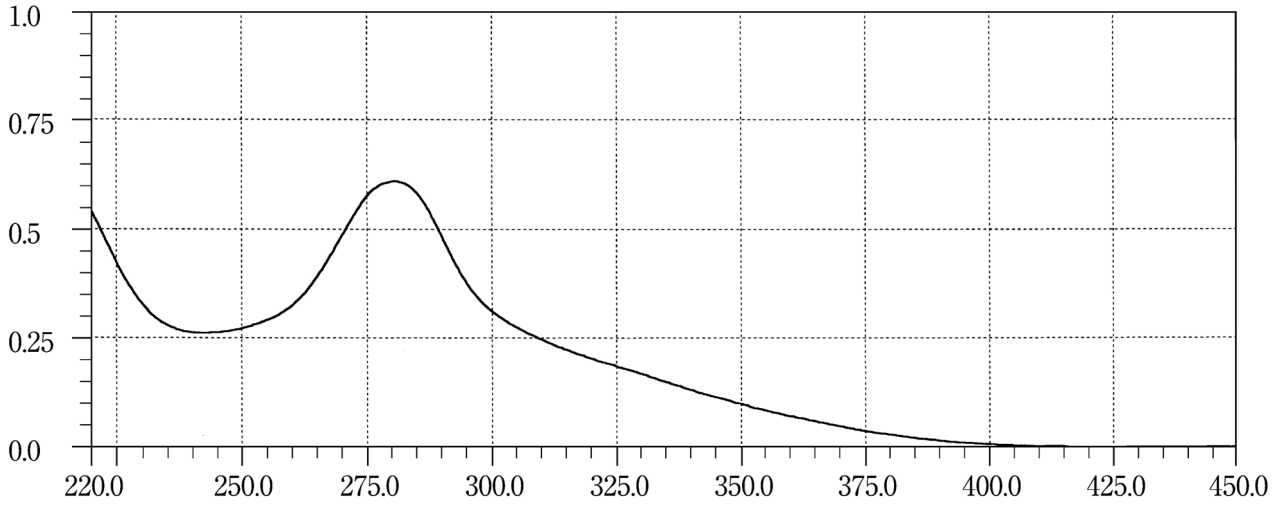
Atenolol



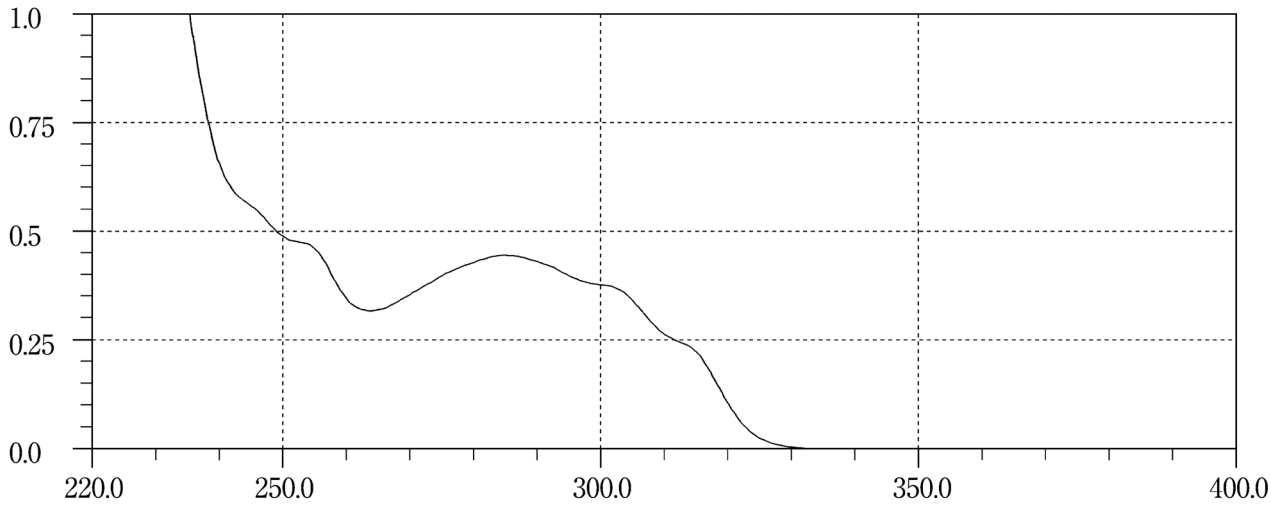
Atorvastatin Calcium Hydrate



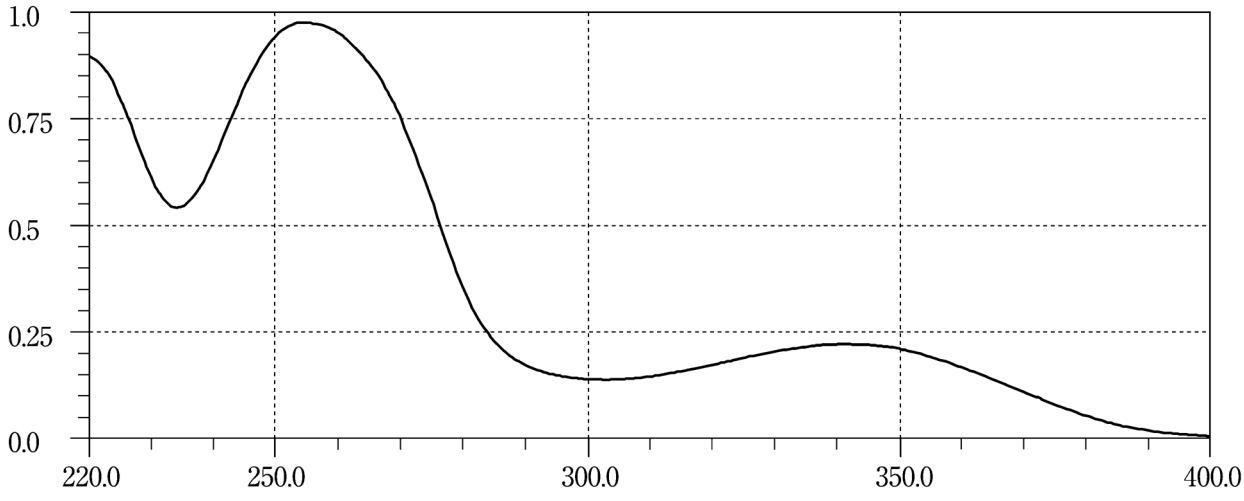
Azathioprine



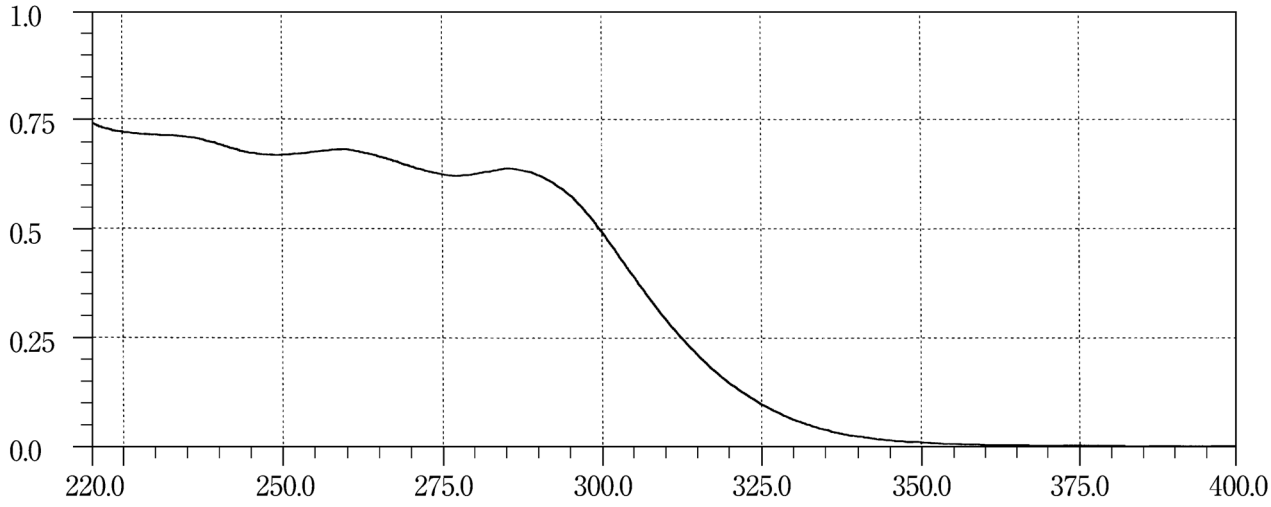
Azelastine Hydrochloride



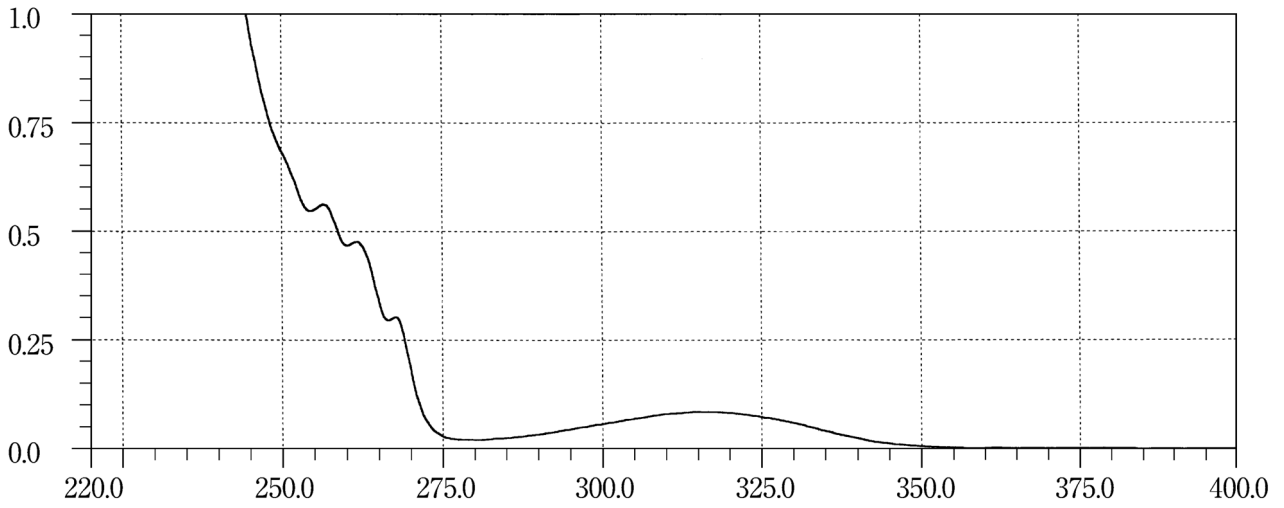
Azelnidipine



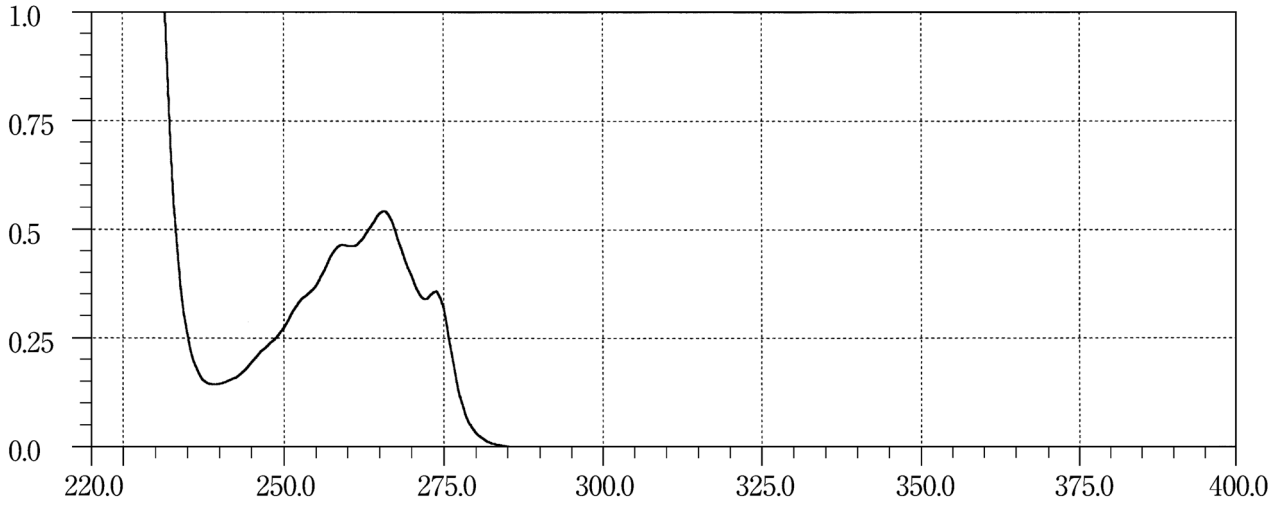
Aztreonam



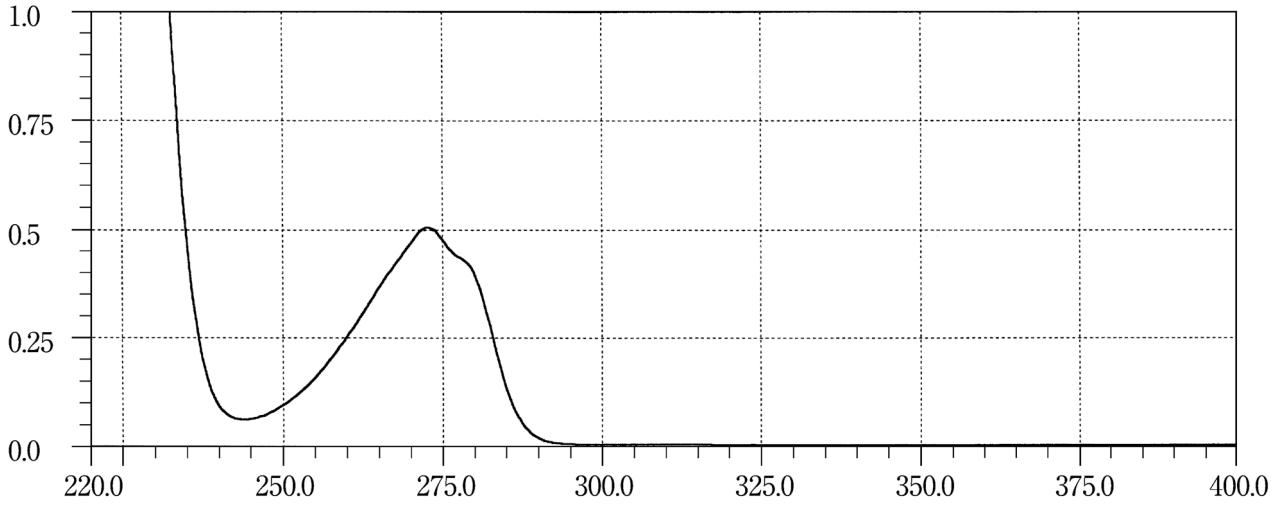
Bacampicillin Hydrochloride



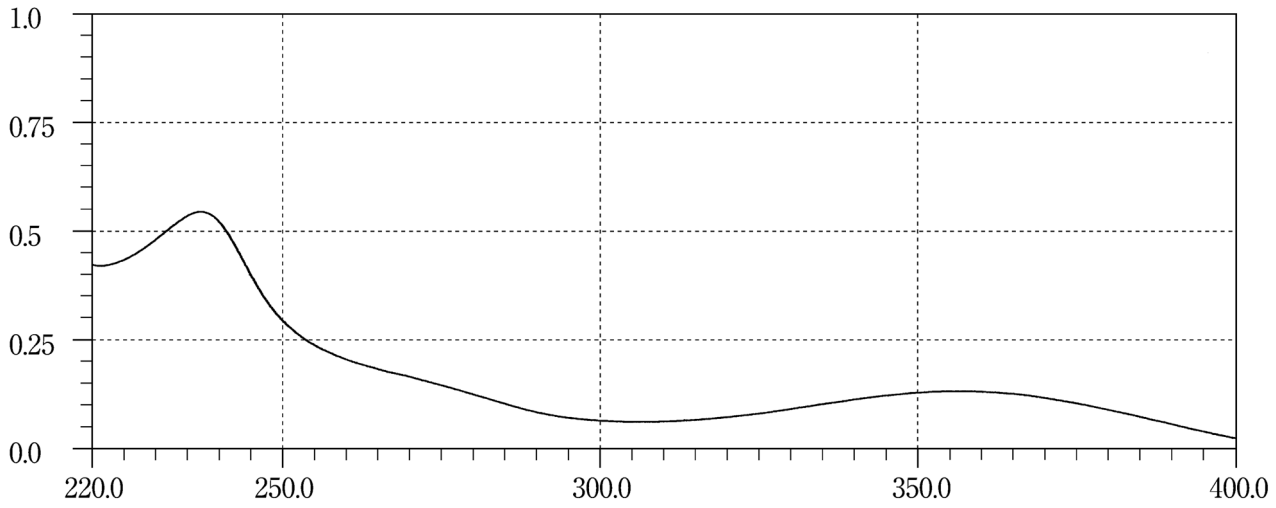
Baclofen



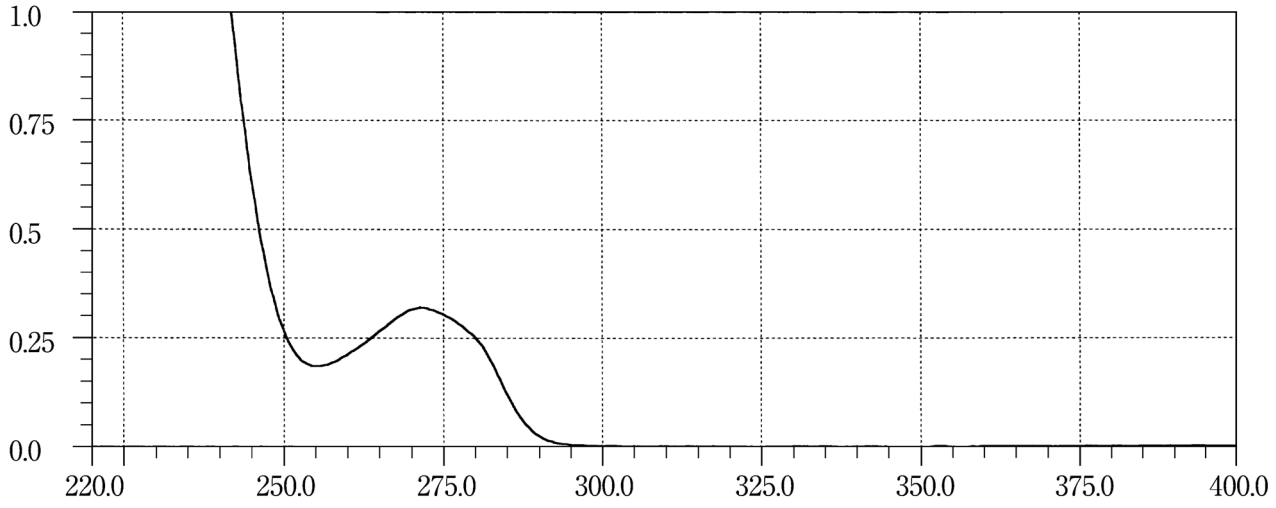
Bamethan Sulfate



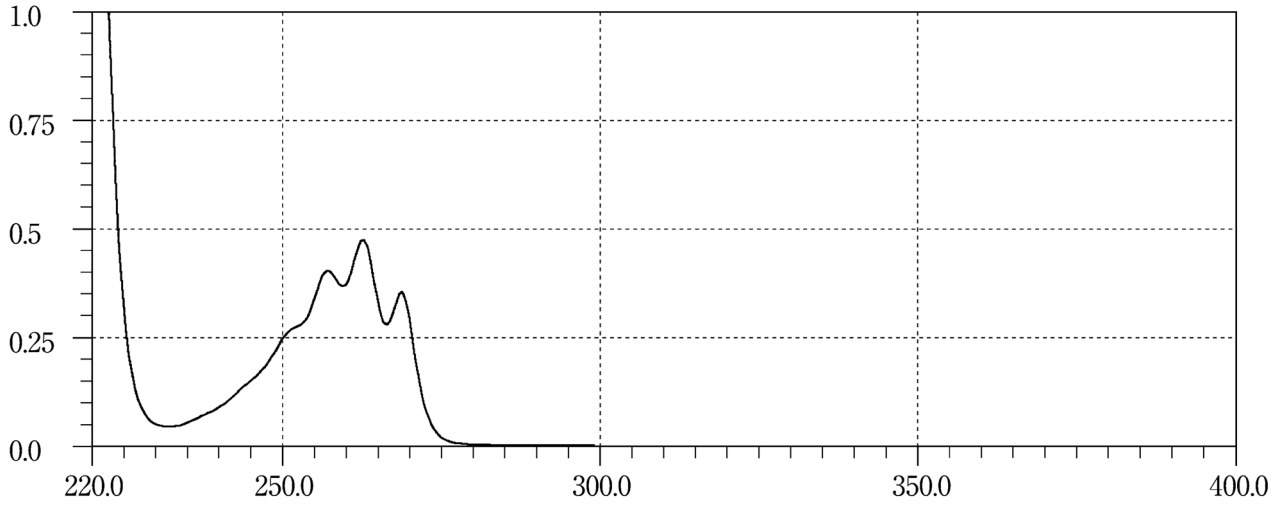
Benidipine Hydrochloride



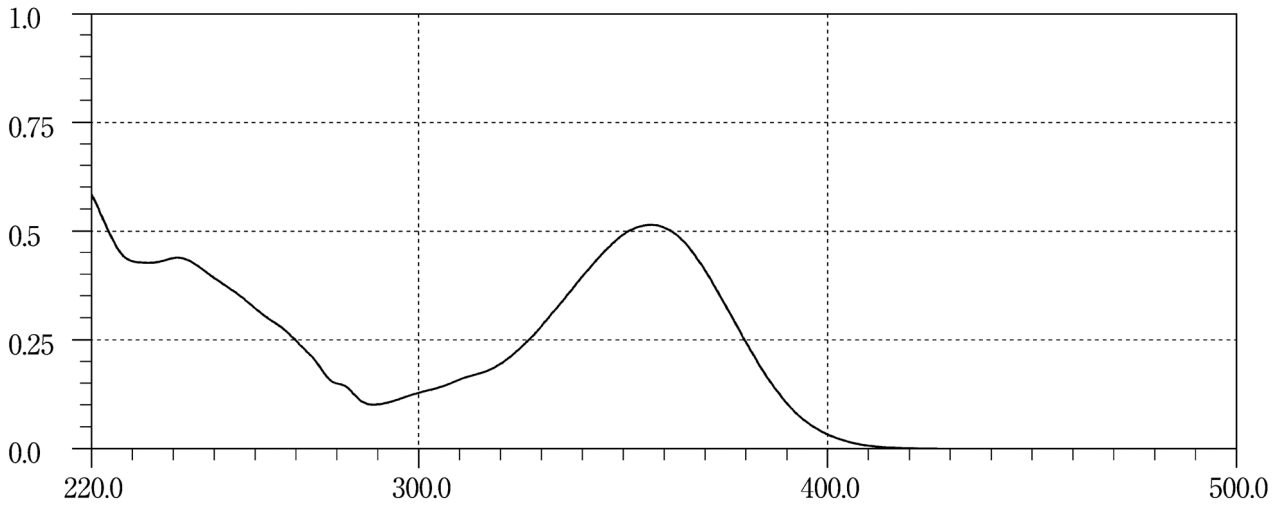
Benserazide Hydrochloride



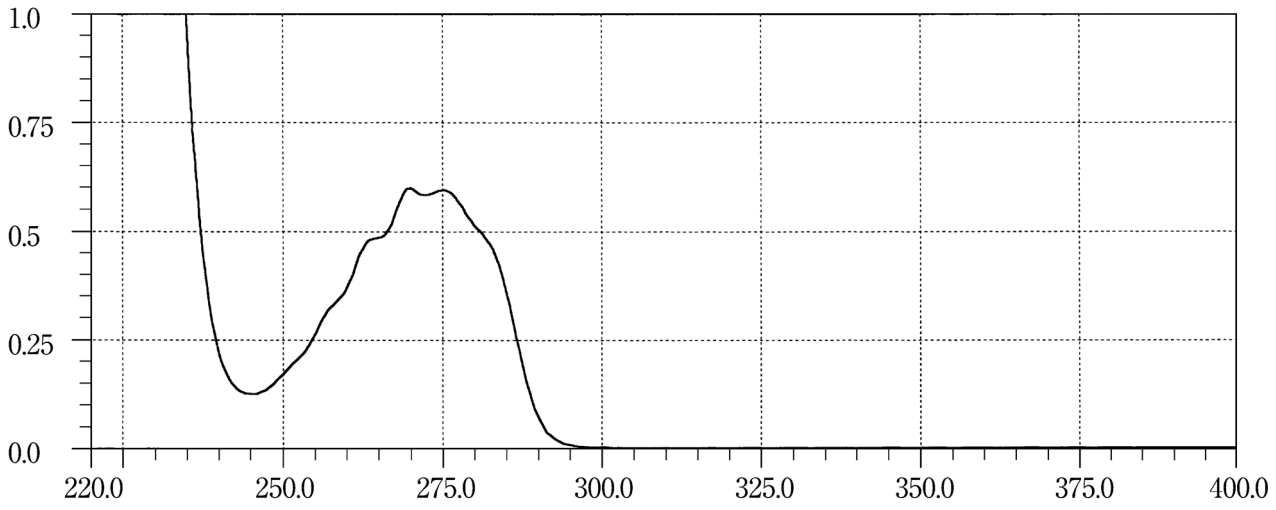
Benzalkonium Chloride



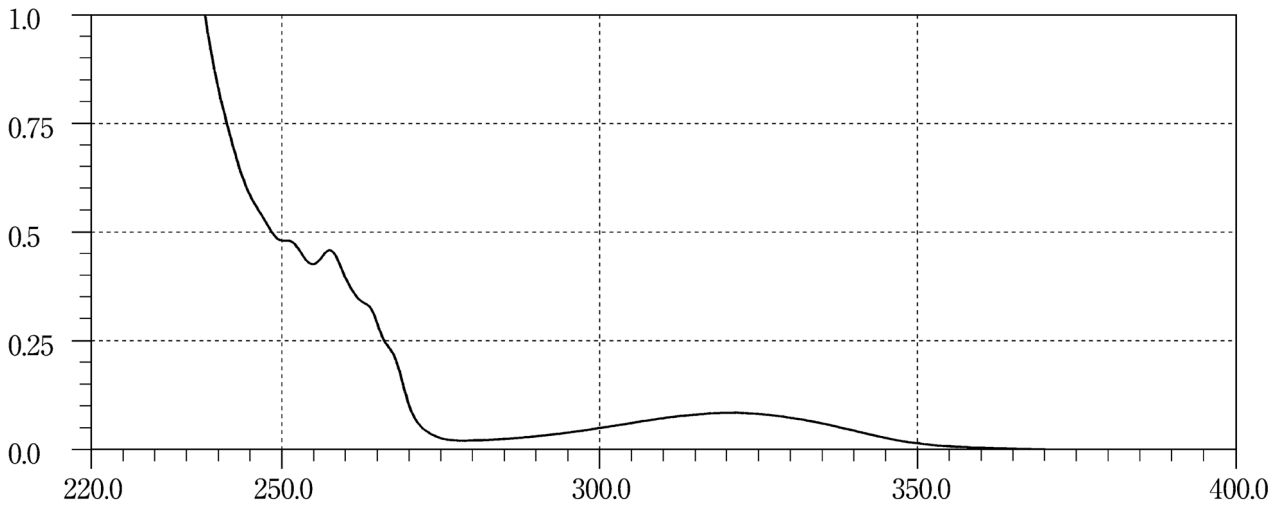
Benzbromarone



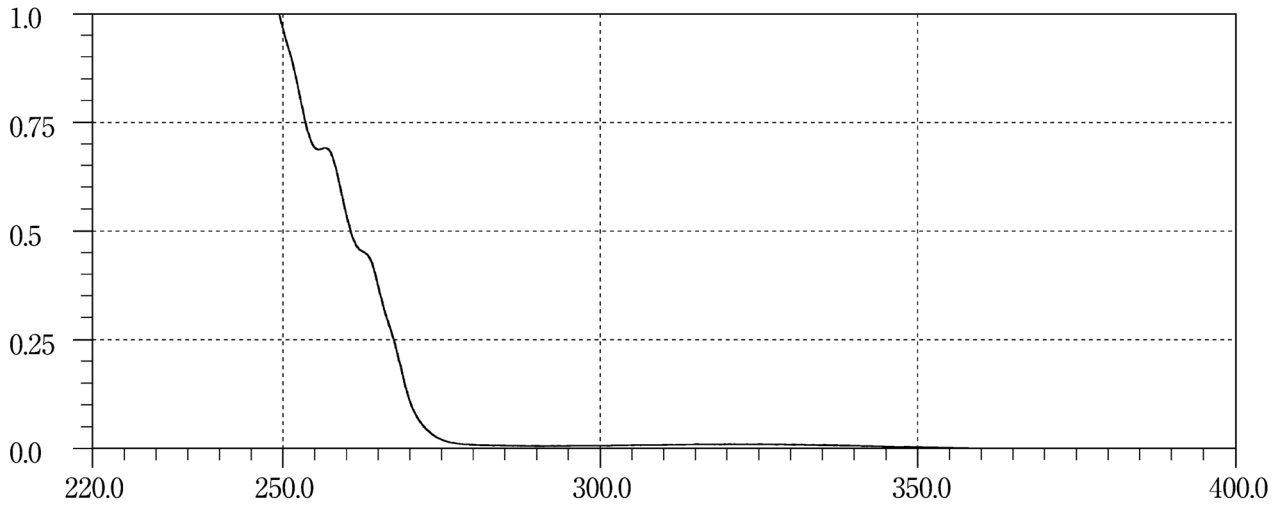
Benzethonium Chloride



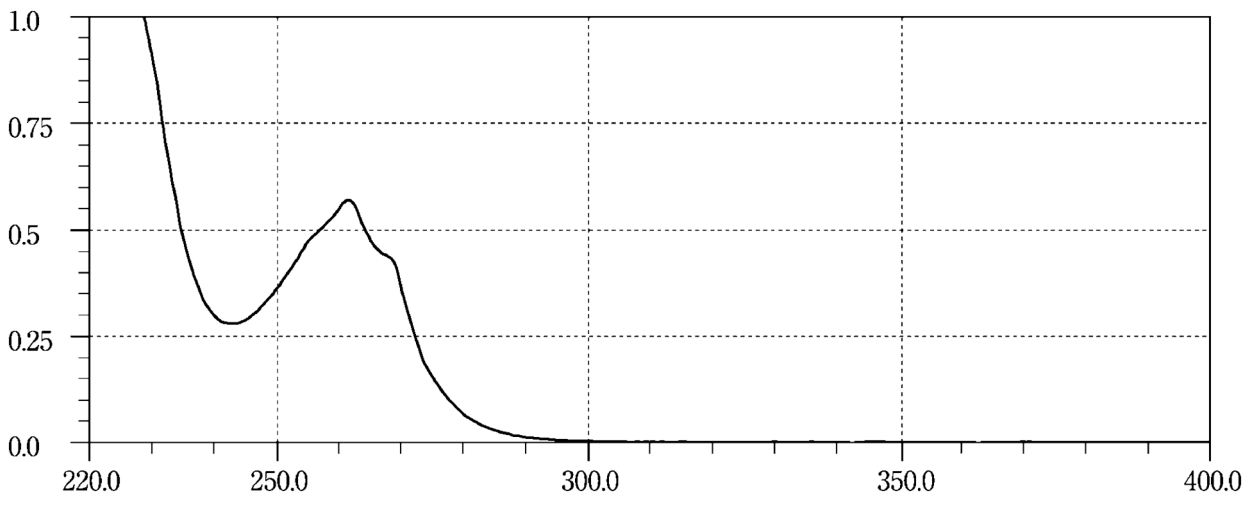
Benzylpenicillin Benzathine Hydrate



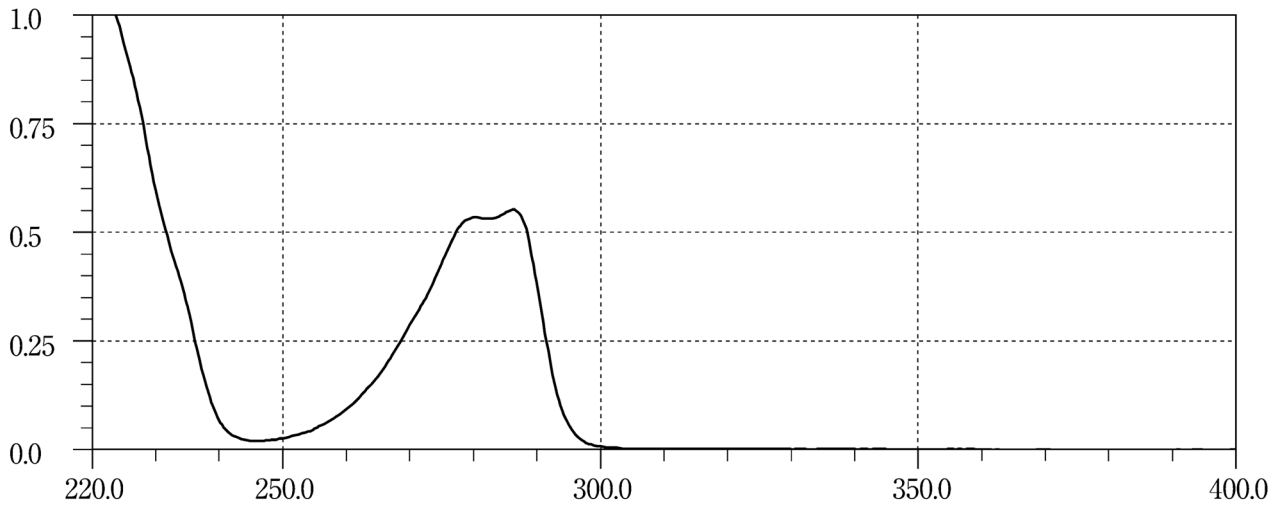
Benzylpenicillin Potassium



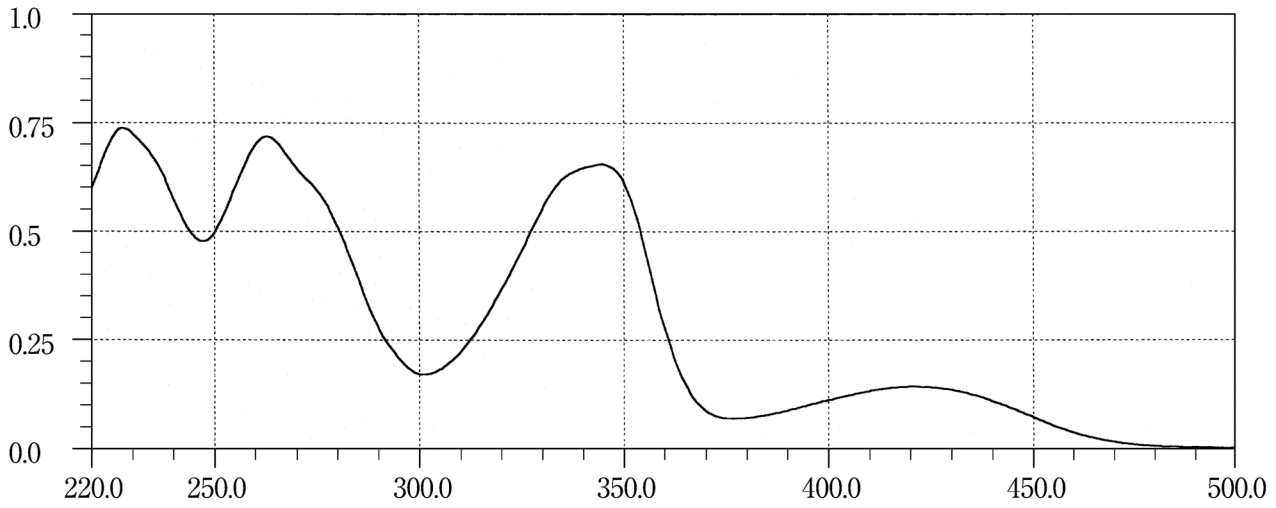
Bepotastine Besilate



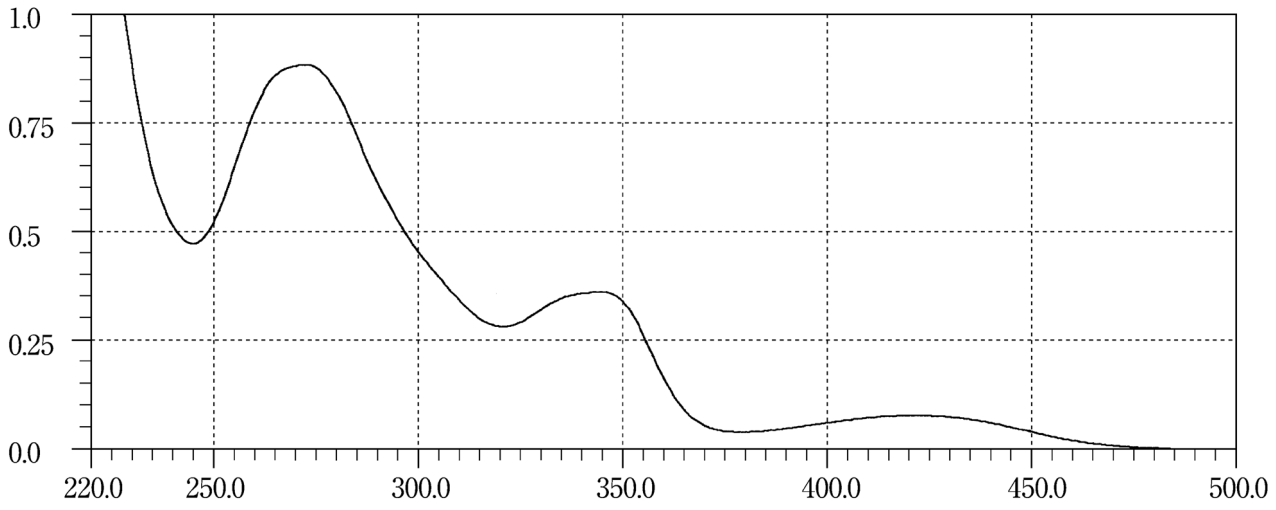
Beraprost Sodium



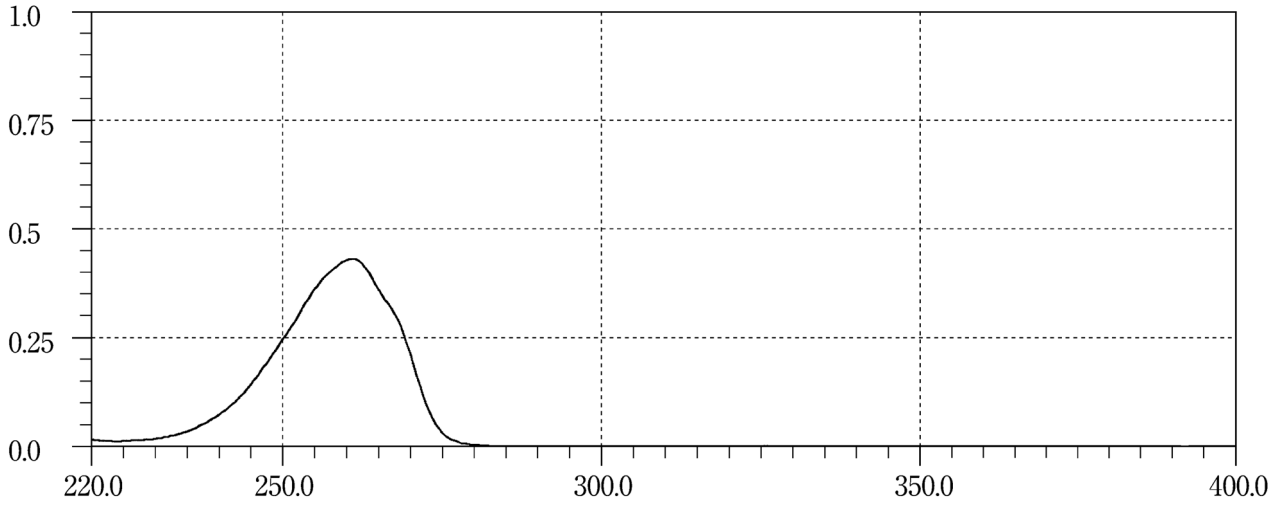
Berberine Chloride Hydrate



Berberine Tannate

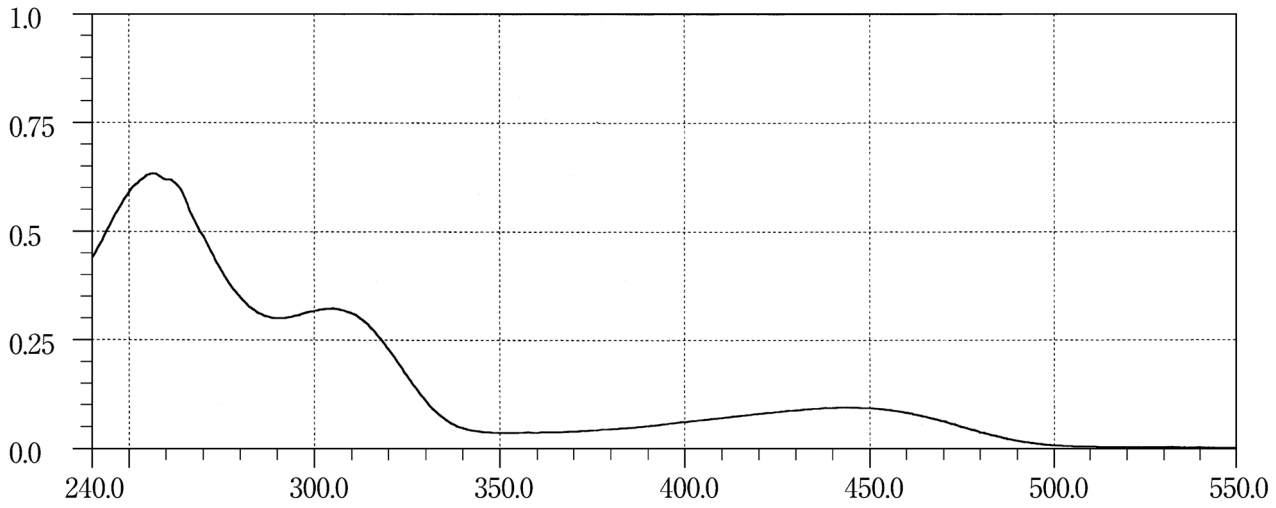


Betahistine Mesilate

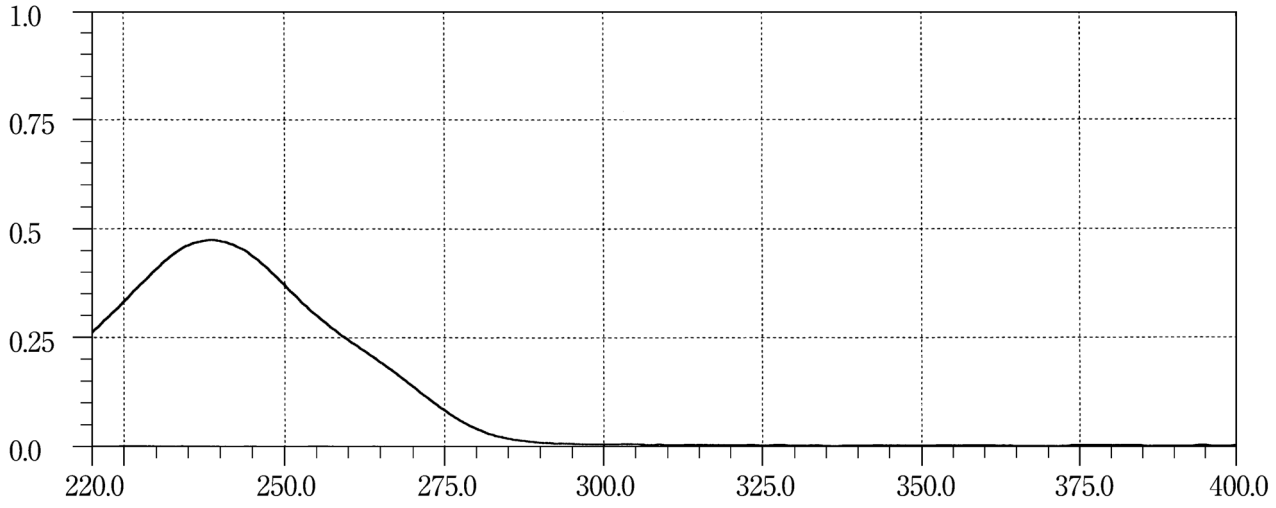


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

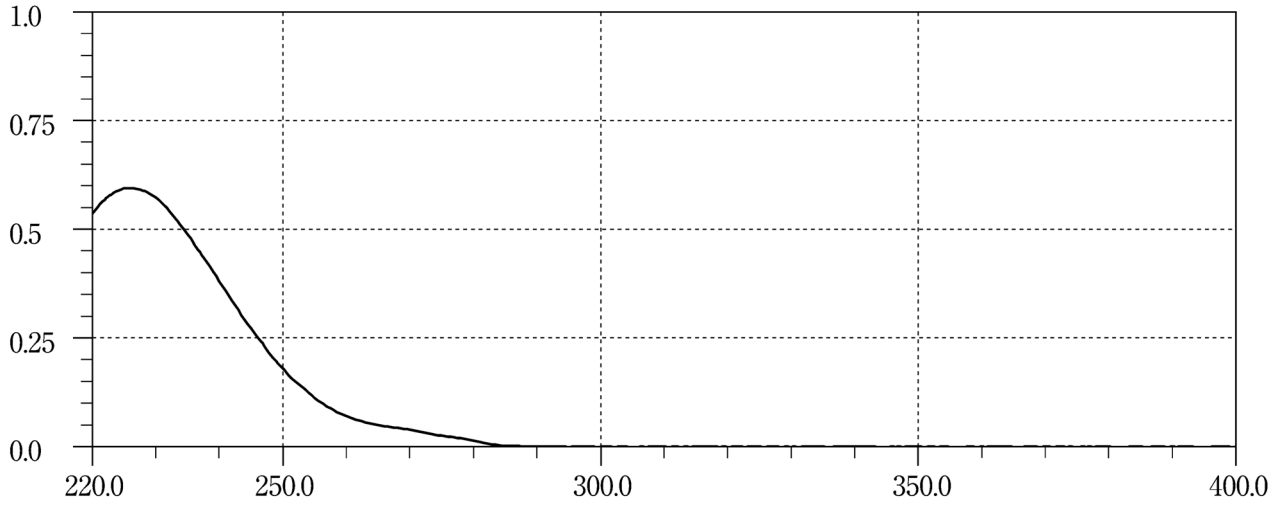
Betamethasone



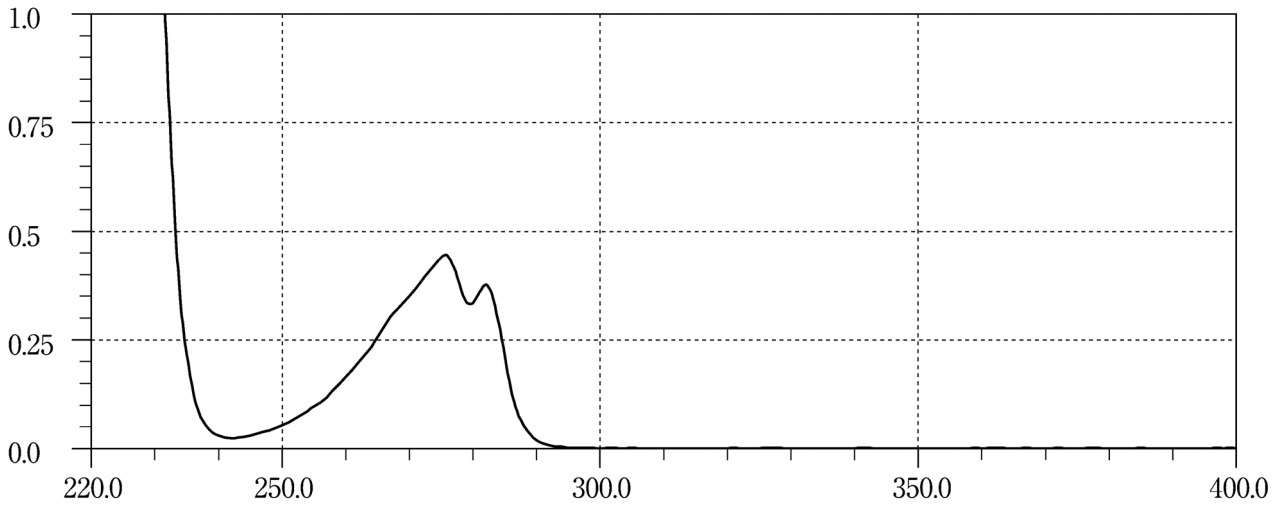
Betamethasone Dipropionate



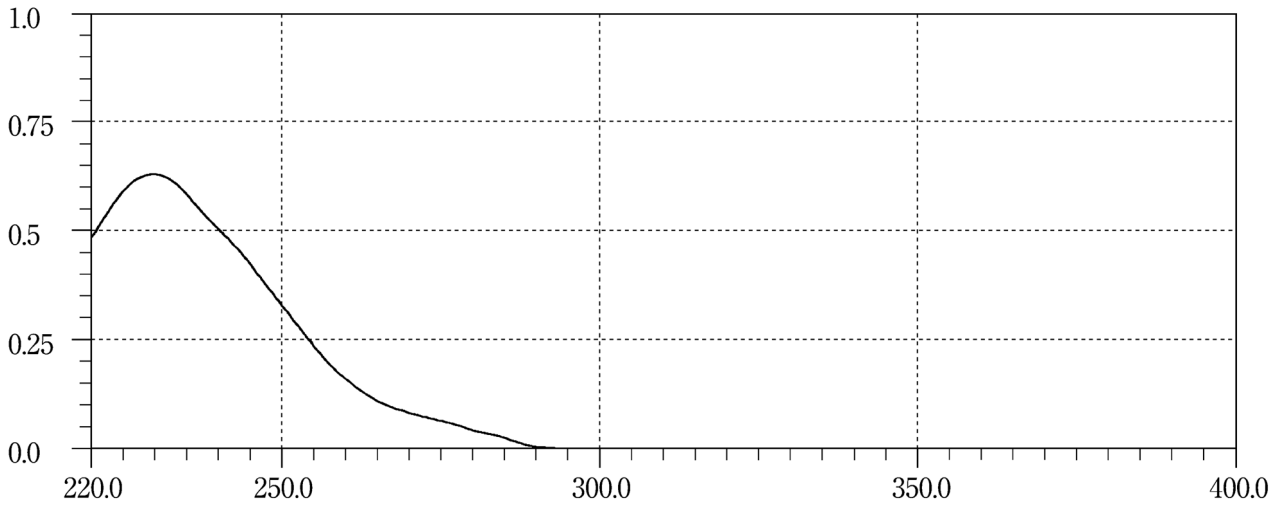
Betamipron



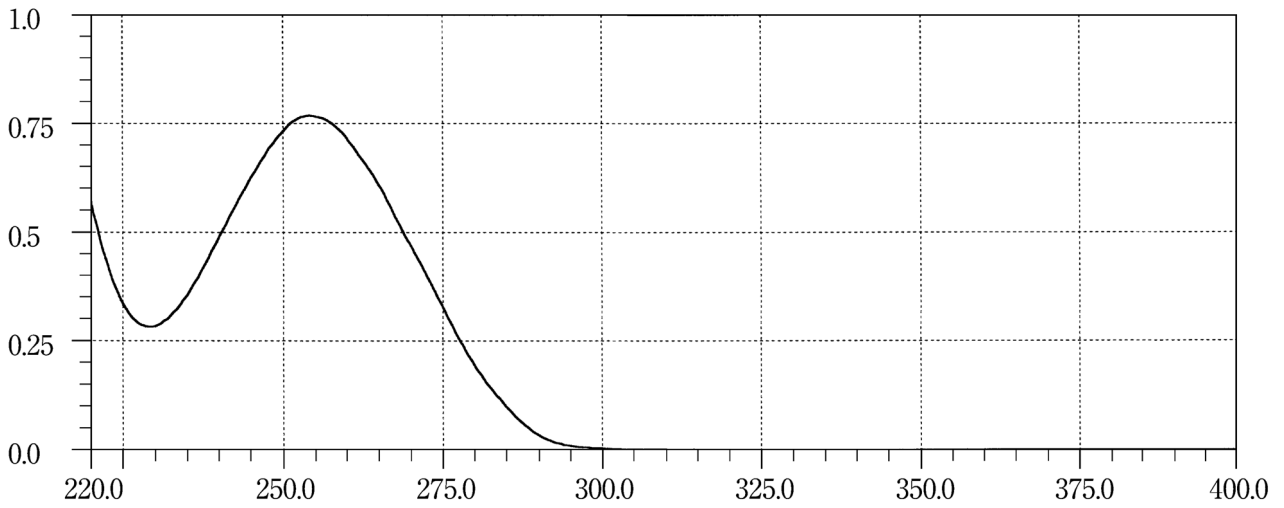
Betaxolol Hydrochloride



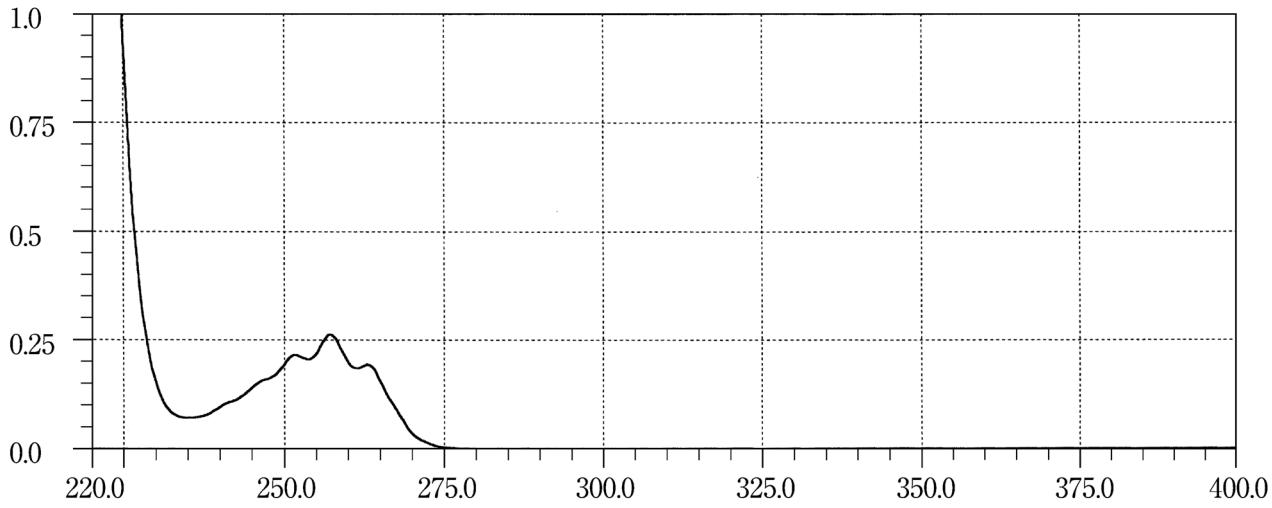
Bezafibrate



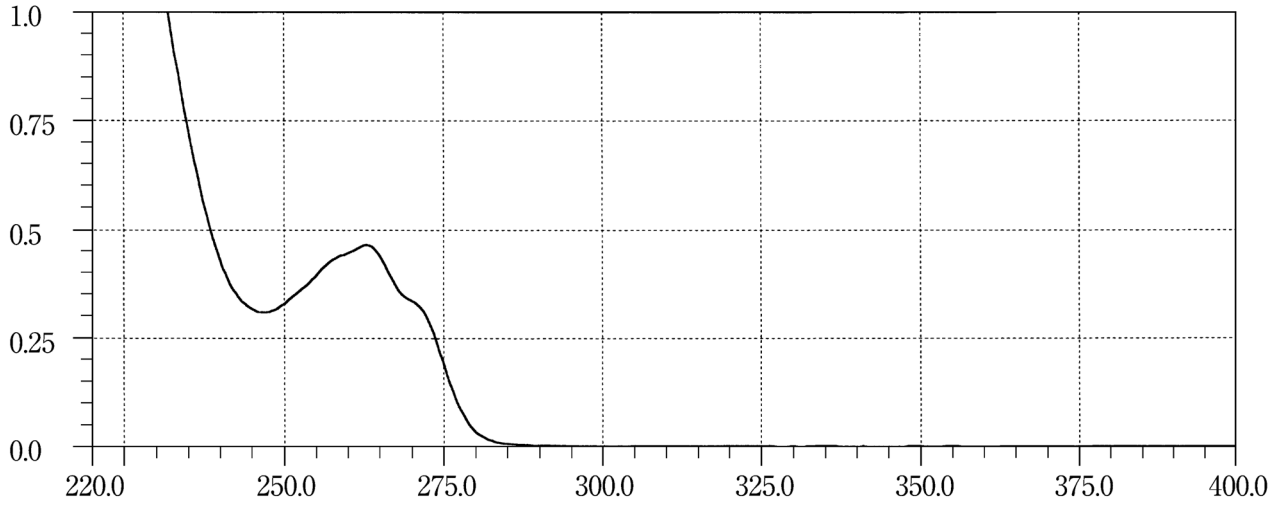
Bifonazole



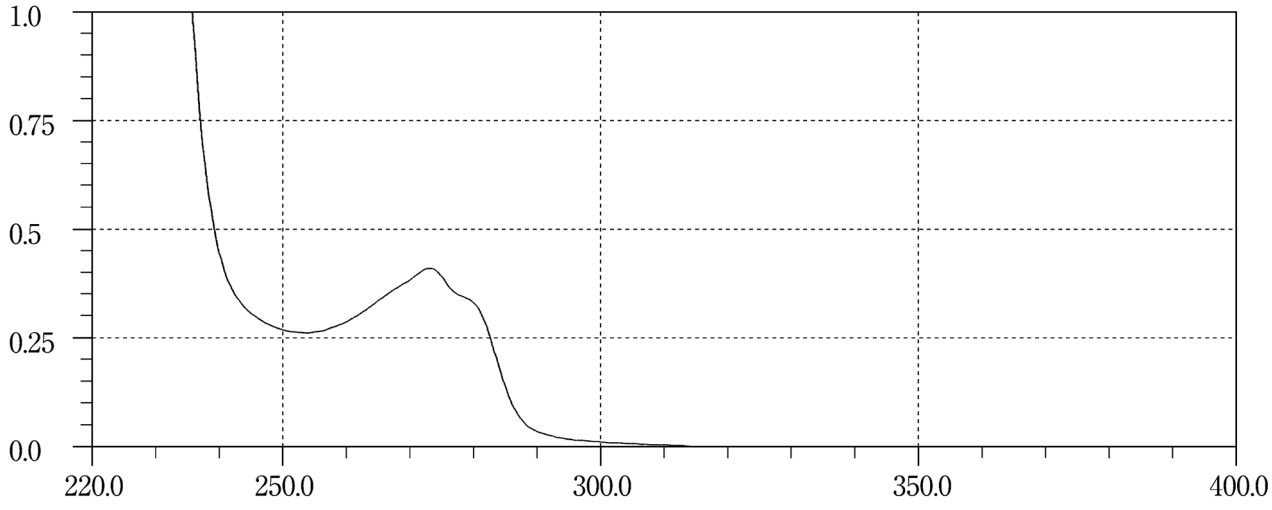
Biperiden Hydrochloride



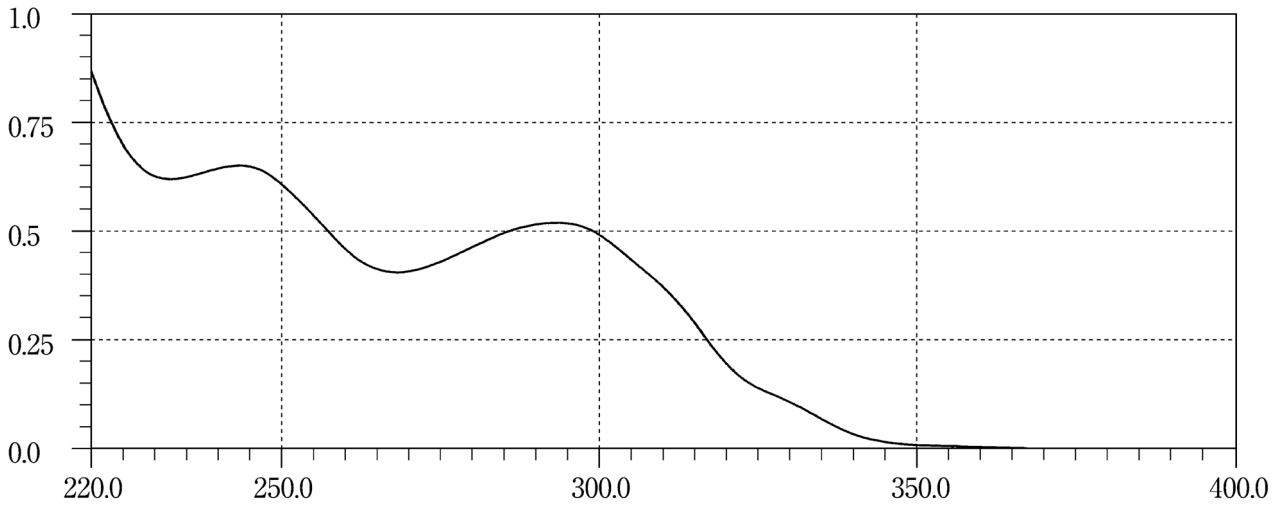
Bisacodyl



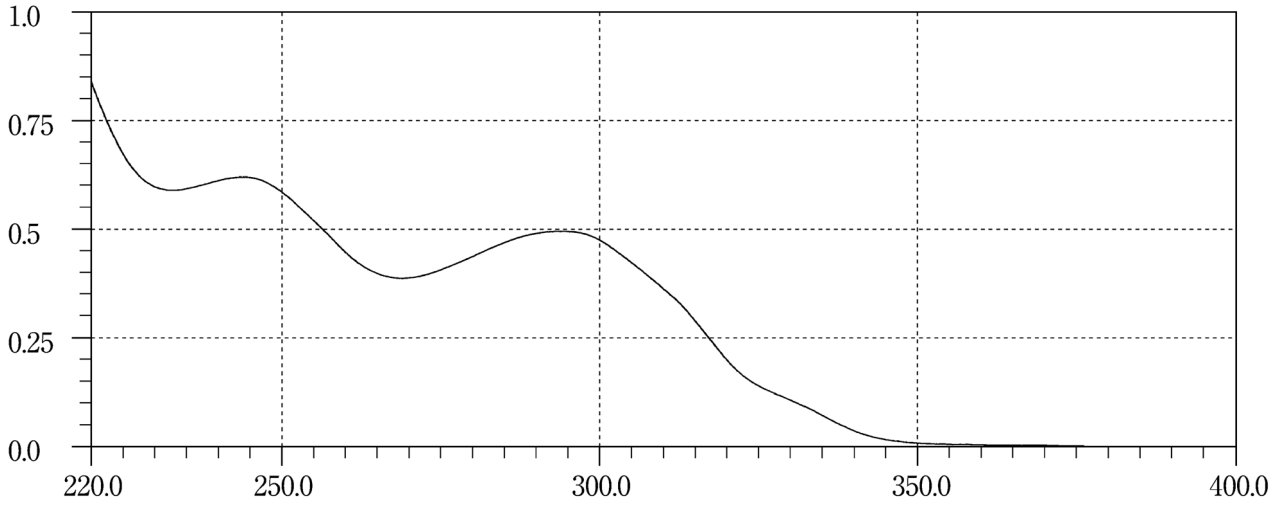
Bisoprolol Fumarate



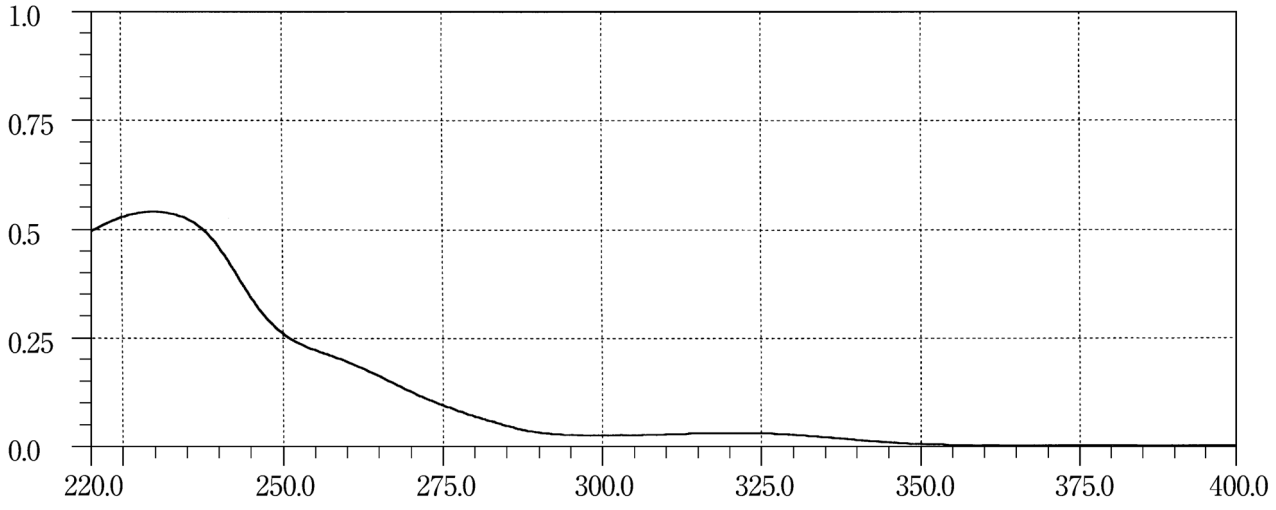
Bleomycin Hydrochloride



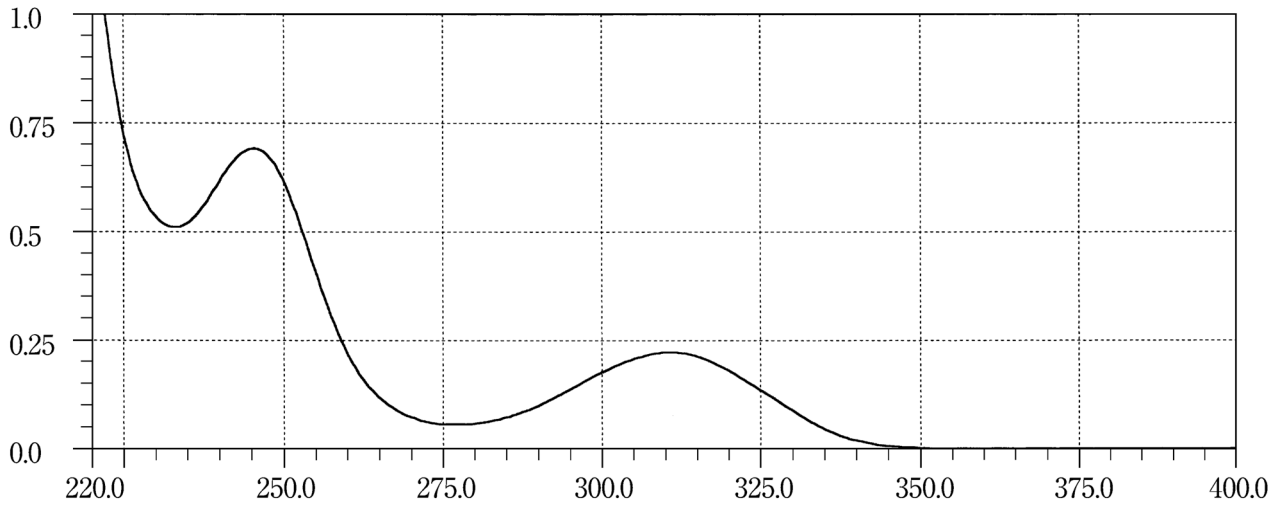
Bleomycin Sulfate



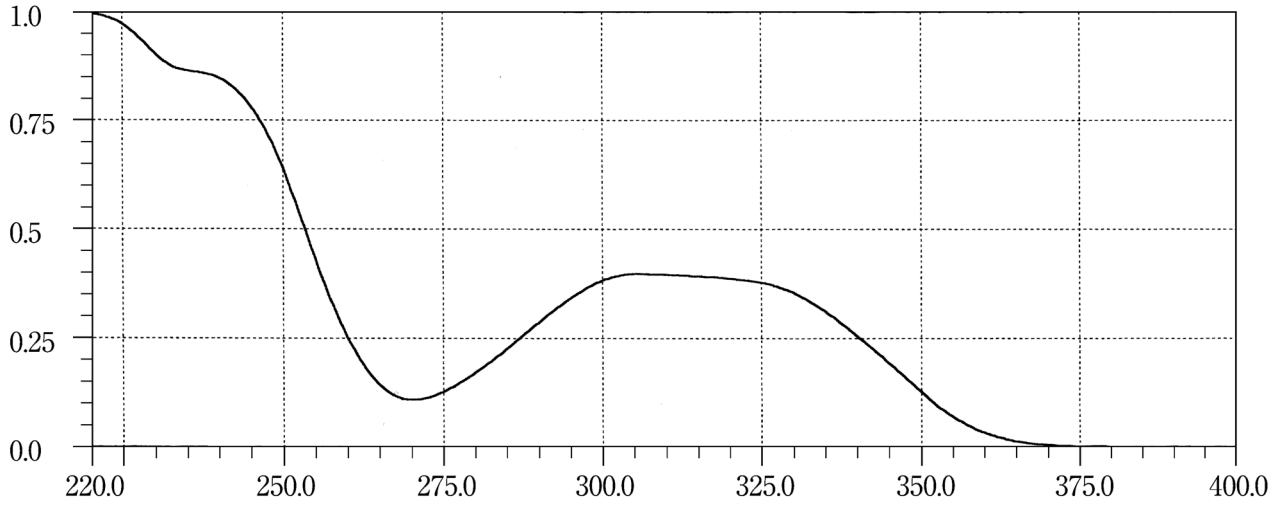
Bromazepam



Bromhexine Hydrochloride



Bromocriptine Mesilate



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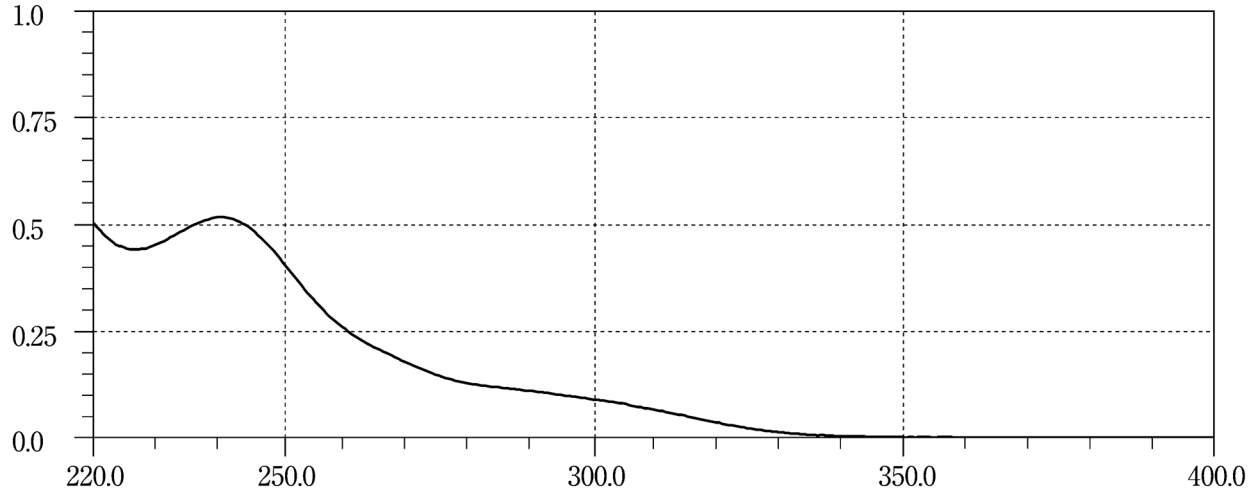
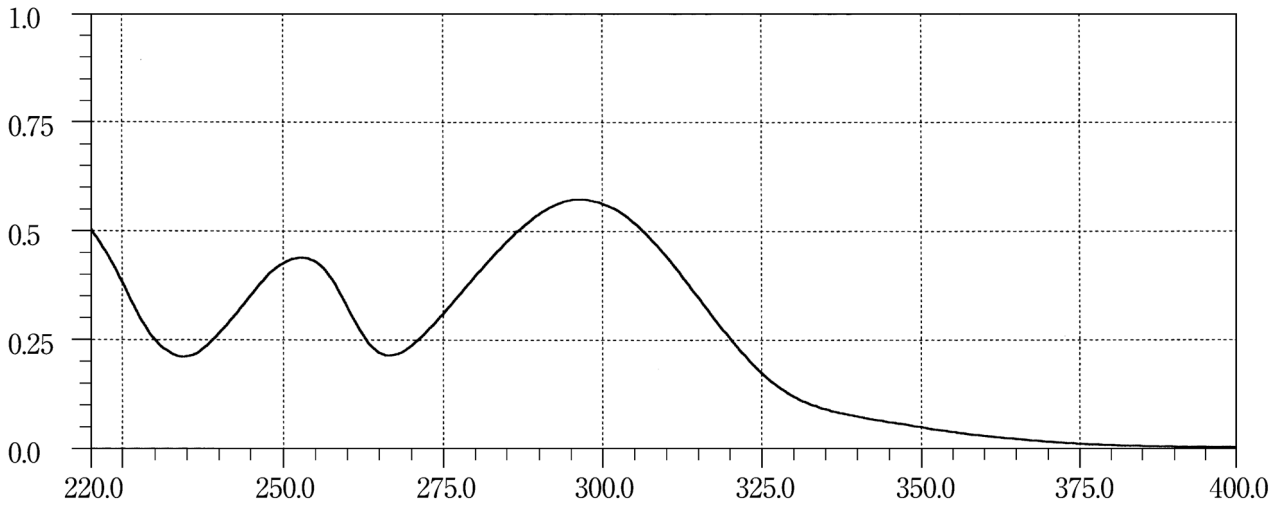
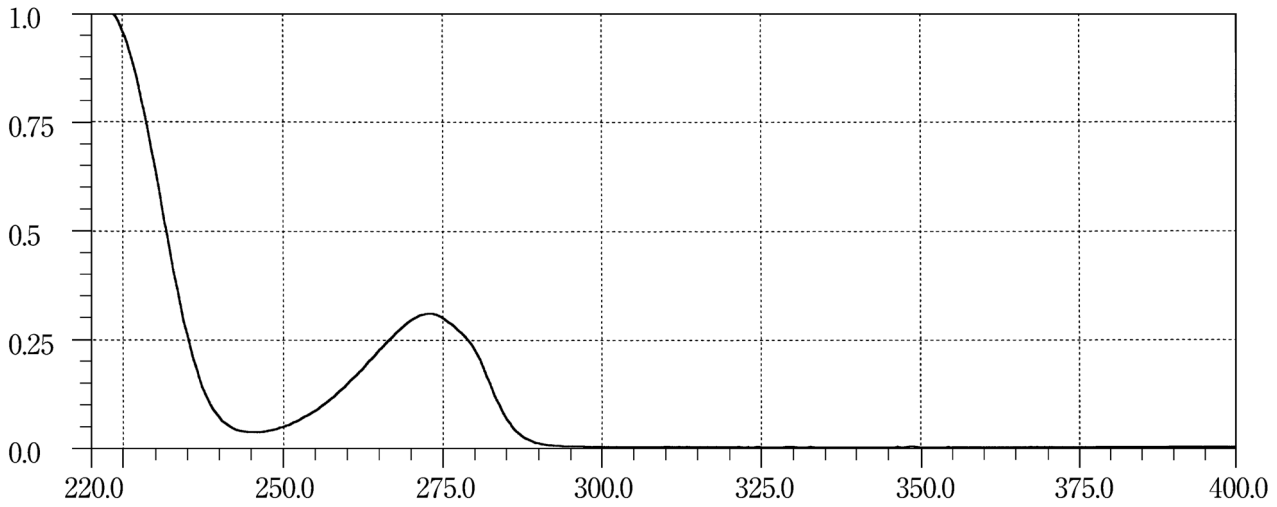


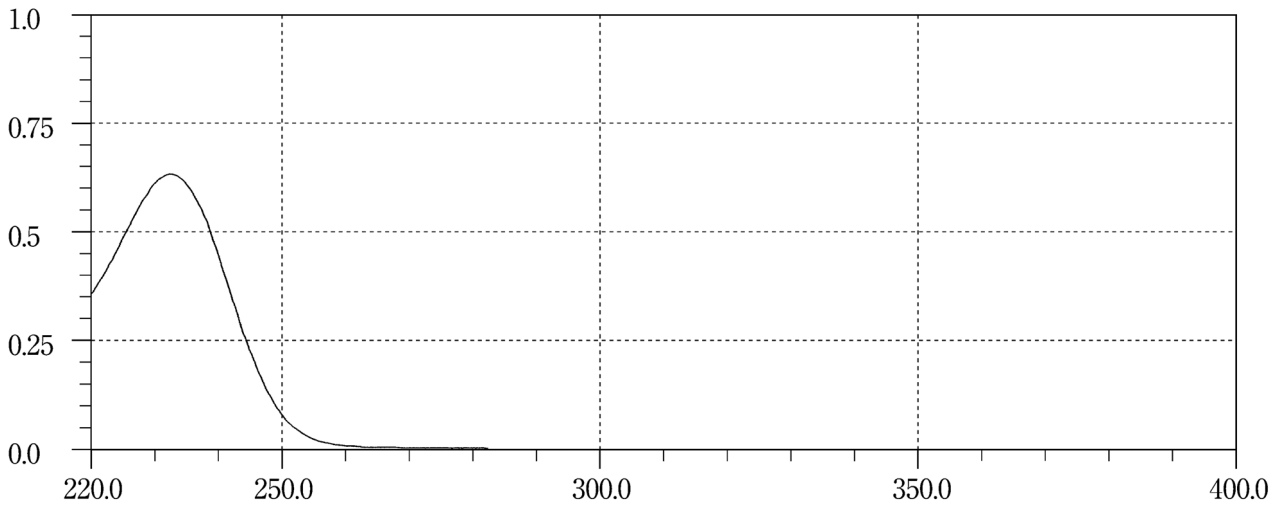
Bucumolol Hydrochloride



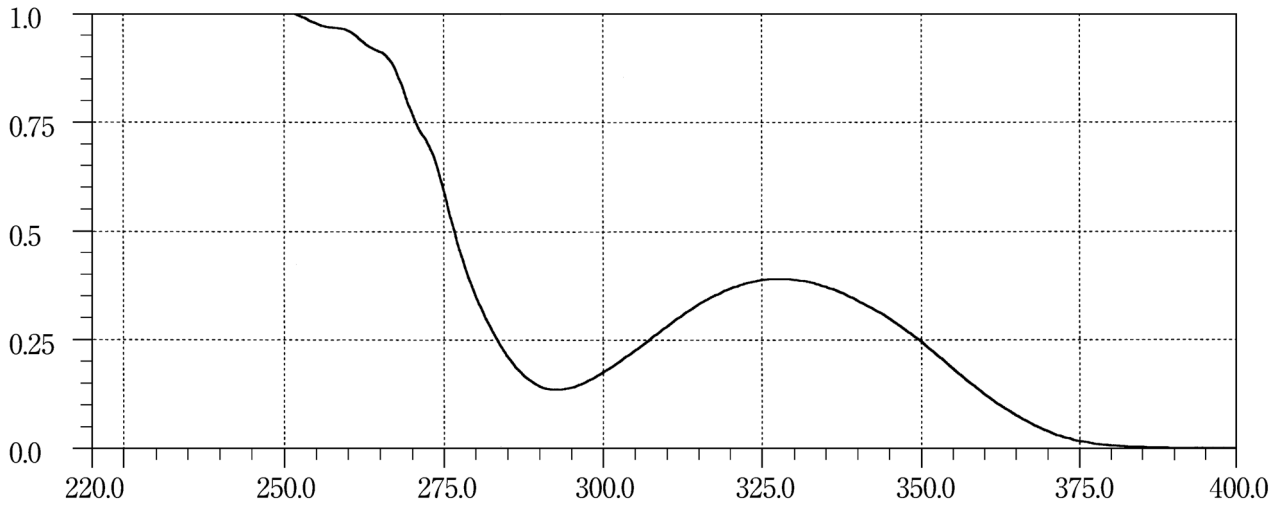
Bufetolol Hydrochloride



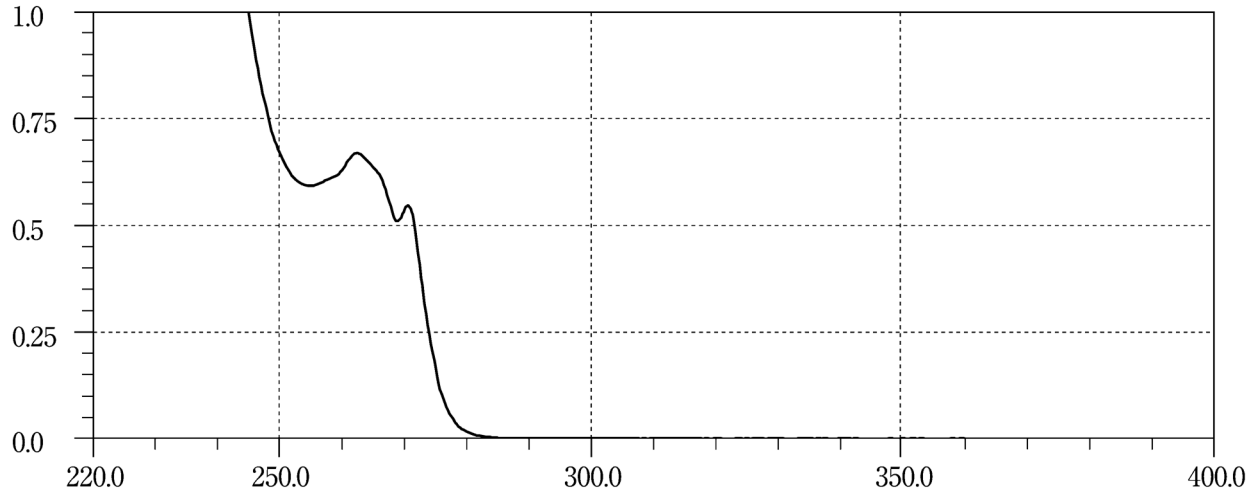
Buformin Hydrochloride



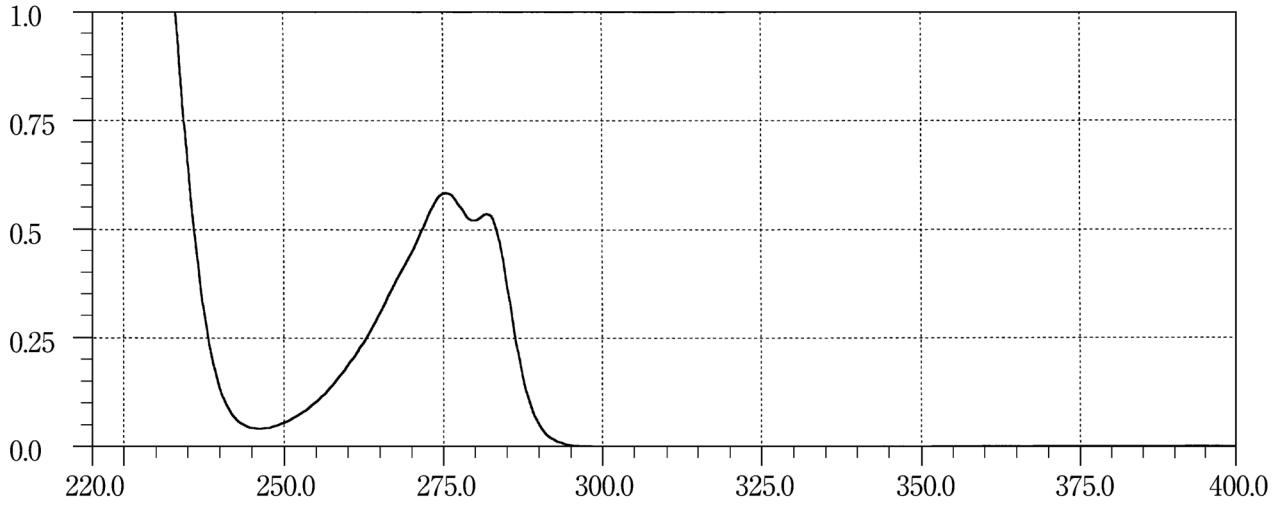
Bumetanide



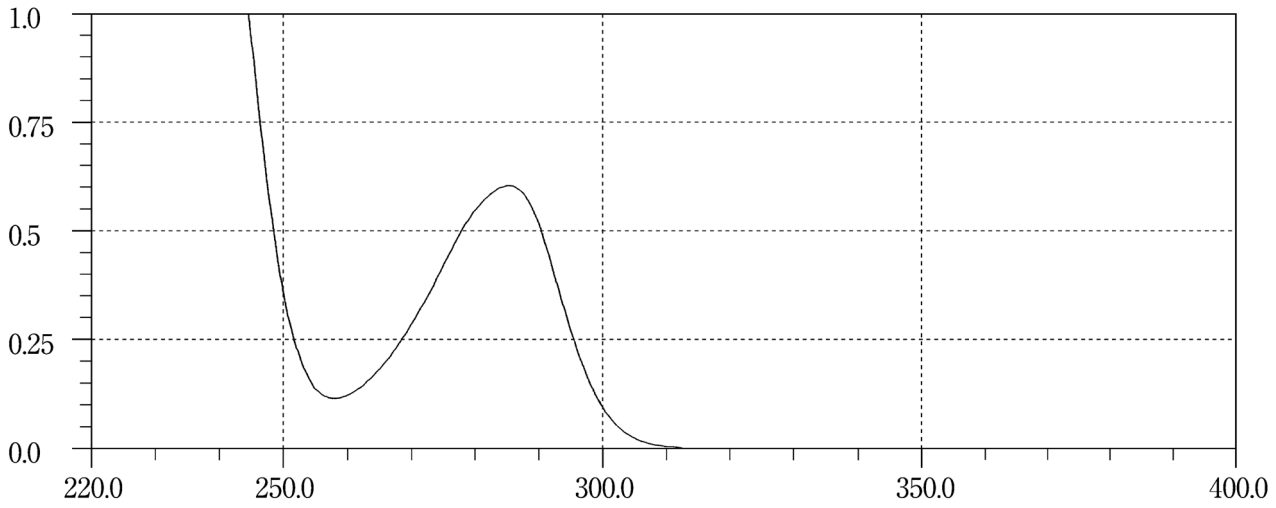
Bupivacaine Hydrochloride Hydrate



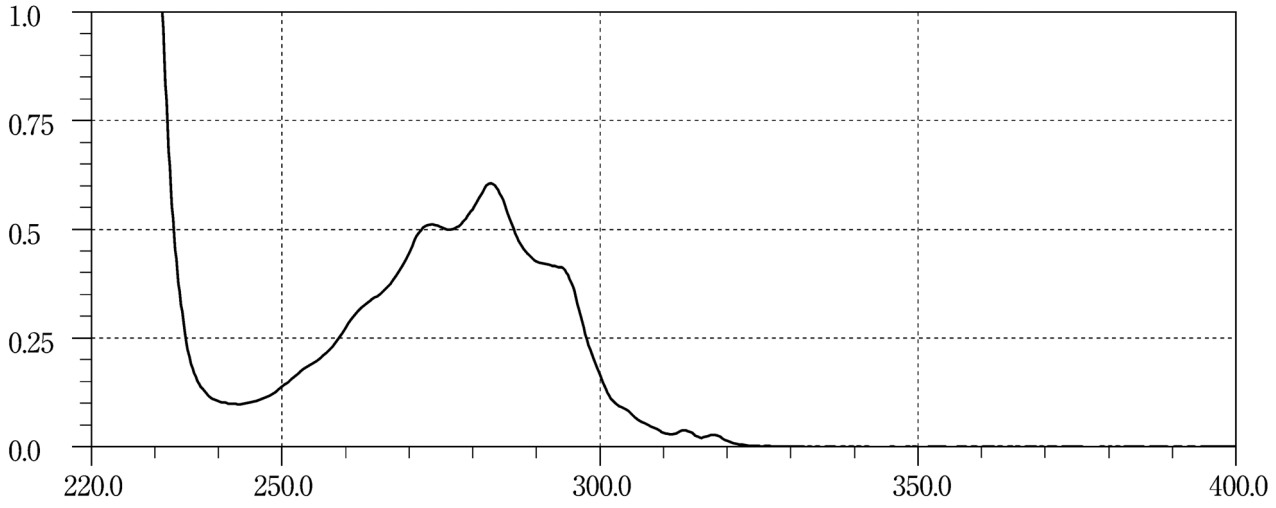
Bupranolol Hydrochloride



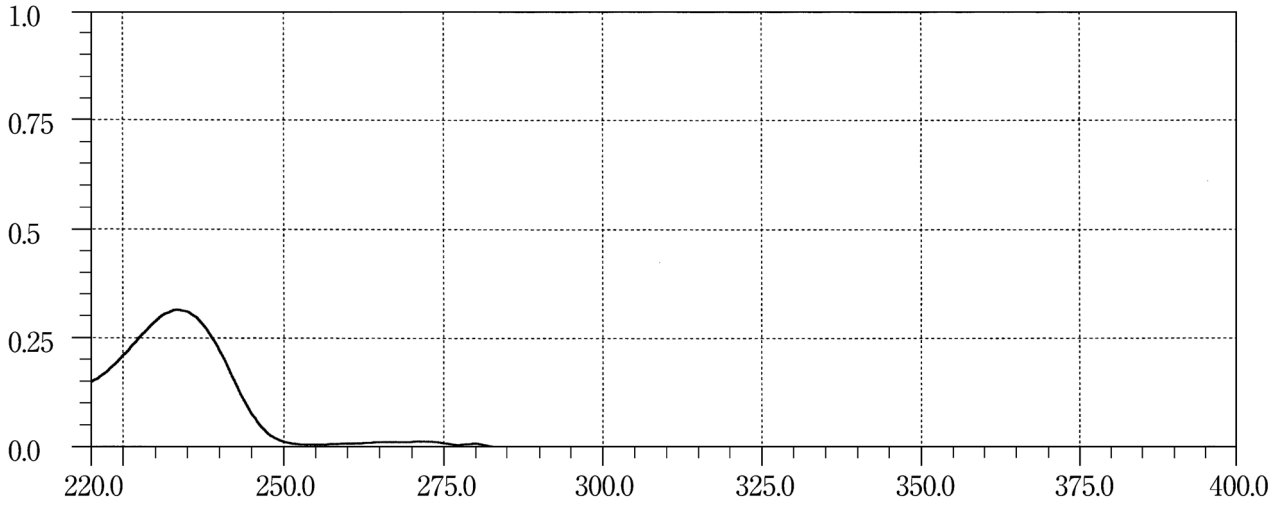
Buprenorphine Hydrochloride



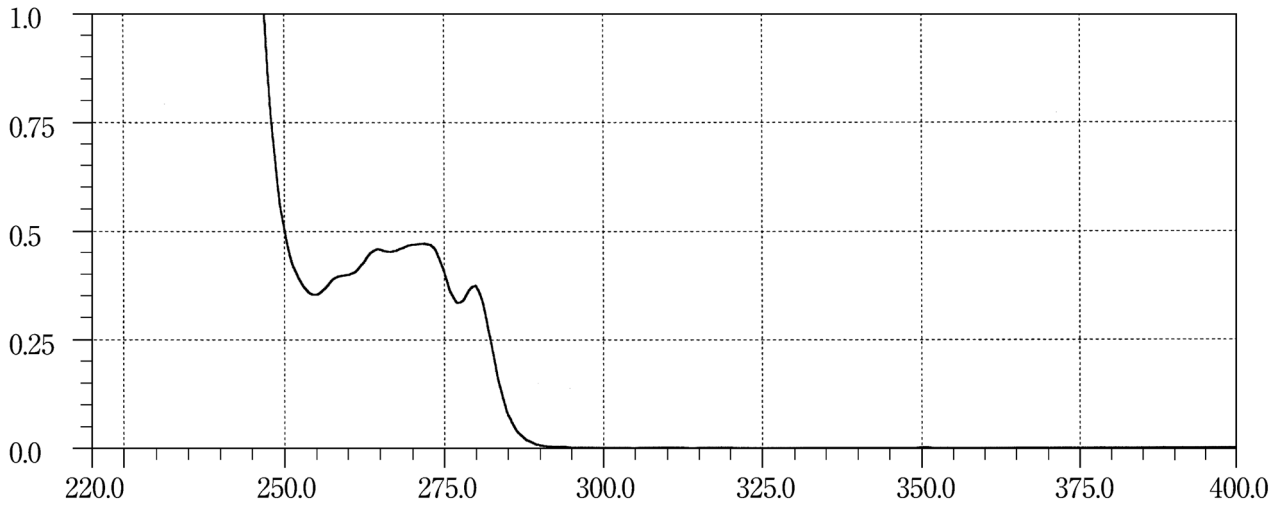
Butenafine Hydrochloride



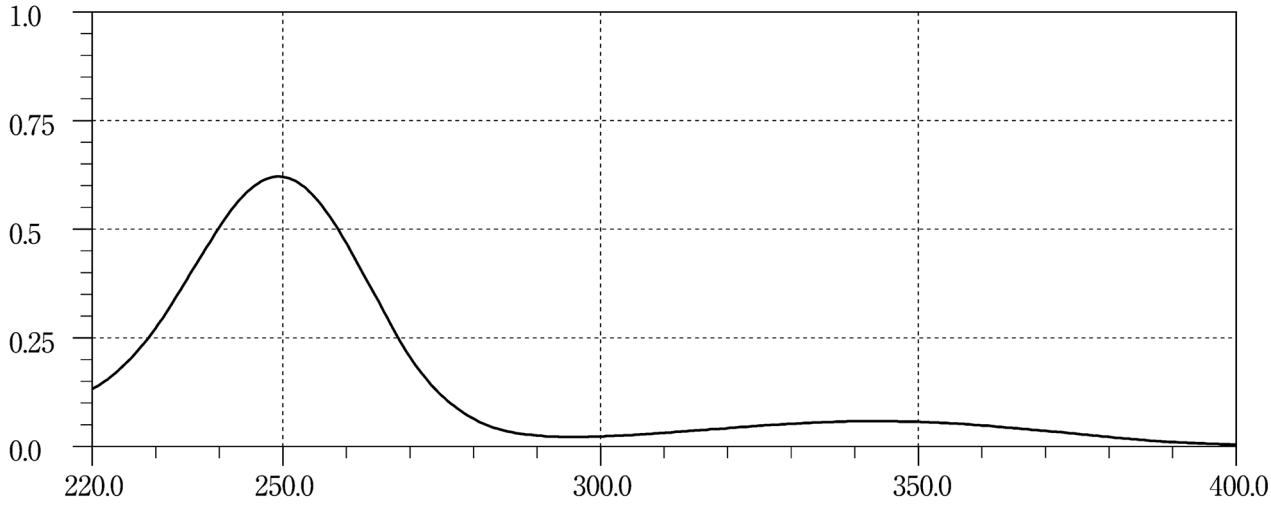
Butropium Bromide 1



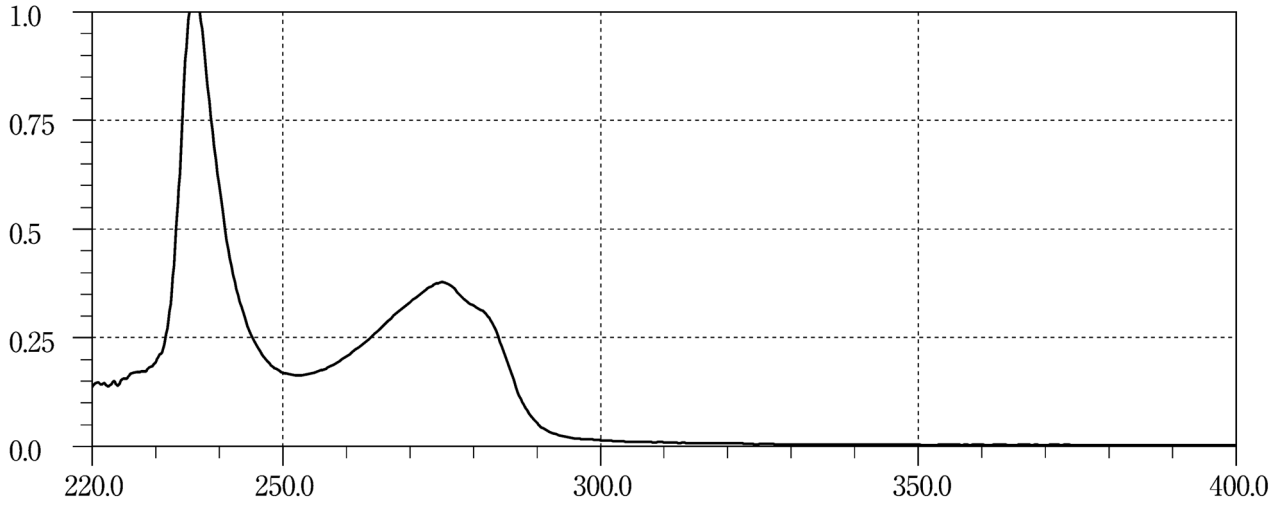
Butropium Bromide 2



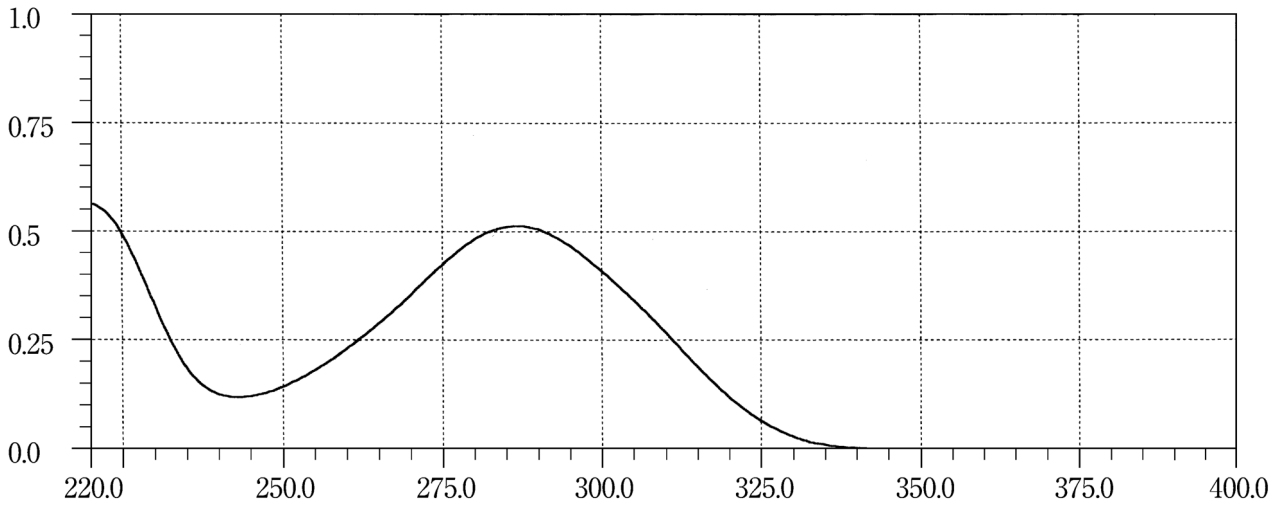
Cadralazine



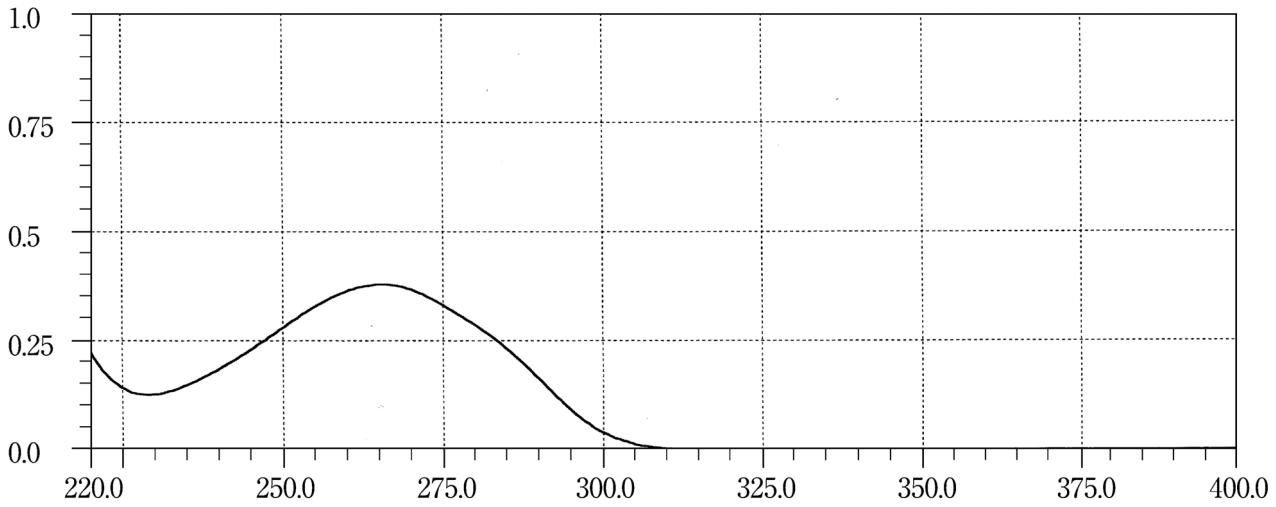
Calcitonin Salmon



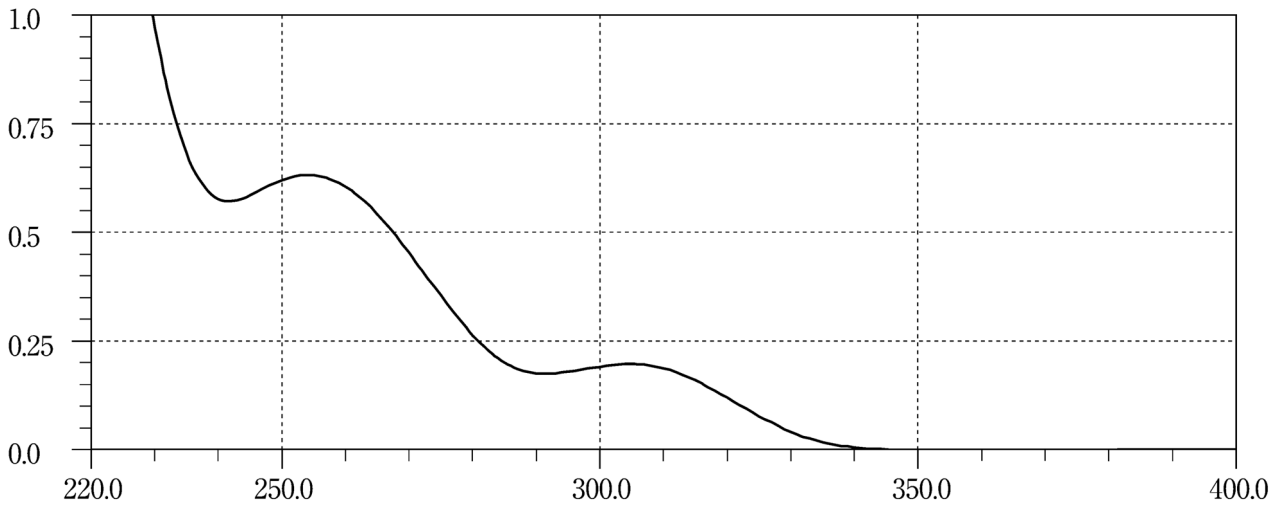
Calcium Folinate



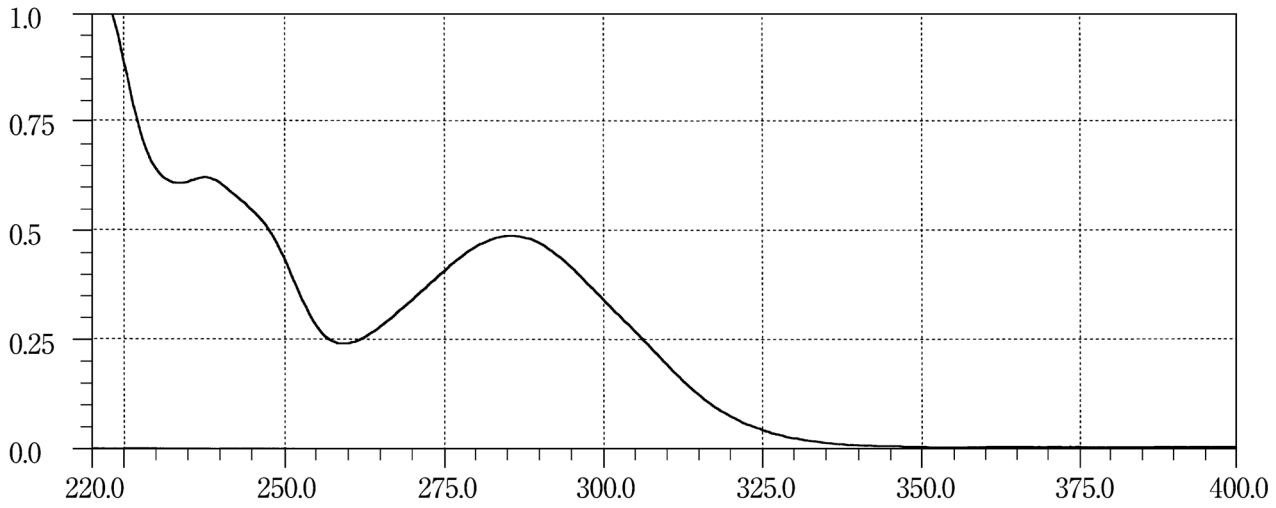
Camostat Mesilate



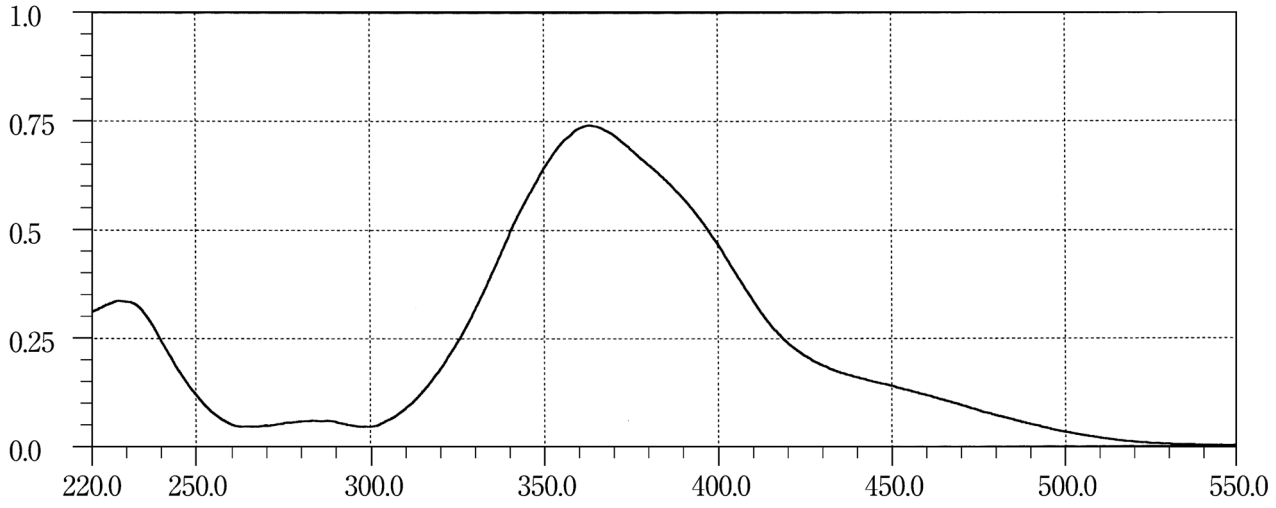
Candesartan Cilexetil



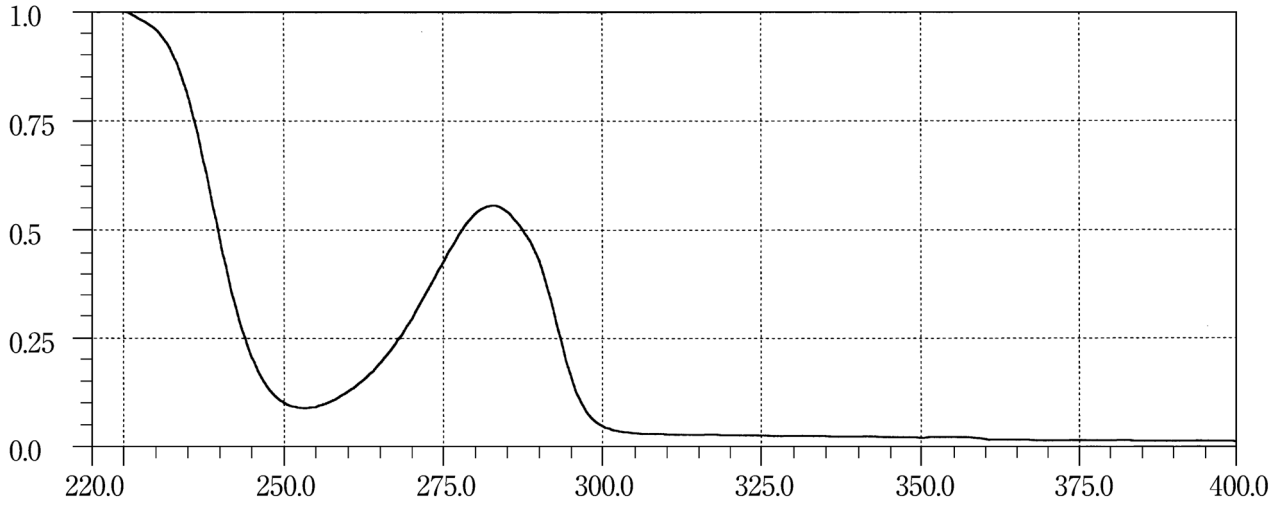
Carbamazepine



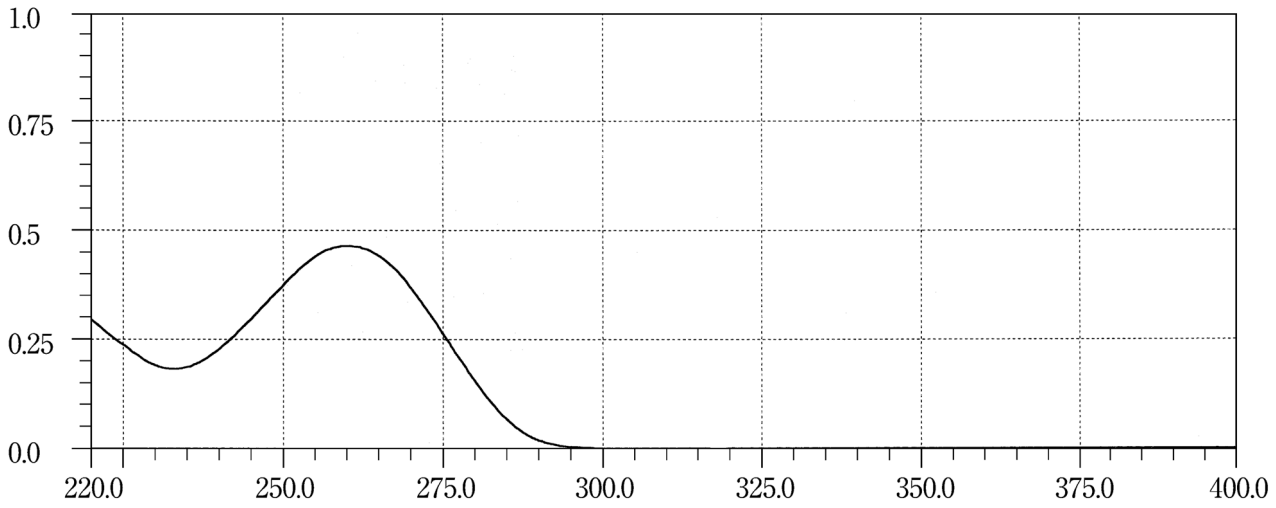
Carbazochrome Sodium Sulfonate Hydrate



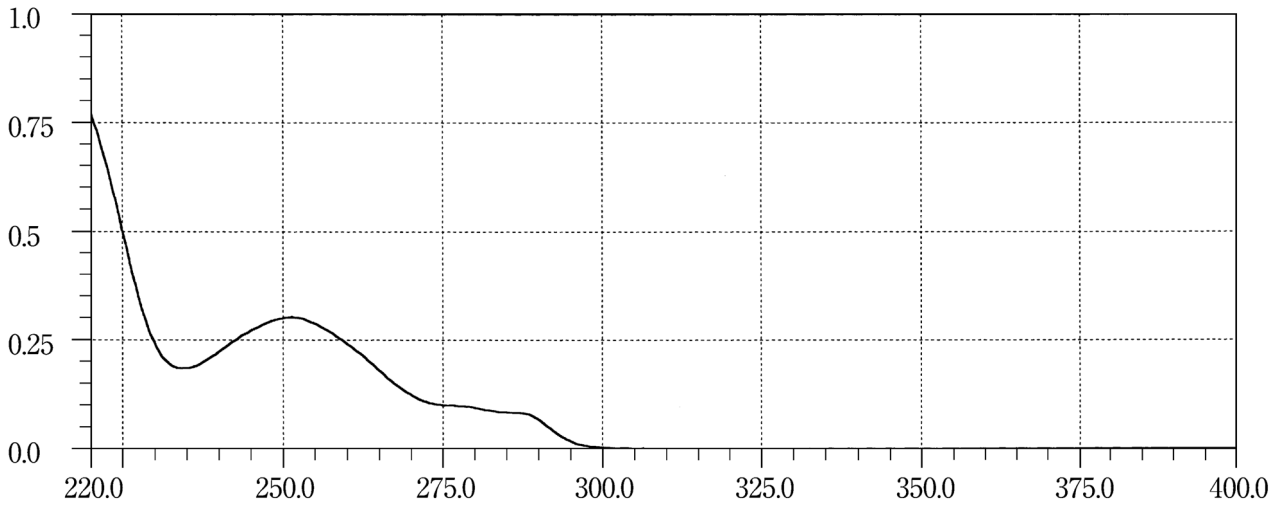
Carbidopa Hydrate



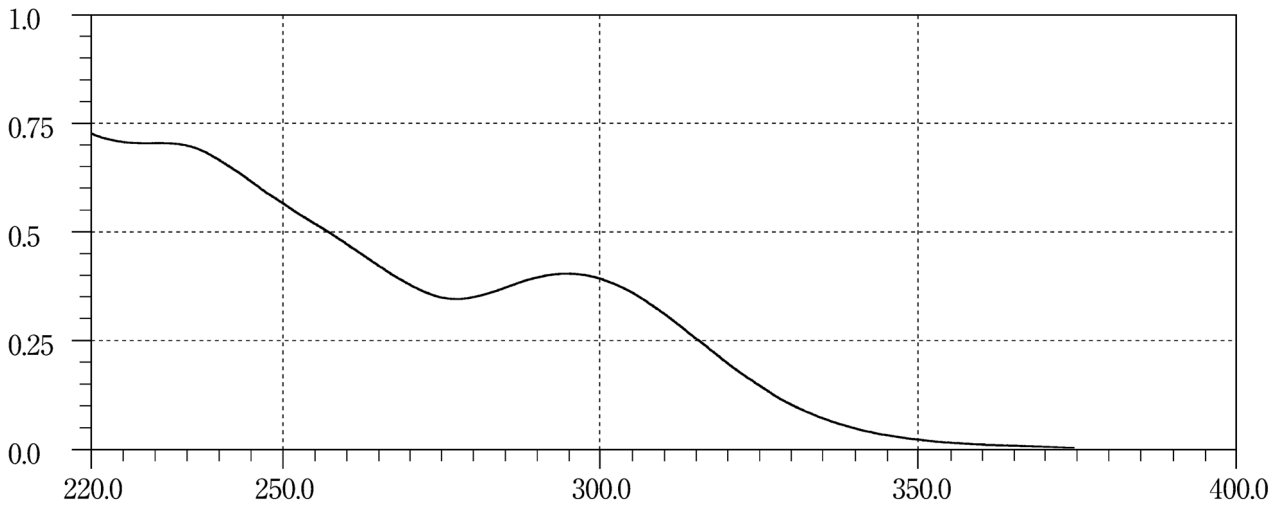
Carmofur



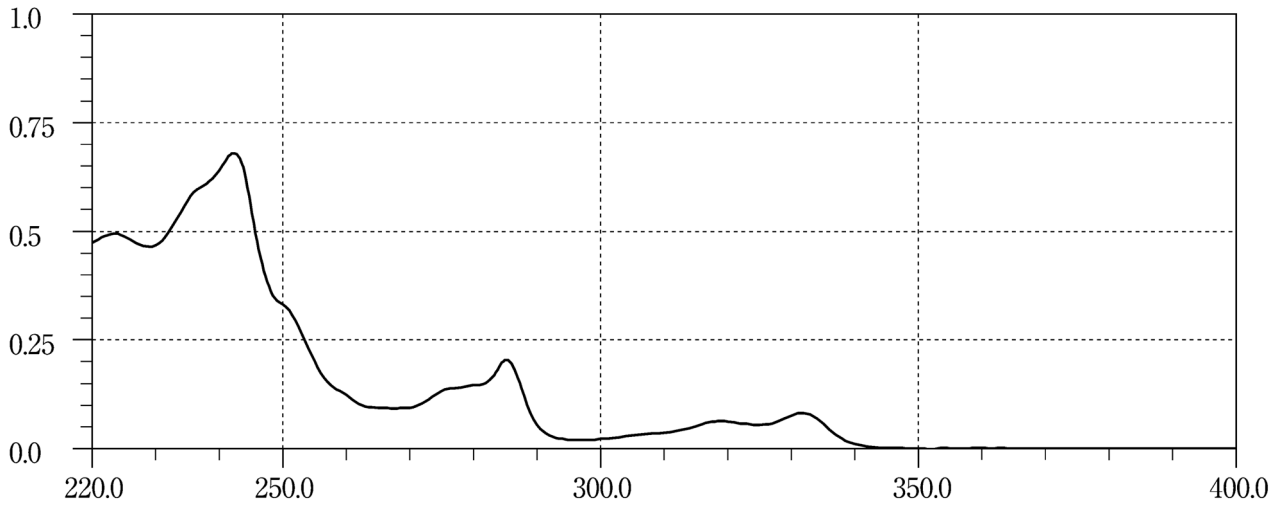
Carteolol Hydrochloride



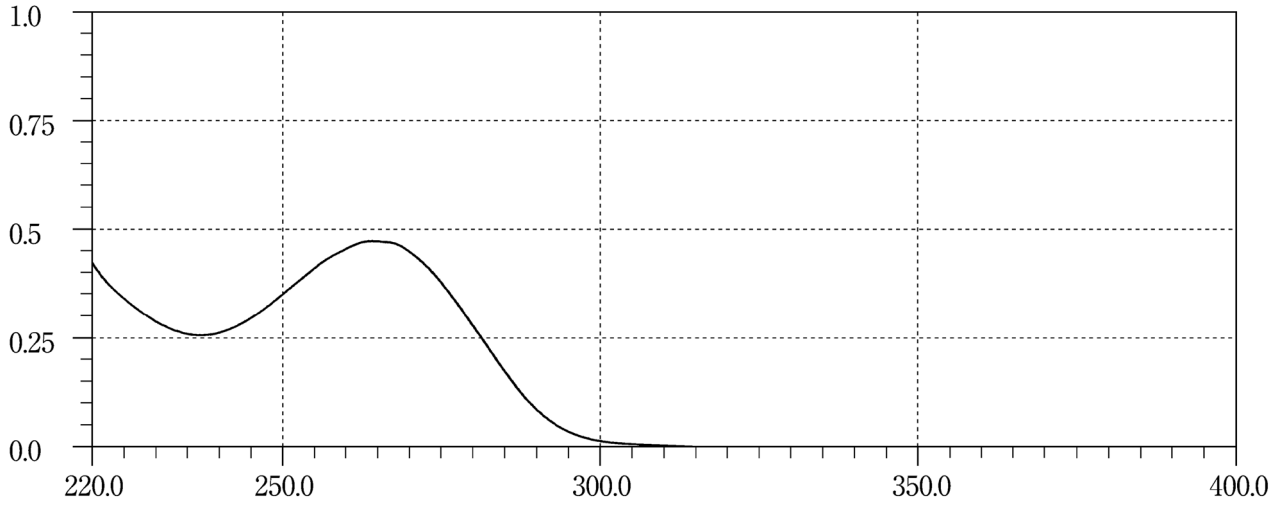
Carumonam Sodium



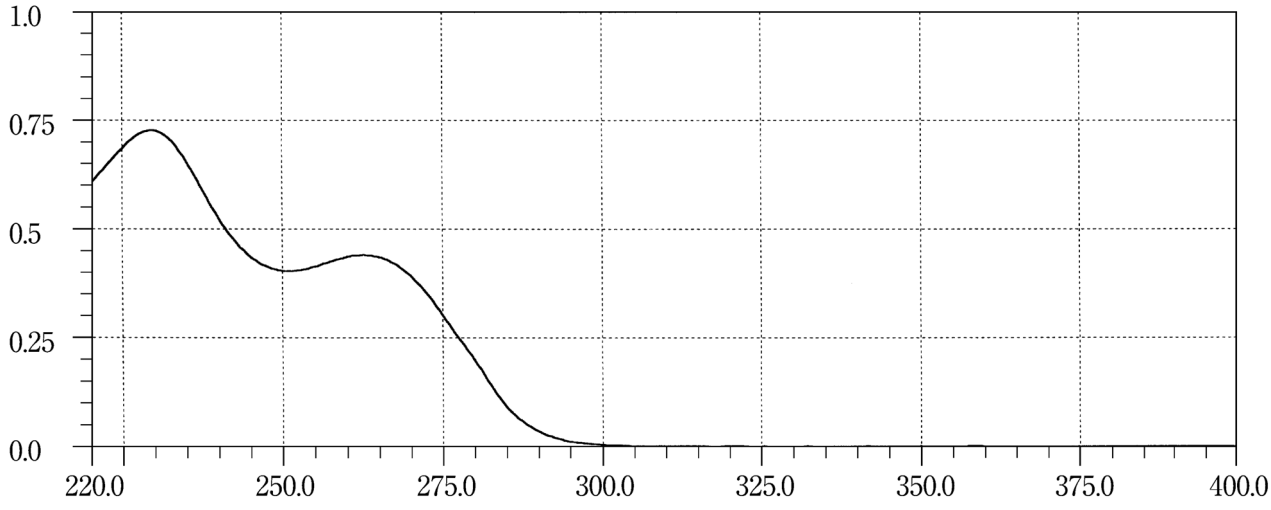
Carvedilol



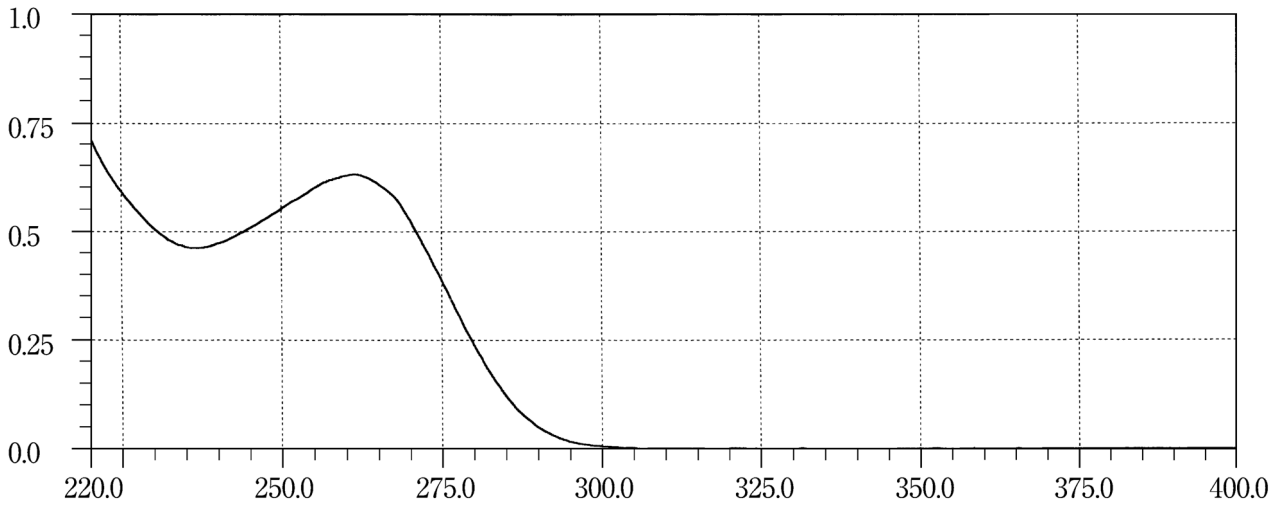
Cefaclor



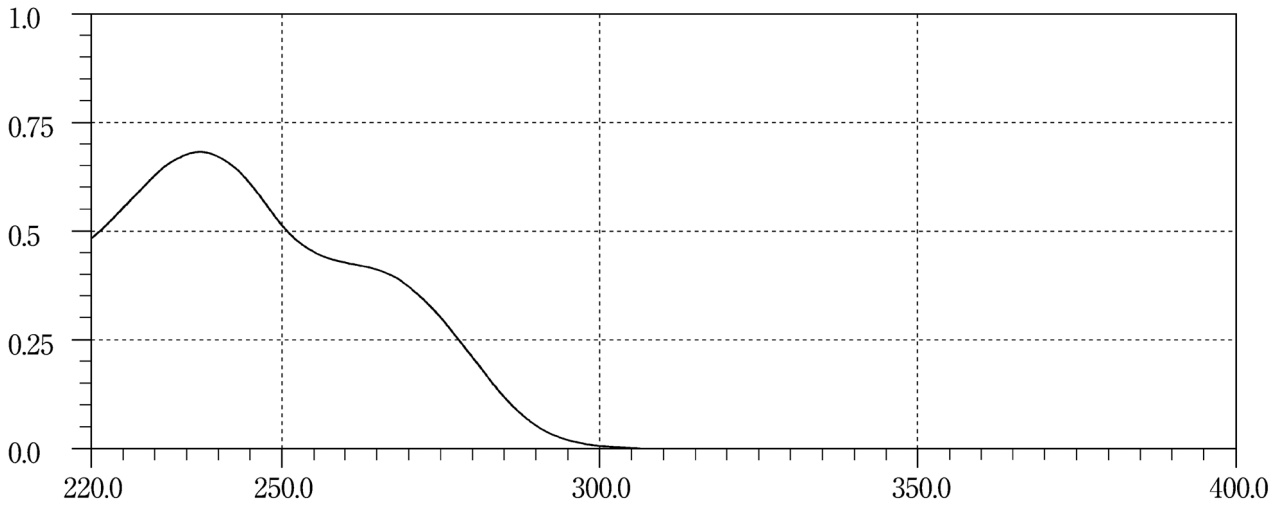
Cefadroxil



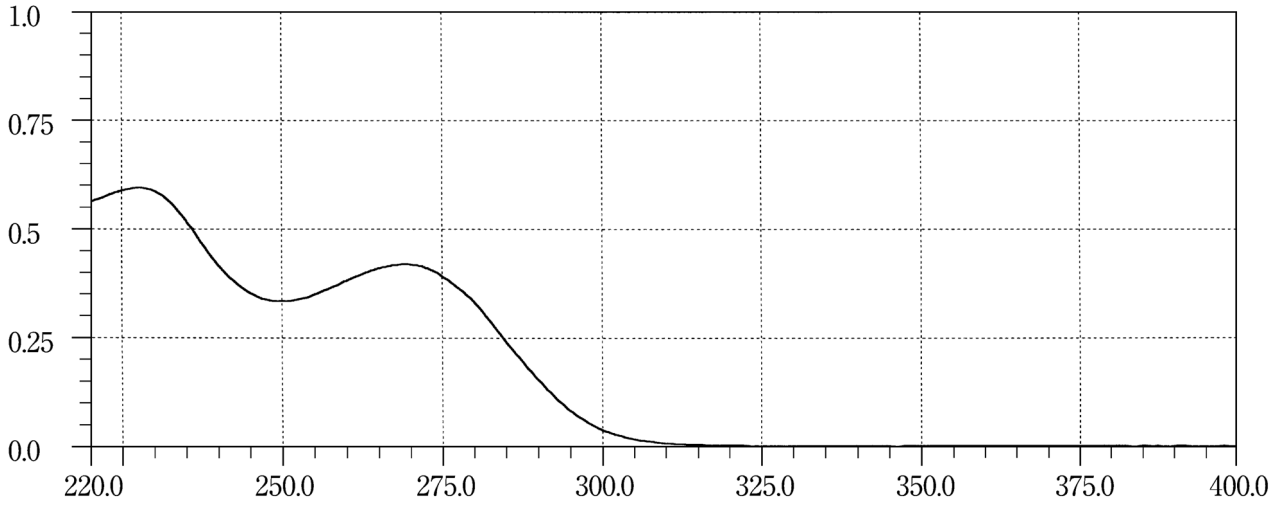
Cefalexin



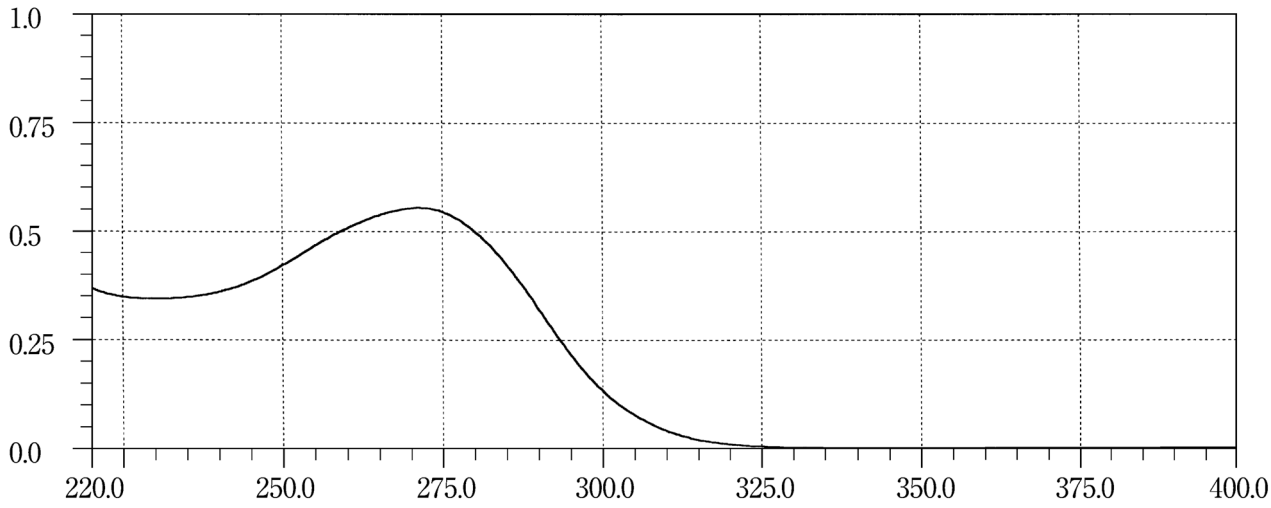
Cefalotin Sodium



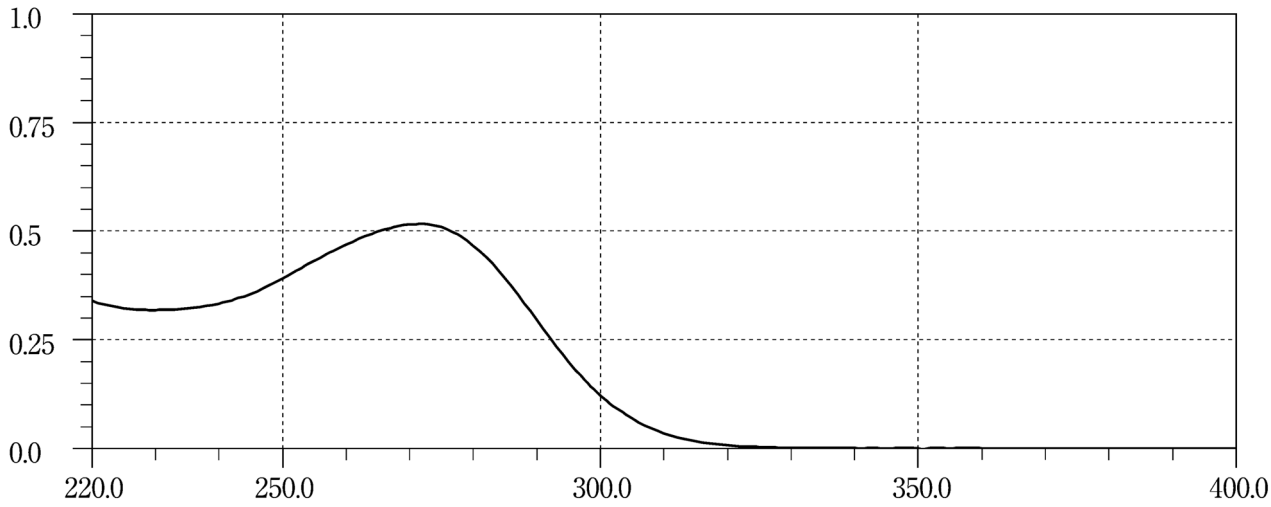
Cefatrizine Propylene Glycolate



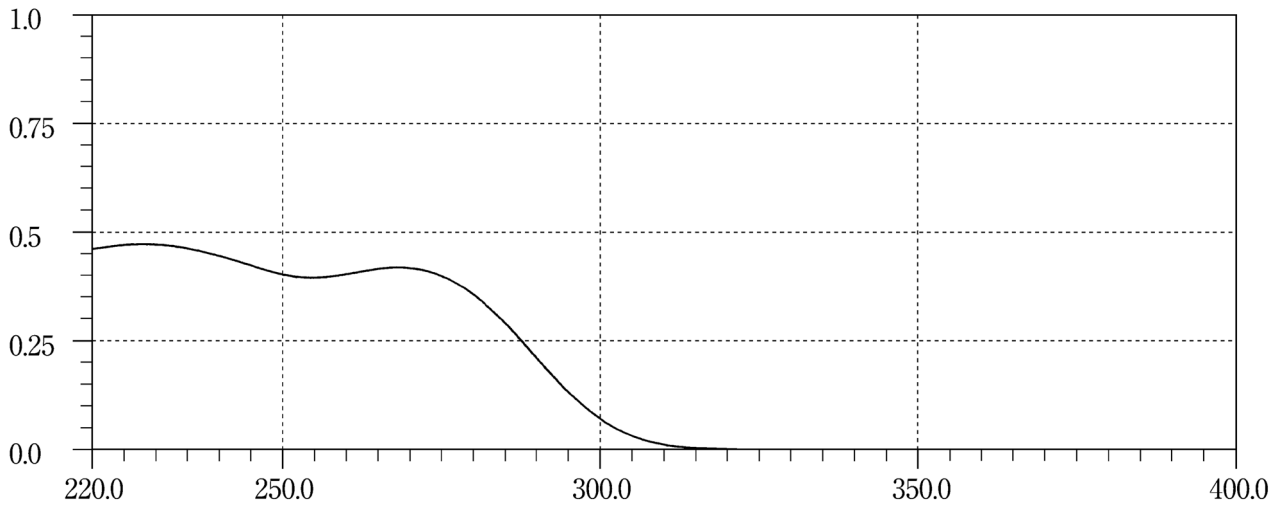
Cefazolin Sodium



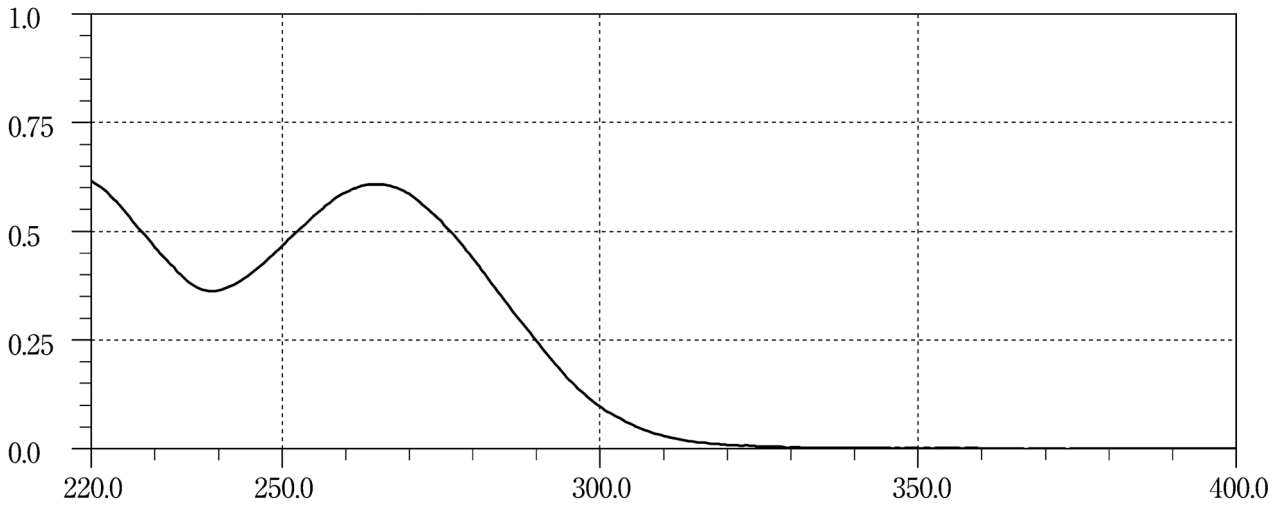
Cefazolin Sodium Hydrate



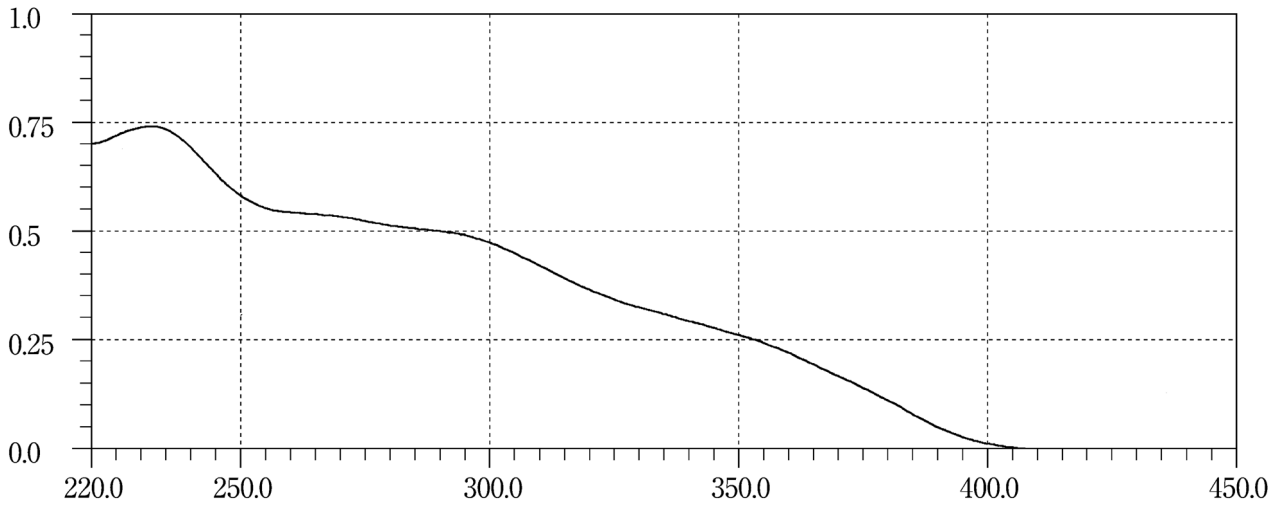
Cefbuperazone Sodium



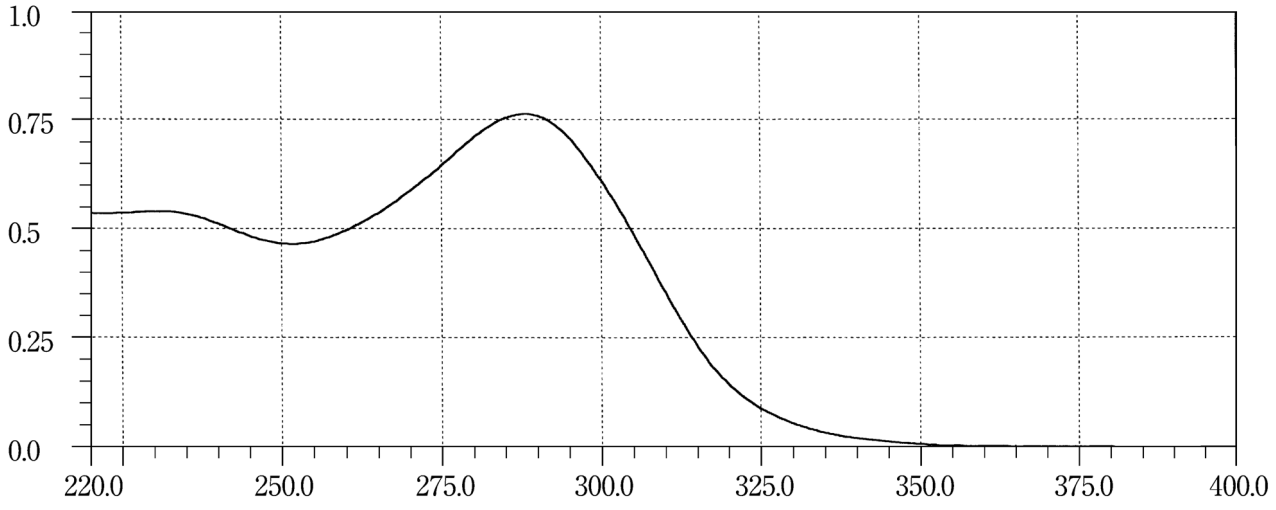
Cefcapene Pivoxil Hydrochloride Hydrate



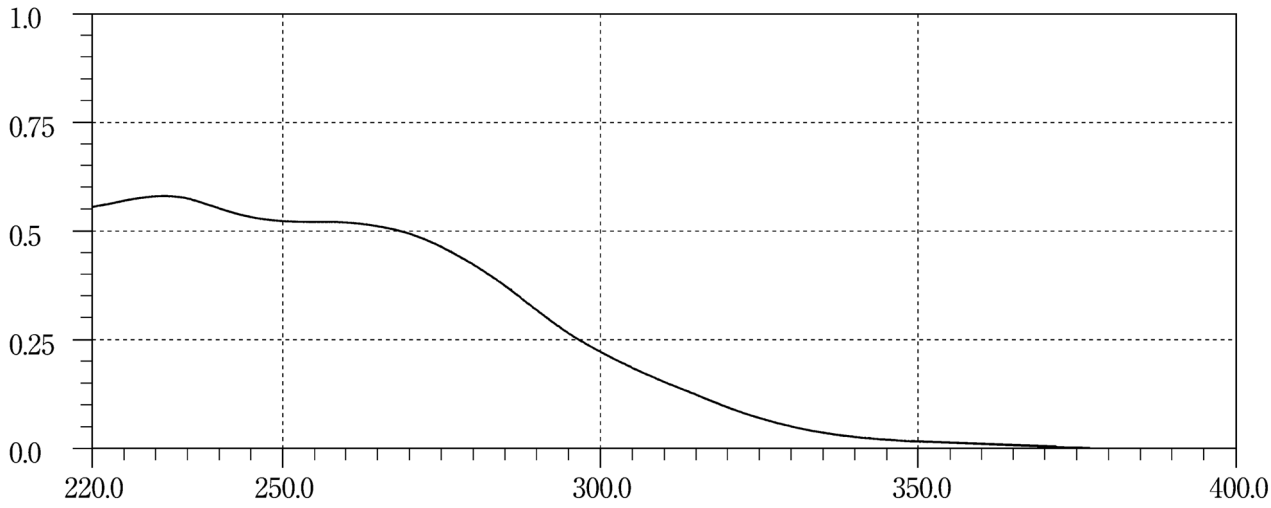
Cefditoren Pivoxil



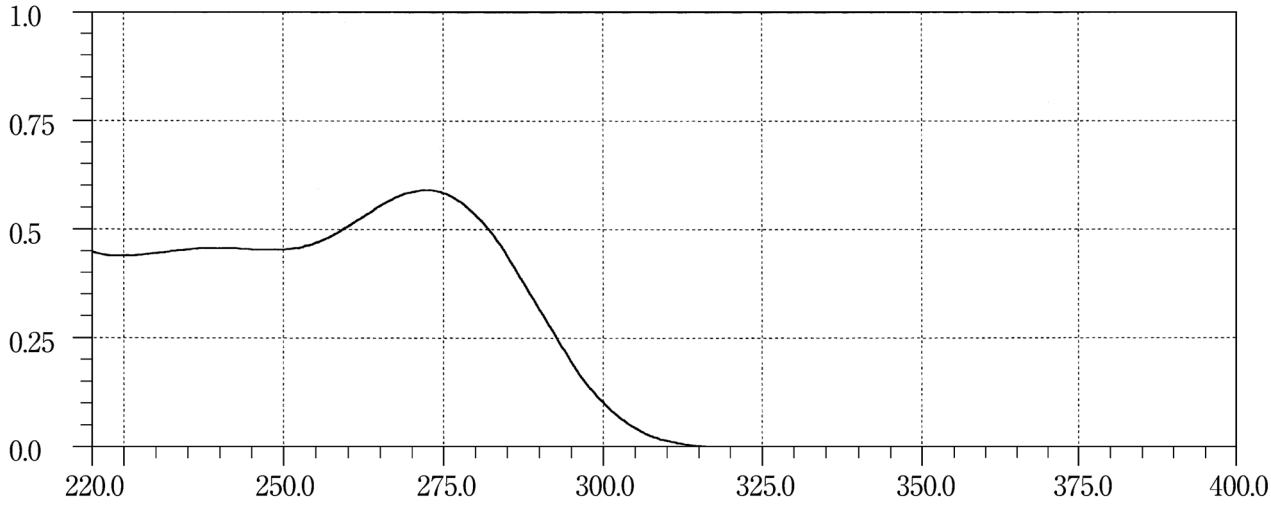
Cefixime Hydrate



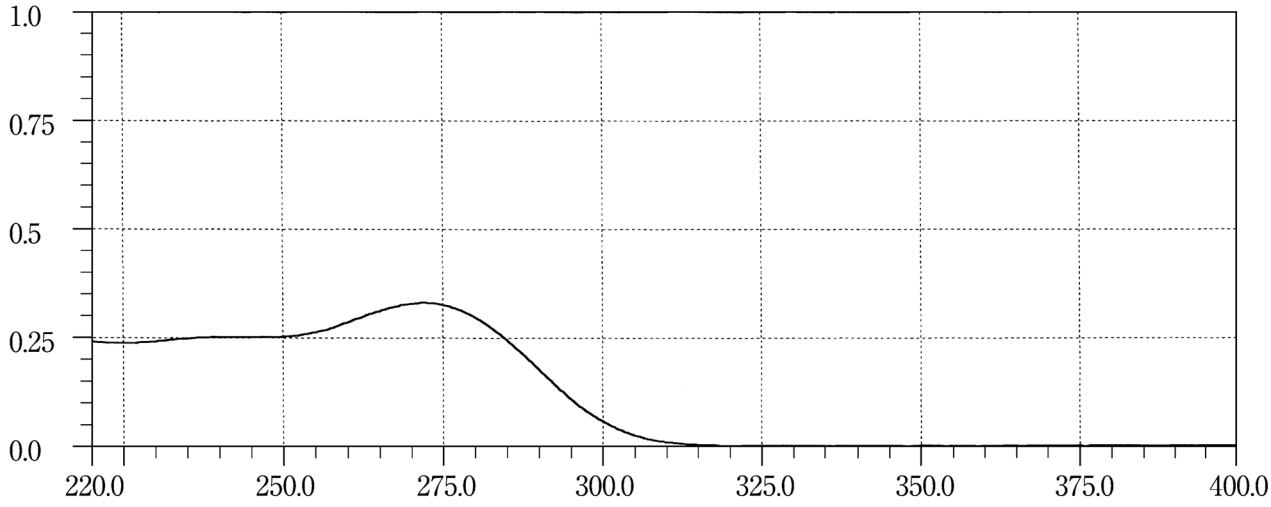
Cefmenoxime Hydrochloride



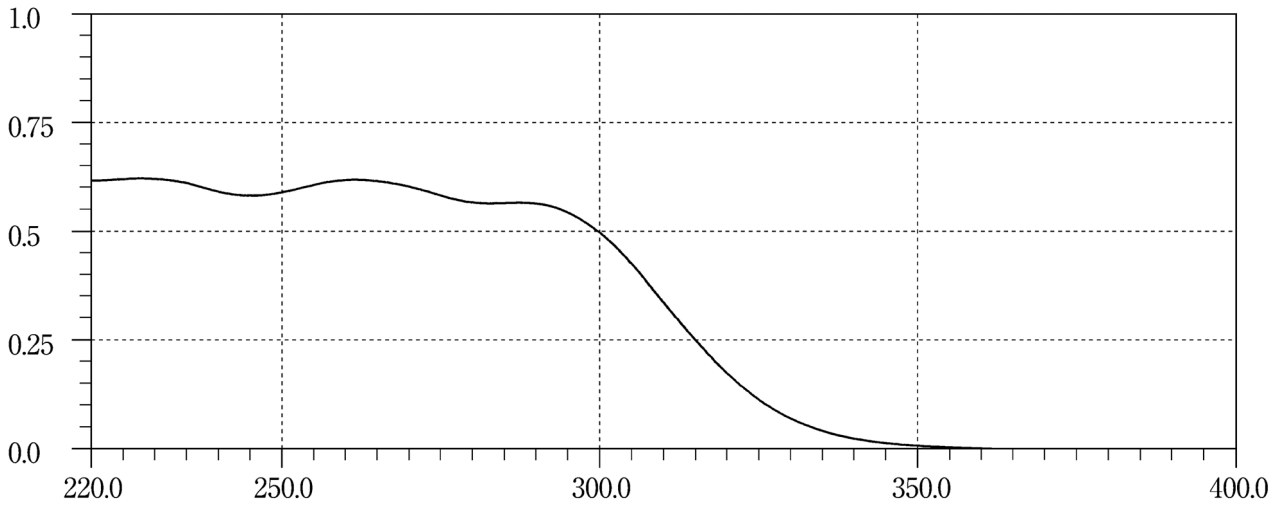
Cefmetazole Sodium



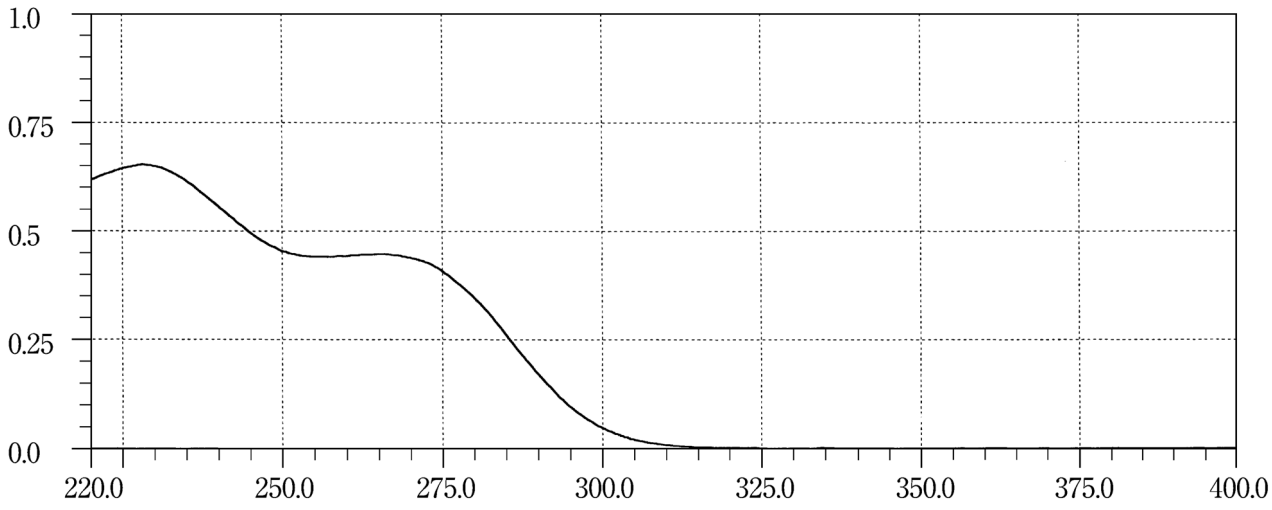
Cefminox Sodium Hydrate



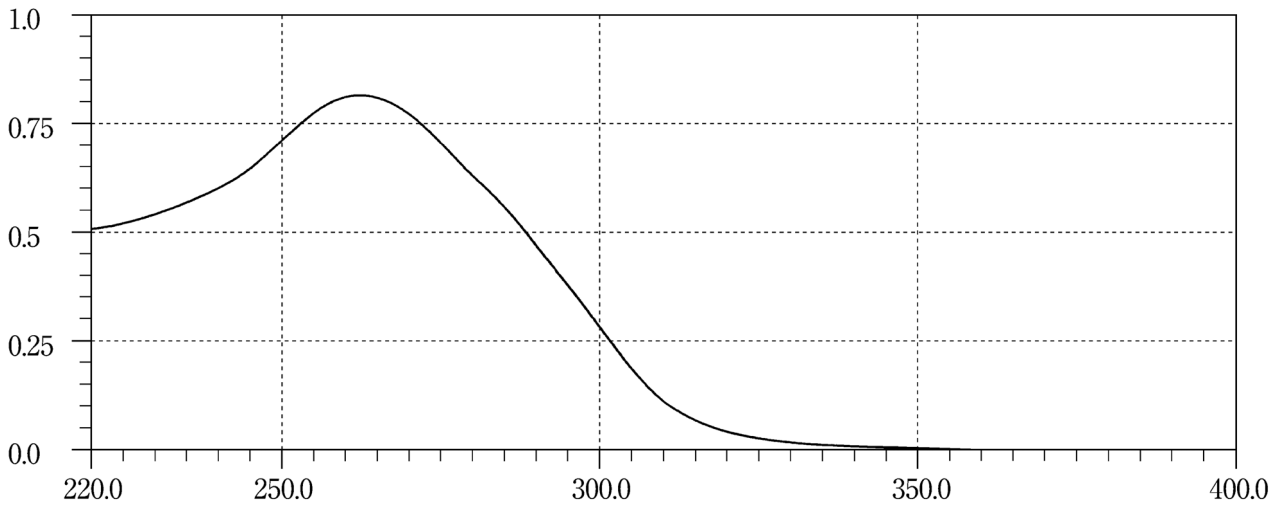
Cefodizime Sodium



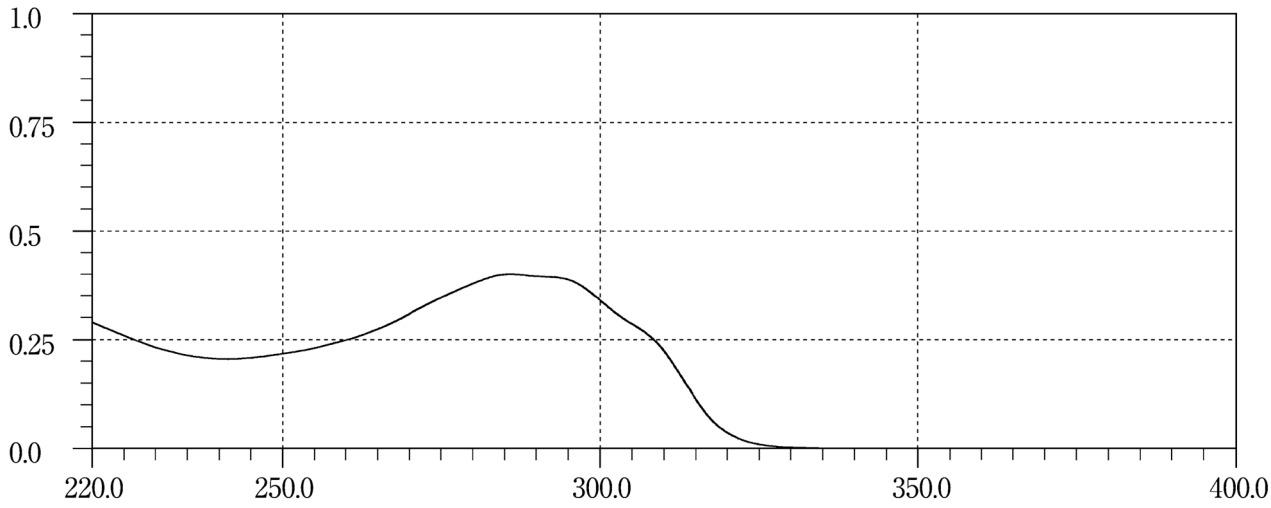
Cefoperazone Sodium



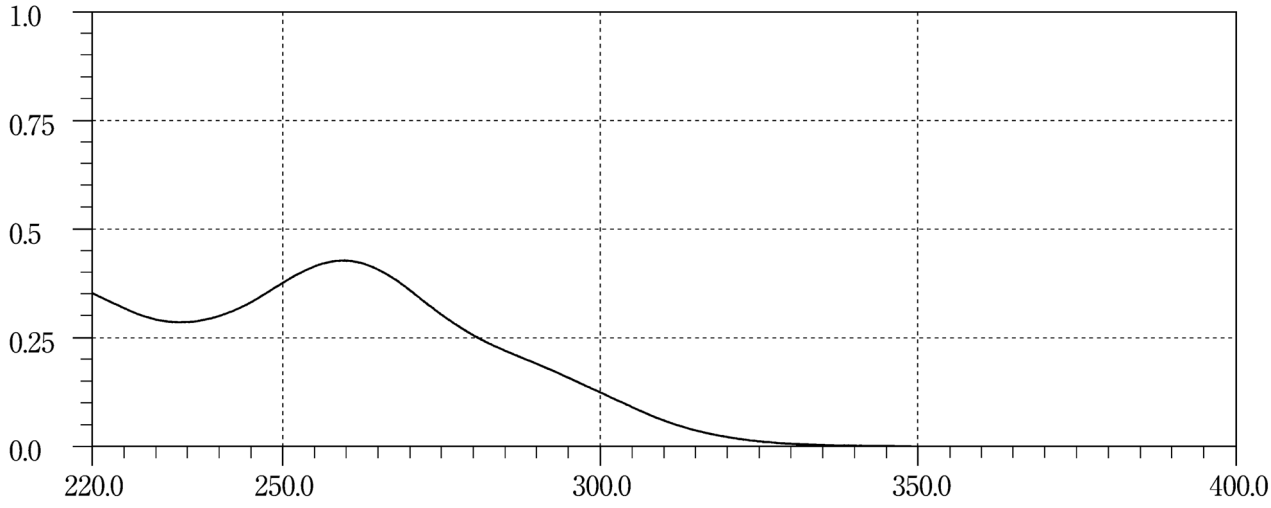
Cefotaxime Sodium



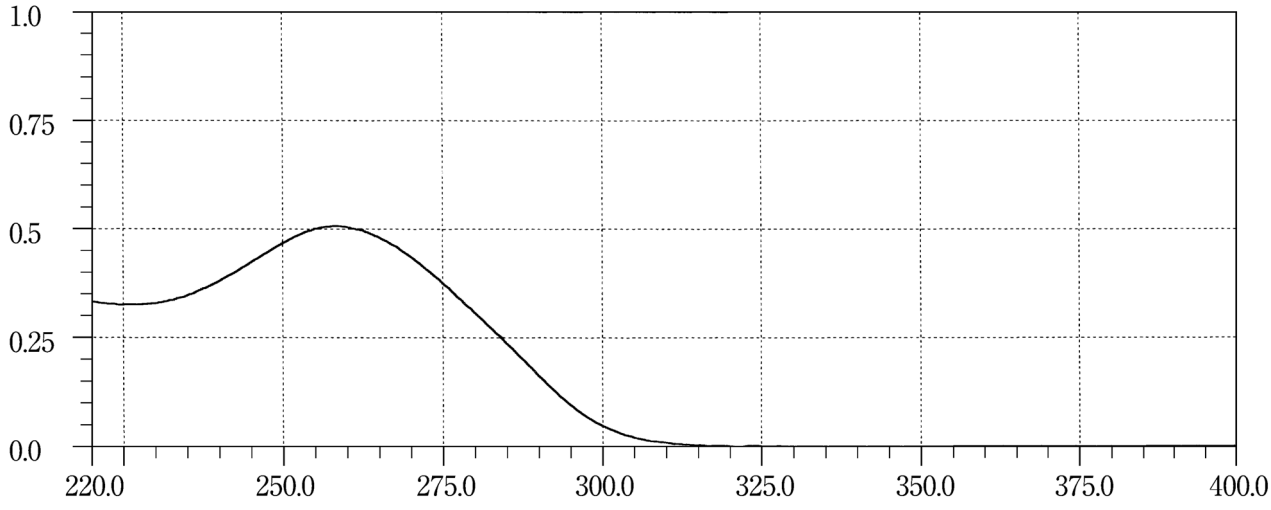
Cefotetan



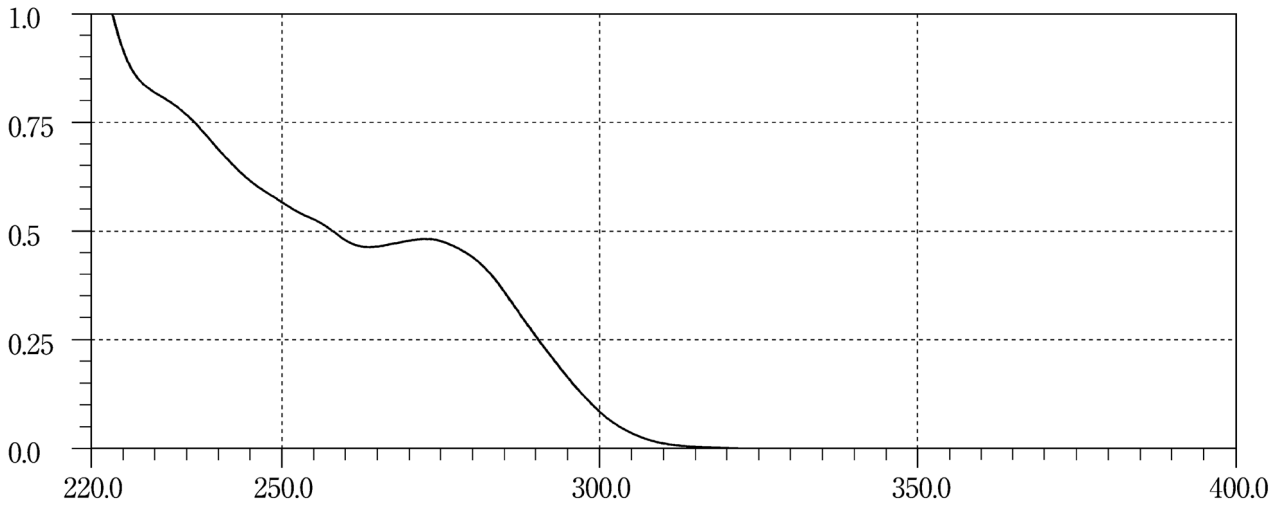
Cefotiam Hexetil Hydrochloride



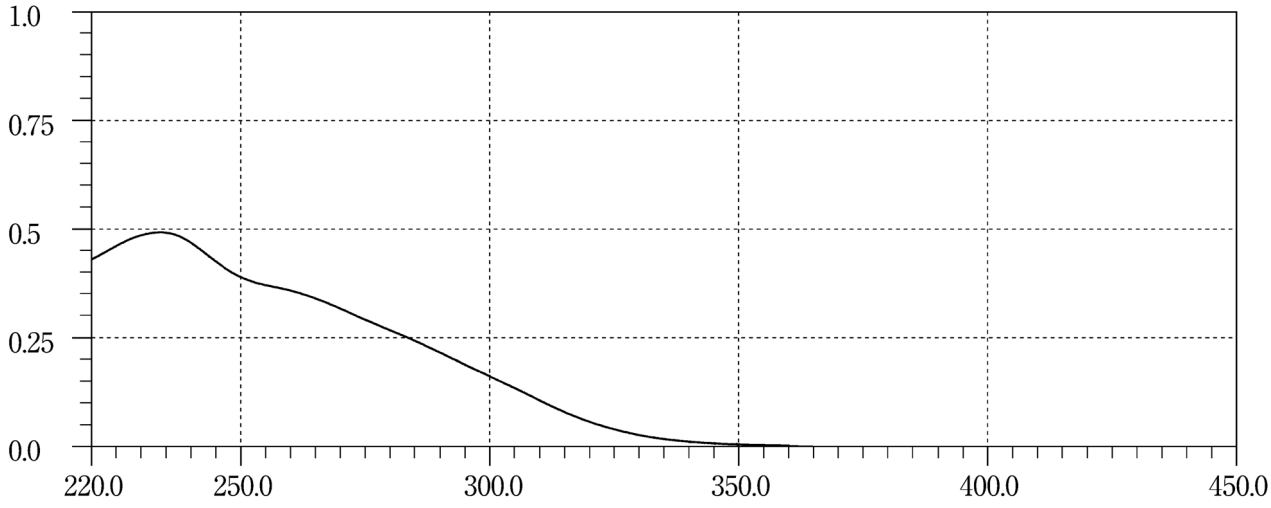
Cefotiam Hydrochloride



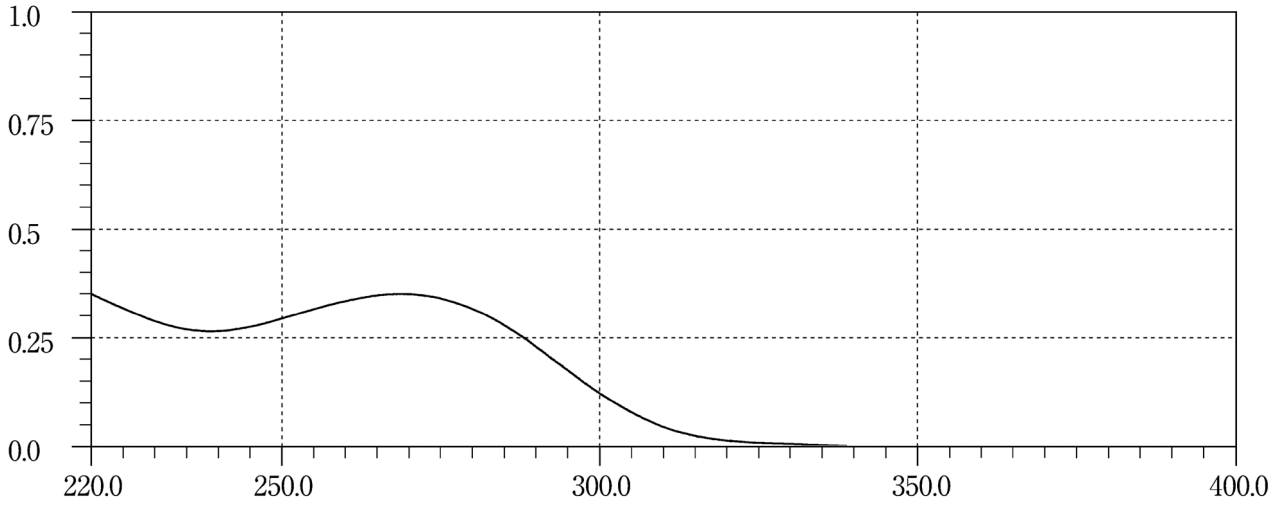
Cefpiramide Sodium



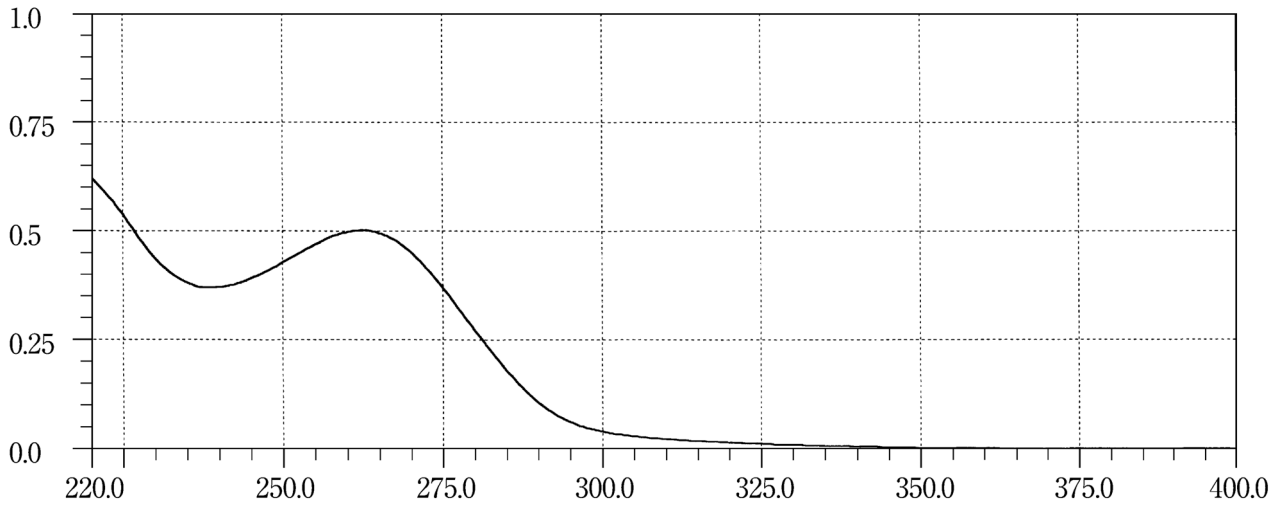
Cefpodoxime Proxetil



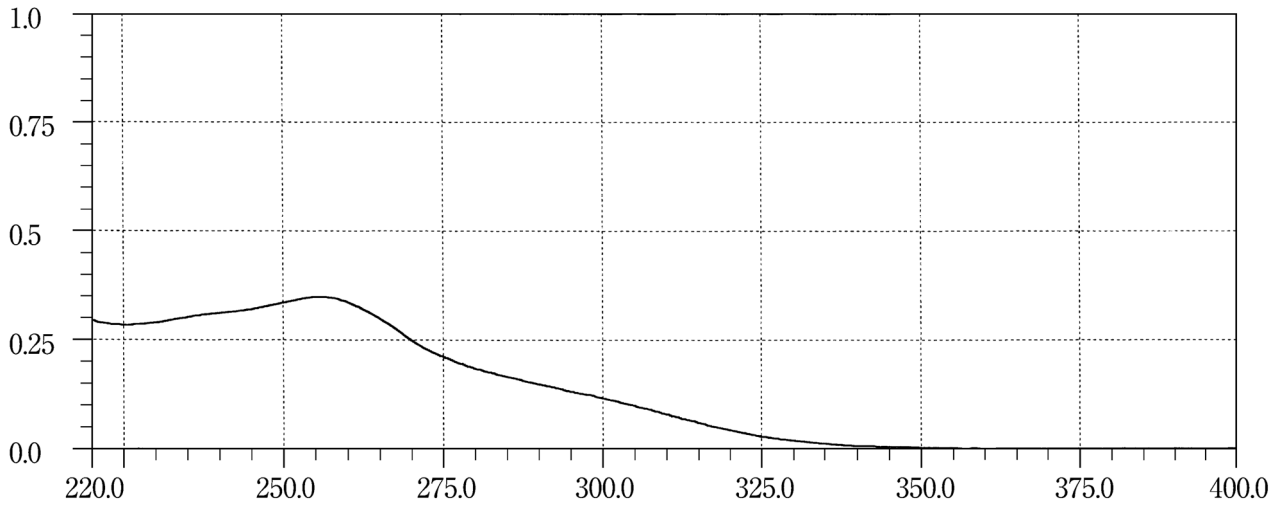
Cefroxadine Hydrate



Cefsulodin Sodium



Ceftazidime Hydrate



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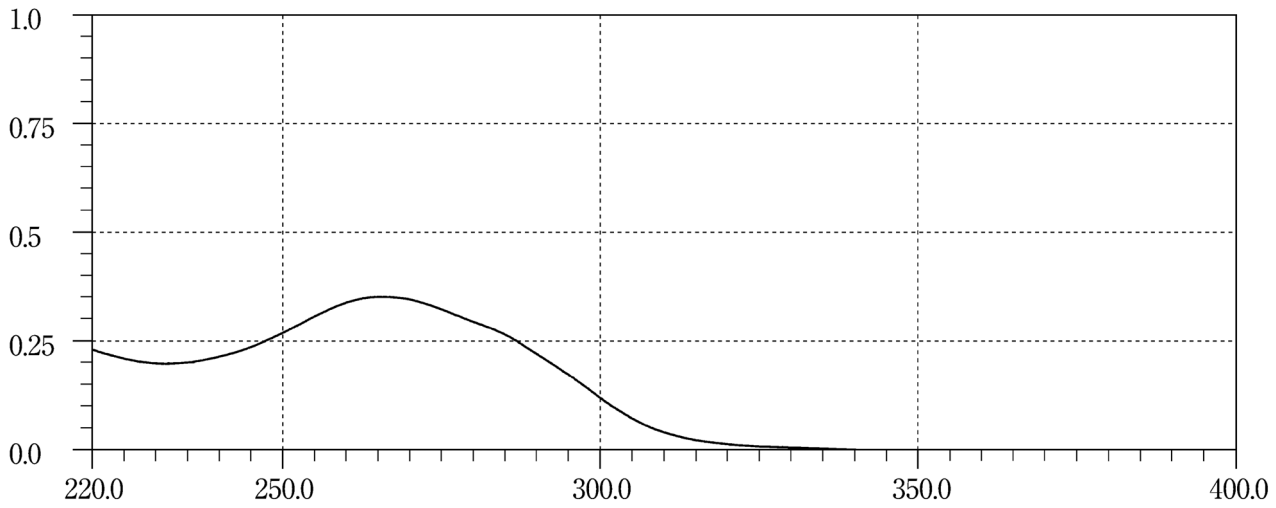
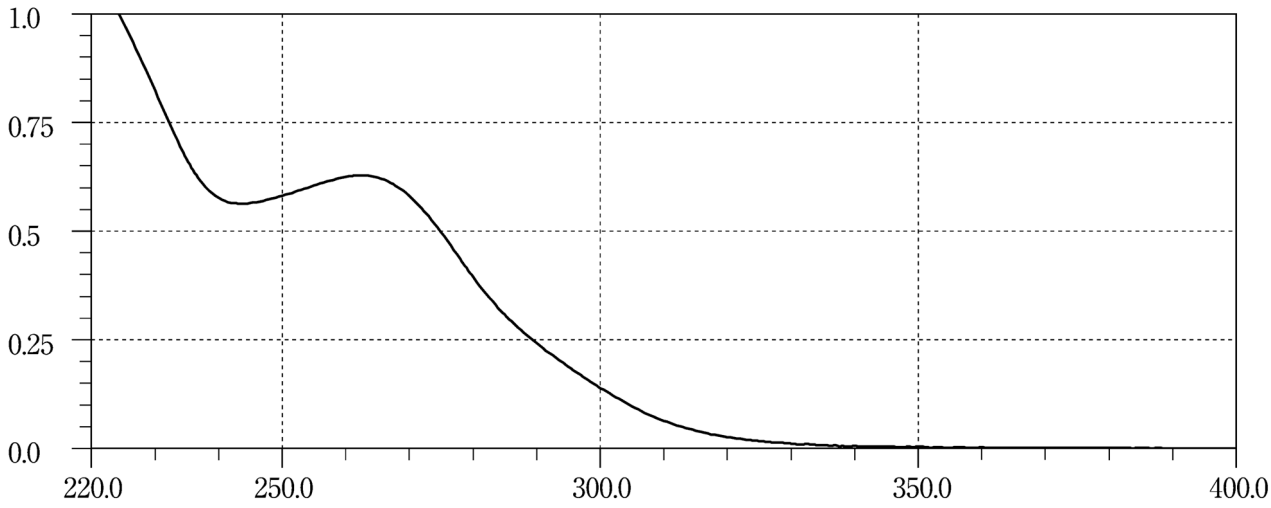
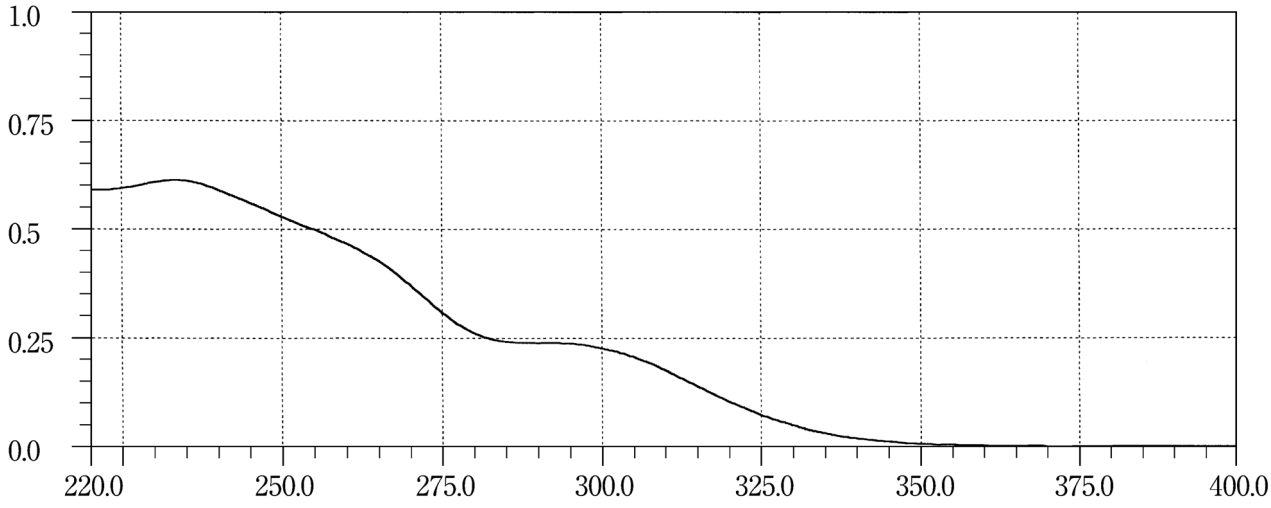


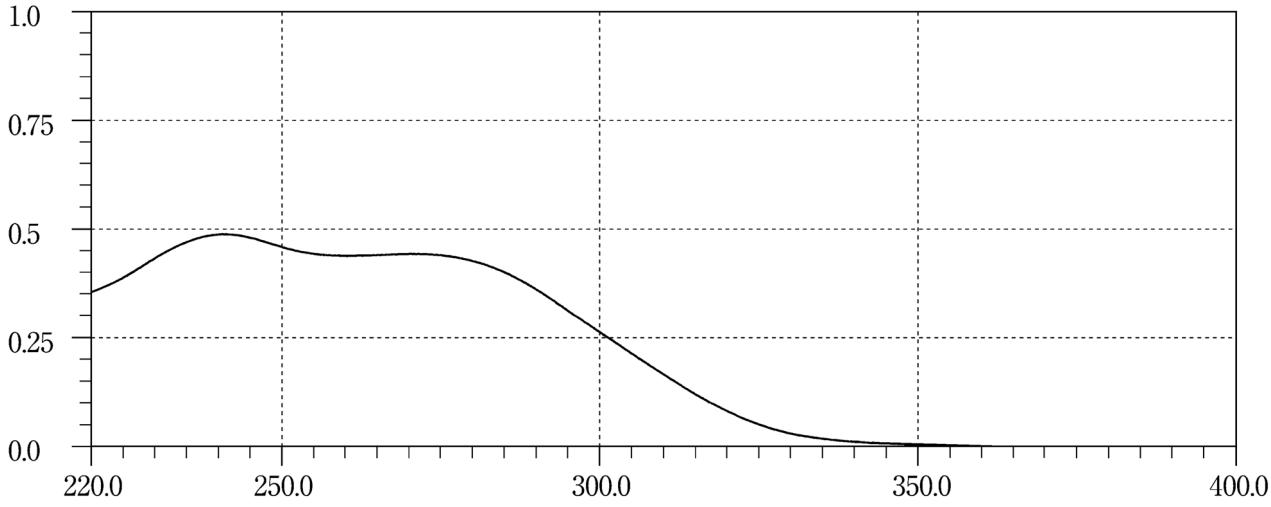
Ceftibuten Hydrate



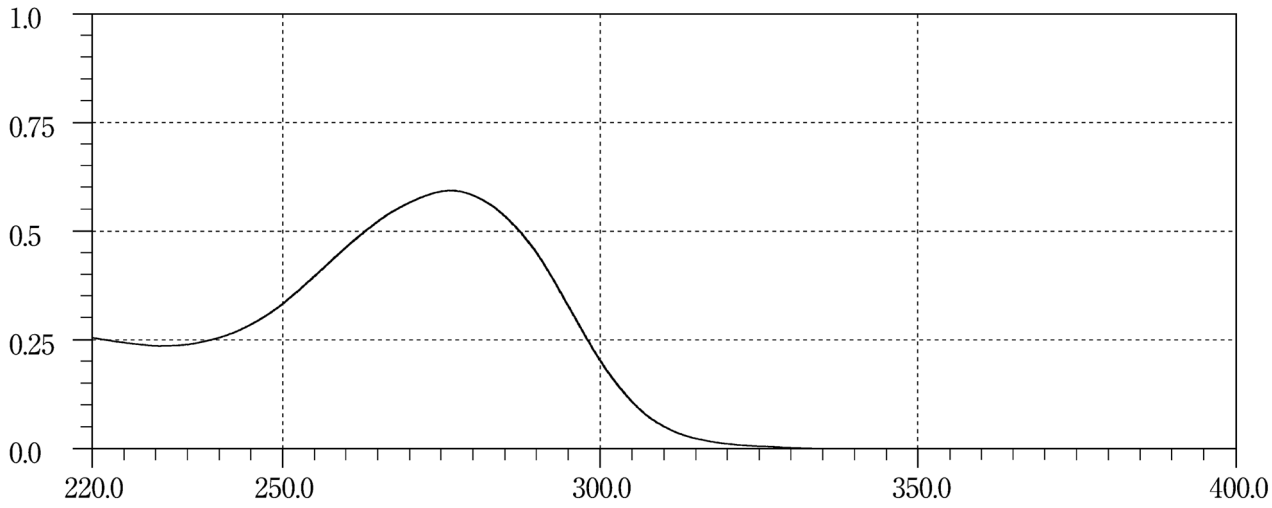
Ceftizoxime Sodium



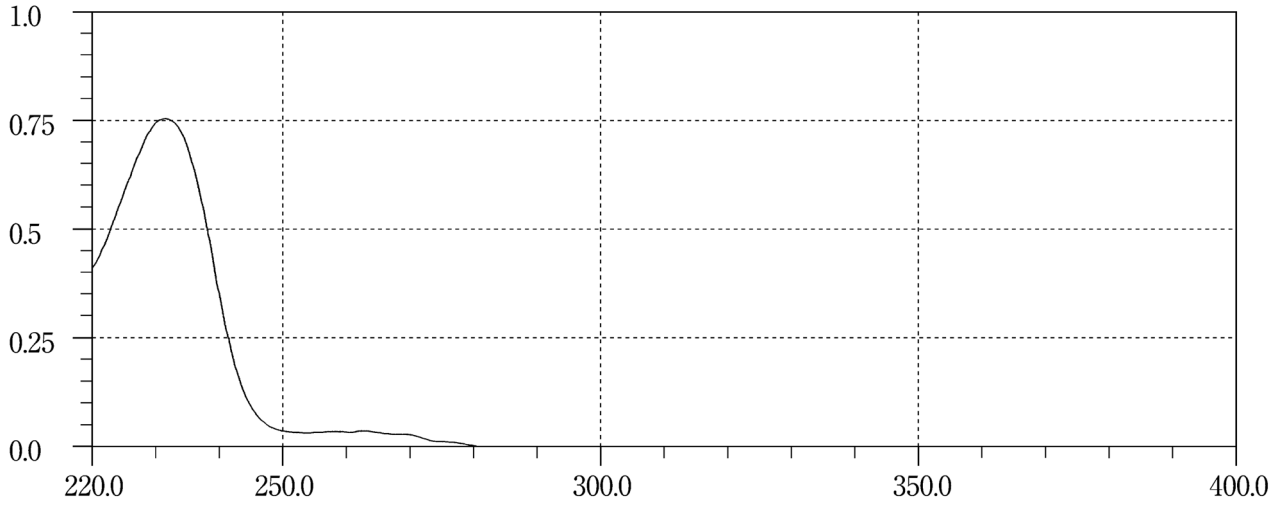
Ceftriaxone Sodium Hydrate



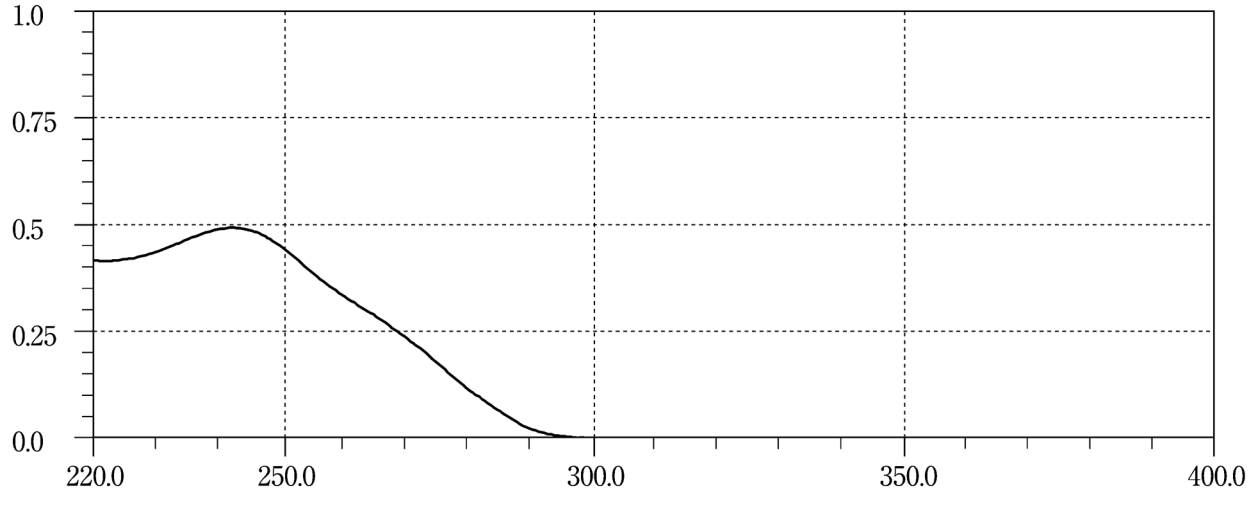
Cefuroxime Axetil



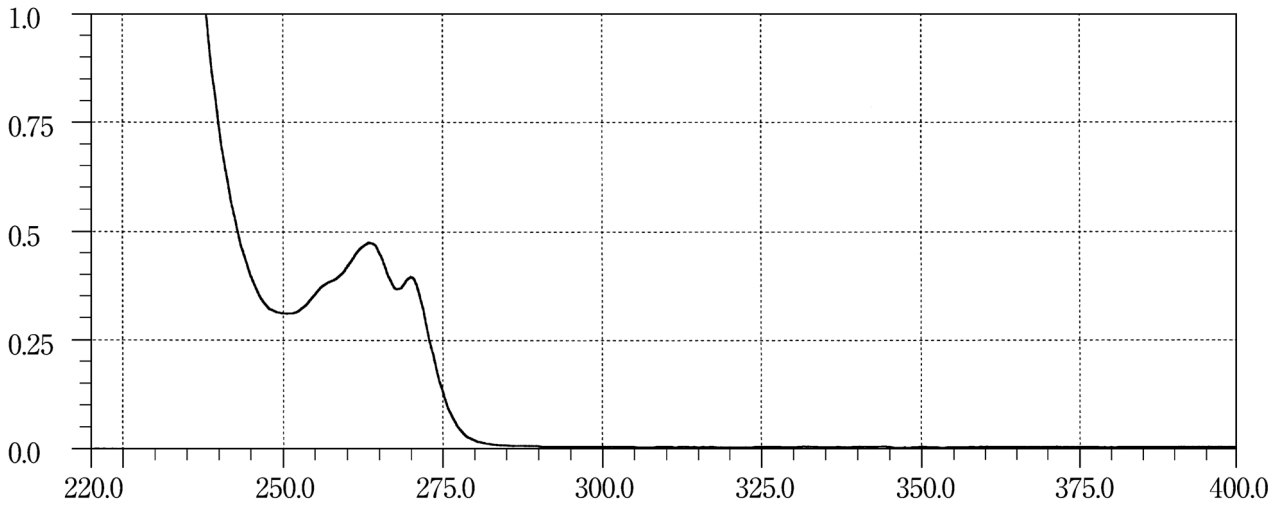
Cetirizine Hydrochloride



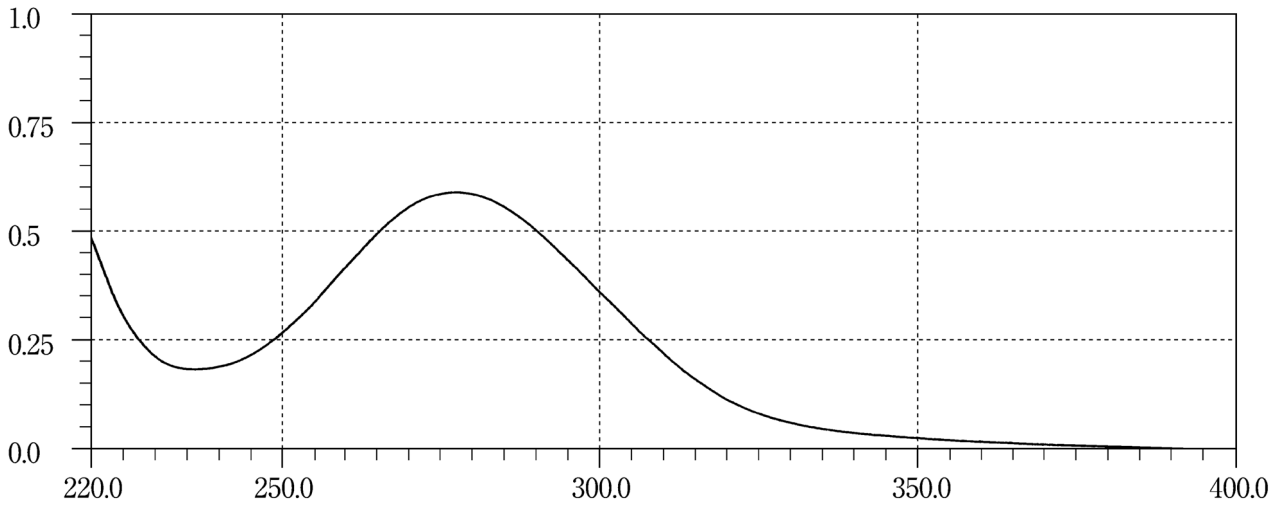
Cetotiamine Hydrochloride Hydrate



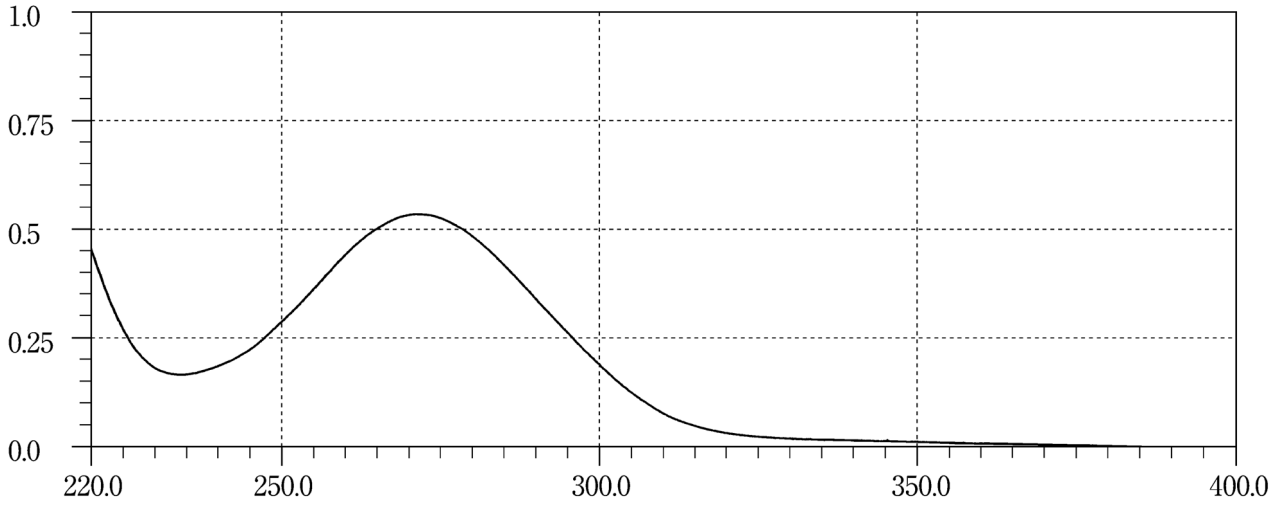
Cetraxate Hydrochloride



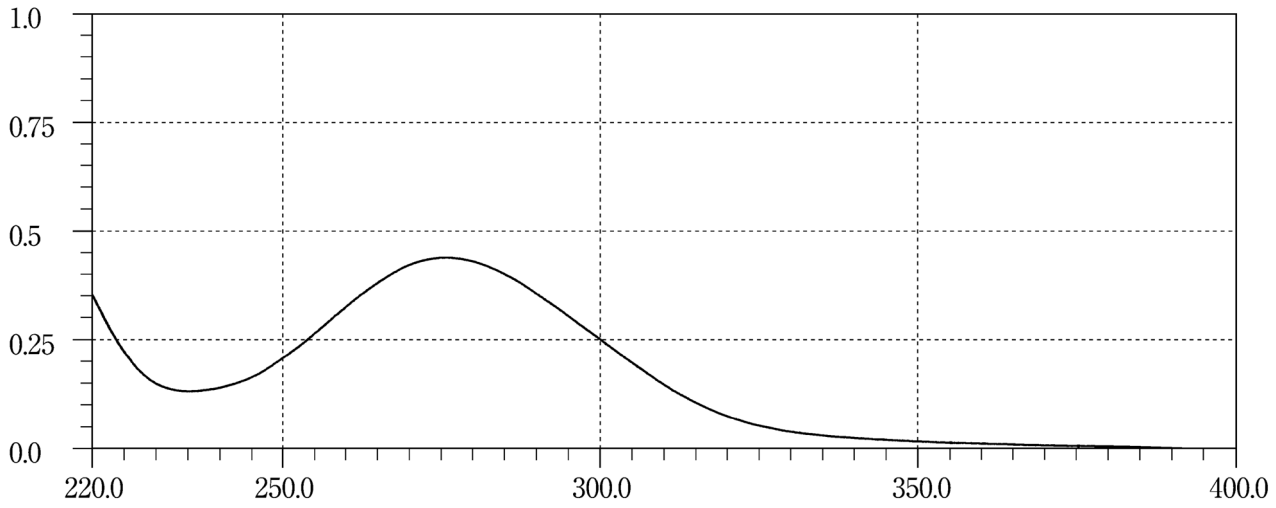
Chloramphenicol



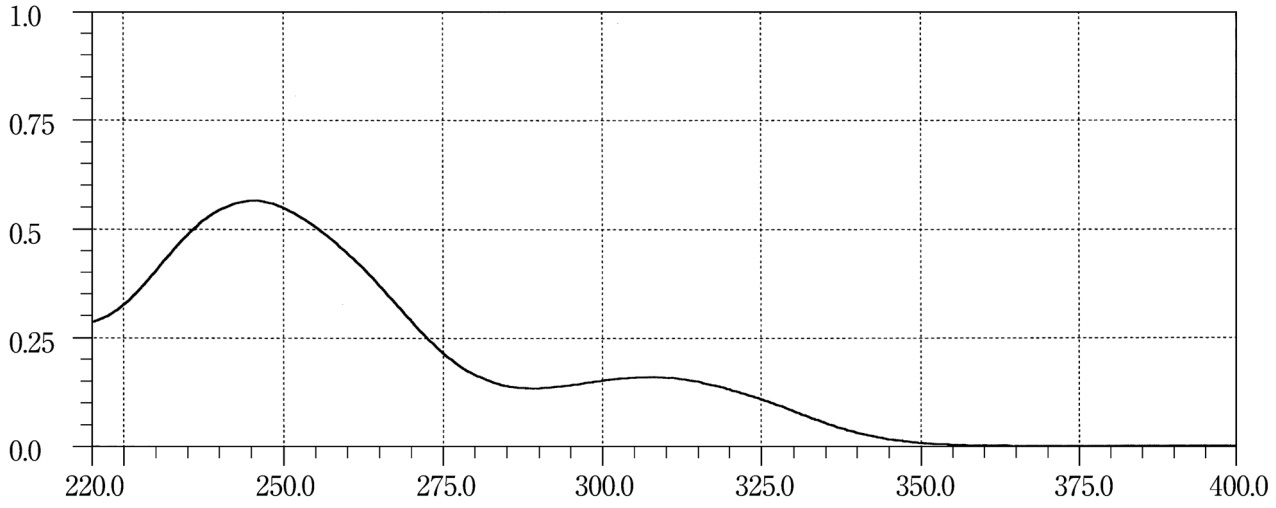
Chloramphenicol Palmitate



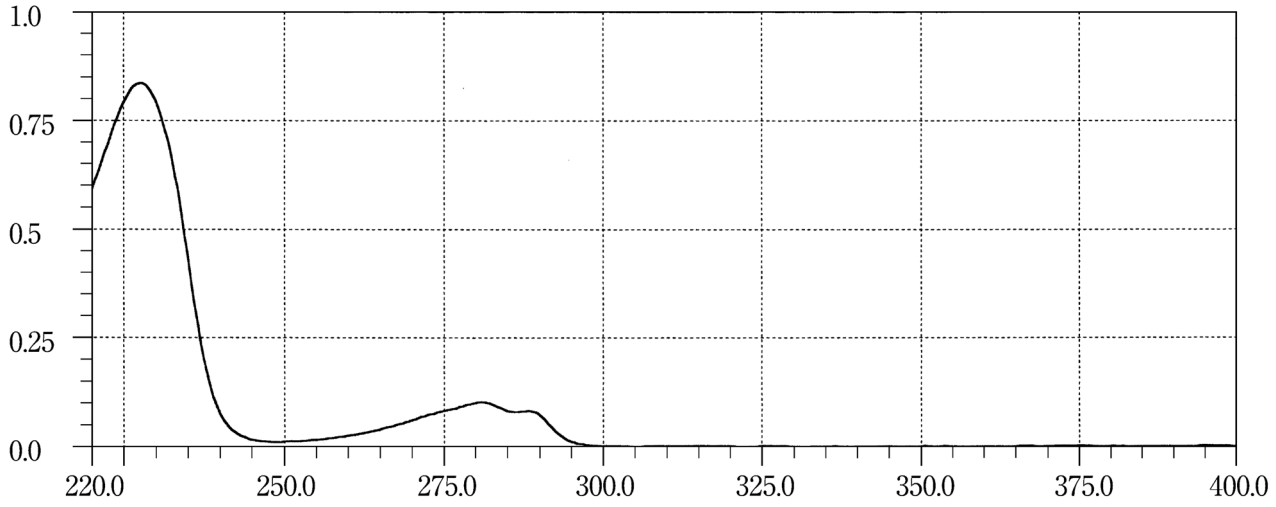
Chloramphenicol Sodium Succinate



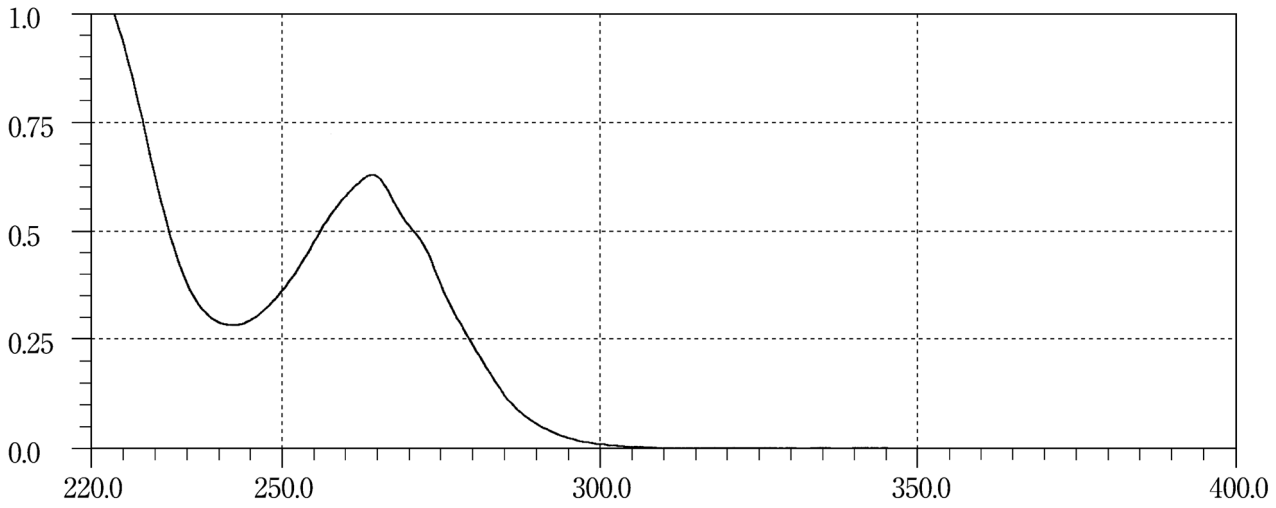
Chlordiazepoxide



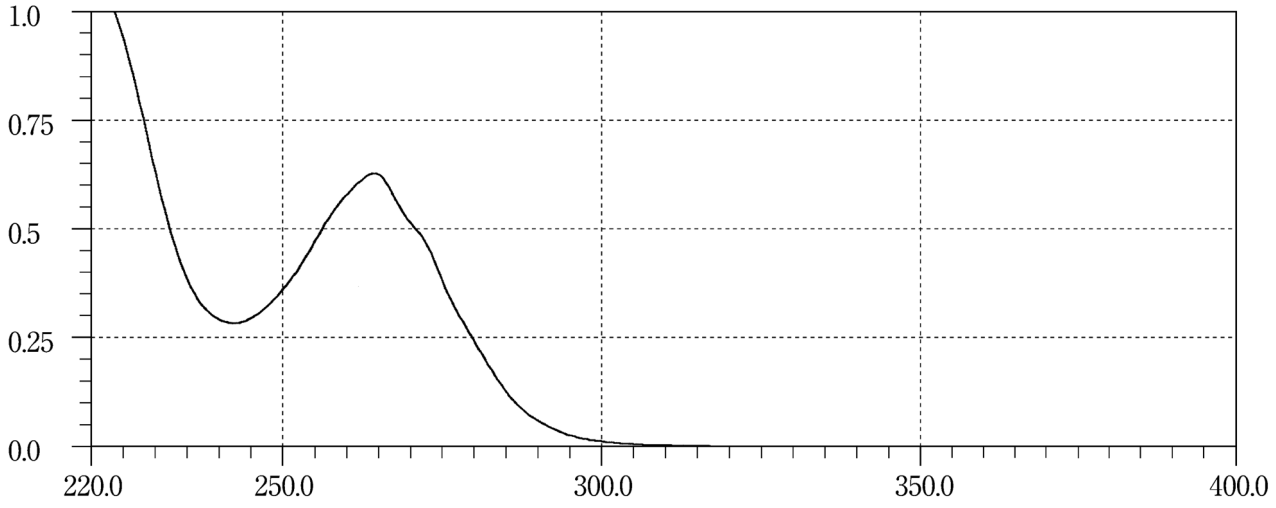
Chlorphenesin Carbamate



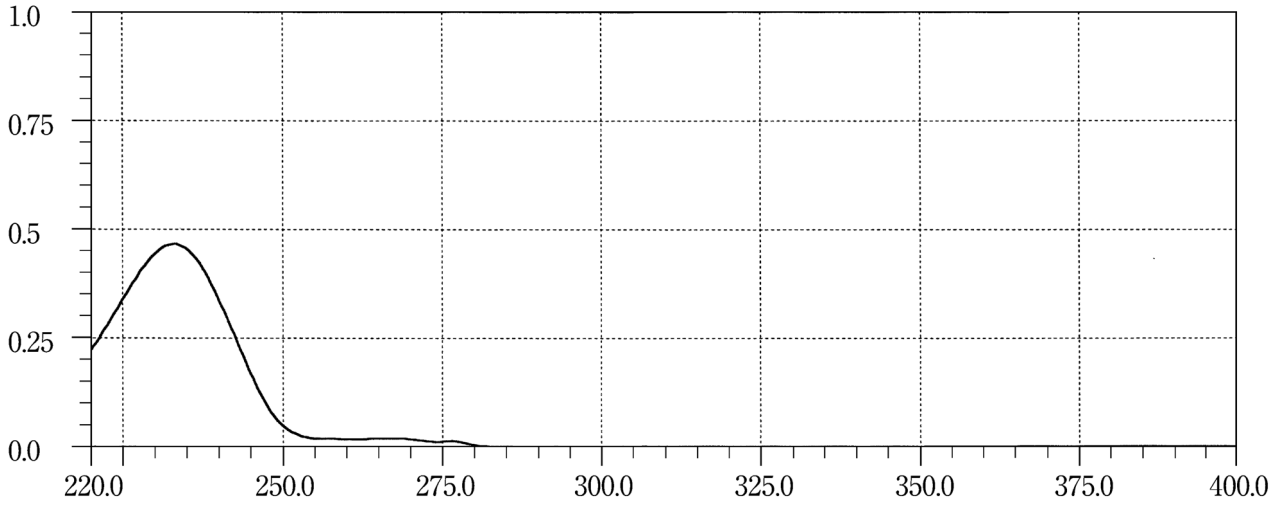
Chlorpheniramine Maleate



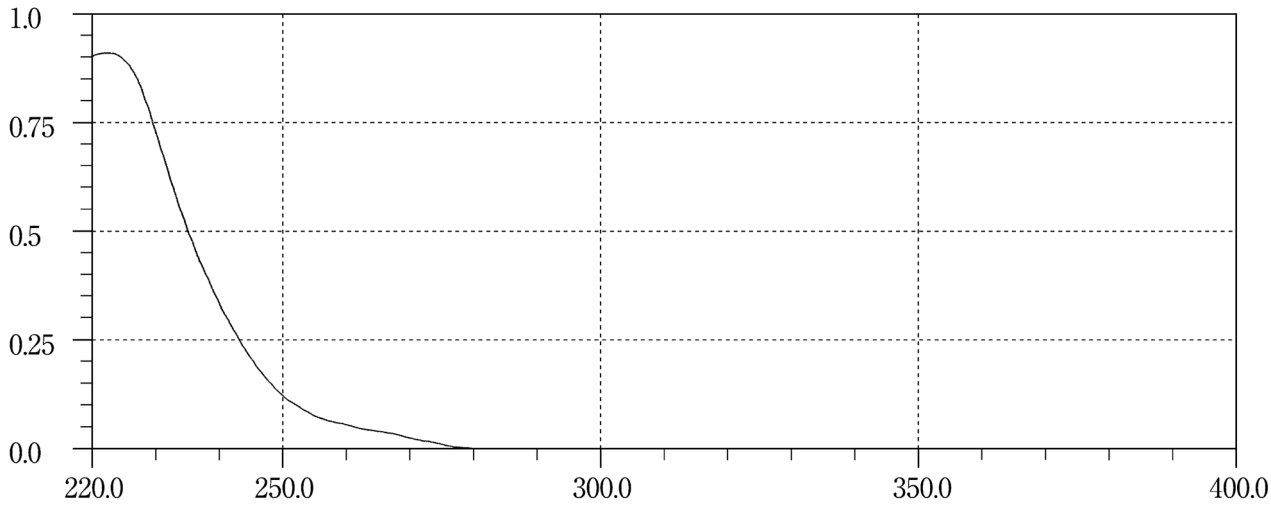
***d*-Chlorpheniramine Maleate**



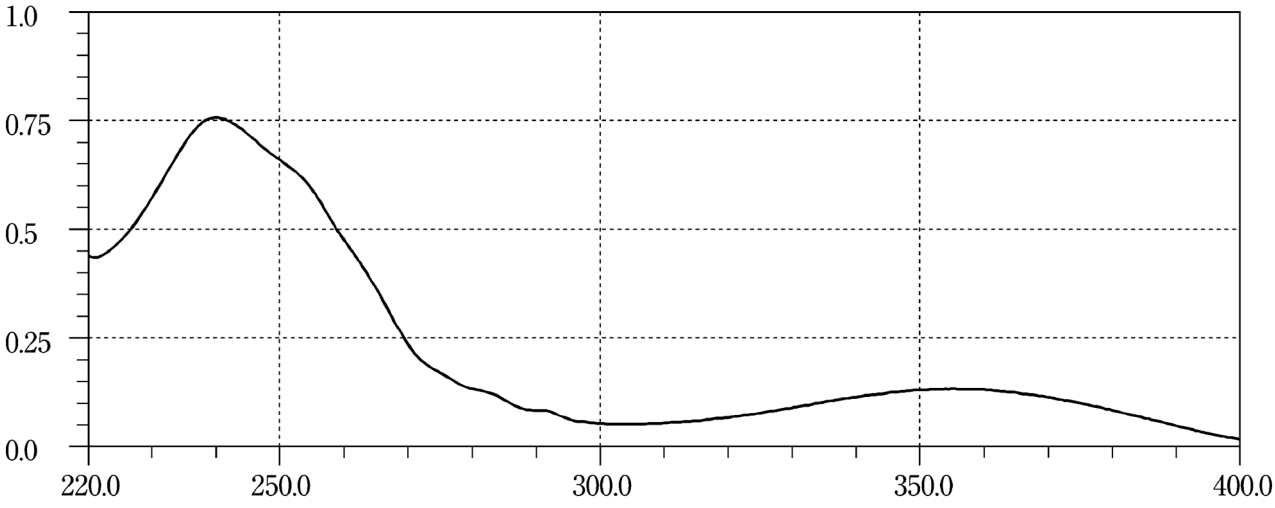
Chlorpropamide



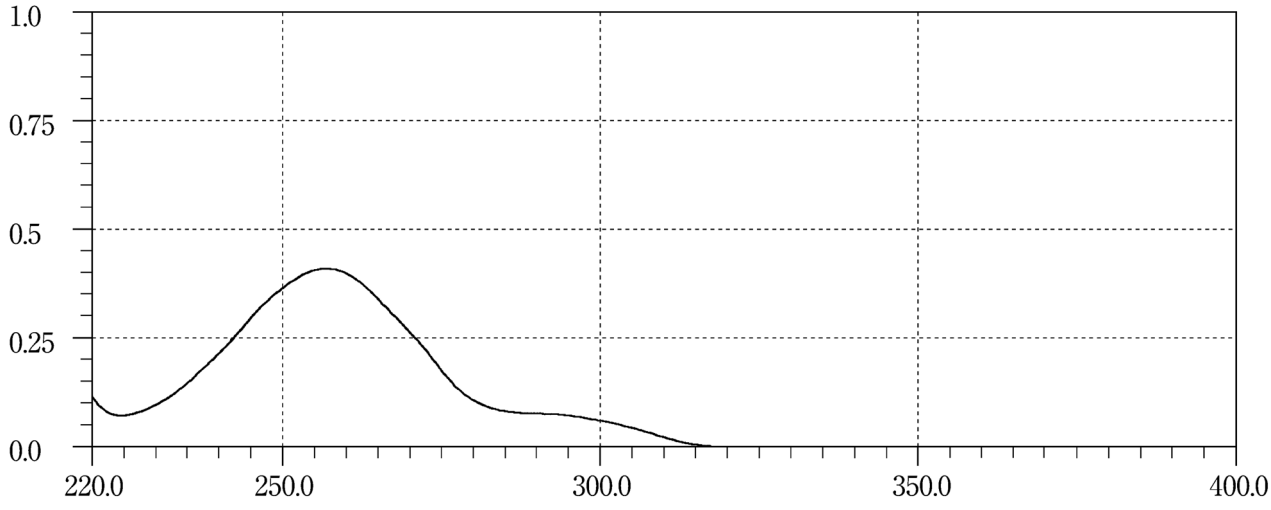
Cibenzoline Succinate



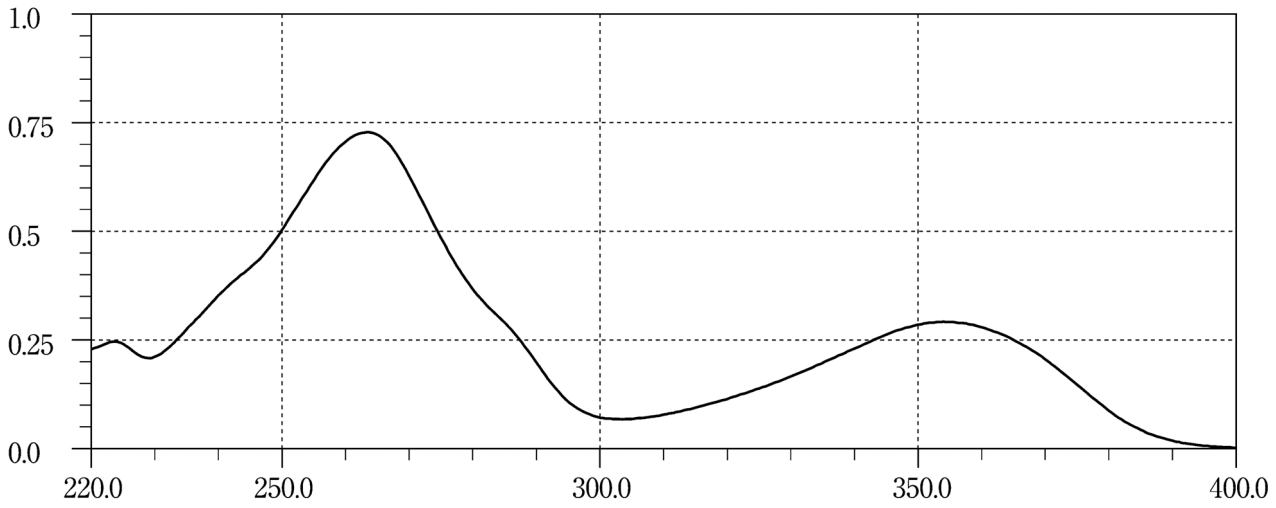
Cilnidipine



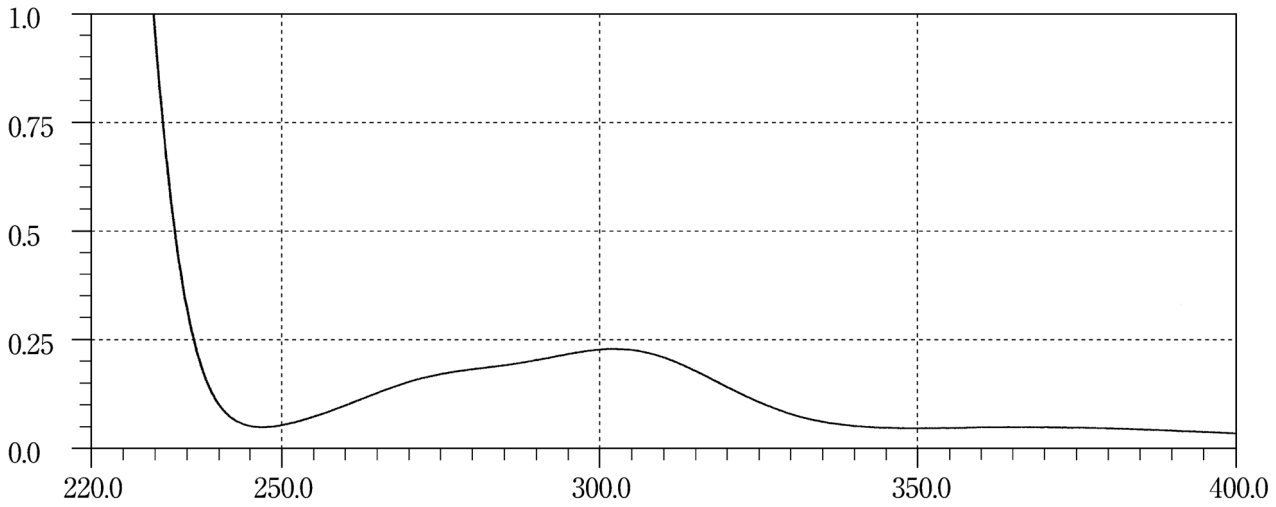
Cilostazol



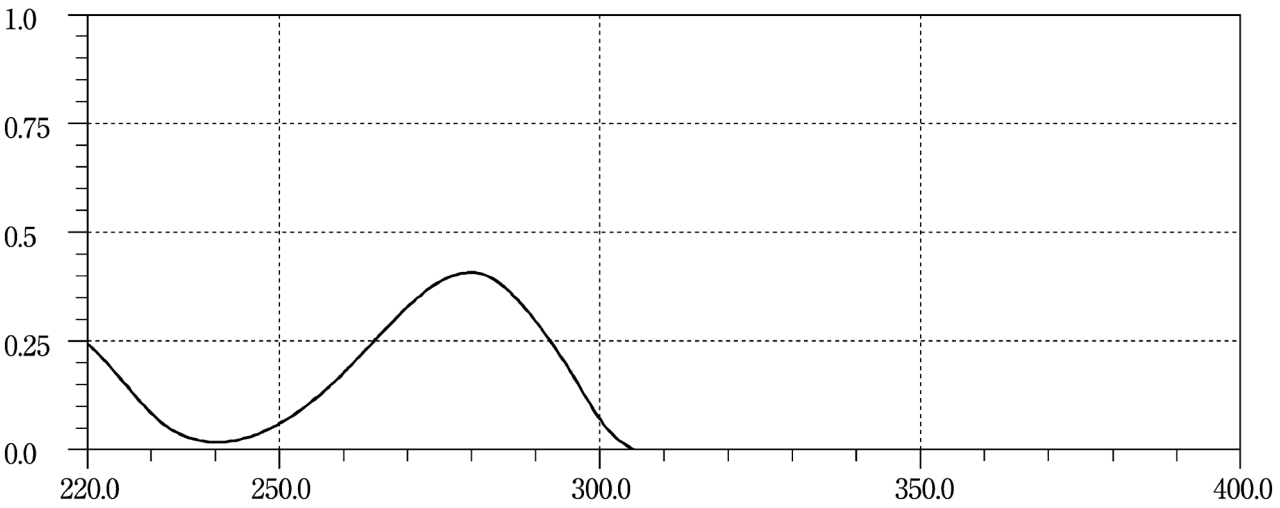
Cinoxacin



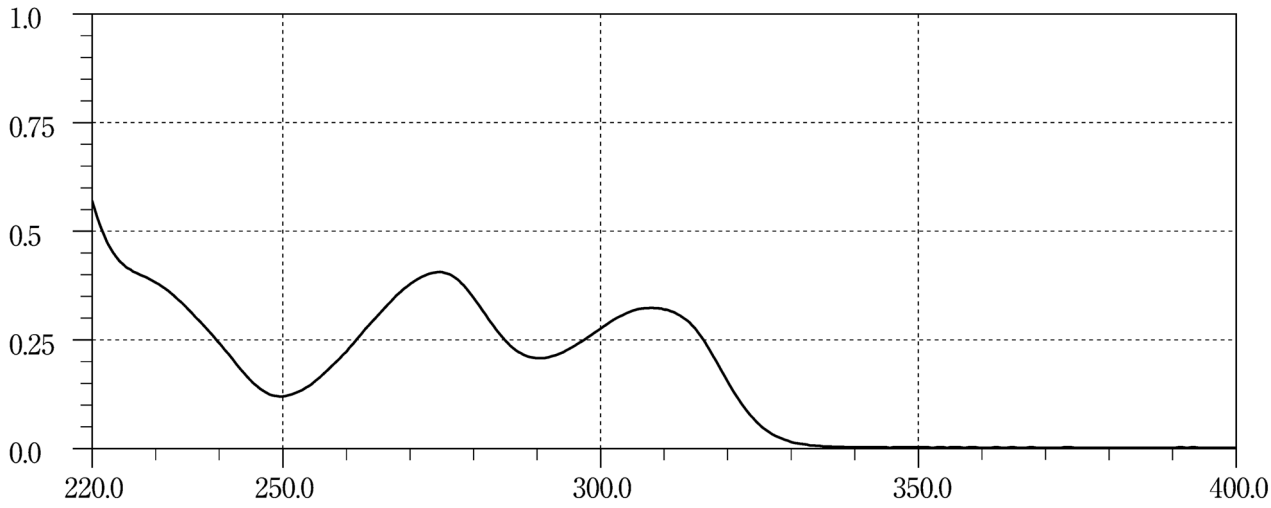
Cisplatin



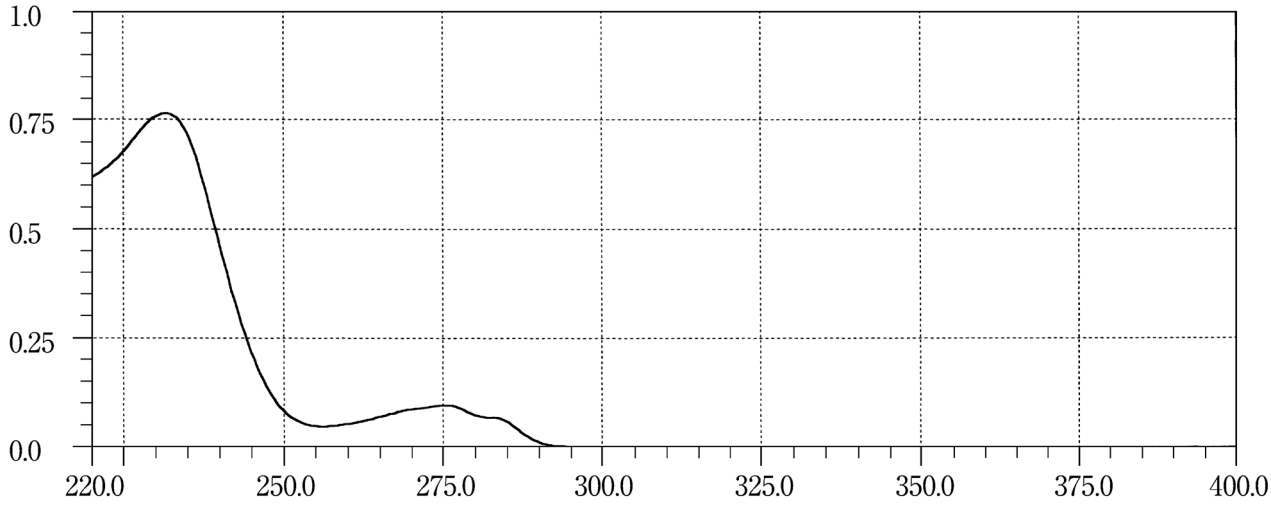
Citicoline



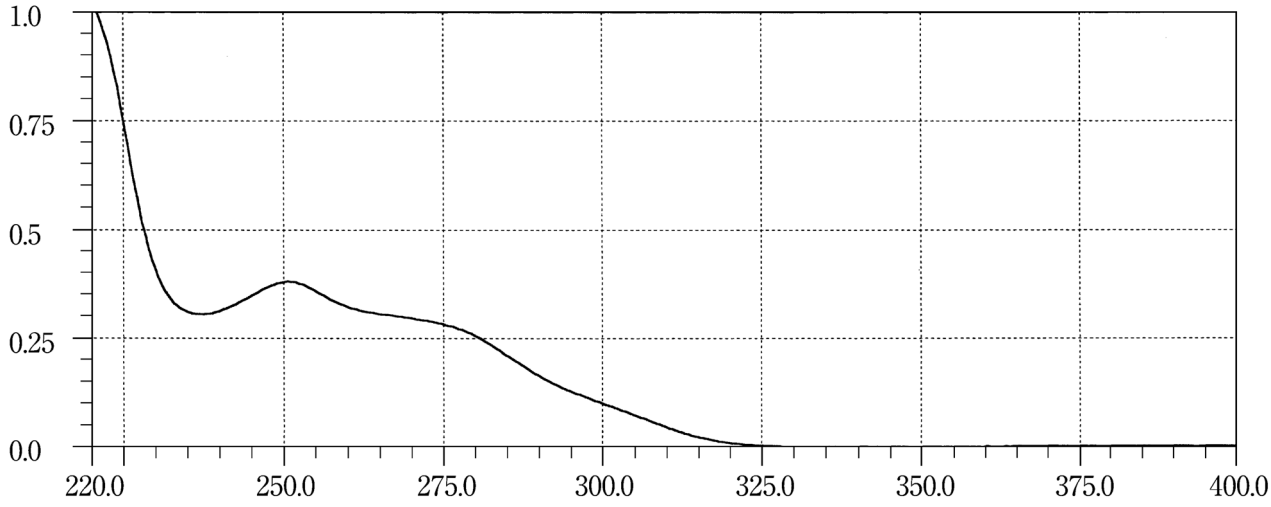
Clebopride Malate



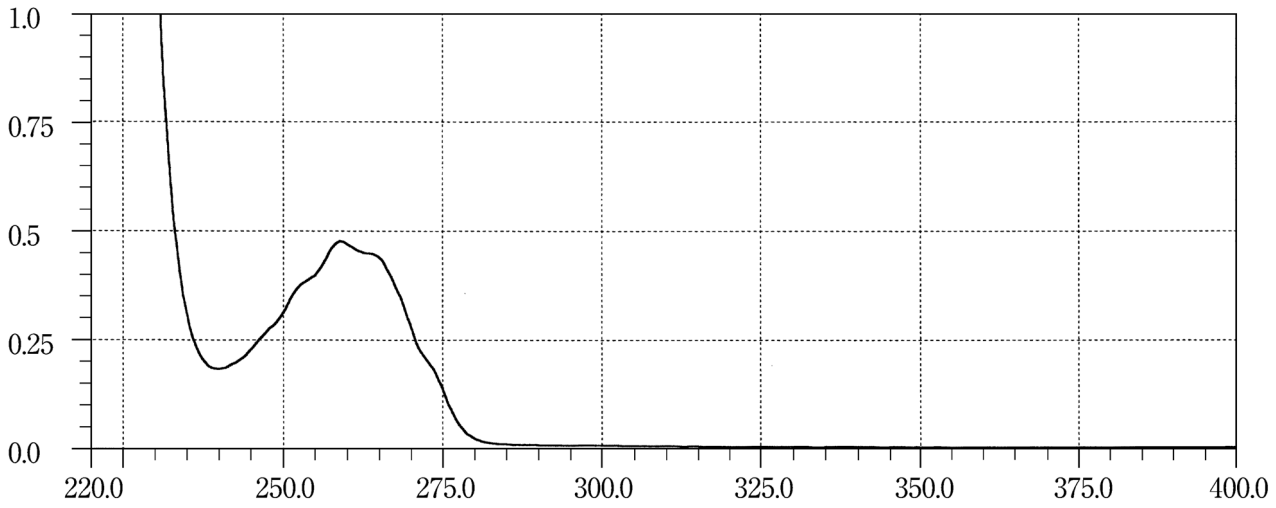
Clinofibrate



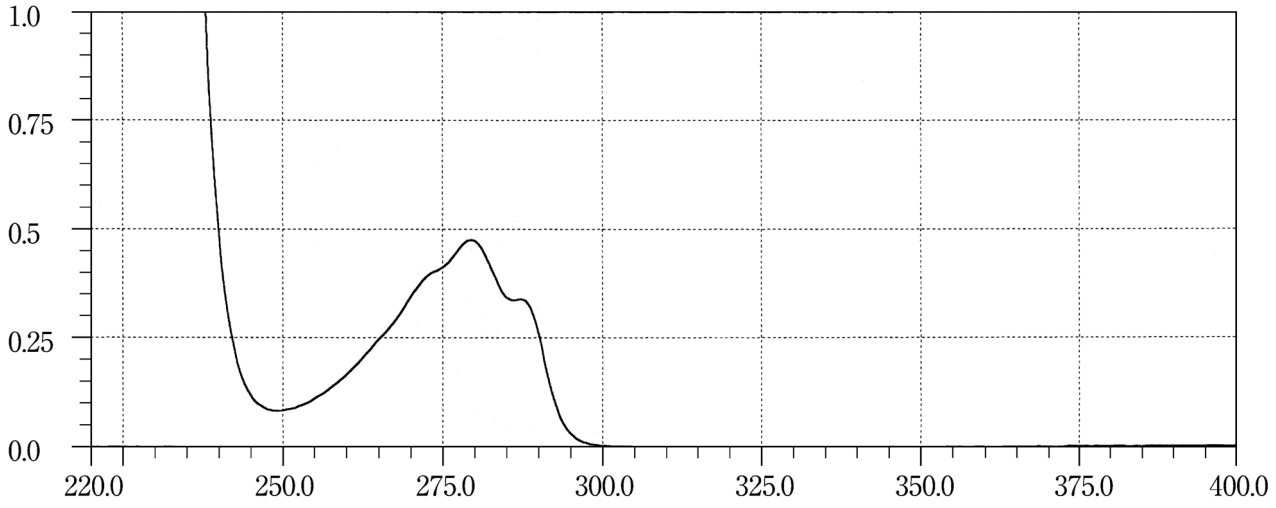
Clocapramine Hydrochloride Hydrate



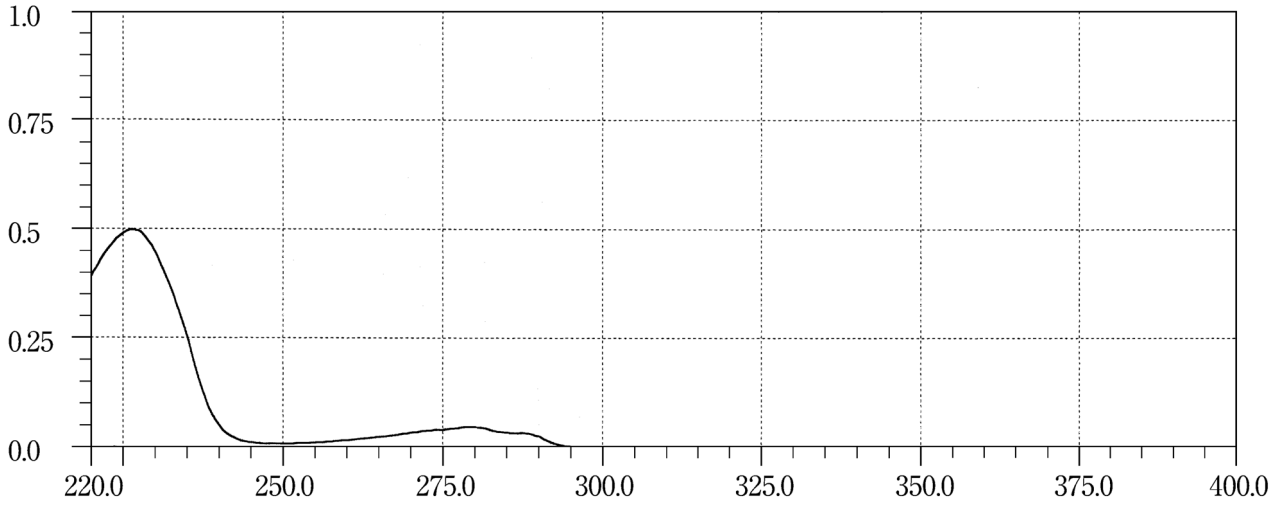
Clofedanol Hydrochloride



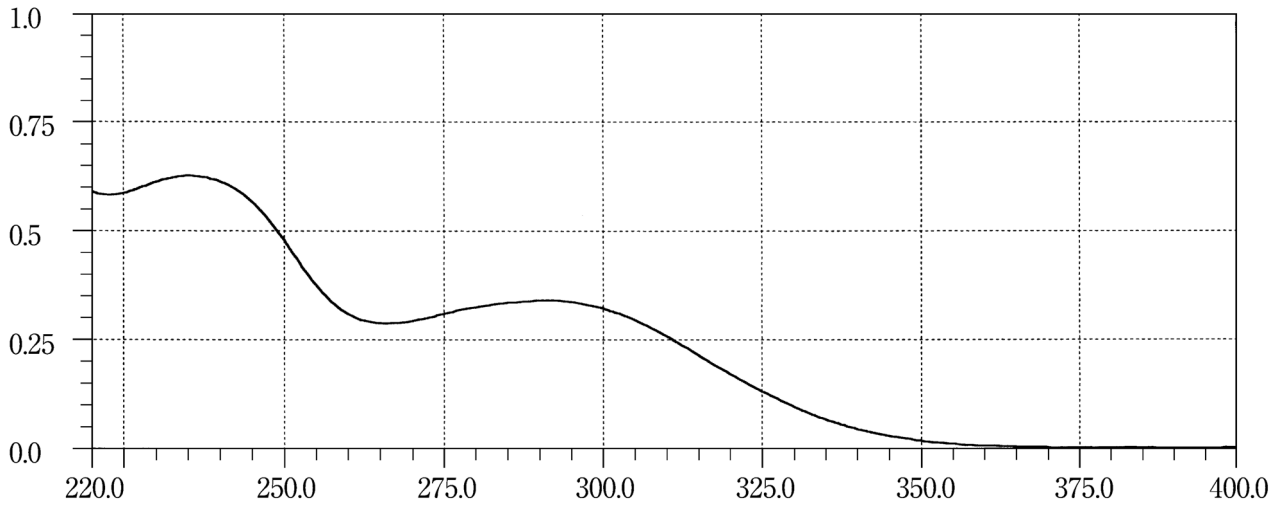
Clofibrate 1



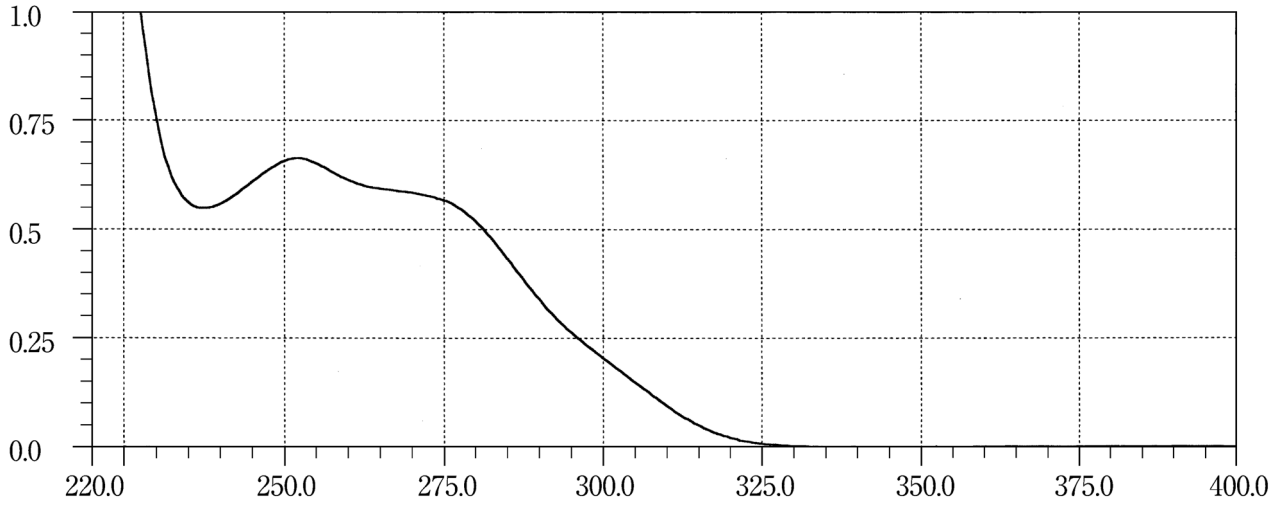
Clofibrate 2



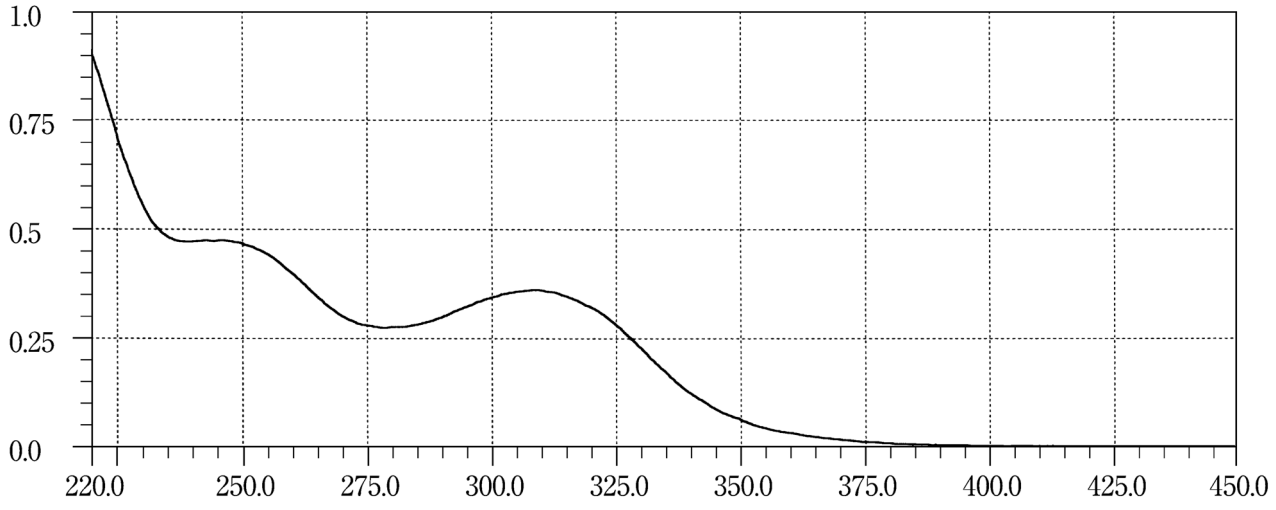
Clomifene Citrate



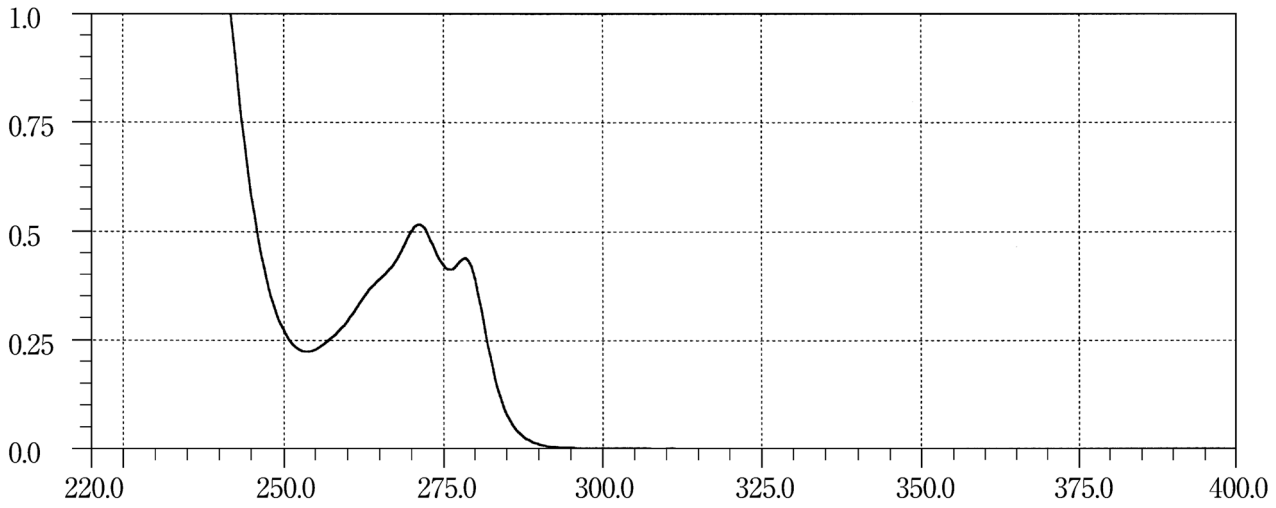
Clomipramine Hydrochloride



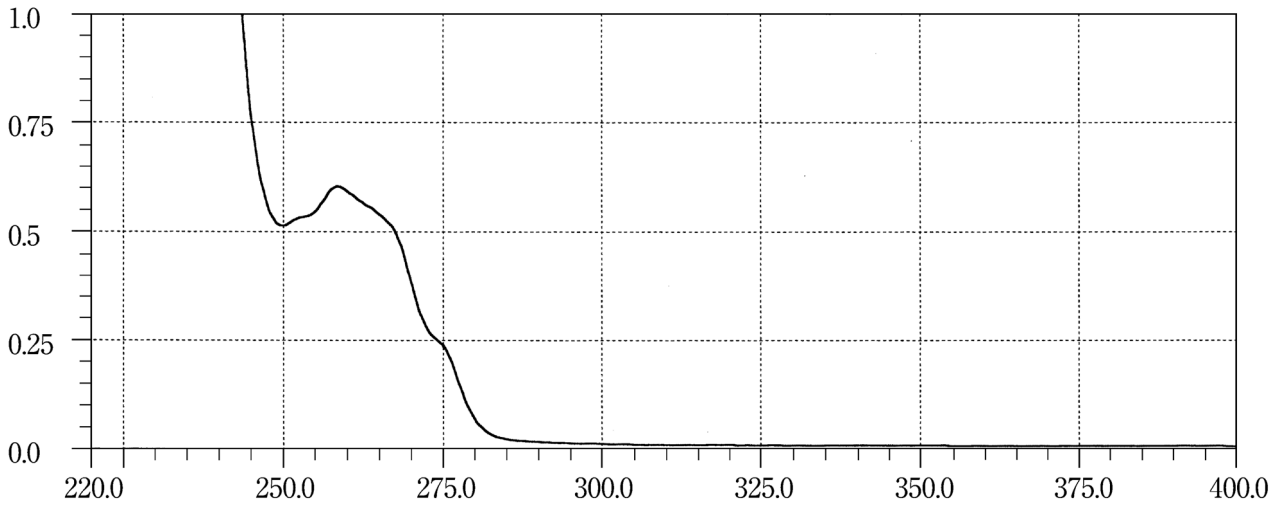
Clonazepam



Clonidine Hydrochloride



Cloperastine Hydrochloride 1



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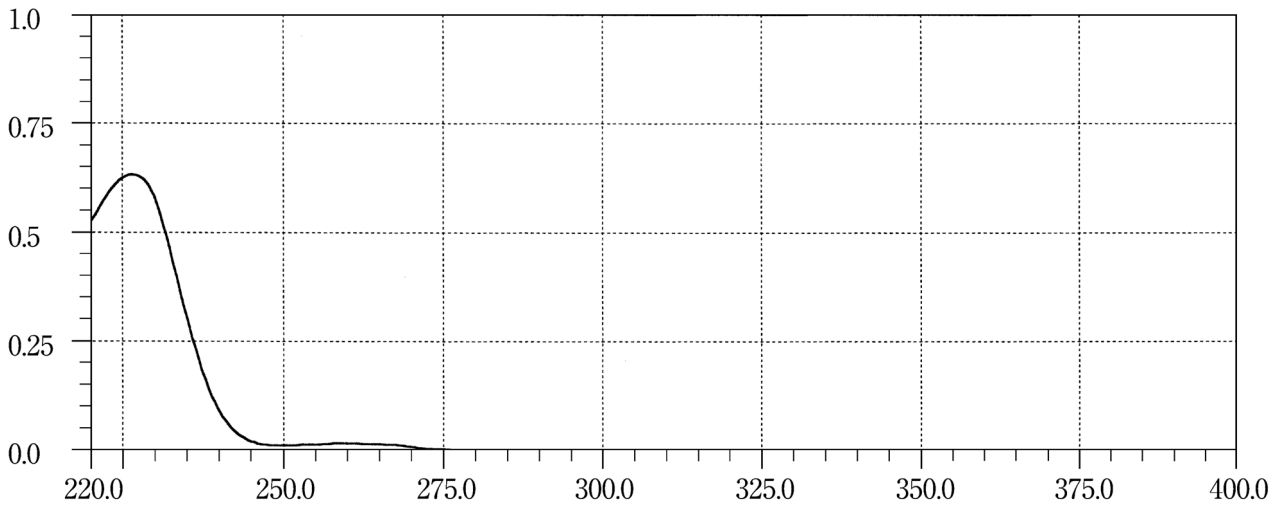
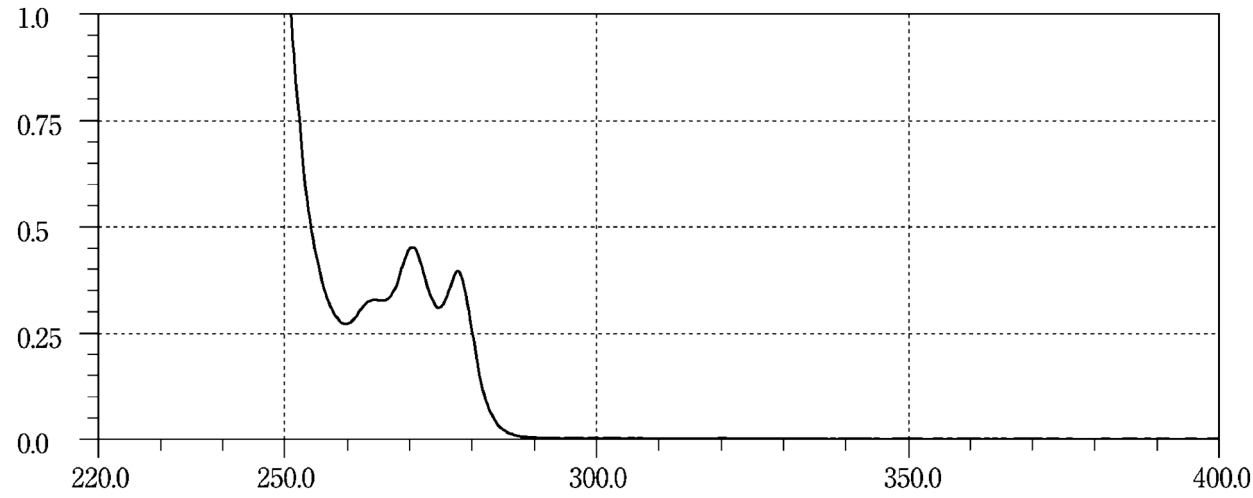
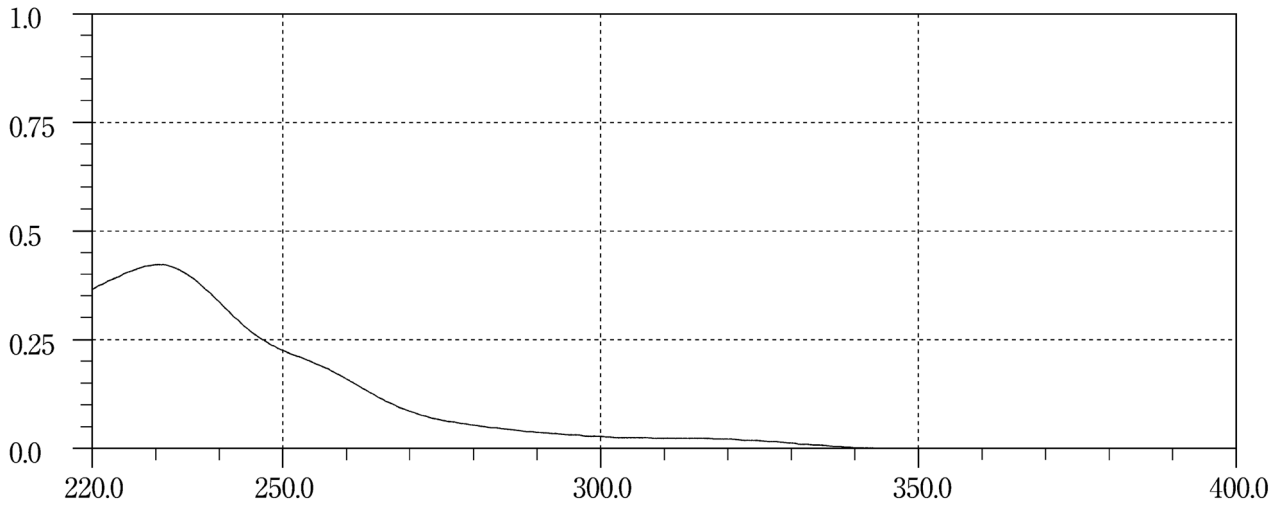


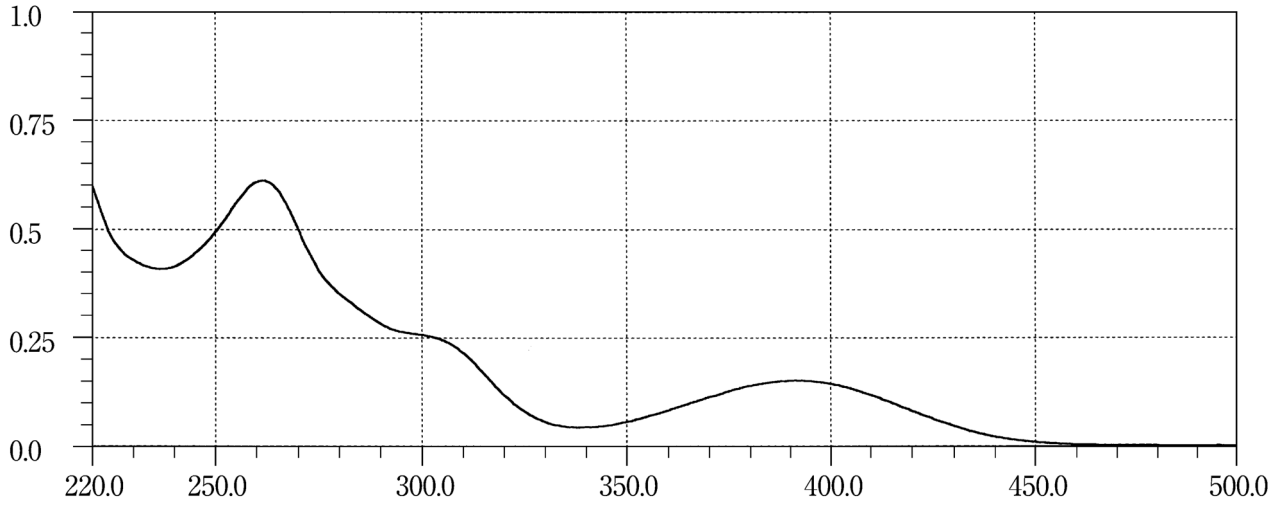
Clopidogrel Sulfate



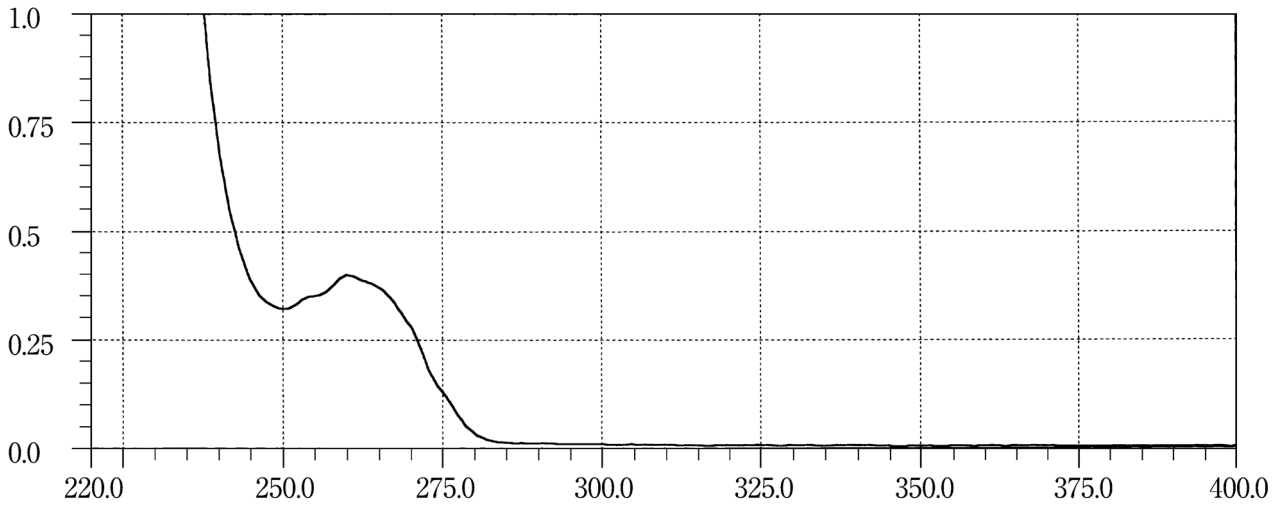
Clorazepate Dipotassium



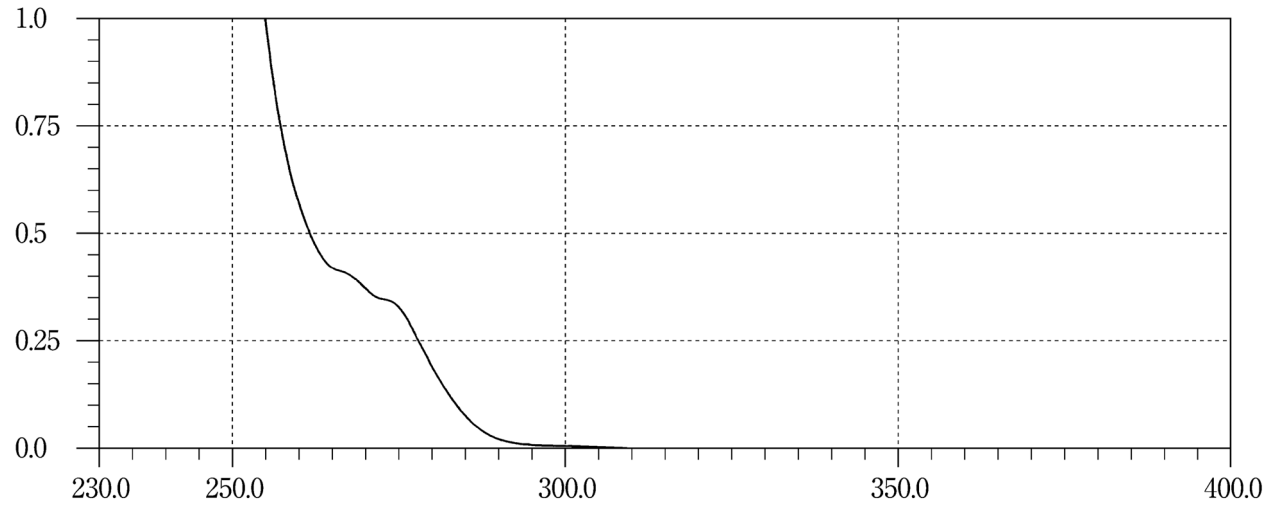
Clotiazepam



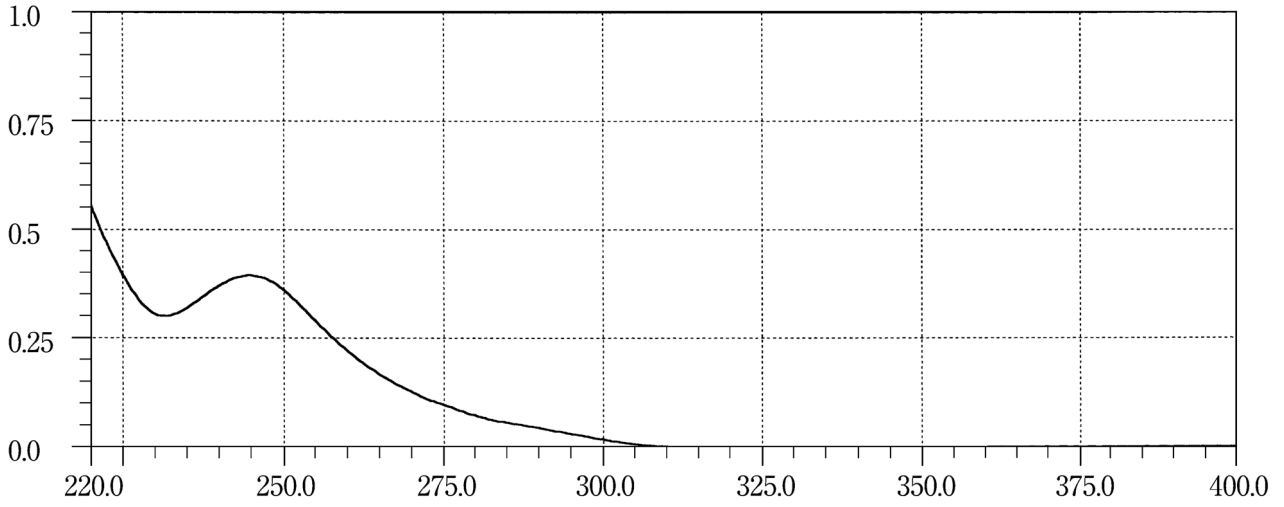
Clotrimazole



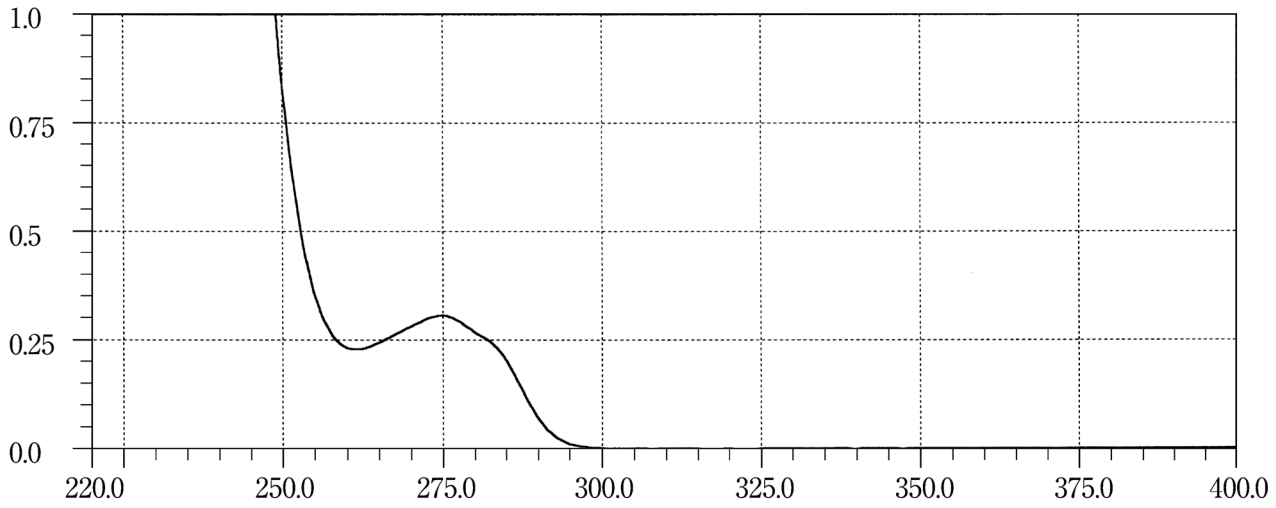
Cloxacillin Sodium Hydrate



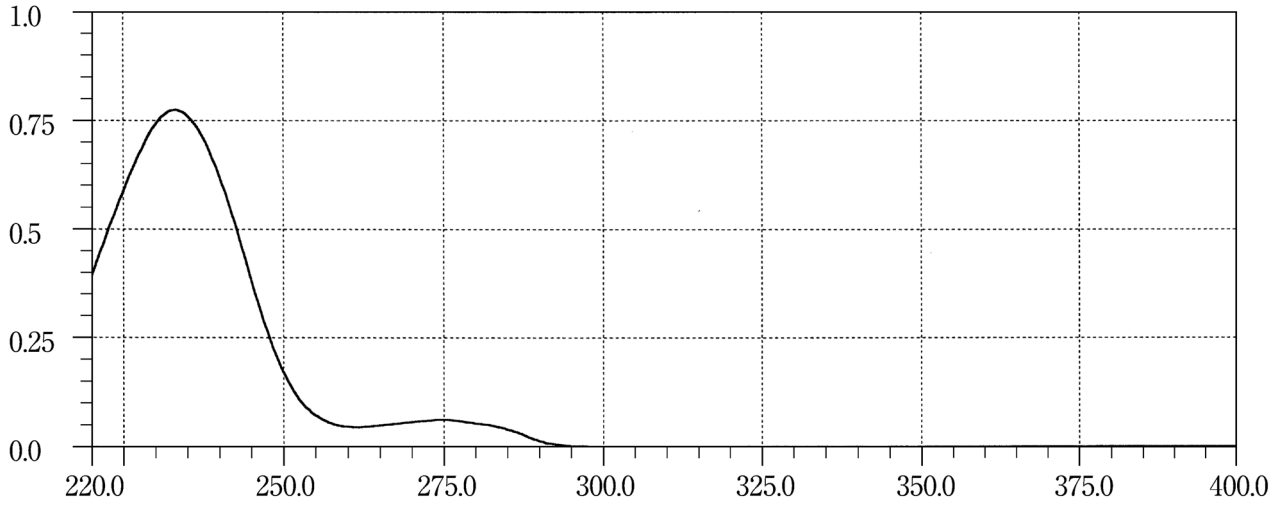
Cloxazolam



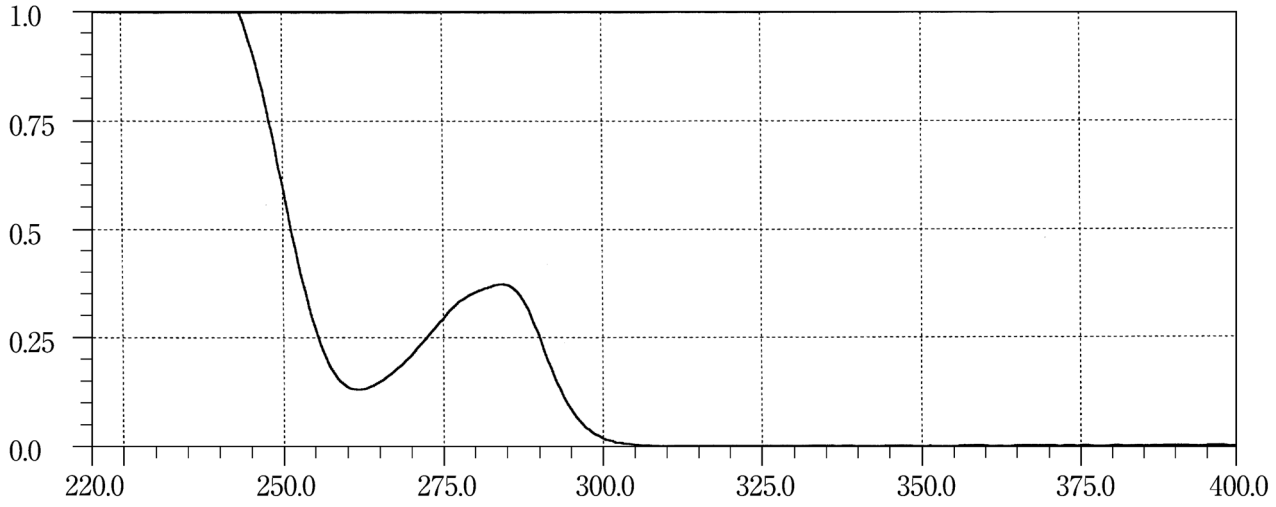
Cocaine Hydrochloride 1



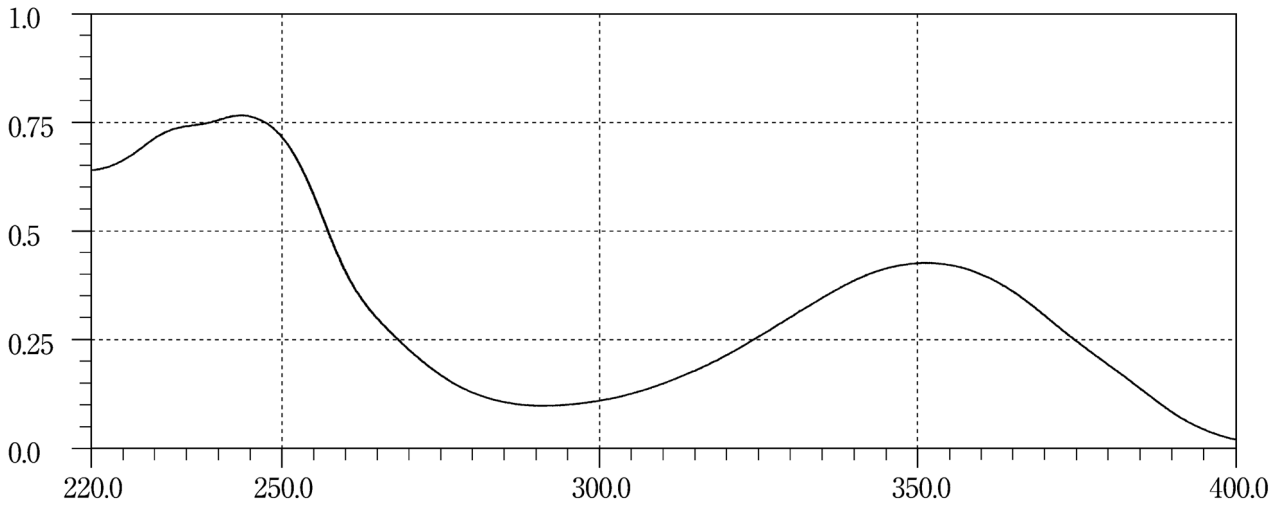
Cocaine Hydrochloride 2



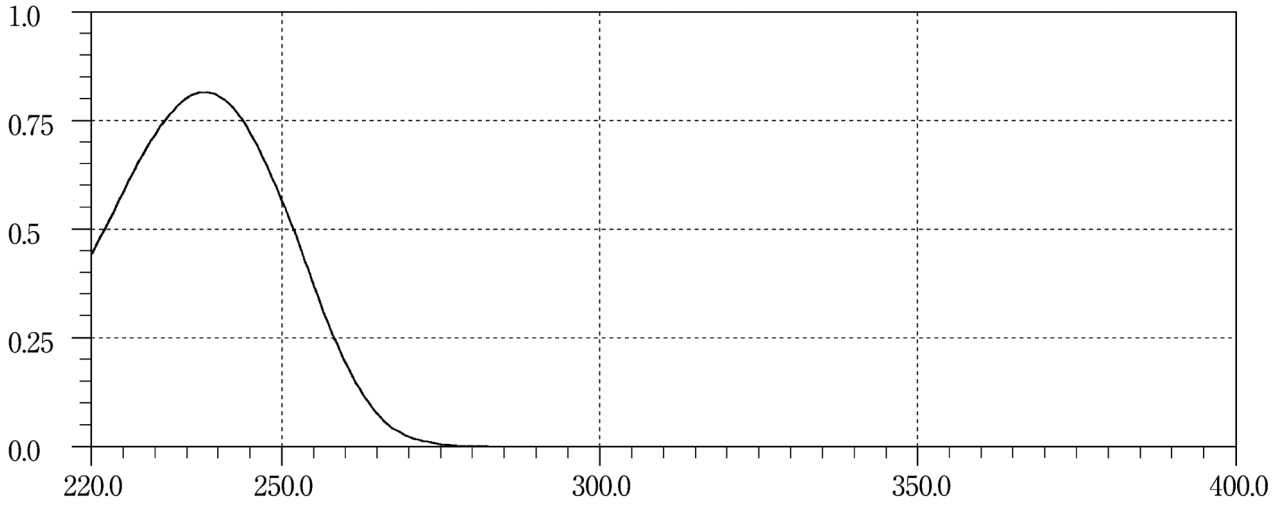
Codeine Phosphate Hydrate



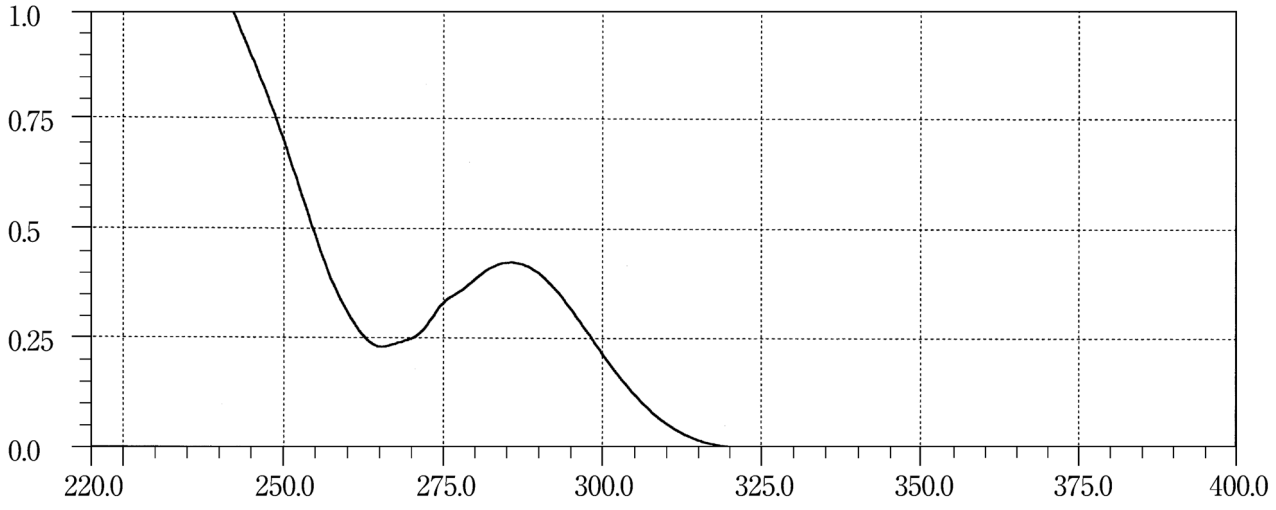
Colchicine



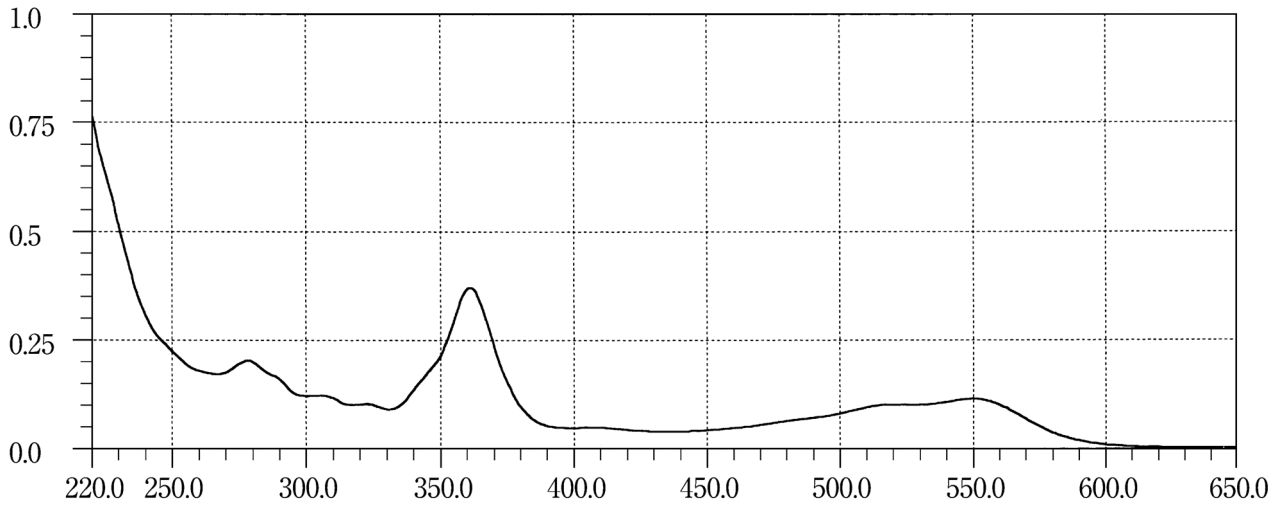
Cortisone Acetate



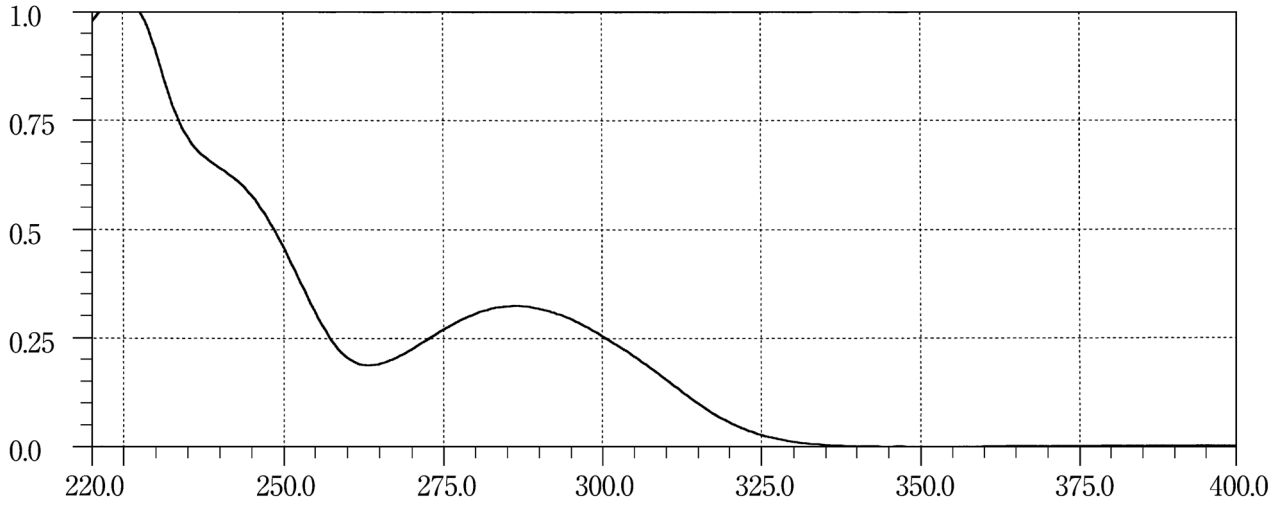
Croconazole Hydrochloride



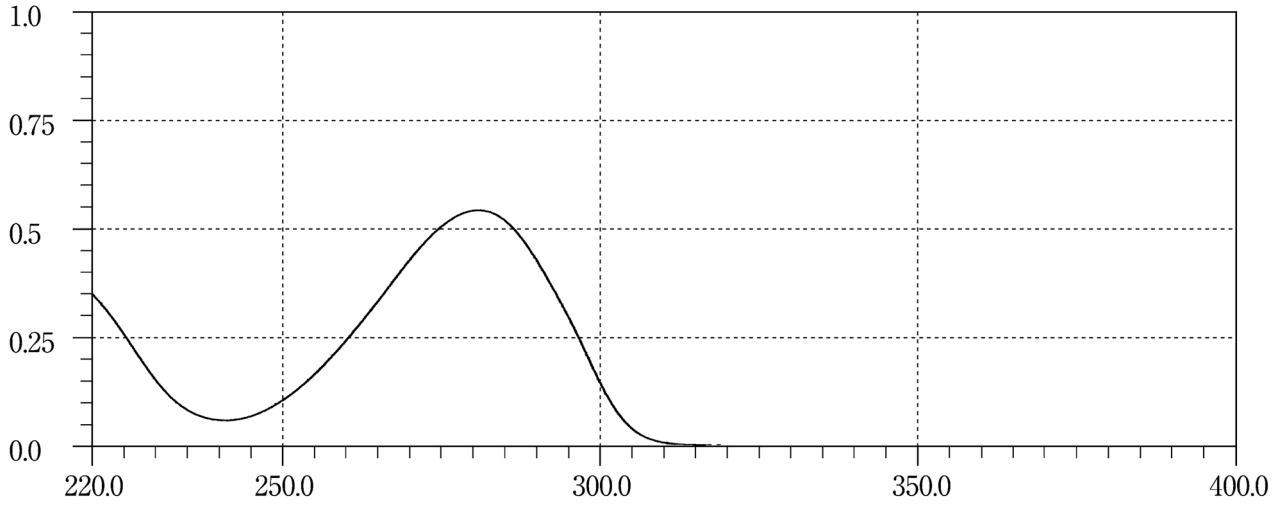
Cyanocobalamin



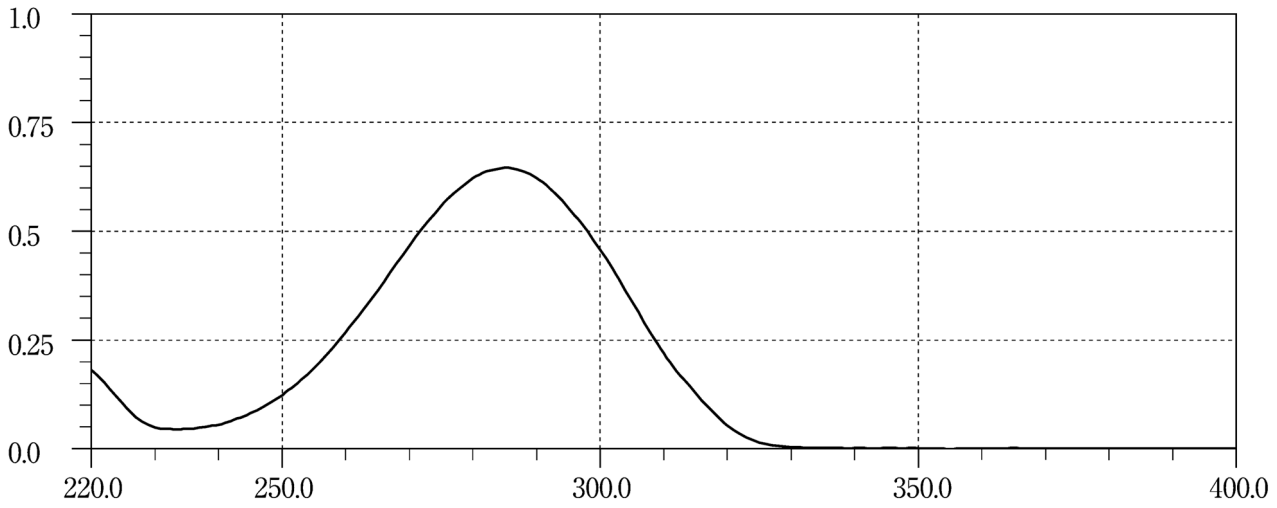
Cyproheptadine Hydrochloride Hydrate



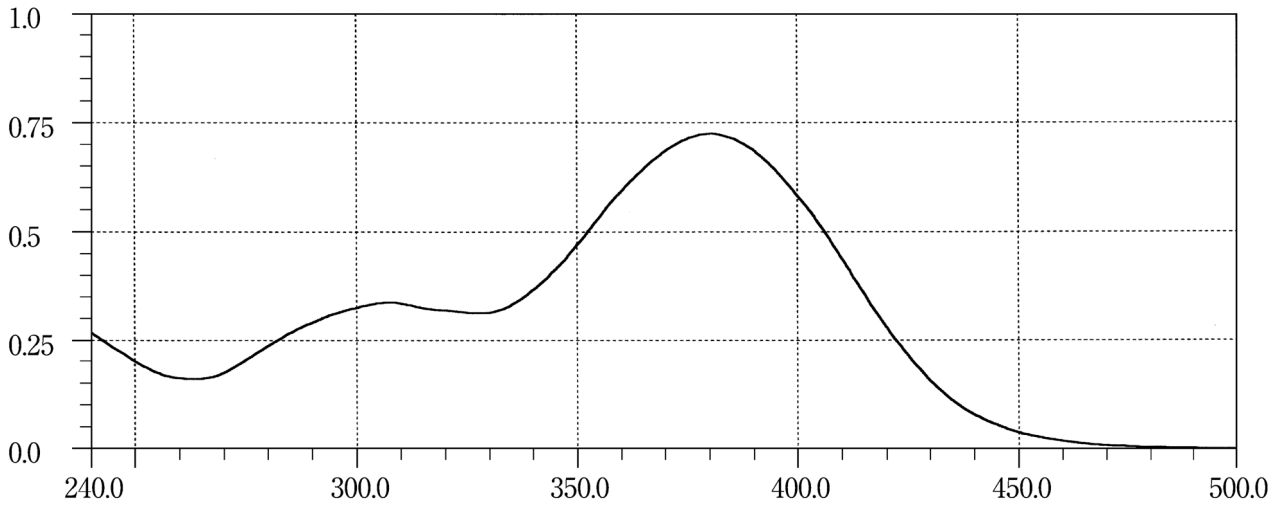
Cytarabine



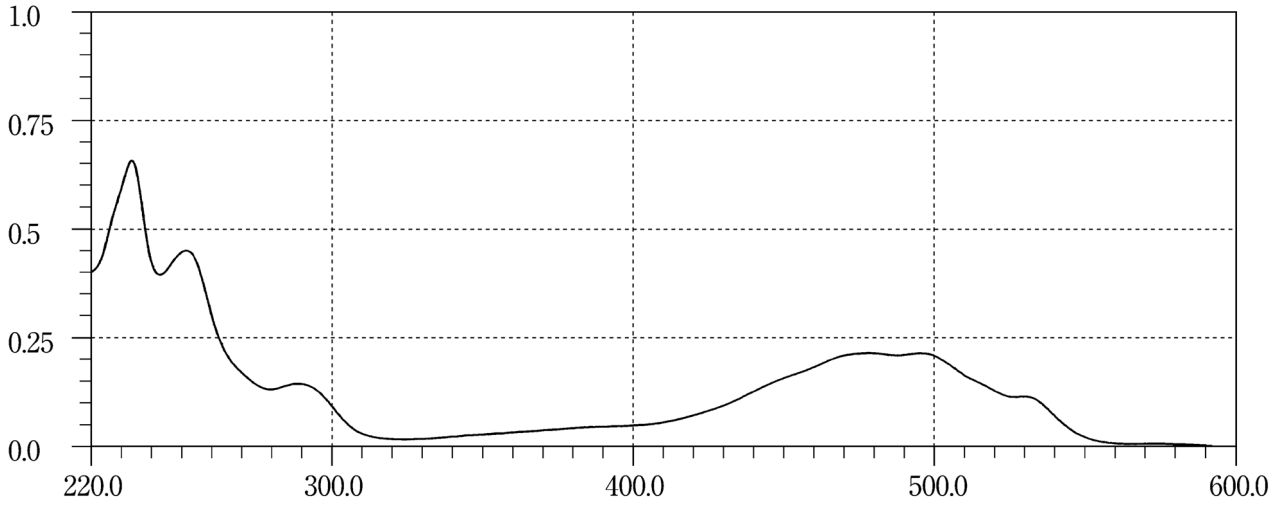
Danazol



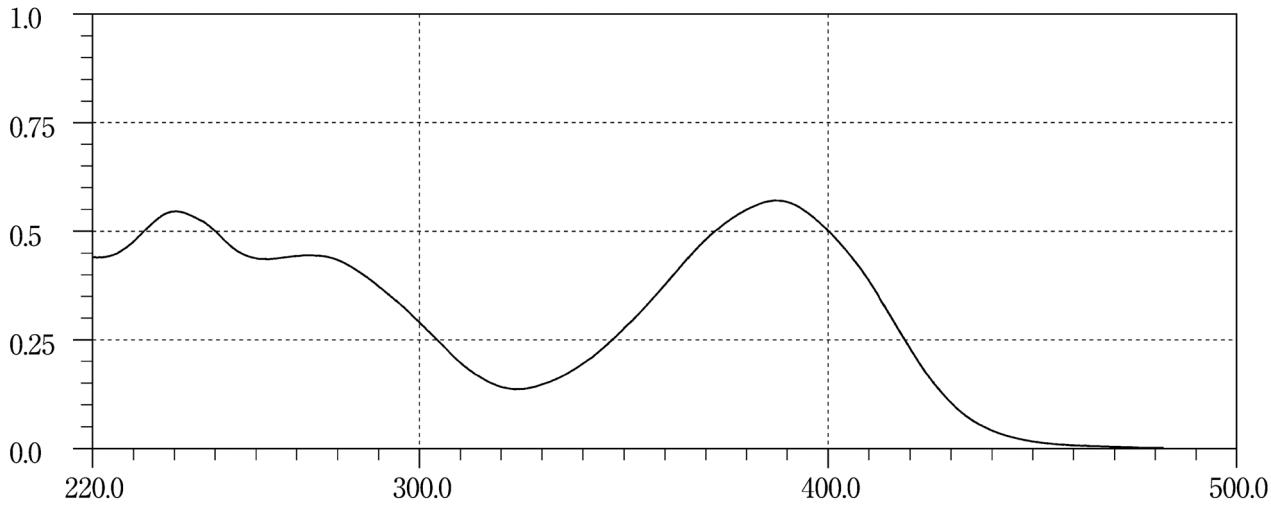
Dantrolene Sodium Hydrate



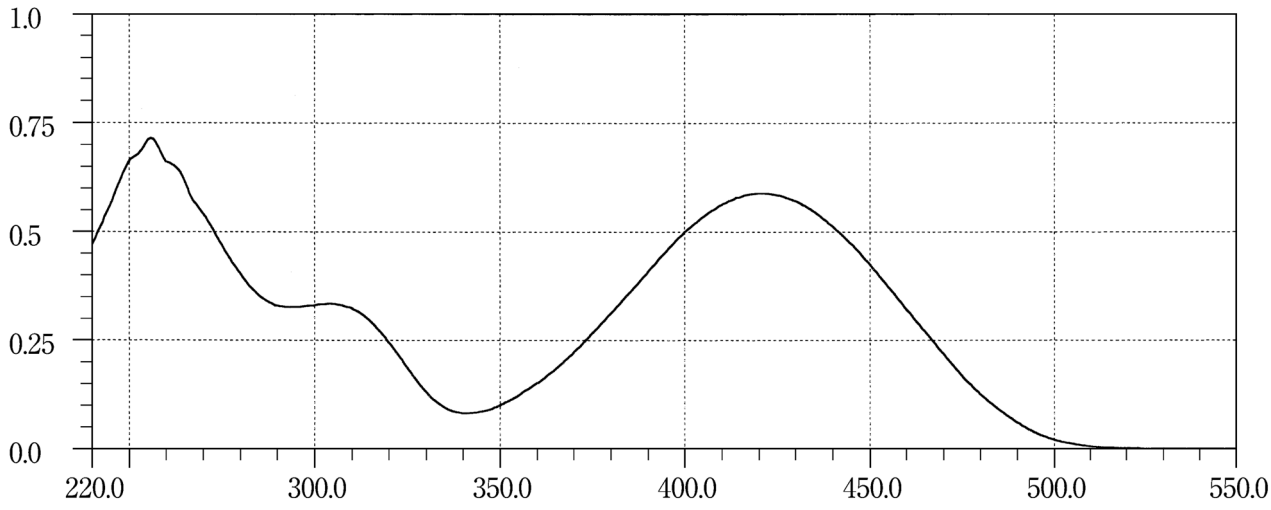
Daunorubicin Hydrochloride



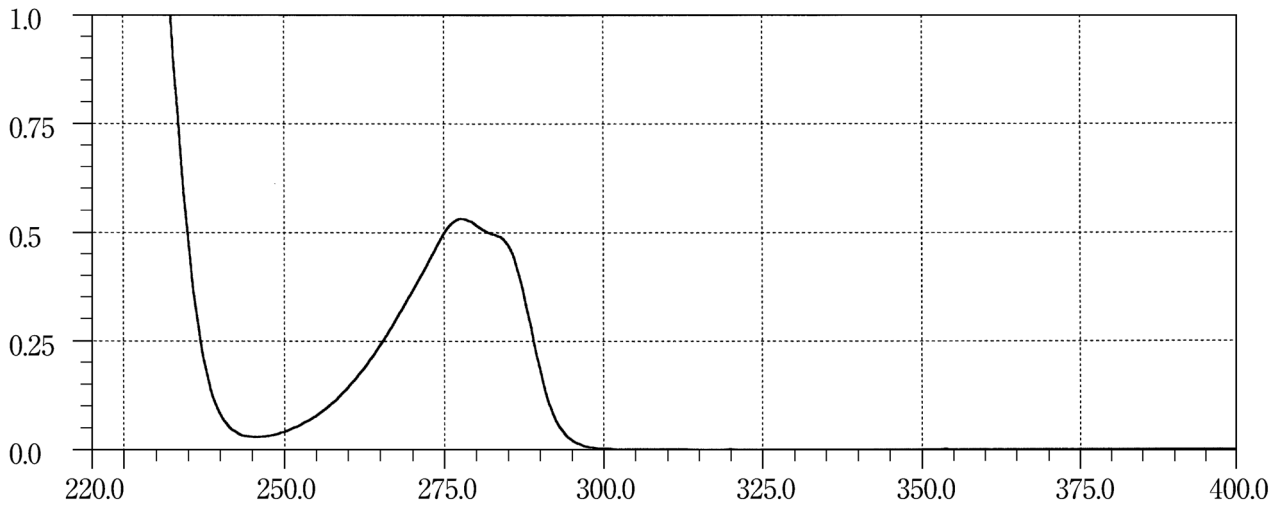
Demethylchlortetracycline Hydrochloride



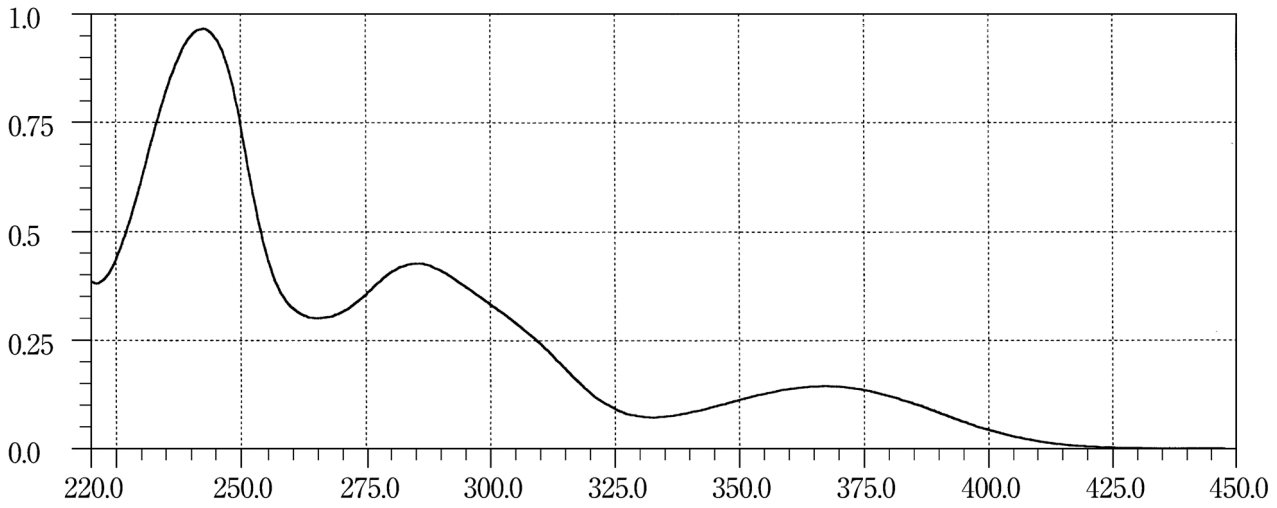
Dexamethasone



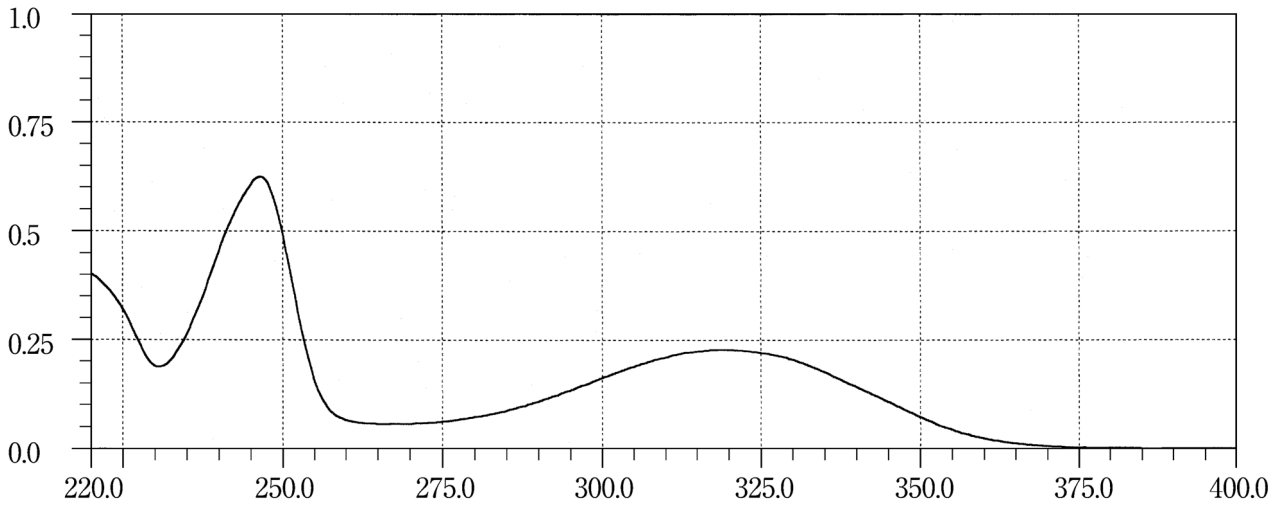
Dextromethorphan Hydrobromide Hydrate



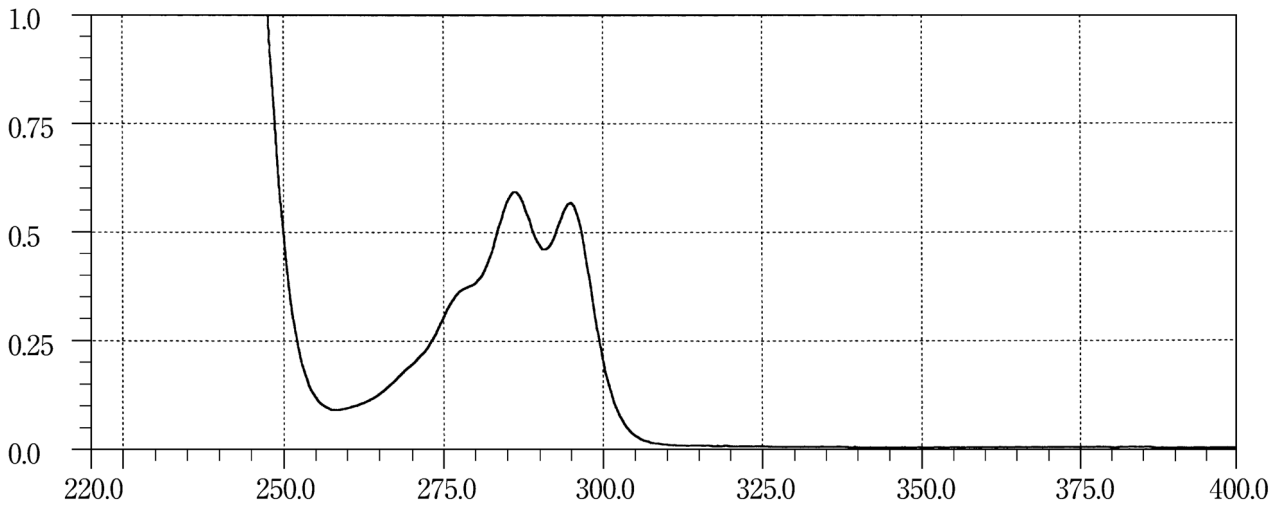
Diazepam



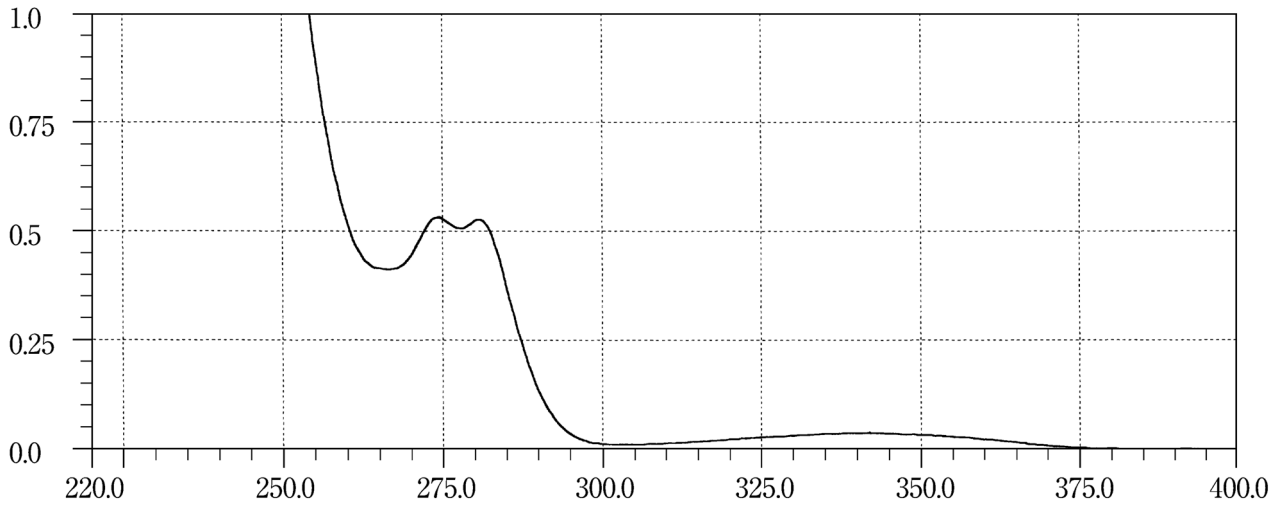
Dibucaine Hydrochloride



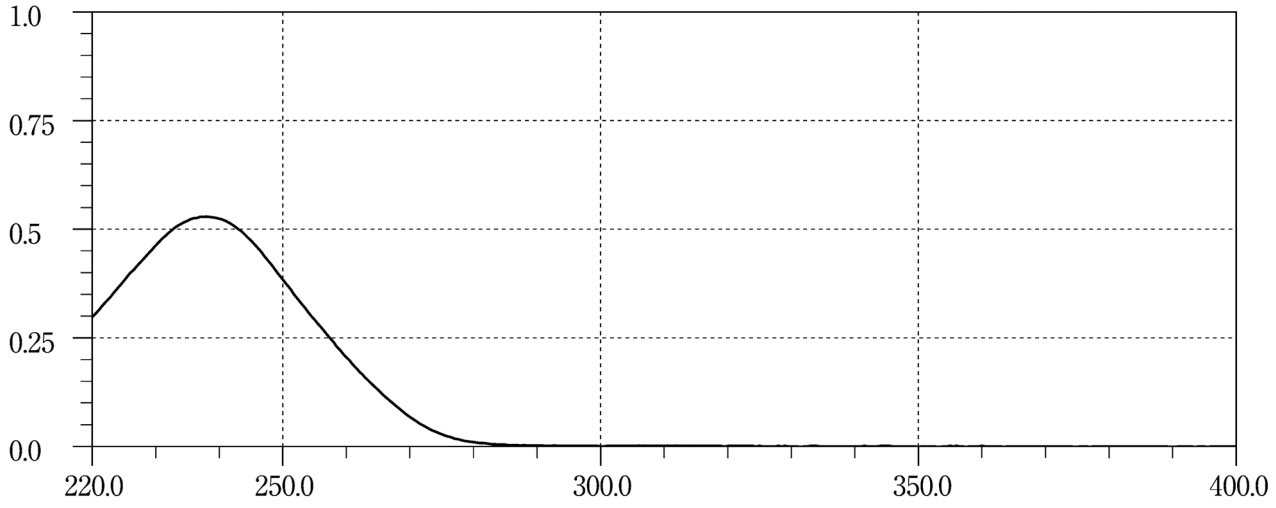
Diclofenamide



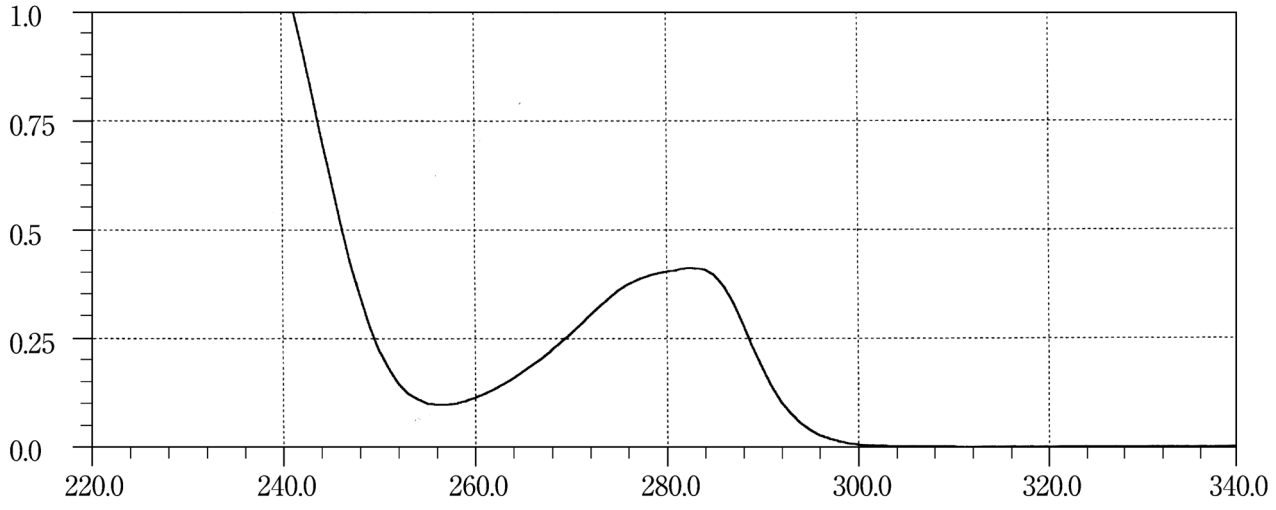
Dicloxacillin Sodium Hydrate



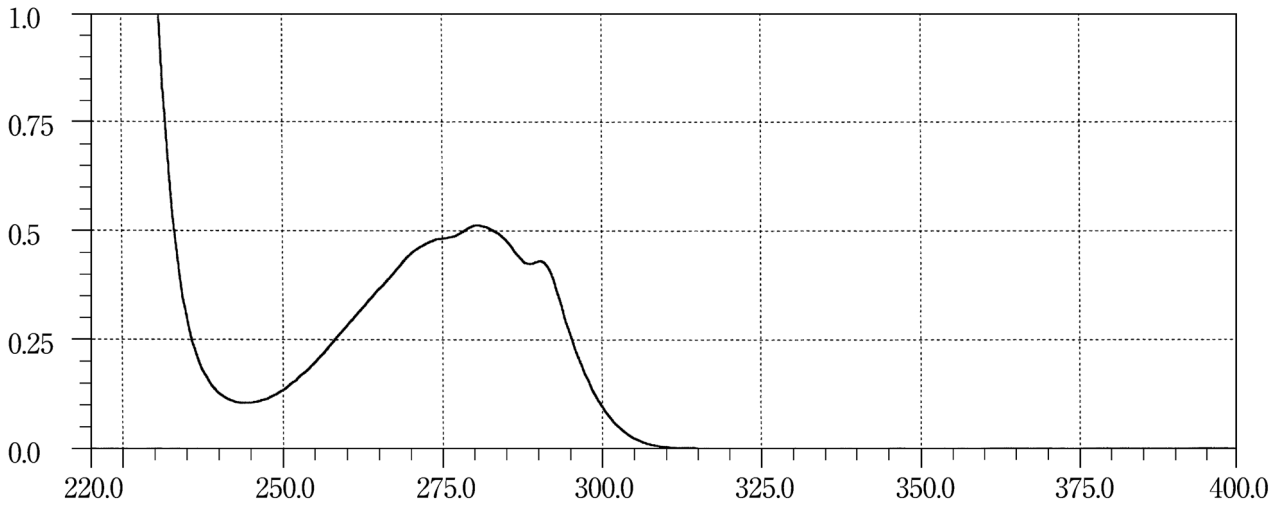
Diflucortolone Valerate



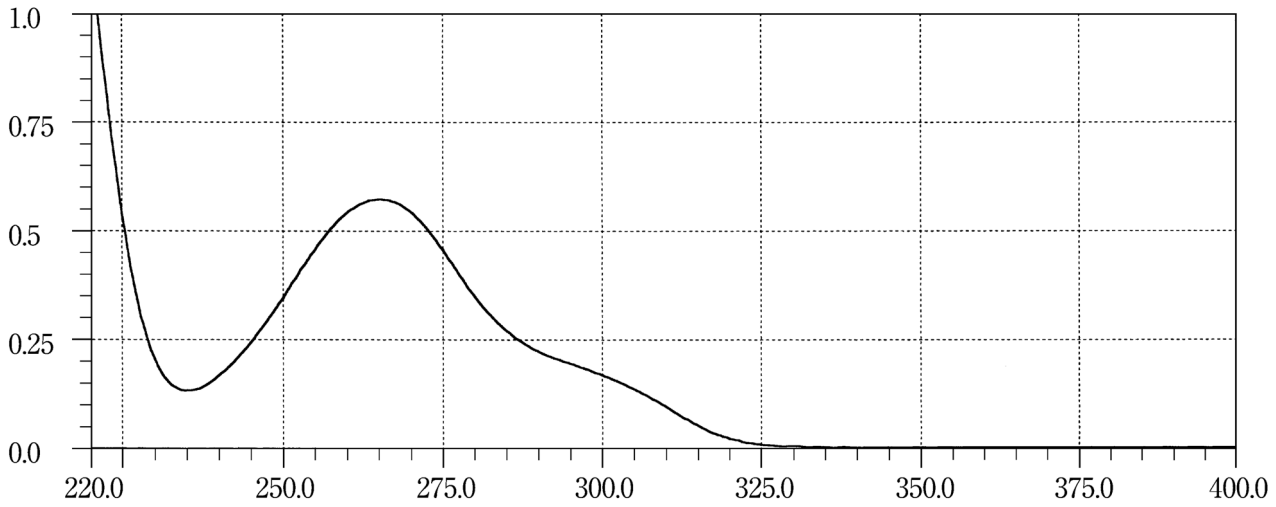
Dihydrocodeine Phosphate



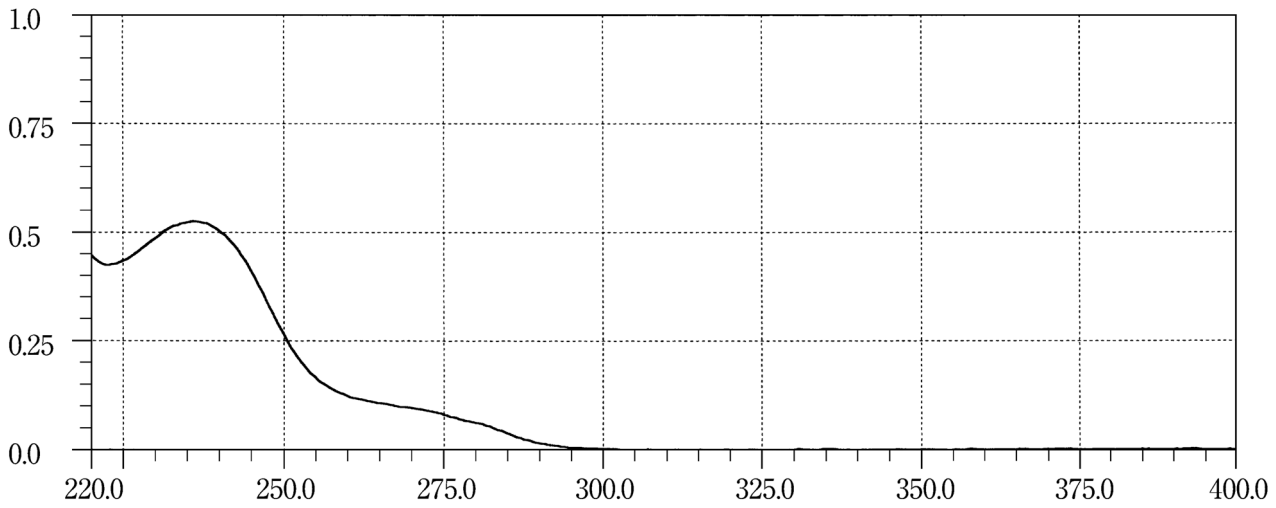
Dihydroergotamine Mesilate



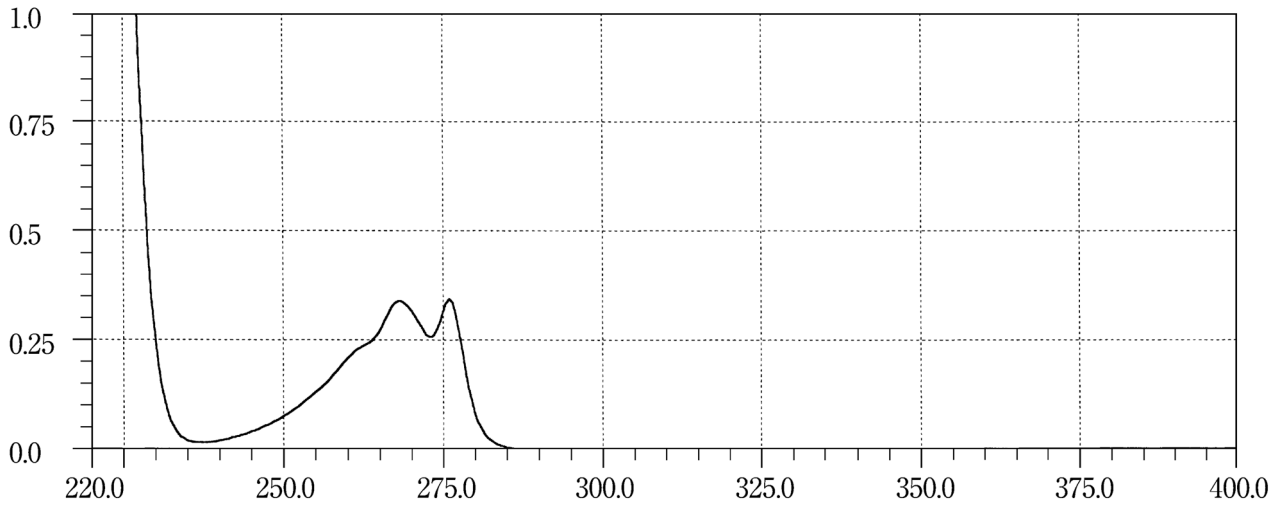
Dilazep Hydrochloride Hydrate



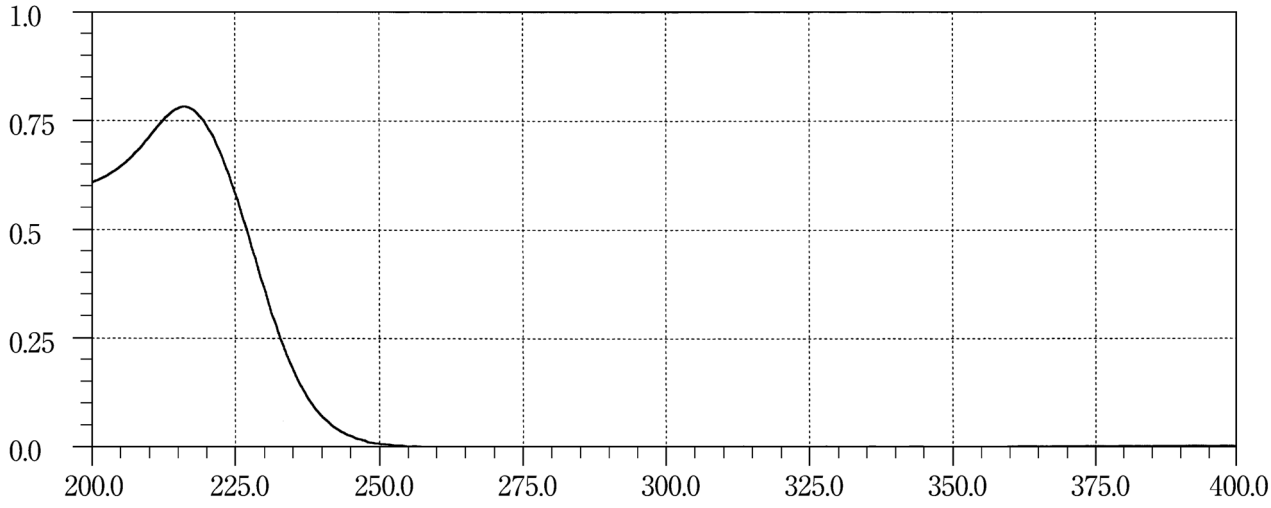
Diltiazem Hydrochloride



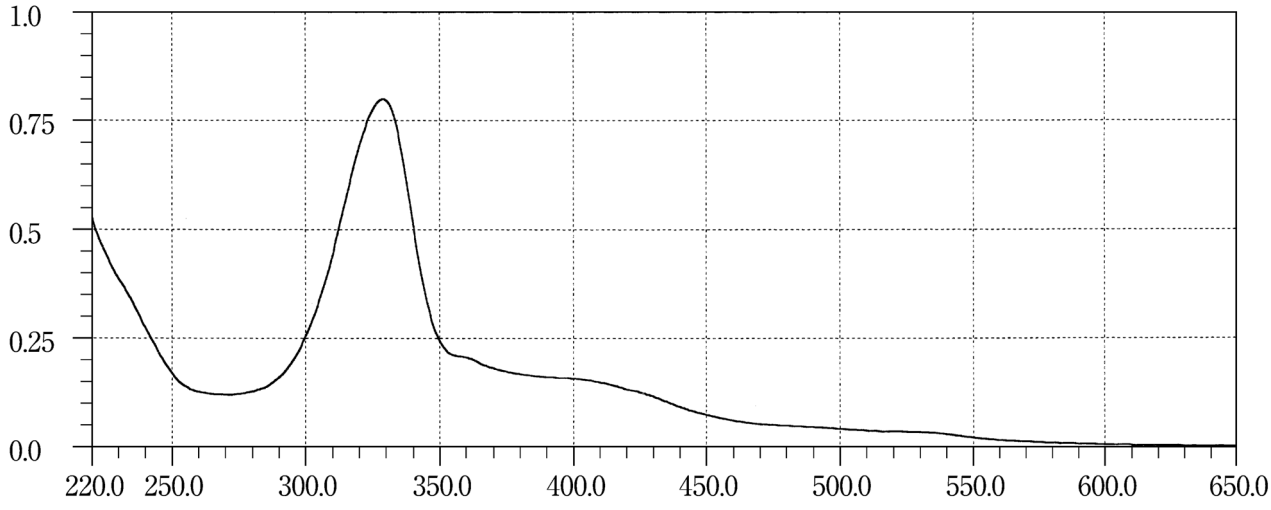
Dimemorfan Phosphate



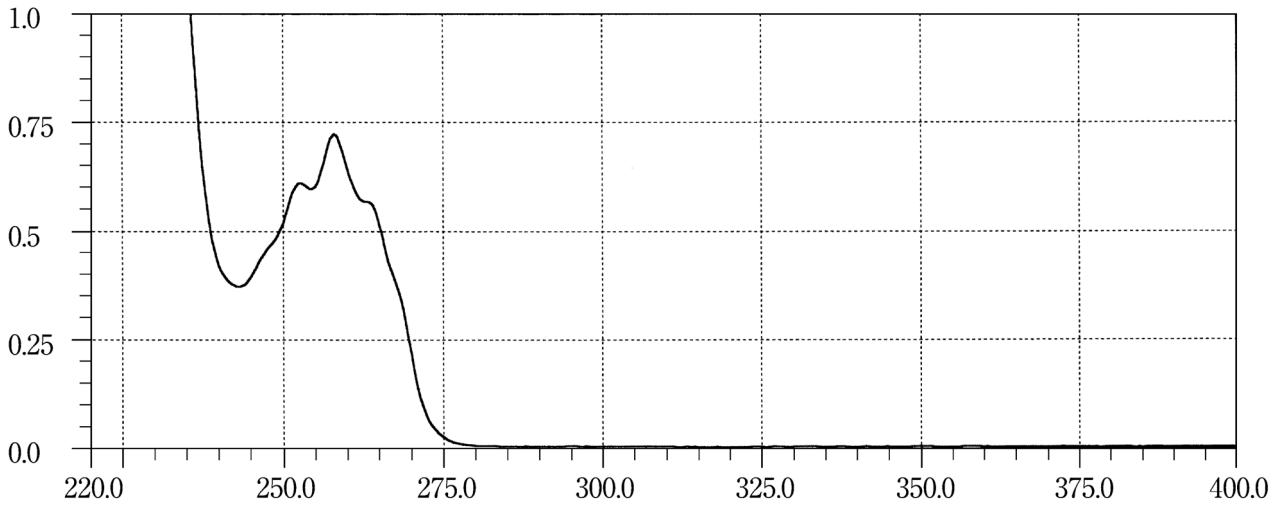
Dimorpholamine



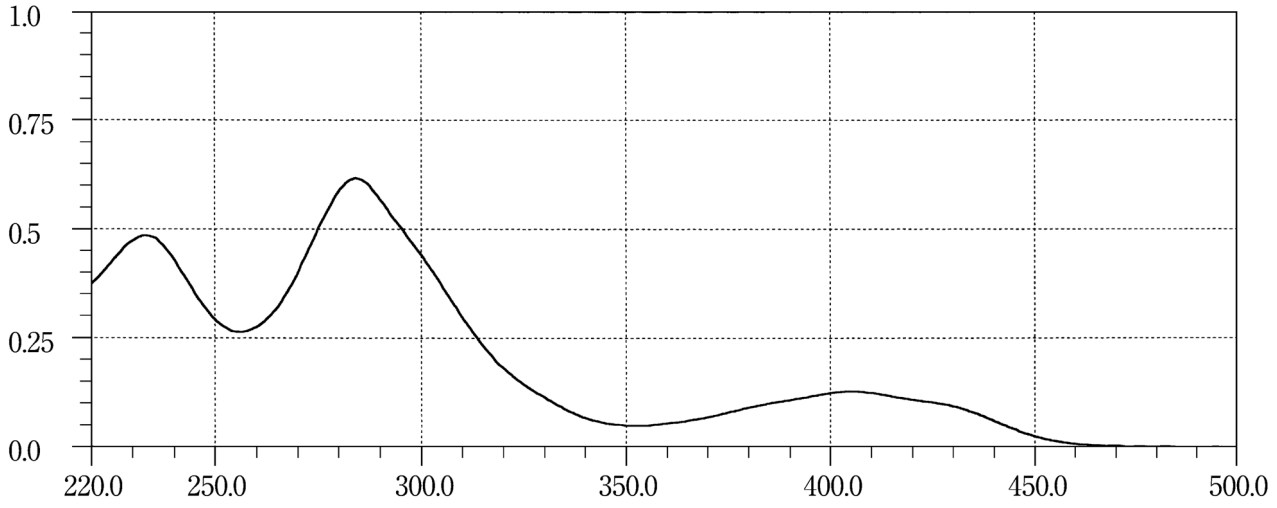
Dinoprost



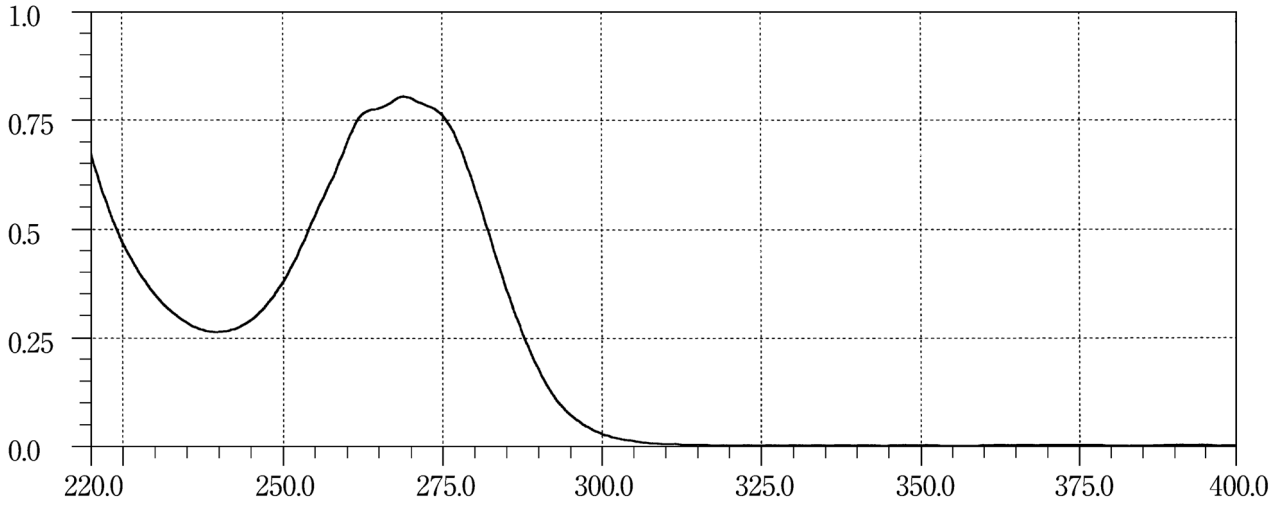
Diphenhydramine Hydrochloride



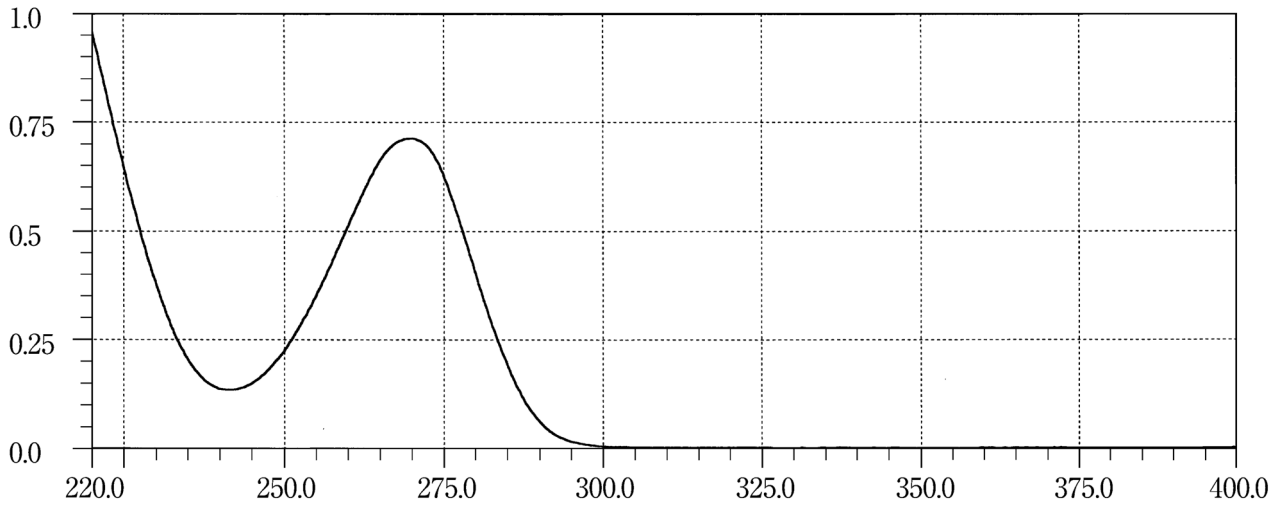
Dipyridamole



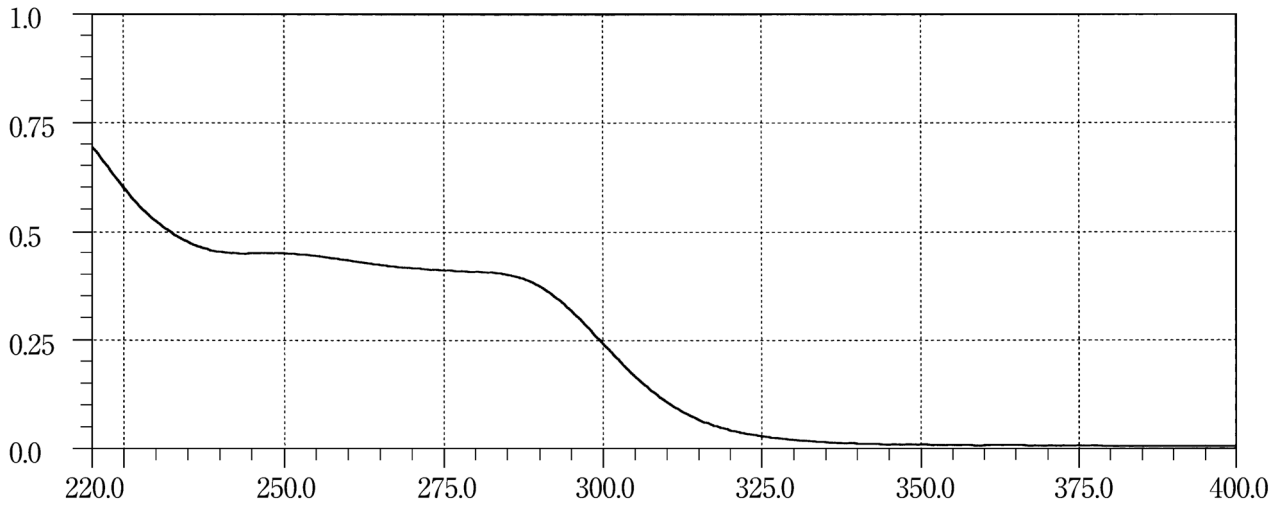
Disopyramide



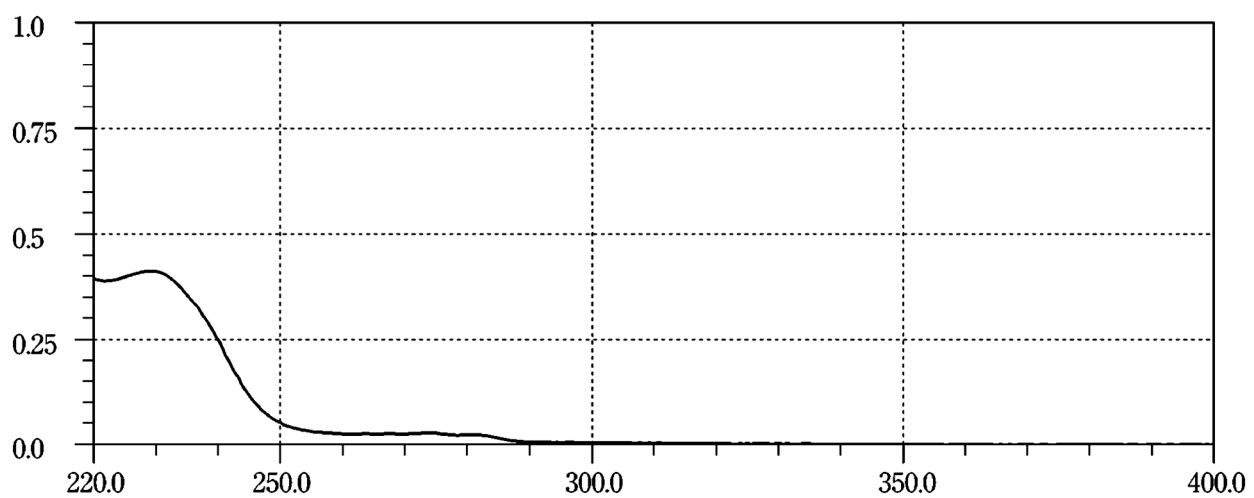
Distigmine Bromide



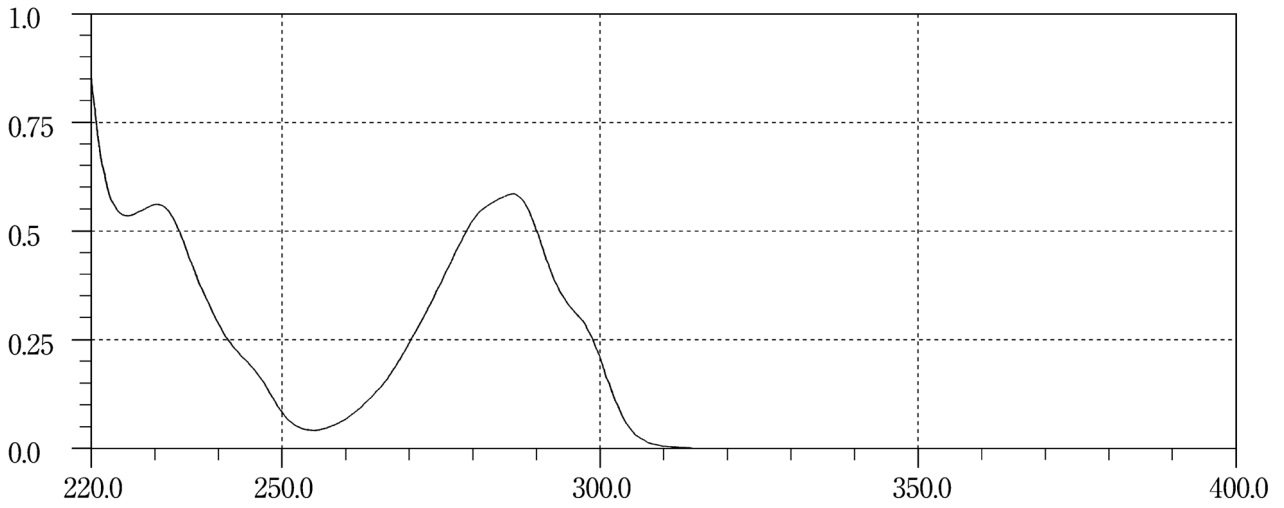
Disulfiram



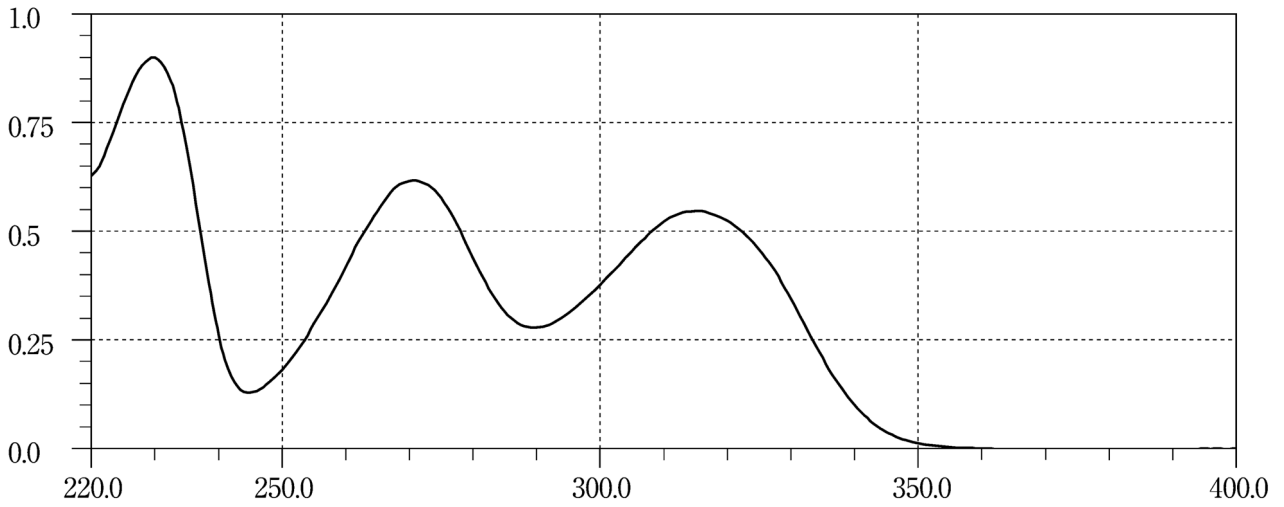
Docetaxel Hydrate



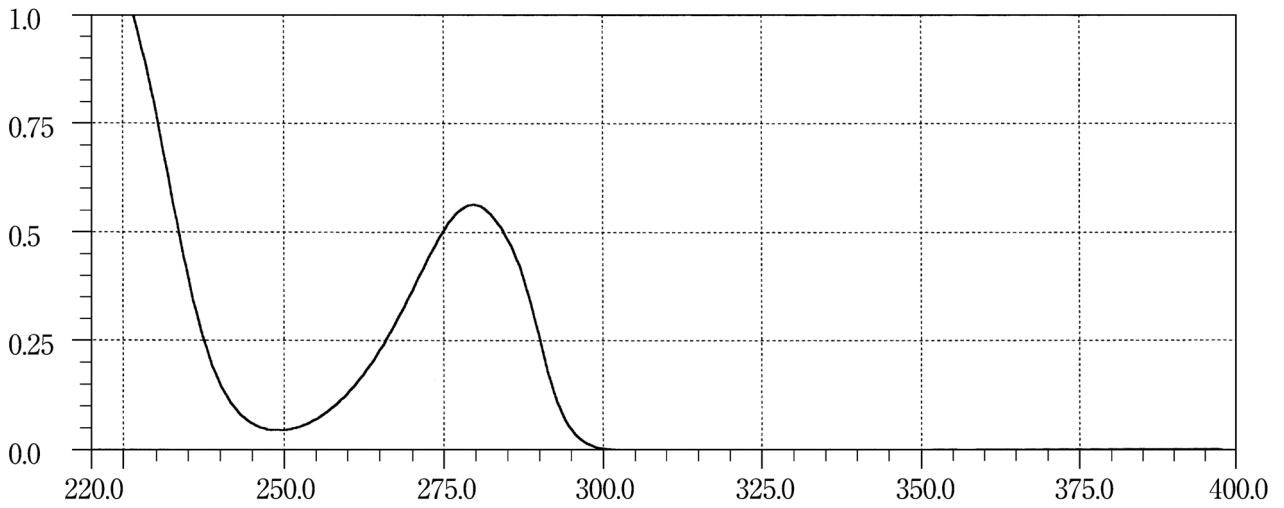
Domperidone



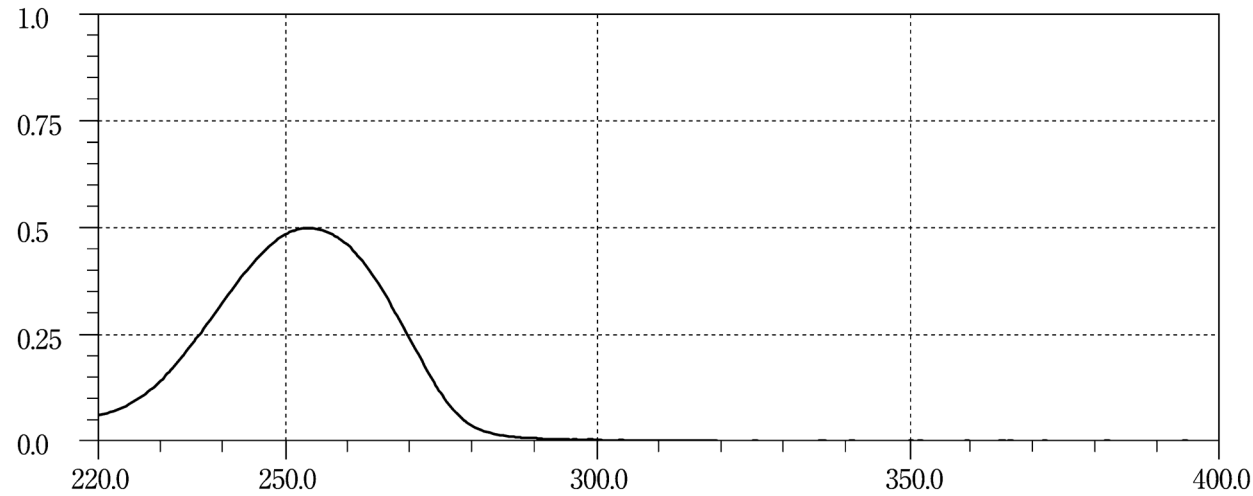
Donepezil Hydrochloride



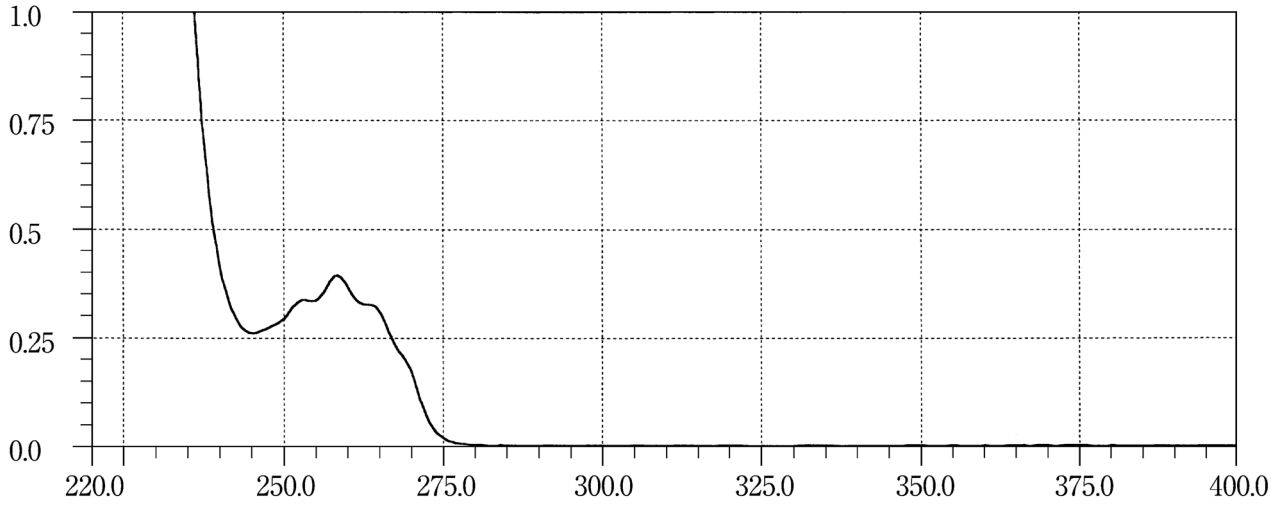
Dopamine Hydrochloride



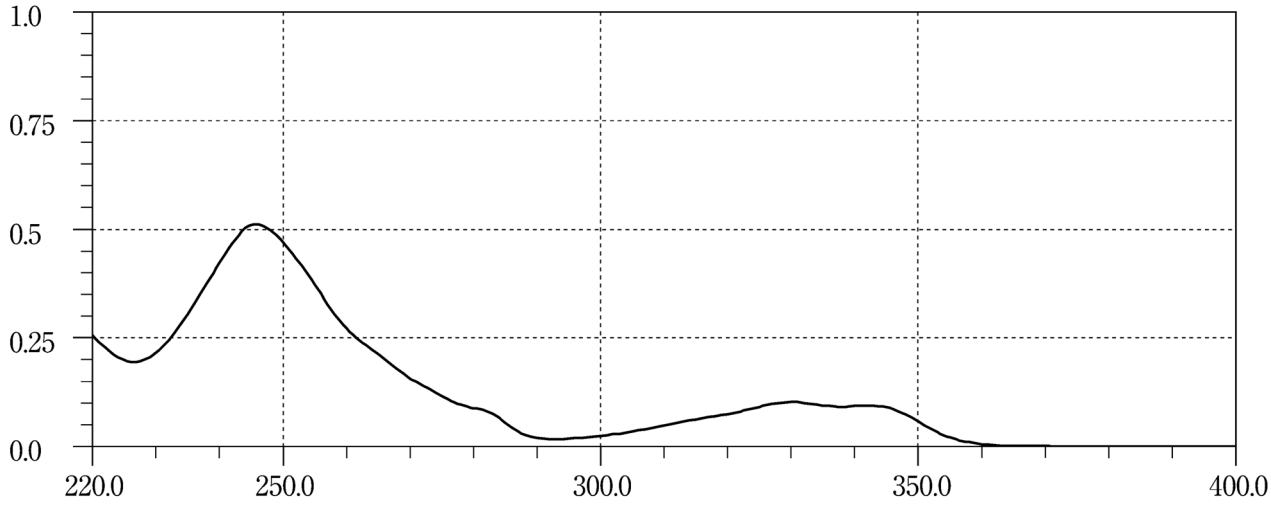
Dorzolamide Hydrochloride



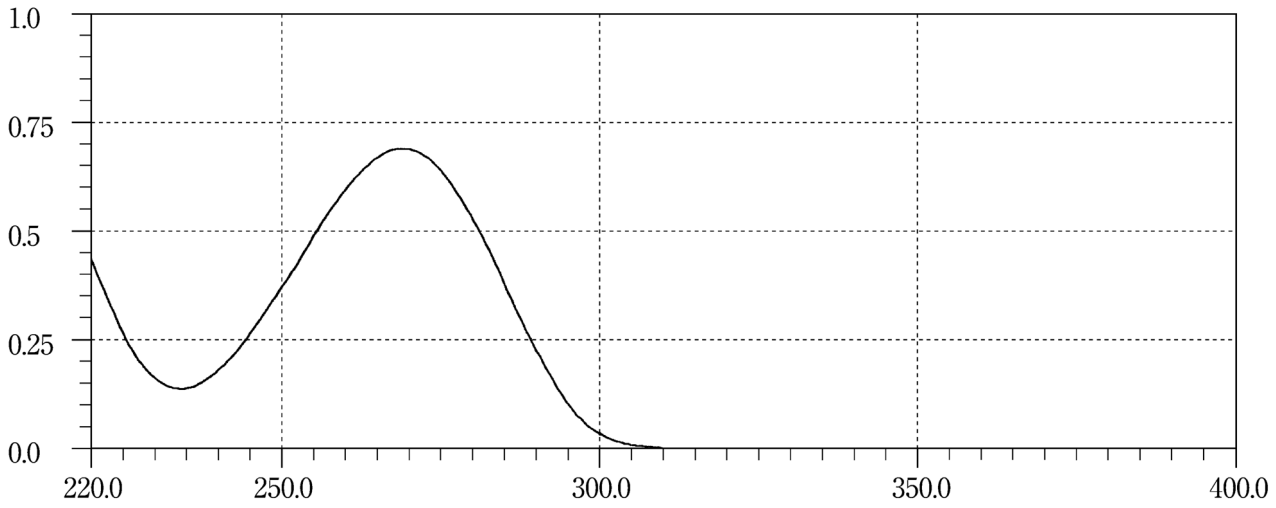
Doxapram Hydrochloride Hydrate



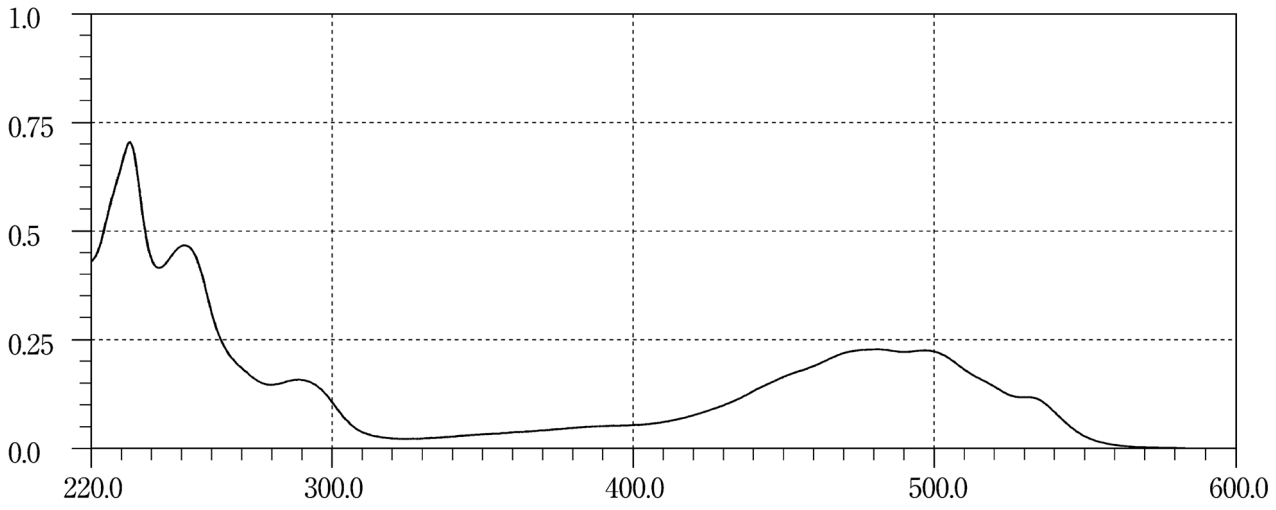
Doxazosin Mesilate



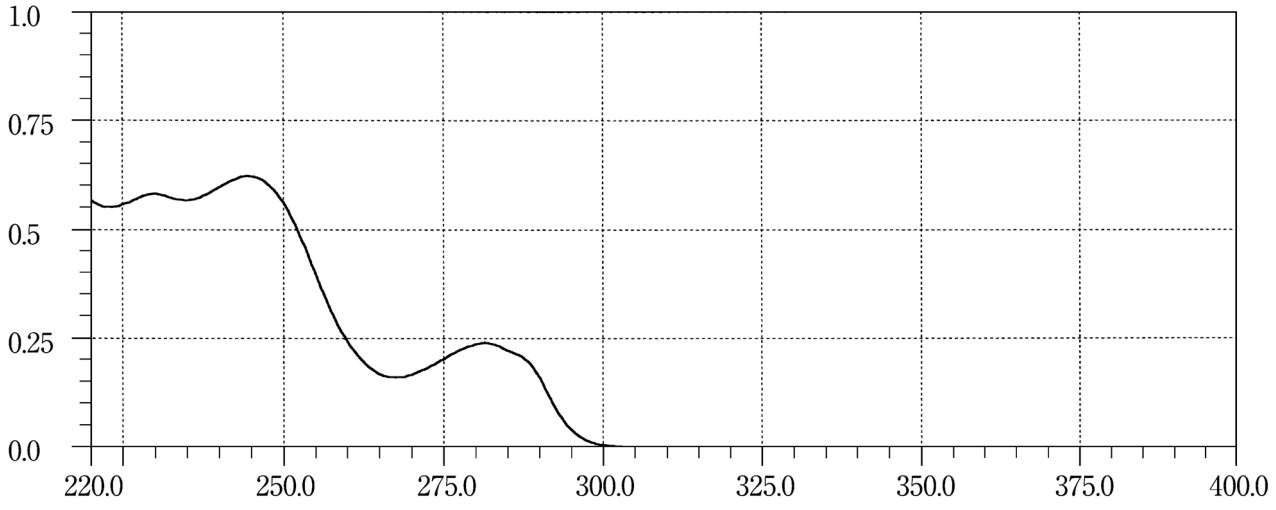
Doxifluridine



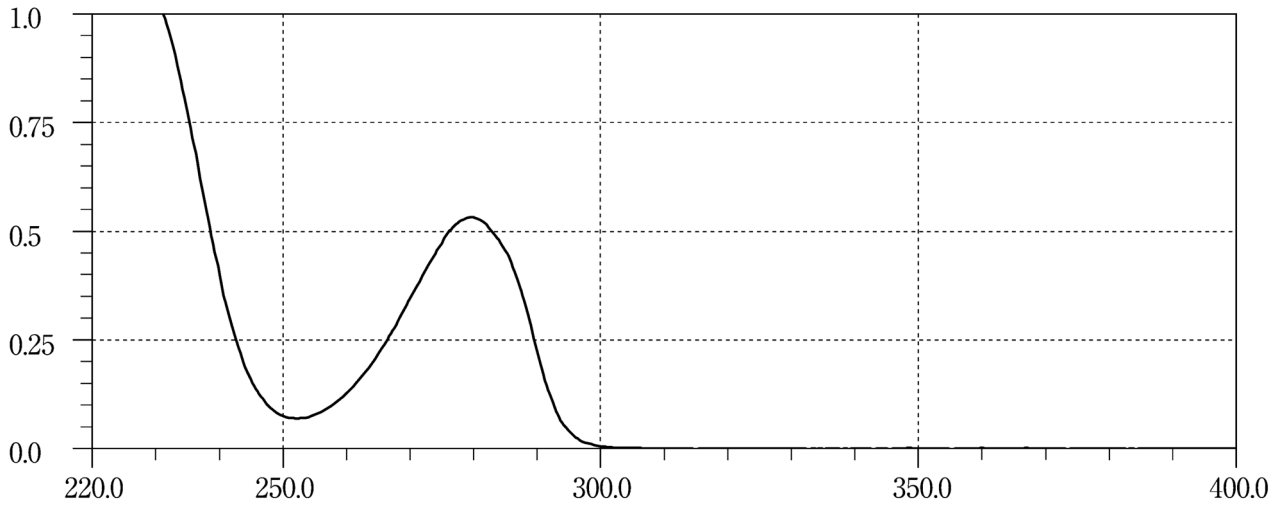
Doxorubicin Hydrochloride



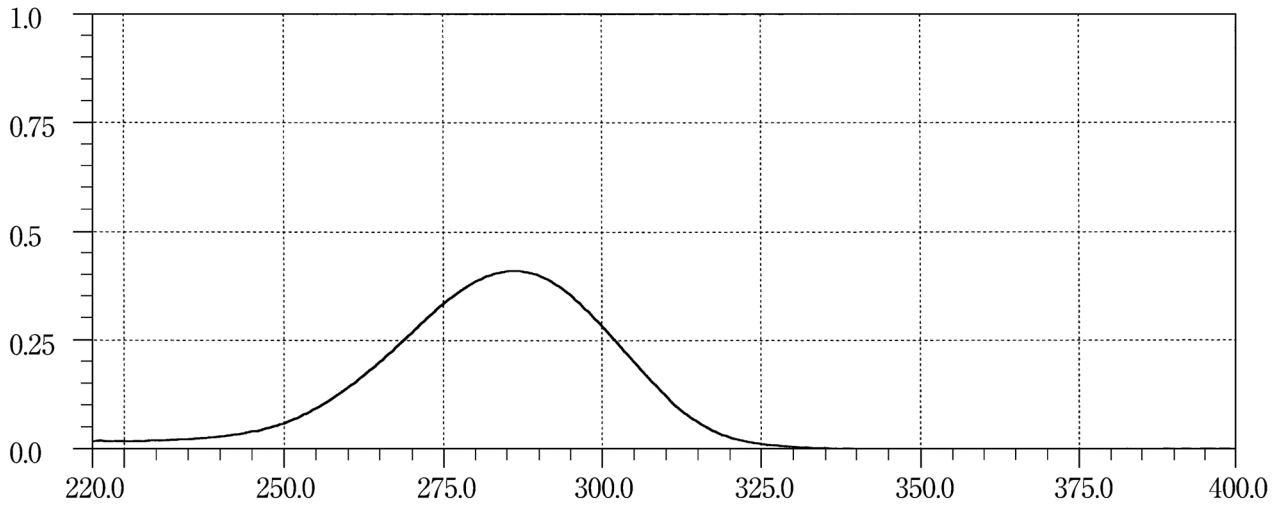
Droperidol



Droxidopa



Dydrogesterone



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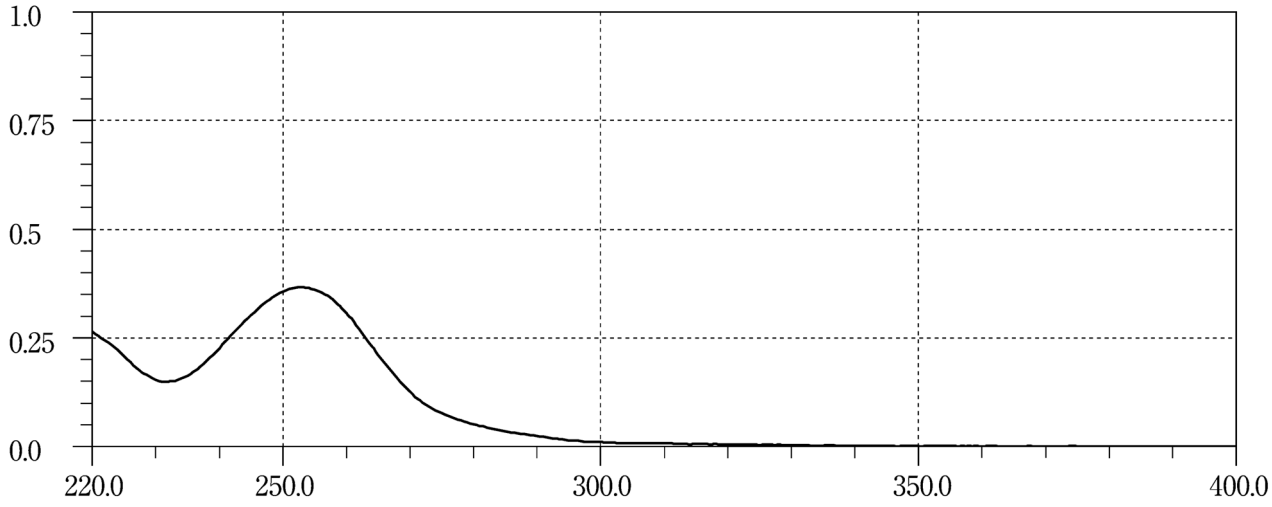

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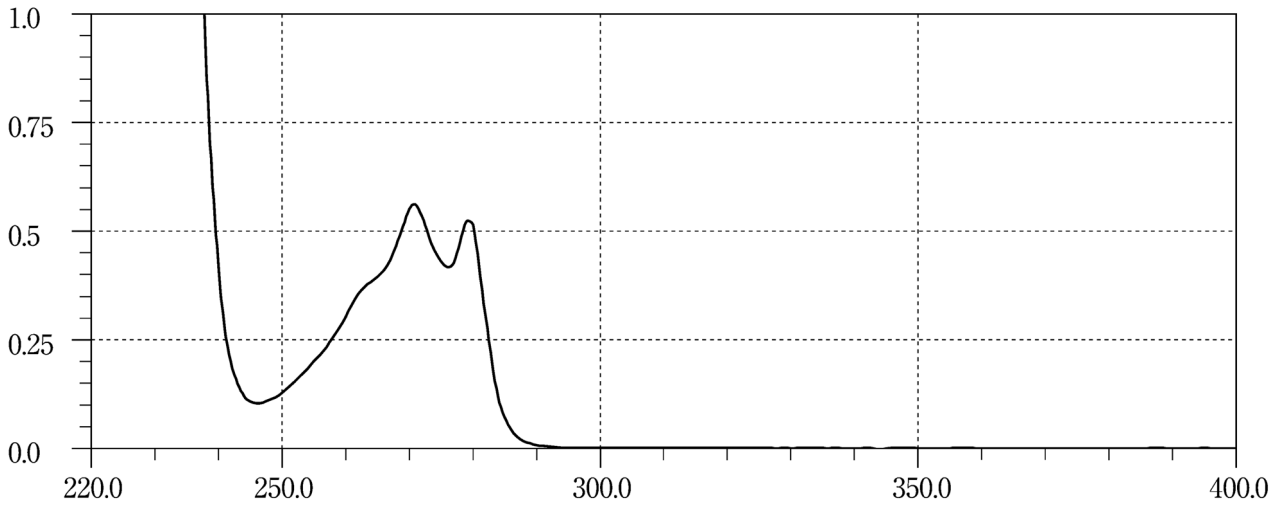

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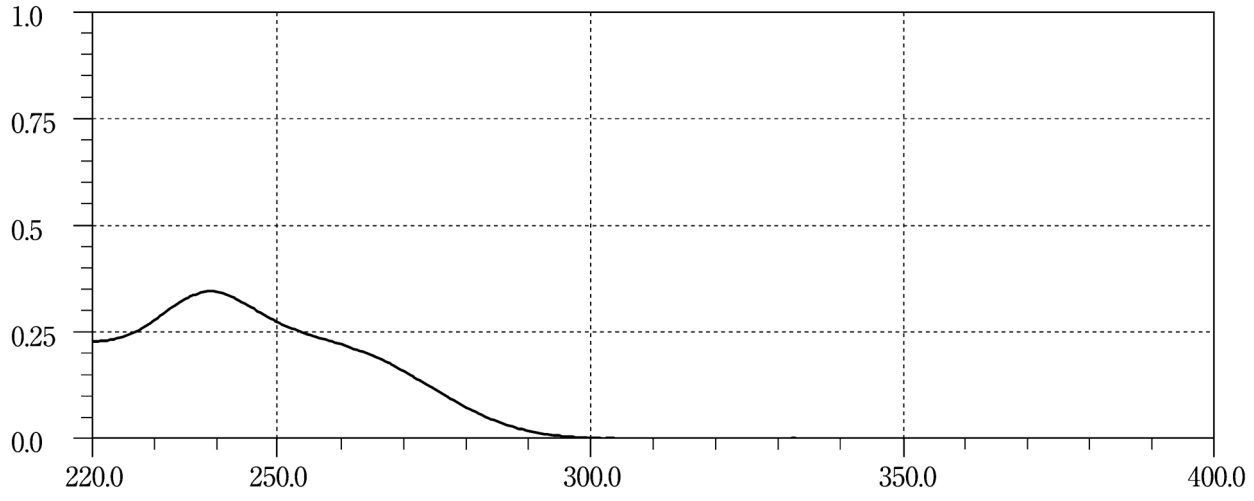

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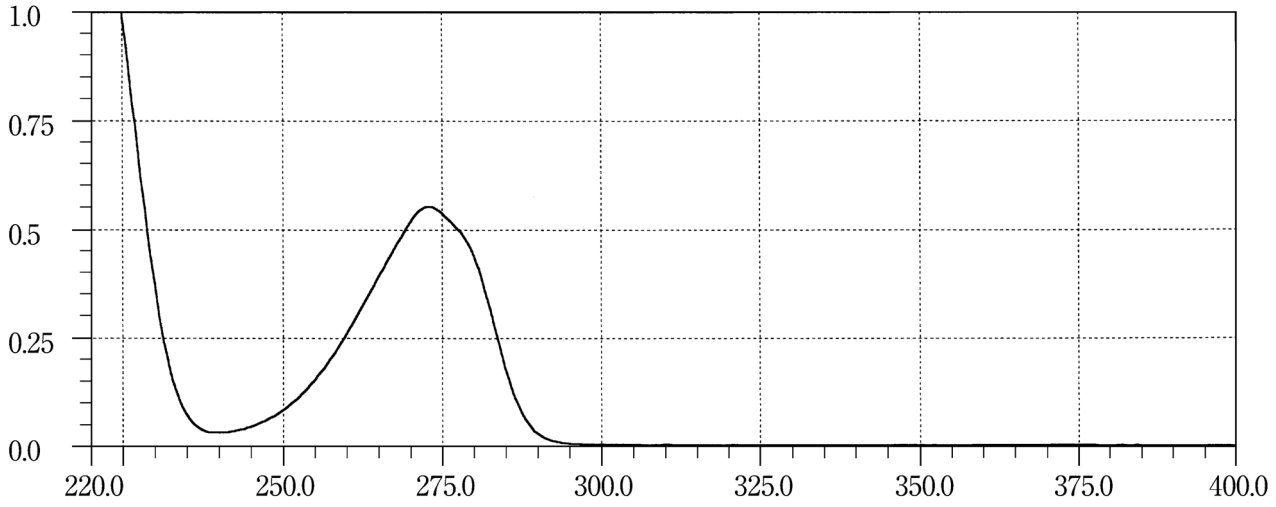

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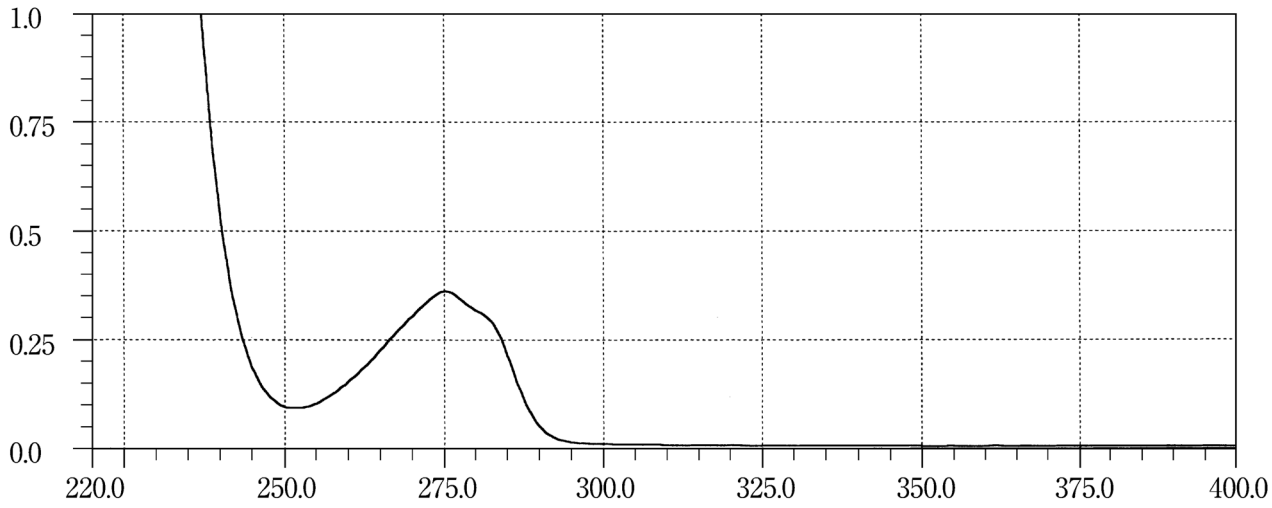

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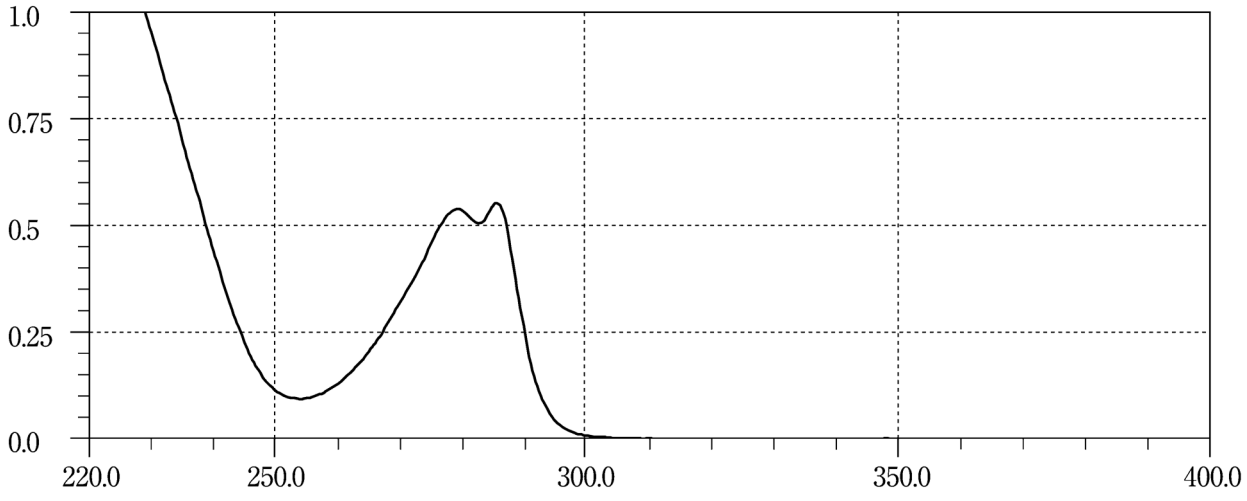

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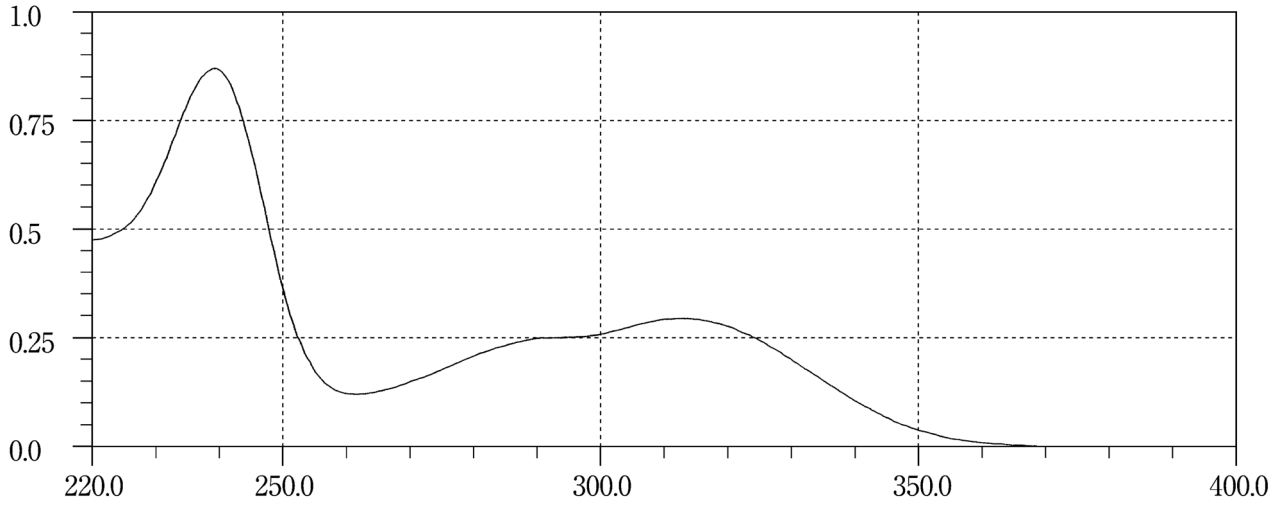
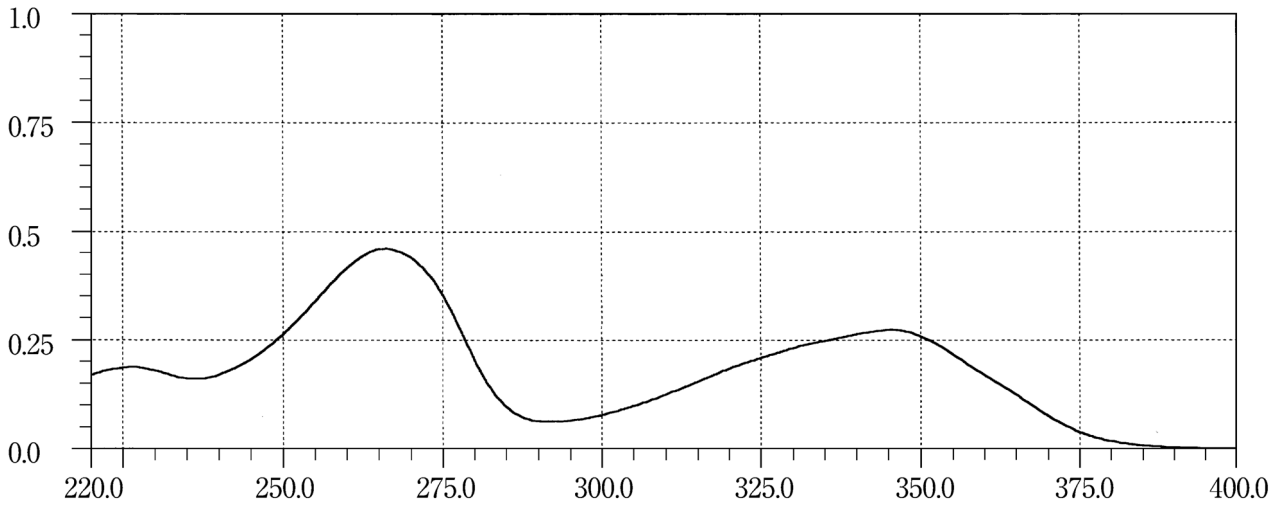


Enoxacin Hydrate



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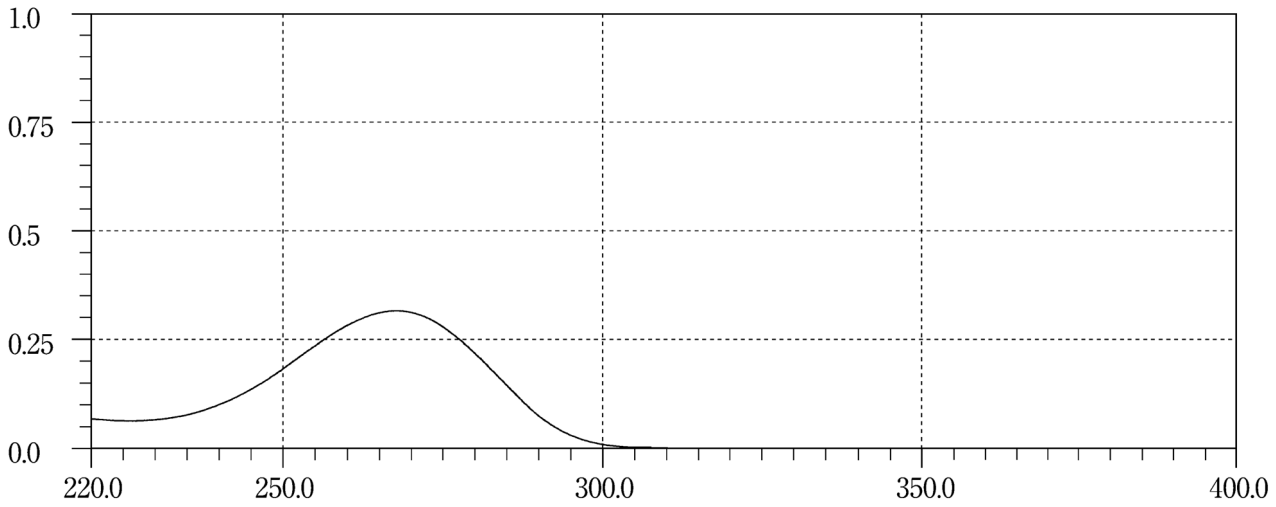

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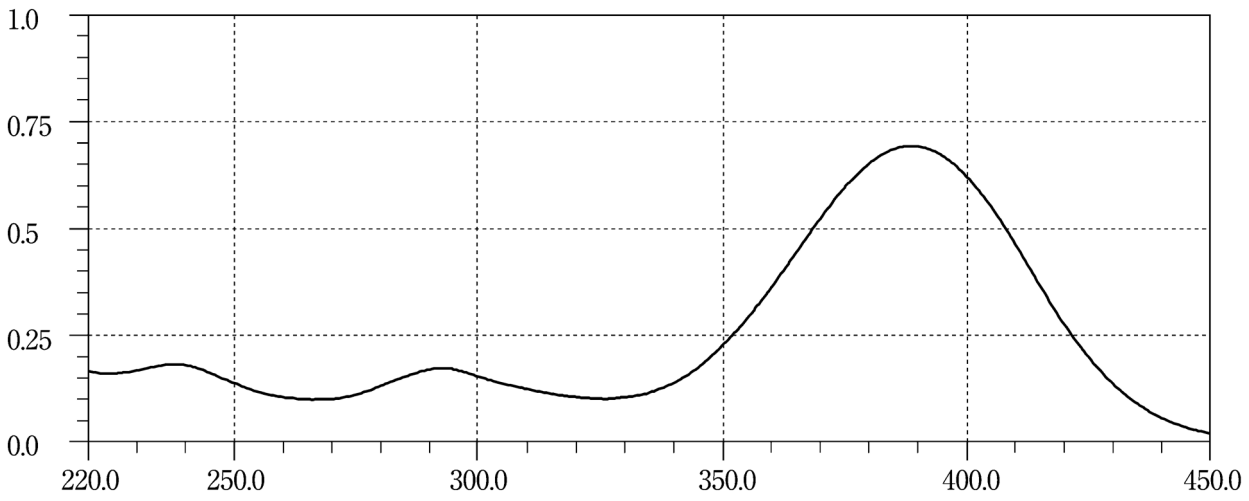
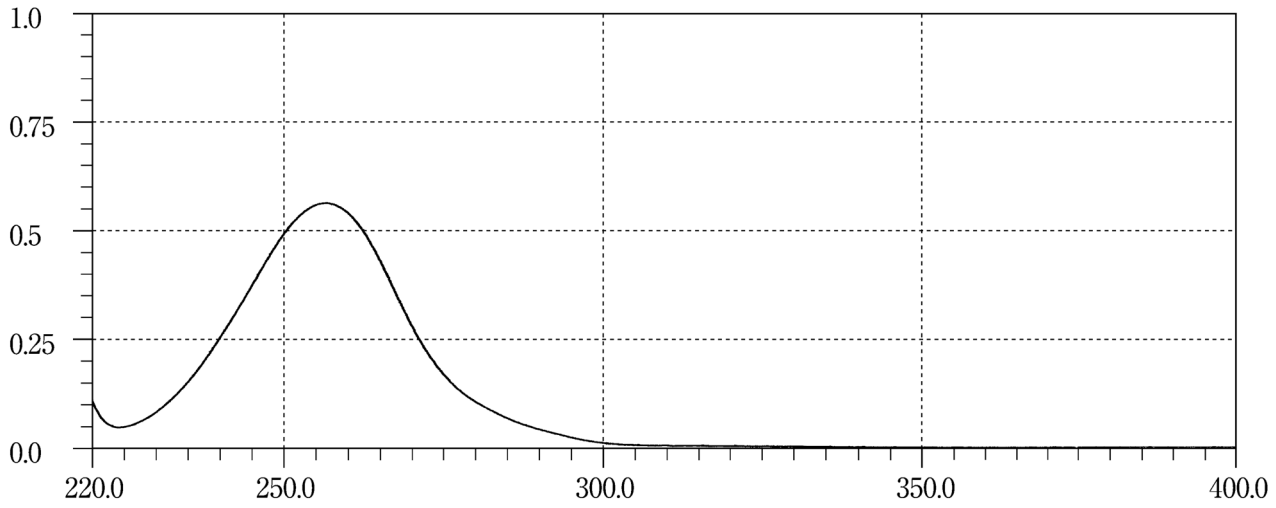
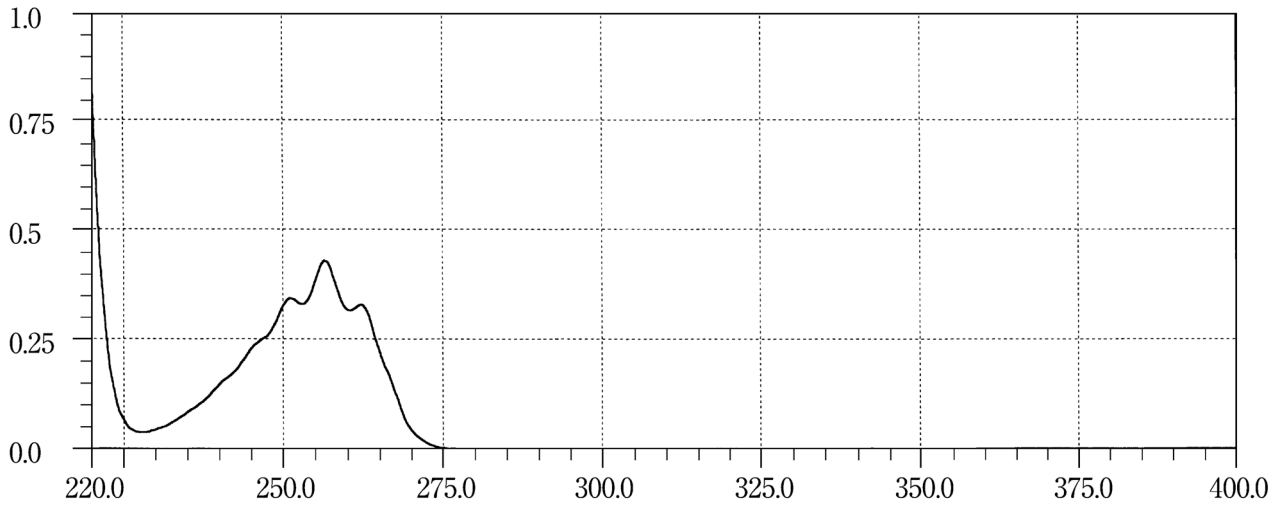


Eperisone Hydrochloride



Ephedrine Hydrochloride



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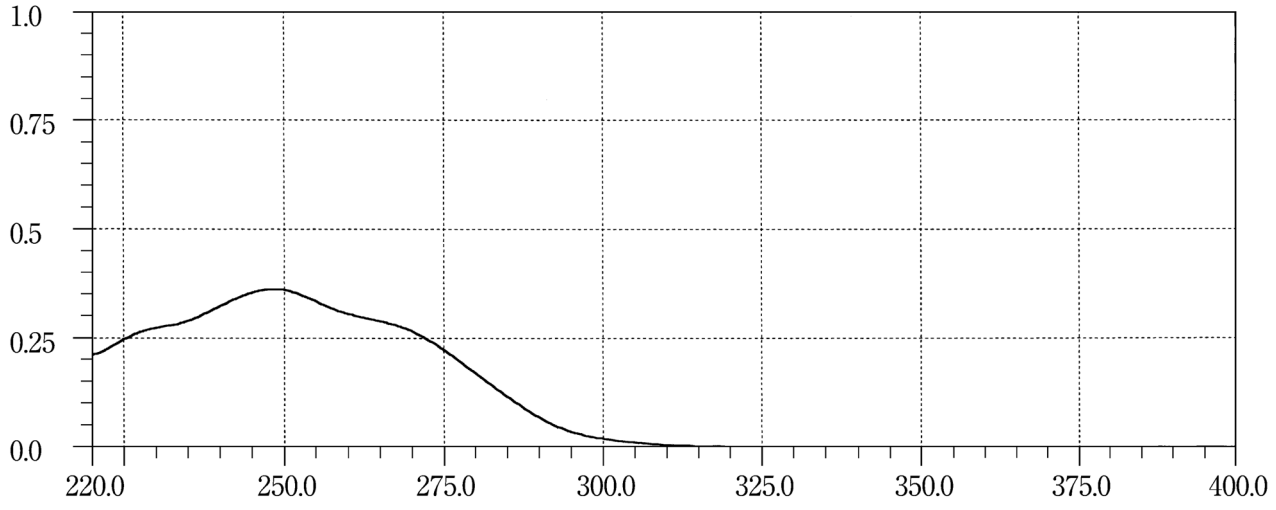
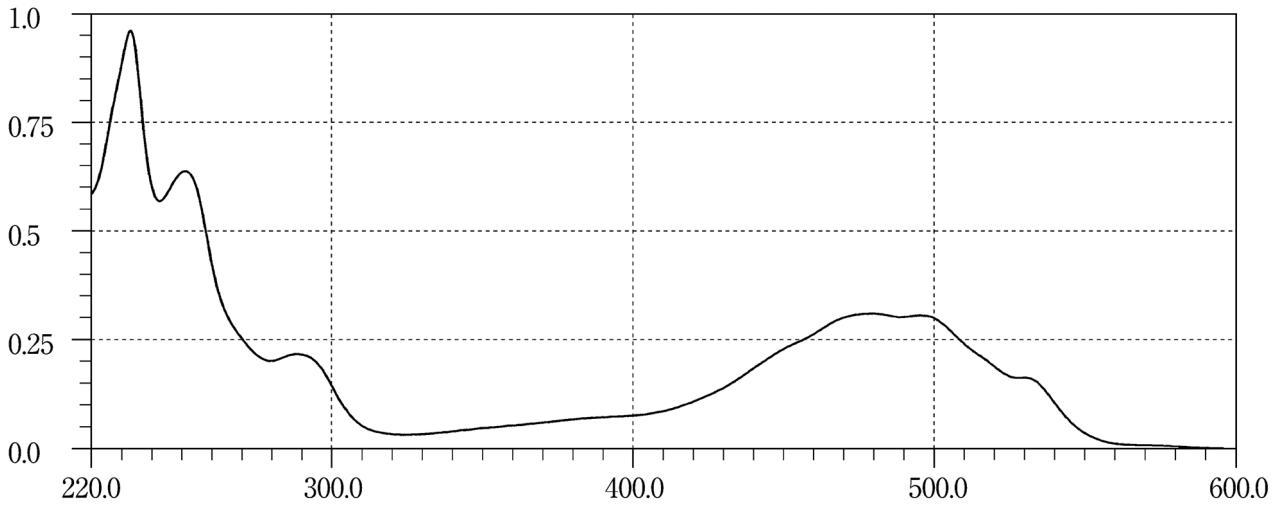


Epirubicin Hydrochloride



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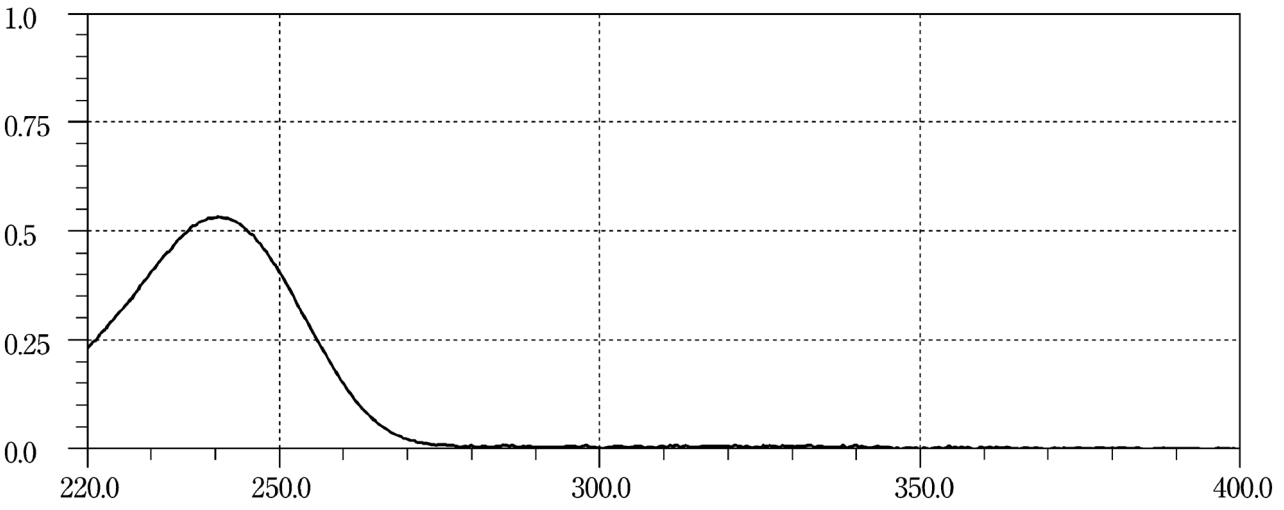

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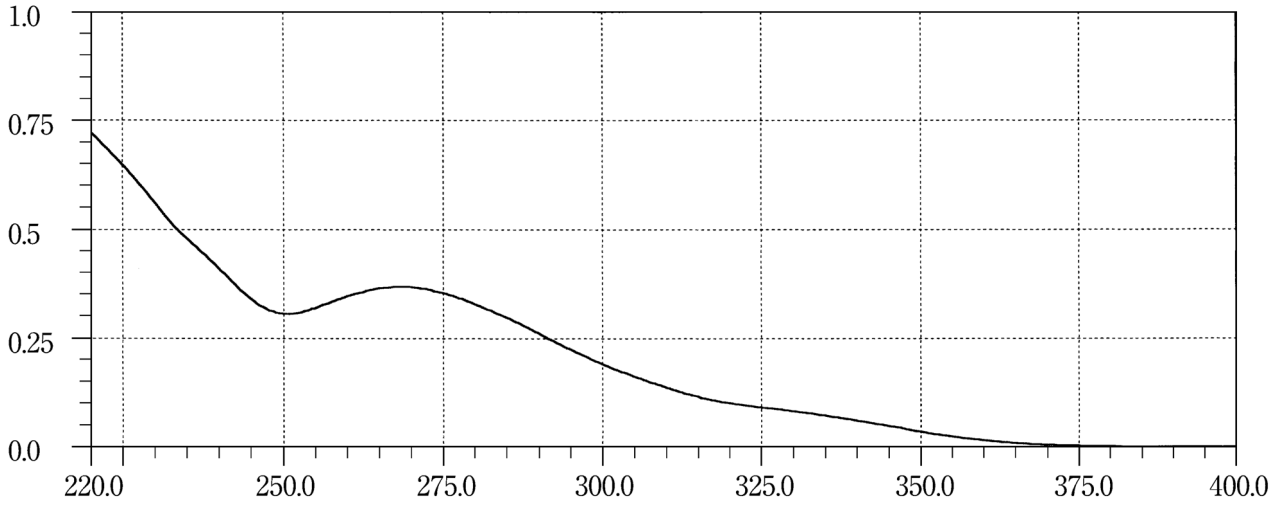

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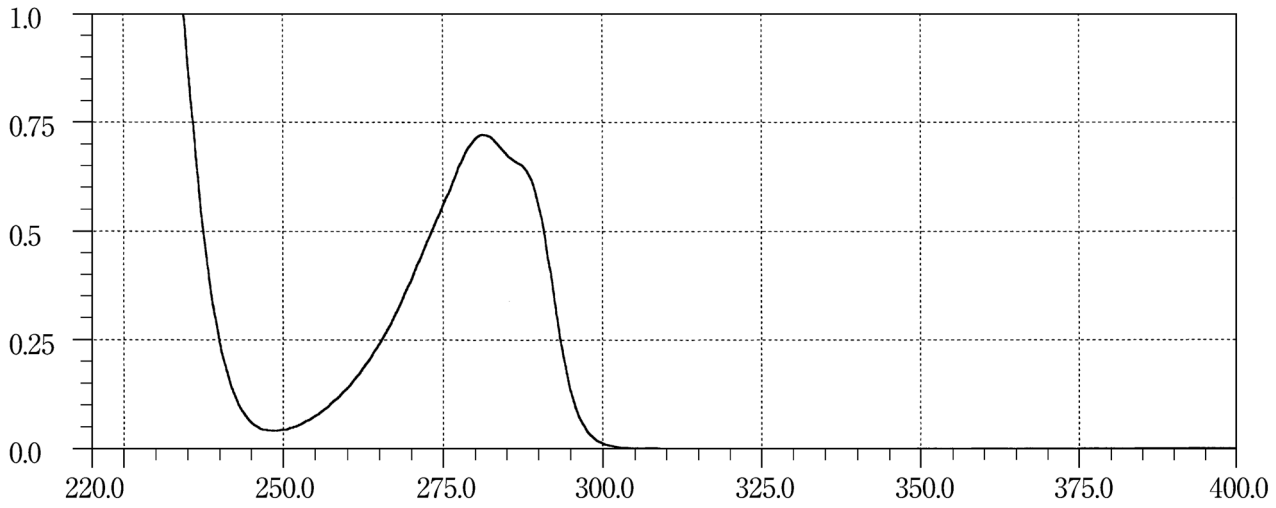
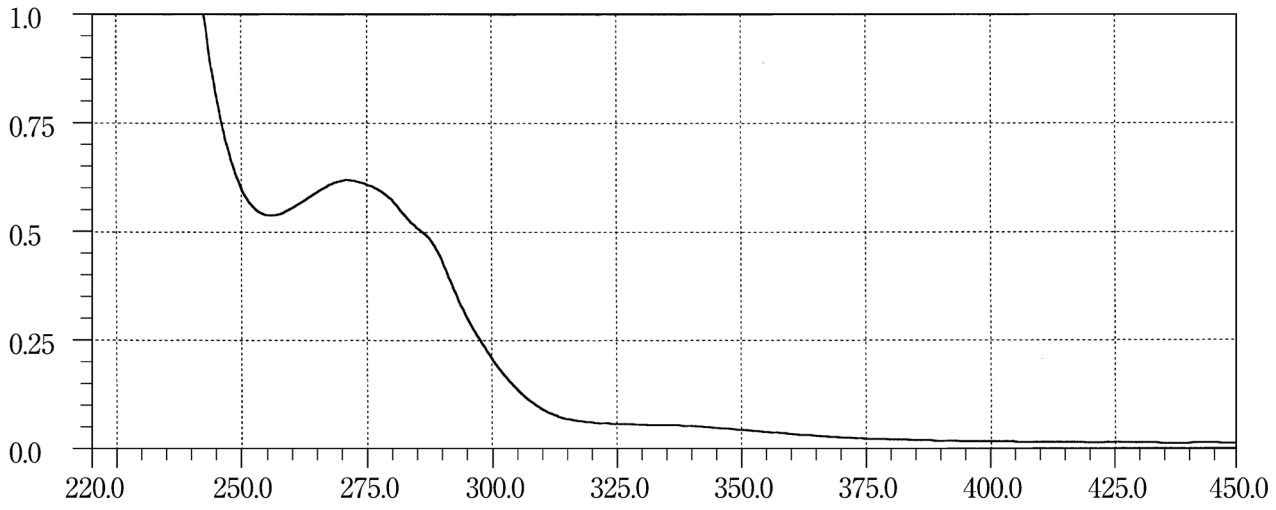


Etacrynic Acid



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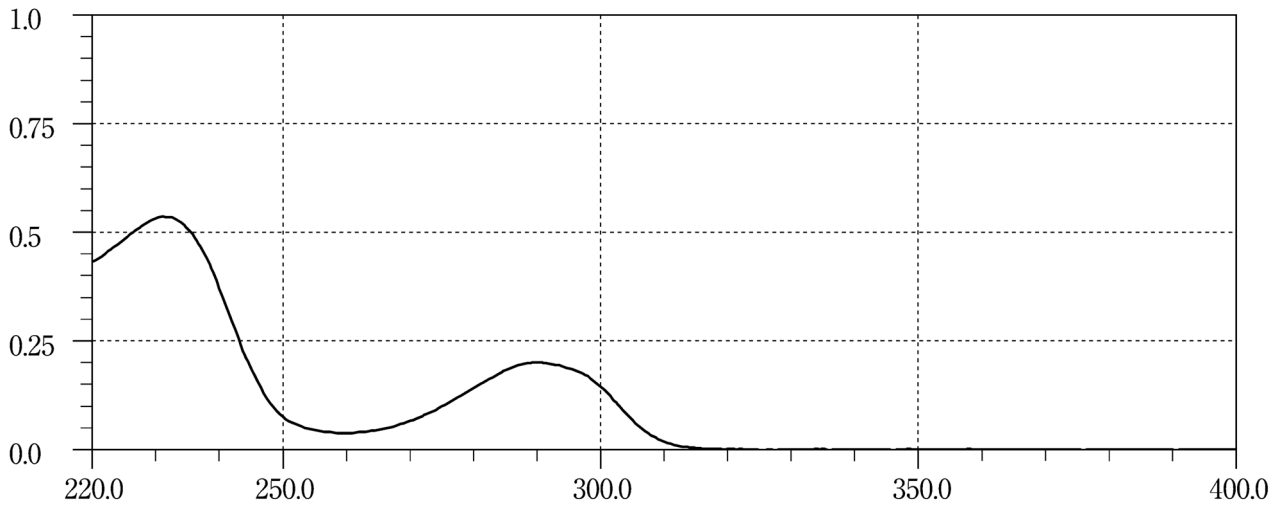
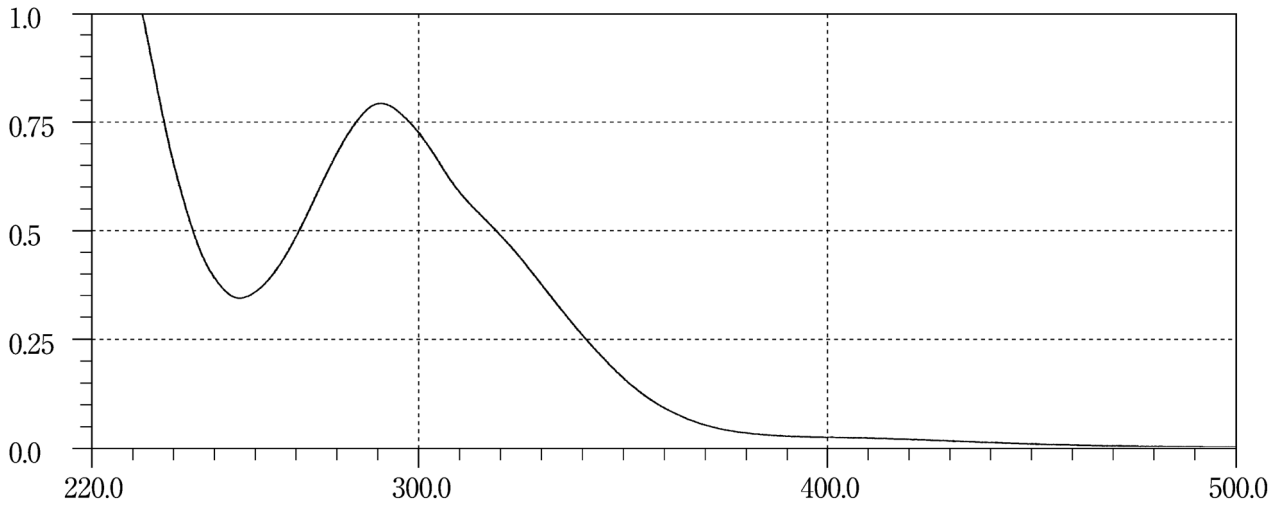


Ethionamide



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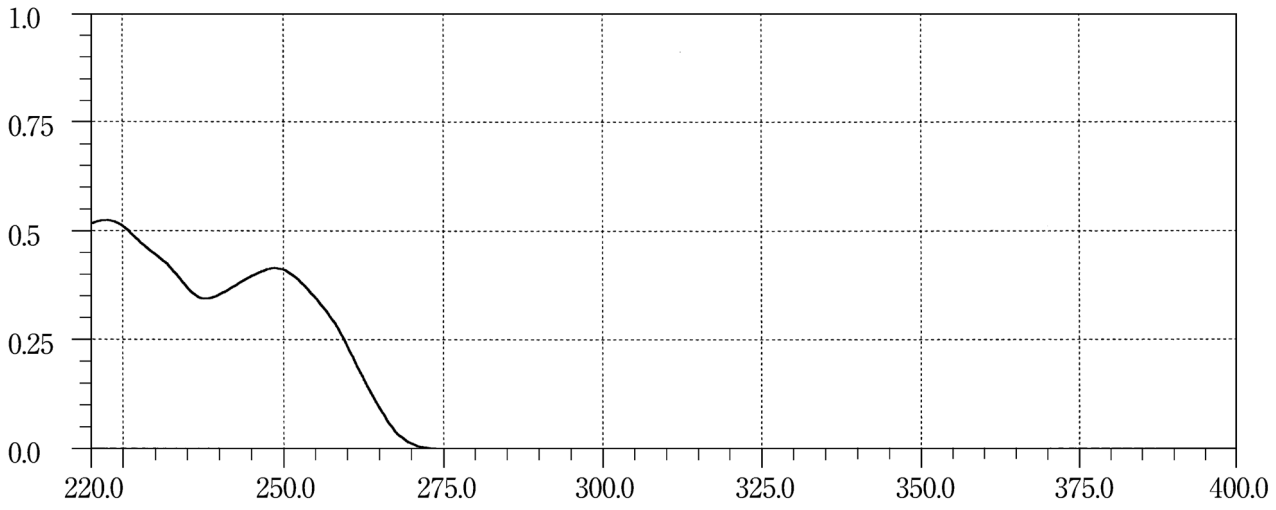
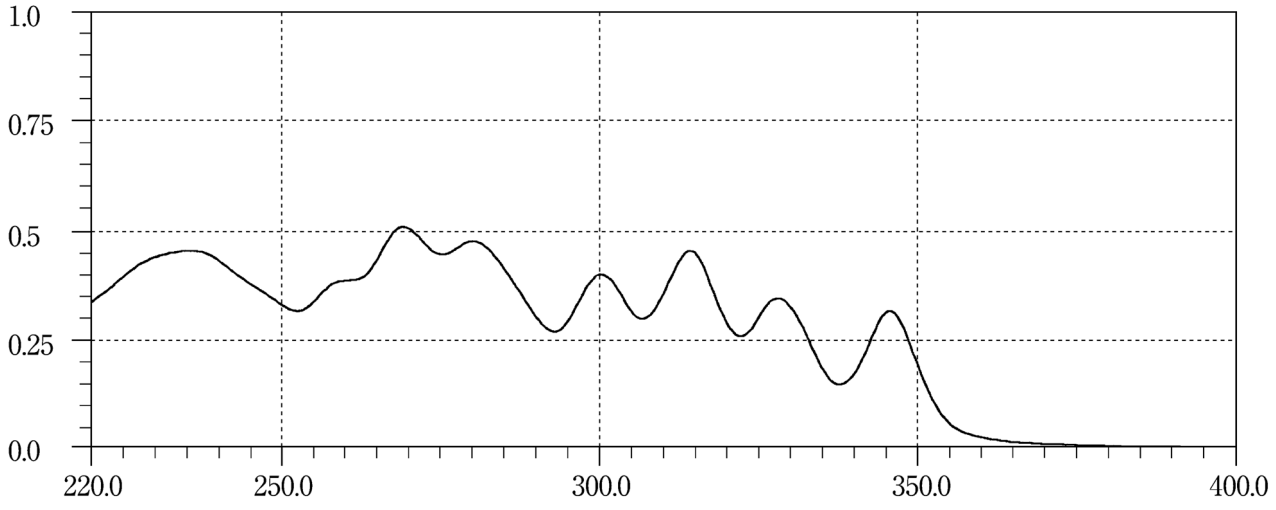
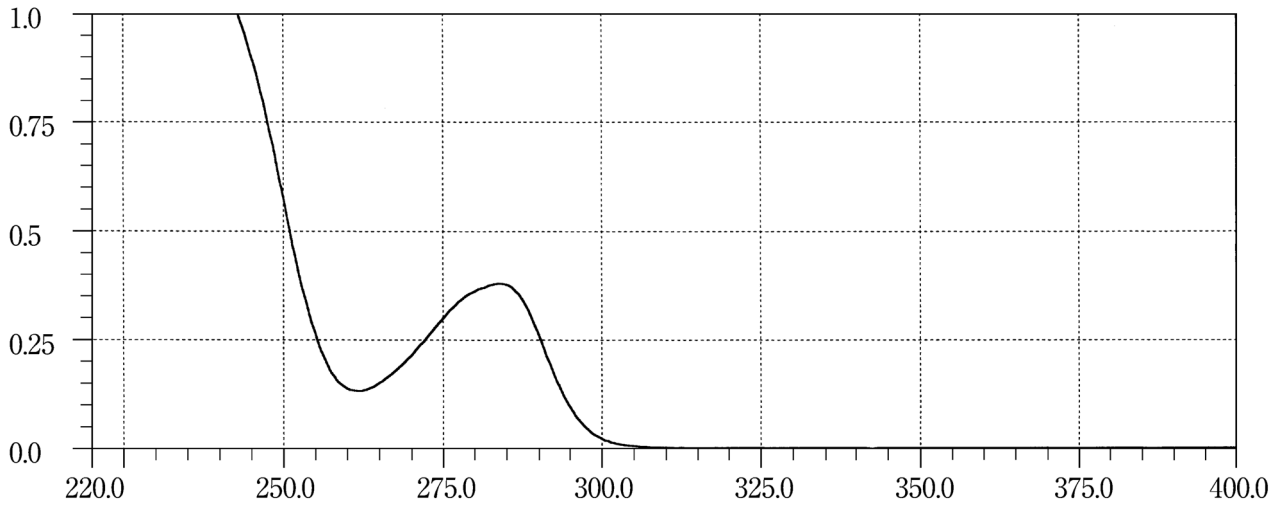


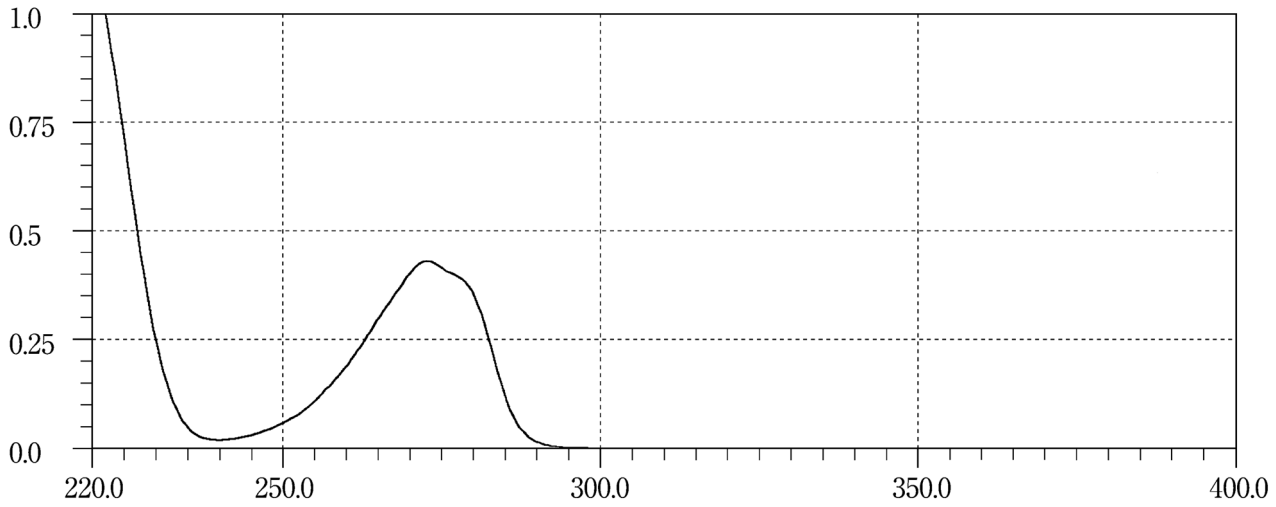
Ethyl Icosapentate



Ethylmorphine Hydrochloride Hydrate



Etilefrine Hydrochloride



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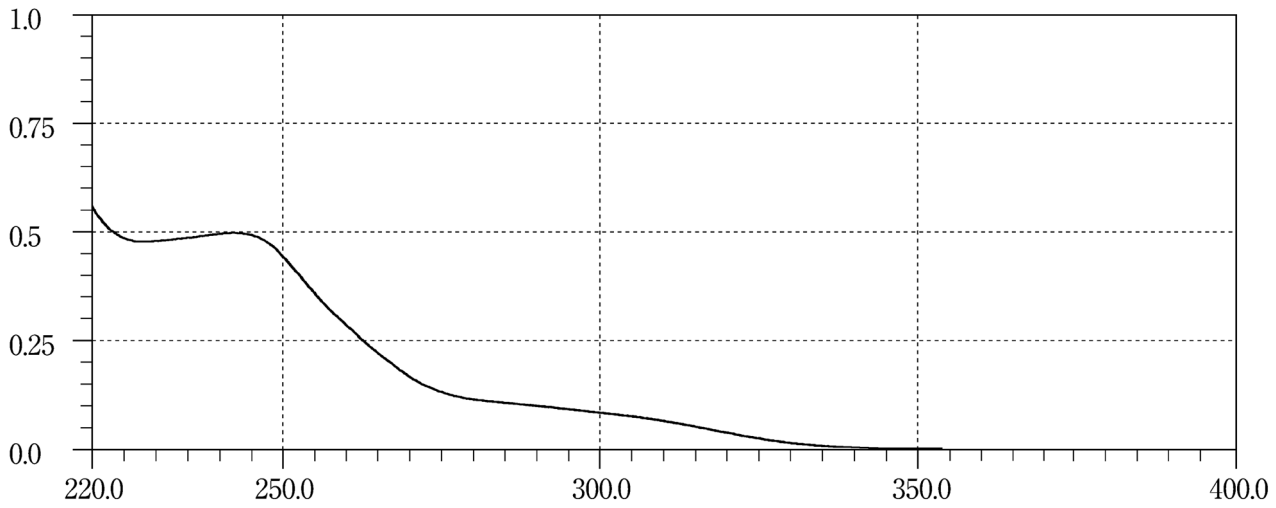
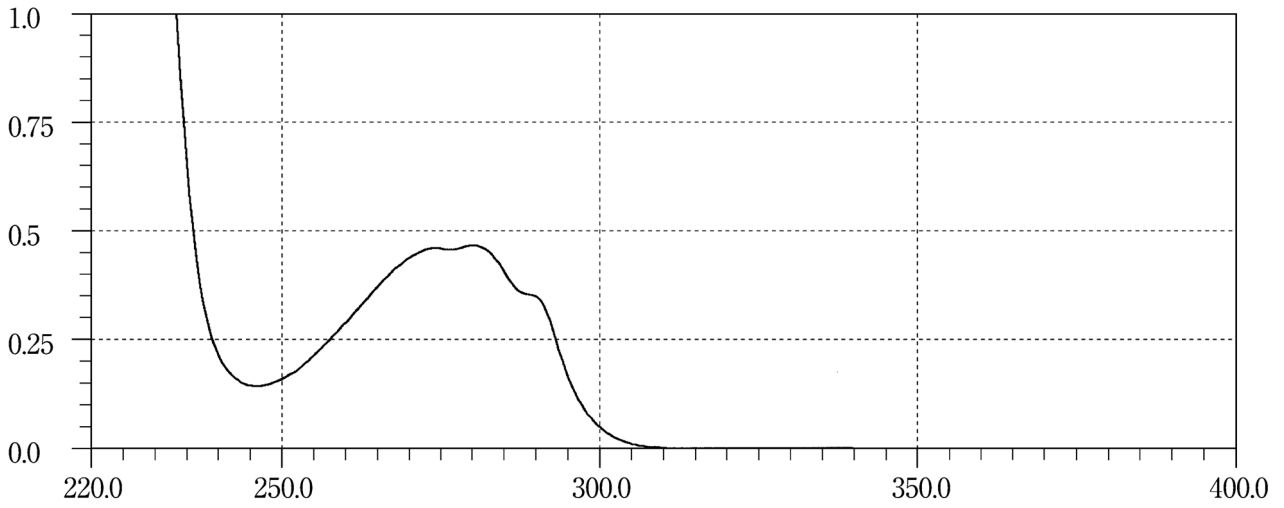


Etodolac



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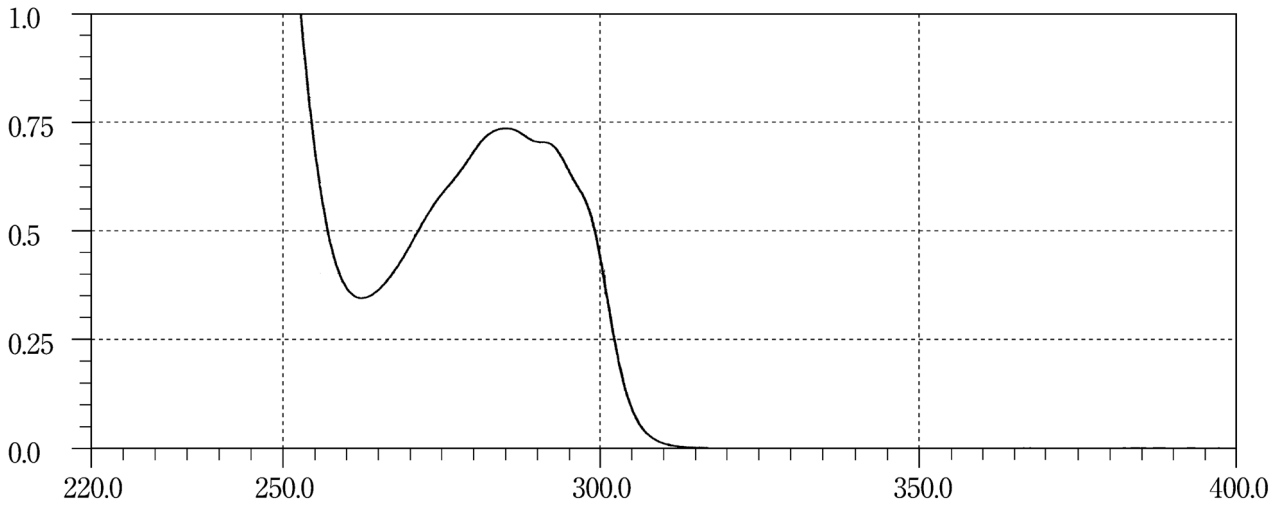

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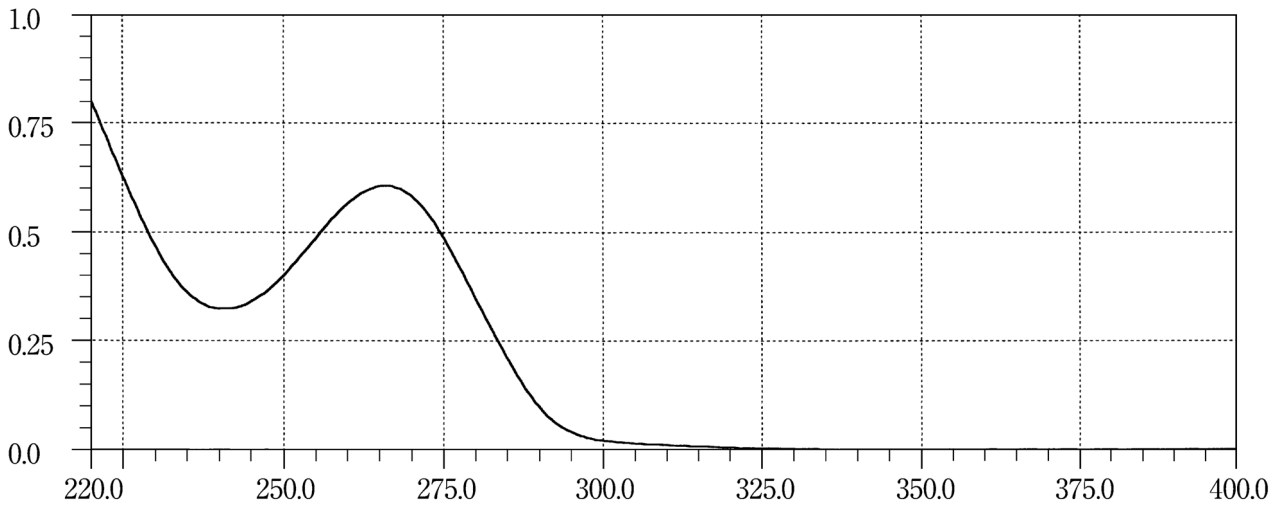
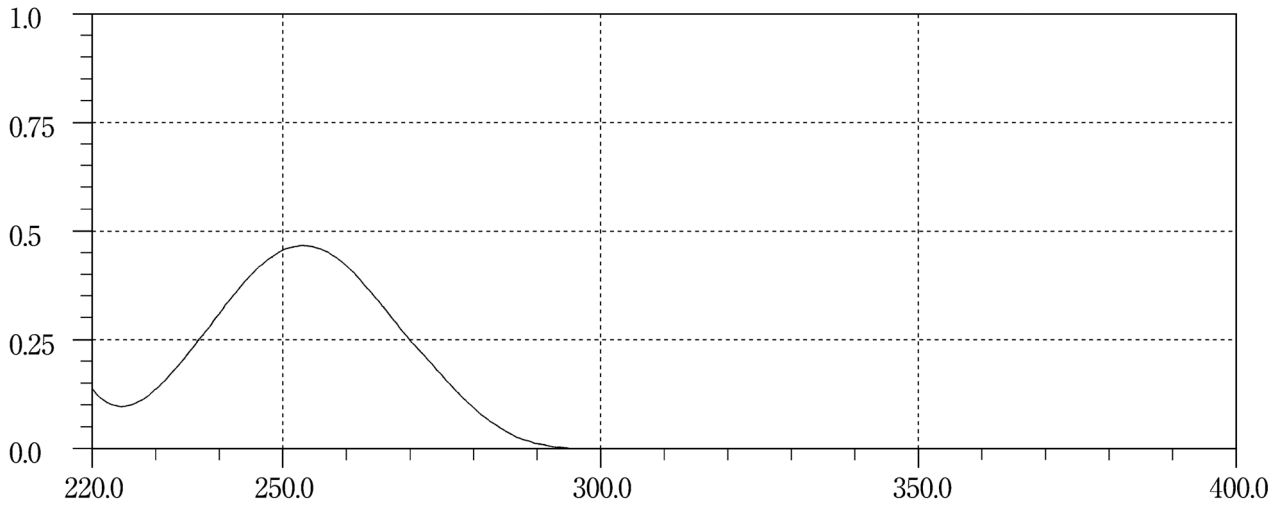


Felbinac



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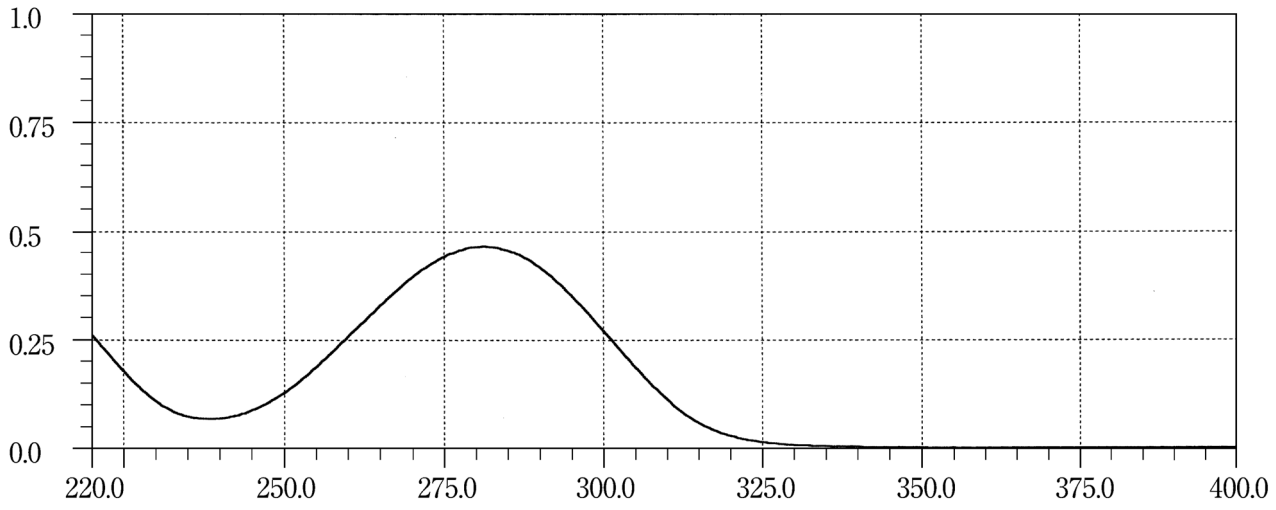
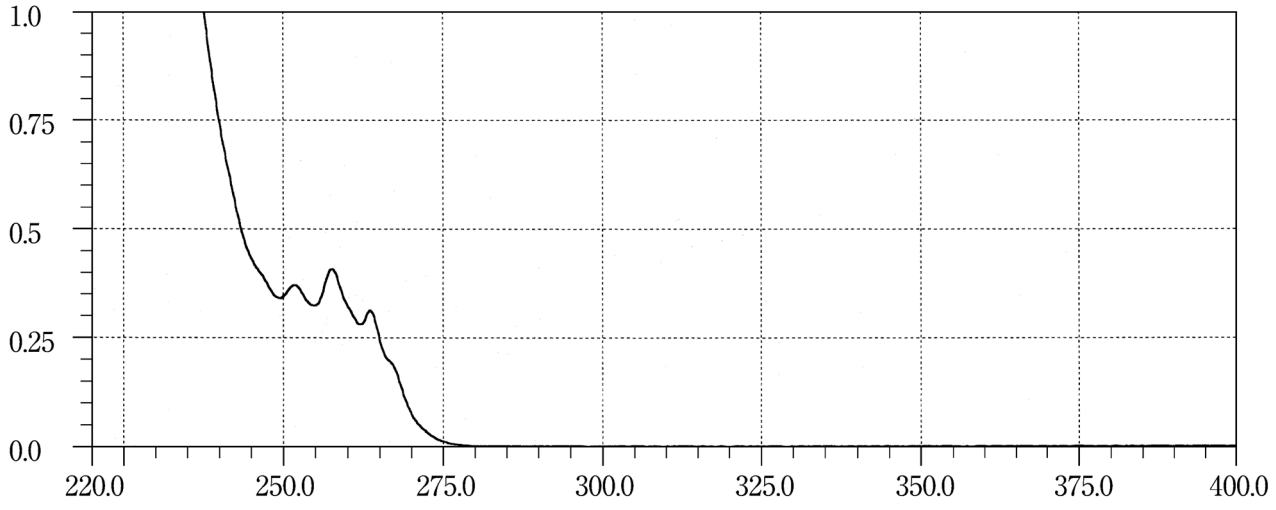
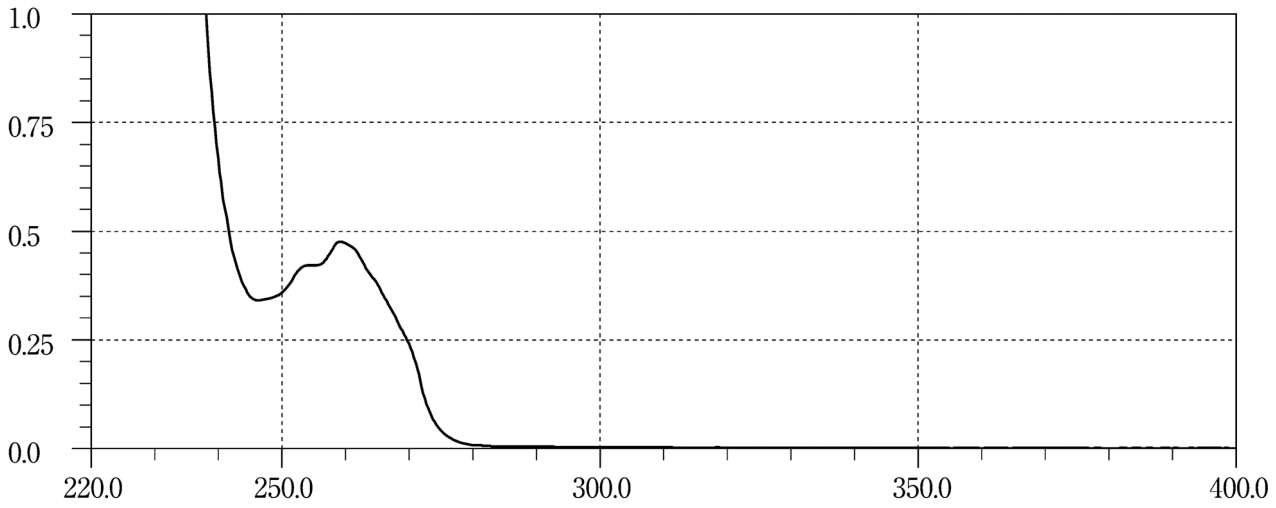


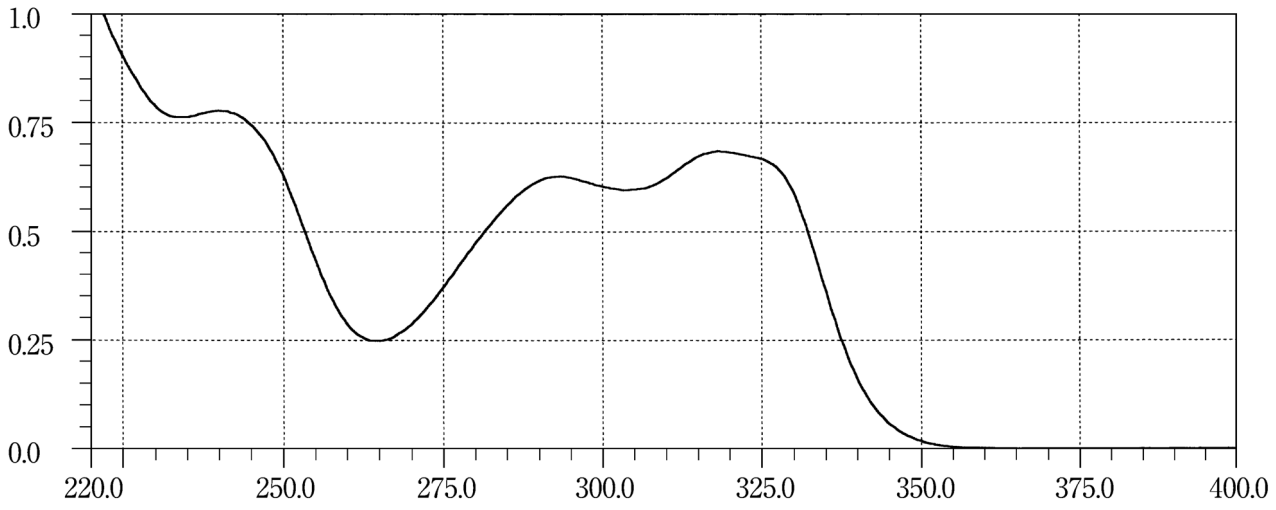
Fentanyl Citrate



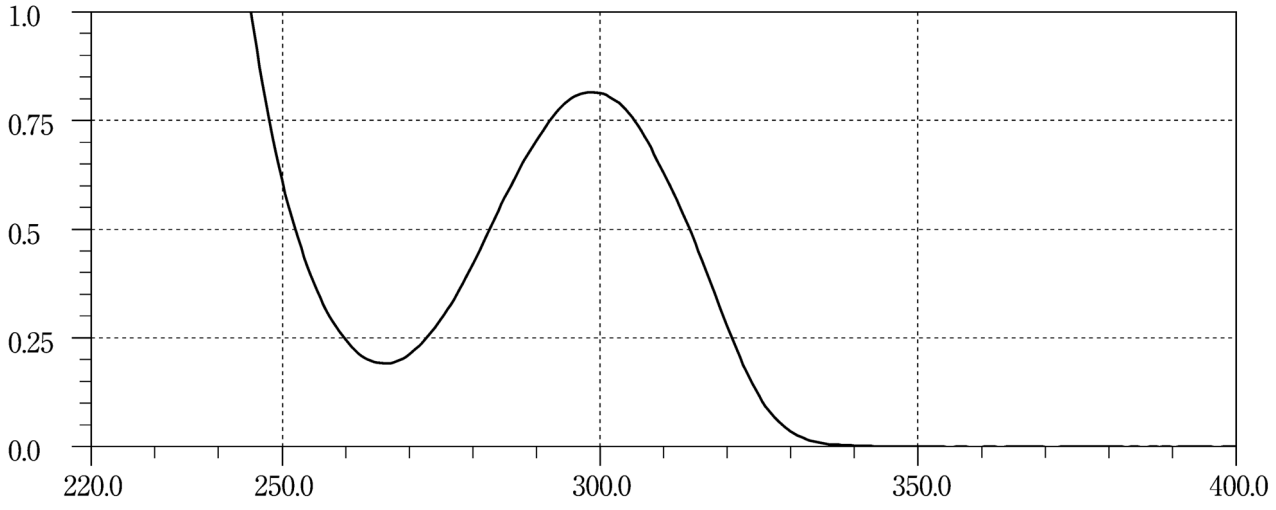
Fexofenadine Hydrochloride



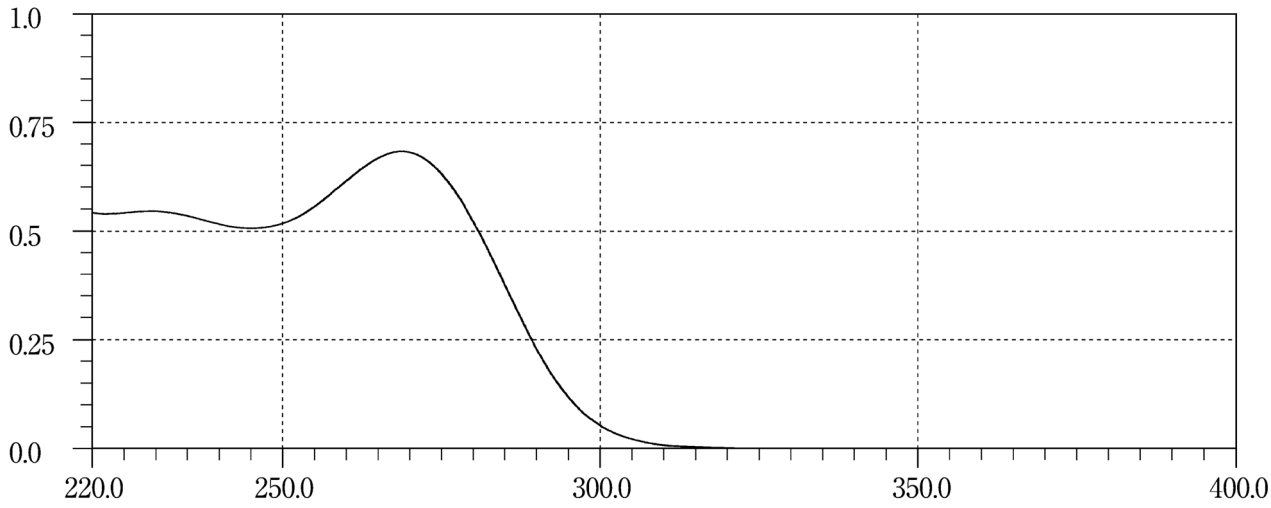
Flavoxate Hydrochloride



Flecainide Acetate



Flomoxef Sodium



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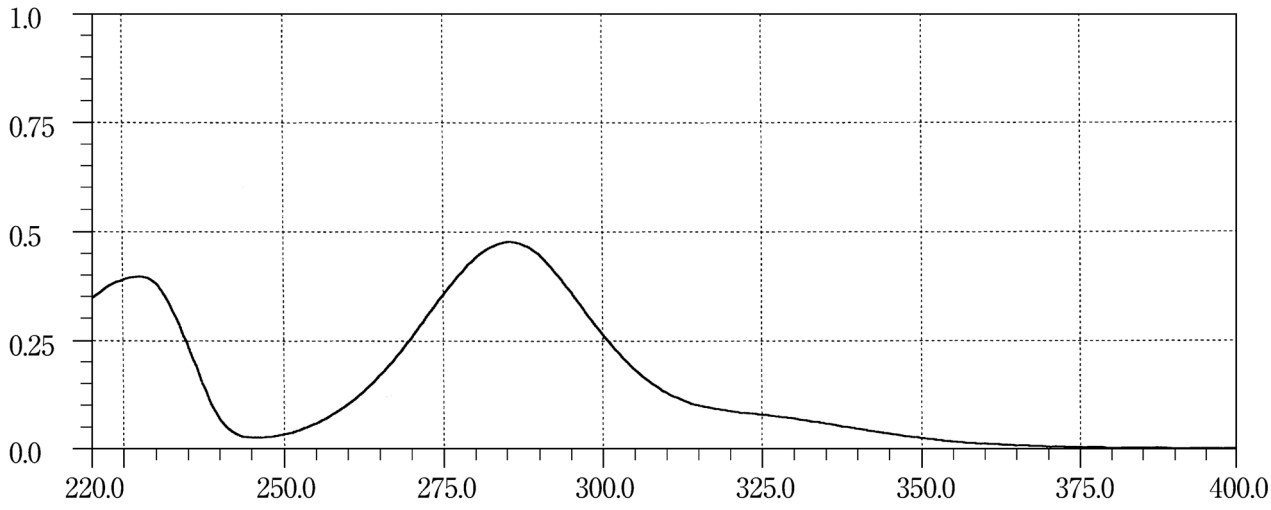

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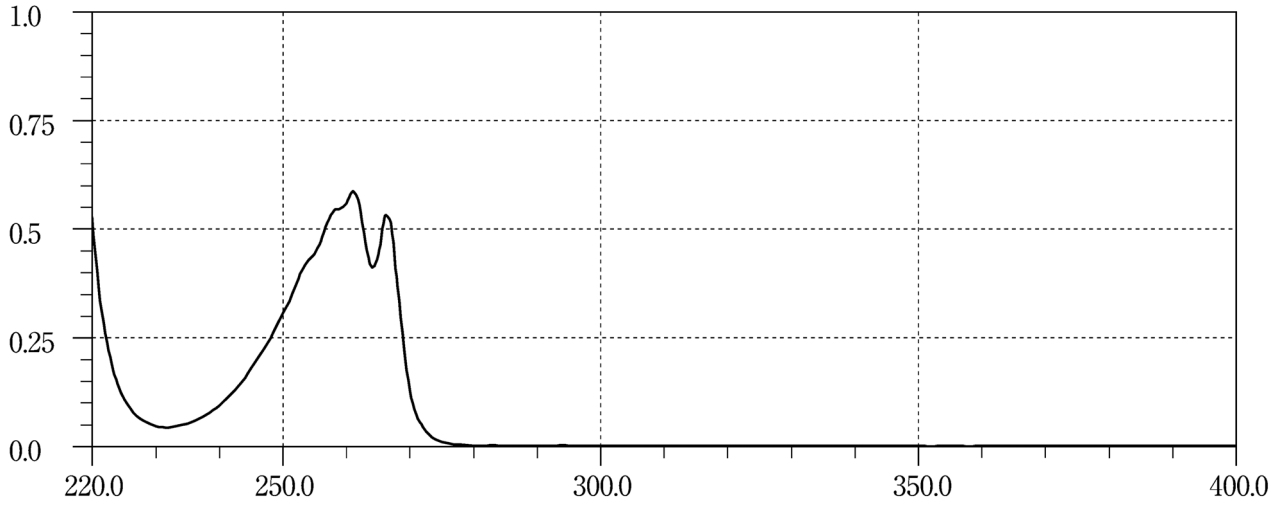
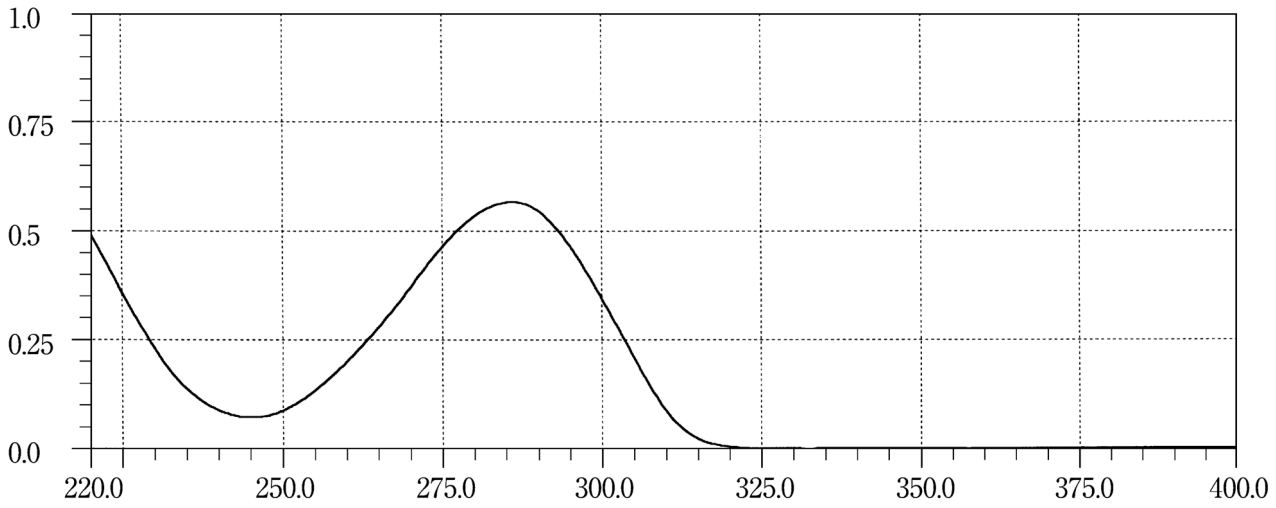
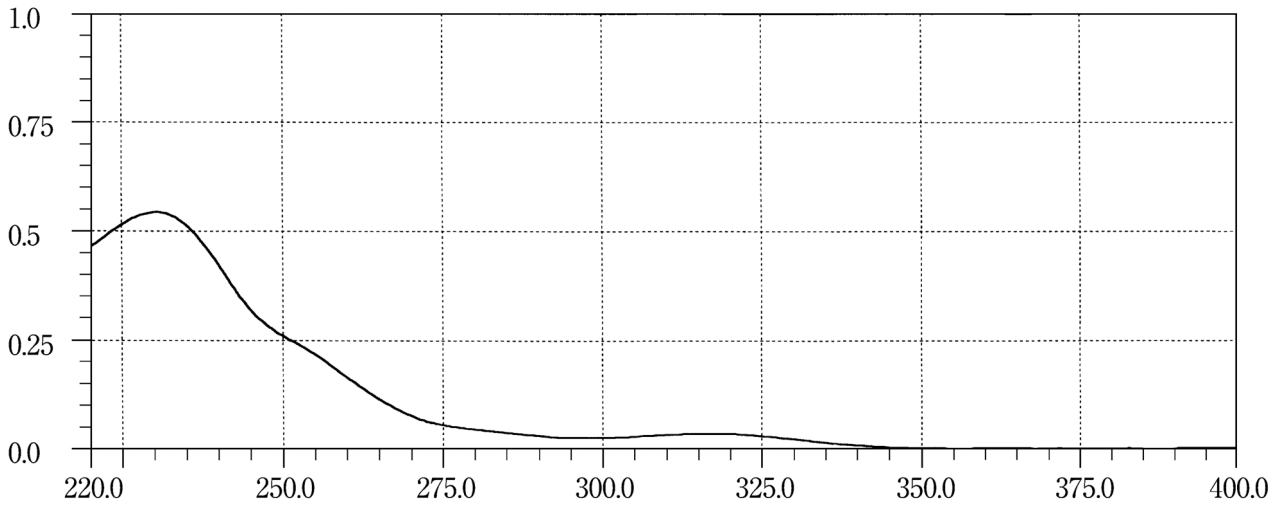


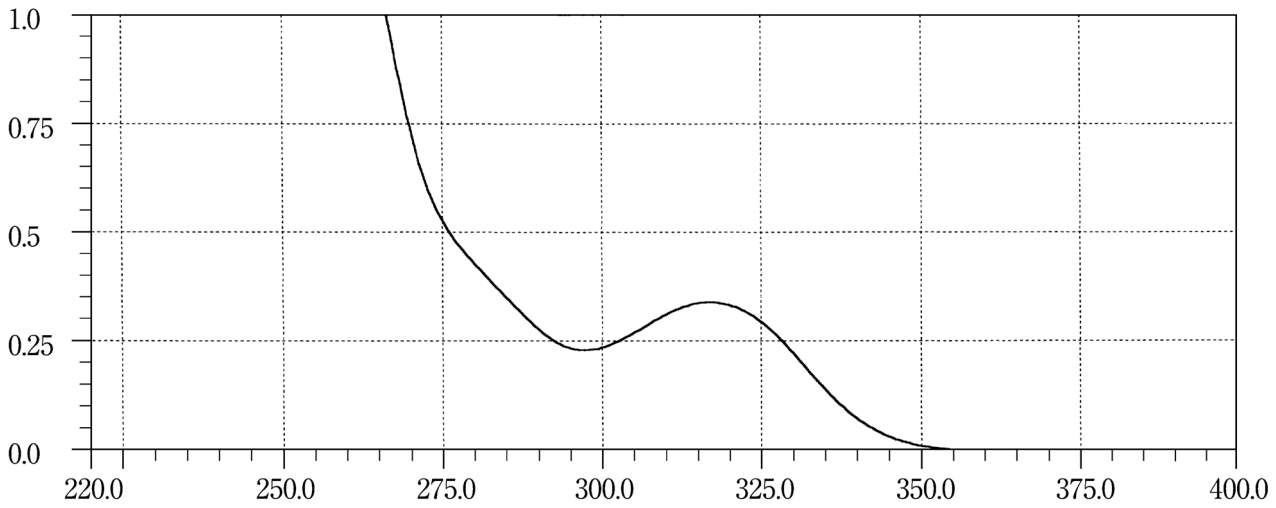
Flucytosine



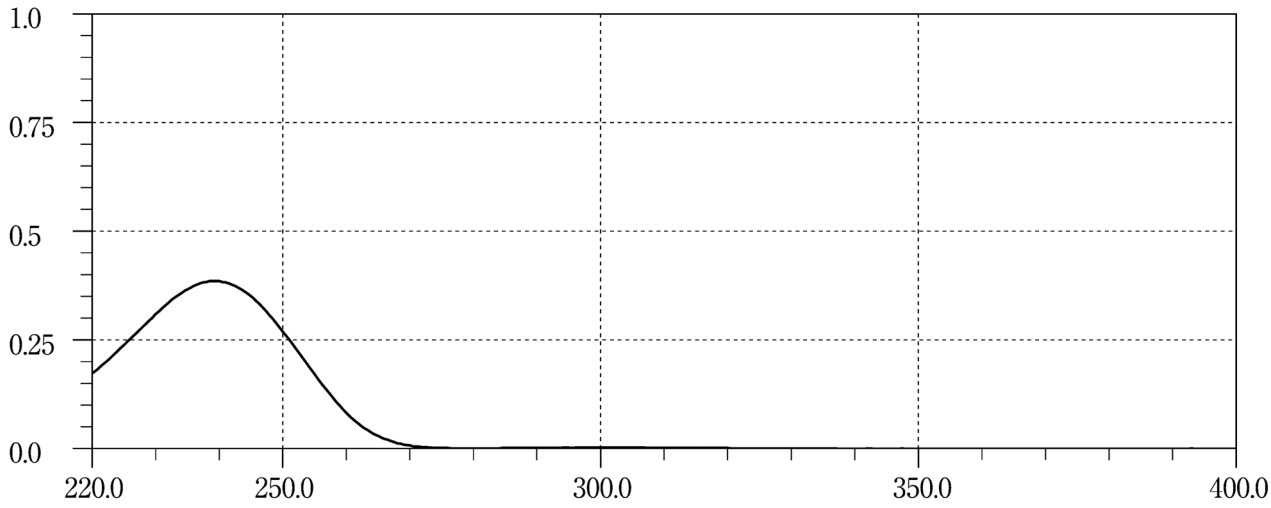
Fludiazepam 1



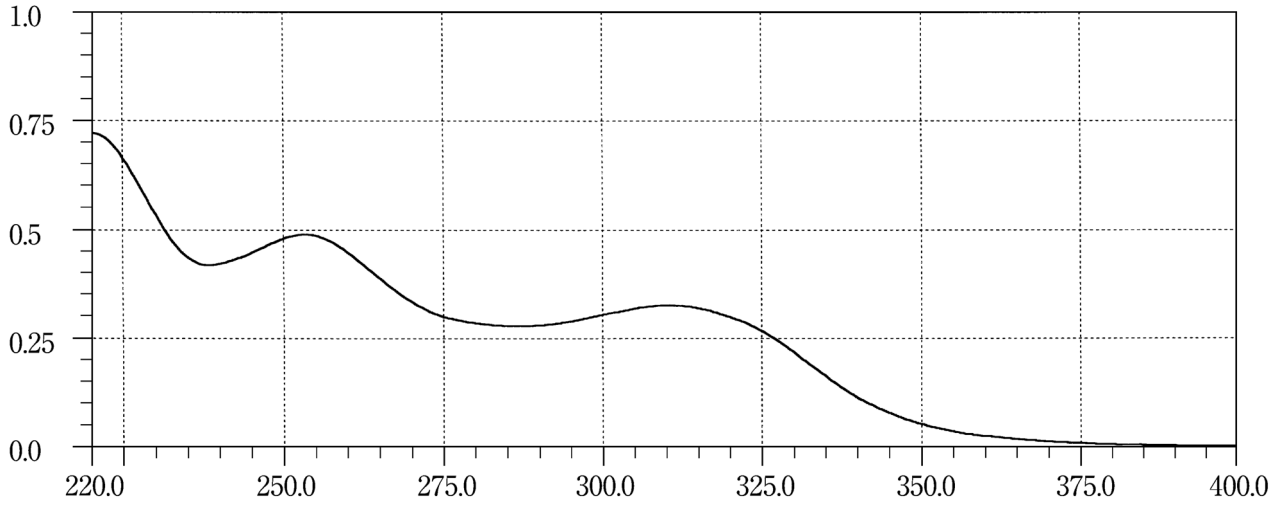
Fludiazepam 2



Fludrocortisone Acetate



Flunitrazepam



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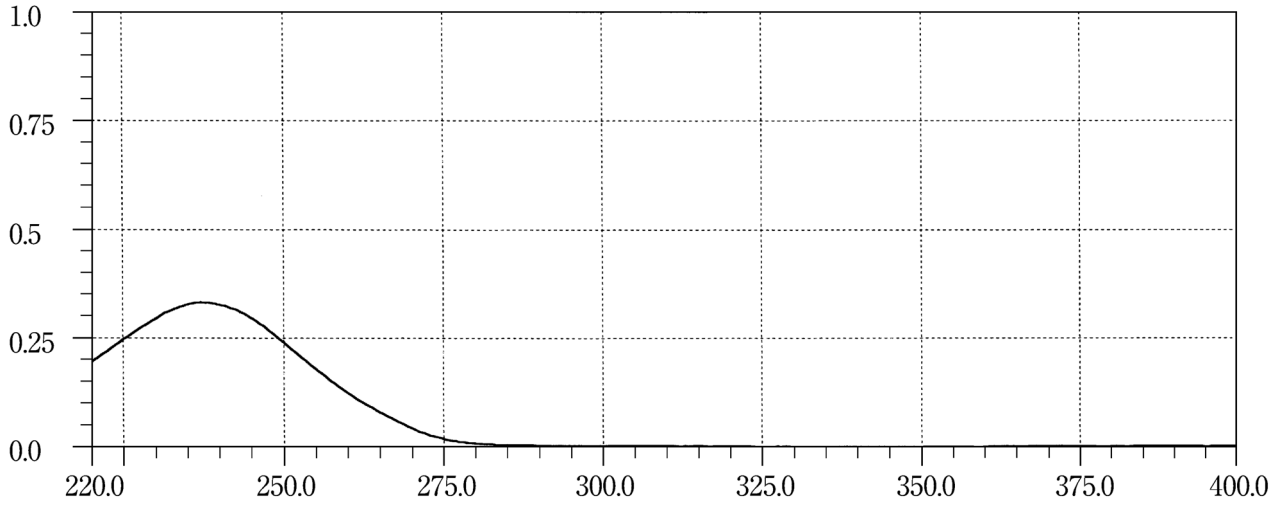

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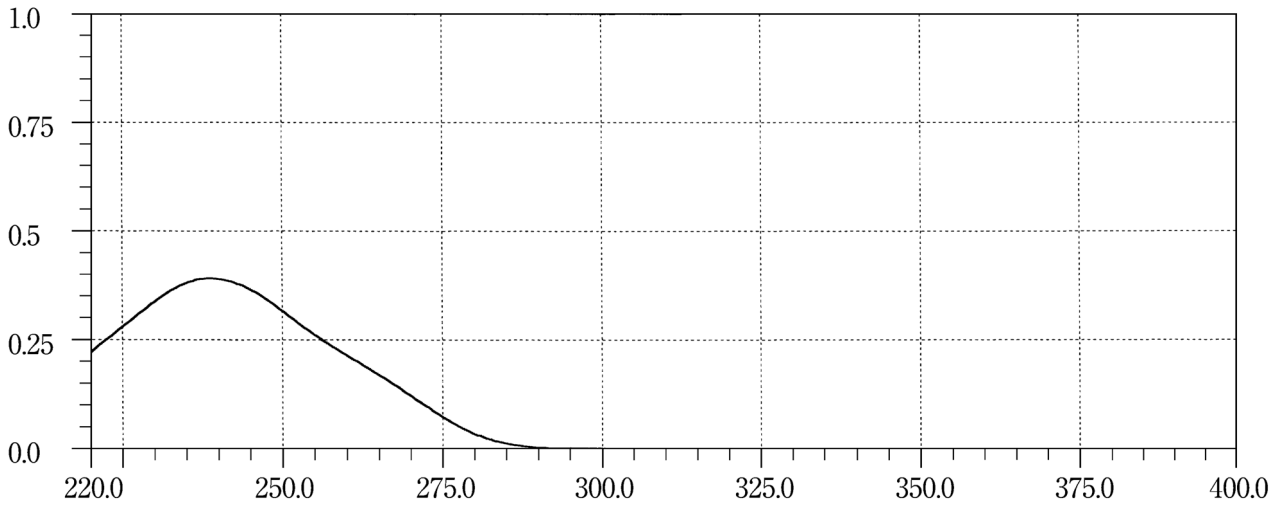

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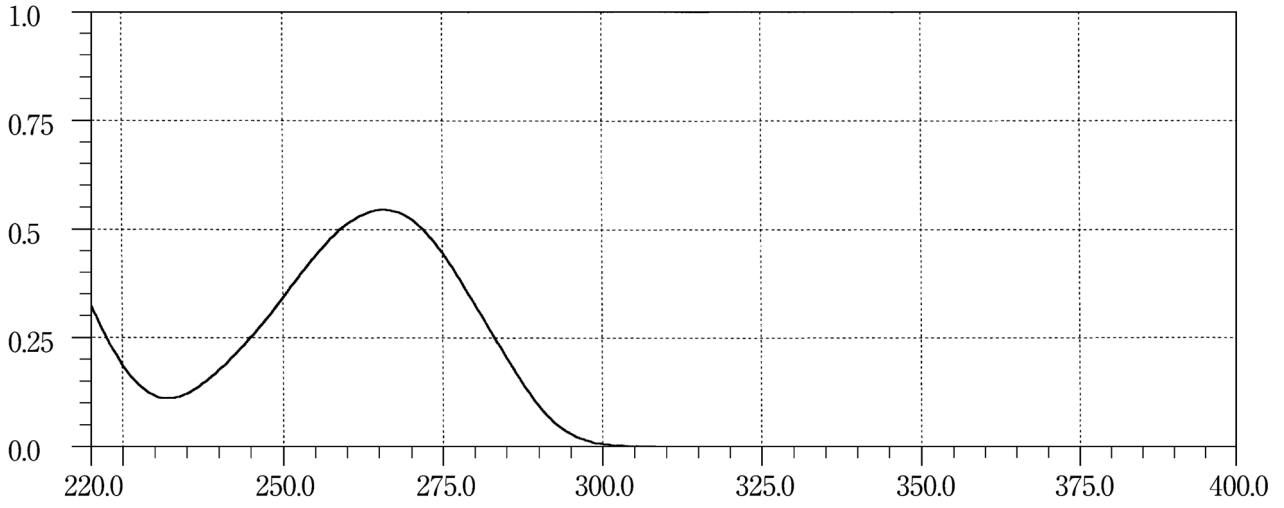
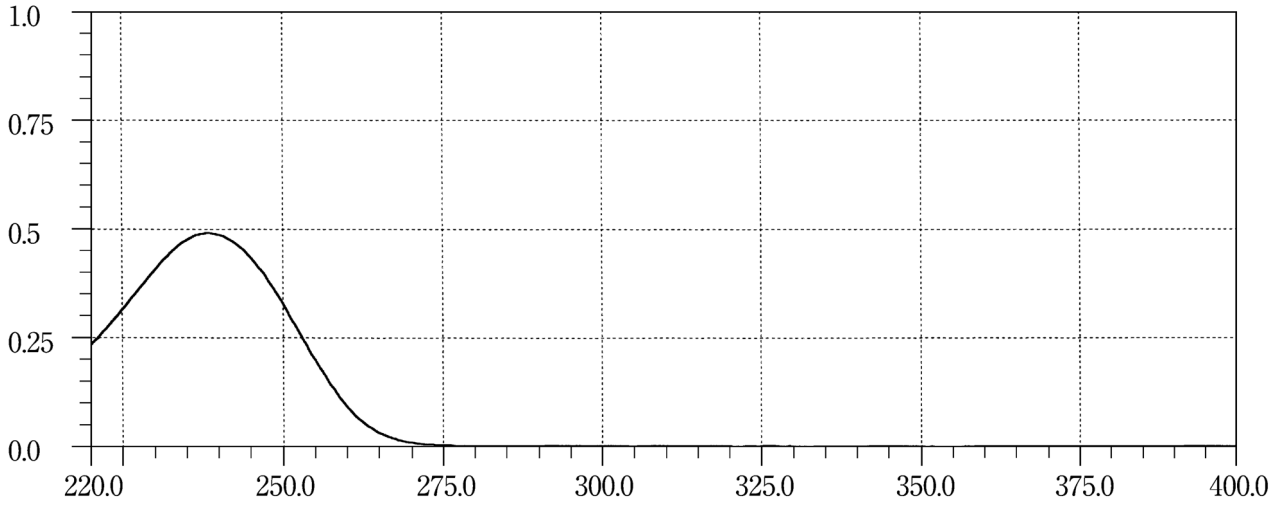
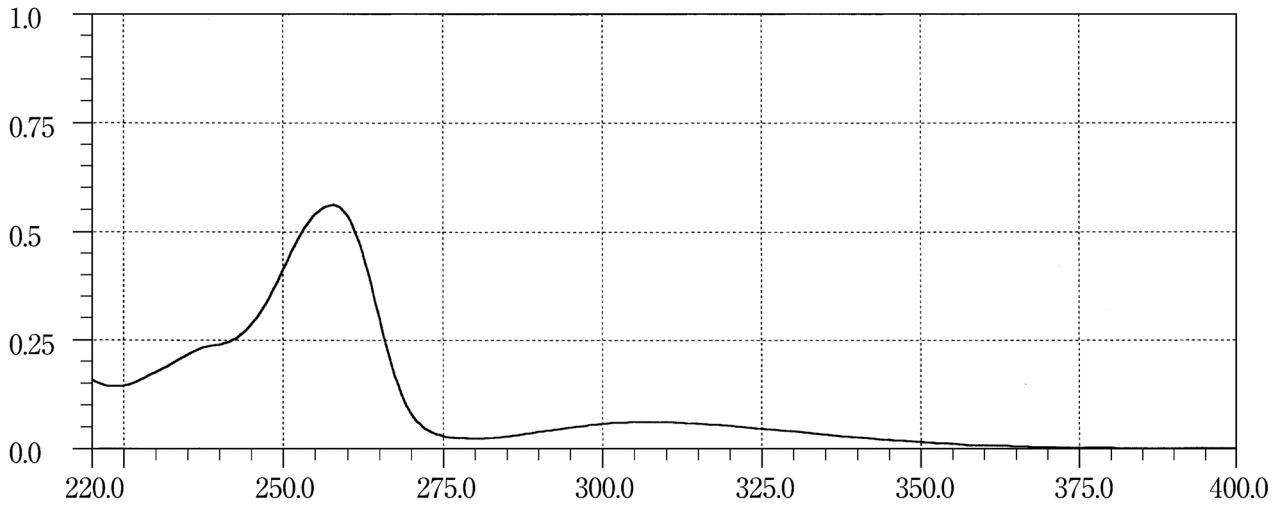


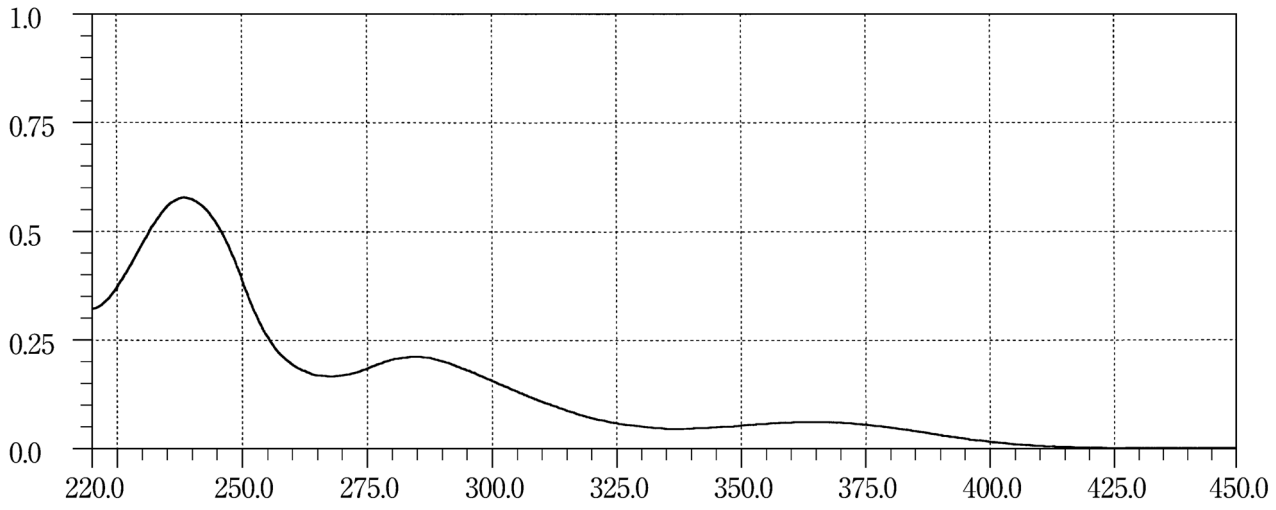
Fluoxymesterone



Fluphenazine Enanthate



Flurazepam Hydrochloride



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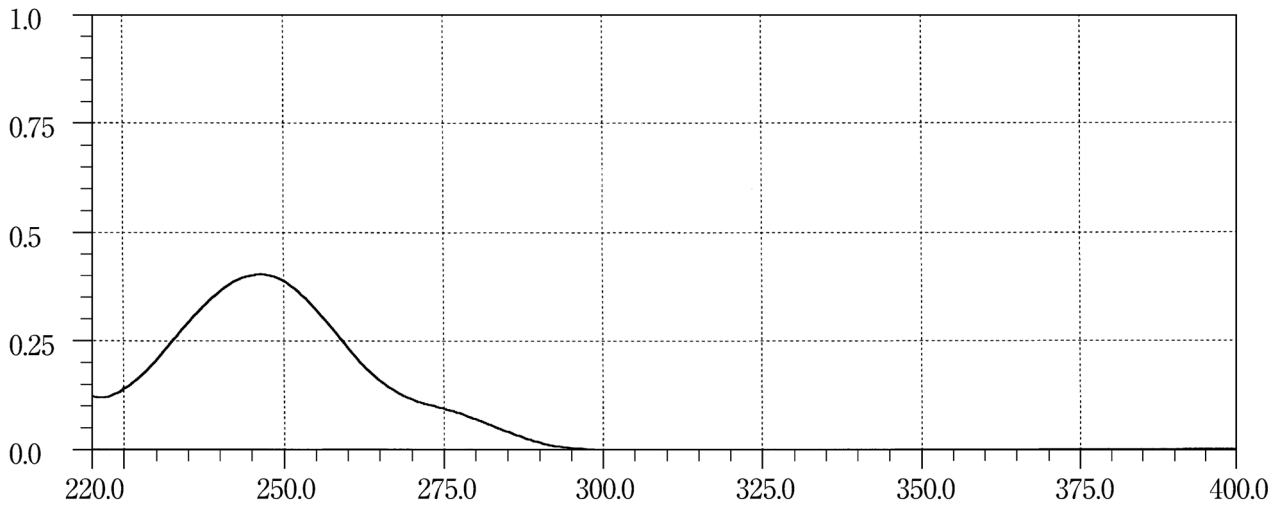
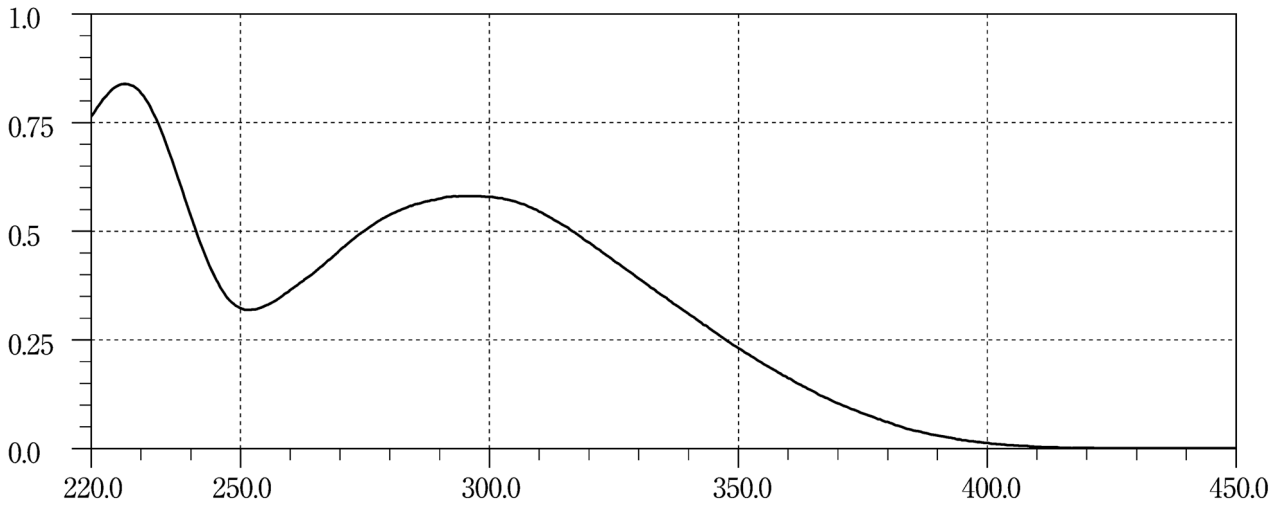
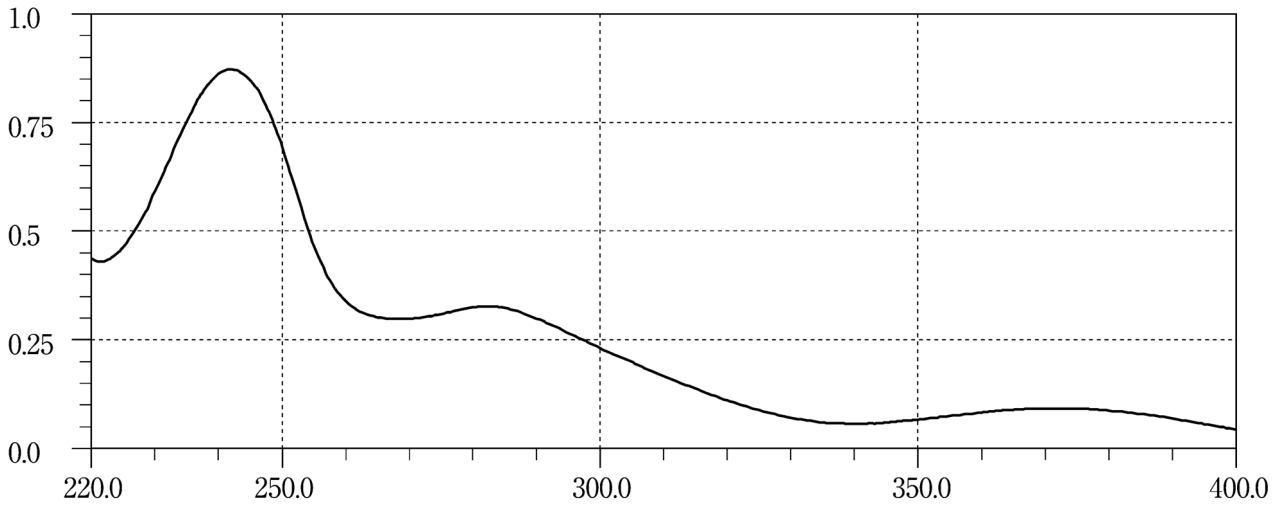


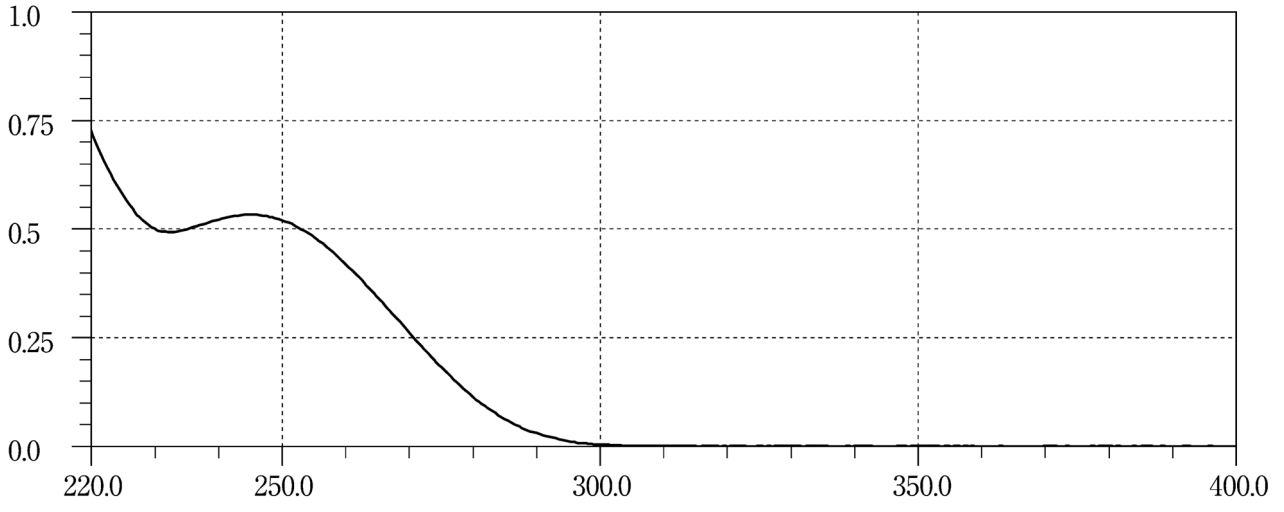
Flutamide



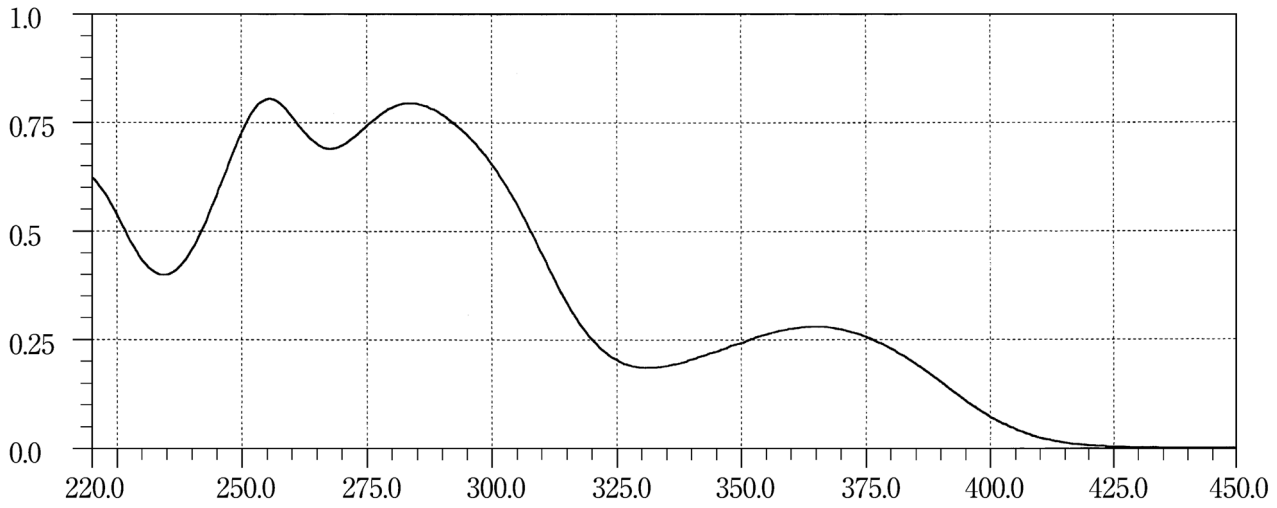
Flutoprazepam



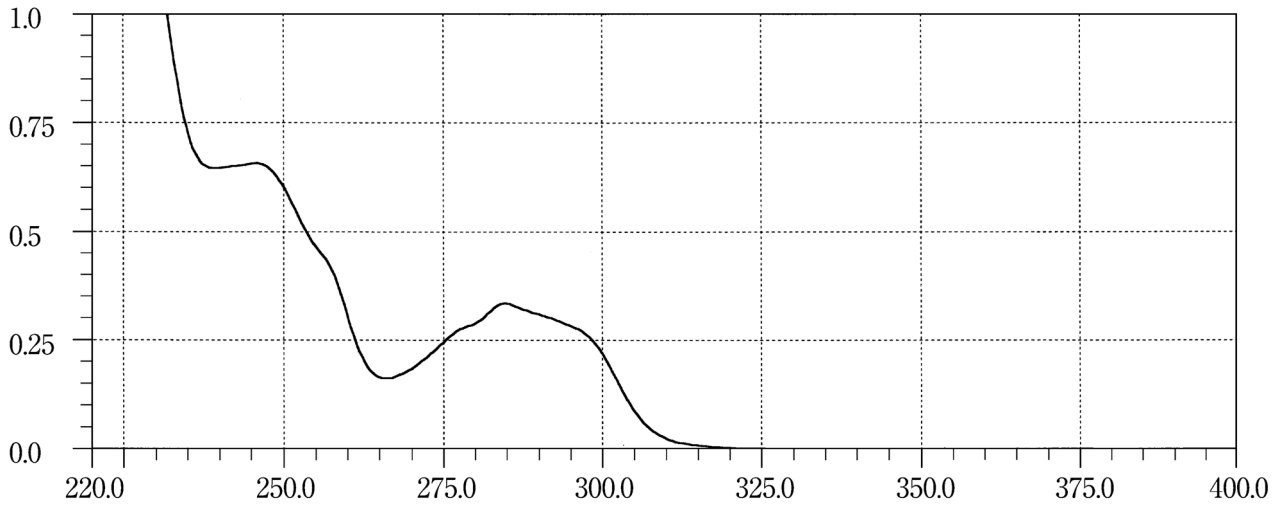
Fluvoxamine Maleate



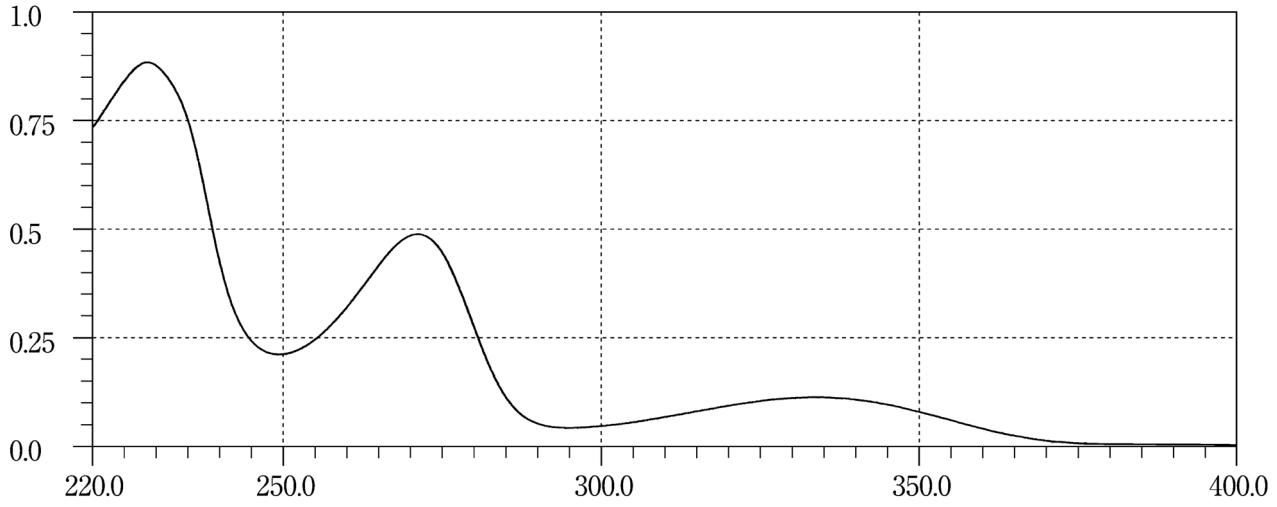
Folic Acid



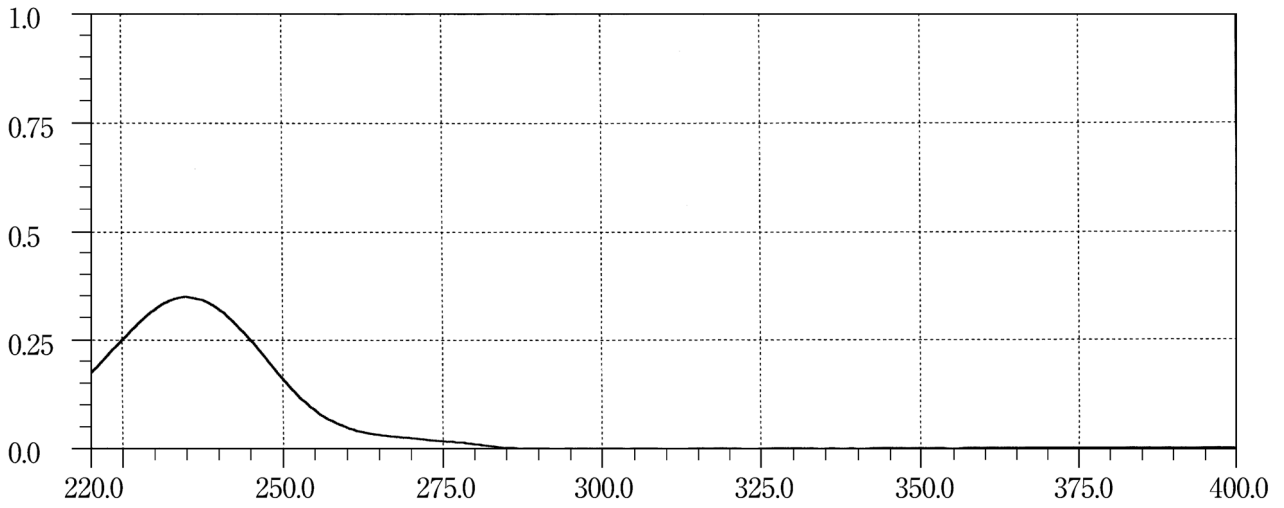
Formoterol Fumarate Hydrate



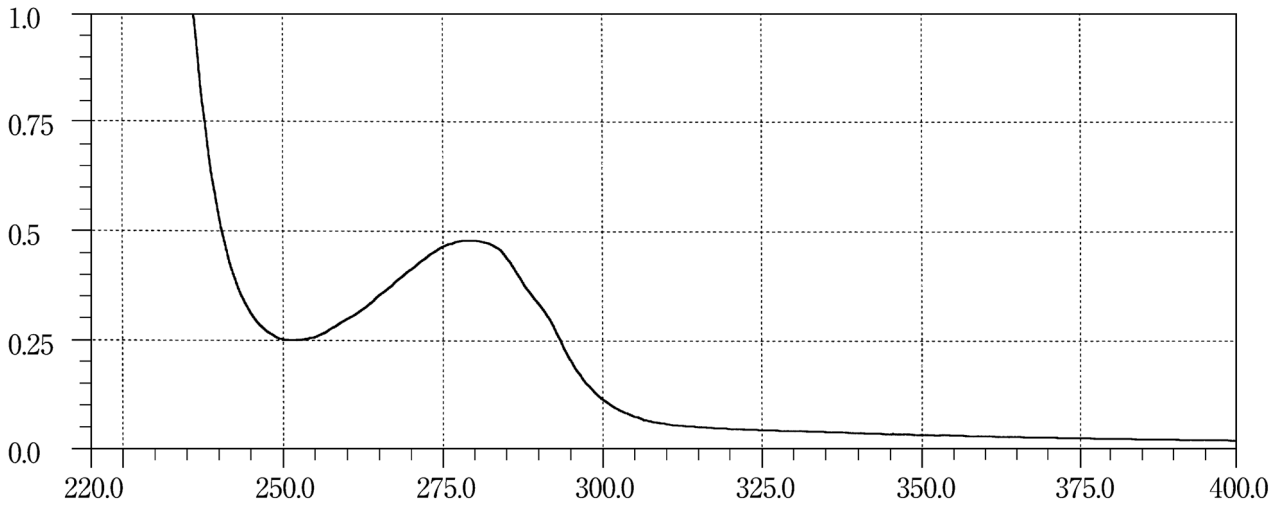
Furosemide



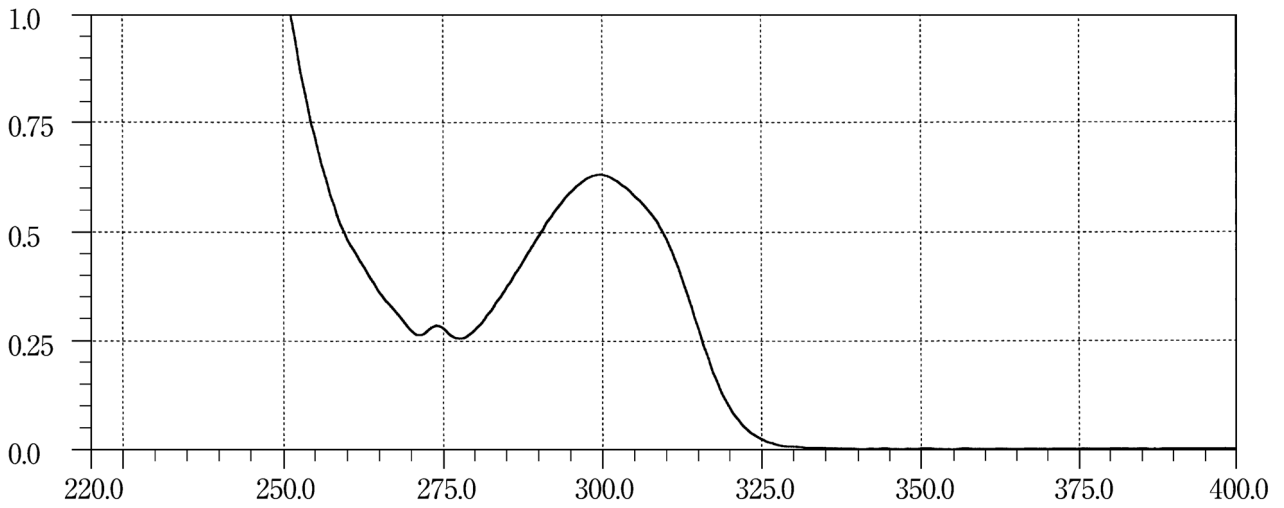
Gabexate Mesilate



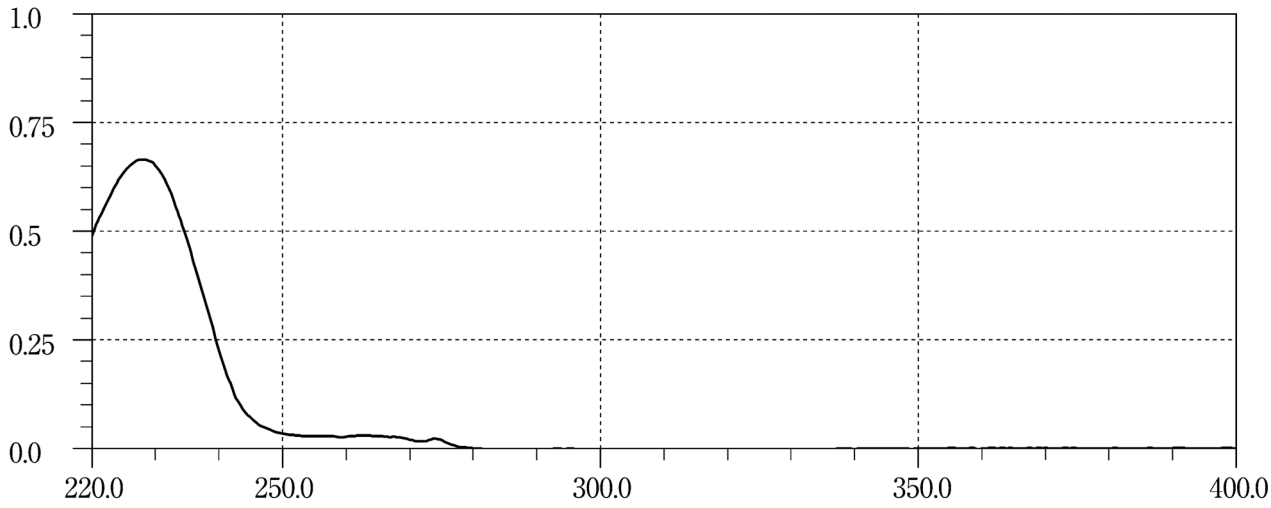
β-Galactosidase (Aspergillus)



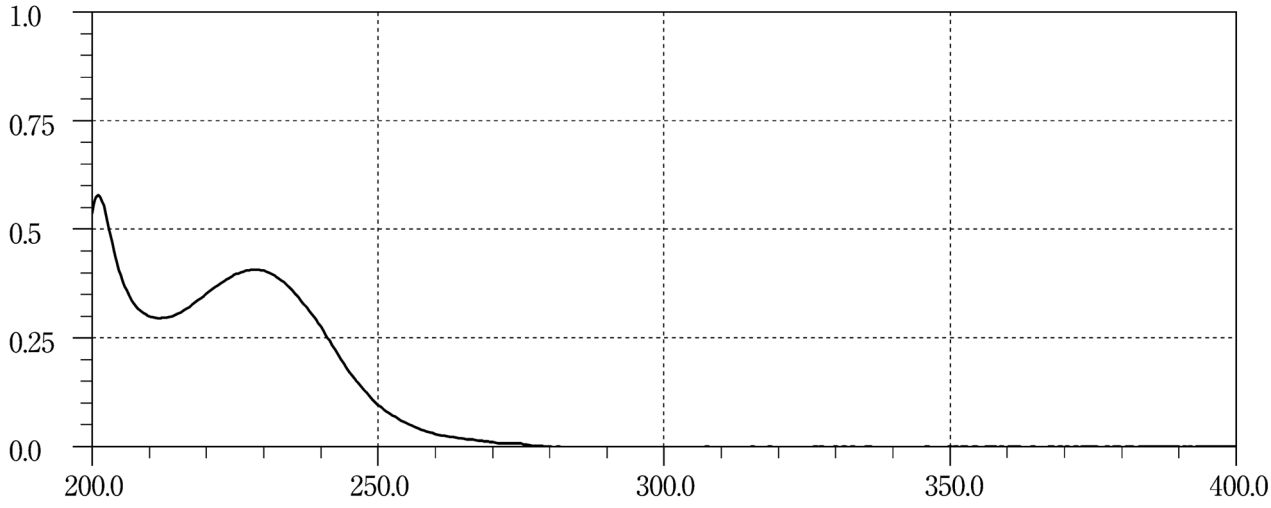
Glibenclamide



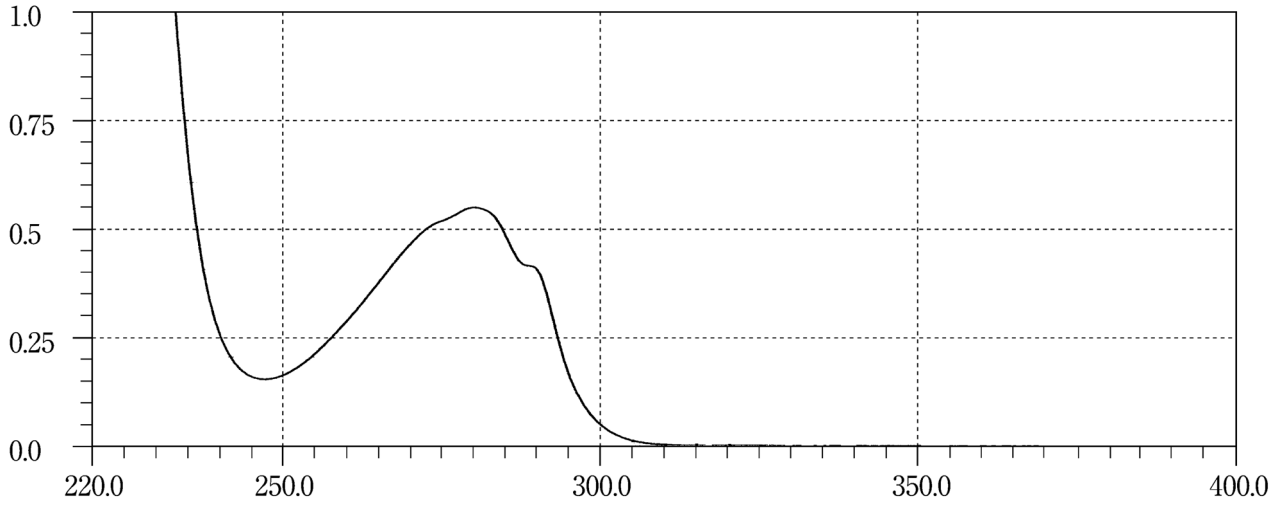
Gliclazide



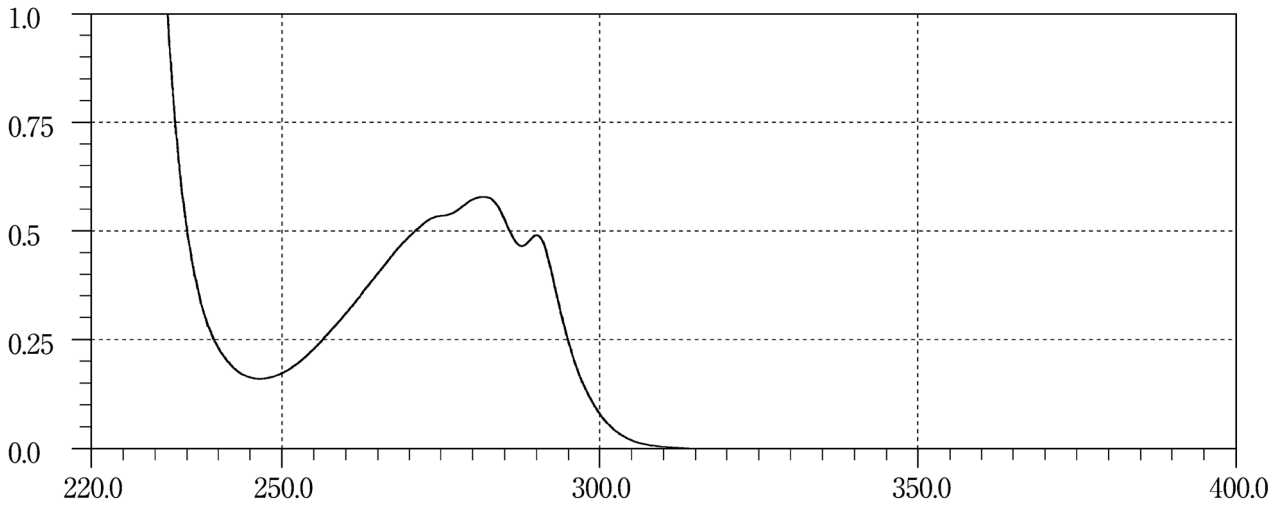
Glimepiride



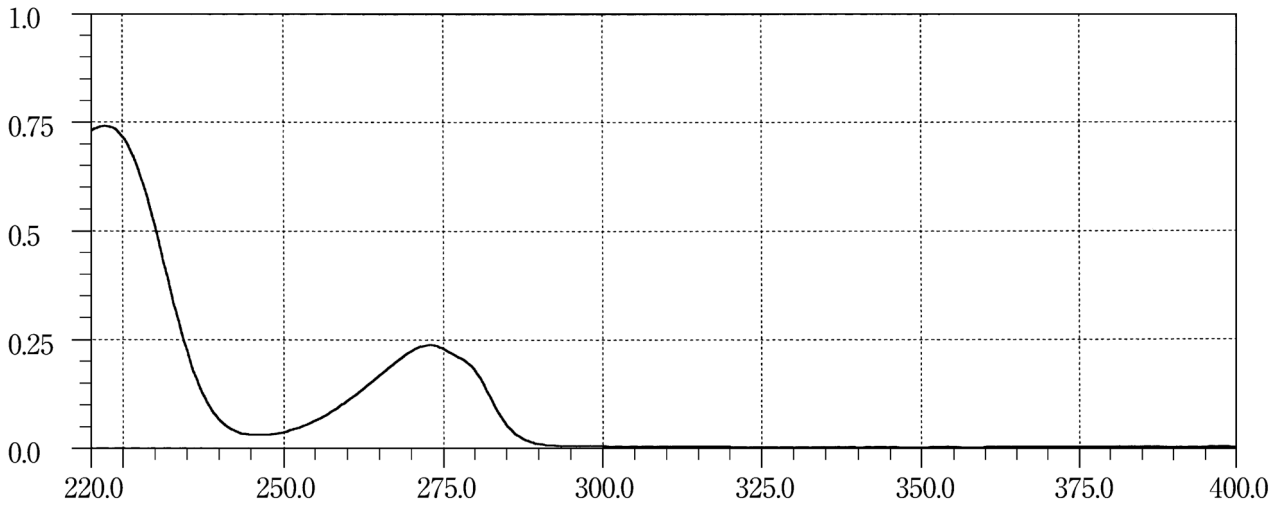
Gonadorelin Acetate



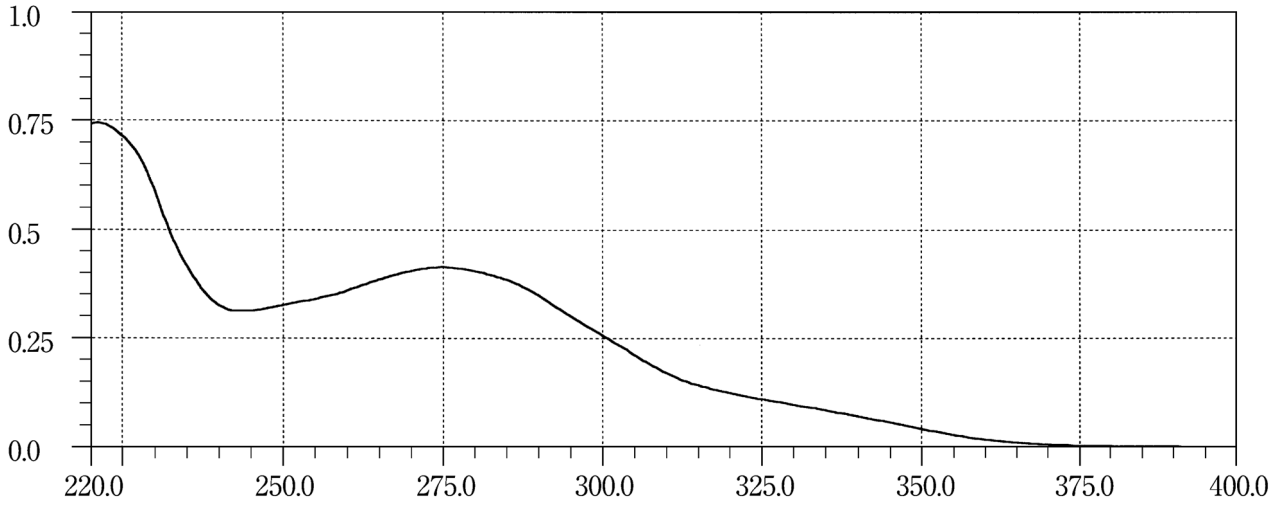
Gramicidin



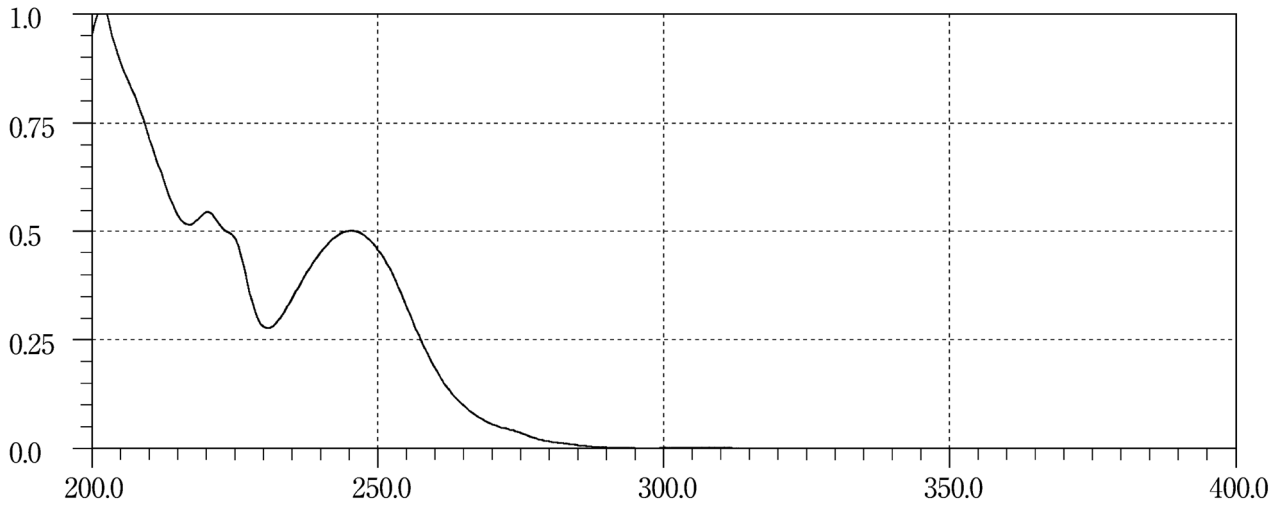
Guaifenesin



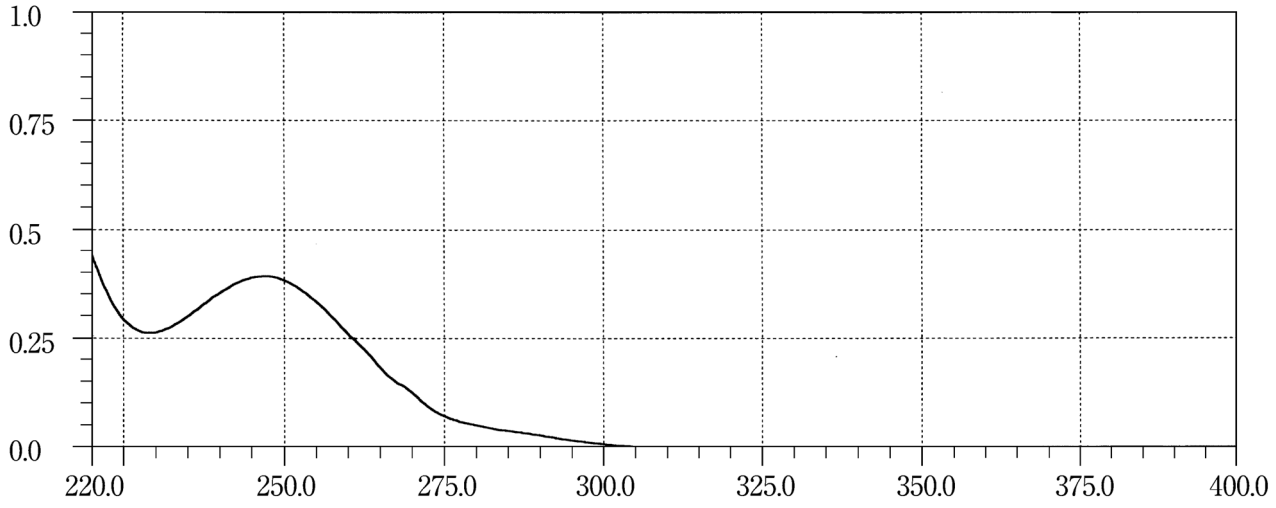
Guanabenz Acetate



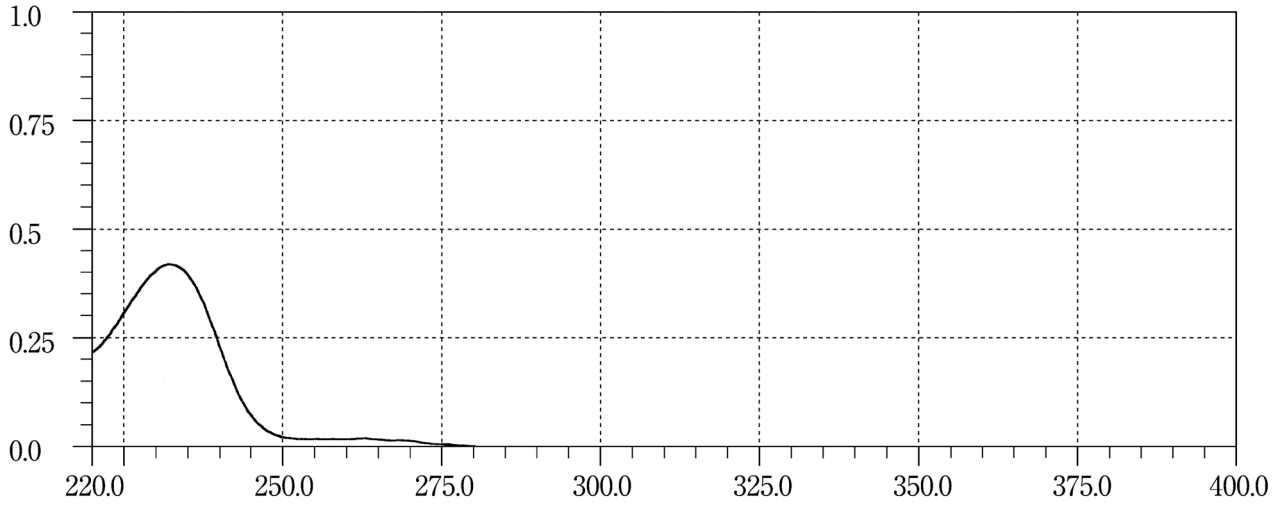
Haloperidol



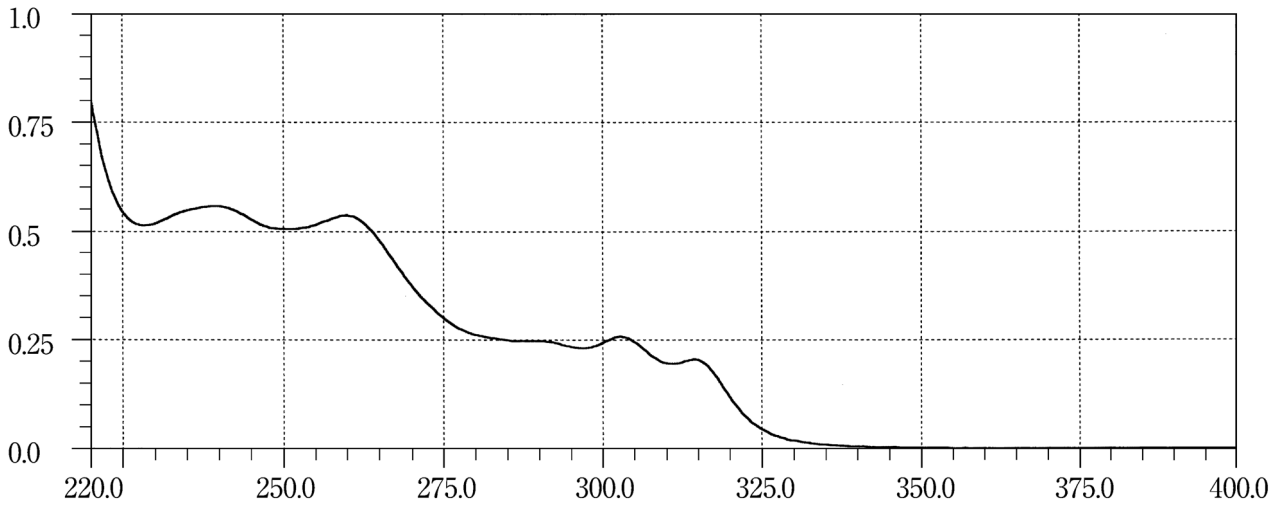
Haloxazolam



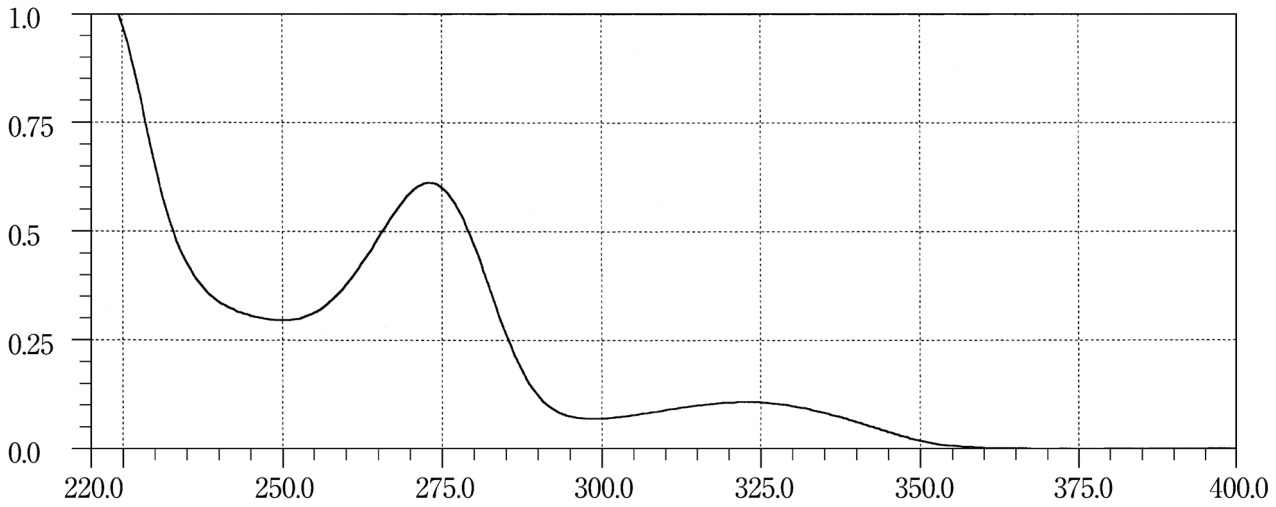
Homochlorcyclizine Hydrochloride



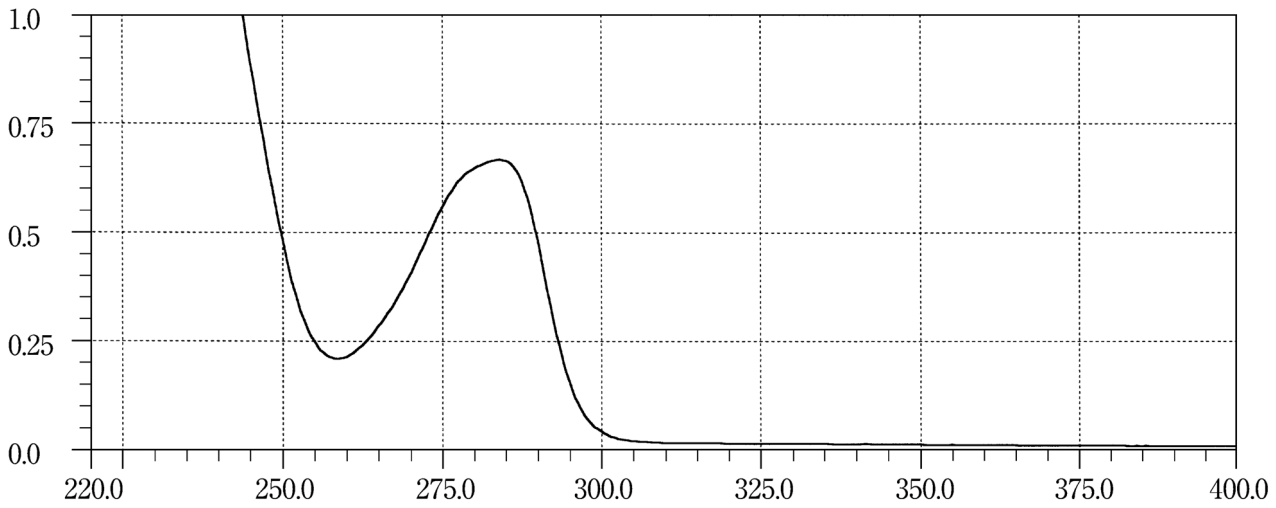
Hydralazine Hydrochloride



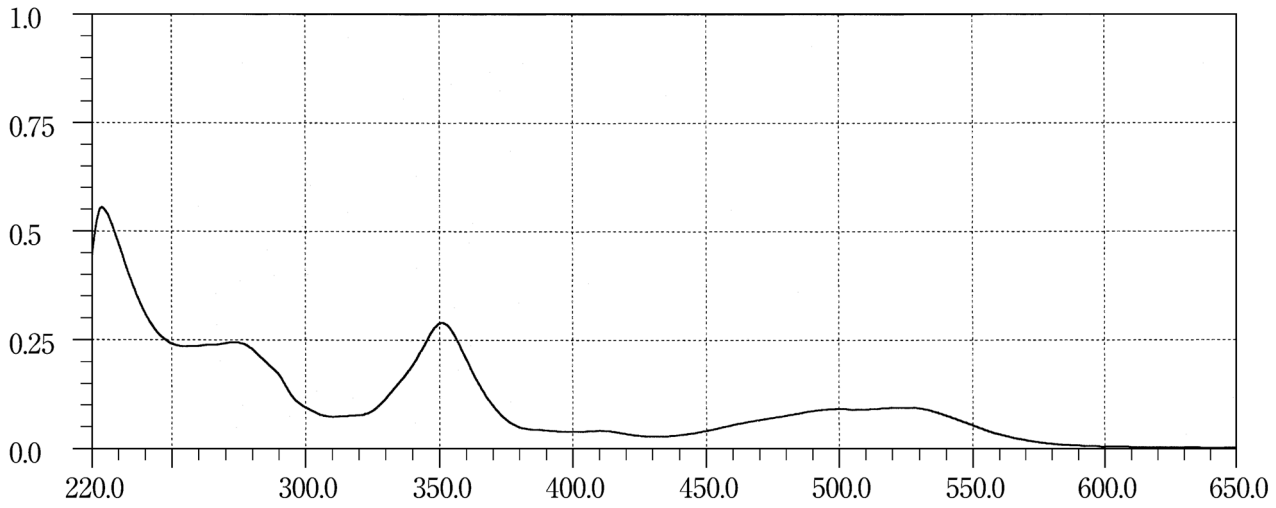
Hydrochlorothiazide



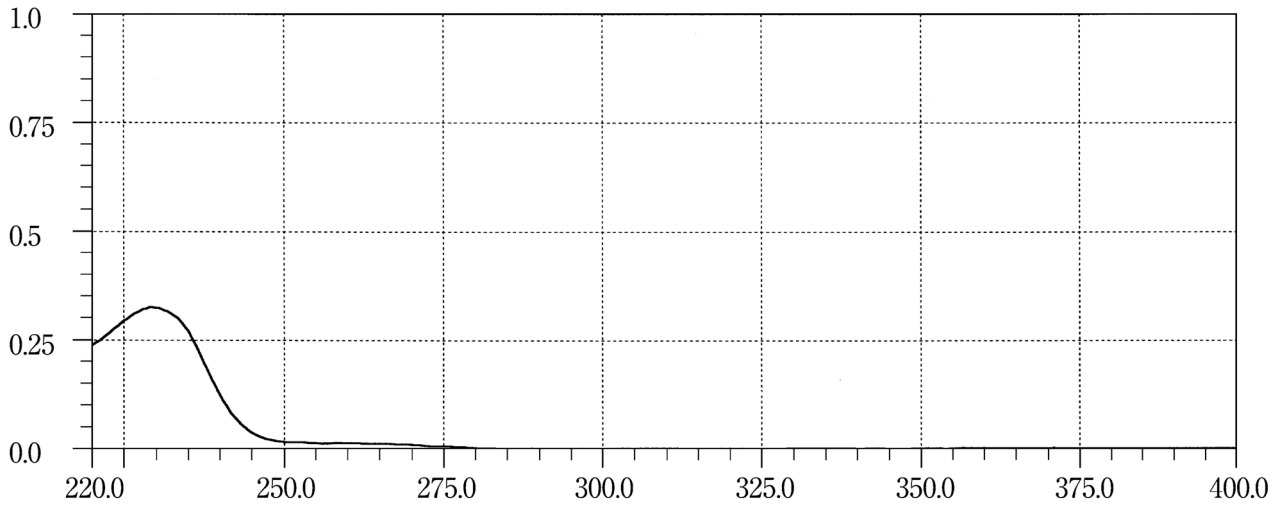
Hydrocotarnine Hydrochloride Hydrate



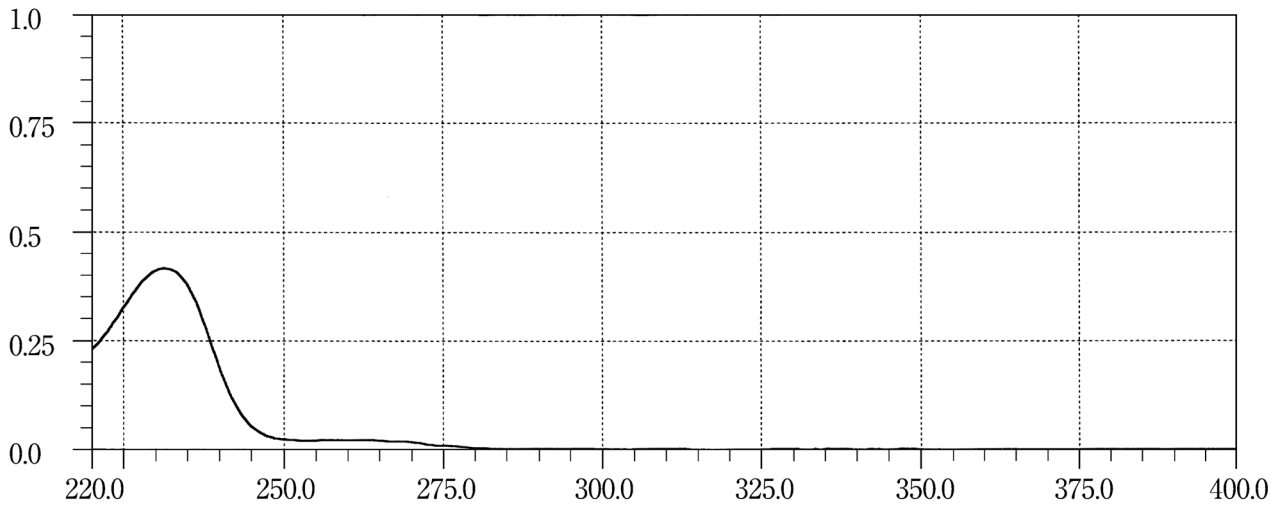
Hydroxocobalamin Acetate



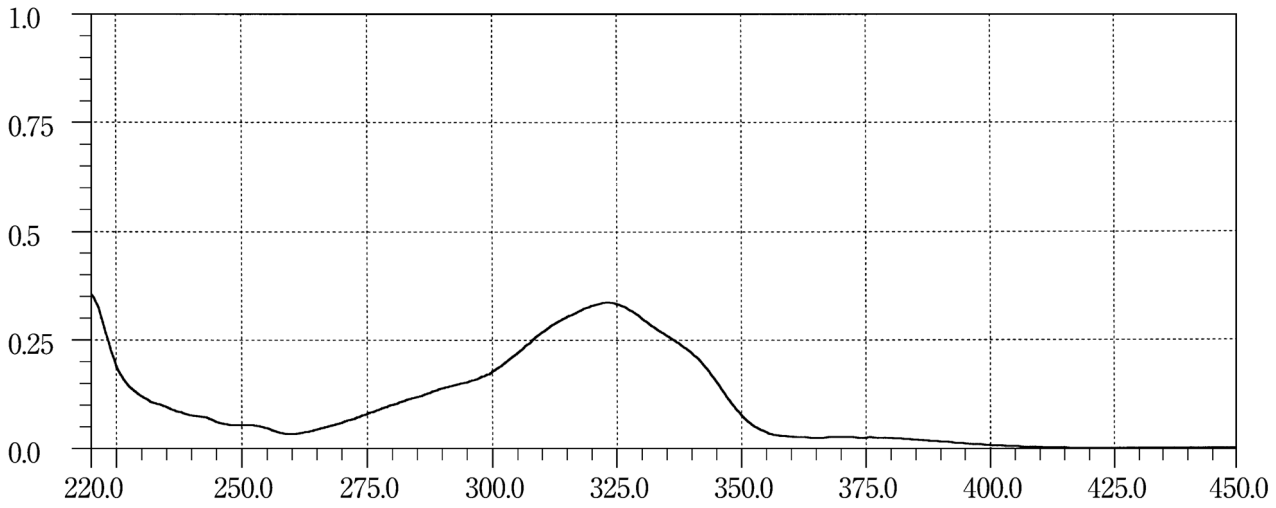
Hydroxyzine Hydrochloride



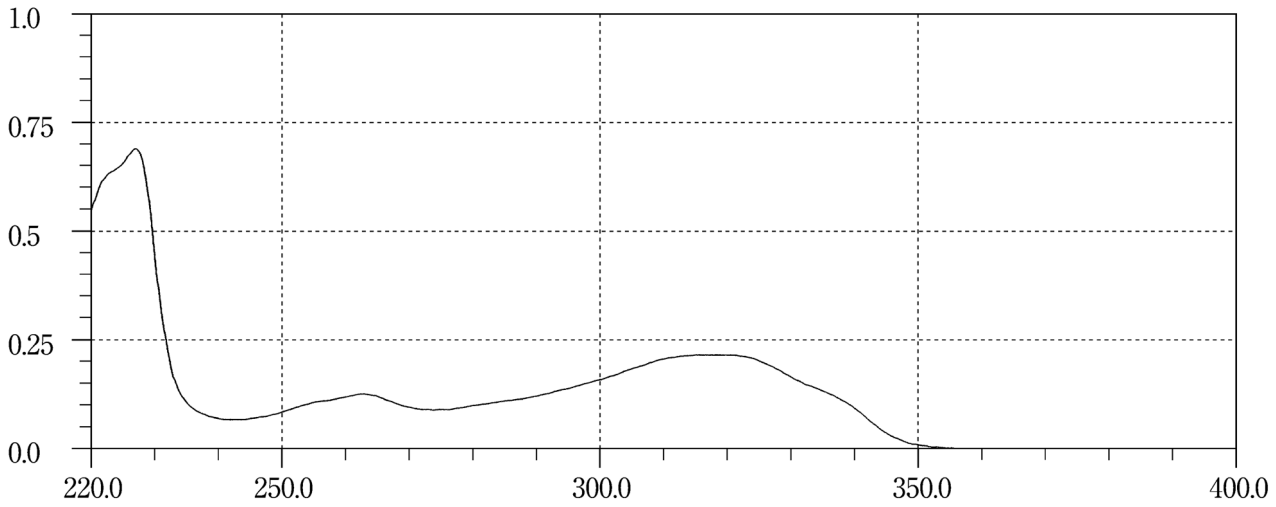
Hydroxyzine Pamoate



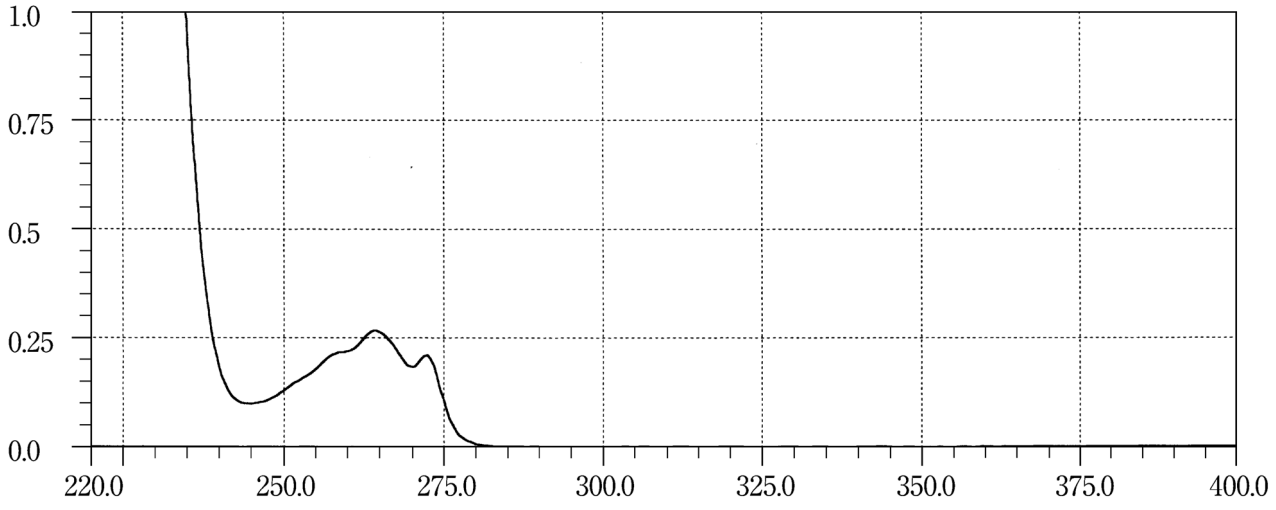
Hymecromone



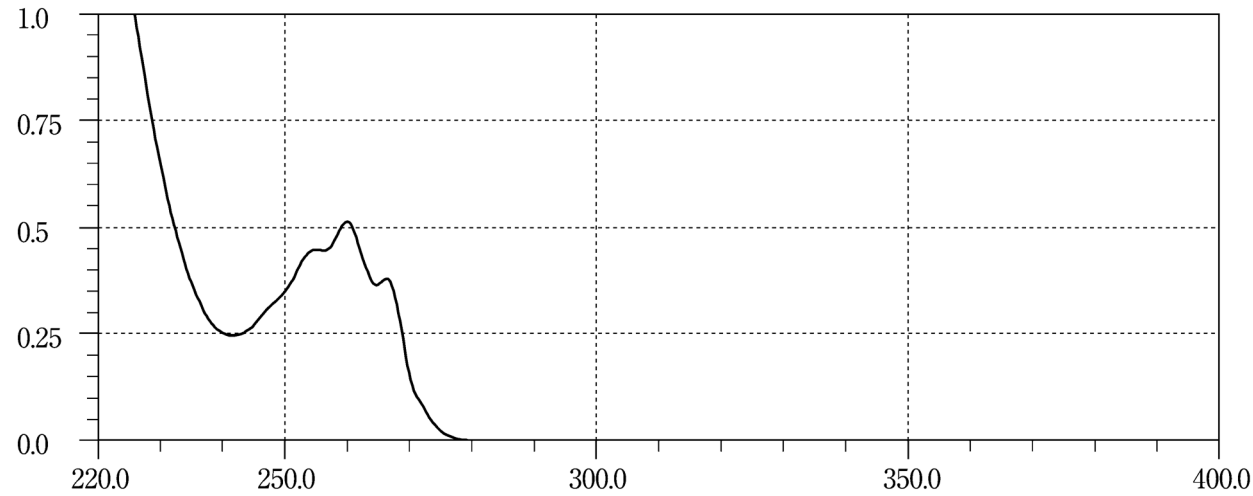
Ibudilast



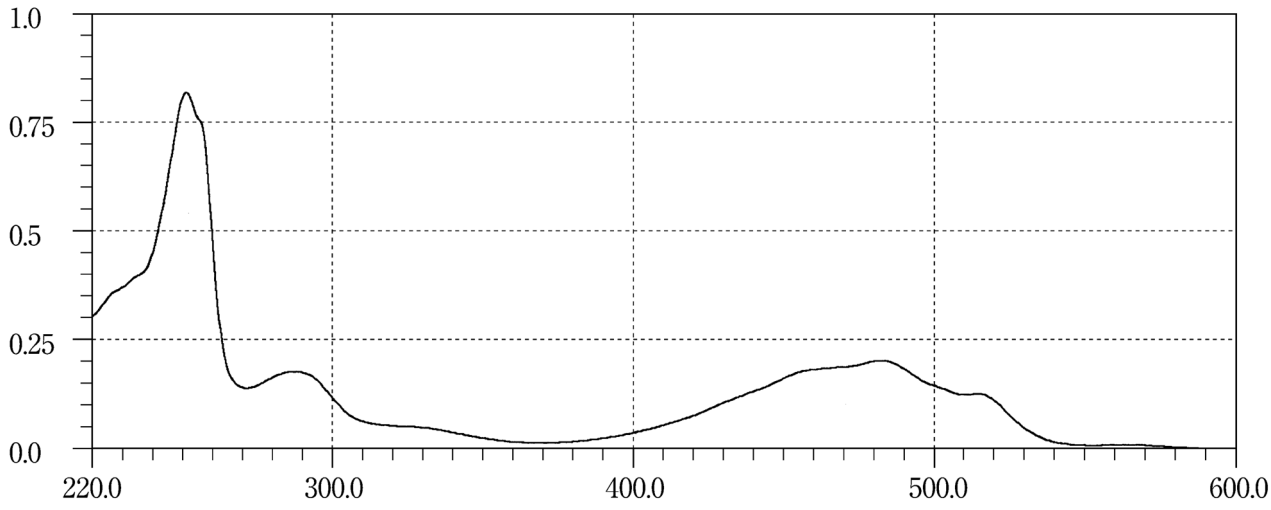
Ibuprofen



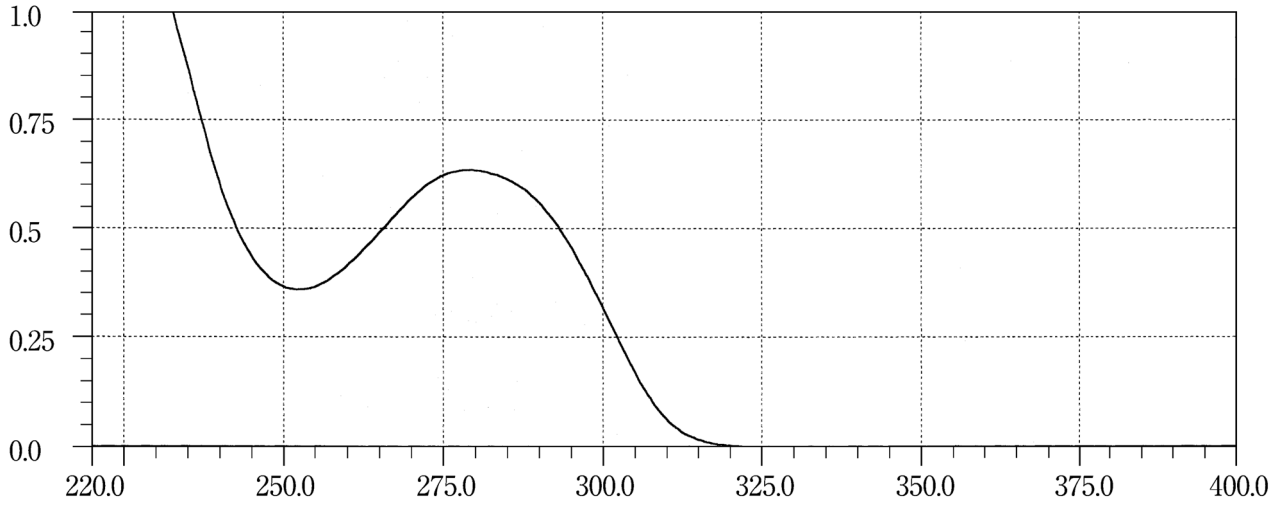
Ibuprofen Piconol



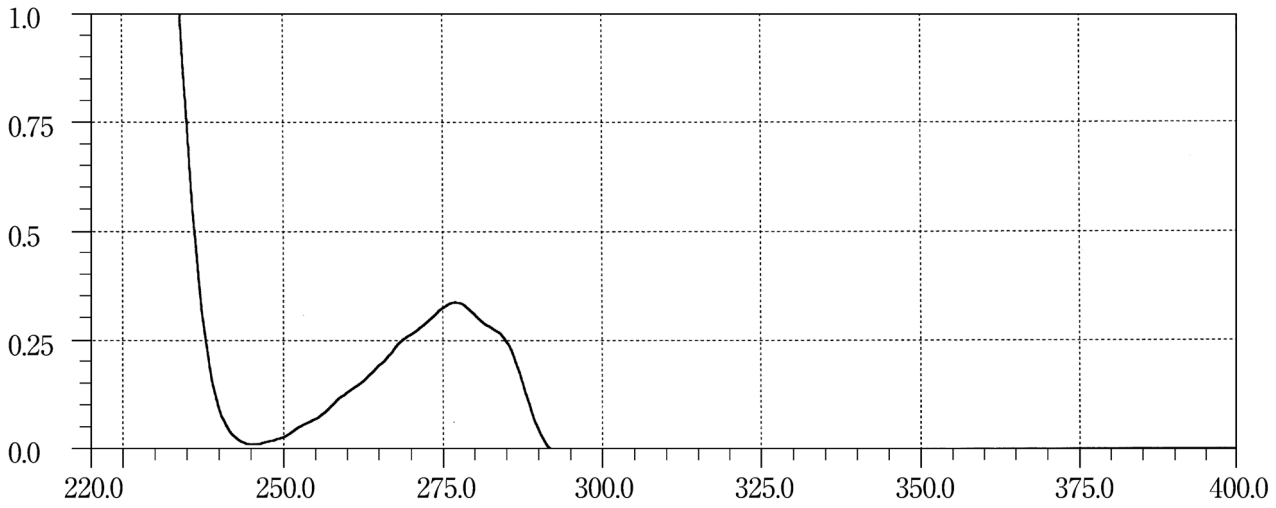
Idarubicin Hydrochloride



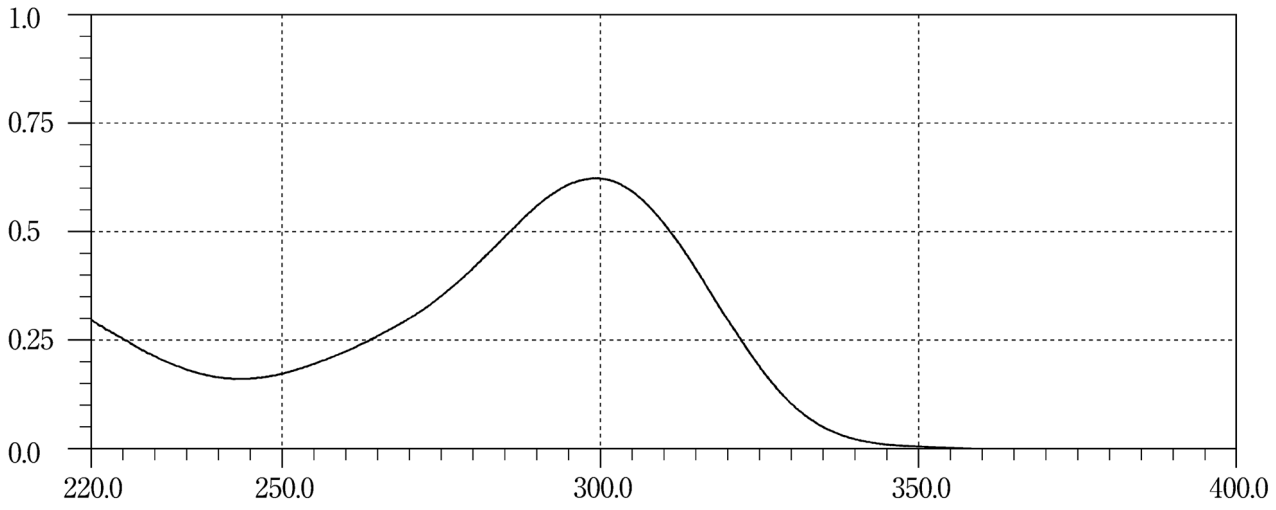
Idoxuridine



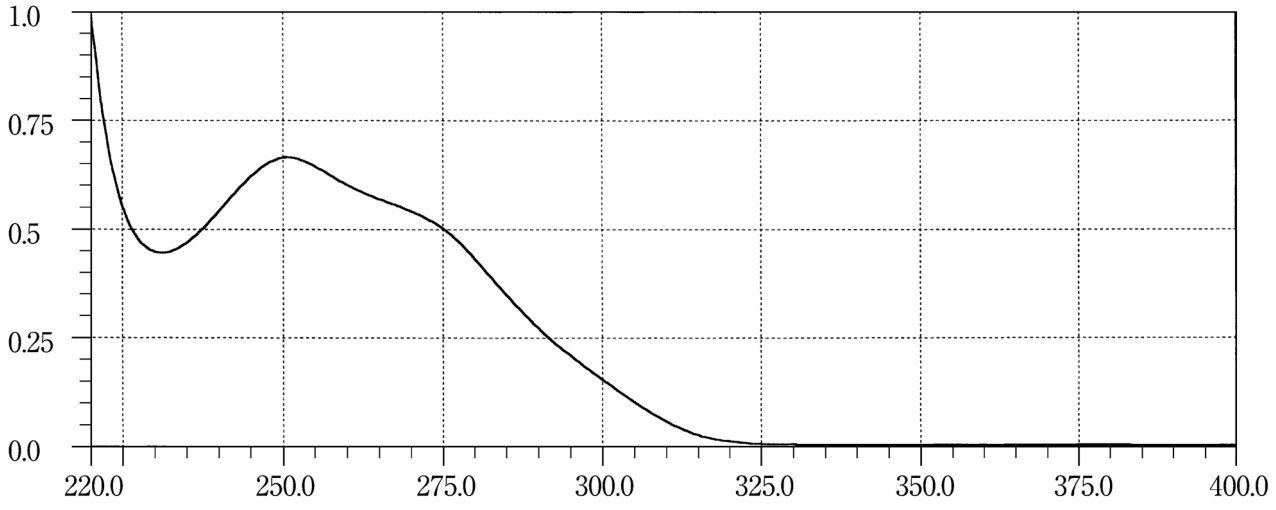
Ifenprodil Tartrate



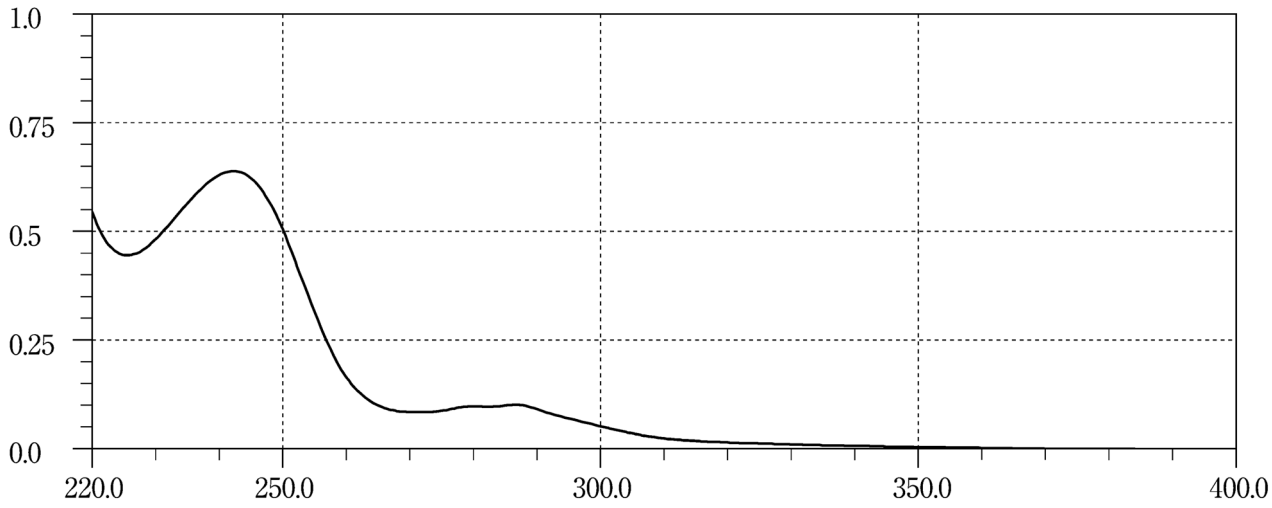
Imipenem Hydrate



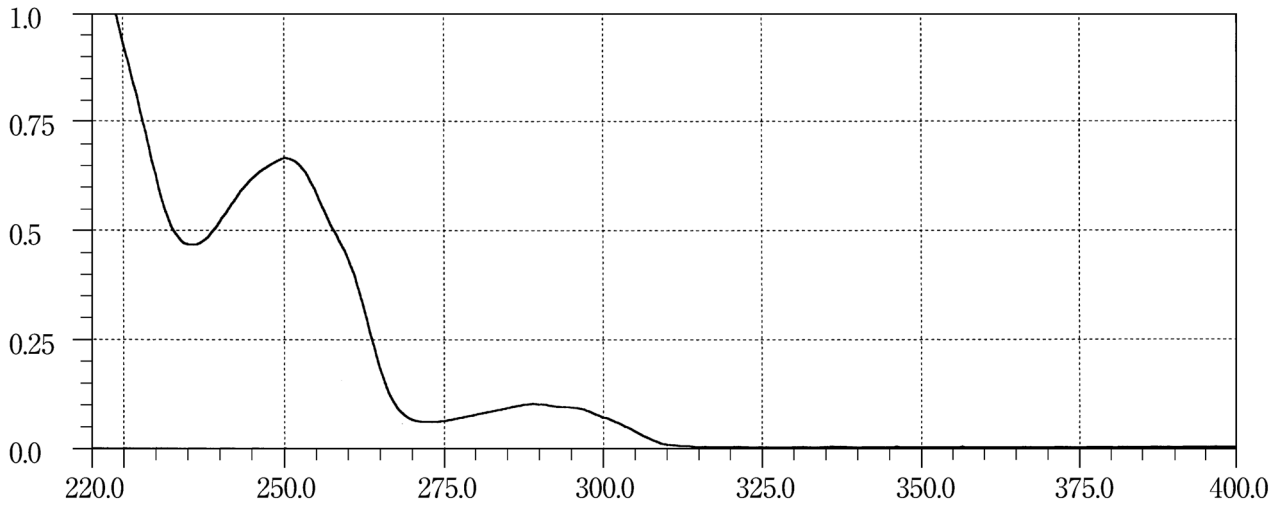
Imipramine Hydrochloride



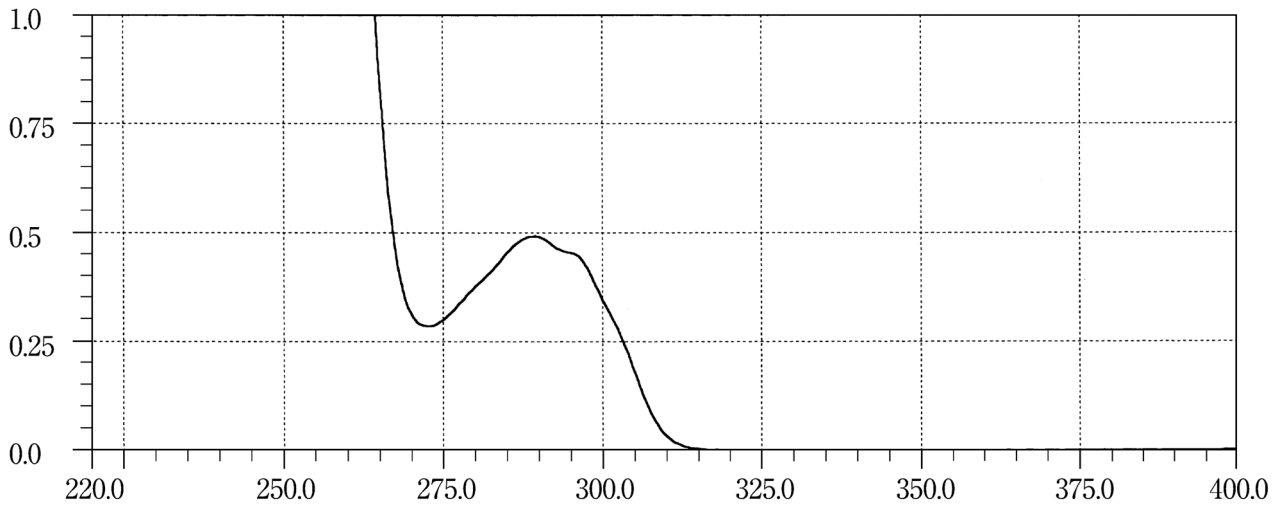
Indapamide



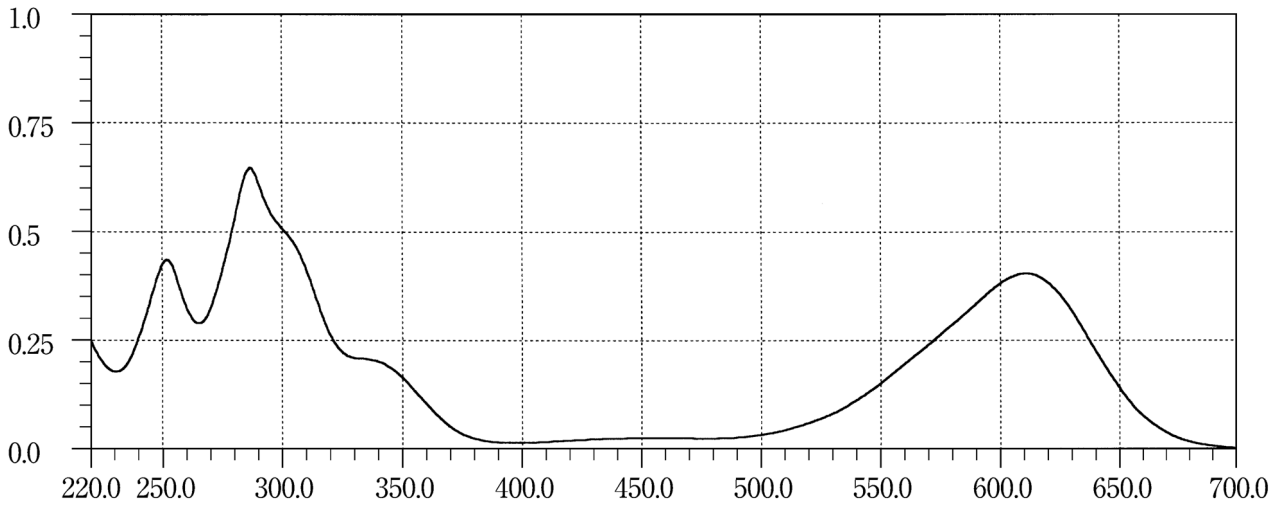
Indenolol Hydrochloride 1



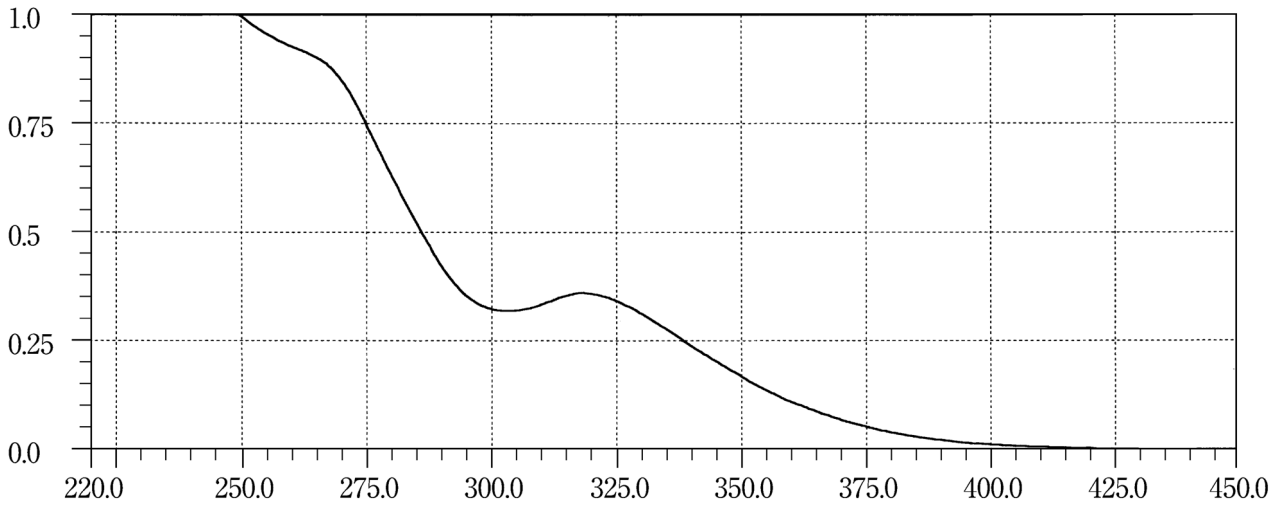
Indenolol Hydrochloride 2



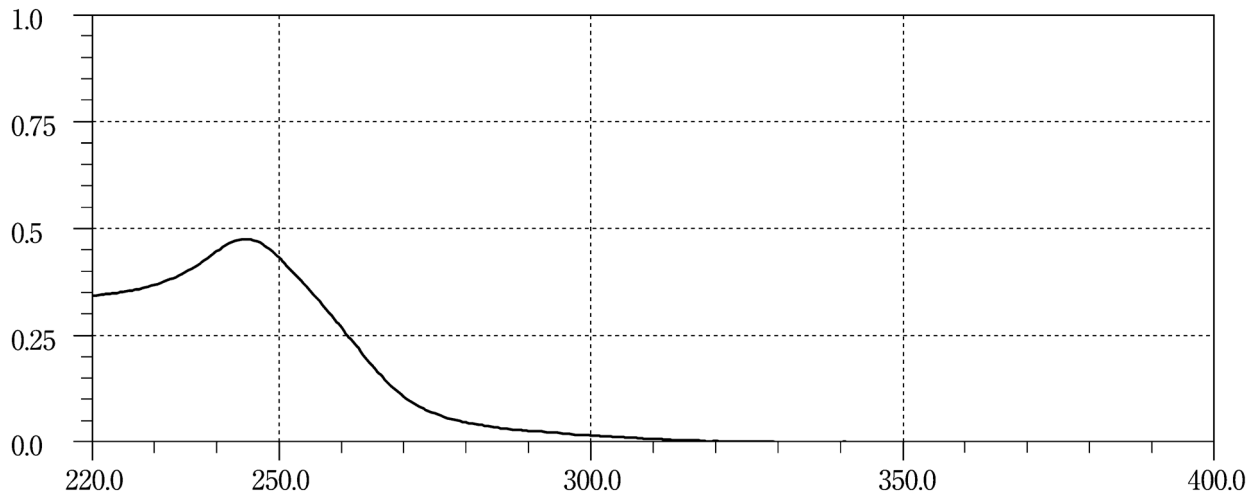
Indigocarmine



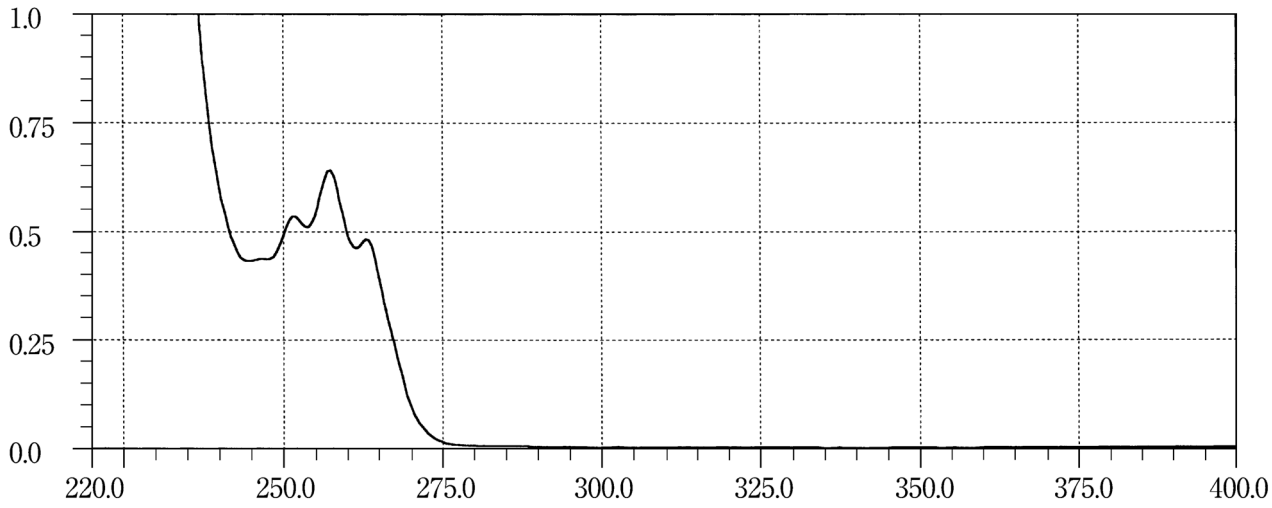
Indometacin



Iohexol



Ipratropium Bromide Hydrate



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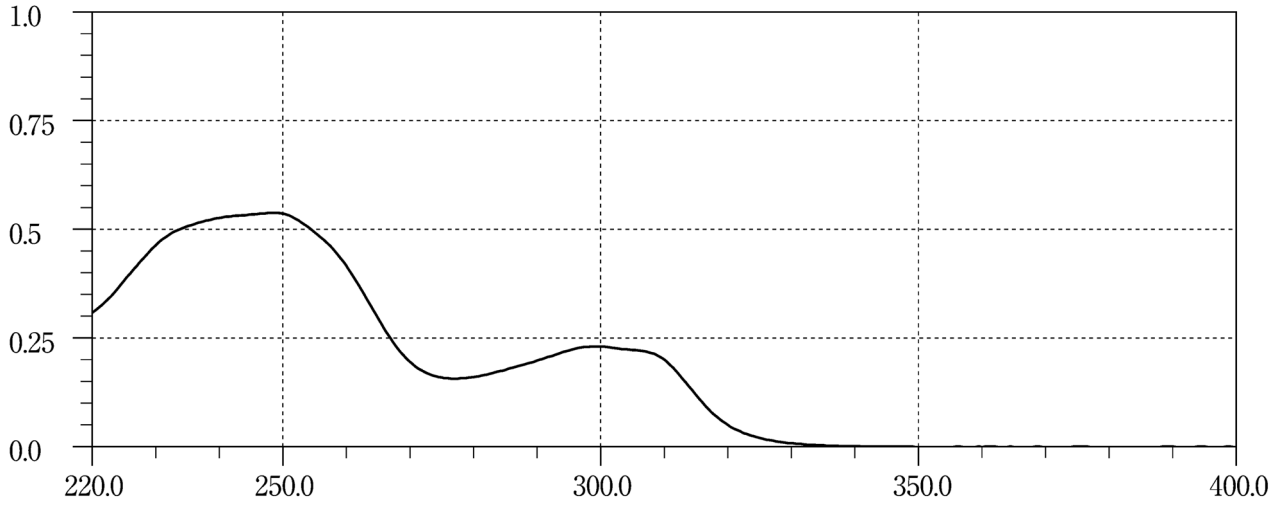

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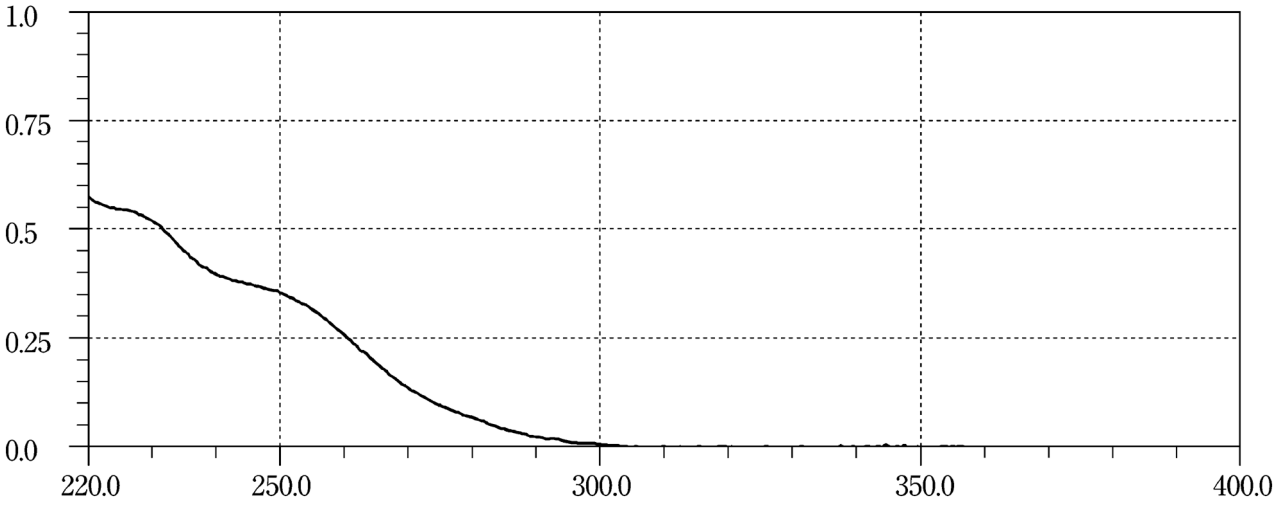

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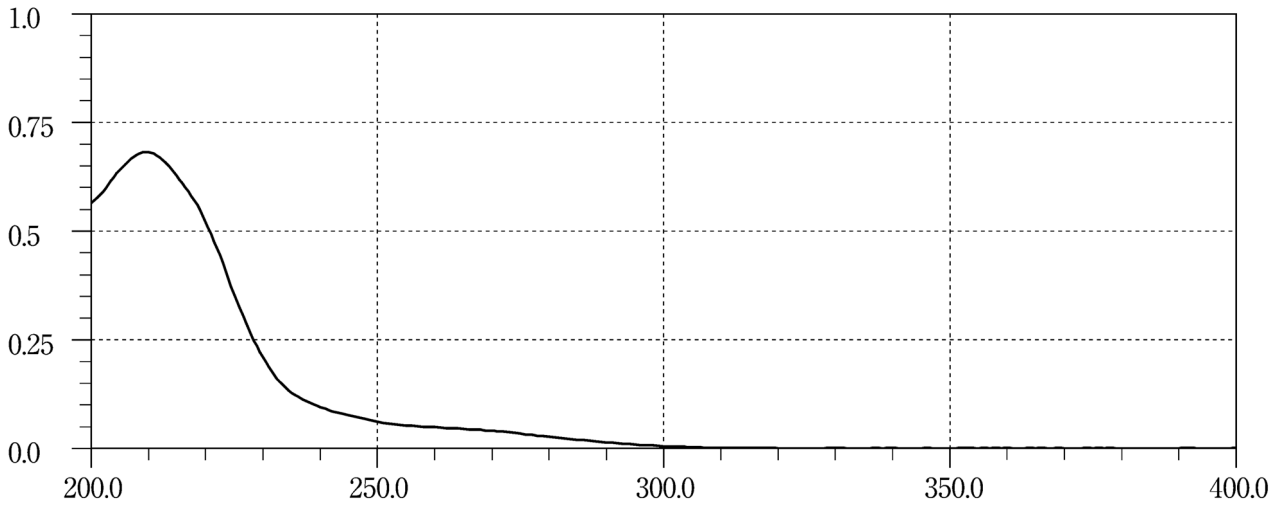

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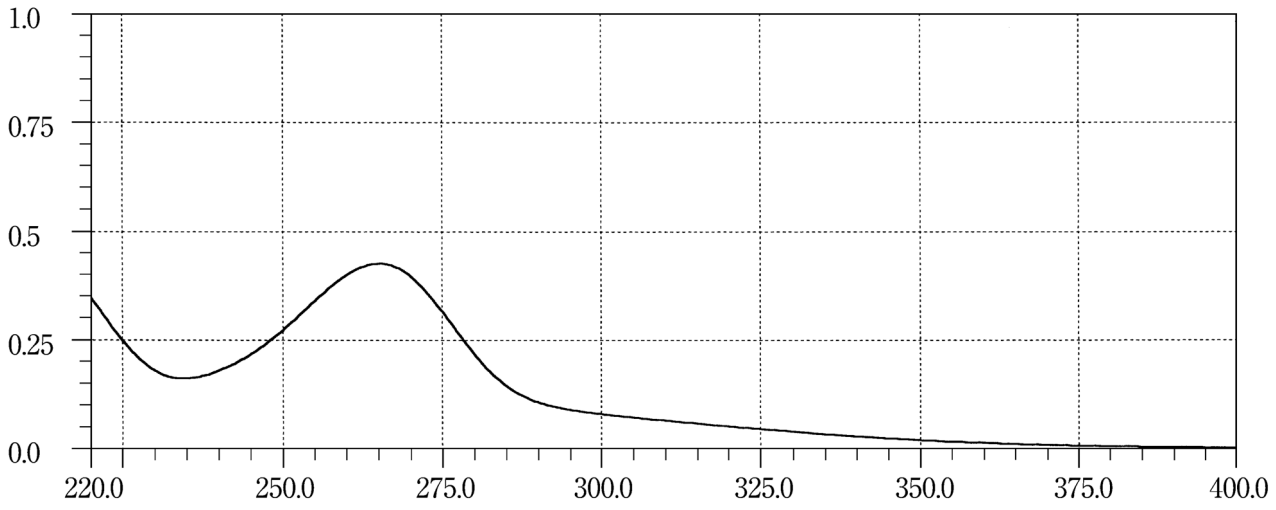

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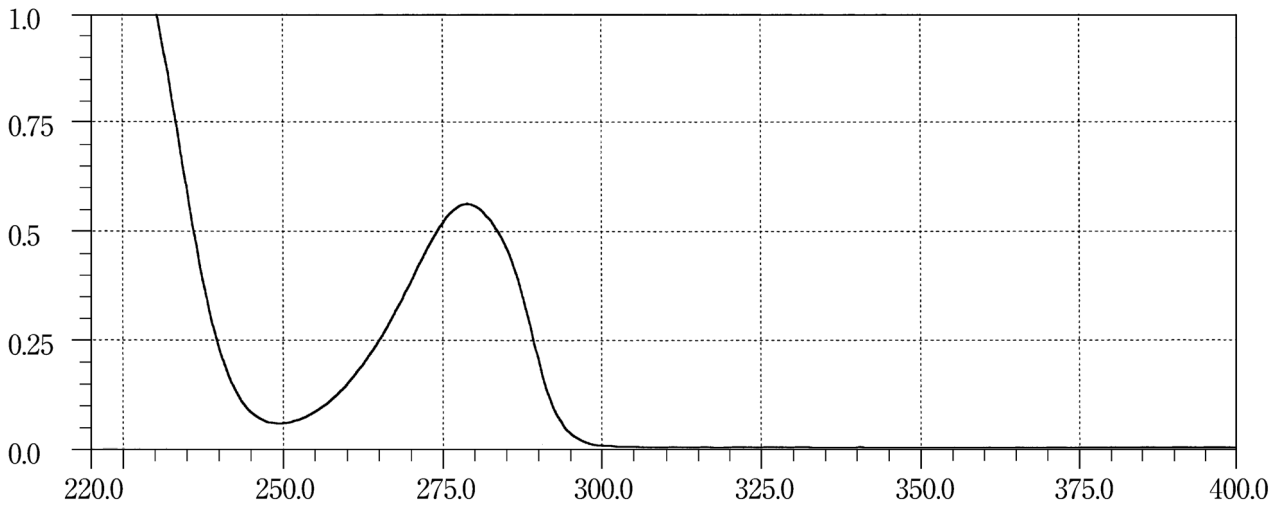
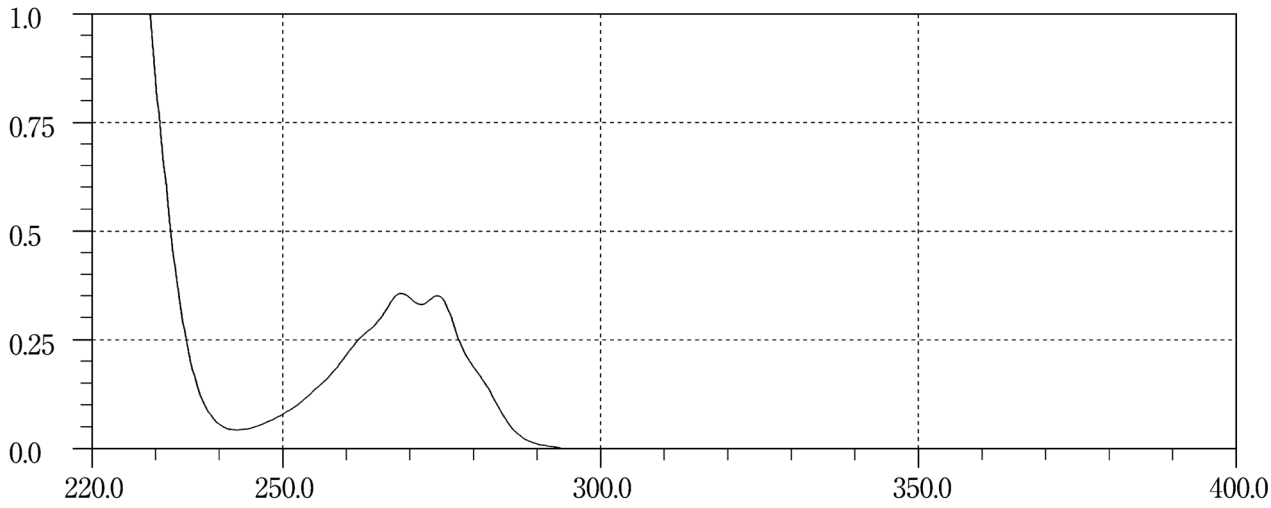


Isoxsuprine Hydrochloride



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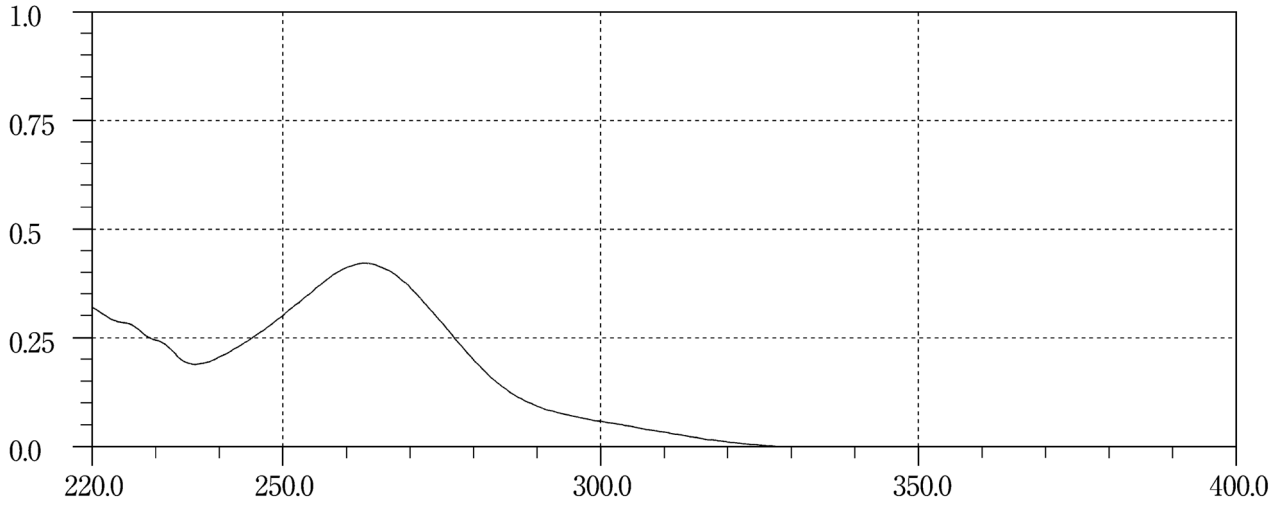

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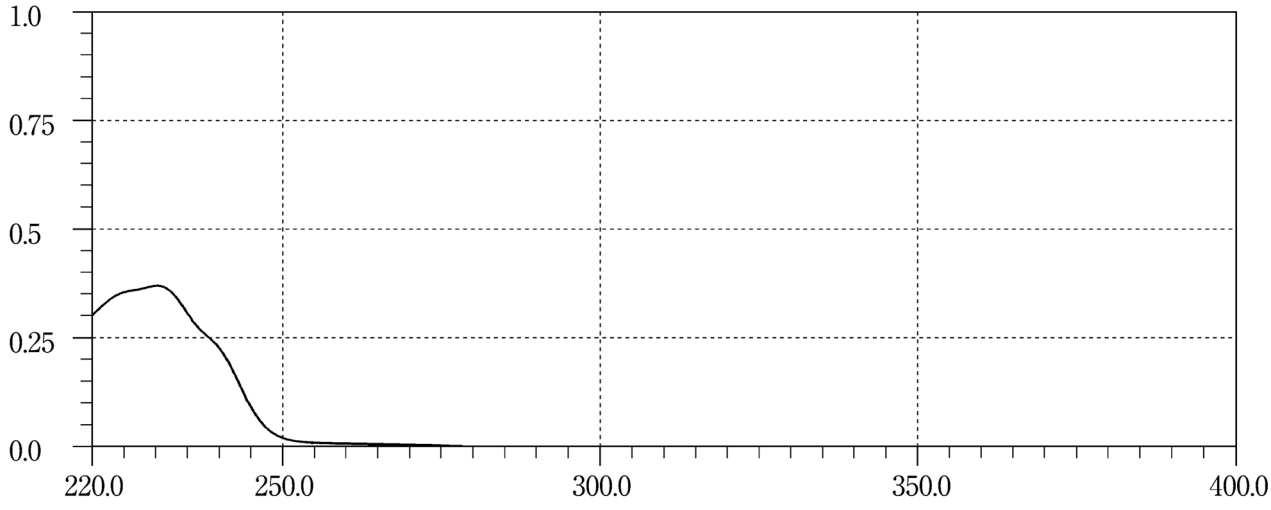
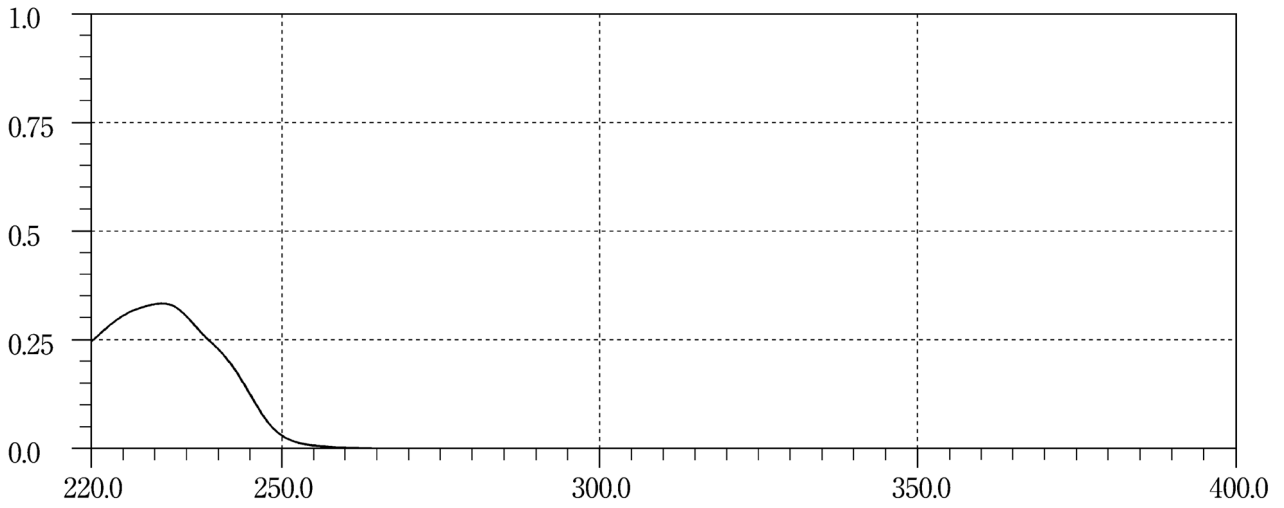
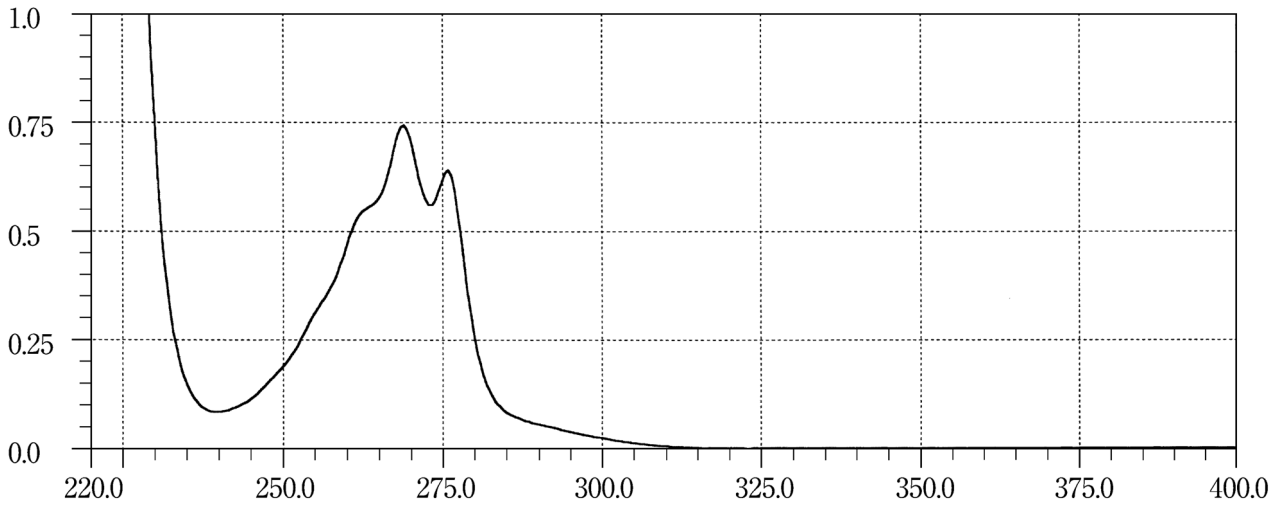


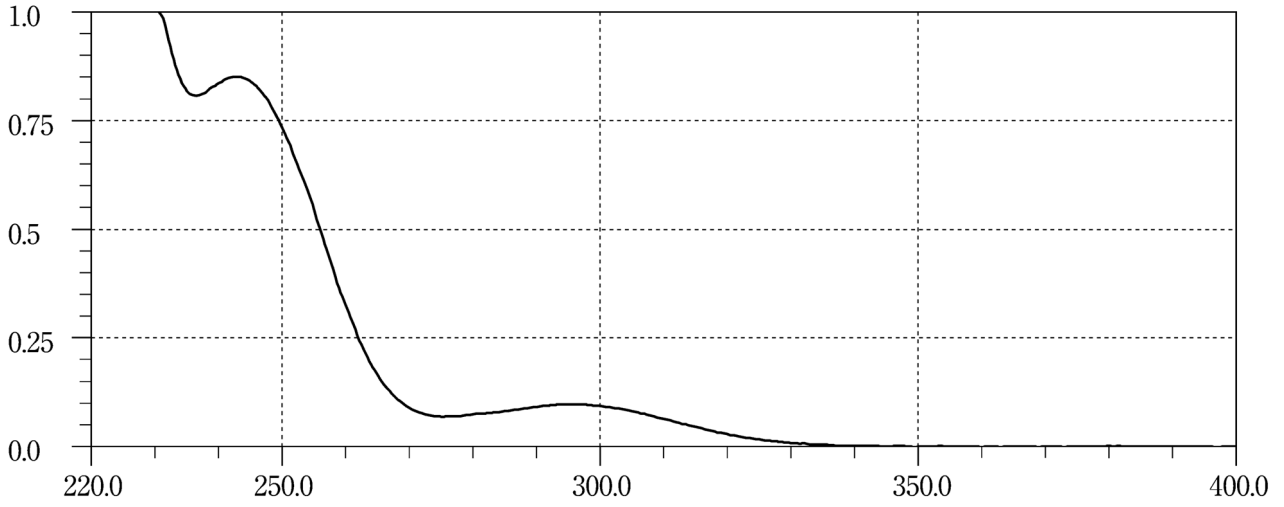
Josamycin Propionate



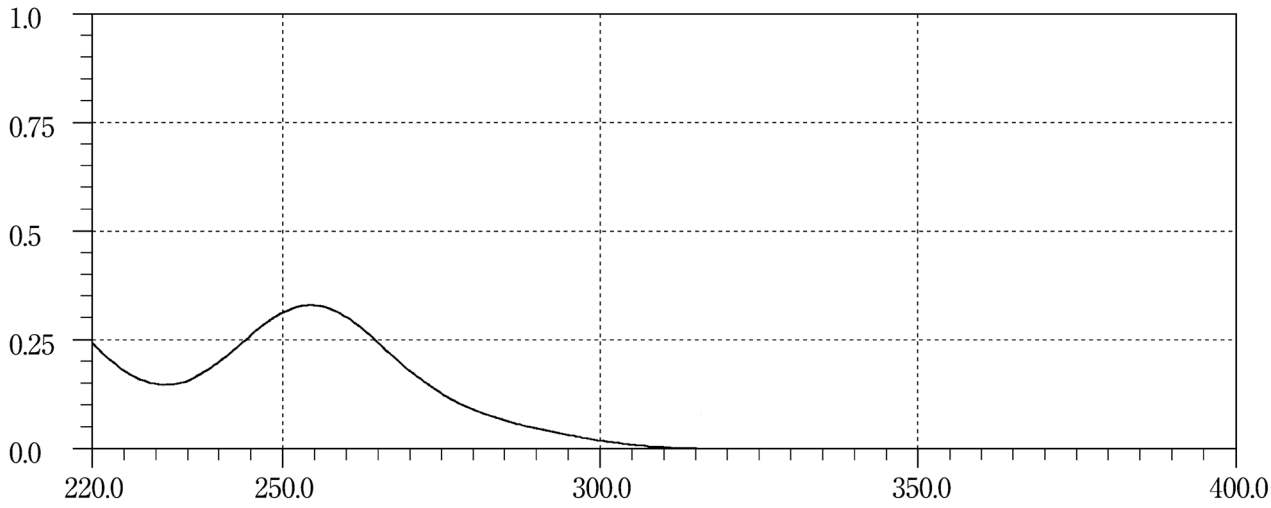
Ketamine Hydrochloride



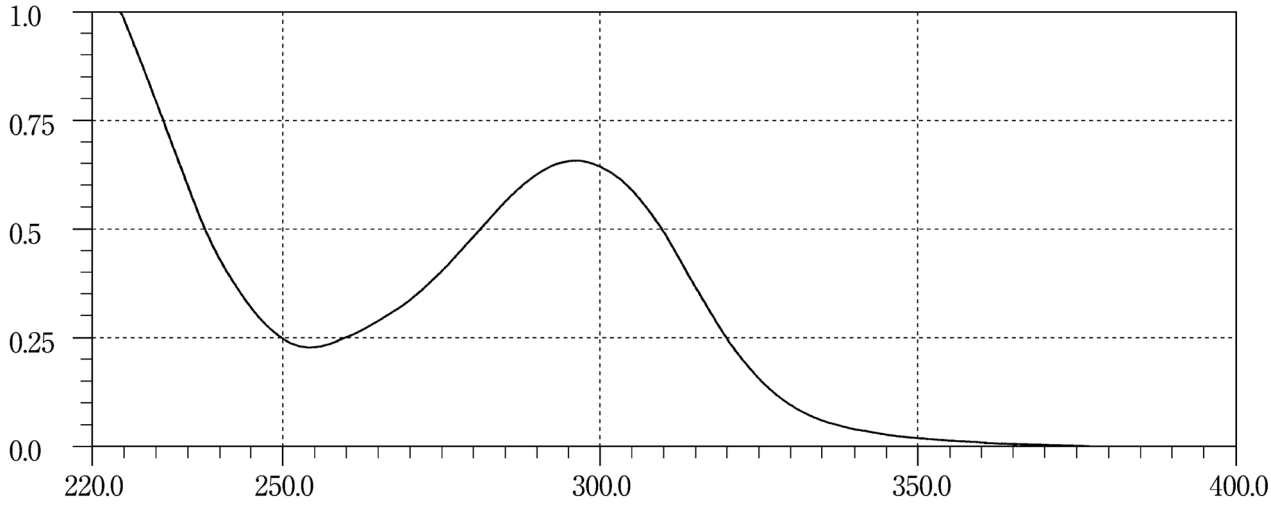
Ketoconazole



Ketoprofen



Ketotifen Fumarate



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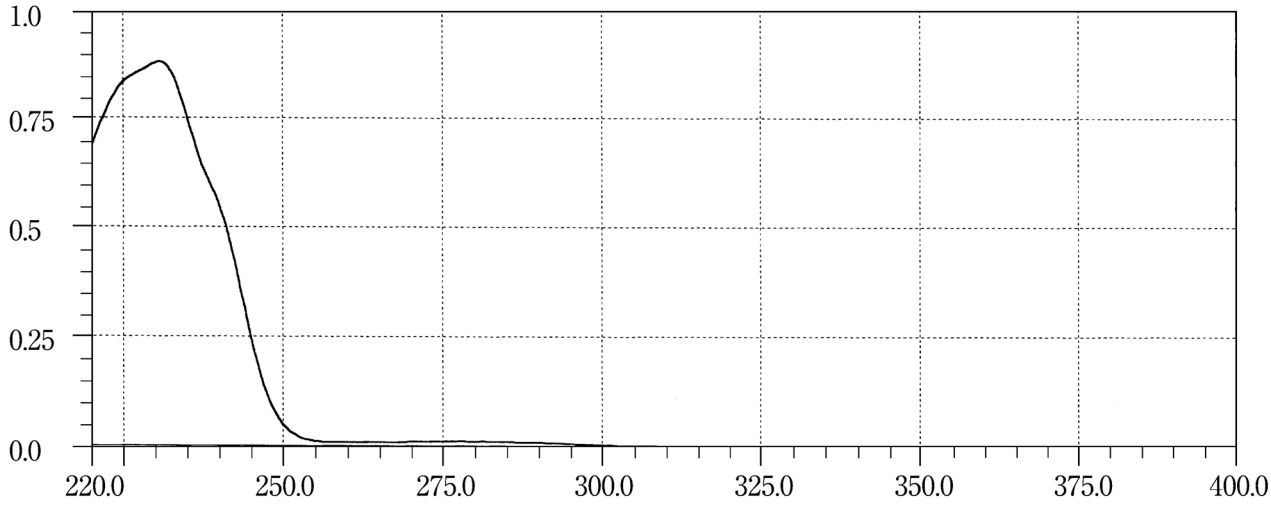
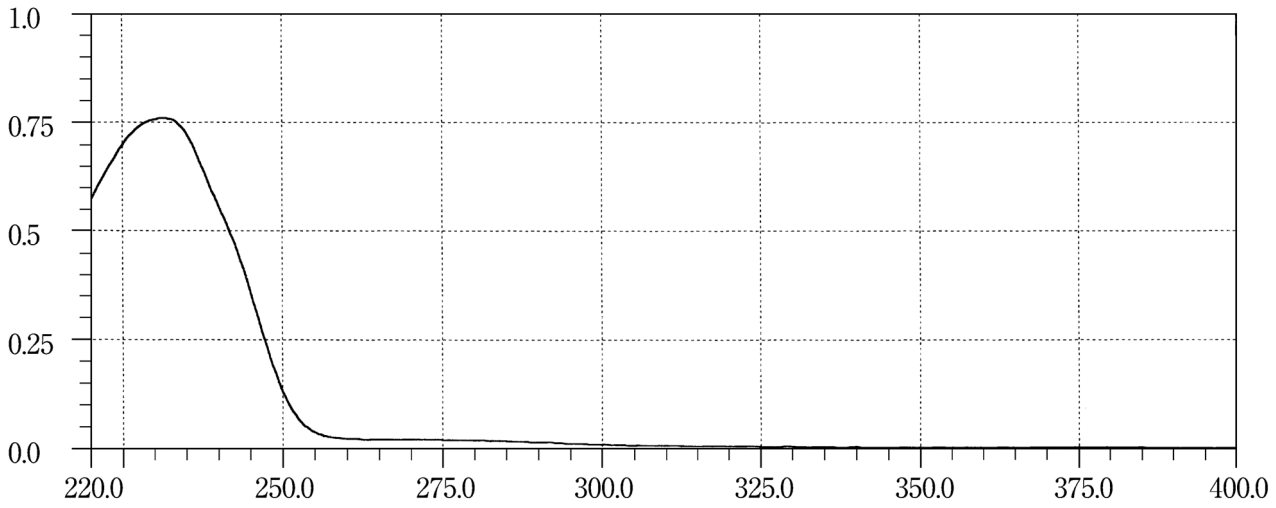
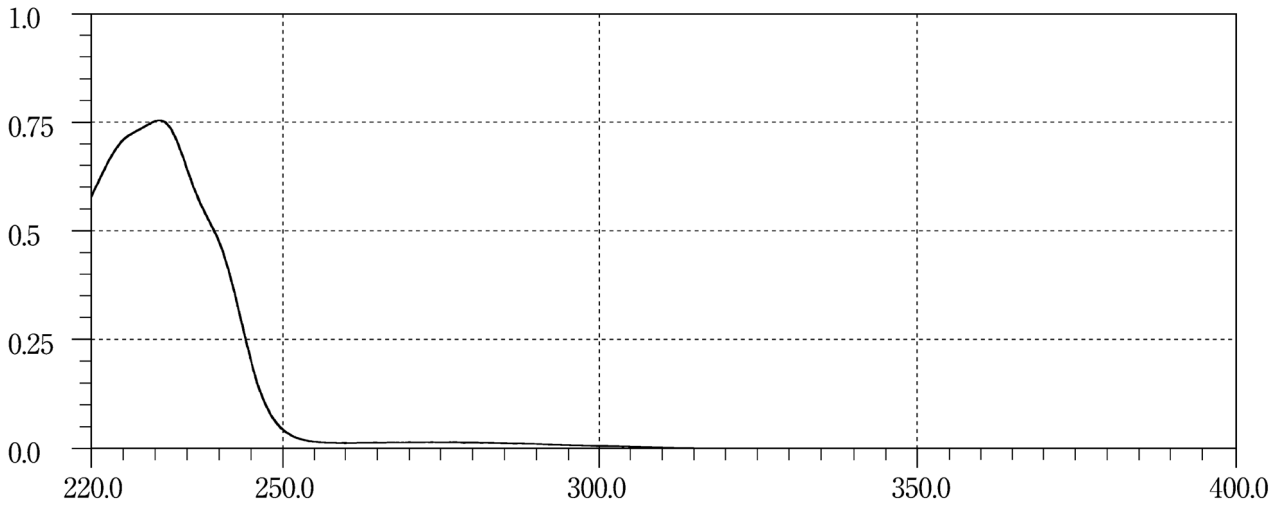


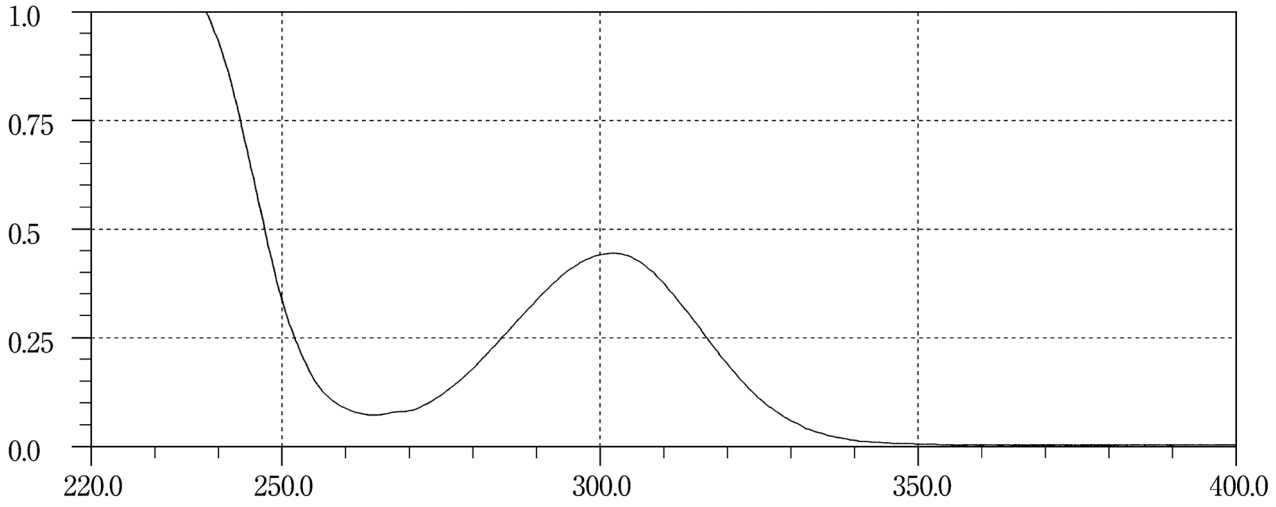
Kitasamycin Acetate



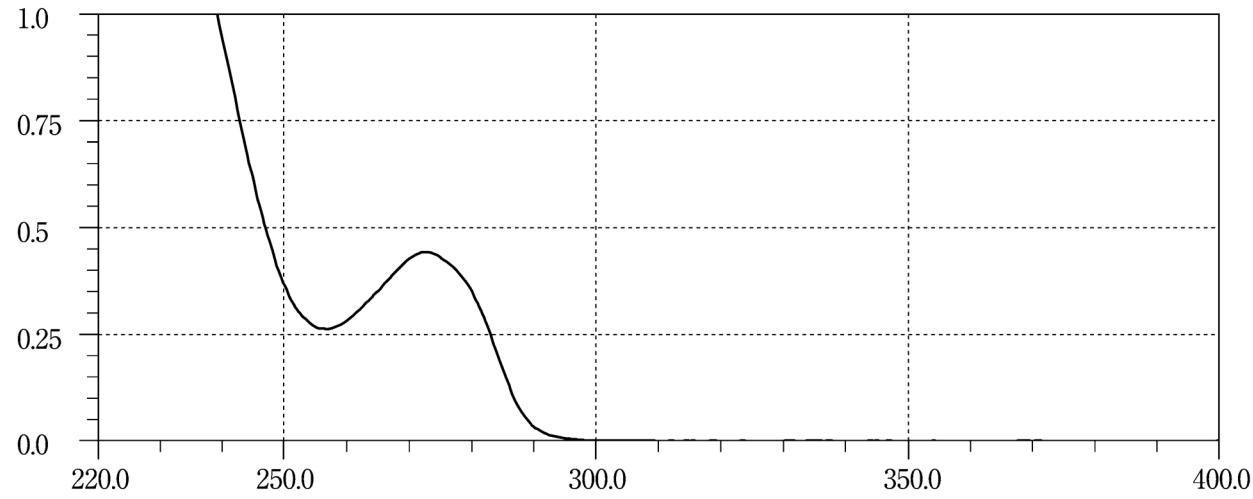
Kitasamycin Tartrate



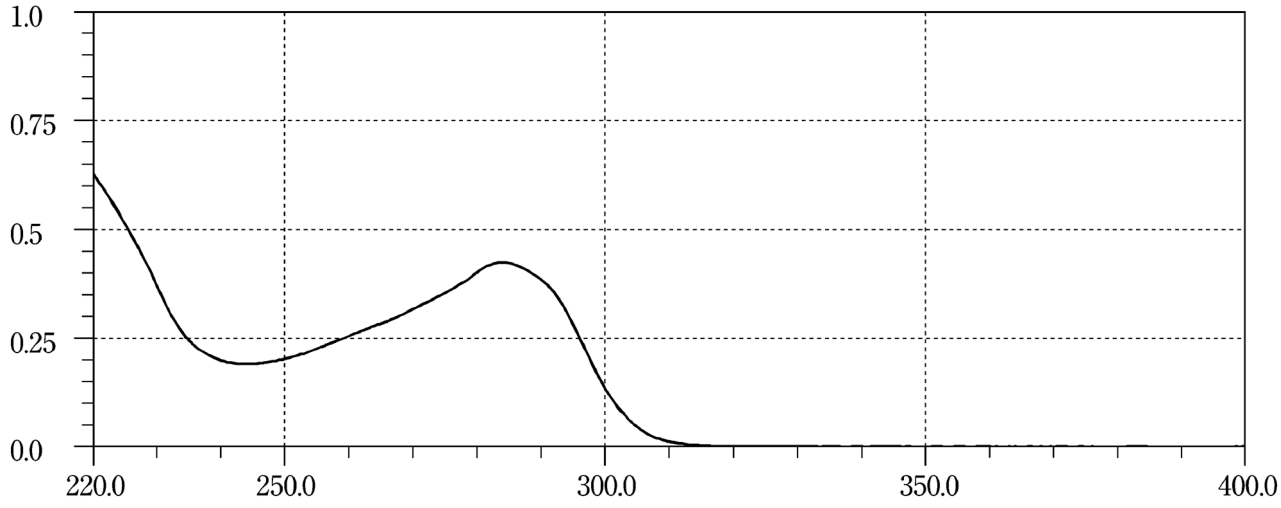
Labetalol Hydrochloride



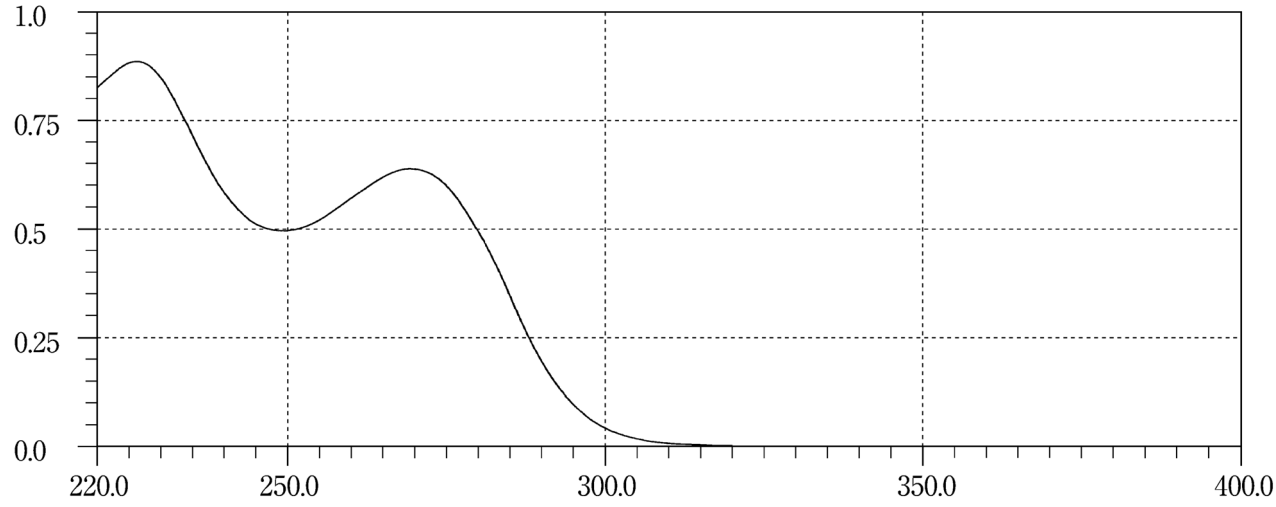
Lafutidine



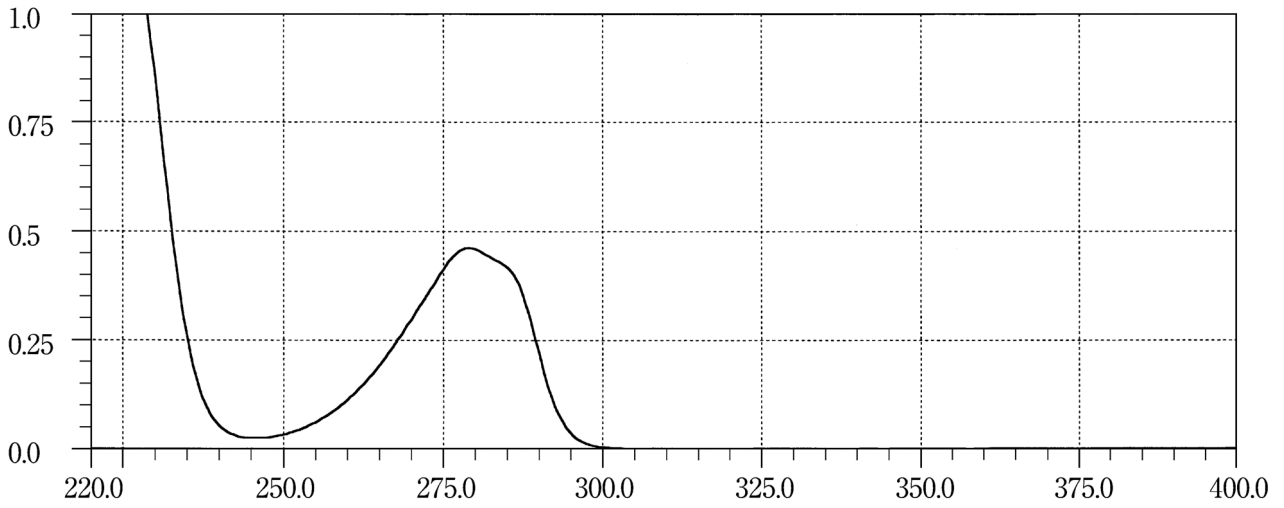
Lansoprazole



Latamoxef Sodium



Levallorphan Tartrate



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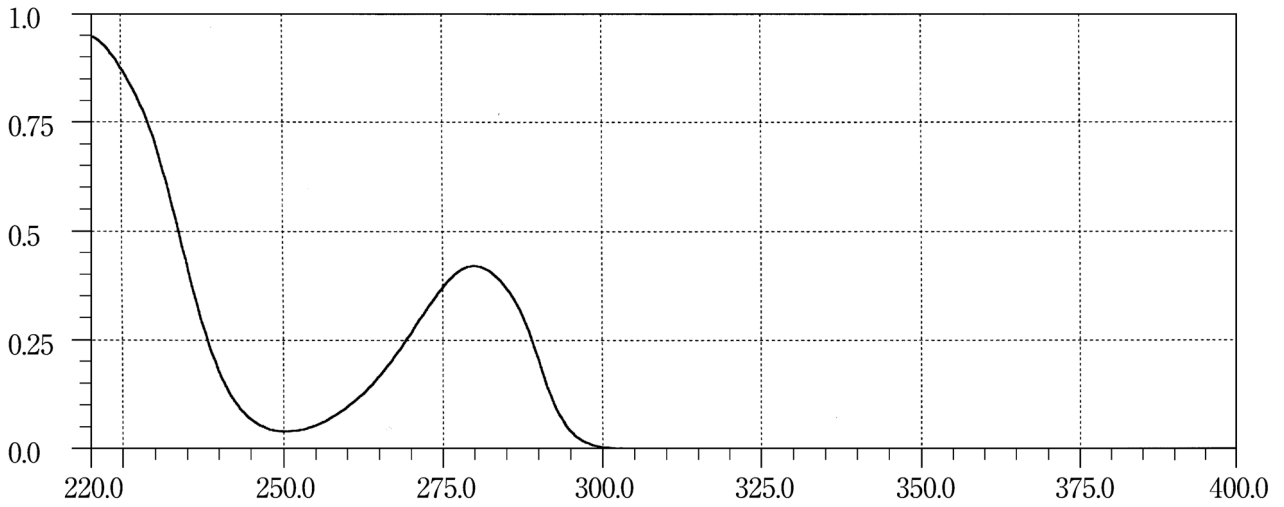
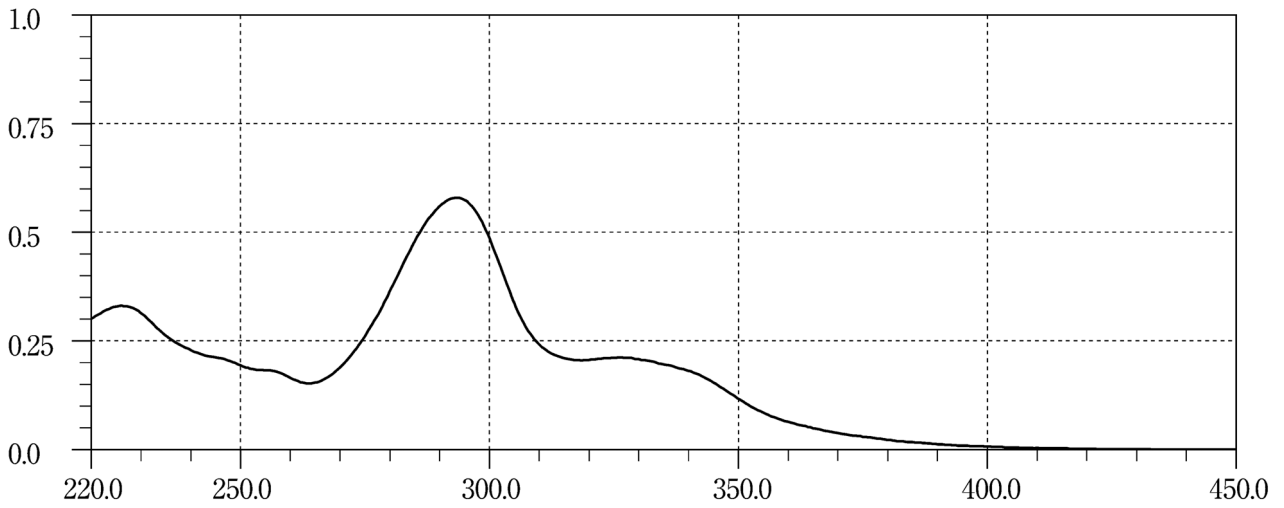
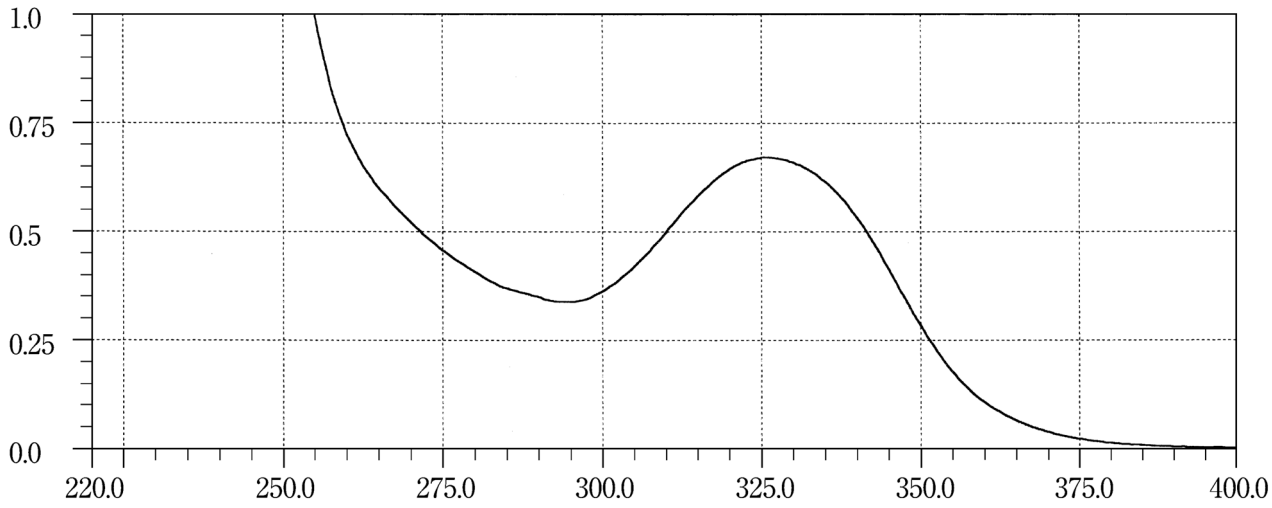


Levofloxacin Hydrate



Levothyroxine Sodium Hydrate



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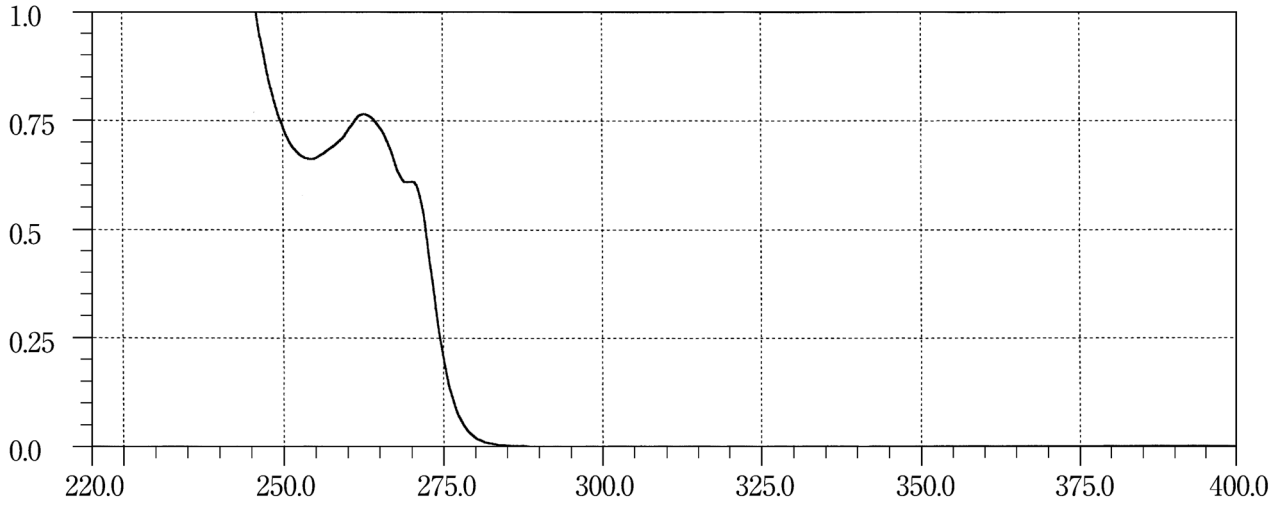

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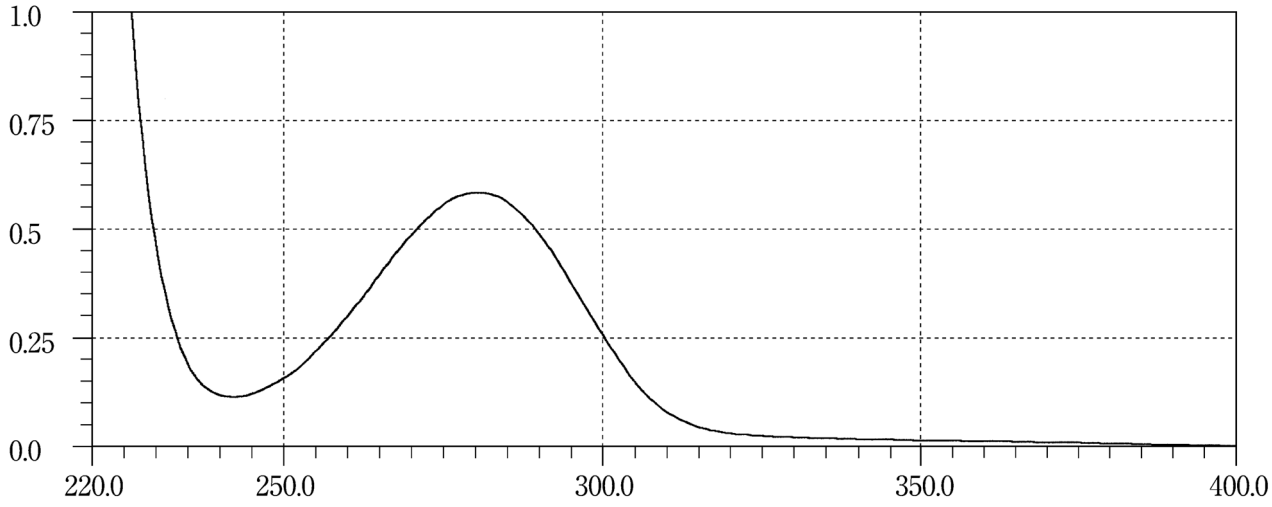
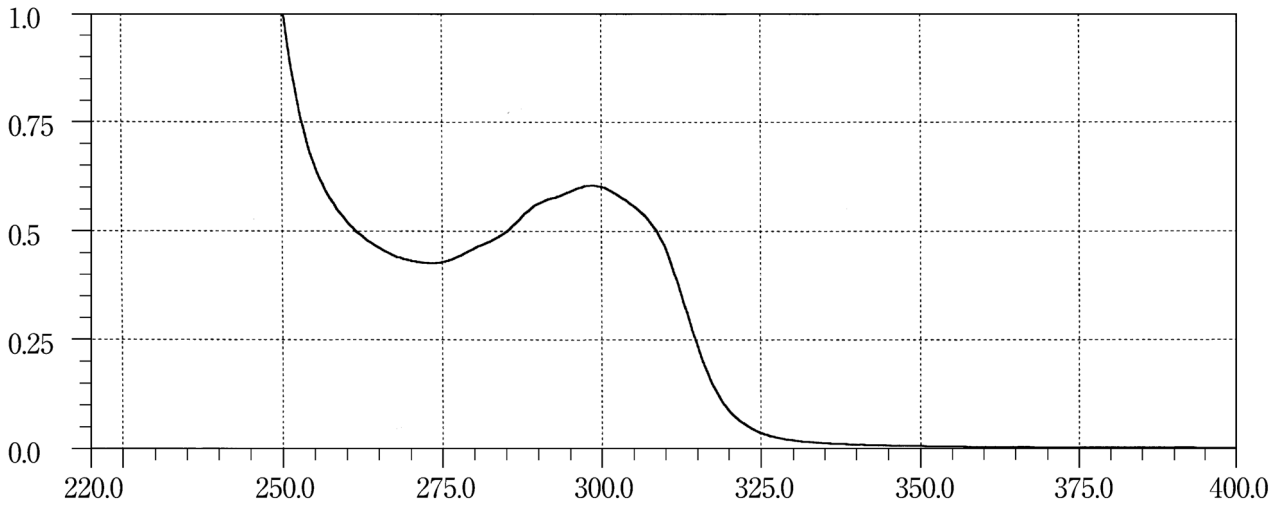
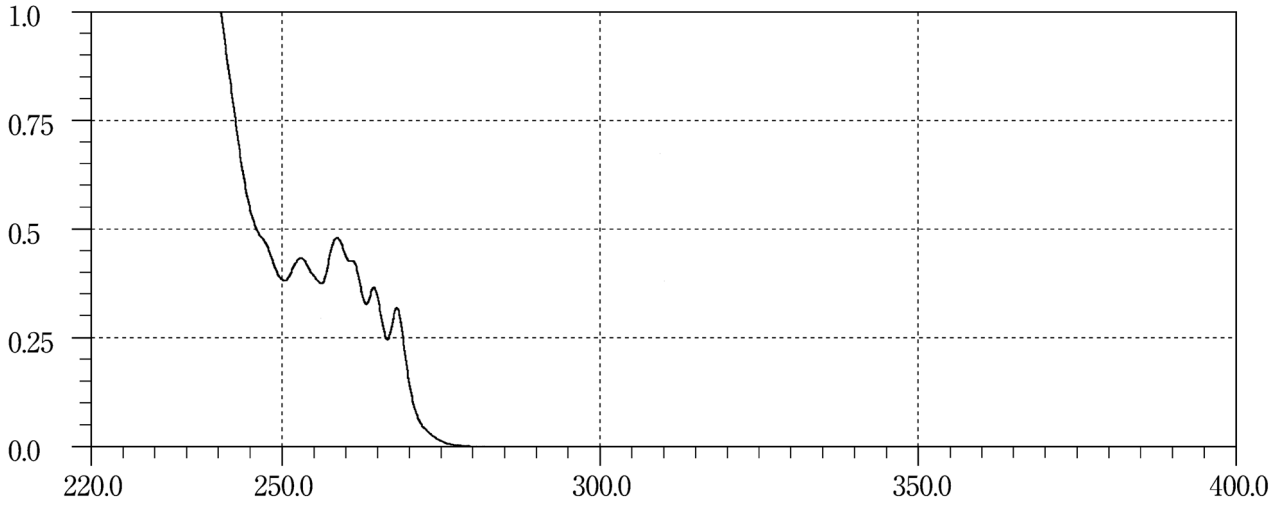


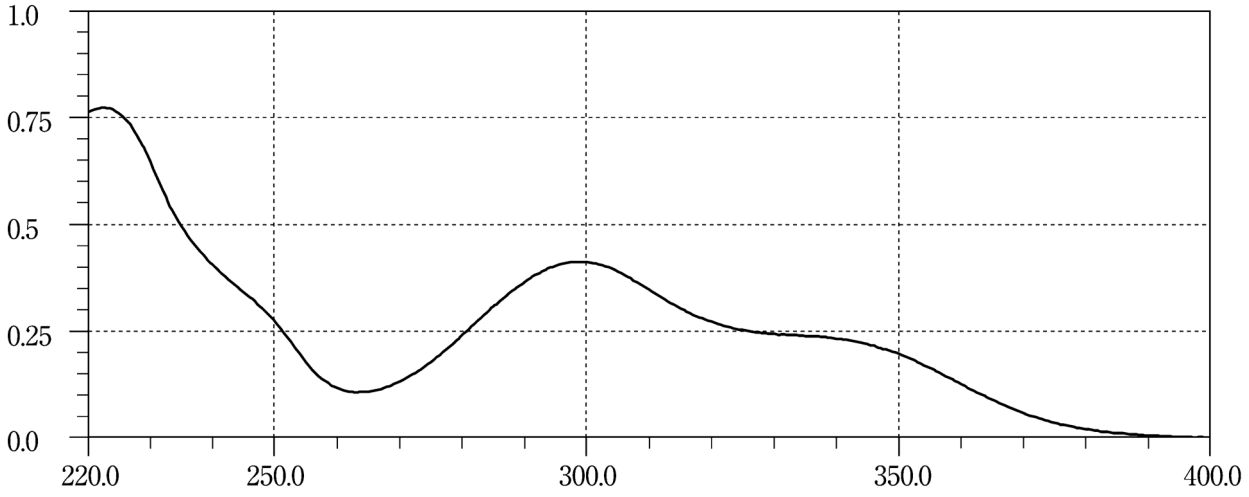
Liothyronine Sodium



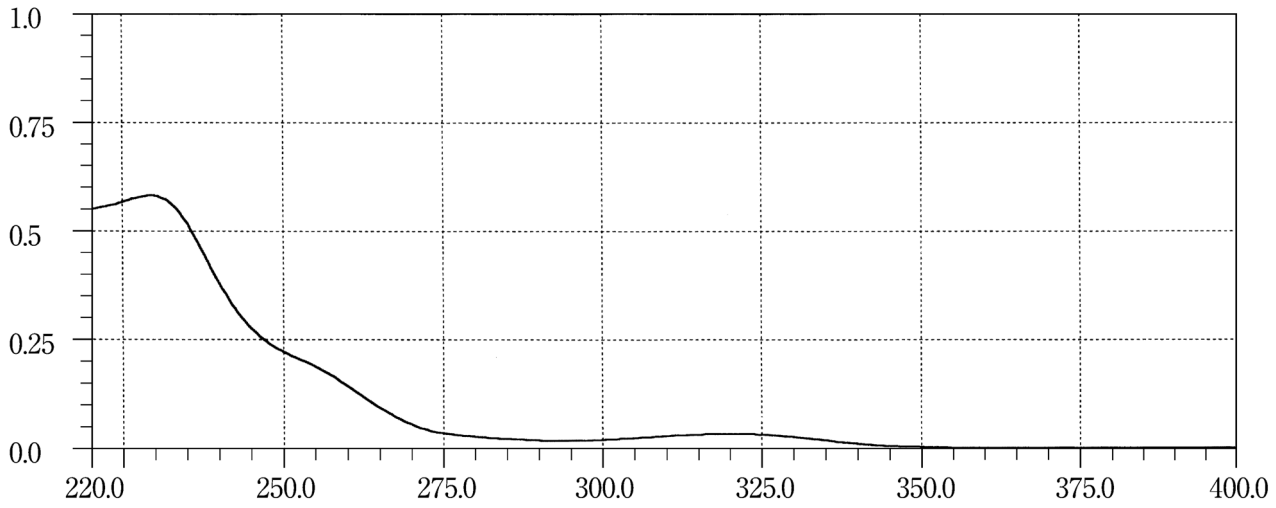
Lisinopril Hydrate



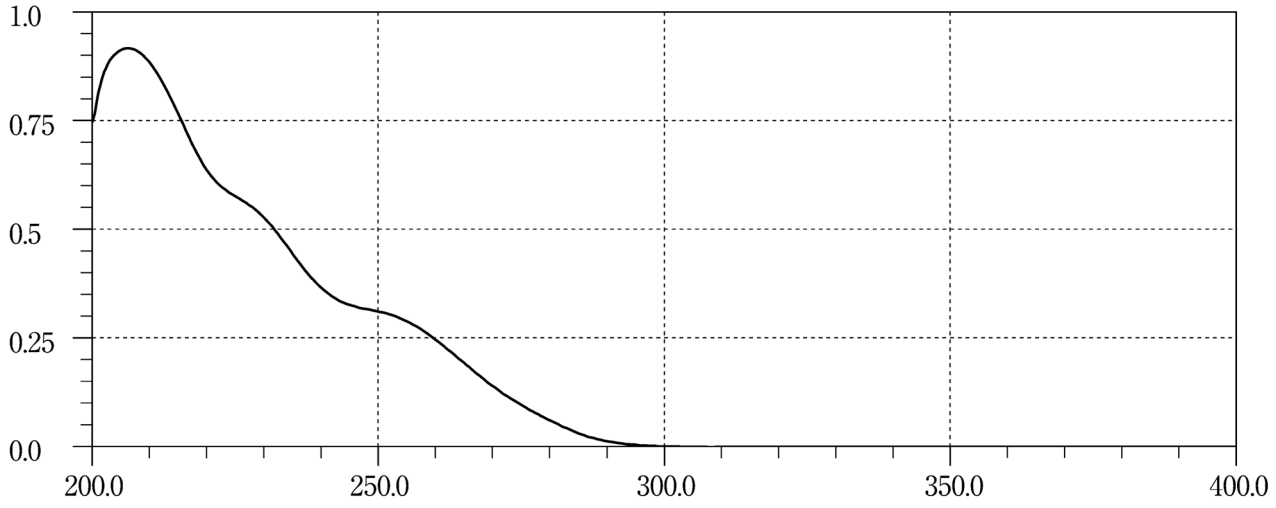
Lobenzarit Sodium



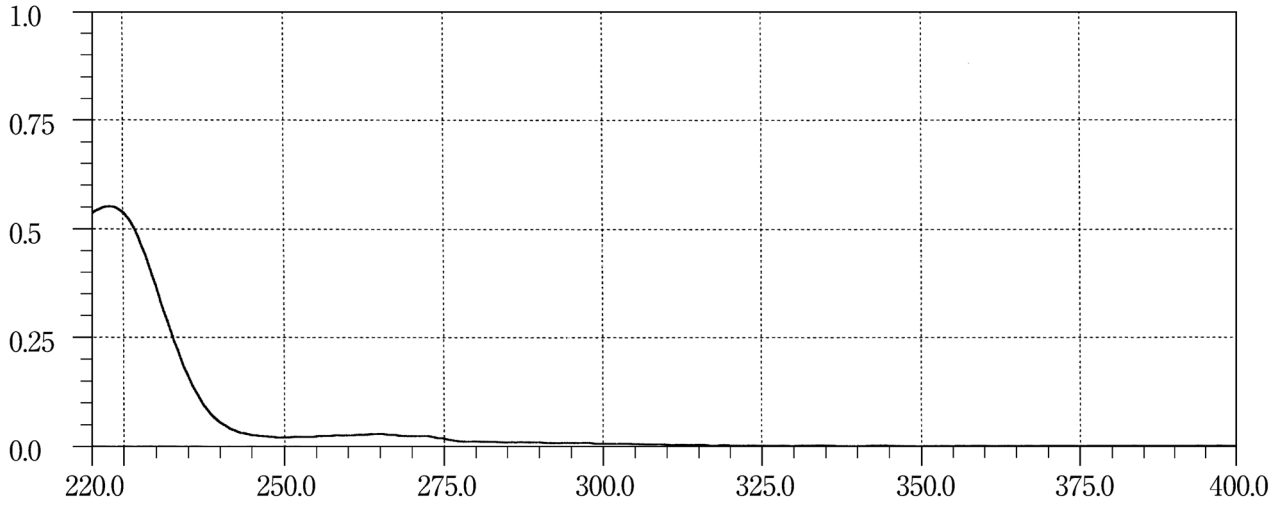
Lorazepam



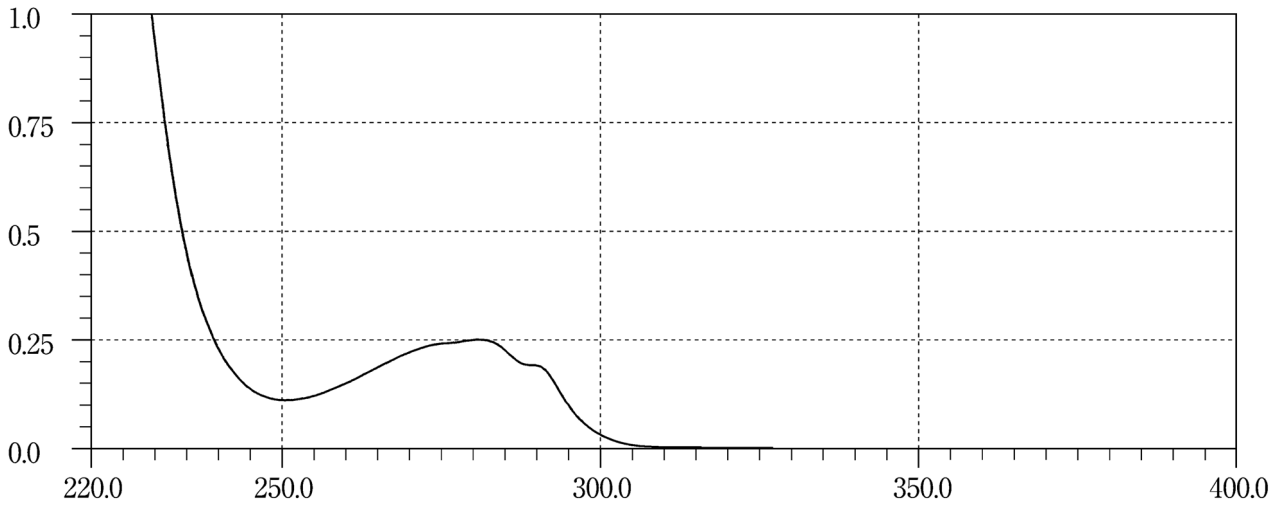
Losartan Potassium



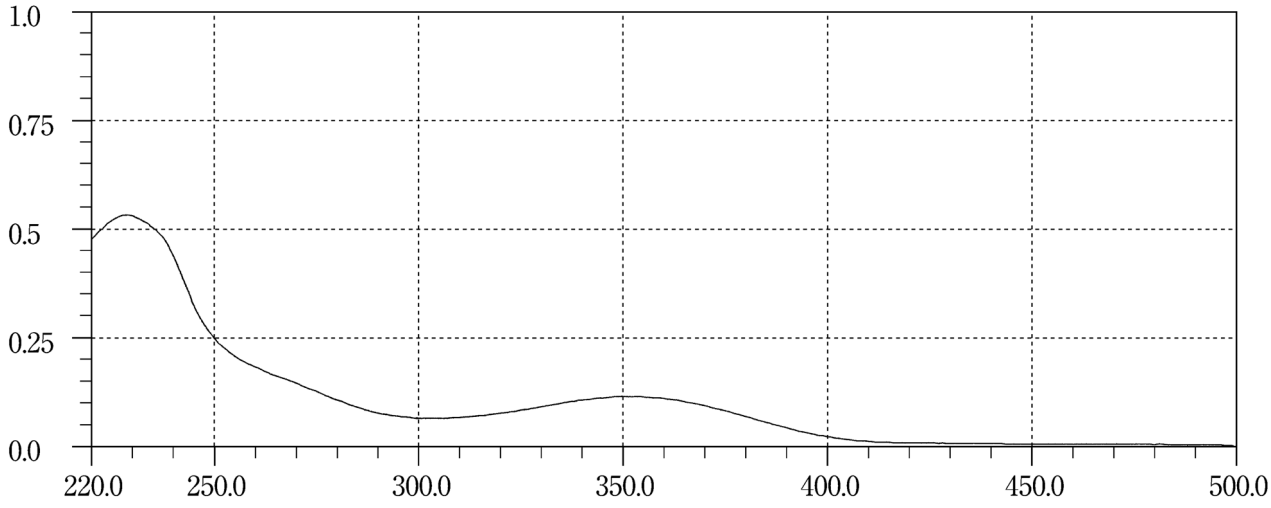
Loxoprofen Sodium Hydrate



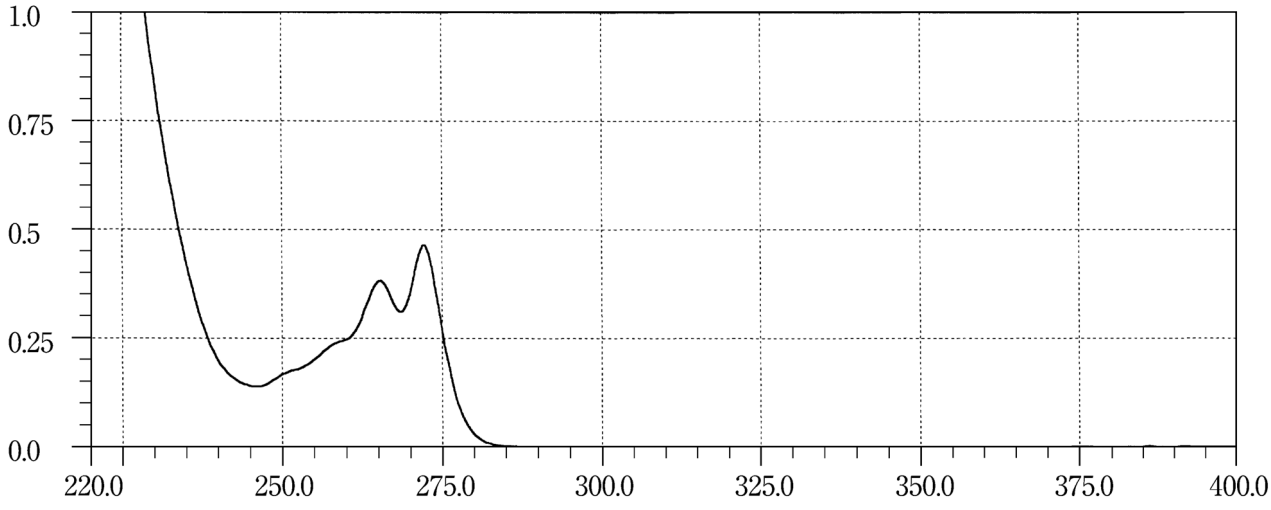
Lysozyme Hydrochloride



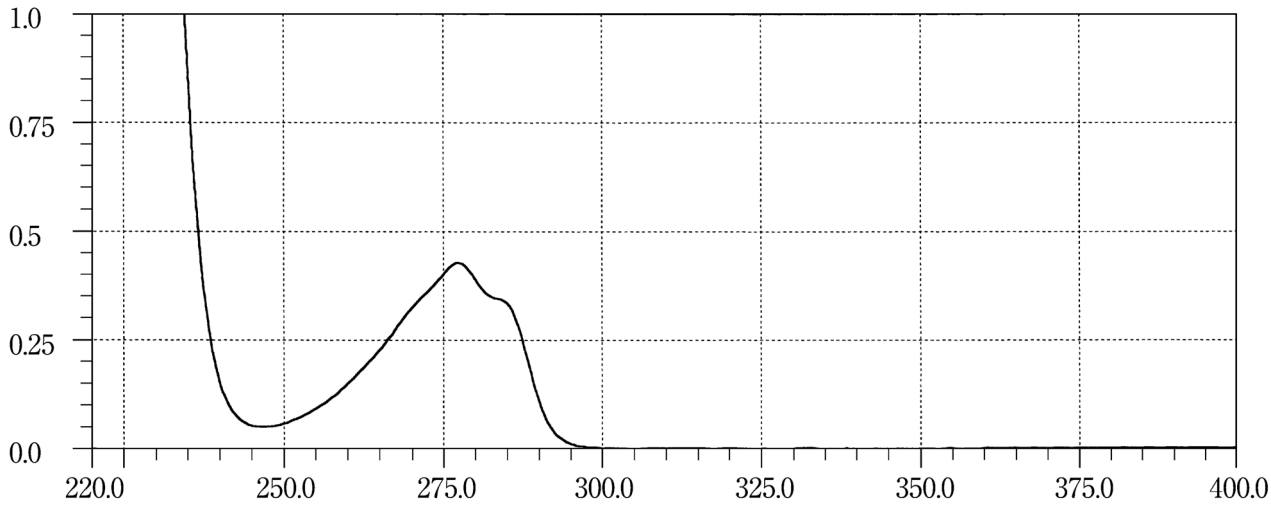
Manidipine Hydrochloride



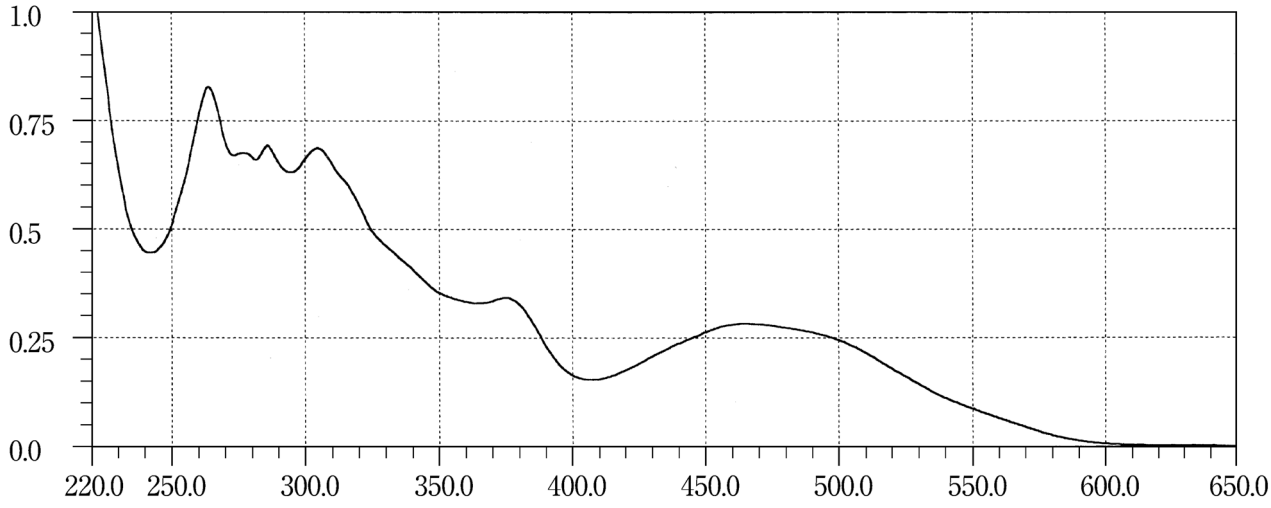
Maprotiline Hydrochloride



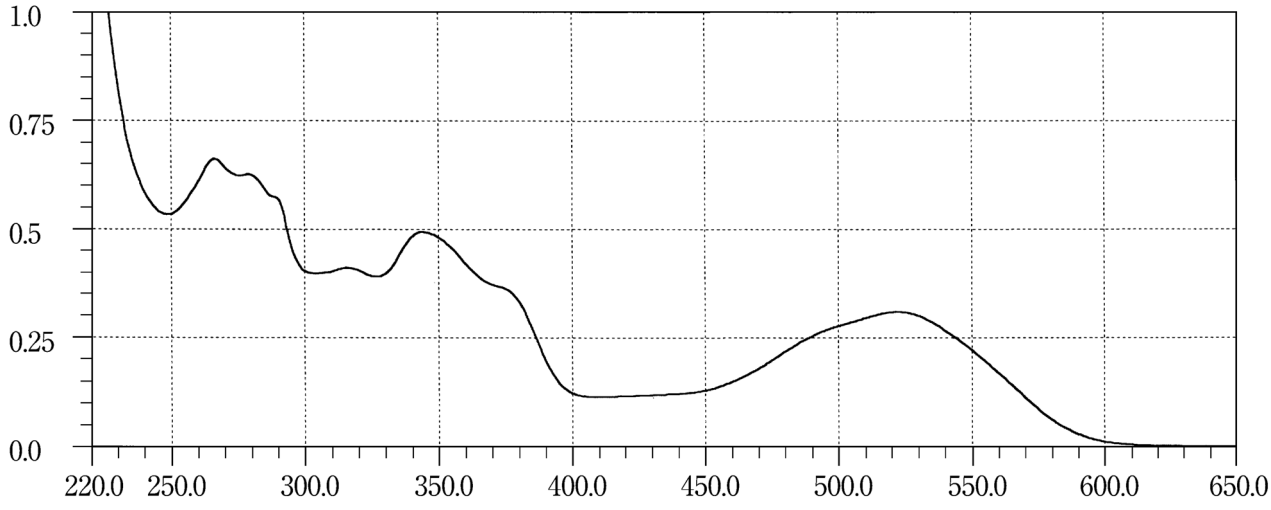
Meclofenoxate Hydrochloride



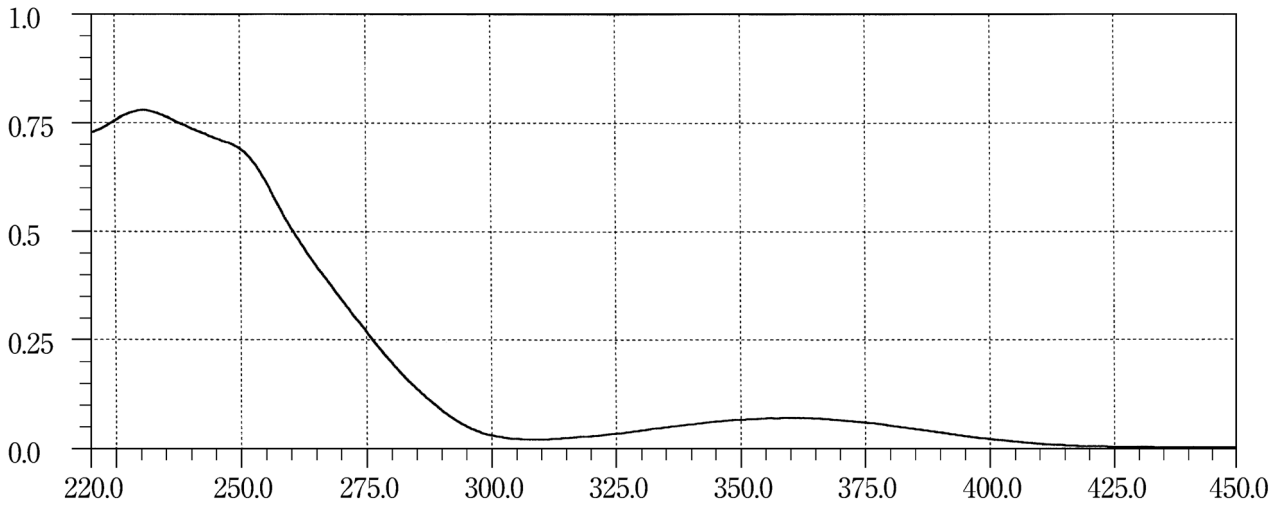
Mecobalamin 1



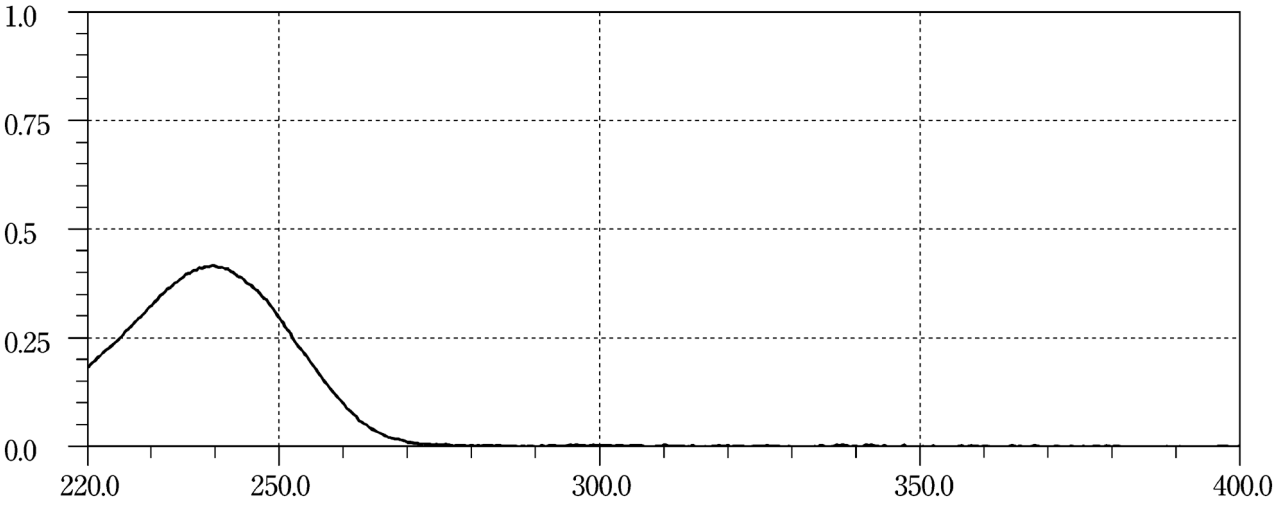
Mecobalamin 2



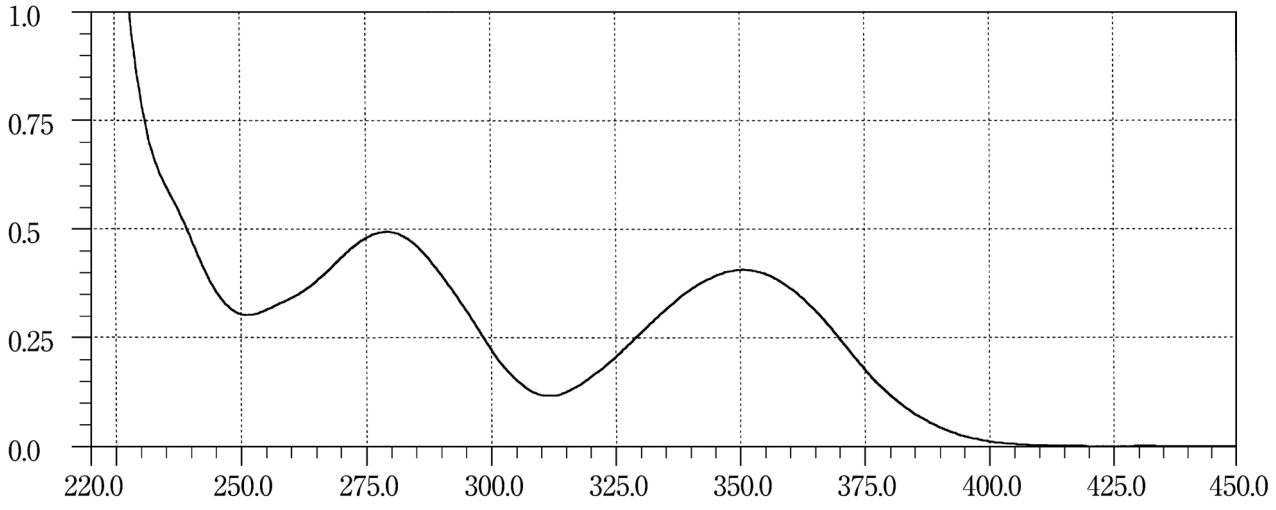
Medazepam



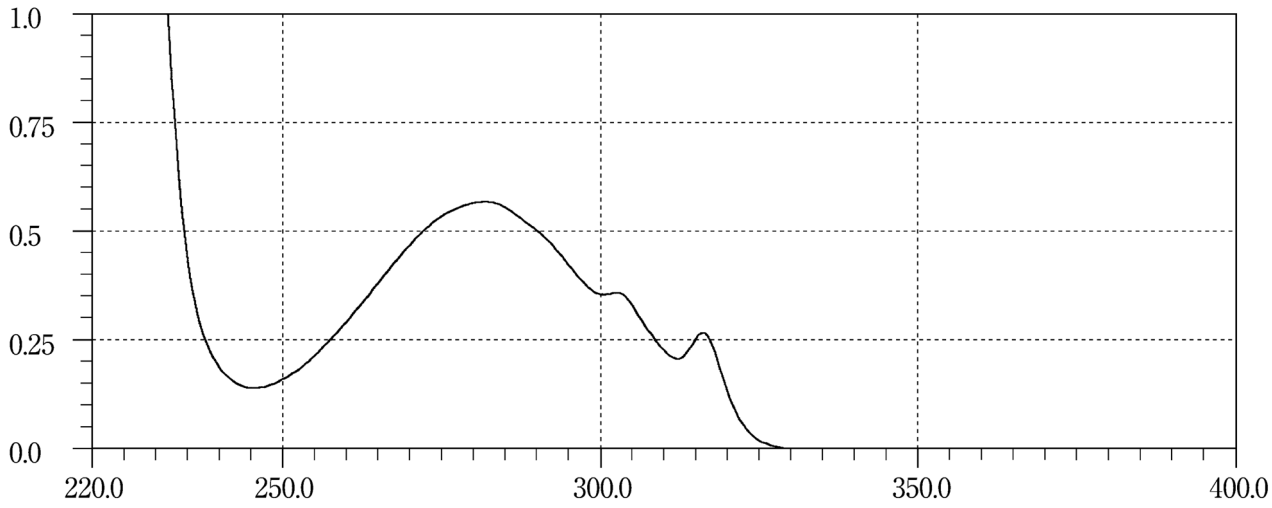
Medroxyprogesterone Acetate



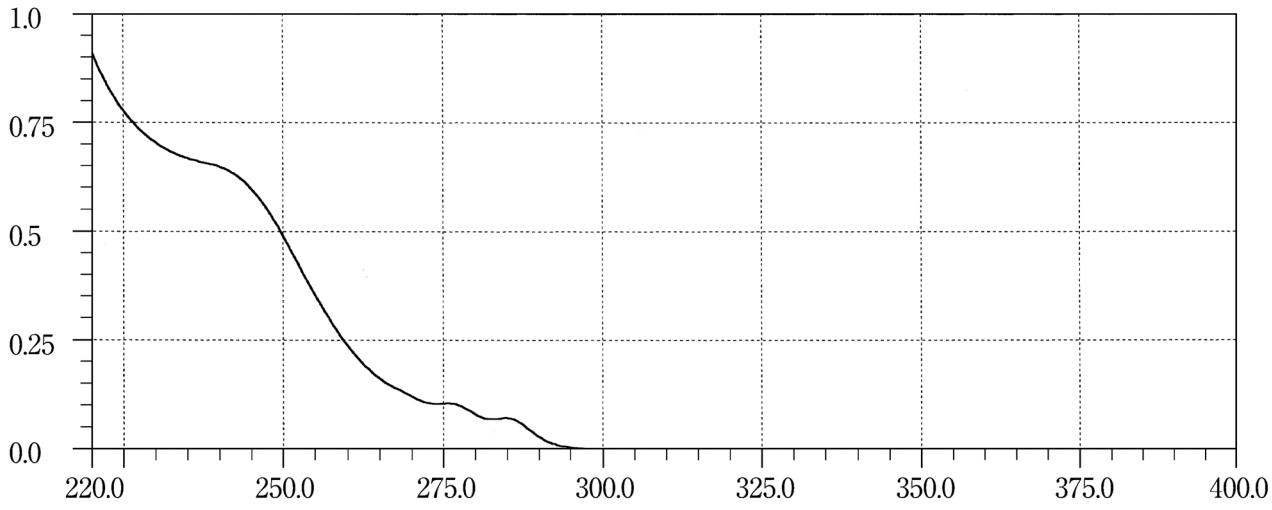
Mefenamic Acid



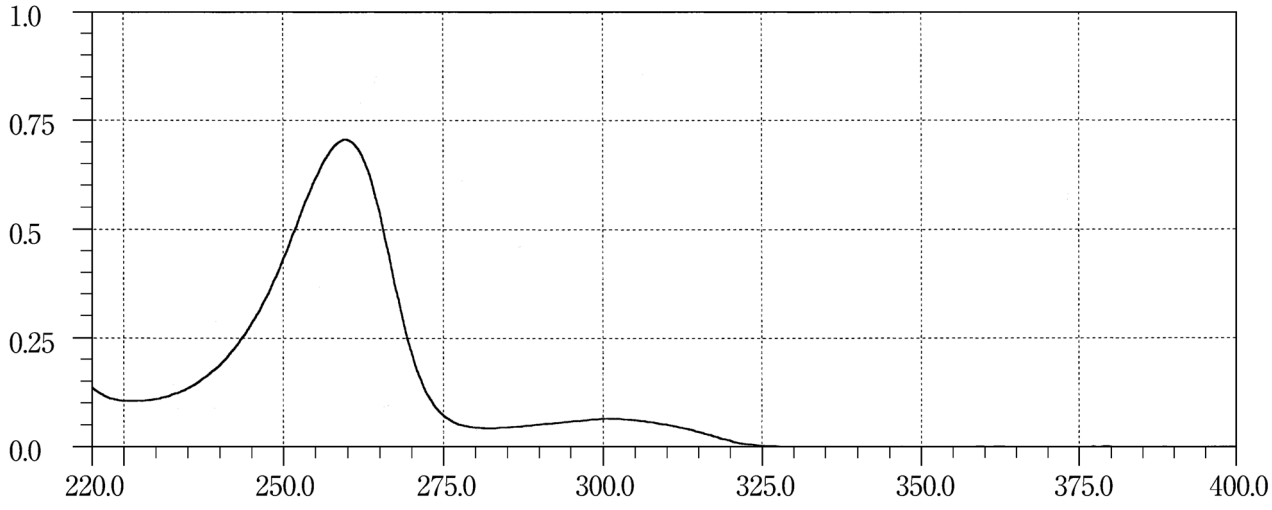
Mefloquine Hydrochloride



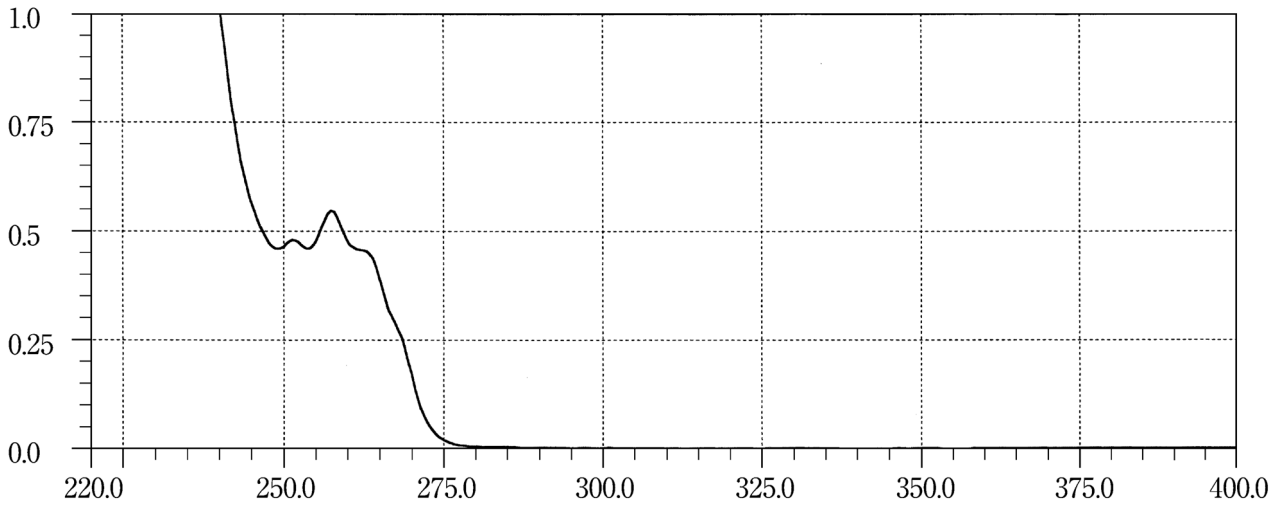
Mefruside



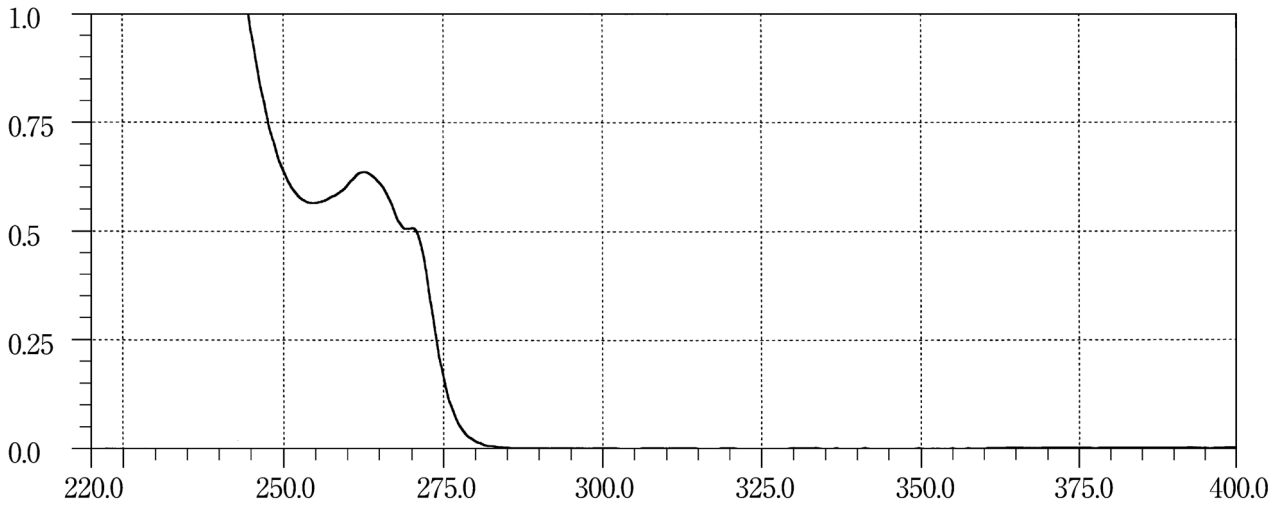
Melphalan



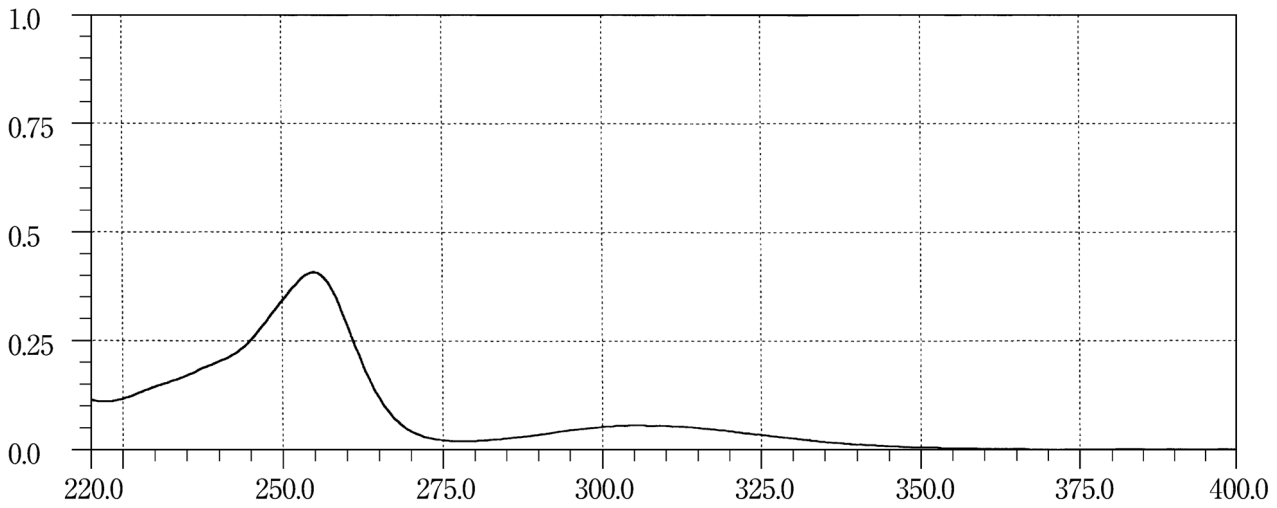
Mepenzolate Bromide



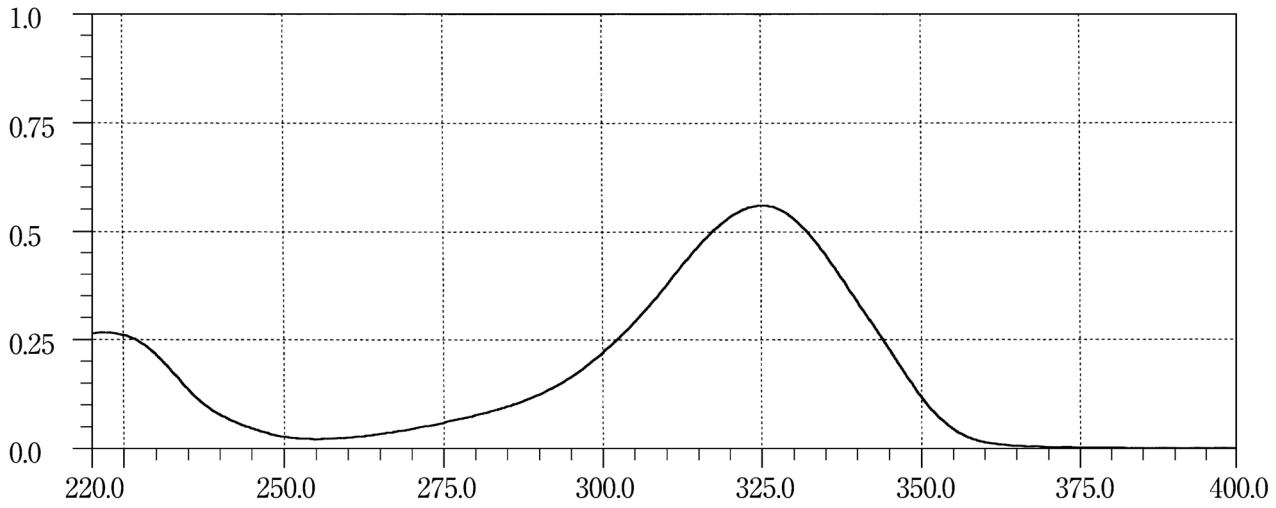
Mepivacaine Hydrochloride



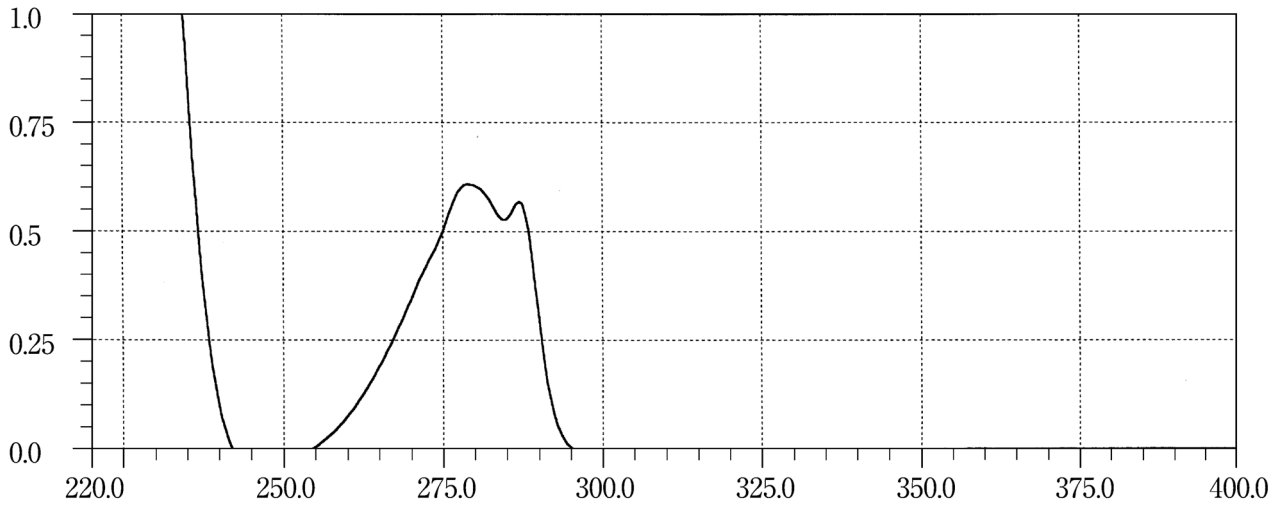
Mequitazine



Mercaptopurine Hydrate



Mestranol



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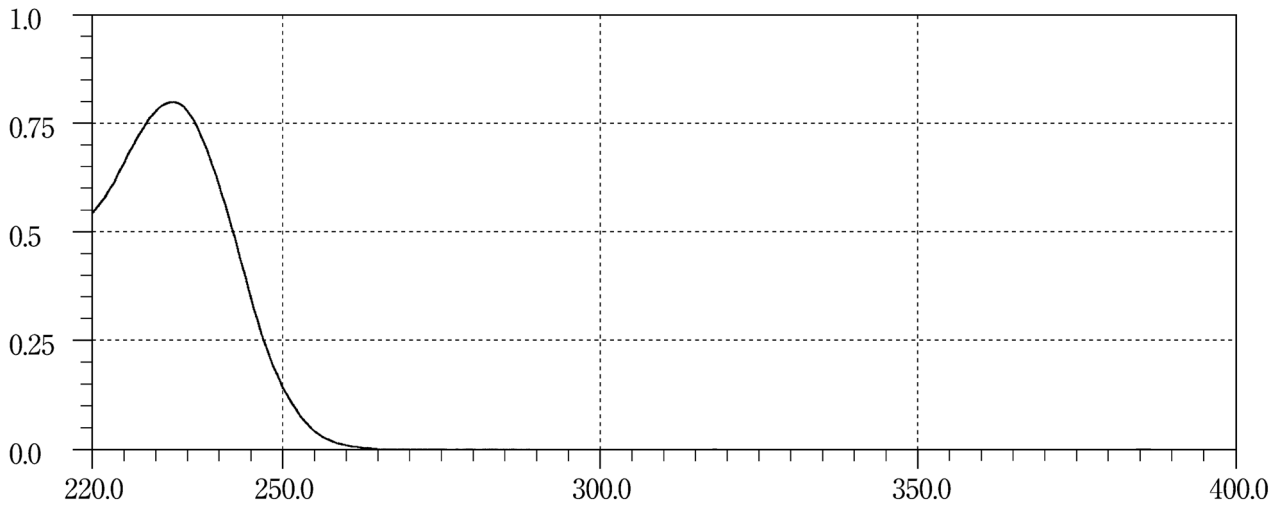

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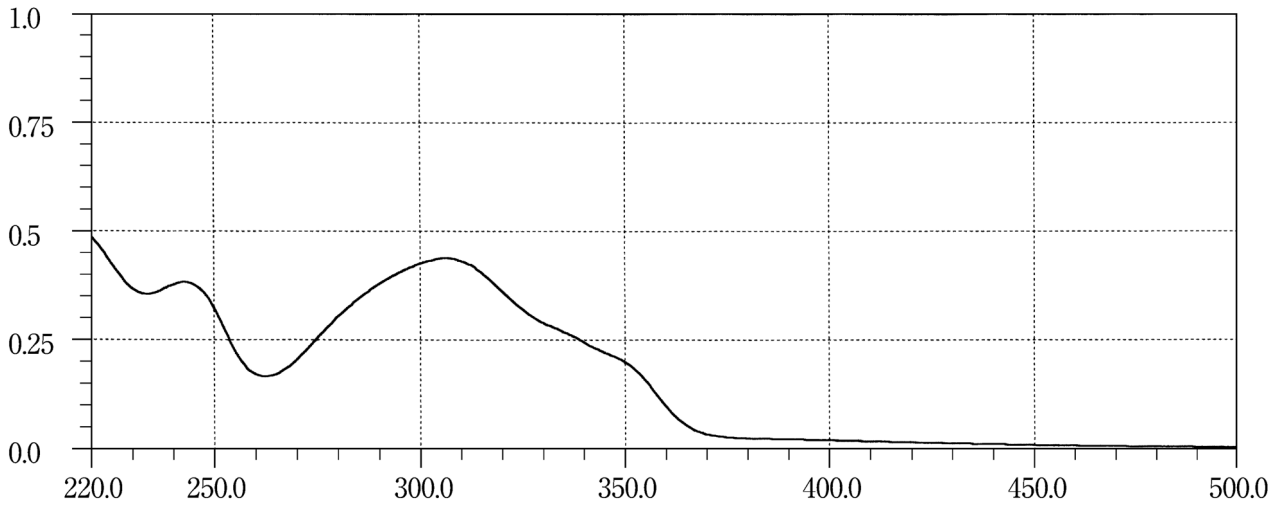

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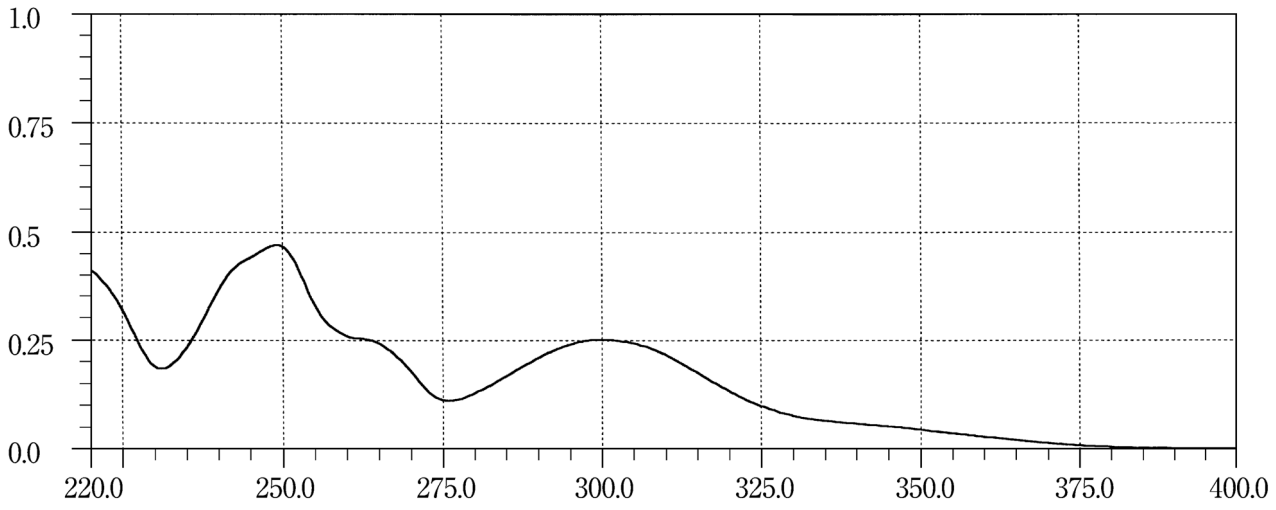

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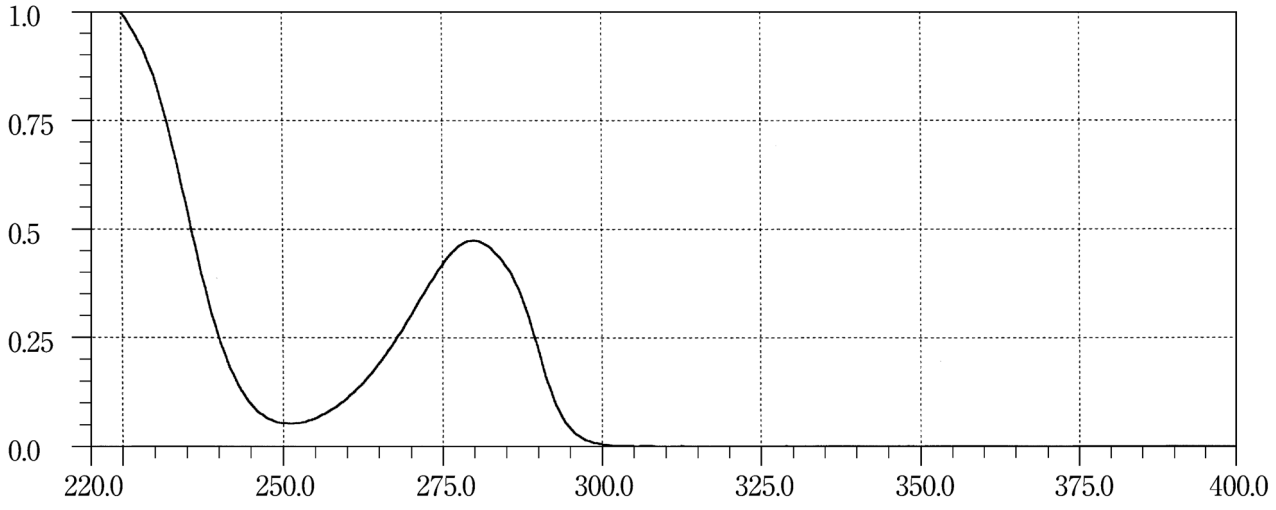
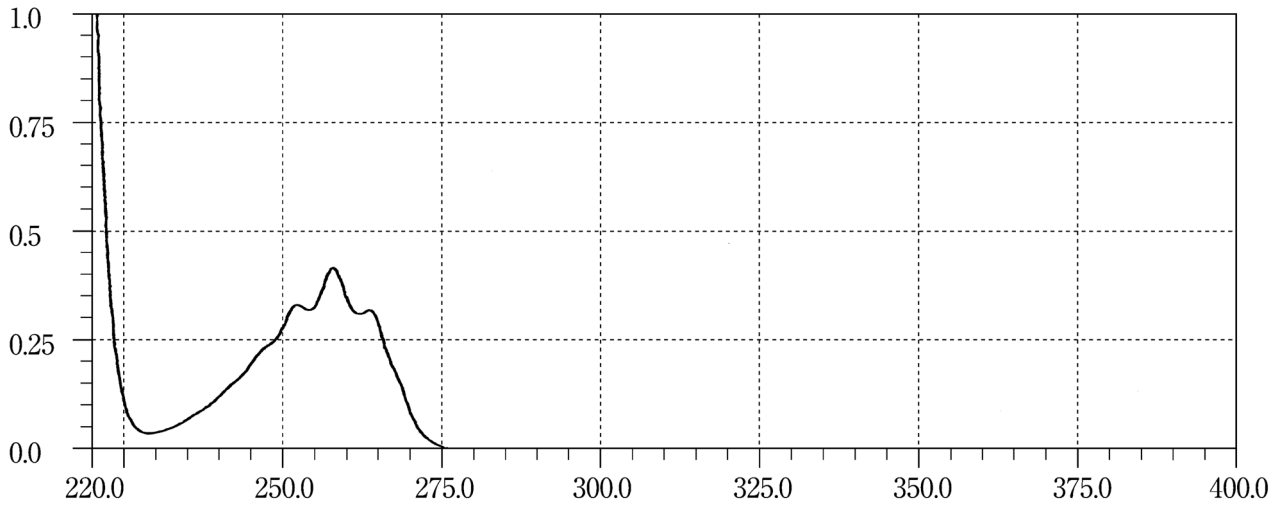


dl-Methylephedrine Hydrochloride



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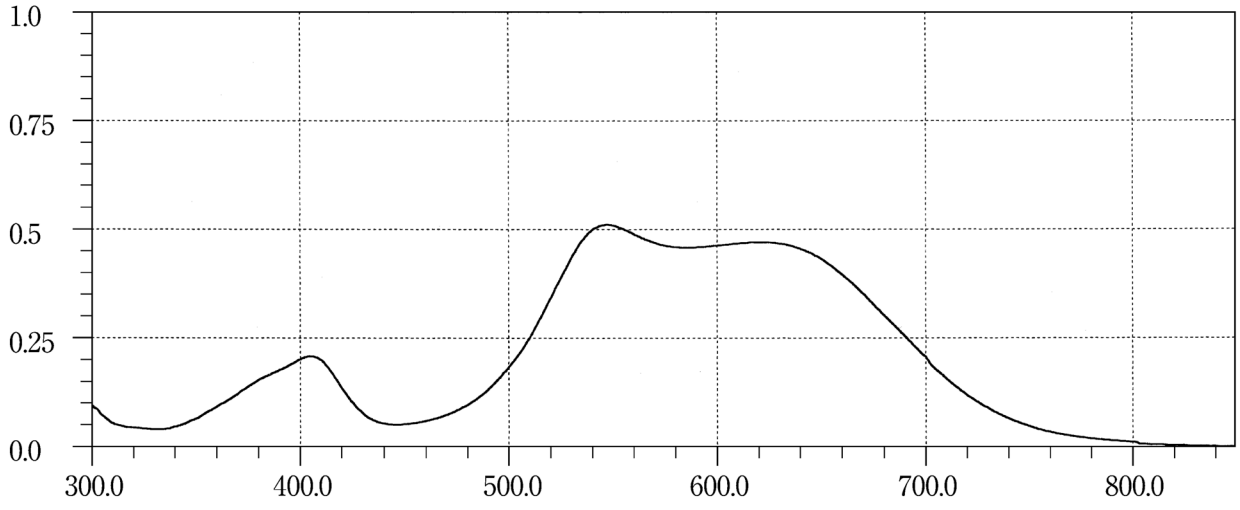
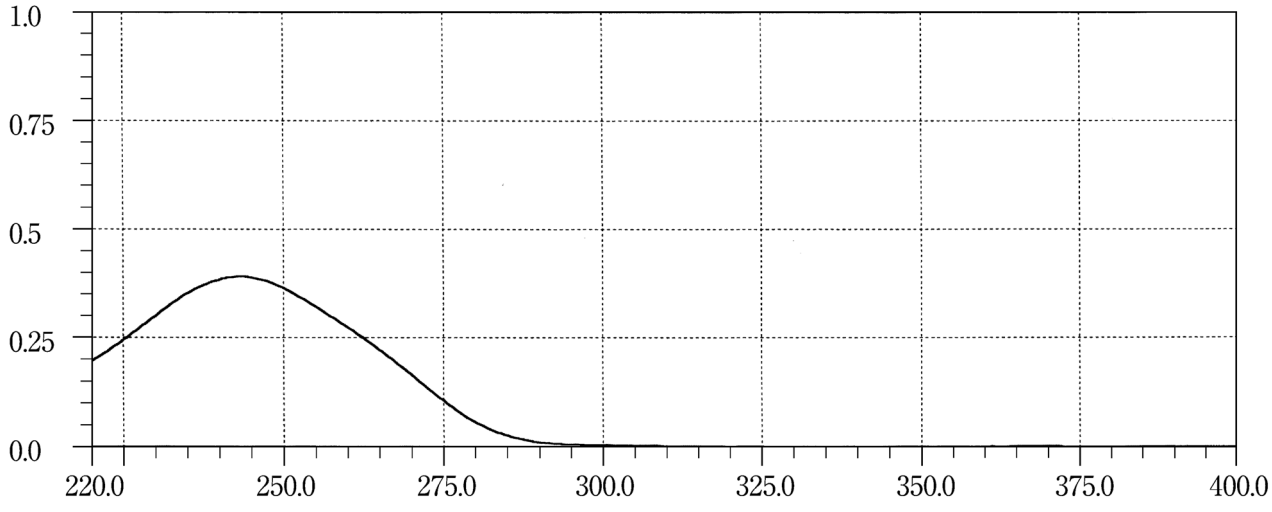
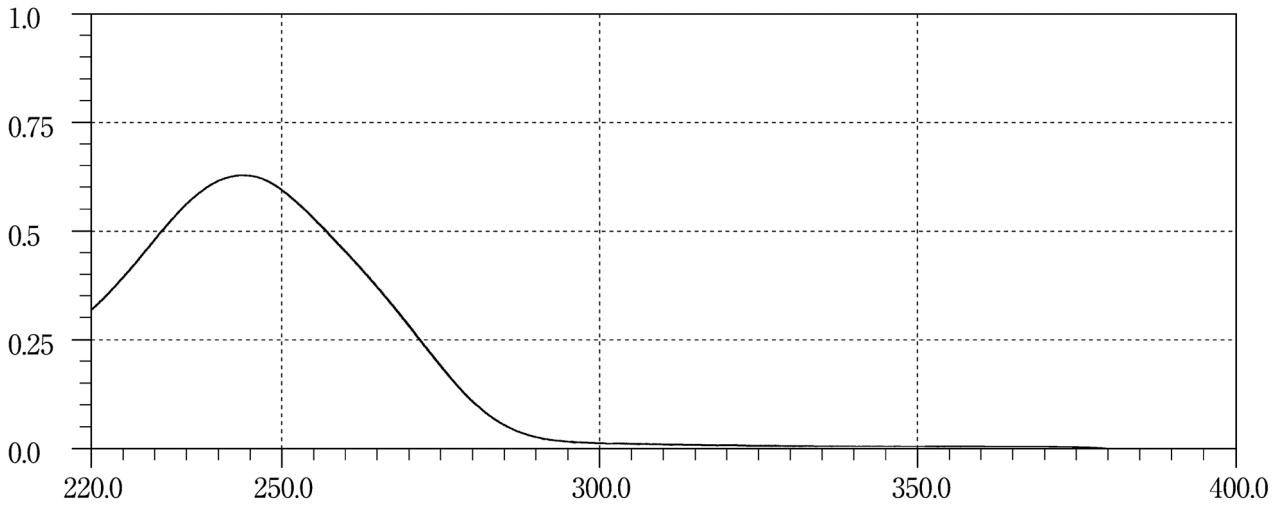


Methylprednisolone



Methylprednisolone Succinate



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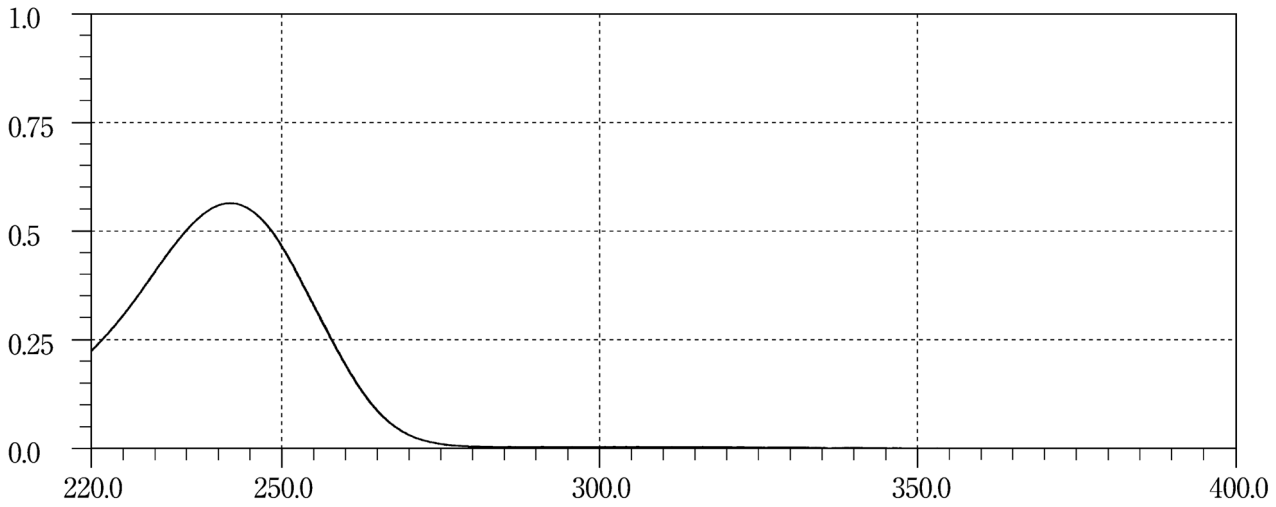

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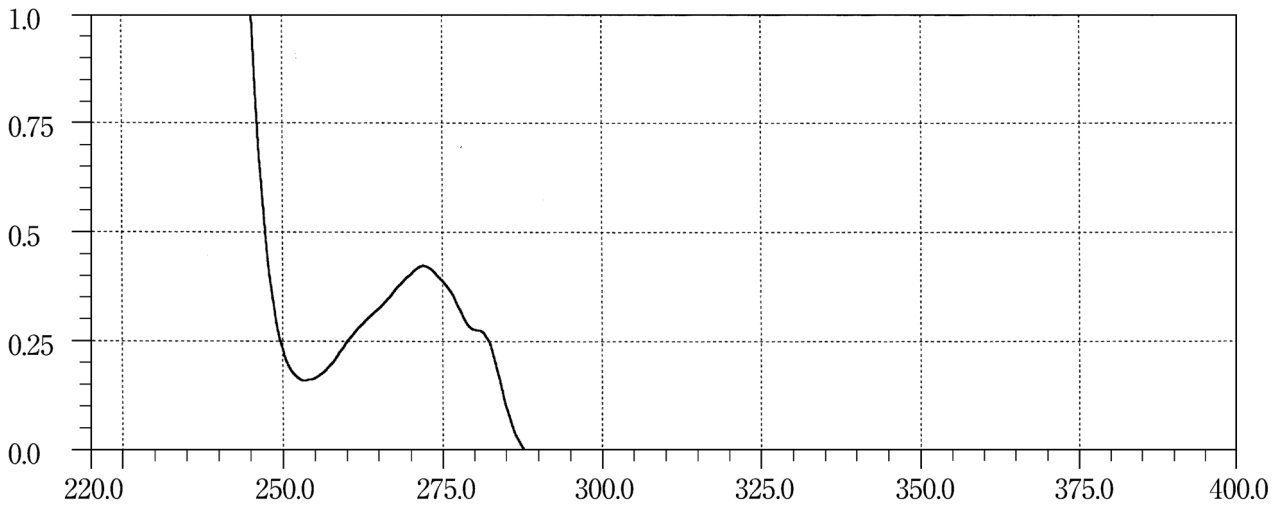

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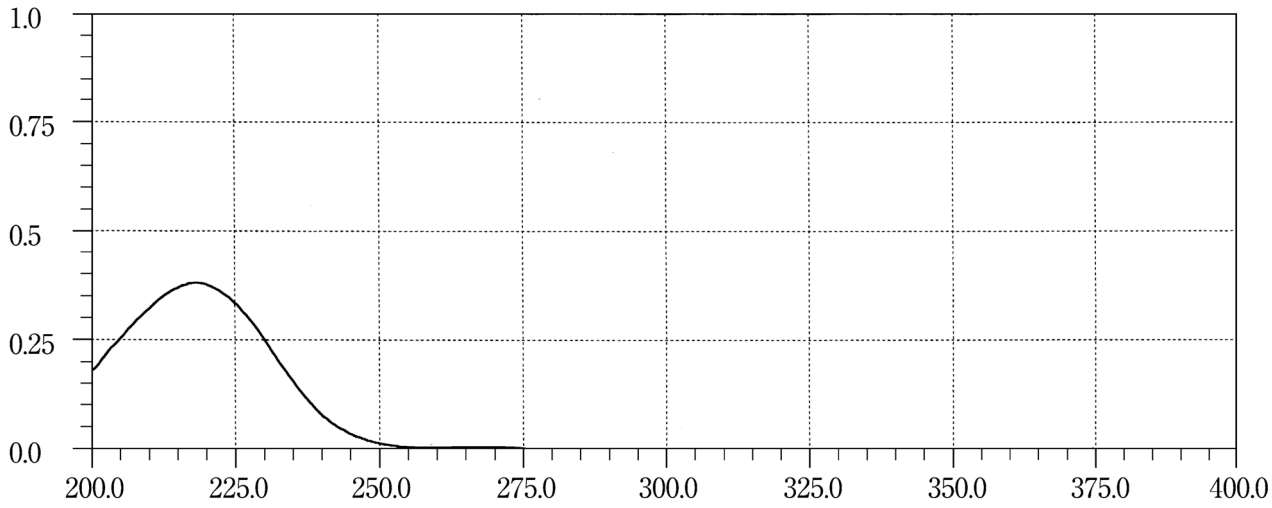

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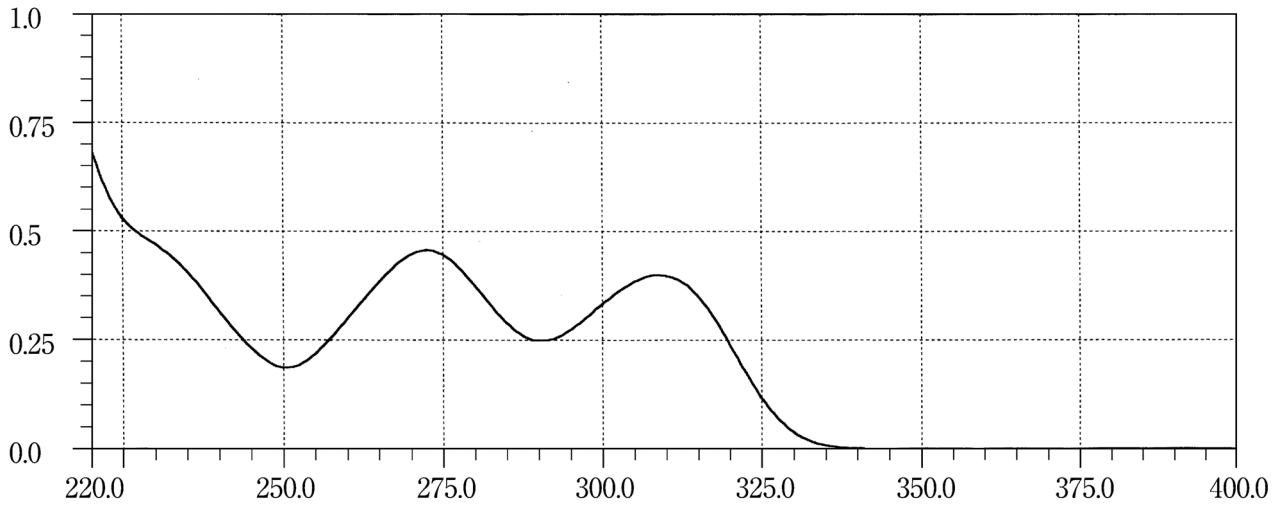

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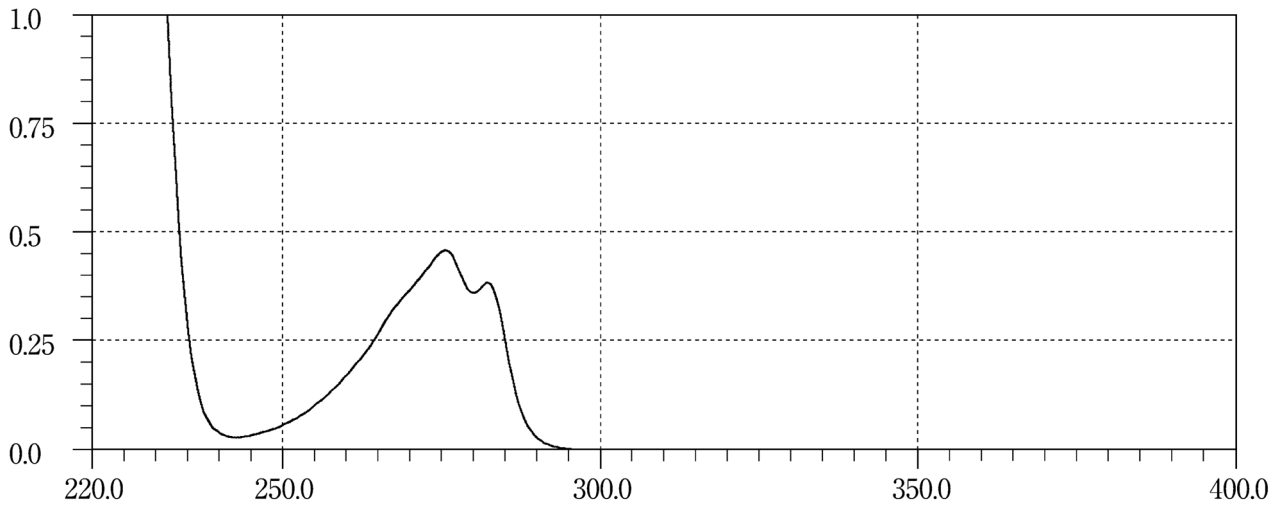

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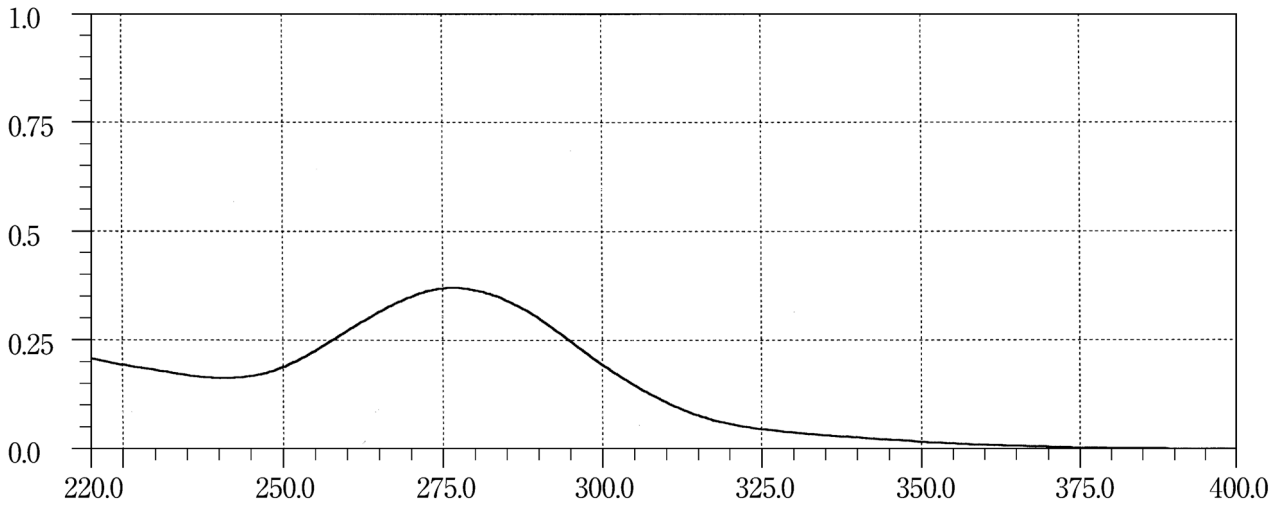

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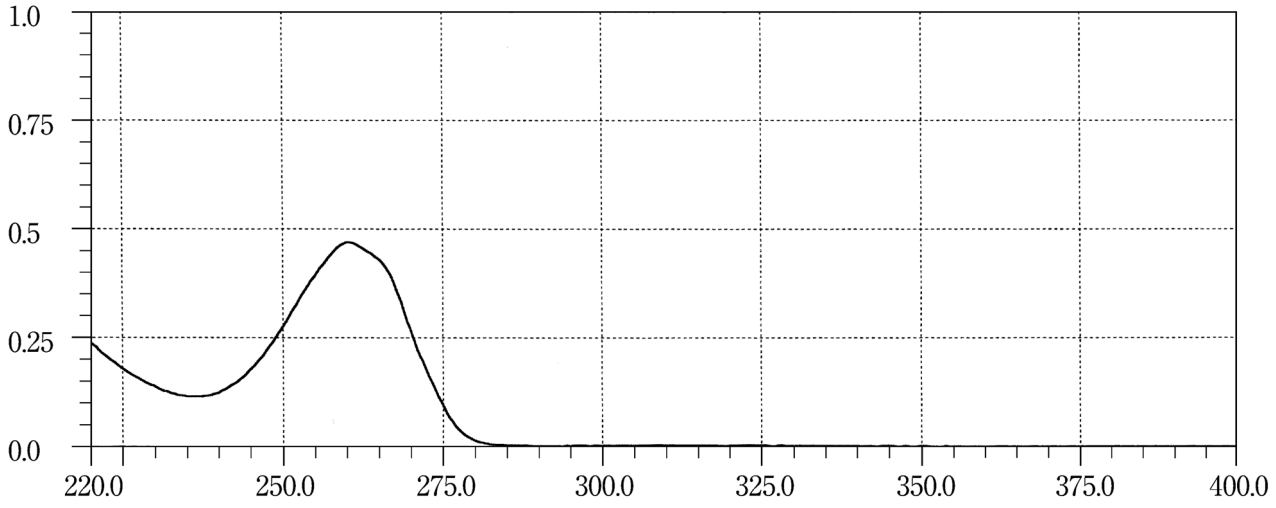
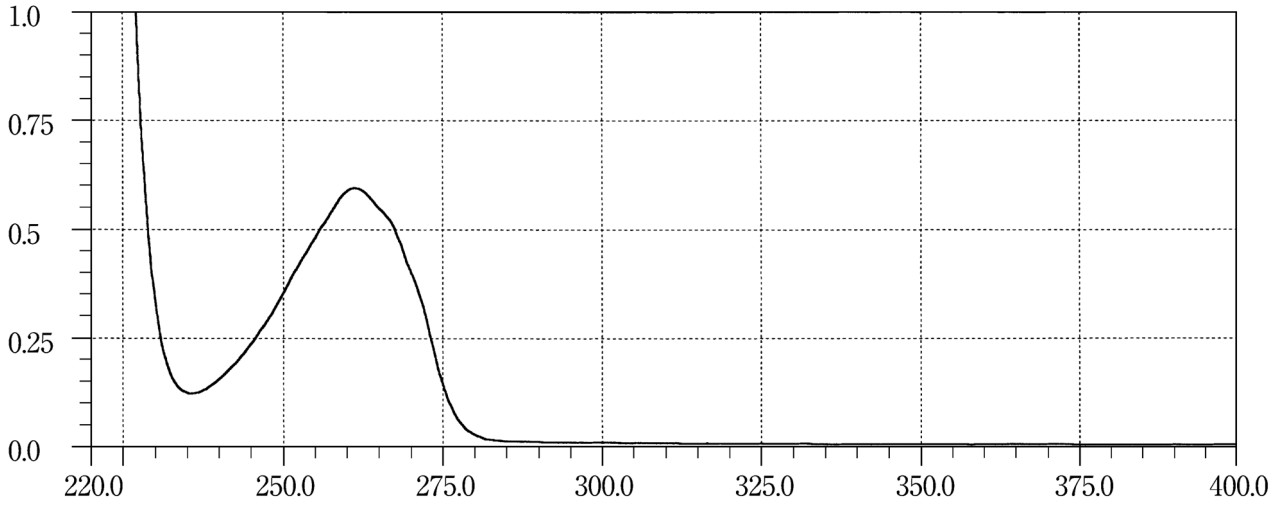


Mexiletine Hydrochloride



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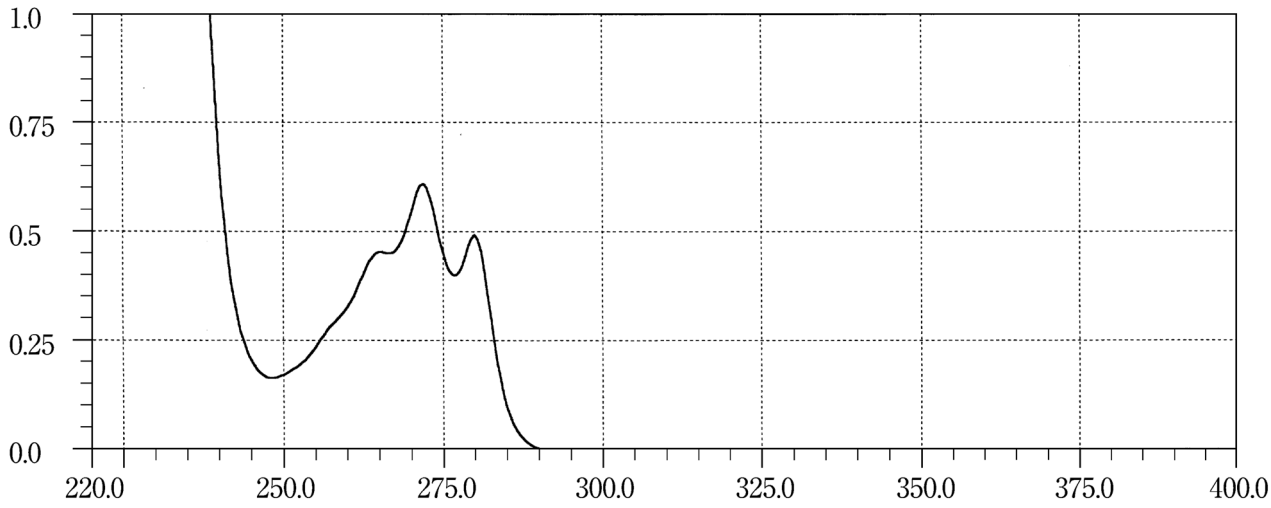
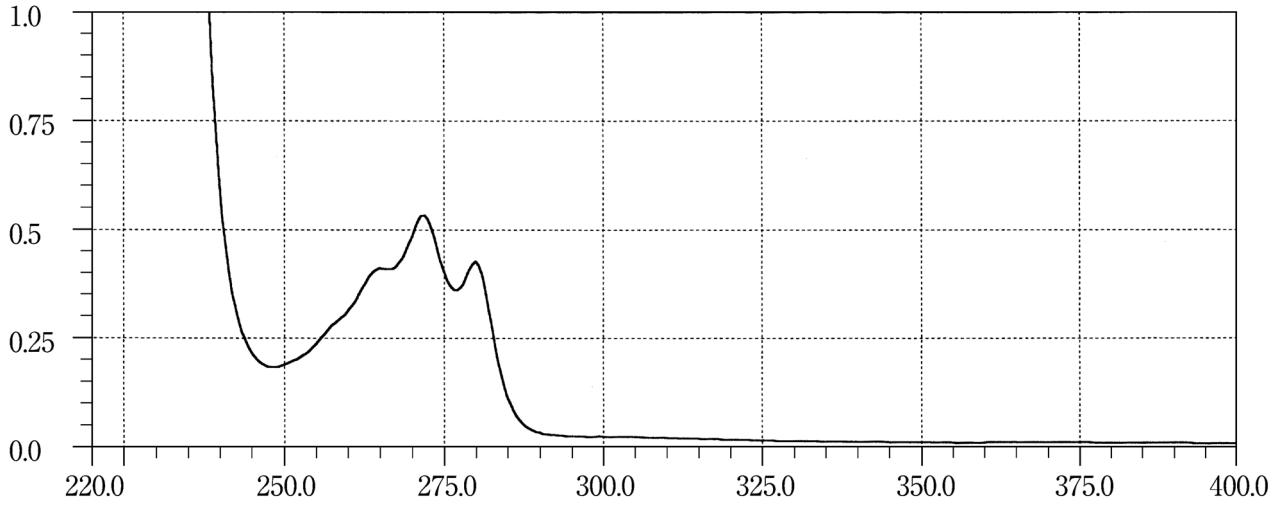


Miconazole Nitrate



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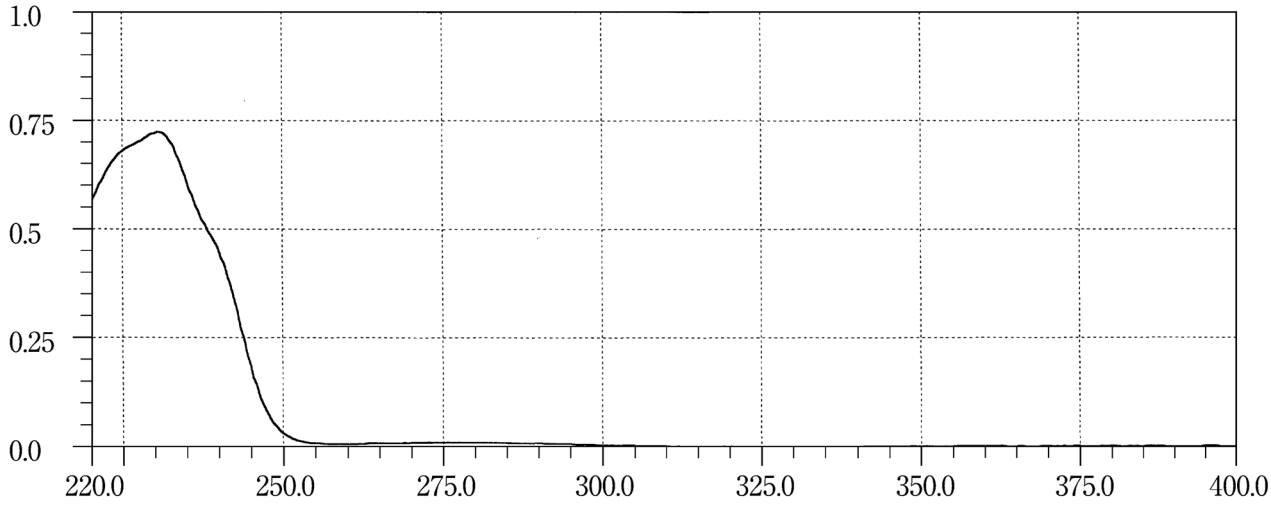
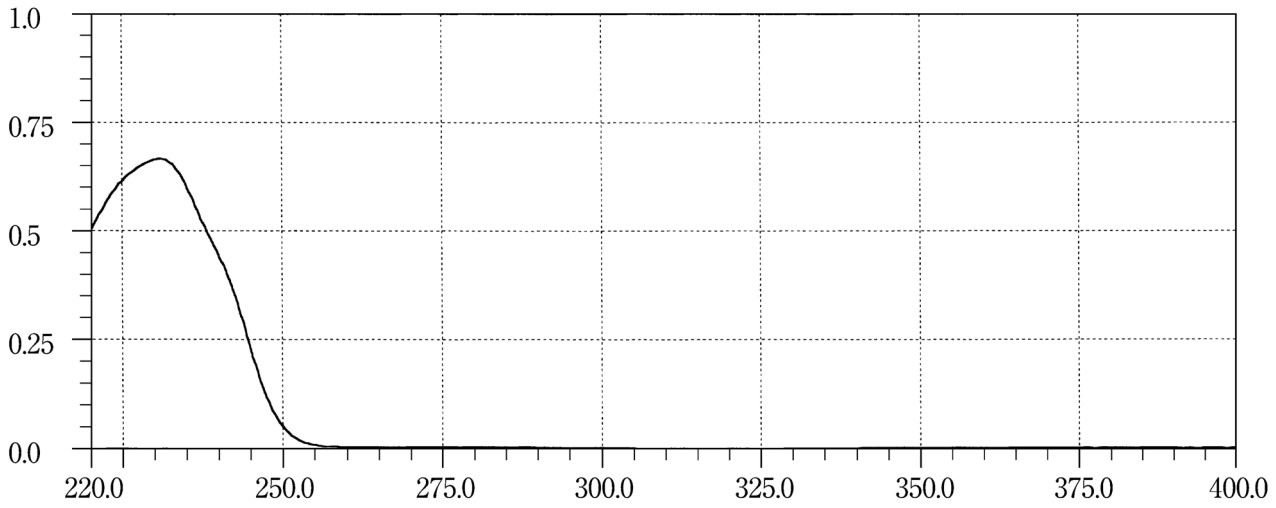
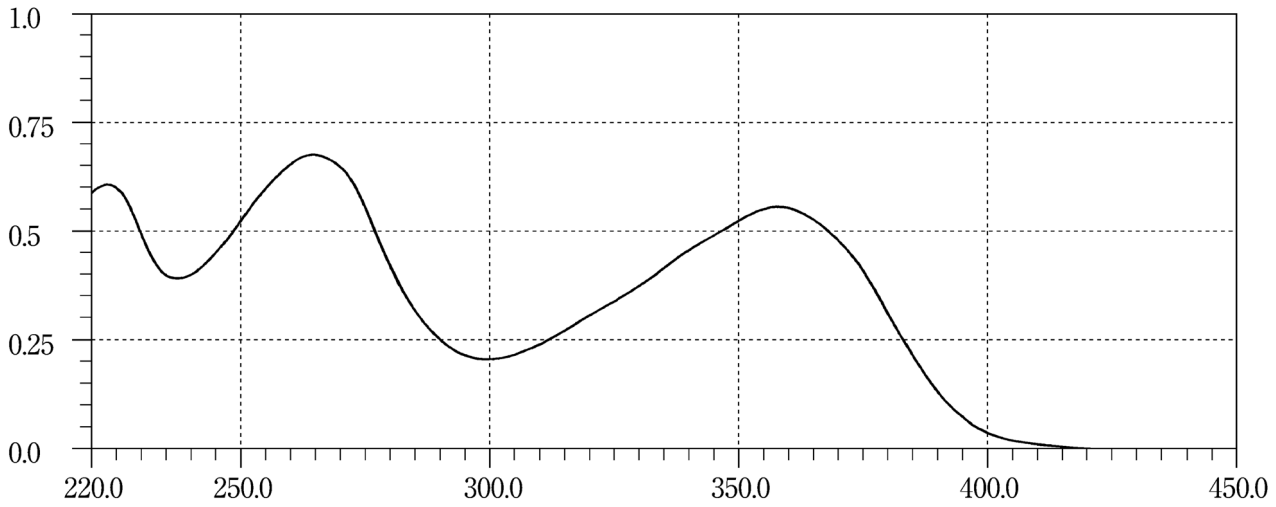


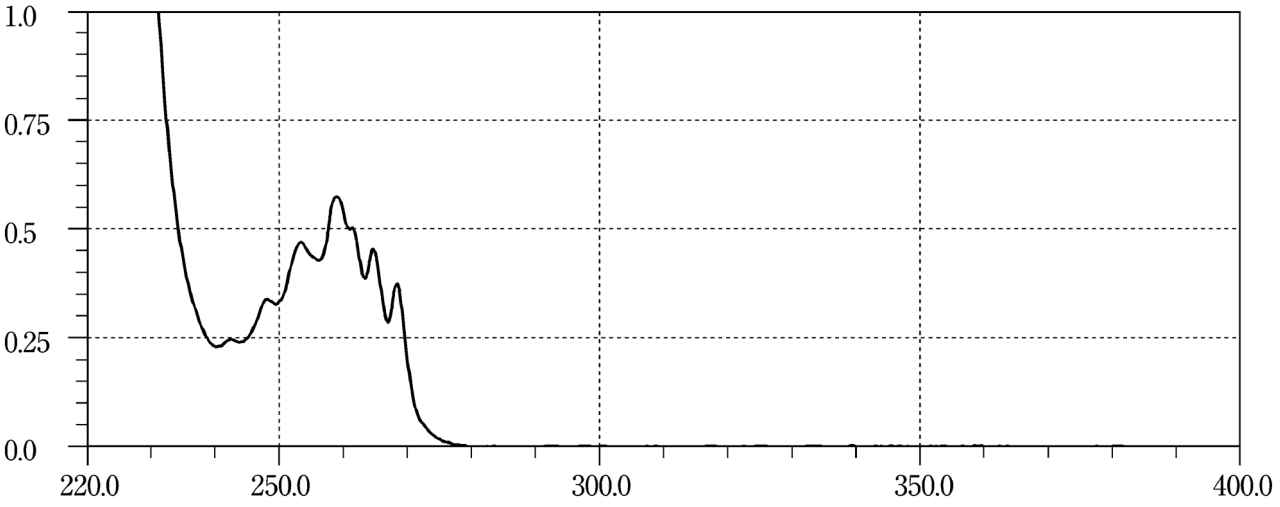
Midecamycin Acetate



Minocycline Hydrochloride



Mitiglinide Calcium Hydrate



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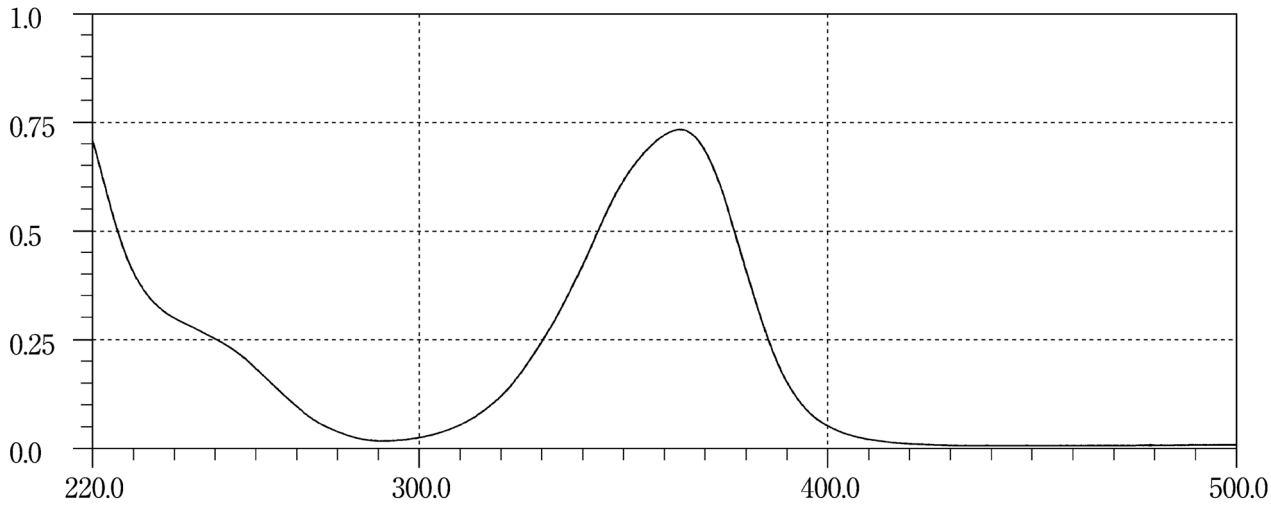

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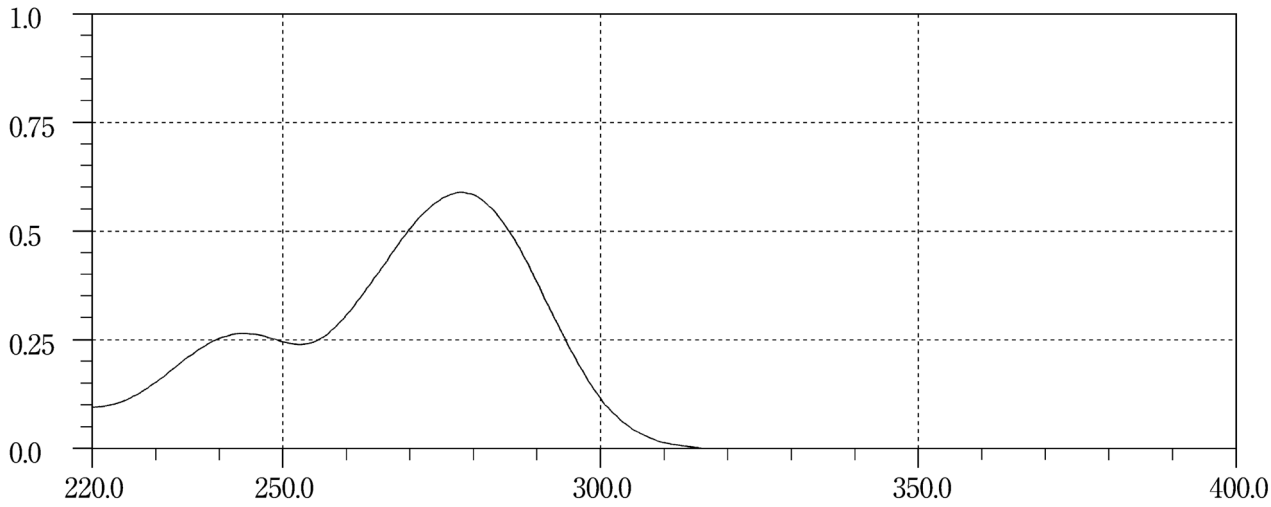

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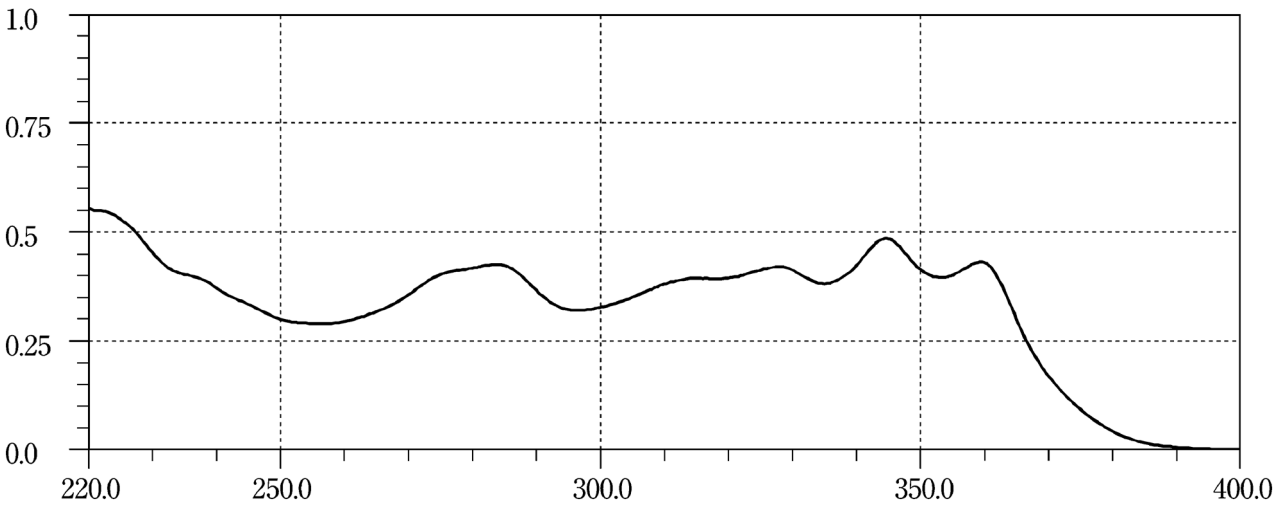
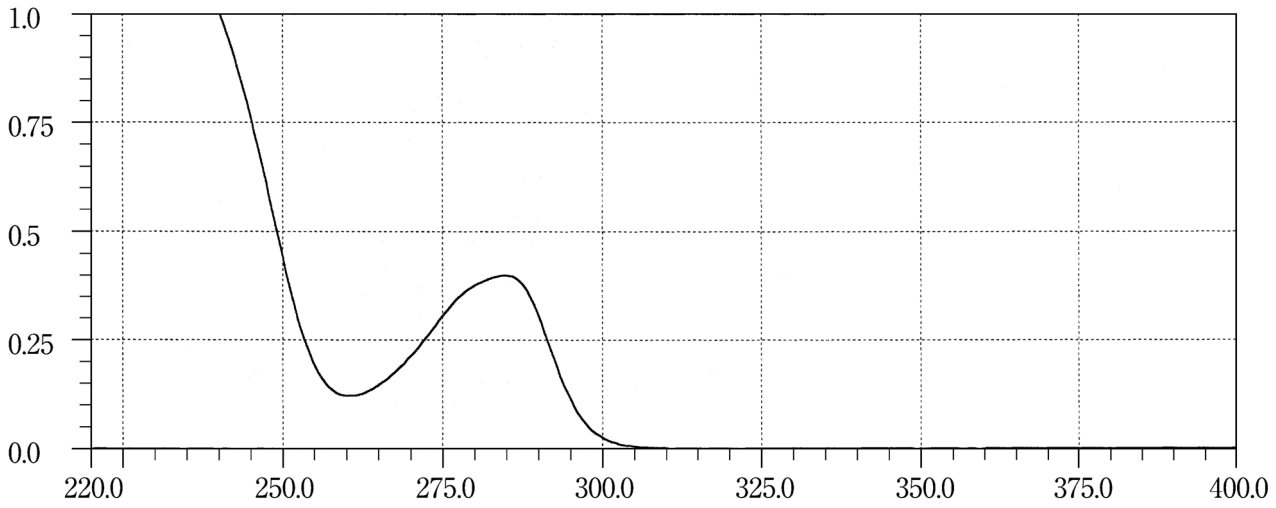
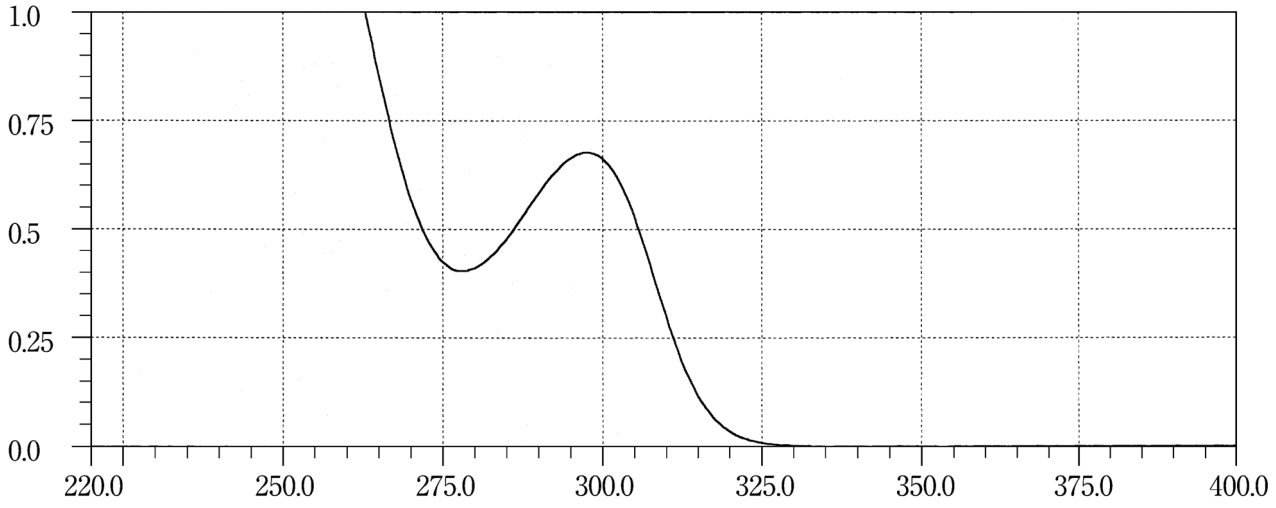


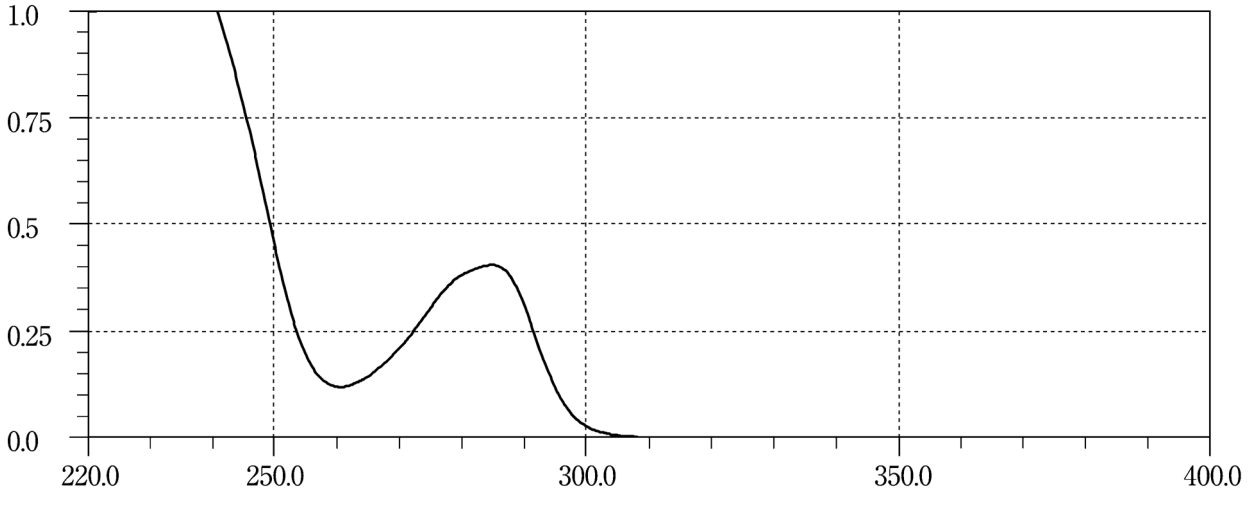
Morphine Hydrochloride Hydrate 1



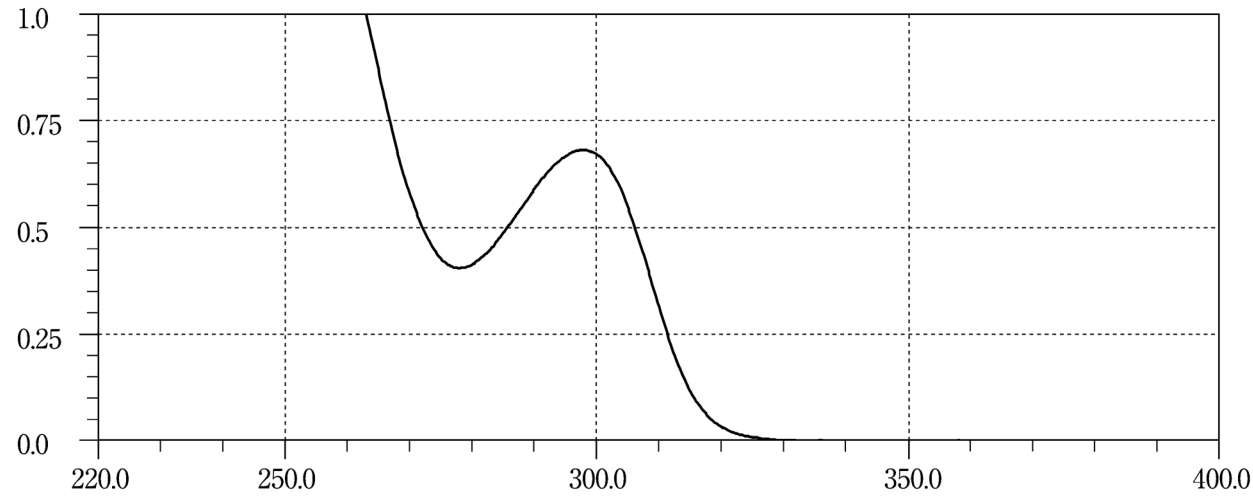
Morphine Hydrochloride Hydrate 2



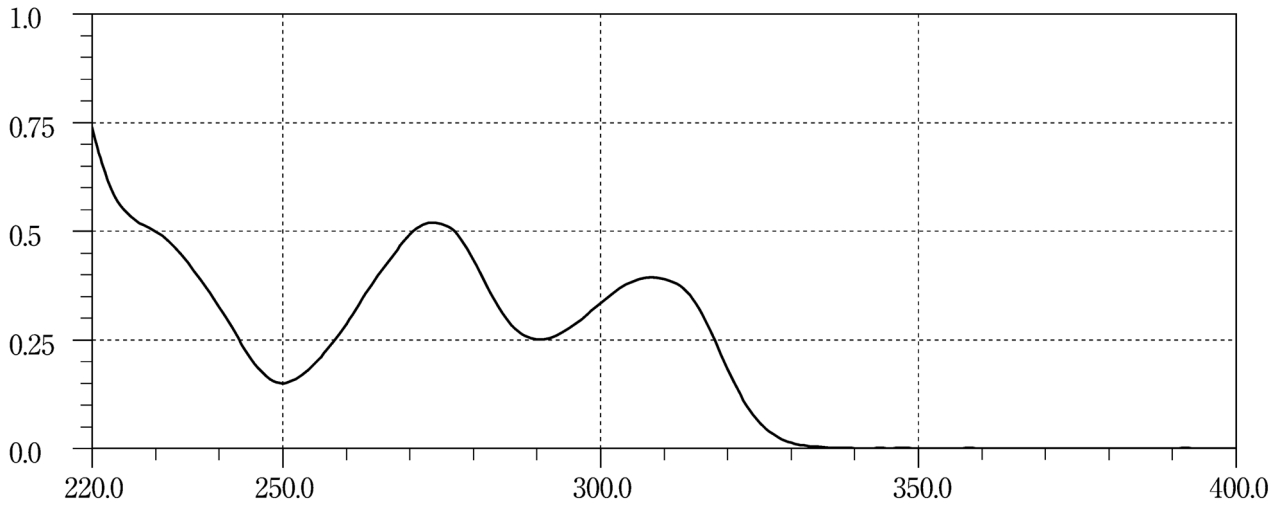
Morphine Sulfate Hydrate 1



Morphine Sulfate Hydrate 2



Mosapride Citrate Hydrate



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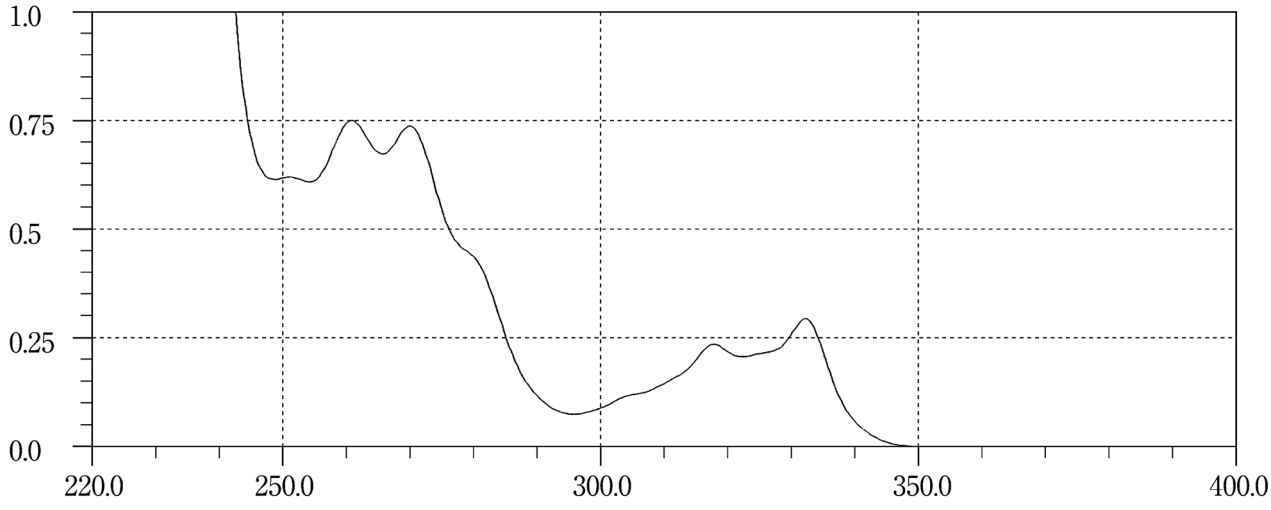

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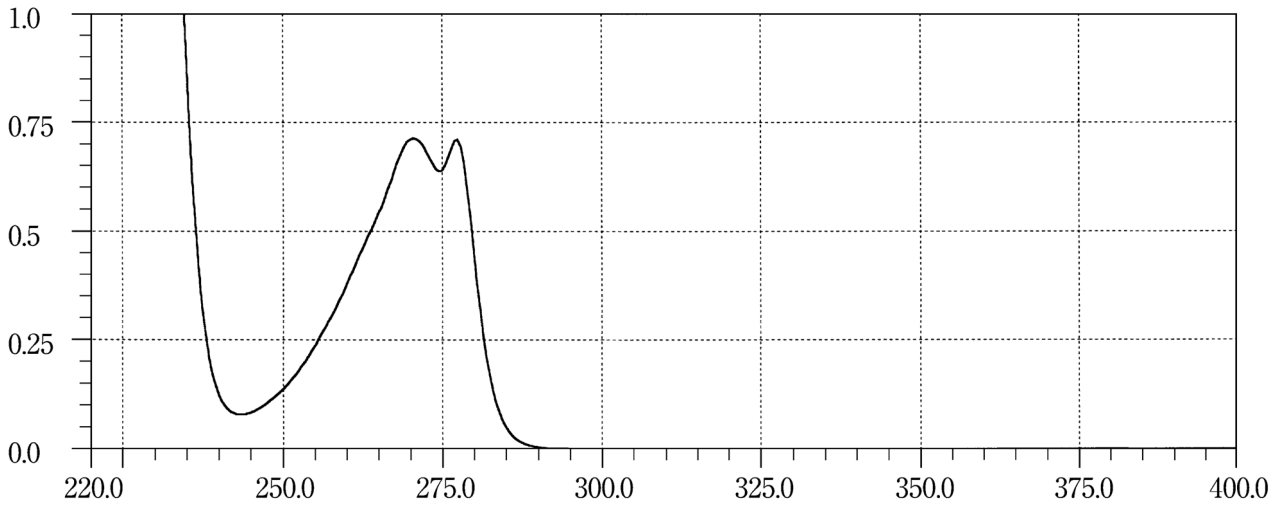

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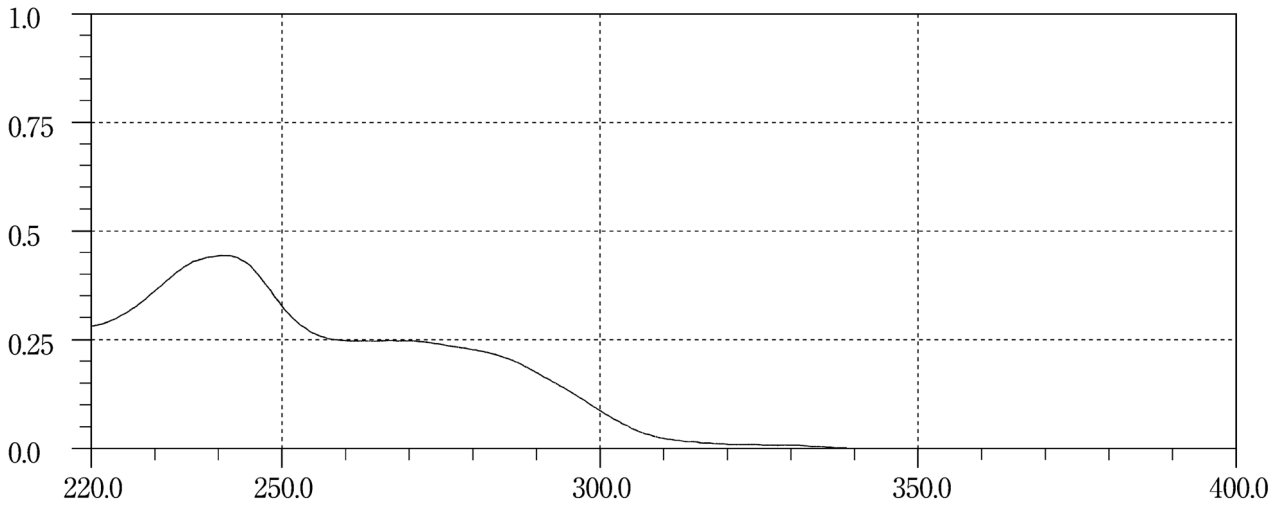

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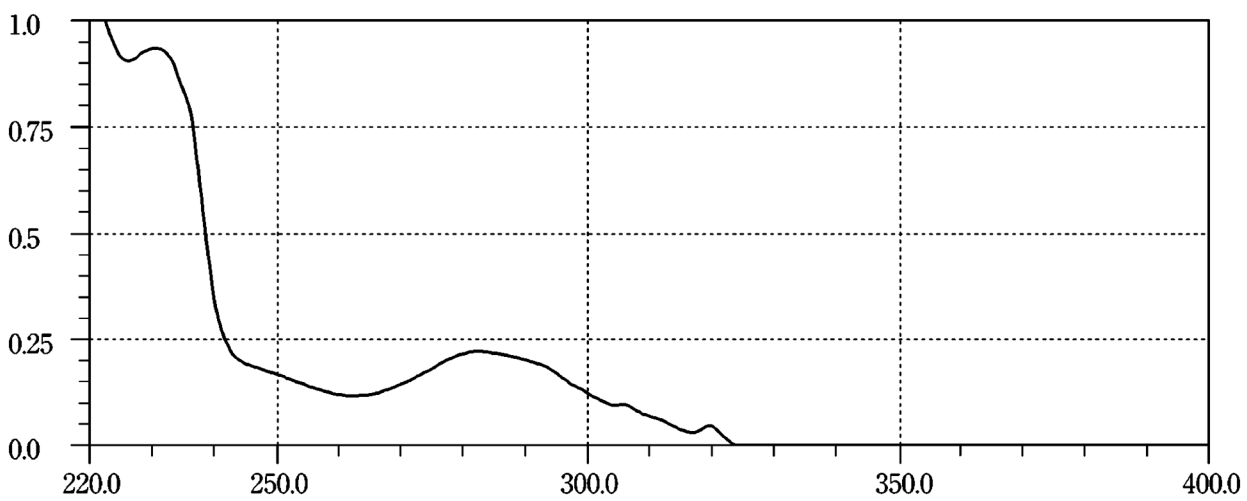
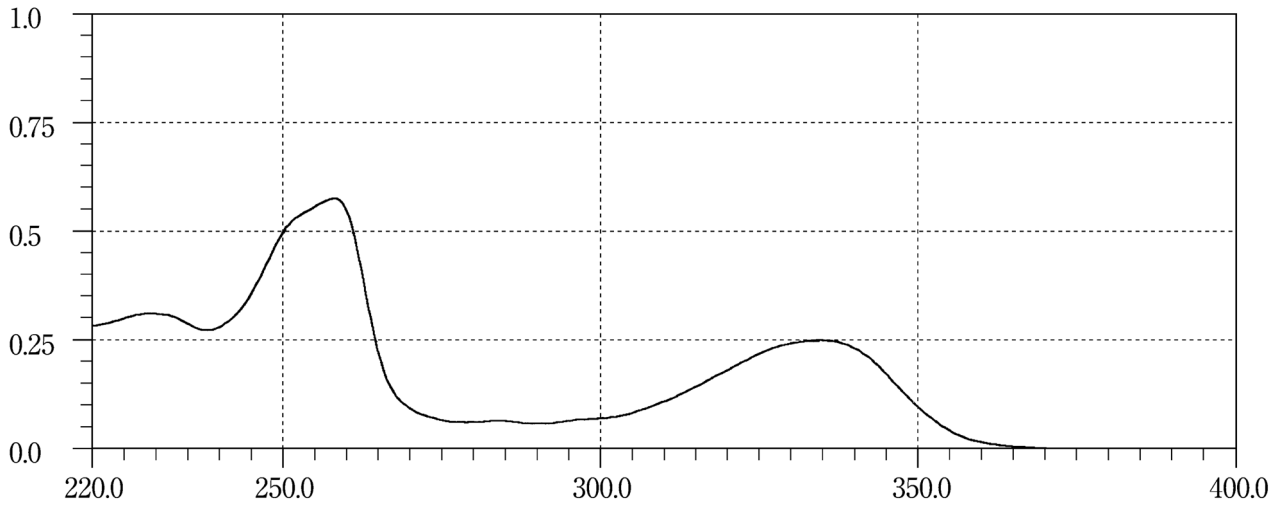
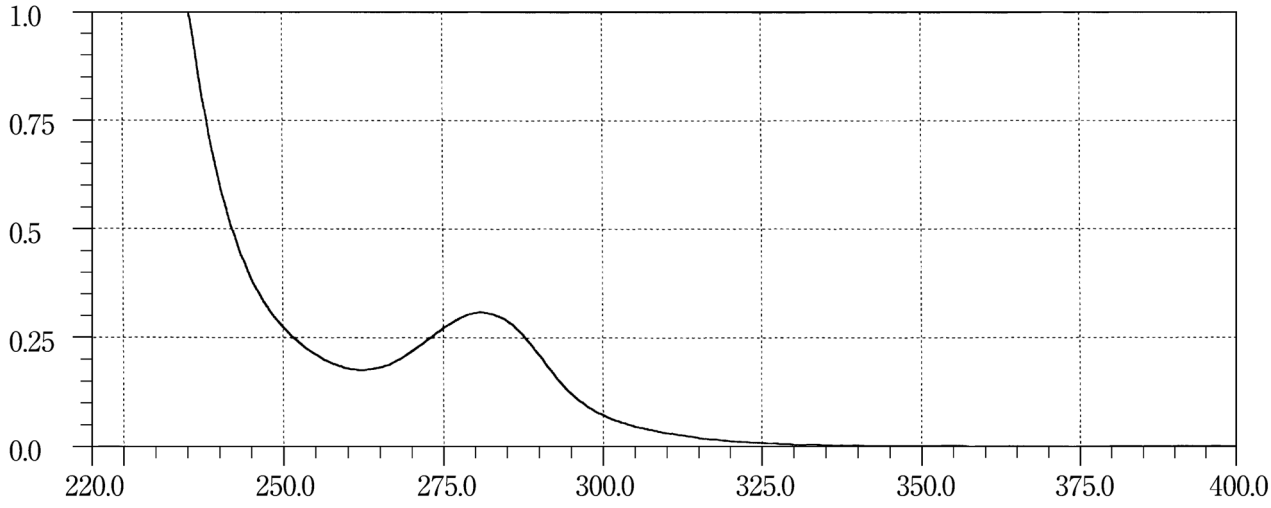


Nalidixic Acid



Naloxone Hydrochloride



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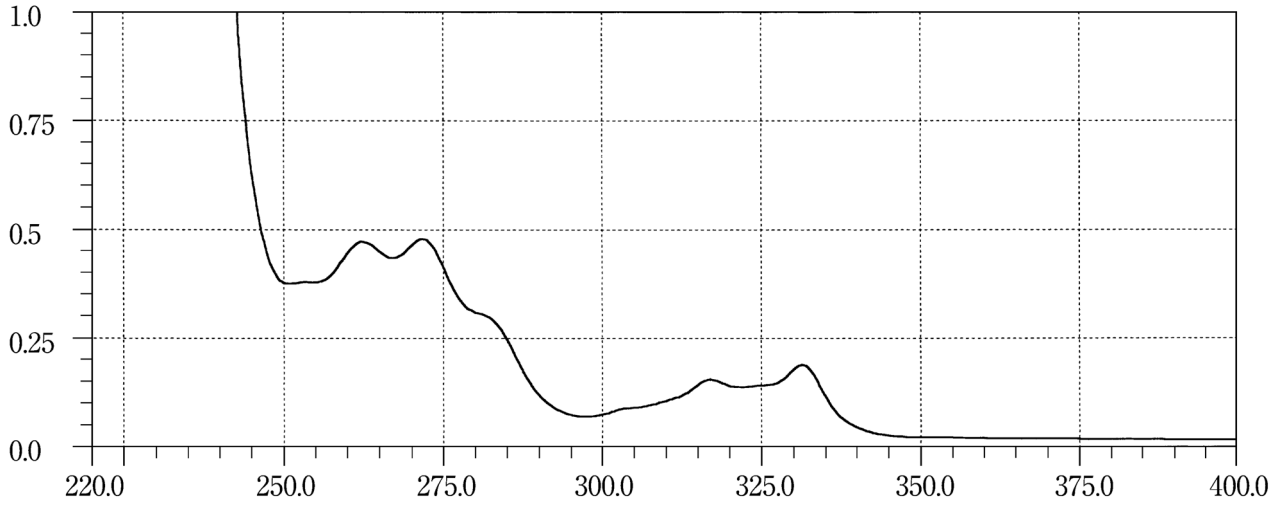
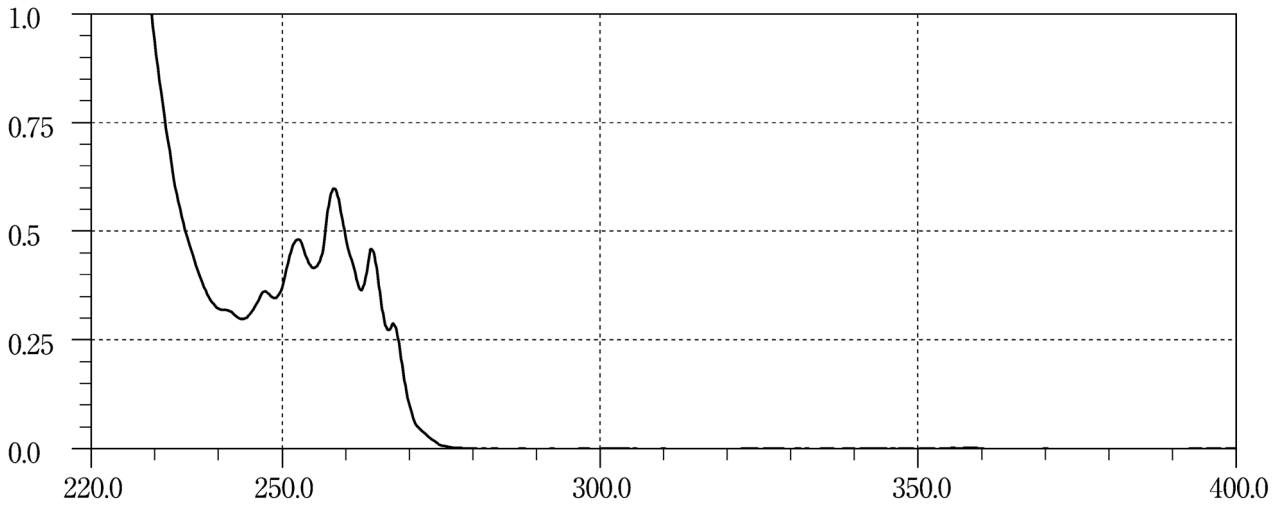
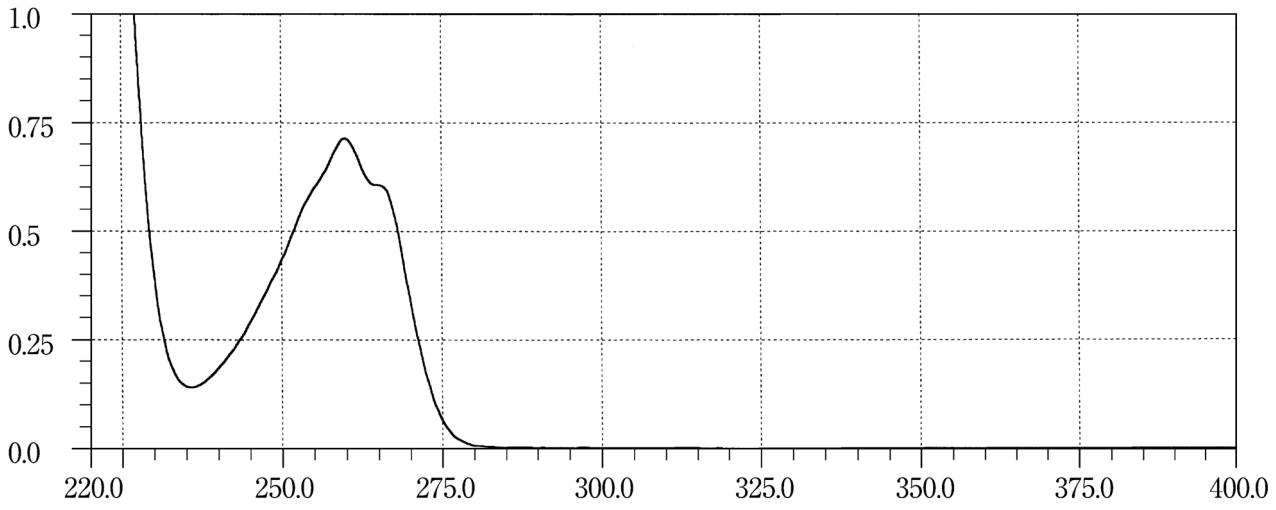


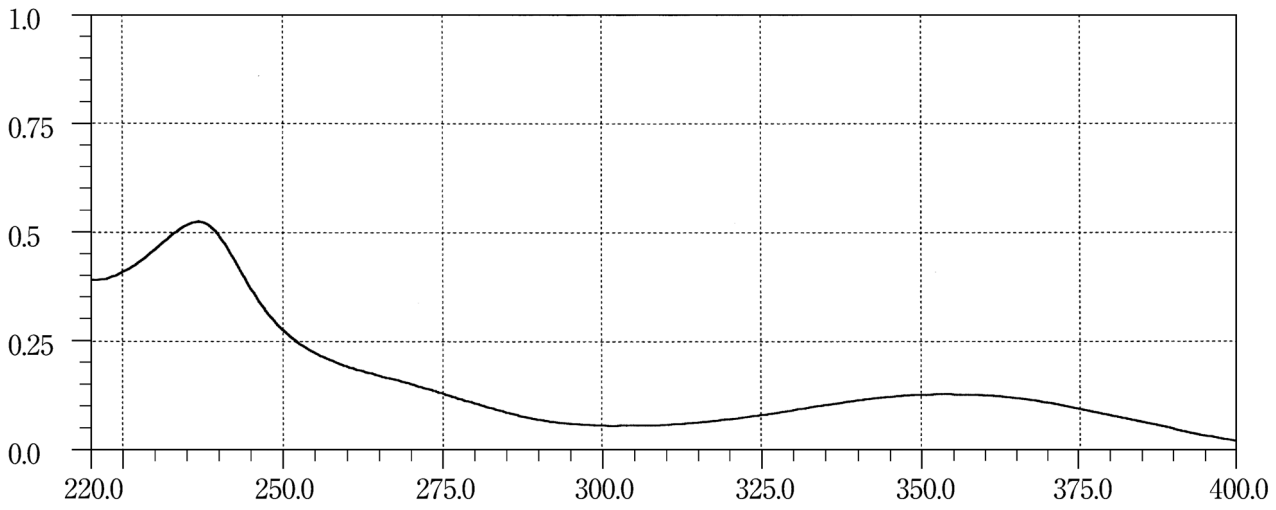
Nateglinide



Neostigmine Methylsulfate



Nicardipine Hydrochloride



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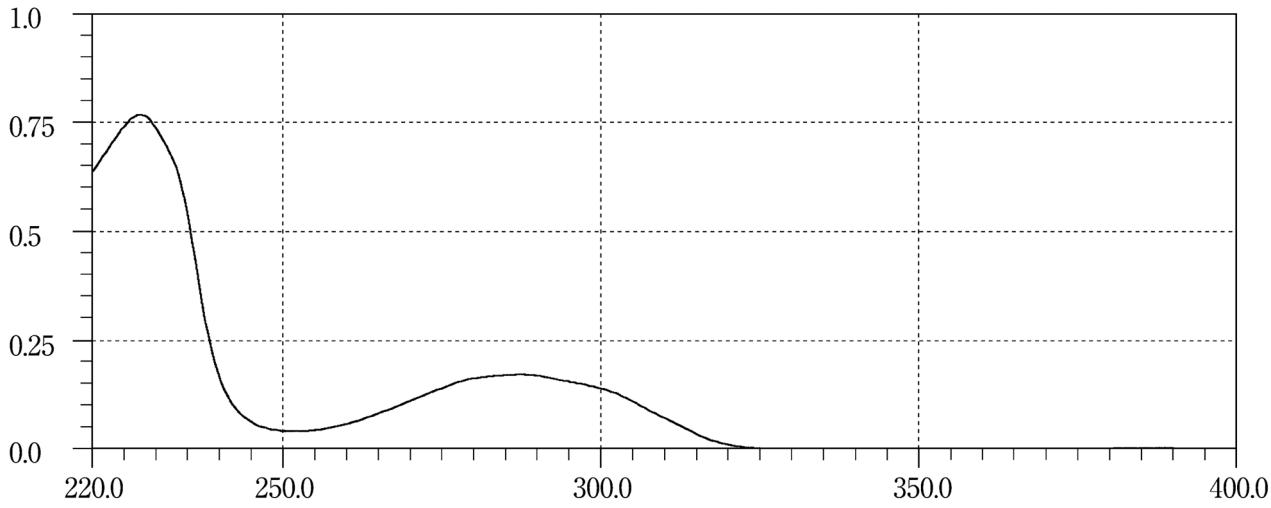

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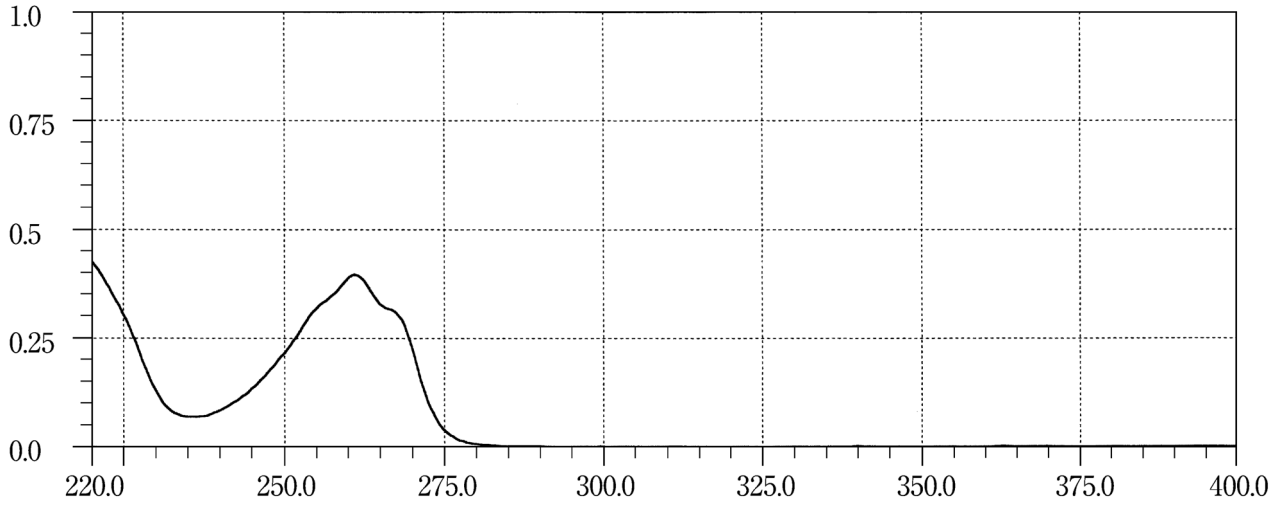

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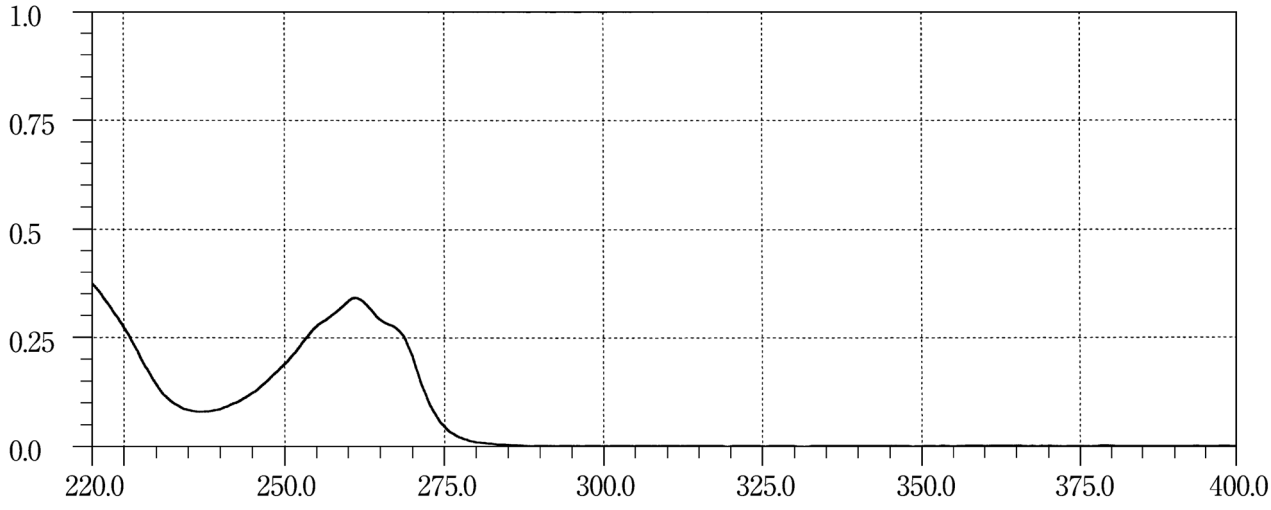

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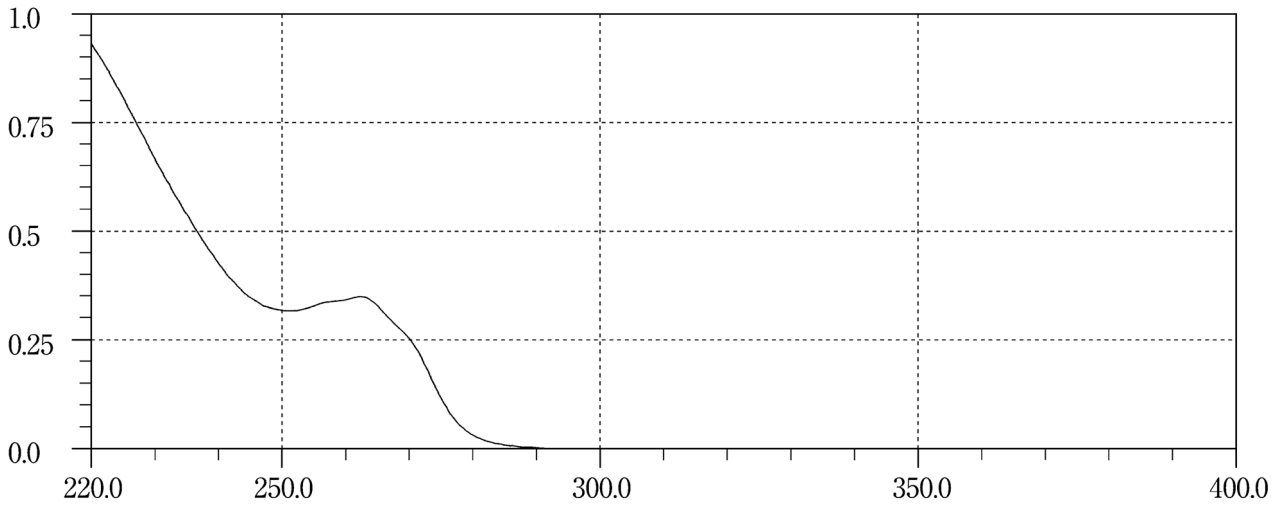

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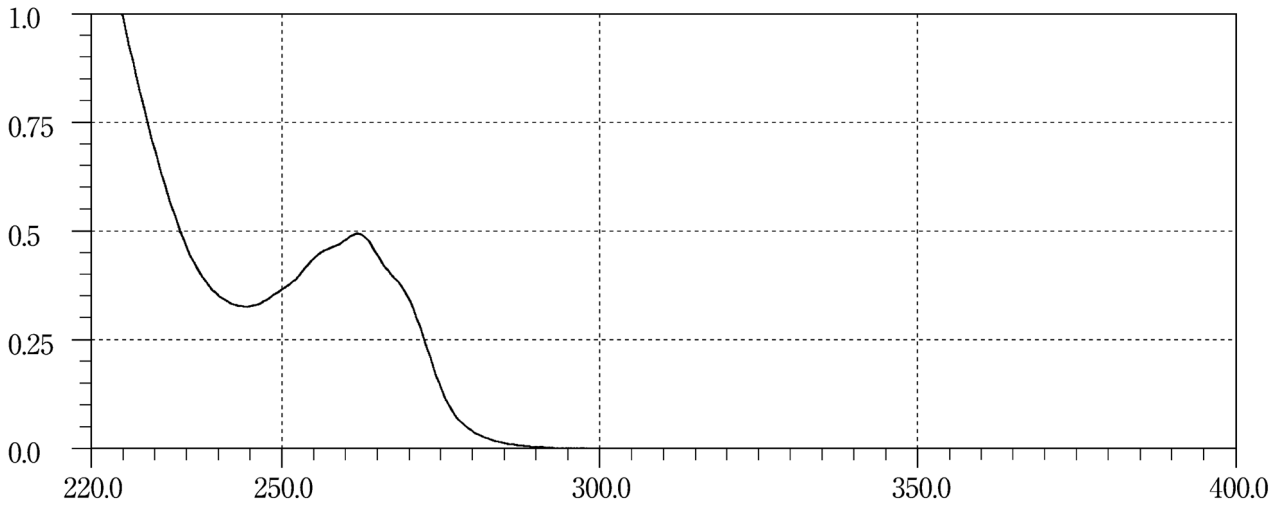
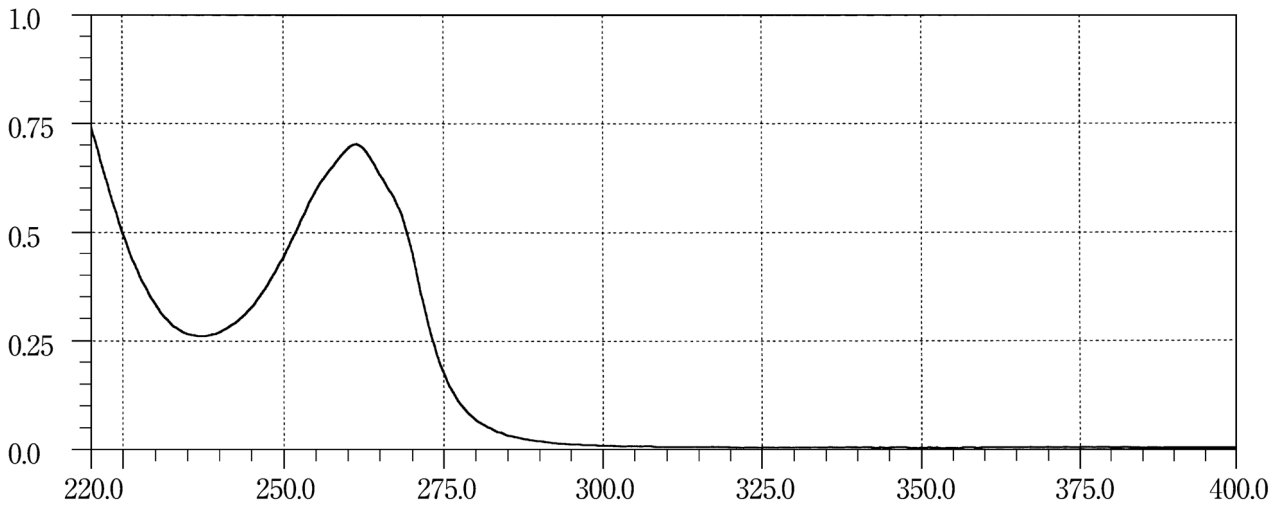
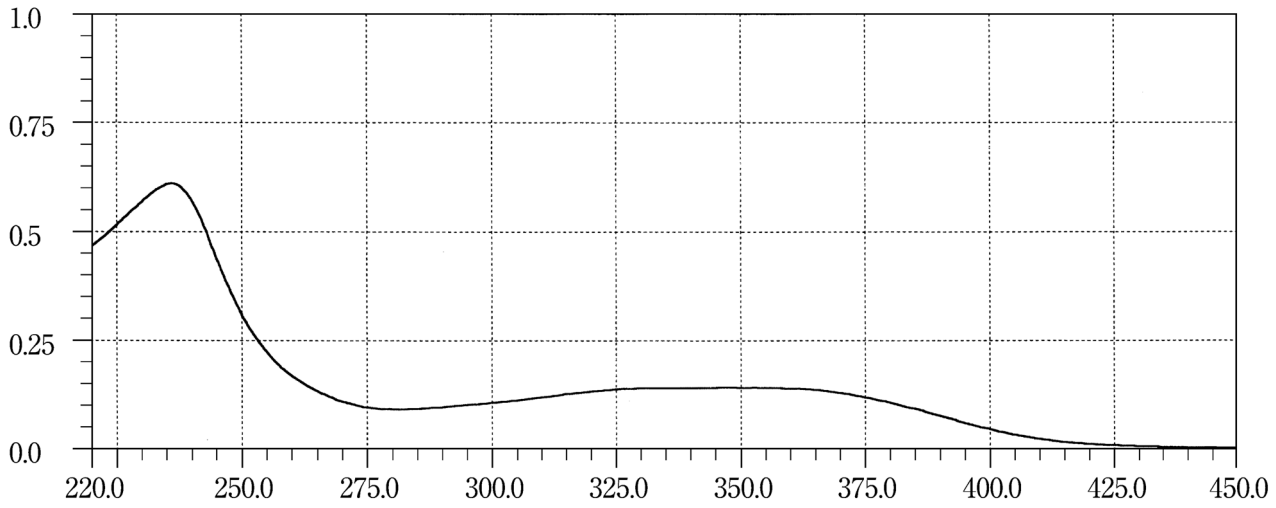


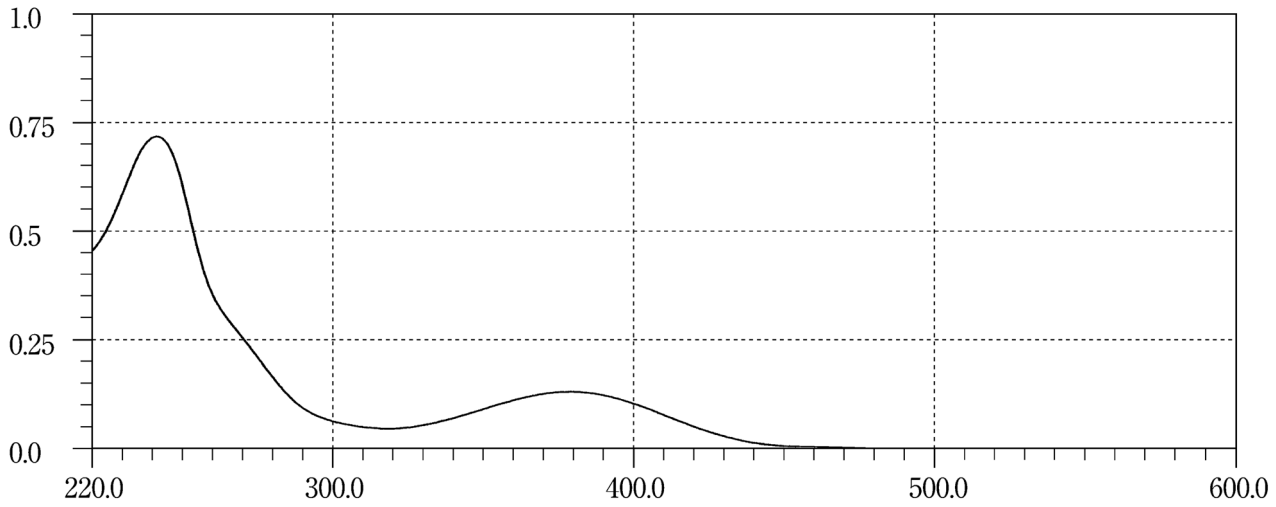
Nicotinic Acid



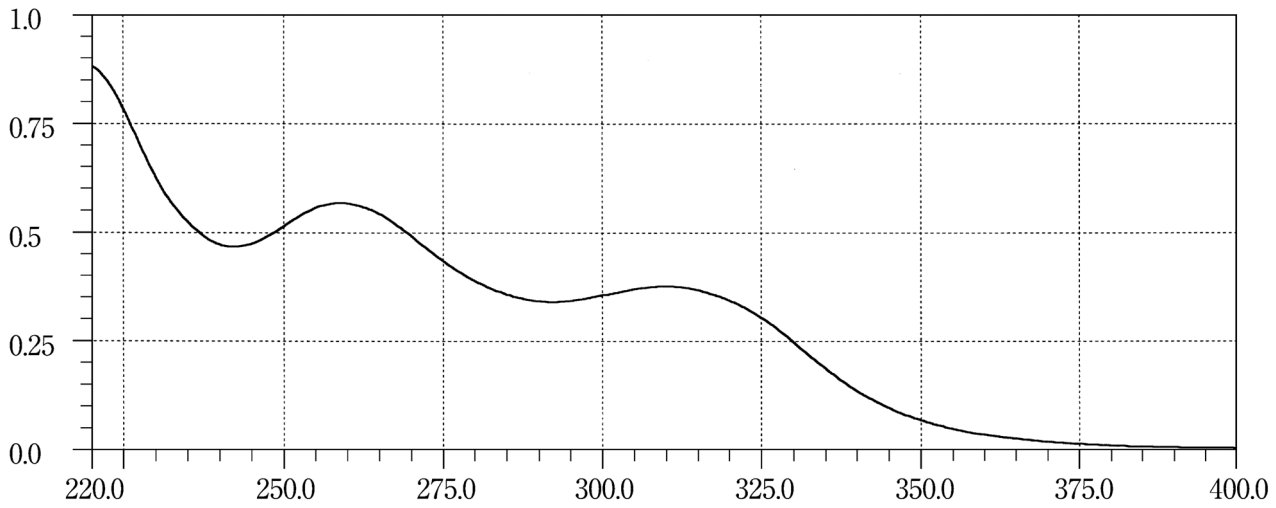
Nifedipine



Nilvadipine



Nitrazepam



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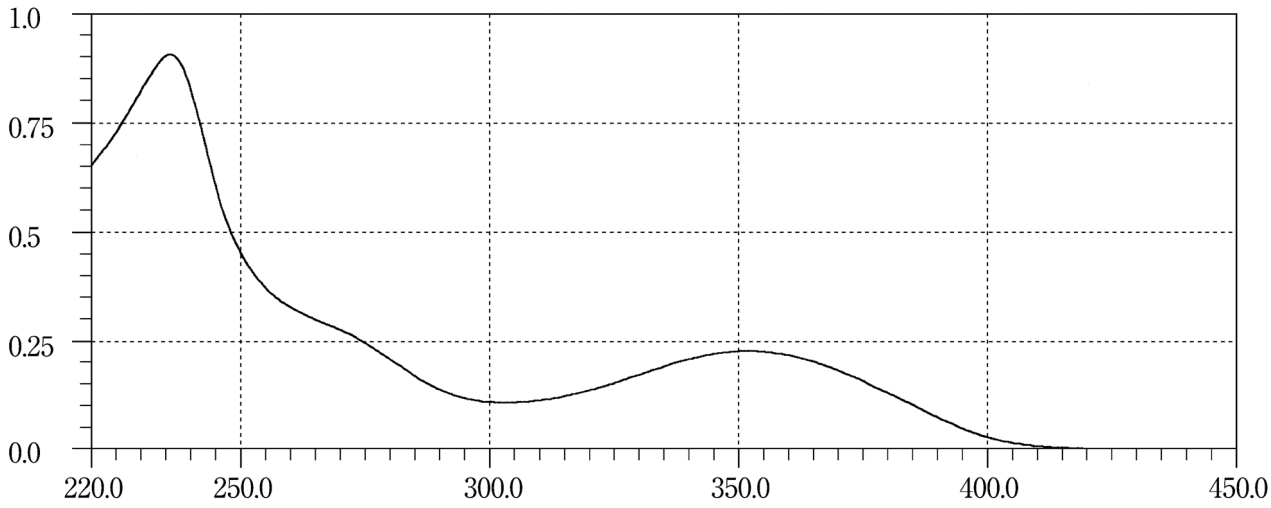
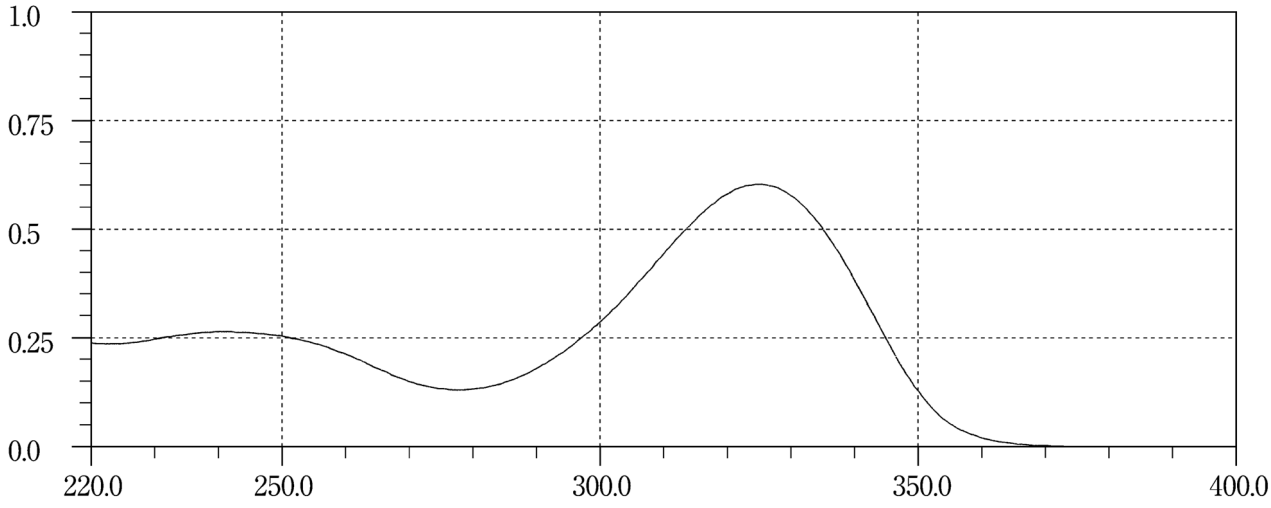
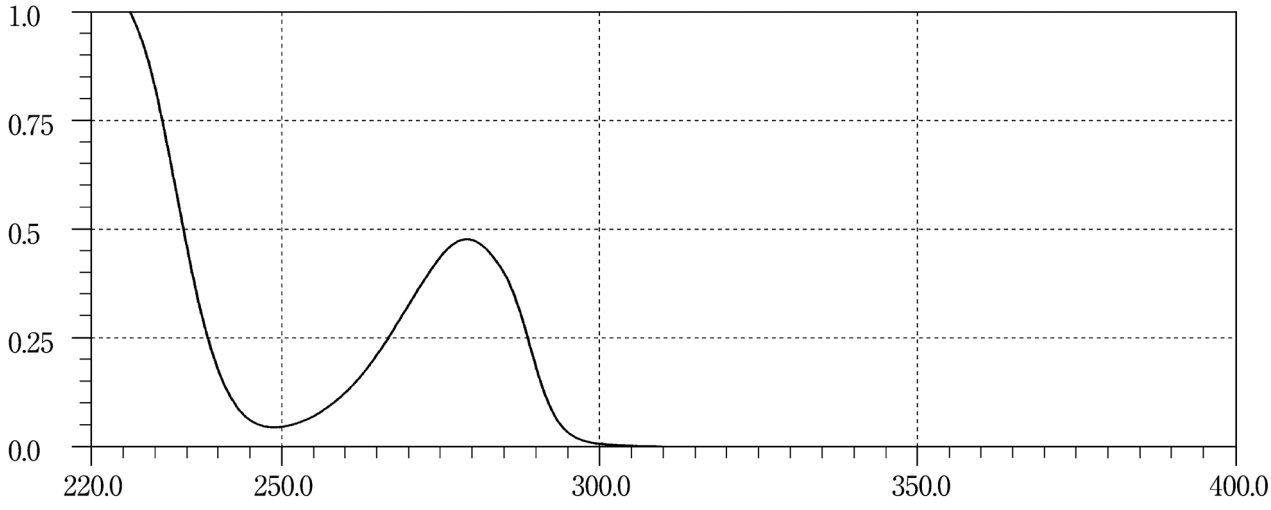


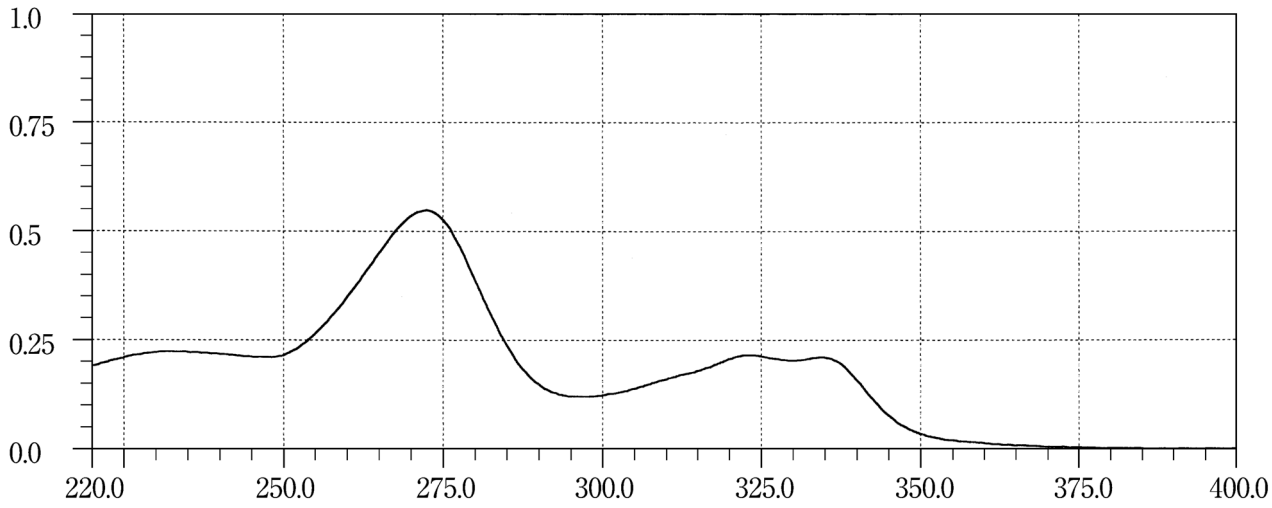
Nizatidine



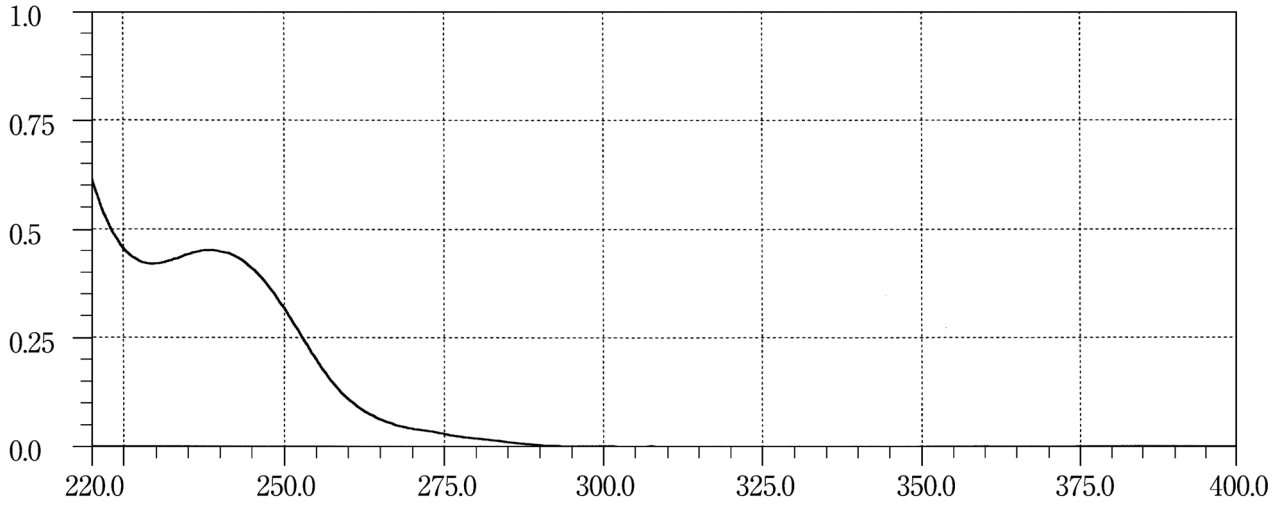
Noradrenaline



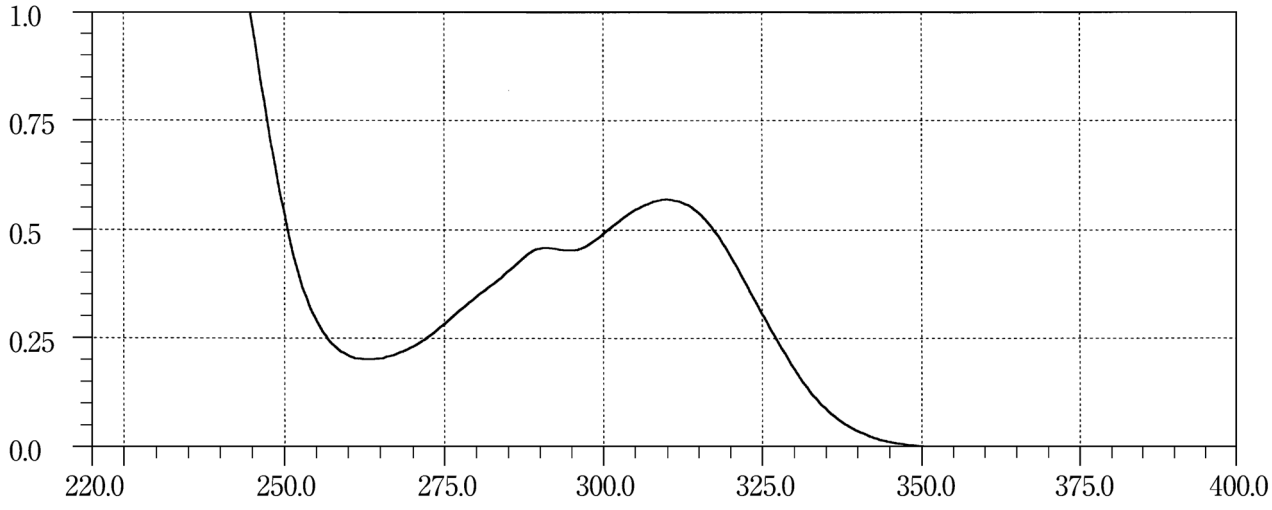
Norfloxacin



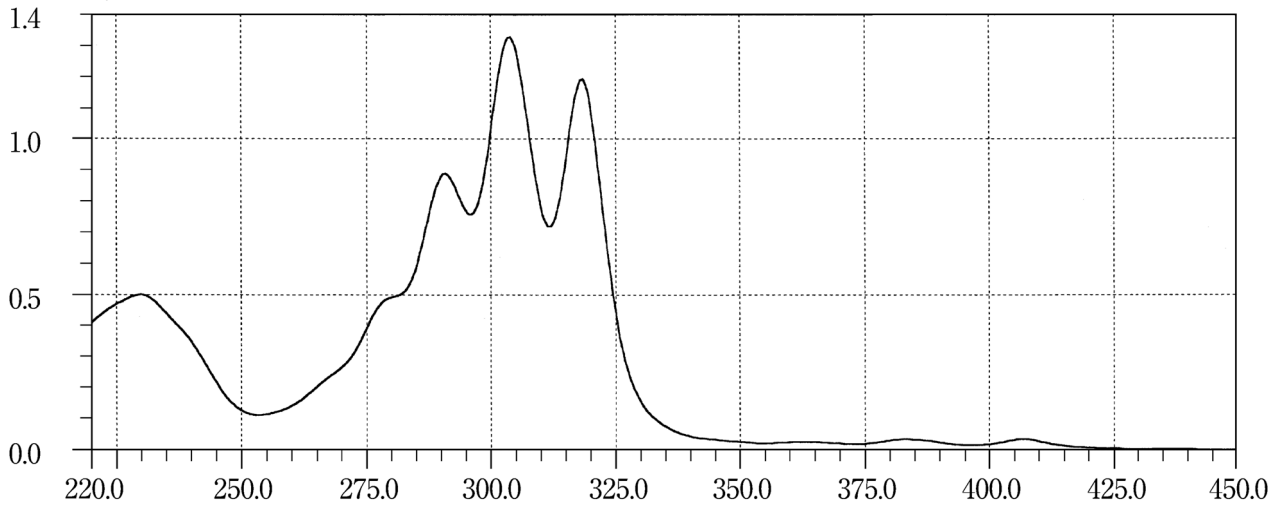
Nortriptyline Hydrochloride



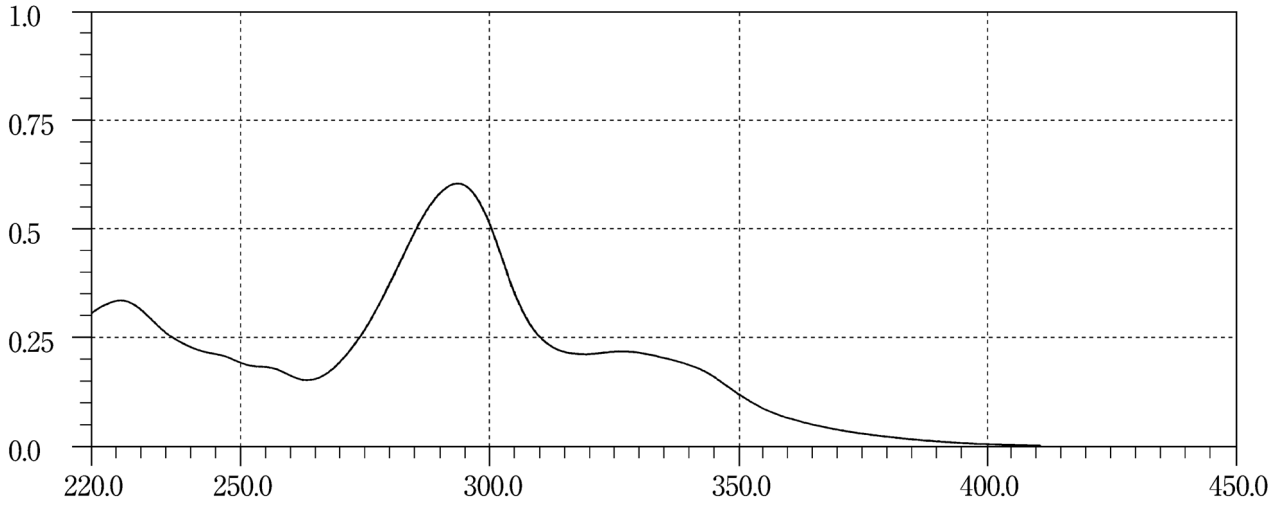
Noscapine



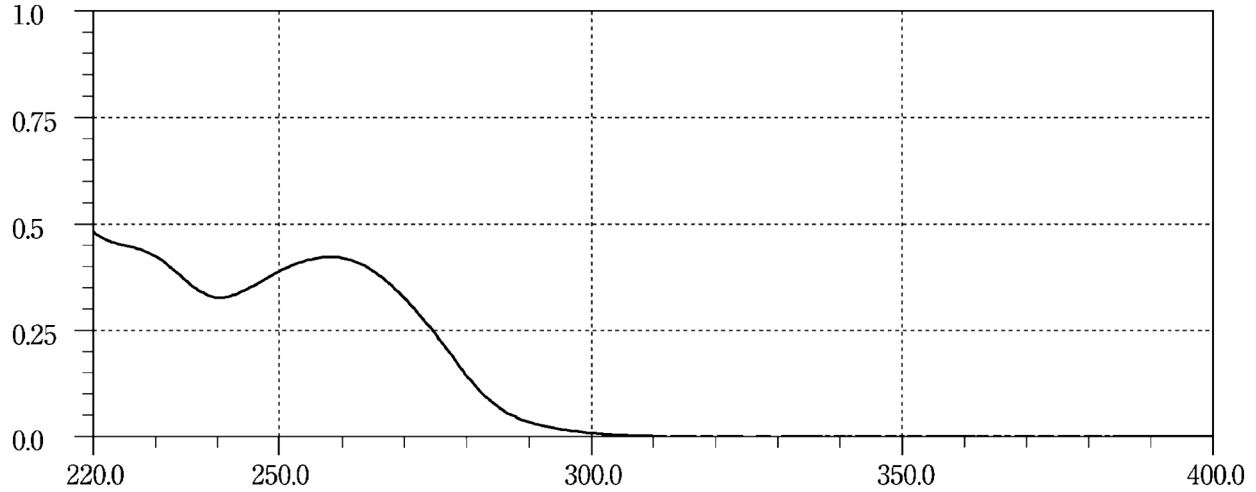
Nystatin



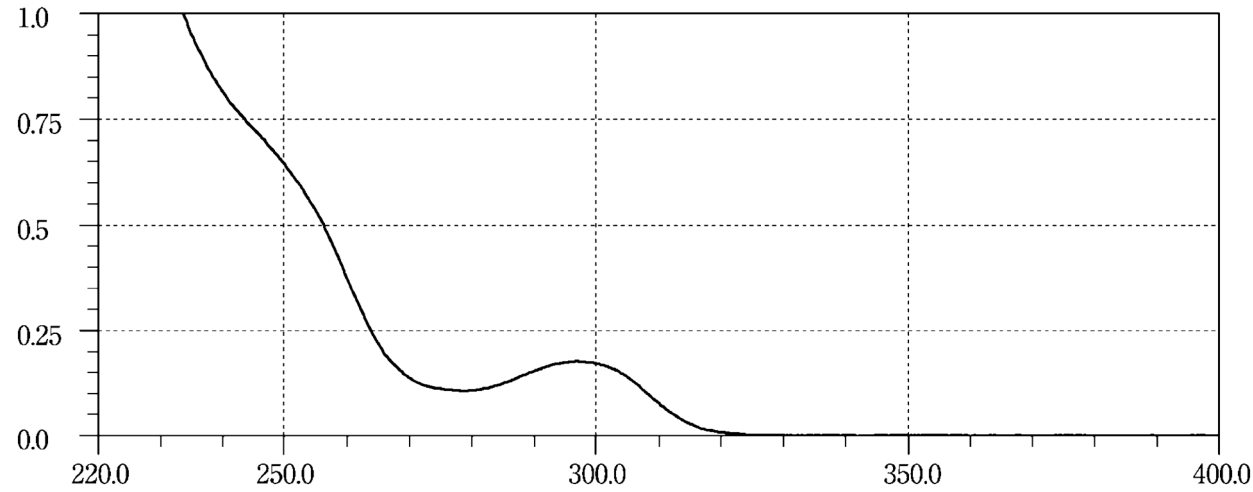
Ofloxacin



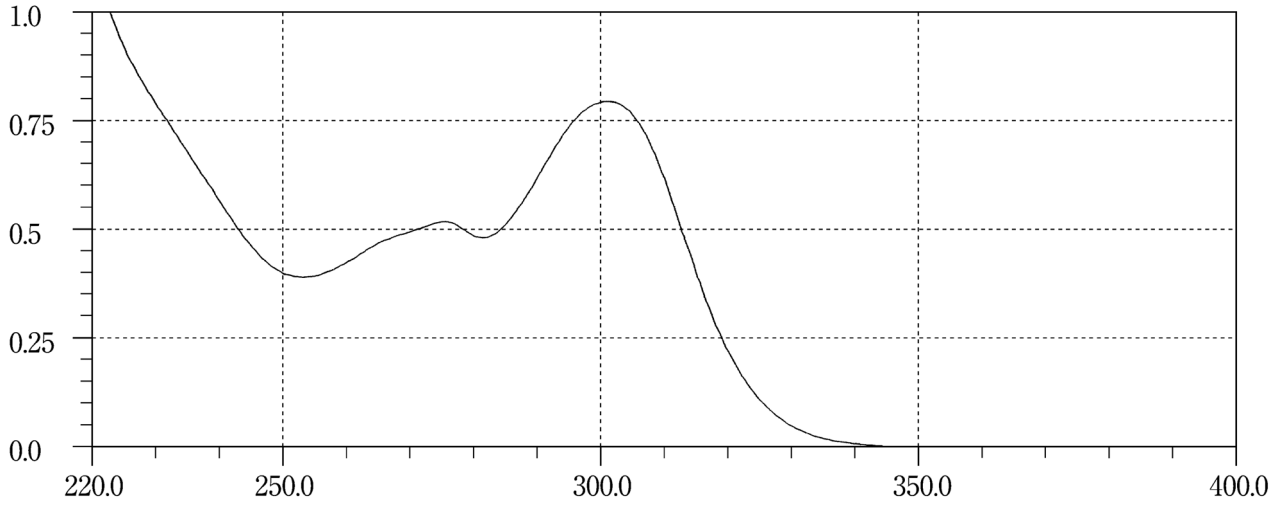
Olmesartan Medoxomil



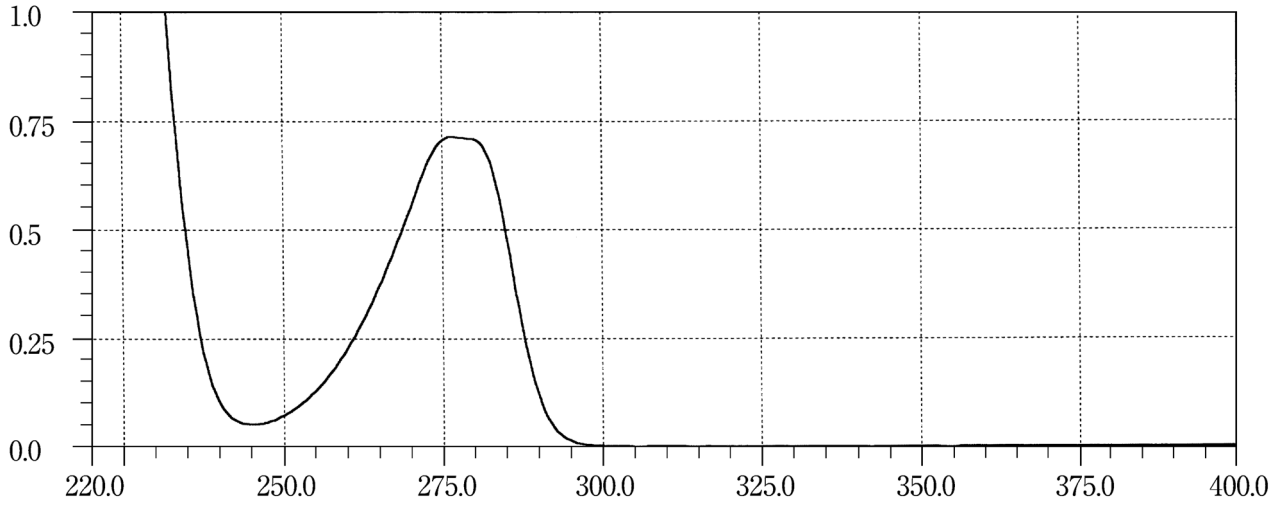
Olopatadine Hydrochloride



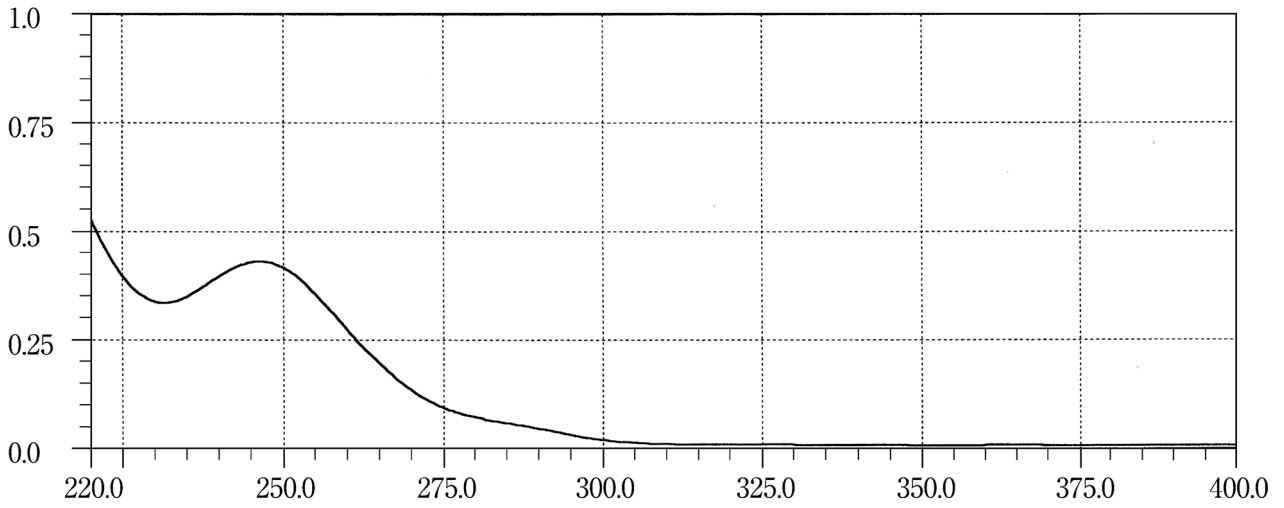
Omeprazole



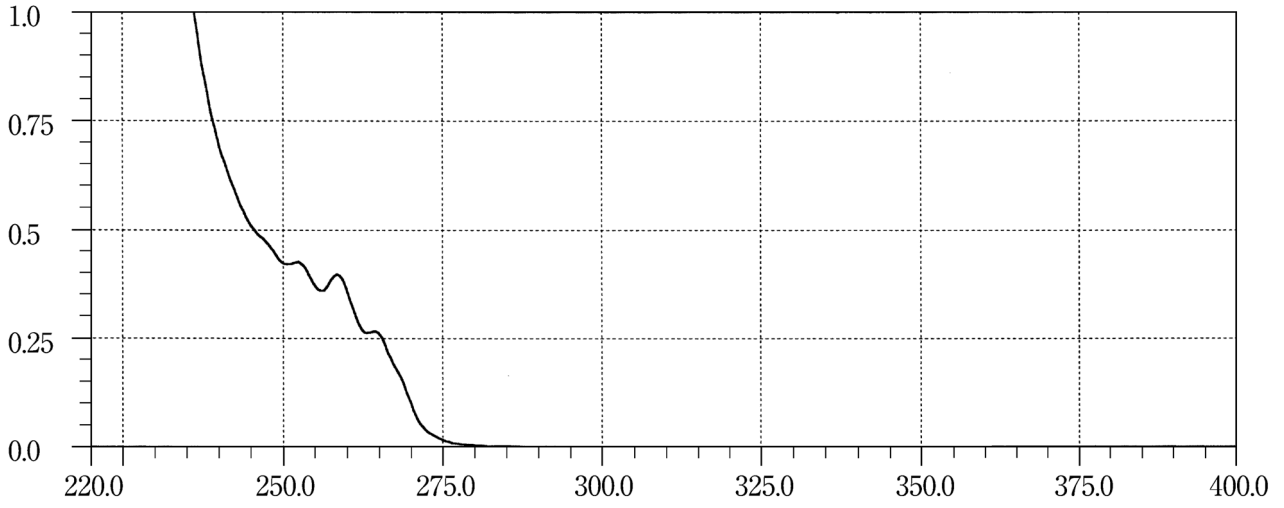
Orciprenaline Sulfate



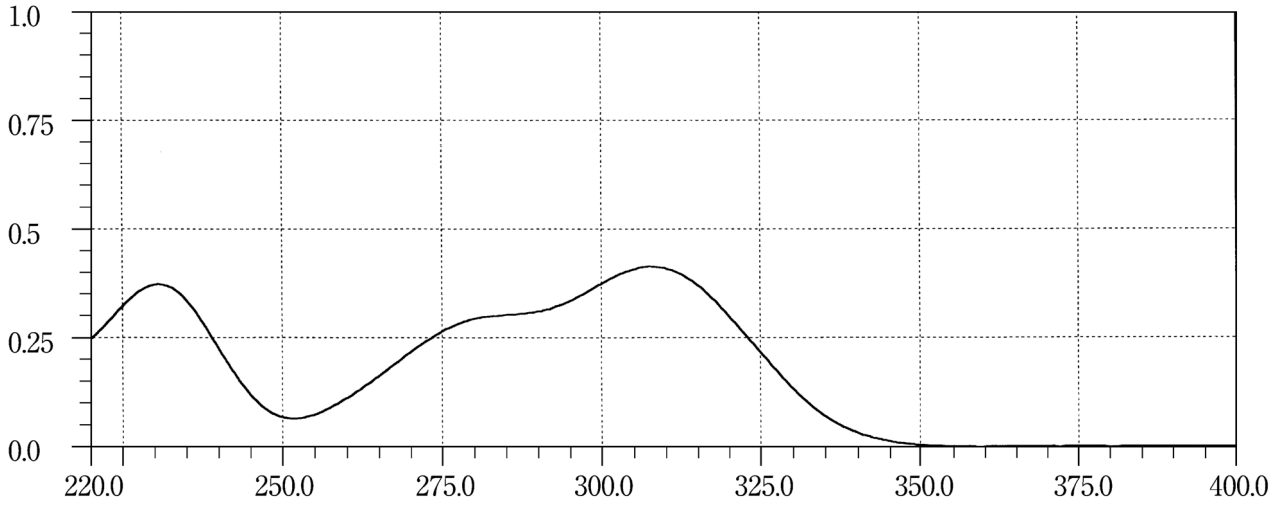
Oxazolam



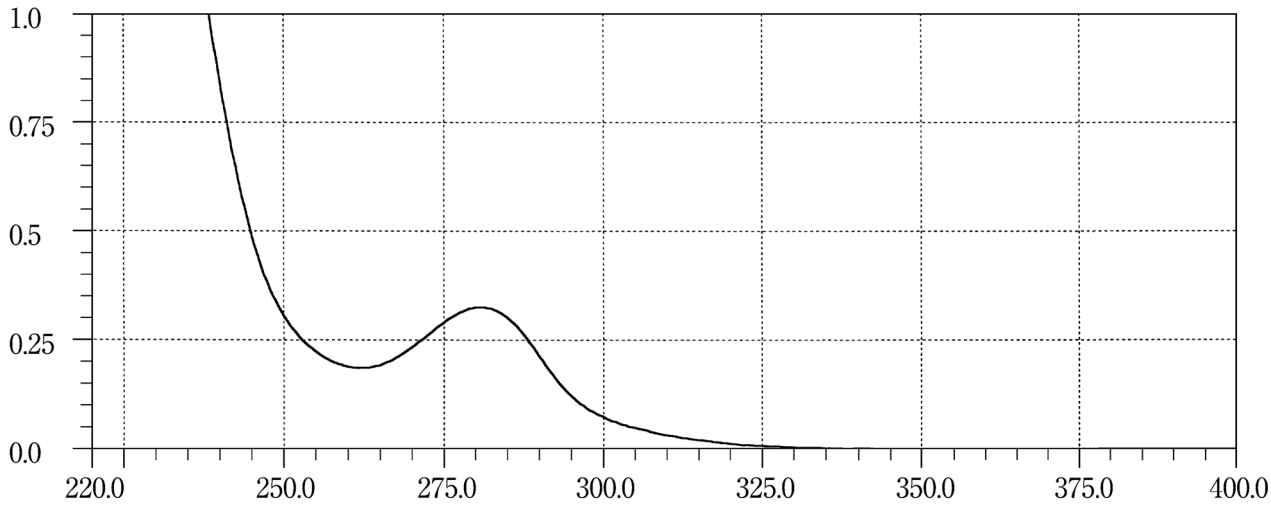
Oxethazaine



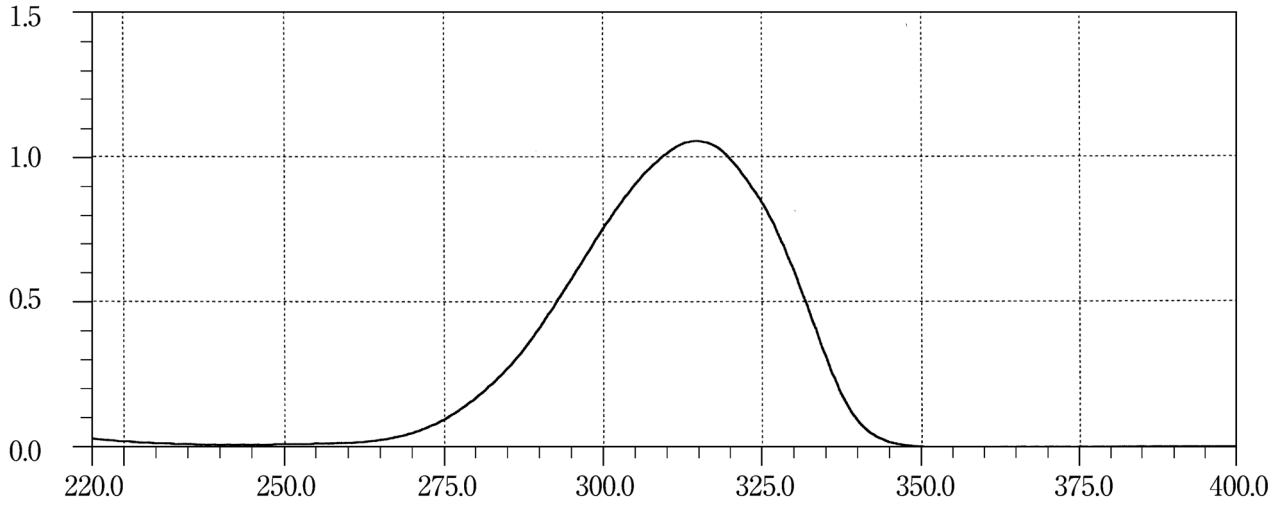
Oxybuprocaine Hydrochloride



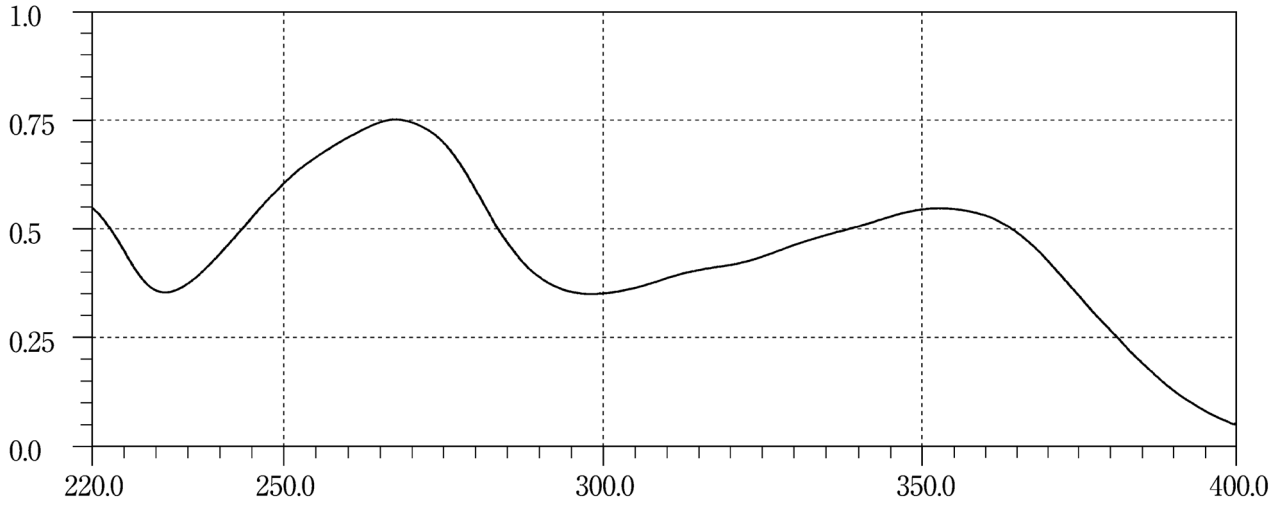
Oxycodone Hydrochloride Hydrate



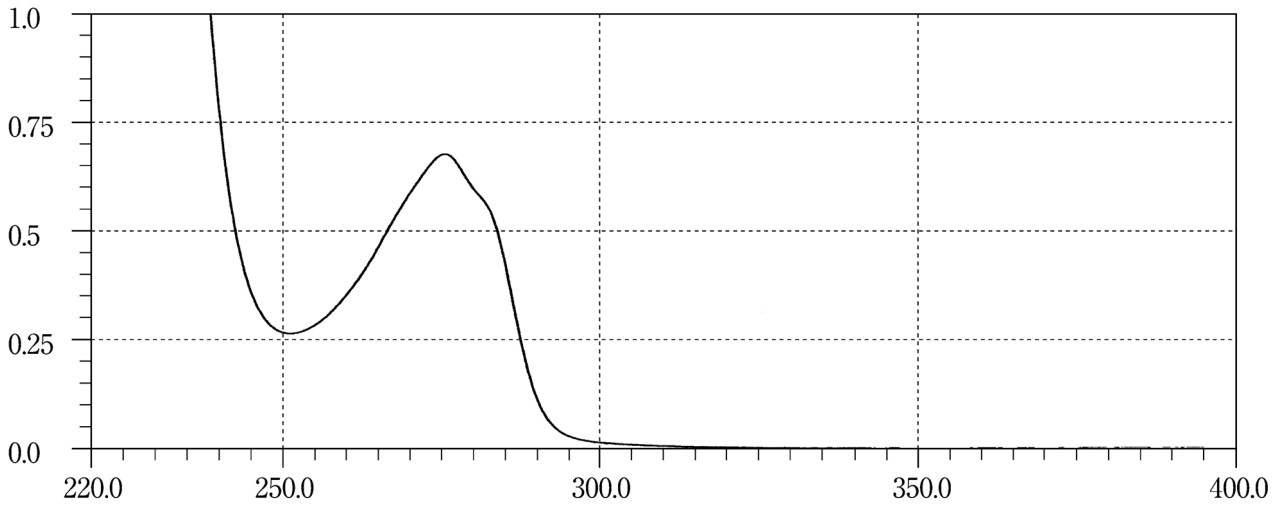
Oxymetholone



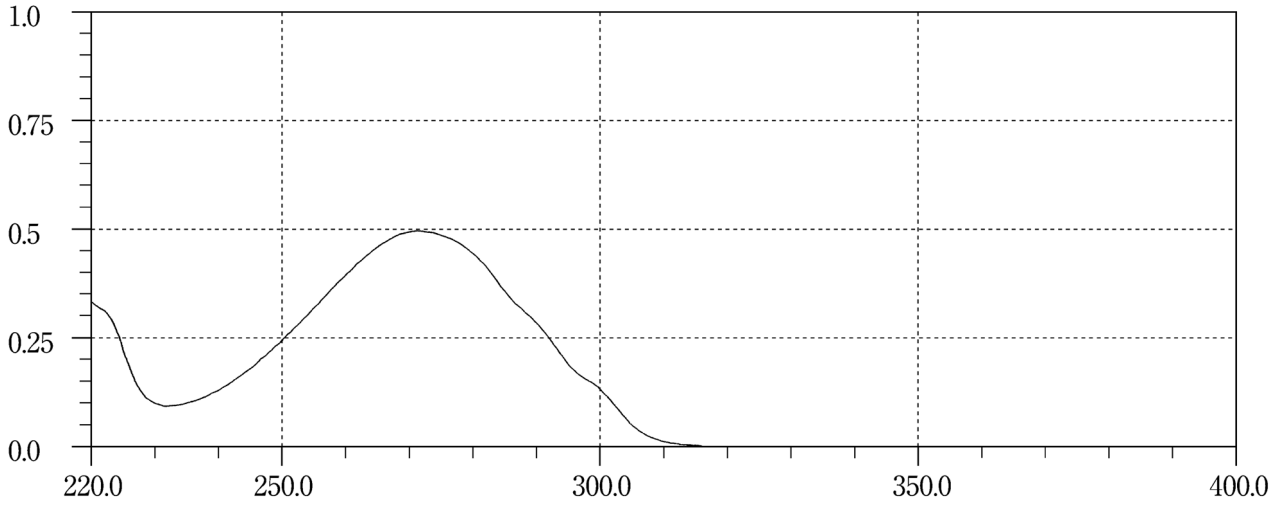
Oxytetracycline Hydrochloride



Oxytocin



Ozagrel Sodium



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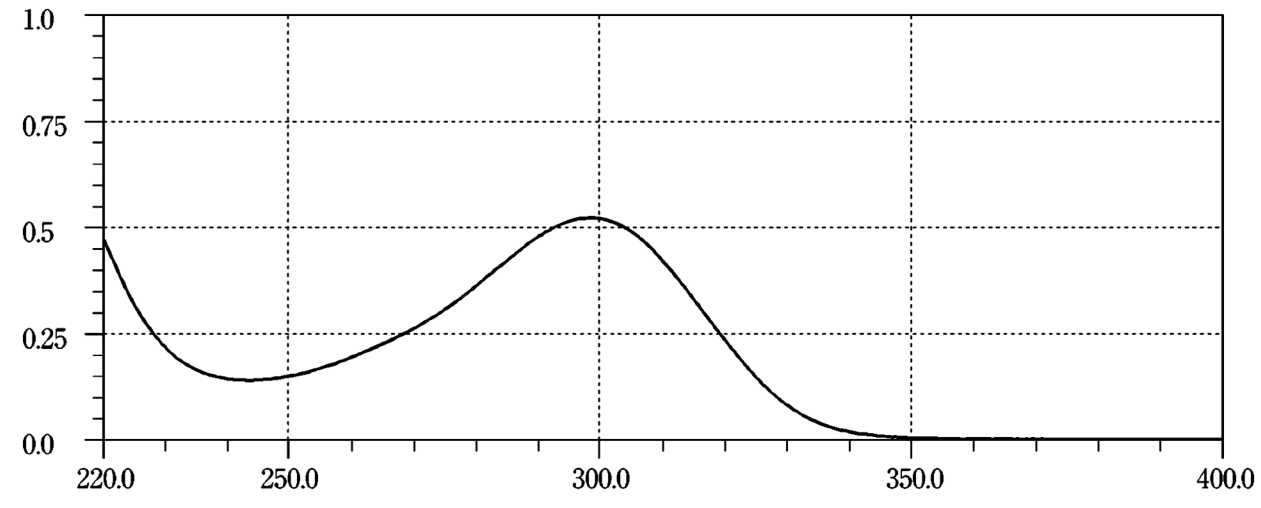
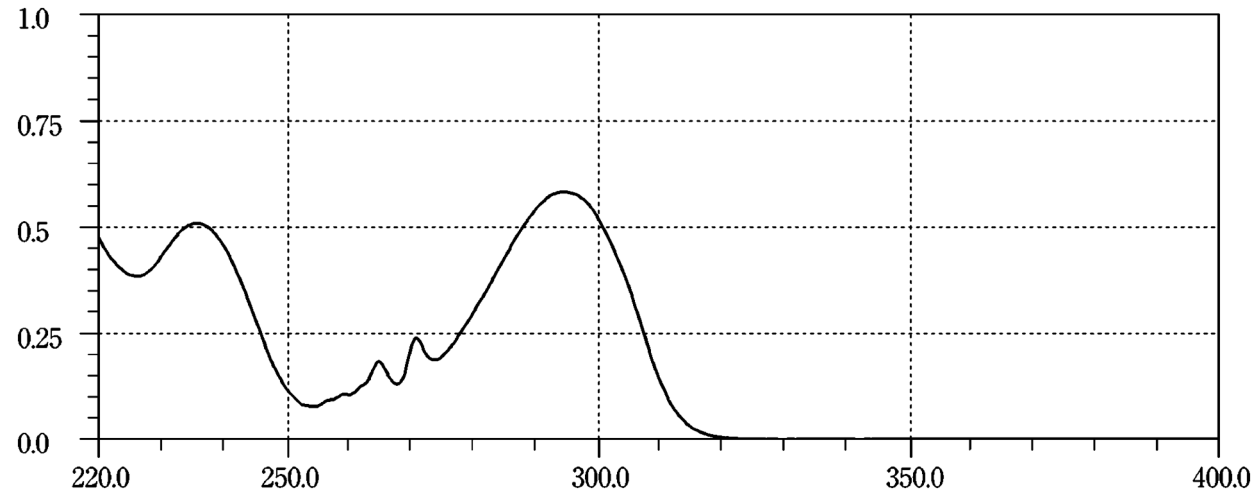
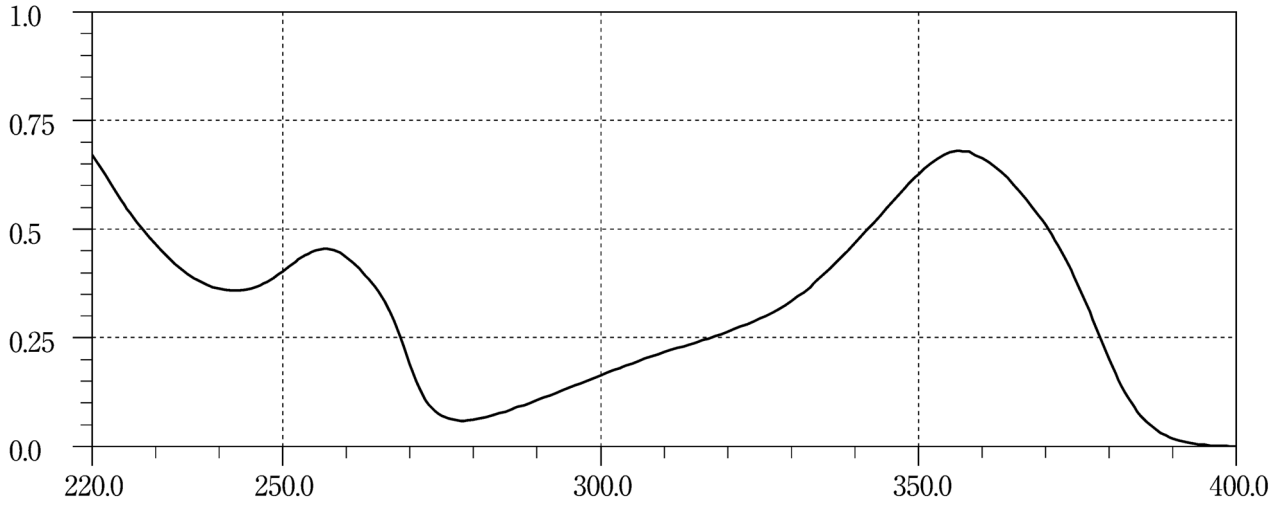


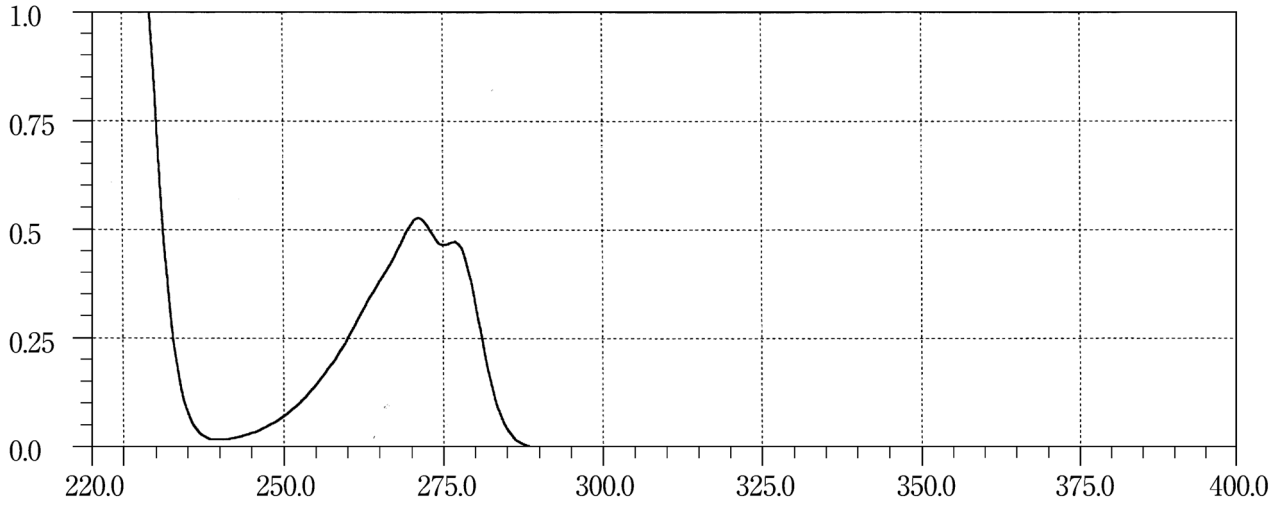
Paroxetine Hydrochloride Hydrate



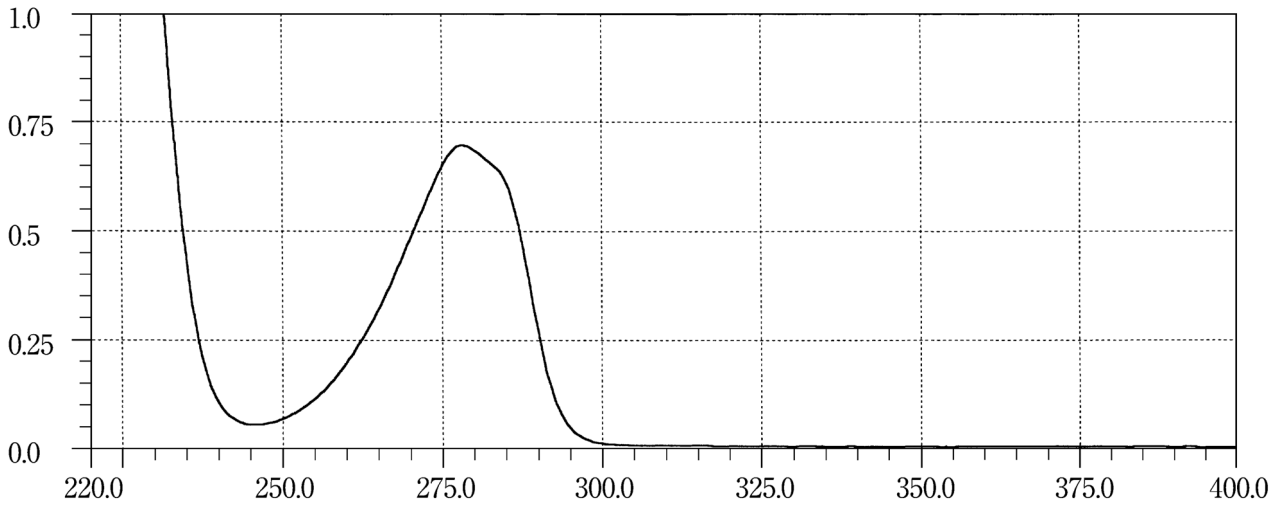
Pemirolast Potassium



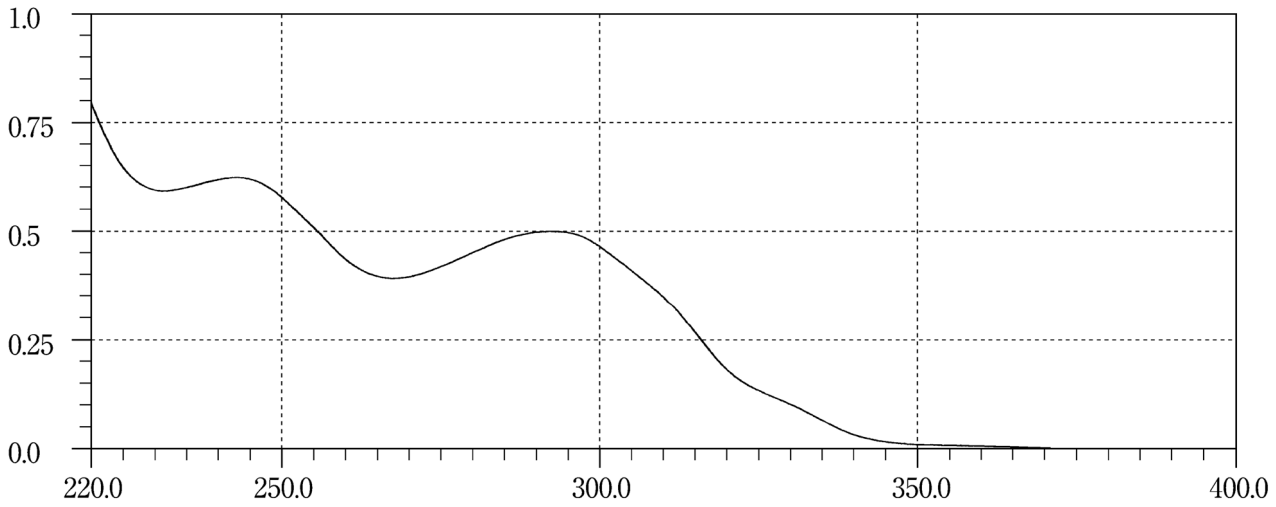
Penbutolol Sulfate



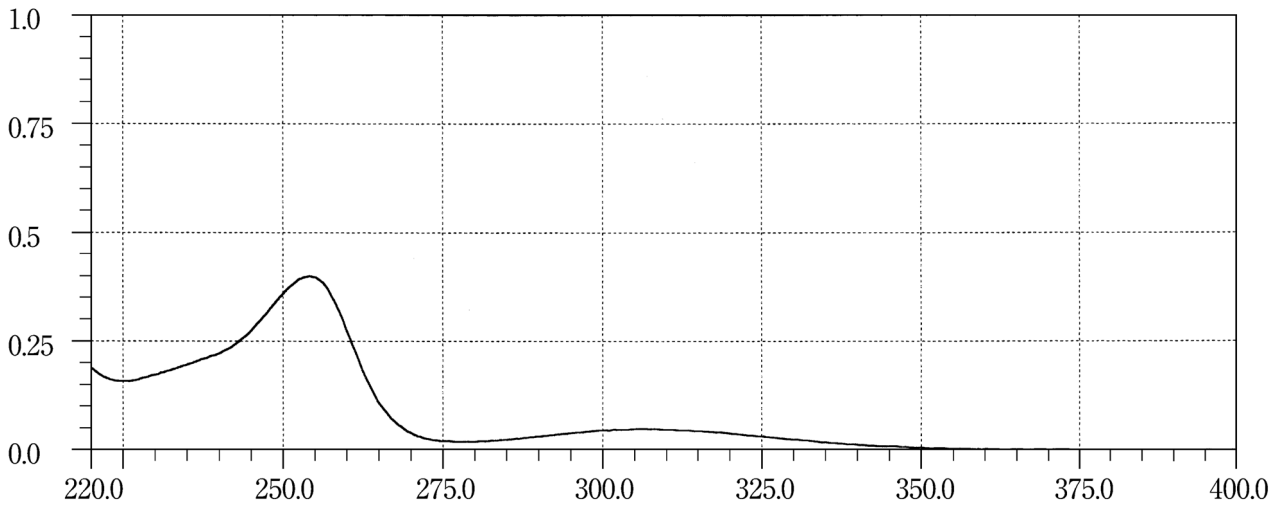
Pentazocine



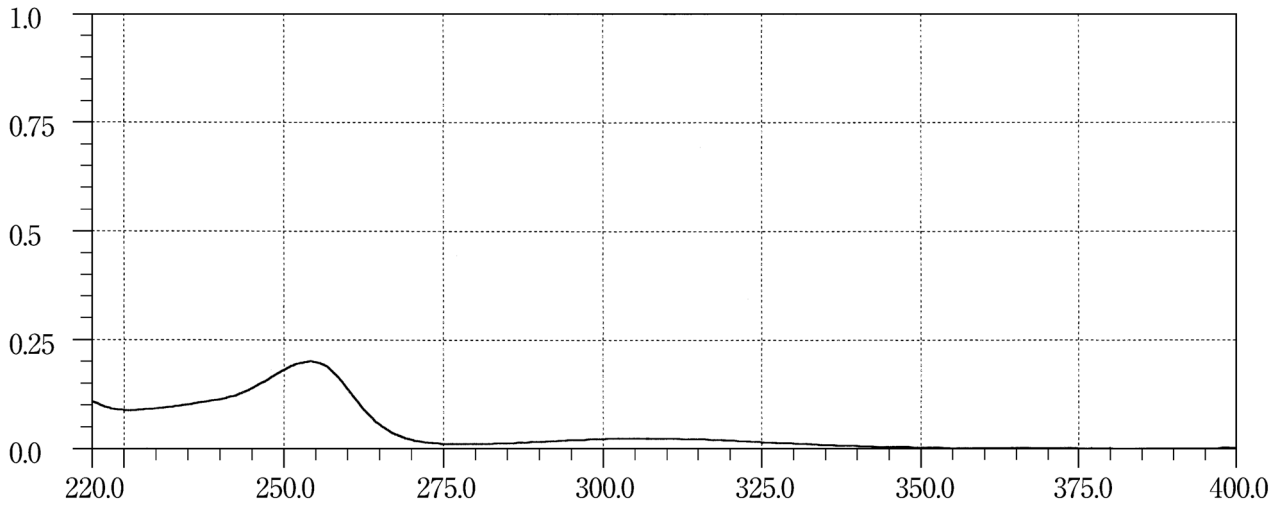
Peplomycin Sulfate



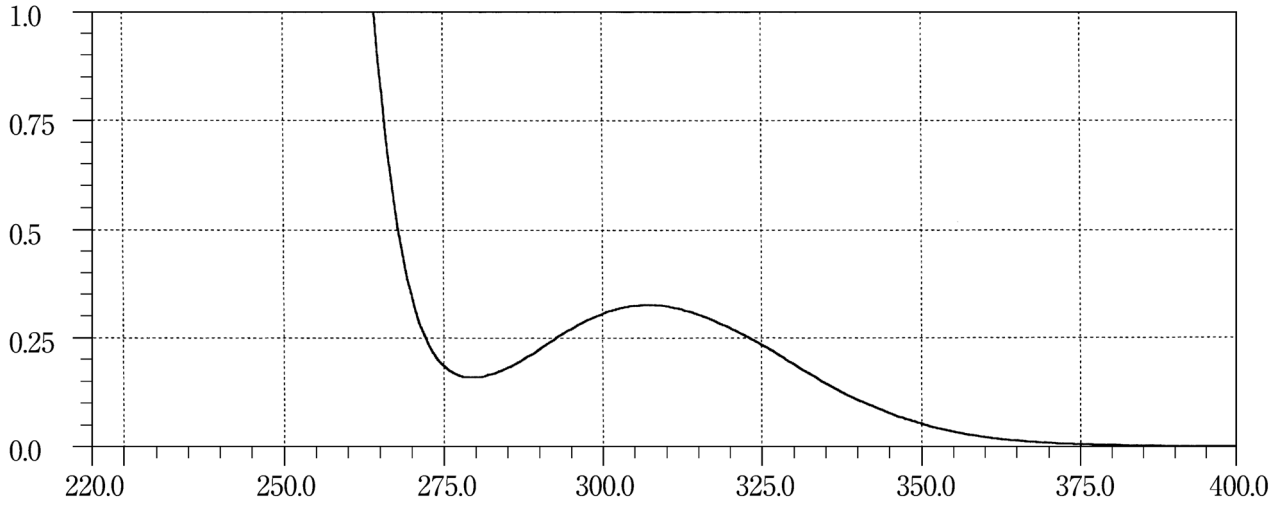
Perphenazine 1



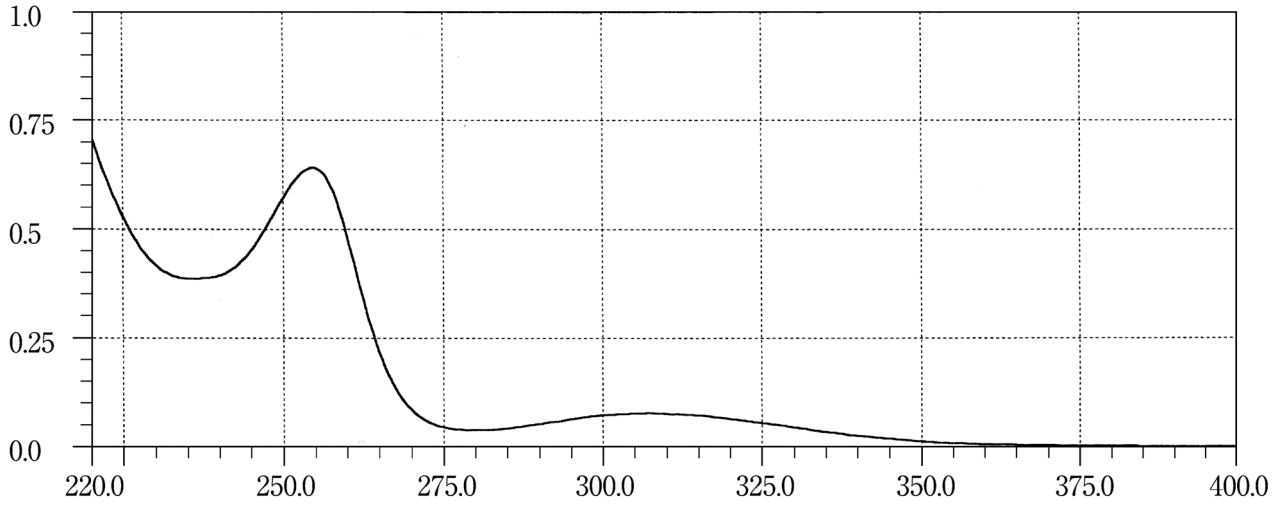
Perphenazine 2



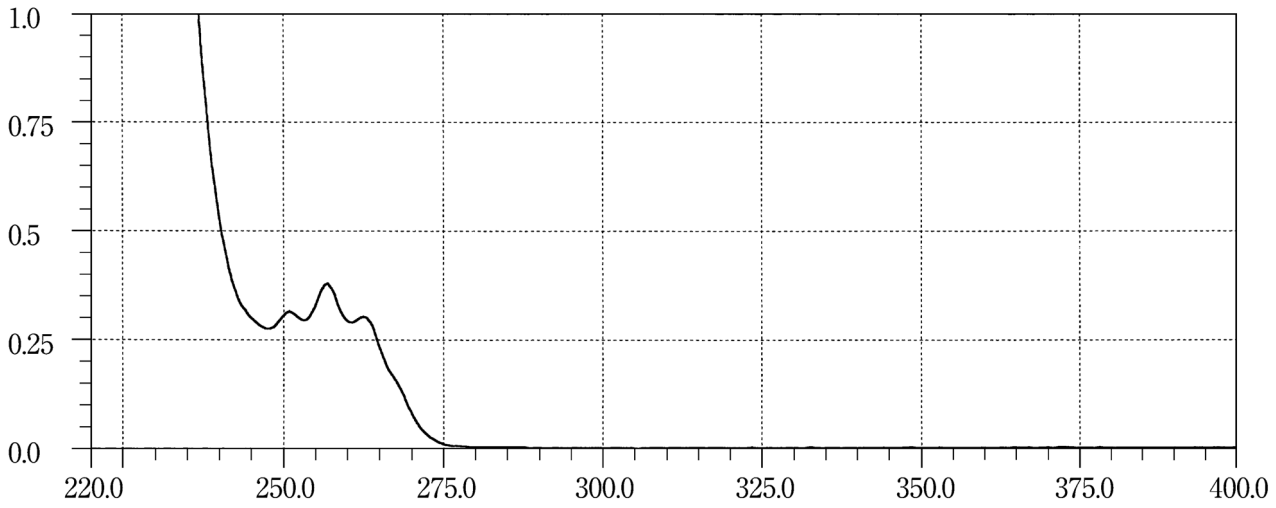
Perphenazine Maleate 1



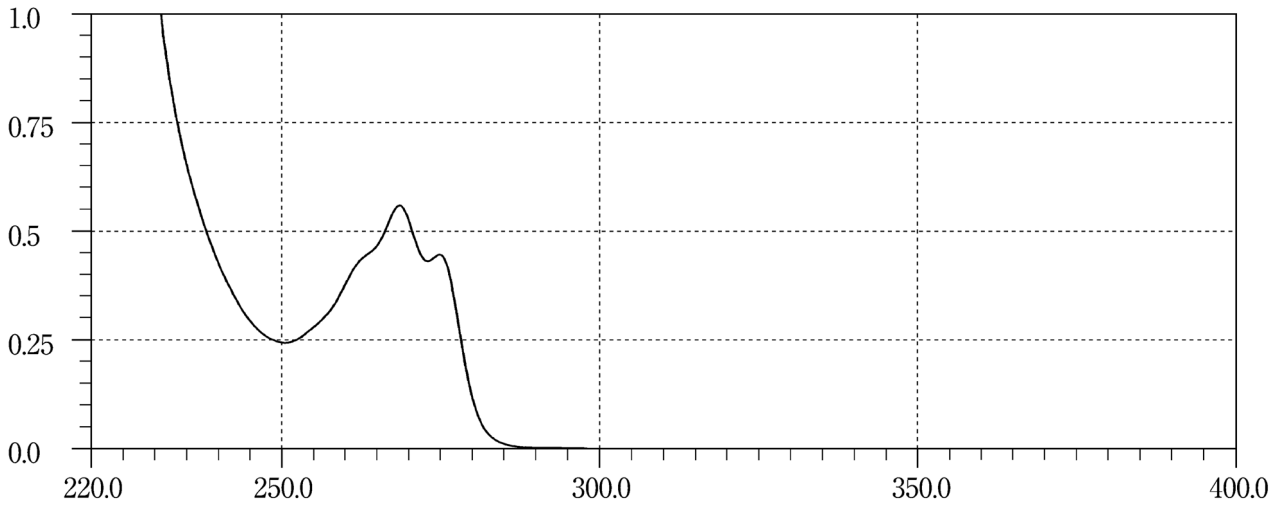
Perphenazine Maleate 2



Pethidine Hydrochloride



Phenethicillin Potassium



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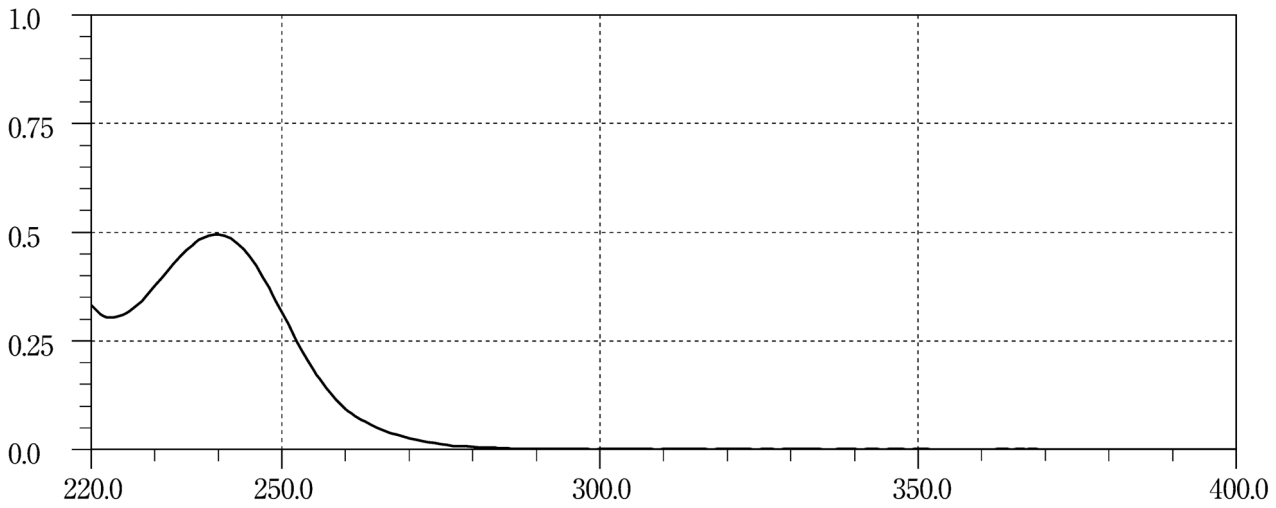
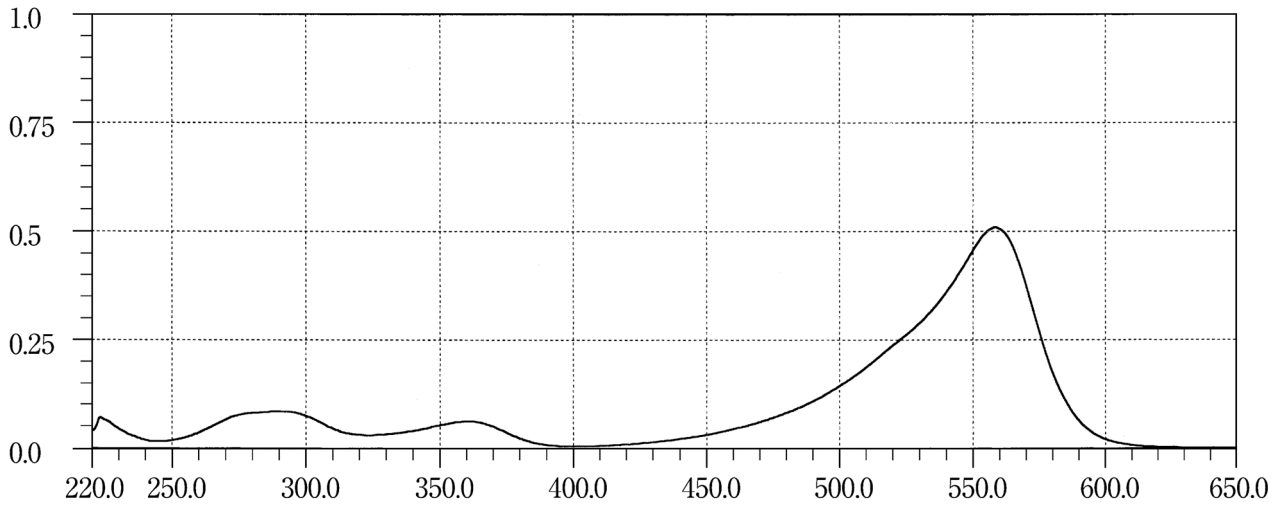
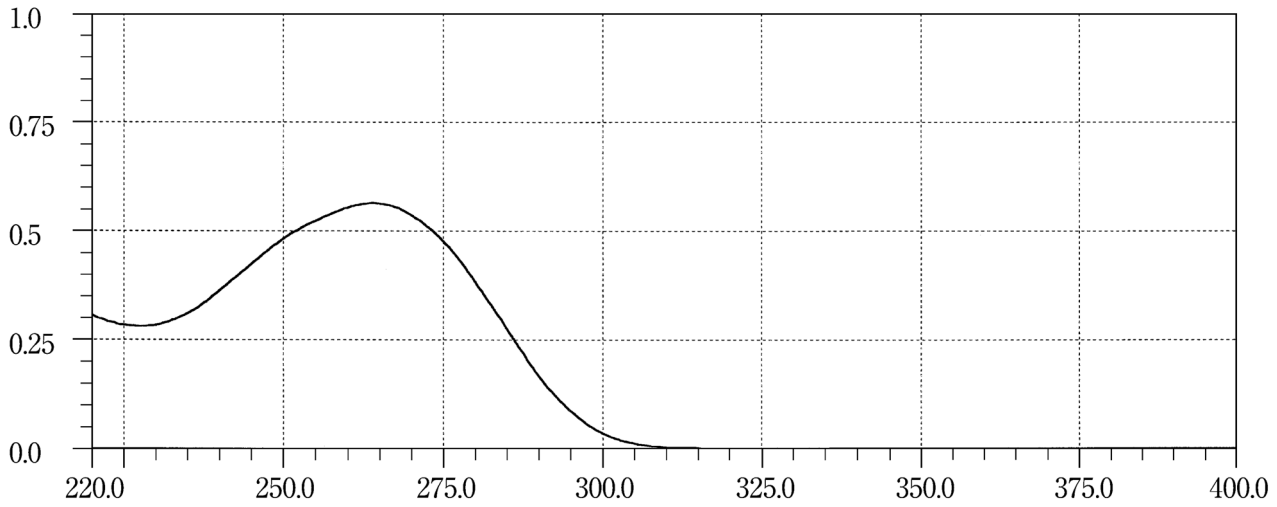


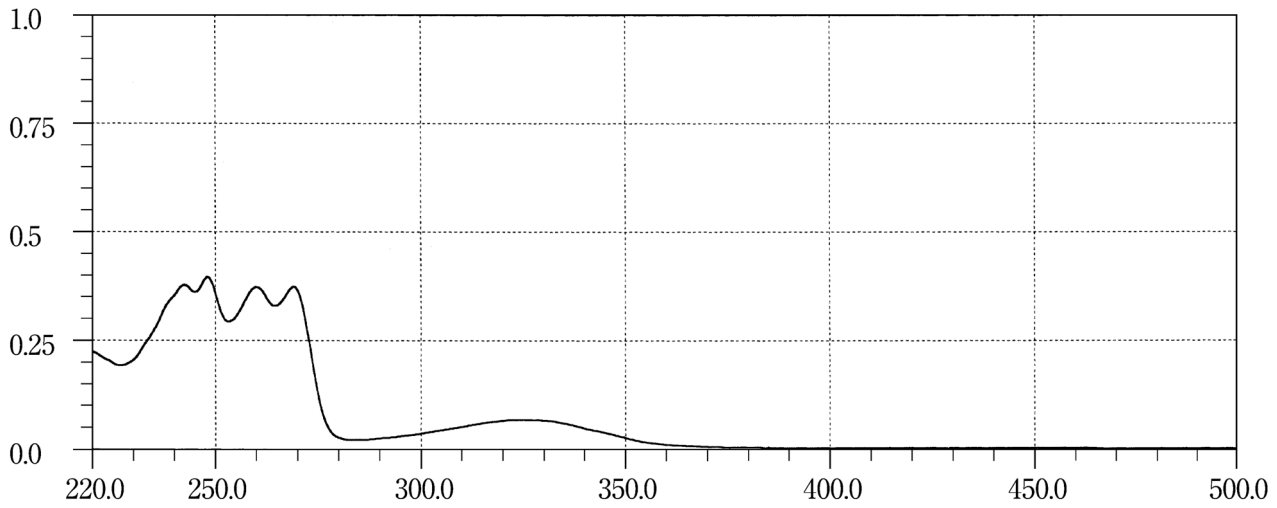
Phenolsulfonphthalein



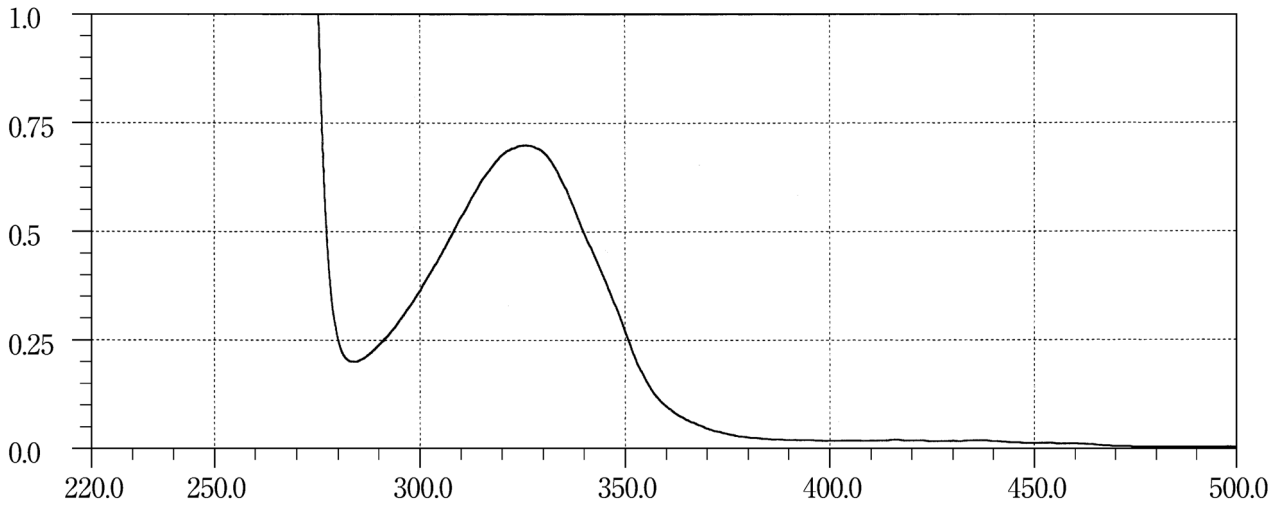
Phenylbutazone



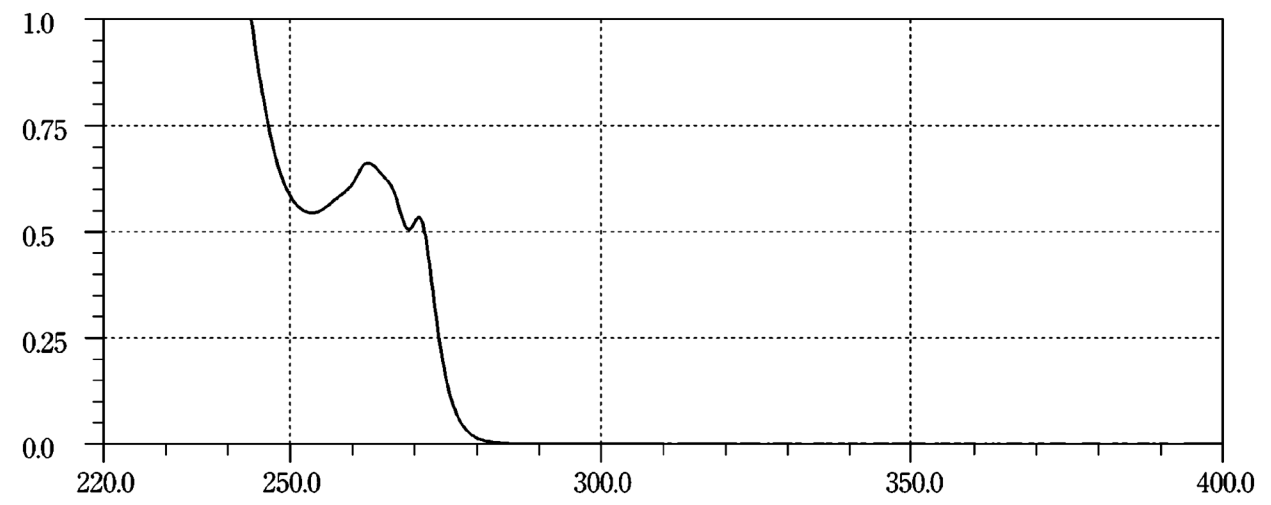
Phytonadione 1



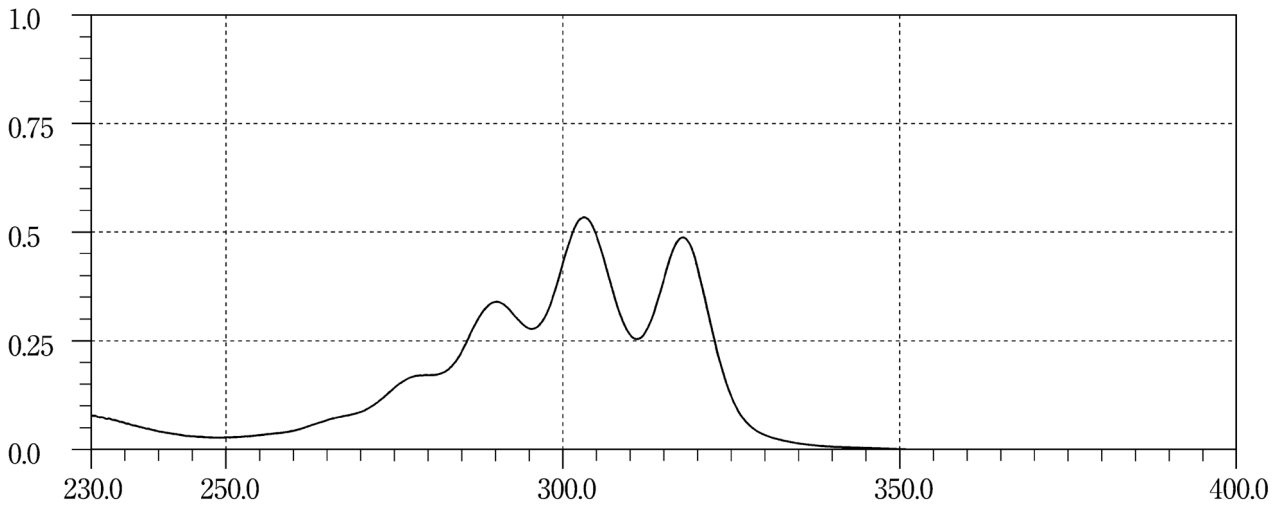
Phytonadione 2



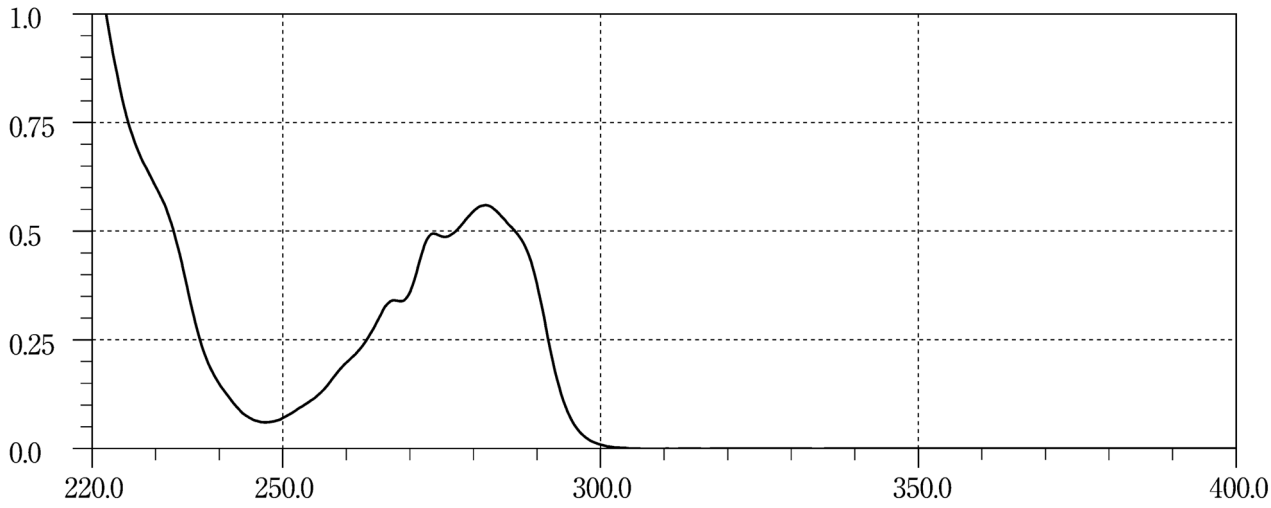
Pilsicainide Hydrochloride Hydrate



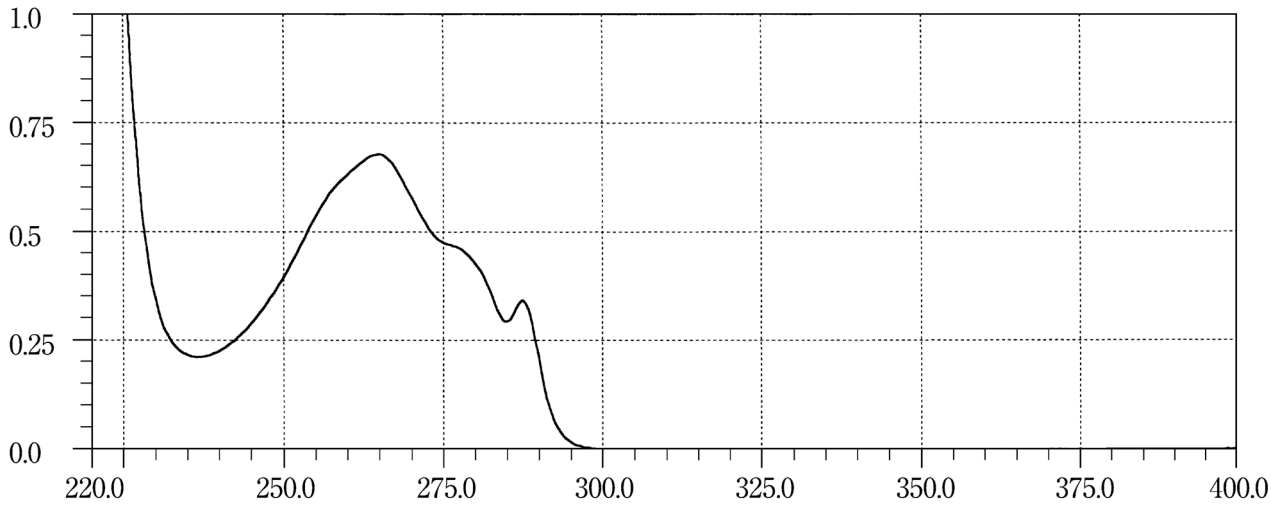
Pimaricin



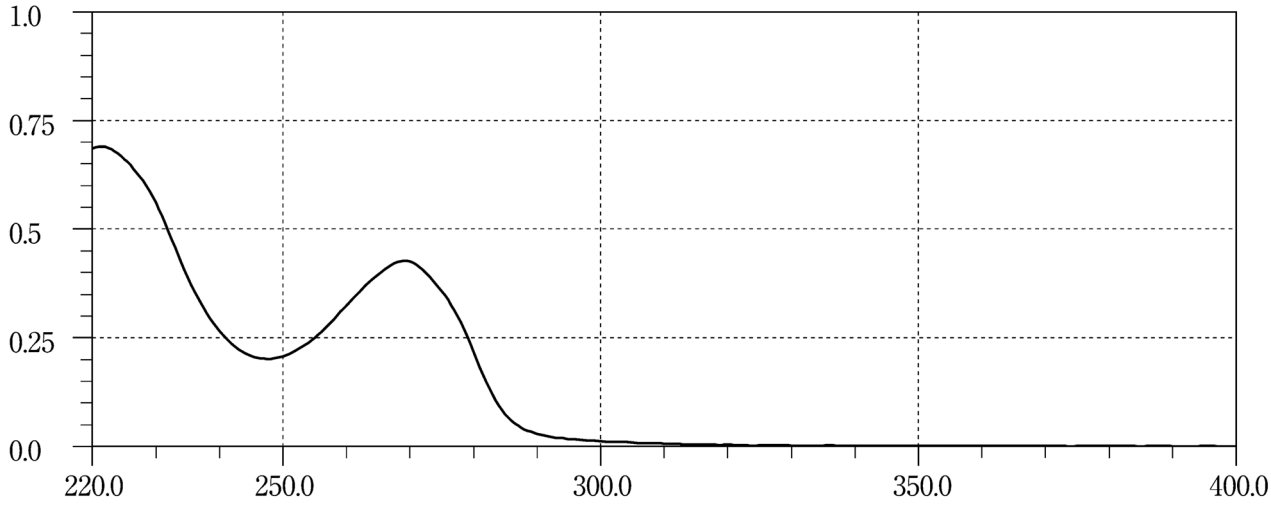
Pimozide



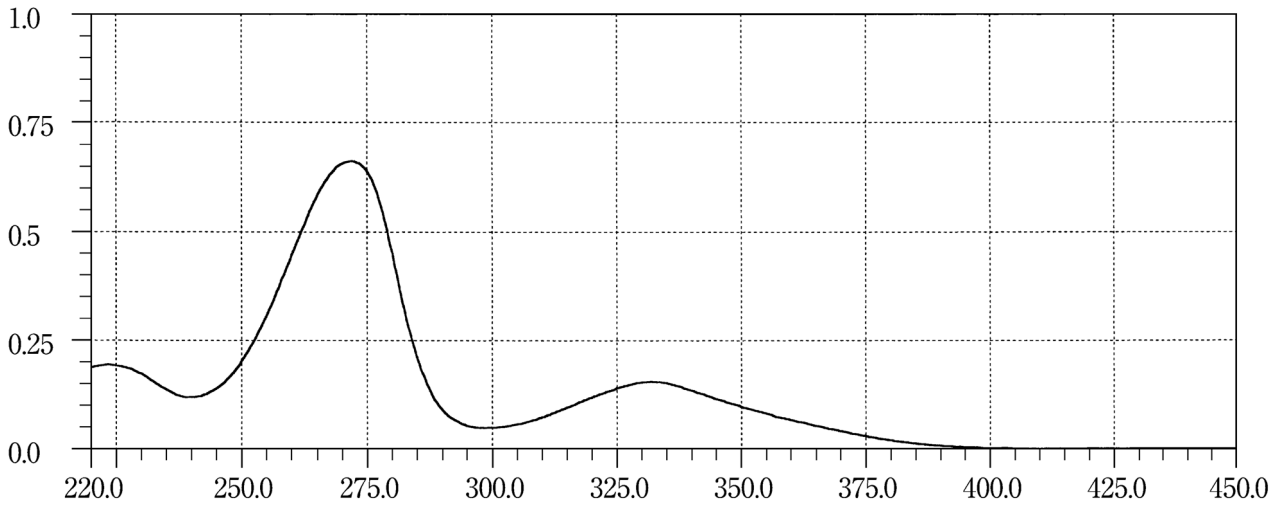
Pindolol



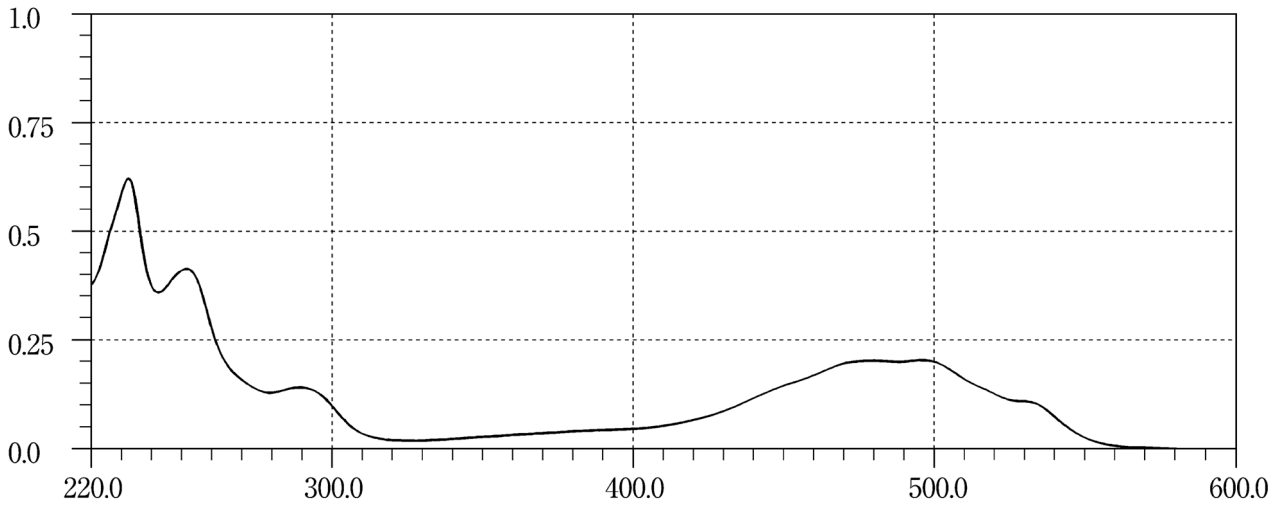
Pioglitazone Hydrochloride



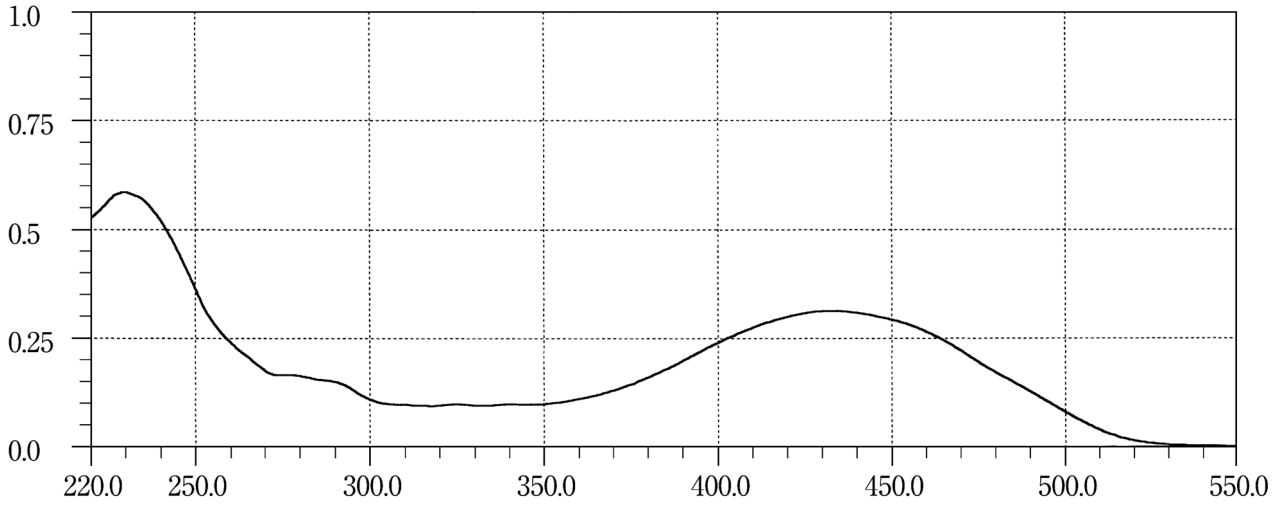
Pipemidic Acid Hydrate



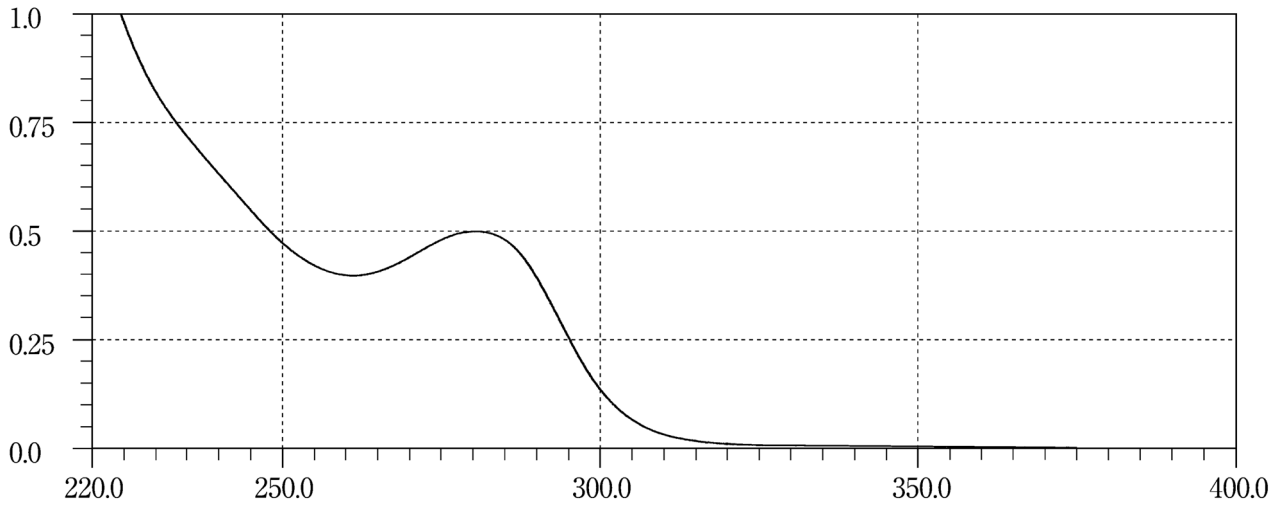
Pirarubicin



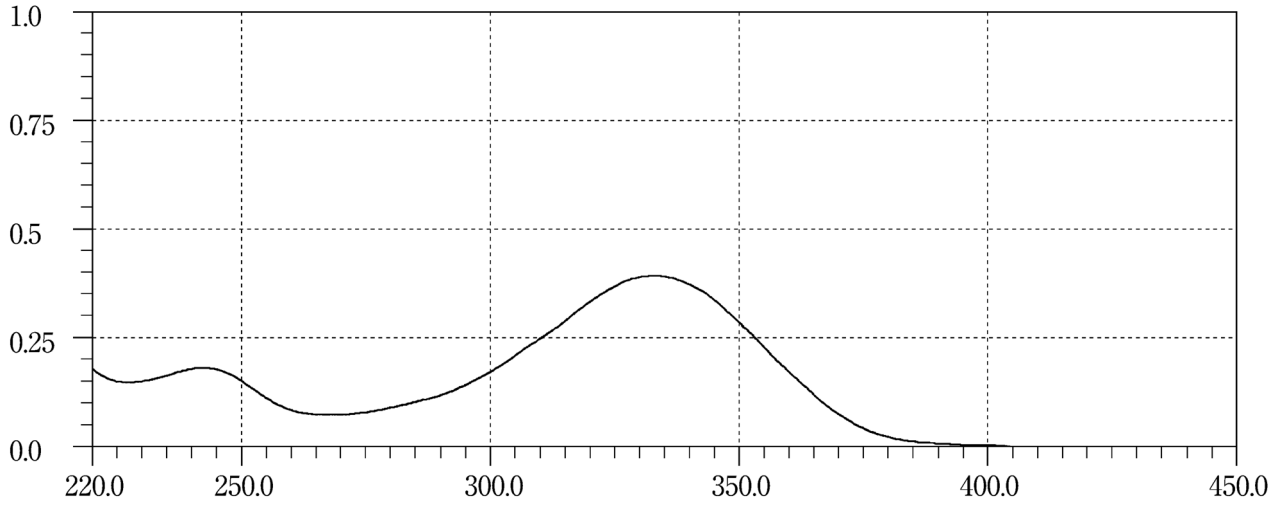
Pirenoxine



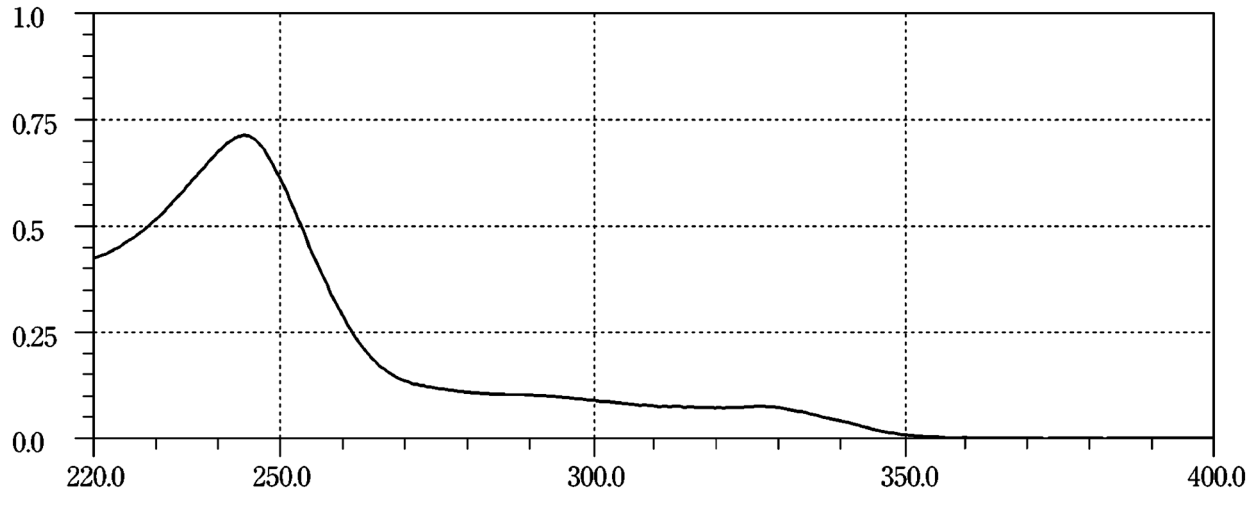
Pirenzepine Hydrochloride Hydrate



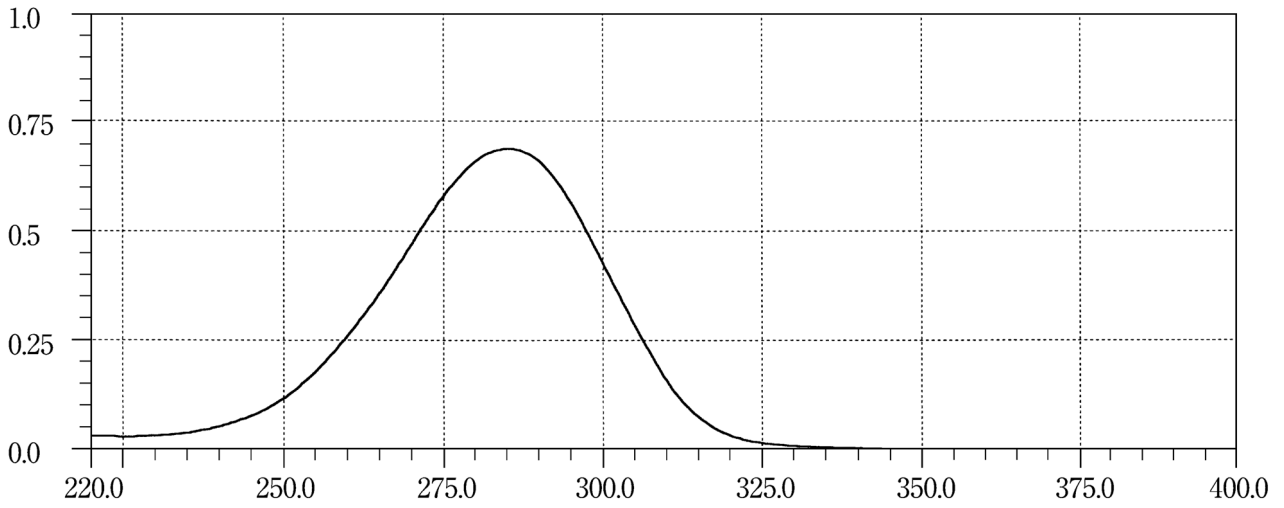
Piroxicam



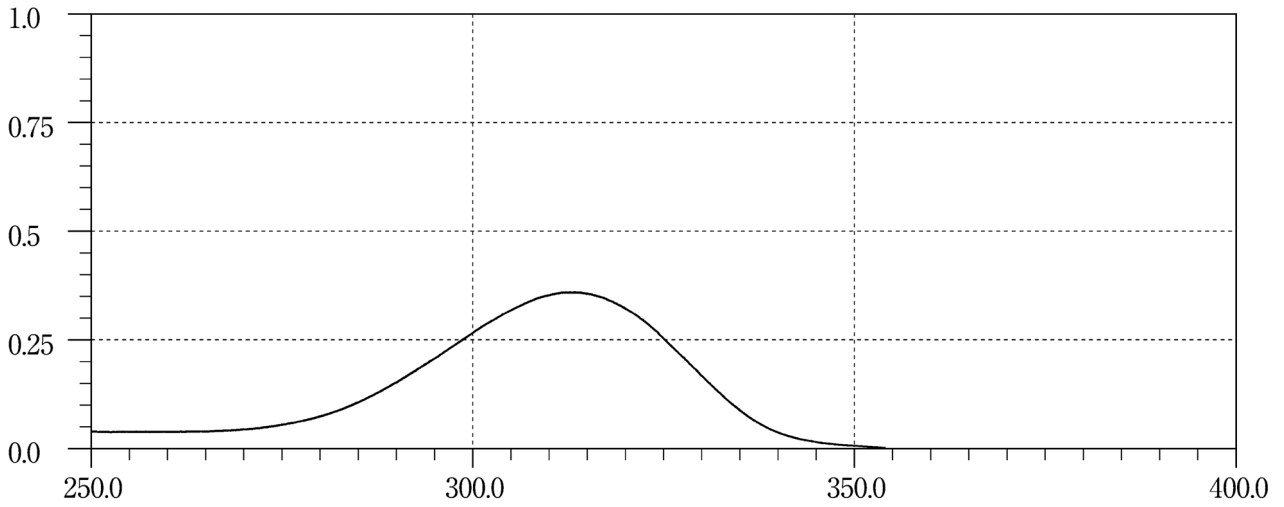
Pitavastatin Calcium Hydrate



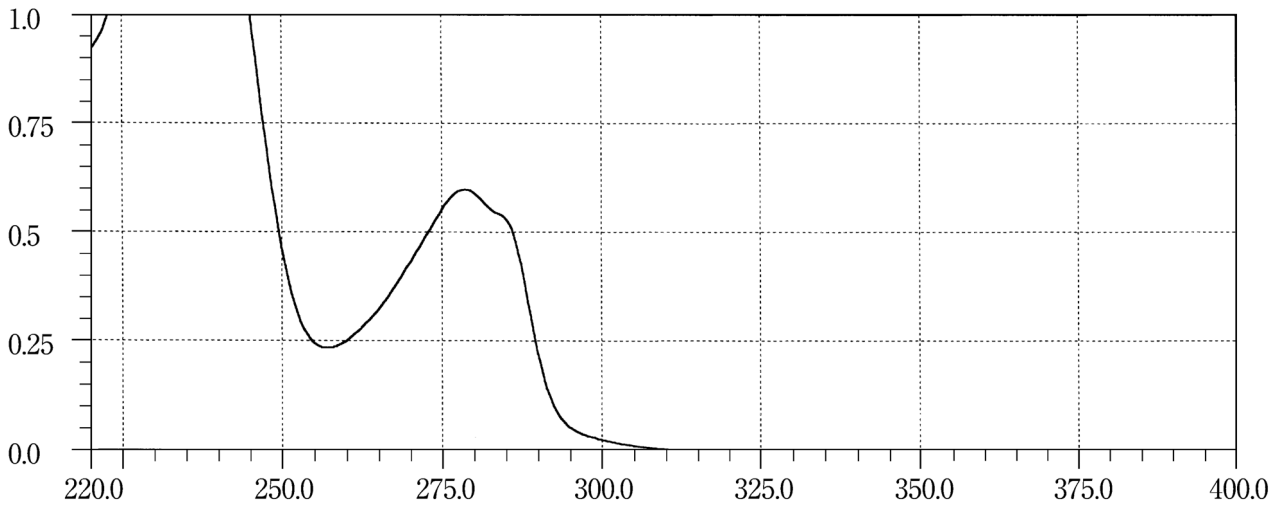
Potassium Canrenoate



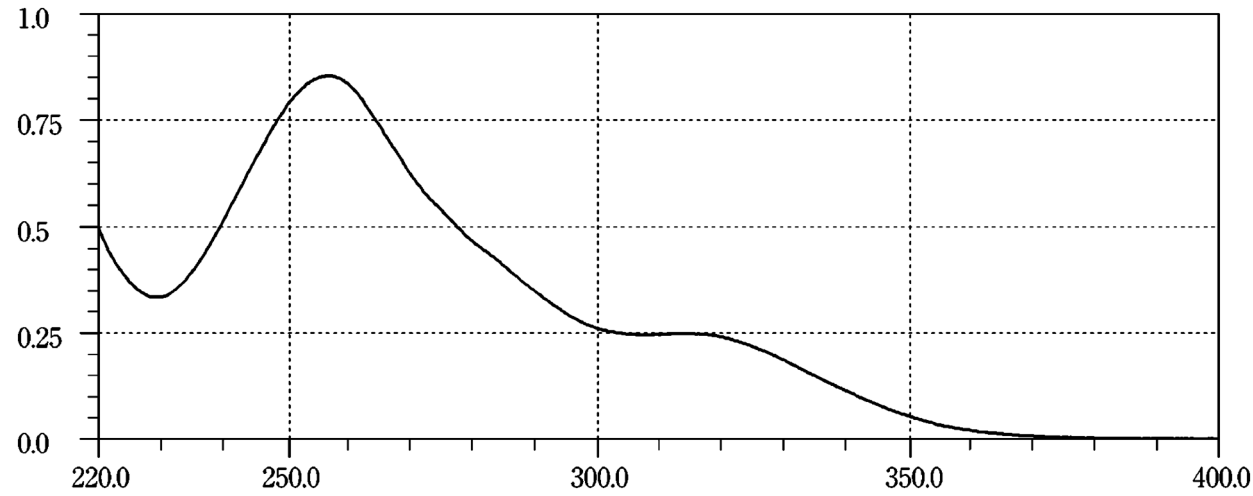
Potassium Clavulanate



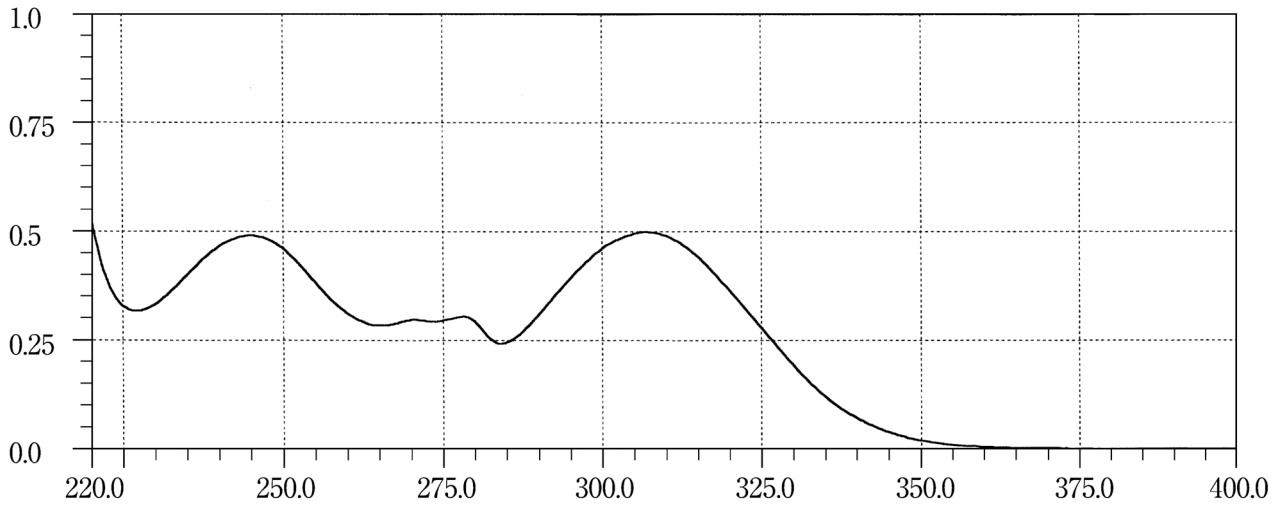
Potassium Guaiacolsulfonate



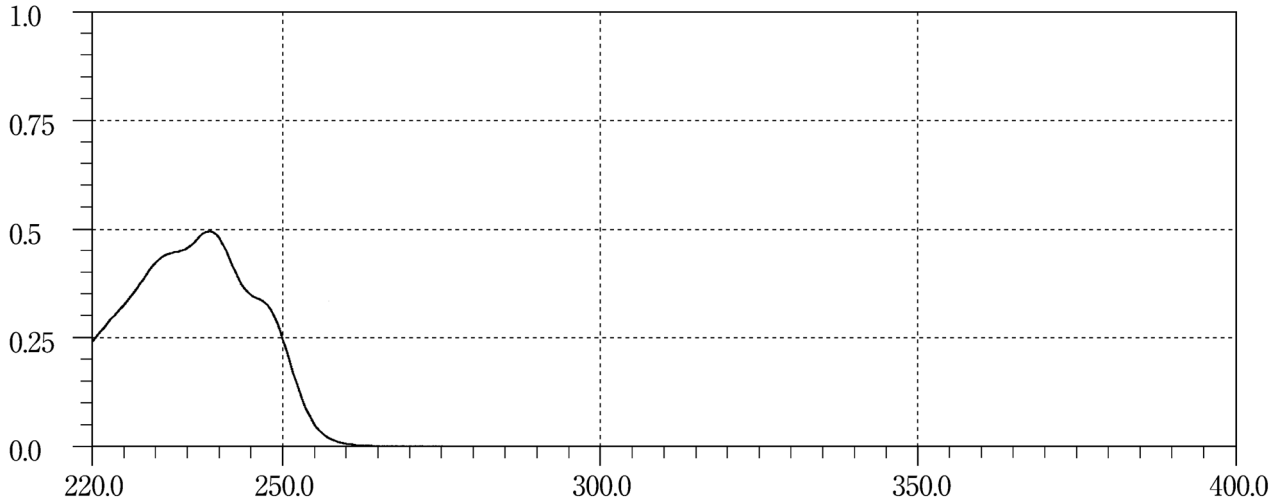
Pranlukast Hydrate



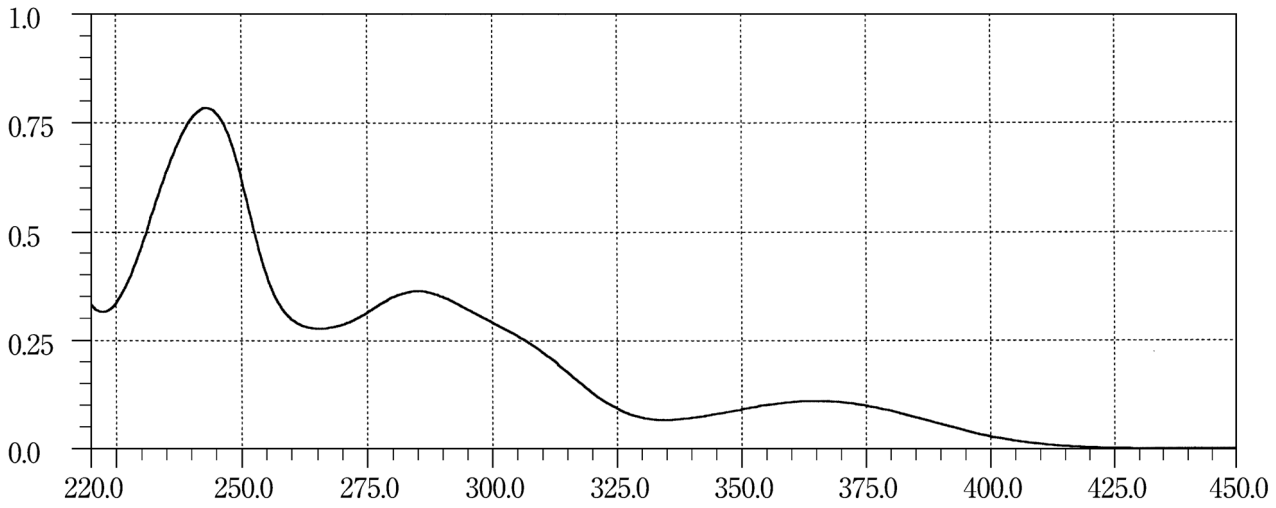
Pranoprofen



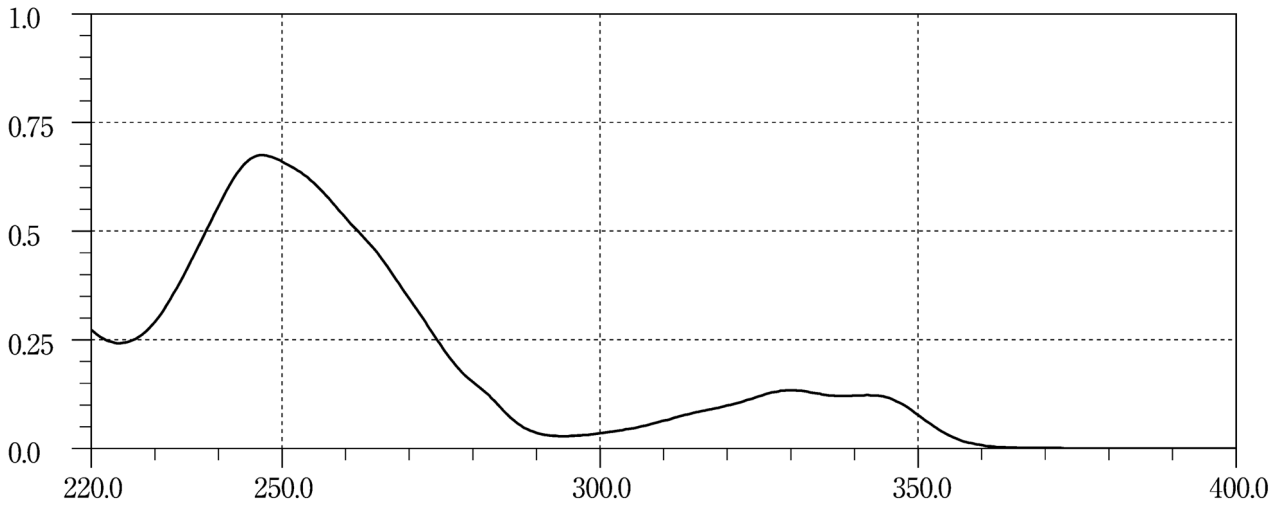
Pravastatin Sodium



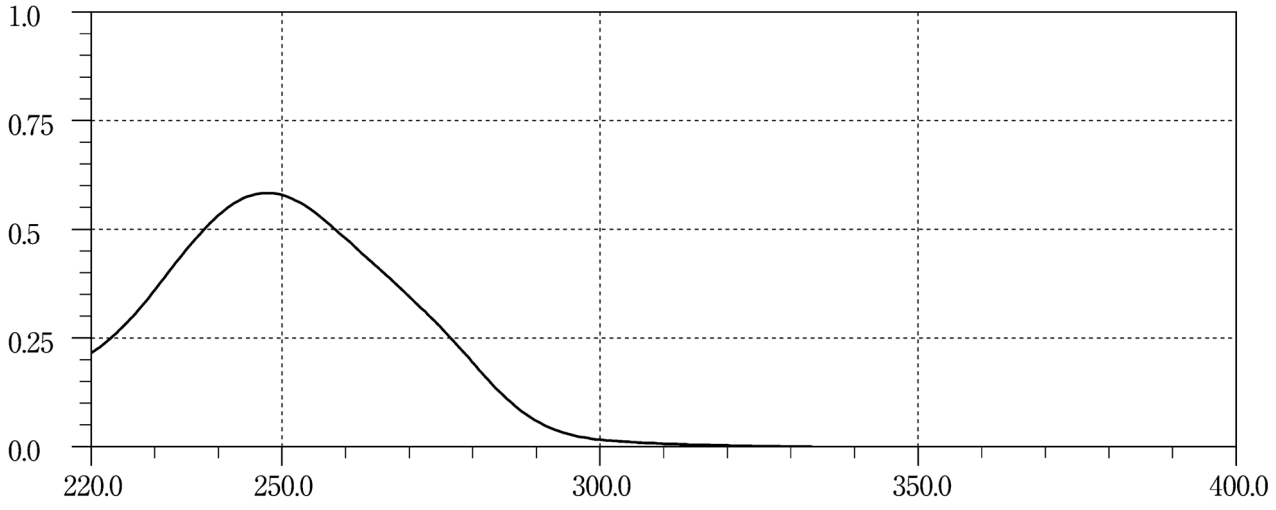
Prazepam



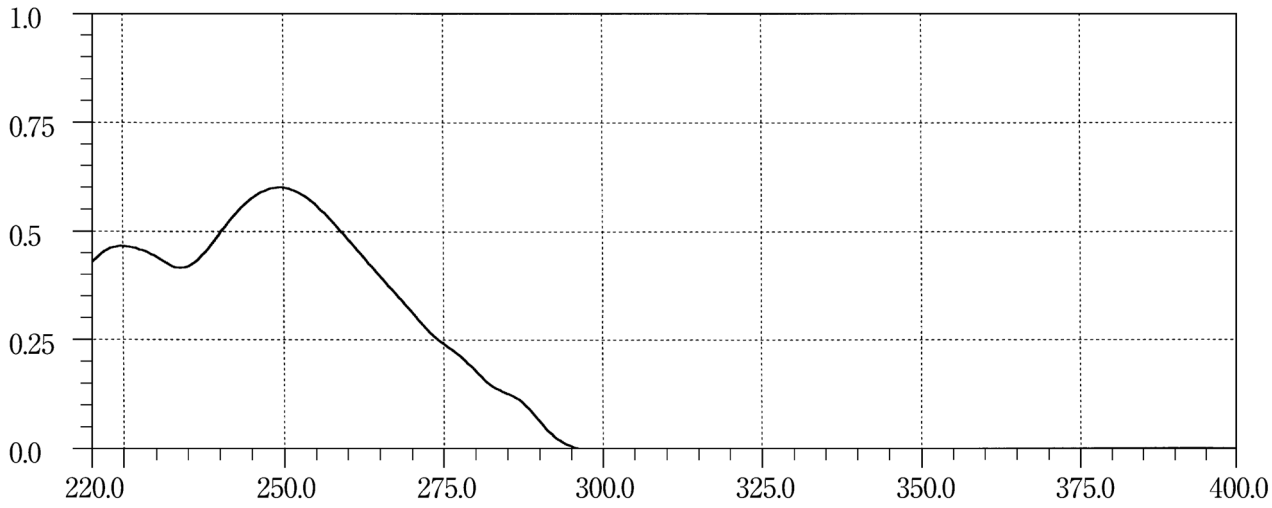
Prazosin Hydrochloride



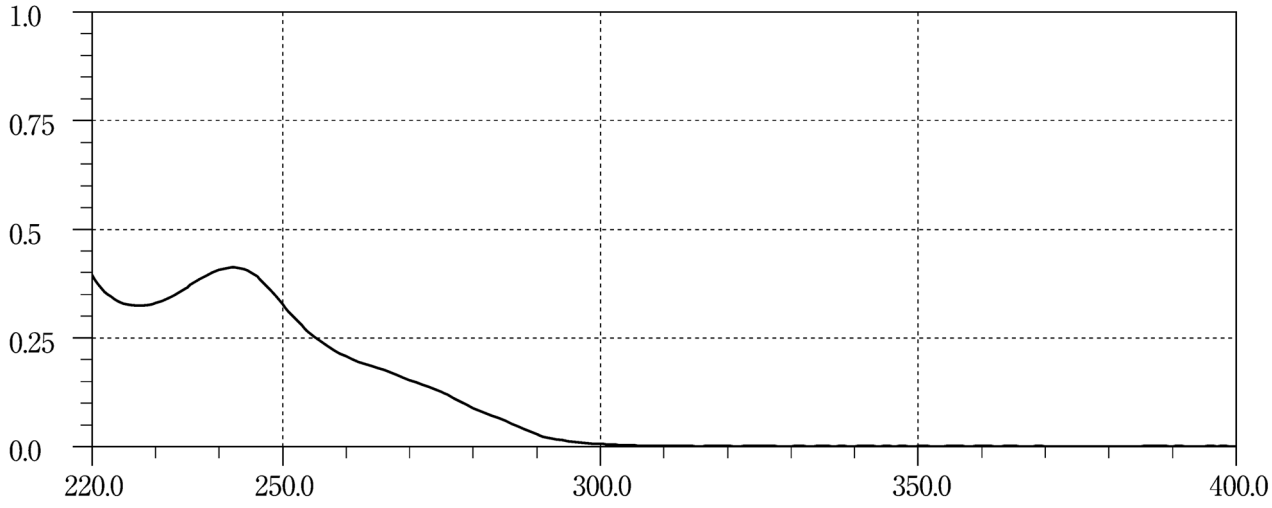
Prednisolone Sodium Phosphate



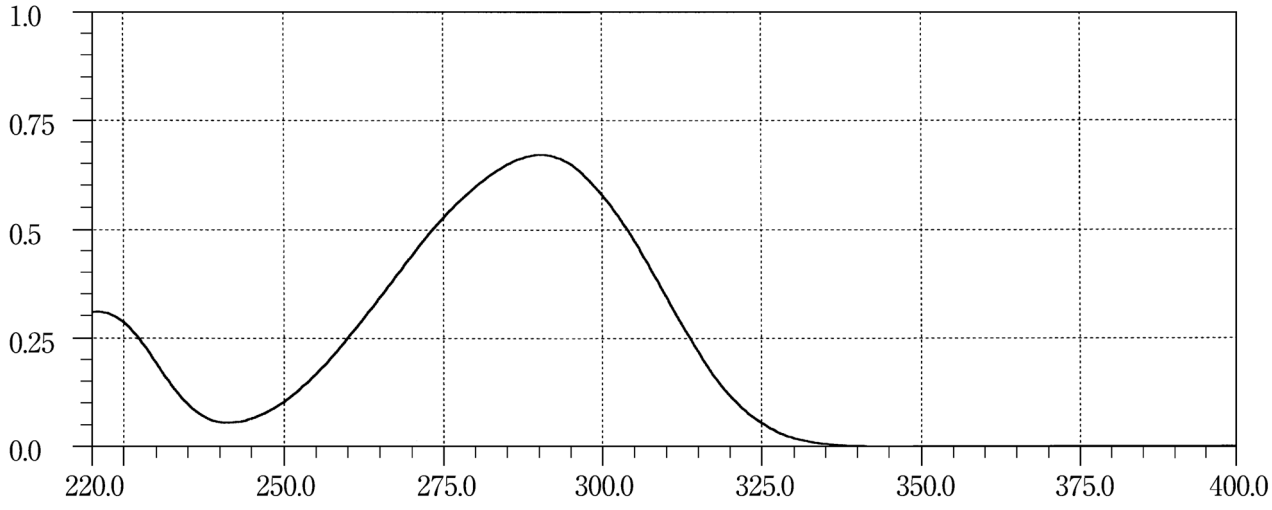
Probenecid



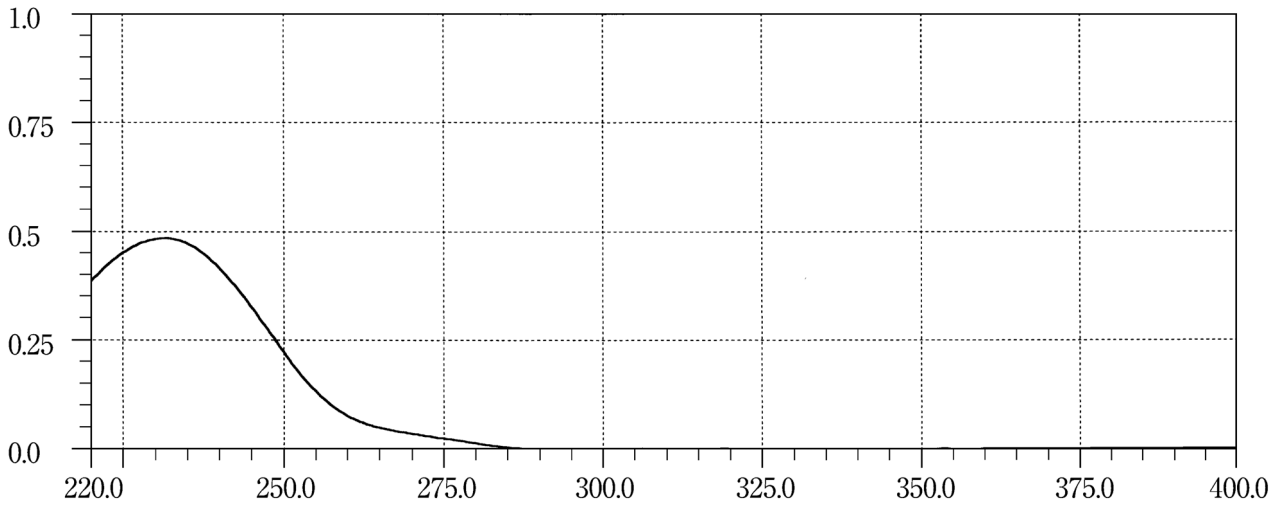
Probucol



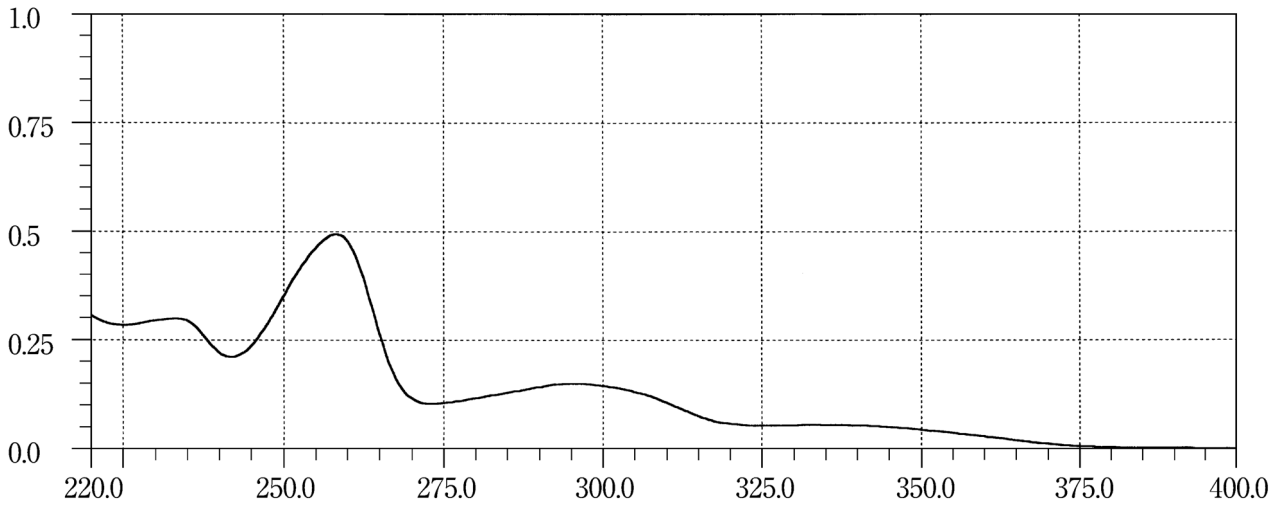
Procaine Hydrochloride



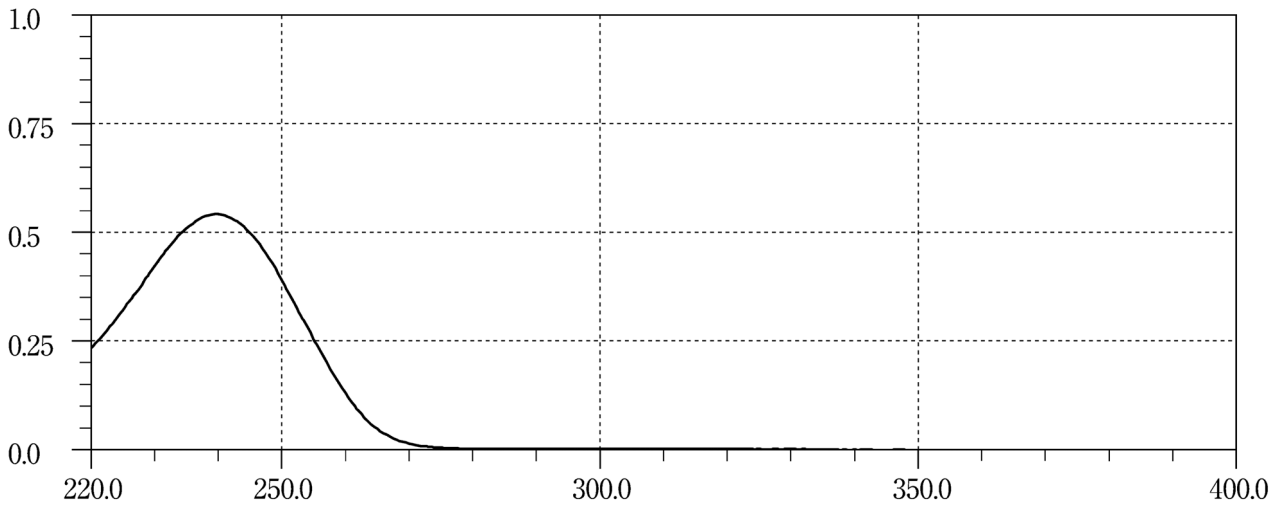
Procarbazine Hydrochloride



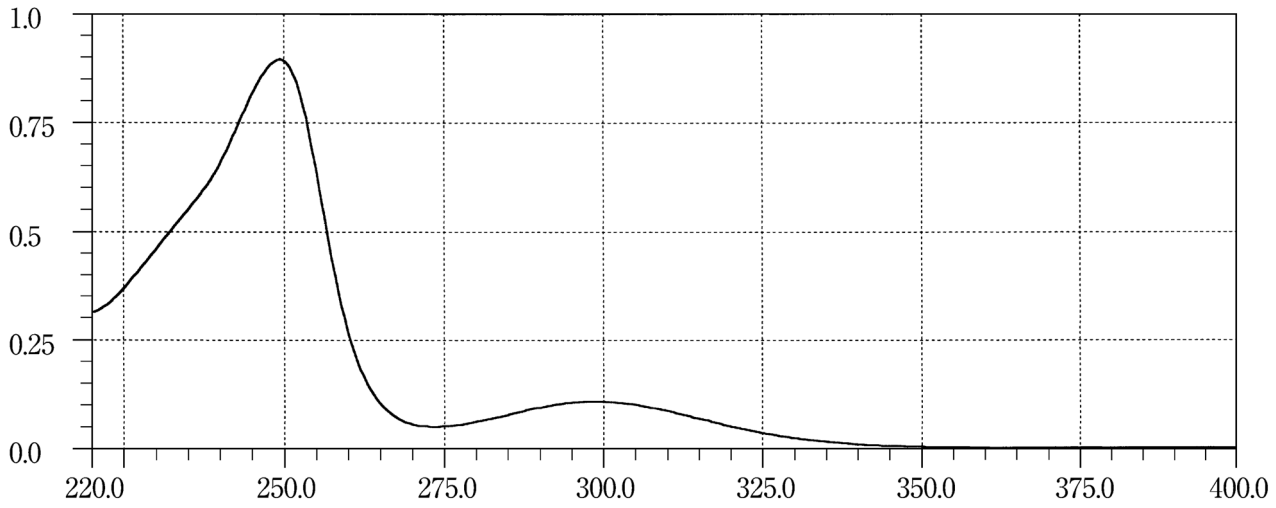
Procaterol Hydrochloride Hydrate



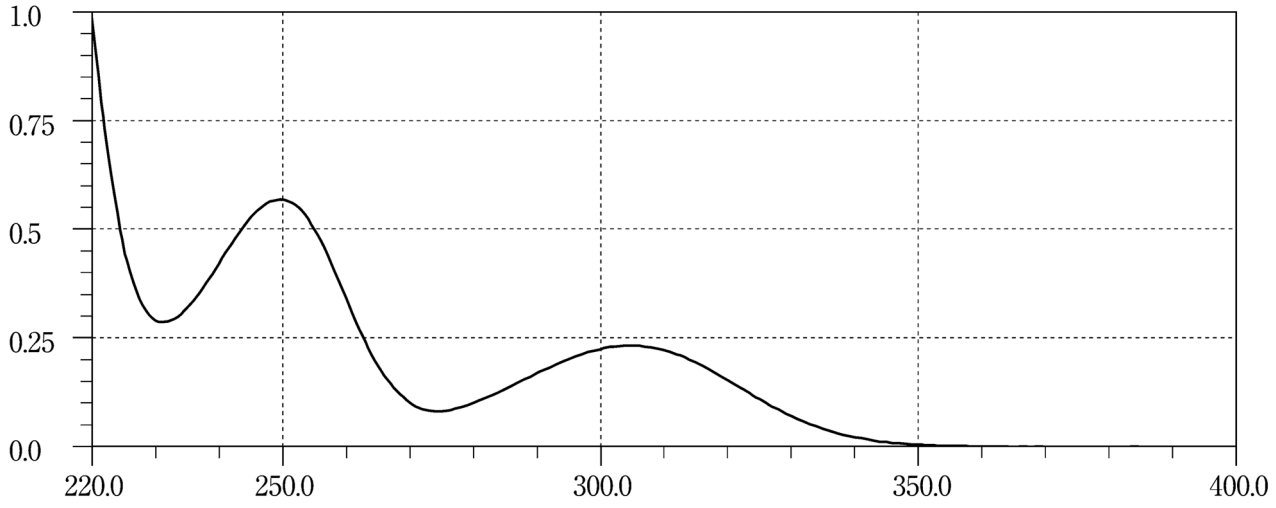
Progesterone



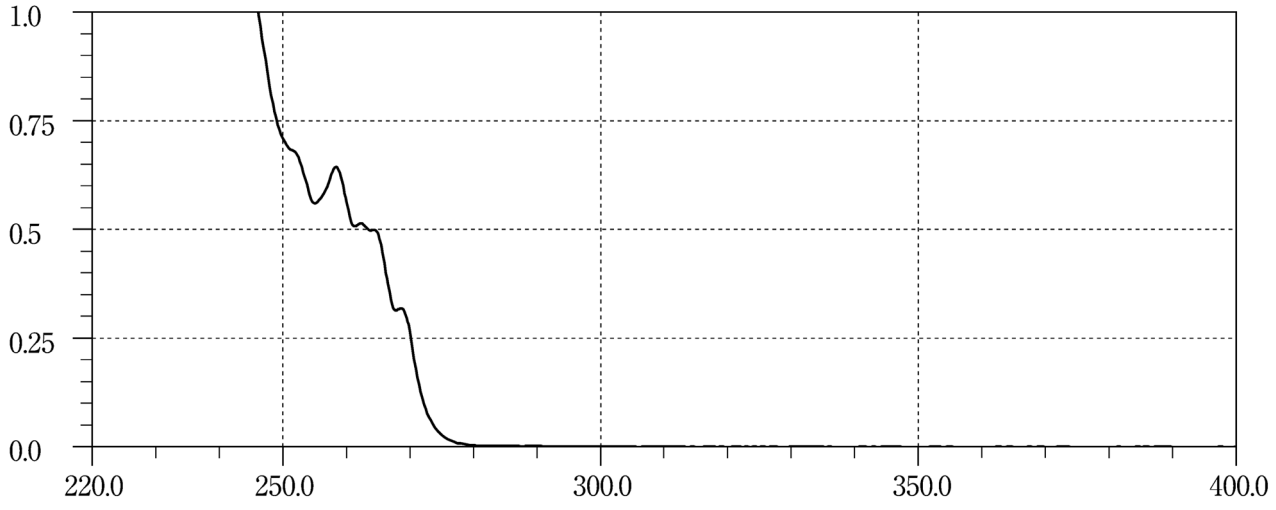
Promethazine Hydrochloride



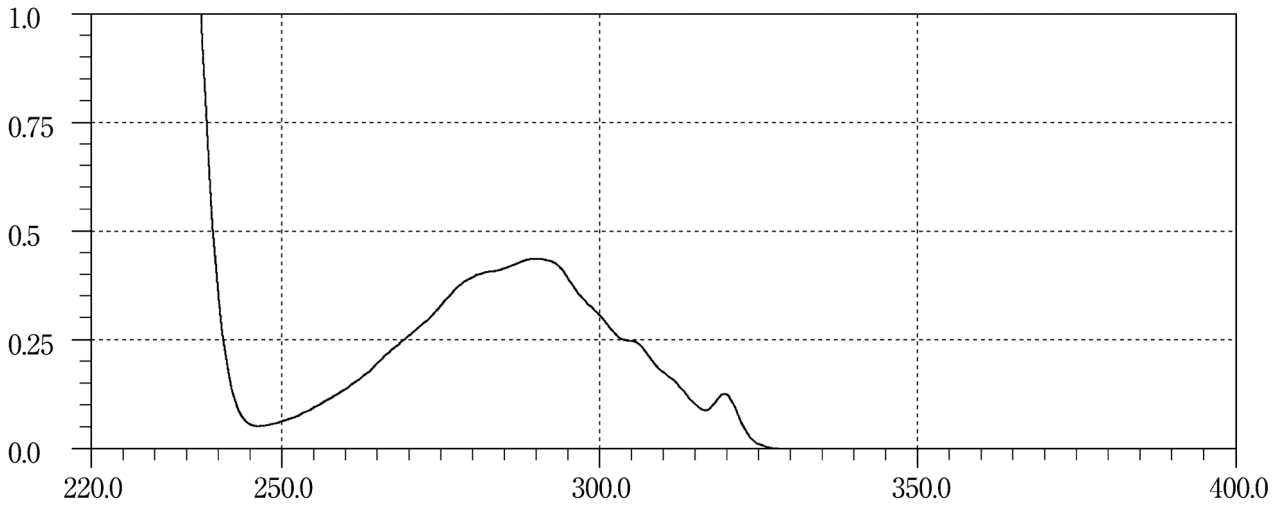
Propafenone Hydrochloride



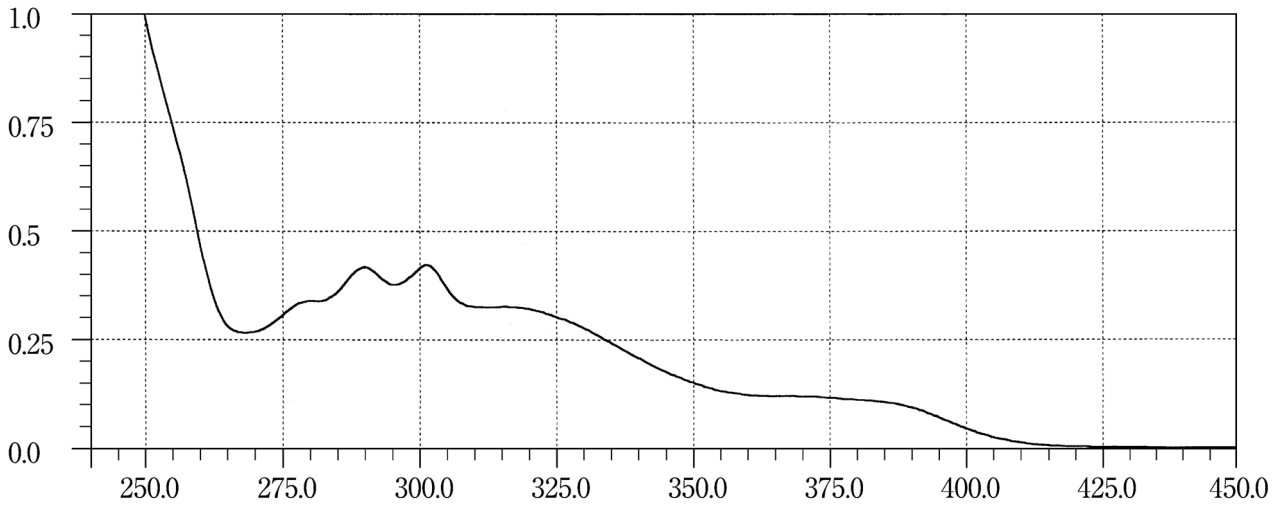
Propiverine Hydrochloride



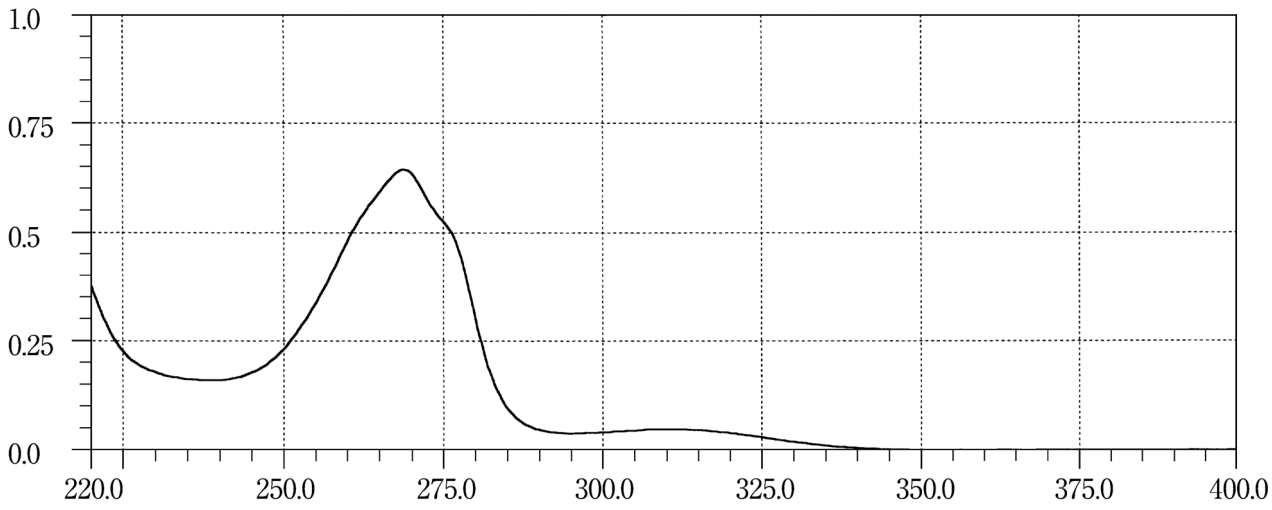
Propranolol Hydrochloride



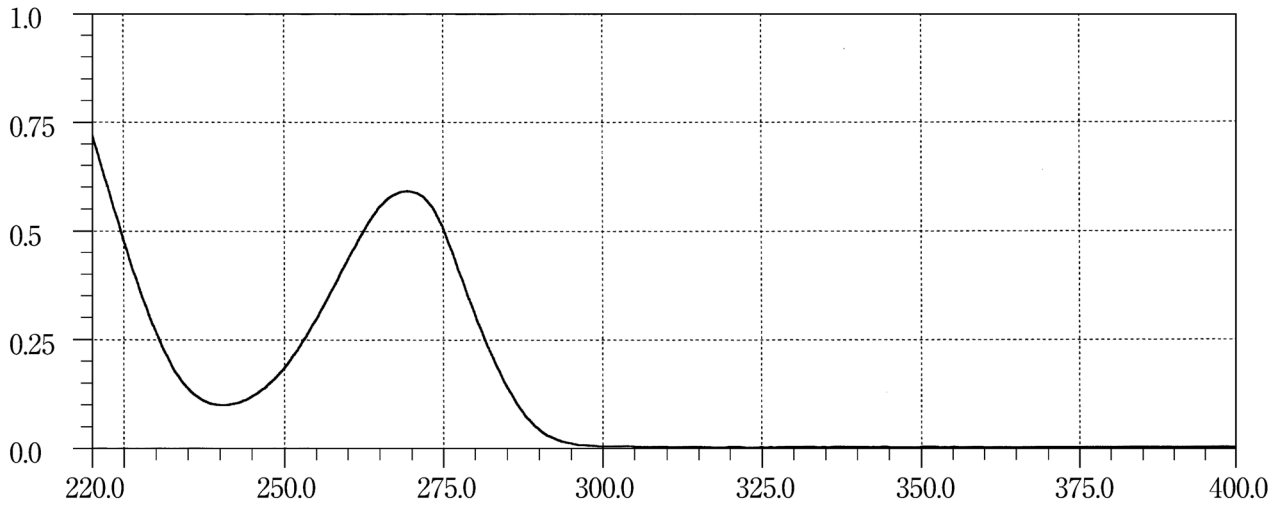
Pyrantel Pamoate



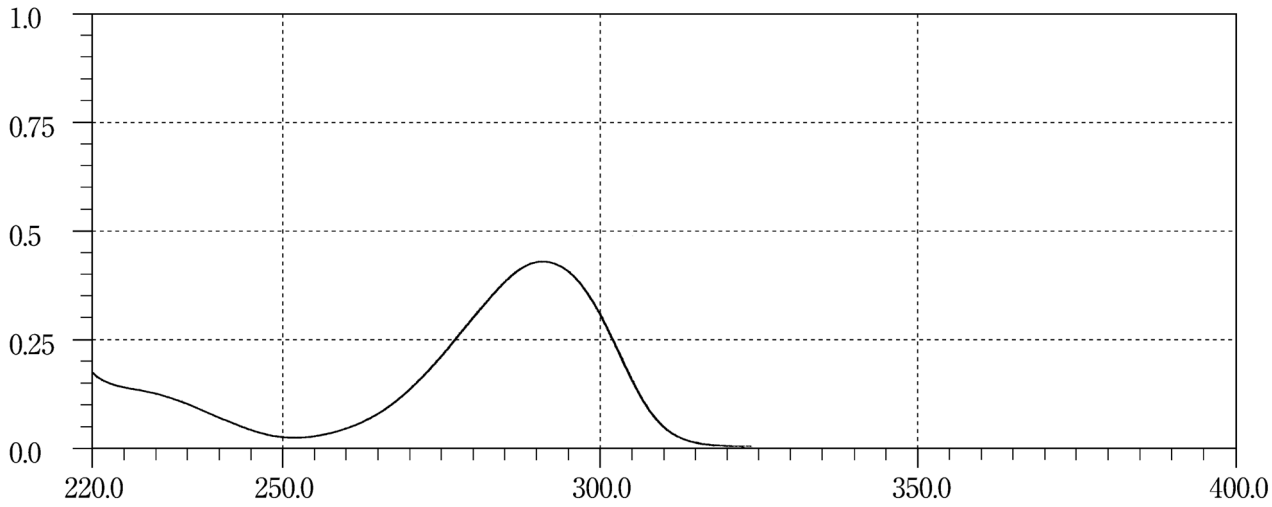
Pyrazinamide



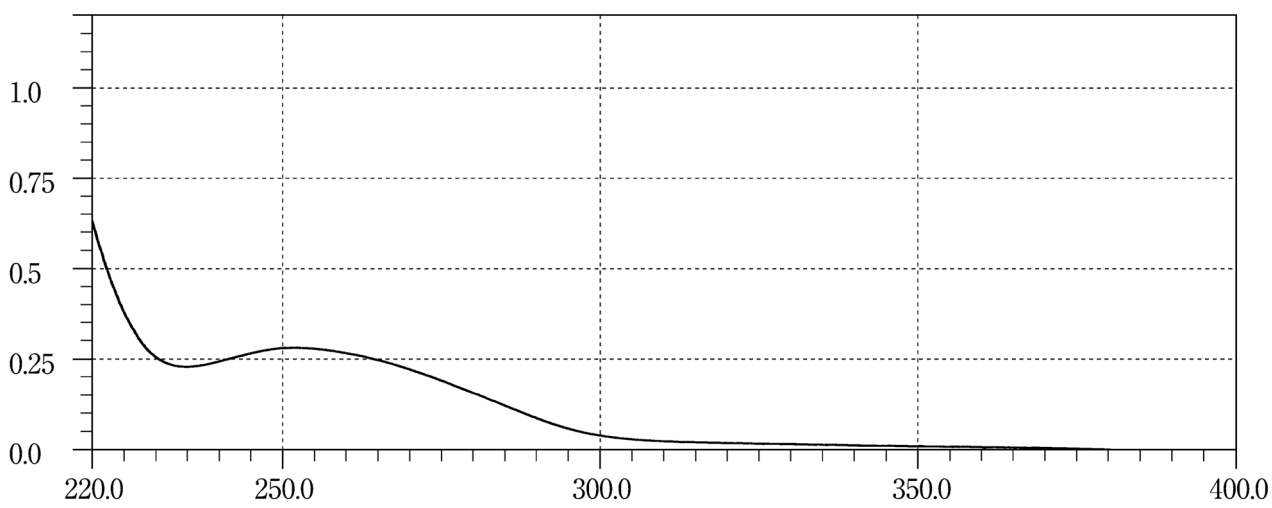
Pyridostigmine Bromide



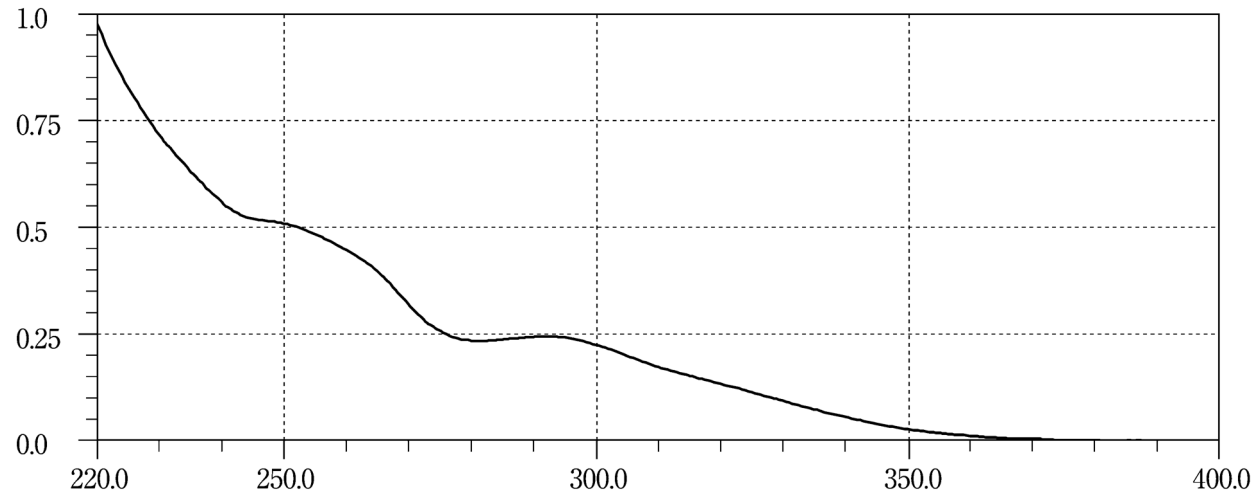
Pyridoxine Hydrochloride



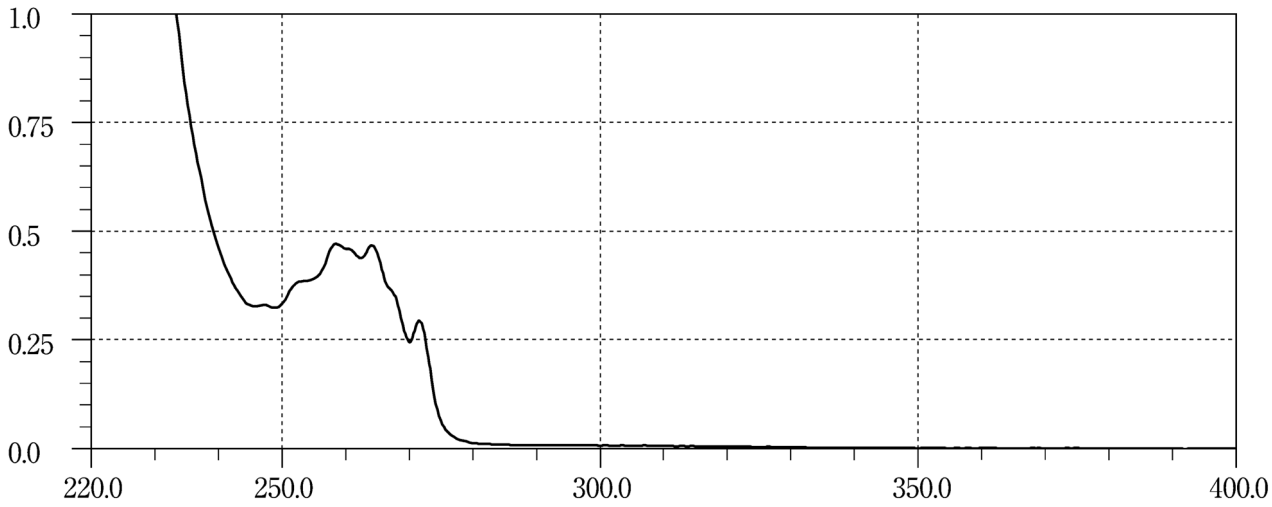
Pyrrolnitrin



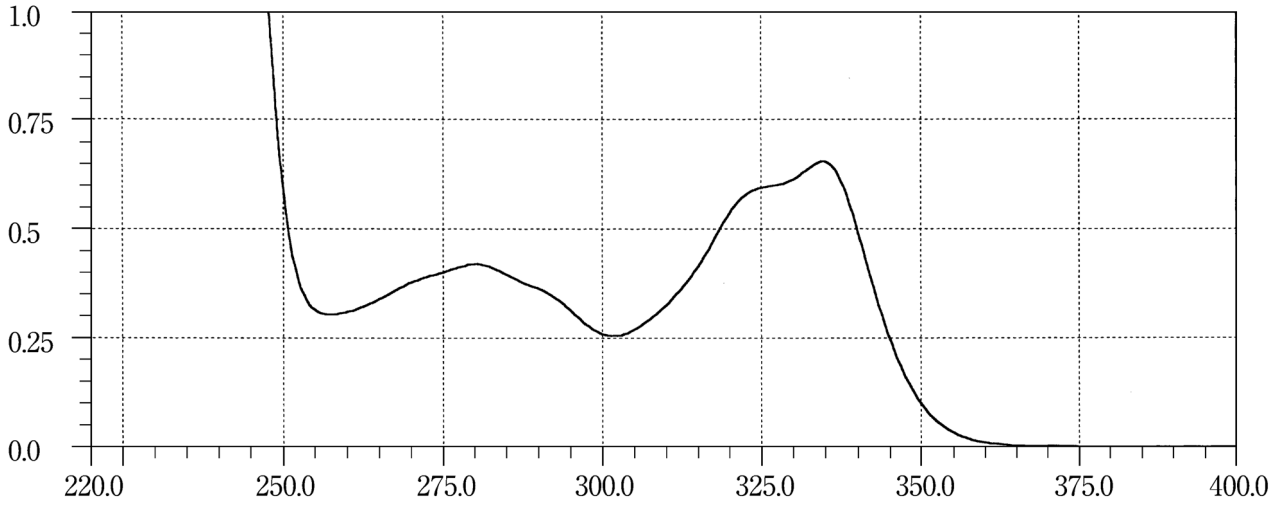
Quetiapine Fumarate



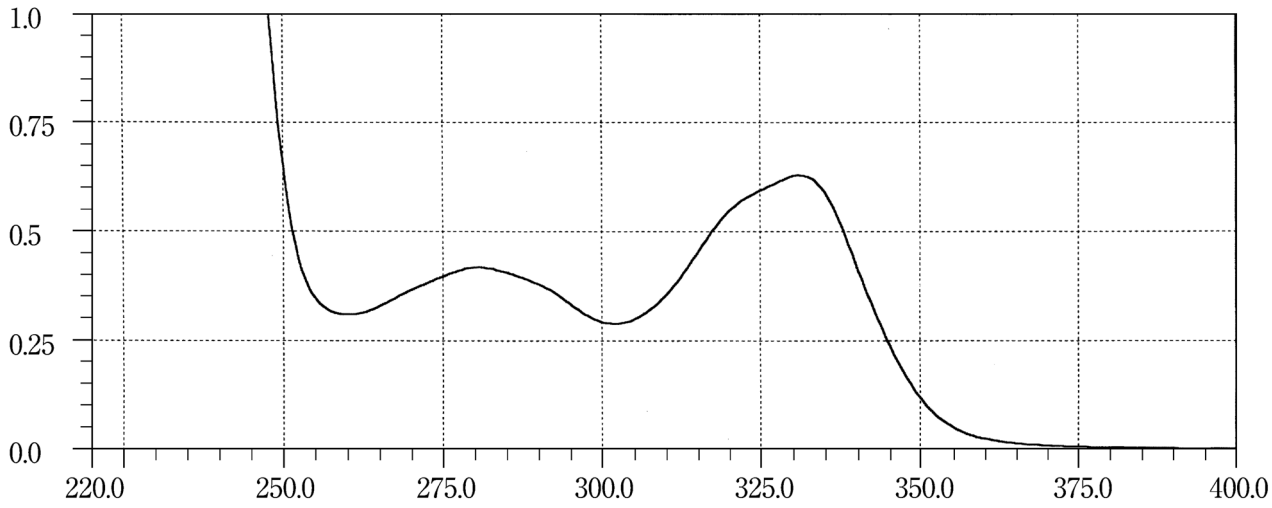
Quinapril Hydrochloride



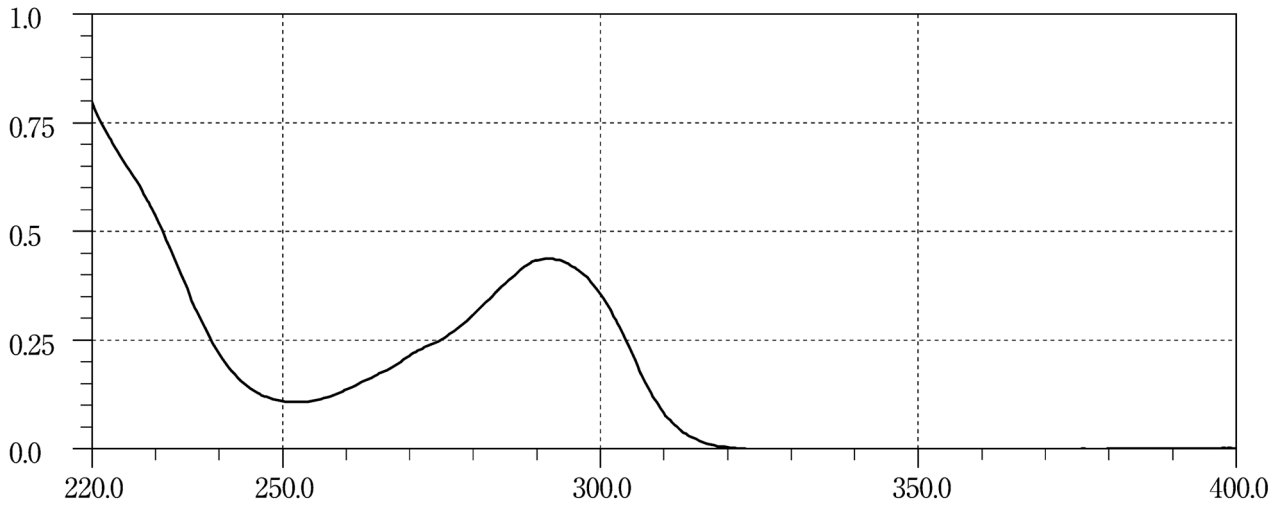
Quinine Ethyl Carbonate



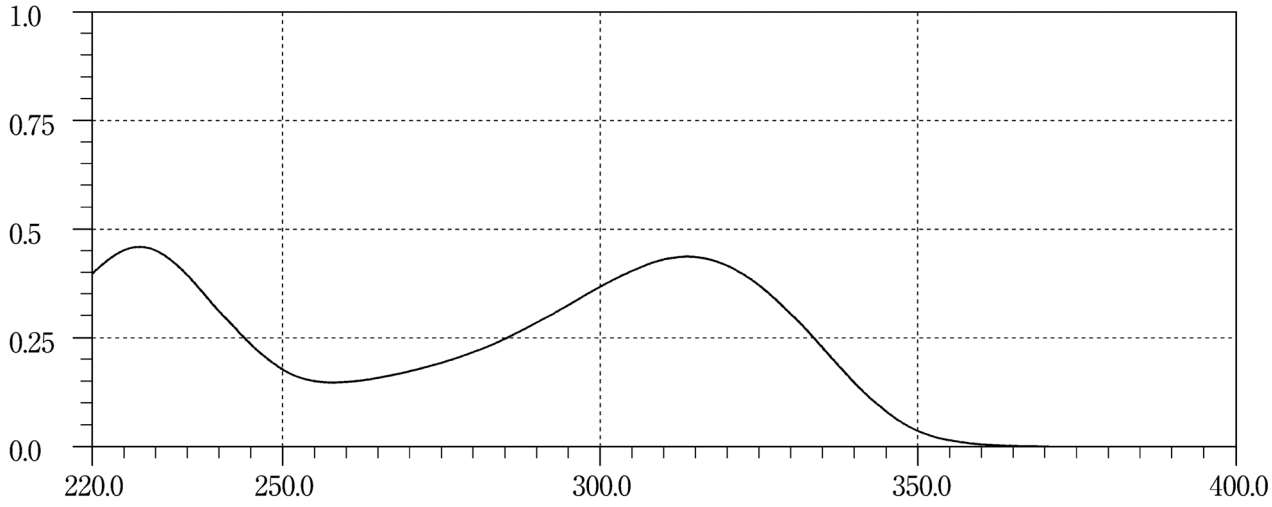
Quinine Sulfate Hydrate



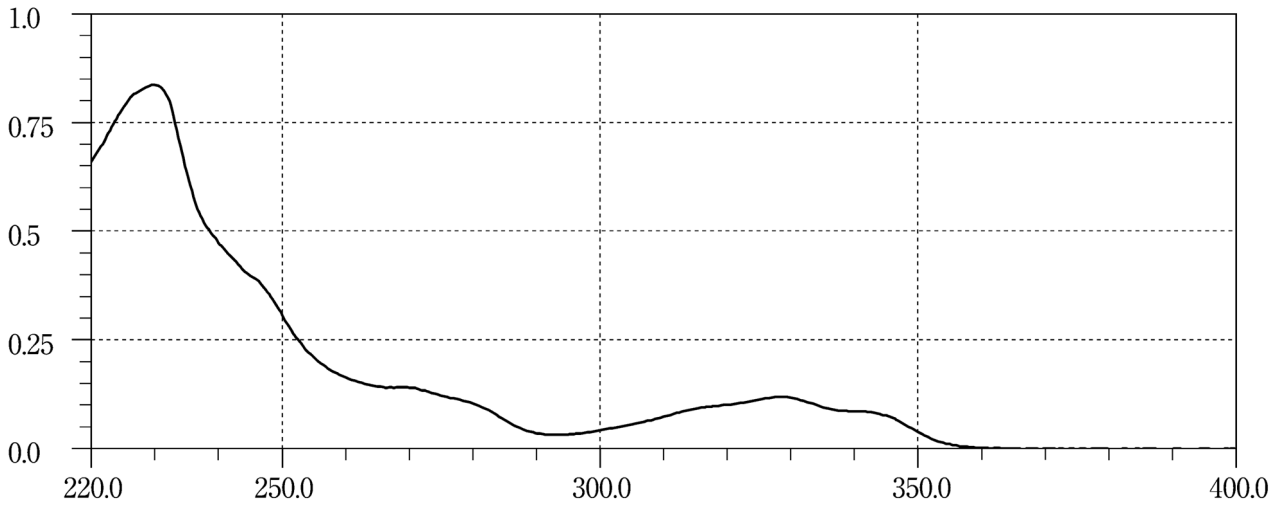
Rabeprazole Sodium



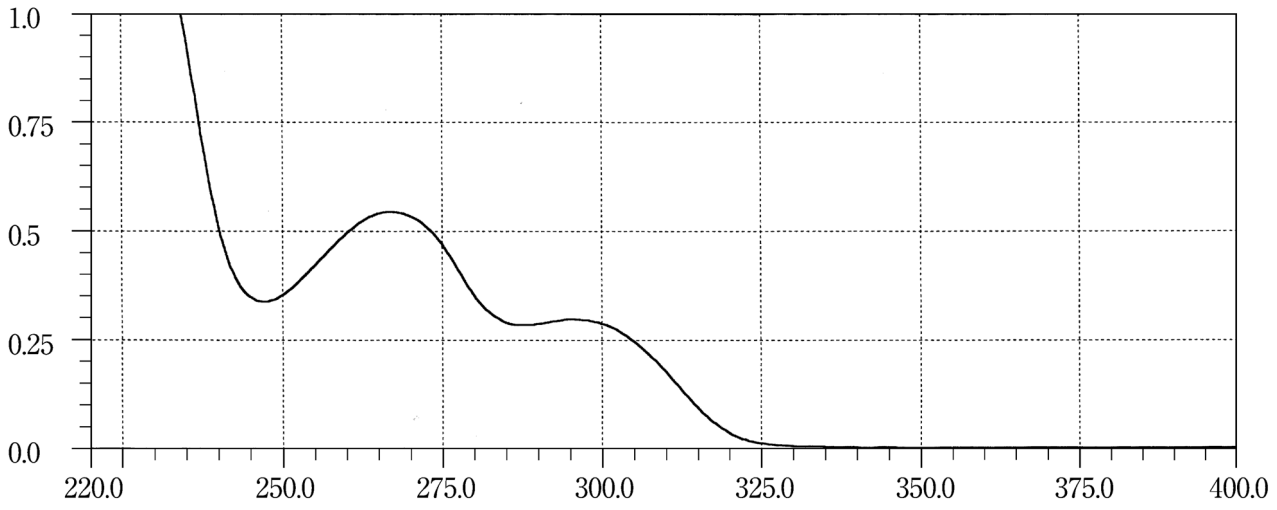
Ranitidine Hydrochloride



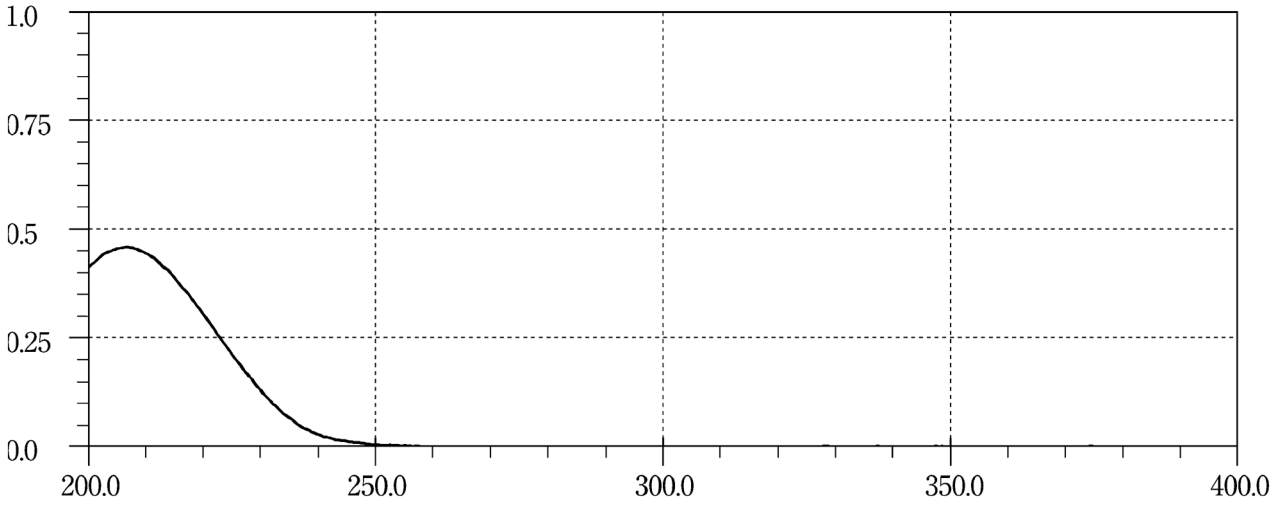
Rebamipide



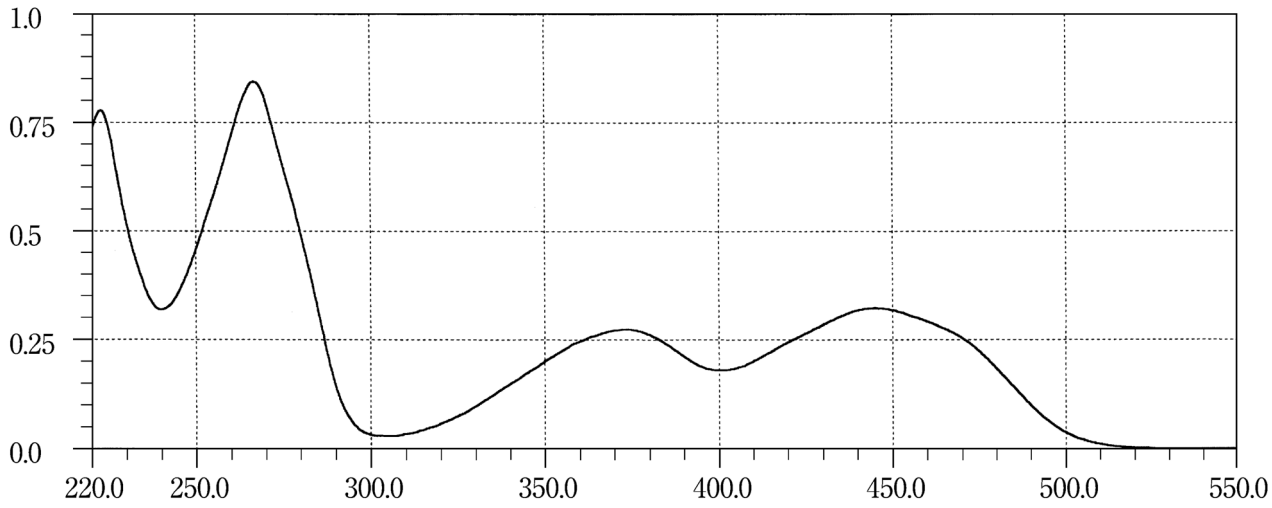
Reserpine



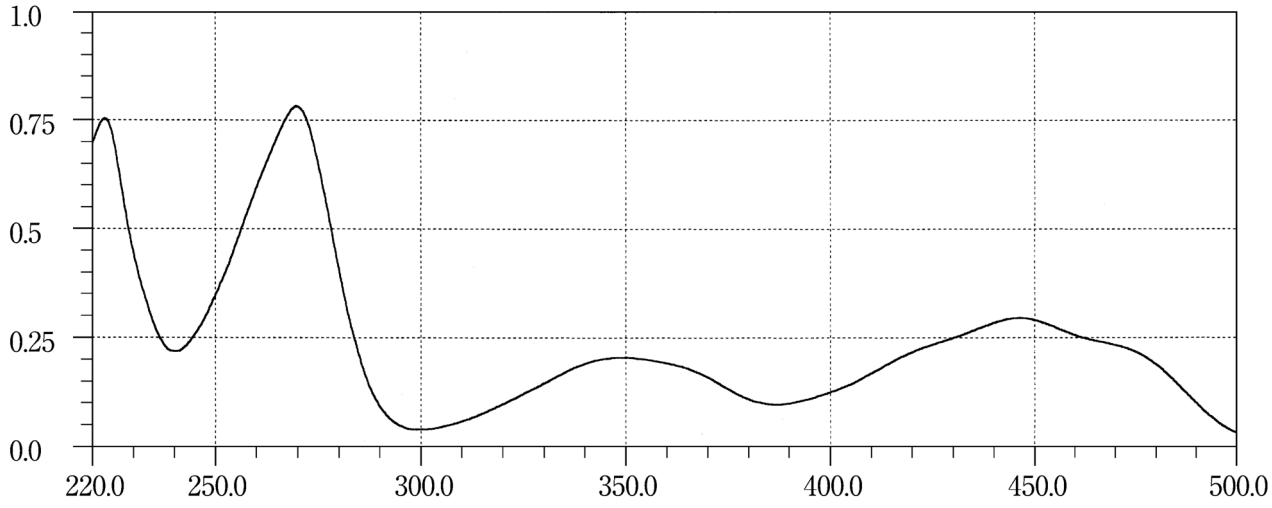
Ribavirin



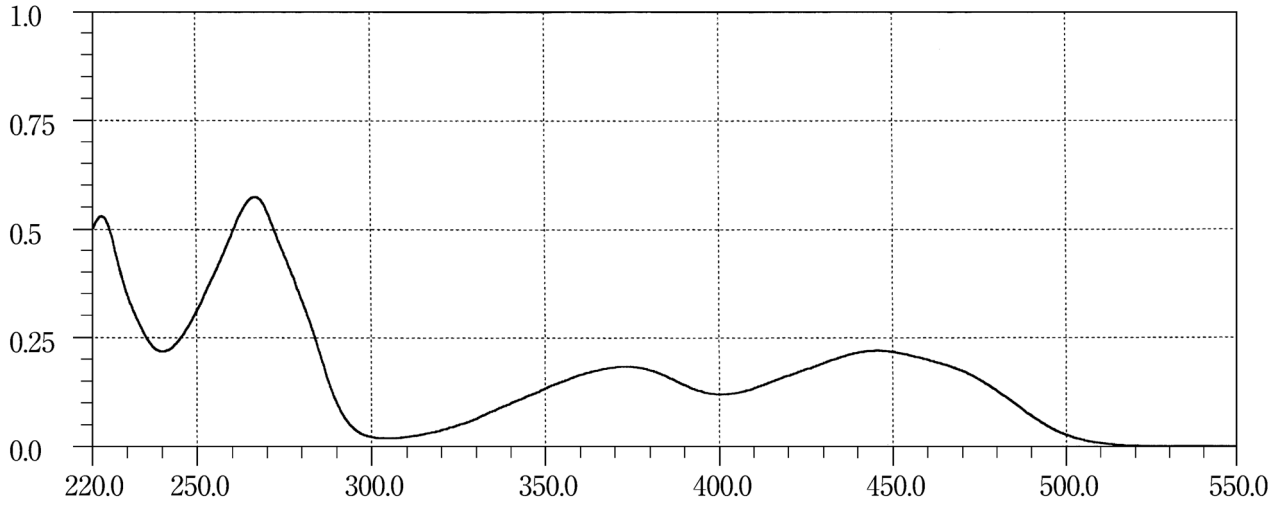
Riboflavin



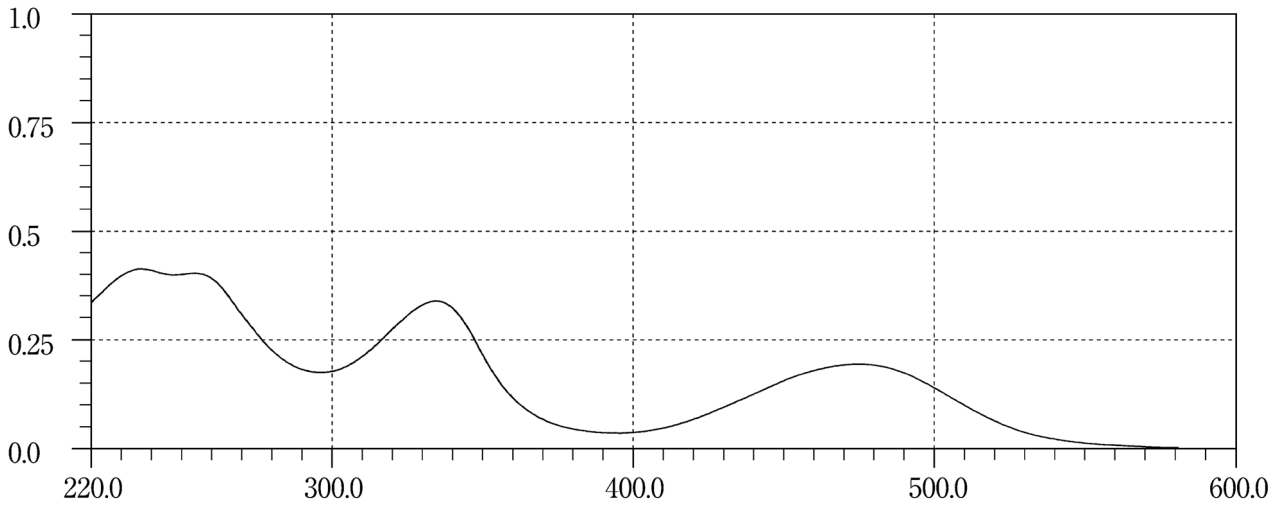
Riboflavin Butyrate



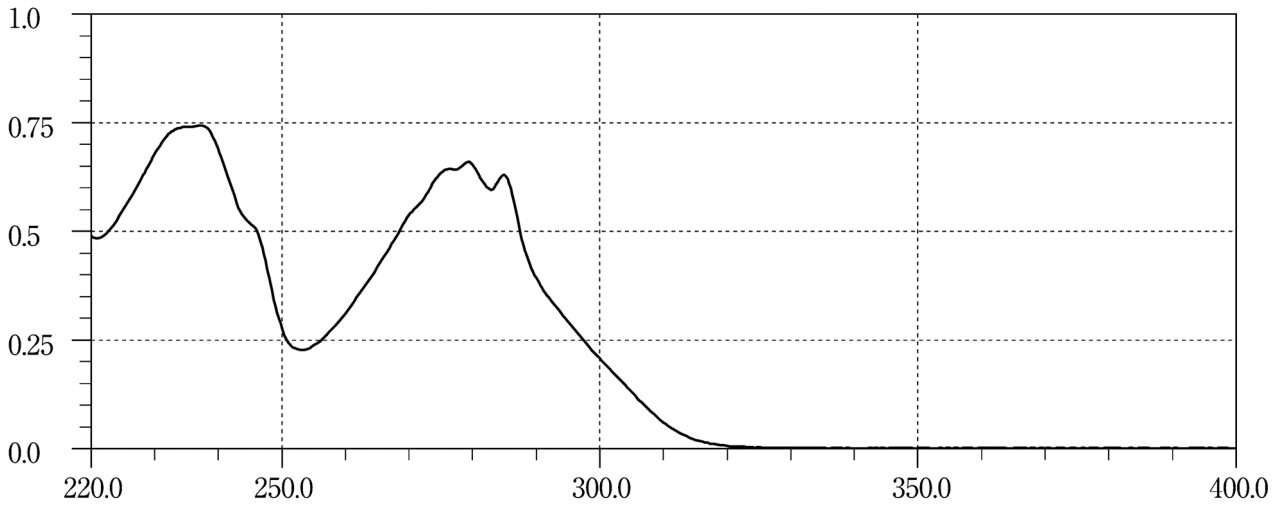
Riboflavin Sodium Phosphate



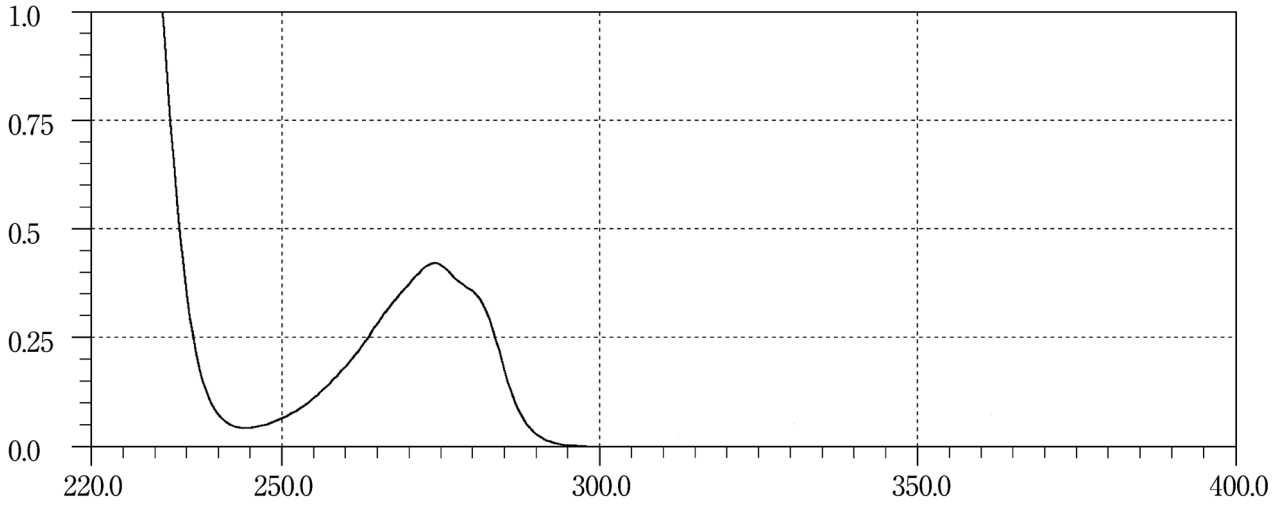
Rifampicin



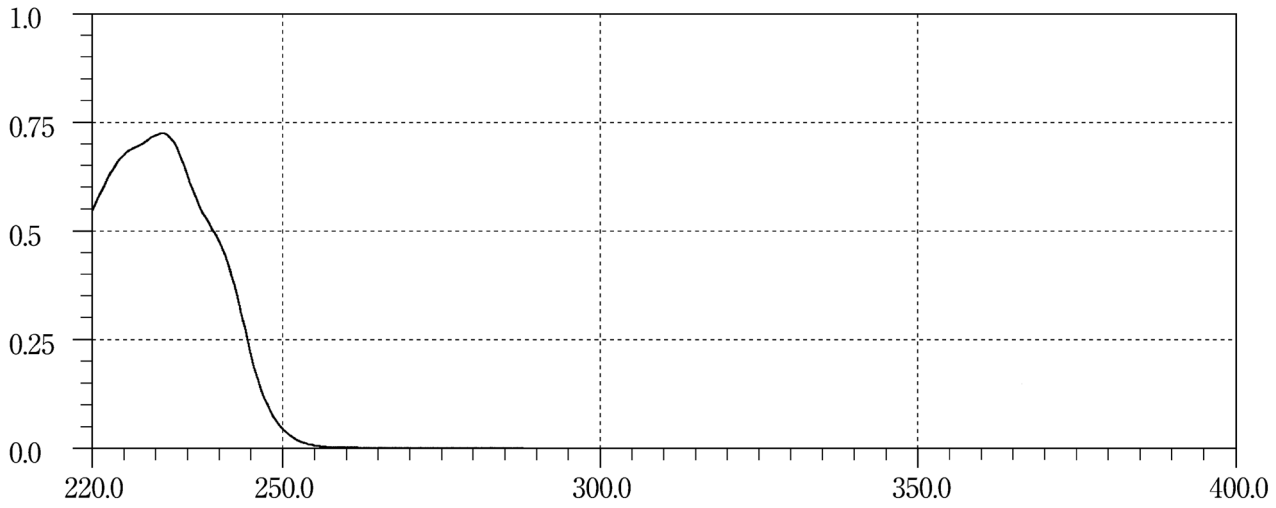
Risperidone



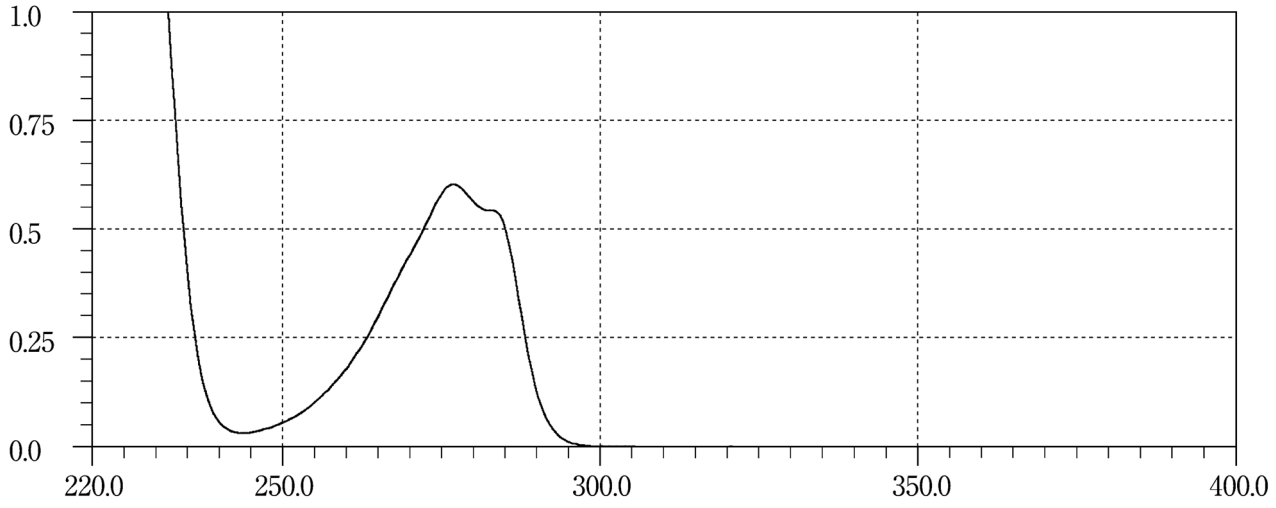
Ritodrine Hydrochloride



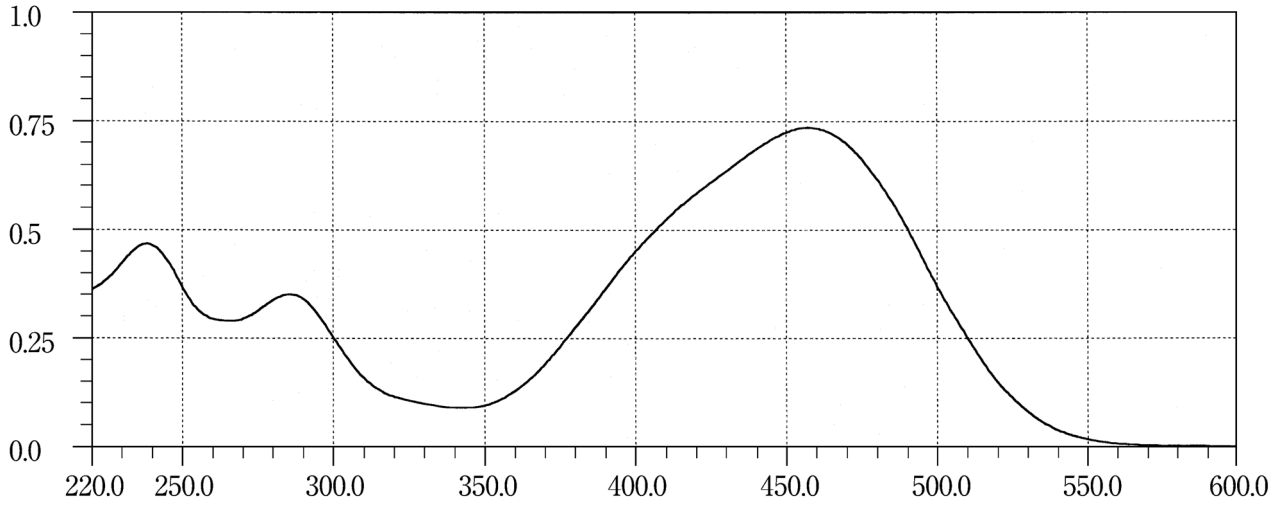
Rokitamycin



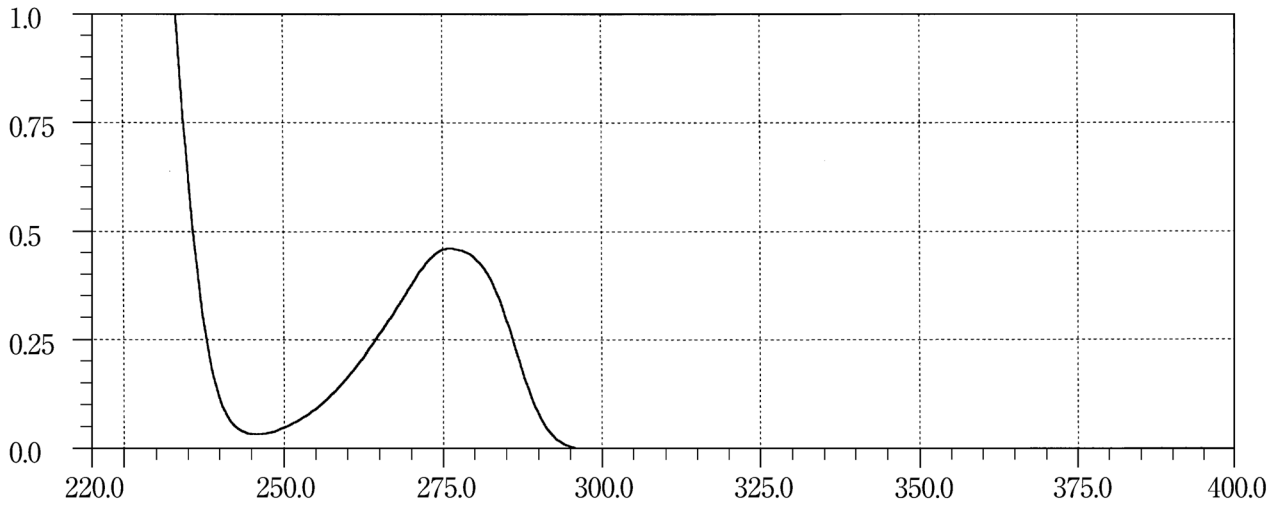
Roxatidine Acetate Hydrochloride



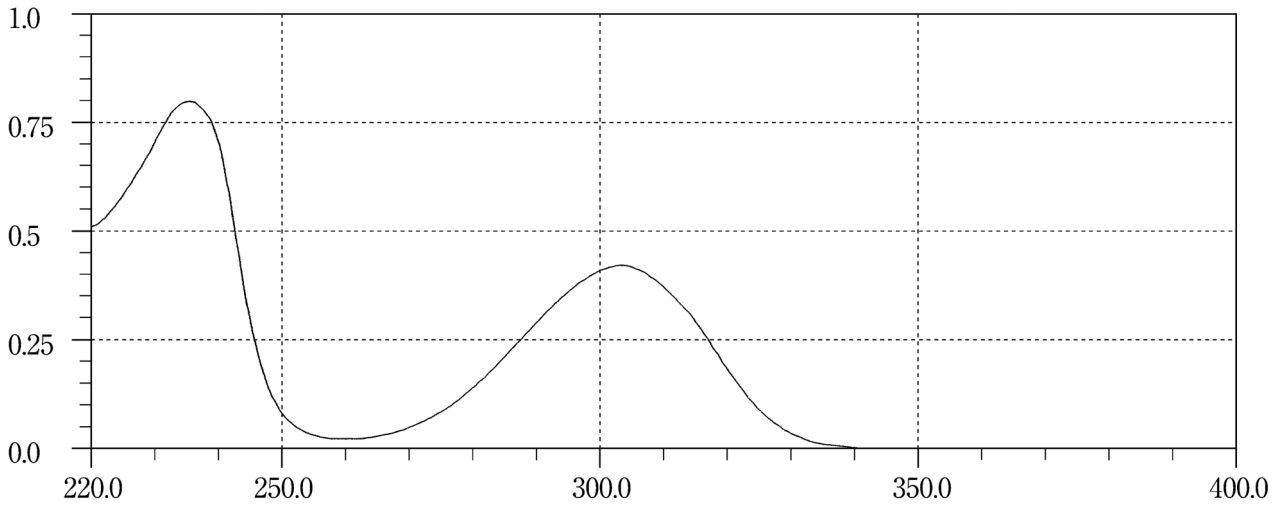
Salazosulfapyridine



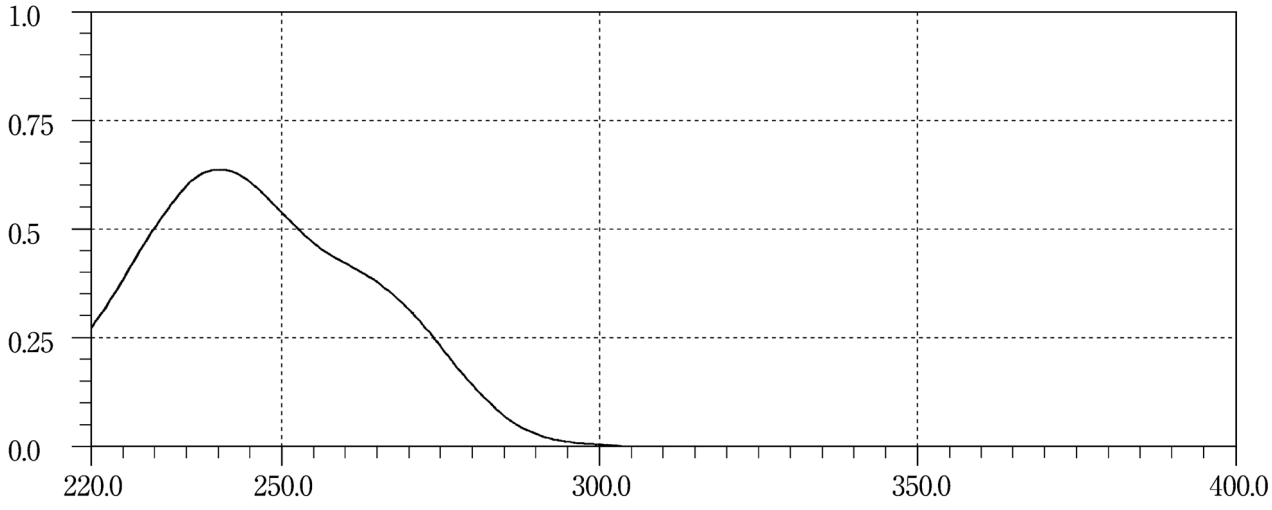
Salbutamol Sulfate



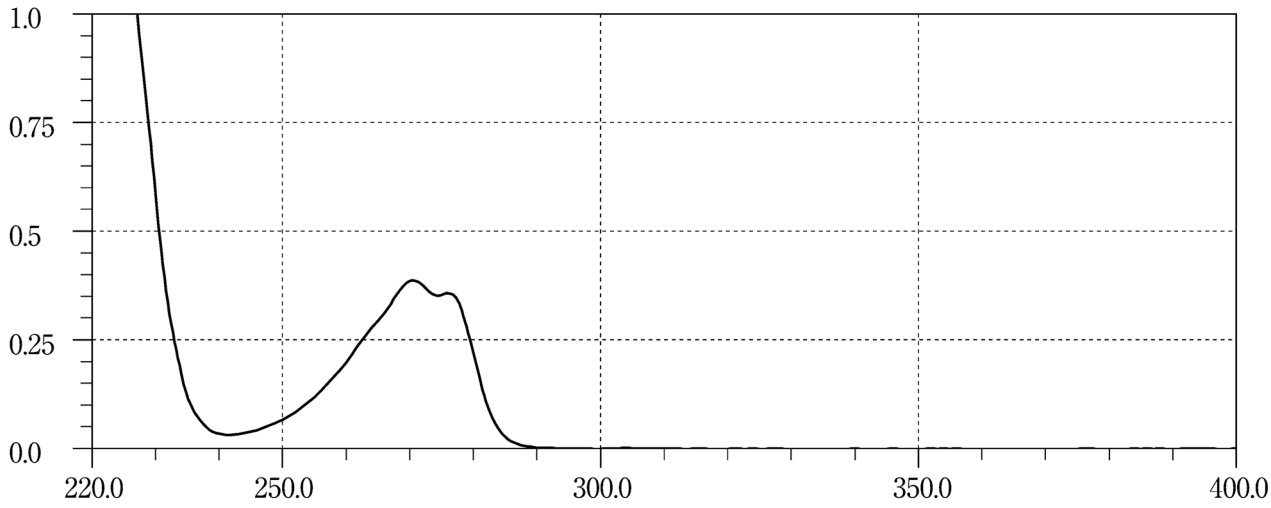
Salicylic Acid



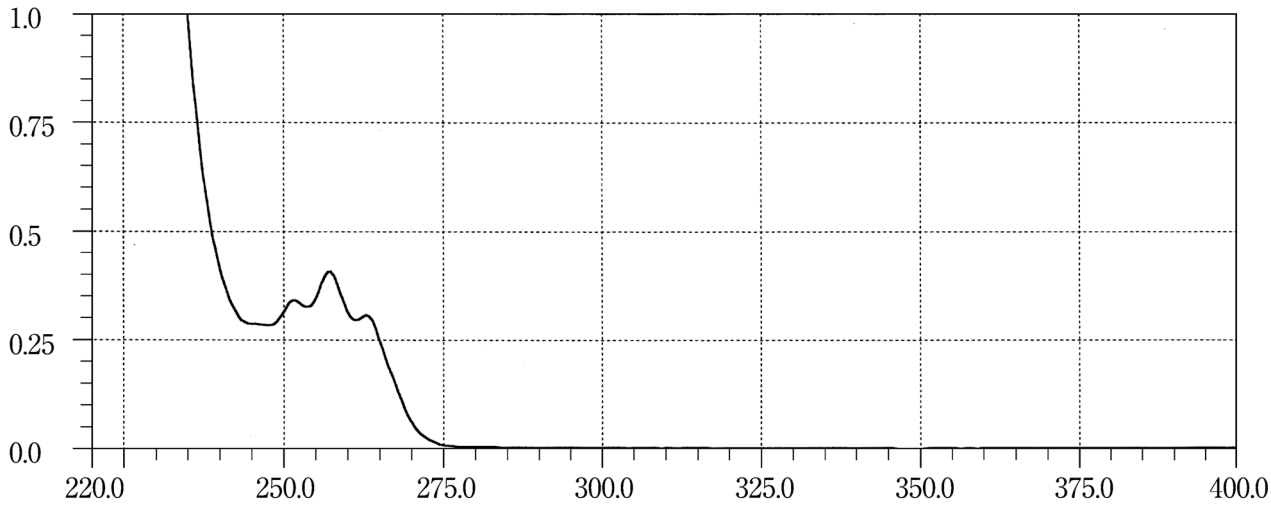
Santonin



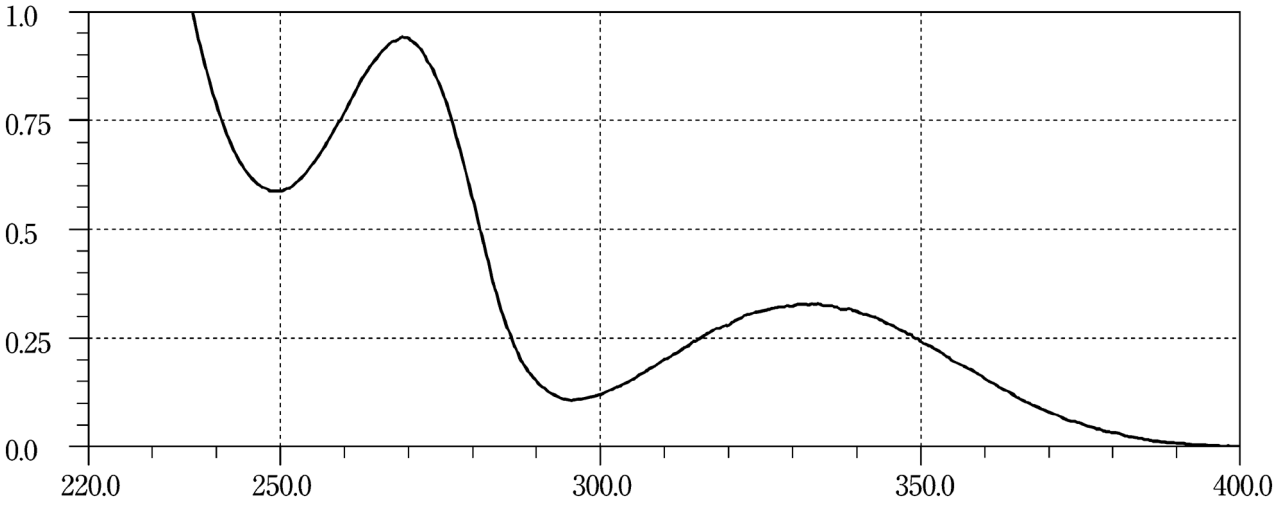
Sarpogrelate Hydrochloride



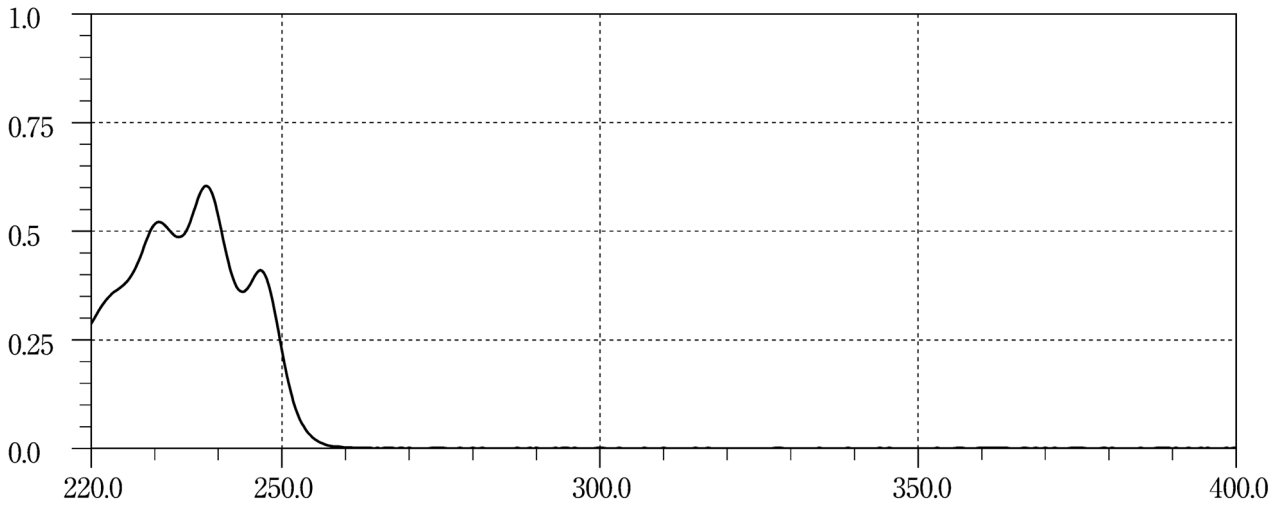
Scopolamine Butylbromide



Silodosin



Simvastatin



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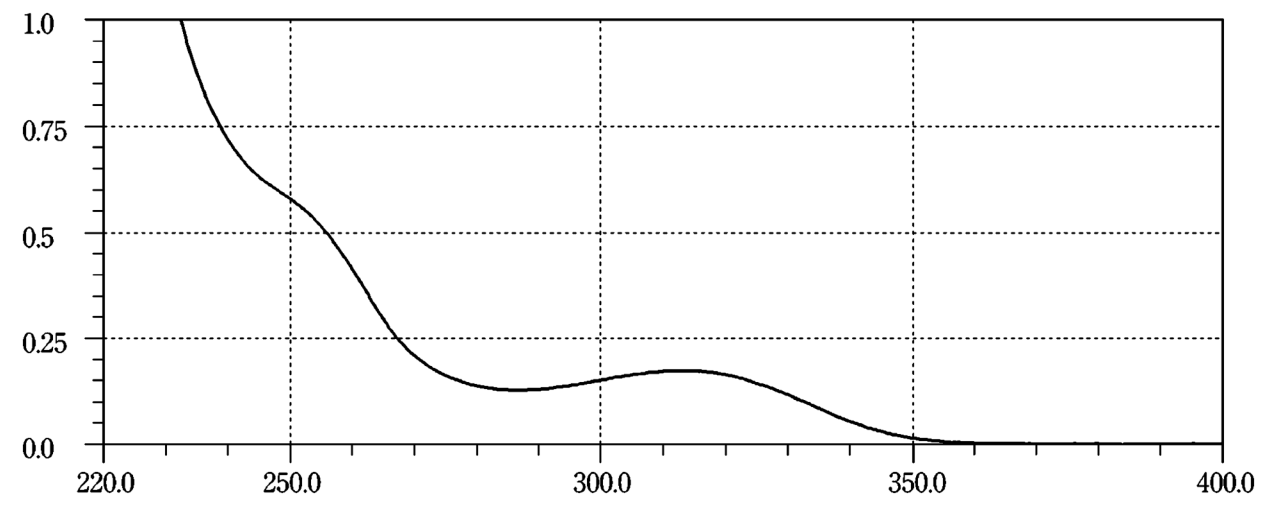

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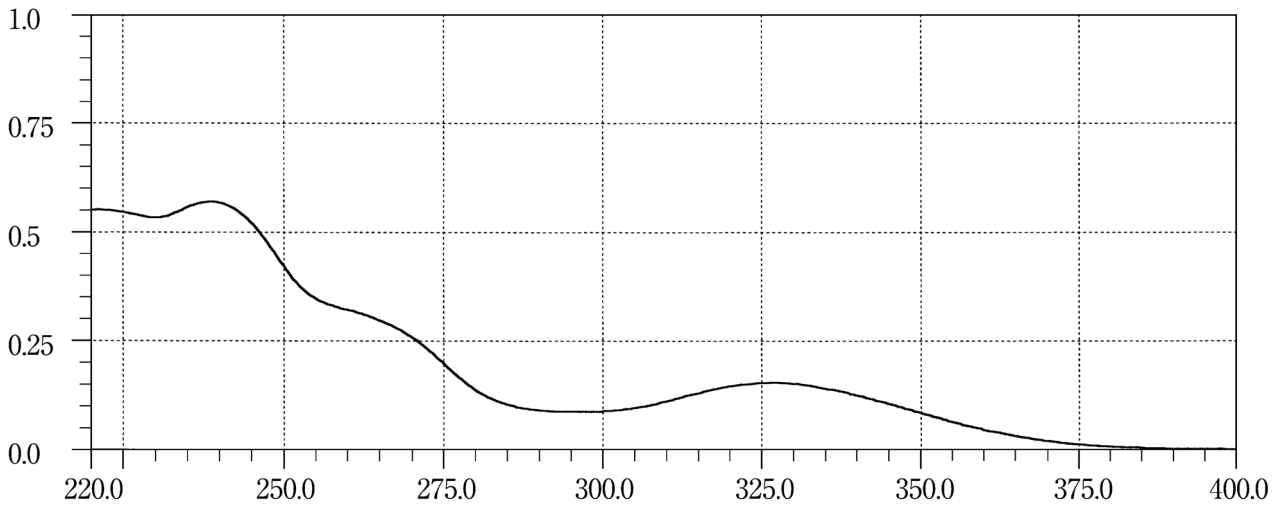
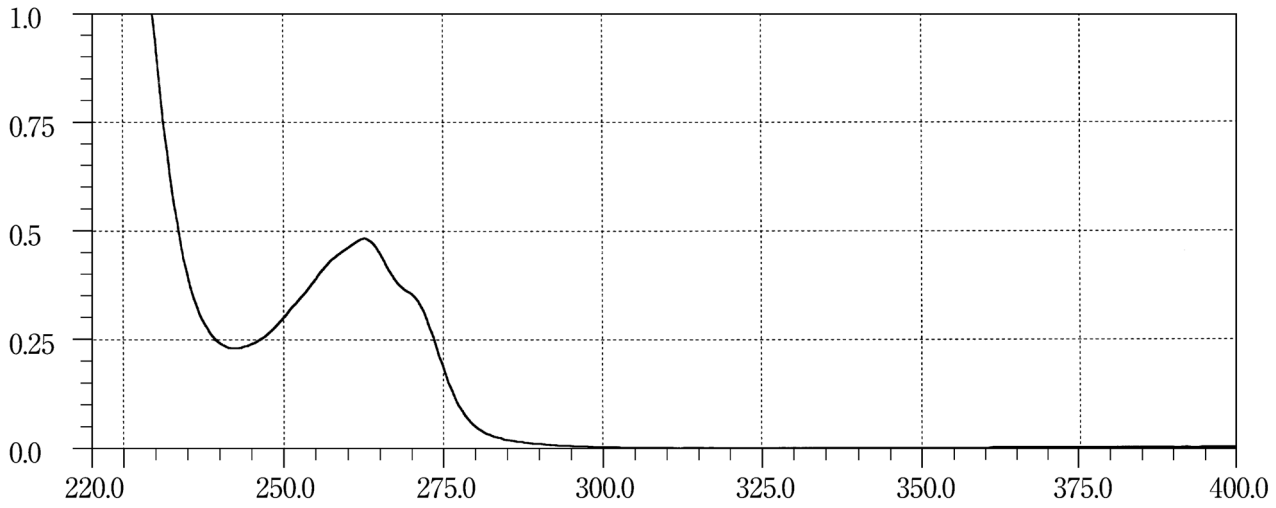
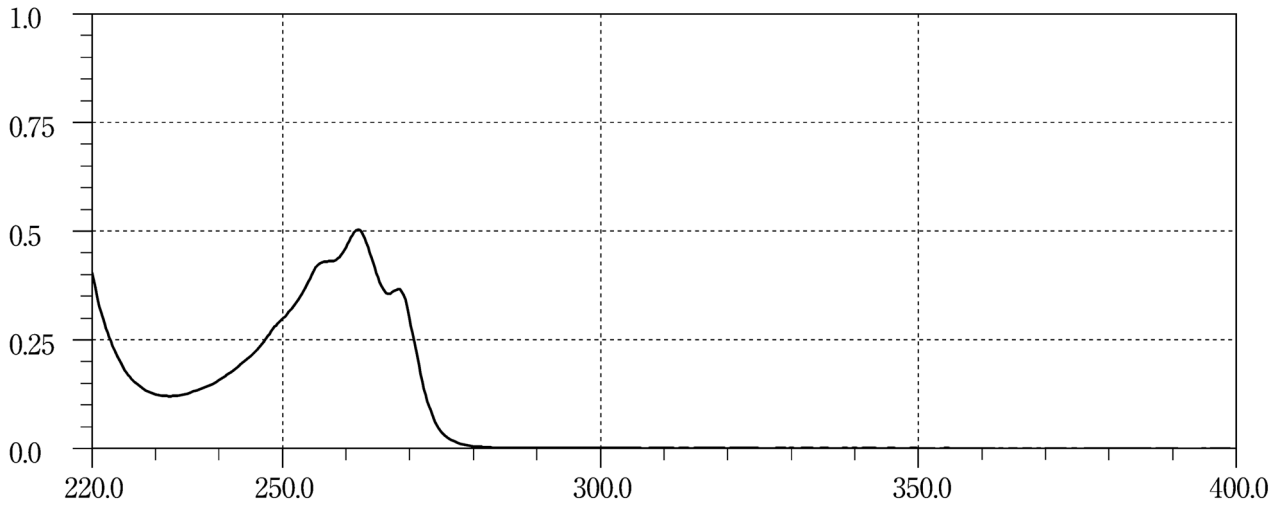


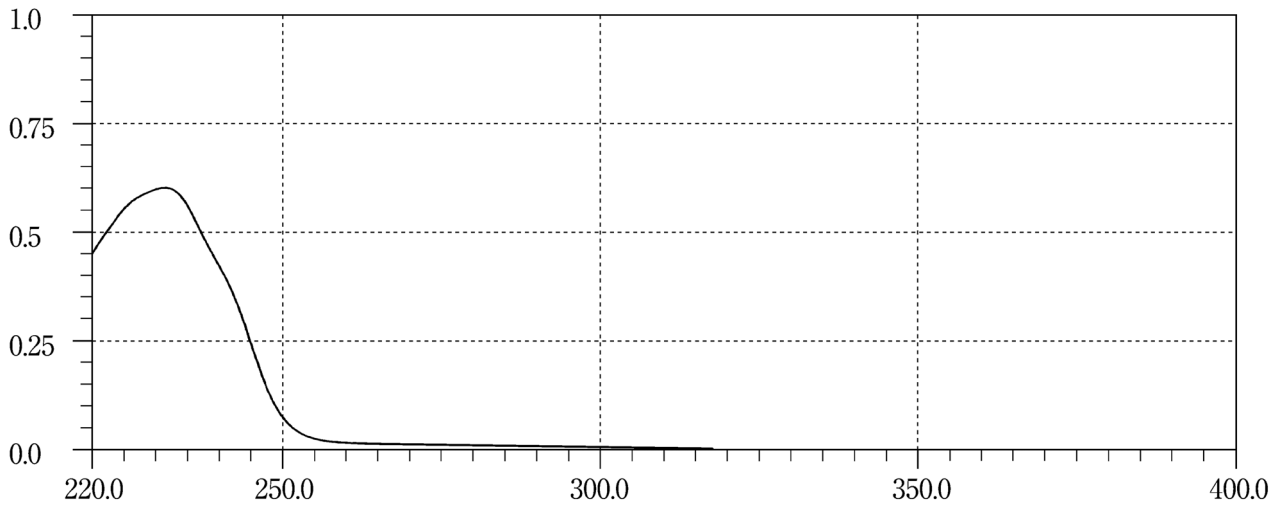
Sodium Picosulfate Hydrate



Sodium Risedronate Hydrate



Spiramycin Acetate



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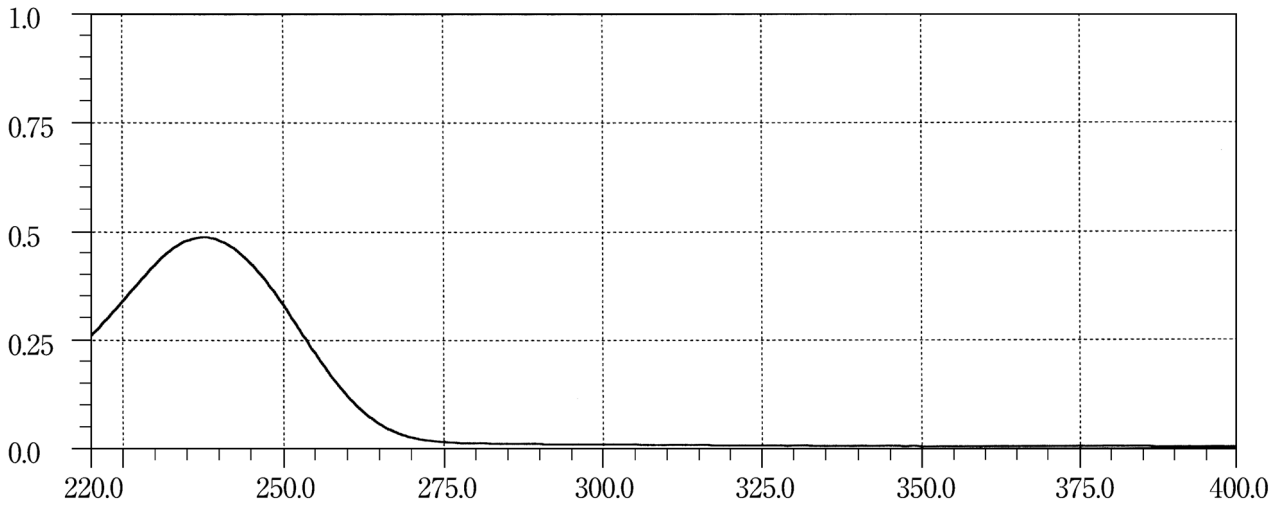

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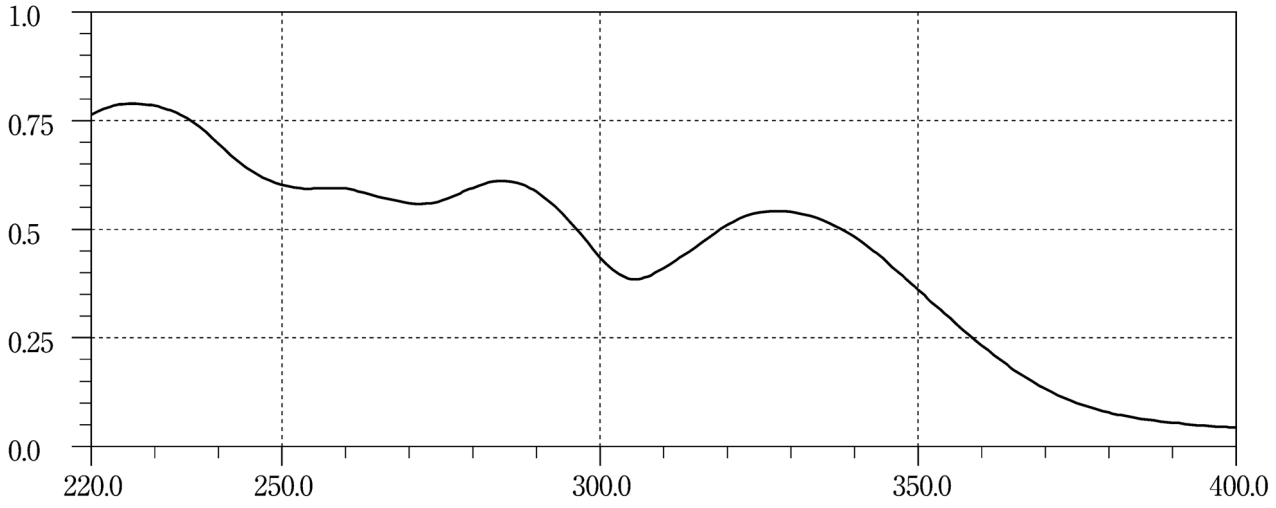
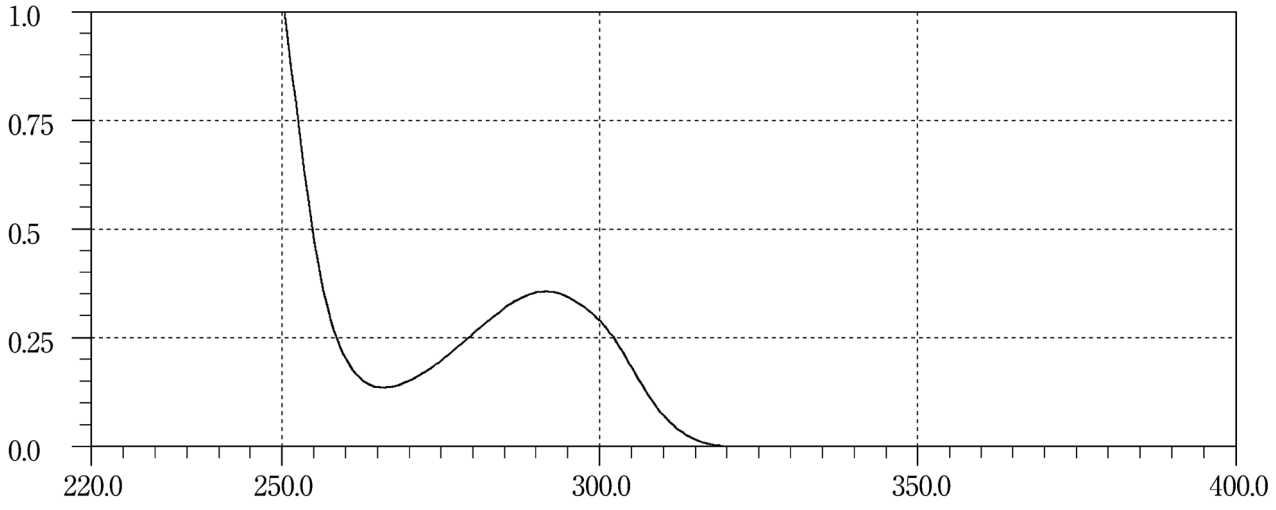


Sulpiride



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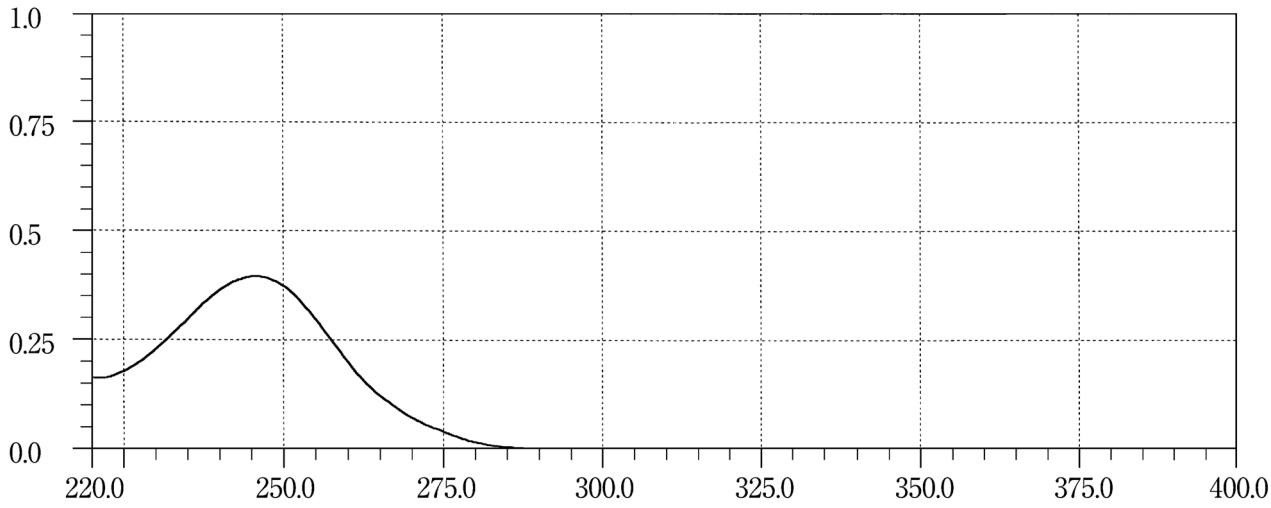
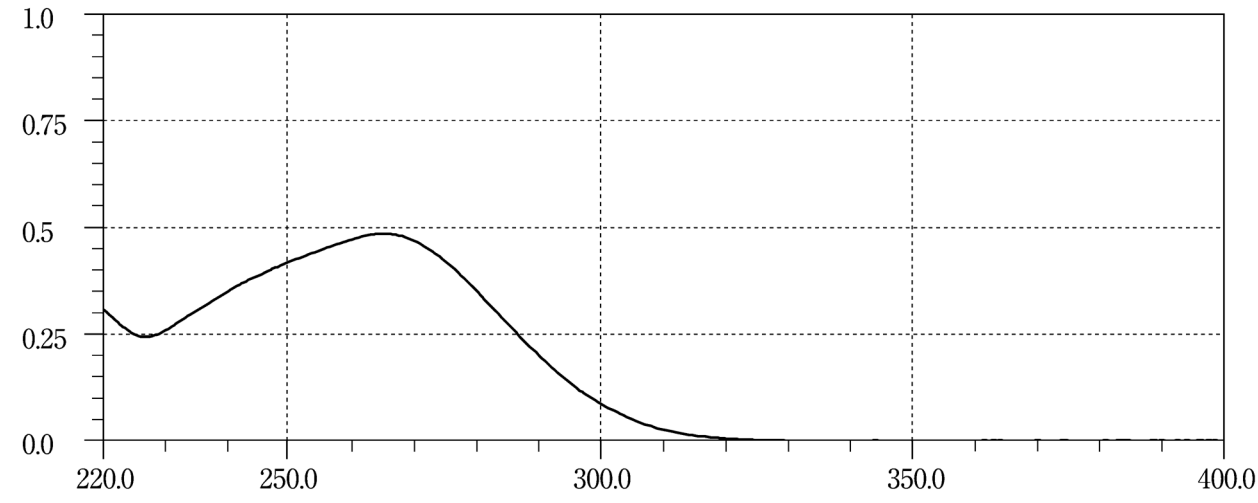
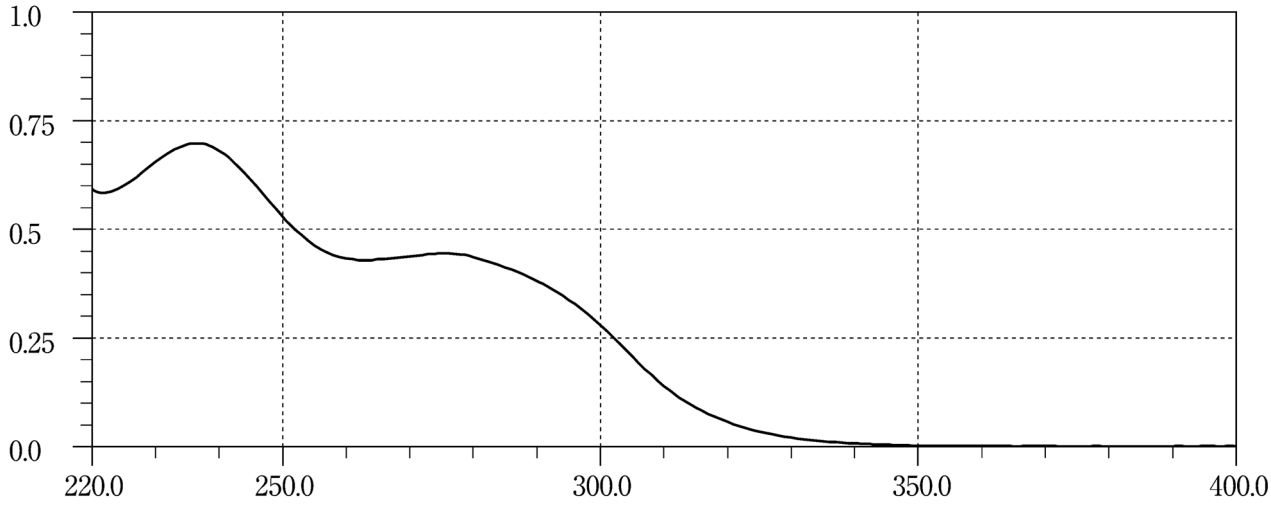


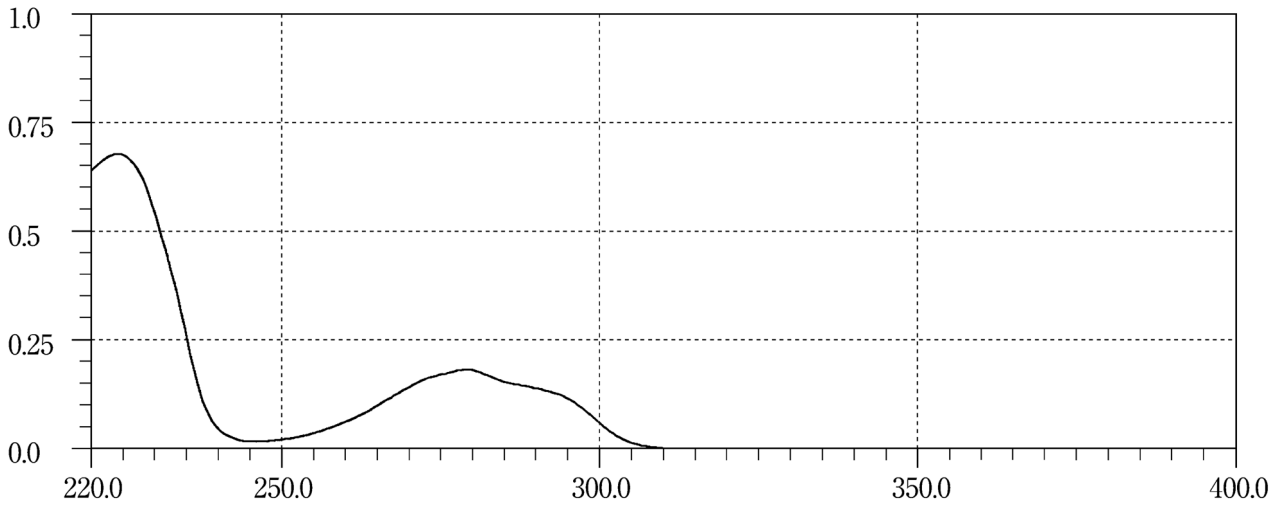
Tacalcitol Hydrate



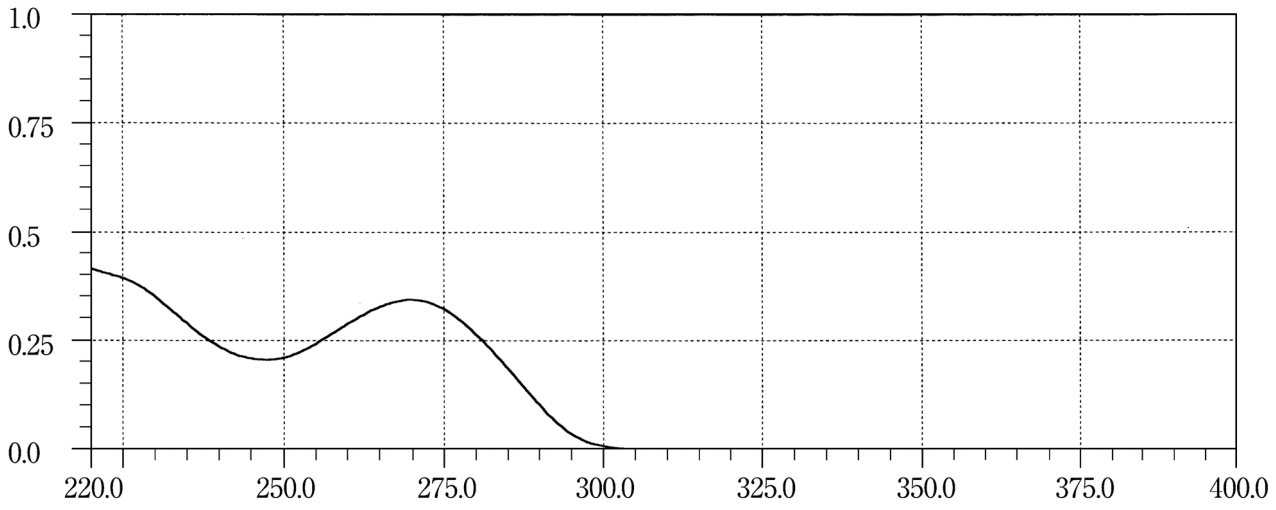
Tamoxifen Citrate



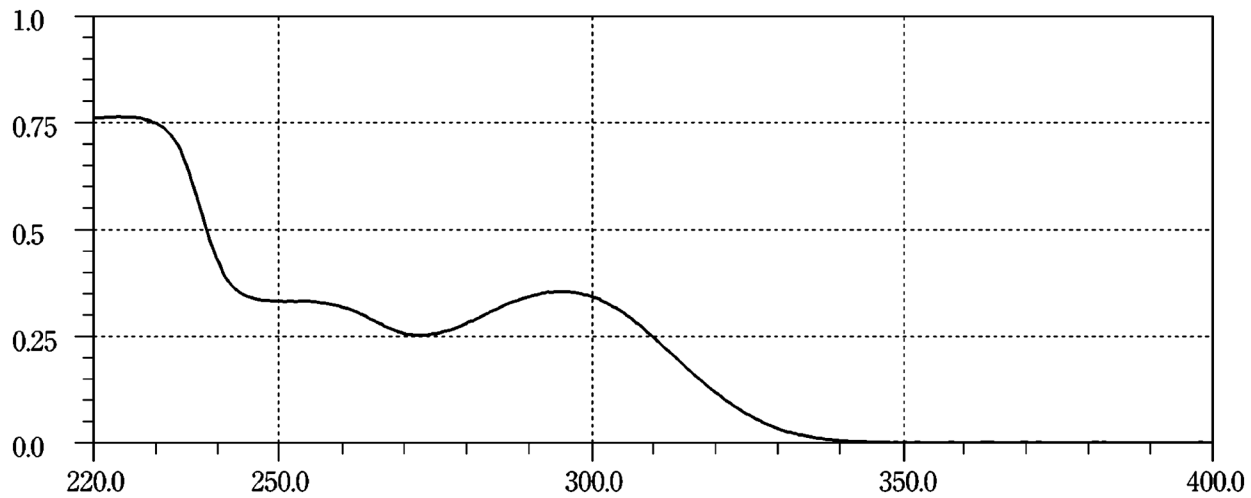
Tamsulosin Hydrochloride



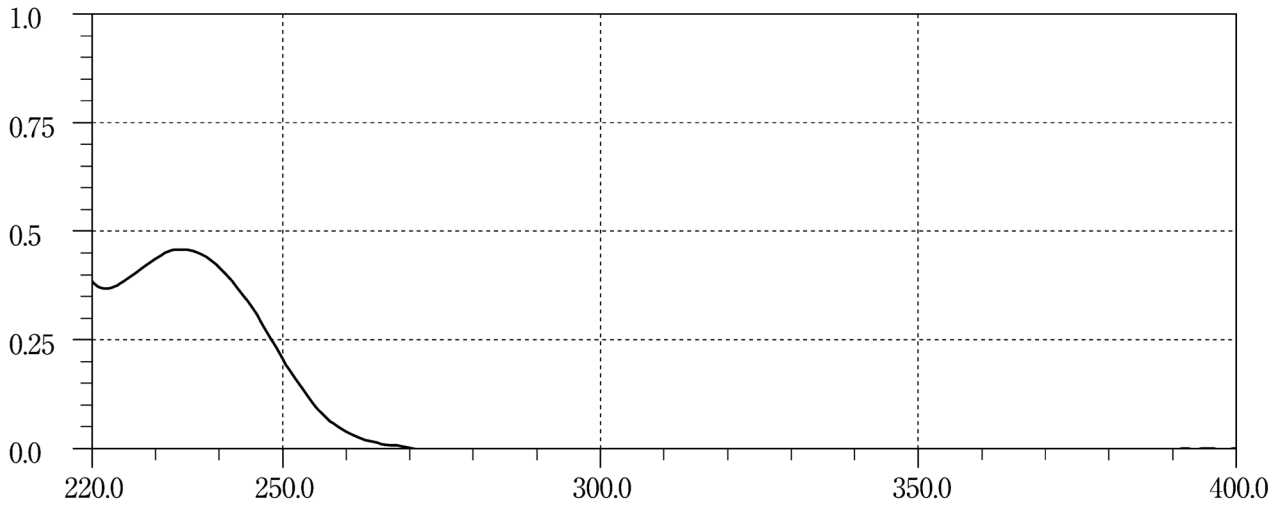
Tegafur



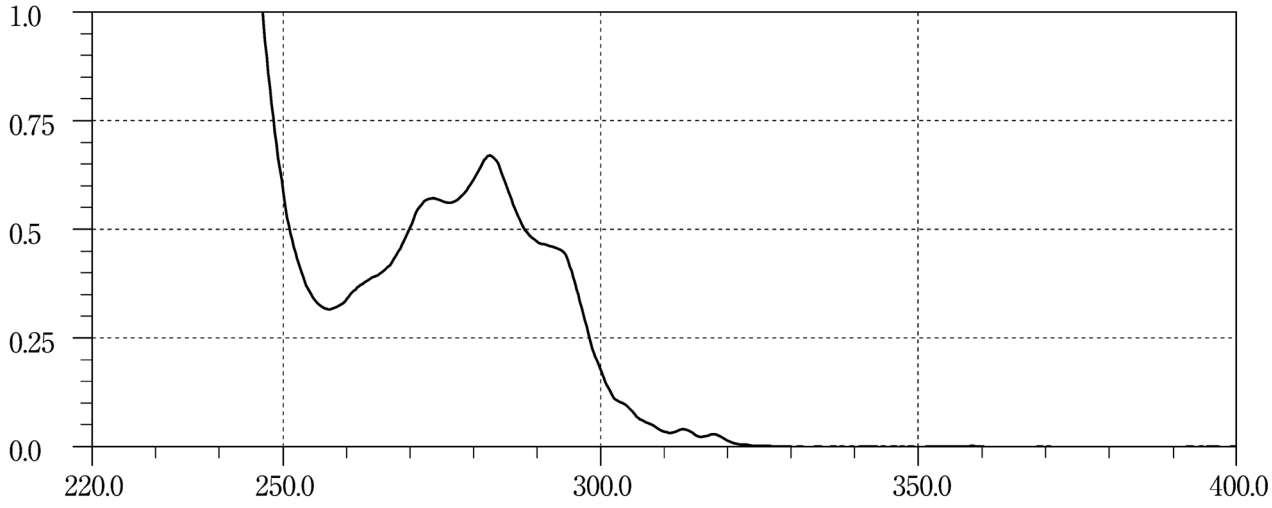
Telmisartan



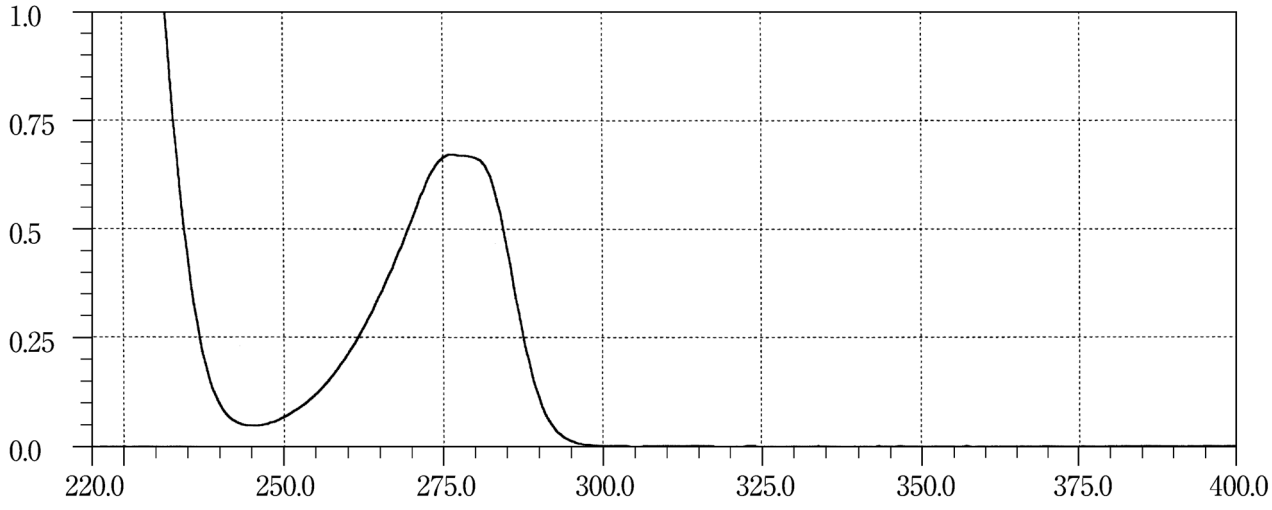
Temocapril Hydrochloride



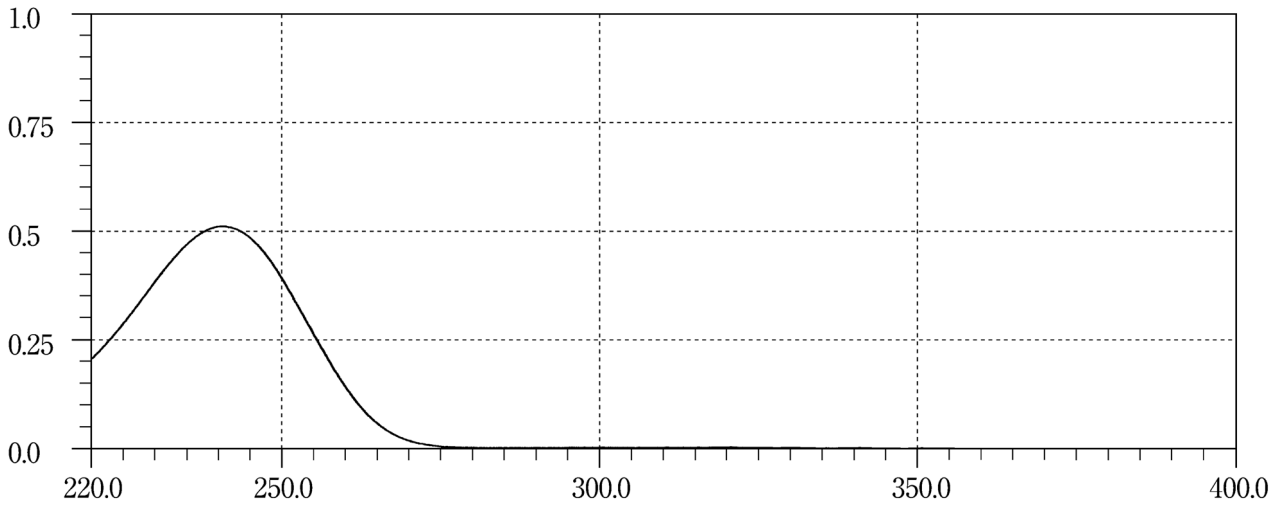
Terbinafine Hydrochloride



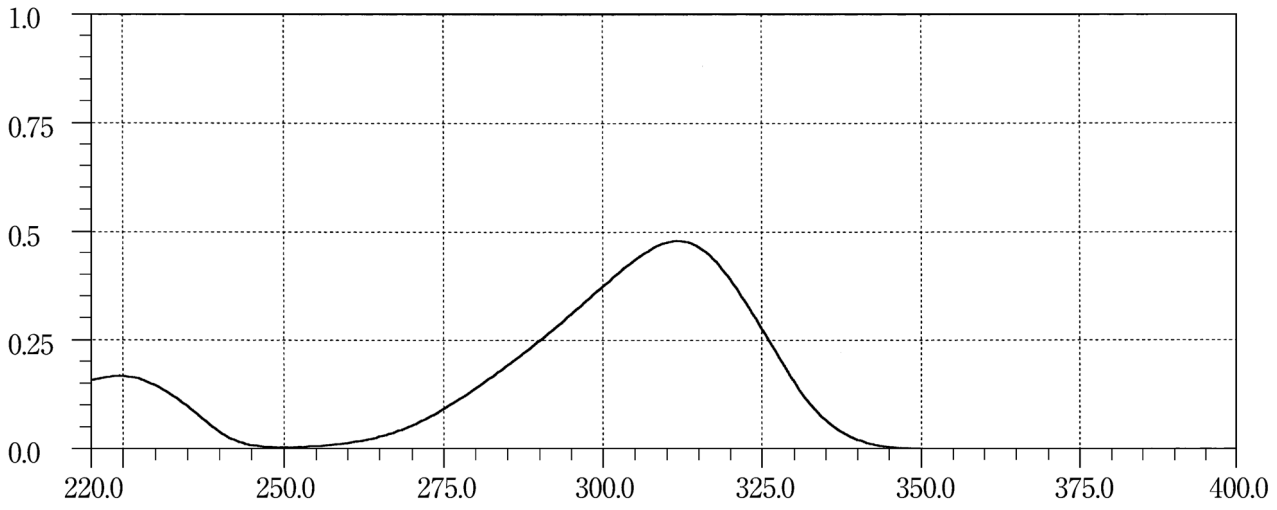
Terbutaline Sulfate



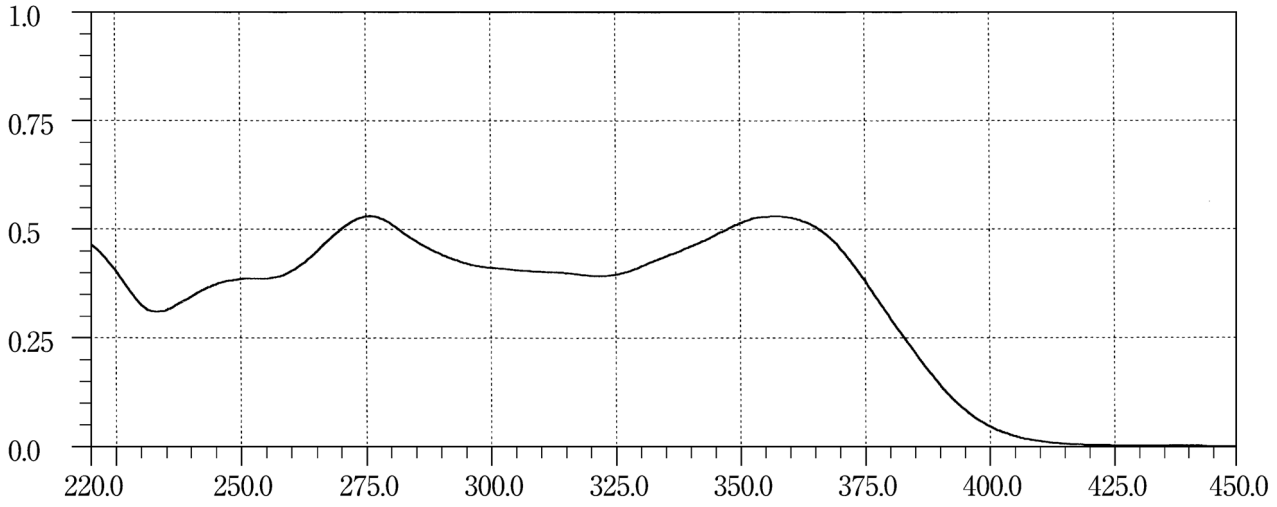
Testosterone Propionate



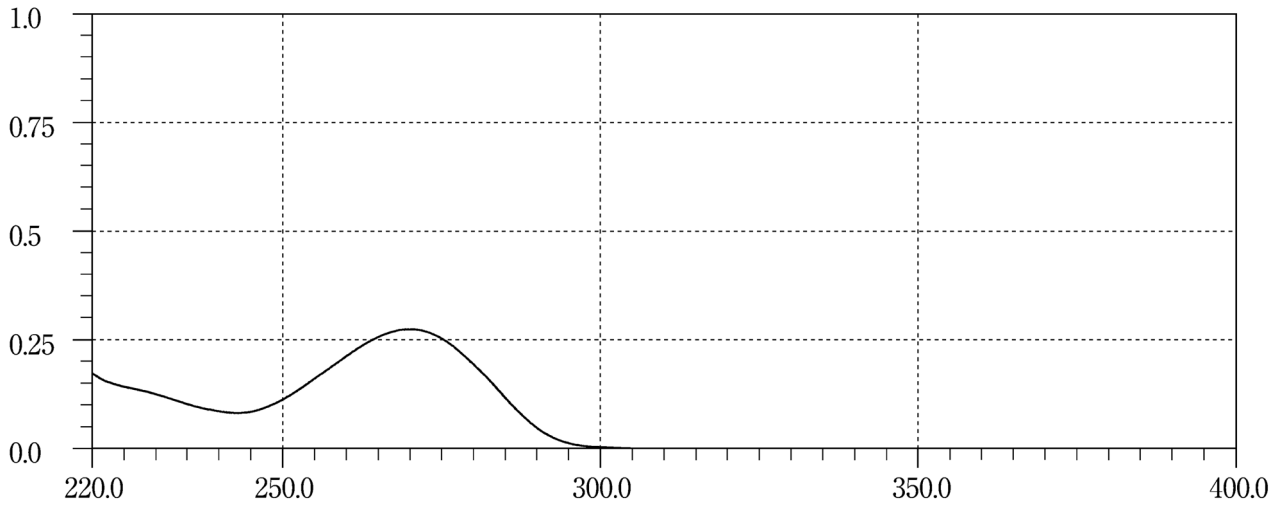
Tetracaine Hydrochloride



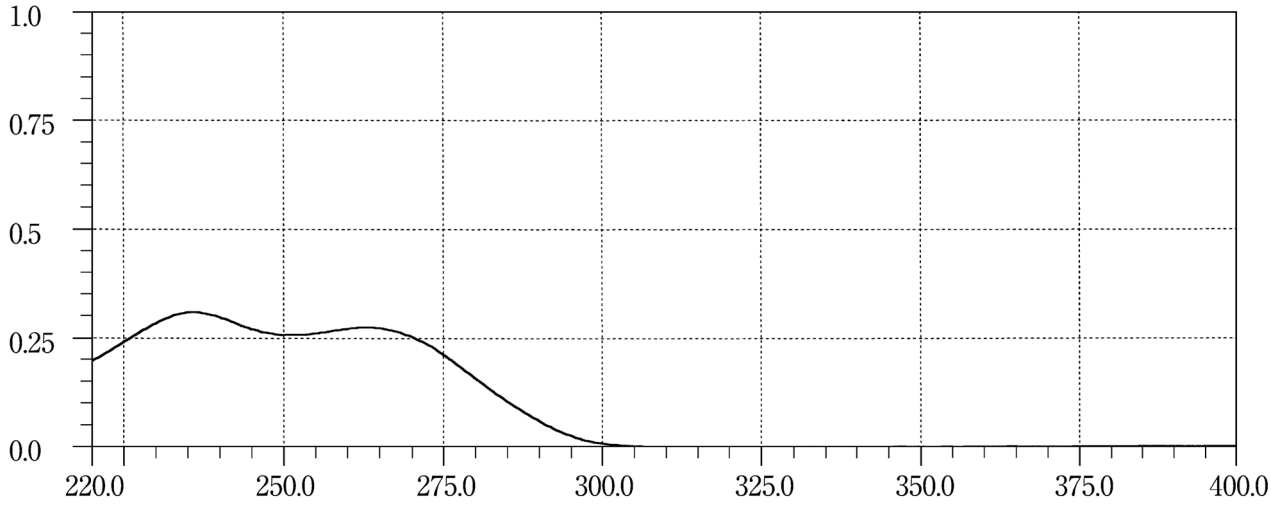
Tetracycline Hydrochloride



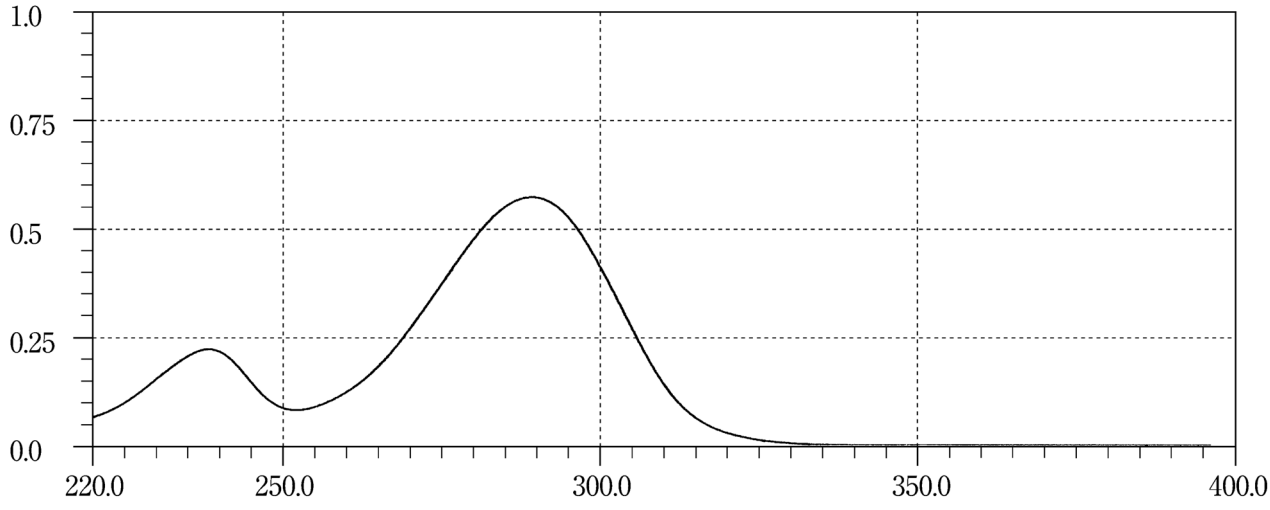
Theophylline



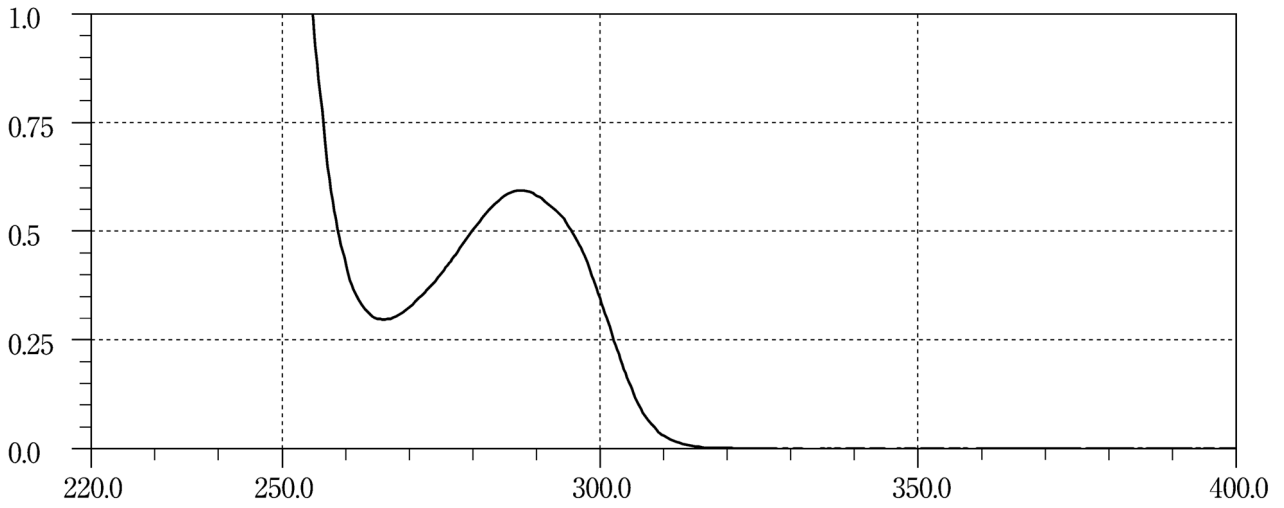
Thiamine Chloride Hydrochloride



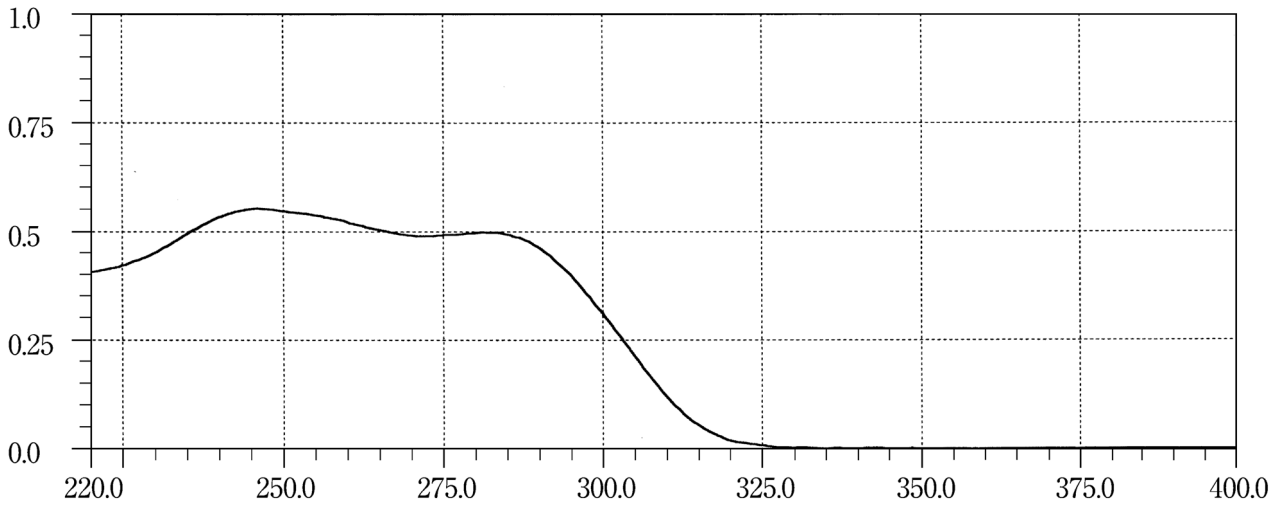
Thiamylal Sodium



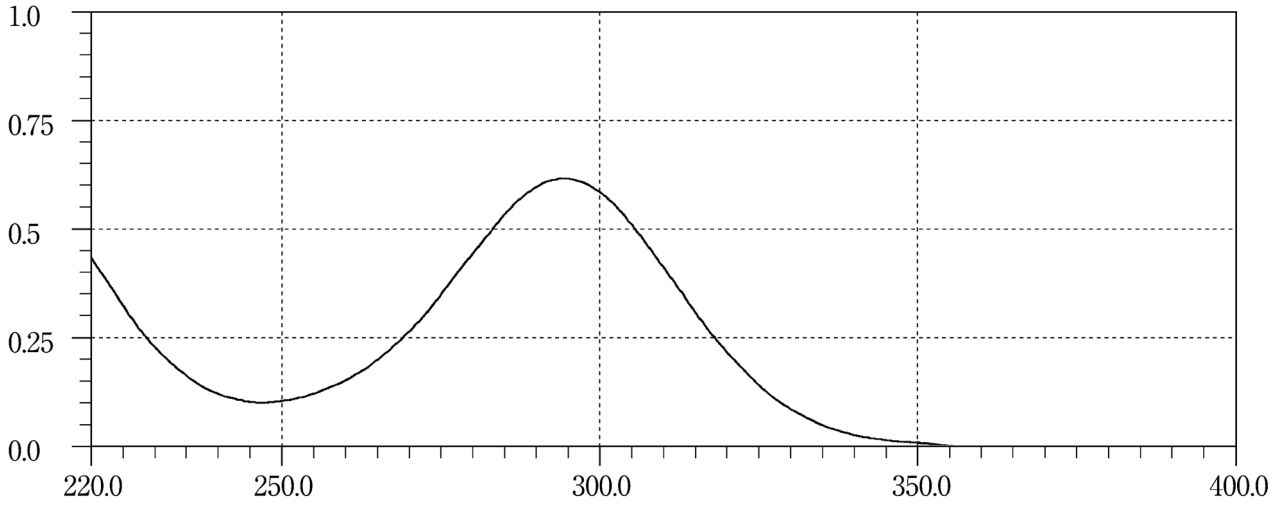
Tiapride Hydrochloride



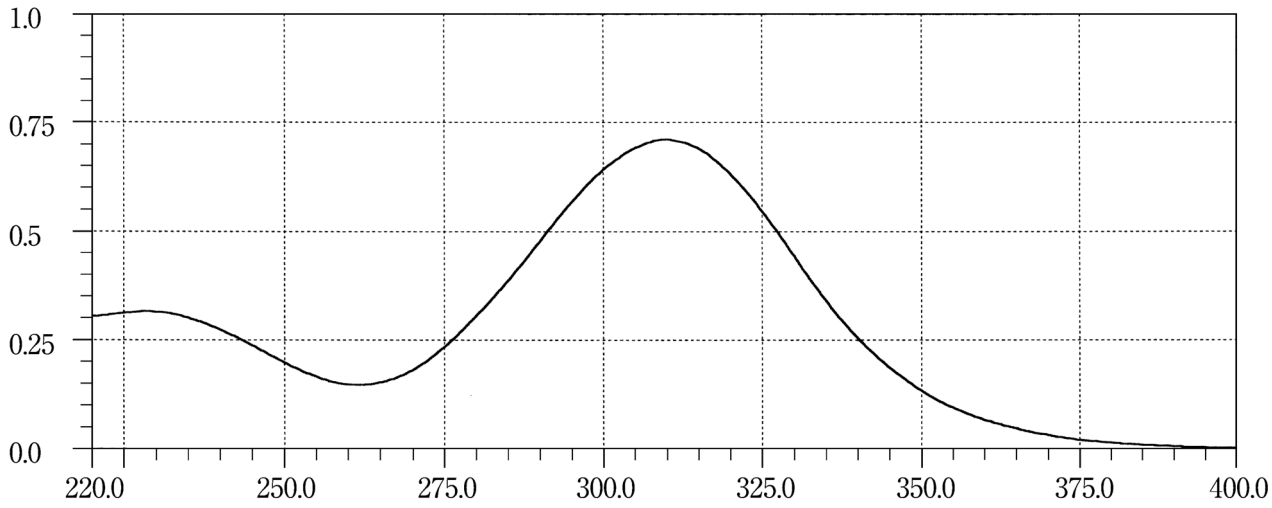
Timepidium Bromide Hydrate



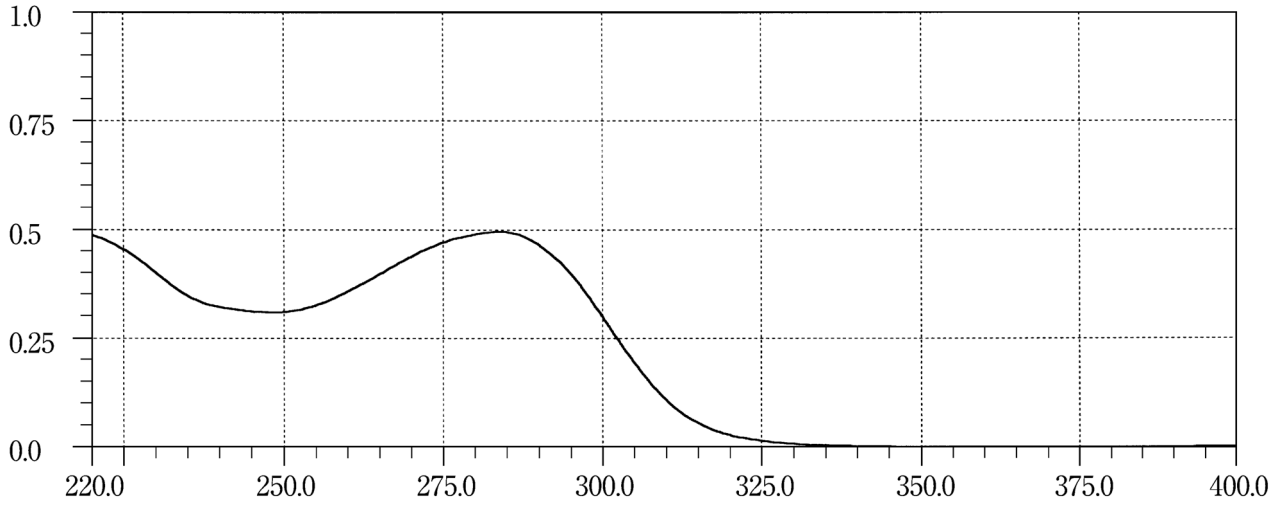
Timolol Maleate



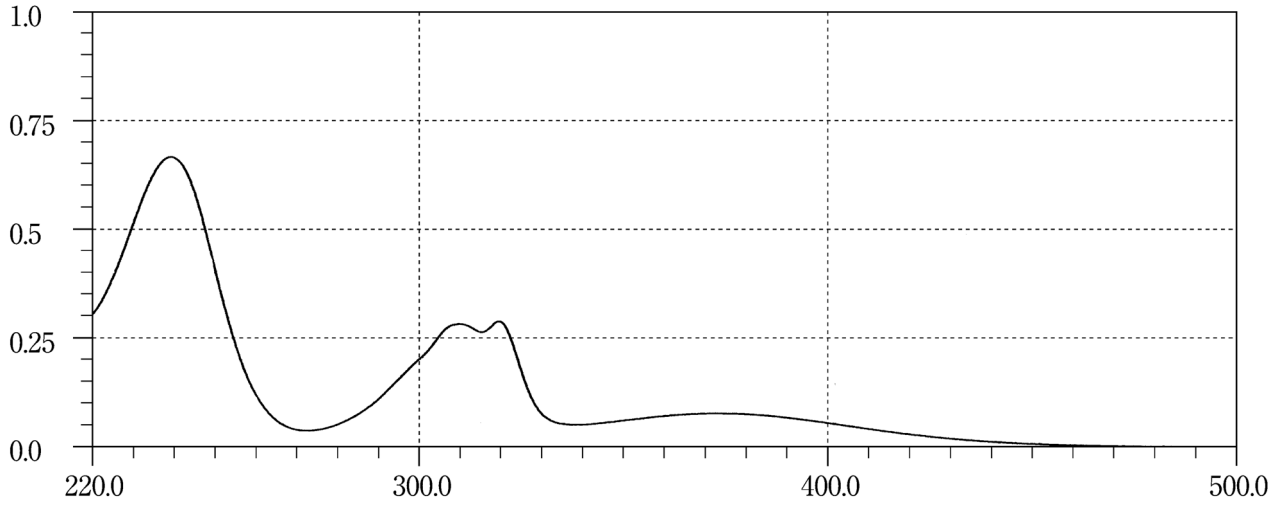
Tinidazole



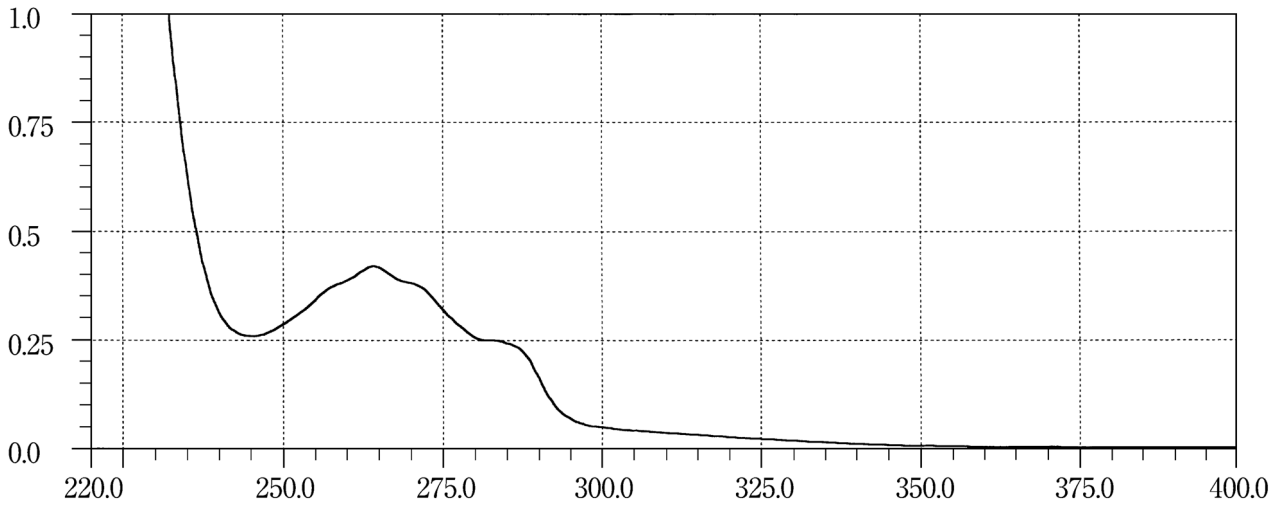
Tipepidine Hibenzate



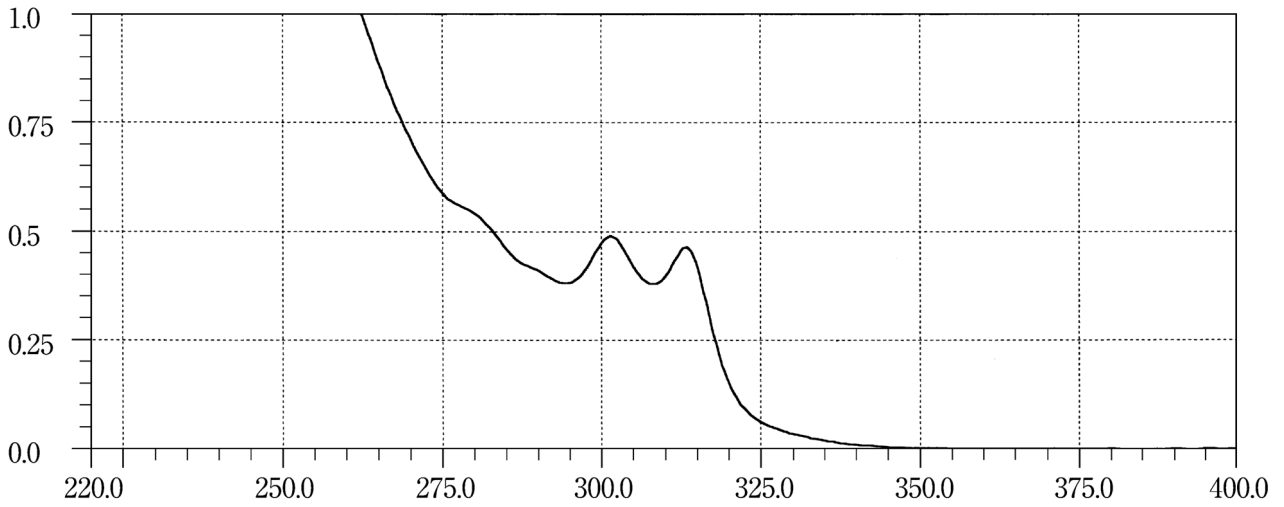
Tizanidine Hydrochloride



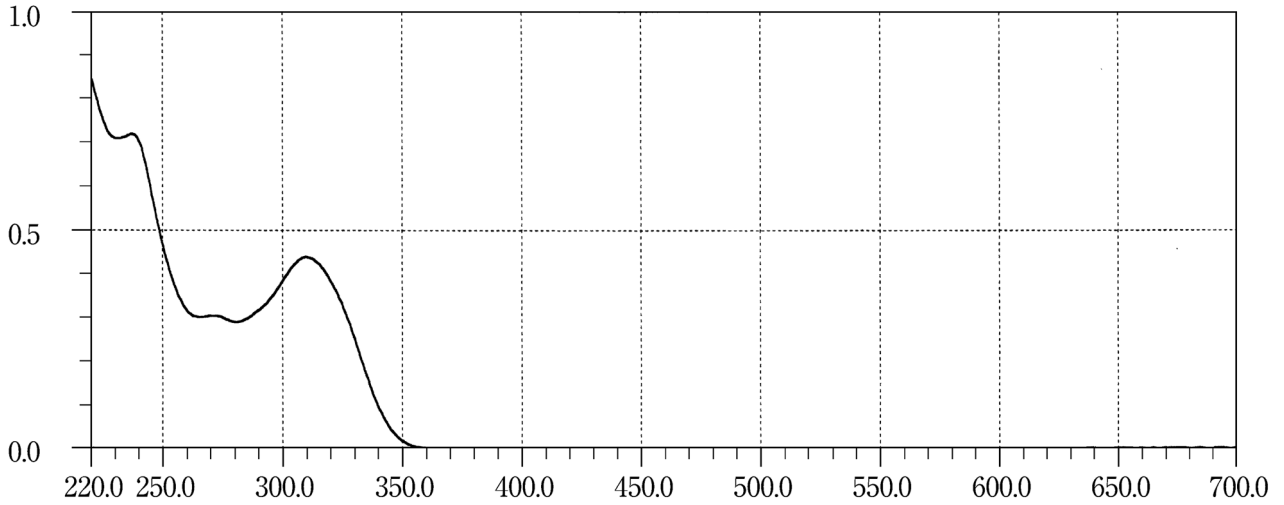
Tocopherol Nicotinate



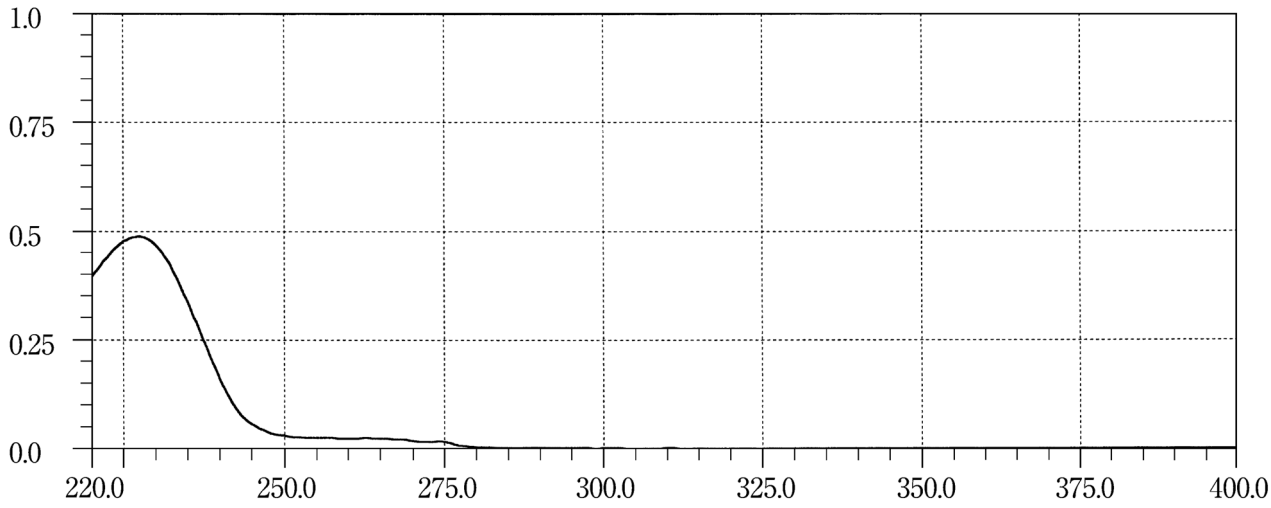
Todralazine Hydrochloride Hydrate



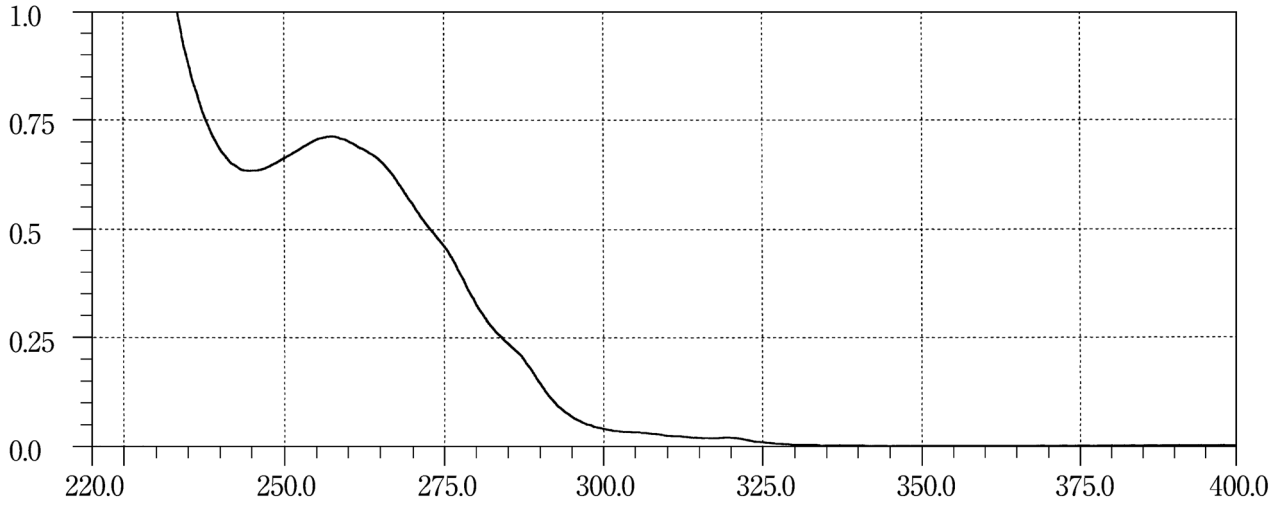
Tofisopam



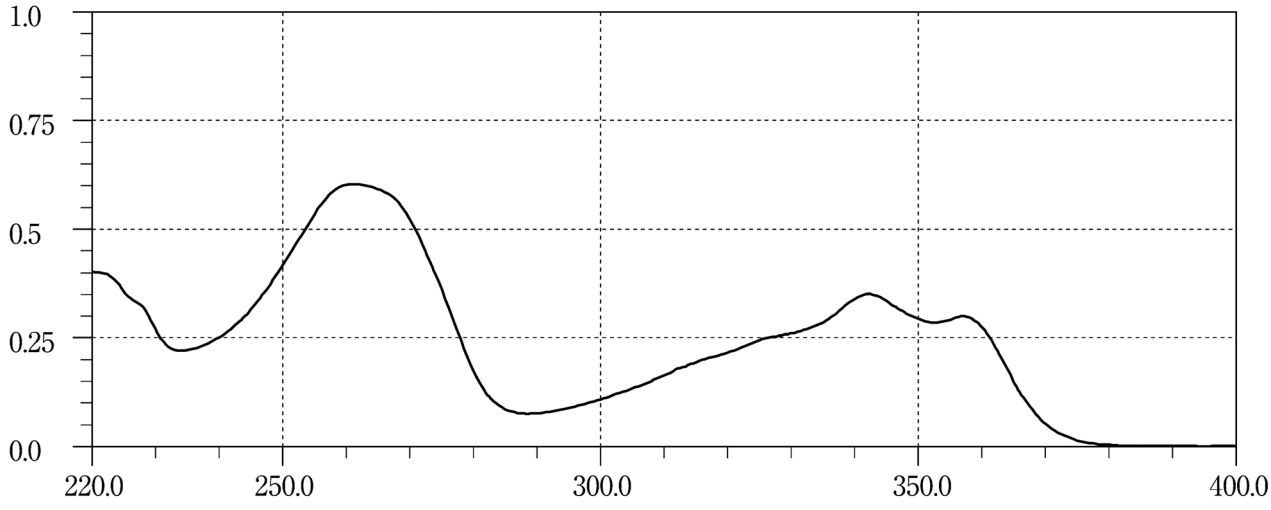
Tolazamide



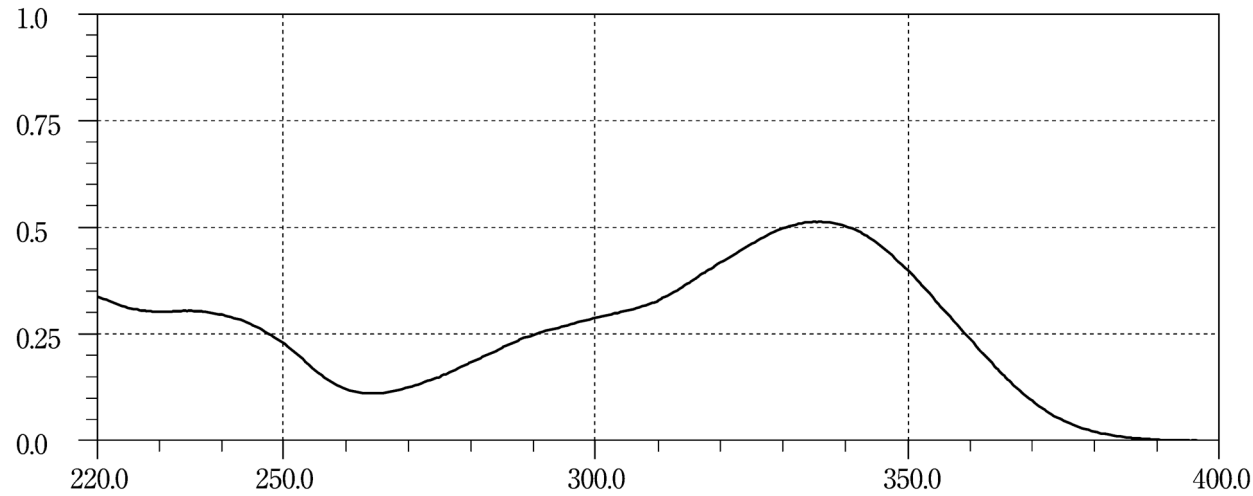
Tolnaftate



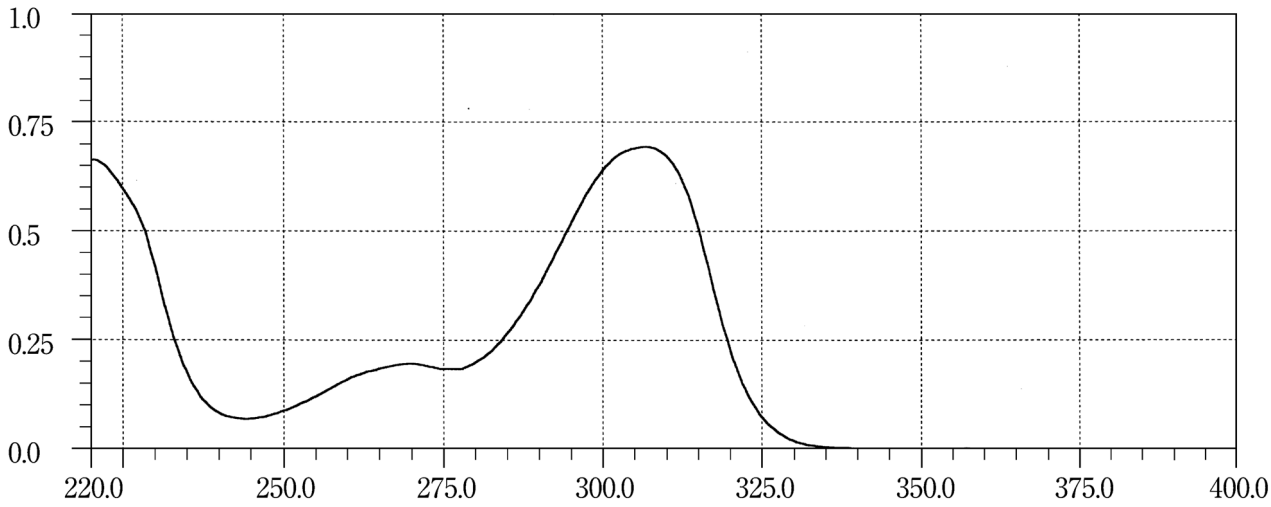
Tosufloxacin Tosilate Hydrate



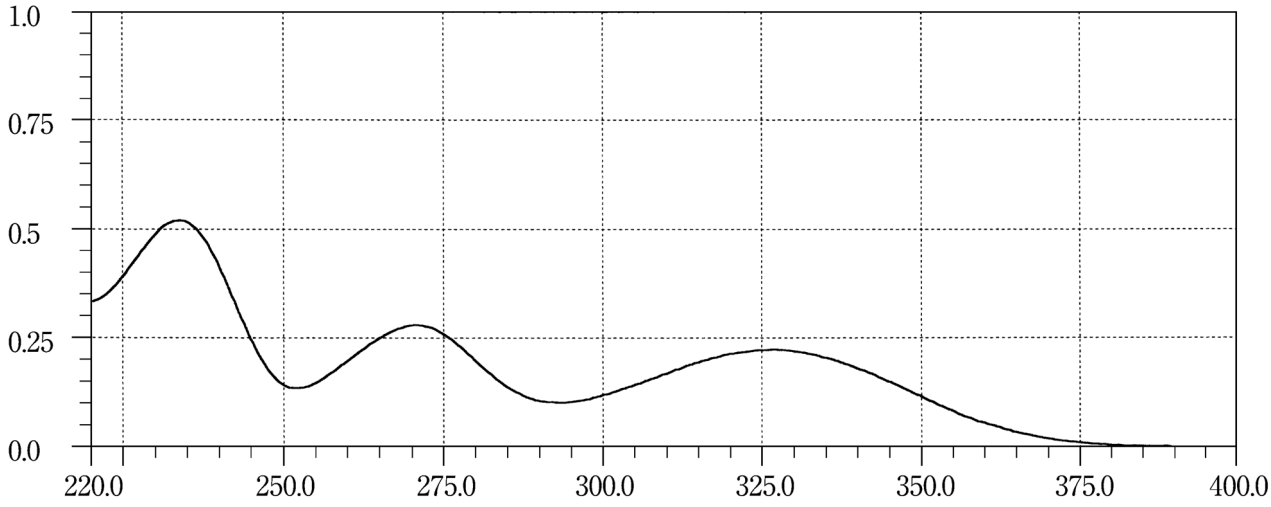
Tranilast



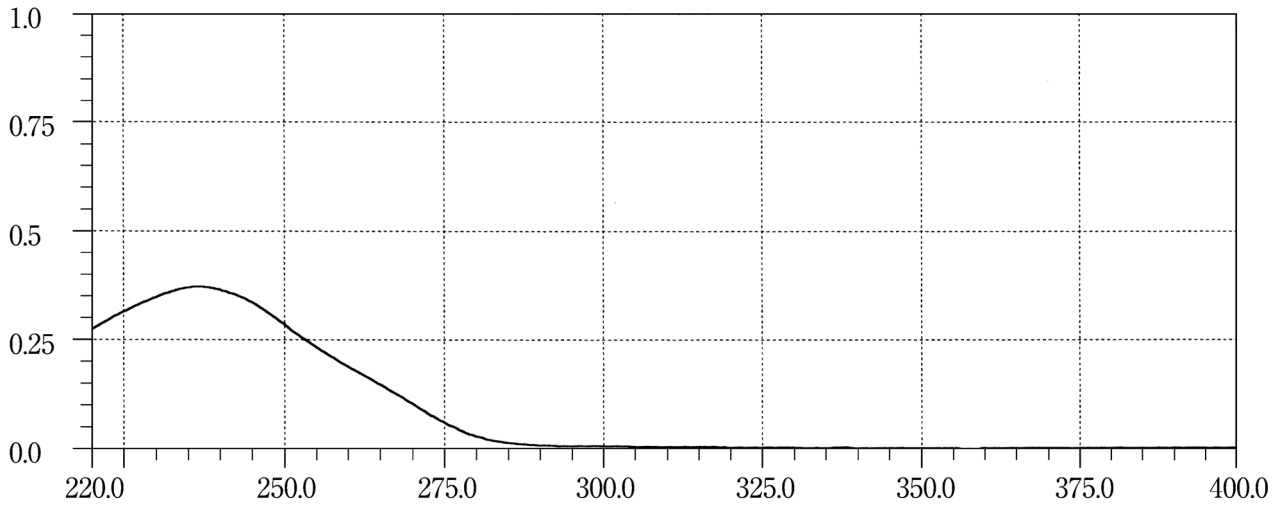
Trapidil



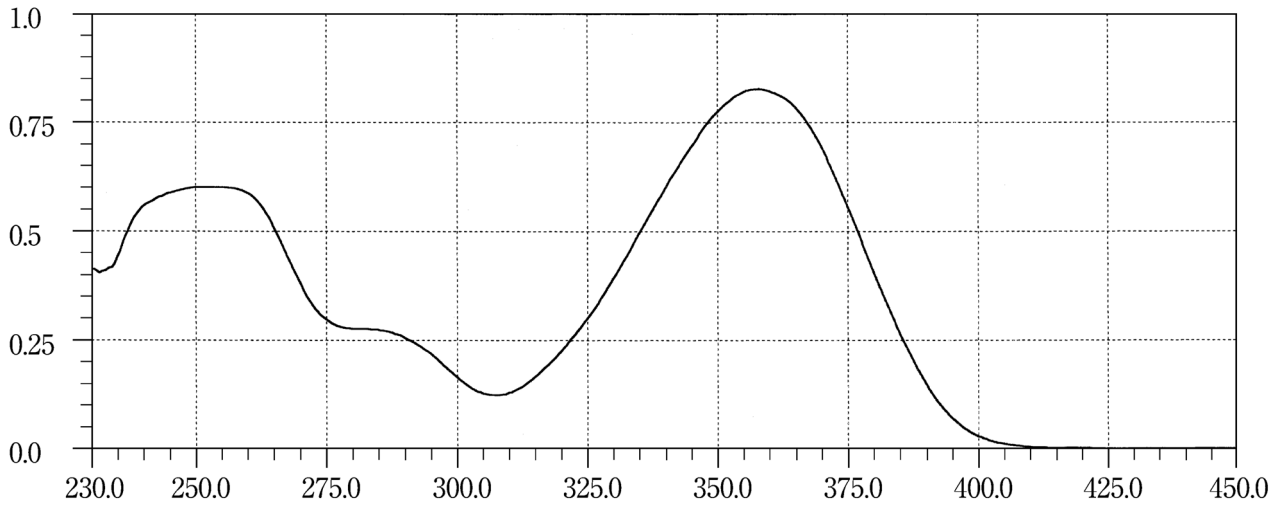
Trepibutone



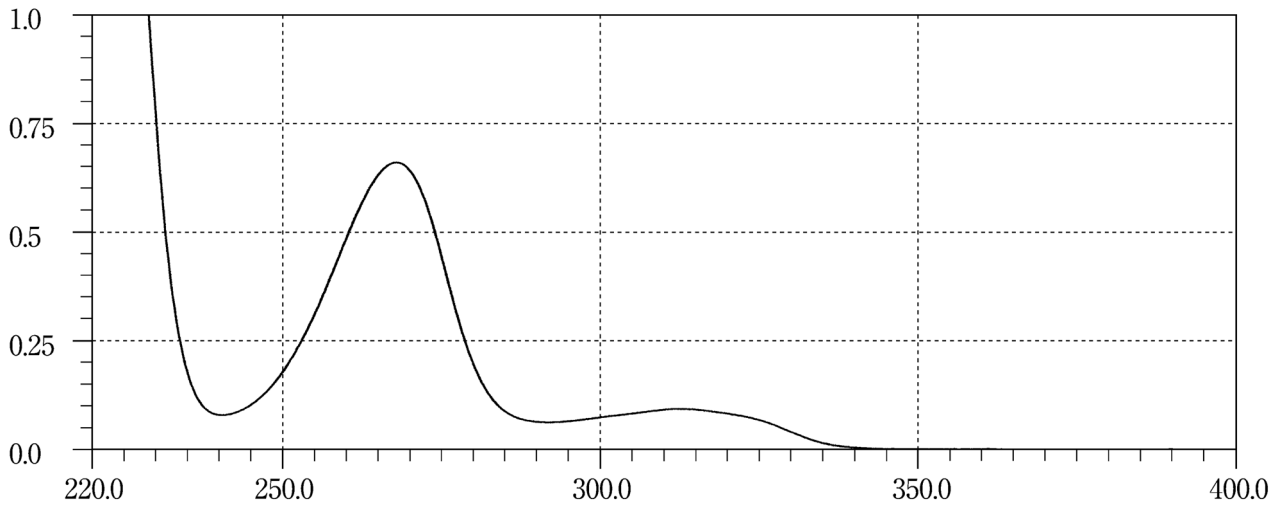
Triamcinolone Acetonide



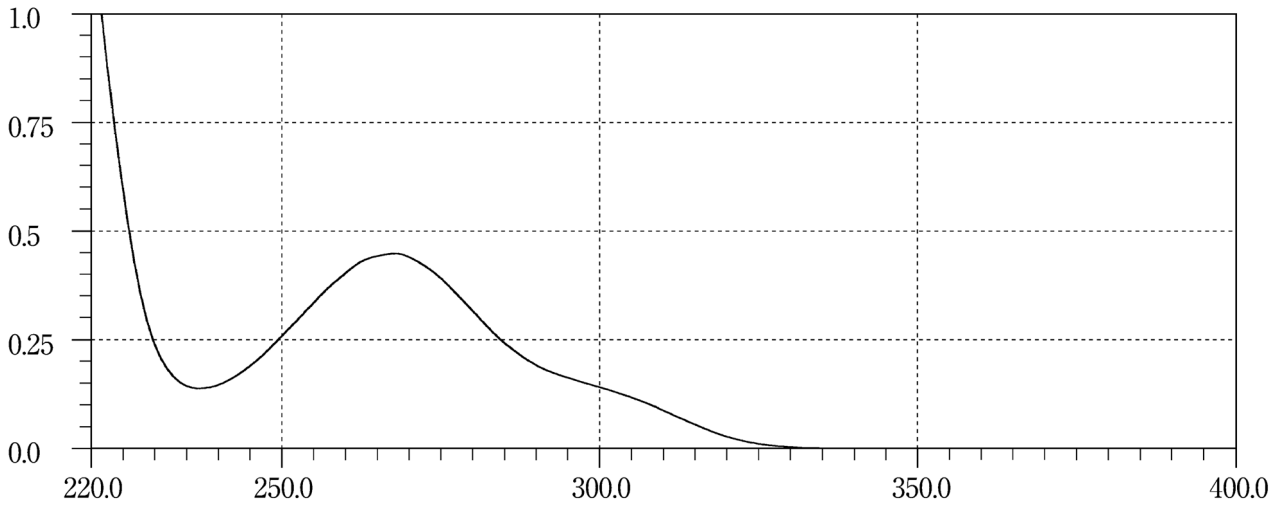
Triamterene



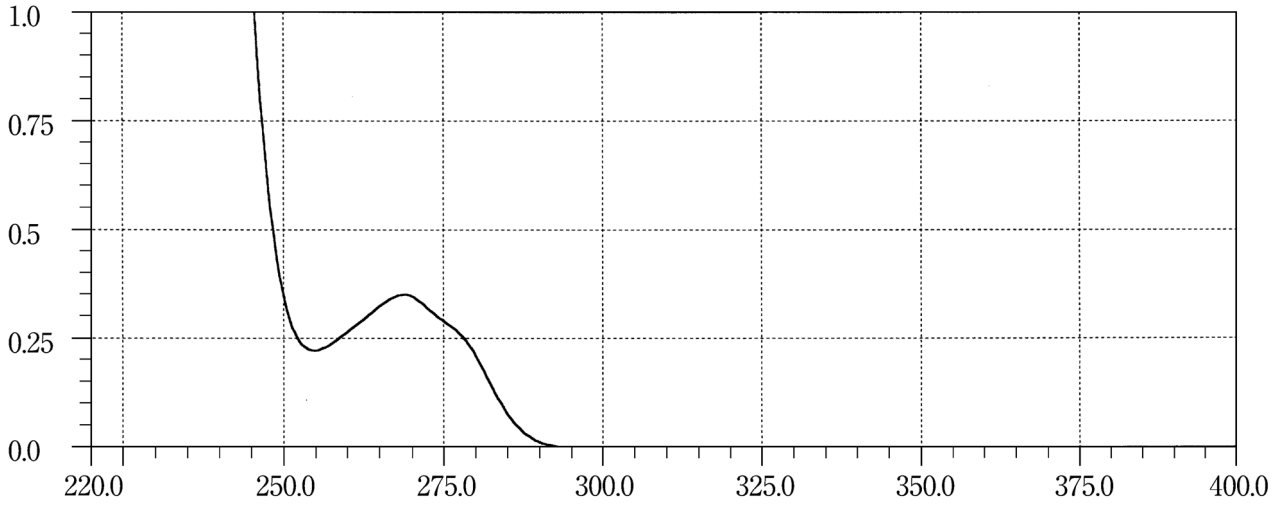
Trichlormethiazide



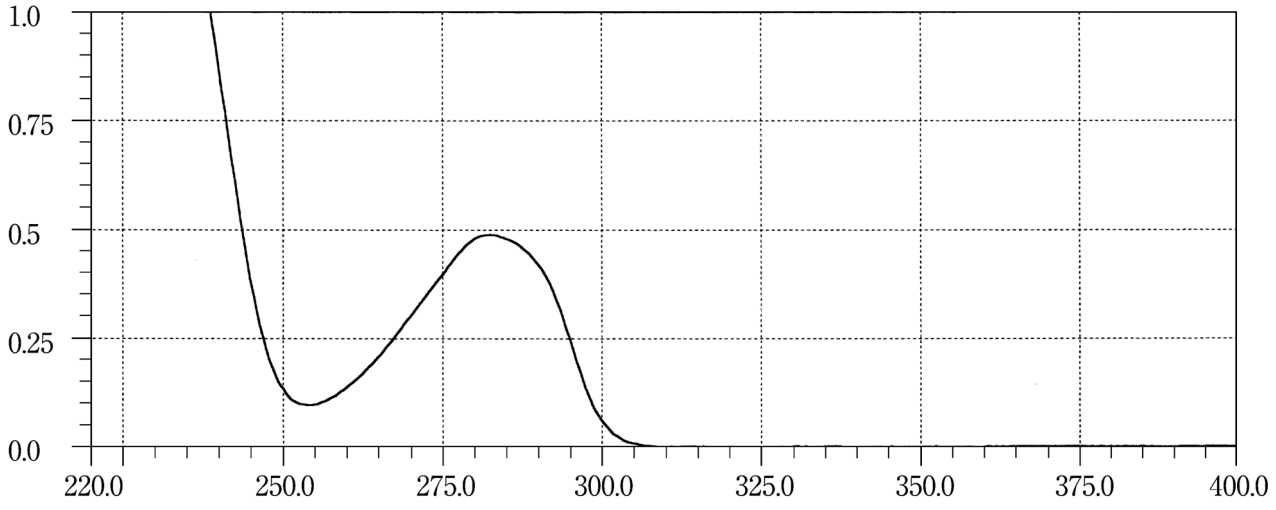
Trimebutine Maleate



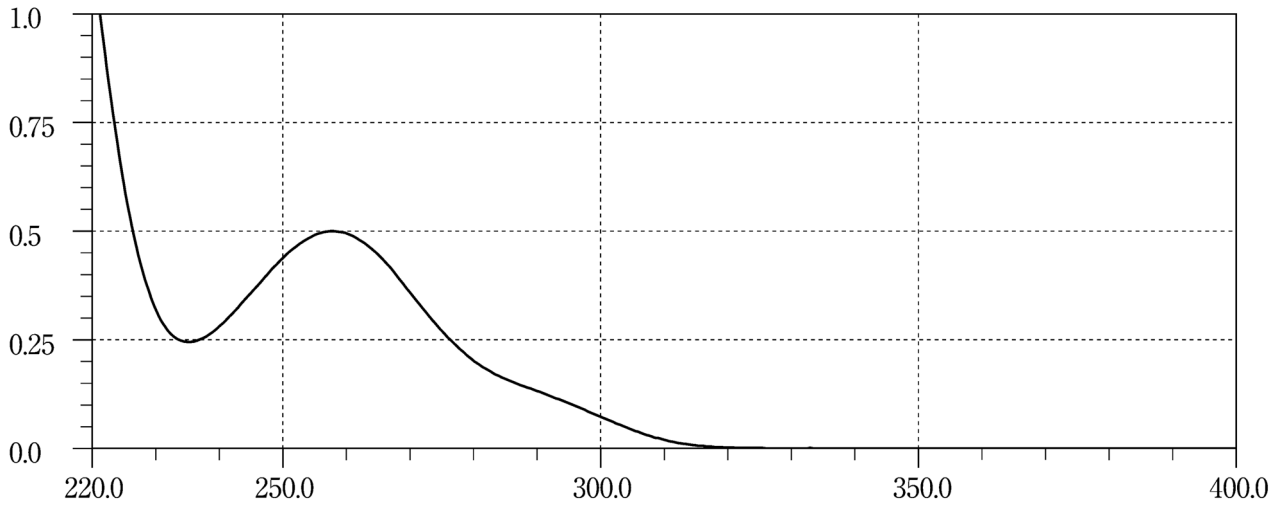
Trimetazidine Hydrochloride



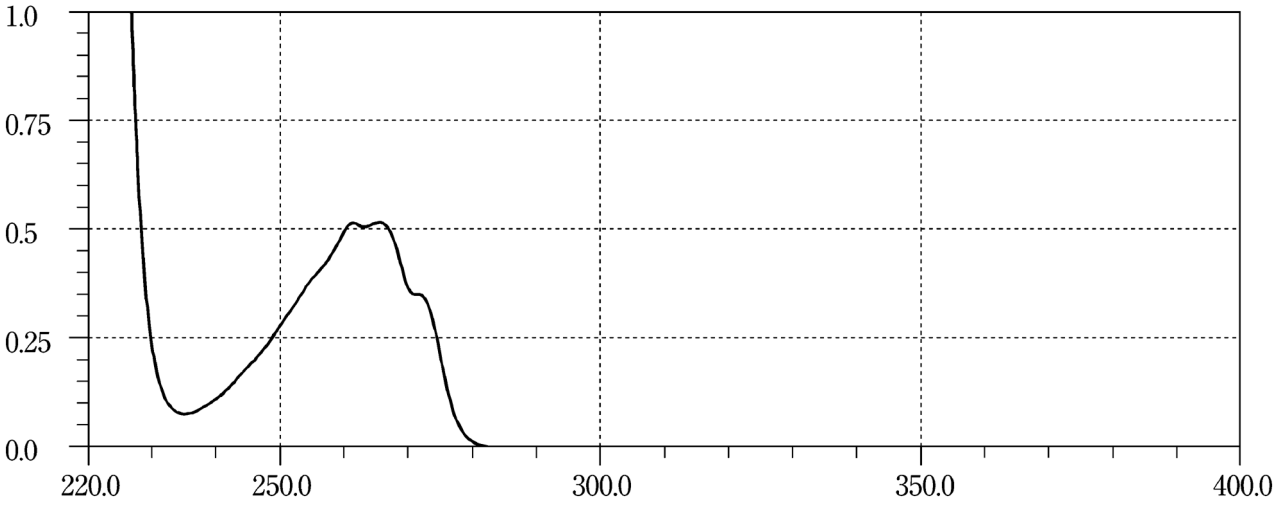
Trimetoquinol Hydrochloride Hydrate



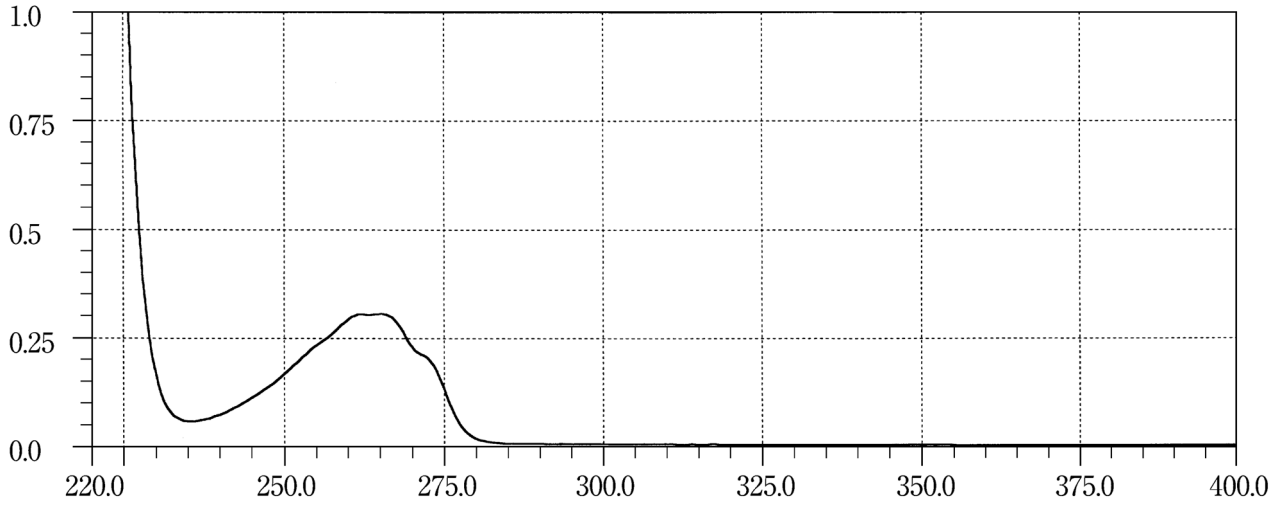
Troxipide



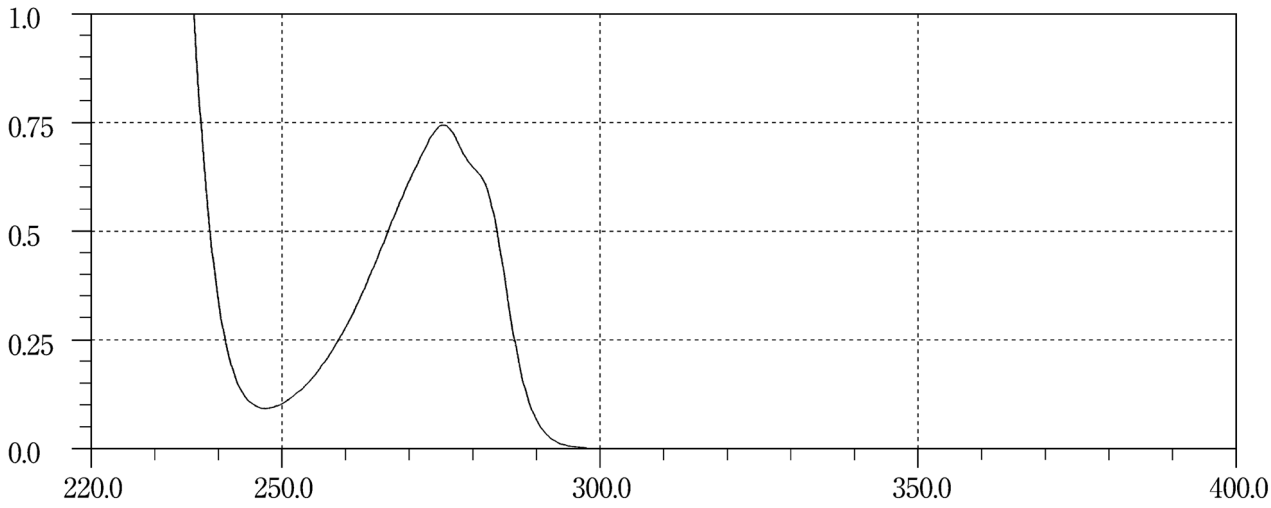
Tulobuterol



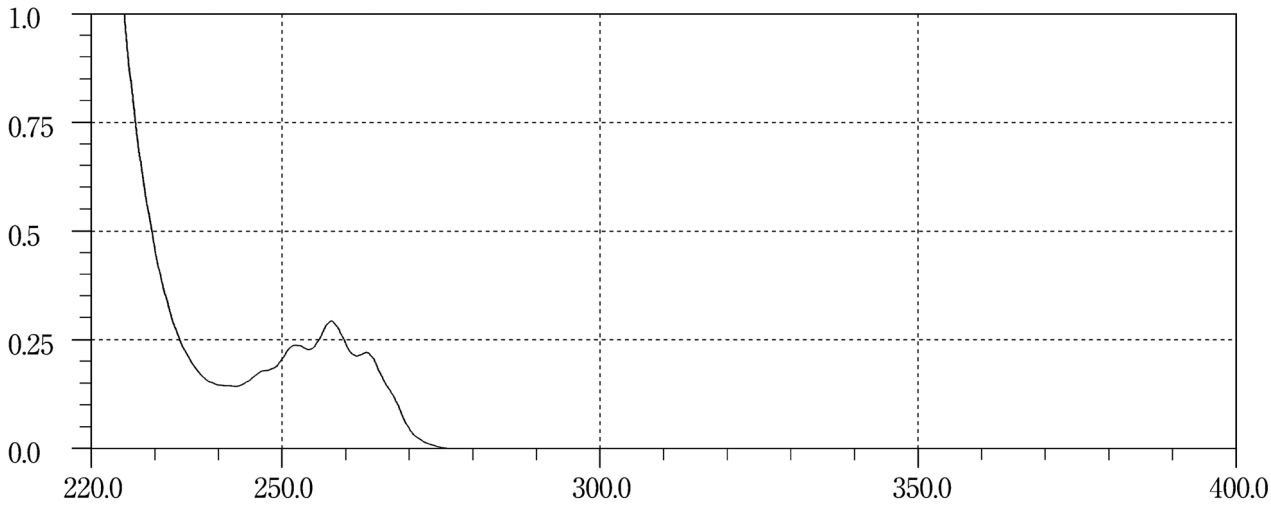
Tulobuterol Hydrochloride



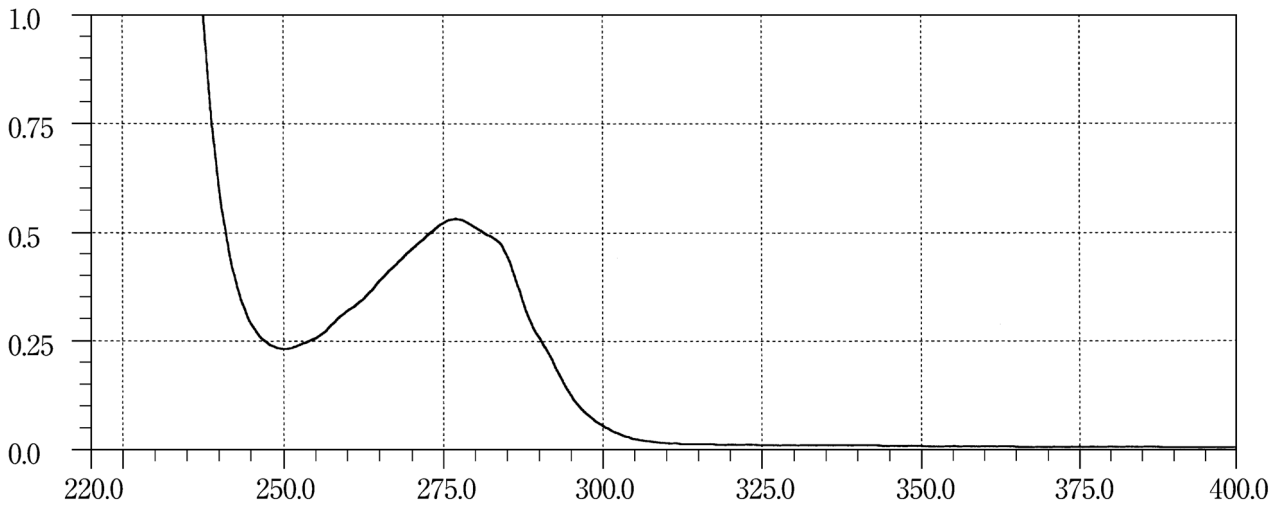
L-Tyrosine



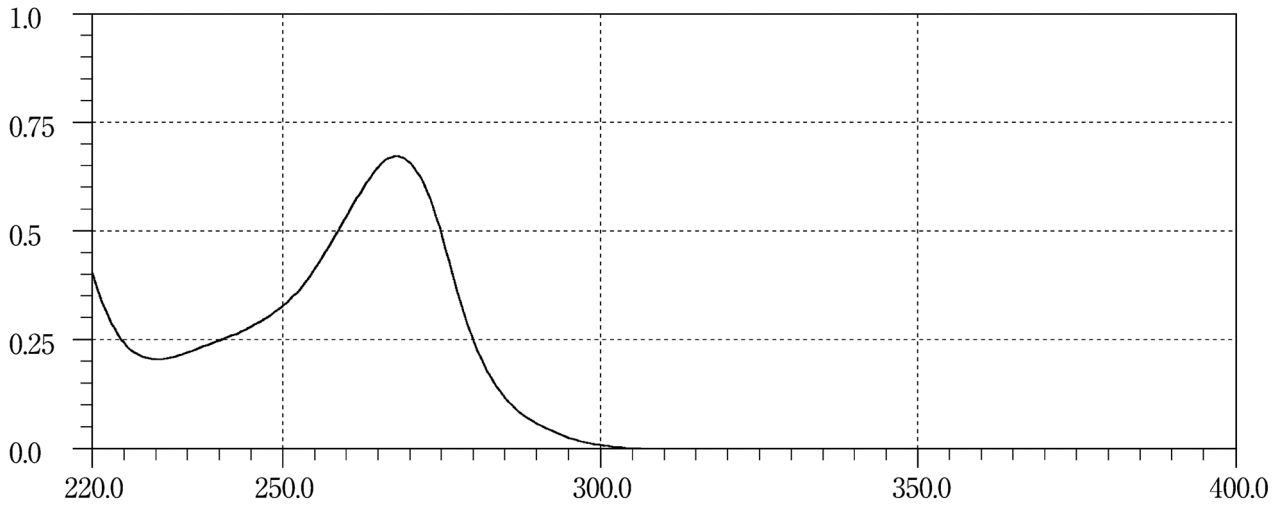
Ubenimex



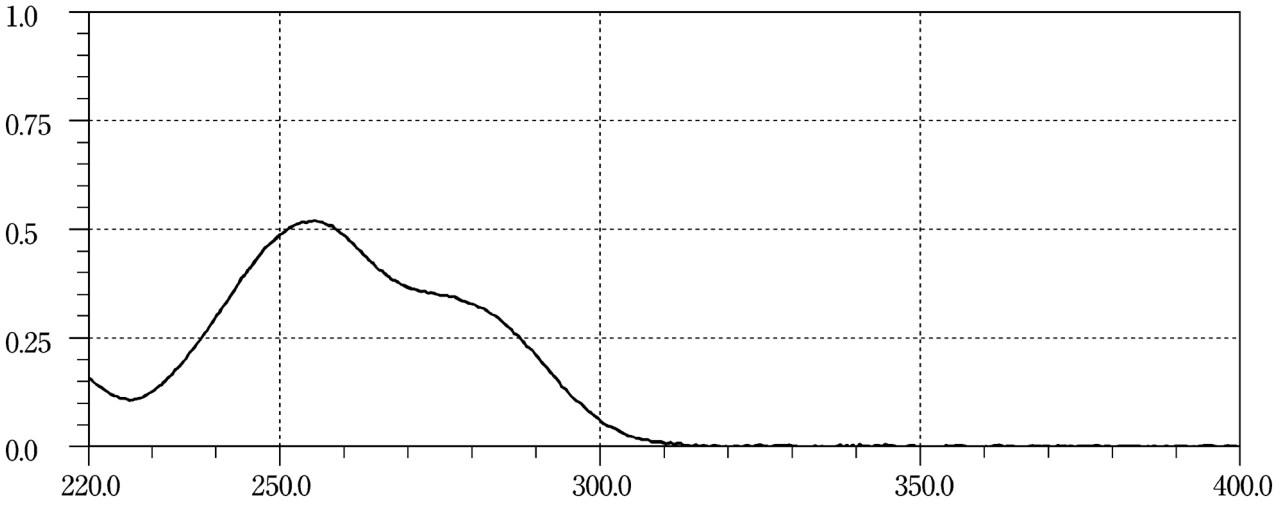
Ulinastatin



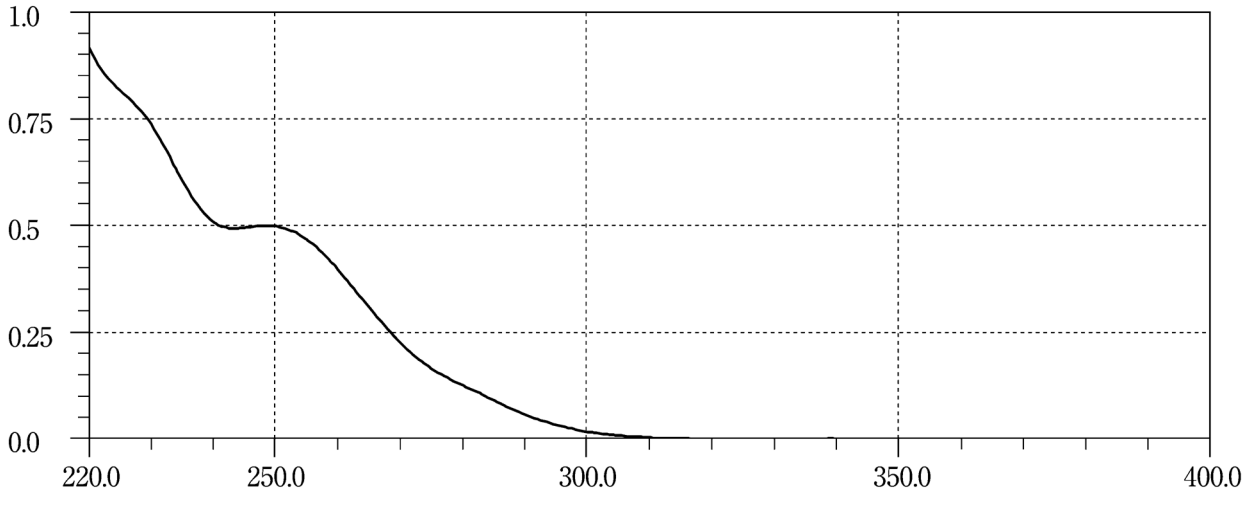
Urapidil



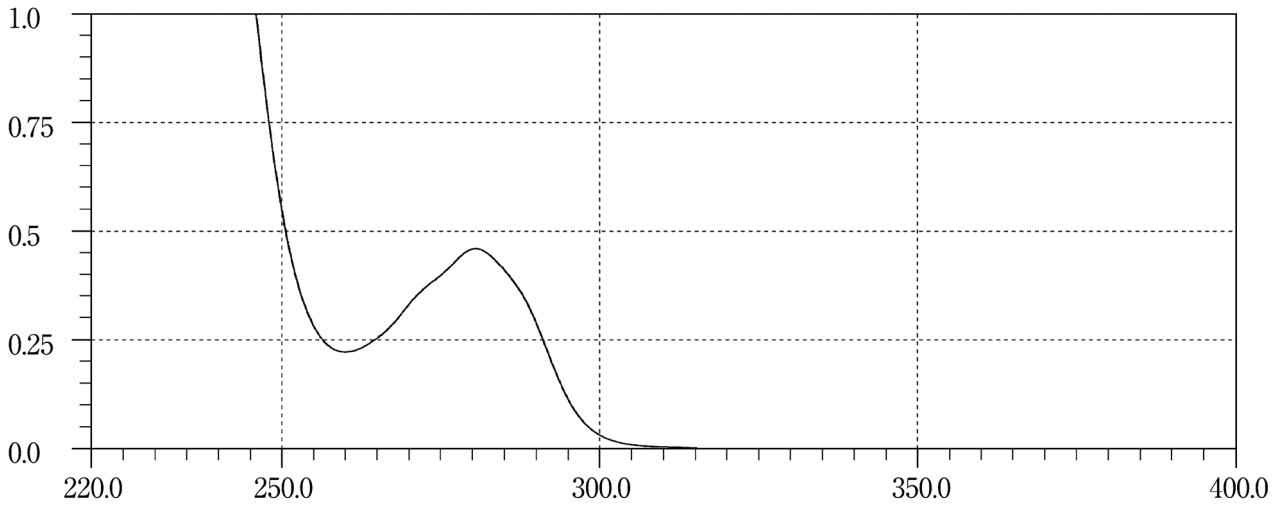
Valaciclovir Hydrochloride



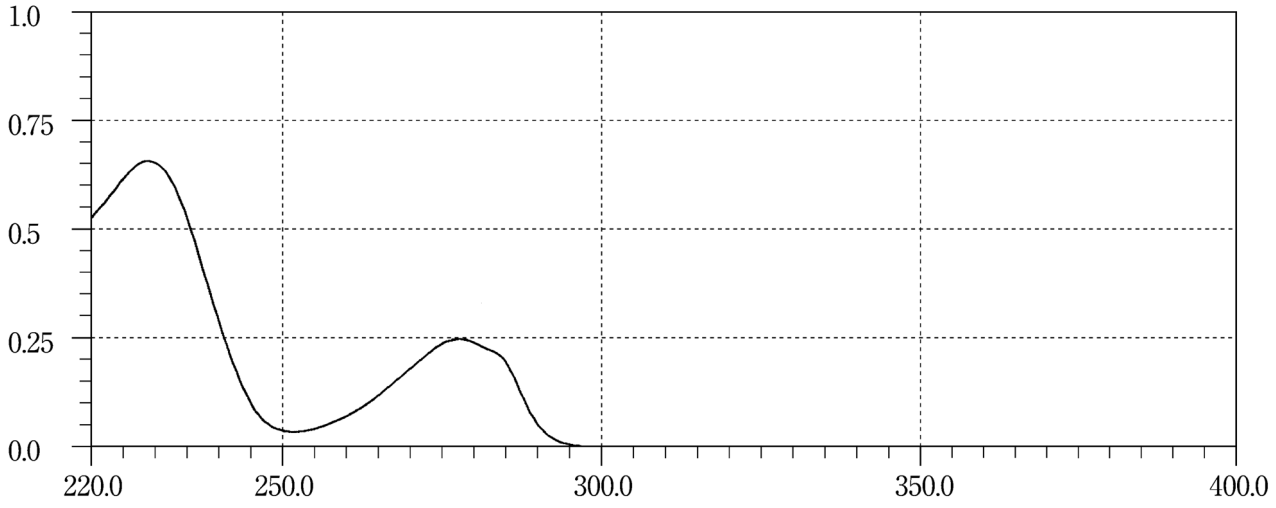
Valsartan



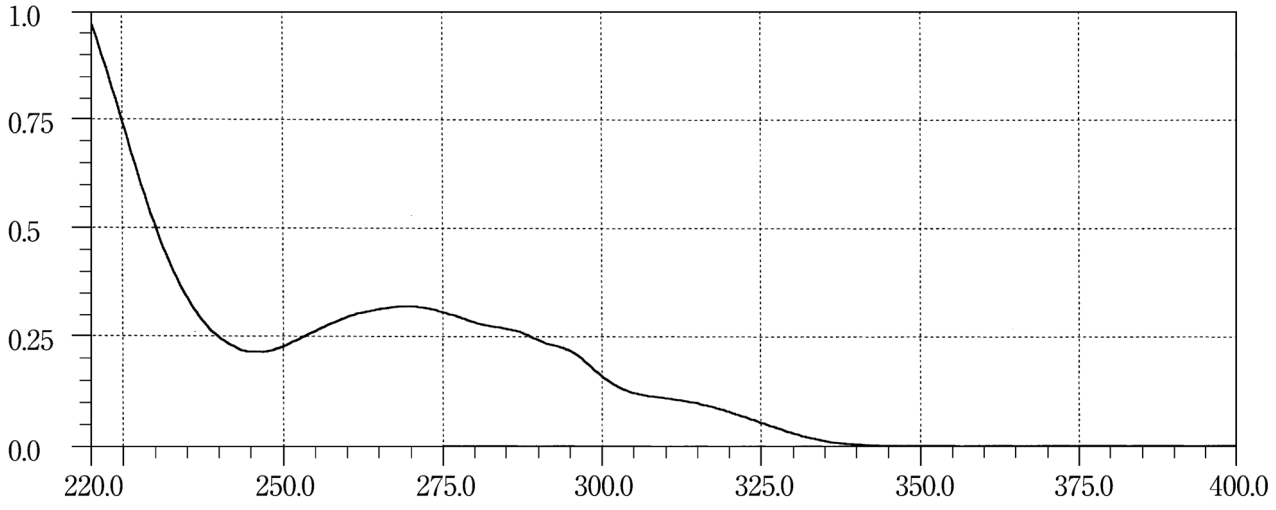
Vancomycin Hydrochloride



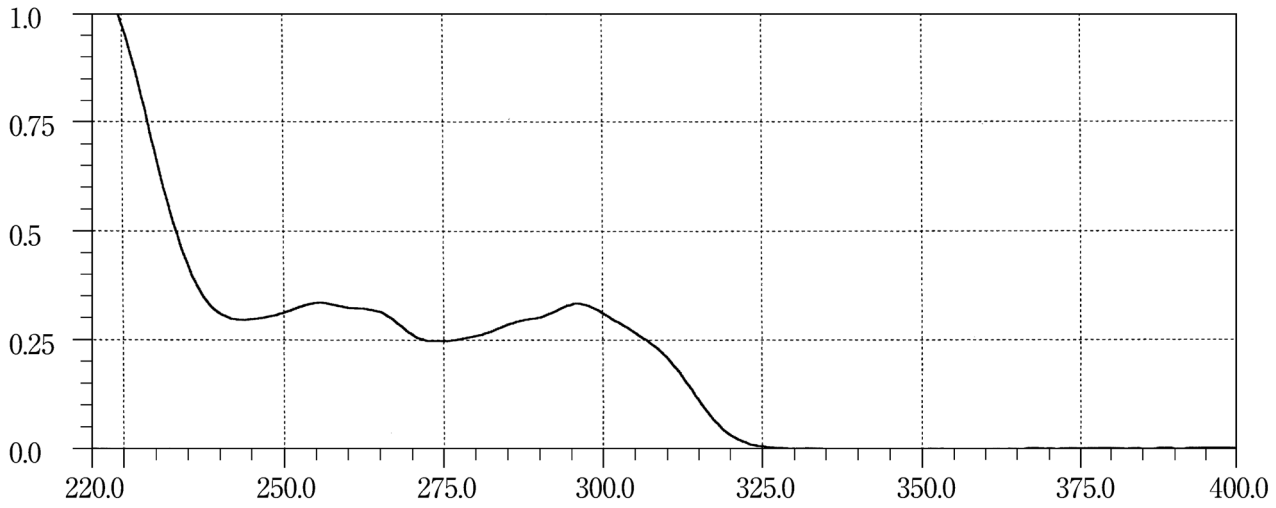
Verapamil Hydrochloride



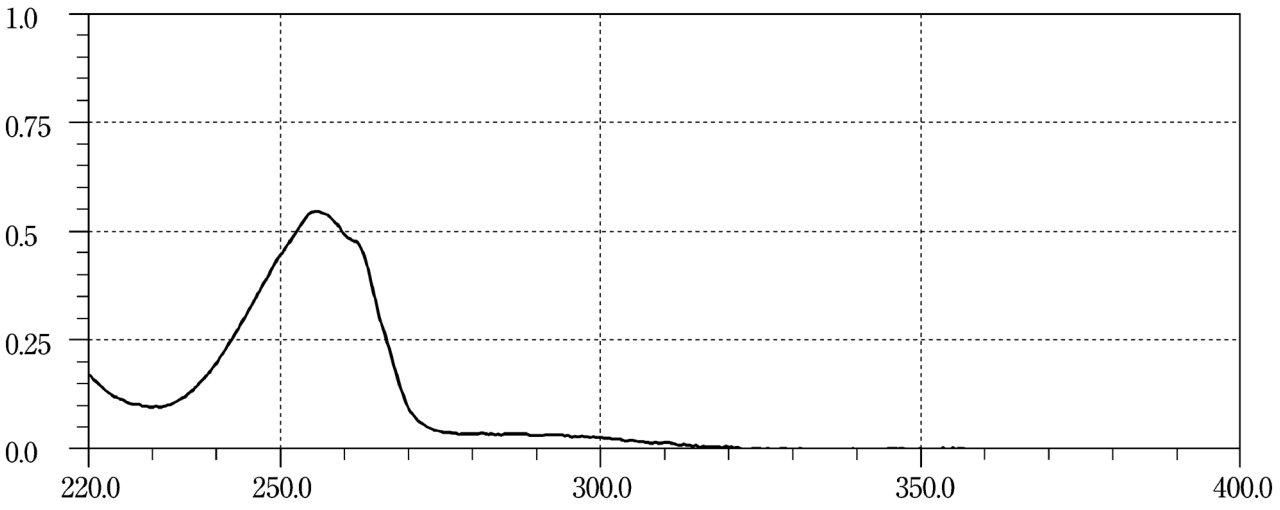
Vinblastine Sulfate



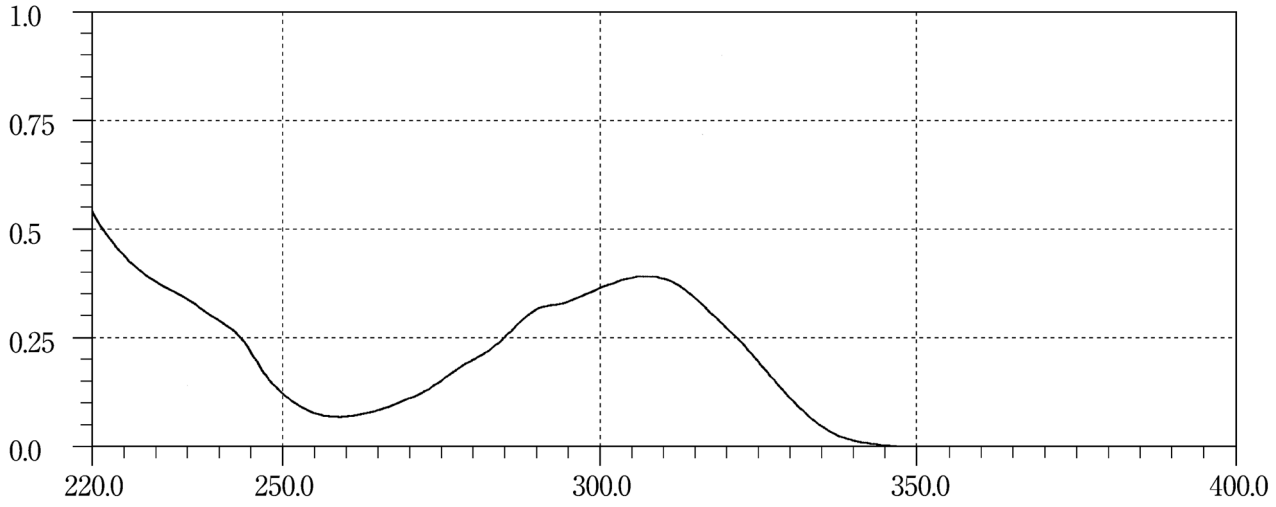
Vincristine Sulfate



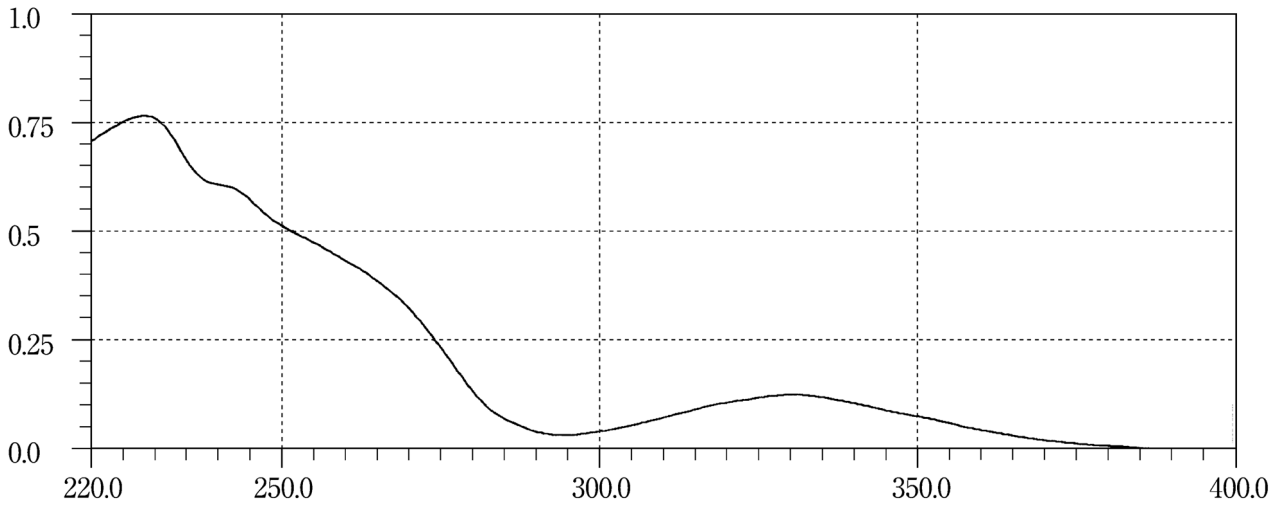
Voriconazole



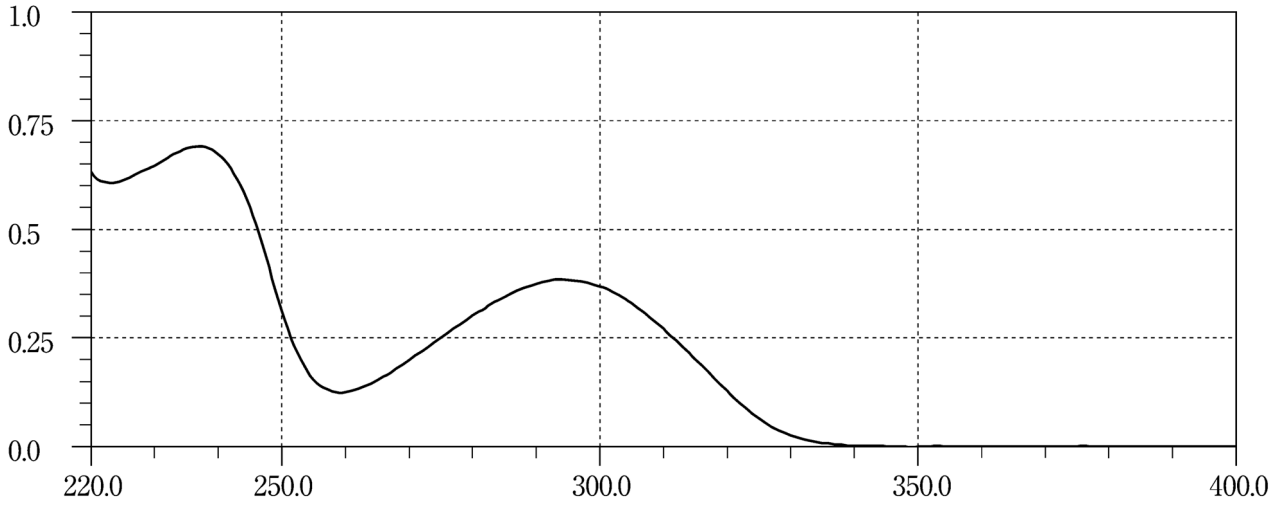
Warfarin Potassium



Zaltoprofen



Zolpidem Tartrate



General Information

GENERAL INFORMATION

G1 Physics and Chemistry

Near Infrared Spectrometry

Near infrared spectrometry (NIR) is one of spectroscopic methods used to qualitatively and quantitatively evaluate substances from analysis of data obtained by determining their absorption spectrum of light in the near-infrared range.

The near-infrared range lies between the visible light and infrared light, typically of wavelengths (wave numbers) between 750 and 2500 nm (13,333 – 4000 cm^{-1}). The absorption of near-infrared light occurs due to harmonic overtones from normal vibration or combination tones in the infrared range (4000 to 400 cm^{-1}), primarily absorption of O-H, N-H, C-H and S-H that involve hydrogen atoms, in particular. For instance the asymmetrical stretching vibration of N-H occurs in the vicinity of 3400 cm^{-1}, but the absorption due to the first harmonic overtone occurs in the vicinity of 6600 cm^{-1} (wavelength 1515 nm), which is near double 3400 cm^{-1}.

Absorption in the near-infrared range is far weaker than absorption due to normal vibration that occurs in the infrared range. Furthermore, in comparison with visible light, near-infrared light has longer wavelength, which makes it possible for the light to penetrate to a depth of several mm into solid specimens including fine particles. This method is often utilized as a nondestructive analysis, as changes occurring with absorbed light spectrum (transmitted light or reflected light) in this process provide physical and chemical information pertaining to specimens.

Conventional spectrometry, such as calibration curve method, is used as a method for analyzing near-infrared absorption spectrum whenever applicable. Ordinarily, however, chemometrics methods are used for analysis. Chemometrics ordinarily involve quantification of chemical data, as well as numerical and statistical procedures for computerization of information. Chemometrics for near-infrared spectrometry includes various types of multivariate analysis such as multiple regression analysis, to perform qualitative or quantitative evaluation of active substances.

Near-infrared spectrometry is used as a rapid and nondestructive method of analysis that replaces conventional and established analysis methods for water determinations or substance verifications. It is necessary to perform a comparison test to evaluate this method against an existing analysis method, to verify that this method is equivalent to such existing analysis method, before using this analysis method as a quality evaluation test method in routine tests.

Applications of near-infrared spectrometry in the pharmaceutical field include qualitative or quantitative evaluation of ingredients, additives or water contents of active substances or preparations. Furthermore, near-infrared spectrometry can also be used for evaluation of physical conditions of substances, such as crystal forms, crystallinity, particle diameters. It is also possible to perform spectrometry on samples that are located in a remote location away from equipment main units, without sampling, by using optical fibers. It can therefore be used as an effective means to perform pharmaceutical manufacturing process control online (or in-line).

1. Equipment

Near-infrared spectrophotometers can either be a distributed near-infrared spectrophotometer or a Fourier transform near-infrared spectrophotometer[1]. Interference filter-type near-infrared spectrophotometers that use interference filter in the spectrometry section are also available, however, this type of equipment is hardly used in the field of pharmaceutical quality control.

1.1. Distributed near-infrared spectrophotometer

This equipment is comprised of light source section, sample section, spectrometry section, photometry section, signal processing section, data processing section, display-record-output section. Halogen lamps, tungsten lamps, light emitting diodes and other such devices that can emit high intensity near-infrared light in a stable manner are used in the light source section. The sample section is comprised of a sample cell and a sample holder. Equipment that have an optical fiber section that is comprised of optical fibers and a collimator are equipped with a function for transmitting light to sample section, which is remotely located away from the spectrophotometer main unit. Quartz is ordinarily used as material for optical fibers.

The spectrometry section is intended to extract light of required wavelength, using dispersive devices and is comprised of slits, mirrors and dispersive devices. Potential dispersive devices include prisms, diffraction grating, acousto-optical tunable filters (AOTF), or liquid crystal tunable filters (LCTF). The photometry section is comprised of detectors and amplifiers. Sensors include semiconductor detectors (silicon, lead sulfide, indium-gallium-arsenic, indium-antimony), as well as photomultiplier tubes. Detecting methods that use semiconductor detectors generally perform detections with single elements, but there are also occasions where arraytype detectors that use multiple elements are used. Such detectors are capable of simultaneously detecting multiple wavelengths (wave numbers). The signal processing section separates signals required for measurements from output signals fed by amplifiers and then outputs such isolated signals. The data processing section performs data conversions and spectral analysis, etc. The display-record-output section outputs data, analysis results and data processing results to a printer.

1.2. Fourier transform near-infrared spectrophotometer

The configuration of the equipment is fundamentally same as that of the distributed-type equipment described in Section 1.1., except for the spectrometry section and the signal processing section.

The spectrometry section is comprised of interferometers, sampling signal generators, detectors, amplifiers, A/D conversion devices, etc. Interferometers include Michelson interferometers, transept interferometers and polarization interferometers. The signal processing section is equipped with functions that are required for spectrometer, as well as a function for translating acquired interference waveform (interferogram) into absorption spectrum by Fourier transformation.

2. Determination

There are three types of measurement methods that are

used with near-infrared spectrometry: transmittance method, diffuse reflectance method and transmittance reflectance method. The section of measurement methods relies on the shape of samples and applications. The transmittance method or diffuse reflectance method is used for solid samples, including fine particles. The transmittance method or transmittance reflectance method is used for liquid samples.

2.1. Transmittance method

The degree of decay for incident light intensity as the light from a light source passes through a sample, is represented as transmittance rate T (%) or absorbance A with the transmittance method. A sample is placed in the light path between a light source and a detector, the arrangement of which is ordinarily same as that of the spectroscopic method.

$$T = 100t$$
$$t = I/I_0 = 10^{-\alpha cl}$$

I_0: Incident light intensity
I: Transmitted light intensity
α: Absorptivity
c: Solution concentration
l: Layer length (sample thickness)

$$A = -\log t = \log (1/t) = \log (I_0/I) = \alpha cl$$

This method is applied for taking measurements of samples that are liquids and solutions. Quartz glass cells and flow cells are used, with the layer length of 1 – 5 mm along. Furthermore, this method can also be applied for taking measurements of samples that are solids, including fine particles. It is also known as diffuse transmittance method. Selecting appropriate layer length is critical for this method, since the transmitted light intensity varies depending on grain sizes and surface condition of samples.

2.2. Diffuse reflectance method

The ratio of the reflection light intensity I, emitted from the sample in a wide reflectance range and a control reflection light intensity I_r emitted from surface of a substance, is expressed as reflectance R (%) with the diffuse reflectance method. The near-infrared light penetrates to a depth of several mm into solid samples, including fine particles. In that process, transmission, refraction, reflection and dispersion are repeated, and diffusion takes place, but a portion of the diffused light is emitted again from the surface of the sample and captured by a detector. The spectrum for the diffuse reflectance absorbance (A_r) can ordinarily be obtained by plotting logarithm of inverse numbers for reflectance ($1/r$) against wavelengths (wave numbers).

$$R = 100r$$
$$r = I/I_r$$

I: Reflection light intensity of light, diffuse reflected off the sample
I_r: Control reflection light intensity of light emitted from surface of reference substance

$$A_r = \log (1/r) = \log (I_r/I)$$

The intensity of diffuse reflectance spectrum can also be expressed with the Kubelka-Munk (K-M) function. The K-M function is derived, based on the existence of a sample with sufficient thickness, and expressed in terms of light scattering coefficient, which is determined by absorptivity, grain size, shape and fill condition (compression).

This method is applied to solid samples, including fine particles, and requires a diffuse reflector.

2.3. Transmittance reflectance method

The transmittance reflectance method is a combination of the transmittance method and reflectance method. A mirror is used to re-reflect a light that has passed through a sample in order to take a measurement of transmittance reflectance rate, T^* (%). Light path must be twice the thickness of the sample. On the other hand, the light reflected by a mirror and being introduced into a detector is used as the control light. When this method is applied to suspended samples, however, a metal plate or a ceramic reflector with rough surface that causes diffuse reflectance is used instead of a mirror.

Transmittance reflectance absorbance (A^*) is obtained by the following formula with this method:

$$T^* = 100t^*$$
$$t^* = I/I_T$$

I: Intensity of transmitted and reflected light, in cases where a sample is placed
I_T: Intensity of reflected light, in cases where is no sample

$$A^* = \log (1/t^*)$$

This is a method that is applied to solid samples, including fine particles, as well as liquids and suspended samples. The thickness of a sample must be adjusted when applying this method to a solid sample. Ordinarily adjustment is made by setting absorbance to 0.1 – 2 (transmittance of 79 – 1%), which provides the best linearity and SN ratio of detector. A cell with appropriate layer length, according to the grain size of the fine particle, must be selected when applying the method to a fine particle sample.

3. Factors that affect spectrum

Following items must be considered as factors that can affect spectrum when applying near-infrared spectrometry, particularly when conducting quantitative analysis.

(i) Sample temperature: A significant change (wavelength shift, for example) can occur when the temperature varies by a several degree (°C). Care must be taken, particularly when the sample is a solution or contains water.

(ii) Water or residual solvent: Water or residual solvent contents of a sample, as well as water (humidity) in the environment wherein measurements are taken, can significantly affect absorption band of the near-infrared range.

(iii) Sample thickness: The thickness of a sample is a factor for spectral changes and therefore needs to be controlled at a certain thickness. A sample may be considered to be of adequate thickness for the diffuse reflectance method, however, if the thickness is less than a certain amount, for example, the sample may have to be placed on a support plate with high reflectance to take measurements by the transmittance reflectance method.

(iv) Fill condition of sample: The condition of sample fill can potentially affect spectrum, when taking measurements of samples that are solids or fine particles. Care must be taken with filling samples in a cell, to ensure that a certain amount is filled through a specific procedure.

(v) Optical characteristics of samples: When a sample is physically, chemically or optically uneven, relatively large beam size must be used, multiple samples must be used, measurements must be taken at multiple points on the same samples, or a sample must be pulverized to ensure averaging of sample. Grain size, fill condition, as well as roughness of surface can also affect fine particle samples.

(vi) Crystal forms: Variations in crystal structures (crystal forms) can also affect spectrum. In cases where multiple crystal forms exist, it is necessary to have consideration for characteristics of samples to be considered and care must be taken to ensure that even standard samples for calibration

curve method have diversified distributions similar to that of samples that are subject to analysis.

(vii) Temporal changes in characteristics of samples: Samples can potentially undergo chemical, physical or optical property changes, due to passing of time or storage after sampling, and such changes affect spectrum in a subtle manner. For instance even with identical samples, if elapsed times differ, then their characteristics of near-infrared spectrum can vary significantly. In creating calibration curves, therefore, the samples to be used must be prepared with adequate considerations for reducing the time to be measured, such as the measurement is carried out offline in a laboratory or online in manufacturing process (or inline).

4. Control of equipment performance[2,3]

4.1. Accuracy of wavelengths (wave numbers)

The accuracy of wavelengths (wave numbers) of an equipment is derived from the deviation of substances for which peak absorption wavelengths (wave numbers) have been defined, such as polystyrene, mixture of rare earth oxides (dysprosium, holmium and erbium; 1:1:1) or steam, from the figures indicated on the equipment. Tolerance figures in the vicinity of 3 peaks are ordinarily set in the following manner, though appropriate tolerance figures can be set, depending on the intended purpose:

1200 ± 1 nm (8300 ± 8 cm^{-1})
1600 ± 1 nm (6250 ± 4 cm^{-1})
2000 ± 1.5 nm (5000 ± 4 cm^{-1})

Since the location of absorption peaks vary, depending on the substance used as reference, absorption peaks of wavelengths (wave numbers) that are closest to the above 3 peaks are selected for suitability evaluations. A mixture of rare earth oxides, for instance, would indicate characteristic absorption peaks at 1261 nm, 1681 nm and 1971 nm.

Absorption peaks at 1155 nm, 1417 nm, 1649 nm, 2352 nm (layer length: 1.0 nm) can be used, when taking measurements with transmittance method that involve the use of dichloromethane as reference. The absorption peak of steam at 7306.7 cm^{-1} can be used with a Fourier transformation-type spectrophotometer, as its wave number resolution ability is high.

Other substances can also be used as reference, so long as their adequacy for the purpose can be verified.

4.2. Spectroscopic linearity

Appropriate standard plates, such as plate-shaped polymer impregnated with varying concentrations of carbon (Carbon-doped polymer standards), can be used to evaluate spectroscopic linearity. In order to verify linearity, however, standard plates with no less than 4 levels of concentration within the reflectance of 10 – 90% must be used. When measurements are expected to be taken with absorbance of no less than 1.0, it is necessary to add standard plates with reflectance of either 2% or 5% or both.

In order to plot absorbance (A_{OBS}) of such standard plates at locations in the vicinity of wavelengths 1200 nm, 1600 nm and 2000 nm against absorbance (A_{REF}) assigned to each standard plate, verifications must be made to ensure that the gradient of linearity obtained are ordinarily within the range 1.0 ± 0.05 for each of these wavelengths and 0 ± 0.05 for ordinate intercept. Depending on the intended purpose, appropriate tolerance figures can be set.

4.3. Spectrophotometric noise

The spectrophotometric noise of the equipment can be checked using appropriate reflectance standard plates, such as white-colored reflecting ceramic tiles or reflective thermoplastic resin (such as polytetrafluoroethylene).

4.3.1. High flux noise

Spectrophotometric noise is evaluated by using standard plates with high reflectance, such as reflectance of 99%. Standard plates are used to take measurements for both samples and control samples. Generally, the average value obtained from calculation of mean square root (*RMS*) of noise for each 100 nm segments in the wavelength range of 1200 – 2200 nm ordinarily must not be more than 0.3×10^{-3} and individual values must not exceed 0.8×10^{-3}. Depending on the intended purpose, appropriate tolerance figures can be set.

$$RMS = \{1/N \cdot \Sigma (A_i - A_m)^2\}^{1/2}$$

N: Number of measurement points per segment
A_i: Absorbance at each measurement point of segment
A_m: Average absorbance for segment

4.3.2. Low flux noise

Spectrophotometric noise is evaluated by using standard plates with low reflectance, such as reflectance of 10%, when the amount of light is low. In such cases, light source, optical system, detector and electronic circuit systems all have some impact on noise. Similar to the cases of high flux noise, generally, the average value obtained from calculation of *RMS* for each 100 nm segments in the wavelength range of 1200 – 2200 nm ordinarily must not be more than 1.0×10^{-3} and individual values must not exceed 2.0×10^{-3}. Depending on the intended purpose, appropriate tolerance figures can be set.

5. Application of qualitative or quantitative analysis

Unlike in the infrared range, mainly harmonic overtones and combinations manifest as spectrum in the near-infrared range. Such absorbance spectrums are often observed as overlay of absorption bands of functional groups and atomic groups. The near-infrared spectrometry, therefore, differs from conventional analysis methods that correspond to each application, by preparing model analysis methods using methodologies of chemometrics, such as multivariate analysis.

Characteristics of near-infrared absorption spectrum must be emphasized and effects of complexities of spectrums, as well as overlay of absorption bands must be reduced by performing mathematical preprocesses, such as primary or secondary spectral differentiation processes or normalizations, which becomes one of vital procedures in establishing analysis methods that use methodologies of chemometrics. While there are many chemometrics methodologies and mathematical preprocessing methods for data, appropriate combinations must be selected that suit the purposes of intended analysis.

Evaluation of validity based on analysis parameters is ordinarily required for the analysis validation when establishing a near-infrared analysis method. Selection of parameters that are appropriate for applications must be made for its intended use. Furthermore, following issues must be considered, in conformity with attributes of the near-infrared spectrometry.

(i) Whether or not wavelengths (wave numbers) intended for the particular analysis method, are suitable for evaluation of characteristics of a sample in performing analysis under given conditions.

(ii) Whether or not the method is adequately robust to deal with variables such as handling of samples (for instance fill condition for fine particle samples, etc.) and configuration matrix.

(iii) Whether or not about the same level of accuracy or precision can be obtained, in comparison with the existing

and established analysis methods, which are available as standards.

(iv) Sustaining and managing performance of an analysis method, once established, are critical. Continuous and systematic maintenance and inspection work must therefore be implemented. Furthermore, it must be determined whether or not appropriate evaluation procedures are available to deal with change controls or implementation of re-validation on changes made in manufacturing processes or raw materials, as well as changes arising from replacement of major components in equipment.

(v) Whether or not there are appropriate evaluation procedures in place to verify validity of transferring implementation of an analysis, which presupposed the use of a specific equipment, from such originally intended equipment to another equipment (model transfer) for the purpose of sharing the analysis method.

5.1. Qualitative analysis

Qualitative analysis, such as verification of substances, is performed after preparing a reference library that includes inter-lot variations within tolerance range and chemometrics methodologies, such as multivariate analysis, have been established. Minute quality characteristic variations between lots can also be established by using this method.

Furthermore, multivariate analysis includes direct analysis methods that consider wavelengths (wave numbers) and absorption as variables, such as wavelength correlation method, residual sum of squares, range sum of squares, along with factor analysis method, cluster analysis method, discriminant analysis method, as well as SIMCA (Soft independent modeling of class analogy).

It is also possible to consider the overall near-infrared absorption spectrum as a single pattern and to identify parameters obtained by applying multivariate analysis methods or characteristic wavelength (wave number) peaks of the sample substance as indices for monitoring, for the purpose of manufacturing process control for active substances or preparations.

5.2. Quantitative analysis

Quantitative analysis uses spectrums of sample groups and analysis values obtained through the existing and established analysis methods, to obtain quantitative models with methodologies of chemometorics. These are used to calculate concentrations of individual ingredients and material values of samples being measured, using conversion formulas. Chemometrics methodologies for obtaining quantitative models include multiple regression analysis method, main ingredient regression analysis method and PLS (Partial least squares) regression analysis method.

In cases where the composition of a sample is simple, concentrations of ingredients in the sample that are subject to analysis can be calculated, by plotting a calibration curve using the absorbance of a specific wavelength (wave number) or the correlating relationship between the parameters and concentration, using samples for preparation of calibration curves with known concentrations (calibration curve method).

References

1) General Rules for Near-infrared Spectrophotometric Analysis, JIS K 0134 (2002), Japanese Industrial Standards
2) Near-Infrared Spectrophotometry, 2.2.40, European Pharmacopoeia 5.0 (2005)
3) Near-Infrared Spectrophotometry, <*1119*>, US Pharmacopoeia 30 (2007)

pH Test for Gastrointestinal Medicine

In this test, medicine for the stomach and bowels, which is said to control stomach acid, is stirred in a fixed amount of the 0.1 mol/L hydrochloric acid for a fixed duration, and the pH value of this solution is measured. The pH value of a stomach medicine will be based on the dose and the dosage of the medicine (when the dosage varies, a minimum dosage is used) and expressed in the pH value obtained from the test performed by the following procedure.

1. Preparation of Sample

Solid medicine which conforms to the general regulations for medicine (the powdered medicine section) can be used as a sample. When the medicine is in separate packages, the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and mixed evenly to make a sample. For granules and similar types in separate packages, among the solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and is then powdered to make sample. For granules and similar types not in separate packages, among solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), 20 doses or more are powdered to make a sample. For capsules and tablets, 20 doses or more are weighed accurately to calculate the average mass for one dose or average mass and then powdered to make a sample.

Liquid medicine is generously mixed to make a sample.

2. Procedure

Put 50 mL of the 0.1 mol/L hydrochloric acid with the molarity coefficient adjusted to 1.000, or equivalent 0.1 mol/L hydrochloric acid with its volume accurately measured in a 100-mL beaker. Stir this solution with a magnetic stirrer and a magnetic stirrer rotator (35 mm length, 8 mm diameter) at the speed of about 300 revolutions per minute. While stirring, add the accurately weighed one-dose sample. After 10 minutes, measure the pH value of the solution using the pH Determination. The solution temperature should be maintained at 37 ± 2°C throughout this operation.

System Suitability

In order to ensure the reliability on the results of drug analyses, it is essential to verify that the test method to be applied to the test, including the method prescribed in the Japanese Pharmacopoeia (JP), can give the results adequate for its intended use using the analytical system in the laboratory in which the test is to be performed, then to carry out system suitability testing for confirming that the analytical system maintains the state suitable for the quality test.

1. Definition and role of system suitability

''System Suitability'' is the concept for ensuring that the performance of the analytical system is as suitable for the analysis of the drug as was at the time when the verification of the test method was performed using the system. Usually, system suitability testing should be carried out at every series of drug analysis. The test procedures and acceptance criteria of system suitability testing must be prescribed in the test methods of drugs. The results of drug analyses are not ac-

ceptable unless the requirements of system suitability have been met.

System suitability testing is an integral part of test methods using analytical instruments, and based on the concept that the equipments, electronic data processing systems, analytical operations, samples to be analyzed and operators constitute an integral system that can be evaluated, when the test procedures and acceptance criteria of system suitability testing are prescribed in the test methods.

2. Points to consider in setting system suitability

Parameters of system suitability testing to be prescribed in the test method depend on the intended use and type of analytical method. Since system suitability testing is to be carried out in a routine manner, it is preferable to select the parameters necessary for ensuring that the analytical system maintains the state suitable for the analysis of the drug and to prescribe its test procedure able to carry out easily and rapidly.

For example, in the case of quantitative purity tests using liquid chromatography or gas chromatography, the evaluation of parameters such as ''System performance'' (to confirm the ability to analyze target substance specifically), ''System repeatability'' (to confirm that the degree of variation in the analytical results of target substance in replicate injections is within the allowable limit) and ''Test for required detectability'' (to confirm the linearity of chromatographic response around the specification limit) are usually required.

The followings are supplements to the section of system suitability prescribed in ''Liquid Chromatography''.

2.1. System repeatability of HPLC and GC

2.1.1. Allowable limit of system repeatability

It is described in the section of system suitability in ''Liquid Chromatography'' that ''In principle, total number of replicate injections should be 6'', and ''The allowable limit of ''System repeatability'' should be set at an appropriate level based on the data when suitability of the method for the evaluation of quality of the drug was verified, and the precision necessary for the quality test''.

Based on the above description, an allowable limit of system repeatability for 6 replicate injections should be set in consideration with the following descriptions. However, in the case that the test method prescribed in the JP monograph is used for the test, the allowable limit of system repeatability prescribed in the monograph should be applied.

(i) Assay for drug substance (for drug substance with the content nearby 100%): An adequate allowable limit should be set at the level that the chromatographic system is able to give the precision suitable for the evaluation of variation in the content of active ingredient within and among the batches of drug substance. For example, the allowable limit of ''not more than 1.0%'' is usually recommended for the drug substances whose width of content specification are not more than 5%, as is in the case of content specification of 98.0 – 102.0% which is often observed in the assay using liquid chromatography.

(ii) Assay for drug products: An adequate allowable limit should be set considering the width of content specification of the drug product and the allowable limit prescribed in the assay of drug substance (when the drug product is analyzed by a method with the same chromatographic conditions as those used for the analysis of drug substance).

(iii) Purity test for related substances: An adequate allowable limit should be set considering the concentration of active ingredients in the solution used for the system suitability testing. In the case that a solution with active ingredient concentration of 0.5 – 1.0% is used for the test of system repeatability, an allowable limit of ''not more than 2.0%'' is usually recommended.

Recommendations for allowable limits described above should not be applicable to gas chromatography.

2.1.2. Method for decreasing the number of replicate injections without losing the quality of system repeatability testing

It is described in the section of system suitability in ''Liquid Chromatography'' that ''In principle, total number of replicate injections should be 6. However, in the case that a long time is necessary for one analysis, such as the analysis using the gradient method, or the analysis of samples containing late eluting components, it may be acceptable to decrease the number of replicate injections by adopting new allowable limit of ''System repeatability'' which can guarantee a level of ''System repeatability'' equivalent to that at 6 replicate injections.''

In consideration of the above description, a method for decreasing the number of replicate injections without losing the quality of system repeatability testing is adopted. One can set the test for system repeatability with reduced number of replicate injections by utilizing this method, if necessary, and can also apply it as an alternative for the method prescribed in a monograph.

The following table shows the allowable limits to be attained in the test at 3 – 5 replicate injections ($n = 3 - 5$) to keep the quality test equivalent to that of test at $n = 6$.

However, it should be kept in mind that since decrease in the number of replicate injections results in increase in the weight of each injection, it becomes more important to perform the test by the experienced operator, and to maintain the equipment in a suitable state.

3. Points to consider at the change of analytical system (Change control of analytical system)

When the test method and analytical system verified is continuously used for the quality test without any change, it is sufficient to confirm the compliance to the requirements of system suitability at every series of drug analysis.

However, when the test is performed for a long period, a situation in which some changes in the analytical system are inevitable, may occur. These changes don't affect the quality of the product itself, but they affect the scale in the evaluation of product quality. If the change in the analytical system may induce a significant deviation of the scale, it may lead to the acceptance of products with inadequate quality and/or the rejection of products with adequate quality. Thus, at the time of change in the analytical system, it is necessary to check whether the change is appropriate or not, to avoid the deviation of the scale in the evaluation of product quality.

In the case of the change of test method, it is required to perform an adequate validation depending on the extent of the change.

On the other hand, in the case of the change of analytical system in a laboratory, such as renewal of apparatus or column of liquid chromatography, and the change of operator, it is necessary to perform at least system suitability testing using the system after change, and to confirm that the equivalency of the results before and after change.

In the case that equivalent results would not be obtained after change, for example, when a renewal of column of liquid chromatograph may induce a significant change of elution pattern, such as the reversal of elution order between target ingredient of the test and substance for checking resolution, it is required to perform a revalidation of the analytical system for the test using new column, since it is uncertain

Table Allowable limits to be attained in the test at 3 – 5 replicate injections (n = 3 – 5) to keep the quality of test equivalent to that of test at n = 6*

		Allowable limit (RSD)					
Allowable limit prescribed in the test of n = 6		1%	2%	3%	4%	5%	10%
Allowable limit to be attained	n = 5	0.88%	1.76%	2.64%	3.52%	4.40%	8.81%
	n = 4	0.72%	1.43%	2.15%	2.86%	3.58%	7.16%
	n = 3	0.47%	0.95%	1.42%	1.89%	2.37%	4.73%

* The probability for inadequate analytical systems to meet the requirements of system suitability testing, is supposed to be 5%.

whether the specificity and/or other validation characteristics necessary for estimating target ingredient is kept or not.

Test for Trace Amounts of Aluminum in Total Parenteral Nutrition (TPN) Solutions

Total parenteral nutrition solutions (TPNs) are nutrient preparations for intravenous injection. Since toxic effects to the central nervous system, bone, etc. due to trace amounts of aluminum have recently been reported in several countries, testing methods for trace amounts of aluminum contaminating TPNs are required for the official standard. The following three analytical methods are available: High-Performance Liquid Chromatography using a fluorescence photometric detector (HPLC with fluorescence detection), Inductivity Coupled Plasma-Atomic Emission Spectrometry (ICP-AES method), Inductivity Coupled Plasma-Mass Spectrometry (ICP-MS method). Detection sensitivity by HPLC with fluorescence detection is about 1 μg/L (ppb), while ICP-AES fitted with special apparatus and ICP-MS have higher sensitivity.

Since TPNs are nutrient preparations, they contain many nutrients such as sugars, amino acids, electrolytes, etc., in various compositions. Thus, care is needed in the selection of a suitable analytical method, because these coexisting components may affect the measurement of trace amounts of aluminum.

In view of the general availability of HPLC apparatus, the present general information describes procedures for the determination of trace levels of aluminum in TPNs by means of HPLC with a fluorescence photometric detector, using two kinds of fluorescent chelating agents, i.e., Quinolinol complexing method, Lumogallion complexing method.

1. Quinolinol complexing method

After forming a complex of aluminum ion in the sample solution with quinolinol, the assay for aluminum by HPLC fitted with a fluorescence photometer is performed.

1.1. Preparation of sample solution

Pipet 1 mL of the sample (TPNs) exactly, and after adding 10 μL of water for aluminum test, make up the sample solution to 10 mL exactly by adding the mobile phase.

1.2. Preparation of a series of standard solutions for calibration curve

Pipet 1 mL of water for aluminum test exactly, and after adding 10 μL each of standard solutions of aluminum (1)–(5), make up the standard solutions for calibration curve to 10 mL (Aluminum concentration: 0, 1.25, 2.5, 5.0, and 10.0 ppb).

1.3. Standard testing method

Pipet 0.1 mL each of the sample solution and standard solutions, and perform the test by HPLC under the following conditions. Calculate the aluminum cotent in the sample solution using a calibration curve method.

Operating conditions—

Detector: A fluorescence photometer (excitation wavelength: 380 nm, emission wavelength: 520 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 8-quinolinol in acetonitrile (3 in 100) and diluted 0.5 mol/L ammonium acetate TS (2 in 5) (1:1).

Flow rate: Adjust so that the retention time of aluminum/8-quinolinol complex is about 9 minutes.

System suitability—

The correlation coefficient of the calibration curve, which is prepared using a series of standard solutions, is not less than 0.99.

Furthermore there is an alternative method, in which the chelating agent 8-quinolinol is not included in the mobile phase. In this method also, aluminum is detected as a complex with 8-quinolinol in the sample solution by using HPLC fitted with a fluorescence photometer. But it is necessary to form a more stable aluminum/8-quinolinol complex in the sample solution, because the chelating agent is not included in the mobile phase. Further, since the analytical wavelength for the fluorescence detection is different from that in the standard method, excitation WL: 370 nm, emission WL: 504 nm, the detection sensitivity is different. Thus, it is appropriate to obtain the calibration curve between 0 – 25 ppb of aluminum. Other than the above- mentioned differences, the size of column, column temperature, and the mobile phase are also different from those used in the standard method, so suitable analytical conditions should be established for performing precise and reproducible examinations of trace amounts of aluminum in the sample specimen.

2. Lumogallion complexing method

After forming a complex of aluminum ion in the sample specimen with the fluorescent reagent of lumogallion, the solution is examined by HPLC fitted with a fluorescence photometer.

2.1. Preparation of sample solution

Pipet 70 μL of the sample specimen (TPN) exactly, add

0.15 mL of lumogallion hydrochloric acid TS and 0.6 mL of buffer solution for aluminum test (pH 7.2) exactly, then mix the solution. After this solution has been allowed to stand for 4 hours at 40°C, it can be used for the measurement as a sample solution.

2.2. Preparation of a series of standard solutions for calibration curve

Pipet 1 mL each of standard aluminum solutions (1) – (5) exactly, and add diluted nitric acid for aluminum test (1 in 100) to make exactly 100 mL. Pipet 70 μL each of these solutions exactly, and add exactly 0.15 mL of lumogallion hydrochloric acid TS and exactly 0.6 mL of buffer solution for aluminum test (pH 7.2) then allow to stand for 4 hours at 40°C to make a series of standard solutions for obtaining the calibration curve (Aluminum: 0, 1.07, 2.13, 4.27, and 8.54 ppb).

2.3. Standard examination method

Take 0.1 mL each of the sample solution and standard aluminum solutions for the calibration curve, and perform HPLC analysis under the following conditions. Calculate the aluminum content in the sample solution by using a calibration curve method.

Operating conditions—

Detector: A fluorescence photometer(excitation wavelength 505 nm, emission wavelength 574 nm)

Column: A stainless steel column 6.0 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Take 100 mL of 2-propanol, and add a diluted 1 mol/L acetic acid-sodium acetate buffer solution of pH 5.0 (1 in 10) to make 1000 mL.

Flow rate: Adjust so that the retention time of aluminum/lumogallion complex is about 5 minutes.

System suitability—

The correlation coefficient of the calibration curve, which is prepared using a series of standard solutions, is not less than 0.99.

3. Notes

(i) Regarding water, solvents, reagents, vessels and other tools used for the examination, select those not contaminated with aluminum. Further, keep the testing environment clean and free from dust in the testing room.

(ii) Before the measurement, it is necessary to confirm that the characteristic properties of the sample do not affect the formation of the complex.

(iii) Reference substances of river water for analysis of trace elements, distributed by the Japan Society for Analytical Chemistry, contain certified amounts of aluminum: JSAC 0301-1 and JSAC 0302 (a known amount of aluminum is artificially added to JSAC 0301-1).

4. Standard Solutions, Reagents and Test Solutions

Other than the standard solutions, reagents and test solutions specified in the Japanese Pharmacopoeia, those described below can be used in this test.

(i) ***N*,*N*-Bis(2-hydroxyethyl)-2-aminoethane sulfonic acid** $C_6H_{15}NO_5S$ White crystals or powder.

(ii) **Hydrochloric acid for aluminum test** Same as the reagent *Hydrochloric acid*. Further, it contains not more than 1 ppb of aluminum.

(iii) **Lumogallion** [5-Chloro-2-hydroxy-3-(2,4-dihydroxyphenylazo)benzenesulfonic acid] $C_{12}H_9ClN_2O_6S$ Red-brown to dark brown powder. Further, it contains not more than 1 ppm of aluminum.

(iv) **Lumogallion hydrochloric acid TS** Dissolve 0.86 g of lumogallion in 300 mL of 2-propanol, and add 350 mL of diluted *Hydrochloric acid for aluminum test* (9 in 50) and *Water for aluminum test* to make 1000 mL exactly.

(v) **Nitric acid for aluminum test** Same as the reagent *Nitric acid*. Further, it contains not more than 1 ppb of aluminum.

(vi) **pH buffer solution for aluminum test (pH 7.2)** Dissolve 106.6 g of *N*,*N*-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid in 800 mL of *Water for aluminum test*, adjust the pH 7.2 by using *Tetramethylammonium hydroxide aqueous solution*, and add *Water for aluminum test* to make 1000 mL.

(vii) **Standard aluminum solution** Pipet a constant volume each of *Water for aluminum test* or the *Standard aluminum stock solution*, dilute and adjust the aluminum concentration to 0, 1.25, 2.5, 5.0, and 10 ppm by using diluted *Nitric acid for aluminum test* (1 in 100), to make Standard aluminum solutions (1) – (5).

(viii) **Tetramethylammonium hydroxide TS** [$(CH_3)_4NOH$] It is a 25% aqueous solution, prepared for aluminum test. Further, it contains not more than 1 ppb of aluminum.

(ix) **Water for aluminum test** It contains not more than 1 ppb of aluminum.

Validation of Analytical Procedures

The validation of an analytical procedure is the process of confirming that the analytical procedure employed for a test of pharmaceutics is suitable for its intended use. In other word, the validation of an analytical procedure requires us to demonstrate scientifically that risks in decision by testing caused by errors from analytical steps are acceptably small. The performance of an analytical procedure is established by various kinds of validation characteristics. The validity of a proposed analytical procedure can be shown by demonstrating experimentally that the validation characteristics of the analytical procedure satisfy the standards set up according to the acceptable limits of testing.

When an analytical procedure is to be newly carried in the Japanese Pharmacopoeia, when a test carried in the Japanese Pharmacopoeia is to be revised, and when the test carried in the Japanese Pharmacopoeia is to be replaced with a new test according to regulations in general notices, analytical procedures employed for these tests should be validated according to this document.

1. Required data for analytical procedures to be carried in the Japanese Pharmacopoeia

1.1. Outline

This section should provide a brief explanation of the principle of a proposed analytical procedure, identify the necessity of the analytical procedure and its advantage compared with other procedures, and summarize the validation. When an analytical procedure is revised, the limitation of the current analytical procedure and the advantage offered by the new analytical procedure should be described.

1.2. Analytical procedure

This section should contain a complete description of the analytical procedure to enable skilled persons to evaluate correctly the analytical procedure and replicate it if necessary. Analytical procedures include all important operating procedures for performing analyses, the preparation of standard samples, reagents and test solutions, precautions, procedures to verify system suitability (e.g. the verification of the separating performance of a chromatographic sys-

tem), formulas to obtain results, the number of replications and so forth. Any instruments and apparatus that are not stated in the Japanese Pharmacopoeia should be described in detail. The physical, chemical or biological characteristics of any new reference standards should be clarified and their testing methods should be established.

1.3. Data showing the validity of analytical procedures

This section should provide complete data showing the validity of the analytical procedures. This includes the experimental designs to determine the validation characteristics, experimental data, calculation results and results of hypothesis tests.

2. Validation characteristics

The definition of typical validation characteristics to be assessed in validation of analytical procedures and examples of assessing procedures are given below.

The terminology and definitions of the validation characteristics may possibly vary depending upon the fields to which analytical procedures are applied. The terminology and definitions shown in this document are established for the purpose of the Japanese Pharmacopoeia. Typical methods for assessing the validation characteristics are shown in the item of assessment. Various kinds of methods to determine the validation characteristics have been proposed and any methods that are widely accepted will be accepted for the present purpose. However, since values of the validation characteristics may possibly depend upon methods of determination, it is required to present the methods of determining the validation characteristics, the data and calculation methods in sufficient detail.

Although robustness is not listed as a validation characteristic, it should be considered during the development of analytical procedures. Studying the robustness may help to improve analytical procedures and to establish appropriate analytical conditions including precautions.

2.1. Accuracy/Trueness

2.1.1. Definition

The accuracy is a measure of the bias of observed values obtained by an analytical procedure. The accuracy is expressed as the difference between the average value obtained from a large series of observed values and the true value.

2.1.2. Assessment

The estimate of accuracy of an analytical method is expressed as the difference between the total mean of observed values obtained during investigation of the reproducibility and the true value. The theoretical value is used as the true value (e.g., in the case of titration methods, etc.). When there is no theoretical value or it is difficult to obtain a theoretical value even though it exists, a certified value or a consensus value may be used as the true value. When an analytical procedure for a drug product is considered, the observed value of the standard solution of the drug substance may be used as the consensus value.

It may be inferred from specificity data that an analytical procedure is unbiased.

The estimate of accuracy and a 95% confidence interval of the accuracy should be calculated using the standard error based on the reproducibility (intermediate precision). It should be confirmed that the confidence interval includes zero or that the upper or lower confidence limits are within the range of the accuracy required of the analytical procedure.

2.2. Precision

2.2.1. Definition

The precision is a measure of the closeness of agreement between observed values obtained independently from multiple samplings of a homogenous sample and is expressed as the variance, standard deviation or relative standard deviation (coefficient of variation) of observed values.

The precision should be considered at three levels with different repetition conditions; repeatability, intermediate precision and reproducibility.

(i) Repeatability/Intra-assay precision

The repeatability expresses the precision of observed values obtained from multiple samplings of a homogenous sample over a short time interval within a laboratory, by the same analyst, using the same apparatus and instruments, lots of reagents and so forth (repeatability conditions).

(ii) Intermediate precision

The intermediate precision expresses the precision of observed values obtained from multiple samplings of a homogenous sample by changing a part of or all of the operating conditions including analysts, experimental dates, apparatus and instruments and lots of reagents within a laboratory (intermediate precision condition).

(iii) Reproducibility

The reproducibility expresses the precision of observed values obtained from multiple samplings of a homogenous sample in different laboratories (reproducibility condition).

2.2.2. Assessment

A sufficient volume of a homogenous sample should be prepared before studying the precision. The solution is assumed to be homogenous. When it is difficult to obtain a homogenous sample, the following samples may be used as homogenous samples; e.g., a large amount of drug products or mixture of drug substance and vehicles that are crushed and mixed well until they can be assumed to be homogenous.

Suitable experimental designs such as one-way layout may be employed when more than one level of precision is to be investigated simultaneously. A sufficient number of repetitions, levels of operating conditions and laboratories should be employed. Sources of variations affecting analytical results should be evaluated as thoroughly as possible through the validation.

It is required to show the variance, standard deviation and relative standard deviation (coefficient of variation) of each level of precision. The 90% confidence interval of the variance and corresponding intervals of the standard deviation and relative standard deviation should also be established. The validity of the proposed analytical procedure for its intended use may be confirmed by comparing obtained values with the required values of the analytical procedure. Whether the proposed analytical procedure is acceptable may normally be decided based on the reproducibility.

2.3. Specificity

2.3.1. Definition

The specificity is the ability of an analytical procedure to measure accurately an analyte in the presence of components that may be expected to be present in the sample matrix. The specificity is a measure of discriminating ability. Lack of specificity of an analytical procedure may be compensated by other supporting analytical procedures.

2.3.2. Assessment

It should be confirmed that the proposed analytical procedure can identify an analyte or that it can accurately measure the amount or concentration of an analyte in a sample. The method to confirm the specificity depends very much upon the purpose of the analytical procedure. For example, the specificity may be assessed by comparing analytical results obtained from a sample containing the analyte only with results obtained from samples containing excipients, related substances or degradation products, and including or excluding the analyte. If reference standards of impurities are un-

available, samples that are expected to contain impurities or degradation products may be used (e.g. samples after accelerated or stress tests).

2.4. Detection limit

2.4.1. Definition

The detection limit is the lowest amount or concentration of the analyte in a sample that is detectable, but not necessarily quantifiable.

2.4.2. Assessment

The detection limit should be normally determined so that producer's and consumer's risks are less than 5%. The detection limit may be calculated using the standard deviation of responses of blank samples or samples containing an analyte close to the detection limit and the slope of the calibration curve close to the detection limit. The following equation is an example to determine the detection limit using the standard deviation of responses of blank samples and the slope of the calibration curve.

$$DL = 3.3\sigma/slope$$

DL: detection limit
σ: the standard deviation of responses of blank samples
slope: slope of the calibration curve

The noise level may be used as the standard deviation of responses of blank samples in chromatographic methods. It should be ensured that the detection limit of the analytical procedure is lower than the specified limit for testing.

2.5. Quantitation limit

2.5.1. Definition

The quantitation limit is the lowest amount or concentration of the analyte in a sample that can be determined. The precision expressed as the relative standard deviation of samples containing an analyte at the quantitation limit is usually 10%.

2.5.2. Assessment

The quantitation limit may be calculated using the standard deviation of responses of blank samples or samples containing an analyte close to the quantitation limit and the slope of the calibration curve close to the quantitation limit. The following equation is an example to determine the quantitation limit using the standard deviation of responses of blank samples and the slope of the calibration curve.

$$QL = 10\sigma/slope$$

QL: quantitation limit
σ: the standard deviation of responses of blank samples
slope: slope of the calibration curve

The noise level may be used as the standard deviation of responses of blank samples in chromatographic methods. It should be ensured that the quantitation limit of the analytical procedure is lower than the specified limit for testing.

2.6. Linearity

2.6.1. Definition

The linearity is the ability of an analytical procedure to elicit responses linearly related to the amount or concentration of an analyte in samples. A well-defined mathematical transformation may sometimes be necessary to obtain a linear relationship.

2.6.2. Assessment

Responses are obtained after analyzing samples with various amounts or concentrations of an analyte according to described operating procedures. The linearity may be evaluated in terms of the correlation coefficient, and the slope and y-intercept of the regression line. It may be also helpful for evaluating the linearity to plot residual errors from the regression line against the amount or concentration and to confirm that there is no particular tendency in the graph. Samples with five different amounts or concentrations of an analyte should be usually investigated.

2.7. Range

2.7.1. Definition

The range for the validation of analytical procedures is the interval between the lower and upper limits of the amount or concentration of an analyte providing sufficient accuracy and precision. The range for the validation of analytical procedures for an analytical procedure with linearity is the interval between the lower and upper limits providing sufficient accuracy, precision and linearity.

2.7.2. Assessment

When the range for the validation of analytical procedures is investigated, 80 to 120% of specified limits of testing should be usually considered. The accuracy, precision and linearity should be evaluated using samples containing the lower and upper limits and in the middle of the range.

3. Categories of tests employing analytical procedures

Tests covered with this document are roughly classified into three categories shown below according to their purposes. The table lists the normally required validation characteristics to be evaluated in the validation of analytical procedures used in these tests. This list should be considered to represent typical validation characteristics. A different approach to validating analytical procedures should be considered depending upon the characteristics of analytical procedures and their intended use.

(i) Type I Identification. Tests for identifying major components in pharmaceuticals according to their characteristics.

(ii) Type II Impurity tests. Tests for determination of impurities in pharmaceuticals.

(iii) Type III Tests for assaying drug substances, active ingredients, and major components in pharmaceuticals. (Additives such as stabilizing agents and preservatives are included in major components.) Tests for determining performance of pharmaceuticals, such as dissolution testing.

Table Lists of validation characteristics required to be evaluated in tests of each type

Validation characteristics \ Type of test	Type I	Type II		Type III
		Quantitation test	Limit test	
Accuracy/Trueness	−	+	−	+
Precision				
Repeatability	−	+	−	+
Intermediate precision	−	−*	−	−*
Reproducibility	−	+*	−	+*
Specificity**	+	+	+	+
Detection limit	−	−	+	−
Quantitation limit	−	+	−	−
Linearity	−	+	−	+
Range	−	+	−	+

− Usually need not to be evaluated.
\+ Usually need to be evaluated.
* Either intermediate precision or reproducibility should be evaluated depending upon circumstances in which analytical procedures or tests are performed. The latter should be normally evaluated in the validation of analytical procedures proposed to be included in the Japanese Pharmacopoeia.
** The lack of the specificity of an analytical procedure may be compensated by other relevant analytical procedures

4. Terminology used in the validation of analytical procedures

(i) Analytical procedure: This document covers analytical procedures applied to identification, and ones that provides responses depending upon the amount or concentration of analytes in samples.

(ii) Laboratory: The laboratory means an experimental room or facility where tests are performed. In this document different laboratories are expected to perform an analytical procedure using different analysts, different experimental apparatus and instruments, different lots of reagents and so forth.

(iii) Number of replications: The number of replications is one that is described in analytical procedures. An observed value is often obtained by more than one measurement in order to achieve good precision of analytical procedures. Analytical procedures including the number of replications should be validated. This is different from repetition in the validation of analytical procedures to obtain accuracy or precision.

(iv) Observed value: The value of a characteristic obtained as the result of performing an analytical procedure.

(v) Consumer's risk: This is the probability that products out of the specification of tests are decided to be accepted after testing. It is usually expressed as β, and is called the probability of type II error or the probability of false negative in impurity tests.

(vi) Producer's risk: This is the probability that products satisfying the specification of tests are decided to be rejected after testing. It is usually expressed as α, and is called the probability of type I error or the probability of false positive in impurity tests.

(vii) Robustness: The robustness is a measure of the capacity to remain unaffected by small but deliberate variations in analytical conditions. The stability of observed values may be studied by changing various analytical conditions within suitable ranges including pH values of solutions, reaction temperature, reaction time or amount of reagents added. When observed values are unstable, the analytical procedure should be improved. Results of studying robustness may be reflected in the developed analytical procedure as precautions or significant digits describing analytical conditions.

(viii) Test: Tests mean various tests described in general tests and official monographs in the Japanese Pharmacopoeia such as impurity tests and assay. They includes sampling methods, specification limits and analytical procedures.

G2 Solid-state Properties

Laser Diffraction Measurement of Particle Size

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The laser light diffraction technique used for the determination of particle-size distribution is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light. Historically, the early laser diffraction instruments only used scattering at small angles. However, the technique has since been broadened to include laser light scattering in a wider range and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by single particles and scattering by clusters of primary particles, i.e. by agglomerates or aggregates. As most particulate samples contain agglomerates or aggregates and as the focus of interest is generally on the size distribution of primary particles, the clusters are usually dispersed into primary particles before measurement.

For non-spherical particles, an equivalent sphere-size distribution is obtained because the technique assumes spherical particles in its optical model. The resulting particle-size distribution may differ from those obtained by methods based on other physical principles (e.g. sedimentation, sieving).

This chapter provides guidance for the measurement of size distributions of particles in different dispersed systems, for example, powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids, through analysis of their angular light-scattering patterns. It does not address specific requirements of particle-size measurement of specific products.

1. Principle

A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for subsequent analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

2. Instrument

The instrument is located in an environment where it is not affected by electrical noise, mechanical vibrations, temperature fluctuations, humidity or direct bright light. An example of a set-up of a laser light diffraction instrument is given in Fig. 1. Other equipment may be used.

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in 2 positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus, in a converging beam. The advantage of the conventional set-up is that a reasonable path length for the sample is allowed within the working distance of the lens. The second set-up allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present.

The interaction of the incident light beam and the ensemble of dispersed particles results in a scattering pattern with different light intensities at various angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens or a series of lenses. These lenses create a scattering pattern that, within limits, does not depend on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.

It is assumed that the measured scattering pattern of the particle ensemble is identical to the sum of the patterns from

all individual single scattering particles presented in random relative positions. Note that only a limited angular range of scattered light is collected by the lens(es) and, therefore, by the detector.

3. Development of the method

The measurement of particle size by laser diffraction can give reproducible data, even in the sub-micron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g. dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 μm to 3 mm. Because of recent advances in lens and equipment design, newer instruments are capable of exceeding this range routinely. With the validation report the user demonstrates the applicability of the method for its intended use.

3.1. Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle-size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

3.2. Evaluation of the dispersion procedure

Inspect the sample to be analyzed, visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as far as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method it is highly advisable to check that comminution of the particles does not occur, and conversely, that dispersion of particles or clusters is satisfactory. This can usually be done by changing the dispersing energy and monitoring the change of the particle-size distribution. The measured size distribution must not change significantly when the sample is well dispersed and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g. crystallization, milling) of the material has changed, the applicability of the method must by verified (e.g. by microscopic comparison).

Sprays, aerosols and gas bubbles in a liquid should be measured directly, provided that their concentration is adequate, because sampling or dilution generally alters the particle-size distribution.

In other cases (such as emulsions, pastes and powders), representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g. agitation, sonication) are often applied for deagglomeration or deaggregation of clusters and stabilization of the dispersion. For these liquid dispersions, a recirculating system is most commonly used, consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing. Non-recirculating, stirred cells are useful when only small amounts of a sample are available or when special dispersion liquids are used.

Dry powders can also be converted into aerosols through the use of suitable dry powder dispersers, which apply mechanical force for deagglomeration or deaggregation. Generally, the dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol, which is blown through the measuring zone, usually into the inlet of a vacuum unit that collects the particles. However, for free flowing, coarser particles or granules the effect of gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving and the mass and percentage of removed material are reported. However, after pre-sieving, note that the sample is no longer representative, unless otherwise proven.

3.3. Optimization of the liquid dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must:

(i) be transparent at the laser wavelength and practically free from air bubbles or particles;

(ii) have a refractive index that differs from that of the test material;

(iii) be non-solvent of the test material (pure liquid or pre-filtered, saturated solution);

(iv) not alter the size of the test materials (e.g. by solubility, solubility enhancement, or recrystallization effects);

(v) favor easily formation and stability of the dispersion;

(vi) be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.);

(vii) possess a suitable viscosity to facilitate recirculation, stirring and filtration.

Surfactants and/or dispersing aids are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH respectively can assist in identifying a suitable dispersant.

A preliminary check of the dispersion quality can be performed by visual or microscopic inspection. It is also possible to take fractional samples out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

3.4. Optimization of the gas dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water and particles may be used. To remove such materials from the compressed gas, a dryer with a filter can be used. Any vacuum unit should be located away from the measurement zone, so that its output does not disturb the measurement.

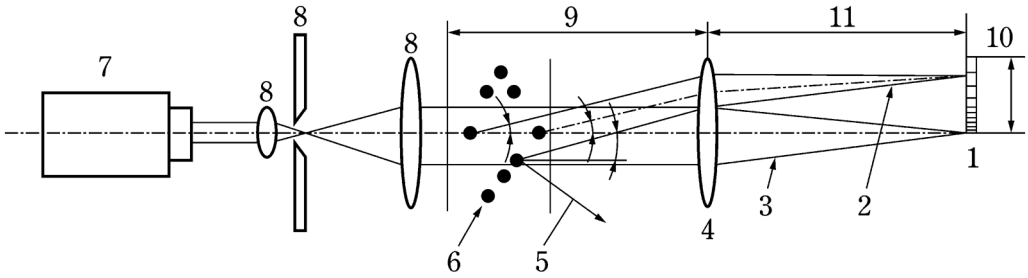
3.5. Determination of the concentration range

In order to produce an acceptable signal-to-noise ratio in the detector, the particle concentration in the dispersion must exceed a minimum level. Likewise, it must be below a maximum level in order to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropriate concentration range for any typical sample of material. (Note: in different instruments, particle concentrations are usually represented by differently scaled and differently named numbers, e.g. obscuration, optical concentration, proportional number of total mass).

3.6. Determination of the measuring time

The time of measurement, the reading time of the detector and the acquisition frequency is determined experimentally



1: Absorption (obscuration) detector
2: Scattered beam
3: Direct beam
4: Fourier lens
5: Scattered light not collected by lens 4
6: Particle ensemble
7: Laser light source
8: Beam processing unit
9: Working distance of lens 4
10: Multi-element detector
11: Focal distance of lens 4

Fig. 1 Example of a set-up of a laser light diffraction instrument

in accordance with the required precision. Generally, the time for measurement permits a large number of detector scans or sweeps at short time intervals.

3.7. Selection of an appropriate optical model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are sometimes applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the different assumptions (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.) made for the test material. If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly larger amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, since small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about $0.01 - 0.1i$) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g. shape, surface roughness and porosity) bear upon the final result.

3.8. Validation

Typically, the validity of a procedure may be assessed by the evaluation of its specificity, linearity, range, accuracy, precision and robustness. In particle-size analysis by laser light diffraction, specificity as defined by ICH is not applicable as it is not possible to discriminate different components into a sample, as is neither possible to discriminate between agglomerates from dispersed particles unless properly complemented by microscopic techniques. Exploring a linear relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Rather than evaluating linearity, this method requires the definition of a concentration range within which the result of the measurements does not vary significantly. Concentrations below that range produce an error due to a poor signal to noise ratio, while concentrations above that range produce an error due to multiple scattering. The range depends mostly in the instrument hardware. Accuracy should be confirmed through an appropriate instrument qualification and comparison with microscopy, while precision may be assessed by means of a repeatability determination.

The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim at acceptance criteria for repeatability such as RSD (%) $\leqq$ 10% [n = 6] for any central value of the distribution (e.g. for x_{50}). Values at the sides of the distribution (e.g. x_{10} and x_{90}) are oriented towards less stringent acceptance criteria such as RSD $\leqq$ 15% [n = 6]. Below 10 μm, these values must be doubled. Robustness may be tested during the selection and optimization of the dispersion media and forces. The change of the dispersing energy may be monitored by the change in the particle-size distribution.

4. Measurement

4.1. Precautions

(i) never look into the direct path of the laser beam or its reflections;

(ii) earth all instrument components to prevent ignition of solvents or dust explosions;

(iii) check the instrument set-up (e.g. warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight);

(iv) in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent air-flow in the case of dry dispersions; such effects can cause erroneous particle-size distributions.

4.2. Measurement of the light scattering of dispersed sample(s)

After proper alignment of the optical part of the instrument, a blank measurement of the particle-free dispersion medium must be performed using the same method as that used for the measurement of the sample. The background signal must be below an appropriate threshold. The detector data are saved in order to substract them later from the data obtained with the sample. The sample dispersion is measured according to the developed method.

For each detector element, an average signal is calculated,

sometimes together with its standard deviation. The magnitude of the signal from each detector element depends upon the detection area, the light intensity and the quantum efficiency. The co-ordinates (size and position) of the detector elements together with the focal distance of the lens determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The ratio of the intensity of a dispersed sample to that in its absence (the blank measurement) indicates the proportion of scattered light and hence the particle concentration.

4.3. Conversion of scattering pattern into particle-size distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important as most algorithms use the mathematical solution for scattering from spherical particles. Furthermore, the measured data always contain some random and systematic errors, which may vitiate the size distributions. Several mathematical procedures have been developed for use in the available instruments. They contain some weighting of deviations between measured and calculated scattering patterns (e.g. least squares), some constraints (e.g. non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each make and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

4.4. Replicates

The number of replicate measurements (with individual sample preparations) to be performed, depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

5. Reporting of results

The particle-size distribution data are usually reported as cumulative undersize distribution and/or as density distribution by volume. The symbol x is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere. Q3(x) denotes the volume fraction undersize at the particle size x. In a graphical representation, x is plotted on the abscissa and the dependent variable Q3 on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10%, 50%, and 90% (denoted as x_{10}, x_{50}, and x_{90} respectively) are frequently used. x_{50} is also known as the median particle size. It is recognized that the symbol d is also widely used to designate the particle size, thus the symbol x may be replaced by d.

Moreover, sufficient information must be documented about the sample, the sample preparation, the dispersion conditions, and the cell type. As the results depend on the particular instrument, data analysis program, and optical model used, these details must also be documented.

6. Control of the instrument performance

Use the instrument according to the manufacturer's instructions and carry out the prescribed qualifications at an appropriate frequency, according to the use of the instrument and substances to be tested.

6.1. Calibration

Laser diffraction systems, although assuming idealized properties of the particles, are based on first principles of laser light scattering. Thus, calibration in the strict sense is not required. However, it is still necessary to confirm that the instrument is operating correctly. This can be undertaken using any certified reference material that is acceptable in industrial practice. The entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It is essential that the total operational procedure is fully described.

The preferred certified reference materials consist of spherical particles of a known distribution. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

The response of a laser diffraction instrument is considered to meet the requirements if the mean value of x_{50} from at least 3 independent measurements does not deviate by more than 3% from the certified range of values of the certified reference material. The mean values for x_{10} and x_{90} must not deviate by more than 5% from the certified range of values. Below 10 μm, these values must be doubled.

Although the use of materials consisting of spherical particles is preferable, non-spherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analysis performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same non-spherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

6.2. Qualification of the system

In addition to the calibration, the performance of the instrument must be qualified at regular time intervals or as frequently as appropriate. This can be undertaken using any suitable reference material as mentioned in the previous paragraph.

The qualification of the system is based on the concept that the equipment, electronics, software and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in the individual monograph, the response of a laser diffraction instrument is considered to meet the requirements if the x_{50} value does not deviate by more than 10% from the range of values of the reference material. If optionally the values at the sides of the distribution are evaluated (e.g. x_{10} and x_{90}), then these values must not deviate by more than 15% from the certified range of values. Below 10 μm, these values must be doubled.

Note 1: For calibration of the instrument stricter requirements are laid down in 6.1. Calibration.

Note 2: Laser Diffraction Measurement of Particle Size complies with ISO13320-1 (1999) and 9276-1 (1998).

Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering

This method is used for measuring average particle diameter and particle diameter distribution of submicron-sized particles dispersed in a liquid by means of dynamic light scattering.

The average particle diameter and the particle diameter distribution obtained by this method are important characteristics mainly of colloidal dispersion formulations, such as emulsion injections, suspension injections, and liposome formulations.

There are two ways of analyzing the detected signals in dynamic light scattering: photon correlation spectroscopy and frequency analysis. Dynamic light scattering is applied to the analysis of particles whose diameters range from nm scale to approximately 1 μm or particles free from the influence of sedimentation.

1. Principle

When particles in Brownian motion in solution or in suspension are irradiated with laser light, scattered light from the particles fluctuates depending on their diffusion coefficients. The intensity of the scattered light from larger particles fluctuates more slowly, because the larger particles move more slowly. On the other hand, the intensity of the scattered light from smaller particles fluctuates more rapidly, because they move faster.

In dynamic light scattering measurements, the particle diameter is determined by applying the Stokes-Einstein equation to analysis of the detected fluctuations of scattered light intensity, which reflect the diffusion coefficient of the particles.

$$d = \frac{kT}{3\pi\eta D} \times 10^{12}$$

d: Particle diameter (nm)
k: Boltzmann constant (1.38×10^{-23} J·K^{-1})
T: Absolute temperature (K)
η: Viscosity (mPa·s)
D: Diffusion coefficient (m^2·s^{-1})

In photon correlation spectroscopy, the time-dependent changes (fluctuation) in the scattered light intensity, namely the observed signals of the scattered light intensity, are transmitted to the correlator. The average particle diameter and the polydispersity index are obtained from the autocorrelation function of the scattered light intensity, which is calculated based on the data processed by the correlator.

In frequency analysis, the average particle diameter and the polydispersity index are obtained from the frequency power spectrum, which is the Fourier transform of the frequency components included in the signals of the scattered light intensity.

Major terms used in this method are as follows.

(i) Average particle diameter: dynamic light scattering harmonic intensity-weighted arithmetic averaged particle diameter, whose unit is nanometer (nm).

(ii) Polydispersity index: dimensionless indicator of the broadness of the particle diameter distribution.

(iii) Scattering volume: observation volume defined by the light-receiving optics and the incident laser light. This value may be given in the specifications of the instrument. Its order of magnitude is typically 10^{-12} m^3.

(iv) Count rate: number of the photon pulses per second detected in the light-receiving optics in photon correlation spectroscopy. This value is proportional to the detected scattered light intensity. The unit is cps (count per second).

(v) Signal of scattered light fluctuation: signal detected by the light-receiving optics in the frequency analysis. The signal is proportional to the scattered light intensity, and includes frequency components depending on the distribution of the particle diameter.

2. Apparatus

2.1. Constitution of the apparatus

The measuring apparatus generally consists mainly of a laser, sample holder, light-receiving optics and detector, and correlator or spectrum analyzer. There are two types of optical detection according to the optical arrangements: (a) homodyne detection in which only the scattered light is measured, and (b) heterodyne detection in which the scattered light and a portion of the incident light are measured simultaneously.

(i) Laser: a monochromatic laser polarized with its electric field component perpendicular to the plane formed by the incident light and light-receiving optical axes (vertical polarization).

(ii) Sample holder: a holder whose temperature can be measured and controlled within an accuracy of ±0.3°C.

(iii) Measuring cell: a rectangular or cylindrical cell made of optical glass or optical plastic, which can be placed in the sample holder. The cell is integrated with the sample holder in some apparatus.

(iv) Light-receiving optics and detector: light optics and detector which capture the scattered light from the sample at a single scattering angle between 90° to 180° and convert the captured light to a photon pulse (digitized signal). In the case that a polarization analyzer is included, it shall be positioned so that the transmittance of the vertically polarized light is maximized.

(v) Correlator: a device which calculates the autocorrelation function from the number of photon pulses in a certain time.

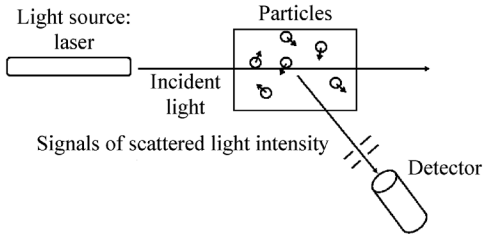


Fig. 1 Schematic illustration of the measurement principle

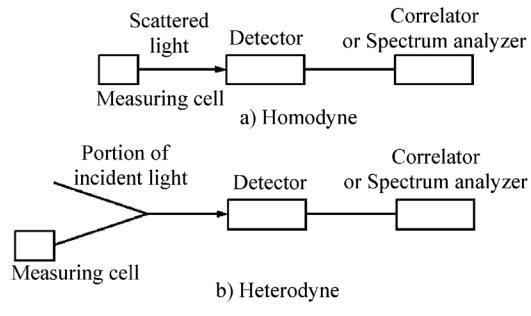


Fig. 2 Different optical arrangements of the apparatus

(vi) Spectrum analyzer: a device which calculates the frequency power spectrum by performing Fourier transformation of the frequency components present in the scattered light fluctuation signals.

(vii) Computation unit: data processor for determining the particle diameter distribution from the autocorrelation function obtained by the correlator or from the frequency power spectrum. Some computation units also function as a correlator or spectrum analyzer.

2.2. Validation and reproducibility of the instrument

Because the particle diameter obtained by dynamic light scattering is not a relative value calculated using standard particles but an absolute value based on a fundamental principle, calibration of the value is unnecessary.

However, it is necessary to confirm the performance of the instrument by using particles with certified diameter, when the instrument is first installed or if abnormal performance is suspected. In addition, it is desirable to confirm the proper performance of the instrument at least every year thereafter.

As standard particles of known diameter, polystyrene latex particles with a narrow distribution of diameter shall be used, whose average particle size is certified to be approximately 100 nm as determined by dynamic light scattering. The measured average diameter of these particles must be within 2% of the stated diameter range, and the relative standard deviation must be less than 2%. In addition, the measured polydispersity index must be less than 0.1.

3. Measurement

3.1. Choice of the dispersion liquid

The dispersion liquid shall fulfill all of the following requirements.

(i) It shall be non-absorbing at the wavelength of the laser.

(ii) It shall not cause damage such as corrosion to the materials of the instrument.

(iii) It shall not dissolve, swell or coagulate the particles.

(iv) It shall have a refractive index different from that of the particulate material.

(v) Its refractive index and viscosity shall be known within an accuracy of 0.5%.

(vi) It shall be clean enough not to interfere with the measurements.

3.2. Cleaning the measuring cell

The degree of cell washing required depends on the conditions of the measurement.

When an individually packaged clean disposable cell is used, cleaning by blowing off dust with compressed clean air is sufficient. When a cell is intended to be washed rigorously, the cell is fully rinsed beforehand with water to remove water-rinsable adhesion substances and is washed with a nonabrasive detergent.

3.3. Sample preparation

It is necessary to prepare a sample whose concentration is within an appropriate range to eliminate the influence of the multiple scattering of light. In addition, it is important to remove dust, which may affect the measurement, and to prevent their re-introduction during the preparation.

When the sample is shaken, dust-laden air is entrapped in the sample and air is dissolved in the solvent. The invisible small air bubbles scatter light more strongly than do the sample particles to be measured. It is necessary not to shake the sample violently after preparation, but to swirl it gently. A homogeneous sample solution can be prepared quickly by adding diluent to the concentrated sample droplet rather than dropping the sample droplet into the diluent.

3.4. Measurement procedure

1) Switch the instrument on and allow it to warm up. A period of approximately 30 minutes is typically required for stabilizing the laser intensity and bringing the sample holder to the desired temperature.
2) Choose the appropriate dispersion liquid, and record the count rate or the amplitude of the signals of scattered light fluctuation from the dispersion liquid.
3) Place the sample containing the dispersed particles in the instrument, and wait until temperature equilibrium is established between the sample and the sample holder. It is desirable to control and measure the temperature within an accuracy of ±0.3°C.
4) Perform a preliminary measurement of the sample, and set the particle concentration within the appropriate range based on 5.2.
5) Perform the measurement with the appropriate measuring time and number of integrations.
6) Record the average particle diameter and the polydispersity index for each measurement.
7) If the measured values are dependent on the particle concentration, adopt the extrapolated infinite dilution values of the average particle diameter and the polydispersity index (or the measured values at the lowest particle concentration).
8) Confirm that no significant sedimentation has occurred in the sample at the end of the measurement. The presence of sediment indicates that the sample may have aggregated or precipitated, or that the sample may be unsuitable for measurement by dynamic light scattering.
9) Perform the measurement for each sample at least three times.

3.5. Repeatability

The repeatability of the determination of the average particle diameter, evaluated in terms of relative standard deviation, must be less than 5%.

4. Data analysis

The dispersion that is the target for the measurement is irradiated with the laser light. Phases of the light scattered by each particle fluctuate because the dispersed particles are in Brownian motion. The observed scattered intensity, which is the sum of the scattered light (result of interference), fluctuates along the time axis. Analyzing the fluctuation of the scattered light intensity as a function of time provides information on the motion of the dispersed particles.

Analysis by photon correlation spectroscopy is performed using the autocorrelation function of the scattered light intensity. This autocorrelation function depend only on the time difference (correlation time) and is independent of the time at which the measurement is started. For a large number of monodisperse particles in Brownian motion in a scattering volume, the autocorrelation function of the scattered light intensity is basically an exponential decay function of the correlation time. Polydispersity index is a parameter indicating the distribution of the decay constant, and is also a scale indicating the broadness of the distribution of particle diameter.

Frequency analysis is performed using the frequency power spectrum calculated from the scattered light intensity. The amplitude of the frequency power spectrum is proportional to the scattered light intensity and the concentration of the sample, and the characteristic frequency is inversely proportional to the particle diameter. The decay constant and the characteristic frequency are related to the translational diffusion coefficient of homogeneous spherical particles in Brow-

nian motion. The diffusion constant of the spherical particles dispersed in the dispersion liquid is related to the particle diameter according to the Stokes-Einstein equation in the absence of inter-particle interaction. The polydispersity index determined by frequency analysis is a measure of the broadness of the particle diameter distribution calculated from the particle diameter distribution based on the scattered light intensity, and might differ from the polydispersity index determined by photon correlation spectroscopy.

Records of data shall include the average particle diameter and polydispersity index, and in addition, shall also state the principle of measurement (photon correlation spectroscopy or frequency analysis), optical configuration (homodyne or heterodyne), observation angle, temperature of the sample, refractive index and viscosity of the dispersion liquid, measuring time or number of integrations, and sample concentration.

5. Points to note regarding the measurement

5.1. Shape of particles

The particles are assumed to be homogeneous and spherical in the data analysis of dynamic light scattering.

5.2. Particle concentration

For measurement, it is necessary to prepare a sample whose concentration falls in the range satisfying the following conditions.

(i) The sample consists of dispersion liquid and particles well-dispersed in the liquid.

(ii) The range of the particle concentration is determined so that consistent results can be obtained in particle diameter measurements. The range is determined beforehand based on measurements of systematically diluted samples.

5.3. Purification of the dispersion liquid

Scattered light signals from the dispersion liquid used for sample dilution must normally be undetected or very weak. If the situations described in cases (i) or (ii) below are found, particulate substances are likely to have become mixed in the sample, and in such cases the dispersion liquid shall be further purified (by filtration, distillation, and so on) before use. The lower limit of the particle concentration is determined mainly so that scattered light from the dispersion liquid and contaminating substances will not affect the measurement. When water is chosen as the dispersion liquid, use of fresh distilled water (prepared by quartz-glass distillation) or desalted and filtered (pore size 0.2 μm) water is recommended.

(i) Large fluctuations of the count rate or of the amplitude of the scattered light fluctuation signals, accompanied by abnormally strong signals, are recorded.

(ii) Light spots appear in the path of the laser light in the sample.

5.4. Others

(i) When particles are highly charged with electricity, long-range interactions between the particles may affect the measurement result, and in such cases, a small amount of salt (for example, sodium chloride: around 10^{-2} mol/L) may be added to the dispersion liquid to reduce the effect.

(ii) Traceable polystyrene latex particles for use in the validation of the instrument are commercially available.

Reference

1) JIS Z8826: 2005 Particle size analysis—Photon correlation spectroscopy
2) ISO 13321: 1996 Particle size analysis—Photon correlation spectroscopy
3) ISO 22412: 2008 Particle size analysis—Dynamic light scattering (DLS)

Powder Fineness

This classification is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

A simple descriptive classification of powder fineness is provided in this chapter. Sieving is most suitable where a majority of the particles are larger than about 75 μm, although it can be used for some powders having smaller particle sizes where the method can be validated. Light diffraction is also a widely used technique for measuring the size of a wide range of particles.

Where the cumulative distribution has been determined by analytical sieving or by application of other methods, particle size may be characterized in the following manner:

x_{90}: Particle size corresponding to 90% of the cumulative undersize distribution
x_{50}: Median particle size (ie: 50% of the particles are smaller and 50% of the particles are larger)
x_{10}: Particle size corresponding to 10% of the cumulative undersize distribution

It is recognized that the symbol d is also widely used to designate these values. Therefore, the symbols d_{90}, d_{50}, d_{10} may be used.

The following parameters may be defined based on the cumulative distribution.

$Q_r(x)$: cumulative distribution of particles with a dimension less than or equal to x where the subscript r reflects the distribution type

r	Distribution type
0	Number
1	Length
2	Area
3	Volume

Therefore, by definition:

$Q_r(x) = 0.90$ when $x = x_{90}$
$Q_r(x) = 0.50$ when $x = x_{50}$
$Q_r(x) = 0.10$ when $x = x_{10}$

An alternative but less informative method of classifying powder fineness is by use of the descriptive terms in the following table.

Classification of powders by fineness		
Descriptive term	x_{50} (μm)	Cumulative distribution by volume basis, $Q_3(x)$
Coarse	>355	$Q_3(355)<0.50$
Moderately fine	180–355	$Q_3(180)<0.50$ and $Q_3(355)\geqq 0.50$
Fine	125–180	$Q_3(125)<0.50$ and $Q_3(180)\geqq 0.50$
Very fine	$\leqq 125$	$Q_3(125)\geqq 0.50$

Powder Flow

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

The widespread use of powders in the pharmaceutical industry has generated a variety of methods for characterizing powder flow. Not surprisingly, scores of references appear in the pharmaceutical literature, attempting to correlate the various measures of powder flow to manufacturing properties. The development of such a variety of test methods was inevitable; powder behavior is multifaceted and thus complicates the effort to characterize powder flow. The purpose of this chapter is to review the methods for characterizing powder flow that have appeared most frequently in the pharmaceutical literature. In addition, while it is clear that no single and simple test method can adequately characterize the flow properties of pharmaceutical powders, this chapter proposes the standardization of test methods that may be valuable during pharmaceutical development.

Four commonly reported methods for testing powder flow are (1) angle of repose, (2) compressibility index or Hausner ratio, (3) flow rate through an orifice, and (4) shear cell. In addition, numerous variations of each of these basic methods are available. Given the number of test methods and variations, standardizing the test methodology, where possible, would be advantageous.

With this goal in mind, the most frequently used methods are discussed below. Important experimental considerations are identified and recommendations are made regarding standardization of the methods. In general, any method of measuring powder flow should be practical, useful, reproducible, sensitive, and yield meaningful results. It bears repeating that no one simple powder flow method will adequately or completely characterize the wide range of flow properties experienced in the pharmaceutical industry. An appropriate strategy may well be the use of multiple standardized test methods to characterize the various aspects of powder flow as needed by the pharmaceutical scientist.

1. Angle of repose

The angle of repose has been used in several branches of science to characterize the flow properties of solids. Angle of repose is a characteristic related to interparticulate friction, or resistane to movement between particles. Angle of repose test results are reported to be very dependent upon the method used. Experimental difficulties arise due to segregation of material and consolidation or aeration of the powder as the cone is formed. Despite its difficulties, the method continues to be used in the pharmaceutical industry, and a number of examples demonstrating its value in predicting manufacturing problems appear in the literature.

The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a cone-like pile of material formed by any of several different methods (described briefly below).

1.1. Basic methods for angle of repose

A variety of angle of repose test methods are reported in the literature. The most common methods for determining the static angle of repose can be classified based on two important experimental variables:

(i) The height of the ''funnel'' through which the powder passes may be fixed relative to the base, or the height may be varied as the pile forms.

(ii) The base upon which the pile forms may be of fixed diameter or the diameter of the powder cone may be allowed to vary as the pile forms.

1.2. Variations in angle of repose methods

In addition to the above methods, variations of them have been used to some extent.

(i) Drained angle of repose: This is determined by allowing an excess quantity of material positioned above a fixed diameter base to ''drain'' from the container. Formation of a cone of powder on the fixed diameter base allows determination of the drained angle of repose.

(ii) Dynamic angle of repose: This is determined by filling a cylinder (with a clear, flat cover on one end) and rotating it at a specified speed. The dynamic angle of repose is the angle (relative to the horizontal) formed by the flowing powder. The internal angle of kinetic friction is defined by the plane separating those particles sliding down the top layer of the powder and those particles that are rotating with the drum (with roughened surface).

1.3. Angle of repose general scale of flowability

While there is some variation in the qualitative description of powder flow using the angle of repose, much of the pharmaceutical literature appears to be consistent with the classification by Carr[1)], which is shown in Table 1. There are examples of formulations with an angle of repose in the range of 40 to 50 degrees that manufactured satisfactorily. When the angle of repose exceeds 50 degrees, the flow is rarely acceptable for manufacturing purposes.

Table 1 Flow properties and corresponding angles of repose[1)]

Flow property	Counter measure to prevent crosslink	Angle of repose (degrees)
Excellent		25 – 30
Good		31 – 35
Fair	aid not needed	36 – 40
Passable	may hang up	41 – 45
Poor	most agitate, vibrate	46 – 55
Very poor		56 – 65
Very, very poor		> 66

1.4. Experimental considerations for angle of repose

Angle of repose is not an intrinsic property of the powder, that is to say, it is very much dependent upon the method used to form the cone of powder. On this subject, the existing literature raises these important considerations:

(i) The peak of the cone of powder can be distorted by the impact of powder from above. By carefully building the powder cone, the distortion caused by impact can be minimized.

(ii) The nature of the base upon which the powder cone is formed influences the angle of repose. It is recommended that the powder cone be formed on a ''common base'', which can be achieved by forming the cone of powder on a layer of powder. This can be done by using a base of fixed diameter with a protruding outer edge to retain a layer of powder upon which the cone is formed.

1.5. Recommended procedure for angle of repose

Form the angle of repose on a fixed base with a retaining lip to retain a layer of powder on the base. The base should be free of vibration. Vary the height of the funnel to carefully build up a symmetrical cone of powder. Care should be taken to prevent vibration as the funnel is moved. The funnel height should be maintained approximately 2 – 4 cm from the top of the powder pile as it is being formed in order to minimize the impact of falling powder on the tip of the

cone. If a symmetrical cone of powder cannot be successfully or reproducibly prepared, this method is not appropriate. Determine the angle of repose by measuring the height of the cone of powder and calculating the angle of repose, α, from the following equation:

$$\tan \alpha = \text{height}/(\text{diameter of base} \times 0.5)$$

2. Compressibility index and Hausner ratio

In recent years the compressibility index and the closely related Hausner ratio have become the simple, fast and popular methods of predicting powder flow characteristics. The compressibility index bas been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials because all of these can influence the observed compressibility index. The compressibility index and the Hausner ratio are determined by measuring both the bulk volume and tapped volume of a powder.

2.1. Basic methods for compressibility index and Hausner ratio

While there are some variations in the method of determining the compressibility index and Hausner ratio, the basic procedure is to measure (1) the unsettled apparent volume, V_o, and (2) the final tapped volume, V_f, of the powder after tapping the material until no further volume changes occur. The compressibility index and the Hausner ratio are calculated as follows:

$$\text{Compressibility Index} = (V_o - V_f)/V_o \times 100$$
$$\text{Hausner Ratio} = V_o/V_f$$

Alternatively, the compressibility index and Hausner ratio may be calculated using measured values for bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) as follows:

$$\text{Compressibility Index} = (\rho_{tapped} - \rho_{bulk})/\rho_{tapped} \times 100$$
$$\text{Hausner Ratio} = \rho_{tapped}/\rho_{bulk}$$

In a variation of these methods, the rate of consolidation is sometimes measured rather than, or in addition to, the change in volume that occurs on tapping. For the compressibility index and the Hausner ratio, the generally accepted scale of flowability is given in Table 2.

Table 2 Scale of flowability[1)]

Compressibility index (%)	Flow character	Hausner ratio
≦10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very poor	1.46 – 1.59
>38	Very, very poor	>1.60

2.2. Experimental considerations for the compressibility index and Hausner ratio

Compressibility index and Hausner ratio are not intrinsic properties of the powder, that is to say, they are dependent upon the methodology used. The existing literature points out several important considerations affecting the determination of the (1) unsettled apparent volume, V_o, (2) the final tapped volume, V_f, (3) the bulk density, ρ_{bulk}, and (4) the tapped density, ρ_{tapped}:

(i) The diameter of the cylinder used
(ii) The number of times the powder is tapped to achieve the tapped density
(iii) The mass of material used in the test
(iv) Rotation of the sample during tapping

2.3. Recommended procedure for compressibility index and Hausner ratio

Use a 250-mL volumetric cylinder with a test sample weight of 100 g. Smaller weights and volumes may be used, but variations in the method should be described with the results. An average of three determinations is recommended.

3. Flow through an orifice

The flow rate of a material depends upon many factors, some of which are particle-related and some related to the process. Monitoring the rate of flow of material through an orifice has been proposed as a better measure of powder flowability. Of particular significance is the utility of monitoring flow continuously since pulsating flow patterns have been observed even for free flowing materials. Changes in flow rate as the container empties can also be observed. Empirical equations relating flow rate to the diameter of the opening, particle size, and particle density have been determined. However, determining the flow rate through an orifice is useful only with free-flowing materials.

The flow rate through an orifice is generally measured as the mass per time flowing from any of a number of types of containers (cylinders, funnels, hoppers). Measurement of the flow rate can be in discrete increments or continuous.

3.1. Basic methods for flow through an orifice

There are a variety of methods described in the literature. The most common for determining the flow rate through an orifice can be classified based on three important experimental variables:

(1) The type of container used to contain the powder. Common containers are cylinders, funnels and hoppers from production equipment.

(2) The size and shape of the orifice used. The orifice diameter and shape are critical factors in determining powder flow rate.

(3) The method of measuring powder flow rate. Flow rate can be measured continuously using an electronic balance and with some sort of recording device (strip chart recorder, computer). It can also be measured in discrete samples (for example, the time it takes for 100 g of powder to pass through the orifice to the nearest tenth of a second or the amount of powder passing through the orifice in 10 seconds to the nearest tenth of a gram).

3.2. Variations in methods for flow through an orifice

Either mass flow rate or volume flow rate can be determined. Mass flow rate is the easier of the methods, but it biases the results in favor of high-density materials. Since die fill is volumetric, determining volume flow rate may be preferable. A vibrator is occasionally attached to facilitate flow from the container, however, this appears to complicate interpretation of results. A moving orifice device has been proposed to more closely simulate rotary press conditions. The minimum diameter orifice through which powder flows can also be identified.

3.3. General scale of flowability for flow through an orifice

No general scale is available because flow rate is critically dependent on the method used to measure it. Comparison between published results is difficult.

3.4. Experimental considerations for flow through an orifice

Flow rate through an orifice is not an intrinsic property of the powder. It is very much dependent upon the methodology used. The existing literature points out several important

considerations affecting these methods:

(i) The diameter and shape of the orifice
(ii) The type of container material (metal, glass, plastic)
(iii) The diameter and height of the powder bed.

3.5. Recommended procedure for flow through an orifice

Flow rate through an orifice can be used only for materials that have some capacity to flow. It is not useful for cohesive materials. Provided that the height of the powder bed (the 'head' of powder) is much greater than the diameter of the orifice, the flow rate is virtually independent of the powder head. Use a cylinder as the container because the cylinder material should have little effect on flow. This configuration results in flow rate being determined by the movement of powder over powder rather than powder along the wall of the container. Powder flow rate often increases when the height of the powder column is less than two times the diameter of the column. The orifice should be circular and the cylinder should be free of vibration. General guidelines for dimensions of the cylinder are as follows:

(i) Diameter of opening >6 times the diameter of the particles
(ii) Diameter of the cylinder >2 times the diameter of the opening

Use of a hopper as the container may be appropriate and representative of flow in a production situation. It is not advisable to use a funnel, particularly one with a stem, because flow rate will be determined by the size and length of the stem as well as the friction between the stem and the powder. A truncated cone may be appropriate, but flow will be influenced by the powder—wall friction coefficient, thus, selection of an appropriate construction material is important.

For the opening in the cylinder, use a flat-faced bottom plate with the option to vary orifice diameter to provide maximum flexibility and better ensure a powder-over-powder flow pattern. Rate measurement can be either discrete or continuous. Continuous measurement using an electronic balance can more effectively detect momentary flow rate variations.

4. Shear cell methods

In an effort to put powder flow studies and hopper design on a more fundamental basis, a variety of powder shear testers and methods that permit more thorough and precisely defined assessment of powder flow properties have been developed. Shear cell methodology has been used extensively in the study of pharmaceutical materials. From these methods, a wide variety of parameters can be obtained, including the yield loci representing the shear stress-shear strain relationship, the angle of internal friction, the unconfined yield strength, the tensile strength, and a variety of derived parameters such as the flow factor and other flowability indices. Because of the ability to more precisely control experimental parameters, flow properties can also be determined as a function of consolidation load, time, and other environmental conditions. The methods have been successfully used to determine critical hopper and bin parameters.

4.1. Basic methods for shear cell

One type of shear cell is the cylindrical shear cell which is split horizontally, forming a shear plane between the lower stationary base and the upper movable portion of the shear cell ring. After powder bed consolidation in the shear cell (using a well-defined procedure), the force necessary to shear the powder bed by moving the upper ring is determined. Annular shear cell designs offer some advantages over the cylindrical shear cell design, including the need for less material. A disadvantage, however, is that because of its design, the powder bed is not sheared as uniformly because material on the outside of the annulus is sheared more than material in the inner region. A third type of shear cell (plate-type) consists of a thin sandwich of powder between a lower stationary rough surface and an upper rough surface that is moveable.

All of the shear cell methods have their advantages and disadvantages, but a detailed review is beyond the scope of this chapter. As with the other methods for characterizing powder flow, many variations are described in the literature. A significant advantage of shear cell methodology in general is a greater degree of experimental control. The methodology generally is rather time-consuming and requires significant amounts of material and a well-trained operator.

4.2. Recommendations for shear cell

The many existing shear cell configurations and test methods provide a wealth of data and can be used very effectively to characterize powder flow. They are also helpful in the design of equipment such as hoppers and bins.

Because of the diversity of available equipment and experimental procedures, no specific recommendations regarding methodology are presented in this chapter. It is recommended that the results of powder flow characterization using shear cell methodology include a complete description of equipment and methodology used.

References

1) Carr R.L.: Evaluating flow properties of solids. *Chem. Eng*. 1965; 72: 163–168.

Solid and Particle Densities

Density of a solid or a powder as a state of aggregation has different definitions depending on the way of including of the interparticulate and intraparticulate voids that exist between the particles or inside the powder. Different figures are obtained in each case, and there are different practical meanings. Generally, there are three levels of definitions of the solid or powder density.

(1) Crystal density: It is assumed that the system is homogeneous with no intraparticulate void. Crystal density is also called true density.

(2) Particle density: The sealed pores or the experimentally non-accessible open pores is also included as a part of the volumes of the solid or the powder.

(3) Bulk density: The interparticulate void formed in the powder bed is also included as a part of the volumes of the solid or the powder. Bulk density is also called apparent density. Generally, the powder densities at loose packing and at tapping are defined as the bulk density and the tapped density, respectively.

Generally, the densities of liquid or gas are affected only by temperature and pressure, but the solid or powder density is affected by the state of aggregation of the molecules or the particles. Therefore, the solid or powder densities naturally vary depending on crystal structure or crystallinity of the substance concerned, and also varies depending on the method of preparation or handling if the sample is amorphous form or partially amorphous. Consequently, even in a case that two solids or powders are chemically identical, it may be possible that the different figures of density are obtained if their crystal structures are different. As the solid or powder particle densities are important physical properties for the powdered pharmaceutical drugs or the powdered raw materials of drugs, the Japanese Pharmacopoeia specifies

each density determination as ''Powder Particle Density Determination'' for the particle density and as ''Determination of Bulk and Tapped Densities'' for the bulk density.

The solid or powder densities are expressed in mass per unit volume (kg/m^3), and generally expressed in g/cm^3 (1 g/cm^3 = 1000 kg/m^3).

Crystal Density

The crystal density of a substance is the average mass per unit volume, exclusive of all voids that are not a fundamental part of the molecular packing arrangement. It is an intrinsic property concerning the specific crystal structure of the substance, and is not affected by the method of determination. The crystal density can be determined either by calculation or by simple measurement.

A. The calculated crystal density is obtained using:
   1) For example, the crystallographic data (volume and composition of the unit cell) obtained by indexing the perfect crystal X-ray diffraction data from single crystal or the powder X-ray diffraction data.
   2) Molecular mass of the substance.

B. The measured crystal density is obtained as the mass to volume ratio after measuring the single crystal mass and volume.

Particle Density

The particle density takes account both the crystal density and the intraparticulate porosity (sealed and/or experimentally non-accessible open pores) as a part of the particle volume. The particle density depends on the value of the volume determined, and the volume in turn depends on the method of measurement. Particle density can be determined either by gas displacement pycnometry or mercury porosimetry, but the Japanese Pharmacopoeia specifies the pycnometry as the ''Powder Particle Density Determination''.

A. The pycnometric density is obtained by assuming that the volume of the gas displaced, which is measured with the gas displacement pycnometer, is equivalent to that of a known mass of the powder. In pycnometric density measurements, any volume with the open pores accessible to the gas is not included as a part of volume of the powder, but the sealed pores or pores inaccessible to the gas is included as a part of the volume of the powder. Due to the high diffusivity of helium which can penetrate to most open pores, it is recommendable as the measurement gas of particle density. Therefore, the pycnometric particle density of a finely milled powder is generally not very different from the crystal density. Hence, the particle density by this method is the best estimate of the true density of an amorphous or partially crystalline sample, and can be widely used for manufacturing control of the processed pharmaceutical powder samples.

B. The mercury porosimetric density is also called granular density. This method also includes the sealed pores as a part of the volumes of the solid or the powder, but excludes the volume only from the open pores larger than some size limit. This pore size limit or minimal access diameter depends on the maximal mercury intrusion pressure applied during the measurement and under normal operating pressure, the mercury does not penetrate the finenest pores accessible to helium. Since this method is capable of measuring the density which corresponds to the pore size limit at each mercury intrusion pressure, the various granular densities can be obtained from one sample.

Bulk Density and Tapped Density

The bulk density of a powder includes the contribution of interparticulate void volume as a part of the volume of the powder. Therefore, the bulk density depends on both the powder particle density and the space arrangement of particles in the power bed. Further, since the slightest disturbance of the bed may result in variation of the space arrangement, it is often very difficult to determine the bulk density with good reproducibility. Therefore, it is essential to specify how the determination was made upon reporting the bulk density.

The Japanese Pharmacopoeia specifies ''Determination of Bulk and Tapped Densities''.

A. The bulk density is determined by measuring the apparent volume of a known mass of powder sample that has been passed through a screen in a graduated cylinder (constant mass method). Separately, the Pharmacopoeia specifies the method of determining bulk density by measuring the mass of powder in a vessel having a known volume (constant volume method).

B. The tapped density is obtained by mechanically tapping a measuring cylinder containing a powder sample. After determining the initial bulk volume, carry out tapping under a fixed measurement condition (tapping rate and drop height), and the measurement is carried out repeatedly until the bulk volume variation obtained at consecutive two measurements is within an acceptable range (constant mass method). Separately, the Pharmacopoeia specifies the method of determining the tapped density by measuring the mass of a fixed volume of the tapped powder (constant volume method).

G3 Biotechnological/Biological Products

Amino Acid Analysis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Amino acid analysis refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Proteins and peptides are macromolecules consisting of covalently bonded amino acid residues organized as a linear polymer. The sequence of the amino acids in a protein or peptide determines the properties of the molecule. Proteins are considered large molecules that commonly exist as folded structures with a specific conformation, while peptides are smaller and may consist of only a few amino acids. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

Apparatus

Methods used for amino acid analysis are usually based on

a chromatographic separation of the amino acids present in the test sample. Current techniques take advantage of the automated chromatographic instrumentation designed for analytical methodologies. An amino acid analysis instrument will typically be a low-pressure or high-pressure liquid chromatograph capable of generating mobile phase gradients that separate the amino acid analytes on a chromatographic column. The instrument must have postcolumn derivatization capability, unless the sample is analyzed using precolumn derivatization. The detector is usually an ultraviolet-visible or fluorescence detector depending on the derivatization method used. A recording device (e.g., integrator) is used for transforming the analog signal from the detector and for quantitation. It is preferred that instrumentation be dedicated particularly for amino acid analysis.

General Precautions

Background contamination is always a concern for the analyst in performing amino acid analysis. High purity reagents are necessary (e.g., low purity hydrochloric acid can contribute to glycine contamination). Analytical reagents are changed routinely every few weeks using only high-pressure liquid chromatography (HPLC) grade solvents. Potential microbial contamination and foreign material that might be present in the solvents are reduced by filtering solvents before use, keeping solvent reservoirs covered, and not placing amino acid analysis instrumentation in direct sunlight.

Laboratory practices can determine the quality of the amino acid analysis. Place the instrumentation in a low traffic area of the laboratory. Keep the laboratory clean. Clean and calibrate pipets according to a maintenance schedule. Keep pipet tips in a covered box; the analysts may not handle pipet tips with their hands. The analysts may wear powder-free latex or equivalent gloves. Limit the number of times a test sample vial is opened and closed because dust can contribute to elevated levels of glycine, serine, and alanine.

A well-maintained instrument is necessary for acceptable amino acid analysis results. If the instrument is used on a routine basis, it is to be checked daily for leaks, detector and lamp stability, and the ability of the column to maintain resolution of the individual amino acids. Clean or replace all instrument filters and other maintenance items on a routine schedule.

Reference Standard Material

Acceptable amino acid standards are commercially available for amino acid analysis and typically consist of an aqueous mixture of amino acids. When determining amino acid composition, protein or peptide standards are analyzed with the test material as a control to demonstrate the integrity of the entire procedure. Highly purified bovine serum albumin has been used as a protein standard for this purpose.

Calibration of Instrumentation

Calibration of amino acid analysis instrumentation typically involves analyzing the amino acid standard, which consists of a mixture of amino acids at a number of concentrations, to determine the response factor and range of analysis for each amino acid. The concentration of each amino acid in the standard is known. In the calibration procedure, the analyst dilutes the amino acid standard to several different analyte levels within the expected linear range of the amino acid analysis technique. Then, replicates at each of the different analyte levels can be analyzed. Peak areas obtained for each amino acid are plotted versus the known concentration for each of the amino acids in the standard dilution. These results will allow the analyst to determine the range of amino acid concentrations where the peak area of a given amino acid is an approximately linear function of the amino acid concentration. It is important that the analyst prepare the samples for amino acid analysis so that they are within the analytical limits (e.g., linear working range) of the technique employed in order to obtain accurate and repeatable results.

Four to six amino acid standard levels are analyzed to determine a response factor for each amino acid. The response factor is calculated as the average peak area or peak height per nmol of amino acid present in the standard. A calibration file consisting of the response factor for each amino acid is prepared and used to calculate the concentration of each amino acid present in the test sample. This calculation involves dividing the peak area corresponding to a given amino acid by the response factor for that amino acid to give the nmol of the amino acid. For routine analysis, a single-point calibration may be sufficient; however, the calibration file is updated frequently and tested by the analysis of analytical controls to ensure its integrity.

Repeatability

Consistent high quality amino acid analysis results from an analytical laboratory require attention to the repeatability of the assay. During analysis of the chromatographic separation of the amino acids or their derivatives, numerous peaks can be observed on the chromatogram that correspond to the amino acids. The large number of peaks makes it necessary to have an amino acid analysis system that can repeatedly identify the peaks based on retention time and integrate the peak areas for quantitation. A typical repeatability evaluation involves preparing a standard amino acid solution and analyzing many replicates (i.e., six analyses or more) of the same standard solution. The relative standard deviation (RSD) is determined for the retention time and integrated peak area of each amino acid. An evaluation of the repeatability is expanded to include multiple assays conducted over several days by different analysts. Multiple assays include the preparation of standard dilutions from starting materials to determine the variation due to sample handling. Often the amino acid composition of a standard protein (e.g., bovine serum albumin) is analyzed as part of the repeatability evaluation. By evaluating the replicate variation (i.e., RSD), the laboratory can establish analytical limits to ensure that the analyses from the laboratory are under control. It is desirable to establish the lowest practical variation limits to ensure the best results. Areas to focus on to lower the variability of the amino acid analysis include sample preparation, high background spectral interference due to quality of reagents and/or laboratory practices, instrument performance and maintenance, data analysis and interpretation, and analyst performance and habits. All parameters involved are fully investigated in the scope of the validation work.

Sample Preparation

Accurate results from amino acid analysis require purified protein and peptide samples. Buffer components (e.g., salts, urea, detergents) can interfere with the amino acid analysis and are removed from the sample before analysis. Methods that utilize postcolumn derivatization of the amino acids are generally not affected by buffer components to the extent seen with precolumn derivatization methods. It is desirable to limit the number of sample manipulations to reduce potential background contamination, to improve analyte recovery, and to reduce labor. Common techniques used to remove buffer components from protein samples include the following methods: (1) injecting the protein sample onto a reversed-phase HPLC system, eluting the protein with a

volatile solvent containing a sufficient organic component, and drying the sample in a vacuum centrifuge; (2) dialysis against a volatile buffer or water; (3) centrifugal ultrafiltration for buffer replacement with a volatile buffer or water; (4) precipitating the protein from the buffer using an organic solvent (e.g., acetone); and (5) gel filtration.

Internal Standards

It is recommended that an internal standard be used to monitor physical and chemical losses and variations during amino acid analysis. An accurately known amount of internal standard can be added to a protein solution prior to hydrolysis. The recovery of the internal standard gives the general recovery of the amino acids of the protein solution. Free amino acids, however, do not behave in the same way as protein-bound amino acids during hydrolysis because their rates of release or destruction are variable. Therefore, the use of an internal standard to correct for losses during hydrolysis may give unreliable results. It will be necessary to take this point under consideration when interpreting the results. Internal standards can also be added to the mixture of amino acids after hydrolysis to correct for differences in sample application and changes in reagent stability and flow rates. Ideally, an internal standard is an unnaturally occurring primary amino acid that is commercially available and inexpensive. It should also be stable during hydrolysis, its response factor should be linear with concentration, and it needs to elute with a unique retention time without overlapping other amino acids. Commonly used amino acid standards include norleucine, nitrotyrosine, and α-aminobutyric acid.

Protein Hydrolysis

Hydrolysis of protein and peptide samples is necessary for amino acid analysis of these molecules. The glassware used for hydrolysis must be very clean to avoid erroneous results. Glove powders and fingerprints on hydrolysis tubes may cause contamination. To clean glass hydrolysis tubes, boil tubes for 1 hour in 1 mol/L hydrochloric acid or soak tubes in concentrated nitric acid or in a mixture of concentrated hydrochloric acid and concentrated nitric acid (1:1). Clean hydrolysis tubes are rinsed with high-purity water followed by a rinse with HPLC grade methanol, dried overnight in an oven, and stored covered until use. Alternatively, pyrolysis of clean glassware at 500°C for 4 hours may also be used to eliminate contamination from hydrolysis tubes. Adequate disposable laboratory material can also be used.

Acid hydrolysis is the most common method for hydrolyzing a protein sample before amino acid analysis. The acid hydrolysis technique can contribute to the variation of the analysis due to complete or partial destruction of several amino acids. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Application of adequate vacuum (≦less than 200 μm of mercury or 26.7 Pa) or introduction of an inert gas (argon) in the headspace of the reaction vessel can reduce the level of oxidative destruction. In peptide bonds involving isoleucine and valine the amido bonds of Ile-Ile, Val-Val, Ile-Val, and Val-Ile are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively. The loss of tryptophan, asparagine, and glutamine during an acid hydrolysis limits quantitation to 17 amino acids. Some of the hydrolysis techniques described are used to address these concerns. Some of the hydrolysis techniques described (i.e., *Methods 4-11*) may cause modifications to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis.

A time-course study (i.e., amino acid analysis at acid hydrolysis times of 24, 48, and 72 hours) is often employed to analyze the starting concentration of amino acids that are partially destroyed or slow to cleave. By plotting the observed concentration of labile amino acids (i.e., serine and threonine) versus hydrolysis time, the line can be extrapolated to the origin to determine the starting concentration of these amino acids. Time-course hydrolysis studies are also used with amino acids that are slow to cleave (e.g., isoleucine and valine). During the hydrolysis time course, the analyst will observe a plateau in these residues. The level of this plateau is taken as the residue concentration. If the hydrolysis time is too long, the residue concentration of the sample will begin to decrease, indicating destruction by the hydrolysis conditions.

An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. The amino acid in free form may not completely represent the rate of destruction of labile amino acids within a peptide or protein during the hydrolysis. This is especially true for peptide bonds that are slow to cleave (e.g., Ile-Val bonds). However, this technique will allow the analyst to account for some residue destruction. Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. The optimal conditions for microwave hydrolysis must be investigated for each individual protein/peptide sample. The microwave hydrolysis technique typically requires only a few minutes, but even a deviation of one minute may give inadequate results (e.g., incomplete hydrolysis or destruction of labile amino acids). Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

Note: During initial analyses of an unknown protein, experiments with various hydrolysis time and temperature conditions are conducted to determine the optimal conditions.

Method 1

Acid hydrolysis using hydrochloric acid containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol.

Procedure—

Liquid Phase Hydrolysis Place the protein or peptide sample in a hydrolysis tube, and dry. [Note: The sample is dried so that water in the sample will not dilute the acid used for the hydrolysis.] Add 200 μL of *Hydrolysis Solution* per 500 μg of lyophilized protein. Freeze the sample tube in a dry ice-acetone bath, and flame seal in vacuum. Samples are typically hydrolyzed at 110°C for 24 hours in vacuum or inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g., 48 and 72 hours) are investigated if there is a concern that the protein is not completely hydrolyzed.

Vapor Phase Hydrolysis This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available. Contamination of the sample from the acid reagent is also minimized by using vapor phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of *Hydrolysis Solution*. The *Hydrolysis*

Solution does not come in contact with the test sample. Apply an inert atmosphere or vacuum (≦less than 200 μm of mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110°C for a 24-hour hydrolysis time. Acid vapor hydrolyzes the dried sample. Any condensation of the acid in the sample vials is minimized. After hydrolysis, dry the test sample in vacuum to remove any residual acid.

Method 2

Tryptophan oxidation during hydrolysis is decreased by using mercaptoethanesulfonic acid (MESA) as the reducing acid.

Hydrolysis Solution 2.5 mol/L MESA solution.

Vapor Phase Hydrolysis About 1 to 100 μg of the protein/peptide under test is dried in a hydrolysis tube. The hydrolysis tube is placed in a larger tube with about 200 μL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 μm of mercury or 6.7 Pa) to vaporize the *Hydrolysis Solution*. The hydrolysis tube is heated to 170°C to 185°C for about 12.5 minutes. After hydrolysis, the hydrolysis tube is dried in vacuum for 15 minutes to remove the residual acid.

Method 3

Tryptophan oxidation during hydrolysis is prevented by using thioglycolic acid (TGA) as the reducing acid.

Hydrolysis Solution A solution containing 7 mol/L hydrochloric acid, 10% of trifluoroacetic acid, 20% of thioglycolic acid, and 1% of phenol.

Vapor Phase Hydrolysis About 10 to 50 μg of the protein/peptide under test is dried in a sample tube. The sample tube is placed in a larger tube with about 200 μL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 μm of mercury or 6.7 Pa) to vaporize the TGA. The sample tube is heated to 166°C for about 15 to 30 minutes. After hydrolysis, the sample tube is dried in vacuum for 5 minutes to remove the residual acid. Recovery of tryptophan by this method may be dependent on the amount of sample present.

Method 4

Cysteine-cystine and methionine oxidation is performed with performic acid before the protein hydrolysis.

Oxidation Solution The performic acid is prepared fresh by mixing formic acid and 30 percent hydrogen peroxide (9:1), and incubated at room temperature for 1 hour.

Procedure The protein/peptide sample is dissolved in 20 μL of formic acid, and heated at 50°C for 5 minutes; then 100 μL of the *Oxidation Solution* is added. The oxidation is allowed to proceed for 10 to 30 minutes. In this reaction, cysteine is converted to cysteic acid and methionine is converted to methionine sulfone. The excess reagent is removed from the sample in a vacuum centrifuge. This technique may cause modifications to tyrosine residues in the presence of halides. The oxidized protein can then be acid hydrolyzed using *Method 1* or *Method 2*.

Method 5

Cysteine-cystine oxidation is accomplished during the liquid phase hydrolysis with sodium azide.

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.2% of phenol, to which is added sodium azide to obtain a final concentration of 0.2% (w/v). The added phenol prevents halogenation of tyrosine.

Liquid Phase Hydrolysis The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the sodium azide present in the *Hydrolysis Solution*. This technique allows better tyrosine recovery than *Method 4*, but it is not quantitative for methionine. Methionine is converted to a mixture of the parent methionine and its two oxidative products, methionine sulfoxide and methionine sulfone.

Method 6

Cysteine-cystine oxidation is accomplished with dimethyl sulfoxide (DMSO).

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol, to which DMSO is added to obtain a final concentration of 2% (v/v).

Vapor Phase Hydrolysis The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the DMSO present in the *Hydrolysis Solution*. As an approach to limit variability and compensate for partial destruction, it is recommended to evaluate the cysteic acid recovery from oxidative hydrolyses of standard proteins containing 1 to 8 mol of cysteine. The response factors from protein/peptide hydrolysates are typically about 30% lower than those for nonhydrolyzed cysteic acid standards. Because histidine, methionine, tyrosine, and tryptophan are also modified, a complete compositional analysis is not obtained with this technique.

Method 7

Cysteine-cystine reduction and alkylation is accomplished by a vapor phase pyridylethylation reaction.

Reducing Solution Transfer 83.3 μL of pyridine, 16.7 μL of 4-vinylpyridine, 16.7 μL of tributylphosphine, and 83.3 μL of water to a suitable container, and mix.

Procedure Add the protein/peptide (between 1 and 100 μg) to a hydrolysis tube, and place in a larger tube. Transfer the *Reducing Solution* to the large tube, seal in vacuum (about 50 μm of mercury or 6.7 Pa), and incubate at about 100°C for 5 minutes. Then remove the inner hydrolysis tube, and dry it in a vacuum desiccator for 15 minutes to remove residual reagents. The pyridylethylated protein/peptide can then be acid hydrolyzed using previously described procedures. The pyridylethylation reaction is performed simultaneously with a protein standard sample containing 1 to 8 mol of cysteine to improve accuracy in the pyridylethylcysteine recovery. Longer incubation times for the pyridylethylation reaction can cause modifications to the α-amino terminal group and the ε-amino group of lysine in the protein.

Method 8

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase pyridylethylation reaction.

Stock Solutions Prepare and filter three solutions: 1 mol/L Tris hydrochloride (pH 8.5) containing 4 mmol/L disodium dihydrogen ethylendiamine tetraacetate (*Stock Solution A*), 8 mol/L guanidine hydrochloride (*Stock Solution B*), and 10% of 2-mercaptoethanol in water (*Stock Solution C*).

Reducing Solution Prepare a mixture of *Stock Solution B* and *Stock Solution A* (3:1) to obtain a buffered solution of 6 mol/L guanidine hydrochloride in 0.25 mol/L Tris hydrochloride.

Procedure Dissolve about 10 μg of the test sample in 50 μL of the *Reducing Solution*, and add about 2.5 μL of *Stock Solution C*. Store under nitrogen or argon for 2 hours at room temperature in the dark. To achieve the pyridylethylation reaction, add about 2 μL of 4-vinylpyridine to the protein solution, and incubate for an additional 2 hours at room temperature in the dark. The protein/peptide is desalted by collecting the protein/peptide fraction from a reversed-phase

HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis.

Method 9

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase carboxymethylation reaction.

Stock Solutions Prepare as directed for *Method 8*.

Carboxymethylation Solution Prepare a solution containing 100 mg of iodoacetamide per mL of ethanol (95).

Buffer Solution Use the *Reducing Solution*, prepared as directed for *Method 8*.

Procedure Dissolve the test sample in 50 μL of the *Buffer Solution*, and add about 2.5 μL of *Stock Solution C*. Store under nitrogen or argon for 2 hours at room temperature in the dark. Add the *Carboxymethylation Solution* in a ratio 1.5 fold per total theoretical content of thiols, and incubate for an additional 30 minutes at room temperature in the dark. [Note: If the thiol content of the protein is unknown, then add 5 μL of 100 mmol/L iodoacetamide for every 20 nmol of protein present.] The reaction is stopped by adding excess of 2-mercaptoethanol. The protein/peptide is desalted by collecting the protein/peptide fraction from a reversed-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis. The *S*-carboxyamidomethyl-cysteine formed will be converted to *S*-carboxymethylcysteine during acid hydrolysis.

Method 10

Cysteine-cystine is reacted with dithiodiglycolic acid or dithiodipropionic acid to produce a mixed disulfide. [Note: The choice of dithiodiglycolic acid or dithiodipropionic acid depends on the required resolution of the amino acid analysis method.]

Reducing Solution A solution containing 10 mg of dithiodiglycolic acid (or dithiodipropionic acid) per mL of 0.2 mol/L sodium hydroxide.

Procedure Transfer about 20 μg of the test sample to a hydrolysis tube, and add 5 μL of the *Reducing Solution*. Add 10 μL of isopropyl alcohol, and then remove all of the sample liquid by vacuum centrifugation. The sample is then hydrolyzed using *Method 1*. This method has the advantage that other amino acid residues are not derivatized by side reactions, and the sample does not need to be desalted prior to hydrolysis.

Method 11

Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, during acid hydrolysis. Asparagine and aspartic acid residues are added and represented by *Asx*, while glutamine and glutamic acid residues are added and represented by *Glx*. Proteins/peptides can be reacted with bis(1,1-trifluoroacetoxy)iodobenzene (BTI) to convert the asparagine and glutamine residues to diaminopropionic acid and diaminobutyric acid residues, respectively, upon acid hydrolysis. These conversions allow the analyst to determine the asparagine and glutamine content of a protein/peptide in the presence of aspartic acid and glutamic acid residues.

Reducing Solutions Prepare and filter three solutions: a solution of 10 mmol/L trifluoroacetic acid (*Solution A*), a solution of 5 mol/L guanidine hydrochloride and 10 mmol/L trifluoroacetic acid (*Solution B*), and a freshly prepared solution of *N*,*N*-dimethylformamide containing 36 mg of BTI per mL (*Solution C*).

Procedure In a clean hydrolysis tube, transfer about 200 μg of the test sample, and add 2 mL of *Solution A* or *Solution B* and 2 mL of *Solution C*. Seal the hydrolysis tube in vacuum. Heat the sample at 60°C for 4 hours in the dark. The sample is then dialyzed with water to remove the excess reagents. Extract the dialyzed sample three times with equal volumes of *n*-butyl acetate, and then lyophilize. The protein can then be acid hydrolyzed using previously described procedures. The α,β-diaminopropionic and α,γ-diaminobutyric acid residues do not typically resolve from the lysine residues upon ion-exchange chromatography based on amino acid analysis. Therefore, when using ion-exchange as the mode of amino acid separation, the asparagine and glutamine contents are the quantitative difference in the aspartic acid and glutamic acid content assayed with underivatized and BTI-derivatized acid hydrolysis. [Note: The threonine, methionine, cysteine, tyrosine, and histidine assayed content can be altered by BTI derivatization; a hydrolysis without BTI will have to be performed if the analyst is interested in the composition of these other amino acid residues of the protein/peptide.]

Methodologies of Amino Acid Analysis General Principles

Many amino acid analysis techniques exist, and the choice of any one technique often depends on the sensitivity required from the assay. In general, about one-half of the amino acid analysis techniques employed rely on the separation of the free amino acids by ion-exchange chromatography followed by postcolumn derivatization (e.g., with ninhydrin or *o*-phthalaldehyde). Postcolumn detection techniques can be used with samples that contain small amounts of buffer components, such as salts and urea, and generally require between 5 and 10 μg of protein sample per analysis. The remaining amino acid techniques typically involve precolumn derivatization of the free amino acids (e.g., phenyl isothiocyanate; 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate or *o*-phthalaldehyde; (dimethylamino)azobenzenesulfonyl chloride; 9-fluorenylmethylchloroformate; and, 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole) followed by reversed-phase HPLC. Precolumn derivatization techniques are very sensitive and usually require between 0.5 and 1.0 μg of protein sample per analysis but may be influenced by buffer salts in the samples. Precolumn derivatization techniques may also result in multiple derivatives of a given amino acid, which complicates the result interpretation. Postcolumn derivatization techniques are generally influenced less by performance variation of the assay than precolumn derivatization techniques.

The following *Methods* may be used for quantitative amino acid analysis. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic systems, etc. Specific parameters may vary according to the exact equipment and procedure used. Many laboratories will utilize more than one amino acid analysis technique to exploit the advantages offered by each. In each of these *Methods*, the analog signal is visualized by means of a data acquisition system, and the peak areas are integrated for quantification purposes.

Method 1—Postcolumn Ninhydrin Detection General Principle

Ion-exchange chromatography with postcolumn ninhydrin detection is one of the most common methods employed for quantitative amino acid analysis. As a rule, a Li-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster Na-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accom-

plished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has characteristic purple or yellow color. Amino acids, except imino acid, give a purple color, and show the maximum absorption at 570 nm. The imino acids such as proline give a yellow color, and show the maximum absorption at 440 nm. The postcolumn reaction between ninhydrin and amino acid eluted from column is monitored at 440 and 570 nm, and the chromatogram obtained is used for the determination of amino acid composition.

Detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for proline. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good composition data, samples larger than 1 μg before hydrolysis are best suited for this amino acid analysis of protein/peptide.

Method 2—Postcolumn OPA Fluorometric Detection General Principle

o-Phthalaldehyde (OPA) reacts with primary amines in the presence of thiol compound, to form highly fluorescent isoindole products. This reaction is utilized for the postcolumn derivatization in analysis of amino acids by ion-exchange chromatography. The rule of the separation is the same as *Method 1*. Instruments and reagents for this form of amino acid analysis are available commercially. Many modifications of this methodology exist.

Although OPA does not react with secondary amines (imino acids such as proline) to form fluorescent substances, the oxidation with sodium hypochlorite allows secondary amines to react with OPA. The procedure employs a strongly acidic cation-exchange column for separation of free amino acids followed by postcolumn oxidation with sodium hypochlorite and postcolumn derivatization using OPA and thiol compound such as *N*-acetyl-L-cysteine and 2-mercaptoethanol. The derivatization of primary amino acids are not noticeably affected by the continuous supply of sodium hypochlorite.

Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. After postcolumn derivatization of eluted amino acids with OPA, the reactant passes through the fluorometric detector. Fluorescence intensity of OPA-derivatized amino acids are monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limit is considered to be a few tens of picomole level for most of the amino acid derivatives. Response linearity is obtained in the range of a few picomole level to a few tens of nanomole level. To obtain good compositional data, the starting with greater than 500 ng of sample before hydrolysis is best suited for the amino acid analysis of protein/peptide.

Method 3—Precolumn PITC Derivatization General Principle

Phenylisothiocyanate (PITC) reacts with amino acids to form phenylthiocarbamyl (PTC) derivatives which can be detected with high sensitivity at 245 nm. Therefore, precolumn derivatization of amino acids with PITC followed by a reversed-phase HPLC separation with UV detection is used to analyze the amino acid composition.

After the reagent is removed under vacuum, the derivatized amino acids can be stored dry and frozen for several weeks with no significant degradation. If the solution for injection is kept cold, no noticeable loss in chromatographic response occurs after three days.

Separation of the PTC-amino acids on a reversed-phase HPLC with ODS column is accomplished through a combination of changes in concentrations of acetonitrile and buffer ionic strength. PTC-amino acids eluted from the column are monitored at 254 nm.

Detection limit is considered to be 1 pmol for most of the amino acid derivatives. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 500 ng of protein/peptide before hydrolysis is best suited for this amino acid analysis of proteins/peptides.

Method 4—Precolumn AQC Derivatization General Principle

Precolumn derivatization of amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by reversed-phase HPLC separation with fluorometric detection is used.

AQC reacts with amino acids to form stable, fluorescent unsymmetric urea derivatives (AQC-amino acids) which are readily amenable to analysis by reversed-phase HPLC. Therefore, precolumn derivatization of amino acids with AQC followed by reversed-phase HPLC separation is used to analyze the amino acid composition.

Separation of the AQC-amino acids on an ODS column is accomplished through a combination of changes in concentrations of acetonitrile and salt. Selective fluorescence detection of the derivatives with excitation wavelength at 250 nm and emission wavelength at 395 nm allows for the direct injection of the reaction mixture with no significant interference from the only major fluorescent reagent by-product, 6-aminoquinoline. Excess reagent is rapidly hydrolyzed ($t_{1/2} < 15$ seconds) to yield 6-aminoquinoline, *N*-hydroxysuccinimide and carbon dioxide, and after 1 minute no further derivatization can take place.

Peak areas for AQC-amino acids are essentially unchanged for at least 1 week at room temperature, and the derivatives have more than sufficient stability to allow for overnight automated chromatographic analysis.

Detection limit is considered to be ranging from ca. 40 to 320 fmol for each amino acid, except for Cys. Detection limit for Cys is approximately 800 fmol. Response linearity is obtained in the range of 2.5 to 200 μmol/L with correlation coefficients exceeding 0.999. Good compositional data could be obtained from the analysis of derivatized protein hydrolysates containing as little as 30 ng of protein/peptide.

Method 5—Precolumn OPA Derivatization General Principle

Precolumn derivatization of amino acids with *o*-phthalaldehyde (OPA) followed by reversed-phase HPLC separation with fluorometric detection is used. This technique does not detect amino acids that exist as secondary amines (e.g., proline).

OPA in conjunction with a thiol reagent reacts with primary amine groups to form highly fluorescent isoindole products. 2-Mercaptoethanol or 3-mercaptopropionic acid can be used as thiol. OPA itself does not fluoresce and consequently produces no interfering peaks. In addition, its solubility and stability in aqueous solution, along with the rapid kinetics for the reaction, make it amenable to automated derivatization and analysis using an autosampler to mix the sample with the reagent. However, lack of reactivity with secondary amino acids has been a predominant drawback. This method does not detect amino acids that exist as secondary amines (e.g., proline). To compensate for this

drawback, this technique may be combined with another technique described in *Method 7* or *Method 8*.

Precolumn derivatization of amino acids with OPA is followed by a reversed-phase HPLC separation. Because of the instability of the OPA-amino acid derivative, HPLC separation and analysis are performed immediately following derivatization. The liquid chromatograph is equipped with a fluorometric detector for the detection of derivatized amino acids. Fluorescence intensity of OPA-derivatized amino acids is monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limits as low as 50 fmol via fluorescence have been reported, although the practical limit of analysis remains at 1 pmol.

Method 6—Precolumn DABS-Cl Derivatization General Principle

Precolumn derivatization of amino acids with (dimethylamino)azobenzenesulfonyl chloride (DABS-Cl) followed by reversed-phase HPLC separation with visible light detection is used.

DABS-Cl is a chromophoric reagent employed for the labeling of amino acids. Amino acids labeled with DABS-Cl (DABS-amino acids) are highly stable and show the maximum absorption at 436 nm.

DABS-amino acids, all 19 naturally occurring amino acids derivatives, can be separated on an ODS column of a reversed-phase HPLC by employing gradient systems consisting of acetonitrile and aqueous buffer mixture. Separated DABS-amino acids eluted from the column are detected at 436 nm in the visible region.

This *Method* can analyze the imino acids such as proline together with the amino acids at the same degree of sensitivity, DABS-Cl derivatization method permits the simultaneous quantification of tryptophan residues by previous hydrolysis of the protein/peptide with sulfonic acids such as mercaptoethanesulfonic acid, *p*-toluenesulfonic acid or methanesulfonic acid described under *Method 2* in ''Protein Hydrolysis''. The other acid-labile residues, asparagine and glutamine, can also be analysed by previous conversion into diaminopropionic acid and diaminobutyric acid, respectively, by treatment of protein/peptide with BTI described under *Method 11* in ''Protein Hydrolysis''.

The non-proteinogenic amino acid, norleucine cannot be used as internal standard in this method, as this compound is eluted in a chromatographic region crowded with peaks of primary amino acids. Nitrotyrosine can be used as an internal standard, because it is eluted in a clean region.

Detection limit of DABS-amino acid is about 1 pmol. As little as 2 to 5 pmol of an individual DABS-amino acid can be quantitatively analysed with reliability, and only 10 to 30 ng of the dabsylated protein hydrolysate is required for each analysis.

Method 7—Precolumn FMOC-Cl Derivatization General Principle

Precolumn derivatization of amino acids with 9-fluorenylmethyl chloroformate (FMOC-Cl) followed by reversed-phase HPLC separation with fluorometric detection is used.

FMOC-Cl reacts with both primary and secondary amino acids to form highly fluorescent products. The reaction of FMOC-Cl with amino acid proceeds under mild conditions in aqueous solution and is completed in 30 seconds. The derivatives are stable, only the histidine derivative showing any breakdown. Although FMOC-Cl is fluorescent itself, the reagent excess and fluorescent side-products can be eliminated without loss of FMOC-amino acids.

FMOC-amino acids are separated by a reversed-phase HPLC using an ODS column. The separation is carried out by gradient elution varied linearly from a mixture of acetonitrile methanol and acetic acid buffer (10:40:50) to a mixture of acetonitrile and acetic acid buffer (50:50), and 20 amino acid derivatives are separated in 20 minutes. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm.

The detection limit is in the low fmol range. A linearity range of 0.1 to 50 μmol/L is obtained for most of the amino acids.

Method 8—Precolumn NBD-F Derivatization General Principle

Precolumn derivatization of amino acids with 7-fluoro-4-nitrobenzo-2-oxa-1.3-diazole (NBD-F) followed by reversed-phase HPLC separation with fluorometric detection is used.

NBD-F reacts with both primary and secondary amino acids to form highly fluorescent products. Amino acids are derivatized with NBD-F by heating to 60°C for 5 minutes.

NBD-amino acid derivatives are separated on an ODS column of a reversed-phase HPLC by employing gradient elution system consisting of acetonitrile and aqueous buffer mixture, and 17 amino acid derivatives are separated in 35 minutes. ε-Aminocaproic acid can be used as an internal standard, because it is eluted in a clean chromatographic region. Each derivative eluted from the column is monitored by a fluorometric detector set at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

The sensitivity of this method is almost the same as for precolumn OPA derivatization method (*Method 5*), excluding proline to which OPA is not reactive, and might be advantageous for NBD-F against OPA. The detection limit for each amino acid is about 10 fmol. Profile analysis was achieved for about 1.5 μg of protein hydrolysates in the final precolumn labeling reaction mixture for HPLC.

Data Calculation and Analysis

When determining the amino acid content of a protein/peptide hydrolysate, it should be noted that the acid hydrolysis step destroys tryptophan and cysteine. Serine and threonine are partially destroyed by acid hydrolysis, while isoleucine and valine residues may be only partially cleaved. Methionine can undergo oxidation during acid hydrolysis, and some amino acids (e.g., glycine and serine) are common contaminants. Application of adequate vacuum ($\leqq$0.0267 kPa) or introduction of inert gas (argon) in the headspace of the reaction vessel during vapor phase hydrolysis can reduce the level of oxidative destruction. Therefore, the quantitative results obtained for cysteine, tryptophan, threonine, isoleucine, valine, methionine, glycine, and serine from a protein/peptide hydrolysate may be variable and may warrant further investigation and consideration.

Calculations

Amino Acid Mole Percent This is the number of specific amino acid residues per 100 residues in a protein. This result may be useful for evaluating amino acid analysis data when the molecular weight of the protein under investigation is unknown. This information can be used to corroborate the identity of a protein/peptide and has other applications. Carefully identify and integrate the peaks obtained as directed for each *Procedure*. Calculate the mole percent for each amino acid present in the test sample by the formula:

$$100r_U/r,$$

in which r_U is the peak response, in nmol, of the amino acid

under test; and r is the sum of peak responses, in nmol, for all amino acids present in the test sample. Comparison of the mole percent of the amino acids under test to data from known proteins can help establish or corroborate the identity of the sample protein.

Unknown Protein Samples This data analysis technique can be used to estimate the protein concentration of an unknown protein sample using the amino acid analysis data. Calculate the mass, in μg, of each recovered amino acid by the formula:

$$mM_W/1000,$$

in which m is the recovered quantity, in nmol, of the amino acid under test; and M_W is the average molecular weight for that amino acid, corrected for the weight of the water molecule that was eliminated during peptide bond formation. The sum of the masses of the recovered amino acids will give an estimate of the total mass of the protein analyzed after appropriate correction for partially and completely destroyed amino acids. If the molecular weight of the unknown protein is available (i.e., by SDS-PAGE analysis or mass spectroscopy), the amino acid composition of the unknown protein can be predicted. Calculate the number of residues of each amino acid by the formula:

$$m/(1000M/M_{WT}),$$

in which m is the recovered quantity, in nmol, of the amino acid under test; M is the total mass, in μg, of the protein; and M_{WT} is the molecular weight of the unknown protein.

Known Protein Samples This data analysis technique can be used to investigate the amino acid composition and protein concentration of a protein sample of known molecular weight and amino acid composition using the amino acid analysis data. When the composition of the protein being analyzed is known, one can exploit the fact that some amino acids are recovered well, while other amino acid recoveries may be compromised because of complete or partial destruction (e.g., tryptophan, cysteine, threonine, serine, methionine), incomplete bond cleavage (i.e., for isoleucine and valine) and free amino acid contamination (i.e., by glycine and serine).

Because those amino acids that are recovered best represent the protein, these amino acids are chosen to quantify the amount of protein. Well-recovered amino acids are, typically, aspartate-asparagine, glutamate-glutamine, alanine, leucine, phenylalanine, lysine, and arginine. This list can be modified based on experience with one's own analysis system. Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the protein content results calculated. The protein content determined for each of the well-recovered amino acids should be evenly distributed about the mean. Discard protein content values for those amino acids that have an unacceptable deviation from the mean. Typically ≧greater than 5% variation from the mean is considered unacceptable. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample. Divide the content of each amino acid by the calculated mean protein content to determine the amino acid composition of the sample by analysis.

Calculate the relative compositional error, in percentage, by the formula:

$$100m/m_S,$$

in which m is the experimentally determined quantity, in nmol per amino acid residue, of the amino acid under test; and m_S is the known residue value for that amino acid. The average relative compositional error is the average of the absolute values of the relative compositional errors of the individual amino acids, typically excluding tryptophan and cysteine from this calculation. The average relative compositional error can provide important information on the stability of analysis run over time. The agreement in the amino acid composition between the protein sample and the known composition can be used to corroborate the identity and purity of the protein in the sample.

Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia

Introduction

The primary role of specification of biotechnological/biological products listed in Japanese Pharmacopoeia (JP) is not only for securing quality control or consistency of the quality but also for assuring their efficacy and safety. In the meantime, the requirements to assure quality and safety of drugs have come to be quite strict recently, and a rigid attitude addressing safety assurance is expected for biotechnological/biological products. The key points for quality and safety assurance of biotechnological/biological products are selection and appropriate evaluation of source material, appropriate evaluation of manufacturing process and maintenance of manufacturing consistency, and control of specific physical properties of the products. Now, how to assure quality and safety of such drugs within a scope of JP has come to be questioned. This General Information describes what sorts of approaches are available to overcome these issues.

It is desired that quality and safety assurance of JP listed products are achieved by state-of-the-art methods and concepts which reflect progress of science and accumulation of experiences. This General Information challenges to show the highest level of current scientific speculation. It is expected that this information will contribute to promotion of scientific understanding of quality and safety assurance of not only JP listed products but also the other biotechnological/biological products and to promotion of active discussion of each Official Monograph in JP.

1. Fundamental measures to ensure viral safety of JP listed biotechnological/biological products

The biotechnological/biological product JP includes the products derived from living tissue and body fluid (urine, blood, etc.) of mammals, etc. Protein drugs derived from cell lines of human or animal origin (e.g., recombinant DNA drug, cell culture drug) are also included. The fundamental measures required for comprehensive viral safety of JP listed biotechnological/biological products are as follows: 1) acquaintance of possible virus contamination (source of contamination); 2) careful examination of eligibility of raw materials and their sources, e.g. human/animal, and thorough analysis and screening of the sample chosen as a substrate for drug production (e.g., pooled body fluid, cell bank, etc.) to determine any virus contamination and determination of type and nature of the virus, if contaminated; 3) evaluation to determine virus titer and virus-like particles hazardous to human, if exists; 4) selection of production related material

(e.g., reagent, immune antibody column) free from infectious or pathogenic virus; 5) performance of virus free test at an appropriate stage of manufacturing including the final product, if necessary; 6) adoption of effective viral clearance method in the manufacturing process to remove/inactivate virus. Combined method sometimes achieves higher level of clearance; 7) development of a deliberate viral clearance scheme; 8) performance of the test to evaluate viral removal and inactivation. It is considered that the stepwise and supplemental adoption of the said measures will contribute to ensure viral safety and its improvement.

2. Safety assurance measures described in the Official Monograph and this General Information

As mentioned in above 1, this General Information describes, in package, points to be concerned with and concrete information on the measures taken for viral safety of JP listed products. Except where any specific caution is provided in Official Monograph of a product in question, Official Monograph provides in general that ''Any raw material, substrate for drug production and production related material used for production of drug should be derived from healthy animals and should be shown to be free of latent virus which is infectious or pathogenic to human'', ''Cell line and culture method well evaluated in aspects of appropriateness and rationality on viral safety are used for production, and the presence of infectious or pathogenic latent virus to human in process related materials derived from living organisms should be denied''. and ''biotechnological/biological drug should be produced through a manufacturing process which is capable of removing infectious or pathogenic virus'', etc., to raise awareness on viral safety and on necessity to conduct test and process evaluation for viral safety.

3. Items and contents described in this General Information

As for viral safety of protein drug derived from cell line of human or animal origin, there is a Notice in Japan entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin'' (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare) to reflect the internationally harmonized ICH Guideline, and as for blood plasma protein fraction preparations, there is a document entitled ''Guideline for ensuring viral safety of blood plasma protein fraction preparations''. This General Information for ensuring viral safety of JP listed biotechnological/biological products has been written, referencing the contents of those guidelines, to cover general points and their details to be concerned for ensuring viral safety of not only JP listed biotechnological/biological products but also all products which would be listed in JP in future, i.e., biological products derived from living tissue and body fluid, such as urine, and protein drugs derived from cell line of human or animal origin (Table 1).

Table 1 Items described in General Information for viral safety assurance of JP listed biotechnological/biological products

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2. Safety assurance measures described in the Official Monograph and this General Information
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VIII. Measurement for viral clearance studies
1. Measurement of virus infective titer
2. Testing by nucleic-acid amplification test (NAT)

IX. Reporting and preservation

X. Others

3.1. Purpose

The purpose of this document is to propose the comprehensive concepts of the measures to be taken for ensuring viral safety of biotechnological/biological products derived from living tissue or body fluid of mammals, etc. and of protein drugs derived from cell lines of human or animal origin. That is to say, this document describes the measures and the points of concern on the items, such as ① consideration of the source of virus contamination; ② appropriate evaluation on eligibility at selecting the raw material and on qualification of its source, e.g. human or animal; ③ virus test, and its analysis and evaluation at a stage of cell substrate for drug production; ④ appropriate evaluation to choose product related materials derived from living organisms (e.g. reagent, immune antibody column, etc.); ⑤ conduct of necessary virus test on the product at an appropriate stage of manufacturing; ⑥ development of viral clearance test scheme; ⑦ performance and evaluation of viral clearance test. This document is also purposed to comprehensively describe in details that supplemental and combining adoption of the said measures will contribute to secure viral

safety and its improvement.

3.2. Background

One of the most important issues to be cautioned for safety of a biological product, which is directly derived from human or animal, or of a protein drug, which is derived from cell line of human or animal origin (recombinant DNA derived product, cell culture derived product, etc.), is a risk of virus contamination. Virus contamination may cause serious situation at clinical use once it occurs. Virus contamination may be from a raw material or from a cell substrate for drug production, or may be from an adventitious factor introduced to the manufacturing process.

JP listed biological drugs or protein drugs derived from cell line have achieved drastic contribution to the medical society, and to date, there has not been any evidence of any safety problem on them caused by virus. But, social requirement of health hazard prevention is strong, and it is now very important to prevent accidental incidence, taking security measures carefully supported by scientific rationality. It is always great concern among the persons involved that under what sort of viewpoint and to what extent we have to pursue for ensuring viral safety of a biotechnological/biological product. Before discussing these issues, two fundamental points have to be reconfirmed. One is that; we have to consider scientific, medical, and social profiles a drug has. In other words, ''Medicine is a social asset which is utilized in medical practice paying attention to the risk and benefit from the standpoints of science and society''. It is the destination and the mission of the medical/pharmaceutical society to realize prompt and stable supply of such a social asset, drug, among the medical work front to bring gospel to the patients.

The other is that; issue of viral safety is independent from safety of the components of a drug per se (narrow sense of safety). It is important to consider that this is the matter of general safety of drug (broad sense of safety). In case of a drug which has been used for a long time in the medical front, such as a JP listed product, its broad sense of safety is considered to have been established epidemiologically, and its usage past records have a great meaning. However, different from safety of drug per se (its components), taking into account any possibility of virus contamination, we have to say that only the results accumulated can not always assure viral safety of a drug used in future. Accordingly, the basis for securing broad sense of viral safety of JP listed biotechnological/biological products is to pay every attention to the measures to take for prevention, while evaluating the accumulated results.

Adopting strict regulations and conducting tests at maximum level to the extent theoretically considered may be the ways off assuring safety, but applying such way generally, without sufficient scientific review of the ways and evaluation of usage results, causes excessive requirement of regulation and test not having scientific rationality. As the results, effective and prompt supply of an important drug, already having enough accumulation of experiences, to the medical work front will be hampered, and the drug, a social asset, may not to be utilized effectively. Medicine is a sword used in medical field having double-edge named effectiveness and safety. Effectiveness and safety factors have to be derived as the fruits of leading edge of science, and relatively evaluated on a balance sheet of usefulness. Usefulness evaluation should not be unbalanced in a way that too much emphasis is placed on safety concern without back-up of appropriate scientific rationality. A drug can play an important role as a social asset only when well balanced appropriate scientific usefulness evaluation in addition to social concern of the age are given. In other words, drug is a common asset utilized by society for medication as a fruit of science of the age, and the key point of its utilization lies on a balance of risk and benefit produced from scientific and social evaluation. So, those factors have to be taken into account when target and pursuance levels for ensuring viral safety of a JP listed biotechnological/biological product are reviewed.

And, in general, the risk and benefit of drugs should be considered with the relative comparison to alternative drugs or medical treatment. The usefulness of certain drug should be reviewed finally after the competitive assessment on the risk and benefit on the alternative drugs, relevant drugs and/or alternative medical treatment.

Under such background, the purpose of this article is to describe the scientific and rational measures to be taken for ensuring viral safety of JP listed biotechnological/biological products. Giving scientific and rational measures mean that; appropriate and effective measures, elaborated from the current scientific level, are given to the issues assumable under the current scientific knowledge. In other words, possible contaminant virus is assumed to have the natures of genus, morph, particle size, physical/chemical properties, etc. which are within the range of knowledge of existing virology, and is those assumed to exist in human and animal, tissue and body fluid, which are the source of biotechnological/biological product, reagent, material, additives, etc. Accordingly, viral clearance studies using a detection method which target those viruses have to be designed.

3.3. Unknown risk on the measures taken for ensuring viral safety

There are known and unknown risks.

It is easy to determine a test method and an evaluation standard on the known risk, which exists in the drug per se (pharmaceutical component) or inevitably exists due to a quality threshold, and quantification of such risk is possible. In other words, it is easy to evaluate the known risk on a balance sheet in relation to the benefit, and we can say that valuation even in this respect has been established to some extent.

On the other hand, as for the unknown risk which is inevitable for ensuring viral safety, the subject of the risk can not be defined and quantitative concept is hard to introduce, and, therefore, taking a counter measure and evaluating its effect are not so easy. Therefore, this is the subject to be challenged calling upon wisdom of the related parties among the society of drug.

Talking about the unknown risk, there are view points that say ''It is risky because it is unknown.'' and ''What are the unknowns, and how do we cope with them in ensuring safety?''.

The view of ''It is risky because it is unknown.'' is already nothing but a sort of evaluation result, and directly connects to a final decision if it can be used as a drug. Such evaluation/decision has to be made based upon a rational, scientific or social judgment.

For example, in the case that ''In a manufacturing process of drug, virus, virus-like particle or retrovirus was detected, but its identification could not be confirmed, and, therefore, its risk can not be denied.'', the evaluation of ''It is risky because it is unknown.'' is scientifically rational and reasonable. On the other hand, however, if we reach a decision of ''It is risky because it is unknown.'' due to the reason that ''In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a 'concern' that something unknown may exist.'', it can not be said that such evaluation is based upon a rational, scientific or social judgment. It goes without saying that the utmost care has to

be taken for viral safety, but the substance of 'concern' has to be at least clearly explainable. Otherwise, the 'concern' may result in causing contradiction in the meaningful mission to utilize a social asset, drug, in medical practice.

From scientific view point, we should not be narrow minded by saying ''it is risky'' because ''there is a 'concern' that something unknown may exists'', but challenge to clarify the subject of ''What is unknown, and how to cope with it for ensuring safety'' using wisdom. What is important at the time is to define ''what is unknown'' based upon current scientific knowledge. Only through this way, is it possible for us to elaborate the measures for ensuring safety.

Once we chase up the substance of unknown risk for viral safety without premises of ''what is unknown'', ''unknown'' will be an endless question because it theoretically remains unresolved forever. If this kind of approach is taken, the issue and the measure can not be scientifically connected to each other, which will result in the excessive requirement of regulation and of test to be conducted. Yet, it is unlikely that the measure which has no relation with science will be effective to the subject of ''What is unknown is unknown.''

For example, ''what is unknown'' at the ''evaluation of a purification process which can completely clear up every virus that contaminated in a manufacturing process'' should be the subject of ''what sort of existing virus that contaminated is unknown'', not on the subject of ''what sort of virus that exist in the world is unknown. In the former subject, the premise of the study is based on all the knowledge on viruses including DNA/RNA-virus, virus with/without envelope, particle size, physical/chemical properties, etc. The premise is that the virus contaminated should be within range of existing wisdom and knowledge of virus such as species, type, nature, etc., even though the virus that contaminated is unknown. Under such premise, when evaluation is made on a purification process to decide its capability of clearing a derived virus, which is within the range of existing wisdom and learning, specific viral clearance studies designed to combine a few model viruses with different natures, such as type of nucleic acid, with/without envelope, particle size, physical/chemical properties, etc., would be enough to simulate every sort of the virus already known, and will be a ''good measure for ensuring safety''.

The issue of ''the sort of viruses that exist in the world is unknown'' may be a future study item, but it is not an appropriate subject for the viral clearance test. Further, even if the subject of ''unknown viruses, which have a particle size smaller than that of currently known viruses, may exists'' or ''unknown viruses, which have special physical/chemical properties that can not be matched to any of the currently known viruses, may exists'' is set up as an armchair theory, any experimental work can not be pursued under the current scientific level, since such virus model is not available. Further, any viral clearance test performed by using the currently available methods and technologies will be meaningless ''for ensuring safety'', since particle size or natures of such speculated virus are unknown. Likewise, any counter measures can not be taken on the subject of ''unknown virus, which can not be detected by currently available screening method, may exist'', and conducting any virus detection test at any stage will be useless ''for ensuring safety''.

The requirement of regulations or tests excessively over scientific rationality will raise human, economical and temporal burden to the pharmaceutical companies, and will adversely affect prompt, effective and economical supply of a drug to the medical front. As drug is a sort of social asset, which has to be scientifically evaluated, how to assure maximization of its safety by means of scientifically rational approach at minimum human, economical and time resources is important.

It is also important to reconfirm that achievement of those issues is on the premise that appropriate measures are taken on the supply source of drugs. For example, in a case of ''In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a 'concern' that something unknown may exist.'', appropriateness of the test, which resulted in the judgment that ''virus, virus-like particle or retrovirus was not detected in a process of drug production'', should be a prerequisite premise when judged by science standard ate the time. If there is any question on the premise, it is quite natural that the question of ''there is a 'concern' that unknown something may exist.'' will be effective.

3.4. Applicable range

This General Information is on JP listed biological products, derived from living tissue or body fluid, and protein drugs, derived from human or animal cell line, that in Japan. In the case of protein drugs derived from human or animal cell line, the products developed and approved after enforcement of the Notice Iyakushin No. 329 entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin'' should have been treated under the Notice, and it is inevitable that some products approved before the Notice might not have been sufficiently treated. It is expected that such biodrug will be sufficiently examined to meet such General Information before being listed in JP. On the other hand, blood preparations listed in the biological products standard and covered by ''Guideline for securing safety of blood plasma protein fraction preparations against virus'', are out of the scope of this General Information. Further, in case of a relatively lower molecular biogenous substance, such as amino acid, saccharide and glycerin, and of gelatin, which is even classified as infectious or pathogenic polymer, there are cases that viral contamination can not be considered due to its manufacturing or purification process, and that potent viral inactivation/removal procedure that can not be applied to protein, can be used, and, therefore, it is considered reasonable to omit such substances from the subject for application. However, some part of this General Information may be used as reference. Further, a comprehensive assurance measure for viral safety is recommendable on a biotechnological/biological product not listed in JP using this document as a reference so long as it is similar to the biotechnological/biological product JP.

3.5. Possible viral contamination to a JP listed biotechnological/biological product (source of virus contamination)

Promoting awareness of virus contamination to a JP listed biotechnological/biological product (source of virus contamination) and citing countermeasure are important for eradicating any possible virus contamination and raising probability of safety assurance. Many biotechnological/biological products are produced from a ''substrate'' which is derived from human or animal tissue, body fluid, etc. as an origin/raw material, and in purification or pharmaceutical processing of such products column materials or additives, which are living organism origin, are occasionally used. Accordingly, enough safety measures should be taken against diffusion of the contaminant virus. Further, as mentioned in Notice Iyakushin No. 329, any protein drug derived from cell lines of human or animal origin should be carefully examined with respect to the risk of virus contamination through the cell line, the cell substrate for drug production, and through the manufacturing process applied thereafter.

''Substrate for drug production'' is defined as a starting

material which is at a stage where it is deemed to be in a position to ensure quality/safety of an active substance. The ''substrate for drug production'' is sometimes tissue, body fluid, etc. of human or animal per se and pooled material such as urine, and sometimes a material after some treatment. In many cases, it is considered rational that starting point of full-scale test, evaluation and control should be at the stage of ''substrate for drug production''. The more strict levels of test, evaluation and control achieved at the stage of ''substrate for drug production'' can more rationalize evaluation and control of the raw material or individual level of upper stream. On the contrary, strict evaluation and control of the raw material or individual level at an upper stream stage can rationalize tests, evaluation or quality control at the stage of ''substrate for drug production''.

The measures taken for ensuring viral safety on a biotechnological/biological product currently listed in JP can be assumed from the provisions of manufacturing method, specification and test methods of each preparation. However, unitary principles or information with respect to the measures to be taken for ensuring viral safety, totally reviewing the entire process up to the final product rationally and comprehensively, including source/raw material/substrate, purification process, etc. have not been clarified. The most important thing for ensuring viral safety is to take thorough measures to eliminate the risk of virus contamination at any stage of source animal, raw material and substrate. Although not the cases of a biotechnological/biological product, known examples of a virus contamination from a raw material/substrate for drug production in old times are Hepatitis A Virus (HAV) or Hepatitis C Virus (HCV) contamination in blood protein fraction preparations. It is also well known that Human Immunodeficiency Virus (HIV) infection caused by blood plasma protein fraction preparations occurred in 1980s. The aim of this General Information is to show concrete guidelines for comprehensive viral safety assurance of the JP listed biotechnological/biological products. The pathogenic infectious viruses, currently known to contaminate to raw materials, etc. of drug and have to be cautioned, are HIV, HAV, Hepatitis B Virus (HBV), HCV, Human T-Lymphotropic Virus (HTLV-I/II), Human Parvovirus B19, Cytomegalovirus (CMV), etc. Biotechnological/biological products produced from raw material/cell substrate derived from tissue or body fluid of human or animal origin always have a risk of contamination of pathogenic or other latent virus. Therefore, safety measures should be thoroughly taken. There is also the case that a material, other than the biological component such as raw material/substrate, causes virus contamination. Using enzymatic or monoclonal antibody column or using albumin etc. as a stabilizer, is the example of the case, in which caution has to be taken on risk of virus contamination from the source animal or cell. Further, there is a possibility of contamination from environment or personnel in charge of production or at handling of the product. So, caution has to be taken on these respects as well.

In case of protein drugs derived from cell line of human or animal origin, there may be cases where latent or persistent infectious viruses (e.g., herpesvirus) or endogenous retroviruses exist in the cell. Further, adventitious viruses may be introduced through the routes such as: 1) derivation of a cell line from an infected animal; 2) use of a virus to drive a cell line; 3) use of a contaminated biological reagent (e.g., animal serum components); 4) contamination during cell handling. In the manufacturing process of drug, an adventitious virus may contaminate the final product through the routes, such as 1) contamination through a reagent of living being origin, such as serum component, which is used for culturing, etc.; 2) use of a virus for introduction of a specific gene expression to code an objective protein; 3) contamination through a reagent used for purification such as monoclonal antibody affinity column; 4) contamination through an additive used for formulation production; 5) contamination at handling of cell and culture media, etc. It is reported that monitoring of cell culture parameter may be helpful for early detection of an adventitious viral contamination.

3.6. Basis for ensuring viral safety

Viral safety of a biotechnological/biological product produced from a raw material/substrate, which derived from tissue, body fluid, cell line, etc. of human or animal origin, can be achieved by supplemental and appropriate adoption of the following plural methods.

(1) Acquaintance of possible virus contamination (source of contamination).
(2) Careful examination of eligibility of the raw material and its source, i.e., human or animal, thorough analysis and screening of the sample chosen as the substrate for drug production to determine virus contamination and through examination of the type of virus and its nature, if contaminated.
(3) Evaluation to determine hazardous properties of the virus or virus-like particle to human, if exists.
(4) Choosing a product related material of living organism origin (e.g., reagent, immune anti-body column, etc.) which is free from infectious or pathogenic virus.
(5) Conduct virus free test at an appropriate stage of manufacturing including the final product, if necessary.
(6) Adoption of an effective method to remove/inactivate the virus in the manufacturing process for viral clearance. Combined processes sometimes achieve higher level of viral clearance.
(7) Develop a deliberate viral clearance scheme.
(8) Conduct test and evaluation to confirm removal/inactivation of the virus.

Manufacturer is responsible for explaining rationality of the way of approach adopted among the comprehensive strategy for viral safety on each product and its manufacturing process. At the time, the approach described in this General Information shall be applicable as far as possible.

3.7. Limit of virus test

Virus test has to be conducted to define existence of virus, but it should be noted that virus test alone can not reach a conclusion of inexistence of virus nor sufficient to secure safety of the product. Examples of a virus not being detected are as follows: 1) Due to statistical reason, there is an inherent quantitative limit, such as detection sensitivity at lower concentration depends upon the sample size. 2) Generally, every virus test has a detection limit, and any negative result of a virus test can not completely deny existence of a virus. 3) A virus test applied is not always appropriate in terms of specificity or sensitivity for detection of a virus which exists in the tissue or body fluid of human or animal origin.

Virus testing method is improved as science and technology progress, and it is important to apply scientifically the most advanced technology at the time of testing so that it can be possible to raise the assurance level of virus detection. It should be noted, however, that the limit as mentioned above can not always be completely overcome. Further, risk of virus contamination in a manufacturing process can not be completely denied, and, therefore, it is necessary to elaborate the countermeasure taken these effects into account.

Reliable assurance of viral free final product can not be

obtained only by negative test results on the raw material/substrate for drug production or on the product in general, it is also necessary to demonstrate inactivation/removal capability of the purification process.

3.8. Roles of viral clearance studies

Under the premises as mentioned in the preceding clause that there is a limit of a virus test, that there is a possibility of existence of latent virus in a raw material/substrate for drug production and that there is a risk of entry of a non-endogenous virus in a manufacturing process, one of the important measures for viral safety is how to remove or inactivate the virus, which exists in a raw material, etc. and can not be detected, or the virus, which is contingently contaminated in a manufacturing process. The purpose of viral clearance study is to experimentally evaluate the viral removal/inactivation capability of a step that mounted in a manufacturing process. So, it is necessary to conduct an experimental scale spike test using an appropriate virus that is selected by taking account the properties, such as particle size, shape, with or without envelope, type of nucleic acid (DNA type, RNA type), heat and chemical treatment tolerance, etc., with an aim to determine removal/inactivation capability of the virus that can not be detected in a raw material or contingently contaminated.

As mentioned above, the role of viral clearance study is to speculate removal/inactivation capability of a process through a model test, and it contributes to give scientific basis to assure that a biotechnological/biological product of human or animal origin has reached an acceptable level in aspect of viral safety.

At a viral clearance study, it is necessary to adopt an appropriate approach method which is definitive and rational and can assure viral safety of a final product, taking into consideration the source and the properties of the raw material/substrate as well as the manufacturing process.

4. Raw material/substrate for drug production

4.1. Issues relating to animal species and its region as a source of raw material/substrate for drug production and countermeasures to be taken thereto

For manufacturing JP listed biotechnological/biological products, which require measures for viral safety, a raw material/substrate derived mainly from human, bovine, swine or equine is used, and it is obvious that such human and animal has to be healthy nature. A wild animal should be avoided, and it is recommended to use animals derived from a colony controlled by an appropriate SPF (Specific Pathogen-Free) condition and bred under a well designed hygienic control, including appropriate control for prevention of microbial contamination and contamination monitoring system. If a meat standard for food is available, an animal meeting this standard has to be used. The type of virus to be concerned about depend on animal species, but it may be possible to narrow down the virus for investigation by means of examining the hygiene control, applicability of a meat standard for food, etc. On the other hand, even with the animals of the same species, a different approach may be necessary depending upon the region where the specimen for a raw material/substrate is taken. For example, in case of obtaining raw material/substrate from blood or other specific region, it is necessary to be aware of the risk level, virus multiplication risk, etc. which may specifically exists depending upon its region. Such approach may be different from those applied to body waste such as urine, milk, etc. as a source of raw material/substrate. Further, caution has to be taken on transmissible spongiform encephalopathy (TSE) when pituitary gland, etc. is used as a raw material. This report does not include detailed explanation on TSE, but recommendations are to use raw material derived from 1) animals originated in the countries (area) where incidence of TSE has not been reported; 2) animals not infected by TSE; or 3) species of animal which has not been reported on TSE. It is recommended to discuss the matters concerned with TSE with the regulatory authority if there is any unclear point.

Followings are the raw material/substrate used for manufacturing biotechnological/biological products in Japan.

(1) Biological products derived from human

Blood plasma, placenta, urine, etc. derived from human are used as the sources of raw material of biotechnological/biological product. As for these raw materials, there are two cases: 1) Appropriateness can be confirmed by interview or by examination of the individual who supplies each raw material, and 2) Such sufficient interview or examination of the individual can not be made due to type of raw material. In case that sufficient examination of individual level is not possible, it is necessary to perform the test to deny virus contamination at an appropriate manufacturing stage, for example, the stage to decide it as a substrate for drug production.

(2) Biological products derived from animal besides human

Heparin, gonadotropin, etc. are manufactured from blood plasma or from various organs of bovine, swine and equine.

(3) Protein drug derived from cell line of human or animal origin

In the case of protein drugs derived from cell line of human or animal origin, a cell line of human or animal is the raw material per se, and the substrate for drug production is a cell bank prepared from cloned cell line (master cell bank or working cell bank). Examination at cell bank level is considered enough for viral safety qualification, but it goes without saying that the more appropriate and rational qualification evaluation test of cell bank can be realized when more information is available on the virus of the source animal or on prehistory of driving the cell line, the base of cell bank.

4.2. Qualification evaluation test on human or animal as a source of raw material/substrate for drug production

(1) Biological products derived from human

Body fluid etc. obtained from healthy human must be used for biological products production. Further, in case that interview or examination of the individual, who supplies the raw material, can be possible and is necessary, interview under an appropriate protocol and a serologic test well evaluated in aspects of specificity, sensitivity and accuracy have to be performed, so that only the raw material, which is denied latent HBV, HCV and HIV, will be used. In addition to the above, it is necessary to test for gene of HBV, HCV and HIV by a nucleic amplification test (NAT) well evaluated in aspects of specificity, sensitivity and accuracy.

In case of the raw material (e.g., urine), which can not be tested over the general medical examination of the individual who supplies the material, or of the raw material which is irrational to conduct individual test, the pooled raw material, as the substrate for drug production, has to be conducted at least to deny existence of HBV, HCV and HIV, using a method well evaluated in aspects of specificity, sensitivity and accuracy, such as the antigen test or NAT.

(2) Biological products derived from animal besides human

The animal used for manufacturing biological product has to be under appropriate health control, and has to be confirmed of its health by various tests. Further, it is necessary that the population, to which the animal belongs, has been

under an appropriate breeding condition, and that no abnormal individual has been observed in the population. Further, it is necessary to demonstrate information or scientific basis which can deny known causes infection or disease to human, or to deny such animal inherent latent virus by serologic test or by nucleic amplification test (NAT). The infectious virus that is known to be common between human and animal, and known to cause infection in each animal are tentatively listed in Table 2. It is necessary that the table is completed under careful examination, and denial of all of them, by means of tests on individual animal, tissue, body fluid, etc. as a raw material, or on pooled raw material (as a direct substrate for drug production), is not always necessary. Table 2 can be used as reference information, in addition to the other information, such as; source of animal, health condition, health and breeding control, conformity to the meat standard for food, etc., to elaborate to which virus what kind of test has to be performed, and for which virus it is not always necessary to test for, etc. It is important to clarify and record the basis of choosing the virus and the test conducted thereof.

(3) Protein drug derived from cell line of human or animal origin

It is important to conduct thorough investigation on latent endogenous and non-endogenous virus contamination in a master cell bank (MCB), which is the cell substrate for drug production, in accordance with the Notice Iyakushin No. 329 entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin''. Further, it is necessary to conduct an appropriate adventitious virus test (e.g., *in vitro* and *in vivo* test) and a latent endogenous virus test on the cell at the limit of *in vitro* cell age (CAL) for drug production. Each WCB as a starting cell substrate for drug production should be tested for adventitious virus either by direct testing or by analysis of cells at the CAL, initiated from the WCB. When appropriate non-endogenous virus tests have been performed on the MCB and cells cultured up to or beyond the CAL have been derived from the WCB and used for testing for the presence of adventitious viruses, similar tests need not be performed on the initial WCB.

5. Points of concern with respect to manufacturing and virus testing

To ensure viral safety of a biological product derived from tissue, body fluid etc. of human or animal origin, it is necessary to exclude any possibility of virus contamination from a raw material, such as tissue and body fluid, or a substrate, paying attention to the source of virus contamination as mentioned in above 3.5, and to adopt appropriate manufacturing conditions and technologies in addition to enhancement of manufacturing environment, so that virus contamination in the course of process and handling and from operators, facilities and environment can be minimized.

In addition to the above, effective virus test and viral inactivation/removal technology, which are reflected by rapid progress of science, have to be introduced. Adoption of two or more steps with different principles is recommended for virus inactivation/removal process. Further, it is important to minimize any possible virus derivation by using a reagent, which quality is equivalent to that of a drug. Examples of virus inactivation/removal processes are ① heating (It is reported that almost viruses are inactivated by heating at 55 – 60°C for 30 minutes with exceptions of hepatitis virus, etc. and that dry heating at 60°C for 10 – 24 hours is effective in case of the products of blood or urine origin.), ② treatment with organic solvent/surfactant (S/D treatment), ③ membrane filtration (15 – 50 nm), ④ acid treatment, ⑤ irradiation (γ-irradiation, etc.), ⑥ treatment with column chromatograph (e.g. affinity chromatography, ion-exchange chromatography), ⑦ fractionation (e.g. organic solvent or ammonium sulfate fractionation), ⑧ extraction.

5.1. Virus test conducted in advance of purification process

(1) Biological products derived from human

In many cases, samples for virus test before purification process are body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. As mentioned in 4.2 (1), it is necessary to deny latent HBV, HCV and HIV by the test evaluated enough in aspects of specificity, sensitivity and accuracy. Even in a case that a non-purified bulk before purification process is produced from a substrate, it is not always necessary to conduct virus test again at the stage before purification, so long as the presence of any latent virus can be denied at the stage of substrate by an appropriate virus test, with cases where the non-purified bulk is made from the substrate by adding any reagent etc. of living organisms origin are an exception.

(2) Biological products derived from animal besides human

Similar to (1) above, samples for virus test before purification process are, in many cases, body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. In these cases, it is necessary to have a data, which can deny latent virus of probable cause of human infection or disease as mentioned in the above 4.2 (2), or to have a result of serologic test or nucleic amplification test (NAT) evaluated enough in aspects of specificity, sensitivity and accuracy. The concept, which is applied to a case that non-purified bulk before purification process is produced from substrate, is the same as those provided in the above 4.2 (1).

(3) Protein drug derived from cell line of human or animal origin

Generally, substrate in this case is cell bank, and the sample for testing before purification process is a harvested cell after cell culturing or unprocessed bulk which consists of single or pooled complex culture broth. The unprocessed bulk may be sometimes culture broth without cell. Denial of latent virus, which is determined by virus test at a MCB or WCB level, does not always deny latent virus in unprocessed bulk after culturing. Further, it is noted that the viral test at the CAL is meaningful as a validation but can not guarantee definite assurance of latent virus denial, since the test is generally performed only once. In case of using a serum or a component of blood origin in a culture medium, definite denial of latent virus at the level of unprocessed bulk can not be assured so long as the viral test has not been conducted on each lot at the CAL, since lot renewal can be a variable factor on viral contamination.

A representative sample of the unprocessed bulk, removed from the production reactor prior to further processing, represents one of the most suitable levels at which the possibility of adventitious virus contamination can be determined with a high probability of detection. Appropriate testing for viruses should be performed at the unprocessed bulk level unless virus testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic). In certain instances it may be more appropriate to test a mixture consisting of both intact and disrupted cells and their cell culture supernatants removed from the production reactor prior to further processing.

In case of unprocessed bulk, it is required to conduct virus test on at least 3 lots obtained from pilot scale or commercial

Table 2 Infectious viruses known to be common between human and animal and known to cause infection to each animal

	bovine	swine	sheep	goat	equine
Cowpox virus	◎				
Paravaccinia virus	◎	◎	◎	◎	
Murray valley encephalitis virus	◎	◎			
Louping ill virus	◎	◎	◎	◎	
Wesselsbron virus			◎		
Foot-and-mouth disease virus	◎	◎			
Japanese encephalitis virus		◎			
Vesicular stomatitis virus		◎			
Bovine papular stomatitis virus	◎				
Orf virus			◎		
Borna disease virus			◎		◎
Rabies virus	◎	◎	◎	◎	◎
Influenza virus		◎			
Hepatitis E virus		◎			
Encephalomyocarditis virus	◎	◎			
Rotavirus	◎				
Eastern equine encephalitis virus					◎
Western equine encephalitis virus					◎
Venezuelan equine encephalitis virus					◎
Morbillivirus					◎
Hendra virus					◎
Nipah virus		◎			
Transmissible gastroenteritis virus		◎			
Porcine respiratory coronavirus		◎			
Porcine epidemic diarrhea virus		◎			
Hemagglutinating encephalomyelitis virus		◎			
Porcine respiratory and reproductive syndrome virus		◎			
Hog cholera virus		◎			
Parainfluenza virus Type 3		◎			
Talfan/Teschen disease virus		◎			
Reovirus		◎			
Endogenous retrovirus		◎			
Porcine adenovirus		◎			
Porcine circovirus		◎			
Porcine parvovirus		◎			
Porcine poxvirus		◎			
Porcine cytomegalovirus		◎			
Pseudorabies virus		◎			
Russian spring-summer encephalitis virus			◎	◎	
Rift Valley fever virus			◎	◎	
Crimean-Congo hemorrhagic fever virus (Nairovirus)	◎		◎	◎	
Torovirus	◎				

scale production. It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material sources and results of viral clearance studies. Screening *in vitro* tests, using one or several cell lines, are generally employed to test unprocessed bulk. If appropriate, a NAT test or other suitable methods may be used.

Generally, harvest material in which adventitious virus has been detected should not be used to manufacture the product. If any adventitious viruses are detected at this level, the process should be carefully checked to determine the cause of the contamination, and appropriate actions taken.

5.2. Virus test as an acceptance test of an intermediate material, etc.

When a biological product is manufactured from tissue, body fluid etc. of human or animal origin, there are cases that an intermediate material, partially processed as a raw material or substrate by outside manufacturer, is purchased and used for manufacturing. In such case, if any test to meet this General Information has been conducted by such outside manufacturer, it is necessary for the manufacturer of the biological product, who purchased the intermediate material, to examine what sort of virus test has to be conducted as acceptance tests, and to keep record on the basis of rationality including the details of the test conducted.

On the other hand, if no test to meet this General Information has been conducted by such outside manufacturer of the raw material, all necessary virus free test has to be conducted to meet this General Information on the intermediate material regarding it as the direct substrate for drug production.

5.3. Virus test on a final product

Virus tests to be conducted on a final product (or on a product to reach the final product) has to be defined under comprehensive consideration of the type of raw material or substrate, the result of virus test conducted on raw material/substrate, the result of evaluation on viral removal/inactivation process, any possibility of virus contamination in the manufacturing process, etc. Comprehensive viral safety assurance can only be achieved by appropriate selection of the raw material/substrate, an appropriate virus test conducted on the raw material/substrate/intermediate material, the virus test conducted at an appropriate stage of manufacturing, an appropriate viral clearance test, etc. However, there are cases of having specific backgrounds, such as 1) use of the raw material derived from unspecified individual human, 2) possible existence of virus at window period, 3) specific detection limit of virus test, etc. and in these cases, virus contamination to the final product may occur if there is any deficiency on the manufacturing process (e.g., damage of membrane filter) or any mix-up of the raw materials, etc. To avoid such accidental virus contamination, it may be recommended to conduct nucleic amplification test (NAT) on the final product focusing on the most risky virus among those that may possibly to exist in the raw material.

6. Process evaluation on viral clearance

6.1. Rationale, objective and general items to be concerned with respect to viral clearance process evaluation

Evaluation of a viral inactivation/removal process is important for ensuring safety of a biological product derived from tissue or body fluid of human or animal origin. Conducting evaluation on viral clearance is to assure, even to some extent, elimination of the virus, which may exist in a raw material, etc. or may be derived to the process due to unexpected situation. Viral clearance studies should be made by a carefully designed appropriate method, and has to be rationally evaluated.

The objective of viral clearance studies is to assess process step(s) that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the process. This should be achieved by the deliberate addition (''spiking'') of significant amounts of a virus at different manufacturing/purification steps and demonstrating its removal or inactivation during the subsequent steps. It is not necessary to evaluate or characterize every step of a manufacturing process if adequate clearance is demonstrated by the use of fewer steps. It should be borne in mind that other steps in the process may have an indirect effect on the viral inactivation/removal achieved. Manufacturers should explain and justify the approach used in studies for evaluating viral clearance.

The reduction of virus infectivity may be achieved by removal of virus particles or by inactivation of viral infectivity. For each production step assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal. For inactivation steps, the study should be planned in such a way that samples are taken at different times and an inactivation curve constructed.

6.2. Selection of virus

To obtain broad range of information of viral inactivation/removal, it is desirable that a model virus used for viral clearance studies should be chosen from the viruses with broad range of characteristics in aspects of DNA/RNA, with or without envelope, particle size, significant resistance to physical/chemical treatment, etc. and it is necessary to combine about 3 model viruses to cover these characteristics.

At choice of a model virus, there are also the ways to choose a virus closely related to or having the same characteristics of the virus known to exist in the raw material. In such case, it is in principle recommendable to choose a virus which demonstrates a higher resistance to inactivation/removal treatment if two or more candidate viruses are available for choice. Further, a virus which can grow at a high titer is desirable for choice, although this may not always be possible. In addition to the above, choosing a virus, which will provide effective and reliable assay result at each step, is necessary, since sample condition to be tested at each step of a production process may influence the detection sensitivity. Consideration should also be given to health hazard which may pose to the personnel performing the clearance studies.

For the other items taken for consideration at choice of virus, the Notice, Iyakushin No. 329 can be used as a reference. Examples of the virus which have been used for viral clearance studies are shown in Table 3 which was derived from Iyakushin No. 329. However, the Notice, Iyakushin No. 329, is on viral safety of a product derived cell line of human or animal origin, and a more appropriate model virus has to be chosen taking into account the origin/raw material of biological products.

6.3. Design of viral clearance studies

The purpose of viral clearance studies is to quantitatively evaluate removal or inactivation capability of a process, in which a virus is intentionally spiked to a specific step of a manufacturing process.

Following are the precautions to be taken at planning viral clearance studies.

(1) Care should be taken in preparing the high-titer virus to avoid aggregation which may enhance physical removal and decrease inactivation thus distorting the correlation with actual production.

(2) Virus detection method gives great influence to viral clearance factor. Accordingly, it is advisable to gain detection sensitivity of the methods available in advance, and use a method with a detection sensitivity as high as possible. Quantitative infectivity assays should have adequate sensitivity and reproducibility in each manufacturing process, and should be performed with sufficient replicates to ensure adequate statistical validity of the result. Quantitative assays not associated with infectivity may be used if justified. Appropriate virus controls should be included in all infectivity assays to ensure the sensitivity of the method. Also, the statistics of sampling virus when at low concentrations (for example, number of virus is 1-1000/L) should be considered.

(3) Viral clearance studies are performed in a miniature size system that simulates the actual production process of the biotechnological/biological product used by the manufacturer. It is inappropriate to introduce any virus into a production facility because of GMP constraints. Therefore, viral clearance studies should be conducted in a separate laboratory equipped for virological work and performed by staff with virological expertise in conjunction with production personnel involved in designing and preparing a scaled-down version of the purification process. The viral clearance studies should be performed under the basic concept of GLP.

(4) Each factor on a viral clearance study of a process, which is performed in miniature size, should reflect that of actual manufacturing as far as possible, and its rationality should be clarified. In case of chromatograph process, length of column bed, linear velocity, ratio of bed volume per velocity (in other words, contact time), buffer, type of column packing, pH, temperature, protein concentration, salt concentration and concentration of the objective

Table 3 Example of viruses which have been used for viral clearance studies

Virus	Family	Genus	Natural host	Genome	Env	Size (nm)	Shape	Resistance
Vesicular Stomatitis Virus	Rhabdo	Vesiculovirus	Equine Bovine	RNA	yes	70×150	Bullet	Low
Parainfluenza Virus	Paramyxo	Type 1,3 Respirovirus Type 2,4 Rubulavirus	Various	RNA	yes	100 – 200+	Pleo-Spher	Low
MuLV	Retro	Type C oncovirus	Mouse	RNA	yes	80 – 110	Spherical	Low
Sindbis Virus	Toga	Alphavirus	Human	RNA	yes	60 – 70	Spherical	Low
BVDV	Flavi	Pestivirus	Bovine	RNA	yes	50 – 70	Pleo-Spher	Low
Pseudorabies Virus	Herpes	Varicellovirus	Swine	DNA	yes	120 – 200	Spherical	Med
Poliovirus Sabin Type 1	Picorna	Enterovirus	Human	RNA	no	25 – 30	Icosahedral	Med
Encephalomyocarditis Virus	Picorna	Cardiovirus	Mouse	RNA	no	25 – 30	Icosahedral	Med
Reovirus Type 3	Reo	Orthoreovirus	Various kind	RNA	no	60 – 80	Spherical	Med
SV 40	Papova	Polyomavirus	Monkey	DNA	no	40 – 50	Icosahedral	Very high
Parvovirus: canine, porcine	Parvo	Parvovirus	Canine Porcine	DNA	no	18 – 24	Icosahedral	Very high

product are all correspondent to those of the actual production. Further, similarity of elution profile should be achieved. For the other process, similar concept should be applied. If there is any factor which can not reflect the actual production, its effect to the result should be examined.

(5) It is desirable that two or more inactivation/removal processes of different principles are selected and examined.

(6) As for the process which is expected to inactivate/remove virus, each step should be evaluated in aspect of clearance capability, and carefully determined if it is the stage of inactivation, removal or their combination for designing the test. Generally, in viral clearance test, a virus is spiked in each step which is the object of the test, and after passing through the process in question, the reduction level of infectivity is evaluated. But, in some case, it is accepted that a high potential virus is spiked at a step of the process, and virus concentration of each succeeding step is carefully monitored. When removal of virus is made by separation or fractionation, it is desirable to investigate how the virus is separated or fractionated (mass balance).

(7) For assessment of viral inactivation, unprocessed crude material or intermediate material should be spiked with infectious virus and the reduction factor calculated. It should be recognized that virus inactivation is not a simple, first order reaction and is usually more complex, with a fast ''phase 1'' and a slow ''phase 2''. The study should, therefore, be planned in such a way that samples are taken at different times and an inactivation curve constructed. It is recommended that studies for inactivation include at least one time point less than the minimum exposure time and greater than zero, in addition to the minimum exposure time. The reproducible clearance should be demonstrated in at least two independent studies. When there is a possibility that the virus is a human pathogen, it is very important that the effective inactivation process is designed and additional data are obtained. The initial virus load should be determined from the virus which can be detected in the spiked starting material. If this is not possible, the initial virus load may be calculated from the titer of the spiking virus preparation. Where inactivation is too rapid to plot an inactivation curve using process conditions, appropriate controls should be performed to demonstrate that infectivity is indeed lost by inactivation.

(8) If antibody against virus exists in an unprocessed material, caution should be taken at clearance studies, since it may affect the behavior of virus at viral removal or inactivation process.

(9) Virus spiked in unprocessed material should be sufficient enough to evaluate viral removal or inactivation capability of the process. However, the virus ''spike'' to be added to the unprocessed material should be as small as possible in comparison with the sample volume of the unprocessed material so as not to cause characteristic change of the material by addition of the virus nor to cause behavioral change of the protein in the material.

(10) It is desirable that the virus in the sample is subject for quantitative determination without applying ultracentrifuge, dialysis, storage, etc. as far as possible. However, there may be a case that any handling before quantitative test, such as remove procedure of inhibitor or toxic substance, storage for a period to realize test at a time, etc., is inevitable. If any manipulation, such as dilution, concentration, filtration, dialysis, storage, etc., is applied for preparation of the sample for testing, a parallel control test, which passes through a similar manipulation, should be conducted to assess infectivity variance at the manipulation.

(11) Buffers and product (desired protein or other component contained therein) should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus might be necessary. If the product itself has anti-viral activity, the clearance study may need to be performed without the product in a ''mock'' run, although omitting the product or substituting a similar protein that does not have anti-viral activity could affect the behaviour of the virus in some production steps.

(12) Many purification schemes use the same or similar buffers or columns, repetitively. The effects of this approach should be taken into account when analyzing the data. The effectiveness of virus elimination by a particular process may vary with the stage in manufacture at which it is used.

(13) Overall reduction factors may be underestimated where production conditions or buffers are too cytotoxic or virucidal and should be discussed on a case-by-case basis. Overall reduction factors may also be overestimated due to inherent limitations or inadequate design of viral clearance studies.

(14) It has to be noted that clearance capability of viral removal/inactivation process may vary depending upon the type of virus. The viral removal/inactivation process, which displays viral clearance by specific principle or mechanism, may be quite effective to the virus, which meets such mechanism of action, but not effective to the other type of viruses. For example, S/D (Solvent/Detergent) treatment is generally effective to the virus with lipid membrane, but not effective to the non-enveloped virus. Further, some virus is resistant to the general heating process (55 – 60°C, 30 minutes). When clearance is expected for such virus, introduction of a further severe condition or process, which has different principle or mechanism, is necessary. Virus removal by membrane filtration, which is different from S/D or heat treatment in aspect of principle, is effective to a broad range of virus that can not pass through the membrane. Affinity chromatography process, which specifically absorbs the objective protein, can thoroughly wash out the materials other than the objective protein including virus etc. and is generally effective for viral removal. Separation/fractionation of a virus from an objective protein is sometimes very difficult, but there are not so rare that ion exchange chromatography, ethanol fractionation, etc. is effective for clearance of a virus which can not be sufficiently inactivated or removed by the other process.

(15) Effective clearance may be achieved by any of the following: multiple inactivation steps, multiple complementary separation steps, or combinations of inactivation and separation steps. Separation methods may be dependent on the extremely specific physico-chemical properties of a virus which influence its interaction with gel matrices and precipitation properties. However, despite these potential variables, effective removal can be obtained by a combination of complementary separation steps or combinations of inactivation and separation steps. Well designed separation steps, such as chromatographic procedures, filtration steps and extractions, can be also effective virus removal steps provided that they are performed under appropriately controlled conditions.

(16) An effective virus removal step should give reproducible reduction of virus load shown by at least two independent studies.

(17) Over time and after repeated use, the ability of chromatography columns and other devices used in the purification scheme to clear virus may vary. Some estimate of the stability of the viral clearance after several uses may provide support for repeated use of such columns.

(18) The Notice, Iyakushin No. 329, would be used as a reference when viral clearance studies on biological products are designed.

6.4. Interpretation of viral clearance studies

6.4.1. Evaluation on viral clearance factor

Viral clearance factor is a logarithm of reduction ratio of viral amount (infectious titer) between each step applied for viral clearance of a manufacturing process. Total viral clearance factor throughout the process is sum of the viral clearance factor of each step appropriately evaluated.

Whether each and total viral clearance factor obtained are acceptable or should not be evaluated in aspects of every virus that can be realistically anticipated to derive into the raw material or the manufacturing process, and its rationality should be shown.

In case that existence of any viral particle is recognized in a substrate for drug production, e.g., a substrate of rodent origin for biodrug production, it is important not only to demonstrate removal or inactivation of such virus, but also to demonstrate that the purification process has enough capability over the required level to assure safety of the final product at an appropriate level. The virus amount removed or inactivated in a manufacturing process should be compared with the virus amount assumed to exist in the substrate etc. used for manufacturing drug, and for this purpose, it is necessary to obtain the virus amount in the raw materials/substrate, etc. Such figure can be obtained by measuring infectious titer or by the other method such as transmission electron microscope (TEM). For evaluation of overall process, a virus amount, far larger than that assumed to exist in the amount of the raw materials/substrate which is equivalent to single administration of the final product, has to be removed. It is quite rare that existence of virus can be assumed in a substrate for drug production, with the exception of the substrate of rodent origin, and such suspicious raw material/substrate should not be used for manufacturing drug with a special exceptional case that the drug in question is not available from the other process and is clinically indispensable, and that the information including infectious properties of the virus particle assumed to exist has been clarified. Any virus contaminations in the substrates used for production of biotechnological/biological products are usually denied by some tests or examinations. In such case, a specific virus that can possibly contaminate may be used as a model. However, in general, it would be necessary to perform a viral clearance test by choosing a combination of appropriate model viruses that can show the capability for clearance of a wide variety of viruses in the process, as indicated in 6.2. In this case, a common numerical goal cannot be established on the viral clearance. Therefore, the validity of viral clearance factor of the process should be taken into account, considering various information on factual possibility of virus contaminations of the substrates and others, detection sensitivity of the virus free test, and other cases in publications.

6.4.2. Calculation of viral clearance index

Viral clearance factor, ''R'', for viral removal/inactivation process can be calculated by the following formula.

$$R = \log[(V_1 \times T_1)/(V_2 \times T_2)]$$

In which

R: Logarithm of reduction ratio
V_1: Sample volume of the unprocessed material
T_1: Virus amount (titer) of the unprocessed material
V_2: Sample volume of the processed material
T_2: Virus amount (titer) of the processed material

At the calculation of viral clearance factor, it is recommendable to use the virus titer detected in the sample preparation of the unprocessed material after addition of virus, not the viral titer added to the sample preparation wherever possible. If this is not possible, loaded virus amount is calculated from virus titer of the solution used for spike.

6.4.3. Interpretation of results and items to be concerned at evaluation

At the interpretation and the evaluation of the data on ef-

fectiveness of viral inactivation/removal process, there are various factors to be comprehensively taken into account, such as ① appropriateness of the virus used for the test, ② design of the viral clearance studies, ③ virus reduction ratio shown in logarithm, ④ time dependence of inactivation, ⑤ factors/items which give influence to the inactivation/removal process, ⑥ sensitivity limit of virus assay method, ⑦ possible effect of the inactivation/removal process which is specific to certain class of viruses.

Additional items to be concerned at appropriate interpretation and evaluation of the viral clearance data are as follows:

(1) Behavior of virus used to the test

At interpretation of the vial clearance results, it is necessary to recognize that clearance mechanism may differ depending upon the virus used for the test. Virus used for a test is generally produced in tissue culture, but behavior of the virus prepared in the tissue culture may be different from that of the native virus. Examples are possible differences of purity and degree of aggregation between the native and the cultured viruses. Further, change of surface properties of a virus, e.g., addition of a sucrose chain which is ascribed to specific nature of a separation process, may give effect to the separation. These matters should be also considered at interpretation of the results.

(2) Design of test

Viral clearance test should have been designed taking into account variation factors of the manufacturing process and scaling down, but there still remain some variance from actual production scale. It is necessary to consider such variance at the interpretation of the data and limitation of the test.

(3) Acceptability of viral reduction data

Total viral clearance factor is expressed as a sum of logarithm of reduction ratio obtained at each step. The summation of the reduction factor of multiple steps, particularly of steps with little reduction (e.g., below 1 $\log_{10}$), may overestimate viral removal/inactivation capability of the overall process. Therefore, virus titer of the order of 1 $\log_{10}$ or less has to be ignored unless justified. Further, viral clearance factor achieved by repeated use of the same or similar method should be ignored for calculation unless justified.

(4) Time dependence of inactivation

Inactivation of virus infectivity frequently shows biphasic curve, which consists of a rapid initial phase and subsequent slow phase. It is possible that a virus not inactivated in a step may be more resistant to the subsequent step. For example, if an inactivated virus forms coagulation, it may be resistant to any chemical treatment and heating.

(5) Evaluation of viral reduction ratio shown in logarithm

Viral clearance factor shown in logarithm of reduction ratio of virus titer can demonstrate drastic reduction of residual infectious virus, but there is a limit that infectious titer can never be reduced to zero. For example, reduction in infectivity of a preparation containing 8 $\log_{10}$ infectious unit per mL by a factor of 8 $\log_{10}$ leaves zero $\log_{10}$ per mL or one infectious unit per mL, taking into account the detection limit of assay.

(6) Variable factor of manufacturing process

Minor variance of a variation factor of a manufacturing process, e.g., contact time of a spiked sample to a buffer or a column, will sometimes give influence to viral removal or inactivation effect. In such case, it may be necessary to investigate to what extent such variance of the factor has given influence to the process concerned in aspect of viral inactivation.

(7) Existence of anti-viral antiserum

Anti-viral antiserum that exists in the sample preparation used for a test may affect sensitivity of distribution or inactivation of a virus, which may result in not only defusing the virus titer but complicating interpretation of the test result. So, existence of anti-viral antiserum is one of the important variable factors.

(8) Introduction of a new process for removal/inactivation

Viral clearance is an important factor for securing safety of drug. In case that an achievement level of infective clearance of a process is considered insufficient, a process which is characterized by inactivation/removal mechanism to meet the purpose or an inactivation/removal process which can mutually complement to the existence process has to be introduced.

(9) Limit of viral clearance studies

Viral clearance studies are useful for contributing to the assurance that an acceptable level of safety in the final product is achieved but do not by themselves establish safety. However, a number of factors in the design and execution of viral clearance studies may lead to an incorrect estimate of the ability of the process to remove virus infectivity, as described above.

7. Statistics

The viral clearance studies should include the use of statistical analysis of the data to evaluate the results. The study results should be statistically valid to support the conclusions reached.

7.1. Statistical considerations for assessing virus assays

Virus titrations suffer the problems of variation common to all biological assay systems. Assessment of the accuracy of the virus titrations and reduction factors derived from them and the validity of the assays should be performed to define the reliability of a study. The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

Assay

1. Assay methods may be either semiquantitative or quantitative. Both semiquantitative and quantitative assays are amenable to statistical evaluation.

2. Variation can arise within an assay as a result of dilution errors, statistical effects and differences within the assay system which are either unknown or difficult to control. These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay variation).

3. The 95% confidence limits for results of within-assay variation normally should be on the order of ± 0.5 $\log_{10}$ of the mean. Within-assay variation can be assessed by standard textbook methods. Between-assay variation can be monitored by the inclusion of a reference preparation, the estimate of whose potency should be within approximately 0.5 $\log_{10}$ of the mean estimate established in the laboratory for the assay to be acceptable. Assays with lower precision may be acceptable with appropriate justification.

7.2. Reproducibility and confidence limit of viral clearance studies

An effective virus inactivation/removal step should give reproducible reduction of virus load shown by at least two independent studies. The 95% confidence limits for the reduction factor observed should be calculated wherever possible in studies of viral clearance. If the 95% confidence limits for the viral assays of the starting material are $\pm s$, and for the viral assays of the material after the step are $\pm a$, the 95% confidence limits for the reduction factor are

$\pm\sqrt{s^2 + a^2}$.

8. Re-evaluation of viral clearance

Whenever significant changes in the production or purification process are made, the effect of that change, both direct and indirect, on viral clearance should be re-evaluated as needed. Changes in process steps may also change the extent of viral clearance.

9. Measurement for viral clearance studies

9.1. Measurement of virus infective titer

Assay methods may be either semiquantitative or quantitative. Semiquantitative methods include infectivity assays in animals or in cultured cell infections dose (CCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titers are then measured by the proportion of animals or culture infected. In quantitative methods, the infectivity measured varies continuously with the virus input. Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit. Both quantal and quantitative assays are amenable to statistical evaluation.

9.2. Testing by nucleic-acid-amplification test (NAT)

NAT can detect individual or pooled raw material/cell substrate or virus genome at a high sensitivity even in a stage that serum test on each virus is negative. Further, it can detect HBV or HCV gene, which can not be measured in culture system. Window period can be drastically shortened at the test on HBV, HCV and HIV, and the method is expected to contribute as an effective measure for ensuring viral safety. However, depending upon a choice of primer, there may be a case that not all the subtype of objective virus can be detected by this method, and, therefore, it is recommendable to evaluate, in advance, if subtype of a broad range can be detected.

NAT will be an effective evaluation method for virus removal capability for viral clearance. However, in case of viral inactivation process, viral inactivation obtained by this method may be underrated, since there is a case that inactivated virus still shows positive on nucleic acid. Further, at introduction of NAT, cautions should be taken on rationality of detection sensitivity, choice of a standard which is used as run-control, quality assurance and maintenance of a reagent used for primer, interpretation of positive and negative results, etc.

10. Reporting and preservation

All the items relating to virus test and viral clearance studies should be reported and preserved.

11. Others

The Notice, Iyakushin No. 329, should be used as a reference at virus test and viral clearance studies.

Conclusion

As mentioned at the Introduction, assurance of quality/safety etc. of JP listed drugs should be achieved by state-of-the-art methods and concepts reflecting the progress of science and accumulation of experiences.

The basis for ensuring viral safety of JP listed biotechnological/biological products is detailed in this General Information. What is discussed here is that an almost equal level of measures are required for both development of new drugs and for existing products as well, which means that similar level of concerns should be paid on both existing and new products in aspect of viral safety. This document is intended to introduce a basic concept that quality and safety assurance of JP listed product should be based upon the most advanced methods and concepts. This document has been written to cover all conceivable cases, which can be applied to all biotechnological/biological products. Therefore, there may be cases that it is not so rational to pursue virus tests and viral clearance studies in accordance with this document on each product, which has been used for a long time without any safety issue. So, it will be necessary to elaborate the most rational ways under a case-by-case principle taking into due consideration source, origin, type, manufacturing process, characteristics, usages at clinical stage, accumulation of the past usage record, etc. relating to such biotechnological/biological products.

Capillary Electrophoresis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution, under the influence of a direct-current electric field.

The migration velocity of an analyte under an electric field of intensity E, is determined by the electrophoretic mobility of the analyte and the electroosmotic mobility of the buffer inside the capillary. The electrophoretic mobility of a solute (μ_{ep}) depends on the characteristics of the solute (electric charge, molecular size and shape) and those of the buffer in which the migration takes place (type and ionic strength of the electrolyte, pH, viscosity and additives). The electrophoretic velocity (v_{ep}) of a solute, assuming a spherical shape, is given by the equation:

$$v_{ep} = \mu_{ep} E = \left(\frac{q}{6\pi\eta r}\right)\left(\frac{V}{L}\right)$$

q: Effective charge of the solute,
η: Viscosity of the electrolyte solution,
r: Stoke's radius of the solute,
V: Applied voltage,
L: Total length of the capillary.

When an electric field is applied through the capillary filled with buffer, a flow of solvent is generated inside the capillary, called electroosmotic flow. The velocity of the electroosmotic flow depends on the electroosmotic mobility (μ_{eo}) which in turn depends on the charge density on the capillary internal wall and the buffer characteristics. The electroosmotic velocity (v_{eo}) is given by the equation:

$$v_{eo} = \mu_{eo} E = \left(\frac{\varepsilon\zeta}{\eta}\right)\left(\frac{V}{L}\right)$$

ε: Dielectric constant of the buffer,
ζ: Zeta potential of the capillary surface.

The velocity of the solute (v) is given by:

$$v = v_{ep} + v_{eo}$$

The electrophoretic mobility of the analyte and the electroosmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute. In normal capillary electrophoresis, anions will migrate in the opposite direction to the electroosmotic flow and their velocities will be smaller than the electroosmotic velocity. Cations will migrate in the same direction as the electroosmotic flow and their velocities will be greater than the electroosmotic velocity. Under conditions in which there is a fast electroosmotic velocity with respect to the electrophoretic velocity of the solutes, both cations and anions can be separated in the

same run.

The time (t) taken by the solute to migrate the distance (l) from the injection end of the capillary to the detection point (capillary effective length) is given by the expression:

$$t = \frac{l}{v_{ep} + v_{eo}} = \frac{l \times L}{(\mu_{ep} + \mu_{eo})V}$$

In general, uncoated fused-silica capillaries above pH 3 have negative charge due to ionized silanol groups in the inner wall. Consequently, the electroosmotic flow is from anode to cathode. The electroosmotic flow must remain constant from run to run if good reproducibility is to be obtained in the migration velocity of the solutes. For some applications, it may be necessary to reduce or suppress the electroosmotic flow by modifying the inner wall of the capillary or by changing the concentration, composition and/or pH of the buffer solution.

After the introduction of the sample into the capillary, each analyte ion of the sample migrates within the background electrolyte as an independent zone, according to its electrophoretic mobility. Zone dispersion, that is the spreading of each solute band, results from different phenomena. Under ideal conditions the sole contribution to the solute-zone broadening is molecular diffusion of the solute along the capillary (longitudinal diffusion). In this ideal case the efficiency of the zone, expressed as the number of theoretical plates (N), is given by:

$$N = \frac{(\mu_{ep} + \mu_{eo}) \times V \times l}{2 \times D \times L}$$

D: Molecular diffusion coefficient of the solute in the buffer.

In practice, other phenomena such as heat dissipation, sample adsorption onto the capillary wall, mismatched conductivity between sample and buffer, length of the injection plug, detector cell size and unlevelled buffer reservoirs can also significantly contribute to band dispersion.

Separation between two bands (expressed as the resolution, R_S) can be obtained by modifying the electrophoretic mobility of the analytes, the electroosmotic mobility induced in the capillary and by increasing the efficiency for the band of each analyte, according to the equation:

$$R_S = \frac{\sqrt{N}(\mu_{epb} - \mu_{epa})}{4(\bar{\mu}_{ep} + \mu_{eo})}$$

μ_{epa} and μ_{epb}: Electrophoretic mobilities of the two analytes separated,

$\bar{\mu}_{ep}$: Mean electrophoretic mobility of the two analytes $\bar{\mu}_{ep} = \frac{1}{2}(\mu_{epb} + \mu_{epa})$.

Apparatus

An apparatus for capillary electrophoresis is composed of:

(1) a high-voltage, controllable direct-current power supply,

(2) two buffer reservoirs, held at the same level, containing the prescribed anodic and cathodic solutions,

(3) two electrode assemblies (the cathode and the anode), immersed in the buffer reservoirs and connected to the power supply,

(4) a separation capillary (usually made of fused-silica) which, when used with some specific types of detectors, has an optical viewing window aligned with the detector. The ends of the capillary are placed in the buffer reservoirs. The capillary is filled with the solution prescribed in the monograph,

(5) a suitable injection system,

(6) a detector able to monitor the amount of substances of interest passing through a segment of the separation capillary at a given time. It is usually based on absorption spectrophotometry (UV and visible) or fluorometry, but conductimetric, amperometric or mass spectrometric detection can be useful for specific applications. Indirect detection is an alternative method used to detect non-UV-absorbing and non-fluorescent compounds,

(7) a thermostatic system able to maintain a constant temperature inside the capillary is recommended to obtain a good separation reproducibility,

(8) a recorder and a suitable integrator or a computer.

The definition of the injection process and its automation are critical for precise quantitative analysis. Modes of injection include gravity, pressure or vacuum injection and electrokinetic injection. The amount of each sample component introduced electrokinetically depends on its electrophoretic mobility, leading to possible discrimination using this injection mode.

Use the capillary, the buffer solutions, the preconditioning method, the sample solution and the migration conditions prescribed in the monograph of the considered substance. The employed electrolytic solution is filtered to remove particles and degassed to avoid bubble formation that could interfere with the detection system or interrupt the electrical contact in the capillary during the separation run. A rigorous rinsing procedure should be developed for each analytical method to achieve reproducible migration times of the solutes.

1. Capillary Zone Electrophoresis

In capillary zone electrophoresis, analytes are separated in a capillary containing only buffer without any anticonvective medium. With this technique, separation takes place because the different components of the sample migrate as discrete bands with different velocities. The velocity of each band depends on the electrophoretic mobility of the solute and the electroosmotic flow in the capillary (see General Principles). Coated capillaries can be used to increase the separation capacity of those substances adsorbing on fused-silica surfaces.

Using this mode of capillary electrophoresis, the analysis of both small ($M_r < 2000$) and large molecules ($2000 < M_r < 100{,}000$) can be accomplished. Due to the high efficiency achieved in capillary zone electrophoresis, separation of molecules having only minute differences in their charge-to-mass ratio can be effected. This separation mode also allows the separation of chiral compounds by addition of chiral selectors to the separation buffer.

Optimization

Optimization of the separation is a complex process where several separation parameters can play a major role. The main factors to be considered in the development of separations are instrumental and electrolytic solution parameters.

Instrumental parameters

(1) *Voltage*: A Joule heating plot is useful in optimizing the applied voltage and column temperature. Separation time is inversely proportional to applied voltage. However, an increase in the voltage used can cause excessive heat production, giving rise to temperature and, as a result thereof, viscosity gradients in the buffer inside the capillary. This effect causes band broadening and decreases resolution.

(2) *Polarity*: Electrode polarity can be normal (anode at the inlet and cathode at the outlet) and the electroosmotic flow will move toward the cathode. If the electrode polarity is reversed, the electroosmotic flow is away from the outlet and only charged analytes with electroosmotic mobilities

greater than the electroosmotic flow will pass to the outlet.
(3) *Temperature*: The main effect of temperature is observed on buffer viscosity and electrical conductivity, and therefore on migration velocity. In some cases, an increase in capillary temperature can cause a conformational change in proteins, modifying their migration time and the efficiency of the separation.
(4) *Capillary*: The dimensions of the capillary (length and internal diameter) contribute to analysis time, efficiency of separations and load capacity. Increasing total length can decrease the electric fields (working at constant voltage), and increasing both effective length and total length increase migration time. For a given buffer and electric field, heat dissipation, and hence sample band-broadening, depend on the internal diameter of the capillary. The latter also affects the detection limit, depending on the sample volume injected and the detection system employed.

Since the adsorption of the sample components on the capillary wall limits efficiency, methods to avoid these interactions should be considered in the development of a separation method. In the specific case of proteins, several strategies have been devised to avoid adsorption on the capillary wall. Some of these strategies (use of extreme pH and adsorption of positively charged buffer additives) only require modification of the buffer composition to prevent protein adsorption. In other strategies, the internal wall of the capillary is coated with a polymer, covalently bonded to the silica, that prevents interaction between the proteins and the negatively charged silica surface. For this purpose, ready-to-use capillaries with coatings consisting of neutral-hydrophilic, cationic and anionic polymers are available.

Electrolytic solution parameters

(1) *Buffer type and concentration*: Suitable buffers for capillary electrophoresis have an appropriate buffer capacity in the pH range of choice and low mobility to minimize current generation.

Matching buffer-ion mobility to solute mobility, whenever possible, is important for minimizing band distortion. The type of sample solvent used is also important to achieve on-column sample focusing, which increases separation efficiency and improves detection.

An increase in buffer concentration (for a given pH) decreases electroosmotic flow and solute velocity.
(2) *Buffer pH*: The pH of the buffer can affect separation by modifying the charge of the analyte or additives, and by changing the electroosmotic flow. In protein and peptide separation, changing the pH of the buffer from above to below the isoelectric point (pI) changes the net charge of the solute from negative to positive. An increase in the buffer pH generally increases the electroosmotic flow.
(3) *Organic solvents*: Organic modifiers (methanol, acetonitrile, etc.) may be added to the aqueous buffer to increase the solubility of the solute or other additives and/or to affect the degree of ionization of the sample components. The addition of these organic modifiers to the buffer generally causes a decrease in the electroosmotic flow.
(4) *Additives for chiral separations*: For the separation of optical isomers, a chiral selector is added to the separation buffer. The most commonly used chiral selectors are cyclodextrins, but crown ethers, polysaccharides and proteins may also be used. Since chiral recognition is governed by the different interactions between the chiral selector and each of the enantiomers, the resolution achieved for the chiral compounds depends largely on the type of chiral selector used. In this regard, for the development of a given separation it may be useful to test cyclodextrins having a different cavity size (α-, β-, or γ-cyclodextrin) or modified cyclodextrins with neutral (methyl, ethyl, hydroxyalkyl, etc.) or ionizable (aminomethyl, carboxymethyl, sulfobutyl ether, etc.) groups. When using modified cyclodextrins, batch-to-batch variations in the degree of substitution of the cyclodextrins must be taken into account since it will influence the selectivity. Other factors controlling the resolution in chiral separations are concentration of chiral selector, composition and pH of the buffer and temperature. The use of organic additives, such as methanol or urea can also modify the resolution achieved.

2. Capillary Gel Electrophoresis

In capillary gel electrophoresis, separation takes place inside a capillary filled with a gel that acts as a molecular sieve. Molecules with similar charge-to-mass ratios are separated according to molecular size since smaller molecules move more freely through the network of the gel and therefore migrate faster than larger molecules. Different biological macromolecules (for example, proteins and DNA fragments), which often have similar charge-to-mass ratios, can thus be separated according to their molecular mass by capillary gel electrophoresis.

Characteristics of Gels

Two types of gels are used in capillary electrophoresis: permanently coated gels and dynamically coated gels. Permanently coated gels, such as cross-linked polyacrylamide, are prepared inside the capillary by polymerization of the monomers. They are usually bonded to the fused-silica wall and cannot be removed without destroying the capillary. If the gels are used for protein analysis under reducing conditions, the separation buffer usually contains sodium dodecyl sulfate and the samples are denatured by heating a mixture of sodium dodecyl sulfate and 2-mercaptoethanol or dithiothreitol before injection. When non-reducing conditions are used (for example, analysis of an intact antibody), 2-mercaptoethanol and dithiothreitol are not used. Separation in cross-linked gels can be optimized by modifying the separation buffer (as indicated in the capillary zone electrophoresis section) and controlling the gel porosity during the gel preparation. For cross-linked polyacrylamide gels, the porosity can be modified by changing the concentration of acrylamide and/or the proportion of cross-linker. As a rule, a decrease in the porosity of the gel leads to a decrease in the mobility of the solutes. Due to the rigidity of these gels, only electrokinetic injection can be used.

Dynamically coated gels are hydrophilic polymers, such as linear polyacrylamide, cellulose derivatives, dextran, etc., which can be dissolved in aqueous separation buffers giving rise to a separation medium that also acts as a molecular sieve. These separation media are easier to prepare than cross-linked polymers. They can be prepared in a vial and filled by pressure in a wall-coated capillary (with no electroosmotic flow). Replacing the gel before every injection generally improves the separation reproducibility. The porosity of the gels can be increased by using polymers of higher molecular mass (at a given polymer concentration) or by decreasing the polymer concentration (for a given polymer molecular mass). A reduction in the gel porosity leads to a decrease in the mobility of the solute for the same buffer. Since the dissolution of these polymers in the buffer gives low viscosity solutions, both hydrodynamic and electrokinetic injection techniques can be used.

3. Capillary Isoelectric Focusing

In isoelectric focusing, the molecules migrate under the influence of the electric field, so long as they are charged, in a pH gradient generated by ampholytes having pI values in a wide range (poly-aminocarboxylic acids), dissolved in the

separation buffer.

The three basic steps of isoelectric focusing are loading, focusing and mobilization.

(1) Loading step: Two methods may be employed:

(i) loading in one step: the sample is mixed with ampholytes and introduced into the capillary either by pressure or vacuum;

(ii) sequential loading: a leading buffer, then the ampholytes, then the sample mixed with ampholytes, again ampholytes alone and finally the terminating buffer are introduced into the capillary. The volume of the sample must be small enough not to modify the pH gradient.

(2) Focusing step: When the voltage is applied, ampholytes migrate toward the cathode or the anode, according to their net charge, thus creating a pH gradient from anode (lower pH) to cathode (higher pH). During this step the components to be separated migrate until they reach a pH corresponding to their isoelectric point (pI) and the current drops to very low values.

(3) Mobilization step: If mobilization is required for detection, use one of the following methods. Three methods are available:

(i) in the first method, mobilization is accomplished during the focusing step under the effect of the electroosmotic flow; the electroosmotic flow must be small enough to allow the focusing of the components;

(ii) in the second method, mobilization is accomplished by applying positive pressure after the focusing step;

(iii) in the third method, mobilization is achieved after the focusing step by adding salts to the cathode reservoir or the anode reservoir (depending on the direction chosen for mobilization) in order to alter the pH in the capillary when the voltage is applied. As the pH is changed, the proteins and ampholytes are mobilized in the direction of the reservoir which contains the added salts and pass the detector.

The separation achieved, expressed as ΔpI, depends on the pH gradient (dpH/dx), the number of ampholytes having different pI values, the molecular diffusion coefficient (D), the intensity of the electric field (E) and the variation of the electrophoretic mobility of the analyte with the pH ($-d\mu/dpH$):

$$\Delta pI = 3\sqrt{\frac{D(dpH/dx)}{E(-d\mu/dpH)}}$$

Optimization

The main parameters to be considered in the development of separations are:

(1) Voltage: Capillary isoelectric focusing utilises very high electric fields, 300 V/cm to 1000 V/cm in the focusing step.

(2) Capillary: The electroosmotic flow must be reduced or suppressed depending on the mobilization strategy (see above). Coated capillaries tend to reduce the electroosmotic flow.

(3) Solutions: The anode buffer reservoir is filled with a solution with a pH lower than the pI of the most acidic ampholyte and the cathode reservoir is filled with a solution with a pH higher than the pI of the most basic ampholyte. Phosphoric acid for the anode and sodium hydroxide for the cathode are frequently used.

Addition of a polymer, such as methylcellulose, in the ampholyte solution tends to suppress convective forces (if any) and electroosmotic flow by increasing the viscosity. Commercial ampholytes are available covering many pH ranges and may be mixed if necessary to obtain an expanded pH range. Broad pH ranges are used to estimate the isoelectric point whereas narrower ranges are employed to improve accuracy. Calibration can be done by correlating migration time with isoelectric point for a series of protein markers.

During the focusing step precipitation of proteins at their isoelectric point can be prevented, if necessary, using buffer additives such as glycerol, surfactants, urea or zwitterionic buffers. However, depending on the concentration, urea denatures proteins.

4. Micellar Electrokinetic Chromatography (MEKC)

In micellar electrokinetic chromatography, separation takes place in an electrolyte solution which contains a surfactant at a concentration above the critical micellar concentration (*cmc*). The solute molecules are distributed between the aqueous buffer and the pseudo-stationary phase composed of micelles, according to the partition coefficient of the solute. The technique can therefore be considered as a hybrid of electrophoresis and chromatography. It is a technique that can be used for the separation of both neutral and charged solutes, maintaining the efficiency, speed and instrumental suitability of capillary electrophoresis. One of the most widely used surfactants in MEKC is the anionic surfactant sodium dodecyl sulfate, although other surfactants, for example cationic surfactants such as cetyltrimethylammonium salts, are also used.

The separation mechanism is as follows. At neutral and alkaline pH, a strong electroosmotic flow is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulfate is employed as the surfactant, the electrophoretic migration of the anionic micelle is in the opposite direction, towards the anode. As a result, the overall micelle migration velocity is slowed down compared to the bulk flow of the electrolytic solution. In the case of neutral solutes, since the analyte can partition between the micelle and the aqueous buffer, and has no electrophoretic mobility, the analyte migration velocity will depend only on the partition coefficient between the micelle and the aqueous buffer. In the electropherogram, the peaks corresponding to each uncharged solute are always between that of the electroosmotic flow marker and that of the micelle (the time elapsed between these two peaks is called the separation window). For electrically charged solutes, the migration velocity depends on both the partition coefficient of the solute between the micelle and the aqueous buffer, and on the electrophoretic mobility of the solute in the absence of micelle.

Since the mechanism in MEKC of neutral and weakly ionized solutes is essentially chromatographic, migration of the solute and resolution can be rationalized in terms of the retention factor of the solute (k'), also referred to as mass distribution ratio (D_m), which is the ratio of the number of moles of solute in the micelle to those in the mobile phase. For a neutral compound, k' is given by:

$$k' = \frac{t_R - t_0}{t_0\left(1 - \frac{t_R}{t_{mc}}\right)} = K\frac{V_S}{V_M}$$

t_R: Migration time of the solute,

t_0: Analysis time of an unretained solute (determined by injecting an electroosmotic flow marker which does not enter the micelle, for instance methanol),

t_{mc}: Micelle migration time (measured by injecting a micelle marker, such as Sudan III, which migrates while continuously associated in the micelle),

K: Partition coefficient of the solute,

V_S: Volume of the micellar phase,
V_M: Volume of the mobile phase.

Likewise, the resolution between two closely-migrating solutes (R_S) is given by:

$$R_S = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k'_b}{k'_b + 1} \times \frac{1 - \left(\frac{t_0}{t_{mc}}\right)}{1 + \left(\frac{t_0}{t_{mc}}\right) k'_a}$$

N: Number of theoretical plates for one of the solutes,
α: Selectivity,
k'_a and k'_b: Retention factors for both solutes, respectively ($k'_b > k'_a$).

Similar, but not identical, equations give k' and R_S values for electrically charged solutes.

Optimization

The main parameters to be considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

Instrumental parameters

(1) *Voltage*: Separation time is inversely proportional to applied voltage. However, an increase in voltage can cause excessive heat production that gives rise to temperature gradients and viscosity gradients of the buffer in the cross-section of the capillary. This effect can be significant with high conductivity buffers such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution.

(2) *Temperature*: Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelles, the critical micellar concentration and the viscosity of the buffer. These parameters contribute to the migration time of the solutes. The use of a good cooling system improves the reproducibility of the migration time for the solutes.

(3) *Capillary*: As in capillary zone electrophoresis, the dimensions of the capillary (length and internal diameter) contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electric fields (working at constant voltage), increase migration time and improve the separation efficiency. The internal diameter controls heat dissipation (for a given buffer and electric field) and consequently the sample band broadening.

Electrolytic solution parameters

(1) *Surfactant type and concentration*: The type of surfactant, in the same way as the stationary phase in chromatography, affects the resolution since it modifies separation selectivity. Also, the log k' of a neutral compound increases linearly with the concentration of surfactant in the mobile phase. Since resolution in MEKC reaches a maximum when k' approaches the value of $\sqrt{t_m/t_0}$, modifying the concentration of surfactant in the mobile phase changes the resolution obtained.

(2) *Buffer pH*: Although pH does not modify the partition coefficient of non-ionized solutes, it can modify the electroosmotic flow in uncoated capillaries. A decrease in the buffer pH decreases the electroosmotic flow and therefore increases the resolution of the neutral solutes in MEKC, resulting in a longer analysis time.

(3) *Organic solvents*: To improve MEKC separation of hydrophobic compounds, organic modifiers (methanol, propanol, acetonitrile, etc.) can be added to the electrolytic solution. The addition of these modifiers usually decreases migration time and the selectivity of the separation. Since the addition of organic modifiers affects the critical micellar concentration, a given surfactant concentration can be used only within a certain percentage of organic modifier before the micellization is inhibited or adversely affected, resulting in the absence of micelles and, therefore, in the absence of partition. The dissociation of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible; in some cases the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically.

(4) *Additives for chiral separations*: For the separation of enantiomers using MEKC, a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Micelles that have a moiety with chiral discrimination properties include salts of *N*-dodecanoyl-L-amino acids, bile salts, etc. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions which contain micellized achiral surfactants.

(5) *Other additives*: Several strategies can be carried out to modify selectivity, by adding chemicals to the buffer. The addition of several types of cyclodextrins to the buffer can also be used to reduce the interaction of hydrophobic solutes with the micelle, thus increasing the selectivity for this type of compound.

The addition of substances able to modify solute-micelle interactions by adsorption on the latter, is used to improve the selectivity of the separations in MEKC. These additives may be a second surfactant (ionic or non-ionic) which gives rise to mixed micelles or metallic cations which dissolve in the micelle and form co-ordination complexes with the solutes.

Quantification

Peak areas must be divided by the corresponding migration time to give the corrected area in order to:

(1) compensate for the shift in migration time from run to run, thus reducing the variation of the response,
(2) compensate for the different responses of sample constituents with different migration times.

Where an internal standard is used, verify that no peak of the substance to be examined is masked by that of the internal standard.

Calculations

From the values obtained, calculate the content of the component or components being examined. When prescribed, the percentage content of one or more components of the sample to be examined is calculated by determining the corrected area(s) of the peak(s) as a percentage of the total of the corrected areas of all peaks, excluding those due to solvents or any added reagents (normalization procedure). The use of an automatic integration system (integrator or data acquisition and processing system) is recommended.

System Suitability

In order to check the behavior of the capillary electrophoresis system, system suitability parameters are used. The choice of these parameters depends on the mode of capillary electrophoresis used. They are: retention factor (k') (only for micellar electrokinetic chromatography), apparent number of theoretical plates (N), symmetry factor (A_S) and resolution (R_S). In previous sections, the theoretical expressions for N and R_S have been described, but more practical equations that allow these parameters to be calculated from the electropherograms are given below.

Apparent Number of Theoretical Plates

The apparent number of theoretical plates (N) may be calculated using the expression:

$$N = 5.54\left(\frac{t_R}{w_h}\right)^2$$

t_R: Migration time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,

w_h: Width of the peak at half-height.

Resolution

The resolution (R_S) between peaks of similar height of two components may be calculated using the expression:

$$R_S = \frac{1.18(t_{R2} - t_{R1})}{w_{h1} + w_{h2}}$$

$$t_{R2} > t_{R1}$$

t_{R1} and t_{R2}: Migration times or distances along the baseline from the point of injection to the perpendiculars dropped from the maxima of two adjacent peaks,

w_{h1} and w_{h2}: Peak widths at half-height.

When appropriate, the resolution may be calculated by measuring the height of the valley (H_v) between two partly resolved peaks in a standard preparation and the height of the smaller peak (H_p) and calculating the peak-to-valley ratio:

$$p/v = \frac{H_p}{H_v}$$

Symmetry Factor

The symmetry factor (A_S) of a peak may be calculated using the expression:

$$A_S = \frac{w_{0.05}}{2d}$$

$w_{0.05}$: Width of the peak at one-twentieth of the peak height,

d: Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Tests for area repeatability (standard deviation of areas or of the area/migration-time ratio) and for migration time repeatability (standard deviation of migration time) are introduced as suitability parameters. Migration time repeatability provides a test for the suitability of the capillary washing procedures. An alternative practice to avoid the lack of repeatability of the migration time is to use migration time relative to an internal standard.

A test for the verification of the signal-to-noise ratio for a standard preparation (or the determination of the limit of quantification) may also be useful for the determination of related substances.

Signal-to-noise Ratio

The detection limit and the quantification limit are equivalent to signal-to-noise ratios of 3 and 10, respectively. The signal-to-noise ratio (S/N) is calculated using the expression:

$$S/N = \frac{2H}{h}$$

H: Height of the peak corresponding to the component concerned, in the electropherogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to twenty times the width at half-height,

h: Range of the background in an electropherogram obtained after injection of a blank, observed over a distance equal to twenty times the width at the half-height of the peak in the electropherogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

Isoelectric Focusing

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

General Principles

Isoelectric focusing (IEF) is a method of electrophoresis that separates proteins according to their isoelectric point. Separation is carried out in a slab of polyacrylamide or agarose gel that contains a mixture of amphoteric electrolytes (ampholytes). When subjected to an electric field, the ampholytes migrate in the gel to create a pH gradient. In some cases gels containing an immobilized pH gradient, prepared by incorporating weak acids and bases to specific regions of the gel network during the preparation of the gel, are used. When the applied proteins reach the gel fraction that has a pH that is the same as their isoelectric point (pI), their charge is neutralized and migration ceases. Gradients can be made over various ranges of pH, according to the mixture of ampholytes chosen.

Theoretical Aspects

When a protein is at the position of its isoelectric point, it has no net charge and cannot be moved in a gel matrix by the electric field. It may, however, move from that position by diffusion. The pH gradient forces a protein to remain in its isoelectric point position, thus concentrating it; this concentrating effect is called ''focusing''. Increasing the applied voltage or reducing the sample load result in improved separation of bands. The applied voltage is limited by the heat generated, which must be dissipated. The use of thin gels and an efficient cooling plate controlled by a thermostatic circulator prevents the burning of the gel whilst allowing sharp focusing. The separation R is estimated by determining the minimum pI difference (ΔpI), which is necessary to separate 2 neighboring bands:

$$R\text{: } \Delta pI = 3\sqrt{\frac{D(\mathrm{d}pH/\mathrm{d}x)}{E(-\mathrm{d}\mu/\mathrm{d}pH)}}$$

D: Diffusion coefficient of the protein

$\mathrm{d}pH/\mathrm{d}x$: pH gradient

E: Intensity of the electric field, in volts per centimeter

$-\mathrm{d}\mu/\mathrm{d}pH$: Variation of the solute mobility with the pH in the region close to the pI

Since D and $-\mathrm{d}\mu/\mathrm{d}pH$ for a given protein cannot be altered, the separation can be improved by using a narrower pH range and by increasing the intensity of the electric field. Resolution between protein bands on an IEF gel prepared with carrier ampholytes can be quite good. Improvements in resolution may be achieved by using immobilized pH gradients where the buffering species, which are analogous to carrier ampholytes, are copolymerized within the gel matrix. Proteins exhibiting pIs differing by as little as 0.02 pH units may be resolved using a gel prepared with carrier ampholytes while immobilized pH gradients can resolve proteins differing by approximately 0.001 pH units.

Practical Aspects

Special attention must be paid to sample characteristics and/or preparation. Having salt in the sample can be problematic and it is best to prepare the sample, if possible, in deionized water or 2 per cent ampholytes, using dialysis or gel filtration if necessary.

The time required for completion of focusing in thin-layer polyacrylamide gels is determined by placing a colored protein (e.g. hemoglobin) at different positions on the gel surface and by applying the electric field: the steady state is reached when all applications give an identical band pattern. In some protocols the completion of the focusing is indicated by the time elapsed after the sample application.

The IEF gel can be used as an identity test when the migration pattern on the gel is compared to a suitable standard preparation and IEF calibration proteins, the IEF gel can be used as a limit test when the density of a band on IEF is compared subjectively with the density of bands appearing in a standard preparation, or it can be used as a quantitative test when the density is measured using a densitometer or similar instrumentation to determine the relative concentration of protein in the bands subject to validation.

Apparatus

An apparatus for IEF consists of:

—a controllable generator for constant potential, current and power. Potentials of 2500 V have been used and are considered optimal under a given set of operating conditions. Supply of up to 30 W of constant power is recommended,

—a rigid plastic IEF chamber that contains a cooled plate, of suitable material, to support the gel,

—a plastic cover with platinum electrodes that are connected to the gel by means of paper wicks of suitable width, length and thickness, impregnated with solutions of anodic and cathodic electrolytes.

Isoelectric Focusing in Polyacrylamide Gels: Detailed Procedure

The following method is a detailed description of an IEF procedure in thick polyacrylamide slab gels, which is used unless otherwise stated in the monograph.

Preparation of the Gels

Mould The mould (see Figure) is composed of a glass plate (A) on which a polyester film (B) is placed to facilitate handling of the gel, one or more spacers (C), a second glass plate (D) and clamps to hold the structure together.

7.5% Polyacrylamide gel Dissolve 29.1 g of acrylamide and 0.9 g of *N,N′*-methylenebisacrylamide in 100 mL of water. To 2.5 volumes of this solution, add the mixture of ampholytes specified in the monograph and dilute to 10 volumes with water. Mix carefully and degas the solution.

Preparation of the mould Place the polyester film on the lower glass plate, apply the spacer, place the second glass plate and fit the clamps. Place 7.5% polyacrylamide gel prepared before use on a magnetic stirrer, and add 0.25 volumes of a solution of ammonium persulfate (1 in 10) and 0.25 volumes of *N,N,N′,N′*-tetramethylethylenediamine. Immediately fill the space between the glass plates of the mould with the solution.

Method

Dismantle the mould and, making use of the polyester film, transfer the gel onto the cooled support, wetted with a few millilitres of a suitable liquid, taking care to avoid forming air bubbles. Prepare the test solutions and reference solutions as specified in the monograph. Place strips of paper for sample application, about 10 mm × 5 mm in size, on the gel

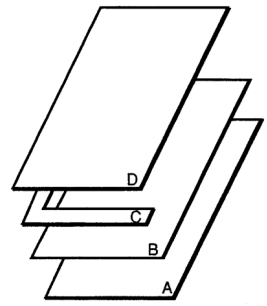


Figure. Mould

and impregnate each with the prescribed amount of the test and reference solutions. Also apply the prescribed quantity of a solution of proteins with known isoelectric points as pH markers to calibrate the gel. In some protocols the gel has pre-cast slots where a solution of the sample is applied instead of using impregnated paper strips. Cut 2 strips of paper to the length of the gel and impregnate them with the electrolyte solutions: acid for the anode and alkaline for the cathode. The compositions of the anode and cathode solutions are given in the monograph. Apply these paper wicks to each side of the gel several millimetres from the edge. Fit the cover so that the electrodes are in contact with the wicks (respecting the anodic and cathodic poles). Proceed with the isoelectric focusing by applying the electrical parameters described in the monograph. Switch off the current when the migration of the mixture of standard proteins has stabilized. Using forceps, remove the sample application strips and the 2 electrode wicks. Immerse the gel in fixing solution for isoelectric focusing in polyacrylamide gel. Incubate with gentle shaking at room temperature for 30 minutes. Drain off the solution and add 200 mL of destaining solution. Incubate with shaking for 1 hour. Drain the gel, add coomassie staining TS. Incubate for 30 minutes. Destain the gel by passive diffusion with destaining solution until the bands are well visualized against a clear background. Locate the position and intensity of the bands in the electropherogram as prescribed in the monograph.

Variations to the Detailed Procedure (Subject to Validation)

Where reference to the general method on isoelectric focusing is made, variations in methodology or procedure may be made subject to validation. These include:

(1) the use of commercially available pre-cast gels and of commercial staining and destaining kits,
(2) the use of immobilized pH gradients,
(3) the use of rod gels,
(4) the use of gel cassettes of different dimensions, including ultra-thin (0.2 mm) gels,
(5) variations in the sample application procedure, including different sample volumes or the use of sample application masks or wicks other than paper,
(6) the use of alternate running conditions, including variations in the electric field depending on gel dimensions and equipment, and the use of fixed migration times rather than subjective interpretation of band stability,
(7) the inclusion of a pre-focusing step,
(8) the use of automated instrumentation,
(9) the use of agarose gels.

Validation of Iso-Electric Focusing Procedures

Where alternative methods to the detailed procedure are employed they must be validated. The following criteria may be used to validate the separation:

(1) formation of a stable pH gradient of desired characteristics, assessed for example using colored pH markers of known isoelectric points,
(2) comparison with the electropherogram provided with the chemical reference substance for the preparation to be examined,
(3) any other validation criteria as prescribed in the monograph.

Specified Variations to the General Method

Variations to the general method required for the analysis of specific substances may be specified in detail in monographs. These include:

(1) the addition of urea in the gel (3 mol/L concentration is often satisfactory to keep protein in solution but up to 8 mol/L can be used): some proteins precipitate at their isoelectric point. In this case, urea is included in the gel formulation to keep the protein in solution. If urea is used, only fresh solutions should be used to prevent carbamylation of the protein,
(2) the use of alternative staining methods,
(3) the use of gel additives such as non-ionic detergents (e.g. octylglucoside) or zwitterionic detergents (e.g., 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) or 3-[(3-Cholamidopropyl)dimethy lammonio]-2-hydroxy-1-propanesulfonate (CHAPSO)), and the addition of ampholyte to the sample, to prevent proteins from aggregating or precipitating.

Points to Consider

Samples can be applied to any area on the gel, but to protect the proteins from extreme pH environments samples should not be applied close to either electrode. During method development the analyst can try applying the protein in 3 positions on the gel (i.e. middle and both ends); the pattern of a protein applied at opposite ends of the gel may not be identical.

A phenomenon known as cathodic drift, where the pH gradient decays over time, may occur if a gel is focused too long. Although not well understood, electroendoosmosis and absorption of carbon dioxide may be factors that lead to cathodic drift. Cathodic drift is observed as focused protein migrating off the cathode end of the gel. Immobilized pH gradients may be used to address this problem.

Efficient cooling (approximately 4°C) of the bed that the gel lies on during focusing is important. High field strengths used during isoelectric focusing can lead to overheating and affect the quality of the focused gel.

Reagents and Solutions—

Fixing solution for isoelectric focusing in polyacrylamide gel Dissolve 35 g of 5-sulfosalicylic acid dihydrate and 100 g of trichloroacetic acid in water to make 1000 mL.

◆**Coomassie staining TS** Dissolve 125 mg of coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

Destaining solution A mixture of water, methanol and acetic acid (100) (5:4:1).◆

Mass Spectrometry of Peptides and Proteins

Mass spectrometry (MS) is based on the ionization of molecules and separation of the electrically charged ions according to the dimensionless quantity, m/z value, which is obtained by dividing the relative mass (m) of the ion to unified atomic mass unit by the charge number (z) of the ion. The unified atomic mass unit is defined as one twelfth of the mass of ground state ^{12}C and used to express the mass of atom, molecule and ion. The results are expressed as a mass spectrum with m/z values of the ions on the x-axis and signal intensity of the ions on the y-axis. The mass of the molecule is calculated from the m/z values and z. Tandem mass spectrometry (MS/MS) is based on the fragmentation of the precursor ion selected in the first stage mass analysis and measurement of the product ions in the second stage mass analysis. This technique provides useful information for structural analysis of the molecule. Information obtained in MS is qualitative and is sometimes used for qualification. MS and MS/MS are useful for measuring masses of peptides and proteins and for confirming amino acid sequences and post-translational modifications. Both methods are therefore used for identification of pharmaceutical peptides and proteins.

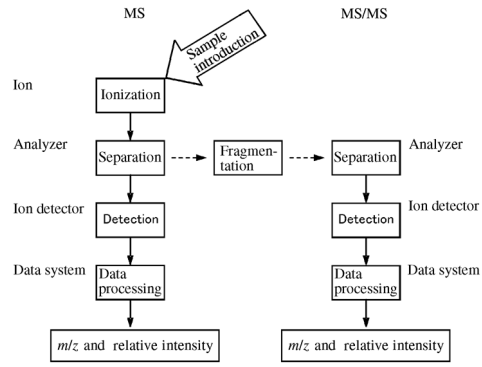


Fig. 1 Schematic diagram of mass spectrometry (MS) and tandem mass spectrometry (MS/MS)

1. Instrument

A mass spectrometer is composed of an ion source, an analyzer, an ion detector, and a data system (Fig. 1). A peptide and protein sample introduced into the ion source is ionized by soft-ionization methods, such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). The charged and gas phased ions are sorted according to the m/z ratio under a vacuum in the analyzer, which may be a quadrupole, time-of-flight, ion trap or Fourier transform ion cyclotron resonance analyzer. The ion flux collected in the detector is converted to an electric signal. Then the signal is recorded as a mass spectrum. MS/MS is carried out by using two mass spectrometers connected in series, an ion-trap mass spectrometer and Fourier transform ion cyclotron resonance mass spectrometer. The precursor ions are generally fragmented by collision-induced dissociation, post-source decay, electron capture dissociation, etc.

2. Analytical mode

2.1. MS

There are two useful modes for MS:

(1) Total ion monitoring

The signals of the entire ion are acquired over the chosen range of m/z value. This mode provides information on the masses of the molecule of interest and different species.

(2) Selected ion monitoring

The signals of the ion at chosen m/z value are acquired. This mode is useful for the sensitive measurement of the chosen molecule.

2.2. MS/MS

There are four essential modes for MS/MS:

(1) Product ion analysis

The signals of all the product ions produced from the precursor at chosen *m/z* value are acquired. This mode provides structural information on the substrates and various co-existing species.

(2) Precursor ion scan mode

The signals of the precursor that yields the product ion at chosen *m/z* value are monitored. This mode is used for sorting the molecules containing a component of interest.

(3) Constant neutral loss scan mode

The signals of the precursor that loses the fragment at chosen *m/z* value are monitored. This mode is useful to sort the molecules containing a component of interest.

(4) Selected reaction monitoring

The signals of product ions at chosen *m/z* value that are produced from the precursor at chosen *m/z* value are monitored. This mode allows for sensitive and selective measurement and is used for quantification of a molecule in a complex mixture.

3. Analytical procedure

3.1. MS

In advance, it should be conformed if the detectability and the difference between the calculated mass and observed mass meet the criteria stated in the monograph by mass measuring using a test solution specified in the system suitability in the monograph. If they do not meet the criteria, the system should be optimized by adjustment of the voltage of the ion source, analyzer and detector, as well as by calibration using appropriate mass calibrator. MS is performed according to the sample preparation and operating conditions indicated in the monograph. The general procedure is described as follows.

(1) Matrix-assisted laser desorption/ionization (MALDI)

A desalted peptide and protein sample is dissolved in an appropriate solvent, e.g., an aqueous solution of trifluoroacetic acid. A suitable matrix, such as α-ciano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, or sinapic acid, is dissolved in an aqueous solution containing acetonitrile and trifluoroacetic acid. A mixture of sample solution and matrix solution is deposited on a sample plate and dried. The sample on the plate is set in the ion source, and ionized by a laser beam at suitable intensity.

(2) Electrospray ionization (ESI)

A desalted peptide and protein sample is dissolved in a suitable solvent, such as an aqueous solution containing acetic acid and methanol or acetonitrile. The sample solution is sprayed through a needle tip and held at a potential of several kilovolts. The sample is introduced by using a syringe or HPLC.

3.2. MS/MS

System suitability is tested by MS/MS of the test sample specified in the monograph. The detectability and system performance should be confirmed based on the detection of the product ions specified in the monograph. The sample is ionized in the same way as for MS, and the chosen precursor is fragmented by the suitable conditions specified in the monograph. The signals are recorded as a mass spectrum. A peptide containing disulfide bonds is generally reduced by dithiothreitol, 2-mercaptethanol and tris(2-carboxyethyl) phosphine. The reduced peptides are derivatized with monoiodoacetic acid, iodoacetamide, and 4-vinylpyridine.

4. Identification test

4.1. Mass of the molecule

The monoisotopic mass of the peptide is preferably acquired. If the monoisotopic mass is not detectable, the average mass is calculated from the top of the ion peak. Deconvolution is effective for calculating the average mass of multiply-charged ions from proteins. The mass should meet the criteria specified in the monograph.

4.2. Amino acid sequence

After measuring the mass of the sample peptide, the presence of the specified product ions that arise from the selected precursor is confirmed according to the conditions indicated in the monograph. Digestion of sample proteins with a suitable enzyme followed by MS/MS is sometimes effective for sequencing of the high-molecular weight proteins which provide insufficient product ions. Details of the digestion procedure are provided in the section on peptide mapping.

5. Glossary

Ion-trap (IT)

Ion-trap refers to the quadrupole ion trap mass analyzer in a restricted sense. Ions stored in the analyzer by applying radio frequency voltage to ring electrodes are separated by subsequent ejection of the ions from the analyzer by varying the voltage on the ring electrodes. This allows multiple stage MS in which a selected ion is repeatedly trapped, fragmented and ejected.

Electrospray ionization (ESI)

The sample in solution is sprayed through a needle tip and held at high-voltage at atmospheric pressure. The sample is ionized by a formation of charged liquid droplets. High-molecular mass proteins are detected as multiply-charged ions. The analyzer can be connected with HPLC.

Quadrupole (Q)

The analyzer is composed of four parallel electrodes which have a hyperboloidal or corresponding cross-section. The ions transmitted to the analyzer are separated by varying the potential of direct and radio frequency components applied to the rods so that the filter for sorting the *m/z* values of ions is changed.

Collision-induced dissociation (CID)

When an ion collides with a neutral atom or molecule (He, Ar, N_2 and so on), some of the translational energy of the collision is converted into internal energy, thereby causing dissociation. The terms low-energy CID and high-energy CID refer to those CIDs for which the translational energy of the precursor ions is lower than 1000 eV and higher than 1000 eV, respectively.

Electron capture dissociation (ECD)

Multiply-charged positive ions interact with low energy electrons producing charge-reduced radical ions, which readily dissociate. This method is primarily used for MS/MS in FT-ICR MS or IT MS.

Time-of-flight (TOF)

The ionized sample is accelerated at high-voltage and separated based on the time required for an ion to travel to the detector. There are two types of analyzer, a linear type in which ions travel linearly from the ion source to the detector, and a reflectron type where ions are inverted by a reflectron. The latter type allows high-resolution measurement by correction of the variation in the initial energy of ions.

Fourier transform ion cyclotron resonance (FT-ICR)

The analyzer is based on the principle that the cyclotron frequency of the ions in a magnetic field is inversely proportional to its *m/z* value. Ions are excited to a larger radius orbit using radio frequency energy and their image current is detected on receiver plates. The resulting data are devolved by applying a Fourier transform to give a mass spectrum.

Post-source decay (PSD)

Metastable ion decay occurs by excess internal energy and collision with residual gas during ion acceleration out of the MALDI ion source and prior to reaching the detector. This method is used for MS/MS by using MALDI-TOF MS with a reflectron mode.

Matrix-assisted laser desorption/ionization (MALDI)

The sample, which is mixed with a suitable matrix and deposited on a target plate, is ionized by irradiation with nanosecond laser pulses. Proteins, carbohydrates, oligonucleotides, and lipids can be ionized without any dissociation. Singly-charged ions are mainly detected.

Monosaccharide Analysis and Oligosaccharide Analysis/ Oligosaccharide Profiling

Glycosylation analysis is a method to confirm the consistency of the oligosaccharides attached to glycoprotein drug substance, product or material. When oligosaccharides attached to the glycoprotein affect efficacy and safety or the possibility cannot be denied, oligosaccharides are considered as critical quality attribute, and strategy should be designed in order to ensure the consistency of glycosylation. One of the strategies is glycosylation analysis, which involves 1) analysis of released monosaccharides (monosaccharide analysis), 2) analysis of released oligosaccharides (oligosaccharide analysis/oligosaccharide profiling), 3) analysis of glycopeptides (glycopeptide analysis), and 4) analysis of intact glycoprotein (glycoform analysis). These methods provide monosaccharide compositions, oligosaccharide identities and distribution in whole glycoprotein, site-specific glycosylation identities and distribution, and overall glycosylation characteristics and distribution of glycoprotein, respectively. In the setting specification of glycosylation analysis, proper methods should be selected and used alone or in combination, in consideration of the relationship between the oligosaccharide structures and functions, such as biological activity, pharmacodynamics, pharmacokinetics, immunogenicity, stability, and solubility. Glycosylation consistency may be ensured not only by oligosaccharide analysis but also at manufacturing process. Glycosylation analysis can be also used as in-process test, and as method to confirm glycosylation consistency during process development. Methods and general consideration of monosaccharide analysis and oligosaccharide analysis/oligosaccharide profiling are described below. For glycopeptide analysis, General Test <*2.62*> Mass Spectrometry, and General Information Peptide Mapping and Mass Spectrometry of Peptides and Proteins would be helpful, and for glycoform analysis, General Information Isoelectric Focusing and Capillary Electrophoresis, and General Test <*2.62*> Mass Spectrometry would be helpful.

1. Monosaccharide analysis

Monosaccahrides are released by cleavage of glycosidic bond using acid hydrolysis, exoglycosidase or methanolysis. Released monosaccharides are dried and purified if needed, and then analyzed using liquid chromatography, gas chromatography, or capillary electrophoresis. Internal standard method or absolute calibration method are used for quantitative measurement. The analytical results are typically expressed as molar ratio of individual monosaccharides to glycoprotein.

1.1. Isolation and purification of glycoprotein

Monosaccharide analysis is generally performed after glycoprotein is isolated and purified in an appropriate manner, because excipients and salts may affect hydrolysis, derivatization of monosaccharides, and chromatographic separation. When purification of the glycoprotein is required, the procedure is specified in the specific monograph.

1.2. Release of monosaccharide

1.2.1. Acid hydrolysis

Acid hydrolysis is the most common procedure to release neutral and amino sugars. In general, monosaccarides may be released by acid hydrolysis of glycosidic bond under conditions such as 2 to 7 mol/L trifluoroacetic acid at about 100°C. Since amino sugar residue directly attached to protein is difficult to release, for accurate quantification of amino sugars, acid hydrolysis should be performed separately under conditions such as 2 to 6 mol/L hydrochloride at 100°C. Because hydrolysis rate is dependent on the identity of the monosaccharide, the anomeric configuration, and position of the glycosidic linkage, it is recommended that release and degradation of individual monosaccharides are confirmed by time-course study. Because N-acetyl groups of amino sugars are removed under acid hydrolysis conditions, re-N-acetylation may be performed if needed. Since sialic acid is labile, sialic acids are released separately under conditions such as 0.1 mol/L hydrochloride, 0.1 mol/L sulfuric acid, or 2 mol/L acetic acid at 80°C.

1.2.2. Enzymatic treatment

Exoglycosidase digestion is also used for release of sialic acids from glycoprotein. Typically, sialidases with broad specificity, such as those derived from *Arthrobacter ureafaciens* or *Clostridium perfringens* are used. Digestion conditions should be optimized in consideration of the identity of sialic acids, linkage, O-acetylation and others. Other enzymes with high specificity may be used to distinguish different types of linkage.

1.2.3. Methanolysis

Dried sample is heated under methanolic hydrogen chloride. Monosaccharides are released as methyl glycosides. Degradation of released monosaccharides is low compared to acid hydrolysis.

1.3. Quantification of the released monosaccharides

1.3.1. High-pH anion-exchange chromatography with pulsed amperometric detection

Acid is removed from the hydrolysate if needed. Monosaccharides can be separated and quantified without derivatization using high-pH anion-exchange chromatography with pulsed amperometric detection. Monosaccharides have about 12 to 14 of acid dissociation constant (pKa). They are ionized under high pH conditions (pH 12 to 13), and can be separated by strong anion-exchange chromatography using polymer-based stationary phase containing quaternary ammonium groups. Amperometric detection is a method to detect electrochemically active ions by measuring the current when analyte is oxidized or reduced at electrodes. Sugar is ionized at high pH and can be selectively detected by amperometry. Because oxidized products of sugars foul the electrodes and reduce the signals, pulsed amperometry, where electrode surface is cleaned by changing positive and negative potentials after data acquisition, is used. Since amino acids are also detectable by amperometry, it is noted that analysis may be interfered in the case of the glycoprotein with low oligosaccharide contents. This analytical method can be used for oligosaccharide analysis as well as for analysis of neutral and amino sugars, and sialic acids.

1.3.2. Derivatization and liquid chromatography

(1) Neutral and amino sugars

Monosaccharides obtained by acid hydrolysis are treated to remove the acid, re-N-acetylated if needed, then reductively aminated with 2-aminobenzoic acid, 2-aminopyridine, or ethyl-4-aminobenzoate, or derivatized with 3-methyl-1-phenyl-5-pyrazolone. Impurities derived from reagents may interfere the analysis, attention should be paid to the purity of the reagents used. To prevent excessive reagents to affect test results, derivatized monosaccharides are purified if needed. The derivatized monosaccharides may be separated using reversed-phase chromatography, or anion-exchange chromatography with formation of borate complex. Separated monosaccharides are detected by fluorometric or ultraviolet detector. Underivatized monosaccharides may be separated by ion-exchange chromatography, post-column derivatization using such as arginine, and then detected.

(2) Sialic acid

Released sialic acids by mild acid hydrolysis or sialidase digestion are derivatized with 1,2-diamino-4.5-methylenedioxybenzen or 1,2-phenylenediamine, which react with α-keto acid specifically. This reaction proceed in acidic conditions, thus acid hydrolysate can be used for derivatization without removal of the acid. Derivatized slialic acids are separated by reversed-phase chromatography and detected by fluorometric detector.

1.3.3. Gas chromatography

There are several methods for gas chromatography; monosaccharides obtained by methanolysis are re-N-acetylated and trimethylsilylated (trimethylsilyl derivatives), and the monosaccharides obtained by acid hydrolysis are reduced and then acetylated (alditol acetate derivatives). The former can quantitate sialic acids simultaneously without degradation, but each sugar gives several peaks due to α- and β-anomers and isomers, and chromatogram becomes complex.

Methylation analysis provides the structural information or the glycosidic linkage of individual monosaccharides. After all hydroxy groups in the oligosaccharide are methylated, permethylated oligosaccharide is subject to acid hydrolysis and resultant partially methylated monosaccharides are reduced and acetylated. Partially methylated alditol acetates are separated and quantified using gas chromatography.

1.4. Acceptance criteria

Confirmation of compliance of the test material is typically achieved by demonstrating that contents of individual monosaccharides per protein are within a specified range. In order to set acceptance criteria properly, it is needed to consider the relationship between characteristics of glycosylation, and efficacy and safety.

1.5. Monosaccharide reference materials

Monosaccharides to be analyzed are often used as reference materials for monosaccharide analysis. In this case, monosaccharide reference material mixtrure is prepared as mixing each monosaccharide equally, or at similar ratio expected in test substance.

1.6. System suitability

The solution for system suitability test should be prepared properly using monosaccharide reference materials. It may be difficult to separate each monosaccharide completely due to its similar physical property. Acceptance criteria should be set properly.

2. Oligosaccharide analysis/oligosaccharide profiling

Oligosaccharides are released from glycoprotein by enzymatic or chemical treatment, and then released oligosaccharides are analyzed or profiled by liquid chromatography <*2.01*>, capillary electrophoresis, mass spectrometry <*2.62*>, or combination of them. Analysis result is obtained as oligosaccharide profile, which provides the information on the identity and the distribution of oligosaccharide.

2.1. Separation and purification of glycoprotein

Interfering substance, such as excipients, salts and surfactant, are removed if needed. When purification of the glycoprotein is required, the procedure is specified in the specific monograph.

2.2. Release and isolation of oligosaccharides

Release of N-linked oligosaccharides from glycoprotein is performed by enzymatic treatment or hydrazinolysis. Release of O-linked oligosaccharides is performed by alkali β-elimination, hydrazinolysis, and O-glycanase digestion. The releasing conditions must be optimized in order to release and recover all oligosaccharides attached to the glycoprotein reproducibly, independent of their structure and their individual position in the protein. Table 1 give a non-exhaustive list of enzymatic cleavage agents and their specificity. Released oligosaccharides may be purified prop-

Table 1 Examples of enzymatic cleavage agents

Enzyme	Specificity
N-linked oligosaccharide release	
Peptide-N^4-(*N*-acetyl-β-glucosaminyl) asparagine amidase (EC 3.5.1.52)	Hydrolysis of peptide-N^4-(*N*-acetyl-β-D-glucosaminyl) asparagine residue in which the glucosamine residue may be further glycosylated, to yield a (substituted) *N*-acetyl-β-D-glucosaminylamine and a peptide containing an aspartate residue
—Peptide N-glycosidase F (PNGase F)	Release of N-linked oligosaccharides but no release of N-linked oligosaccharides containing (α1,3)-linked core fucose
—Peptide N-glycosidase A	Release of N-linked oligosaccharides including those containing (α1,3)-linked core fucose
Mannosyl-glycoprotein endo-β-*N*-acetyl-glucosaminidase (EC 3.2.1.96)	Endohydrolysis of the *N*,*N*′-diacetylchitobiosyl unit in high-mannose glycopeptides/glycoproteins containing the—[Man(GlcNAc)$_2$]Asn structure [Man(GlcNAc)$_2$]Asn
—Endo-β-*N*-acetylglucosaminidase F (endo F)	Release of high-mannose, hybrid, and complex oligosaccharides
—Endo-β-*N*-acetylglucosaminidase H (endo H)	Release of high-mannose and hybrid oligosaccharides
O-linked oligosaccharide release	
Glycopeptide α-*N*-acetylgalactosaminidase (EC 3.2.1.97)*	Release of D-galactose-(α1,3)-*N*-acetylgalactosamine α-linked to serine/threonine residue

* This enzyme has limited usage because of its high substrate specificity.

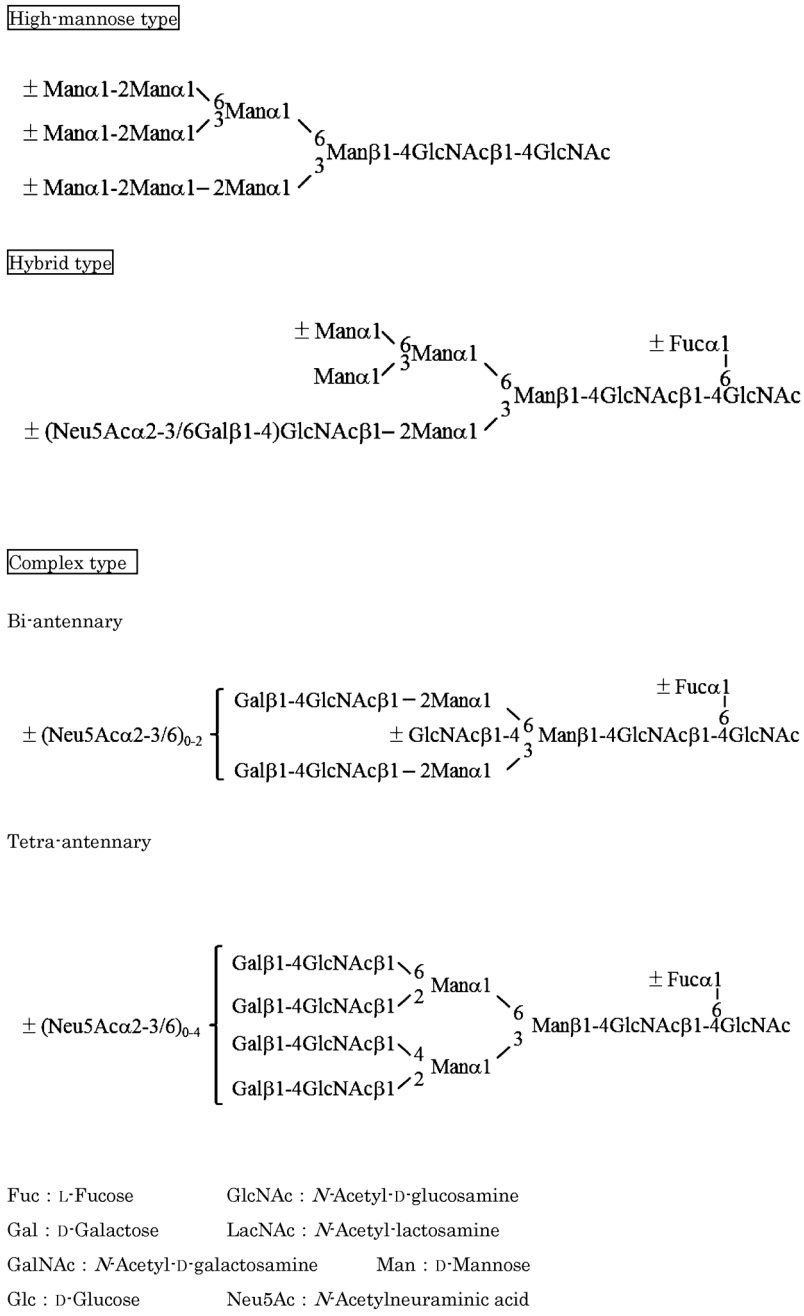


Fig. Common types of N-linked oligosaccharides

erly if needed.

2.2.1. Enzymatic release

For the relesase of N-linked oligosaccharides, peptide N-glycosidase F (PNGase F) derived from *Flavobacterium meningosepticum* or peptide N-glycosidase A (PNGase A) derived from almonds are available. These enzyme hydrolyze the amide bond between asparagine residue and *N*-acetyl-glucosamine residue at reducing end of oligosaccharides to produce glycosylamine derivative and aspartic acid residue. Glycosylamine derivative is subsequently hydrolyzed non-enzymatically under weak acidic conditions to ammonia and free oligosaccharide. O-glycanase from *Diplococcus pneumoniae* is available to release O-linked oligosaccharides, but specificity of this enzyme is too narrow.

2.2.1.1. PNGase F digestion

PNGase F have an optimum pH 7 to 9. Glycoprotein is treated directly or under presence of reducing agent, surfactant, and/or denaturing agent. Glycoprotein may be treated with PNGase F after reduced and alkylated, or after digested into glycopeptides. Glycoproteins from some insect cells and plants may have a (α1,3)-linked fucose attached to the proximal GlcNAc of the core chitobiose, and N-linked oligosaccharides containing this structure are not released by this enzyme.

2.2.1.2. PNGase A digestion

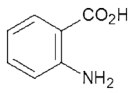
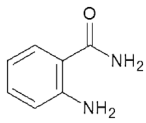
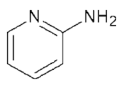
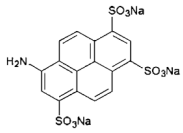
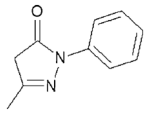
PNGase A have an optimum pH 4 to 6. Since this enzyme is difficult to release oligosaccharides directly from whole glycoprotein, glycoprotein sample is digested with proteolytic agent, such as endoprotease, and then glycopeptides are treated with this enzyme to release oligosaccharides.

2.2.2. Chemical cleavage

2.2.2.1. Hydrazinolysis

Well-dried glycoprotein is added anhydrous hydrazine and heated. Hydrazine cleaves glycosylamine linkage between oligosaccharide and peptide as well as peptide bond. With careful control of reaction conditions, selective release of N-

Table 2 Examples of derivatizing agents and suitable analytical techniques.

Agent	Structure	Acronym	Analytical techniques	Fluorescent or UV detection
2-Aminobenzoic acid		2-AA	LC, CE, MS	Ex: 360 nm, Em: 425 nm Ex: 325 nm, Em: 405 nm
2-Aminobenzamide		2-AB	LC, MS	Ex: 330 nm, Em: 420 nm
2-Aminopyridine		2-AP	LC, MS	Ex: 310 nm, Em: 380 nm Ex: 320 nm, Em: 400 nm
Trisodium 8-aminopyrene-1,3,6-trisulfonic salt		APTS	CE	Ex: 488 nm, Em: 520 nm
3-methyl-1-phenyl-5-pyrazolone		PMP	LC, MS	UV 245 nm

linked oligosaccharide and/or O-linked oligosaccharides can be achieved. Because de-N-acetylation of amino sugar and sialic acid in oligosaccharides also occurs, amino groups are re-N-acetylated after removing hydrazine. Attention should be paid to the possibility of loss of sialic acid, and successive degradation from reducing end of released O-linked oligosaccharides (peeling reaction).

2.2.2.2. Alkali β-elimination

Heating glycoprotein under alkaline conditions results in release of O-linked oligosaccharides. To prevent peeling reactions, reaction is conducted in the presence of a reducing agent, such as sodium tetrahydroborate. It is noted that reducing end of obtained O-linked oligosaccharides is already reduced, thus, cannot be derivatized. There is a method to release the oligosaccharides and simultaneously to derivatize with 3-methyl-1-phenyl-5-pyrazolone.

2.3. Analysis of released oligosaccharides

Oligosaccharides are analyzed directly or after derivatized. Table 2 gives a non-exhaustive list of commonly used fluorescent labels and suitable analytical techniques. Analysis method is needed to separate and detect individual oligosaccharides or family of oligosaccharides with the structure which affects efficacy and safety.

2.3.1. Liquid chromatography *<2.01>*

2.3.1.1. Derivatization and liquid chromatography/fluorometric or UV detection

Profiling of derivatized oligosaccharides by liquid chromatography is the most common methods. Oligosaccharides derivatized with 2-aminobenzamide, 2-aminobenzoic acid, 2-aminopyridine or others are separated by hydrophilic interaction, reversed-phase, ion-exchange, or mixed-mode chromatography, and then detected by fluorometry. Oligosaccharides derivatized with 3-methyl-1-phenyl-5-pyrazolone are separated by reversed-phase chromatography, and then detected by UV spectrometry. Hydrophilic interaction liquid chromatography separates oligosaccharides on the basis of hydrophilicity (i.e. size, the number of sialic acid, ...). Reversed-phase chromatography separates oligosaccharides on the basis of hydrophobilicy (i.e. type of oligosaccharide, branching, number of sialic acid, ...). Ion-exchange chromatography separates oligosaccharides on the basis of number of charges. Mix mode of ion-exchange and hydrophilic interaction separates oligosaccharides on the basis of structure as well as number of charges.

2.3.1.2. High-pH anion-exchange chromatography/pulsed amperometric detection

Released oligosaccharides are separated by strong anion-exchange chromatography using polymer-based stationaly phase containing quaternary ammonium goups, and detected by pulsed amperometry. This method can separate and detect sialo-oligosaccharides according to the number of sialic acids and linkage differences. This method has often been used for profiling of sialo-oligosaccharides because of no need of derivatization (no loss of sialic acid and no loss of oligosaccharides during procedure) and high resolution. Because response factors of individual oligosaccharides are not equal, relative peak response does not directly reflect the molar ratio of individual oligosaccharides.

2.3.2. Capillary electrophoresis

Derivatized oligosaccharides are separated by capillary zone electrophoresis using appropriate electrolyte buffer, and then detected by laser-induced fluorometric detector. Oligosaccharide is separated based on the sample properties such as charge, size, or shape. In general, capillary is used with the inner wall surface modified using neutral polymers covalently or dynamically in order to prevent electroosmotic flow. Derivatizing agent, and pH and additives of the electrolyte buffer are selected to achieve good separation. Capillary electrophoresis has high resolution separations and requires small amounts of sample.

2.3.3. Mass spectrometry *<2.62>*

Mass spectrometry is used for underivatized oligosaccharides as well as derivatized oligosaccharides. Monosaccharide compositions of oligosaccharides can be deduced from observed molecular mass of oligosaccharides. For ionization methods for oligosaccharides, soft ionization techniques, such as electrospray ionization and matrix-assisted laser desorption/ionization are used. It is noted that oligosaccharides containing sialic acid are susceptible to loss of sialic acid during mass spectrometry.

2.4. Assignment or identification of the peak

Identification of the oligosaccharides attached to the glycoprotein is important for test method development and evaluation of oligosaccharide profile. In general, structure

Table 3 Examples of exoglycosidases and endoglycsidase useful for structure assignment.

Enzyme	Origin	Specificity
Exo-α-sialidase (EC 3.2.1.18)	*Arthrobactor ureafaciens*	α2-3,6,8,9
	Vibrio cholerae	α2-3,6,8
	Clostridium perfringens	α2-3,6,8
	Newcastle disease virus	α2-3
	Streptococcus pneumoniae	α2-3
β-Galactosidase (EC 3.2.1.23)	Bovine testes	β1-3,4
	Streptomyces pneumoniae	β1-4
α-L-Fucosidase (EC 3.2.1.51)	Almond meal	α1-3
	Xanthomonas sp.	α1-3,4
	Bovine kidney	α1-2,3,4,6
α-Mannosidase (EC 3.5.1.24)	Jack Bean	α1-2,3,6
α-Galactosidase (EC 3.2.1.22)	Green coffee beans	α1-3,4,6
Keratan-sulfate endo-1,4-β-galactosidase) (EC 3.2.1.103)	*Bacteroides fragilis*	β1-3,4/poly LacNAc

of oligosaccharides may be deduced based on the molecular mass determined by mass spectrometry, the pattern of fragment ions obtained by tandem mass spectrometry, sensitivity to exoglycosidases or endoglycosidases with high specificity, comparison of chromatogram or electropherogram with well-characterized oligosaccharide standards, methylation analysis, and knowledge on the oligosaccharide patterns biosynthesized in the used cell line. Table 3 give a non-exhaustive list of exoglycosidases and endoglycosidases for structural assignment. During routine application, the identity of oligosaccharide peaks may be confirmed by comparison with oligosaccharide profile obtained from the reference material.

2.5. Acceptance criteria

Oligosaccharide profile obtained from the test material is compared with that obtained in parallel using reference material, and then peak position and response ratio of individual oligosaccharides are comparable. Or peak ratio of individual oligosaccharide to total response (peak area percentage method) or relative peak response meets the acceptance criteria. In order to set specification properly, it is important to identify the oligosaccharide structure to be controlled in consideration of the relationship between oligosaccharide structure, and efficacy and safety.

2.6. Reference materials

It is important that reference material has been validated for glycosylation analysis.

2.7. System suitability

System suitability is developed depending on the purpose of oligosaccharide test. Acceptance criteria, e.g. presence of specific peaks, resolution between two adjacent peaks, the number of detectable peaks, and/or conformance to reference oligosaccharide profile may be set for oligosaccharide profile, obtained from standard material, or well-characterized glycoprotein with similar property by treating similarly. Otherwise, oligosaccharide reference material, e.g. oligosaccharide standard prepared from the substance being tested and demonstrated to be suitable, or system suitability oligosaccharide marker, is similarly treated. Similar acceptance criteria described above may be set for obtained oligosaccharide profile.

Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products

This document describes the currently available methods of mycoplasma testing that should be performed for cell substrates that are used in the manufacture of biotechnological/biological products.

Methods suggested for detection of mycoplasma are, A. culture method, B. indicator cell culture method, and C. nucleic acid amplification test (NAT) method.

Mycoplasma testing should be performed on the master cell bank (MCB) and the working cell bank (WCB), as well as on the cell cultures used during the manufacturing process of the product. For the assessment of these cells, mycoplasma testing should be performed using both methods A and B. Note that method C may be used as an alternative to methods A and/or B (one or both of other methods) after suitable validation.

Prior to mycoplasma testing by methods A or B, the sample should be tested to detect the presence of any factors inhibiting the growth of mycoplasma. If such growth-inhibiting factors are detected, they should be neutralized or eliminated by an appropriate method, such as centrifugation or cell passage.

If the test will be performed within 24 hours of obtaining the sample, the sample should be stored at 2 – 8°C. If more than 24 hours will elapse before the test is performed, the sample should be stored at −60°C or lower.

If mycoplasma is detected, additional testing to identify the species may be helpful in determining the source of contamination.

A. Culture Method

1. Culture Medium

Both agar plates and broth are used. Each batch of agar and broth medium should be free of antibiotics except for penicillin. Refer to the Minimum Requirements for Biological Products regarding selection of the culture media. Other culture media may be used if they fulfill the requirements described in the following section 2.

2. Suitability of Culture Medium

Each batch of medium should be examined for mycoplasma growth-promoting properties. To demonstrate the capacity of the media to detect known mycoplasma, each test should include control cultures of at least two known species or strains of mycoplasma, one of which should be a dextrose fermenter (i.e., *Mycoplasma pneumoniae* ATCC 15531, NBRC 14401 or equivalent species or strains) and one of which should be an arginine hydrolyser (i.e., *Mycoplasma orale* ATCC 23714, NBRC 14477 or equivalent species or strains). The mycoplasma strains used for the positive control tests should be those with a low number of passages obtained from an official or suitably accredited agency, and handled appropriately. Inoculate the culture medium with 100 colony-forming units (CFU) or 100 color-changing units (CCU) or less.

3. Culture and Observation

1) Inoculate no less than 0.2 mL of test sample (cell suspension) in evenly distributed amounts over the surface of each of two or more agar plates. After the surfaces of the inoculated plates are dried, the plates should be incubated in an atmosphere of nitrogen containing 5 – 10% carbon diox-

ide and adequate humidity at 35 – 37°C for no less than 14 days.

2) Inoculate no less than 10 mL of the test sample (cell suspension) into each of one or more vessels containing 100 mL of broth medium, and incubate at 35 – 37°C.

If the culture medium for the sample cells contains any growth-inhibiting factors, such as antibiotics, these factors should be removed. Refer to the Validation tests for growth-inhibiting factors described in the Minimum Requirements for Biological Products for the detection of growth-inhibiting factors.

3) Subculture 0.2 mL of broth culture from each vessel on the 3rd, 7th, and 14th days of incubation onto two or more agar plates. Observe the broth media every 2 or 3 days and if a color change occurs, subculture. The plates should be incubated in nitrogen containing 5 – 10% carbon dioxide and adequate humidity at 35 – 37°C for no less than 14 days.

4) Examination of all plates for mycoplasma colonies should be done microscopically on the 7th and 14th day at 100 times magnification or greater.

B. Indicator Cell Culture Method

Using Vero cell culture substrate, pretest the suitability of the method using an inoculum of 100 CFU or 100 CCU or less of *Mycoplasma hyorhinis* (ATCC 29052, ATCC 17981, NBRC 14858 or equivalent species or strains) and *M. orale* (ATCC 23714, NBRC 14477 or equivalent species or strains).

Indicator cell substrate equivalent to Vero cells and suitable mycoplasma strains may be acceptable if data demonstrate at least equal sensitivity for the detection of known mycoplasma contaminants. The mycoplasma strains should be those with a low number of passages obtained from an official or suitably accredited agency, and handled appropriately, and the unit of inoculation should be determined before use. The cell substrate used should be obtained from a qualified cell bank and certified to be mycoplasma free. The acquired cells should be carefully cultured and propagated, and sufficient volumes of seed stock should be prepared with the proper precautions to avoid mycoplasma contamination. The stock should be tested for mycoplasma contamination using at least one of the methods described in this document, then frozen for storage. For each test a new container from the stock should be thawed and used within 6 passages.

Indicator cell cultures should be grown on cover slips submerged in culture dishes or equivalent containers for one day. Inoculate no less than 1 mL of the test sample (cell culture supernatant) into two or more of the culture dishes.

The test should include a negative (non-infected) control and two positive mycoplasma controls, such as *M. hyorhinis* (ATCC 29052, ATCC 17981, NBRC 14858 or equivalent species or strains) and *M. orale* (ATCC 23714, NBRC 14477 or equivalent species or strains). Use an inoculum of 100 CFU or 100 CCU or less for the positive controls.

Incubate the cell cultures for 3 – 6 days at 35 – 38°C in an atmosphere of air containing 5% carbon dioxide.

Examine the cell cultures after fixation for the presence of mycoplasma by epifluorescence microscopy (400 to 600 times magnification or greater) using a DNA-binding fluorochrome, such as bisbenzimide or an equivalent stain. Compare the microscopical appearance of the test cultures with that of the negative and positive controls.

Procedure

1) Aseptically place a sterilized glass cover slip into each cell culture dish (35 mm diameter).

2) Prepare Vero cell suspension in Eagle's minimum essential medium containing 10% fetal calf serum at a concentration of 1×10^4 cells per mL. The fetal calf serum should be tested and confirmed to be free from mycoplasma prior to use.

3) Inoculate aliquots of 2 mL of the Vero cell suspension into each culture dish. Ensure that the cover slips are completely submerged, and not floating on the surface of the culture medium. Incubate the cultures at 35 – 38°C in an atmosphere of air containing 5% carbon dioxide for one day, so that the cells are attached to the glass cover slip.

4) Replace 2 mL of the culture medium with fresh medium, then add 0.5 mL of the test sample (cell culture supernatant) to each of two or more culture dishes. Perform the same procedure for the positive (2 types of mycoplasmas, such as *M. hyorhinis* (ATCC 29052, ATCC 17981, NBRC 14858 or equivalent species or strains) and *M. orale* (ATCC 23714, NBRC 14477 or equivalent species or strains) and negative controls.

5) Incubate the cultures for 3 – 6 days at 35 – 38°C in an atmosphere of air containing 5% carbon dioxide.

6) Remove the culture medium from the culture dishes, and add 2 mL of a mixture of acetic acid (100) and methanol (1:3) (fixative) to each dish; then, allow them to stand for 5 minutes.

7) Remove the fixative from each dish, then add the same amount of fixative again, and leave the dishes to stand for 10 minutes.

8) Remove the fixative and then completely air-dry all the dishes.

9) Add 2 mL of bisbenzimide fluorochrome staining solution to each culture dish. Cover the dishes and let them stand at room temperature for 30 minutes.

10) Aspirate the staining solution and rinse each dish with 2 mL of distilled water 3 times. Take out the glass cover slips and dry them.

11) Mount each cover slip with a drop of a mounting fluid. Blot off surplus mounting fluid from the edges of the cover slips.

12) Examine by epifluorescence microscopy at 400 to 600 times magnification or greater.

13) Compare the microscopic appearance of the test sample with that of the negative and positive controls.

14) The test result is judged to be positive if there are more than 5 cells per 1000 (0.5%) that have minute fluorescent spots that appear to surround, but are outside, the cell nucleus.

C. Nucleic Acid Amplification Test (NAT)

Nucleic acid amplification test (NAT) is a detection method of genes or mRNA transcribed from genes of target cells or viruses by enzymatic amplification with specific primers for target nucleic acid sequences, and the amplified products are detected by several ways. When NAT is used for detection of mycoplasma, high sensitivity detection is expected for the presence or absence of the target sequence derived from mycoplasma by amplification of nucleic acid extracted from a test sample (cell suspension or cell culture supernatant) with specific primers/probes. NAT indicates the presence of a target sequence and not necessarily the presence of viable mycoplasmas.

A number of different NAT methods are available. This general information does not prescribe a particular method. NAT method applied should be validated for sufficient sensitivity, specificity, and robustness of results that remain unaffected by small variations in extraction method parameters or in composition of the reaction mix. Any NAT method is available if the specificity and the sensitivity is properly validated as described in this section. Where a commercial kit is used, certain elements of the validation may be carried out

by the manufacturer and information provided to the user. However, it should be remembered that the different results might be obtained by user depending on the instrument used and the target cells tested. The user should confirm the manufacturer's validation results by own facilities. Especially, when the target cell substrate is different from cells validated by manufacturer, detection limit and reproducibility of the kit should be confirmed with the cells of interest. When the user's extraction method or instruments used for detection etc. are different from the method or the instrument specified by manufacturer, the employed method or the instrument should be validated.

In addition, when the information on the primers/probes or the kit reagents may not be available from the manufacturer, countermeasure is required to obtain the information from the manufacturer about the modification of the kit production when modified. If the composition of the kit reagents is modified, user should confirm that the detection limit and the detection accuracy of the modified kit for target mycoplasma is comparable to the previous one, as needed. On the other hand, appropriate alternative method should be considered, since the production of the kit may be discontinued.

Basically, cell suspension but not cell culture supernatant will be used as a test sample, since mycoplasma contaminated in cell culture mainly growth in cell-dependent manner. When cell culture supernatant is used as test samples, validation is required that the method employed is able to fully detect the mycoplasma contamination in cell cultures.

NAT may be used instead of methods A and/or B, after suitable validation described below, and the validation revealed sufficient sensitivity for all of the listed mycoplasma species.

In order to increase the detection sensitivity of nucleic acid derived from infectious mycoplasma, it is possible to perform NAT after enrichment of mycoplasma that may be present in test samples by culturing with Vero cells. In this case, again, validation is required to show sufficient sensitivity for all of the listed mycoplasma species.

C-1. Mycoplasma testing by NAT

The tests should include both a positive control (run control) (such as *M. hyorhinis* (ATCC 17981, NBRC 14858 or equivalent species or strains) of 100 CFU or 100 CCU or less) and a negative control. The mycoplasma strains used for the positive control tests should be those within a low number of passages obtained from an official or suitably accredited agency, and handled appropriately. The unit of inoculation should be determined before use. When cell suspension is used as a test sample, preliminary test is required for the effect of cellular nucleic acid to NAT with the cells confirmed to be mycoplasma-free as a negative control, and confirm that no positive signals are obtained from the negative control. The test result is judged to pass the test if no mycoplasma sequences are amplified from the test sample.

C-2. Precautions for the test

Because NAT enables the detection of trace amounts of nucleic acid, false positive results may be obtained by contamination of the facilities, instruments and reagents etc. with amplified products. To prevent the risk of contamination, wherever possible, each step of the storage and preparation of reagents, the extraction of nucleic acid, the amplification of nucleic acid, and the detection of amplified products should be performed in separate facilities or equipments with special precautions for handling. To exclude false-positive results by contamination of carry-over amplified products, Uracil-N-glycosylase (UNG) procedure may be available. To exclude the false-negative results by low efficiency of extraction or interfering substances for NAT in test samples, simultaneously detection of house-keeping genes of the test cells as internal control is recommended.

On the other hand, if automatic closed system from extraction to amplification is used to prevent cross-contamination, segregation of the area is not always required. However, measures to prevent contamination are required when disposing the amplified products from the automatic system.

C-3. Validation of NAT for the detection of mycoplasmas

NAT methods for the detection of target sequences are either qualitative or quantitative tests. To detect mycoplasma contamination of cell substrates, qualitative tests are adequate and may be considered to be limit tests. This section describes methods to validate qualitative NAT analytical procedures for assessing mycoplasma contamination. These validation methods may also be applicable for quantitative NAT with optimal cut-off point.

The most important parameters for validation of the analytical procedure are the specificity and the detection limit. In addition, the robustness of the analytical procedure should be evaluated. Note that for the purpose of this document, validation of NAT method is defined as the complete procedure from extraction of nucleic acid to detection of the amplified products.

Where commercial kits are used for a part or all of the analytical procedure, documented full validation data already covered by the kit manufacturer can replace validation data by the user, and a full validation by the user is unnecessary. Nevertheless, the performance of the kit with respect to its intended use and user's test system should be demonstrated by the user (e.g. specificity, detection limit).

NAT may be used as:

- a test for in-process control purposes;
- an alternative method to replace methods A and/or B.

This section will thus separate these 2 objectives by presenting first a guideline for the validation of the NAT themselves, and second, a guideline for a comparability study between NAT and methods A or B.

Mycoplasma reference strains evaluated for concentration either in CFUs or equivalent copies are required at various stages during validation of specificity or detection limit of NAT. During routine application of the test, mycoplasma reference strains or the test sample calibrated for concentration using reference strains are used as positive controls. In the test, mycoplasma or mycoplasma nucleic acid (e.g. plasmid) may be used as a positive control. Mycoplasma is required for validation of the procedure including extraction efficiency.

1) Evaluation parameters

Three parameters should be evaluated: specificity, detection limit, and robustness.

2) Specificity

Specificity of NAT is the ability to unequivocally detect target nucleic acid in the presence of test samples that may be expected to be present. The specificity of NAT is dependent on the choice of primers/probes and the strictness of the test conditions (both of the amplification and the detection steps).

It is important to use primers/probes by choosing nucleic acid sequences that are specific and well conserved for a wide range of mycoplasmas (the bacterial class *Mollicutes* such as the genus *Mycoplasma* and related genera such as *Ureaplasma*, *Spiroplasma*, *Acholeplasma* etc.). The ability of NAT to detect a large panel of mycoplasma species should be demonstrated by experimental results using reference mycoplasmas described in 3), and evaluated only by the the-

oretical analysis of primers/probes comparing with databases is not recommended.

3) Detection limit

The detection limit of an individual analytical procedure is the lowest amount of target nucleic acid in a sample that can be detected but not necessarily quantitated as an exact value. For establishment of the detection limit, a positive cut-off point should be determined for the nucleic acid amplification analytical procedure. The positive cut-off point is the target sequence copies per volume of sample that can be detected in 95% of test runs. This positive cut-off point is influenced by the nucleic acid sequences of target mycoplasma in the individual samples being tested and by factors such as enzyme efficiency, and can result in different 95% cut-off values for individual analytical test runs. To determine the positive cut-off point, a dilution series of characterized and calibrated (either in CFUs or nucleic acid copies) mycoplasma reference strains or international standards should be tested on different days to examine variation between test runs.

For validation of the limit of detection, the following species should be used. These species represent an optimal selection in terms of the frequency of occurrence as contaminants of mammalian culture cells used for production of biotechnological/biological products, phylogenetic relationships, and animal-derived components used during culture and production processes. Note that the list is only for validation of NAT and not for used as positive run control in routine tests.

- *Acholeplasma laidlawii* (ATCC 23206, NBRC 14400 or equivalent strains)
- *Mycoplasma arginini* (ATCC 23838 or equivalent strains)
- *Mycoplasma fermentans* (ATCC 19989, NBRC 14854 or equivalent strains)
- *Mycoplasma hyorhinis* (ATCC 17981, NBRC 14858 or equivalent strains)
- *Mycoplasma orale* (ATCC 23714, NBRC 14477 or equivalent strains)
- *Mycoplasma pneumoniae* (ATCC 15531, NBRC 14401 or equivalent strains)
- *Mycoplasma salivarium* (ATCC 23064, NBRC 14478 or equivalent strains)

Where there is use of insect or plant cells during production, mycoplasma strains derived from insect or plant (e.g. *Spiroplasma citri*) should be tested in addition to the above list. Where there is use of or exposure to avian cells or materials during production, mycoplasma species derived from avian should be tested whether avian mycoplasmas (e.g. *Mycoplasma synoviae*) can be detected.

For establishment of the detection limit, appropriate dilution series (10-fold or $10^{0.5}$-fold dilution) should be prepared from the undiluted mycoplasma evaluated for concentration (CFU etc.), and performed NAT for each dilution. Based on the dilution factor that shows the limit of the detection, a positive cut-off point should be determined as the minimum number of CFUs of target sequences in the test sample. In case amplified products are separated by electrophoresis and the positive band is detected by fluorescent staining, confirmation is required whether no positive band is appeared from the test sample of mycoplasma-free cells. Detection using quantitative real-time PCR requires to set an adequate cut-off point of amplification cycles, and the setting of the cut-off point should be validated. Since extraction efficiency of nucleic acid from the test sample affects the detection sensitivity, detection of mycoplasma in cell suspension should be evaluated.

For each mycoplasma reference strain described above, at least 3 independent 10-fold dilution series should be tested, with a sufficient number of replicates at each dilution to give a total number of 24 test results for each dilution, to enable a statistical analysis of the results. For example, a laboratory may test 3 dilution series on different days with 8 replicates for each dilution, 4 dilution series on different days with 6 replicates for each dilution, or 6 dilution series on different days with 4 replicates for each dilution. In order to keep the number of dilutions at a manageable level, a preliminary test should be performed to obtain a preliminary value for the positive cut-off point (i.e. the highest dilution giving a positive signal). The range of dilutions can then be chosen around the determined preliminary cut-off point. The concentration of mycoplasmas (CFUs, etc.) that can be detected in 95% of test runs can then be calculated using an appropriate statistical evaluation. These results may also serve to evaluate the variability of the analytical procedure.

4) Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage.

The evaluation of robustness should be considered during the development phase. It should show the reliability of the analytical procedure with respect to deliberate variations in method parameters. For NAT, small variations in the method parameters can be crucial. However, the robustness of the method can be demonstrated during its development when small variations in the concentrations of reagents (e.g. $MgCl_2$, primers, and deoxyribonucleotides) are tested. Modifications of extraction kits or extraction procedures as well as different thermal cycler types may also be evaluated.

5) Use of NAT instead of methods A and/or B

NAT may be used instead of methods A (culture method) and/or B (indicator cell culture method). In this case, a comparability study should be carried out. This comparability study should include mainly a comparison of the respective detection limits of the NAT and methods A and/or B. However, specificity (mycoplasma panel detected, putative false positive results) should also be considered.

For the detection limit, acceptability criteria are defined as follows:

- if the alternative method is proposed to replace method A (the culture method), the NAT system should be shown to detect 10 CFU/mL for each mycoplasma test species described in 3).
- if the alternative method is proposed to replace method B (the indicator cell culture method), the NAT system should be shown to detect 100 CFU/mL for each mycoplasma test species described in 3).

For both cases, suitable standards calibrated for the number of CFUs may be used for establishing that these acceptability criteria are reached.

One of the following 2 strategies can be used to perform this comparability study:

- perform the NAT alternative method in parallel with the methods A or B to evaluate simultaneously the detection limit of both methods using the same samples of calibrated strains with CFUs.
- compare the performance of the NAT alternative method using previously obtained data from methods A or B. In this case, calibration of CFUs of reference strains used for both validations as well as their stabilities should be described carefully.

Alternatively, comparability of detection limit may be demonstrated by detection of nucleic acid copies or etc. of mycoplasma in test samples. In this case, the relation be-

tween CFUs and nucleic acid copies for the reference preparations should be previously established.

6) Controls

• Internal controls: For validation, internal controls are useful to confirm appropriate amplification without effect of inhibitory substances derived from test samples. Internal controls are also necessary for routine verification of extraction and absence of inhibition to NAT. The internal control may contain the primer binding-site, or some other suitable sequence may be used. It is preferably added to the test material before isolating the nucleic acid and therefore acts as an overall control for extraction, reverse transcription, amplification, and detection. Cellular genes derived from test samples may also be used as the internal control.

• External controls: The external positive control contains a defined number of target-sequence copies or CFUs from one or more suitable species of mycoplasma chosen from those used during validation of the test conditions. One of the positive controls is set close to the positive cut-off point to demonstrate that the expected sensitivity is achieved. The external negative control contains no target sequence but does not necessarily represent the same matrix as the test article.

7) Interpretation of results

The primers/probes used may also amplify non-mycoplasma nucleic acid, leading to false-positive results. Procedures are established at the time of validation for dealing with confirmation of positive results, where necessary.

C-4 Method of cultivating mycoplasma with Vero cells

1) Use at least two cell culture dishes for each of the test sample, positive control and negative control.

2) Into each cell culture dish (35 mm diameter), inoculate 2 mL of the Vero cell suspension (1×10^4 cells per mL) in Eagle's minimum essential medium containing 10% fetal calf serum (tested in advance using the NAT method to verify that it does not contain any detectable mycoplasma DNA). Incubate the cultures at 35 – 38°C in an atmosphere of air containing 5% carbon dioxide for one day.

3) Replace the culture media with fresh media, and add 0.5 mL of the test sample (cell culture supernatant) to each of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or 100 CCU or less of *M. hyorhinis* (ATCC 17981, NBRC 14858 or equivalent species or strains)) and negative controls.

4) Incubate the Vero cell culture dishes for the test sample, positive and negative controls for 3 – 6 days at 35 – 38°C in an atmosphere of air containing 5% carbon dioxide.

Peptide Mapping

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Peptide mapping is an identity test for proteins, especially those obtained by r-DNA technology. It involves the chemical or enzymatic treatment of a protein, resulting in the formation of peptide fragments, followed by separation and identification of the fragments in a reproducible manner. It is a powerful test that is capable of identifying single amino acid changes resulting from events such as errors in the reading of complementary DNA (cDNA) sequences or point mutations. Peptide mapping is a comparative procedure because the information obtained, compared to a reference standard or reference material similarly treated, confirms the primary structure of the protein, is capable of detecting whether alterations in structure have occurred, and demonstrates process consistency and genetic stability. Each protein presents unique characteristics which must be well understood so that the scientific and analytical approaches permit validated development of a peptide map that provides sufficient specificity.

This chapter provides detailed assistance in the application of peptide mapping and its validation to characterize the desired protein product, to evaluate the stability of the expression construct of cells used for recombinant DNA products, to evaluate the consistency of the overall process and to assess product stability as well as to ensure the identity of the protein product, or to detect the presence of protein variant.

1. Peptide Map

Peptide mapping is not a general method, but involves developing specific maps for each unique protein. Although the technology is evolving rapidly, there are certain methods that are generally accepted. Variations of these methods will be indicated, when appropriate, in specific monographs.

A peptide map may be viewed as a fingerprint of a protein and is the end product of several chemical processes that provide a comprehensive understanding of the protein being analyzed. Four major steps are necessary for the development of the procedure: isolation and purification of the protein, if the protein is part of a formulation; selective cleavage of the peptide bonds; chromatographic separation of the peptides; and analysis and identification of the peptides. A test sample is digested and assayed in parallel with a reference standard or a reference material. Complete cleavage of peptide bonds is more likely to occur when enzymes such as endoproteases (e.g., trypsin) are used, instead of chemical cleavage reagents. A map should contain enough peptides to be meaningful. On the other hand, if there are too many fragments, the map might lose its specificity because many proteins will then have the same profiles.

2. Isolation and Purification

Isolation and purification are necessary for analysis of bulk drugs or dosage forms containing interfering excipients and carrier proteins and, when required, will be specified in the monograph. Quantitative recovery of protein from the dosage form should be validated.

3. Selective Cleavage of Peptide Bonds

The selection of the approach used for the cleavage of peptide bonds will depend on the protein under test. This selection process involves determination of the type of cleavage to be employed —enzymatic or chemical— and the type of cleavage agent within the chosen category. Several cleavage agents and their specificity are shown in Table 1. This list is not all-inclusive and will be expanded as other cleavage agents are identified.

3.1. Pretreatment of Sample

Depending on the size or the configuration of the protein, different approaches in the pretreatment of samples can be used. For monoclonal antibodies, the heavy and light chains will need to be separated before mapping. If trypsin is used as a cleavage agent for proteins with a molecular mass greater than 100,000 Da, lysine residues must be protected by citraconylation or maleylation; otherwise, too many peptides will be generated.

3.2. Pretreatment of the Cleavage Agent

Pretreatment of cleavage agents —especially enzymatic agents— might be necessary for purification purposes to ensure reproducibility of the map. For example, trypsin used as a cleavage agent will have to be treated with tosyl-L-phenylalanine chloromethyl ketone to inactivate chymotrypsin. Other methods, such as purification of trypsin by HPLC

Table 1 Examples of cleavage agents

Type	Agent	Specificity
Enzymatic	Trypsin (EC 3.4.21.4)	C-terminal side of Arg and Lys
	Chymotrypsin (EC 3.4.21.1)	C-terminal side of hydrophobic residues (e.g., Leu, Met, Ala, aromatics)
	Pepsin (EC 3.4.23.1 & 2)	Nonspecific digest
	Lysyl endopeptidase (Lys-C endopeptidase) (EC 3.4.21.50)	C-terminal side of Lys
	Glutamyl endopeptidase (from *S. aureus* strain V8) (EC 3.4.21.19)	C-terminal side of Glu and Asp
	Peptidyl-Asp metallo endopeptidase (Endoproteinase Asp-N) (EC 3.24.33)	N-terminal side of Asp
	Clostripain (EC 3.4.22.8)	C-terminal side of Arg
Chemical	Cyanogen bromide	C-terminal side of Met
	2-Nitro-5-thio-cyanobenzoic acid	N-terminal side of Cys
	o-Iodosobenzoic acid	C-terminal side of Trp and Tyr
	Dilute acid	Asp and Pro
	BNPS-skatole	Trp

or immobilization of enzyme on a gel support, have been successfully used when only a small amount of protein is available.

3.3. Pretreatment of the Protein

Under certain conditions, it might be necessary to concentrate the sample or to separate the protein from added substances and stabilizers used in formulation of the product, if these interfere with the mapping procedure. Physical procedures used for pretreatment can include ultrafiltration, column chromatography, and lyophilization. Other pretreatments, such as the addition of chaotropic agents (e.g., urea) can be used to unfold the protein prior to mapping. To allow the enzyme to have full access to cleavage sites and permit some unfolding of the protein, it is often necessary to reduce and alkylate the disulfide bonds prior to digestion.

Digestion with trypsin can introduce ambiguities in the tryptic map due to side reactions occurring during the digestion reaction, such as nonspecific cleavage, deamidation, disulfide isomerization, oxidation of methionine residues, or formation of pyroglutamic groups created from the deamidation of glutamine at the N-terminal side of a peptide. Furthermore, peaks may be produced by autohydrolysis of trypsin. Their intensities depend on the ratio of trypsin to protein. To avoid autohydrolysis, solutions of proteases may be prepared at a pH that is not optimal (e.g., at pH 5 for trypsin), which would mean that the enzyme would not become active until diluted with the digest buffer.

3.4. Establishment of Optimal Digestion Conditions

Factors that affect the completeness and effectiveness of digestion of proteins are those that could affect any chemical or enzymatic reactions.

(i) pH: The pH of the digestion mixture is empirically determined to ensure the optimal performance of the given cleavage agent. For example, when using cyanogen bromide as a cleavage agent, a highly acidic environment (e.g., pH 2, formic acid) is necessary; however, when using trypsin as a cleavage agent, a slightly alkaline environment (pH 8) is optimal. As a general rule, the pH of the reaction milieu should not alter the chemical integrity of the protein during the digestion and should not change during the course of the fragmentation reaction.

(ii) Temperature: A temperature between 25°C and 37°C is adequate for most digestions. The temperature used is intended to minimize chemical side reactions. The type of protein under test will dictate the temperature of the reaction milieu, because some proteins are more susceptible to denaturation as the temperature of the reaction increases. For example, digestion of recombinant bovine somatropin is conducted at 4°C, because at higher temperatures it will precipitate during digestion.

(iii) Time: If sufficient sample is available, a time course study is considered in order to determine the optimum time to obtain a reproducible map and avoid incomplete digestion. Time of digestion varies from 2 to 30 hours. The reaction is stopped by the addition of an acid which does not interfere in the tryptic map or by freezing.

(iv) Amount of Cleavage Agent: Although excessive amounts of cleavage agent are used to accomplish a reasonably rapid digestion time (i.e., 6 to 20 hours), the amount of cleavage agent is minimized to avoid its contribution to the chromatographic map pattern. A protein to protease ratio between 20:1 and 200:1 is generally used. It is recommended that the cleavage agent can be added in two or more stages to optimize cleavage. Nonetheless, the final reaction volume remains small enough to facilitate the next step in peptide mapping —the separation step. To sort out digestion artifacts that might be interfering with the subsequent analysis, a blank determination is performed, using a digestion control with all the reagents, except the test protein.

4. Chromatographic Separation

Many techniques are used to separate peptides for mapping. The selection of a technique depends on the protein being mapped. Techniques that have been successfully used for separation of peptides are shown in Table 2. In this section, a most widely used reverse-phase high performance liquid chromatography (RP-HPLC) is described as one of the procedures of chromatographic separation.

The purity of solvents and mobile phases is a critical factor in HPLC separation. HPLC-grade solvents and water that are commercially available are recommended for RP-HPLC. Dissolved gases present a problem in gradient systems where the solubility of the gas in a solvent may be less in a mixture than in a single solvent. Vacuum degassing and agitation by sonication are often used as useful degassing procedures. When solid particles in the solvents are drawn into the HPLC system, they can damage the sealing of pump valves or clog the top of the chromatographic column. Both pre- and post-pump filtration is also recommended.

4.1. Chromatographic Column

The selection of a chromatographic column is empirically determined for each protein. Columns with 100 Å or 300 Å pore size with silica support can give optimal separation. For smaller peptides, octylsilane chemically bonded to totally porous silica articles, 3 to 10 μm in diameter (L7) and octadecylsilane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 μm in diameter (L1) column packings are more efficient than the butyl silane chemically bonded to totally porous silica particles, 5 to 10 μm in diameter

Table 2 Techniques used for the separation of peptides

Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)
Ion-Exchange Chromatography (IEC)
Hydrophobic Interaction Chromatography (HIC)
Polyacrylamide Gel Electrophoresis (PAGE), nondenaturating
SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)
Capillary Electrophoresis (CE)
Paper Chromatography-High Voltage (PCHV)
High-Voltage Paper Electrophoresis (HVPE)

(L26) packing.

4.2. Solvent

The most commonly used solvent is water with acetonitrile as the organic modifier to which less than 0.1% trifluoroacetic acid is added. If necessary, add isopropyl alcohol or n-propyl alcohol to solubilize the digest components, provided that the addition does not unduly increase the viscosity of the components.

4.3. Mobile Phase

Buffered mobile phases containing phosphate are used to provide some flexibility in the selection of pH conditions, since shifts of pH in the 3.0 to 5.0 range enhance the separation of peptides containing acidic residues (e.g., glutamic and aspartic acids). Sodium or potassium phosphates, ammonium acetate, phosphoric acid, and a pH between 2 and 7 (or higher for polymer-based supports) have also been used with acetonitrile gradients. Acetonitrile containing trifluoroacetic acid is used quite often.

4.4. Gradient Selection

Gradients can be linear, nonlinear, or include step functions. A shallow gradient is recommended in order to separate complex mixtures. Gradients are optimized to provide clear resolution of one or two peaks that will become ''marker'' peaks for the test.

4.5. Isocratic Selection

Isocratic HPLC systems using a single mobile phase are used on the basis of their convenience of use and improved detector responses. Optimal composition of a mobile phase to obtain clear resolution of each peak is sometimes difficult to establish. Mobile phases for which slight changes in component ratios or in pH significantly affect retention times of peaks in peptide maps should not be used in isocratic HPLC systems.

4.6. Other Parameters

Temperature control of the column is usually necessary to achieve good reproducibility. The flow rates for the mobile phases range from 0.1 to 2.0 mL per minute, and the detection of peptides is performed with a UV detector at 200 to 230 nm. Other methods of detection have been used (e.g., postcolumn derivatization), but they are not as robust or versatile as UV detection.

4.7 Validation

This section provides an experimental means for measuring the overall performance of the test method. The acceptance criteria for system suitability depend on the identification of critical test parameters that affect data interpretation and acceptance. These critical parameters are also criteria that monitor peptide digestion and peptide analysis. An indicator that the desired digestion endpoint was achieved is by the comparison with a reference standard or reference material, which is treated exactly as the article under test. The use of a reference standard or reference material in parallel with the protein under test is critical in the development and establishment of system suitability limits. In addition a specimen chromatogram should be included with the reference standard or reference material for additional comparison purposes. Other indicators may include visual inspection of protein or peptide solubility, the absence of intact protein, or measurement of responses of a digestion-dependent peptide. The critical system suitability parameters for peptide analysis will depend on the particular mode of peptide separation and detection and on the data analysis requirements.

When peptide mapping is used as an identification test, the system suitability requirements for the identified peptides covers selectivity and precision. In this case, as well as when identification of variant protein is done, the identification of the primary structure of the peptide fragments in the peptide map provides both a verification of the known primary structure and the identification of protein variants by comparison with the peptide map of the reference standard/reference material for the specified protein. The use of a digested reference standard or reference material for a given protein in the determination of peptide resolution is the method of choice. For an analysis of a variant protein, a characterized mixture of a variant and a reference standard or reference material can be used, especially if the variant peptide is located in a less-resolved region of the map. The index of pattern consistency can be simply the number of major peptides detected. Peptide pattern consistency can be best defined by the resolution of peptide peaks. Chromatographic parameters —such as peak-to-peak resolution, maximum peak width, peak area, peak tailing factors, and column efficiency— may be used to define peptide resolution. Depending on the protein under test and the method of separation used, single peptide or multiple peptide resolution requirements may be necessary.

The replicate analysis of the digest of the reference standard or reference material for the protein under test yields measures of precision and quantitative recovery. Recovery of the identified peptides is generally ascertained by the use of internal or external peptide standards. The precision is expressed as the relative standard deviation (RSD). Differences in the recovery and precision of the identified peptides are expected; therefore, the system suitability limits will have to be established for both the recovery and the precision of the identified peptides. These limits are unique for a given protein and will be specified in the individual monograph.

Visual comparison of the relative retention times, the peak responses (the peak area or the peak height), the number of peaks, and the overall elution pattern is completed initially. It is then complemented and supported by mathematical analysis of the peak response ratios and by the chromatographic profile of a 1:1 (v/v) mixture of sample and reference standard or reference material digest. If all peaks in the sample digest and in the reference standard or reference material digest have the same relative retention times and peaks response ratios, then the identity of the sample under test is confirmed.

If peaks that initially eluted with significantly different relative retention times are then observed as single peaks in the 1:1 mixture, the initial difference would be an indication of system variability. However, if separate peaks are observed in the 1:1 mixture, this would be evidence of the nonequivalence of the peptides in each peak. If a peak in the 1:1 mixture is significantly broader than the corresponding peak in the sample and reference standard or reference material digest, it may indicate the presence of different peptides. The use of computer-aided pattern recognition software for the analysis of peptide mapping data has been proposed and applied, but issues related to the validation of the computer

software preclude its use in a compendial test in the near future. Other automated approaches have been used that employ mathematical formulas, models, and pattern recognition. Such approaches are, for example, the automated identification of compounds by IR spectroscopy and the application of diode-array UV spectral analysis for identification of peptides. These methods have limitations due to inadequate resolutions, co-elution of fragments, or absolute peak response differences between reference standard or reference material and sample fragments.

The numerical comparison of the retention times and peak areas or peak heights can be done for a selected group of relevant peaks that have been correctly identified in the peptide maps. Peak areas can be calculated using one peak showing relatively small variation as an internal reference, keeping in mind that peak area integration is sensitive to baseline variation and likely to introduce error in the analysis. Alternatively, the percentage of each peptide peak height relative to the sum of all peak heights can be calculated for the sample under test. The percentage is then compared to that of the corresponding peak of the reference standard/reference material. The possibility of auto-hydrolysis of trypsin is monitored by producing a blank peptide map, that is, the peptide map obtained when a blank solution is treated with trypsin.

The minimum requirement for the qualification of peptide mapping is an approved test procedure that includes system suitability as a test control. In general, early in the regulatory process, qualification of peptide mapping for a protein is sufficient. As the regulatory approval process for the protein progresses, additional qualifications of the test can include a partial validation of the analytical procedure to provide assurance that the method will perform as intended in the development of a peptide map for the specified protein.

5. Analysis and Identification of Peptides

This section gives guidance on the use of peptide mapping during development in support of regulatory applications.

The use of a peptide map as a qualitative tool does not require the complete characterization of the individual peptide peaks. However, validation of peptide mapping in support of regulatory applications requires rigorous characterization of each of the individual peaks in the peptide map. Methods to characterize peaks range from N-terminal sequencing of each peak followed by amino acid analysis to the use of mass spectroscopy (MS).

For characterization purposes, when N-terminal sequencing and amino acids analysis are used, the analytical separation is scaled up. Since scale-up might affect the resolution of peptide peaks, it is necessary, using empirical data, to assure that there is no loss of resolution due to scale-up. Eluates corresponding to specific peptide peaks are collected, vacuum-concentrated, and chromatographed again, if necessary. Amino acid analysis of fragments may be limited by the peptide size. If the N-terminus is blocked, it may need to be cleared before sequencing. C-terminal sequencing of proteins in combination with carboxypeptidase digestion and MALDI-TOF MS can also be used for characterization purposes.

The use of MS for characterization of peptide fragments is by direct infusion of isolated peptides or by the use of on-line LC-MS for structure analysis. In general, it includes electrospray and MALDI-TOF MS analyzer as well as fast atom bombardment (FAB). Tandem MS has also been used to sequence a modified protein and to determine the type of amino acid modification that has occurred. The comparison of mass spectra of the digests before and after reduction provides a method to assign the disulfide bonds to the various sulfhydryl-containing peptides.

If regions of the primary structure are not clearly demonstrated by the peptide map, it might be necessary to develop a secondary peptide map. The goal of a validated method of characterization of a protein through peptide mapping is to reconcile and account for at least 95% of the theoretical composition of the protein structure.

Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices of Japanese Pharmacopoeia and Other Standards

Introduction

The Official Gazette issued on March 29, 2002 announced that General Notices of the Japanese Pharmacopoeia and other standards were amended to add a provision that ''When a drug product or a drug substance which is used to manufacture a drug product, is manufactured from a raw material of animal origin, the animal in question should be in principle a healthy subject, if not otherwise provided.''.

The Notice Iyaku-hatsu No. 0329001, which was issued on the same date, provided that ''Healthy subject herein provided is the animal which does not cause any disease or any infection to human being at an appropriate production process and use of the drug product, and as for the oral or external drug for example, the animal, as its raw material of animal origin, should be confirmed at this stage to meet the Food Standard. It has to be noted that this standard of healthy subject has to be revised timely taking into account the up-to-date information with respect to the amphixenosis infections common between human beings and animals.''.

This General Information describes safety assurance against infection associated with the use of drugs, which are manufactured from raw materials of animal origin, to follow up the Notice as mentioned above.

1. Basic concept

When drugs derived from raw materials of animal origin including human are used, it is important to take into account any possibility that communicable disease agents such as virus may cause infectious disease or any possible hazards to patients. In such case, it goes without saying that the primary subject that has to be considered is the absence of any infectious agents such as virus in the raw materials of animal origin including human as the source of the drug. More important point is whether there is any possibility of transmission of infectious agents when the drugs containing such infectious agents are administered to patient. The eligibility of animals including human, as the source of raw materials of drugs, in other words ''the subject which is free from any disease or transmission of infectious agents that is infectious to human being at an appropriate production process and use of the drug product'' is that ''The drug should be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animals including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indications of the final product.''

2. Animals including human as the source of raw materials of drugs

The most clear and appropriate preventive measures against infection to human being due to administration of drugs which are derived from animals including human are to assure the absence of any infectious agents such as virus in its raw materials or an appropriate critical raw material by either of the following: (1) the use of raw materials of healthy animal origin, which are proved to be free from communicable disease agents to human, or (2) the use of appropriate critical raw materials (e.g., cell substrate, blood plasma, pooled urine after some treatments) for drug production, which are proved to be free from communicable disease agents after certain appropriate processing on raw materials of animal origin.

As for raw materials of drugs of human origin, cell, tissue, blood, placenta, urine, etc. are used. Whenever it is possible for each donor of such raw materials to be asked or inspected about his (her) health condition, the appropriateness as a donor should be confirmed at this stage from the standpoint of safety concerning communicable disease agents such as virus.

For example, ''Basic concept on handling and use of a drug product, etc. which is derived from cell/tissue'' (Attachment 1 of the Notice Iyaku-Hatsu No. 1314 dated December 26, 2000) issued by the Director-General of the Medicinal Safety Bureau, Ministry of Health and Welfare, states that since the cell/tissue supplied by a human donor comes to be applied to patients without processing through any sufficient inactivation or removal of communicable disease agents, the selection and qualification criteria on such donor has to be established. These criteria are to be composed with the respect to the check items on the case history and the physical conditions as well as the test items on the various transmission of infectious agents through cell/tissue, and that the appropriateness of these criteria has to be clarified. Hepatitis Type-B (HBV), Hepatitis Type-C (HCV), Human Immune Deficiency Viral infections (HIV), Adult T-Cell Leukemia and Parvovirus B19 Infections should be denied through the interview to the donor and the tests (serologic test, nucleic-acid amplification test, etc.). Further, if necessary, Cytomegalovirus infection and EB Virus infection should be denied by tests. ''Infections caused by bacteria such as Treponema pallidum, Chlamydia, Gonococci, Tubercule bacillus, etc.'', ''septicemia and its suspicious case'', ''vicious tumor'', ''serious metabolic or endocrine-related disorders'', ''collagenosis and haematological disorder'', ''hepatic disease'' and ''dementia (transmissible spongiform encephalopathies and its suspicious case)'' should be checked on the case history or by the interview, etc. and the experience of being transfused or/and transplanted should be checked to confirm eligibility as a donor. The most appropriate check items and test methods then available are to be used, which need to be reconsidered at appropriate timing taking into account the updated knowledge and the progress of the science and the technologies. At screening of a donor, reexaminations has to be made at appropriate timing using the eligible check items and the test methods taking into account the window period (Initial period after infection, in which antibody against bacteria, fungi or virus is not detected.)

In the case of plasma derivatives produced from the donated blood in Japan, the donor should be checked by means of self-assessed report about health conditions, and a serologic check and a nucleic acid amplification test (NAT) on mini pooled plasma targeted for HBV, HCV and HIV should be performed at the stage of donated blood. Further, the plasma material for fractionation should be stored 4 months in minimum so that the arrangement could be taken based on the information available after collection of the blood and the blood infusion to exclude the possibility of using any critical raw material which might cause infection to patients.

On the other hand, as for the materials such as urine which are taken from the unspecified number of the donors and come to be critical raw materials for drug production after some treatments, it is unrealistic and not practical to conduct the tests of virus infection, etc. on the individual donor. Consequently, appropriate tests such as virus test has to be performed on such critical raw materials for drug production.

In the case of the animals besides human, the wild ones should be excluded. Only the animals, which are raised under well sanitarily controlled conditions taken to prevent bacterial contamination or under the effective bacterial pollution monitoring systems, have to be used, and it is recommended that the animals from a colony appropriately controlled under specific pathogen-free (SPF) environment are to be used as far as possible. Further, for the animals regulated under the Food Standard, only the animals that met this standard should be used. It should be confirmed by appropriate tests that the animals were free from pathogen, if necessary.

The concrete measures to avoid transmittance or spread of infectivity of prion, which is considered to be the pathogen of transmissible spongiform encephalopathies (TSEs), as far as possible are the followings: ① avoidance of use of animals, which are raised in the areas where high incidence or high risk of TSEs (Scrapie in sheep and goat, bovine spongiform encephalopathies (BSE) in cattle, chronic wasting disease (CWD) in deer, new type of Creutzfeldt-Jacob-Disease (CJD) in human, etc.) is reported, and humans, who have stayed long time (more than 6 months) in such areas, as raw materials or related substances of drugs; ② avoidance of use of any substances that are derived from the individual infected with scrapie, BSE, CJD, etc.; ③ avoidance of using a material derived from organ, tissue and cell, etc. of high risk of TSEs; and ④ taking appropriate measures basing on the information collected, which includes incidence of TSEs, the results of epidemiological investigation and the experimental research on prion, and incidence of tardive infection on donors after collecting raw materials, etc.

3. Human or animal cells which are used as critical raw materials for drug production

Cell substrates derived from humans or animals are used for drug production. In such case, it is desirable that the humans or the animals, which are the origins of the cell substrates, are healthy subjects. However, it is considered practical that viral safety of the drugs derived from the cell substrates are evaluated on the cells, which are so called critical raw materials for production of such drugs. In such case, the safety should be confirmed through the test and analysis on established cell bank thoroughly with respect to virus etc., as far as possible. The items and the methods of the tests that have been followed in this case are described in detail in the Notice of Japanese version on the internationally accepted ICH Guideline entitled ''Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin'' (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare). In the meantime, it is important how to handle the cell in case that any virus has been detected under the

cell level tests. This Notice describes how to cope with this situation as follows: ''It is recognised that some cell lines used for the manufacture of product will contain endogenous retroviruses, other viruses or viral sequences. In such circumstances, the action plan recommended for manufacturer is described in Section V (Rationale and action plan for viral clearance studies and virus tests on purified bulk) of the Notice. The acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or for causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk.'' For example, it is well known that Type A-, R- and C-endogenous particles like retrovirus are observed in the cells of the rodents used most often for drug production. It is also known that they are not infectious to human and is not dangerous, and CHO cells are generally used for drug production. The established cell lines (e.g., NAMALWA Cell, BALL-1 Cell, etc.) derived from cancer patients are sometimes used, but through the thorough virus tests, etc., their safety are confirmed. The established cell lines are assumed to be safer than the primary cultured cells which are hard to conduct the thorough virus test.

4. Establishment and control of appropriate production process and adherence to the clinical indication of final product for safety assurance

Safety assurance against potential infections at only the level of animals that are source of raw materials of drugs is limited. Further, ''health of animal'' can not be defined univocally, and the various factors have to be taken into account. The final goal of this subject is to protect human from any infectious disease caused by drugs. Achieving this goal, the establishment and control of appropriate production processes of each drug and the adherence to the clinical indications of the final product are important.

As mentioned above, the rodent cells used most often for the production of the drugs are known to have endogenous retrovirus-like particles sometimes. The reason why such cells can be used for the production of the drugs is that multiple measures are applied for safety in the purification stages which include appropriate inactivation or removal processes. There are cases in which the production procedure involves intentional use of a virus or a microorganism. In this case, relevant measures capable of removing or inactivating of such virus or microorganism are appropriately incorporated in the purification process, so that the risk of infection to human can be fully denied and its safety can be assured when it is used as a drug. Further, even in the case that it is difficult to clarify the risk of contamination of the infectious agents or that the raw material are contaminated by viruses etc., the raw material in question may be used for the production of drugs so long as appropriate inactivation or removal processes are introduced, their effectiveness can be confirmed and the safety can be assured by appropriate control of the manufacturing processes under GMP, etc.

5. Conclusion

The qualification of animals including human, as the source of raw materials of drugs, in other words ''the subject which does not cause any infectious diseases to human being at an appropriate production process and use of the drug product'' is that ''the drug has to be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animal including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indication of the final product.''

To cope with this subject, the advanced scientific measures, which actually reflect the updated knowledge and progress of the science and the technology about infectious diseases in human and infection of animal origin, have to be taken into account timely.

SDS-Polyacrylamide Gel Electrophoresis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)—Uniform Percentage Gels

Polyacrylamide gel electrophoresis is used for the qualitative characterisation of proteins in biological preparations, for control of purity and for quantitative determinations.

Analytical gel electrophoresis is an appropriate method with which to identify and to assess the homogeneity of proteins in pharmaceutical preparations. The method is routinely used for the estimation of protein subunit molecular masses and for determination of the subunit compositions of purified proteins.

Ready-to-use gels and reagents are commercially available and can be used instead of those described in this text, provided that they give equivalent results and that they meet the validity requirements given below under Validation of the test.

1. Characteristics of Polyacrylamide Gels

The sieving properties of polyacrylamide gels are established by the three-dimensional network of fibres and pores which is formed as the bifunctional bisacrylamide cross-links adjacent polyacrylamide chains. Polymerisation is usually catalysed by a free radical-generating system composed of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED).

As the acrylamide concentration of a gel increases, its effective pore size decreases. The effective pore size of a gel is operationally defined by its sieving properties; that is, by the resistance it imparts to the migration of macromolecules. There are limits on the acrylamide concentrations that can be used. At high acrylamide concentrations, gels break much more easily and are difficult to handle. As the pore size of a gel decreases, the migration rate of a protein through the gel decreases. By adjusting the pore size of a gel, through manipulating the acrylamide concentration, the resolution of the method can be optimised for a given protein product. Thus, a given gel is physically characterised by its respective composition of acrylamide and bisacrylamide.

In addition to the composition of the gel, the state of the protein is an important component to the electrophoretic mobility. In the case of proteins, the electrophoretic mobility is dependent on the pK value of the charged groups and the size of the molecule. It is influenced by the type, the concentration and the pH of the buffer, by the temperature and the field strength, and by the nature of the support material.

2. Denaturing Polyacrylamide Gel Electrophoresis

The method cited as an example is limited to the analysis of monomeric polypeptides with a mass range of 14,000 to

100,000 daltons. It is possible to extend this mass range by various techniques (e.g. gradient gels, particular buffer system). For instance, tricine sodium dodecyl sulfate (SDS) gels, using tricine as the trailing ion in the electrophoresis running buffer (instead of glycine as in the method described here), can separate very small proteins and peptides under 10,000-15,000 daltons.

Denaturing polyacrylamide gel electrophoresis using glycine SDS (SDS-PAGE) is the most common mode of electrophoresis used in assessing the pharmaceutical quality of protein products and will be the focus of the example method. Typically, analytical electrophoresis of proteins is carried out in polyacrylamide gels under conditions that ensure dissociation of the proteins into their individual polypeptide subunits and that minimise aggregation. Most commonly, the strongly anionic detergent SDS is used in combination with heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind to SDS, become negatively charged and exhibit a consistent charge-to-mass ratio regardless of protein type. Because the amount of SDS bound is almost always proportional to the molecular mass of the polypeptide and is independent of its sequence, SDS-polypeptide complexes migrate through polyacrylamide gels with mobilities dependent on the size of the polypeptide.

The electrophoretic mobilities of the resultant detergent-polypeptide complexes all assume the same functional relationship to their molecular masses. SDS complexes will migrate toward the anode in a predictable manner, with low molecular mass complexes migrating faster than larger ones. The molecular mass of a protein can therefore be estimated from its relative mobility in calibrated SDS-PAGE and the intensity of a single band relative to other undesired bands in such a gel can be a measure of purity.

Modifications to the polypeptide backbone, such as N- or O-linked glycosylation, can change the apparent molecular mass of a protein since SDS does not bind to a carbohydrate moiety in a manner similar to a polypeptide; therefore, a consistent charge-to-mass ratio is not maintained.

Depending on the extent of glycosylation and other post-translational modifications, the apparent molecular mass of proteins may not be a true reflection of the mass of the polypeptide chain.

2.1. Reducing conditions

Polypeptide subunits and three-dimensional structure are often maintained in proteins by the presence of disulfide bonds. A goal of SDS-PAGE analysis under reducing conditions is to disrupt this structure by reducing disulfide bonds. Complete denaturation and dissociation of proteins by treatment with 2-mercaptoethanol (2-ME) or dithiothreitol (DTT) will result in unfolding of the polypeptide backbone and subsequent complexation with SDS. Using these conditions, the molecular mass of the polypeptide subunits can reasonably be calculated by linear regression (or, more closely, by non linear regression) in the presence of suitable molecular mass standards.

2.2. Non-reducing conditions

For some analyses, complete dissociation of the protein into subunit peptides is not desirable. In the absence of treatment with reducing agents such as 2-ME or DTT, disulfide covalent bonds remain intact, preserving the oligomeric form of the protein. Oligomeric SDS-protein complexes migrate more slowly than their SDS-polypeptide subunits. In addition, non-reduced proteins may not be completely saturated with SDS and, hence, may not bind the detergent in a constant mass ratio. Moreover, intra-chain disulphide bonds constrain the molecular shape, usually in such a way as to reduce the Stokes radius of the molecule, thereby reducing the apparent molecular mass M_r. This makes molecular mass determinations of these molecules by SDS-PAGE less straightforward than analyses of fully denatured polypeptides, since it is necessary that both standards and unknown proteins be in similar configurations for valid comparisons.

3. Characteristics of Discontinuous Buffer System Gel Electrophoresis

The most popular electrophoretic method for the characterisation of complex mixtures of proteins uses a discontinuous buffer system involving two contiguous, but distinct gels: a resolving or separating (lower) gel and a stacking (upper) gel. The two gels are cast with different porosities, pH, and ionic strengths. In addition, different mobile ions are used in the gel and electrode buffers. The buffer discontinuity acts to concentrate large volume samples in the stacking gel, resulting in improved resolution. When power is applied, a voltage drop develops across the sample solution which drives the proteins into the stacking gel. Glycinate ions from the electrode buffer follow the proteins into the stacking gel. A moving boundary region is rapidly formed with the highly mobile chloride ions in the front and the relatively slow glycinate ions in the rear. A localised high-voltage gradient forms between the leading and trailing ion fronts, causing the SDS-protein complexes to form into a thin zone (stack) and migrate between the chloride and glycinate phases. Within broad limits, regardless of the height of the applied sample, all SDS-proteins condense into a very narrow region and enter the resolving gel as a well-defined, thin zone of high protein density. The large-pore stacking gel does not retard the migration of most proteins and serves mainly as an anti-convective medium. At the interface of the stacking and resolving gels, the proteins undergo a sharp increase in retardation due to the restrictive pore size of the resolving gel and the buffer discontinuity, which also contributes to focusing of the proteins. Once in the resolving gel, proteins continue to be slowed by the sieving of the matrix. The glycinate ions overtake the proteins, which then move in a space of uniform pH formed by the 2-amino-2-hydroxymethyl-1,3-propanediol and glycine. Molecular sieving causes the SDS-polypeptide complexes to separate on the basis of their molecular masses.

4. Preparing Vertical Discontinuous Buffer SDS Polyacrylamide Gels

This section describes the preparation of gels using particular instrumentation. This does not apply to pre-cast gels. For pre-cast gels or any other commercially available equipment, the manufacturer's instructions must be used for guidance.

The use of commercial reagents that have been purified in solution is recommended. When this is not the case and where the purity of the reagents used is not sufficient, a pre-treatment is applied. For instance, any solution sufficiently impure to require filtration must also be deionised with a mixed bed (anion/cation exchange) resin to remove acrylic acid and other charged degradation products. When stored according to recommendations, acrylamide/bisacrylamide solutions and solid persulfate are stable for long periods.

4.1. Assembling the gel moulding cassette

Clean the two glass plates (size: e.g. 10 cm × 8 cm), the polytetrafluoroethylene comb, the two spacers and the silicone rubber tubing (diameter e.g. 0.6 mm × 35 cm) with mild detergent and rinse extensively with water, followed by dehydrated alcohol, and allow the plates to dry at room temperature. Lubricate the spacers and the tubing with non-silicone grease. Apply the spacers along each of the two short

sides of the glass plate 2 mm away from the edges and 2 mm away from the long side corresponding to the bottom of the gel. Begin to lay the tubing on the glass plate by using one spacer as a guide. Carefully twist the tubing at the bottom of the spacer and follow the long side of the glass plate. While holding the tubing with one finger along the long side twist again the tubing and lay it on the second short side of the glass plate, using the spacer as a guide. Place the second glass plate in perfect alignment and hold the mould together by hand pressure. Apply two clamps on each of the two short sides of the mould. Carefully apply four clamps on the longer side of the gel mould thus forming the bottom of the gel mould. Verify that the tubing is running along the edge of the glass plates and has not been extruded while placing the clamps. The gel mould is now ready for pouring the gel.

4.2. Preparation of the gel

In a discontinuous buffer SDS polyacrylamide gel, it is recommended to pour the resolving gel, let the gel set, and then pour the stacking gel since the composition of the two gels in acrylamide-bisacrylamide, buffer and pH are different.

4.2.1. Preparation of the resolving gel

In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide for the resolving gel, using the values given in Table 1. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and the TEMED, filter the solution if necessary under vacuum through a cellulose acetate membrane (pore diameter 0.45 μm). Keep the solution under vacuum, while swirling the filtration unit, until no more bubbles are formed in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED as indicated in Table 1, swirl and pour immediately into the gap between the two glass plates of the mould. Leave sufficient space for the stacking gel (the length of the teeth of the comb plus 1 cm). Using a tapered glass pipette, carefully overlay the solution with water-saturated isobutanol. Leave the gel in a vertical position at room temperature to allow polymerisation.

4.2.2. Preparation of the stacking gel

After polymerisation is complete (about 30 min), pour off the isobutanol and wash the top of the gel several times with water to remove the isobutanol overlay and any unpolymerised acrylamide. Drain as much fluid as possible from the top of the gel, and then remove any remaining water with the edge of a paper towel.

In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide, using the values given in Table 2. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and the TEMED, filter the solution if necessary under vacuum through a cellulose acetate membrane (pore diameter: 0.45 μm). Keep the solution under vacuum, while swirling the filtration unit, until no more bubbles are formed in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED as indicated in Table 2. Swirl and pour immediately into the gap between the two glass plates of the mould directly onto the surface of the polymerised resolving gel. Immediately insert a clean polytetrafluoroethylene comb into the stacking gel solution, being careful to avoid trapping air bubbles. Add more stacking gel solution to fill the spaces of the comb completely. Leave the gel in a vertical position and allow to polymerise at room temperature.

4.3. Preparation of the sample

Unless otherwise specified in the specific monograph the samples can be prepared as follows:

Sample solution (non-reducing conditions). Mix equal volumes of: a mixture comprising water plus the preparation or the reference solutions, and concentrated SDS-PAGE sample buffer.

Sample solution (reducing conditions). Mix equal volumes of: a mixture comprising water plus the preparation or the reference solutions, and concentrated SDS-PAGE sample buffer for reducing conditions containing 2-ME (or DTT) as reducing agent.

The concentration prescribed in the monograph can vary depending on the protein and staining method.

Sample treatment: keep for 5 min in a boiling water bath or in a block heater set at 100°C, then chill. (Note that temperature and time may vary in the monograph since protein cleavage may occur during the heat treatment.)

4.4. Mounting the gel in the electrophoresis apparatus and electrophoretic separation

After polymerisation is complete (about 30 min), remove the polytetrafluoroethylene comb carefully. Rinse the wells immediately with water or with the SDS-PAGE running buffer to remove any unpolymerised acrylamide. If necessary, straighten the teeth of the stacking gel with a blunt hypodermic needle attached to a syringe. Remove the clamps on one short side, carefully pull out the tubing and replace the clamps. Proceed similarly on the other short side. Remove the tubing from the bottom part of the gel. Mount the gel in the electrophoresis apparatus. Add the electrophoresis buffers to the top and bottom reservoirs. Remove any bubbles that become trapped at the bottom of the gel between the glass plates. This is best done with a bent hypodermic needle attached to a syringe. Never pre-run the gel before loading the samples, since this will destroy the discontinuity of the buffer systems. Before loading the sample carefully rinse each well with SDS-PAGE running buffer. Prepare the test and reference solutions in the recommended sample buffer and treat as specified in the individual monograph. Apply the appropriate volume of each solution to the stacking gel wells. Start the electrophoresis using the conditions recommended by the manufacturer of the equipment. Manufacturers of SDS-PAGE equipment may provide gels of different surface area and thickness and electrophoresis running time and current/voltage may vary in order to achieve optimal separation. Check that the dye front is moving into the resolving gel. When the dye is near the bottom of the gel, stop the electrophoresis. Remove the gel assembly from the apparatus and carefully separate the glass plates. Remove the spacers, cut off and discard the stacking gel and immediately proceed with staining.

4.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)—Gradient concentration gels

Gradient gels (resolving gels) are prepared with an increasing concentration of acrylamide from the top to the bottom. Preparation of gradient gels requires a gradient forming apparatus. Ready-to-use gradient gels are commercially available with specific recommended protocols.

Gradient gels offer some advantages over fixed concentration gels. Some proteins which co-migrate on fixed concentration gels can be resolved within gradient gels. During electrophoresis the proteins migrate until the pore size stops further progress and therefore a stacking effect occurs, resulting in sharper bands. Per the table below, gradient gels also allow separation of a wider range of proteins molecular masses than on a single fixed concentration gel.

The table below gives suggested compositions of the linear gradient, relating the range of acrylamide concentrations to the appropriate protein molecular ranges. Note that other gradient shapes (e.g. concave) can be prepared for specific

applications.

Acrylamide (%)	Protein range (kDa)
5–15	20–250
5–20	10–200
10–20	10–150
8–20	8–150

Gradient gels are also used for molecular mass determination and protein purity determination.

4.6. Detection of proteins in gels

Coomassie and silver staining are the most common protein staining methods and are described in more detail below. Several other commercial stains, detection methods and commercial kits are available. For example, fluorescent stains are visualised using a fluorescent imager and often provide a linear response over a wide range of protein concentrations, often several orders of magnitude depending on the protein.

Coomassie staining has a protein detection level of approximately 1 to 10 μg of protein per band. Silver staining is the most sensitive method for staining proteins in gels and a band containing 10 ng to 100 ng can be detected. These figures are considered robust in the context of these gels. Improved sensitivity of one or two orders of magnitude has sometimes been reported in the literature.

Coomassie staining responds in a more linear manner than silver staining; however the response and range depend on the protein and development time. Both Coomassie and silver staining can be less reproducible if staining is stopped in a subjective manner, i.e. when the staining is deemed satisfactory. Wide dynamic ranges of reference proteins are very important to use since they help assess the intra-experimental sensitivity and linearity. All gel staining steps are done while wearing gloves, at room temperature, with gentle shaking (e.g. on an orbital shaker platform) and using any convenient container.

4.6.1. Coomassie staining

Immerse the gel in a large excess of Coomassie staining solution and allow to stand for at least 1 h. Remove the staining solution.

Destain the gel with a large excess of destaining solution. Change the destaining solution several times, until the stained protein bands are clearly distinguishable on a clear background. The more thoroughly the gel is destained, the smaller is the amount of protein that can be detected by the method. Destaining can be speeded up by including a few grams of anion-exchange resin or a small sponge in the destaining solution.

The acid-alcohol solutions used in this procedure do not completely fix proteins in the gel. This can lead to losses of some low-molecular-mass proteins during the staining and destaining of thin gels. Permanent fixation is obtainable by allowing the gel to stand in a mixture of 1 volume of trichloroacetic acid, 4 volumes of methanol and 5 volumes of water for 1 h before it is immersed in the Coomassie staining solution.

4.6.2. Silver staining

Immerse the gel in a large excess of fixing solution and allow to stand for 1 h. Remove the fixing solution, add fresh fixing solution and incubate either for at least 1 h or overnight, if convenient. Discard the fixing solution and wash the gel in a large excess of water for 1 h. Soak the gel for 15 min in a 1% *V/V* solution of glutaraldehyde. Wash the gel twice for 15 min in a large excess of water. Soak the gel in fresh silver nitrate reagent for 15 min, in darkness. Wash the gel three times for 5 min in a large excess of water. Immerse the gel for about 1 min in developer solution until satisfactory staining has been obtained. Stop the development by incubation in the blocking solution for 15 min. Rinse the gel with water.

4.7. Recording of the results

Gels are photographed or scanned while they are still wet or after an appropriate drying procedure. Currently, ''gel scanning'' systems with data analysis software are commercially available to photograph and analyse the wet gel immediately.

Depending on the staining method used, gels are treated in a slightly different way. For Coomassie staining, after the destaining step, allow the gel to stand in a 100 g/L solution of glycerol for at least 2 h (overnight incubation is possible). For silver staining, add to the final rinsing a step of 5 min in a 20 g/L solution of glycerol.

Drying of stained SDS Polyacrylamide gels is one of the methods to have permanent documentation. This method frequently results in the ''cracking of gel'' during drying between cellulose films.

Immerse two sheets of porous cellulose film in water and incubate for 5 min to 10 min. Place one of the sheets on a drying frame. Carefully lift the gel and place it on the cellulose film. Remove any trapped air bubbles and pour a few millilitres of water around the edges of the gel. Place the second sheet on top and remove any trapped air bubbles. Complete the assembly of the drying frame. Place in an oven or leave at room temperature until dry.

4.8. Molecular mass determination

Molecular masses of proteins are determined by comparison of their mobilities with those of several marker proteins of known molecular weight. Mixtures of pre-stained and unstained proteins with precisely known molecular masses blended for uniform staining are available for calibrating gels. They are available in various molecular mass ranges. Concentrated stock solutions of proteins of known molecular mass are diluted in the appropriate sample buffer and loaded on the same gel as the protein sample to be studied.

Immediately after the gel has been run, the position of the bromophenol blue tracking dye is marked to identify the leading edge of the electrophoretic ion front. This can be done by cutting notches in the edges of the gel or by inserting a needle soaked in India ink into the gel at the dye front. After staining, measure the migration distances of each protein band (markers and unknowns) from the top of the resolving gel. Divide the migration distance of each protein by the distance travelled by the tracking dye. The normalised migration distances are referred to as the relative mobilities of the proteins (relative to the dye front), or *R*f. Construct a plot of the logarithm of the relative molecular masses (M_r) of the protein standards as a function of the *R*f values. Unknown molecular masses can be estimated by linear regression analysis (more accurately by non-linear regression analysis) or interpolation from the curves of log M_r against *R*f if the values obtained for the unknown samples are positioned along the approximately linear part of the graph.

4.9. Validation of the test

The test is not valid unless the target resolution range of the gel has been demonstrated by the distribution of appropriate molecular mass markers e.g. across 80% of the length of the gel. The separation obtained for the expected proteins must show a linear relationship between the logarithm of the molecular mass and the *R*f. If the plot has a sigmoidal shape then only data from the linear region of the curve can be used in the calculations. Additional validation

Table 1 Preparation of resolving gel

Solution components	Component volumes (mL) per gel mould volume of							
	5 mL	10 mL	15 mL	20 mL	25 mL	30 mL	40 mL	50 mL
6% Acrylamide								
Water	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
Acrylamide solution(1)	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 M Tris (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% Acrylamide								
Water	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
Acrylamide solution(1)	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
1.5 M Tris (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10% Acrylamide								
Water	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
Acrylamide solution(1)	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
1.5 M Tris (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% Acrylamide								
Water	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
Acrylamide solution(1)	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
1.5 M Tris (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
14% Acrylamide								
Water	1.4	2.7	3.9	5.3	6.6	8.0	10.6	13.8
Acrylamide solution(1)	2.3	4.6	7.0	9.3	11.6	13.9	18.6	23.2
1.5 M Tris (pH 8.8)(2)	1.2	2.5	3.6	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% Acrylamide								
Water	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
Acrylamide solution(1)	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
1.5 M Tris (pH 8.8)(2)	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS(3)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS(4)	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED(5)	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution.
(2) 1.5 M Tris (pH 8.8): 1.5 M tris-hydrochloride buffer solution (pH 8.8).
(3) 100 g/L SDS: a 100 g/L solution of sodium dodecyl sulfate.
(4) 100 g/L APS: a 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes rapidly, fresh solutions must be prepared daily.
(5) TEMED: *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

requirements with respect to the test sample may be specified in individual monographs.

Sensitivity must also be validated. A reference protein control corresponding to the desired concentration limit that is run in parallel with the test samples can serve as a system suitability of the experiment.

4.10. Quantification of impurities

SDS-PAGE is often used as a limit test for impurities. When impurities are quantified by normalisation to the main band using an integrating densitometer or image analysis, the responses must be validated for linearity. Note that depending on the detection method and protein as described in the introduction of the section ''Detection of proteins in gels'' the linear range can vary but can be assessed within each run by using one or more control samples containing an appropriate range of protein concentration.

Where the impurity limit is specified in the individual monograph, a reference solution corresponding to that level of impurity should be prepared by diluting the test solution. For example, where the limit is 5%, a reference solution

Table 2 Preparation of stacking gel

Solution components	Component volumes (mL) per gel mould volume of							
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	8 mL	10 mL
Water	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
Acrylamide solution[1]	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.0 M Tris (pH 6.8)[2]	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
100 g/L SDS[3]	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
100 g/L APS[4]	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED[5]	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution.
(2) 1.0 M Tris (pH 6.8): 1 M tris-hydrochloride buffer solution (pH 6.8).
(3) 100 g/L SDS: a 100 g/L solution of sodium dodecyl sulfate.
(4) 100 g/L APS: a 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes rapidly, fresh solutions must be prepared daily.
(5) TEMED: *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

would be a 1:20 dilution of the test solution. No impurity (any band other than the main band) in the electropherogram obtained with the test solution may be more intense than the main band obtained with the reference solution.

Under validated conditions impurities may be quantified by normalisation to the main band using an integrating densitometer or by image analysis.

5. Reagents

(i) 30% acrylamide/bisacrylamide (29:1) solution: Prepare a solution containing 290 g of acrylamide and 10 g of methylenebisacrylamide per litre of water. Filter.

(ii) SDS-PAGE running buffer: Dissolve 151.4 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 721.0 g of glycine and 50.0 g of sodium dodecyl sulfate in water and dilute to 5000 mL with the same solvent. Immediately before use, dilute to 10 times its volume with water and mix. Measure the pH of the diluted solution. The pH is between 8.1 and 8.8.

(iii) SDS-PAGE sample buffer (concentrated): Dissolve 1.89 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium dodecyl sulfate and 50 mg of bromophenol blue in water. Add 25.0 mL of glycerol and dilute to 100 mL with water. Adjust the pH to 6.8 with hydrochloric acid, and dilute to 125 mL with water.

(iv) SDS-PAGE sample buffer for reducing conditions (concentrated): Dissolve 3.78 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.0 g of sodium dodecyl sulfate and 100 mg of bromophenol blue in water. Add 50.0 mL of glycerol and dilute to 200 mL with water. Add 25.0 mL of 2-mercaptoethanol. Adjust to pH 6.8 with hydrochloric acid, and dilute to 250.0 mL with water. Alternatively, dithiothreitol may be used as reducing agent instead of 2-mercaptoethanol. In this case prepare the sample buffer as follows: dissolve 3.78 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.0 g of sodium dodecyl sulfate and 100 mg of bromophenol blue in water. Add 50.0 mL of glycerol and dilute to 200 mL with water. Adjust to pH 6.8 with hydrochloric acid, and dilute to 250.0 mL with water. Immediately before use, add dithiothreitol to a final concentration of 100 mM.

(v) Coomassie staining solution: A 1.25 g/L solution of acid blue 83 in a mixture consisting of 1 volume of glacial acetic acid, 4 volumes of methanol and 5 volumes of water. Filter.

(vi) Developer solution: Dilute 2.5 mL of a 20 g/L solution of citric acid and 0.27 mL of formaldehyde to 500.0 mL with water.

(vii) Fixing solution: To 250 mL of methanol, add 0.27 mL of formaldehyde and dilute to 500.0 mL with water.

(viii) Silver nitrate reagent: To a mixture of 3 mL of concentrated ammonia and 40 mL of 1 M sodium hydroxide, add 8 mL of a 200 g/L solution of silver nitrate, dropwise, with stirring. Dilute to 200 mL with water.

(ix) Destaining solution: A mixture consisting of 1 volume of glacial acetic acid, 4 volumes of methanol and 5 volumes of water.

(x) 1.5 M tris-hydrochloride buffer solution (pH 8.8): Dissolve 90.8 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 400 mL of water. Adjust the pH with hydrochloric acid and dilute to 500.0 mL with water.

(xi) Blocking solution: A 10% *V*/*V* solution of acetic acid.

Surface Plasmon Resonance

Surface plasmon resonance (SPR) optical detection is a method for detecting changes in mass on a sensor chip as changes in the angle at which the reflected light disappears by SPR. This method is used to analyze the binding specificity and binding affinity between substances, and to measure the concentration of analytes in samples.

The devices designed to measure interactions between substances by means of surface plasmon resonance usually adopt a prism-based Kretschmann configuration (Fig. 1). If polarized light is applied in a manner allowing total reflection on the metallic film surface of the sensor chip, an SPR signal (a reduction in intensity in a portion of the reflected light) is observed. The angle at which the SPR signal is produced varies depending on the mass placed on the sensor chip. Thus, the angle at which the SPR signal is produced is changed by binding or dissociation between the molecule immobilized on the sensor chip (the ligand) and the molecule added (the analyte) (Fig. 1). The results of measurement are in the form of a sensorgram presenting changes over time in the SPR signal-producing angle or the response unit (RU) converted from changes in the angle. If the thus-obtained binding and dissociation sensorgram is fitted to the theoretical curve, the ligand/analyte binding rate constant (k_a), dissociation rate constant (k_d) and dissociation constant (K_D =

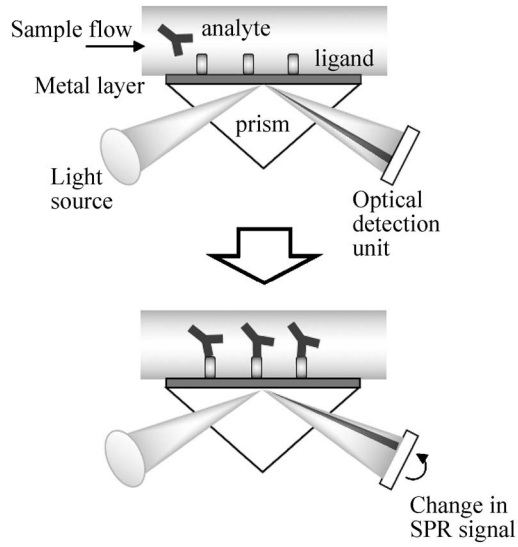


Fig. 1 Principle of SPR measurement (Kretschmann configuration)

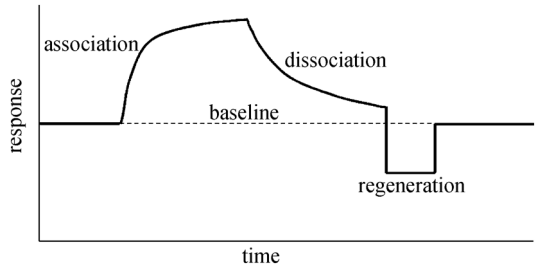


Fig. 2 Example of an SPR sensorgram

k_d/k_a) can be determined. If the response of the analyte in a given sample is compared to the response of the analyte of known concentration, the analyte concentration in a given sample can be determined.

1. Instrument

The instrument usually used for SPR optical detection (a continuous flow system) consists of a light source, an optical detector, a fluid delivery system, a sensor chip insertion port and a data accumulating unit. A sensor chip conjugated with carboxymethyl dextran is usually employed. An appropriate sensor chip tailored to the characteristics of the molecule to be immobilized should be selected. If the sensor chip is set on the device, multiple flow cells are formed on the sensor chip surface, allowing the ligand to be immobilized on each flow cell.

2. Measurement

SPR optical detection is used for testing the binding specificity between the ligand and the analyte, analyzing the binding affinity between the ligand and the analyte, or measuring the analyte concentration. Usually, SPR signals are observed over time while applying the buffer solution to the flow cell and injecting the analyte, and a sensorgram illustrating the binding of the analyte to the ligand immobilized on the sensor chip is obtained. In kinetic analysis designed to analyze the binding affinity from the shape of the sensorgram, a running buffer free of the analyte is applied after the end of analyte injection in order to obtain a dissociation sensorgram. After measurement, a regeneration buffer is applied so that the analyte bound to the ligand can be removed completely (Fig. 2).

2.1. Sample and buffer solution

(1) Analyte solution

Depending on the objective of analysis and the affinity between the molecules to be measured, the sample is diluted to an optimal concentration with the running buffer to yield an analyte solution. If the sample contains insoluble contaminants, they need to be removed by an appropriate method such as centrifugation or filtration using a low protein adsorptive filter.

(2) Running buffer

A buffer appropriate for the ligand and analyte is selected. Addition of salts or detergents to the buffer can be useful in stabilizing the ligand and the analyte. If appropriate, the buffer is filtrated and degassed prior to use. If non-specific binding to the reference flow cell is observed at the time of analyte injection, the buffer needs to be optimized by changing its pH, ionic strength, or other conditions.

(3) Regeneration buffer

As regeneration solution, a buffer with low pH, high pH, or high ionic strength, or a buffer containing surfactants, nonpolar reagents, or chelating agents can be used. The type of buffer to be used varies depending on the material of the flow path of the device. Thus, it is necessary to check the chemical resistance of the device. The ideal setting for regeneration is the one under which complete dissociation of the bound analyte can be achieved without altering the nature of the ligand on the sensor chip surface. If the regeneration buffer is suitable, the baseline after regeneration returns to the baseline recorded before addition of the analyte and it is possible to avoid a reduction in the binding response during repeated measurement. If the setting for regeneration is inappropriate, the level of binding to the ligand decreases during the cycles of measurement, thus affecting the reproducibility of measurement. If the dissociation rate is sufficiently high, the flow of the buffer allows dissociation of the analyte from the ligand, thus making it unnecessary to apply the buffer for regeneration.

2.2. Preparation of the sensor chip used for the measurement

Two methods of binding the ligand to the sensor chip are available: a direct method (direct immobilization of the ligand), and a capture method (capture immobilization of the ligand). In both methods, it is essential to immobilize the ligand while retaining its biological activity and minimizing the impact on binding to the analyte. A ligand of high purity level must be used for the immobilization.

The amount of the ligand to be immobilized is determined with reference to the equation given below.

$$\text{Ligand immobilization quantity} = \frac{\text{Required } R_{max}}{\text{Valency of the ligand}} \times \frac{\text{molecular weight of the ligand}}{\text{molecular weight of the analyte}}$$

The R_{max} (response in the case of maximal binding of the analyte to the ligand) needed for measurement is determined depending on the sensitivity of the instrument used. For analysis of binding affinity, R_{max} needs to be low to avoid steric effects, aggregation and mass transport limitation (a condition under which the amount of analyte supplied serves as a rate-limiting factor for changes in the binding amount due to a shortage in the amount of analyte against the excess ligand). For measurement of the analyte concentration, high R_{max} is desirable to induce a mass transport limitation which increases the dependency of the analyte-binding amount on the concentration and improves the linearity of the calibration curve.

Usually, a control flow cell free of bound ligand is prepared on the sensor chip and is used to detect nonspecific

bindings. The following can serve as the control flow cell: (1) an untreated flow cell, (2) a flow cell having undergone a chemical treatment identical to that for ligand immobilization, and (3) a flow cell having undergone immobilization of a ligand-like molecule having no potential for binding to the analyte. In the case that the ligand is immobilized by the capture method, a flow cell having undergone immobilization of the capturing molecule serves as the control flow cell.

If the immobilized ligand is stable, it is possible to store the sensor chip apart from the device. Such storage is used under conditions such as dry environments or immersion in a buffer solution at low temperature.

Ligand immobilization methods

(1) Direct method

The ligand is immobilized directly via the amino group, thiol group, carboxyl group, aldehyde group or hydroxyl group of the ligand, or via the hydrophobicity of the ligand. The sensor chip usually possesses a layer containing carboxyl groups, which can be used for immobilization. Thus, the ligand is immobilized by the covalent bond. In the case of direct immobilization, the surface often becomes inhomogeneous due to the lack of a uniform direction in the ligand.

(2) Capture method

A capturing molecule having the potential to bind to the ligand is immobilized on the sensor chip so that the ligand can be captured on the chip by binding to the capturing molecule. Capturing molecules include the antibody to the ligand, and antibody to the specific tag sequence allocated to the ligand. If the ligand is an antibody drug, protein A and protein G serve as capturing molecules. If the ligand is a biotinylated molecule, streptavidin is used as a capturing molecule. By the capture method, the direction in the ligand is likely to become uniform. It is important that dissociation of the ligand from the capturing molecule does not occur during measurement. If ligand capturing is performed at each cycle of measurement, there is no need to determine the conditions of regeneration for individual ligands, thus making it easy to set the conditions for measurement.

2.3. Setting the conditions for measurement

(1) Checking the baseline

Before starting the measurement, the stability of the baseline needs to be confirmed. If the baseline is not stable, stabilization should be attempted by the following procedures: administration of several infusions of buffer solution, high ion intensity solution or surfactant solution, application of buffer at a high flow rate, and repetition of the sequence of analyte binding and regeneration.

(2) Flow rate

For analysis of the binding affinity, it is necessary to set the flow rate high in order to suppress the mass transport limitation. For measurement of the analyte concentration, the flow rate must be set low to facilitate the mass transport limitation.

(3) Duration of the analysis

The time needed for analysis in each step (binding, dissociation, etc.) varies depending on the type of measurement. When specific binding is to be tested, the time for binding is set as the time allowing sufficient observation of changes in response. In the case of binding affinity analysis by means of kinetic analysis, a sufficient amount of time should be allowed for dissociation if the response involves slow dissociation. In the case of affinity analysis by means of steady state analysis, the time sufficient for the binding level to reach the equilibrium must be set. In the case of concentration measurement, the time will suffice if it covers the points of measurement capable of yielding an appropriate calibration curve.

(4) Checking R_{max}

If the R_{max} measured exceeds the theoretical R_{max} calculated from the molecular weight of the ligand and analyte toghether with the ligand immobilization quantity and the ligand's binding valency, the following reasons can be considered: inappropriate binding valency, analyte aggregation, or non-specific binding. In such cases, the conditions for measurement or analysis need to be modified.

(5) Checking the reproducibility of measurement

The reproducibility of measurement may be affected if the conditions for measurement are not optimal, and if the ligand is inactivated during repetition of the measuring cycle. Furthermore, if a sensor chip that has been kept stored is used, the reproducibility may be affected by the storage. When the conditions for measurement are set, close attention needs to be paid to reproducibility. The acceptable repeated number of measurements and the acceptable storage period should be set in advance.

2.4. Methods of measurement

2.4.1. Analysis of binding specificity

The analyte is added, and its binding to the ligand is tested on the basis of the binding responses. An appropriate control experiment should be carried out (e.g., demonstrating lack of binding of other analytes to the immobilized ligand), to confirm that the binding observed in the measurement was specific to the analyte.

2.4.2. Analysis of binding affinity

(1) Kinetic analysis

The analyte is injected and its binding is measured. Then, fluid free of the analyte is applied and dissociation of the analyte is measured. Thereafter, complete dissociation of the analyte is achieved by the regeneration step, followed by measurement of the next analyte solution. Another method is analysis of the binding affinity through successive application of analyte solutions at varying concentration levels without interposing a regeneration step. Usually, measurement is performed using the analyte at 5 or more concentrations (between 1/10 of K_D and 10 × K_D).

(2) Steady state analysis

If binding and dissociation take place rapidly, making kinetic analysis or model fitting difficult, steady state analysis is performed. The analyte injection is continued for a period of time until the analyte binding reaches equilibrium. The responses upon reaching equilibrium are recorded. Dissociation of the bound analyte is achieved by regeneration and the next analyte solution is measured. With this method, K_D is calculated as an analyte concentration which yields 1/2 R_{max}. Thus, the analyte concentration needs to be set so that binding to the ligand at the highest analyte concentration is close to saturation.

2.4.3. Measurement of concentration

If measurement is done under the conditions facilitating mass transport limitation, the linearity of the calibration curve is improved, allowing increased accuracy of measurement over a wide range. For this reason, the analyte is injected into the flow cell immobilized with a large amount of ligand, and binding is measured under this setting. Then, dissociation of the analyte is achieved by regeneration, and the next analyte solution is measured. A calibration curve is prepared from the results of measurement of the analyte at known concentration levels. Then, the analyte concentration is calculated. Another available method attempts to calculate the analyte concentration by making use of the proportional relationship between the analyte concentration and diffusion rate, without using a calibration curve.

3. Data analysis

When analysis is performed, the unnecessary part of the sensorgram (e.g., corresponding to capture of the ligand by the capturing molecule, and the regeneration step) is removed, and the response of the control flow cells is subtracted from the response of the ligand-bound flow cells. In addition, the sensorgram baseline is adjusted to 0. As needed, the sensorgram yielded by injection of the buffer for measurement alone is subtracted from the sensorgram yielded by analyte injection.

3.1. Analysis of binding affinity

(1) Kinetic analysis

Kinetic analysis is intended to calculate the parameters for the approximate formula (k_a, k_d, K_D, R_{max}, etc.) from the sensorgram with the use of the reaction rate equation derived from the ligand/analyte binding model. If the ligand binds to the analyte at a ratio of 1:1, the reaction rate equation for association phase is as follows:

$$dR/dt = k_a \times C \times (R_{max} - R) - k_d \times R$$

The reaction rate equation for the dissociation phase is as follows:

$$dR/dt = -k_d \times R$$

(C: analyte concentration; R: response).

A reaction rate equation involving a term corresponding to mass transport limitation or fluid effect can also be used.

The dissociation constant (K_D) serving as an indicator of binding affinity is defined as follows.

$$K_D = k_d/k_a$$

The reaction models employed for analysis of binding affinity include: ① a model of 1:1 ligand/analyte binding, ② a model of 2:1 ligand/analyte binding, like antigen/antibody binding, ③ a model of competitive binding of two analytes to the ligand, ④ a model of one analyte to the ligand possessing two binding sites of different affinity levels, and ⑤ a model of conformational change after 1:1 complex formation. A model theoretically considered as appropriate should be selected, with the results of other biochemical experiments being taken into account.

After the kinetic analysis, an evaluation is needed to determine the appropriateness of the fitting performed. This is accomplished by evaluating the residual plot between the sensorgram obtained and the theoretical curve, or statistical parameters such as χ^2 (mean squared residual, demonstrating the difference between the measured data and the calculated theoretical curve).

Poor fitting to the theoretical curve may be attributable to the following factors: (1) low purity of the reagent, (2) inappropriate method or density of immobilization, (3) inappropriate analyte concentration, (4) nonspecific binding, (5) reduced ligand activity, and (6) inappropriate selection of a reaction model. Thus, the conditions for measurement and the reaction model need to be reviewed. If the RI (refractive index) calculated as a response of the buffer components in the sample is excessively high during analysis of the data on reactions involving rapid binding/dissociation, fitting is performed by fixing the RI to 0. In the case of a poor fitting, the fit might be improved by setting the initial values close to the anticipated values of k_a and k_d.

(2) Steady state analysis

Steady state analysis is as follows. The response reaching equilibrium at each analyte concentration (Y axis) is plotted against the analyte concentration (X axis). Then, regression is performed using the following equation:

Steady state response equation:

$$\text{Equilibrium level at analyte concentration} = \text{analyte concentration} \times \frac{R_{max}}{\text{analyte concentration} + K_D}$$

In this way, the K_D shown by the response of 1/2 R_{max} is determined. The K_D calculated with this equation is the value when 1:1 ligand/analyte binding is assumed. If the actually measured response converges at R_{max}, good analysis is possible. However, if it is in a range lower than R_{max}, the analytical data are less reliable and it is desirable to repeat the measurement by expanding the range of measured concentrations to cover the higher concentration levels.

3.2. Measurement of concentration

From the sensorgram derived by injecting an analyte of known concentration, the slope of the sensorgram near the start of injection or the response at a certain time after starting the injection is determined and plotted against the analyte concentration. A calibration curve is prepared with an appropriate formula for approximation (e.g., the formula for 4-parameter logistic regression, linear regression). Then, the slope or the response is determined from measurement of the sample as an analyte, followed by calculation of the sample concentration from the calibration curve.

4. Application to various tests

4.1. Example of an application to the identification test

This test is aimed at confirming binding of the sample to the ligand by testing the specific binding as described in 2.4.1. To evaluate the system performance, measurement is performed on the reference material and a negative control (a substance distinguishable from the ligand in terms of the ligand-binding activity) to confirm the specificity of the binding.

4.2. Example of application to binding affinity test

This test is aimed at determining the K_D of the reference material and the sample, making use of the binding affinity analysis described in section 2.4.2. The criterion value related to the binding affinity may be set as the K_D or relative K_D (sample K_D/reference material K_D).

Regarding system suitability, the system performance and system repeatability are set. For example, concerning the system performance, it is confirmed that the ligand immobilization quantity is within the predetermined range; that the calculated K_D of known ligand-binding affinity samples is consistent with the order of affinity levels; and that χ^2 is not more than the predetermined level. System repeatability is confirmed by checking that the relative standard deviation for K_D during repeated measurement is not more than the predetermined level.

4.3. Example of application to measurement of specific activity based on the binding quantity to the target molecule

When specific activity is calculated with the quantity of binding to the target molecule, the measurement is performed using the concentration measuring method described in 2.4.3. On the basis of the calibration curve prepared from the reference material, the relative potency to the reference material is calculated using the data on the response of the sample solution, and then the potency is divided by the protein concentration to yield the specific activity.

Regarding system suitability, the system performance and system repeatability are confirmed. For example, concerning system performance, it is confirmed that the ligand immobilization quantity is within the predetermined range; and that the correlation coefficient or determination coefficient of the calibration curve is not less than the predetermined level. System repeatability is confirmed by checking that the

relative standard deviation of the response during repeated measurement is not more than the predetermined level.

Total Protein Assay

This test is harminized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

The following procedures are provided as illustrations of the determination of total protein content in pharmacopoeial preparations. Other techniques, such as HPLC, are also acceptable if total protein recovery is demonstrated. Many of the total protein assay methods described below can be performed successfully using kits from commercial sources.

Note: Where water is required, use distilled water.

Method 1 (UV method)

Protein in solution absorbs UV light at a wavelength of 280 nm, due to the presence of aromatic amino acids, mainly tyrosine and tryptophan. This property is the basis of this method. Protein determination at 280 nm is mainly a function of the tyrosine and tryptophan content of the protein. If the buffer used to dissolve the protein has a high absorbance relative to that of water, there is an interfering substance in the buffer. This interference can be compensated for when the spectrophotometer is adjusted to zero buffer absorbance. If the interference results in a large absorbance that challenges the limit of sensitivity of the spectrophotometer, the results may be compromised. Furthermore, at low concentrations protein can be absorbed onto the cuvette, thereby reducing the content in solution. This can be prevented by preparing samples at higher concentrations or by using a nonionic detergent in the preparation.

Note: Keep the Test Solution, the Standard Solution, and the buffer at the same temperature during testing.

Standard Solution Unless otherwise specified in the individual monograph, prepare a solution of the reference standard or reference material for the protein under test in the same buffer and at the same concentration as the Test Solution.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration of 0.2 to 2 mg per mL.

Procedure Concomitantly determine the absorbances of the Standard Solution and the Test Solution in quartz cells at a wavelength of 280 nm, with a suitable spectrophotometer, using the buffer as the blank. To obtain accurate results, the response should be linear in the range of protein concentrations to be assayed.

Light-Scattering The accuracy of the UV spectroscopic determination of protein can be decreased by the scattering of light by the test specimen. If the proteins in solution exist as particles comparable in size to the wavelength of the measuring light (250 to 300 nm), scattering of the light beam results in an apparent increase in absorbance of the test specimen. To calculate the absorbance at 280 nm due to light-scattering, determine the absorbances of the Test Solution at wavelengths of 320, 325, 330, 335, 340, 345, and 350 nm. Using the linear regression method, plot the log of the observed absorbance versus the log of the wavelength, and determine the standard curve best fitting the plotted points. From the graph so obtained, extrapolate the absorbance value due to light-scattering at 280 nm. Subtract the absorbance from light-scattering from the total absorbance at 280 nm to obtain the absorbance value of the protein in solution. Filtration with a filter having a 0.2-μm porosity or clarification by centrifugation may be performed to reduce the effect of light-scattering, especially if the solution is noticeably turbid.

Calculations Calculate the concentration, C_U, of protein in the test specimen by the formula:

$$C_U = C_S (A_U/A_S),$$

in which C_S is the concentration of the Standard Solution; and A_U and A_S are the corrected absorbances of the Test Solution and the Standard Solution, respectively.

Method 2 (Lowry method)

This method, commonly referred to as the Lowry assay, is based on the reduction by protein of the phosphomolybdictungstic mixed acid chromogen in the Folin-Ciocalteu's phenol reagent, resulting in an absorbance maximum at 750 nm. The Folin-Ciocalteu's phenol reagent (Folin's TS) reacts primarily with tyrosine residues in the protein, which can lead to variation in the response of the assay to different proteins. Because the method is sensitive to interfering substances, a procedure for precipitation of the protein from the test specimen may be used. Where separation of interfering substances from the protein in the test specimen is necessary, proceed as directed below for Interfering Substances prior to preparation of the Test Solution. The effect of interfering substances can be minimized by dilution provided the concentration of the protein under test remains sufficient for accurate measurement. Variations of the Lowry test that are indicated in national regulatory documents[1)] can be substituted for the method described below.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 5 and 100 μg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions. An appropriate buffer will produce a pH in the range of 10 to 10.5.

Blank Use the buffer used for the Test Solution and the Standard Solutions.

Reagents and Solutions—

Copper Sulfate Reagent Dissolve 100 mg of copper (II) sulfate pentahydrate and 200 mg of sodium tartrate dihydrate in water, dilute with water to 50 mL, and mix. Dissolve 10 g of anhydrous sodium carbonate in water to a final volume of 50 mL, and mix. Slowly pour the sodium carbonate solution into the copper sulfate solution with mixing. Prepare this solution fresh daily.

5% SDS TS Dissolve 5 g of sodium dodecyl sulfate in water, and dilute with water to 100 mL.

Alkaline Copper Reagent Prepare a mixture of 5% SDS TS, Copper Sulfate Reagent, and Sodium Hydroxide Solution (4 in 125) (2:1:1). This reagent may be stored at room temperature for up to 2 weeks.

Diluted Folin's TS Mix 10 mL of Folin's TS with 50 mL of water. Store in an amber bottle, at room temperature.

Procedure To 1 mL of each Standard Solution, the Test Solution, and the Blank, add 1 mL of Alkaline Copper Reagent, and mix. Allow to stand at room temperature for 10 minutes. Add 0.5 mL of the Diluted Folin's TS to each solution, and mix each tube immediately after the addition, and allow to stand at room temperature for 30 minutes. Deter-

mine the absorbances of the solutions from the Standard Solutions and the Test Solution at the wavelength of maximum absorbance at 750 nm, with a suitable spectrophotometer, using the solution from the Blank to set the instrument to zero.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Interfering Substances In the following procedure, deoxycholate-trichloroacetic acid is added to a test specimen to remove interfering substances by precipitation of proteins before testing. This technique also can be used to concentrate proteins from a dilute solution.

Sodium Deoxycholate Reagent Prepare a solution of sodium deoxycholate in water having a concentration of 150 mg in 100 mL.

Trichloroacetic Acid Reagent Prepare a solution of trichloroacetic acid in water having a concentration of 72 g in 100 mL.

Procedure Add 0.1 mL of Sodium Deoxycholate Reagent to 1 mL of a solution of the protein under test. Mix on a vortex mixer, and allow to stand at room temperature for 10 minutes. Add 0.1 mL of Trichloroacetic Acid Reagent, and mix on a vortex mixer. Centrifuge at 3000 × g for 30 minutes, decant the liquid, and remove any residual liquid with a pipet. Redissolve the protein pellet in 1 mL of Alkaline Copper Reagent. Proceed as directed for the Test Solution. [Note: Color development reaches a maximum in 20 to 30 minutes during incubation at room temperature, after which there is a gradual loss of color. Most interfering substances cause a lower color yield; however, some detergents cause a slight increase in color. A high salt concentration may cause a precipitate to form. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.]

Method 3 (Bradford method)

This method, commonly referred to as the Bradford assay, is based on the absorption shift from 470 nm to 595 nm observed when Coomassie brilliant blue G-250 binds to protein. The Coomassie brilliant blue G-250 binds most readily to arginyl and lysyl residues in the protein, which can lead to variation in the response of the assay to different proteins.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 100 μg and 1 mg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Coomassie Reagent Dissolve 100 mg of Coomassie brilliant blue G-250[2)] in 50 mL of ethanol (95). [Note: Not all dyes have the same brilliant blue G content, and different products may give different results.] Add 100 mL of phosphoric acid, dilute with water to 1000 mL, and mix. Filter the solution through filter paper (Whatman No.1 or equivalent), and store the filtered reagent in an amber bottle at room temperature. [Note: Slow precipitation of the dye will occur during storage of the reagent. Filter the reagent before use.]

Procedure Add 5 mL of the Coomassie Reagent to 100 μL of each Standard Solution, the Test Solution, and the Blank, and mix by inversion. Avoid foaming, which will lead to poor reproducibility. Determine the absorbances of the solutions from the Standard Solutions and the Test Solution at 595 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero.

[Note: Do not use quartz (silica) spectrophotometer cells: the dye binds to this material. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.] There are relatively few interfering substances, but detergents and ampholytes in the test specimen should be avoided. Highly alkaline specimens may interfere with the acidic reagent.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Method 4 (Bicinchoninic acid method)

This method, commonly referred to as the bicinchoninic acid or BCA assay, is based on reduction of the cupric (Cu^{2+}) ion to cuprous (Cu^{+}) ion by protein. The bicinchoninic acid reagent is used to detect the cuprous ion. The method has few interfering substances. When interfering substances are present, their effect may be minimized by dilution, provided that the concentration of the protein under test remains sufficient for accurate measurement.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 10 and 1200 μg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Reagents and Solutions—

BCA Reagent Dissolve about 10 g of bicinchoninic acid, 20 g of sodium carbonate monohydrate, 1.6 g of sodium tartrate dihydrate, 4 g of sodium hydroxide, and 9.5 g of sodium hydrogen carbonate in water. Adjust, if necessary, with sodium hydroxide or sodium hydrogen carbonate to a pH of 11.25. Dilute with water to 1000 mL, and mix.

Copper Sulfate Reagent Dissolve about 2 g of copper (II) sulfate pentahydrate in water to a final volume of 50 mL.

Copper-BCA Reagent Mix 1 mL of Copper Sulfate Reagent and 50 mL of BCA Reagent.

Procedure Mix 0.1 mL of each Standard Solution, the Test Solution, and the Blank with 2 mL of the Copper-BCA Rea-

gent. Incubate the solutions at 37°C for 30 minutes, note the time, and allow to come to room temperature. Within 60 minutes following the incubation time, determine the absorbances of the solutions from the Standard Solutions and the Test Solution in quartz cells at 562 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero. After the solutions are cooled to room temperature, the color intensity continues to increase gradually. If substances that will cause interference in the test are present, proceed as directed for Interfering Substances under Method 2. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Method 5 (Biuret method)

This method, commonly referred to as the Biuret assay, is based on the interaction of cupric (Cu^{2+}) ion with protein in an alkaline solution and the resultant development of absorbance at 545 nm.

Standard Solutions Unless otherwise specified in the individual monograph, prepare a solution of Albumin Human for which the protein content has been previously determined by nitrogen analysis (using the nitrogen-to-protein conversion factor of 6.25) or of the reference standard or reference material for the protein under test in sodium chloride solution (9 in 1000). Dilute portions of this solution with sodium chloride solution (9 in 1000) to obtain not fewer than three Standard Solutions having concentrations between 0.5 and 10 mg per mL, the concentrations being evenly spaced. [Note: Low responses may be observed if the sample under test has significantly different level of proline than that of Albumin Human. A different standard protein may be employed in such cases.]

Test Solution Prepare a solution of the test protein in sodium chloride solution (9 in 1000) having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use sodium chloride solution (9 in 1000).

Biuret Reagent Dissolve about 3.46 g of copper (II) sulfate pentahydrate in 10 mL of water, with heating if necessary, and allow to cool (Solution A). Dissolve about 34.6 g of sodium citrate dihydrate and 20.0 g of anhydrous sodium carbonate in 80 mL of water, with heating if necessary, and allow to cool (Solution B). Mix Solutions A and B, and dilute with water to 200 mL. This Biuret Reagent is stable at room temperature for 6 months. Do not use the reagent if it develops turbidity or contains any precipitate.

Procedure To one volume of the Standard Solutions and a solution of the Test Solution add an equal volume of sodium hydroxide solution (6 in 100), and mix. Immediately add a volume of Biuret Reagent equivalent to 0.4 volume of the Test Solution, and mix. Allow to stand at a temperature between 15°C and 25°C for not less than 15 minutes. Within 90 minutes after the addition of the Biuret Reagent, determine the absorbances of the Standard Solutions and the solution from the Test Solution at the wavelength of maximum absorbance at 545 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero. [Note: Any solution that develops turbidity or a precipitate is not acceptable for calculation of protein concentration.]

Calculations Using the least-squares linear regression method, plot the absorbances of the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points, and calculate the correlation coefficient for the line. [Note: Within the given range of the standards, the relationship of absorbance to protein concentration is approximately linear.] A suitable system is one that yields a line having a correlation coefficient of not less than 0.99. From the standard curve and the absorbance of the Test Solution, determine the concentration of protein in the test specimen, making any necessary correction.

Interfering Substances To minimize the effect of interfering substances, the protein can be precipitated from the initial test specimen as follows. Add 0.1 volume of 50% trichloroacetic acid to 1 volume of a solution of the test specimen, withdraw the supernatant layer, and dissolve the precipitate in a small volume of 0.5 mol/L sodium hydroxide TS. Use the solution so obtained to prepare the Test Solution.

Comments This test shows minimal difference between equivalent IgG and albumin samples. Addition of the sodium hydroxide and the Biuret Reagent as a combined reagent, insufficient mixing after the addition of the sodium hydroxide, or an extended time between the addition of the sodium hydroxide solution and the addition of the Biuret Reagent will give IgG samples a higher response than albumin samples. The trichloroacetic acid method used to minimize the effects of interfering substances also can be used to determine the protein content in test specimens at concentrations below 500 μg per mL.

Method 6 (Fluorometric method)

This fluorometric method is based on the derivatization of the protein with *o*-phthalaldehyde (OPA), which reacts with the primary amines of the protein (i.e., NH_2-terminal amino acid and the ε-amino group of the lysine residues). The sensitivity of the test can be increased by hydrolyzing the protein before testing. Hydrolysis makes the α-amino group of the constituent amino acids of the protein available for reaction with the OPA reagent. The method requires very small quantities of the protein.

Primary amines, such as tris(hydroxymethyl)aminomethane and amino acid buffers, react with OPA and must be avoided or removed. Ammonia at high concentrations will react with OPA as well. The fluorescence obtained when amine reacts with OPA can be unstable. The use of automated procedures to standardize this procedure may improve the accuracy and precision of the test.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 10 and 200 μg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Reagents and Solutions—

Borate Buffer Dissolve about 61.83 g of boric acid in water, and adjust with potassium hydroxide to a pH of 10.4.

Dilute with water to 1000 mL, and mix.

Stock OPA Reagent Dissolve about 120 mg of *o*-phthalaldehyde in 1.5 mL of methanol, add 100 mL of Borate Buffer, and mix. Add 0.6 mL of polyoxyethylene (23) lauryl ether, and mix. This solution is stable at room temperature for at least 3 weeks.

OPA Reagent To 5 mL of Stock OPA Reagent add 15 μL of 2-mercaptoethanol. Prepare at least 30 minutes prior to use. This reagent is stable for one day.

Procedure Adjust each of the Standard Solutions and the Test Solution to a pH between 8.0 and 10.5. Mix 10 μL of the Test Solution and each of the Standard Solutions with 100 μL of OPA Reagent, and allow to stand at room temperature for 15 minutes. Add 3 mL of 0.5 mol/L sodium hydroxide TS, and mix. Using a suitable fluorometer, determine the fluorescent intensities of solutions from the Standard Solutions and the Test Solution at an excitation wavelength of 340 nm and an emission wavelength between 440 and 455 nm. [Note: The fluorescence of an individual specimen is read only once because irradiation decreases the fluorescent intensity.]

Calculations The relationship of fluorescence to protein concentration is linear. Using the linear regression method, plot the fluorescent intensities of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the fluorescent intensity of the Test Solution, determine the concentration of protein in the test specimen.

Method 7 (Nitrogen method)

This method is based on nitrogen analysis as a means of protein determination. Interference caused by the presence of other nitrogen-containing substances in the test protein can affect the determination of protein by this method. Nitrogen analysis techniques destroy the protein under test but are not limited to protein presentation in an aqueous environment.

Procedure A Determine the nitrogen content of the protein under test as directed elsewhere in the Pharmacopoeia. Commercial instrumentation is available for the Kjeldahl nitrogen assay.

Procedure B Commercial instrumentation is available for nitrogen analysis. Most nitrogen analysis instruments use pyrolysis (i.e., combustion of the sample in oxygen at temperatures approaching 1000°C), which produces nitric oxide (NO) and other oxides of nitrogen (NO_x) from the nitrogen present in the test protein. Some instruments convert the nitric oxides to nitrogen gas, which is quantified with a thermal conductivity detector. Other instruments mix nitric oxide (NO) with ozone (O_3) to produce excited nitrogen dioxide (NO_2^*) which emits light when it decays and can be quantified with a chemiluminescence detector. A protein reference standard or reference material that is relatively pure and is similar in composition to the test proteins is used to optimize the injection and pyrolysis parameters and to evaluate consistency in the analysis.

Calculations The protein concentration is calculated by dividing the nitrogen content of the sample by the known nitrogen content of the protein. The known nitrogen content of the protein can be determined from the chemical composition of the protein or by comparison with the nitrogen content of the appropriate reference standard or reference material.

◆1) Example: Minimum Requirements for Biological Products and individual monograph in JP.◆

2) Purity of the reagent is important.

G4 Microorganisms

Decision of Limit for Bacterial Endotoxins

The endotoxin limit for injections is to be decided as follows:

$$\text{Endotoxin limit} = K/M$$

where K is a threshold pyrogenic dose of endotoxin per kg body mass (EU/kg), and depending on the administration route, values for K are set as in the following table.

Intended route of administration	K (EU/kg)
Intravenous	5.0
Intravenous, for radiopharmaceuticals	2.5
Intraspinal	0.2

M is equal to the maximum bolus dose of product per kg body mass. When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period. M is expressed in mL/kg for products to be administered by volume, in mg/kg or mEq/kg for products to be administered by mass, and in Unit/kg for products to be administered by biological units. Notes:

1) For products to be administered by mass or by units, the endotoxin limit should be decided based on the labeled amount of the principal drug.

2) Sixty kg should be used as the average body mass of an adult when calculating the maximum adult dose per kg.

3) The pediatric dose per kg body mass should be used when this is higher than the adult dose.

4) The K values for the intravenous route are applicable to drugs to be administered by any route other than those shown in the table.

Disinfection and Decontamination Methods

This chapter describes procedures for reducing the number of microorganisms to a predetermined level using chemical agents during hygiene control of structures and facilities in clean areas or aseptic processing areas that require cleanliness control, as well as of personnel involved in manufacturing control and manufacturing operations in those areas, in pharmaceutical product manufacturing plants.

Appropriate action should be taken based on this chapter when performing microbiological tests specified in the monographs, when taking measures needed to prevent microbial contamination of products and materials used in the manufacture of pharmaceutical products, and when microbial control is needed in pharmacies.

1. Terms and Definitions

The terms used in this chapter are defined as follows.

• Microorganisms: Generally it is a term for bacteria, fungi, protozoa, viruses, and the like. In this chapter, the

term means bacteria and fungi.

• Disinfection: Generally it is a term for harmful microorganisms such as pathogens are removed, killed, or detoxified. In this chapter, the term means microorganisms on an object or in local areas such as the surface of an object are reduced.

• Decontamination: To reduce microorganisms in structures and facilities such as work space and work rooms to a predetermined microbial count level.

• Logarithmic reduction value: The difference in log values for microbial count before and after certain treatments.

• Disinfectant rotation: A disinfection program for when microorganisms that are resistant to a using disinfectant are discovered. In which a disinfectant with different efficacy is used until those microorganisms are no longer detected, or disinfectants with different mechanisms of action are alternately used for certain periods of time in turn. The effectiveness of this method should be evaluated before its implementation.

2. Disinfection Methods

This includes methods such as wiping, spraying, or immersion using chemical agents to reduce microorganisms on equipment, floors, walls, or containers that are carried into clean areas or aseptic processing areas, and the local surfaces such as the wrapping materials of environmental monitoring media. The disinfectants in Table 1 are commonly used, in single or in combination, upon due consideration of the nature of the surface to which the method is applied, such as corrosion by disinfectants, as well as the extent of contamination, such as the types and counts of microorganisms. Although this method does not kill or remove all microorganisms on an object or local surface, disinfectants of proven efficacy should be used when this method is employed. The effects of chemical agents used as disinfectants on microorganisms will differ depending on factors such as the applied concentration, temperature, contact time, and level of surface contamination. When using this method, attention should be paid to disinfectant expiration dates, microbial contamination, the effect of chemical residue on pharmaceutical product quality, and deterioration such as discoloration, deformation and corrosion of the materials being treated.

2.1. Disinfectants

Table 1 presents examples of commonly used disinfectants and their concentrations, and the microbial effects of these disinfectants. Disinfectants and concentrations proven to be safe and effective other than those shown here can also be used.

2.2. Evaluation Methods

When disinfection methods are applied to clean areas and aseptic processing areas, the effectiveness of the conditions should be checked upon due consideration of factors such as the disinfectant concentration, contact time, material of the surface being disinfected, and type of microorganisms that are to be reduced with the disinfectant. Examples of evaluation methods are provided below. Methods other than those shown in the examples may be used if they can be demonstrated to be scientifically appropriate.

2.2.1. Test Microorganism Suspension Method

The diluent that is actually used (purified water, tap water, etc.) should be used to adjust the disinfectant to the actually used concentration. Inoculate 1 mL of the prepared disinfectant with 10^5 to 10^6 CFU test microorganisms. Allow the disinfectant to take effect for the prescribed time (usually 5 to 15 minutes), then dilute or remove (filter) the disinfectant. In the diluent or filtered wash solution, neutralize[1] the disinfectant using a solution containing an inactivator such as lecithin, polysorbate 80, or sodium thiosulfate as needed. Count the number of the microorganisms used for inoculation and the number after disinfection under conditions meeting the requirements in Microbiological Examination of Non-sterile Products <*4.05*> I. 3.4 Suitability of the counting method in the presence of product. Calculate the logarithmic decrement from the test microorganism counts before and after disinfectant treatment. A 3 log or greater decrease in bacteria or fungi and a 2 log or greater decrease in spores will indicate that the disinfection of each target microorganism is effective. The required test microorganism species for evaluating efficacy should be selected with reference to Table 2. These test microorganisms should be used in the evaluation by being cultured and diluted under the conditions described in the Microbiological Examination of Non-sterile Products <*4.05*>. However, *Bacillus subtilis* should be used in the evaluation after a spore suspension has been pre-

Table 1 Types of disinfectants, concentrations, and mechanisms of action

Classification	Types of Disinfectant	Concentration	Mechanism of action
Oxidant	Peracetic acid	0.3 w/v%	Oxidizing action
	Hydrogen peroxide	3 w/v%	
	Sodium hypochlorite	0.02 to 0.05%	
Alcohol-based	Isopropanol	50 to 70%	Protein and nucleic acid denaturation
	Ethanol	76.9 to 81.4 vol%	
Surfactant-based	Benzalkonium chloride Benzethonium chloride	0.05 to 0.2%	Protein denaturation
	Alkyldiamino-ethylglycine hydrochloride	0.05 to 0.5%	Cell membrane function impairment, protein coagulation/ denaturation
Biguanide-based	Chlorhexidine gluconate	0.05 to 0.5%	Interruption of bacterial enzyme or alteration/ disruption of cytoplasmic membrane

Table 2 Test microorganisms

Classification	Test microorganism
Bacteria	*Escherichia coli* ATCC 8739, NBRC 3972 *Staphylococcus aureus* ATCC 6538, NBRC 13276 *Pseudomonas aeruginosa* ATCC 9027, NBRC 13275 *Bacillus subtilis* ATCC 6633, NBRC 3134
Fungi	*Candida albicans* ATCC 10231, NBRC 1594 *Aspergillus brasiliensis* ATCC 16404, NBRC 9455

Table 3 Examples of materials to be disinfected

Material	Example of application
Stainless steel	Workbenches, tanks, machines
Glass	Windows, screens
Polycarbonate	Screens, containers
Decorative calcium silicate board	Walls, ceiling
Epoxy resin coating	Floors
Vinyl chloride	Floors, curtains, vinyl bags
Rigid urethane rubber	Floors
Nitrile rubber	Gloves

pared with reference to Microbial Assay for Antibiotics ⟨*4.02*⟩. Species that are suitable for the purpose of the test can be used.

2.2.2. Hard Surface Carrier Method

Prepare each type of surface material carrier (approximately 5 cm × 5 cm) in a quantity resulting in the appropriate precision. Inoculate a broad range of carriers with 10^5 to 10^6 CFU test microorganisms, allow to dry, and then add disinfectant dropwise in the actually used concentration. Allow the disinfectant to take effect for the prescribed time (usually 5 to 15 minutes), then collect the test microorganisms on the carriers by diluting in the collected solution. In the collected solution, neutralize[1)] the disinfectant using a solution containing an inactivator such as lecithin, polysorbate 80, or sodium thiosulfate as needed. The stomach method, shaking method, swab method, or the like can be used as the method of collection with reference to JIS T11737-1.[2)] Count the number of the test microorganisms used for inoculation and the number of the recovered microorganisms under test conditions meeting the requirements in Microbiological Examination of Non-sterile Products ⟨*4.05*⟩ I. Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests, 3.4 Suitability of the counting method in the presence of product. Calculate the logarithmic decrement from the test microorganism counts before and after disinfectant treatment. Conditions showing the same results as the decreases specified in 2.2.1 Test Organism Suspension Method will indicate that the disinfection of each target microorganism is effective. In addition to the selection of the required test microorganism species for evaluating efficacy with reference to Table 2, one or two representative microorganisms which are frequently isolated in environmental monitoring should be added. Species that are suitable for the purpose of the test can be used. The test microorganisms should be cultured and diluted, etc., as specified in 2.2.1 Test Microorganism Suspension Method. Examples of various surface materials used in clean areas or aseptic processing areas are given in Table 3, but other materials can be added for evaluation as needed depending on the circumstances of actual use.

3. Decontamination Methods

In these methods, decontamination is achieved, for example, by vaporizing or spraying chemical agents to reduce the number of microorganisms to a predetermined level in isolators and RABS (restricted access barrier systems) employed in sterile pharmaceutical product manufacturing processes, or structures and facilities such as work spaces and work rooms in clean areas or aseptic processing areas.

When this method is applied to structures and facilities for manufacturing sterile pharmaceutical products, the efficacy of the decontaminants and decontamination conditions must be validated, and worker safety must be ensured.

3.1. Decontaminants

Commonly used decontaminants are shown below. Decontaminants proven to be safe and effective other than those shown here can also be used.

3.1.1. Hydrogen Peroxide

Decontamination is achieved when hydrogen peroxide (30) is volatilized and allowed to spread. This is a method in which hydrogen peroxide that has been vaporized using a heater is allowed to spread inside an isolator or work room to kill microorganisms through the oxidative power of hydrogen peroxide. When a high degree of microbiological cleanliness must be achieved, as when decontaminating the interior of an isolator for sterilization operations, conditions should ensure a 6 log or greater decrease in the spores of the biological indicator, and when work rooms are decontaminated, conditions should ensure a 3 log or greater decrease. Although the method can be used at ambient temperature, the suitability of the method must be investigated beforehand because the potent oxidative power of hydrogen peroxide may cause deterioration such as discoloration, deformation, and corrosion of the materials exposed, depending on the nature of the materials, and the hydrogen peroxide itself may be degraded by the contact of the materials. If surfaces that are in contact with the product exist inside the isolator, it will be necessary to simultaneously decontaminate the interior and ensure the sterility assurance of the surfaces that are in contact with the product. In such cases, the pre-sterilization bioburden, parameters, utilities, and the like should be controlled in terms of the sterilization method with reference to the chapter ''Sterilization Methods and Sterilization Indicators.''

3.1.2. Peracetic Acid

Decontamination can be achieved, for example, when 0.2% peracetic acid aqueous solution is sprayed in the form of a mist and is allowed to spread. This method is used to clean work rooms, with conditions ensuring at least a 3 log decrease in the spores of the biological indicator. This is a method in which microorganisms are killed through the oxidative power of peracetic acid. Although the method can be used at ambient temperature, the suitability of the method must be investigated beforehand because the potent oxidative power of peracetic acid may result in deterioration such as discoloration, deformation, or corrosion of some materials.

3.1.3. Formaldehyde

Decontamination is achieved when formalin, an aqueous solution containing 36.0 to 38.0% formaldehyde, is vaporized by being heated, or when paraformaldehyde is sublimated by being heated, and allowed to spread. This is a method in which microorganisms are killed through the denaturation of protein by the aldehyde group (-CHO) in the formaldehyde molecule. This method is used to clean work rooms, with conditions ensuring at least a 3 log decrease in the spores of the biological indicator. As formaldehyde is harmful to the human body and has been designated a deleterious substance in the Poisonous and Deleterious Substances Control Act, it must be handled in work spaces equipped with a power exhaust device. It must also be detoxified when disposing of chemical waste.

3.2. Evaluation Methods

Methods using biological indicators to evaluate the effects of decontamination are generally used. Biological indicators that are resistant to decontaminants are commonly placed in various locations in structures and facilities such as work spaces and work rooms prior to decontamination. After decontamination, the biological indicators are commonly

collected and are cultured to check for survivor microorganisms. In addition to culturing, faster methods, for example, that are equal to or greater than culturing can be used. When the decontamination of an isolator with hydrogen peroxide needs to be verified by inactivating 6 log or greater spore count after the use of 10^6 CFU biological indicators, it is not necessary to demonstrate complete destruction of the spores in the isolator after decontamination. Decontamination conditions suitable for a 6 log reduction in spores can also be established by statistical analysis or a method for evaluating the effects of decontamination by collecting the biological indicator and counting the number of survivor microorganisms by culturing to calculate the logarithmic decrement of the biological indicator.

The spores of *Geobacillus stearothermophilus* ATCC 7953 and 12980 are known to be resistant to hydrogen peroxide and formaldehyde, and can thus be used as indicator organisms. As representative environmental microorganisms, the spores of *Bacillus atrophaeus* ATCC 9372 can also be used as a biological indicator for the decontamination of work rooms.

4. Points to Consider

4.1. Worker Safety

Disinfectants and decontaminants often have an effect on the human body. That is, they are toxic. Therefore, when they are used, the method and amounts used must be strictly observed, protective gear must be properly used as needed, and the residue level must be checked.

4.2. Selection of Disinfectants and Decontaminants Used in Pharmaceutical Product Manufacturing Environments

When selecting disinfectants and decontaminants to be used in pharmaceutical product manufacturing environments, the following should be taken into consideration to select the appropriate ones depending on the purpose for which they are being used. The following items (1) through (13) must also be taken into consideration in order to ensure safer and more appropriate use of disinfectants and decontaminant.

(1) Type and number of microorganisms to be treated
(2) Antimicrobial spectrum
(3) Method of use, concentration, contact time, and expiration period of chemical agents
(4) Method for preparing decontaminant, including sterilization, when used in aseptic processing areas
(5) Suitability of materials being treated with disinfectants and decontaminants (such as extent of deterioration)
(6) Effects in the presence of organic substances such as protein
(7) Effective time dulation
(8) Effect on human body (safety)
(9) Suitability with cleansers
(10) Necessity of disinfectant rotation, and the method, if needed
(11) Necessary procedures for preventing contamination of pharmaceutical products by chemical agents (such as method of inactivation and checking residue level)
(12) Ease of waste disposal (neutralization, inactivation)
(13) Environmental effects of waste disposal

References

1) US Pharmacopeia 37 (2014), <1072> DISINFECTANTS AND ANTISEPTICS
2) Japanese Industrial Standards JIS T 11737 – 1 (2013), Sterilization of medical devices—Microbiological methods, Part 1: Determination of a population of microorganisms on products (ISO 11737 – 1: 2006)

Media Fill Test (Process Simulation)

The media fill test is one of the process validations employed to evaluate the propriety of the aseptic processing of pharmaceutical products using sterile media, etc. instead of actual products. Therefore, media fill should be conducted under conditions that simulate routine manufacturing procedures, e.g. filling and sealing, operating environment, processing operation, number of personnel involved, etc., and include permissible worst case conditions. Process simulation can be applied to the other aseptic manufacturing processes in addition to aseptic manufacturing processes for finished drug products such as ''filling'' and ''sealing''.

1. Frequency of media fills

1.1. Initial performance qualification

Initial performance qualification should be conducted for each new facility, item of equipment, filling line, and container design (except for multiple sizes of the same container design), etc. As referring to Table 1, a sufficient number of units should be used to simulate aseptic manufacturing process. A minimum of three consecutive separate successful runs should be performed on each separate day. However, the action as shown in Table 1 may be performed at the time when the contamination is found.

1.2. Periodic performance requalification

1) As referring to Table 2, a sufficient number of units should be used to simulate aseptic manufacturing process. Media fill run should be conducted at least on semi-annual base for each shift and processing line. All personnel working in the critical processing area should be trained about aseptic processing and participate in a media fill run at least once a year.

2) When filling lines have not been used for over six months, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to the use of the filling lines.

3) In cases of facility and equipment modification (interchanging standard parts may not require requalification),

Table 1 Initial performance qualification

Minimum number of simulations	Number of units filled per simulation	Contaminated units in any of the three simulations	Action
3	<5000	$\geqq 1$	Investigation, corrective measures, restart validation
3	5000 – 10000	1	Investigation, consideration of repeat of one media fill
		>1	Investigation, corrective measures, restart validation
3	>10000	1	Investigation
		>1	Investigation, corrective measures, restart validation

Table 2 Periodic performance requalification

Minimum number of simulations	Number of units filled per simulation	Contaminated units	Action
Every half year	< 5000	1	Investigation, revalidation
	5000 – 10000	1	Investigation, consideration of repeat media fill
		> 1	Investigation, corrective measures, revalidation
	> 10000	1	Investigation
		> 1	Investigation, corrective measures, revalidation

major changes in personnel working in critical aseptic processing, anomalies in environmental monitoring results, or a product sterility test showing contaminated products, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to the scheduled media fills.

2. Acceptance criteria of media fills

Both in initial performance qualification and periodic performance qualification, the target should be zero growth regardless of number of units filled per simulation. Where contaminated units are found, action shown in Tables 1 and 2 should be taken.

2.1. Investigation of positive units

Where contaminated units are found in media fill, an investigation should be conducted regarding the cause, taking into consideration the following points:

1) Microbial monitoring data
2) Particulate monitoring data
3) Personnel monitoring data (microbial monitoring data on gloves, gowns, etc. at the end of work)
4) Sterilization cycle data for media, commodities, equipment, etc.
5) Calibration data of sterilization equipment
6) Storage conditions of sterile commodities
7) HEPA filter evaluation (integrity tests, velocity, etc.)
8) Pre and post filter integrity test data (including filter housing assembly)
9) Air flow patterns and pressures
10) Unusual events that occurred during the media fill run
11) Characterization of contaminants
12) Hygienic control and training programs
13) Gowning procedures and training programs
14) Aseptic processing technique and training programs
15) Operator's health status (especially coughing, sneezing, etc., due to respiratory diseases)
16) Other factors that affect sterility

3. Data guidance for media fills

Each media fill run should be fully documented and the following information recorded:

1) Data and time of media fill
2) Identification of filling room and filling line used
3) Container/closure type and size
4) Volume filled per container
5) Filling speed
6) Filter type and integrity test result (in case of filtration)
7) Type of media filled
8) Number of units filled
9) Number of units not incubated and reason
10) Number of units incubated
11) Number of units positive
12) Incubation time and temperature
13) Procedures used to simulate any step of a normal production fill (e.g., mock lyophilization or substitution of vial headspace gas)
14) Microbiological monitoring data obtained during the media fill set-up and run
15) List of personnel who took part in the media fill
16) Growth promotion results of the media (in case of powder fill, an antimicrobial activity test for the powder is necessary)
17) Identification and characterization of the microorganisms from any positive units
18) Product(s) covered by the media fill
19) Investigation of runs with a positive unit or failed runs
20) Management review

4. Media fill procedures

Methods to validate aseptic processing of liquid, powder and freeze-dried products are described. Basically, it is possible to apply media fill procedures for liquid products to other dosage forms.

4.1. Media selection and growth promotion

Soybean-casein digest medium or other suitable media are used. When strains listed in the Microbiological Examination of Non-sterile Products <*4.05*> and, if necessary, one or two representative microorganisms which are frequently isolated in environmental monitoring are inoculated under the specified conditions, each strain should show obvious growth.

4.2. Sterile medium preparation

The medium is sterilized according to the pre-validated method.

4.3. Incubation and inspection of media filled units

Leaking or damaged units should be removed and recorded prior to incubation of media filled units. Units should be incubated at 20 – 35°C for not less than 14 days. The use of another temperature range should be justified. If two temperatures are used for incubation, the units are typically incubated for at least 7 days at each temperature starting with the lower temperature. Established temperature should be kept within ±2.5°C. Observe the media filled units for growth of microorganisms on the last day of the incubation. Microorganisms in contaminated units should be followed to identification and characterization. For the identification of contaminants, ''Rapid Identification of Microorganisms Based on Molecular Biological Method'' shown in General Information or appropriate commercial kit for identification of microorganisms may be applicable.

A. Liquid products

Media fill procedure

Media fill should include normal facility/equipment operations and clean-up routines. Containers, closures, parts of the filling machine, trays, etc. are washed and sterilized according to the standard operating procedures. Media fills should be conducted under processing conditions that include ''worst case'' conditions, e.g., correction of line stoppage, repair or replacement of filling needles/tubes, replacement of on-line filters, permitted interventions, duration and

size of run, number of personnel involved, etc.

It is not necessary to put all worst-case scenarios in a single media fill run, but all worst-case scenarios should be evaluated intentionally. While advanced processing lines are highly automated, often operated at relatively high speeds, and are designed to limit operator intervention, there are processing lines showing frequent human interventions. Although the most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production run, other appropriate models can be justified.

An appropriate volume of medium is filled into sterilized containers at a predetermined filling speed and the containers are sealed. The media are contacted with all interior surfaces in the containers by an appropriate method, and then incubated at the predetermined temperature.

B. Powder products

B.1 Powder selection and antimicrobial activity test

Actual products or placebo powder are used. In general, lactose monahydrate, D-mannitol, polyethylene glycol 6000, carboxymethyl cellulose salts or media powder, etc. are used as placebo powders. Prior to employing any of the powders, evaluate whether the powder has antimicrobial activity. Media powders are dissolved in water and other powders in liquid medium, and the solutions are inoculated with less than 100 CFU microorganisms of each kind, shown in 4.1, for the growth promotion test. If obvious growth appears in the medium incubated at the predetermined temperature for 5 days, the powder has no antimicrobial activity and is available for the media fill test.

B.2 Sterilization of powders

Powders are bagged in suitable containers (e.g. double heat-sealed polyethylene bags), and are subjected to radiation sterilization.

B.3 Sterility of filling powders

The powders must pass the Sterility Test. However, if the sterilization is fully validated, sterility testing of the powders can be omitted.

B.4 Media fill procedures

Chose a suitable procedure from among the following procedures.

1) Fill sterilized liquid media into containers by suitable methods, and then fill actual products or sterilized placebo powder with the powder filling machine. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

2) Distribute liquid media into containers, and then sterilize them in an autoclave. Remove the containers to the filling area, and then fill actual products or sterilized placebo powder into the containers with the powder filling machine.

3) Fill actual products or sterilized placebo powder into containers with the powder filling machine, and then fill sterilized liquid media into the containers by appropriate methods. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

C. Lyophilized products

In the case of lyophilized products, it may be impossible to conduct a media fill run in the same way as used for actual processing of lyophilized products. The process of freezing and lyophilization of the solution may kill contaminant organisms and change the characteristics of the media too. The use of inert gas as a blanket gas may inhibit the growth of aerobic bacteria and fungi. Therefore, in general, the actual freezing and lyophilization process should be avoided and air is used as the blanket gas. For products manufactured under an anaerobic atmosphere, process simulation should be performed with the use of anaerobic growth media and the inert gas such as nitrogen gas.

Media fill procedures

Use the following method or other methods considered to be equivalent to these methods.

1) After filling of the media into containers by the filling machine, cap the containers loosely and collect them in presterilized trays.

2) After placing the trays in the lyophilizer, close the chamber door, and conduct lyophilization according to the procedures for production operation. Hold them without freezing and boiling-over under weak vacuum for the predetermined time.

3) After the vacuum process, break the vacuum, and seal the stoppers.

4) Contact the media with all product contact surfaces in the containers by appropriate methods, and then cultivate them at the predetermined temperature.

References

ISO 13408-1 (2008): Aseptic processing of health care products: General requirements.

Microbial Attributes of Non-sterile Pharmaceutical Products

This chapter is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

The presence of certain micro-organisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have therefore to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practice during the manufacture, storage and distribution of pharmaceutical preparations. ◆This chapter provides guidelines for acceptable limits of viable microorganisms (bacteria and fungi) existing in raw materials and non-sterile pharmaceutical products.◆ Microbial examination of non-sterile products is performed according to the methods given in the Microbiological Examination of Non-sterile Products <*4.05*> on Microbiological Examination of Non-sterile Products: I. Microbial Enumeration Tests and II. Tests for Specified Micro-organisms. ◆When these tests are carried out, a microbial control program must be established as an important part of the quality management system of the product. Personnel responsible for conducting the tests should have specialized training in microbiology, biosafety measures and in the interpretation of the testing results.◆

◆1. Definitions

(i) Non-sterile pharmaceutical products: Non-sterile drugs shown in monographs of the JP and non-sterile finished dosage forms.

(ii) Raw materials: All materials, including raw ingredients and excipients, used for the preparation of drugs, except for water and gases.

(iii) Bioburden: Number and type of viable micro-organisms existing in non-sterile pharmaceutical products.

(iv) Action levels: Established bioburden levels that require immediate follow-up and corrective action if they are exceeded.

(v) Alert levels: Established bioburden levels that give early warning of a potential drift from normal bioburden level, but which are not necessary grounds for definitive corrective action, though they may require follow-up investigation.

(vi) Quality management system: The procedures, operation methods and organizational structure of a manufacturer (including responsibilities, authorities and relationships between these) needed to implement quality management.

2. Scope

In general, Microbial Enumeration Tests is not applied to drugs containing viable micro-organisms as an active ingredient.

3. Sampling plan and frequency of testing

3.1. Sampling methods

Microbial contaminants are usually not uniformly distributed throughout the batches of non-sterile pharmaceutical products or raw materials. A biased sampling plan, therefore, cannot be used to estimate the real bioburden in the product. A sampling plan which can properly reflect the status of the product batch should be established on the basis of the bioburden data obtained by retrospective validation and/or concurrent validation. In general, a mixture of samples randomly taken from at least different three portions, almost the same amount for each portion, is used for the tests of the product. When the sampling is difficult in a clean area, special care is required during sampling to avoid introducing microbial contamination into the product or affecting the nature of the product bioburden. If it is confirmed that the product bioburden is stable for a certain period, as in the case of non-aqueous or dried products, it is not necessary to do the tests, immediately after the sampling.

3.2. Testing frequency

The frequency of the tests should be established on the basis of a variety of factors unless otherwise specified. These factors include:

(i) Dosage forms of non-sterile pharmaceutical products (usage);

(ii) Manufacturing processes;

(iii) Manufacturing frequency;

(iv) Characteristics of raw materials (natural raw material, synthetic compound, etc.);

(v) Batch sizes;

(vi) Variations in bioburden estimates (changes in batches, seasonal variations, etc.);

(vii) Changes affecting the product bioburden (changes in manufacturing process, supplier of raw materials, batch number of raw materials, etc.);

(viii) Others.

In general, the tests may be performed at a high frequency during the initial production of a drug to get information on the microbiological attributes of the product or raw materials used for the production. However, this frequency may be reduced as bioburden data are accumulated through retrospective validation and/or concurrent validation. For example, the tests may be performed at a frequency based on time (e.g., weekly, monthly or seasonally), or on alternate batches.

4. Microbial control program

When the ''Microbiological Examination of Non-sterile Products *<4.05>*'' is applied to a non-sterile pharmaceutical product, the methods for the recovery, cultivation and estimation of the bioburden from the product must be validated and a ''Microbial control program'' covering the items listed below must be prepared.

(i) Subject pharmaceutical name (product name);

(ii) Frequency of sampling and testing;

(iii) Sampling methods (including responsible person, quantity, environment, etc. for sampling);

(iv) Transfer methods of the samples to the testing area (including storage condition until the tests);

(v) Treatment of the samples (recovery methods of microbial contaminants);

(vi) Enumeration of viable micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);

(vii) Detection of specified micro-organisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);

(viii) Estimation of the number of and characterization of microbial contaminants;

(ix) Establishment of ''Microbial acceptance criteria'' (including alert level and action level);

(x) Actions to be taken when the levels exceed ''Microbial acceptance criteria'';

(xi) Persons responsible for the testing and evaluation, etc.;

(xii) Other necessary items.◆

5. Microbial acceptance criteria for non-sterile pharmaceutical products

By establishing ''Microbial acceptance criteria'' for non-sterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC), ◆it is possible to evaluate at the initial processing stage of the product whether the microbiological quality of the raw materials is adequate or not. Furthermore, it is then possible to implement appropriate corrective action as needed to maintain or improve the microbiological quality of the product.◆ The target limits of microbial levels for raw materials (synthetic compounds and minerals) are shown in Table 1.

◆In general, synthetic compounds have low bioburden levels due to the high temperatures, organic solvents, etc., used in their manufacturing processes. Raw materials originated from plants and animals in general have higher bioburdens than synthetic compounds.

The microbial quality of the water used in the processing of active ingredients or non-sterile pharmaceuticals may have a direct effect on the quality of the finished dosage form. This means it is necessary to keep the level of microbial contaminants in the water as low as possible.◆

Acceptance criteria for microbiological quality for non-sterile finished dosage forms are shown in Table 2. ◆These microbial limits are based primarily on the type of dosage form, water activity, and so on. For oral liquids and pharmaceutical products having a high water activity, in general, low microbial acceptance criteria are given.◆

Table 2 includes a list of specified micro-organisms for which acceptance criteria are set. The list is not necessarily

Table 1 Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use

	Total Aerobic Microbial Count (CFU/g or CFU/mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/mL)
Substances for pharmaceutical use	10^3	10^2

Table 2 Acceptance criteria for microbiological quality of non-sterile dosage forms

Route of administration	Total Aerobic Microbial Count (CFU/g or CFU/mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/mL)	Specified Micro-organism
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	—
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Candida albicans* (1 g or 1 mL)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (more rigorous requirements apply to liquid preparations for nebulization)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of bile-tolerant gram-negative bacteria (1g or 1 mL)

◆**Table 3** Acceptance criteria for crude drugs and crude drug-containing preparations

	Total Aerobic Microbial Count (CFU/g or CFU/mL)	Total Combined Yeasts/Moulds Count (CFU/g or CFU/mL)	Specified Micro-organism
Category 1	Acceptance criterion: 10^7 Maximum limit: 50,000,000	Acceptance criterion: 10^5 Maximum limit: 500,000	Acceptance criterion for *Escherichia coli*: 10^3 (1 g or 1 mL) Absence of *Salmonella* (10 g or 10 mL)
Category 2	Acceptance criterion: 10^5 Maximum limit: 500,000	Acceptance criterion: 10^4 Maximum limit: 50,000	Acceptance criterion for bile-tolerant gram-negative bacteria: 10^4 (1 g or 1 mL) Absence of *Escherichia coli* (1 g or 1 mL) Absence of *Salmonella* (10 g or 10 mL)

◆

exhaustive and for a given preparation it may be necessary to test for other micro-organisms depending on the nature of the starting materials and the manufacturing process.

If it has been shown that none of the prescribed tests will allow valid enumeration of micro-organisms at the level prescribed, a validated method with a limit of detection as close as possible to the indicated acceptance criterion is used.

In addition to the micro-organisms listed in Table 2, the significance of other micro-organisms recovered should be evaluated in terms of:

(i) the use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
(ii) the nature of the product: does the product support growth, does it have adequate antimicrobial preservation?
(iii) the method of application;
(iv) the intended recipient: risk may differ for neonates, infants, the debilitated;
(v) use of immunosuppressive agents, corticosteroids;
(vi) presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and the interpretation of microbiological data.

For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:
—10^1 CFU: maximum acceptable count = 20,
—10^2 CFU: maximum acceptable count = 200,
—10^3 CFU: maximum acceptable count = 2000, and so forth.

◆6. Acceptance criteria for crude drugs and crude drug-containing preparations

Target limits of microbial contamination for crude drugs and crude drug-containing preparations are shown in Table 3. Category 1 includes crude drugs and crude drug preparations which are used for extraction by boiling water or to which boiling water is added before use. Category 2 includes crude drugs which are taken directly without extraction process and directly consumed crude drug preparations containing powdered crude drugs. In this guideline, bile-tolerant gram-negative bacteria, *Escherichia coli* and *Salmonella* are mentioned as specified micro-organisms, but other micro-organisms (such as certain species of *Bacillus cereus*, *Clostridia*, *Pseudomonas*, *Burkholderia*, *Staphylococcus aureus*, *Asperigillus* and *Enterobacter* species) are also necessary to be tested depending on the origin of raw materials for crude drugs or the preparation method of crude drug-containing preparations. The target limit of microbial contamination for the raw materials is to be set based on the risk assessment being taken into account the provided process of those materials or the desired quality specification for them.◆

Microbiological Environmental Monitoring Methods of Processing Areas for Sterile Pharmaceutical Products

This chapter describes the methods for the evaluation of air-cleanliness and the recommended limits for environmental microorganisms. The main purposes of this chapter are 1) to confirm that the designed cleanliness levels and microbial limits are attained and maintained in processing areas for sterile pharmaceutical products, and 2) to confirm that the number of particulates and microorganisms are appropriately controlled in the processing environment for sterile pharmaceuticals.

In reference to the evaluation methods and the recommended limits described in this chapter, a risk assessment should be performed for each manufacturing facility and acceptance criteria should be established based on the identified risks. Alternative measuring methods can be applied on rational grounds.

1. Definitions

For the purposes of this chapter, the following definitions apply:

(i) Action level: An established number of objects to be monitored (and species of microorganisms, if appropriate) that requires immediate investigation and corrective action based on the investigation when exceeded.

(ii) Alert level: An established number of objects to be monitored (and species of microorganisms, if appropriate) that gives early warning of potential problems.

(iii) Aseptic processing: Filling of sterile products and other operations performed under the environmental conditions in which air supply, materials, equipment, and personnel are regulated to control microbial and particulate contamination to acceptable levels.

(iv) Aseptic processing area: The classified part of a facility in which air supply, materials, equipment, and personnel are highly regulated to control microbial and particulate contamination to acceptable levels. The area is classified into two categories: Grade A and Grade B.

(v) Microorganisms: General term for bacteria, fungi, protozoa, viruses, etc. In this chapter, microorganisms indicate only bacteria and fungi.

(vi) Shift: Scheduled period of work or production during which operations are conducted by a single or defined group of workers.

(vii) Risk assessment: A series of processes including identification, analysis, and evaluation of hazards that may cause harm in accordance with ICH Q9, ''Quality Risk Management.'' In this chapter, ''harm'' indicates contamination of products or manufacturing areas; ''hazards'' indicates possible causes of the contamination, such as personnel, environment, or operations carried out. Risk is expressed as a combination of the probable incidence and severity of the harm.

(viii) Calibration: The act of establishing the relationship between values indicated by a measuring instrument and the values represented by a material measure, by comparison with the corresponding known values of a standard instrument or a standard reference material and of adjusting the accuracy of the measuring instrument for the proper use.

(ix) At rest: The state in which production equipment is installed and operating, with no operating personnel present.

(x) In operation: The state in which the installed equipment is functioning in the defined operating mode with the specified number of personnel working.

2. Processing areas

Processing areas refer to areas in which actions such as cultivation, extraction/purification, washing and drying of containers and stoppers, weighing of raw materials, preparation of solutions, sterilization, filling, sealing, and packaging are performed, including the gowning area.

The processing areas for sterile pharmaceutical products are maintained and controlled to prevent containers, raw materials, and in-process products from microbial and particulate contamination.

Personnel engaged in such activities in the areas should receive necessary training in hygiene, microbiology, manufacturing technology, and clothing.

2.1. Classification of processing areas

(i) Grade A: A local area in which operational activities to prevent contamination risks of products at a high level are conducted. For pharmaceutical products prepared aseptically, the area is designed to preserve sterility of sterilized pharmaceutical products, containers, and closures that are exposed within it. In this area, manipulations of sterile materials prior to filling operation (e.g. aseptic connections, sterile ingredient additions), filling, and closing operations are conducted.

(ii) Grade B: A multipurpose area in which operational activities to prevent contamination risks of products at a comparably high level are conducted. For pharmaceutical products prepared aseptically, the area is used as a route to load sterilized containers, raw materials, and in-process products that are stored to preserve sterility. Areas in which

personnel, equipment and apparatuses that directly come into aseptic processing areas exist are also classed as Grade B. In a general clean room, this is the surrounding environment for the Grade A area. When contamination risks of microorganisms derived from the environment are low, for example, where isolators are installed so that the levels of human intervention and exposure are low, the surrounding area dose not necessary qualify as Grade B.

(iii) Grades C, D: Areas to prevent contamination risks of products at a comparably low level. Activities conducted in such areas include operational activities of non-sterile containers, raw materials, and in-process products that are exposed to the surrounding environment, and cleaning of equipment and apparatuses for aseptic processing. When contamination risks of microorganisms derived from the environment are low, for example, where isolators are installed so that the levels of human intervention and the exposure are low, these areas can be used as the surrounding areas.

2.2. Environmental control level by processing area

Airborne particulates in areas used for processing of pharmaceutical products may be a key indicator to monitor performance of air-conditioning systems. They may act physically as a source of insoluble particulates in the products, and biologically as a carrier of microorganisms.

In areas used for the processing of pharmaceutical products, therefore, the number of airborne particulates, as well as the number of microorganisms, should be controlled within the specified limits. Air volume, airflow pattern, frequency of ventilation, and material and personnel flow are appropriately designed so that airborne particulates that exist in the areas can be effectively discharged.

The air-cleanliness and the recommended limits for environmental microorganisms for each grade area are shown in Table 1 and Table 2.

Compared with the classifications in ISO/DIS 14644-1 (2010), the maximum number of airborne particulates in Grade A, B, and C (in operation) are almost identical to those of ISO 5, ISO 7, and ISO 8, respectively.

When the number of sampling points is determined in order to classify manufacturing areas based on the defined cleanliness levels, Table 3 can be used as a reference. Sampling points that are evenly distributed throughout the area to be monitored should be selected. The height at which operational activities are conducted in the area should be also considered. Addition of sampling points can also be effective, based on the risks.

The sampling points specified in ISO/DIS 14644-1 (2010) are shown in Table 3.

Table 1 Air-cleanliness

Grade	Maximum permitted number of airborne particulates (number/m^3)			
	at rest*1		in operation	
Size	≥0.5 μm	≥5.0 μm	≥0.5 μm	≥5.0 μm
A	3520	20	3520	20
B	3520	29	352000	2900
C	352000	2900	3520000	29000
D	3520000	29000	....*2	....*2

*1 The number of particulates given in the table for the ''at rest'' condition should be achieved 15 – 20 minutes after the completion of operations.

*2 The number will depend on the nature of the operation carried out.

Table 3 Minimum sampling points based on an area of clean rooms

Area of clean rooms (m^2) equal to or less than	Minimum sampling points
1	1
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14
76	15
104	16
108	17
116	18
148	19
156	20
192	21
232	22
276	23
352	24
436	25
500	26

Table 2 Recommended limits for environmental microorganisms (in operation)*1

Grade	Airborne microorganisms		Microorganisms on surfaces	
	air sample (CFU/m^3)	settle plates*2 (CFU/plate)	contact plates (CFU/24 – 30 cm^2)	gloves (CFU/5 fingers)
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	....
D	200	100	50	....

*1 These are average values.

*2 The exposure time of each plate should be less than four hours. Monitoring should be performed throughout operations.

For design qualification of Grade A area, a minimum sample volume of 1 m^3 should be taken per measurement.

The monitoring of $\geq 5.0\ \mu m$ airborne particulates and airborne microorganisms on settle plates are performed, if necessary.

3. Environmental monitoring program

For the manufacture of sterile pharmaceutical products, it is necessary to predict potential deterioration of the processing environment before it occurs and to prevent any adverse effect on the quality of products. The environmental monitoring program should include all necessary items to verify whether air-cleanliness in each production area is constantly maintained. The items included in the program should be determined in reference to Sections 3.1 to 3.6 in this chapter. An environmental monitoring program should be prepared for each facility. All personnel engaged in the environmental monitoring program should receive sufficient training in hygiene control, microbiology, measurement principles, measurement procedures, and gowning procedures.

3.1. Applicability

Microorganisms and airborne particulates should be monitored. Microorganisms to be monitored are bacteria and fungi, and airborne particulates to be monitored are those $\geq 0.5\ \mu m$ in size.

3.2. Frequency of monitoring

In the processing areas used for sterile pharmaceutical products, monitoring of airborne particulates and microorganisms is required. The Grade A area, in which sterile products are in contact with environmental air, should be monitored during every operational shift. Recommended frequencies of monitoring during operation are given in Table 4. The frequencies are set for general and conventional aseptic processing. In individual cases, appropriate monitoring frequency should be determined based on the results of risk assessment. In particular, the risks of contamination of products should be considered when determining monitoring frequency of airborne microorganisms in Grade A and B areas. The monitoring frequency should be adequate for the assessment of potential effect. When high contamination risks of products are concerned, for example, where products are exposed to the environment for a long time or operational activities are frequently performed in a Grade A area, such areas should be more frequently monitored.

In contrast, in manufacturing operations in which isolator, RABS (Restricted Access Barrier System), or brow/fill/seal units are used, monitoring frequency may be reduced due to lower contamination risks to the products from human and the environment.

3.3. Monitoring points

The items to be monitored include air, floors, walls, equipment surfaces, gloves, and gowns in the processing areas. When selecting monitoring points to be included in the environmental monitoring program, the following points should be included: the points where critical operations are performed, where a contamination risk is considered high, and points that represent the cleanliness levels of the manufacturing area.

Regular monitoring points in the manufacturing area should be determined based on the risk assessment and the data obtained in monitoring for cleanliness classification; e.g., the near vicinity (e.g. within 30 cm) of a site where products are exposed to the surrounding environment, a site that is prone to potential sources of contamination due to frequent human interventions and traffic or due to susceptibility to lower cleanliness levels, or a site regarded as worst-point based on the airflow analysis.

3.4. Monitoring methods

Appropriate methods should be selected according to the items to be monitored. Consideration should be given to potential contamination risks increased by interventions of personnel who are involved in sampling and disturbance of airflow during sampling.

For monitoring of airborne microorganisms, there are two types of microbial sampling methods: active sampling methods and passive sampling methods. Various types of culture medium and culture methods are available for different types of microorganisms to be monitored. For details, refer to Section 5, ''Measurement of microorganisms'' in this chapter.

3.5. Environmental control criteria

Establishment of an alert level for each item to be monitored can lead to early detection of performance degradation of facilities. It is also useful to control risks. In environmental monitoring, it is important to evaluate whether the specified cleanliness level for a monitored object is constantly maintained.

The measured values obtained by environmental monitoring are averaged. The contamination risks are evaluated based on the averaged values and should not be underestimated. In a case where bacteria are detected in a Grade A area, assessment of potential effect on the products should be carried out. Surfaces and personnel should be monitored

Table 4 Recommended Frequency of Environmental Monitoring

Grade		airborne particulates	airborne microorganisms	microorganisms on surfaces	
				instruments, walls etc.	gloves, gowns
A		In operation	For each shift	At the completion of each operation	At the completion of each operation
B		In operation	For each shift	At the completion of each operation	At the completion of each operation
C, D*	Areas in which products and containers are exposed to the surrounding environment	Once a month	Twice a week	Twice a week	----
	Other areas	Once a month	Once a week	Once a week	----

* When a contamination risk is low, for example, where products are not exposed to the surrounding environment, monitoring frequency may be reduced accordingly.

after the completion of critical operations.

Measurement of $\geq 5.0\,\mu m$ airborne particulates in Grade A and B areas is useful for early detection of abnormalities in the environment. When $\geq 5.0\,\mu m$ airborne particulates are continuously or frequently detected, even if the number is low, further investigation should be encouraged due to possible abnormalities that may have impact on the environment.

3.6. Evaluation of monitoring data and measures to be taken when the limit is exceeded

Environmental monitoring data should be evaluated both in the short-term and the long-term. The following items should be included in the evaluation:

(i) Changes in numbers of microorganisms and airborne particulates over a period of time

(ii) Changes in detected species of microorganisms

(iii) Changes of monitoring points

(iv) Review of the validity of alert and action levels

(v) Review of frequency of positive results from each operator

(vi) Changes that may impact the monitoring results during the monitoring periods

Trend analysis of environmental monitoring data will provide information required to predict potential deterioration of the manufacturing environment before it occurs and to determine its probable causes. Information that may impact the environment, such as monitoring location, date and time, product manufactured during the monitoring period, batch number, personnel in operation, is also important.

In the event of any deviation found in the environmental monitoring data, actions to be taken for the products manufactured and measures to be taken to recover the required cleanliness of the environment should be determined with consideration of the nature of activities performed at the time, distance between the product and the site where the deviation was found, and the severity of the deviation.

4. Measurement of particulates

For measurement of particulates, particle counters that can detect particulates of different sizes are used. In general, a particle counter is composed of an air suction pump, a sensor that discerns particle size from variations in the reflection of a laser beam, and a converter unit. When there is distance between a counter and a sampling point, sampling tubing is used. To measure distribution of particulates precisely, the inlet of the sampling probe is positioned parallel to airflow, and air is aspirated at the same velocity as the airflow.

For measurement of particulates, calibrated devices should be used. Consideration should be given to length, diameter of the tubing, and radii of any bends in the tubing, as well as to the device itself. Calibration items include flow rate, counting efficiency, false counts, and counting loss.

There are three types of methods for particulates monitoring; an independent particle counter is placed at individual monitoring point; a network of sequentially accessed monitoring points is connected by manifold to a single particle counter; or a combination of the two. In any method, the concentration of particulates in the predetermined particulate size range according to the cleanliness level of the area to be monitored should be indicated or recorded. When monitoring $\geq 5.0\,\mu m$ particulates, a short length of sample tubing should be used, because of the relatively higher rate of precipitation of particulates of large size. For particulate monitoring, the selection of the monitoring system may take into account any health risks of operators presented by the materials used in the manufacturing operation (e.g. pathogens, radiopharmaceuticals, or strong sensitizers).

In general, continuous monitoring is recommended in a Grade A area. Sampling volumes that can be accurately converted to volume per m^3 should be applied.

5. Measurement of microorganisms

Measurement methods of microorganisms for environmental monitoring include active microbial sampling methods, measurement methods for microorganisms on surfaces, and settling plates. Various types of sampling devices and measurement methods are available for the sampling and measurement of microorganisms in the air and on surfaces. Appropriate samplers and measuring methodology should be selected according to the purpose of monitoring and the items to be monitored.

5.1. Measurement by cultivation

5.1.1. Active microbial sampling methods

Methods in which a fixed volume of air is aspirated and the number of microorganisms in the air sampled is counted. There are filtration-type sampling devices and impact-type sampling devices.

Both methods have advantages and disadvantages. Capabilities of an air sampling device (air sample volume capacity, performance of microorganism collection, etc.) should be confirmed before use. When a device is used in a Grade A area, the following points needs to be confirmed before use: the device can effectively collect air; it is easily decontaminated or sterilized; it does not disturb unidirectional airflow.

An appropriate air sampling volume for active microbial measurement should be comprehensively determined on reasonable grounds, such as cleanliness level of the area to be monitored and monitoring frequency, etc. In a Grade A area, the air sample volume should be 1 m^3 at each sampling.

(i) Impact-type sampling method: When an impact-type sampling device is used, the speed at which the collected air strikes the culture medium surface must be sufficient to capture the microorganisms, but must not have an adverse effect on the collected microorganisms. The volume of air collected must not cause a significant change in the physical or chemical properties of the culture medium.

The most commonly used samplers are as follows: i) slit sampler, ii) Andersen sampler, iii) pinhole sampler, and iv) centrifugal sampler. Each sampler has specific characteristics. The slit sampler is a device to trap microorganisms in a known volume of air that is passed through a standardized slit. The air is impacted on a slowly revolving Petri dish containing a nutrient agar. The rotation rate of the Petri dish and the distance from the slit to the agar surface are adjustable, and it is possible to estimate the number of microorganisms in the air that passes through the device for a period of up to 1 hr. The Andersen sampler consists of a perforated cover and several Petri dishes containing a nutrient agar, and a known volume of air that is passed through the perforated cover impacts on the agar medium in the Petri dishes. The sampler is suitable for the determination of the distribution of size ranges of microorganism particulates in the air. The pinhole sampler resembles the slit sampler, but has pinholes in place of the slit. Microorganisms are collected by spraying a known volume of air through several pinholes onto agar medium in a slowly revolving Petri dish. The centrifugal sampler consists of a propeller that pulls a known volume of air into the device and then propels the air outward to impact on a tangentially placed nutrient agar strip. The sampler is portable and can be used anywhere, but the sampling volume of air is limited.

(ii) Filter-type sampling method: With the filter-type sampling devices, the desired volume of air can be collected by

appropriately changing the air intake rate or the filter size. However, care must be taken to ensure that sterility is maintained while the filter is placed in and removed from the holder. There are two types of filters: wet-type with gelatin filters and dry-type with membrane filters. With the dry-type filters, static electricity effects can make it impossible to quantitatively collect microorganisms on the filter.

5.1.2. Measurement methods for microorganisms on surfaces

The area to be surface-sampled should be designated according to the condition and shape of the object to be monitored.

(i) Contact plates: A contact plate is used with an appropriate contact surface and sufficient area. In principle, the recommended sampling area for equipment or apparatuses is 24 – 30 cm^2.

The culture medium surface should be brought into contact with the sampling site for several seconds by applying uniform pressure without circular or linear movement. After contact and removal, the plates are covered, and as soon as possible, incubated under appropriate culture conditions. After a contact plate has been used, the site to which the plate was applied must be wiped aseptically to remove any adherent culture medium.

(ii) Swabs: A sterilized, lint-free swab that is suitable for collecting microorganisms is premoistened with an appropriate rinse fluid, and then sampling is conducted by swabbing the defined sampling area in a slow circular movement or in closely parallel strokes while changing direction. After sampling, the swab is agitated in a specified amount of an appropriate sterilized rinse fluid, and the rinse fluid is assayed for viable organisms according to Section 4.05 of the Microbiological Examination of Non-sterile Products.

(iii) Adhesive sheets: An adhesive sheet for sampling is evenly applied to the surface of the item to be monitored and removed. This process should be repeated several times for one sampling area. Microorganisms captured on the adhesive sheet are counted in an appropriate manner. Ultrasonic treatment can be applied to recover microorganisms into solution.

5.1.3. Settling plates (passive microbial sampling method)

Petri dishes of a specified diameter (petri dishes 9 cm in diameter are commonly used) containing a suitable culture medium are placed at the measurement location, and the cover is removed. The plates are exposed for a specified time and the microorganisms deposited from the air onto the agar surface are enumerated after incubation. This method is not effective for quantitative monitoring of total airborne microorganisms, because it does not detect microorganisms that do not settle onto the surface of the culture media, and the settling velocity of aggregates of microorganisms is affected by air currents and disturbances in airflow. Although the results obtained by the settle plate method are only qualitative or semi-quantitative, this method is suitable for long-term evaluation of possible contamination of products or devices by airborne microorganisms.

Before using this method, it should be ensured that the growth of microorganisms is not inhibited due to dryness of the agar surface after lengthy exposure. The data obtained by settling plates can be useful when considered in combination with results from active sampling methods.

5.1.4. Cultivation

Culture conditions under which microorganisms can grow with high reproducibility should be applied in environmental monitoring. Growth promotion testing should be performed on all lots of prepared media. Inactivating agents may be used to negate or inhibit the effects of disinfectants or antibacterial agents that are used or manufactured in the area to be monitored.

Culture media and conditions will depend on types of target microorganisms. Examples of culture media and conditions are shown in Table 5. Liquid media as well as agar media listed in the table can be used according to the measurement method.

Media and extraction liquids should be sterilized in an appropriate manner.

In general, minimum incubation time is 5 days. If the counted number of colonies in shorter incubation time is reliable, the number may be adopted for viable count.

For detection of anaerobes, an appropriate culture medi-

Table 5 Media (examples)

Microorganisms to be detected	Media	Temperature and Incubation Time
Aerobes, Yeast and fungi	SCD agar medium SCDLP agar medium SCDL agar medium	25 – 30°C More than 5 days
Aerobes	SCD agar medium SCDLP agar medium SCDL agar medium	30 – 35°C More than 5 days
Yeast and fungi	SCD agar medium SCDLP agar medium SCDL agar medium Sabouraud glucose agar medium Potato dextrose agar medium Glucose peptone agar medium	20 – 25°C More than 5 days
Anaerobes	Reinforced clostridial agar medium SCD agar medium	30 – 35°C More than 5 days (under an anaerobic culture condition)
Extraction Liquids	Saline solution Phosphate buffered saline solution Phosphate buffered solution (pH 7.2) Buffered sodium chloride-peptone solution (pH 7.0) Peptone saline solution Peptone solution	

um and conditions should be applied.

(i) SCDLP agar medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Lecithin	1.0 g
Polysorbate 80	7.0 g
Agar	15.0 g
Water	1000 mL

Adjust pH to 7.1 – 7.5 at 25°C after sterilization in an autoclave using a validated cycle.

(ii) SCDL agar medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Lecithin	1.0 g
Agar	15 g
Water	1000 mL

Adjust pH to 7.1 – 7.5 at 25°C after sterilization in an autoclave using a validated cycle.

(iii) Glucose peptone agar medium

Peptone	5.0 g
Yeast extract	2.0 g
Glucose	20.0 g
Magnesium sulfate heptahydrate	0.5 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Adjust pH to 5.6 – 5.8 at 25°C after sterilization in an autoclave using a validated cycle.

(iv) Reinforced clostridial agar medium

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Dextrose monohydrate	5.0 g
Cystein hydrochloride	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	15.0 g
Water	1000 mL

Adjust pH to 6.6 – 7.0 at 25°C after sterilization in an autoclave using a validated cycle.

(v) Phosphate buffered saline

Potassium dihydrogen phosphate	0.0425 g
Sodium chloride	8.5 g
Water	1000 mL

(vi) Peptone saline

Peptone	1.0 g
Sodium chloride	8.5 g
Water	1000 mL

(vii) Peptone solution

Peptone	10.0 g
Sodium chloride	5.0 g
Water	1000 mL

5.1.5. Identification

Identification of microorganisms detected in Grade A and B areas to the species level is recommended. Genotypic methods are more accurate and precise than traditional biochemical and phenotypic techniques. These results can be used for investigations into contaminants found in sterility tests or process simulations. See ''Rapid Identification Method of Microorganisms by Gene Analysis'' for additional information on gene analysis.

5.2. Rapid test methods

Rapid test methods can provide results in a shorter time compared with traditional culture methods.

In general, scientifically validated devices should be used for the following aspects of the identification process:

(i) Collecting method (filtration, impact, adhesion, or air aspiration etc.)

(ii) Detection signal (fluorescence, luminescence etc.)

(iii) Detection device

In many cases, the detection thresholds in the rapid test methods are higher than those in traditional methods. Sufficient consideration should be given to qualification of equipment and calibration methods when introducing rapid test methods. In addition, as the measurement principles are different from those in cultural methods, acceptance criteria for each method should be established based on scientific rationale. The acceptance criteria for rapid test methods should be equivalent to or more stringent than those for traditional methods.

6. References

(i) PIC/S GUIDE TO GOOD MANUFACTURING PRCTICE FOR MEDICAL PRODUCTS ANNEXES: Annex 1-Manufacture of sterile medicinal products (March 2014)

(ii) ISO/DIS 14644-1 (2010): Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration

Parametric Release of Terminally Sterilized Pharmaceutical Products

The pharmaceuticals and medical devices to which terminal sterilization can be applied generally must be sterilized so that a sterility assurance level of 10^{-6} or less is obtained. The sterility assurance level of 10^{-6} or less can be proven by using a sterilization process validation based on physical and microbiological methods, but cannot be proven by Sterility Tests ⟨*4.06*⟩.

In Japan, parametric release has been required since 1997 for sterile medical devices that have been sterilized by methods such as moist-heat sterilization or ionizing radiation.[1)] The same sterilization validation, sterility assurance levels, and the like that are used for sterile medical devices have also been applied to sterile pharmaceutical products produced by terminal sterilization, but the use of parametric release is not widespread.

This chapter deals with the necessary requirements for the appropriate management of the critical sterilization parameters, including validation and routine control, of the sterilization process for ''parametric release'' ensuring a sterility assurance level of 10^{-6} or less in products, without performing the Sterility Tests ⟨*4.06*⟩ with low probability of contamination detection on products which have been subjected to terminal sterilization. At such times, the critical sterilization parameters to be controlled should be selected and validated according to the risk posed to product quality, and the selection should be based on the results of the sterilization process and a full understanding of microbial control during the manufacturing process. This will allow parametric release based on parameter control to be implemented.

Because of the limited experience with the actual adoption of parametric release for terminally sterilized pharmaceutical products in Japan, the adoption of parametric release may

tend to require unconventional sterilization equipment and techniques. In this chapter, these points have been sorted anew in order to recommend and expedite the adoption of parametric release.

For general matters such as process validation of manufacturing control and quality control of sterile pharmaceutical products, refer to the laws, notifications, administrative notices, and the like in which they are described in greater detail.

1. Definitions

The definitions of the terminology used in this chapter are provided below.

1.1. Parametric release: A release procedure based on an evaluation of information collected in the course of production, including critical parameters of the sterilization process (temperature, humidity, pressure, time, radiation dose, etc.), based on the results of validation and confirmation of compliance with GMP requirements, in lieu of release based on testing results of the final product.

1.2. Terminal sterilization: A process whereby a product is placed in a container and is then sterilized, and which permits the measurement and evaluation of quantifiable microbial lethality. The process should normally be carried out under conditions ensuring a sterility assurance level of 10^{-6} using, for example, which is a suitable sterilization indicator.

1.3. Sterility assurance level (SAL): Probability of a viable microorganism being present in a sterilized product, expressed as 10^{-n}.

1.4. Critical sterilization parameter: Physical parameters in the sterilization process, whose variability affects the sterility assurance level.

1.5. Sterilization indicator: Indicators are introduced into a loaded product that is to be sterilized for confirmation of the sterilization of the product to be sterilized or auxiliary use for each sterilization batch. Indicators include physical indicators (such as dosimeters), chemical indicators (CI), and biological indicators (BI).

1.6. F_0 value: Assume a value of 10°C for the Z value defined as the number of degrees of temperature required for a 10-fold change in the D value when the reference temperature is 121.1°C. The F_0 value indicates the time (minutes) required to give the equivalent lethality at T_b of the sterilization heat obtained by integrating the lethality rate (L) over an entire heating cycle.

$$L = \log^{-1}\frac{T_0 - T_b}{Z} = 10^{\frac{T_0 - T_b}{Z}}$$

T_0: Temperature inside the chamber or inside the product to be sterilized

T_b: Reference temperature (121.1°C)

$$F_0 = \int_{t_0}^{t_1} L \, dt$$

$t_1 - t_0$ = Processing time (minutes)

1.7. Risk assessment: A systematic process for organizing information to support decisions on risk during the risk management process. The process consists of identifying hazards, and analyzing and assessing risks associated with exposure to such hazards. In this method, ''harm'' refers to terminally sterilized pharmaceutical products, including the integrity of container closure systems, that do not meet the desired sterility assurance level. Hazards refer to latent factors that give rise to such harm.

2. Release of sterilized product

Concepts such as sterilization validation, process control methods including critical sterilization parameters, and sterility assurance levels for parametrically released terminally sterilized pharmaceutical products are the same as for conventional terminally sterilized pharmaceutical products. The greatest difference is that release is not based on the results of sterility tests with low probability of decontamination detection. Parametric release is also based on records of critical sterilization parameters. A procedure for release based upon confirmation of sterilization within the permissible range of predefined critical sterilization parameters must be established and documented.

The sterility of parametrically released products should be confirmed with the inclusion of the following.

1) Batch production records must be checked.

2) The recorded results for critical sterilization parameters must be within permissible range.

3) Products must be sterilized in the specified loading format.

4) If sterilization indicators (such as BI or CI) are used, the results must be appropriate.

5) The bioburden of the product before sterilization must be within permissible range.

6) Microorganism evaluation data of the production environment must be within permissible range as needed.

If the results of examination and verification are out of permissible range, the product cannot be released, even if the sterility test results are acceptable.

3. Applicable sterilization methods and control points

In methods of sterilization applied to parametric release, the mechanism by which microorganisms are sterilized must be clearly defined, the critical control points must also be clearly specified, and the sterilization process must be able to be validated by appropriate physical and microbiological methods. In this chapter, the moist-heat sterilization and radiation (gamma ray radiation sterilization and electron beam radiation sterilization) in ''Sterilization and Sterilization Indicators'' are presented as basic sterilization methods, but other sterilization methods can also be used as long as they can control the critical sterilization parameters and can consistently ensure a sterility assurance level of 10^{-6} or less. Regulatory approval must be obtained to apply parametric release to approved terminally sterilized pharmaceutical products.

3.1. Moist-heat sterilization

Moist-heat sterilization includes widely used saturated steam sterilization and other types of moist-heat sterilization.

Critical sterilization parameters in this method are temperature, pressure, and exposure time at the specified temperature. Therefore, the temperature, pressure, and exposure time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications. The control points and control frequency for the critical sterilization parameters in moist-heat sterilization are provided as reference in Table 1.

3.2. Radiation

Radiation refers to methods of killing microorganisms through exposure to ionizing radiation. The types of ionizing radiation used are gamma-rays (γ-rays) emitted from a radioisotope such as ^{60}Co, or electron beams and bremsstrahlung (X-rays) generated from an electron accelerator. In the case of γ-rays, the cells are killed by secondarily-generated electrons, while in the case of the electron beam,

Table 1 Control points and control frequency for parametric release of terminally sterilized pharmaceutical products in moist-heat sterilization (reference)

Control point		Control frequency
Critical sterilization parameter	—Temperature (the validity of the control point should be pre-validated)* —Pressure in sterilization chamber* —Exposure time at specified temperature* —Heating history (usually indicated by F0 value)	Each batch
Critical process characteristics	—Loading format of product being sterilized* —Vacuum deaeration profile (if applicable)	Each batch
Quality of sterilization medium (for saturated steam)	—Degree of superheat —Dryness —Non-condensable gas concentration —Chemical purity (add to control points as needed)	Recommended frequency: once or twice year
Quality of sterilization medium (for vapor-air mixture, hot water)	—Chemical purity (add to control points as needed)	Recommended frequency: once or twice year
Common utilities	—Quality of the air introduced into the sterilization chamber to restore pressure, etc. (add to control points as needed) —Quality of water used for cooling (add to control points as needed)	Recommended frequency: once or twice year
Sterilizer	—Calibration of critical instruments (thermometers, pressure gages, timers, recorders, etc.)* —Air-tightness of cans —Vacuum performance (add to control points as needed) —Temperature distribution in unloaded state —Other maintenance points that are necessary for machinery and equipment	Recommended frequency: once or twice year

* Control requirements needed for any sterilization cycles for which parametric release is applicable

the cells are killed by electrons generated directly from the electron accelerator. For this reason, the processing time for electron beam sterilization is generally shorter than that for γ-ray sterilization; however, it is inferior to γ-rays in terms of penetration, therefore appropriate consideration must be paid to the density and thickness of the product being sterilized when choosing a method. In radiation, sterilization process control is mainly performed by measuring the dose, therefore the process is also called dosimetric release. The critical sterilization parameters, and the utilities and control devices that should be controlled in radiation sterilization are provided as reference in Table 2.

4. Sterilization validation

In order to adopt parametric release, critical sterilization parameters that can scientifically prove a sterility assurance

Table 2 Critical sterilization parameters, utilities, and control devices in radiation sterilization (reference)

	γ-ray radiation sterilization	Electron beam radiation sterilization
Critical sterilization parameters	—Absorbed dose —Loading pattern (density) of product being sterilized —Exposure time —Other requirements	—Absorbed dose —Loading pattern (density) of product being sterilized —Electron beam characteristics (average electron beam current, electron energy, scan width) —Exposure time (conveyor speed or cycle time) —Other requirements
Utilities and control devices that should be controlled	—Dose measurement system —Others	—Dose measurement device —Belt conveyor —Dose measurement system —Other

Table 3 ISO and JIS specifications related to sterilization[2–12]

Sterilization	ISO specifications	JIS specifications
Radiation sterilization	ISO 11137-1: 2006 ISO 11137-2: 2006 ISO 11137-3: 2006	JIS T 0806-1: 2010 JIS T 0806-2: 2010 JIS T 0806-3: 2010
Moist heat sterilization	ISO 17665-1: 2006	JIS T 0816-1: 2010
CI	ISO 11140-1: 2005	JIS T 11140-1: 2013
BI	ISO 11138-1: 2006 ISO 11138-3: 2006	
Packaging materials	ISO 11607-1: 2006 ISO 11607-2: 2006	JIS T 0841-1: 2009 JIS T 0841-2: 2009
Microbiological tests	ISO 11737-1: 2006 ISO 11737-2: 2009	JIS T 11737-1: 2013 JIS T 11737-2: 2013

level of 10^{-6} or less and their permissible ranges should be determined by validation using qualified sterilization chambers and sterilizers. It should be checked on a routine basis that sterilization is being achieved under conditions meeting these permissible ranges, and the results should be regularly examined.

1) All equipment needed for sterilization must undergo design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ).

2) In order to confirm that the equipment can be operated under typical sterilization conditions, OQ should be performed to check on temperature distribution, temperature uniformity, vacuum performance, and pressure regulating capacity in an unloaded state in the case of moist heat sterilization, and to check on dose uniformity, etc., in the case of radiation sterilization.

3) In terms of sterilization method and conditions, appropriate methods and parameters should be established in accordance with the properties of the product. Any of the following methods can be adopted to establish sterilization conditions. The ISO and JIS specifications in Table 3 should be used for reference when implementing sterilization validation.

— Half-cycle method

— Overkill method
— Combination of bioburden and BI
— Absolute bioburden method
— In the case of radiation sterilization, methods based on ISO 11137-2 (JIS T 0806-2)

4) Heat permeability tests, BI challenge tests, and the like should be conducted in PQ to establish loading format and to identify whether hot spots and cold spots do and do not exist. Parameters demonstrating a sterility assurance level of 10^{-6} or less and their permissible ranges should be determined on the basis of the PQ results. PQ may also be performed after products or loading formats have been grouped according to the types and characteristics of the product to be sterilized, the size of sterilization batches, sterilization cycle characteristics, and the like.

5) In case permissible ranges of deviation, recording back-up conditions, and the like in sterilization cycles are set, adequate risk assessment should be performed to confirm its validity.

6) Periodic re-validation should normally be performed once a year. Periodic re-validation should be performed to confirm the efficacy of the parameters set for each sterilizer in view of the expected product or loading format. Re-validation may also be performed after grouping in the same manner as in PQ.

7) When changes potentially affecting product conformance or sterility assurance are made, sterilization validation should be performed in advance to show that a sterility assurance level of 10^{-6} or less can be proven even after the change. Changes that affect sterility assurance can include changes in the composition, volume, packaging configuration, and loading format of the pharmaceutical product to be sterilized, conditions for supplying the sterilization medium, and the sterilizer structure and bioburden.

5. Routine control

5.1. General requirements of routine control

1) Appropriate measures must be taken for the product to be sterilized in order to avoid cross-contamination of unsterilized and sterilized products.

2) Measures should be taken as needed to prevent recontamination of sterilized products.

3) Sterilization-related process control, maintenance, supply of gas, air, and water, etc., procedures and control points for confirming sterilization, and the like should be fully documented.

4) Based on the results of validation performed to establish terminal sterilization conditions, detailed procedures for implementing the sterilization process should be established, documented, and complied. These procedures should include the followings.

1. Critical sterilization parameters, control points, and their permissible ranges necessary for routine sterilization control
2. Methods and criteria for determining that the sterilization process meets these requirements
3. Procedural provisions for various records and their storage
4. Measures to be taken in the event of deviations
5. Loading format for each product (except for continuous sterilizers)
6. Confirmation that sterilization is initiated within the prescribed time range after the preparation of drug solution or after the filtration of drug solution, if filtration is also used

5) Periodic re-validation, maintenance, calibration, equipment test parameters, and the like should be documented with detailed procedures and frequency.

6) Methods for detecting microorganisms that are strongly resistant to bioburden test methods and applicable sterilization methods should be established and documented.

7) Methods of action to take when microorganisms strongly resistant to applicable sterilization methods are isolated should be established and documented.

8) Sterilization indicators that are suitable for checking the process may be used. The specifications, efficacy, validity of methods of use and the like for using sterilization indicators should be validated and documented.

5.2. Methods of routine control

1) Routine control should be implemented for each sterilization batch in accordance with established procedures.

2) All data showing that the sterilization process was achieved within the specified permissible ranges should be recorded. The records should also be checked and approved by the persons responsible.

1. The date on which the sterilization process was carried out, and the times when the process was started and completed
2. The sterilization equipment that was used
3. The sterilization conditions that were adopted
4. Records of the history of physical parameters in the sterilization process
5. Sterilization criteria and results
6. Attributes and loading pattern of product to be sterilized
7. Names of personnel implementing the sterilization process

3) Appropriate action should be taken in accordance with established procedures for deviations from established procedures, alert levels, action levels, permissible ranges for parameters, and the like.

4) Records of the sterilization process and the maintenance of the sterilization process support system should be prepared and kept.

5) Equipment used in the control, measurement, and recording of critical sterilization parameters in the sterilization cycle should be calibrated, the calibration frequency and acceptable error should be established, and calibration should be performed with standard devices associated with formal standards. Control and measurement devices supporting the sterilization process should also be similarly handled.

6) Storage must not compromise the quality of sterilized products. Storage locations, methods, environments, times and the like should be predefined and appropriately controlled accordingly.

5.3. Microorganism control program

For sterile pharmaceutical products, it is important to understand, evaluate, and control the bioburden of the product before sterilization, the presence or absence of microbes that are heat-resistant to applicable sterilization methods, and the resistance of isolated microorganisms. That is, the bioburden test referred to here is intended to measure the bioburden count until before the start of sterilization using the Microbial Enumeration Tests in Microbiological Examination of Non-sterile Products <*4.05*> or an alternative method via a previously specified method and frequency, and, if needed, to survey the characteristics of isolated microorganisms to investigate the presence or absence of heat-resistant microbes or their resistance to the applicable sterilization method. A bioburden test of the product before sterilization should be conducted for each batch. However, if the overkill method is adopted, the bioburden test may be conducted at an appropriately

established frequency.

5.3.1. Microbial enumeration tests

This test should be conducted in view of the time from the adoption of the test until the start of the sterilization process for the applicable pharmaceutical product based on the Microbial Enumeration Tests in Microbiological Examination of Non-sterile Products ⟨*4.05*⟩. A predetermined quantity of the applicable pharmaceutical product before sterilization should be tested. The test should be conducted by the membrane filter method using all specified sample units under aseptically controlled conditions. If it is difficult to use the entire sample or to conduct the test using the membrane filter method, another method should be used after clarification of the reasons.

5.3.2. Heat-resistant microorganism test

This is a screening test which should be conducted as needed to confirm the presence or absence of heat-resistant microorganisms (spores) in the product before sterilization. A predetermined quantity of the applicable pharmaceutical product before sterilization should be tested. The test should be conducted using all specified sample units under aseptically controlled conditions. Samples should be heated to between 80°C and 100°C for 10 to 15 minutes in a water bath. All of the sample should be tested by the membrane filter method. If it is difficult to use the entire sample or to conduct the test using the membrane filter method, another method should be used after clarification of the reasons. The culture conditions should be based on the Microbial Enumeration Tests in Microbiological Examination of Non-sterile Products ⟨*4.05*⟩.

5.3.3. Identification of microbial species

Microorganisms isolated in the Microbial enumeration tests or heat-resistant microorganism test should be identified. Strongly sterilization-resistant microorganisms will be spore-forming, and it is essential that the spores can be accurately identified. Identification methods include methods based on phenotype, methods using simplified identification kits, and methods using the molecular structure or genetic information of microbial components (chemical classification, genetic analysis). The identification should at least reveal the genus, and information on the characteristics should be captured. The results of identification should be utilized for control purposes in sterilization resistance tests, estimation of routes of contamination, and bioburden reduction.

5.3.4. Sterilization resistance test

When heat-resistant microorganisms are isolated in the heat-resistant microorganism test, select an appropriate spore-forming medium to allow the microorganisms to form spores. A spore solution should be prepared using the spores to measure the *D* value (Z value, if needed) as an indicator of sterilization resistance in the product. The *D* value should be measured for the sterilization temperature of the product based on ISO11138 (as reference). If it is known in advance that a solution gives a value higher than the *D* value of the product, that solution may be used to measure the *D* value.

If it is difficult to measure the *D* value, the reasons should be clarified, a solution containing at least 10^6 spores per product should be prepared and heated for no more than half the sterilization time of the sterilization conditions for the product, and it should then be verified that the results of the membrane filter method in Part 5.1 of the Sterility Tests ⟨*4.06*⟩ (however, soybean casein digest medium should be used in the case of culturing) are negative, thereby ensuring that the sterility assurance lever of 10^{-6} or less has been met.

5.4. Sterilization indicators

BI, CI, dosimeters and the like are used as indices of sterility, and are used as a parameter to monitor the sterilization process. When a BI, CI, or dosimeter is used for daily process control, an appropriate one that responds to critical parameters should be used. It must also be verified that the loading pattern on the product or simulated product is equal to or greater than that used for operation qualification

References

1) Medical Device Manufacturing Control and Quality Control Regulations, MHLW Ordinance No. 40, August 26, 1995.
2) JIS T 0806-1: 2010, Sterilization of healthcare products —Radiation—Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices (ISO11137-1: 2006 Sterilization of health care products—Radiation—Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices)
3) JIS T 0806-2: 2014, Sterilization of health care products—Radiation—Part 2: Establishing the sterilization dose (ISO11137-2: 2013 Sterilization of health care products—Radiation—Part 2: Establishing the sterilization dose)
4) JIS T 0806-3: 2010, Sterilization of health care products—Radiation—Part 3: Guidance on dosimetric aspects (ISO11137-3: 2006 Sterilization of health care products—Radiation—Part 3: Guidance on dosimetric aspects)
5) JIS T 0816-1: 2010, Sterilization of health care products—Moist heat—Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices (ISO17665-1: 2006 Sterilization of health care products—Moist heat—Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices)
6) JIS T 11140-1: 2013, Sterilization of health care products—Chemical indicators—Part 1: General requirements (ISO11140-1: 2014 Sterilization of health care products—Chemical indicators—Part 1: General requirements)
7) ISO11138-1: 2006 Sterilization of health care products —Biological indicators—Part 1: General requirements
8) ISO11138-3: 2006 Sterilization of health care products —Biological indicators—Part 3: Biological indicators for moist heat sterilization processes
9) JIS T 0841-1: 2009, Packaging for terminally sterilized medical devices—Part 1: Requirements for materials, sterile barrier systems and packaging systems (ISO11607-1: 2006 Packaging for terminally sterilized medical devices—Part 1: Requirements for materials, sterile barrier systems and packaging systems)
10) JIS T 0841-2: 2009, Packaging for terminally sterilized medical devices—Part 2: Validation requirements for forming, sealing and assembly processes (ISO11607-2: 2006 Packaging for terminally sterilized medical devices—Part 2: Validation requirements for forming, sealing and assembly processes)
11) JIS T 11737-1: 2013, Sterilization of medical devices—Microbiological methods—Part 1: Determination of a population of microorganisms on products (ISO11737-1: 2006 Sterilization of medical devices—Microbiological methods—Part 1: Determination of a population of microorganisms on products)
12) JIS T 11737-2: 2013, Sterilization of medical devices—Microbiological methods—Part 2: Tests of sterility performed in the definition, validation and maintenance

of a sterilization process (ISO11737-2: 2009 Sterilization of medical devices—Microbiological methods—Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process)

Preservatives-Effectiveness Tests

The purpose of the Preservatives-Effectiveness Tests is to assess microbiologically the preservative efficacy, either due to the action of product components themselves or any added preservative(s), for multi-dose containers[1,2]. The efficacy of the preservatives is assessed by direct inoculation and mixing of the test strains in the product, and determination of survival of the test strains with time.

Water activity in products plays an important role in the growth of microbial contaminant. In the case of article packaged in multiple-dose container, the degeneration change in quality could be occurred during use due to the microbial contamination, and if such contaminated product is used it could cause not only a decreasing in medical effect but also a hazard to the patient from infection. From these reasons, to the products packaged in multiple-dose containers addition of appropriate preservatives is permitted by General Rules for Preparations in the JP.

Preservatives must not be used solely to comply with GMP for drugs or to reduce viable aerobic (bacteria and yeasts/moulds) counts. In addition, preservatives show the toxicity depending on quantity. Therefore, preservatives must not be added to products in amounts which might jeopardize the safety of human beings, and consideration must be given to minimizing the amounts of preservative used. These tests are commonly used to verify that products maintain their preservative effectiveness at the design phase of formulation or in the case of periodic monitoring. Although these tests are not performed for lot release testing, antimicrobial action of the product itself or the efficacy of the preservative added to the product should be verified over the shelf life. Testing for antimicrobial preservative content should normally be performed at release. Under certain circumstances, in-process testing may suffice in lieu of release testing.

1. Products and their Categories

The products have been divided into two categories for these tests (Table 1). Category I products are those made with aqueous bases or vehicles, and having a water activity of not less than 0.6. Category II products are those made with nonaqueous bases or vehicles. Oil-in-water emulsions are considered Category I products, and water-in-oil emulsions Category II products.

2. Test Microorganisms, Growth Promotion Test and Suitability of the Counting Method

2.1. Preparation of test strains

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 2. The strains shown in Table 2 or those considered to be equivalent are used as the test microorganisms.

In addition to these strains designated as test microorganisms, it is desirable to use strains that might contaminate the product and grow on or in it, depending on its characteristics. It is desirable, for example, that *Zygosaccharomyces rouxii* (NCYC 381; IP 2021.92; NBRC 1960) is used for the products like as Syrups containing a high concentration of sugar. The test strains can be harvested by growth on solid agar or liquid media.

Cultures on agar media: Inoculate each of the five test strains on the surface of agar plates or agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Glucose Agar Medium. Incubate bacterial cultures at 30°C to 35°C

Table 1 Product categories

Category	Products
IA	· Injections · Sterile products made by dissolving or suspending in aqueous vehicles (ophthalmic preparations, ear preparations, nasal preparations, etc.)
IB	· Topically used non-sterile products made by dissolving or suspending in aqueous vehicles or by mixing with aqueous bases (ear preparations, nasal preparations, inharations, including those applied to mucous membranes, etc.)
IC	· Preparations for oral administration other than antacids made by dissolving or suspending in aqueous vehicles or by mixing with aqueous bases and those applied to the oral cavity.
ID	· Antacids made with aqueous vehicles or bases
II	· All the dosage forms listed under Category I made with non-aqueous bases or vehicles

Table 2 Test microorganisms and culture conditions

Organism	Strain	Medium	Incubation temperature	Inoculum incubation time
Escherichia coli	ATCC 8739 NBRC 3972	Soybean-Casein Digest Medium Soybean-Casein Digest Agar Medium	30 – 35°C	18 – 24 hours
Pseudomonas aeruginosa	ATCC 9027 NBRC 13275	Soybean-Casein Digest Medium Soybean-Casein Digest Agar Medium	30 – 35°C	18 – 24 hours
Staphylococcus aureus	ATCC 6538 NBRC 13276	Soybean-Casein Digest Medium Soybean-Casein Digest Agar Medium	30 – 35°C	18 – 24 hours
Candida albicans	ATCC 10231 NBRC 1594	Fluid Sabouraud Glucose Medium Sabouraud Glucose Agar Medium	20 – 25°C	44 – 52 hours
Aspergillus brasiliensis	ATCC 16404 NBRC 9455	Sabouraud Glucose Agar Medium	20 – 25°C	6 – 10 days

for 18 to 24 hours, the culture of *Candida albicans* at 20°C to 25°C for 44 to 52 hours and the culture of *Aspergillus brasiliensis* at 20°C to 25°C for 6 to 10 days or until good sporulation is obtained. For the bacteria and *C. albicans*, harvest the cultured cells aseptically. Suspend the collected cells in physiological saline and adjust the viable cell count to about 10^8 CFU/mL. In the case of *A. brasiliensis*, suspend the cultured cells in physiological saline containing 0.05 w/v% of polysorbate 80 and adjust the spore count to about 10^8 CFU/mL. Filter, if needed, the spore suspension through a sterilized gauze or glass wool to remove hyphae. The medium components must be removed from all of the cells so prepared by centrifugation if needed. Use these suspensions as the inocula.

Liquid cultures: After cultivation each of the four strains except for *A. brasiliensis* in Soybean-Casein Digest Medium or in Fluid Sabouraud Glucose Medium, remove the medium by centrifugation. Wash the cells in physiological saline and resuspend them in the same solution with the viable cell count of the inoculum adjusted to about 10^8 CFU/mL.

When strains other than the five listed above are cultured, select a culture medium suitable for growth of the strain concerned. The cell suspension may also be prepared by a method suitable for that strain. If it is not possible to inoculate the microbial suspensions into the test specimens within 2 hours after they have been prepared from the cultivations on agar media or in liquid media, keep them at 2 – 8°C and use within 24 hours. Usually, the spore of *A. brasiliensis* may be stored at 2 – 8°C for up to 7 days. Determine the viable cell count of the inocula immediately before use, and then calculate the theoretical viable cell count per mL or per gram of the product present just after inoculation.

2.2. Growth Promotion of the Media

An appropriate culture medium among Soybean-Casein Digest Ager Medium and Sabouraud Glucose Agar Medium is used for these tests. Other media also may be used if they have similar nutritive ingredients and growth-promoting properties for the microorganisms to be tested. The media to be used should be performed the growth promotion test using the strains specified in Table 2 or those considered to be equivalent. The incubation times are not more than 3 days for Soybean-Casein Digest Agar Medium, and not more than 5 days for Sabouraud Glucose Agar Medium.

By the agar media the colony counts should be obtained at least 50% of the standardized cell counts. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

2.3. Suitability of the Counting Method

Dilute 1 mL or 1 g of the product to be examined with 9 times its mass of physiological saline or other appropriate neutral diluting solution (10^{-1} dilution), and prepare more two dilutions of this solution by serial 10-fold dilution (10^{-2} and 10^{-3} dilutions). Add a suitable count of the test cells to each tube of these dilutions, mix, and inoculate them so as to yield less than 250 CFU/plate for bacteria and *C. albicans* (ideally 25 – 250 CFU) or less than 80 CFU/plate for *A. brasiliensis* (ideally 8 – 80 CFU). This plating should be performed minimally in duplicate (or more to minimize variability in the plate count estimate). A positive control for this procedure is to introduce the same inocula into saline and transfer similar volumes of saline to agar plates. A suitable recovery scheme is the one that provides at least 50% of this saline control count (averaged). In this case, the method is validated to verify its suitability in the presence of test specimen, and if the growth of the cells is inhibited an effective inactivator may be added in the buffer solution or liquid medium to be used for dilution of the test specimen, as well as in the agar plate count medium. However, it is necessary to confirm that the inactivator has no effect on the growth of the microorganisms. When the occurrence of the preservative or the product itself affects determination of the viable cell count and there is no suitable inactivator available, calculate the viable cell counts by the Membrane filtration method in Microbiological Examination of Non-sterile Products <*4.05*>. In the case where any change is occurred in the test material or procedure or in the product to be examined which might give any effects to the test result, the validation must be performed for the test once again. In the validation study, if the cell recovery count is not less than 50% of the inoculated cell counts, the inoculated cell counts at 0 day may be used as the theoretical inoculate cell count. See 3.2 for more information to obtain suitable counting method for the Category II products.

3. Test Procedure

3.1. Category I products

Inject each of the cell suspensions aseptically into five containers containing the product and mix uniformly. Single-strain challenges rather than mixed cultures should be used. When it is difficult to inject the cell suspension into the container aseptically or the volume of the product in each container is too small to be tested, transfer aseptically a sufficient volume of the product into each of alternative sterile containers, and mix the inoculum. When the product is not sterile, incubate additional containers containing the uninoculated product as controls and calculate their viable cell counts. The volume of the suspension mixed in the product is 0.5 – 1.0% of the volume of the product. Generally, the cell suspension is inoculated and mixed so that the concentration of viable cells is 1×10^5 to 1×10^6 CPU per mL or per g of the product. For Category ID products (antacids) inoculate so that the final concentration of viable cells is 1×10^3 to 1×10^4 CFU per mL of the product. Incubate these inoculated containers at 20°C to 25°C with protection from light, and calculate the viable cell count of the test preparations at 0, 7 (Category IA only), 14 and 28 days. Record any marked changes (e.g., changes in color or the development of a bad odor or fungus) when observed in the test preparations during this time. Such changes should be considered when assessing the preservative efficacy of the product concerned. The sequential changes in the viable counts are expressed as changes in term of log reduction against the inoculated cell counts (CFU/mg or g). Determination of the viable cell counts is based, in principle, on the Plate-count methods (Pour-plate methods; Surface-spread method) or the Membrane filtration method in ''Microbiological Examination of Non-sterile Products <*4.05*>''. Alternative microbiological procedures, including an automated method, may be used for the products of Categories I and II, provided that they give a result equal to or better than that of the Pharmacopoeial methods[3)].

3.2. Category II products

The procedures are the same as those described for Category I products, but special procedures and considerations are required for both uniform dispersion of the test microorganism in the product and determination of viable cell counts in the test preparations.

For semisolid ointment bases, heat the test preparation to 45°C to 50°C until it becomes oily, add the cell suspension and disperse the inoculum uniformly with a sterile glass rod or spatula. Surfactants may also be added to achieve uniform dispersion, but it is necessary to confirm that the surfactant added has no effect on survival or growth of the

Table 3 Interpretation criteria by product category

Category	Microorganisms	Interpretation criteria
IA	Bacteria	At 7 days: Not less than 1.0 log reduction from the initial count. At 14 days: Not less than 3.0 log reduction from the initial count. At 28 days: No increase from the 14 day's count.
	Yeasts/Moulds	At 7, 14 and 28 days: No increase from the initial count.
IB	Bacteria	At 14 days: Not less than 2.0 log reduction from the initial count. At 28 days: No increase from the 14 day's count.
	Yeasts/Moulds	At 14 and 28 days: No increase from the initial count.
IC	Bacteria	At 14 days: Not less than 1.0 log reduction from the initial count. At 28 days: No increase from the 14 day's count.
	Yeasts/Moulds	At 14 and 28 days: No increase from the initial count.
ID	Bacteria	At 14 and 28 days: No increase from the initial count.
	Yeasts/Moulds	At 14 and 28 days: No increase from the initial count.
II	Bacteria	At 14 and 28 days: No increase from the initial count.
	Yeasts/Moulds	At 14 and 28 days: No increase from the initial count.

test microorganisms and that it does not potentiate the preservative efficacy of the product. For determination of the viable cell count, a surfactant or emulsifier may be added to disperse the test preparations uniformly in the buffer solution or liquid medium. Sorbitan monooleate, polysorbate 80 or lecithin may be added to improve miscibility between the buffer solution or the liquid medium and semisolid ointments or oils in which test microorganisms were inoculated. These agents serve to inactivate or neutralize many of the most commonly used preservatives.

4. Interpretation

Interpret the preservative efficacy of the product according to Table 3. When the results described in Table 3 are obtained, the product examined is considered to be met the requirement of the test. There is a strong possibility of massive microbial contamination having occurred when microorganisms other than the inoculated ones are found in the sterile product to be examined, and caution is required in the test procedures and/or the control of the manufacturing process of the product. When the contamination level in a nonsterile product to be examined exceeds the microbial enumeration limit specified in ''Microbial Attributes of Nonsterile Pharmaceutical Products'' in General Information, caution is also required in the test procedures and/or the control of the manufacturing process of the product. The statement ''No increase from the initial count'' means not more than 0.5 $\log_{10}$ increase from the initial calculated count.

5. Culture Media

Culture media used for Preservatives Effectiveness Tests are described below. Other media may be used if they have similar nutritive ingredients and selective and growth-promoting properties for the microorganisms to be tested.

(i) Soybean-Casein Digest Medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(ii) Soybean-Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.1 – 7.5 at 25°C. Sterilize in an autoclave using a validated cycle.

(iii) Sabouraud Glucose Agar Medium

Glucose	40.0 g
Peptone (animal tissue and casein 1:1)	10.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.

iv) Fluid Sabouraud Glucose Medium

Glucose	20.0 g
Peptone (animal tissue and casein 1:1)	10.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.4 – 5.8 at 25°C. Sterilize in an autoclave using a validated cycle.

6. References

1) European Pharmacopoeia. 8 (2014), 5.1.3. EFFICACY OF ANTIMICROBIAL PRESERVATION.
2) U.S. Pharmacopeia. 38 (2015), <51> ANTIMICROBIAL EFFECTIVENESS TESTING.
3) General Information ''Rapid Microbial Methods''

Rapid Counting of Microbes using Fluorescent Staining

This chapter provides rapid methods using fluorescence staining for the quantitative estimation of viable microorganisms. Incubation on an agar medium has been widely used for quantitative estimation of viable microorganisms, but a number of environmental microorganisms of interest are not easy to grow in culture under usual conditions, thus new microbial detection methods based on fluorescence or luminescence have been developed. In the fluorescence staining method, microorganisms are stained with fluorescent dye, and can easily be detected and counted with various sorts of apparatus, such as a fluorescence microscope or flow cytometer. Methods are available to detect total microorganisms, including both dead and viable cells, or to

detect only cells with a specified bioactivity by choosing the dye reagent appropriately. Nucleic acid staining reagents, which bind with DNA or RNA, detect all cells containing nucleic acids, whether they are live or dead. This technique is the most fundamental for the fluorescence staining method. On the other hand, fluorescent vital staining methods target the respiratory activity of the microorganism and the activity of esterase, which is present universally in microorganisms. In the microcolony method, microcolonies in the early stage of colony formation are counted. The CFDA-DAPI double staining method and the microcolony method are described below. These methods can give higher counts than the other techniques, because these rapid and accurate techniques provide quantitative estimation of viable microorganisms based on a very specific definition of viability, which may be different from that implicit in other methods. The procedures of these methods described here may be changed as experience with the methods is accumulated. Therefore, other reagents, instruments and apparatus than those described here may also be used if there is a valid reason for so doing.

1. CFDA-DAPI double staining method

Fluorescein diacetate (FDA) reagents are generally used for the detection of microorganisms possessing esterase activity. These reagents are hydrolyzed by intracellular esterase, and the hydrolyzed dye exhibits green fluorescence under blue excitation light (about 490 nm). Modified FDAs such as carboxyfluorescein diacetate (CFDA) are used because of the low stainability of gramnegative bacteria with FDA. The principle of the CFDA-DAPI double staining method, which also employs a nucleic acid staining reagent, 4′,6-diamidino-2-phenylindole (DAPI), is as follows. The nonpolar CFDA penetrates into the cells and is hydrolyzed to fluorescent carboxyfluorescein by intracellular esterase. The carboxyfluorescein is accumulated in the living cells due to its polarity, and therefore green fluorescence due to carboxyfluorescein occurs when cells possessing esterase activity are illuminated with blue excitation light. No fluorescent carboxyfluorescein is produced with dead cells, since they are unable to hydrolyze CFDA. On the other hand, DAPI binds preferentially to the adenine and thymine of DNA after penetration into both viable and dead microorganisms, and consequently all of the organisms containing DNA exhibit blue fluorescence under ultraviolet excitation light. Therefore, this double staining method enables to count specifically only live microorganisms possessing esterase activity under blue excitation light, and also to determine the total microbial count (viable and dead microorganisms) under ultraviolet excitation light.

1.1. Apparatus

1.1.1. Fluorescence microscope or fluorescence observation apparatus

Various types of apparatus for counting fluorescencestained microorganisms are available. Appropriate filters are provided, depending on the fluorescent dye reagents used. A fluorescence microscope, laser microscope, flow cytometer, and various other types of apparatus may be used for fluorescence observation.

1.2. Instruments

(i) Filtering equipment (funnels, suction flasks, suction pumps)

(ii) Membrane filters (poresize: 0.2 μm); A suitable filter that can trap particles on the surface can be used such as polycarbonate filter, alumina filter, etc.

(iii) Glass slide

(iv) Cover glass

(v) Ocular micrometer for counting (with 10 × 10 grids)

1.3. Procedure

An example of the procedure using fluorescence microscope is described below.

1.3.1. Preparation of samples

Prepare samples by ensuring that microbes are dispersed evenly in the liquid (water or buffer solution).

1.3.2. Filtration

Set a membrane filter (poresize: 0.2 μm) on the funnel of the filtering equipment. Filter an appropriate amount of sample to trap microbes in the sample on the filter.

1.3.3. Staining

Pour sufficient amount of buffer solution for CFDA staining, mixed to provide final concentration of 150 μg/mL of CFDA and 1 μg/mL of DAPI, into the funnel of the filtering equipment and allow staining in room temperature for 3 minutes, then filter the liquid by suction. Pour sufficient amount of aseptic water in the funnel, filter by suction, and remove excess fluorescent reagent left on the filter. Thoroughly dry the filter.

1.3.4. Slide preparation

Put one drop of immersion oil for fluorescence microscope on the glass slide. Place the air dried filter over it, with the filtering side on the top. Then put one drop of immersion oil for fluorescence microscope on the surface of the filter, place a cover glass. Put another drop of immersion oil for fluorescence microscope on the cover glass when using an oilimmersion objective lens.

1.3.5. Counting

Observe and count under fluorescence microscope, with 1000 magnification. In case of CFDA-DAPI double staining method, count the microorganisms (with esterase activity) exhibiting green fluorescence under the blue excitation light first to avoid color fading by the ultraviolet light, then count the microorganisms (with DNA) exhibiting blue fluorescence under the ultraviolet excitation light in the same microscopic field. Count the organisms exhibiting fluorescence on more than 20 randomly selected fields among 100 grids observed through an ocular micrometer of the microscope, and calculate the total number of organisms using the following formula. The area of the microscopic field should be previously determined with the ocular and objective micrometers. The amount of the sample to be filtered must be adjusted so that the cell number per field is between 10 and 100. It might be necessary to reprepare the sample in certain instances. (In such case that the average count number is not more than 2 organisms per field, or where more than 5 fields are found which have no organism per field, it is assumed that the microorganism count is below the detection limit.)

Number of microbes (cells/mL)
= {(average number of microbes per visual field) × (area of filtration)}/{(amount of sample filtered) × (area of one microscopic field)}

1.4. Reagents and test solutions

(i) Aseptic water: Filter water through a membrane filter with 0.2 μm pore size to remove particles, then sterilize it by heating in an autoclave at 121°C for 15 minutes. Water for injection may be used.

(ii) CFDA solution, 10 mg/mL: Dissolve 50 mg of CFDA in dimethylsulfoxide to prepare a 5 mL solution. Store at −20°C in light shielded condition.

(iii) Buffer solution for CFDA staining: Dissolve 5 g of sodium chloride with 0.5 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS and diluted disodium hydrogen phosphate TS (1 in 3) to prepare 100 mL of solution. Add sodium dihydrogen phosphate dihydrate solution (1 in 64) to adjust the pH level to 8.5. Filter the solution

through a membrane filter with a pore size of 0.2 μm.

(iv) DAPI solution, 10 μg/mL: Dissolve 10 mg of DAPI in 100 mL of aseptic water. Dilute this solution 10 times with aseptic water and filter through a membrane filter with a pore size of 0.2 μm. Store at 4°C in light shielded condition.

(v) Immersion oil for fluorescence microscope

2. Microcolony method

Microcolonies, which are in early stages of colony formation, are fluorescently stained, then observed and counted under fluorescence microscope or other suitable systems. This method enables to count the number of proliferative microorganisms, with short incubation time. In this method, the organisms are trapped on a membrane filter, the filter is incubated on a medium for a short time, and the microcolonies are counted. By this method, even colonies which are undetectable with the naked eye can be identified, so viable organisms can be counted rapidly and with high precision. Various nucleic acid staining reagents can be used for staining of microcolonies.

2.1. Apparatus

2.1.1. Fluorescence microscope or fluorescence observation apparatus

Various types of apparatus for counting fluorescencestained microorganisms are available. Appropriate filters are provided, depending on the fluorescence dye reagents used. A fluorescence microscope, laser microscope and various other types of apparatus may be used for fluorescence observation.

2.2. Instruments

(i) Filtering equipment (funnels, suction flasks, suction pumps)

(ii) Membrane filters (pore size: 0.2 μm); A suitable filter that can trap particles on the surface can be used such as polycarbonate filter, alumina filter, etc.

(iii) Glass slide

(iv) Cover glass

(v) Filter paper (No. 2)

(vi) Ocular micrometer for counting (with 10 × 10 grids)

2.3. Procedure

An example of the procedure using a fluorescence microscope is described below.

2.3.1. Preparation of samples

Prepare samples by ensuring that microbes are dispersed evenly in the liquid (water or buffer solution).

2.3.2. Filtration

Set a membrane filter (pore size: 0.2 μm) on the funnel of the filtering equipment. Filter an appropriate amount of sample to trap microbes in the sample on the filter.

2.3.3. Incubation

Remove the filter from the filtering equipment and place it with filtering side facing up on a culture medium avoiding formation of airbubbles between the filter and the medium. Incubate at a suitable temperature for appropriate hours in a dark place. It should be noted that the appropriate incubation conditions (such as medium, incubation temperature and/or incubation time) are different, depending on the sample.

2.3.4. Fixation

Soak filter paper with an appropriate amount of neutral buffered formaldehyde test solution, then place the filter that has been removed from the culture medium on top with filtering side up, and allow to remain at room temperature for more than 30 minutes to fix the microcolonies.

2.3.5. Staining

Soak filter paper with an appropriate amount of staining solution (such as 1 μg/mL of DAPI, 2% polyoxyethylenesorbitan monolaurate), then place the filter on top with filtering side up, and then leave at room temperature, light shielded for 10 minutes to stain microcolonies. Wash the filter by placing it with the filtering side facing up on top of a filter paper soaked with aseptic water for 1 minute. Thoroughly air dry the filter.

2.3.6. Slide preparation

Put one drop of immersion oil for fluorescence microscope on the slide glass. Place an air dried filter over it, with the filtering side on the top. Then, put one drop of immersion oil for fluorescence microscope on top, place a cover glass.

2.3.7. Counting

Count the organisms exhibiting fluorescence on more than 20 randomly selected fields among the 100 grids observed through an ocular micrometer of the microscope with 400 or 200 magnification, and calculate the total number of organisms using the following formula. The area of the microscopic field should be previously determined with the ocular and objective micrometers. In such case that the average count number is not more than 2 microcolonies per field, or where more than 5 fields are found which have no microcolony per field, it is assumed that the microorganism count is below the detection limit.

Number of microcolonies (cells/mL)
= {(average number of microcolonies per visual field) × (area of filtration)}/{(amount of sample filtered) × (area of one microscopic field)}

2.4. Reagents and test solutions

(i) Aseptic water: Filter water through a membrane filter with 0.2 μm pore size to remove particles, and sterilize it by heating in an autoclave at 121°C for 15 minutes. Water for injection may be used.

(ii) Staining solution: Dissolve 10 mg of DAPI in 100 mL of aseptic water. Dilute the solution 10 times with aseptic water and filter through a membrane filter with pore size of 0.2 μm. Store at 4°C in light shielded condition. Dissolve polyoxyethylene sorbitan monolaurate to the final concentration of 2%, when using.

(iii) Neutral buffered formaldehyde solution (4w/v% formaldehyde solution; neutrally buffered).

(iv) Immersion oil for fluorescence microscope

Rapid Identification of Microorganisms Based on Molecular Biological Method

This chapter describes the methods for the identification or estimation of microorganisms (bacteria and fungi), found in in-process control tests or lot release tests of pharmaceutical products, at the species or genus level based on their DNA sequence homology. The identification of isolates found in the sterility test or aseptic processing can be helpful for investigating the causes of contamination. Furthermore, information on microorganisms found in raw materials used for pharmaceutical products, processing areas of pharmaceutical products, and so on is useful in designing measures to control the microbiological quality of drugs. For the identification of microorganisms, phenotypic analysis is widely used, based on morphological, physiological, and biochemical features and analysis of components. Commercial kits based on differences in phenotype patterns have been used for the identification of microorganisms, but are

not always applicable to microorganisms found in raw materials used for pharmaceutical products and in processing areas of pharmaceutical products. In general, the identification of microorganisms based on phenotypic analysis needs special knowledge and judgment is often subjective. It is considered that the evolutionary history of microorganisms (bacteria and fungi) is memorized in their ribosomal RNAs (rRNAs), so that systematic classification and identification of microorganisms in recent years have been based on the analysis of these sequences. This chapter presents a rapid method to identify or estimate microorganisms based on partial sequences of divergent regions of the 16S rRNA gene for bacteria and of the internal transcribed spacer 1 (ITS1) region located between 18S rRNA and 5.8S rRNA for fungi, followed by comparison of the sequences with those in the database. Methods described in this chapter do not take the place of usual other methods for the identification, and can be modified based on the examiner's experience, and on the available equipment or materials. Other gene regions besides those mentioned in this chapter can be used if appropriate.

1. Apparatuses

(i) DNA sequencer

Various types of sequencers used a gel board or capillary can be used.

(ii) DNA amplifier

To amplify target DNA and label amplified (PCR) products with sequencing reagents.

2. Procedures

The following procedures are described as an example.

2.1. Preparation of template DNA

It is important to use a pure cultivated bacterium or fungus for identification. In the case of colony samples, colonies are picked up with a sterilized toothpick (in the case of fungi, a small fragment of colony sample is picked up), and suspended in 0.3 mL of DNA releasing solution in a 1.5 mL centrifuge tube. In the case of culture fluid, a 0.5 mL portion of fluid is put in a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 10 min. After removal of the supernatant, the pellet is suspended in 0.3 mL of DNA releasing solution, and then heated at 100°C for 10 min. In general, PCR can be run for bacteria and yeasts heated in DNA releasing solution. For fungi, DNA extraction from culture fluid is better because some of colony samples can disturb PCR reaction.

2.2. PCR

Add 2 μL of template DNA in PCR reaction solution. Use 10F/800R primers (or 800F/1500R primers in the case to analyze also a latter part of 16S rRNA) for bacteria and ITS1F/ITS1R primers for fungi, and then perform 30 amplification cycles at 94°C for 30 sec, 55°C for 60 sec, and 72°C for 60 sec. DNA fragments are amplified about 800 bp in the case of bacteria and about 150 – 470 bp depending on the strain in the case of fungi. Include a negative control (water instead of the test solution) in the PCR.

2.3. Confirmation of PCR products

Mix 5 μL of PCR product with 1 μL of loading buffer solution, place it in a 1.5 w/v% agarose gel well, and carry out electrophoresis with TAE buffer solution (1-fold concentration). Carry out the electrophoresis together with appropriate DNA size markers. After the electrophoresis, observe PCR products on a trans-illuminator (312 nm) and confirm the presence of a single band of the targeted size. If multiple bands are observed, cut the targeted band out of the gel, and extract DNA by using appropriate commercial DNA extraction kit.

2.4. Purification of PCR products

Remove unincorporated PCR primers and deoxynucleoside triphosphates (dNTP) from PCR products by using appropriate purification methods.

2.5. Quantification of purified DNA

When purified DNA is measured by spectrophotometer, calculate 1 $OD_{260\,nm}$ as 50 μg/mL.

2.6. Labeling of PCR products with sequencing reagents

Use an appropriate fluorescence-labeled sequencing reagent suitable for the available DNA sequencer or its program and label the PCR products according to the instructions provided with the reagent.

2.7. Purification of sequencing reagent-labeled PCR products

Transfer the product in 75 μL of diluted ethanol (7 in 10) into a 1.5 mL centrifuge tube, keep in an ice bath for 20 min, and centrifuge at 15,000 rpm for 20 min. After removal of supernatant, add 250 μL of diluted ethanol (7 in 10) to the precipitate and centrifuge at 15,000 rpm for 5 min. Remove the supernatant and dry the precipitate.

2.8. DNA homology analysis

Place sequencing reagent-labeled PCR products in the DNA sequencer and read the nucleotide sequences of the PCR products. Compare the partial nucleotide sequence with those in the BLAST database.

3. Judgment

If sequencing data show over 90% identity with a sequence in the database, in general, judgment may be made as follows.

(i) In the case of bacteria, compare the nucleotides in the product obtained with the 10F primer (the 800F primer when 800F/1500R primers are used) with the BLAST database. Higher ranked species are judged as identified species or closely related species.

(ii) In the case of fungi, compare sequencing data for the product obtained with the ITS1F primer with the BLAST database. Higher ranked species are judged as identified species or closely related species.

4. Reagents, Test Solutions

(i) 0.5 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS: Dissolve 18.6 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 100 mL.

(ii) 1 mol/L Tris buffer solution (pH 8.0): Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in a suitable amount of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

(iii) TE buffer solution: Mix 1.0 mL of 1 mol/L tris buffer solution (pH 8.0) and 0.2 mL of 0.5 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and add water to make 100 mL.

(iv) DNA releasing solution: Divide TE buffer solution containing 1 vol% of polyoxyethylene (10) octylphenyl ether into small amounts and store frozen until use.

(v) PCR reaction solution

10-fold buffer solution*	5 μL
dNTP mixture**	4 μL
10 μmol/L Sense primer	1 μL
10 μmol/L Anti-sense primer	1 μL
Heat-resistant DNA polymerase (1 U/μL)	1 μL
Water	36 μL

* Being composed of 100 mmol/L 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride, pH 8.4, 500 mmol/L potassium chloride, 20 mmol/L magnesium chloride and 0.1 g/L gelatin.

** A solution containing 2.5 mmol/L each of dGTP (sodium 2′-deoxyguanosine 5′-triphosphate), dATP (sodium 2′-

deoxyadenosine 5′-triphosphate), dCTP (sodium 2′-deoxycytidine 5′-triphosphate) and dTTP (sodium 2′-deoxythymidine 5′-triphosphate). Adequate products containing these components as described above may be used.

(vi) Sequencing reagent: There are many kinds of sequencing methods, such as the dye-primer method for labeling of primer, the dye-terminator method for labeling of dNTP terminator and so on. Use an appropriate sequencing reagent kit for the apparatus and program to be used.

(vii) 50-Fold concentrated TAE buffer solution: Dissolve 242 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 57.1 mL of acetic acid (100) and 100 mL of 0.5 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and add water to make 1000 mL.

(viii) 1-Fold concentrated TAE buffer solution: Diluted 50-fold concentrated TAE buffer solution (1 in 50) prepared before use is referred to as 1-fold concentrated TAE buffer solution.

(ix) Agarose gel: Mix 1.5 g of agarose, 2.0 mL of 50-fold concentrated TAE buffer solution, 10 μL of a solution of ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) (1 in 100) and 100 mL of water. After dissolving the materials by heating, cool the solution to about 60°C, and prepare gels.

(x) Loading buffer solution (6-fold concentrated): Dissolve 0.25 g of bromophenol blue, 0.25 g of xylene cyanol FF and 1.63 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 50 mL of water, and add 30 mL of glycerol and water to make 100 mL.

(xi) PCR primers

For	Primer	
Bacteria	10F	5′-GTTTGATCCTGGCTCA-3′
	800R	5′-TACCAGGGTATCTAATCC-3′
	800F	5′-GGATTAGATACCCTGGTA-3′
	1500R	5′-TACCTTGTTACGACTT-3′
Fungi	ITS1F	5′-GTAACAAGGT(T/C)TCCGT-3′
	ITS1R	5′-CGTTCTTCATCGATG-3′

(xii) Polyoxyethylene(10)octylphenyl ether: A pale yellow, viscous liquid.

Rapid Microbial Methods

Advances in science and technology have provided new methods to perform high-precision measurements of bacterial physiological activities, intracellular components, and so on, and consequently new techniques for bacterial detection, enumeration and quantification have appeared. Since the 1980s it has become clear that the majority of bacteria in the natural environment have low growth ability in conventional culture media, and the detection, enumeration and identification of these bacteria are difficult by means of culture methods alone. The bacterial cell counts obtained vary from method to method, and it should be noted that it is difficult to obtain a reliable value even applied with a new method. Moreover, even if type strains exist for method validation, it is not easy to standardize the physiological activity.

Compared to the conventional methods, these new methods are not necessarily superior in every respect, but they usually offer greater speed and accuracy, and can be applied not only to bacteria, but also to fungi and viruses. Therefore, these new methods are very useful to improve the standards of microbial control in critical areas, and to decrease the risk of hazardous microbial contamination.

The conventional cultivation-based methods use colony formation or turbidity change due to cell growth as an indicator, whereas the new methods vary greatly as regards the detection target and the detection principle. The new methods may be more suitable for obtaining a comprehensive understanding of the microbial community, as well as for identifying specific microorganisms. Among these methods, phylogenic analysis based on gene sequences has become popular, and the dramatic development of sequencing techniques in recent years now allows us to analyze the composition of the microbial community in a short time. In this information chapter, the principles of the new methods and their range of applicability are introduced, and key points in the usage of these methods are described.

1. Detection targets and principles

Name	Target	Principles of measurement	Examples of measurement device
1) Direct Method			
Solid phase cytometry	Microorganism	Directly detect the signals from the bacteria trapped onto a filter. The signals on their physiological activities can be obtained by choosing suitable dyes. Autofluorescence may also be used. To selectively detect specific bacteria, gene probe, antibody or fluorescent-labeled phage may be utilized. Various optical devices including a fluorescent microscope and laser microscope are used as detection/measurement apparatus.	Fluorescence microscope, Laser scanning cytometer, etc.
Flow cytometory	Microorganism	Directly detect the signals given by the bacteria passing through fluid or air. The signals on their physiological activities can be obtained by choosing suitable dyes. Autofluorescence may also be used. To selectively detect specific bacteria, gene probe, antibody or fluorescent-labeled phage may be utilized. Various optical devices are used as detection/measurement apparatus.	Flow cytometer, etc.
2) Indirect Method			
Immunological methods	Antigen	React the antigen of bacteria with the specific antibody, and detect the color or fluorescence visually or by a microplate reader. Immunochromatography is a simple and easy method for the purpose.	Immunochromatography, Micro plate reader, etc.
Nucleic acid amplification	Nucleic acid	Amplify a nucleic acid of microorganism by using the primers specific to the target microorganism, and analyze the amplified nucleic acid fragments. Quantitative determination is possible by performing of quantitative PCR.	Electrophoresis apparatus, Quantitative PCR
Bioluminescence/ fluorescence	ATP, etc.	Measure ATP which is released from microorganisms on the basis of luminous or fluorescence phenomena occurred by enzyme reaction.	luminescence detector, fluorescence detector, etc.
Micro colony method	Growth (Micro colony)	Detect and count the micro colony that appears in early stage of colonization. The same culture conditions (medium composition, temperature, etc.) as the plate culture method can be used.	Fluorescence microscopy etc.
Impedance method	Growth (Electrical characteristic)	Utilize the change in electrical properties of medium due to the metabolites produced by the growth of microorganisms.	Electrodes
Gas measuring method	Growth (Gas production, etc.)	Utilize the change in amount of gases caused by CO_2 production, O_2 consumption, etc. with the growth of microorganisms.	Gas measuring instrument Color change of medium
Fatty acid profiles	Fatty acid	Utilize the fatty acid profile of cell components that differs depending on the taxonomic groups of microorganism.	Gas chromatography
Infrared spectroscopy	Cell component	Utilize the pattern of infrared spectrum obtained by infrared light irradiation to whole microorganism.	Fourier transformation infrared spectroscope
Mass spectrometry	Cell component	Measure the cell component by means of a mass spectrometer, and identify it by database.	Mass spectrometry
Genetic fingerprinting method	DNA	Utilize the electrophoresis pattern of DNA fragments obtained by cleaving the DNA extracted from sample with a restriction enzyme. It can be identified by database. Analysis of community structure is possible by T-RFLP.	Electrophoresis apparatus
High throughput sequencing	Nucleic acid	Determine the sequence of nucleic acids extracted from bacteria exist in sample, and analyze the community structure phylogenetically.	Sequencer, etc.

2. Validation

To qualify introduced equipment, a standard component or strain, which represents the target of each method, should be utilized. That is, in direct measurement, type strains should be used, while in indirect measurement, standard components, etc., of the target bacteria are used.

To validate a protocol/procedure, it is required to demonstrate that the detection target is a suitable index/indicator for bacterial number or quantity. It is also important to state whether any special precautions are necessary in applying the protocol/procedure. When using a type strain, the result of validation should be equivalent to or better than that of the conventional method. However, because the detection principles of new methods are usually different from that of conventional methods, the correlation between them is not always required. For detection of environmental bacteria, it is important that the physiological state of the type strain should be maintained as close as possible to that of environmental bacteria, in order to obtain reliable results.

3. Applications and particular considerations:

New methods are expected to find application in a variety of fields. However, since their detection targets and detection protocols/procedures are different from the conventional methods, the resulting data may not show a good correlation with existing data. Although, it is important in principle that a new method should have an equal or greater capability than the conventional method, a new method may be used after verifying their validity, even in the absence of equivalence to conventional methods.

Because the new methods are rapid, product testing, environmental monitoring, bioburden evaluation, raw materials control, etc. can be performed in real-time, and this is highly advantageous for process control, allowing alert levels, action levels and so on to be set up based on trend analysis of the obtained data.

These new rapid methods may be applied to;

- Quality control of pharmaceutical manufacturing water
- Microbial evaluation of processing areas
- Sterility test
- Microbial limit test
- Antimicrobial and preservatives effectiveness test
- Raw material acceptance test

etc.

Sterilization and Sterilization Indicators

Sterilization refers to the destruction or removal of all forms of viable microorganisms in items. This reference information applies to cases where sterilization is required as well as the manufacture of sterile products. When sterilization is applicable, an appropriate sterilization method should be selected in accordance with the items being sterilized (such as products, or equipment, instrumentation, or materials that must be sterilized), including the packaging, after full consideration of the advantages and disadvantages of each sterilization method.

After installation of a sterilizer (including design and development of the sterilization process), an equipment maintenance and inspection program must be established based on qualification evaluation to ensure that the sterilization process is being properly performed as designed on the basis of sufficient scientific evidence. A quality system must also be established for manufacturing in general at manufacturing facilities where sterile pharmaceutical products are manufactured. For example, all operation potentially affecting quality, including sterility after sterilization, must be clearly identified, and any operating procedures that are needed to prevent microbial contamination of products must be established and properly enforced.

In order to establish sterilization conditions and ensure sterility after sterilization, the bioburden before sterilization of the items being sterilized must be evaluated periodically or on the basis of batches. For bioburden test method, refer to 4.05 Microbiological Examination of Non-sterile Products, etc.

Representative sterilization methods are presented in this reference information, but other sterilization methods can also be used, provided that they meet the following requirements and do not have any deleterious affect on the item being sterilized.

- The mechanism of sterilization is well established
- The critical physical parameters of the sterilization process are clear, controllable, and measurable
- The sterilization procedure can be performed effectively and reproducibly

1. Definitions

The terms used in this text are defined as follows.

- Filter integrity test: A non-destructive test which is demonstrated to correlates with the microbial removal performance data of filters.
- Bioburden: Population of viable microorganisms in an item to be sterilized.
- D value: The value represents exposure time (decimal reduction time) to achieve 90% reduction of a population of the test microorganism, and resulted that 10% of the original organisms remain.
- F_H value: The unit of lethality indicating the measure of the microbial inactivation capacity of a process in dry heat sterilization, expressed as the equivalent time (minutes) at 160°C for microbes with a z value (the number of degrees that are required for a 10-fold change in the D value) of 20°C.
- F_0 value: The unit of lethality indicating the measure of the microbial inactivation capacity of a process in moist heat sterilization, expressed as the equivalent time (minutes) at 121.1°C for microbes with a z value (the number of degrees that are required for a 10-fold change in the D value) of 10°C.
- Sterility assurance level (SAL): Probability of a single viable microorganism surviving in a product after sterilization, expressed as 10^{-n}.
- Dose of irradiation (absorbed dose): Quantity of ionizing radiation energy imparted per unit mass of the item, expressed in units of gray (Gy).
- Critical parameter: A measureable parameter that is inherently essential to the sterilization process.
- Loading pattern: A specified combination of the numbers, orientation and distribution of the item(s) to be sterilized within the sterilization chamber or irradiation container.

2. Sterilization

2.1. Heat method

In the heat method, microorganisms are killed by heat.

2.1.1. Moist-heat sterilization

Moist-heat sterilization includes widely used saturated steam sterilization and other types of moist-heat sterilization. The control points, utilities, and control devices in moist-heat sterilization are provided as reference in Table 1.

Saturated steam sterilization is a method for killing microorganisms with high pressure saturated steam. Critical

Table 1 Control points, utilities, and control devices in moist-heat sterilization (reference)

	Saturated steam sterilization	Other types of moist-heat sterilization
Control point	• Temperature profile (usually indicated by F_0 value) • Temperature (drain or the like as needed) • Pressure (in sterilizer) • Exposure time at specified temperature • Loading pattern of items being sterilized • Steam quality (degree of superheat, dryness, non-condensable gas concentration, and chemical purity, as needed) • Quality of air that is introduced to the sterilizer for vacuum break. • Quality of cooling water • Other requirements	• Temperature profile (usually indicated by F_0 value) • Temperature (drain and the like as needed) • Pressure, as needed (in sterilizer) • Exposure time at specified temperature • Loading pattern of item being sterilized • Quality of air that is introduced to the sterilizer for vacuum break. • Quality of cooling water • Other requirements
Utilities and control devices that should be controlled	• Steam • Air introduced to the sterilizer for vacuum break • Cooling water • Temperature control device • Pressure control device • Time control device • Other	• Steam • Hot water • Air introduced to the sterilizer for vacuum break • Cooling water • Temperature control device • Pressure control device • Time control device • Conveyor for when a continuous sterilizer is used • Other

Table 2 Control points, utilities, and control devices in dry-heat sterilization (reference)

	Batch-type dry heat sterilizer	Tunnel-type dry heat sterilizer
Control point	• Temperature profile (usually indicated by F_H value) • Temperature • Exposure time at specified temperature • Pressure differential between inside and outside of container • Loading pattern of items being sterilized • Quality of air (heating air, cooling air) • Other requirements	• Temperature profile (usually indicated by F_H value) • Temperature • Belt speed (exposure time) • Pressure differential between inside and outside of equipment • Loading density • Quality of air (heating air, cooling air) • Other requirements
Utilities and control devices that should be controlled	• Air (heating air, cooling air) • Temperature control device • Time control device • Internal differential pressure gage • HEPA filter • Other	• Air (heating air, cooling air) • Temperature control device • Time control device • Internal differential pressure gage • HEPA filter • Cooler (if needed) • Other

parameters in this method are temperature, pressure, and exposure time at the specified temperature. Therefore, the temperature, pressure, and exposure time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

Other types of moist-heat sterilization may include steam pressurization cycles, water dispersion cycles, water immersion cycles, and the like, which are used when the items being sterilized is sterilized in a hermetically sealed container. Critical parameters in such methods are the temperature in the container and the exposure time at the specified temperature.

2.1.2. Dry-heat sterilization

Dry-heat sterilization is a method for destructing microorganisms with dry heated air. This method is usually conducted in a batch or continuous (tunnel-type) dry heat sterilizer. Attention must be paid to the cleanliness of the air that flows into the sterilizer in either case. The control points, utilities, and control devices in dry-heat sterilization are provided as reference in Table 2. This method is suitable for when the item to be sterilized is highly heat-resistant, such as glass, ceramic or metal, or is thermo-stable, such as mineral oils, fatty oils, or solid pharmaceutical products.

Critical parameters in this method are temperature and the exposure time at the specified temperature (belt speed). Dry-heat sterilization requires higher temperatures and longer exposure times than does moist-heat sterilization even though the sterilization in both methods may be based on the same heating temperature. The temperature and exposure time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

2.1.3. Microwave sterilization

When substances to be sterilized such as chemical solutions are exposed to microwaves, the polar molecules of the substance being sterilized vibrate as they attempt to change

orientation due to the absorbed microwaves, and energy is released by the friction between the molecules. The method of killing microorganisms by the heat (microwave heat) generated at this time is called the microwave sterilization. A frequency of 2450 ± 50 MHz is ordinarily used.

Microwave devices are composed of a heating irradiation component which produces radiofrequency radiation to generate heat using a magnetron, a component for maintaining the sterilization temperature using an infrared heater or the like, and a cooling component for cooling the item being sterilized. Such devices continuously sterilize the item at ordinary pressure. The control points, utilities, and control devices in microwave sterilization are provided as reference in Table 3.

This method is applied to liquid products or products with high water content in hermetically sealed containers.

Critical parameters in this method include the temperature of the items being sterilized and processing time. Therefore, the temperature of the items being sterilized and the processing time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

Table 3 Control points, utilities, and control devices in microwave sterilization (reference)

Control point	• Temperature profile (usually indicated by F_0 value) • Temperature • Processing time • Belt speed • Configuration of items being sterilized • Other requirements
Utilities and control devices that should be controlled	• High frequency control device • External heater (if needed) • Cooler (if needed) • Temperature monitoring device • Time monitoring device • Other

Microwave heating characteristically allows rapid sterilization at high temperatures to be continuously carried out with excellent thermal efficiency and responsiveness. However, the ease of heat transfer in the items being sterilized sometimes makes it difficult to ensure uniform heating. Attention must also be paid to the pressure resistance and uniformity of the containers that are used because the heating takes place at ambient pressure, resulting in increases in internal pressure.

2.2. Gas method

The gas method kills microorganisms through contact with a sterilization gas or vapor. Microorganisms can be sterilized at lower temperatures than in heat methods, and the items being sterilized generally sustain little thermal damage. This method is therefore often applied to plastic containers and the like which are not very resistant to heat.

In the most common gas sterilization methods, adequate washing and drying are important to prevent contamination and moisture from compromising the sterilization effect. The sterilization effect may also be compromised if the gas is absorbed by the item being sterilized.

2.2.1. Ethylene oxide (EO) gas sterilization

EO gas sterilization kills microorganisms by altering the proteins and nucleic acids of microorganisms. Since EO gas is explosive, it is usually diluted 10 to 30% with carbon dioxide. EO gas is also a strongly reactive alkylating agent and therefore cannot be used to sterilize products which are likely to react with or absorb it.

The sterilization process consists of preconditioning, sterilization cycles, and aeration. EO gas is toxic (mutagenic, for example), and the substance being sterilized must therefore be aerated to ensure that the residual concentration of EO gas or other secondarily generated toxic gases (such as ethylene chlorohydrin) is at or below safe levels. Gas emissions must also be treated in compliance with regulations. The control points, utilities, and control devices in EO gas sterilization are provided as reference in Table 4.

Critical parameters in this method include temperature,

Table 4 Control points, utilities, and control devices in EO gas sterilization (reference)

Control point	• Pressure increase, injection time, and final pressure for the injection of sterilization gas • Temperature (in sterilizer and items being sterilized) • Humidity • EO gas concentration (gas concentration in sterilizer should be directly analyzed, but the following alternatives are acceptable when direct analysis is not feasible) i) Weight of gas used ii) Volume of gas used iii) Use of conversion formula based on initial reduced pressure and gas injection pressure • Operating time (exposure time) • Loading pattern of items being sterilized • Biological indicator placement points and cultivation results • Preconditioning conditions (temperature, humidity, time, etc.) • Aeration conditions (temperature, time, etc.) • Other requirements
Utilities and control devices that should be controlled	• EO gas • Injected vapor or water • Air replaced after completion of sterilization • Temperature control device • Pressure control device • Humidity control device • Time control device • Other

humidity, gas concentration (pressure), and time. Therefore, the temperature, humidity, gas concentration (pressure), and time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

2.2.2. Hydrogen peroxide sterilization

Sterilization with hydrogen peroxide is a method for killing microorganisms through the oxidative power of hydrogen peroxide or the oxidation caused by radicals that are produced upon the generation of hydrogen peroxide plasma. Although items can be sterilized at lower temperatures than in heat methods, this method is not suitable for the sterilization of objects that absorb hydrogen peroxides, such as cellulose-based disposable garment and membrane filters because the sterilization effect will be compromised. The control points, utilities, and control devices in hydrogen peroxide sterilization are provided as reference in Table 5.

Critical parameters in this method include the concentration, time, and temperature. The control of a radio frequency device is also important when substances are sterilized with the use of plasma. The residual moisture of the substance being sterilized and the humidity in the sterilization environment may affect sterilization and should therefore be controlled when necessary.

2.3. Radiation method

2.3.1. Radiation sterilization

Radiation sterilization includes γ-ray radiation for killing microorganisms through the exposure of the items that are to be sterilized to γ-rays emitted from ^{60}Co, and electron beam radiation for killing microorganisms through exposure to an electron beam emitted from an electron beam accelerator. To select this method of sterilization, it must first be ensured that it is compatible with the items to be sterilized, including whether the quality of the substance could potentially deteriorate.

In γ-ray radiation sterilization, microorganisms are killed by secondarily produced electrons, whereas in electron beam radiation sterilization, microorganisms are directly killed by electrons. Although this kind of electron-based direct action is available, indirect action is also available, where sterilization is accomplished through the production of radicals and the like and damage to the DNA of microorganisms when γ-rays or electron beams react with water molecules.

Since sterilization can take place at room temperature, both methods can be applied to heat-labile items, and items can be sterilized while packaged because the radiation rays will penetrate the packaging. γ-Ray sterilization is suitable primarily for high density products such as metals, water, and powder because the penetration is better than that of electron beams. Electron beam radiation sterilization has a higher radiation dose per unit time (dose rate) compared with γ-rays and therefore has a shorter processing time. The control points, utilities, and control devices in radiation sterilization are provided as reference in Table 6.

2.4. Filtration method

The filtration method is a method for physically removing microorganisms in liquids or gas using a sterilization filter. It can therefore be applied to items that are unstable against heat or radiation. Filtration sterilization is for microorganisms which can be removed by a 0.2 μm membrane filter, and is not suitable for *Mycoplasma* spp., *Leptospira* spp., or viruses. The control points, utilities, and control devices in filtration sterilization are provided as reference in Table 7.

The critical parameters affecting the removal of microorganisms by the filter in liquid filtration sterilization include filtration time, filtration capacity, filtration flow rate, filtration differential pressure, and temperature. The critical parameters in gas filtration sterilization include filtration differential pressure and temperature. When a liquid is to be sterilized, the removal of microorganisms by a filter will be affected by the physical and chemical properties of the liquid that is undergoing filtration (such as viscosity, pH, and surfactant action). The microbial trapping performance of a sterilizing filter can generally be validated when a sterilizing filter challenged with more than 10^7 CFU microorganisms of

Table 5 Control points, utilities, and control devices in hydrogen peroxide sterilization (reference)

	Hydrogen peroxide sterilization	Hydrogen peroxide low temperature gas plasma sterilization
Control point	• Concentration (the concentration in the sterilizer should be directly analyzed, but a method based on evidence of sterilizer performance uniformity in the chamber is an acceptable alternative when direct analysis is not feasible) • Time • Temperature • Humidity • Pressure • Quality of hydrogen peroxide • Consumption of hydrogen peroxide • Residual moisture of substance being sterilized • Loading pattern of items being sterilized • Biological indicator placement points and cultivation results • Chemical indicator placement points and results • Other requirements	• Concentration (the concentration in the sterilizer should be directly analyzed, but a method based on evidence of sterilizer performance uniformity in the chamber is an acceptable alternative when direct analysis is not feasible) • Time • Temperature • Humidity • Pressure • Quality of hydrogen peroxide • Consumption of hydrogen peroxide • Residual moisture of substance being sterilized • Loading pattern of items being sterilized • Biological indicator placement points and cultivation results • Chemical indicator placement points and results • Other requirements
Utilities and control devices that should be controlled	• Hydrogen peroxide • Pressure gauge • Hydrogen peroxide injector • Other	• Hydrogen peroxide • Pressure gauge • Hydrogen peroxide injector • High frequency generator • Other

Table 6 Control points, utilities, and control devices in radiation sterilization (reference)

	γ-Ray radiation sterilization	Electron beam radiation sterilization
Control point	• Absorbed dose • Loading pattern (density) of items being sterilized • Exposure time (conveyor speed or cycle time) • Other requirements	• Absorbed dose • Loading pattern (density) of items being sterilized • Electron beam properties (mean electron beam current, electron beam energy, scanning width) • Other requirements
Utilities and control devices that should be controlled	• Belt conveyor • Dose measurement system • Other	• Electron beam measurement device • Belt conveyor • Dose measurement system • Other

Table 7 Control points, utilities, and control devices in filtration sterilization (reference)

	Liquid filtration sterilization	Gas filtration sterilization
Control point	• Filtration time • Filtration capacity • Filtration flow rate • Filtration differential pressure • Temperature • Filter integrity • In cases involving multiple use: expiration period and number of times the filter can be used for sterilization • Other requirements	• Filtration differential pressure • Temperature, if needed • Filter integrity • Expiration period • Number of sterilizations of times the filter can be used for sterilization • Direction of gas current (for bidirectional flow) • Other requirements
Utilities and control devices that should be controlled	• Pressure gage • Flow rate meter • Integrity tester • Other	• Pressure gage • Flow rate meter • Integrity tester • Other

a strain of *Brevundimonas diminuta* (ATCC 19146, NBRC 14213), cultured under the appropriate conditions, per square centimeter of effective filter area, provides a sterile effluent.

The bioburden of liquids prior to filtration will affect filtration sterilization performance and should therefore be controlled.

3. Sterilization Indicators

3.1. Biological indicators (BI)

3.1.1. Introduction

A BI is an indicator prepared from the spores of a microorganism resistant to the specified sterilization process, and is used to develop and/or validate a sterilization process.

Indicators are classified based on configuration into the ''paper strip type'', ''the type that is inoculated on or into the surface of metal or the like'', ''liquid type'' and ''the self-contained type in which a medium and paper strip are pre-encapsulated''. They are also classified by carrier, where one type comprises a carrier of paper, glass, stainless steel, plastic or the like that is inoculated with bacterial spores and packaged, and another type comprises the product or simulated product as the carrier, which is inoculated with bacterial spores. Typical examples of indicators by sterilization method are shown in Table 8.

3.1.2. Labeling of commercially available BI

Users of commercially available BI produced in accordance with ISO11138-1 must check the following information provided by the BI manufacturer to users.

- Traceability (microorganism, carrier, labeling, etc.)
- Species name
- Nominal bacterial spore count
- Resistance
- Method used
- Storage conditions (temperature, expiration date, etc.)
- Culture conditions (temperature, time, medium, etc.)
- Disposal method

Parameters determining BI performance include ''species,'' ''resistance,'' and ''bacterial count.'' Resistance varies, even for the same species, depending on the nature and configuration of the carrier or packaging, and evaluation must therefore include the packaging.

3.1.3. Control during use of commercially available BI

BI must be handled in accordance with the storage conditions, time to start of culture after sterilization, culturing conditions, disposal method, and the like provided by the BI manufacturer. Because the storage conditions in particular affect BI performance, precautions must be taken to prevent a BI from being allowed to stand for a long period of time until use after being removed from the packaging.

The BI should be set up to enable evaluation of the entire items being sterilized. The BI should be set up in places where the sterilization effect is expected to be low in any given method, such as cold spots in heat sterilization. Care should be taken to avoid damaging the BI packaging or carrier when recovered. Predetermined procedures for preventing microbial contamination should be in place in case bacteria are released or spread if the packaging does end up becoming damaged.

When using a BI that has been purchased, the user should

Table 8 List of typical indicators by sterilization method

Sterilization method	Species	Strain name	*D* value, etc. (reference)
Moist-heat sterilization	*Geobacillus stearothermophilus*	ATCC 7953, NBRC 13737	≥1.5 min (121°C)
Dry-heat sterilization	*Bacillus atrophaeus*	ATCC 9372, NBRC 13721	≥2.5 min (160°C)
EO gas sterilization	*Bacillus atrophaeus*	ATCC 9372, NBRC 13721	≥2.5 min (54°C) ≥12.5 min (30°C) Gas concentration: 600 mg/L ± 30 mg/L; relative humidity: 60% RH
Hydrogen peroxide sterilization	*Geobacillus stearothermophilus*	ATCC 12980, NBRC 12550 or ATCC 7953, NBRC 13737	—

measure the spore count or the like when received as needed to make sure there are no significant differences with the nominal count provided by the BI manufacturer.

3.1.4. Precautions for when sterilization indicators are prepared by the user

The following must be evaluated prior to use when users prepare indicators themselves using the bioburden collected from the items being sterilized or the manufacturing environment rather than purchasing a BI for use.

- Species name
- Bacterial spore count
- Resistance (*D* value at sterilization temperature or sterilization gas concentration)
- Storage conditions (temperature, expiration date, etc.)
- Culture conditions (temperature, incubation time, medium, etc.)

An evaluation program must be established to continuously show that the resistance of picked bacteria is the most resistant of the bioburden.

3.1.5. Precautions when commercially available BI are modified by users

When a BI that has been purchased is removed from the packaging and is used to inoculate an item such as drug solution or materials, the bacterial spore count or resistance will vary and must therefore be assessed prior to use.

ISO11138 or USP <55> can be used for reference for such evaluation. Resistance can be evaluated by using a biological indicator evaluation resistometer (BIER) or the capillary method with oil bath. When such self-assessment is unfeasible, a third-party testing facility can be used.

3.2. Chemical indicator (CI)

A CI is an indicator that chemically or physically changes due to exposure to heat, gas, radiation, or the like. Such indicators are produced by being applied to or printed on a piece of paper, for example. Because the principals involved in such changes will depend on the sterilization method, a CI that is suitable for the intended sterilization method must be used. CI is classified into the following six classes based on the intended application. The classes shown here are unrelated to level of performance.

A CI indicates the progress of a sterilization step or of a number of critical parameters, but is not used to assure sterilization effect or sterility and therefore cannot be used as an alternative to a BI.

Class 1: Process indicators

These are intended to distinguish whether an item being sterilized has passed through a sterilization step. They respond to one or more critical parameters.

Class 2: Indicators for use in specific tests

These are used in tests of the exhaust capacity and vapor penetration of a vacuum-type high-pressure steam sterilizer as specified in the ISO11140 series. They correspond to the Bowie-Dick type.

Class 3: Single-variable indicators

These respond to only one critical parameter. They show exposure in a sterilization step based on a specified value for the designated parameter.

Class 4: Multi-variable indicators

These respond to two or more critical parameters. They show exposure in a sterilization step based on specified values for the designated parameters.

Class 5: Integrating indicators

These respond to all critical parameters. Their performance is equal to or greater than that required of BI in the ISO11138 series.

Class 6: Emulating indicators

These respond to all critical parameters of a specified sterilization cycle. The specifications are critical parameters of the designated sterilization step.

3.3. Dosimeter

3.3.1. Types of dosimeters

The dosimeter in a radiation process is an instrument or system which reads the absorbed dose based on changes caused by the absorption of the radiation, for which ''reproducibility'' and ''response permitting radiation to be measured'' are required. Most dosimeters are susceptible to environmental conditions (process parameters) such as temperature and dose rate before, during, and after exposure to the facilities being used, and caution is therefore required. The choice of dosimeter and calibration guidelines for radiation processes have been specified (ISO/ASTM 51261) as reference for the selection and use of dosimeters. Dosimeters for measuring the absorbed dose of radiation are shown in Table 9. γ-Ray dosimeters are not normally suitable for sterilization process control involving the use of electron

Table 9 Types of dosimeters

Type of radiation	Dosimeter
γ-ray	Dyed polymethyl methacrylate dosimeter Clear polymethyl methacrylate dosimeter Ceric-cerous dosimeter Alanine – EPR dosimeter
γ-ray, electron beam	Cellulose acetate dosimeter Radiochromic film dosimeter

beams of less than 3 MeV energy.

3.3.2. Dosimeter use

Dosimeters are used when dose distribution is measured to determine the conditions of radiation and to evaluate the absorbed dose of an items being sterilized during ordinary radiation sterilization. In the former, dosimeters are set up in advance in the object being sterilized and are then recovered after radiation for measurement in the measurement system to find the absorbed dose at each location. The dosimeters should be arranged in a broad range of vertical and horizontal directions because it is necessary to determine the relationship between minimum/maximum exposure and the process parameters as well as to verify the appropriateness of the packaging configuration based on the variation in radiation penetration and dose. In the latter, there is no need to arrange the dosimeters in the locations characterized by the maximum or minimum dose in the object being sterilized. Control points where dosimeters are easily arranged and recovered should be selected, and the absorbed dose of the object being sterilized should be ensured based on the absorbed dose at the control points. Therefore, in the measurement of dose distribution, the quantitative relationship between the control points and the locations of maximum/minimum exposure should be determined, and the passing dose range at the control points should also be calculated.

Newly purchased dosimeters should be calibrated prior to use, and dosimeters should be calibrated every time a batch is changed and at least once a year.

4. Establishment of Sterilization Conditions

4.1. Half-cycle method

In the half-cycle method, a sterilization time twice as long as that required to inactivate all of the 10^6 CFU bacteria included in the BI is used, regardless of the bioburden count on the object being sterilized or the resistance of the test microorganisms to sterilization. This method is primarily used to establish the conditions of EO or other gas sterilization.

4.2. Overkill method

In the overkill method, a sterilization condition to achieve an SAL of 10^{-6} or better is used, regardless of bioburden count on the object being sterilized or the resistance of the test microorganisms to sterilization.

This means a level of sterilization of 12 D in steam sterilization. However, a level $\geq F_0$ 12 is also referred to as the overkill method.

4.3. Combination of BI and bioburden method

In the combined bioburden/BI method, the maximum bioburden count is determined based on the results of extensive bioburden analysis, and the sterilization time (or radiation dose) is calculated using an appropriate commercially available BI with a test microorganism count $\geq$ the maximum bioburden count based on the target SAL.

When this procedure is used, the bioburden count of the object being sterilized must be tested on a daily basis, and the resistance of the test microorganisms to sterilization must be periodically measured.

If the bioburden testing reveals a microorganism more resistant than the BI microorganism, it should be used as the indicator. The sterilization conditions must also be revised as needed.

$$\text{Sterilization time (or radiation dose)} = D \times \log (N_0/N)$$

D: D value of BI
N: Target sterility assurance level (SAL)
N_0: Maximum bioburden count in object being sterilized

4.4. Absolute bioburden method

In the absolute bioburden method, the sterilization resistance of the microorganisms found in the object being sterilized or environment is measured, and the sterilization conditions are determined, in the case of moist-heat sterilization, by employing the D value of the most resistant microorganism based on the bioburden count of the object being sterilized.

The bioburden count should be determined by extensive bioburden analysis. When this procedure is used, the microorganism count and the resistance of the detected microorganisms to sterilization must be assessed on a daily basis in routine bioburden control.

Radiation sterilization may be performed in accordance with ISO11137–2.

5. References

- ISO 11138-1 (2006): Sterilization of health care products-Biological indicators-Part1: General requirements
- ISO 11137-2 (2013): Sterilization of health care products-Radiation- Part2: Establishing the sterilization dose
- ISO/ASTM 51261 (2013): Guide for selection and calibration of dosimetry systems for radiation processing
- ISO 11140–1 (2014): Sterilization of health care products-Chemical indicators- Part1: General requirements
- USP 38 (2015) <55> BIOLOGICAL INDICATORS-RESISTANCE PERFORMANCE TEST

G5 Crude Drugs

Analytical Methods for Aflatoxins in Crude Drug and Crude Drug Preparations

Aflatoxins are carcinogenic secondary metabolites produced by some fungal strains[1]. They are found in agricultural products such as cereals, tree nuts and spices. Many countries including Japan have set regulatory limits on aflatoxins in foods[2,3]. Not only foods but also crude drug may be contaminated by aflatoxins because aflatoxin contamination in the ingredients of botanical products has been reported in some foreign countries[4-6]. Therefore, aflatoxin testing in crude drug and preparations containing crude drugs as main ingredient (crude drug preparations) is required to be performed. In this connection, the regulatory limit for aflatoxins (sum of B_1, B_2, G_1 and G_2) has been set at 10 μg/kg for all foods in Japan[3].

1. Summary

The methods based on HPLC with fluorescence detection and LC-MS are used for aflatoxins instrumental analysis[7-10]. Aflatoxin standards should be handled with care because they are highly toxic compounds. (Refer to 4. Points to note) Simple measurement kits are also used for analysis of aflatoxin and some of them enable aflatoxin determination without using aflatoxin standards.

Aflatoxins are generally purified from the samples by cartridge-type columns such as immunoaffinity column and multifunctional column. An immunoaffinity column is useful for purification of aflatoxin from crude drug preparations, but in some cases, a multifunctional column is effective.

In this document, the analytical methods for aflatoxins using simple measurement kits, which are useful for screen-

ing, and HPLC with fluorescence detection are described. The methods should be selected according to the characteristics and aflatoxin contamination levels of the samples. Method optimization and validation are required to be performed. As references, an official method of Japan for aflatoxin analysis in food[7,11], analytical methods described in European and US Pharmacoperia, and WHO guidelines can be used[8-10].

2. Analytical methods

2.1. An analytical method using a qualitative kit

The kit can detect the presence or absence of aflatoxin in the sample by using an antigen-antibody interaction. A kit for the detection of total aflatoxins can be used for qualitative test. The following method can be used for detecting aflatoxins in some extracts (Orengedokuto, Kakkonto, Shoseiryuto, Hachimijiogan, Goshajinkigan, Daiokanzoto and Mukoi-Daikenchuto) listed in Japanese Pharmacopoeia at cut-off levels of 4 ppb[12]. In order to quantify aflatoxins in the positive samples, instrumental analysis is required to be done.

(i) Preparation for sample solution

Weigh accurately about 1 g of the powdered sample, add exactly 4 mL of a mixture of acetonitrile, water and methanol (6:4:1) and then shake for 30 minutes. After centrifugation, dilute exactly 2 mL of the supernatant to 50 mL with phosphate buffered saline (PBS) containing 4% of polysorbate 20. Apply the diluted extract to an immunoaffinity column, which is preequilibrated with PBS. Wash the column with 10 mL of PBS containg 0.01% of polysorbate 20 followed by 10 mL of water. Apply 1 mL of acetonitrile on the column and collect eluate. Wait 5 minutes and apply 2 mL of acetonitrile. Collect applied elution solvent. Evaporate the eluate to dryness under nitrogen. Dissolve the residue in exactly 0.5 mL of diluted methanol (7 in 10), and use this as the sample solution. In this method, 0.5 mL of the sample solution is equivalent to 0.5 g of the sample matrix.

(ii) Measurement and evaluation

Prepare the test strips, the microwells with the inner bottom covered by gold colloid, and the assay diluent attached with the kit. Add exactly 50 μL of the assay diluent to each microwell. Dissolve the coating conjugate in the microwell by pipetting. Add exactly 50 μL of sample extracts to each microwell and mix the content in each well by pipetting it up and down. Put one test strip into one well and allow the test strip to develop color for 5 minutes. Interpret test results from the lines formed in the test zone and the control zone. If the two lines are visible, this indicates the sample contains total aflatoxin less than 4 ppb (negative sample).

2.2. An instrumental method of analysis

Aflatoxins can be detected by a fluorescence detector because they are fluorescent substances. In order to enhance the fluorescent intensities of AFB_1 and AFG_1, the intensities of which are weak in polar solvents, a derivatization step is performed. Precolumn derivatization with trifluoroacetic acid and postcolumn derivatization with a photochemical reactor or an electrochemical cell are known. AFB_2 and AFG_2 are not derivatized by the above methods. The following method can be used for quantification of aflatoxins in some extracts listed in Japanese Pharmacopoeia[12]. This method is an example and the other method can also be used.

(i) Preparation for sample solution

Weigh accurately about 1 g of the powdered sample and add exactly 4 mL of a mixture of acetonitrile, water and methanol (6:4:1) and then shake for 30 minutes. After centrifugation, dilute exactly 2 mL of the supernatant to 50 mL with phosphate buffered saline (PBS) containing 4% of polysorbate 20. Apply the diluted extract to an immunoaffinity column, which is preequilibrated with PBS. Wash the column with 10 mL of PBS containg 0.01% of polysorbate 20 followed by 10 mL of water. Apply 1 mL of acetonitrile on the column and collect eluate. Wait 5 minutes and apply 2 mL of acetonitrile. Collect applied elution solvent. Evaporate the eluate to dryness under nitrogen. Redissolve the residue in exactly 0.5 mL of 70% methanol in water. In this method, 0.5 mL of the sample solution is equivalent to 0.5 g of the sample matrix.

(ii) Measurement and evaluation

Use an ODS column for separation. Aflatoxins can be detected by an HPLC equipped with a fluorescence detector because they are fluorescent (exitation λ = 365 nm, emission λ = 430 nm). Use trifluoroacetic acid (TFA) for derivatization of aflatoxins. Aflatoxins elute in the order of AFG_{2a} (a derivative of AFG_1), AFB_{2a} (a derivative of AFB_1), AFG_2 and AFB_2 when a mixture of acetonitrile, water and methanol (6:3:1) is used as a mobile solvent. Postcolumn derivatization with a photochemical reactor is useful when performing continuous monitoring of aflatoxins. In that case, aflatoxins elute in the order of AFG_2, AFG_{2a}, AFB_2 and AFB_{2a}. Prepare some standard solutions in which aflatoxins are present at the concentrations from 0.5 to 20 μg/L, and verify linearity in the range.

3. Reagents and solutions

Some reagents and solutions prescribed in Japanese Pharmacopoeia and those listed below are used.

(i) PBS containing 0.01% or 4% of polysorbate 20 Dissolve 8.0 g of NaCl, 0.2 g of KCl, 2.9 g of $Na_2HPO_4.12H_2O$, 0.2 g of KH_2PO_4 and 0.1 g (0.01%) or 40 g (4%) of polysorbate 20 in 900 mL of water and adjust to pH 7.4 with 0.1 mol/L hydrochloric acid TS or dilute sodium hydroxide TS, and add water to make 1000 mL. Keep the solution at 2 – 8°C.

(ii) Aflatoxin solution Dilute the aflatoxin standard stock solution with acetonitrile or methanol. Use the commercially available standard stock solution which is precisely prepared in concentration.

4. Points to note

(i) Aflatoxins are highly cardiotoxic compounds, and sufficient caution is required when handling them. Especially in cases when handling a high concentration of aflatoxins, pay maximum attention. Wear a protect coat, gloves, a mask and goggles. All handling should be performed in a fume hood.

(ii) Soak the used labware in 0.5 – 1.0% sodium hypochlorite (NaClO) solution for more than 2 hours before discarding and washing it. Commercially available sodium hypochlorite solutions for disinfection or for food additive may also be used after adjusting the concentration.

(iii) Keep the aflatoxin solution in a dark and cool place. Keep the commercially available standard stock solution under specified conditions.

(iv) Aflatoxins may be absorbed by a glass vessel. In order to avoid absorption, using silanized glass vials may be effective. The vial should be washed with 20% acetonitrile and air-dried before use.

(v) Be cautious that there are no big air bubbles or cracks in the gel of the immunoaffinity column. If there are bubbles or cracks, remove them by applying pressure from the upper part of the column.

(vi) Confirm the performance of the immunoaffinity column by performing a spike and recovery test if necessary.

(vii) In case of using a multifunctional column for prepar-

ing the sample solution, it is necessary to assess its performance beforehand by performing a spike and recovery test.

5. References

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Aristolochic Acid

Aristolochic acid, which occurs in plants of *Aristolochiaceae*, is suspected to cause renal damage. It is also reported to be oncogenic (see References).

Aristolochic acid toxicity will not be a problem if crude drugs of the origin and parts designated in the JP are used, but there may be differences in crude drug nomenclature between different countries, and it is known that crude drug preparations not meeting the specifications of the JP are circulating in some countries. Consequently, when crude drugs or their preparations are used, it is important that the materials should not include any plant containing aristolochic acid.

Since Supplement I to JP14, the test for aristolochic acid I was added to the Purity under Asiasarum Root, which consists of the rhizome and root. Because the aerial part of the plant may contain aristolochic acid and may have been improperly contaminated in Asiasarum Root.

It is considered that Akebia Stem, Sinomenium Stem and Saussurea Root do not contain aristolochic acid, unless plants of origin other than that designated in the JP are used. However, contamination of aristolochic acid might occur, as mentioned above. In this case, the test described in the Purity under Asiasarum Root is useful for checking the presence of aristolochic acid.

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Notification for the Quantitative Marker Constituents of Crude Drugs and Crude Drug Preparations

One of the highly distinctive features of the crude drugs and preparations containing crude drugs as main ingredient (crude drug preparations) is the fact that they are multicomponent systems composed of a prodigious number of compounds. As an example, glycyrrhiza, one of the most important crude drugs worldwide has been demonstrated that they harbor more than 100 kinds of secondary metabolites, and this number could be counted up to more than 1000 when the intermediate occurred in the biosynthetic pathways were included. The whole members of the compounds are supposed to be integrated to reveal the effects of glycyrrhiza.

A wide diversity of constituents in the drugs made from botanical raw materials is fundamentally derived from a wide variety of secondary metabolites occurred in plants. However, there are more reasons for the diversity. The species of plants used as raw materials for drugs are specified in the approval documents of the drugs, though, a variation of their composition derived from the genetic variation of the plants will be produced since each botanical species includes a significant genetic divergence. The secondary metabolites of plants will be further differentiated in their quality and quantity according to the environmental factors such as soil (soil texture, water retentivity, pH, etc.) and weather (amount of precipitation, temperature, humidity, etc.) conditions. Also crude drugs are finally processed by excluding periderm, steaming, roasting, etc. and consequently the constituents are different according to the process method.

The stipulation on the quantitative values of marker constituents in crude drugs and crude drug preparations has very important implications for standardization of the quality of pharmaceutical natural products. The stipulations on crude drugs generally provide the minimum requisite amount of marker compounds. For example, glycyrrhiza of the Japanese Pharmacopoeia is ordered to contain not less than 2.0% of glycyrrhizic acid. Crude drugs are known to exhibit large difference in the content of secondary metabolites among individual plants that caused from multiple reasons described in the previous paragraph. It is reported that the glycyrrhizic acid content of glycyrrhiza in one plant can contain some 10 times the content of another plant when they are grown together in one farm field and harvested simultaneously. Accordingly, it is hard to standardize the ranges of content for marker constituents of crude drugs on a policy of the efficient use of natural resources for crude drugs. However, the crude drug preparations as finished products are required to contain a certain amount of active ingredients from a perspective of the reproducibility of medical treatments. Consequently, the marker constituents of the Kampo formulations and conventional crude drug preparations as the final stage of crude drug preparations are generally specified by the range of content values. Crude drugs containing different amount of marker constituents are appropriately blended to prepare the finished products with a particular amount of marker constituents.

It should be noted that the marker constituents of crude drugs for regulations include a variety of types. Followings are the examples of some different types. Sennosides, the marker constituents of senna leaf are the obvious active ingredients that accounts for the laxative effects of senna leaf to some extent. Other compounds of the anthraquinone-type found in senna leaf, such as rhein and aloe-emodin, are also

potent as laxative, though, because of their large difference in content in senna leaf, a medicinal effect of senna leaf can be standardized by managing the content of sennosides. However, sennosides are likely to be broken down into anthraquinones by heating and other factors that not only sennosides but also the increased rhein may be targeted for standardization in cases of the Kampo formula extracts including rhubarb, one of the sennoside-containing crude drugs, as a component. On the other hand, glycyrrhizic acid, a very famous bioactive component of glycyrrhiza, is an active ingredient that contributes to some parts of the medical effect of glycyrrhiza. Though, many components other than glycyrrhizic acid are also known to play a part in the medical effects of glycyrrhiza. Consequently, glycyrrhizic acid should be regarded as one of the multiple active ingredients of glycyrrhiza that being specified its content as a marker constituent. In other cases of the marker constituents that seemed to have less biological effects such as 10-hydroxy-2-(*E*)-decenoic acid in royal jelly or (*E*)-cinnamic acid in some extracts of Kampo formula, their content are standardized as a distinctive compound of each pharmaceutical product. Referring to the biosyntheses of natural products, the content of these compounds specified as marker constituents are unlikely to be independently changed to stand out from others. The specification values of crude drugs and crude drug preparations are stipulated in order to control the appropriate production process based on the strategy that every crude drug and crude drug preparation will be standardized to a certain level through the content control of marker constituents.

An instance of the relationship between the active ingredient and the marker compound of senna leaf may be a special case among crude drugs and pharmaceutical natural products made from crude drugs. The medicinal effects of the majority of crude drugs and pharmaceutical natural products made from crude drugs are achieved by the cooperative performance of their entire constituents. These drugs are standardized with the quantitative specification of particular constituents designated as the quantitative marker constituents, and this is owing to the fact that the standardization of every constituent consisting the multicomponent system is impossible. Taken all together, it requires particular consideration to the fact that the quantitative marker compounds specified for crude drugs and crude drug preparations are not directly same as the active ingredients in the chemical drugs that absolutely accounts for the medical effects of the drugs.

Purity Tests on Crude Drugs using Genetic Information

The first step in the quality assurance of natural products is the use of raw materials from the right part of the right origin (the right source). Therefore, it is clearly stated in Article 4 of the General Rules For Crude Drugs that the source of a crude drug is the approval or rejection criteria. There are various methods for differentiating the sources of crude drugs, such as morphological methods, organoleptic tests, and chemical methods, and appropriate methods for each are described in the individual monographs. Morphological methods, organoleptic tests, and chemical methods are discrimination methods for species that are based on the phenotypic characteristics of the crude drugs. On the other hand, together with recent progress in molecular biology techniques and the accumulation of genetic information on plants, differentiating methods of crude drugs based on genotypes have been established. Unlike morphological and other methods that are based on phenotypic characteristics, the genotypic methods are not affected by environmental factors. Also, the methods have several advantages, such as specialized expertise and skill for classification are not needed, and objective results are easily obtained.

The evolution of living organisms is accomplished by genetic mutation, and differences among the nucleotide sequences of genes of closely related species reflect the strain relationships between the species. Based on this theory, in recent years methods that classify species phylogenetically using the nucleotide sequence of rDNA that codes for ribosomal RNA (rRNA) on the nuclear genome have been adopted. In the same way, the sequence of this rDNA is also most often used in the classification of higher plants based on the genotype. In particular, it is very easy to classify closely related species using the intergenic transcriber space (ITS) region of the rDNA region, since by comparison with the coded gene region nucleotide substitution is more often undertaken. Furthermore, since the genes on the nuclear genome originate from the parents' genom, there is an advantage that interspecies hybrids can be detected. Higher plants also have mitochondrial genes and chloroplastic genes. Although the genes on these genomes are also often used for classification, interspecies hybrids cannot be confirmed because the genes are normally uniparental inheritance.

The two methods presented here have been developed based on the reported identification methods of Atractylodes Lancea Rhizome and Atractylodes Rhizome[1,2] utilizing the gene sequence of the ITS of rDNA. Inter-laboratory validation study for purity test of Atractylodes Rhizome targeted for Atractylodes Lancea Rhizome have been completed. The plant sources for Atractylodes Lancea Rhizome stipulated in the individual monographs are *Atractylodes lancea* De Candolle or *A. chinensis* Koidzumi (*Compositae*), while those for Atractylodes Rhizome are *A. japonica* Koidzumi ex Kitamura or *A. ovata* De Candolle (*Compositae*). The approval or rejection of the source of Atractylodes Lancea Rhizome is, in principle, determined by the description of the crude drug, including microscopy, while that of Atractylodes Rhizome is determined by the description of the crude drug, including microscopy, together with color reaction, which is an identification test. In the above scientific paper, it was shown that these 4 plant species can be clearly classified by comparing the nucleotide sequences of the ITS mentioned above, and that the species can be easily classified without performing sequence analysis by performing PCR using a species specific primer set or by using a restriction enzyme which recognizes species specific sequence.

In validation studies, the simplicity of the test is given maximum consideration. We examined methods that observe PCR amplification bands using species specific primer sets (Mutant Allele Specific Amplification: Method 1) and that observe DNA fragments produced by restriction enzyme treatment of the PCR products, which are prepared using a primer set common to each plant source (PCR-Restriction Fragment Length Polymorphism: Method 2), and do not involve nucleotide sequence analyses. In these methods based on PCR, an extremely small amount of template DNA is amplified to billions to hundred-billions times. Therefore, when using them as identification tests for powdered crude drugs, the target DNA fragment can be observed even if the vast majority of the crude drug for analysis is not appropriate plant species and there is only a minute amount of powder from a crude drug derived from a suitable plant.

(Consequently, in identification tests, either a cut or a whole crude drug must be used, as long as one is careful to avoid contamination by powder originating from other crude drugs.) On the other hand, when used as a purity test, the form of the crude drug is irrelevant as long as the gene amplification is performed properly and the target gene is not polymorphic, so if DNA fragments of an inappropriate plant are confirmed in the purity test, regardless of the form of the crude drug, it becomes clear there is contamination by an inappropriate crude drug.

The methods shown here are reference information and at the present stage results obtained using the methods do not affect the approval or rejection of the crude drug in each monograph. Furthermore, by performing the sequence analysis outlined in the previous paper, it goes without saying that more accurate decision concerning the source species can be made.

1. DNA Amplification Equipment

DNA amplification equipment is used to amplify the DNA which is extracted from a crude drug and then purified. Since there are slight differences in the methods of temperature control, and so on depending on the equipment used, there may be differences in the intensity, etc. of the PCR amplification bands even if PCR is carried out under the stipulated conditions. Therefore, when judging results based solely on the presence or absence of PCR amplification bands as in 3. Methods 1, confirm that only proper amplification bands are obtained when performing PCR using DNA obtained from samples confirmed beforehand to be the source species. If proper amplification bands are not obtained, the PCR temperature conditions should be slightly adjusted. This equipment can be used for the restriction enzyme treatment in 4. Method 2.

2. General precautions

Crude drugs are different from fresh plants in that they are dried products and a certain amount of time has passed since they were harvested. Therefore, in many cases the DNA has undergone fragmentation. Furthermore, various substances that can block or interfere with the PCR reaction may be present in the plant. For these reasons, the extraction and purification of template DNA is the process that should receive the greatest amount of attention. In the case of Atractylodes crude drugs, the periderm should be removed using a clean scalpel or other clean instrument before pulverizing the sample because very often there are inhibitory substances present in the periderm.

3. Method 1 (Mutant Allele Specific Amplification Method)

Generally, this method is referred to as Mutant Allele Specific Amplification (MASA) or Amplification Refractory Mutation System (ARMS), and it provides nucleotide sequence information of sample-derived template DNA, based upon the presence or absence of DNA amplification in PCR using a species specific primer set.

3.1. Procedure

The following is an example procedure.

3.1.1. Preparation of template DNA

There are various methods with which to extract and purify DNA from the samples. It is recommended that commercially available DNA extraction kits be used when considering their advantages of not using any noxious reagents and not requiring any complicated purification procedures. In this case, attention should be paid to the final amount (concentration) of DNA obtained, and the initial amount of initial sample and the volume of liquid to elute the DNA need to be controlled. When extraction and purification are performed using silica gel membrane type kits stipulated in notifications[3] related to inspection methods of the foods produced by recombinant DNA techniques, it is appropriate to use 200 mg of sample, 1 mL of AP1 buffer solution, 2 μL of RNase A, and 325 μL of AP2 buffer solution. Also, the most important things are that the supernatant loaded on the first column is clear and that there is no need to load 1 mL unreasonably. Furthermore, 50 μL is an appropriate volume used in the final elution of the DNA, and normally the initial eluate is used as the DNA sample stock solution.

3.1.2. Confirmation of purity of DNA in DNA sample stock solution and assay of DNA

The purity of the DNA in the stock solution can be confirmed by the $OD_{260\,nm}/OD_{280\,nm}$ ratio using a spectrophotometer. A ratio of 1.5 indicates that the DNA has been adequately purified. The amount of DNA is calculated using 1 $OD_{260\,nm}$ = 50 μg/mL. The measurement mentioned above is performed using appropriately diluted DNA sample stock solution. Based on the results obtained, dilute with water to the concentration needed for the subsequent PCR reactions, dispense the solution into micro tubes as the sample DNA solution, and if necessary store frozen at not over −20°C. The dispensed DNA sample is used immediately after thawing and any remaining solution should be discarded and not refrozen. If the concentration of the DNA sample stock solution does not reach the concentration stipulated in PCR, it is used as a DNA sample solution.

3.1.3. PCR

When a commercially available PCR enzyme mentioned in the above notification[4] is used, it is appropriate that 25 μL of reaction mixture consisting of 2.5 μL of the PCR buffer solution containing magnesium, dNTP (0.2 mmol/L), 5′ and 3′ primer (0.4 μmol/L), Taq DNA polymerase (1.25 units), and 5 μL of 10 ng/μL sample DNA solution (50 ng of DNA) is prepared on ice. Among them, the PCR buffer solution and dNTP are provided as adjuncts to the enzyme. When conducting purity tests on Atractylodes Lancea Rhizome in Atractylodes Rhizome, the primer sets used are C and D (C is positive with *A. lancea*, D is positive with A. *chinensis*) as described in the paper mentioned above (*J. Nat. Med.* 60, 149 – 156, 2006), however, when a combination of primer A and B is used, it is possible to confirm the source species of each of the respective specimens. In order to confirm that the DNA has been extracted correctly, the reaction solution containing the positive control primer (Pf and Pr) as shown below should be prepared. In addition, the negative control solutions which are not containing DNA sample or either of the primer sets should be prepared and simultaneously conduct PCR.

Pf: 5′-CAT TGT CGA AGC CTG CAC AGC A-3′
Pr: 5′-CGA TGC GTG AGC CGA GAT ATC C-3′

The PCR reaction is performed under the following conditions: starting the reaction at 95°C for 10 minutes, 30 cycles of 0.5 minutes at 95°C and 0.75 minutes at 68°C (69°C only when using the primer set C), terminate reaction at 72°C for 7 minutes, and store at 4°C. The resulting reaction mixture is used for the following process as PCR amplification reaction solution.

3.1.4. Agarose gel electrophoresis and detection of PCR products

After completion of the PCR reaction, mix 5 μL of the PCR amplification reaction solution with an appropriate volume of gel loading buffer solution, add the mixture to the wells of 2 w/v% agarose gel, and then perform electrophoresis using 1-fold TAE buffer solution (refer to General Infor-

mation, Rapid Identification of Microorganisms Based on Molecular Biological Method). Run in parallel an appropriate DNA molecular mass standard. Electrophoresis is terminated when the bromophenol blue dye in the gel loading buffer has advanced to a point corresponding to 1/2 to 2/3 the length of the gel.

Stain the gel after electrophoresis when not using gel stained in advance with ethidium bromide. Place the gel that has undergone electrophoresis and staining in a gel image analyzer, irradiate with ultraviolet light (312 nm), and detected its electrophoresis pattern. Compare this to the DNA molecular mass standard and determine the absence or presence of the target amplification band.

3.2. Judgment

Confirm at first that a 305 bp band is found with the reaction solution to which the positive control primer set has been added, and confirm there are no bands in a solution with no primer sets and a solution with no sample DNA solution. Next, if a 226 bp band is confirmed when the primer set C is added or if a 200 bp band is confirmed when the primer set D is added, the sample is judged to be Atractylodes Lancea Rhizome (in the case of cut crude drug, contamination of Atractylodes Lancea Rhizome is observed) and it is rejected. The sample is judged not to be Atractylodes Lancea Rhizome (in the case of cut crude drug, there is no contamination of Atractylodes Lancea Rhizome) and the purity test is acceptable if a 305 bp band is confirmed with the positive control primer set, bands are not observed in reaction solution without primer and reaction solution without DNA sample solution, and a 226 bp band is not observed with the primer set C and a 200 bp band is not observed with the primer set D. If a band is not observed with the positive control primer, it is to be concluded that the DNA extraction failed and the procedure should be started over again from the DNA extraction step. If bands are confirmed in reaction solutions without primer sets or without DNA sample solution, it should be assumed that there was an error in the PCR procedure and therefore the procedure should be repeated again from the step 3.1.3. PCR.

4. Method 2 (PCR-Restriction Fragment Length Polymorphism)

Generally, this method is referred to as PCR-Restriction Fragment Length Polymorphism (RFLP), and it provides nucleotide sequence information of sample-derived template DNA, based upon the DNA fragment pattern produced by restriction enzyme treatment of the PCR products, which are amplified by using a primer set common to the DNA sequence of the objective plant.

The test is performed with 25 samples randomly taken from a lot, and each sample is designated with a number from 1 to 25. Differentiation of the sources is performed by individual PCR-RFLP measurement of the samples, and decision of the acceptability of the purity is dependent on how many nonconforming samples are present in the first 20 samples, taken in numerical order, for which judgment is possible as described below.

4.1. Procedure

The following is an example procedure.

4.1.1. Preparation of template DNA

There are various methods with which to extract and purify DNA from the samples. It is recommended that commercially available DNA extraction kits be used, when considering their advantages of not using noxious reagents and not requiring complicated purification procedures. Recently, PCR reagents that inhibit the effect of PCR enzyme-inhibiting substances present in samples have become commercially available, and by using these reagents, it is possible to prepare the template DNA from the sample simply by incubating the sample with the DNA extraction reagent. Here, a recommended DNA preparing procedure using such PCR reagents is described for the convenience of experimenters.

Cut 20 mg of the sample into small pieces with a clean knife, add 400 μL of the DNA extraction reagent, and incubate at 55°C overnight (16 – 18 hours). Then heat at 95°C for 5 minutes to inactivate the enzyme in the reagent. Centrifuge to precipitate the sample, and use 50 μL of the supernatant liquid as the template DNA solution. The DNA solution prepared in this method can not be used for concentration measurement based on $OD_{260\,nm}$, because it contains many foreign substances affecting OD_{260} value from the sample.

The composition of the DNA extraction reagent is as follows:

2-Amino-2-hydroxymethyl-1,3-propanediol-hydrochloric acid (pH 8.0)	20 mmol/L
Ethylenediamine tetraacetate	5 mmol/L
Sodium chloride	400 mmol/L
Sodium dodecyl sulfate	0.3%
Proteinase K	200 μg/mL

4.1.2. PCR

In the method using the PCR enzyme and PCR reagent as described[2], the reaction mixture is prepared on an ice bath in a total volume of 20 μL of a solution containing 10.0 μL of 2-fold concentrated PCR reagent, 5′- and 3′-primers (0.5 μmol/L), Taq DNA polymerase (0.5 units) and 0.5 μL of template DNA solution.

The PCR reaction is performed under the following conditions: 95°C for 10 minutes, 40 cycles of 95°C for 0.5 minute, 65°C for 0.25 minute, and 72°C for 0.25 minute and 72°C for 7 minutes. Store the solution at 4°C, and use this solution as the PCR amplified solution. A negative control (containing water instead of the template DNA solution) must be included in the procedure.

The sequence of each primer is as follows:

5′-primer: 5′-GGC ACA ACA CGT GCC AAG GAA AA-3′

3′-primer: 5′-CGA TGC GTG AGC CGA GAT ATC C-3′

4.1.3. Restriction enzyme treatment

The treatment is performed on individual reaction solutions using two enzymes, *Fau* I and *Msp* I. In the case of *Fau* I, to an appropriate amount of the reaction solution, composed of a reaction buffer containing 1.0 unit of enzyme, add 3.0 μL of PCR products while cooling in an ice bath to make 15.0 μL. In the case of *Msp* I, to an appropriate amount of the reaction solution, composed of a reaction buffer containing 20.0 units of enzyme, add 3.0 μL of PCR products while cooling in an ice bath to make 15.0 μL. Incubate these solutions at the temperature recommended by the manufacturer for 2 hours, and then inactivate the enzyme by heating at 72°C for 10 minutes. The negative control of the PCR reaction is also treated in the same manner.

4.1.4. Agarose gel electrophoresis and detection of DNA fragments

After the restriction enzyme treatment, mix the total amount of the reaction solution and an appropriate amount of the gel loading buffer solution, place it in a 4 w/v% agarose gel well, and carry out electrophoresis with 1-fold concentrated TAE buffer solution (see ''Rapid Identification of Microorganisms Based on Molecular Biological Methods'' under General Information). Carry out the electrophoresis together with appropriate DNA molecular mass standard. Stop the electrophoresis when the bromophenol

blue included in the loading buffer solution has moved about 2 cm from the well. The 4 w/v% agarose gel is sticky, difficult to prepare and hard to handle, so that it is better to use a commercially available precast gel.

After the electrophoresis, stain the gel, if it is not already stained, with ethidium bromide, and observe the gel on an illuminating device under ultraviolet light (312 nm) to confirm the electrophoretic pattern.

4.2. Judgment

4.2.1. Judgment of each sample

Confirm that no band is obtained with the negative control of the PCR, other than the primer dimer (about 40 bp) band. A sample treated with *Fau* I, showing bands of about 80 bp and 60 bp, or that treated with *Msp* I, showing bands of about 90 bp and 50 bp, is judged as Atractylodes Lancea Rhizome. A sample not showing any band other than a band at about 140 bp and the primer dimer band is judged as Atractylodes Rhizome. If a sample does not show any band other than the primer dimer band, it is considered that PCR products were not obtained, and judgment is impossible for the sample.

4.2.2. Judgment of the purity

Judgment of the purity is based on the result of the judgment of each sample. If there is no sample that is judged as Atractylodes Lancea Rhizome among 20 samples taken in order of the numbering, excluding any sample for which judgment is impossible, the lot is acceptable for purity. When there is one sample that is judged as Atractylodes Lancea Rhizome among the 20 samples, perform the same test with 25 newly taken samples from the lot, and if there is no sample that is judged as Atractylodes Lancea Rhizome, the lot is acceptable for purity. When there is a sample that is judged as Atractylodes Lancea Rhizome in the second test, or there is more than one sample that is judged as Atractylodes Lancea Rhizome in the first test, the lot is not acceptable for purity.

5. Reference

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4) Notification No. 0629002, 2.1.3.1.1, Director of Food Safety Department, June 2006.

Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy and its Application to Reagents in the Japanese Pharmacopoeia

1. Marker Compounds for the Assay of Crude Drugs in the JP and Establishment of Reference Substances for Quantitative Analyses

When the quantitative assay values are specified in the monographs of crude drugs and extracts of Kampo formulations in the JP, it is more difficult to establish and prepare their JP Reference Standards than those for synthetic chemical pharmaceutical substances, because the marker compounds for their assay are derived from natural sources.

Unlike the synthetic chemical pharmaceutical substances, crude drugs and extracts of Kampo formulations are mixtures of a great deal of compounds. Although it is necessary to choose a substance contained at the level of 0.1% to several % in the crude drugs and the extracts of Kampo formulations as the marker compounds for their quantitative assay, the synthesis of such compounds is not so easy in most cases. Therefore, the marker compound would be separated from natural materials and be isolated to have sufficient purity. However, the preparation of the reference substance in such a way would require high economical cost and a great deal of effort. In addition, the composition of impurities contained in the reference substance prepared in such a way would be different batch by batch according to the difference of raw materials and their processes of extraction, isolation and purification. Accordingly, the difference among batches of reference materials is much larger than that of synthetic substances, and the control of their purity as the official reference standards is very difficult. Furthermore, in many cases of substances of natural origin, the greatest impurity would be water. For determining water contents precisely, it is necessary to use Karl Fischer method, and as the result, a large quantity of the valuable reference standard would be consumed.

Because there are such bottlenecks mentioned above in many cases of monographs of crude drugs and extracts of Kampo formulations, the establishment of the JP Reference Standard is difficult. Instead, reagents, which are commercially available or ready to put into the market, are designated as the reference substances for the quantitative assay, and the method and the content specification using the reagent are specified in monographs of crude drugs and extracts of Kampo formulations. In these cases, the specifications of their marker substances are defined in the section of Reagents and Test Solutions of the JP. However, in a strict sense, since the assay values obtained in this manner are not certified metrologically, the reliability of the analytical value obtained by using them is somewhat ambiguous.

2. Application of Quantitative NMR to Reference Substances Used in the Assay of Crude Drugs and Extracts of Kampo Formulations

The application of quantitative NMR can solve the issue on the purity of reagents derived from natural source. These reagents are used as the reference substances with metrological traceability, when the precise contents of these reagents are determined metrologically by using quantitative NMR based on the idea shown in 10.1 Principle of Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy under <*5.01*> Crude Drugs Test.

Currently, the quantitative NMR is being carried out for these reagents defined for the quantitative assay of crude drugs in the JP and a report in which the points to practically consider at determination of absolute purities of the reagents by using quantitative NMR are discussed has been published.[1)] In addition, a validation study of quantitative NMR has also been performed using the substances which will be used with high possibility as the reference substances for HPLC quantitative analysis. For the analyte compound having molecular mass of around 300, when about 10 mg of the compound was used for the quantitative NMR measurement, it was demonstrated that an accuracy of 2 significant digits for the determined value was achieved at the ordinary laboratory level, even when the error among the NMR instruments used was included.[2)] Usually, the contents of marker compounds for the quantitative assay of crude drugs are several % at the maximum, and the minimum unit for the content specification is at the level of 0.1%. There-

fore, when variability of content in crude drugs is considered, the assurance of 2 significant digits for accuracy seems sufficient for the reference substances, which are used for the quantitative assay of crude drugs.

When discussion above is considered, the ambiguity of analytical values obtained by the use of the reagents derived from natural source as the reference substances for the quantitative assay of crude drugs can be avoided practically, by using the reagents certified by quantitative NMR as the reference substances in HPLC, etc., and by incorporating the certified purity of such reagent into the calculation of the quantitative value of the sample. For example, for Gardenia Fruit in the JP, the content of geniposide is specified at not less than 3.0% based on the HPLC analysis. The report cited above[1] demonstrated that the absolute purity of geniposide used as the reference substance in the quantitative assay of Gardenia Fruit is determined to be about 92% by quantitative NMR. Therefore, in the case that the quantitative value of 3.0% in Gardenia Fruit sample is obtained as a result of HPLC analysis by using this reagent as the reference substance assuming its purity as 100%, the true value for the sample is evaluated to be 2.8% taking it into consideration of the absolute purity determined by quantitative NMR with the assurance of metrological traceability.

3. Supply of Certified Reagents by Using Quantitative NMR

Currently, in the accreditation system of the International Accreditation Japan (IA Japan), the National Institute of Technology and Evaluation (ASNITE), a feasibility study how the accreditation should be given to the organization which performs the assay certification of the reagents using calibrated NMR apparatus has been in progress. In addition, in the IA Japan, addition of''Quantitative NMR''to the test method categories is scheduled. Therefore, in the near future, the reagent manufacturers will become able to perform the assay certification of the reagent after having this accreditation. Under such situation, the user of the reagent would not be required to perform qualitative NMR individually to obtain the purity value with SI traceability. Furthermore, the inter-institutional errors (including inter-instrumental errors) would become negligible, and we will be able to carry out more precise and accurate quantitation assay of the sample by incorporating the labeled certified value on the reagent into the calculation of the quantitative value of the sample.

The certified reference materials (NMIJ CRM) to be used for the SI traceable metrological determination of the internal reference compounds are supplied from the National Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology (NMIJ AIST).

4. Management of Instrument Performance for Quantitative NMR

Quantitative NMR used to determine the purity of reagents for the JP, is an internal standard method that analyte compound and SI traceable reference material in a NMR tube are measured at the same time.[3] In this method, the number of nuclei are measured using NMR phenomenon, which means that the molar quantity of analyte compound in a sample solution is directly calibrated with a reference material.

In the management of instrument performance for quantitative NMR measurements, it should be confirmed that integral value of the targeted signals can be determined correctly within the spectrum where the signals are measured (in general, 0 – 10 ppm). The important point here is not to include the signals derived from impure substances in the quantitative spectrum when integrate. Therefore, to manage instrument performance, a high-purity compound of already known purity (determined by quantitative NMR and not less than 99.0% is preferable) should be used. In addition, signals derived from simpler spin system should be selected and integrated, and the ratio of theoretical number of nuclei among signals should be accurate (for example, when each of the two signals is derived from 1H, the ratio of the integrated values of the both is 0.995 – 1.005).

Considering the excitation bandwidth of a NMR pulse, when an instrument of 800 MHz is used under the following conditions; center of spectrum window around 5 ppm, spectral width at 20 ppm (the conditions stipulated in assay using qNMR in the section of reagents), with a 90° pulse width of 10 microseconds, the excitation efficiency of the pulse in the range of 0 – 10 ppm, where signals of analyte compound are observed, is usually not less than 99.95%. Thus, the instrument, when probe is well tuned and shim is adjusted properly, can assure an accuracy of 2 significant digits at routine level of measurement. Furthermore, when an instrument of 400 MHz is used, similar excitation efficiency can be obtained up to 20 microseconds for 90° pulse width, so quantitative NMR can be measured sufficiently with a standard probe.

5. Reference

1) J. Hosoe, et al., Pharmaceutical and Medical Device Regulatory Science, 41, 960 – 970 (2010)
2) J. Hosoe, et al., Pharmaceutical and Medical Device Regulatory Science, 43, 182 – 193 (2012)
3) J. Hosoe, et al., Pharmaceutical and Medical Device Regulatory Science, 45, 243 – 250 (2014)

On the Scientific Names of Crude Drugs listed in the JP

The notation system of the scientific names for the original plants and animals of crude drugs listed in JP is not necessary the same as the taxonomic system used in the literature. The reason for this is that the JP is not an academic text, but an ordinance. The relationship between the scientific names used in the JP and those generally used taxonomically is indicated in the following table, to avoid misunderstanding by JP users owing to differences in the notation system.

Scientific Names used in the JP and Those being used Taxonomically

Crude Drug	Scientific names used in the JP ＝Scientific names being used taxonomically (Combined notation, Standard form for author or authors) Scientific names that are different from those written in JP but identical to them taxonomically or being regarded as identical, and typical subclassified groups belonging to their species. The names marked with ‘‘*’’ are those being written together in JP.	Family
Acacia アラビアゴム	*Acacia senegal* Willdenow ＝*Acacia senegal* (L.) Willd.	*Leguminosae*
	Other species of the same genus	
Achyranthes Root ゴシツ	*Achyranthes fauriei* Leveille et Vaniot ＝*Achyranthes fauriei* H. Lev. & Vaniot	*Amaranthaceae*
	Achyranthes bidentata Blume	
Agar カンテン	*Gelidium elegans* Kuetzing	*Gelidiaceae*
	Other species of the same genus	
	Red seaweeds of several species	
Akebia Stem モクツウ	*Akebia quinata* Decaisne ＝*Akebia quinata* (Thunb. ex Houtt.) Decne.	*Lardizabalaceae*
	Akebia trifoliata Koidzumi ＝*Akebia trifoliata* (Thunb.) Koidz.	
Alisma Tuber タクシャ	*Alisma orientale* Juzepczuk ＝*Alisma orientale* (Sam.) Juz.	*Alismataceae*
	Alisma plantago-aquatica L. var. *orientale* Sam.	
Aloe アロエ	*Aloe ferox* Miller ＝*Aloe ferox* Mill.	*Liliaceae*
	Hybrid between *Aloe ferox* Miller and *Aloe africana* Miller ＝*Aloe africana* Mill.	
	Hybrid between *Aloe ferox* Miller and *Aloe spicata* Baker	
Alpinia Officinarum Rhizome リョウキョウ	*Alpinia officinarum* Hance	*Zingiberaceae*
Amomum Seed シュクシャ	*Amomum xanthioides* Wallich ＝*Amomum xanthioides* Wall.	*Zingiberaceae*
	Amomum villosum Lour. var. *xanthioides* (Wall.) T. L. Wu et Senjen	
Anemarrhena Rhizome チモ	*Anemarrhena asphodeloides* Bunge	*Liliaceae*
Angelica Dahurica Root ビャクシ	*Angelica dahurica* Bentham et Hooker filius ex Franchet et Savatier ＝*Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav.	*Umbelliferae*
Apricot Kernel キョウニン	*Prunus armeniaca* Linné ＝*Prunus armeniaca* L.	*Rosaceae*
	Prunus armeniaca Linné var. *ansu* Maximowicz ＝*Prunus armeniaca* L. var. *ansu* Maxim.	
	Prunus sibirica Linné ＝*Prunus sibirica* L.	
Aralia Rhizome ドクカツ	*Aralia cordata* Thunberg ＝*Aralia cordata* Thunb.	*Araliaceae*
Areca ビンロウジ	*Areca catechu* Linné ＝*Areca catechu* L.	*Palmae*

Artemisia Capillaris Flower インチンコウ	*Artemisia capillaris* Thunberg ＝*Artemisia capillaris* Thunb.	*Compositae*
Artemisia Leaf ガイヨウ	*Artemisia princeps* Pampanini ＝*Artemisia princeps* Pamp.	*Compositae*
	Artemisia montana Pampanini ＝*Artemisia montana* (Nakai) Pamp.	
Asiasarum Root サイシン	*Asiasarum sieboldii* F. Maekawa ＝*Asiasarum sieboldii* (Miq.) F. Maek.	*Aristolochiaceae*
	Asarum sieboldii Miq.	
	Asarum sieboldii Miq. var. *seoulense* Nakai	
	Asiasarum heterotropoides F. Maekawa var. *mandshuricum* F. Maekawa ＝*Asiasarum heterotropoides* (F. Schmidt) F. Maek. var. *mandshuricum* (Maxim.) F. Maek.	
	Asarum heterotropoides F. Schmidt var. *mandshuricum* (Maxim.) Kitag.	
Asparagus Root テンモンドウ	*Asparagus cochinchinensis* Merrill ＝*Asparagus cochinchinensis* (Lour.) Merr.	*Liliaceae*
Astragalus Root オウギ	*Astragalus membranaceus* Bunge ＝*Astragalus membranaceus* (Fisch.) Bunge	*Leguminosae*
	Astragalus mongholicus Bunge	
	Astragalus membranaceus (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao	
Atractylodes Lancea Rhizome ソウジュツ	*Atractylodes lancea* De Candolle ＝*Atractylodes lancea* (Thunb.) DC.	*Compositae*
	Atractylodes chinensis Koidzumi ＝*Atractylodes chinensis* (DC.) Koidz.	
	Hybrid between above species	
Atractylodes Rhizome ビャクジュツ	*Atractylodes japonica* Koidzumi ex Kitamura ＝*Atractylodes japonica* Koidz. ex Kitam.	*Compositae*
	Atractylodes macrocephala Koidzumi ＝*Atractylodes macrocephala* Koidz.	
	* *Atractylodes ovata* De Candolle ＝*Atractylodes ovata* (Thunb.) DC.	
Bear Bile ユウタン	*Ursus arctos* Linné ＝*Ursus arctos* L.	*Ursidae*
	Other animals of the related genus	
Bearberry Leaf ウワウルシ	*Arctostaphylos uva-ursi* Sprengel ＝*Arctostaphylos uva-ursi* (L.) Spreng.	*Ericaceae*
Beef Tallow 牛脂	*Bos taurus* Linné var. *domesticus* Gmelin ＝*Bos taurus* L. var. *domesticus* Gmelin	*Bovidae*
Yellow Beeswax ミツロウ	*Apis mellifera* Linné ＝*Apis mellifera* L.	*Apidae*
	Apis cerana Fabricius	
Belladonna Extract ベラドンナコン	*Atropa belladonna* Linné ＝*Atropa belladonna* L.	*Solanaceae*
Benincasa Seed トウガシ	*Benincasa cerifera* Savi	*Cucurbitaceae*
	Benincasa hispida (Thunb.) Cogn.	
	Benincasa cerifera Savi forma *emarginata* K. Kimura et Sugiyama ＝*Benincasa cerifera* Savi f. *emarginata* K. Kimura & Sugiyama	

Benzoin アンソッコウ	*Styrax benzoin* Dryander ＝*Styrax benzoin* Dryand.	*Styracaceae*
	Other species of the same genus	
Bitter Cardamon ヤクチ	*Alpinia oxyphylla* Miquel ＝*Alpinia oxyphylla* Miq.	*Zingiberaceae*
Bitter Orange Peel トウヒ	*Citrus aurantium* Linné ＝*Citrus aurantium* L.	*Rutaceae*
	Citrus aurantium Linné var. *daidai* Makino ＝*Citrus aurantium* L. var. *daidai* Makino	
	Citrus aurantium L. 'Daidai'	
Brown Rice コウベイ	*Oryza sativa* Linné ＝*Oryza sativa* L.	*Gramineae*
Bupleurum Root サイコ	*Bupleurum falcatum* Linné ＝*Bupleurum falcatum* L.	*Umbelliferae*
	Bupleurum chinense DC. *Bupleurum scorzonerifolium* Willd.	
Burdock Fruit ゴボウシ	*Arctium lappa* Linné ＝*Arctium lappa* L.	*Compositae*
Cacao Butter カカオ脂	*Theobroma cacao* Linné ＝*Theobroma cacao* L.	*Sterculiaceae*
Calumba コロンボ	*Jateorhiza columba* Miers	*Menispermaceae*
Camellia Oil ツバキ油	*Camellia japonica* Linné ＝*Camellia japonica* L.	*Theaceae*
Capsicum トウガラシ	*Capsicum annuum* Linné ＝*Capsicum annuum* L.	*Solanaceae*
Cardamon ショウズク	*Elettaria cardamomum* Maton	*Zingiberaceae*
Carnauba Wax カルナウバロウ	*Copernicia cerifera* Martius ＝*Copernicia cerifera* Mart.	*Palmae*
Cassia Seed ケツメイシ	*Cassia obtusifolia* Linné ＝*Cassia obtusifolia* L.	*Leguminosae*
	Cassia tora Linné ＝*Cassia tora L.*	
Castor Oil ヒマシ油	*Ricinus communis* Linné ＝*Ricinus communis* L.	*Euphorbiaceae*
Catalpa Fruit キササゲ	*Catalpa ovata* G. Don	*Bignoniaceae*
	Catalpa bungei C. A. Meyer ＝*Catalpa bungei* C. A. Mey.	
Cherry Bark オウヒ	*Prunus jamasakura* Siebold ex Koidzumi ＝*Prunus jamasakura* Siebold ex Koidz.	*Rosaceae*
	Prunus verecunda Koehne ＝*Prunus verecunda* (Koidz.) Koehne	
Chrysanthemum Flower キクカ	*Chrysanthemum morifolium* Ramatuelle ＝*Chrysanthemum morifolium* Ramat.	*Compositae*
	Chrysanthemum indicum Linné ＝*Chrysanthemum indicum* L.	

Cimicifuga Rhizome ショウマ	*Cimicifuga simplex* Turczaninow ＝*Cimicifuga simplex* (DC.) Turcz.	*Ranunculaceae*
	Cimicifuga dahurica Maximowicz ＝*Cimicifuga dahurica* (Turcz.) Maxim.	
	Cimisifuga heracleifolia Komarov ＝*Cimisifuga heracleifolia* Kom.	
	Cimicifuga foetida Linné ＝*Cimicifuga foetida* L.	
Cinnamon Bark ケイヒ	*Cinnamomum cassia* Blume	*Lauraceae*
Cinnamon Oil ケイヒ油	*Cinnamomum cassia* Blume	*Lauraceae*
	Cinnamomum zeylanicum Nees	
Cistanche Herb ニクジュヨウ	*Cistanche salsa* G. Beck ＝*Cistanche salsa* (C.A.Mey.) Beck	*Orobanchaceae*
	Cistanche deserticola Y. C. Ma ＝*Cistanche deserticola* Ma	
	Cistanche tubulosa Wight	
Citrus Unshiu Peel チンピ	*Citrus unshiu* Marcowicz ＝*Citrus unshiu* (Swingle) Marcow.	*Rutaceae*
	Citrus reticulata Blanco 'Unshiu'	
	Citrus reticulata Blanco	
Clematis Root イレイセン	*Clematis chinensis* Osbeck	*Ranunculaceae*
	Clematis mandshurica Ruprecht ＝*Clematis mandshurica* Rupr.	
	Clematis hexapetala Pallas ＝*Clematis hexapetala* Pall.	
Clove チョウジ Clove Oil チョウジ油	*Syzygium aromaticum* Merrill et Perry ＝*Syzygium aromaticum* (L.) Merr. & M. L. Perry	*Myrtaceae*
	* *Eugenia caryophyllata* Thunberg ＝*Eugenia caryophyllata* Thunb. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison	
Cnidium Monnieri Fruit ジャショウシ	*Cnidium monnieri* Cusson ＝*Cnidium monnieri* (L.) Cusson	*Umbelliferae*
Cnidium Rhizome センキュウ	*Cnidium officinale* Makino	*Umbelliferae*
Coconut Oil ヤシ油	*Cocos nucifera* Linné ＝*Cocos nucifera* L.	*Palmae*
Codonopsis Root トウジン	*Codonopsis pilosula* Nannfeldt ＝*Codonopsis pilosula* Nannf.	*Campanulaceae*
	Codonopsis tangshen Oliver ＝*Codonopsis tangshen* Oliv.	
Coix Seed ヨクイニン	*Coix lacryma-jobi* Linné var. *mayuen* Stapf ＝*Coix lacryma-jobi* L. var. *mayuen* (Rom. Caill.) Stapf	*Gramineae*
Condurango コンズランゴ	*Marsdenia cundurango* Reichenbach filius ＝*Marsdenia cundurango* Rchb. f.	*Asclepiadaceae*

Coptis Rhizome オウレン	*Coptis japonica* Makino ＝*Coptis japonica* (Thunb.) Makino	*Ranunculaceae*
	Coptis japonica (Thunb.) Makino var. *dissecta* (Yatabe) Nakai *Coptis japonica* (Thunb.) Makino var. *japonica* *Coptis japonica* (Thunb.) Makino var. *major* (Miq.) Satake	
	Coptis chinensis Franchet ＝*Coptis chinensis* Franch.	
	Coptis deltoidea C. Y. Cheng et Hsiao	
	Coptis teeta Wallich ＝*Coptis teeta* Wall.	
Corn Oil トウモロコシ油	*Zea mays* Linné ＝*Zea mays* L.	*Gramineae*
Cornus Fruit サンシュユ	*Cornus officinalis* Siebold et Zuccarini ＝*Cornus officinalis* Siebold & Zucc.	*Cornaceae*
Corydalis Tuber エンゴサク	*Corydalis turtschaninovii* Besser forma *yanhusuo* Y. H. Chou et C. C. Hsu ＝*Corydalis turtschaninovii* Besser f. *yanhusuo* (W. T. Wang) Y. H. Chou & C. C. Hsu	*Papaveraceae*
	Corydalis yanhusuo W. T. Wang	
Crataegus Fruit サンザシ	*Crataegus cuneata* Siebold et Zuccarini ＝*Crataegus cuneata* Siebold & Zucc.	*Rosaceae*
	Crataegus pinnatifida Bunge var. *major* N. E. Brown ＝*Crataegus pinnatifida* Bunge var. *major* N. E. Br.	
Cyperus Rhizome コウブシ	*Cyperus rotundus* Linné ＝*Cyperus rotundus* L.	*Cyperaceae*
Digenea マクリ	*Digenea simplex* C. Agardh ＝*Digenea simplex* (Wulfen) C. Agardh	*Rhodomelaceae*
Dioscorea Rhizome サンヤク	*Dioscorea japonica* Thunberg ＝*Dioscorea japonica* Thunb.	*Dioscoreaceae*
	Dioscorea batatas Decaisne ＝*Dioscorea batatas* Decne.	
	Dioscorea opposita Thunb.	
Dolichos Seed ヘンズ	*Dolichos lablab* Linné ＝*Dolichos lablab* L.	*Leguminosae*
Eleutherococcus Senticosus Rhizome シゴカ	*Eleutherococcus senticosus* Maximowicz ＝*Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim.	*Araliaceae*
	* *Acanthopanax senticosus* Harms ＝*Acanthopanax senticosus* (Rupr. & Maxim.) Harms	
Ephedra Herb マオウ	*Ephedra sinica* Stapf	*Ephedraceae*
	Ephedra intermedia Schrenk et C. A. Meyer ＝*Ephedra intermedia* Schrenk & C. A. Mey.	
	Ephedra equisetina Bunge	

Epimedium Herb インヨウカク	*Epimedium pubescens* Maximowicz ＝*Epimedium pubescens* Maxim.	*Berberidaceae*
	Epimedium brevicornu Maximowicz ＝*Epimedium brevicornu* Maxim.	
	Epimedium wushanense T. S. Ying	
	Epimedium sagittatum Maximowicz ＝*Epimedium sagittatum* (Siebold & Zucc.) Maxim.	
	Epimedium koreanum Nakai	
	Epimedium grandiflorum Morren var. *thunbergianum* Nakai ＝*Epimedium grandiflorum* Morr. var. *thunbergianum* (Miq.) Nakai	
	Epimedium sempervirens Nakai	
Eucalyptus Oil ユーカリ油	*Eucalyptus globulus* Labillardiere ＝*Eucalyptus globulus* Labill.	*Myrtaceae*
	Allied species	
Eucommia Bark トチュウ	*Eucommia ulmoides* Oliver ＝*Eucommia ulmoides* Oliv.	*Eucommiaceae*
Evodia Fruit ゴシュユ	*Euodia ruticarpa* Hooker filius et Thomson ＝*Euodia ruticarpa* (A. Juss.) Hook. f. & Thomson	*Rutaceae*
	* *Evodia rutaecarpa* Bentham ＝*Evodia rutaecarpa* (A. Juss.) Benth. *Tetradium ruticarpum* (A. Juss.) Hartley	
	Euodia officinalis Dode	
	* *Evodia officinalis* Dode *Evodia rutaecarpa* (A. Juss.) Benth. var. of *ficinalis* (Dode) Huang	
	Euodia bodinieri Dode	
	* *Evodia bodinieri* Dode *Evodia rutaecarpa* (A. Juss.) Benth. var. *bodinieri* (Dode) Huang	
Fennel ウイキョウ	*Foeniculum vulgare* Miller ＝*Foeniculum vulgare* Mill.	*Umbelliferae*
Fennel Oil ウイキョウ油	*Foeniculum vulgare* Miller ＝*Foeniculum vulgare* Mill.	*Umbelliferae*
	Illicium verum Hooker filius ＝*Illicium verum* Hook. f.	*Illiciaceae*
Forsythia Fruit レンギョウ	*Forsythia suspensa* Vahl ＝*Forsythia suspensa* (Thunb.) Vahl	*Oleaceae*
Fritillaria Bulb バイモ	*Fritillaria verticillata* Willdenow var. *thunbergii* Baker ＝*Fritillaria verticillata* Willd. var. *thunbergii* (Miq.) Baker	*Liliaceae*
	Fritillaria thunbergii Miq.	
Gambir アセンヤク	*Uncaria gambir* Roxburgh ＝*Uncaria gambir* (Hunter) Roxb.	*Rubiaceae*
Gardenia Fruit サンシシ	*Gardenia jasminoides* Ellis	*Rubiaceae*
	Gardenia jasminoides Ellis f. *longicarpa* Z. W. Xie & Okada	
Gastrodia Tuber テンマ	*Gastrodia elata* Blume	*Orchidaceae*
Gentian ゲンチアナ	*Gentiana lutea* Linné ＝*Gentiana lutea* L.	*Gentianaceae*

Geranium Herb ゲンノショウコ	*Geranium thunbergii* Siebold et Zuccarini ＝*Geranium thunbergii* Siebold & Zucc.	*Geraniaceae*
Ginger ショウキョウ	*Zingiber officinale* Roscoe	*Zingiberaceae*
Ginseng ニンジン	*Panax ginseng* C. A. Meyer ＝*Panax ginseng* C. A. Mey.	*Araliaceae*
	* *Panax schinseng* Nees	
Glehnia Root ハマボウフウ	*Glehnia littoralis* Fr. Schmidt ex Miquel ＝*Glehnia littoralis* F. Schmidt ex Miq.	*Umbelliferae*
Glycyrrhiza カンゾウ	*Glycyrrhiza uralensis* Fischer ＝*Glycyrrhiza uralensis* Fisch.	*Leguminosae*
	Glycyrrhiza glabra Linné ＝*Glycyrrhiza glabra* L.	
Hedysarum Root シンギ	*Hedysarum polybotrys* Handel-Mazzetti ＝*Hedysarum polybotrys* Hand.-Mazz.	*Leguminosae*
Hemp Fruit マシニン	*Cannabis sativa* Linné ＝*Cannabis sativa* L.	*Moracea*
Honey ハチミツ	*Apis mellifera* Linné ＝*Apis mellifera* L.	*Apidae*
	Apis cerana Fabricius	
Houttuynia Herb ジュウヤク	*Houttuynia cordata* Thunberg ＝*Houttuynia cordata* Thunb.	*Saururaceae*
Immature Orange キジツ	*Citrus aurantium* Linné var. *daidai* Makino ＝*Citrus aurantium* L. var. *daidai* Makino	*Rutaceae*
	Citrus aurantium L. ‘Daidai’	
	Citrus natsudaidai Hayata	
	Citrus aurantium Linné ＝*Citrus aurantium* L.	
	Citrus aurantium L. subsp. *hassaku* (Tanaka) Hiroe ＝*Citrus hassaku* hort. ex Tanaka	
Imperata Rhizome ボウコン	*Imperata cylindrica* Beauvois ＝*Imperata cylindrica* (L.) P. Beauv.	*Gramineae*
	Imperata cylindrica (L.) P. Beauv. var. *major* (Nees) C. E. Hubb.	
Ipecac トコン	*Cephaelis ipecacuanha* A. Richard ＝*Cephaelis ipecacuanha* (Brot.) A. Rich.	*Rubiaceae*
	Cephaelis acuminata Karsten ＝*Cephaelis acuminata* H. Karst.	
Japanese Angelica Root トウキ	*Angelica acutiloba* Kitagawa ＝*Angelica acutiloba* (Siebold & Zucc.) Kitag.	*Umbelliferae*
	Angelica acutiloba Kitagawa var. *sugiyamae* Hikino ＝*Angelica acutiloba* (Siebold & Zucc.) Kitag. var. *sugiyamae* Hikino	
Japanese Gentian リュウタン	*Gentiana scabra* Bunge	*Gentianaceae*
	Gentiana scabra Bunge var. *buergeri* (Miq.) Maxim.	
	Gentiana manshurica Kitagawa ＝*Gentiana manshurica* Kitag.	
	Gentiana triflora Pallas ＝*Gentiana triflora* Pall.	
	Gentiana triflora Pall. var. *japonica* Hara	

Japanese Valerian カノコソウ	*Valeriana fauriei* Briquet ＝*Valeriana fauriei* Briq.	*Valerianaceae*
	Valeriana fauriei Briq. f. *yezoensis* Hara	
Japanese Zanthoxylum Peel サンショウ	*Zanthoxylum piperitum* De Candolle ＝*Zanthoxylum piperitum* (L.) DC.	*Rutaceae*
	Zanthoxylum piperitum (L.) DC. f. *inerme* Makino	
Jujube Seed サンソウニン	*Zizyphus jujuba* Miller var. *spinosa* Hu ex H. F. Chou ＝*Zizyphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou	*Rhamnaceae*
Jujube タイソウ	*Zizyphus jujuba* Miller var. *inermis* Rehder ＝*Zizyphus jujuba* Mill. var. *inermis* (Bunge) Rehder	*Rhamnaceae*
Koi コウイ	*Zea mays* Linné ＝*Zea mays* L.	*Gramineae*
	Manihot esculenta Crantz	*Euphorbiacaea*
	Solanum tuberosum Linné ＝*Solanum tuberosum* L.	*Solanaceae*
	Ipomoea batatas Poiret ＝*Ipomoea batatas* (L.) Poir.	*Convolvulaceae*
	Ipomoea batatas (L.) Lam.	
	Oryza sativa Linné ＝*Oryza sativa* L.	*Gramineae*
Purified Lanolin 精製ラノリン	*Ovis aries* Linné ＝*Ovis aries* L.	*Bovidae*
Lard 豚脂	*Sus scrofa* Linné var. *domesticus* Gray ＝*Sus scrofa* L. var. *domesticus* Gray	*Suidae*
Leonurus Herb ヤクモソウ	*Leonurus japonicus* Houttuyn ＝*Leonurus japonicus* Houtt.	*Labiatae*
	Leonurus sibiricus Linné ＝*Leonurus sibiricus* L.	
Lilium Bulb ビャクゴウ	*Lilium lancifolium* Thunberg ＝*Lilium lancifolium* Thunb.	*Liliaceae*
	Lilium brownii F. E. Brown var. *colchesteri* Wilosn ＝*Lilium brownii* F. E. Br. var. *colchesteri* (Van Houtte) E. H. Wilson ex Elwes	
	Lilium brownii F. E. Brown var. *viridulum* Baker	
	Lilium brownii F. E. Brown ＝*Lilium brownii* F. E. Br.	
	Lilium pumilum De Candolle ＝*Lilium pumilum* DC.	
Lindera Root ウヤク	*Lindera strychnifolia* Fernandez-Villar ＝*Lindera strychnifolia* (Siebold & Zucc.) Fern.-Vill.	*Lauraceae*
	Lindera aggregata (Sims) Kosterm.	
Lithospermum Root シコン	*Lithospermum erythrorhizon* Siebold et Zuccarini ＝*Lithospermum erythrorhizon* Siebold & Zucc.	*Boraginaceae*
Longan Aril リュウガンニク	*Euphoria longana* Lamarck ＝*Euphoria longana* Lam.	*Sapindaceae*
	Dimocarpus longan Lour.	
Lonicera Leaf and Stem ニンドウ	*Lonicera japonica* Thunberg ＝*Lonicera japonica* Thunb.	*Caprifoliaceae*

Loquat Leaf ビワヨウ	*Eriobotrya japonica* Lindley ＝*Eriobotrya japonica* (Thunb.) Lindl.	*Rosaceae*
Lycium Bark ジコッピ	*Lycium chinense* Miller ＝*Lycium chinense* Mill.	*Solanaceae*
	Lycium barbarum Linné ＝*Lycium barbarum* L.	
Lycium Fruit クコシ	*Lycium chinense* Miller ＝*Lycium chinense* Mill.	*Solanaceae*
	Lycium barbarum Linné ＝*Lycium barbarum* L.	
Magnolia Bark コウボク	*Magnolia obovata* Thunberg ＝*Magnolia obovata* Thunb.	*Magnoliaceae*
	* *Magnolia hypoleuca* Siebold et Zuccarini ＝*Magnolia hypoleuca* Siebold & Zucc.	
	Magnolia officinalis Rehder et Wilson ＝*Magnolia officinalis* Rehder & E. H. Wilson	
	Magnolia officinalis Rehder et Wilson var. *biloba* Rehder et Wilson ＝*Magnolia officinalis* Rehder & E. H. Wilson var. *biloba* Rehder & E. H. Wilson	
Magnolia Flower シンイ	*Magnolia salicifolia* Maximowicz ＝*Magnolia salicifolia* (Siebold & Zucc.) Maxim.	*Magnoliaceae*
	Magnolia kobus De Candolle ＝*Magnolia kobus* DC.	
	Magnolia biondii Pampanini ＝*Magnolia biondii* Pamp.	
	Magnolia sprengeri Pampanini ＝*Magnolia sprengeri* Pamp.	
	Magnolia heptapeta Dandy ＝*Magnolia heptapeta* (Buchoz) Dandy	
	* *Magnolia denudata* Desrousseaux ＝*Magnolia denudata* Desr.	
Mallotus Bark アカメガシワ	*Mallotus japonicus* Mueller Argoviensis ＝*Mallotus japonicus* (Thunb.) Müll. Arg.	*Euphorbiaceae*
Malt バクガ	*Hordeum vulgare* Linné ＝*Hordeum vulgare* L.	*Gramineae*
Mentha Herb ハッカ *Mentha Oil* ハッカ油	*Mentha arvensis* Linné var. *piperascens* Malinvaud ＝*Mentha arvensis* L. var. *piperascens* Malinv.	*Labiatae*
	Mentha haplocalyx Briq.	
	Hybrid originated from *Mentha arvensis* L. var. *piperascens* Malinv. as the mother species	
Moutan Bark ボタンピ	*Paeonia suffruticosa* Andrews	*Paeoniaceae*
	* *Paeonia moutan* Sims	
Mulberry Bark ソウハクヒ	*Morus alba* Linné ＝*Morus alba* L.	*Moraceae*
Nelumbo Seed レンニク	*Nelumbo nucifera* Gaertner ＝*Nelumbo nucifera* Gaertn.	*Nymphaeaceae*
Notopterygium Rhizome キョウカツ	*Notopterygium incisum* Ting ex H. T. Chang	*Umbelliferae*
	Notopterygium forbesii Boissieu	

Nuphar Rhizome センコツ	*Nuphar japonicum* De Candolle ＝*Nuphar japonicum* DC.	*Nymphaeaceae*
Nutmeg ニクズク	*Myristica fragrans* Houttuyn ＝*Myristica fragrans* Houtt.	*Myristicaceae*
Nux Vomica ホミカ	*Strychnos nux-vomica* Linné ＝*Strychnos nux-vomica* L.	*Loganiaceae*
Olive Oil オリブ油	*Olea europaea* Linné ＝*Olea europaea* L.	*Oleaceae*
Ophiopogon Root バクモンドウ	*Ophiopogon japonicus* Ker-Gawler ＝*Ophiopogon japonicus* (L. f.) Ker Gawl.	*Liliaceae*
Orange Oil オレンジ油	*Citrus species*	*Rutaceae*
Oriental Bezoar ゴオウ	*Bos taurus* Linné var. *domesticus* Gmelin ＝*Bos taurus* L. var. *domesticus* Gmelin	*Bovidae*
Oyster Shell ボレイ	*Ostrea gigas* Thunberg ＝*Ostrea gigas* Thunb.	*Ostreidae*
Panax Japonicus Rhizome チクセツニンジン	*Panax japonicus* C. A. Meyer ＝*Panax japonicus* C. A. Mey.	*Araliaceae*
Peach Kernel トウニン	*Prunus persica* Batsch ＝*Prunus persica* (L.) Batsch	*Rosaceae*
	Prunus persica Batsch var. *davidiana* Maximowicz ＝*Prunus persica* (L.) Batsch var. *davidiana* (Carrière) Maxim.	
	Prunus davidiana (Carrière) Franch.	
Peanut Oil ラッカセイ油	*Arachis hypogaea* Linné ＝*Arachis hypogaea* L.	*Leguminosae*
Peony Root シャクヤク	*Paeonia lactiflora* Pallas ＝*Paeonia lactiflora* Pall.	*Paeoniaceae*
Perilla Herb ソヨウ	*Perilla frutescens* Britton var. *crispa* W. Deane ＝*Perilla frutescens* (L.) Britton var. *crispa* (Thunb.) W. Deane	*Labiatae*
Peucedanum Root ゼンコ	*Peucedanum praeruptorum* Dunn	*Umbelliferae*
	Angelica decursiva Franchet et Savatier ＝*Angelica decursiva* (Miq.) Franch. & Sav.	
	* *Peucedanum decursivum* Maximowicz ＝*Peucedanum decursivum* (Miq.) Maxim.	
Pharbitis Seed ケンゴシ	*Pharbitis nil* Choisy ＝*Pharbitis nil* (L.) Choisy	*Convolvulaceae*
Phellodendron Bark オウバク	*Phellodendron amurense* Ruprecht ＝*Phellodendron amurense* Rupr.	*Rutaceae*
	Phellodendron amurense Rupr. var. *sachalinense* F. Schmidt *Phellodendron amurense* Rupr. var. *japonicum* (Maxim.) Ohwi *Phellodendron amurense* Rupr. var. *lavallei* (Dode) Sprangue	
	Phellodendron chinense Schneider ＝*Phellodendron chinense* C. K. Schneid.	
Picrasma Wood ニガキ	*Picrasma quassioides* Bennet ＝*Picrasma quassioides* (D. Don) Benn.	*Simaroubaceae*
Pinellia Tuber ハンゲ	*Pinellia ternata* Breitenbach ＝*Pinellia ternata* (Thunb.) Breitenb.	*Araceae*
Plantago Herb シャゼンソウ	*Plantago asiatica* Linné ＝*Plantago asiatica* L.	*Plantaginaceae*

Plantago Seed シャゼンシ	*Plantago asiatica* Linné ＝*Plantago asiatica* L.	*Plantaginaceae*
Platycodon Root キキョウ	*latycodon grandiflorum* A. De Candolle ＝*Platycodon grandiflorum* (Jacq.) A. DC.	*Campanulaceae*
Pogostemon Herb カッコウ	*Pogostemon cablin* Bentham ＝*Pogostemon cablin* (Blanco) Benth.	*Labiatae*
Polygala Root オンジ	*Polygala tenuifolia* Willdenow ＝*Polygala tenuifolia* Willd.	*Polygalaceae*
Polygonatum Rhizome オウセイ	*Polygonatum falcatum* A. Gray	*Liliaceae*
	Polygonatum sibiricum Redouté	
	Polygonatum kingianum Collett et Hemsley ＝*Polygonatum kingianum* Collett & Hemsl.	
	Polygonatum cyrtonema Hua	
Polygonum Root カシュウ	*Polygonum multiflorum* Thunberg ＝*Polygonum multiflorum* Thunb.	*Polygonaceae*
Polyporus Sclerotium チョレイ	*Polyporus umbellatus* Fries ＝*Polyporus umbellatus* (Pers.) Fries	*Polyporaceae*
Poria Sclerotium ブクリョウ	*Wolfiporia cocos* Ryvarden et Gilbertson ＝*Wolfiporia cocos* (Schw.) Ryv. & Gilbn.	*Polyporaceae*
	* *Poria cocos* Wolf ＝*Poria cocos* (Schw.) Wolf	
Powdered Opium アヘン末	*Papaver somniferum* Linné ＝*Papaver somniferum* L.	*Papaveraceae*
Prepared Glycyrrhiza シャカンゾウ	*Glycyrrhiza uralensis* Fischer ＝*Glycyrrhiza uralensis* Fisch.	*Leguminosae*
	Glycyrrhiza glabra Linné ＝*Glycyrrhiza glabra* L.	
Processed Aconite Root ブシ	*Aconitum carmichaeli* Debeaux	*Ranunculaceae*
	Aconitum japonicum Thunberg ＝*Aconitum japonicum* Thunb.	
Processed Ginger カンキョウ	*Zingiber officinale* Roscoe	*Zingiberaceae*
Prunella Spike カゴソウ	*Prunella vulgaris* Linné var. *lilacina* Nakai ＝*Prunella vulgaris* L. var. *lilacina* Nakai	*Labiatae*
Pueraria Root カッコン	*Pueraria lobata* Ohwi ＝*Pueraria lobata* (Willd.) Ohwi	*Leguminosae*
Quercus Bark ボクソク	*Quercus acutissima* Carruthers ＝*Quercus acutissima* Carruth.	*Fagaceae*
	Quercus serrata Murray	
	Quercus mongholica Fischer ex Ledebour var. *crispula* Ohashi ＝*Quercus mongholica* Fisch. ex Ledeb. var. *crispula* (Blume) Ohashi	
	Quercus variabilis Blume	
Rape Seed Oil ナタネ油	*Brassica campestris* Linné subsp. *napus* Hooker fil. et Anderson var. *nippo-oleifera* Makino ＝*Brassica campestris* L. subsp. *napus* Hook. f. et Anders. var. *nippo-oleifera* Makino	*Cruciferae*

Red Ginseng コウジン	*Panax ginseng* C. A. Meyer ＝*Panax ginseng* C. A. Mey.	*Araliaceae*
	* *Panax schinseng* Nees	
Rehmannia Root ジオウ	*Rehmannia glutinosa* Liboschitz var. *purpurea* Makino ＝*Rehmannia glutinosa* Libosch. var. *purpurea* Makino	*Scrophulariaceae*
	Rehmannia glutinosa Liboschitz ＝*Rehmannia glutinosa* Libosch.	
Rhubarb ダイオウ	*Rheum palmatum* Linné ＝*Rheum palmatum* L.	*Polygonaceae*
	Rheum tanguticum Maximowicz ＝*Rheum tanguticum* Maxim.	
	Rheum officinale Baillon ＝*Rheum officinale* Baill.	
	Rheum coreanum Nakai	
	Hybrid between above species	
Rose Fruit エイジツ	*Rosa multiflora* Thunberg ＝*Rosa multiflora* Thunb.	*Rosaceae*
Rosin ロジン	Several plants of *Pinus genus*	*Pinaceae*
Royal Jelly ローヤルゼリー	*Apis mellifera* Linné ＝*Apis mellifera* L.	*Apidae*
	Apis cerana Fabricius	
Safflower コウカ	*Carthamus tinctorius* Linné ＝*Carthamus tinctorius* L.	*Compositae*
Saffron サフラン	*Crocus sativus* Linné ＝*Crocus sativus* L.	*Iridaceae*
Salvia Miltiorrhiza Root タンジン	*Salvia miltiorrhiza* Bunge	*Labiatae*
Saposhnikovia Root ボウフウ	*Saposhnikovia divaricata* Schischkin ＝*Saposhnikovia divaricata* (Turcz.) Schischk.	*Umbelliferae*
Sappan Wood ソボク	*Caesalpinia sappan* Linné ＝*Caesalpinia sappan* L.	*Leguminosae*
Saussurea Root モッコウ	*Saussurea lappa* Clarke ＝*Saussurea lappa* (Decne.) C. B. Clarke	*Compositae*
	Aucklandia lappa Decne.	
Schisandra Fruit ゴミシ	*Schisandra chinensis* Baillon ＝*Schisandra chinensis* (Turcz.) Baill.	*Schisandraceae*
Schizonepeta Spike ケイガイ	*Schizonepeta tenuifolia* Briquet ＝*Schizonepeta tenuifolia* Briq.	*Labiatae*
Scopolia Rhizome ロートコン	*Scopolia japonica* Maximowicz ＝*Scopolia japonica* Maxim.	*Solanaceae*
	Scopolia carniolica Jacquin ＝*Scopolia carniolica* Jacq.	
	Scopolia parviflora Nakai ＝*Scopolia parviflora* (Dunn) Nakai	
Scutellaria Root オウゴン	*Scutellaria baicalensis* Georgi	*Labiatae*

Senega セネガ	*Polygala senega* Linné ＝*Polygala senega* L.	*Polygalaceae*
	Polygala senega Linné var. *latifolia* Torrey et Gray ＝*Polygala senega* L. var. *latifolia* Torr. & A. Gray	
Senna Leaf センナ	*Cassia angustifolia* Vahl	*Leguminosae*
	Cassia acutifolia Delile	
Sesame ゴマ Sesame Oil ゴマ油	*Sesamum indicum* Linné ＝*Sesamum indicum* L.	*Pedaliaceae*
Sinomenium Stem ボウイ	*Sinomenium acutum* Rehder et Wilson ＝*Sinomenium acutum* (Thunb.) Rehder & E. H. Wilson	*Menispermaceae*
Smilax Rhizome サンキライ	*Smilax glabra* Roxburgh ＝*Smilax glabra* Roxb.	*Liliaceae*
Sophora Root クジン	*Sophora flavescens* Aiton	*Leguminosae*
Soybean Oil ダイズ油	*Glycine max* Merrill ＝*Glycine max* (L.) Merr.	*Leguminosae*
Sweet Hydrangea Leaf アマチャ	*Hydrangea macrophylla* Seringe var. *thunbergii* Makino ＝*Hydrangea macrophylla* (Thunb.) Ser. var. *thunbergii* (Siebold) Makino	*Saxifragaceae*
Swertia Herb センブリ	*Swertia japonica* Makino ＝*Swertia japonica* (Shult.) Makino	*Gentianaceae*
Toad Venom センソ	*Bufo bufo gargarizans* Cantor	*Bufonidae*
	Bufo melanostictus Schneider	
Tragacanth トラガント	*Astragalus gummifer* Labillardiére ＝*Astragalus gummifer* Labill.	*Leguminosae*
Tribulus Fruit シツリシ	*Tribulus terrestris* Linné ＝*Tribulus terrestris* L.	*Zygophyllaceae*
Trichosanthes Root カロコン	*Trichosanthes kirilowii* Maximowicz ＝*Trichosanthes kirilowii* Maxim.	*Cucurbitaceae*
	Trichosanthes kirilowii Maximowicz var. *japonicum* Kitamura ＝*Trichosanthes kirilowii* Maxim. var. *japonicum* (Miq.) Kitam.	
	Trichosanthes bracteata Voigt ＝*Trichosanthes bracteata* (Lam.) Voigt	
Turmeric ウコン	*Curcuma longa* Linné ＝*Curcuma longa* L.	*Zingiberaceae*
Turpentine Oil テレビン油	Several plants of *Pinus genus*	*Pinaceae*
Uncaria Hook チョウトウコウ	*Uncaria rhynchophylla* Miquel ＝*Uncaria rhynchophylla* (Miq.) Miq.	*Rubiaceae*
	Uncaria sinensis Haviland ＝*Uncaria sinensis* (Oliv.) Havil.	
	Uncaria macrophylla Wallich ＝*Uncaria macrophylla* Wall.	

Wood Creosote 木クレオソート	Several plants of *Pinus* genus	*Pinaceae*
	Several plants of *Cryptomeria* genus	*Taxodiaceae*
	Several plants of *Fagus* genus	*Fagaceae*
	Several plants of *Afzelia (Intsia)* genus	*Leguminosae*
	Several plants of *Shorea* genus	*Dipterocarpaceae*
	Several plants of *Tectona* genus	*Verbenaceae*
Zedoary ガジュツ	*Curcuma zedoaria* Roscoe	*Zingiberaceae*

When ''Other species of the same genus'' is included as its original plants the scientific name is not written in Monograph, however, it is written in this table.

Reference

Terabayashi S. *et al.*: Pharmaceutical and Medical Device Regulatory Science, 41(5), 407 – 418 (2010).

Thin-layer Chromatography for Crude Drugs and Crude Drug Preparations

Thin-layer chromatography is a method to separate each component by developing with a mobile phase, using a thin-layer consisting of an appropriate stationary phase, and is used for identification, purity test, etc. of substances.

Thin-layer chromatography for crude drugs and preparations containing crude drugs as main ingredient (crude drug preparations) is used for identifying whether characteristic constituents or groups of constituents in crude drugs and extracts based on Kampo formulae are included or not.

1. Instruments and equipment:

Generally, the following instruments and equipment are used.

(i) Thin-layer plate: A smooth and uniformly thick glass plate is coated in advance with an uniform powder of carrier listed in Solid Supports/Column Packings for Chromatography ⟨*9.42*⟩. It is classified into two types. The stationary phase of thin-layer chromatography plates (TLC plates) has a particle size of 10 – 15 μm, and that of high-performance thin-layer chromatography plates (HPTLC plates) has a particle size of 5 – 7 μm. In a case where separation requirements given that the quality of the chromatogram indicated in the individual monograph is ensured, it is possible to use a thin-layer plate with a preadsorbent zone which has been coated in advance and the home-made plate. Alternatively, such thin-layer plates are also available that use plate-like or sheeted hard aluminum and polyester for support medium instead of glass plate. Thin-layer plates are kept while avoiding humidity.

(ii) Application of samples: The sample solution(s) or standard solution(s) at the prescribed volume in the individual monograph, are applied with sample applicators of constant volume, which is generally a special capillary or microsyringe, at a position around 20 mm distance from the lower edge as a starting line of and release at least 10 mm from side to side edges of the thin-layer plate, in the form of circular spots (spot-like) of 2 – 6 mm in diameter or narrow linearly bands (band-shaped) of 4 – 10 mm in width with an appropriate interval of at least about 10 mm and then allowed to dry in air. In a case where separation requirements given that the quality of the chromatogram indicated in the individual monograph is ensured, it is possible to modify position of starting line and sample spots application interval.

(iii) Chromatographic chamber: Generally, a chromatographic chamber made of inert, transparent material and having a lid is used: a flat-bottom or twin trough. Unless otherwise specified, attach a filter paper along with the inside wall of the chamber, and moisten the filter paper with the developing solvent. In the chamber, the developing solvent is placed up to about 10 mm in height from the bottom, seal the chamber closely, and allow it to stand for 1 hour at ordinary temperature. Place a thin-layer plate in the chamber so as only the upper end of the plate is touched to the wall of the chamber, seal the chamber closely, and perform the development at ordinary temperature. A chromatographic chamber shall be of a size appropriate for the thin-layer plate and the developing solvent to be poured into shall be of a volume not to immerse spot(s) or band(s) of samples applied to the thin-layer plate in advance.

(iv) Device for coloring: Glass mister sprayer or electric mister sprayer is used for spraying visualization reagent. Drying the thin-layer plate after development, visualization of components to be tested on the chromatogram is performed by evenly sprayed visualization reagent directly on the thin-layer plate to work the test reagent. The ways to discharge visualization reagent include air supply of compressed gas either by manually or electrically. Further, in case of a heating device, components to be tested which have been separated on the chromatogram are heated after spraying visualization reagent and derivatized for visualization. It is preferable to use a hot plate at a constant temperature to heat a thin-layer plate after spraying visualization reagent. In case of using a thermostatic oven, thin-layer plate is heated on the metal plate heated to a constant temperature in advance. A flat-bottom trough chamber, twin trough chamber and desiccator, could be used during the immersion visualization and the fumigation (exposure to reagent vapor) visualization.

(v) Detection device: It is a camera obscura equipped with visible light, ultraviolet light of wavelength 254 nm and 365 nm, and wide-range wavelength light, and corresponding filter, a dark box or room. The light source is required to meet the requirements for the tests prescribed in the individual monograph. Photographing device to be added to the detecting device is used for taking photographs to be recorded and requires adequate sensitivity, resolution and reproducibility enough to perform the tests.

(vi) Record of TLC images: TLC images are taken by a camera and recorded/stored in a format of film image or electronic image. Except for detection after exposure to ul-

traviolet radiation, it is preferable to take pictures of color samples for reference concurrently in case of recording color tones of chromatogram detected under a visible light. Further, it should be noted that color tones identified visually and those recorded are different in some cases when recording fluorescent spots caused by irradiation with a wavelength of 365 nm. It is also possible to use an image scanner with sufficient resolution to record chromatograms detected under visible light. A TLC scanning device is capable of detecting absorption of light or fluorescence of chromatogram and spots or bands of samples to be tested and recording absorption and fluorescence spectra corresponding to components to be tested.

(vii) TLC scanning device: The device measures absorption by ultraviolet or visible light or fluorescence by excitation light on a developed thin-layer plate and stores records of development patterns by converting them into chromatograms (peak information). Scanning data obtained from chromatograms is used for quantitative analyses.

2. Detection and visualization

Generally, pulling out a thin-layer plate and drying it after development, detection of spots separated on a chromatogram is visually confirmed under visible light directly or after visualized. It is detected as a spot in a form close to circle when applied in a circular form (spot-like) and as a linear band when applied in a narrow linear form (band-shaped). In case of components to be tested having ultraviolet absorptivity, detection is performed using a thin-layer plate containing fluorescent agent (fluorescent indicator) by ultraviolet irradiation with a dominant wavelength of 254 nm. While fluorescent indicator contained in the thin-layer plate emits greenish fluorescence excited by irradiation with a dominant wavelength of 254 nm, spots or bands of components to be tested reduce radiation light emission by absorbing irradiation light to reduce excitation of fluorescent indicator resulting in an observation as raspberry (dark purple) spots or bands. With a property to produce fluorescence on itself excited under ultraviolet irradiation, spots or bands of components to be tested produce fluorescence excited on the thin-layer plate by irradiation of ultraviolet with a dominant wavelength of 365 nm even without using fluorescent indicator. High illumination light source with stable radiation intensity at around 365 nm within the ultraviolet wavelength range includes lamps having a line spectrum with a narrow width at 365 nm and having a line spectrum at 366 nm (within a range from 364 to 367 nm) with more intense radiated signal. Even though light source and wavelength described in specification differs depending on the lamps to be used, light source lamp with a wavelength of 366 nm also includes a light source lamp with a wavelength of 365 nm making it possible to handle as a description of irradiation with a dominant ultraviolet wavelength of 365 nm.

Derivatization reaction based on spraying, immersion and fumigation of an appropriate coloring reagent visualizes spots or bands of components to be tested. In case of some visualization reagents, such derivatization reaction is further visualized by subsequent heating after spraying reagents. In some cases, characteristic fluorescence may be produced by irradiation with a dominant wavelength of 365 nm after spraying or after spraying and heating as well.

3. Operation methods

Generally, unless otherwise specified, operation method shall comply with the following methods. Prepared sample solution and standard solution which is prescribed in the individual monograph shall be applied on the starting line of a thin-layer plate by an indicated volume. Confirming that applied circular form or linear spots or bands are not immersed in the developing solvent and placing a thin-layer plate in a developing container, developments are initiated after closing the lid of the chromatographic chamber. Sending up the developing solvent to a required development distance, the thin-layer plate is taken out to be allowed to dry in air. In addition, starting line (starting point) and mobile phase front are marked before and after development. Then, based on visualization of chromatogram on the thin-layer plate, color tone or *R*f value of circular spots or linear bands of components to be tested is determined (Fig. 1). *R*f value is obtained by the following formula.

$$R\mathrm{f} = \frac{\text{Distance from starting line to center of spot or band}}{\text{Distance from starting line to mobile phase front}} = \frac{b}{a}$$

Development operation and visualization shall be performed in an apparatus such as a draft chamber in which solvent vapor is efficiently removed with sufficient air ventilation.

4. Confirmation and purity test

When using this test method for an identification test, it is confirmed in general that color tone and *R*f value of components to be tested in sample solution is equal to those in standard solution. In case of the identification test of multicomponent system sample solution, it is possible to confirm by color tone and *R*f value of spots when components to be tested are recognized as a single spot clearly showing characteristic fluorescence and coloring. Alternatively, it is also possible to identify by the patterns of spots and bands. Moreover, this test method in combination with spectroscopic method (such as Ultraviolet-visible Spectrophotometry <*2.24*>, Nuclear Magnetic Resonance Spectrophotometry <*2.21*>) and Mass Spectrometry <*2.62*> makes it possible to perform further reliable confirmation.

In case of using this test method for purity test, a standard solution with a concentration corresponding to the limit of impurities in the sample solution is used in general, and purity is confirmed by whether any spot of components to be tested derived from sample solution is detected or whether the magnitude of the spot is lighter than that of standard solution.

5. Semi-quantitative and quantitative measurement

Presumptive quantitative measurement can be made by observation of spots or bands of identical *R*f value and about equal magnitude obtained, respectively, with sample solution and standard solution or indicative component solu-

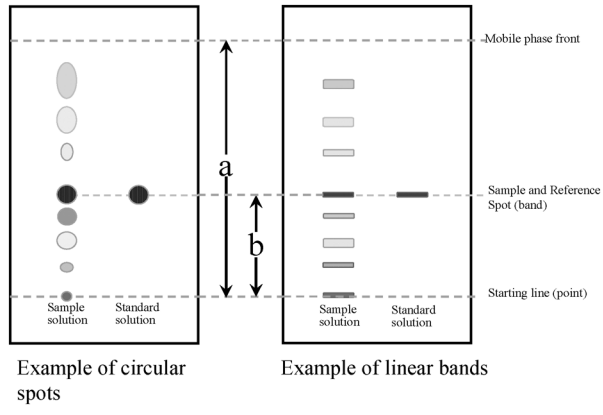


Fig. 1 Pattern diagram of TLC chromatogram

tion by applying the same volume, chromatographed on the same thin-layer plate. A visual comparison of the size or intensity of the spots or bands may serve for semi-quantitative estimation. Quantitative measurements are possible by means of densitometry.

6. Confirmation of suitability of lamp

Objectives of confirmation of suitability are sensitivity, resolution and reproducibility required for securing quality of chromatogram and satisfying separation requirements specified in the individual monograph. Confirmation of suitability in this test method is performed mainly for radiation intensity of line light source used for ultraviolet irradiation. In other words, the test is performed if specified spot (or band) is not recognized by irradiation with a wavelength of line light source specified in the individual monograph or specification of irradiation system was changed. Generally, in case of irradiation with a dominant wavelength of 254 nm to a thin-layer plate containing fluorescent agent, it is confirmed whether the thin-layer plate produce a green fluorescence. Alternatively, in case of irradiation with a dominant wavelength of 365 nm (366 nm), whether bluish white fluorescence is produced is confirmed by 2 μL spotting the 0.5 μg/mL scopoletin for thin-layer chromatography solution on a thin-layer plate.

In case of automated sample application equipment and TLC scanning device using densitometry, specifications of system suitability in Liquid Chromatography <*2.01*> is applied as required.

7. Point to consider regarding test conditions

Among tests prescribed in individual monograph, in identification tests using reagents of the Reference Standard or components to be tested (such as reagents for thin-layer chromatography), it is possible to modify developing temperature, developing distance, composition of developing solvent, developing rate, coloring reagent composition, heating temperature and duration of thin-layer plate within a range of better accuracy and precision than those prescribed is secured. However, such semi-quantitative identification tests are excluded that judgment criteria is based on size and strength of spots. On the other hand, in identification tests which do not use reagents of the Reference Standard or components to be tested, it is possible to modify developing distance, heating temperature and duration of thin-layer plate within a range where separation, *R*f value and color tone prescribed in individual monograph is observed. Further, even in case of identification tests without specification for the Reference Standard or components to be tested, it is possible to make a confirmation based on conformity of color tone for reference and *R*f value using the Reference Standard or components to be tested.

8. Reference

1) The Japanese Pharmacopoeia Sixteenth Edition/General Test Procedures/2. Physical Test Procedures/2.01 Liquid Chromatography/2.03 Thin-layer Chromatography
2) EP 8.0. 2.2.27/THIN-LAYER CHROMATOGRAPHY
3) USP 37 <621> CHROMATOGRAPHY, <201> THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST
4) CP 2010 A-42 Appendix VI VI B Thin-Layer Chromatography (English version)
5) Yukihiro Goda: Dispersion Observed in Fluorescence Spots on Thin-layer Chromatography during UV Lamp (long wave length) Use. The Japanese Journal of Pharmacognosy, 66, 63 (2012).

G6 Drug Formulation

Standard Procedure for Mechanical Calibration of Dissolution Apparatus

This chapter is intended to minimize sources of mechanical variability of apparatus which affect test results and assure the reproducibility of results, for Apparatus 1 (Basket method) and Apparatus 2 (Paddle method), and describes the standard procedure for the mechanical calibration of dissolution apparatus and the recommended specifications.

The validity of the mechanical calibration for qualification of dissolution test is confirmed on the meeting of international harmonization of pharmacopeia about dissolution test, and subsequently. the standard practice for qualification of dissolution apparatus[1)] and the guidance of mechanical calibration[2)] were issued in U.S.A.

Requirements for basic quality of materials and sizes, etc., to be required on dissolution test apparatus, and the suitability of the apparatus conform to the statements of Dissolution Test <*6.10*>. The tolerance of some parameters for the mechanical calibration described in this chapter may be specified strictly compared with that specified in Dissolution Test <*6.10*>. The below table shows the comparison of specified values of each calibration parameter. In order to minimize variability of test results by the mechanical calibration, it is recommended to apply this chapter.

Although qualification of dissolution test results by using Prednisone Tablet RS has been recommended in our country, since it cannot necessarily detect sources of mechanical variability of apparatus, the performance of the mechanical calibration is basically desirable. However the test appropriately performed by using Prednisone Tablet RS is valid for understanding overall factor including degassing state of a test solution and vibration of apparatus undetected by only the calibration of apparatus. Moreover a monitoring by a dissolved oxygen analyzer is valid for confirmation of degassing state of a test solution.

1. Setup of dissolution apparatus and periodic control

Mechanical calibration should be performed on purchase or receipt of dissolution apparatus, after move and after repair which can affect test results, and it is usually desirable to perform the calibration every year. If the instrument is not being used routinely, the mechanical calibration can be performed before performing the first dissolution test after the one year time interval.

2. Procedure of mechanical calibration

2.1. Instruments

Instruments for the mechanical calibration are runout gage, level, centering device and tachometer, etc., as generic ones and it is desirable to use the tools to be traceable to JIS (Japanese Industrial Standards), etc., wherever possible. In addition, special instruments for the mechanical calibration of dissolution apparatus are centering tool, depth gage, plastic ball, etc. Moreover, some dissolution apparatus require the special tools supplied by instrument manufacturers or incorporate automatic mechanical calibration devices within their equipment. These tools and devices may be used provided they follow the general principle of the below procedure.

Table Specified values of calibration parameters of Dissolution Test <6.10> and this chapter

Calibration Parameter	Dissolution Test <6.10>	This chapter
Shaft wobble	rotates smoothly without significant wobble that could affect the results	≦ 1.0 mm total runout
Shaft verticality	—	≦ 0.5° from vertical Bubble should be centered within the lines of the level.
Basket wobble	—	≦ 1.0 mm total runout
Vessel/Shaft centering	≦ 2.0 mm from vertical	≦ 1.0 mm from centerline at upper position and lower position
Vessel verticality	—	≦ 1.0° from vertical
Basket and Paddle depth	25 ± 2 mm	25 ± 2 mm
Rotational speed	± 4% from the specified rate of rotation	± 2% or ± 2 rpm from the specified rate of rotation

2.2. Procedure

Perform the mechanical calibration of dissolution apparatus according to the below specified procedure. If each measured value does not meet the specification, repetitive adjustments and measurements may be necessary.

Confirm that the apparatus is horizontal on the installation table in advance. Also confirm the horizontality of the plate which fix the vessel (vessel plate) by placing a bubble level on the stage and confirming the bubble to be within the lines of the level.

2.2.1. Shaft Wobble

A runout gage is placed on top of the vessel plate, and positioned so that the gage probe touches the shaft about 2 cm above the top of the paddle blade or basket. The absolute value of the difference between the maximum and minimum readings is the wobble. The value of total wobble must not exceed 1.0 mm.

2.2.2. Shaft Verticality

Lower the drive unit to where it would be during an actual dissolution test. If necessary, the shaft verticality may be checked by raising the drive unit. Place an accurate bubble level on the front edge of each of the shafts. The bubble should be within the lines of the level. Rotate the level about 90° so that it touches the side of the shaft, and put it on the side of the shaft. The bubble should again be within the lines of the level.

A digital leveling device may also be used to determine the shaft verticality.

The shaft must be not more than 0.5° from vertical.

2.2.3. Basket Wobble

A runout gage is placed on top of the vessel plate and the drive unit is positioned so that the gage probe touches the bottom rim of the basket. The gage is placed so that the probe slightly presses in on the turning shaft. The value of total wobble must not exceed 1.0 mm.

2.2.4. Vessel Centering

Centering inside the vessel is measured by centering tools for dissolution test apparatus or by an alternative procedure.

When measured by centering tools, two centering tools are used to center the paddle or basket shafts in the vessels and to align the vessels so that their sides are vertical.

As an example of the procedure for the paddle method, the bottom of one centering tool is placed 2 mm above the top of the paddle blade and the bottom of the second centering tool is clamped on the shaft 80 mm above the blade with the both probes positioned in the same direction towards the vessel wall. For the basket method, the bottom of one centering tool is placed 2 mm above the top of the basket and the bottom of the second centering tool is placed 60 mm above the top of the basket with the both probes positioned in the same direction towards the vessel wall. Carefully lower the shaft and centering tools into the vessels so that the paddle blade and the bottom of the basket is about 2.5 cm above the bottom of the vessel. Manually rotate the shaft slowly and check the centering at both levels. If the vessel is not centered at either level, adjust the vessel to center it.

An alternative procedure is to use a mechanical or digital centering device that centers the inside wall of the vessel around the shaft or a surrogate shaft. The centering is measured at two positions inside the vessel in the cylindrical portion, one just below the rim of the vessel and one just above the basket or the paddle in the bottom portion of the vessel.

The shaft must be centered within 1.0 mm from the center line.

2.2.5. Vessel Verticality

The vessel verticality can be calculated as the angle of the vertex of the triangle composed of the two points and the vertical line using the two centering measurements in 2.2.4 Vessel Centering and the difference in height between the two measurements. Or it can be determined using a digital leveling device placed on the inside wall of the vessel. The verticality should be determined at two positions 90° apart.

The vessel must be not more than 1.0° from vertical.

After each vessel has been centered and made vertical, each vessel must be positioned in the exact same position and same direction inside the vessel plate opening.

2.2.6. Basket and Paddle Depth

The actual distance between the bottom of the vessel and the bottom of the basket or paddle is determined. If the depth of the basket/paddle is adjustable, first a depth gage is used to determine the distance between the bottom of the paddle blade or basket and the inside bottom of the vessel. The depth gage is set at 25 mm and placed on the bottom of the vessel. After each shaft is raised into the apparatus drive module, the drive unit is then lowered to its operating position. The paddle or basket is then lowered into the vessel until it touches the top of the depth gage. Instead of a depth gage, sink a plastic ball with a diameter 25 mm ± 2 mm on the bottom of the vessel and the paddle or basket can be lowered into the vessel until it touches the ball. The shafts are locked into this height. Usually the depth of basket and paddle is 25 mm ± 2 mm.

2.2.7. Rotational Speed

A tachometer is used to measure the rotational speed of the paddle or basket. The shafts should be rotating smoothly with a rate within a larger value of ±2% or ±2 rpm of the specified rate.

3. References

1) ASTM E2503 – 13; Standard Practice for Qualification of Basket and Paddle Dissolution Apparatus (2013).
2) FDA Guidance for Industry: The Use of Mechanical Calibration of Dissolution Apparatus 1 and 2—Current Good Manufacturing Practice (CGMP), U.S. Depart-

ment of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), January 2010.

Tablet Friability Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

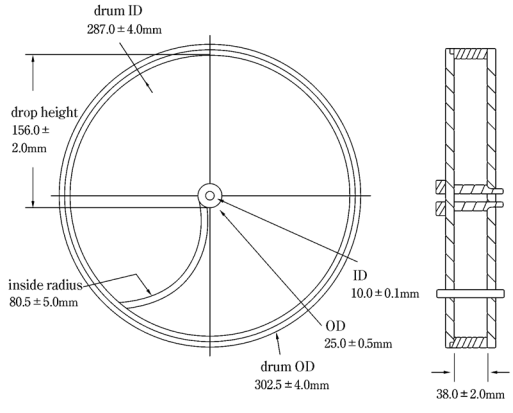
The Tablet Friability Test is a method to determine the friability of compressed uncoated tablets. The test procedure presented in this chapter is generally applicable to most compressed tablets. Measurement of tablets friability supplements other physical strength measurement, such as tablet crushing strength.

Use a drum, with an internal diameter between 283 and 291 mm and a depth between 36 and 40 mm, of transparent synthetic polymer with polished internal surfaces, and subject to minimum static build-up (see figure for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5 and 85.5 mm that extends from the middle of the drum to the outer wall. The outer diameter of the central ring is between 24.5 and 25.5 mm. The drum is attached to the horizontal axis of a device that rotates at 25 ± 1 rpm. Thus, at each turn the tablets roll or slide and fall onto the drum wall or onto each other.

For tablets with a unit mass equal to or less than 650 mg, take a sample of whole tablets *n* corresponding as near as possible to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, and remove the tablets. Remove any loose dust from the tablets as before, and accurately weigh.

Generally, the test is run once. If obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample fails the test. If the results are difficult to interpret or if the mass loss is greater than the targeted value, the test should be repeated twice and the mean of the three tests determined. A maximum mean mass loss from the three samples of not more than 1.0% is considered acceptable for most products.

If tablet size or shape causes irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with the horizontal and the tablets no longer bind together when lying next to each other, which prevents them from falling freely.



Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets, an appropriate humidity-controlled environment is required for testing.

Drums with dual scooping projections, or apparatus with more than one drum, for the running of multiple samples at one time, are also permitted.

G7 Containers and Package

Basic Requirements and Terms for the Packaging of Pharmaceutical Products

This chapter describes the basic requirements for the packaging of pharmaceutical products as well as the terms and their definitions used for the packaging as taking into account the aspects in quality assurance of the pharmaceutical products and the point of view of international harmonization.[1)]

In this chapter, the concept of packaging for pharmaceutical products, or packaging, includes putting or holding the drugs in container. In addition, the information presented as basic requirements shall be a central focus on packaging for drug produsts, as well as for ensuring quality on the transportation and storage of drug substances or additives.

1. Basic requirements of packaging for pharmaceutical products

For the packaging for pharmaceutical products, it is important to settle the requirements of the packaging based on the evaluation of the packaging suitability in the development stage so as to be able to ensure the quality standards of preparations defined over the shelf life of preparations. The suitability of packaging for pharmaceutical products must be maintained through the product life cycle on the basis of the requirements of packaging settled in the development stage.

For the packaging of drug produsts, it is also necessary to consider for the suitability for proper use and for ensuring safe application in addition to quality assurance. The stringency of the evaluation for suitability of packaging for preparations differs depending on the degree of the risk according to the route of administration, such as intravenous administration, oral administration, or dermal administration and the risk due to interaction between the products and the primary packaging according to injections, liquids and solutions, semi-solid or solid dosage forms.

1.1. Suitability evaluation and requirements of packaging in the design stage

The packaging suitability to be evaluated in the design stage includes protection, compatibility, safety, and performance.

The basic items to be evaluated as suitability are described in the course of the packaging design.

1.1.1. Safety of materials used for packaging

Leachables or migrants, such as the monomers of the polymer resins, additives or metal impurities, from the materials used for the primary packaging such as plastic or glass containers should not deteriorate drug safety. The amount of leachable or migratable chemical substances from the primary packaging materials to the contents must be sufficiently small from a safety perspective.

The primary packaging materials of the containers that are

in direct contact with the drugs should be used which quality such as on toxicity has been appropriately evaluated by the suppliers according to ''Basic Requirements for Plastic Containers for Pharmaceutical Use and Rubber Closures for Containers for Aqueous Infusions'' or the like. In the design stage, the information on quality evaluation of the packing materials are desirable to be obtained as far as possible from the suppliers.

1.1.2. Compatibility with the contents

The primary packaging must not reduce the quality of pharmaceutical products over the shelf life of preparations. The contents adsorbing onto the surface of primary packaging, or migrating inside of the materials, must not lead to a drug concentration change of more than a certain level. Moreover, the interaction between the contents and the materials must not lead to degradation of drugs.

The primary packaging should not be deformed, deteriorated, or degraded by the contents.

In the design stage, the compatibility of the primary packaging with the content is examined by the combination of individual candidate material and the content drugs, together with other evaluation items. And chose the applicable material based on the results of the study on the prototype primary packaging for complying with the essential requirements, i.e. the design specifications, for the issues about the protection from moisture and light, the sorption to and leaching from the primary packaging, etc., based on the data from the experiments and/or information from the scientific documentation. When selecting the primary packaging material, the suitability of the material for the secondary packaging is also to be evaluated as needed.

1.1.3. Protection by packaging

The packaging should be able to prevent loss, efflorescence, deliquescence, or evaporation of the contents and to protect the contents by the addition of moisture resistance, light shielding, or a gas barrier, depending on the characteristic of the contents. In the case of not being able to ensure the quality of the contents by the primary packaging alone, it should be ensured by the combination of multiple packaging materials, including the secondary packaging. Furthermore, the containers for injections or ophthalmic solutions are preferable to be made of a high transparency material, so that foreign matter contamination can be observed visually.

For pharmaceutical products susceptible to moisture such as by hydrolysis, the packaging used with desiccants or primary packaging materials with gas-barrier function can be moisture-proof packaging. For preparations susceptible to evaporation of water, gas-barrier materials for the primary packaging can be used. For the pharmaceutical products that are easily oxidized, the packaging with deoxidants or the low-gas-permeability materials can be used for the primary packaging to protect the pharmaceutical products from oxygen in the air.

Protection by the packaging should be evaluated in the packaging design stage, and finally confirmed by stability tests. Resistance to physical shock during transportation is also necessary to be verified.

1.1.4. Container integrity (microbial contamination prevention)

The packaging should be able to protect the contents from microbial contamination, depending on the characteristics of the content drug or dosage form, and especially for the containers used for sterile preparations, the integrity of primary packaging, through the tests such as the fitting compatibility tests for containers and closures, must be confirmed.

In the case of pharmaceutical products that must be sterilized, the primary packaging must meet the above-mentioned suitability for safety, compatibility and protection even after the sterilization. There should not be any residue or generation of toxic substances of more than a certain safety level after the sterilization. In addition, the primary packaging should have a structure and/or material that must prevent any microbial contamination of the pharmaceutical products contained therein during storage and transportation after sterilization.

1.1.5. Packaging performance

The packaging design with consideration for discrimination, usability, and disposal should be performed.

With regard to discrimination, for example, a display should be considered for patients and even for aged patients so that the proper administration and use of the drugs can be ensured. An easy-to-understand display or container for preventing accidental misuse or a prank, such as tamper-resistant packaging and child-resistant packaging, is preferable.

With regard to usability, items such as easy handling of the drug in dispensing, easy dosaging for children, easy removal from the container when the drug is administered or used, successful administration, and preferable storage and portability should be considered for each preparation.

On packaging-related waste, the choice or determination of the containers must be considered for disposal, since paying attention to the effect of use of resources, following the Containers and Packaging Recycling Act and the rules of each local government, and striving to reduce wastes are required. In the primary packaging, the recycled packaging materials that are not assured for material composition must not be used.

1.1.6. Requirements of packaging

Based on the test methods and/or the evaluation techniques used for study of the packaging suitability in the design stage of pharmaceutical preparations, the necessary and sufficient items of the quality control for maintaining the packaging suitability are established. Generally, the requirements of packaging are composed of the control of the material quality, specifications and test methods, in-process tests, and the like.

1.2. Examples of suitability evaluation in the design stage of packaging for pharmaceutical products

The following are the examples of the suitability evaluation in the design stage.

1.2.1. Suitability evaluation of packaging to be used for solid oral dosage forms

For the suitability evaluation of the packaging for solid oral dosage forms, the following tests should be included.

- If bottles are used, the measurement of opening torques with selected stoppers should be performed.
- If PTP packaging or strip packaging is used, the moisture permeability test should be performed.

1.2.2. Suitability evaluation of containers to be used for injections

For the suitability evaluation of containers to be used for injections, the following tests should be included.

- The injections using ampules should perform pinhole tests, and the integrity must be confirmed.
- The injections using vials, rubber closures, or prefilled glass syringes, except ampules, should perform the fitting compatibility tests, and the integrity as a container must be confirmed.
- The plastic containers for pharmaceutical products used for injections (prefilled syringes, plastic bottles, plastic bags, etc.) should be verified as ''tight containers in which microorganisms will not be contaminated'' over the shelf life of preparations.

1.2.3. Suitability evaluation on metal impurities for a container closure system

If leaching of metal impurities from the primary packaging materials used for injections, liquids, or semi-solid preparations is suspected, it is necessary to confirm that the amount of metal impurities contained in preparations is sufficiently low from the viewpoint of safety using Atomic Absorption Spectrophotometry <*2.23*>, Inductively Coupled Plasma Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry <*2.63*>, etc.

1.2.4. Suitability evaluation of kit products

If dispensing devices, such as prefilled syringes, injection cartridges, or metered-dose preparation for inhalation, are used, the accurate dose with reproducibility in conditions as close as possible to the product usage must be verified.

1.2.5. Suitability evaluation of light resistant packaging

If active substances are susceptible to the light and the formulation design alone cannot overcome the effect of light, the light resistant packaging including containers should be considered. A selection of an appropriate light resistant packaging must be verified using a photostability test and the like as severe tests.

1.3. Selection, change control, stability monitoring, etc. of the packaging materials in the packaging process development

To maintain appropriately the quality assurance of pharmaceutical products by packaging, the properness of the requirements of the packaging must be confirmed through the appropriate change control, stability monitoring and the like in the packaging process development and production stages, together with the suitability evaluation performed in the design stage.

For the primary packaging materials used in the manufacture of preparations, whether the quality of material is changed by the supplier must be appropriately managed. Therefore, all information, concerning the manufacturing process of containers including the information about substances added is desirable to be obtained.

The finally selected packaging for pharmaceutical products should be evaluated whether it meets the requirements as designed. If it does not meet the requirements, the packaging form or material must be changed through change control.

1.3.1. Selection or change of packaging materials in the packaging process development

The packaging materials should be selected in consideration of the manufacturability and the prevention capability of adhesion of foreign matters or insects in addition to meeting the requirements determined in the design stage. For the finally selected packaging, it should be verified to withstand against temperature change during storage and transportation, physical shock during transportation, and the like.

The suitability of the selected packaging materials shall be confirmed, using the applicable tests in the General Tests, 7. Test for Containers and Packing Materials. For the tests that are not described in the General Tests, set the applicable test and judge the suitability.

1.3.2. Stability monitoring, etc.

It must be confirmed that the packaging form does not adversely affect the stability of preparations through stability monitoring or reference sample stored. If the packaging is considered to affect the quality of stability of the preparations, the applicable packaging and management should be selected to ensuring quality, where the quality of the lots using this should be monitored, and the packaging should be improved through change control, if necessary.

1.4. Examples of quality control in the packaging process of pharmaceutical products

To maintain the suitability of the packaging of pharmaceutical products, it must be confirmed by performing the test such as process control test before shipment that the packaging meets the requirements.

The following shows the examples.

1.4.1. Examples of solid oral dosage forms

The PTP packaged tablets must be confirmed whether the integrity of the seal as designed is secured in the airtight tests for PTP sheets (e.g. water pressure reduction tests).

1.4.2. Examples of injections

- Aqueous injections using ampules should be checked if it has no pinholes.
- The plastic containers for pharmaceutical products used for injections (prefilled syringes, plastic bottles, plastic bags, etc.) should be checked when they are shipped to the market if they have been produced as tight containers, as designed, where microorganisms are not contaminated.

2. Terms of packaging for pharmaceutical products

2.1. Basic terms

Primary packaging: Any packaging that is in direct contact with active substances, excipients, or preparations, and should not give a physical or chemical change to the contents. The primary packaging holds the quality of the pharmaceutical products and provides better performance including convenience.

For example, the primary packaging includes an ampule that is an ''immediate container'' for injections, and a PTP packaging which is an ''inner bag'' for tablets or capsules.

Outside container or outside wrapper: A container or wrapper that is used to contain or to wrap immediate containers or immediate wrappers for the pharmaceutical products for sale or distribution and has a legal label by ordinance[2] on it.

Tight container: A container that protects the contents from extraneous solids or liquids, from loss of the contents, and from efflorescence, deliquescence, or evaporation under ordinary or customary conditions of handling, shipment, and storage. Where a tight container is specified, it may be replaced by a hermetic container. (General Notices 43)

The tight container includes containers made of plastic resins as the examples of container and packaging often used (bottles, vials, syringes, blister (PTP) packaging, strip packaging, etc.).

Final packaging or marketed packaging: Any packaging that is for pharmaceutical products for sale or distribution and a form of the shipped products to the market by labeling as defined by ordinance[2,3].

The final packaging may be used for irradiation when a radiation sterilization method is used.

Labeling and packaging materials[4]: Product's containers, wrappers, and labeling including package insert.

Immediate wrapper: A container in which pharmaceutical products are contained directly (papers, clothes, plastics, and aluminum bags). It can be sold or distributed as it is by labeling as defined by ordinance[3]. There is an inner bag as an example of immediate wrapper that it is not sold or distributed as it is and does not require a legal label by ordinance[3].

The immediate wrapper in which the pharmaceutical products are contained directly is also referred to as the primary packaging.

Immediate container: A solid container in which the drugs are directly contained (cans, bottles, ampules, vials, tubes, containers for eye drops, boxes, etc.). It can be sold or distributed as it is by labeling as defined by ordinance[3]. In ad-

dition, the paper boxes will be the immediate container, if they use the PTP packaging as an inner bag like tablets.

The immediate container in which the drugs are contained directly is also referred to as a primary packaging.

Inner bag: For example, a plastic bag used under the wrapper for moisture-proof and light resistance and a drug bag that contains each single dose of powder. It is referred as a plastic bag, strip packaging, blister packaging (such as a PTP packaging), and a plastic container for suppositories. In addition, when the pharmaceutical products are contained directly in the inner bag, it corresponds to an immediate packaging, however, if it is not sold or distributed as it is, a legal label by ordinance[3] is not required.

The inner bag in which the drugs are contained directly is also referred to as a primary packaging.

Secondary packaging: Any packaging that is a single or multiple packaging to compensate for a primary packaging and is not in direct contact with active substances, excipients, or preparations. Any secondary packaging can keep the quality of pharmaceutical products and add performance, such as preventing errors and convenience in the use of pharmaceutical products.

Wrapper: A container or parcel made of soft materials such as paper, cloth, plastic, and aluminum bag. As examples of the wrapper for pharmaceutical products, there are medicine envelope, plastic bag, strip packaging, and blister packaging (such as a PTP packaging).

Labeling[5]: A labeling defined by ordinance[2,3], which is a product label and package insert.

Sealing: Sealing from which drugs cannot be taken out unless opened and does not allow its original state to be easily restored after being opened, according to ordinance.[6]

Packaging[7]**:** The appropriate materials, containers or wrappers to keep the quality of pharmaceutical products under ordinary or customary conditions of handling, shipment, storage, or usage, and techniques to hold the products in them, or a packaged state.

Hermetic container: A container that is impervious to air or any other gas under ordinary or customary conditions of handling, shipment, and storage. (General Notices 44)

For the injections, ampules, container closure systems, such as vials/rubber clousers, glass prefilled syringes may be used as this container. For the other dosage forms, blister (PTP) packaging with both sides of aluminum and metal extrusion tubes may be used as this container.

Well-closed container: A container that protects the contents from extraneous solids and from loss of the drug under ordinary or customary conditions of handling, shipment, and storage. Where a well-closed container is specified, it may be replaced by a tight container. (General Notices 42)

The well-closed container includes paper or plastic bags with one opening made of a flexible material, and cans with metal or plastic resins, etc., as the examples of often used.

Container: A device that holds drugs. The stopper or cap is included as a part of the container. The containers have no physical and chemical reactivity affecting the specified description and quality of the contents (General Notices[41]).

As examples of the container for pharmaceutical products, there are cans, bottles, tubes, ampules, vials, and boxes.

Container closure system: A packaging form that consists of the materials used for a primary packaging that is in direct contact with active substances, excipients, or preparations and composed of other materials. A container closure system should be considered in combination with the contents, where the quality cannot be guaranteed with primary packaging alone, the materials used for a secondary packaging should be included.

2.2. Terms of individual packaging or containers

Ampule: A container that is made of a clear or colored glass or plastic that encapsulates drug solution such as injections, or freeze-dried contents. The opening is usually sealed or welded.

Collapsible tube[7]**:** A container that has a nozzle and cap at one end, and the other end is closed, having flexibility to extrude the contents of ointments. This includes metal tubes, plastic tubes, and laminated tubes, etc.

Syringe: A container that is composed of an external cylinder (barrel), a gasket, a pusher (plunger), and a top cap. This may include a needle. It is used for prefilled syringes.

Strip packaging[7]**:** A packaging in which tablets, capsules, powder, and granules are directly tucked between two materials and bonded to the surrounding. It is also referred to as the SP packaging and corresponds to an inner bag or primary packaging as the pharmaceutical products are directly contained.

Vial: A container, a type of bottle, that is made of a clear or colored glass, or plastic used for injections. This is sealed with a rubber closure and aluminum cap.

PTP packaging[7] **(Press through packaging):** Any packaging that is a kind of a blister packaging, using collapsible materials such as aluminum foils to extrude the opening of the plastic forming products. This corresponds to an inner bag or primary packaging as the capsules or tablets are directly contained.

Pillow type packaging[7]**:** Any packaging that is a kind of bag-shaped. For example, in which the vertical central portion is bonded and the top and bottom edges are sealed. Where the primary packaging alone is difficult to ensure quality, a secondary packaging, which consists of composite films laminated with aluminum foils for protection from moisture and lights, is often used.

Plastic bags: A soft container that uses polyethylene or polypropylene resins as single or composite materials, and which has one or more openings. This usually uses a rubber closure as a plug body. It is used as a large volume injection container such as parenteral infusions.

Blister packaging[7]**:** Any packaging where plastic or aluminum foils are heat formed and given one or more pockets and the preparations are put therein, the opening is covered with plastic films, sheets, or aluminum foil, and the periphery to the substrate is bonded or fixed. It refers to the form that is carried out by peeling the film or foil as the preparations are removed, and used for capsules, tablets, prefilled syringes agent, kit products containing a plurality of ampules.

In addition, where the tables, etc. are directly contained, it corresponds to an inner bag or primary packaging.

Single-dose packages: Preparations in single-dose packages. For example, a strip packaging which contains powders or granules for a single dose corresponds to this.

2.3. Terms of packaging performance

Gas barrier packaging[7]**:** Any packaging that gives the function of suppressing gas permeability aimed. This is a low-gas-permeability packaging.

Light resistant container and packaging: A container or packaging which prevents light permeability to protect if the light affects the quality of the contents under ordinary or customary conditions of handling, shipment, and storage. (General Notices 45)

In addition to colored containers to be used, the containers may be covered with shrink films.

Tamper-resistant packaging, tamper-proof packaging[7]**:** Any packaging that is designed to prevent a risk if a person unin-

tentionally handles, or ''plays a prank''.

Child-resistant packaging, childproof packaging[7]**:** Any packaging that is intended for prevention of accidental ingestion by children and may be used for adults properly not to let children open it accidentally.

Moisture-proof packaging[7]**:** Any packaging that uses a material with moisture-proof performance to protect the pharmaceutical products from the effects of moisture, if necessary, using desiccant to keep the inside dry.

3. Reference

1) FDA Guidance for Industry ''Container Closure Systems for Packaging Human Drugs and Biologics'', May 1999.
2) November 25, 2014 enforcement, ''The Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices'' provide in Article 51
3) ''The Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices'' provided in Article 50
4) MHLW Ministerial Ordinance No. 179, ''Ministerial Ordinance on Standards for Manufacturing Control and Quality Control for Drugs and Quasi-drugs'' of December 24, 2004 provided in Article 2 Paragraph 2
5) PFSB/CND Notification No. 0830 – 1 Office Memorandum, ''Ministerial Ordinance on Standards for Manufacturing Control and Quality Control for Drugs and Quasi-drugs'' of August 30, 2013
6) ''The Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices'' provided in Article 58
7) Japanese Industrial Standards JIS Z 0108: 2012 ''Packaging-Vocabulary''

Basic Requirements for Plastic Containers for Pharmaceutical Use and Rubber Closures for Containers for Aqueous Infusions

In this chapter, there describe basic requirements for plastic containers for pharmaceutical use and rubber closures for containers for aqueous infusions, and the methods to evaluate the toxicity of containers at design stage.

Containers for pharmaceutical use should not have the properties to deteriorate the efficacy, safety or stability of the pharmaceutical products to be packed in the container. The compatibility of plastic containers with pharmaceutical products should be judged for each combination of container and the specific pharmaceutical product to be contained therein. Such judgment should be performed through verification that the container for the pharmaceutical preparation can comply with the essential requirements for the container, i.e., the design specifications, based on the data from the experiments on the prototype products of the container and/or information from scientific documentation, etc. In addition, such compatibility must be ensured based upon an appropriate quality assurance system.

1. Basic Requirements in Designing Containers for Pharmaceutical Use

Containers must not deteriorate the quality of the pharmaceutical products during storage. The concentration of the pharmaceuticals must not be decreased by more than a certain level due to the adsorption of the pharmaceuticals on the surface of the container, the migration of the pharmaceuticals into the inside of the material of the container, or the loss of pharmaceuticals through the container. Also, the pharmaceutical products contained therein must not be degraded by an interaction with the material of the container.

The container should not be deformed, should not deteriorate and should not be degraded by the pharmaceutical products contained therein. Unacceptable loss of function of the container should not result from a possible high temperature or low temperature or their repetitions encountered during storage or transportation.

The leachable or migrants from the container should not deteriorate the efficacy or stability of the pharmaceutical products contained therein. In addition, the possible toxic hazards due to the leachable or migrants should not exceed a given level. Furthermore, the amounts of leachable or migratable chemical substances, such as monomers and additives, from the containers to the pharmaceutical products contained therein must be sufficiently small from the viewpoint of safety.

In the case of pharmaceutical products which must be sterilized, it is required to satisfy the above-mentioned essential requirements of the container after the sterilization, if there is a possibility that the quality of the container may change after the sterilization. There should not be any residue or generation of new toxic substances of more than certain risk level after the sterilization. In addition, the container should not have any inappropriate structure and/or material that might result in any bacterial contamination of the pharmaceutical products contained therein during storage and transportation after sterilization.

1.1. Plastic containers for pharmaceutical use

The plastic material used for the container should be of high quality. Therefore, recycled plastic materials, which are of unknown constitution, must not be used.

In the case of pharmaceutical products which are unstable to light, the container should provide a sufficient level of light shielding. In the case of pharmaceutical products which are easily oxidized, the container material should not allow the permeation of oxygen. In the case of aqueous pharmaceutical products and pharmaceutical products that must be kept dry, the container material should not allow the permeation of water vapor. In addition, it is necessary to pay attention to the permeability of solvents other than water through the container.

The container should have a certain level of physical properties such as hardness, flexibility, shock resistance, tensile strength, tear strength, bending strength, heat resistance and the like, in accordance with its intended usage. The container should be of a required level of transparency, when it is necessary to examine foreign insoluble matter and/or turbidity of the pharmaceutical products by visual observation.

Furthermore, in introducing a plastic container, it is desirable that proper disposal method after use is taken into consideration.

1.2. Rubber closures for containers for aqueous infusions

For the rubber closures for container, natural rubber, which has the possibility to cause an allergic response, or recycled rubber material that can not be guaranteed its material composition, should not be used.

As the closure systems, the appropriate materials should be used to prevent the permeation of oxygen, water vapor and solvents.

Further, the rubber closure should have a certain level of physical properties such as air tightness, hermetic seal, penetrability of a needle, coring-resistance and self-sealing

after penetration, in accordance with its intended usage.

2. Toxicity Evaluation of Container at Design Stage

For design verification, the toxicity of the container should be evaluated. For the toxicity evaluation, it is desirable to select appropriate test methods and acceptance criteria for the evaluation, and to explain the rationale for the selection clearly. The tests should be conducted using samples of the whole or a part of the prototype products of the container. If the container consists of plural parts of different materials, each part should be tested separately. Such materials as laminates, composites, and the like are regarded as a single material. To test containers made of such materials, it is recommended to expose the inner surface of the container, which is in contact with the pharmaceutical products contained therein, to the extraction media used in the tests as far as possible.

It is recommended to select the test items and the test methods for the evaluation of the toxicity of the containers, depending on their application site, in accordance with the standard test methods on medical devices and materials published in Japan, a notice entitled Basic Principle of Biological Safety Evaluation Required for Application for Approval to Market Medical Devices (MHLW Notification by Director, OMDE Yakusyokuki 0301 No.20 on March 1, 2012).

3. Test Results to be recorded per Production Unit for Plastic containers for pharmaceutical use and Rubber closures for containers for aqueous Infusions

3.1. Plastic containers for pharmaceutical use

At the commercial production phase, it is required to establish acceptance criteria on at least the test items listed below and to record the test results of each production unit of plastic containers for pharmaceutical products. In addition, it is desirable to explain the rationale for setting the acceptance criteria clearly. However, these requirements should not be applied to orally administered preparations except for liquid preparations.

(i) Combustion Tests: Residue on ignition, heavy metals. If necessary, the amounts of specified metals (lead, cadmium, etc.)

(ii) Extraction Tests: pH, UV spectrum, potassium permanganate-reducing substances, foaming test, residue on evaporation

(iii) Cytotoxicity Test

(iv) Any other tests necessary for the specific container for aqueous infusions.

3.2. Rubber closures for containers for aqueous infusions

At the commercial production phase of rubber closures, it is required to establish acceptance criteria on the test items that should be controlled other than those specified in the general chapter of <*7.03*> Test for Rubber Closure for Aqueous Infusions. And the test results of each production unit of rubber closures for containers for aqueous infusions should be recorded. In addition, it is desirable to explain the rationale for setting the acceptance criteria.

G8 Water

Quality Control of Water for Pharmaceutical Use

Water used for manufacturing pharmaceutical products and for cleaning their containers and equipments used in the manufacture of the products is referred to as ''pharmaceutical water.'' For assuring the quality of pharmaceutical water consistently, it is important to verify through appropriate process validation of water processing system that water with the quality suitable for its intended use is produced and supplied, and to keep the quality of produced water through routine works for controlling the water processing system.

1. Types of Pharmaceutical Water

1.1. *Water*

The specification for ''*Water*'' is prescribed in the Japanese Pharmacopoeia (JP) monograph. It is required for *Water* to meet the Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law. In the case that *Water* is produced at individual facilities using well water or industrial water as source water, it is necessary for produced water to meet the Quality Standards for Drinking Water and an additional requirement for ammonium of ''not more than 0.05 mg/L.'' Furthermore, when *Water* is to be used after storing for a period of time, it is necessary to prevent microbial proliferation.

Water is used as source water for *Purified Water* and *Water for Injection*. It is also used for manufacturing intermediates of active pharmaceutical ingredients (APIs), and for pre-washing of the equipments used in the manufacture of pharmaceutical products.

1.2. *Purified Water*

The specifications for ''*Purified Water*'' and ''*Purified Water in Containers*'' are prescribed in the JP monographs. *Purified Water* is prepared by distillation, ion-exchange, reverse osmosis (RO), ultrafiltration (UF) capable of removing microorganisms and substances with molecular masses of not less than approximately 6000, or a combination of these processes from *Water*, after applying some adequate pretreatments if necessary. For the production of *Purified Water*, appropriate control of microorganisms is required. Particularly, in the case that *Purified Water* is prepared by ion-exchange, RO or UF, it is necessary to apply the treatments adequate for preventing microbial proliferation, or to sanitize the system periodically.

When *Purified Water* is treated with chemical agents for sterilizing, preventing microbial proliferation, or maintaining the endotoxin level within an appropriate control range, a specification suitable for the intended use of treated water should be established individually, and a process control for keeping the quality of treated water in compliance with the specification thus established should be performed.

''*Purified Water in Containers*'' is prepared from *Purified Water* by introducing it in a tight container.

1.3. *Sterile Purified Water*

The specification for ''*Sterile Purified Water in Containers*'' (its alternative name is *Sterile Purified Water*) is prescribed in the JP monograph.

Sterile Purified Water in Container is prepared from *Purified Water* by 1) introducing it into a hermetic container, sealing up the container, then sterilizing the product, or 2)

making it sterile by using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Plastic containers for aqueous injections may be used in place of hermetic containers.

1.4. ***Water for Injection***

The specifications for ''*Water for Injection*'' and ''*Sterile Water for Injection in Containers*'' are prescribed in the JP monographs. *Water for Injection* is prepared by distillation or reverse osmosis and/or ultrafiltration (RO/UF), either: from the water which is obtained by appropriate pretreatments such as ion exchange, RO, etc. on *Water*; or from *Purified Water*.

In the case of water processing systems based on distillation, it is necessary to take care for avoiding contamination of produced water by the impurities accompanied with the entrain.

In the case of water processing system based on RO/UF, it is required to provide water with equivalent quality to that prepared by distillation consistently, based on substantial process validation through long-term operation and elaborate routine control of the system. It is essential to ensure consistent production of water suitable for *Water for Injection* by the entire water processing system including pretreatment facilities, in any systems based on RO/UF. For the water supplied to the system, it is also required to keep the quality suitable as source water through adequate validation and routine control on the water.

For the water processing system based on RO/UF, routine control should be performed by analyzing water specimens, monitoring some quality attributes using in-line apparatus and checking the volume of water passed through the system. In addition, it is recommended to carry out periodical appearance observation and air-leak test on the membranes being currently used. It is also recommended to establish protocols for keeping the performance of membrane modules within appropriate control ranges and for estimating the timing to exchange the modules, through diagnosis on the degree of deterioration based on the results of tensile strength test on the used membrane modules, and visual observation on those modules whether any leakages of membranes have occurred or not, and to what extent they have occurred. Furthermore, it is desirable to establish the frequency of membrane exchange considering with its actual condition of use.

In the case that *Water for Injection* is stored in the water processing system temporarily, a stringent control for microorganisms and endotoxins should be taken. An acceptable criterion of lower than 0.25 EU/mL for endotoxins is specified in the JP monograph of *Water for Injection*.

''*Sterile Water for Injection in Container*'' is prepared from *Water for Injection* by 1) introducing it into a hermetic container, sealing up the container, then sterilizing the product, or 2) making it sterile by using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Plastic containers for aqueous injections may be used in place of hermetic containers.

2. Reverse Osmosis and/or Ultrafiltration (RO/UF)

RO/UF are the methods for refining water by using membrane modules based on either reverse osmosis or ultrafiltration, or the modules combining them, and used as the alternative methods for distillation in the production of *Purified Water* or *Water for Injection*.

When *Water for Injection* is produced by RO/UF, a water processing system equipped with pretreatment facilities, facilities for producing *Water for Injection* and facilities for supplying *Water for Injection* is usually used. The pretreatment facilities are used to remove solid particles, dissolved salts and colloids in source water, and placed before the facilities for producing *Water for Injection* so as to reduce the load on the facilities for producing *Water for Injection*. They consist of apparatus properly selected from aggregation apparatus, precipitation-separation apparatus, filtration apparatus, chorine sterilization apparatus, oxidation-reduction apparatus, residual chlorine-removing apparatus, precise filtration apparatus, reverse osmosis apparatus, ultrafiltration apparatus, ion exchange apparatus, etc., depending on the quality of source water. The facilities for producing *Water for Injection* consist of apparatus for supplying pretreated water, sterilization apparatus with ultraviolet rays, heat exchange apparatus, membrane modules, apparatus for cleaning and sterilizing the facilities, etc. The facilities for supplying *Water for Injection* consist of a reservoir tank for storing *Water for Injection* in the facilities temporarily, pipe lines, heat exchange apparatus, a pump for circulating *Water for Injection* in the facilities, pressure control apparatus, etc.

In the case that *Water for Injection* is stored in the water processing system temporarily, it should usually be circulated in a loop consisting of a reservoir tank and pipe line at a temperature not lower than 80°C for preventing microbial proliferation.

When *Purified Water* is produced by RO/UF, basic composition of water processing system is almost the same as that for *Water for Injection* described above.

When RO/UF is utilized for preparing pharmaceutical water, it is necessary to select the most appropriate combination of membrane modules in consideration of the quality of source water and the quality of produced water required for its intended use. When the ultrafiltration membrane is used to prepare *Purified Water* or *Water for Injection*, membrane modules capable of removing microorganisms and substances with molecular masses not less than approximately 6000 should be used.

3. Selection of Pharmaceutical Water

Depending on the intended use of pharmaceutical water, the water suitable for assuring the quality of final products without causing any trouble during their manufacturing processes, should be selected from the above 4 types (1.1 – 1.4) of pharmaceutical water specified in the JP. Table 1 exemplifies a protocol for such selection (in the case of pharmaceutical water used for the manufacture of active pharmaceutical ingredients and drug products).

Sterile Purified Water in Containers or *Water for Injection* (or *Sterile Water for Injection in Containers*) may be used in place of *Purified Water* (or *Purified Water in Containers*).

3.1. Drug Products

For the manufacture of sterile drug products such as Injections, for which endotoxins together with microorganisms should be severely controlled, *Water for Injection* (or *Sterile Water for Injection in Containers*) should be used. For the manufacture of sterile drug products such as Ophthalmic Preparations and Ophthalmic Ointments, for which contamination with microorganisms should be paid attention, *Purified Water* (or *Purified Water in Containers*), which viable count level is specified at low, can also be used.

For the manufacture of non-sterile drug products, water with a quality not lower than that of *Purified Water* (or *Purified Water in Containers*) should be used. For the Inha-

Table 1 An Exemplified Protocol for Selecting Pharmaceutical Water
(Water Used in the Manufacture of Drug Products or APIs)

Classification	Class of Pharmaceutical Water	Application	Remarks
Drug Product	*Water for Injection* or *Sterile Water for Injection in Containers*	Injections, Dialysis Agents (Peritoneal Dialysis Agents and Hemodialysis Agents)	For Hemodialysis Agents, unless otherwise specified, *Water for Injection*, *Water for Injection in Containers*, or water suitable for the dialysis.
	Purified Water or *Purified Water in Containers*	Ophthalmic Preparations, Ophthalmic Ointments, Inhalations, Ear Preparations, Nasal Preparations	For the sterile drug products, such as Ophthalmic Preparations and Ophthalmic Ointments, for which precautions should be taken against microbial contamination, *Purified Water* (or *Purified Water in Containers*) kept its viable counts at low levels may be used. For Inhalations, Ear Preparations and Nasal Preparations, *Purified Water* (or *Purified Water in Containers*) of which viable counts are controlled at an appropriate level should be used. However, for the Inhalation Liquid Preparations among Inhalations, *Purified Water* (or *Purified Water in Containers*) that vial count is strictly controlled should be used.
		Preparations for Oral Administration, Preparations for Oro-mucosal Application, Preparations for Rectal Application, Preparations for Vaginal Application, Preparations for Cutaneous Application, and Tinctures and Aromatic Waters among Preparations Related to Crude Drugs.	For Liquids and Solutions for Oral Administration, Syrups, Suppositories for Vaginal Use, Ointments, Creams and so on for which precautions should be taken against microbial contamination, *Purified Water* (or *Purified Water in Containers*) adequately controlled from microbiological viewpoints should be used, taking in mind the affection of containing preservatives.
	Water	Among Preparations Related to Crude Drugs: Extracts, Pills, Infusions and Decoctions, Teabags, Fluidextracts	The viable counts in crude drugs and the objective microbial limits of product should be considered in selecting water to be used.
Active Pharmaceutical Ingredient (API)	*Water for Injection* or *Sterile Water for Injection in Containers*	Sterile APIs	
	Purified Water or *Purified Water in Containers*	APIs	In the manufacture of APIs used for products to be rendered sterile in the formulation process and have no subsequent processes capable of removing endotoxins, *Purified Water* (or *Purified Water in Containers*) controlled endotoxins in an appropriate level should be used.
	Water	API Intermediates	

lations, Ear Preparations and Nasal Preparations, appropriately controlled *Purified Water* (or *Purified Water in Containers*) in vial count level should be used, and for Liquids and Solutions among Inhalations, strictly controlled *Purified Water* (or *Purified Water in Containers*) in vial count level should be used. For the Liquids and Solutions for Oral Administration, Syrups, Suppositories for Vaginal Use, Ointments and Creams, which require care against microbiological contamination, *Purified Water* (or *Purified Water in Containers*) adequately controlled from microbiological viewpoints should be used in consideration of the possible impacts of preservatives formulated in the drug products. For the manufacture of products containing crude drugs, it is recommended to select adequate type of water considering viable counts of the crude drugs used for manufacturing the product and microbial limit required for the product.

Water used for pre-washing of containers or equipment surfaces that comes in direct contact with the drug products should have the quality not lower than that of *Water*. Water used for final rinsing should have an equivalent quality to that of water used for manufacturing drug products.

3.2. Active Pharmaceutical Ingredient (API)

Water used for manufacturing active pharmaceutical ingredient (API) should be selected in consideration of the characteristics of drug product for which the API is to be used, and its manufacturing process, so that the quality of the final drug product is assured.

Water used for manufacturing API or for cleaning containers or equipment surfaces that come in direct contact with the raw materials or API intermediates, should have the

quality not lower than that of *Water* adequately controlled from the chemical and microbiological viewpoints, even if the water is used at an earlier stage of synthetic or extraction process in the manufacture of API. Water used in the final purification process should have the quality equal to or higher than that of *Purified Water* (or *Purified Water in Containers*). Water used for final rinsing of containers or equipment surfaces that comes in direct contact with the APIs should have an equivalent quality to that of water used for manufacturing the APIs.

For manufacturing sterile API, *Sterile Purified Water in Containers* or *Water for Injection* (or *Sterile Water for Injection in Containers*) should be used. Similarly, for manufacturing APIs used for drug products where endotoxin control is required and there are no subsequent processes capable of removing endotoxins, *Water for Injection* (or *Sterile Water for Injection in Containers*), or *Purified Water* (or *Purified Water in Containers*) for which endotoxins are controlled at a low level, should be used.

4. Quality Control of Pharmaceutical Water

4.1. Outline

Verification that water with the quality required for its intended use has been produced by the pharmaceutical water processing system through substantial validation studies at an earlier stage of its operation, is the prerequisite for conducting quality control on pharmaceutical water in a routine and periodical manner. If this prerequisite is fulfilled, the following methods are applicable for quality control of pharmaceutical water.

For routine control, it is very useful to control quality of produced water based on the monitoring of electrical conductivity (conductivity) and total organic carbon (TOC). In addition, items to be monitored periodically, such as some specified impurities, viable counts, endotoxins, insoluble particulate matters, etc., should be determined according to the intended use of pharmaceutical water. The frequency of measurement should be determined considering with the variation in the quality of water to be monitored.

The following are points to consider in controlling the quality of produced water from microbiological and physicochemical (conductivity and TOC) viewpoints. It is necessary to monitor other items if necessary, and to confirm that they meet the specifications established individually.

4.2. Sampling

Monitoring should be conducted at an adequate frequency to ensure that the pharmaceutical water processing system is well-controlled and that water with acceptable quality is continuously produced and supplied. Specimens should be collected at the representative locations in the facilities for producing and supplying water, with particular care so that collected specimens reflect the operating condition of the pharmaceutical water processing system. An adequate protocol for the control of microorganisms at the sampling site should be established considering with the situation around the site.

Sampling frequency should be established based on the data from validation studies on the system. For microbiological monitoring, it is adequate to use the water specimens for the test within 2 hours after sampling. In the case that it is not possible to test within 2 hours, the specimens should be kept at 2 – 8°C and be used for the test within 12 hours.

4.3. Alert and Action Levels

In producing pharmaceutical water using a water processing system, microbiological and physicochemical monitoring is usually carried out to assure that water with required quality is being continuously produced when the system is operating as it designed. The operating condition of the system can be estimated by the comparison of monitoring data thus obtained against the alert level, action level, other levels for controlling the system, and acceptance criteria specified for the water required for its intended use, and also by the trend analysis of monitoring data through plotting them in a control chart. In this manner, the alert level and action level are used for controlling the process of water production, and not used for judging pass/fail of produced water.

4.3.1. Definition of Alert Level

''Alert level'' indicates that, when exceeded it, the system is threatening to deviate from its normal operating range. Alert levels are used for giving a warning, and exceeding them does not necessarily require a corrective action. Alert level is generally established either at a mean + 2σ on the basis of past trend analysis, or at a level of 70% (50% for viable counts) of action level, whichever is lower.

4.3.2. Definition of Action Level

''Action level'' indicates that, when exceeded it, the system has deviated from its normal operating range. Exceeding it indicates that corrective action must be taken to bring the system back within its normal operating range.

Alert and action levels should be established within the specified acceptance criteria of the water required for its intended use in consideration of available technologies and the quality required for the water. Consequently, exceeding an alert or action level does not necessarily indicate that the quality of produced water has become inadequate for its intended use.

4.4. Microbiological Monitoring

The main purpose of microbiological monitoring program for pharmaceutical water processing system is to foresee any microbiological quality deterioration of the produced water, and to prevent any adverse effects on the quality of pharmaceutical products. Consequently, detecting all of the microorganisms present in the water to be monitored may not be necessary. However it is required to adopt a monitoring technique able to detect a wide range of microorganisms, including slow growing microorganisms.

The following indicate incubation-based microbiological monitoring techniques for pharmaceutical water processing systems. To adopt a rapid microorganism detection technique, it is necessary to confirm in advance that the microbial counts obtained by such techniques are equivalent to those obtained by the incubation-based monitoring techniques.

4.4.1. Media and Incubation Conditions

There are many mesophilic bacteria of heterotrophic type that are adapted to poor nutrient water environments. Heterotrophic bacteria may form bio-films in many pharmaceutical water processing systems, and to cause quality deterioration of the produced water. Therefore, it is useful to monitor microbiological quality of water by using the R2A Agar Medium, which is excellent for growing bacteria of oligotrophic type.

Table 2 shows examples of measurement methods, minimum sample sizes, media, and incubation periods for estimating viable counts.

The media shown in Table 2 are as follows.

(i) Standard Agar Medium

Casein peptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15 – 20 minutes. pH after sterili-

Table 2 Methods for Assessment of Viable Counts in Pharmaceutical Water

Method	Pharmaceutical Water		
	Water	*Purified Water*	*Water for Injection*
Measurement Method	Pour Plate Method or Membrane Filtration	Pour Plate Method or Membrane Filtration	Membrane Filtration
Minimum Sample Size	1.0 mL	1.0 mL	100 mL
Media	R2A Agar Medium Standard Agar Medium	R2A Agar Medium	R2A Agar Medium
Incubation Period	R2A Agar Medium: 4 – 7 days (or longer) Standard Agar Medium: 48 – 72 hours (or longer)	4 – 7 days (or longer)	4 – 7 days (or longer)
Incubation Temperature	R2A Agar Medium: 20 – 25°C or 30 – 35°C Standard Agar Medium: 30 – 35°C	20 – 25°C or 30 – 35°C	20 – 25°C or 30 – 35°C

zation: 6.9 – 7.1.

(ii) R2A Agar Medium

Peptone (casein and animal tissue)	0.5 g
Casamino acid	0.5 g
Yeast extract	0.5 g
Sodium pyruvate	0.3 g
Glucose	0.5 g
Magnesium sulfate heptahydrate	50 mg
Soluble starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15 – 20 minutes. pH after sterilization: 7.1 – 7.3.

The following reagents should be used for preparing the R2A Agar Medium.

(i) Casamino acid Prepared for microbial test, by the acid hydrolysis of casein.

Loss on drying ⟨*2.41*⟩: Not more than 8% (0.5 g, 105°C, constant mass).

Residue on ignition ⟨*2.44*⟩: Not more than 55% (0.5 g).

Nitrogen content ⟨*1.08*⟩: Not less than 7% (105°C, constant mass, after drying).

4.4.2. Media Growth Promotion Test

In the media growth promotion test with the R2A Agar Medium, use the strains listed below or other strains considered equivalent to these strains. Prior to the test, inoculate these strains into sterile purified water and starve them at 20 – 25°C for 3 days.

Methylobacterium extorquens: NBRC 15911

Pseudomonas fluorescens: NBRC 15842, ATCC 17386, etc.

Dilute the fluid containing the strain starved with sterile purified water to prepare a fluid containing about 5×10^1 – 2×10^2 CFU/mL of viable counts. When pipetting 1 mL of the diluted fluid onto the R2A Agar Medium and incubating at 20 – 25°C for 4 – 7 days, sufficient proliferation of the inoculated strain must be observed.

In the media growth promotion test with the Standard Agar Medium, use the strains listed below or other strains considered equivalent to these strains. Prepare the fluid containing the strain according to the procedure prescribed in the Microbiological Examination of Non-sterile Products ⟨*4.05*⟩. When pipetting 1 mL of the fluid onto the Standard Agar Medium and incubating at 30 – 35°C for 48 hours, sufficient proliferation of the inoculated strain must be observed.

Staphylococcus aureus: ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276

Pseudomonas aeruginosa: ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275

Colon bacillus (*Escherichia coli*): ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972

4.4.3. Action Levels for Microorganisms for Pharmaceutical Water Processing System

The following action levels are considered appropriate and generally applicable to pharmaceutical water processing systems.

Action Levels for viable counts in various types of pharmaceutical water

Water: 10^2 CFU/mL* (Acceptance criterion prescribed in the Quality Standards for Drinking Water provided under the Article 4 of the Water Supply Law)

Purified Water: 10^2 CFU/mL**

Water for Injection: 10^1 CFU/100 mL**

(*Viable counts obtained using the Standard Agar Medium, ** Viable counts obtained using the R2A Agar Medium)

Although the action level for *Purified Water* shown above is set at the same level as that for *Water*, it is recommended for each facility to perform a higher level of microbiological control of water processing system based on the action level established individually.

When actual counts in validation studies or routine control exceed the above action levels, it is necessary to isolate and identify the microorganisms present in the water, and to sanitize or disinfect the affected system.

4.5. Physicochemical Monitoring

Physicochemical monitoring of a pharmaceutical water processing system is usually performed using conductivity and TOC as the indicators for water quality. By monitoring conductivity, total amounts of inorganic salts present in the water can be estimated, and by monitoring TOC, total amount of organic compounds present in the water can be estimated. Normally, the *Conductivity Measurements* ⟨*2.51*⟩ and the *Test for Total Organic Carbon* ⟨*2.59*⟩ specified in the General Tests, Processes and Apparatus of the JP should

be applied to these physicochemical monitoring. However, since tests for monitoring are performed in the situations different from those for judging pass/fail to the acceptance criteria prescribed in the monographs, supplements necessary to cover the situations to which the JP general tests cannot be applied, are described below.

To adopt the monitoring using conductivity and TOC as the indicators for inorganic and organic impurities at individual facility, appropriate alert and action levels, and countermeasures against unexpected apparatus failures should be established for each indicator.

4.5.1. Monitoring of Conductivity as the Indicator for Inorganic Impurities

Measurement of conductivity for monitoring is usually conducted continuously using an in-line apparatus with a flow-through type or pipe-insertion type cell. Alternatively, offline batch testing may be performed using a dip type cell with water specimens taken at point-of-use sites or other appropriate locations of the pharmaceutical water processing system. For the operation control of a pharmaceutical water processing system, guides for judging whether it is adequate to continue the operation of the system or not based on the results from monitoring of conductivity, are shown below, both for the cases of monitoring at the standard temperature (20°C) by applying *Conductivity Measurements* <2.51> of the JP and monitoring at temperatures other than 20°C by applying <645> *WATER CONDUCTIVITY* of the United States Pharmacopeia (USP) with some modifications.

4.5.1.1. Monitoring of Conductivity by applying the *Conductivity Measurements* <2.51> of the JP

When the monitoring of the conductivity of *Purified Water* and *Water for Injection* is performed at the standard temperature (20°C), measure the conductivity after confirming that the measure temperature is within a range of 20 ± 1°C. In this case, the recommended allowable conductivity (action level) for *Purified Water* and *Water for Injection* is as follows.

• Action Level 1.1 $\mu S \cdot cm^{-1}$ (20°C)

Since the above allowable conductivity is established for in-line monitoring, an alternative action level may be used for the monitoring based on offline batch testing.

4.5.1.2. Monitoring of Conductivity by applying the <645> *WATER CONDUCTIVITY* of the USP with some modification

Usually, it is somewhat difficult to control the temperature exactly in in-line conductivity monitoring. Therefore, the following approach can be applied for the monitoring at temperatures other than the standard temperature (20°C) of the JP. This approach is based on the Stages 1 and 2 of the three-stage approach described in ''<645> *WATER CONDUCTIVITY*'' of the USP and in the monographs being associated with water for pharmaceutical use (''Purified Water'', ''Highly Purified Water'' and ''Water for Injections'') of the European Pharmacopoeia (EP).

Stage 1 (In-line Measurement)

(i) Determine the temperature and the conductivity of the water specimens using a non-temperature-compensated conductivity reading.

(ii) From the Table 3, find the temperature value equal to or just lower than the measured temperature. Adopt the corresponding conductivity value on this table as the allowable conductivity at the measured temperature.

(iii) If the observed conductivity is not greater than the allowable conductivity adopted above, the water tested meets the requirement for monitoring conductivity. If the observed conductivity exceeds the allowable conductivity, proceed with Stage 2.

Table 3 Stage 1 Allowable Conductivity for Different Temperatures*

Temperature (°C)	Allowable Conductivity ($\mu S \cdot cm^{-1}$)	Temperature (°C)	Allowable Conductivity ($\mu S \cdot cm^{-1}$)
0	0.6		
5	0.8	55	2.1
10	0.9	60	2.2
15	1.0	65	2.4
20	1.1	70	2.5
25	1.3	75	2.7
30	1.4	80	2.7
35	1.5	85	2.7
40	1.7	90	2.7
45	1.8	95	2.9
50	1.9	100	3.1

* Applicable only to non-temperature-compensated conductivity measurements.

Stage 2 (Off-line Measurement)

(i) Measure the conductivity of the water specimen, by transferring it into a container and agitating it vigorously in order to attain equilibrium between the water specimen and the atmosphere on absorbing/desorbing carbon dioxide.

(ii) Transfer a sufficient amount of water to be tested into a suitable container, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing the conductivity periodically. When the change in conductivity, due to the uptake of atmospheric carbon dioxide, becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity (25°C) of the water specimen.

(iii) If the conductivity of the water specimen at 25°C obtained above is not greater than 2.1 $\mu S \cdot cm^{-1}$, the water tested meets the requirement for monitoring conductivity. If the observed value exceeds 2.1 $\mu S \cdot cm^{-1}$, it should be judged that the water tested does not meet the requirement for monitoring conductivity.

4.5.2. Monitoring of TOC as the Indicator for Organic Impurities

The acceptance criterion of TOC is specified as ''not greater than 0.50 mg/L (500 ppb)'' in the monographs of *Purified Water* and *Water for Injection*. However it is recommended for each facility preparing pharmaceutical water to conduct operation control of pharmaceutical water processing system through TOC monitoring on produced water based on its own alert and action levels for TOC determined individually. The following are the recommended action levels for TOC.

• Action Level: ≦300 ppb (in-line)
≦400 ppb (off-line)

The Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law require that TOC should be ''not greater than 3 mg/L (3 ppm)''. Taking the above recommended action levels into consideration, it is also recommended for each facility to conduct quality control of source water through TOC monitoring based on its own alert and action levels for TOC determined individually.

The JP specifies the *Test for Total Organic Carbon* <2.59>, and normally, TOC measurement should be conducted using

an apparatus which meets the requirements described in the JP method. However, if a TOC apparatus conforms to the apparatus suitability test requirements described in ''<643> *TOTAL ORGANIC CARBON*'' of the USP, or those described in the ''*Methods of Analysis 2.2.44. TOTAL ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE*'' of the EP, the apparatus can be used for the monitoring of pharmaceutical water processing system, if sufficiently pure water not contaminated with ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures, is used as the source water supplied to the system.

A TOC apparatus, characterized by calculating the amount of organic carbon from the difference in conductivity before and after the decomposition of organic substances without separating carbon dioxide from the sample solution, may be influenced negatively or positively, when applied to the water specimens containing ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures. Therefore, the apparatus used for TOC monitoring should be selected appropriately in consideration of the purity of the water to be monitored and the contamination risk in the case of apparatus failure.

4.6. Storage of *Water for Injection*

In storing *Water for Injection* temporarily, adequate measures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature must be taken, and an appropriate storage time should also be established based on the validation studies, in consideration of the risks of contamination and quality deterioration.

5. Points to Consider for Assuring the Quality of Pharmaceutical Water in Containers

There are some specific points to consider for assuring the quality of pharmaceutical water in containers (*Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*), which are available as commercially products.

5.1. Methods for Preparing Sterile Pharmaceutical Water in Containers and Their Sterilization Validation

The following 2 different preparation methods are described in the monographs of *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*.

(i) Introduce *Purified Water* or *Water for Injection* into a hermetic container, seal up the container, then sterilize the product.

(ii) Make *Purified Water* or *Water for Injection* sterile by using a suitable method, introduce the sterilized water into a sterile hermetic container by applying aseptic manipulation, then seal up the container.

For assuring the sterility of pharmaceutical water products, only the validation of final sterilization process is required in the case of preparation method (i), whereas validations of all the processes are indispensable in the case of preparation method (ii), since the latter is based on the idea to assure the sterility of pharmaceutical water products by ''aseptically'' introducing *Purified Water* (or *Water for Injection*) treated in advance with filtration sterilization, etc. into a sterile hermetic container, and sealing it up.

5.2. Deterioration of Water Quality during the Storage in Containers

5.2.1. Inorganic impurities (Conductivity as the indicator)

The conductivity of pharmaceutical water in containers may increase to some higher levels due to the absorption of carbon dioxide from the atmosphere at the time of its preparation and that passed through plastic layer of the containers during storage, and also due to ionic substances released from the containers, even if the conductivity of *Purified Water* or *Water for Injection* used for its production is maintained at the level not more than $1.0\,\mu S \cdot cm^{-1}$. Particularly in the cases of pharmaceutical water products packed in small scale glass containers, it is necessary to pay attention to the change of conductivity during storage.

5.2.2. Organic impurities (Potassium Permanganate-reducing Substances or Total Organic Carbon (TOC) as the indicator)

JP specifies the classical test of potassium permanganate-reducing substances in the monographs of *Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers* for controlling organic impurities in pharmaceutical water in containers. It forms a remarkable contrast to the specifications of *Purified Water* and *Water for Injection*, in which JP requires to control organic impurities in pharmaceutical water in bulk based on the test of TOC (acceptance criterion: not more than 0.5 mg/L (500 ppb)). This is because that it is considered difficult to establish the specification of pharmaceutical water in containers for organic impurities based on the test of TOC from the facts that there were many cases of remarkable increases in TOC values after storage of water in containers. Particularly in the cases of pharmaceutical water products packed in small scale plastic containers, it is necessary to pay attention to the increase of materials released from containers during storage.

The test of potassium permanganate-reducing substances is retained in the specifications of pharmaceutical water in containers, not as the most suitable method for the test of organic impurities present in the water in containers, but as a counter measure for performing the test of the water in containers with the same test method despite of the material (glass, polyethylene, polypropylene, etc.) and the size (0.5 – 2000 mL) of the containers, and the duration of storage. Therefore, it is recommended to adopt the test of TOC as the alternative for the test of potassium permanganate-reducing substances, and to perform quality control of pharmaceutical water in containers based on TOC measurements under the responsibility of each manufacturer, if possible.

In such cases, it is recommended to adopt the following values as the levels preferable to attain.

For products containing not more than 10 mL of water:
TOC not greater than 1500 ppb
For products containing more than 10 mL of water:
TOC not greater than 1000 ppb

As for the pharmaceutical water packed in the plastic containers made of polyethylene, polypropylene, etc., in addition to the concern for the release of materials such as monomer, oligomers, plasticizers, etc. from plastics, it is necessary to pay attention to the storage environment of the products to avoid the contaminations with low molecular volatile organics such as ethanol, or low molecular air pollutants such as nitrogen oxides, since these plastics have the properties of permeating various gases and water.

5.2.3. Microbial Limit (Total Aerobic Viable Counts)

For *Purified Water in Containers*, it is not required to assure the sterility, but it is necessary to produce it by using sanitary or aseptic processes in order to meet the acceptance criterion of ''10^2 CFU/mL'' for total aerobic viable counts throughout the period of their storages. It is also necessary to take special care against microbial contamination during its circulation. In addition, it is recommended to use them as soon as possible after opening their seals.

The acceptance criterion of ''10^2 CFU/mL'' for total aerobic viable counts of *Purified Water in Container* is at the same level as the action level for viable counts in the production of *Purified Water* (in bulk). However, different from the case of microbiological monitoring of *Purified Water*, Soybean-Casein Digest Agar Medium is used for the test of total aerobic viable counts of *Purified Water in Containers* to detect microorganisms contaminated from the surroundings during its storage and circulation.

5.3. Points to consider in the case that commercially available products of pharmaceutical water in containers are used for the manufacture of pharmaceutical products

It is allowable to use commercially available products of pharmaceutical water in containers (*Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*) for the manufacture of pharmaceutical products and products for clinical trial, and for the tests of pharmaceutical products. In such cases, it is necessary to consider the following points.

(i) When such products are used for manufacturing pharmaceutical products, it is recommended to use them soon after confirming their compliances to the requirements of JP monograph from the test results at the time of its receipt or those offered from the supplier of the products.

(ii) In the case that such products are used for manufacturing pharmaceutical products, it is necessary to validate the process in which the water was used as a part of process validation of pharmaceutical products. In the case that they are used for manufacturing products for clinical trial, it is necessary to confirm that the water doesn't give any adverse effects on the quality of the products.

(iii) The products of sterile pharmaceutical water in containers should be used only once after opening their seals, and it must be avoided to use them again after storage.

(iv) It is recommended to prepare a standard operation practice (SOP) adequate for its intended use, considering that the contamination and quality deterioration of the water due to human and laboratory environmental origins might go on rapidly immediately after opening the product seal.

Water to be used in the Tests of Drugs

The water to be used in the tests of drugs is defined as ''the water suitable for performing the relevant test'' in the paragraph 21 under General Notices of the JP. Therefore, it is necessary to confirm that the water to be used in a test of a drug is suitable for the purpose of the relevant test before its use.

Unless otherwise specified in the individual test method, Purified Water, Purified Water in Containers or the water produced by an appropriate process, such as ion exchange or ultrafiltration, may be used for these purposes. Water produced for these purposes at other individual facilities may also be used.

Examples of the water for tests specified in General Tests in the JP are as follows:

- Water for ammonium limit test: <*1.02*> Ammonium Limit Test (Standard Ammonium Solution)
- Water used for measuring organic carbon (water for measurement): <*2.59*> Test for Total Organic Carbon
- Water for ICP analysis: <*2.63*> Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry
- Water for bacterial endotoxins test: <*4.01*> Bacterial Endotoxins Test
- Water for particulate matter test (for injections): <*6.07*> Insoluble Particulate Matter Test for Injections
- Water for particulate matter test (for ophthalmic solutions): <*6.08*> Insoluble Particulate Matter Test for Ophthalmic Solutions
- Water for particulate matter test (for plastic containers): <*7.02*> Test Methods for Plastic Containers

The water for tests specified in General Information in the JP is as follows:

- Water for aluminum test: Test for Trace Amounts of Aluminum in Total Parenteral Nutrition (TPN) Solutions

The term ''water'' described in the text concerning tests of drugs means ''the water to be used in the tests of drugs'' as defined in the paragraph 21 under General Notices.

G9 Reference Standards

Reference Standards and Reference Materials Specified in the Japanese Pharmacopoeia

Reference materials are materials that are used as standards in quantitative and qualitative measurement of drug products, calibration and accuracy confirmation of apparatus, and suitability tests of analytical systems. Reference standards specified in the Japanese Pharmacopoeia (JP) as described in the Reference Standards <*9.01*> are reference materials prepared to the specified quality necessary with regard to their intended use such as quality evaluation tests of drugs, and they are provided with public assurance that the substances have suitable quality for the specified use.

This chapter includes the definitions and explanations of basic terms regarding reference standards, and the particulars of the JP reference standards mainly used for chemical drugs, such as classification by use, requirements for establishment, required quality evaluation items, distribution and precautions for use. The descriptions show only basic policies, and therefore flexible management is required for practical application for the time being.

1. Basic terms of reference standards

- Reference materials: ''The materials used as the standards'' for determination of characteristic values of materials and substances. Reference materials are sufficiently homogeneous and stable with respect to one or more specified properties and are produced so that they are suitable for their intended use in measuring processes [JIS Q 0035:2008]. Reference materials for drug products are materials used as the standards in quantitative and qualitative measurements, calibration and accuracy confirmation of apparatus, and they are prepared so that they are suitable for their intended use.
- Reference standards: The reference materials prepared to a specified quality necessary with regard to their intended use, such as quality evaluation tests of drugs, and they are provided with public assurance that the substances have suitable quality for the specified use.

- JP reference standard: The reference standards specified in the Monographs or General Tests in the JP.
- Certified reference materials: The reference materials with one or more specified characteristic values determined by appropriate metrological procedure. Their qualities are guaranteed by a certificate that describes their characteristic values, uncertainties of the values, and metrological traceability [JIS Q 0035:2008].

2. Classification of the JP reference standards by use

JP reference standards include various types and are used for assays, identification, purity, calibration of apparatus, suitability tests of analytical systems, etc., and they are broadly classified by their use such as quantitative tests, qualitative tests, and calibration of apparatus. These reference standards are further subclassified according to their specific use, such as assays of active ingredients and indicator ingredients, assays of related substances, identification using spectral measurement or chromatography, calibration of apparatus used in tests, determinations and assays that are specified in the General Tests, and suitability tests for analytical systems.

2.1. Reference standards used for quantitative tests

2.1.1. Reference standards for assays of active ingredients etc.: Reference standards used for quantitative assays of products specified in the Monographs, such as chemical agents, antibiotics, additives, and biotechnological/biological products.

2.1.2. Reference standards for assays of indicator ingredients:
Reference standards used for quantitative assays of indicator ingredients of crude drugs specified in the Monographs.

2.1.3. Reference standards for assays of related substances:
Reference standards used for quantitative assays of specific related substances in purity for products specified in the Monographs, such as chemical agents, antibiotics, additives, and biotechnological/biological products.

2.1.4. Other reference standards for assays:
Reference standards needed for quantitative assays specified in the General Tests.

2.2. Reference standards used for qualitative tests

2.2.1. Reference standards for identification:
Reference standards used for identification of products specified in the Monographs, such as chemical agents, antibiotics, additives, biotechnological/biological products, and crude drugs. These identification include the comparison of ultraviolet-visible absorption spectra, infrared absorption spectra, nuclear magnetic resonance spectra, retention time or *R*f values in chromatography, and mobility in electrophoresis.

2.2.2. Reference standards for purity:
Reference standards used for identification of peaks or spots, or for limit tests of related substances, in purity of products specified in the Monographs, such as chemical agents, antibiotics, additives, biotechnological/biological products, and crude drugs.

2.3. Reference standards used for system suitability

2.3.1. Reference standards for system suitability:
Reference standards used for system suitability of products specified in the Monographs, such as chemical agents, antibiotics, additives, biotechnological/biological products, and crude drugs.

2.4. Reference standards used for calibration and suitability confirmation of apparatus

2.4.1. Reference standards for calibration of apparatus:
Reference standards used for secondary calibration of apparatus that are specified in the General Tests.

2.4.2. Reference standards for suitability confirmation of apparatus:
Reference standards used in order to confirm that the measured values gained from an apparatus specified in the General Tests are within the defined range.

3. Names and uses of JP reference standards

JP reference standards are used for tests specified in the Monographs and General Tests, such as Assays, Identification, Purity, calibration of apparatus, and suitability tests of analytical systems. These reference standards include materials with only one specific use and materials with multiple uses. JP reference standards used for assays and quantitative determinations of active ingredients in uniformity of dosage units and dissolution are named in principle by attachment of the phrase ''reference standard'' to the material name. Reference standards used for quantitative tests can be used for other tests such as identification as the reference standards, if possible. Reference standards only used for uses other than quantitative tests are named by attachment of the name of their use. Some possible uses are shown below with examples in brackets.

- For identification (Montelukast Sodium RS for Identification)
- For purity (○○○ RS for Purity)
- For calibration of apparatus (Calcium Oxalate Monohydrate RS for Calibration of Apparatus)
- For system suitability (Montelukast RS for System Suitability)

4. Requirements for establishment of JP reference standards

The JP reference standards have been mainly used as standard substances for quantitative assays of active ingredients so far. On the other hand, the European Pharmacopoeia (Ph. Eur.) and the U.S. Pharmacopeia (USP) have been actively establishing reference standards or reference materials that have specific uses other than the use for quantitative assays of active ingredients. Such specified reference standards include standards for contaminants in purity, for suitability of analytical systems, and for identification. This situation requires JP to change its policy on the reference standards along with the international trends, however, careful deliberation is needed to establish new JP reference standards in consideration of the points shown below.

(1) To adopt a relative determination such as chromatography for a quantitative test, in principle, establish the reference standard for the assay or establish the reference materials indicated purity with metrological traceability as a reagent.

(2) To appoint a comparison method for an identification, such as comparison of ultraviolet-visible absorption spectra, infrared absorption spectra, or nuclear magnetic resonance spectra, comparison of retention time or *R*f values in chromatography, or comparison of electrophoretic mobility, in principle, it is desirable to establish the reference standard for the identification unless the use of some reference standard for assays is applicable.

(3) In a purity where a specific related substance or contaminant is analyzed, it is recommended to establish the reference standard dedicated to the purity if the specific related substance or contaminant cannot be identified from the relative retention time of its peak on the chromatogram, and/or the limit cannot be specified by area normalization method or by comparison of the peaks with the sample solution and with the standard solution derived from the sample solution.

(4) Establish a reference standard for a suitability of an

analytical system, if the system suitability cannot be adequately evaluated by conventional JP methods (determination of the number of theoretical plates and the symmetry factor, etc.)

(5) If the continuous supply of the raw materials for the reference standard is uncertain, a reference standard for a related substance for a purity or a reference standard for a system suitability should not be established.

(6) When a material utilized as standard has the uses other than for quantitative assays and can be obtained as a certified reference material or as a reference material for other tests, that can be specify as the certified reference material and the reference material for other tests in the Reagents, Test Solutions <*9.41*> without establishing the materials as a JP reference standard.

(7) When a material utilized as standard has the uses other than for quantitative assay and can be obtained as a reagent, that can be specify the material in the Reagents, Test Solutions <*9.41*> establishing specifications and test methods according to its use, without establishing the material as a JP reference standard.

5. Quality evaluation items required for JP reference standards

Quality evaluation items required to establish a JP reference standard are shown below. The items are chosen mainly on the assumption that the materials are used as reference standards for chemical agents.

5.1. Quality evaluation items for reference standards used for quantitative assays

(i) Description: color and shape

(ii) Identification: establish a test method to identify or confirm the chemical structure.

i) Ultraviolet-visible absorption spectrum

ii) Infrared absorption spectrum

iii) Nuclear magnetic resonance spectrum (^{1}H)

iv) X-ray powder diffraction image※(when the crystal form is specified)

v) Retention time or *R*f value in chromatography※ (when chromatography is applicable to the identification)

vi) Counter ion※

(iii) Purity

i) Related substances (total amount)

ii) Residual solvents

iii) Other contaminants※

(iv) Characteristic values※

i) Specific rotation

ii) Melting point

(v) Water/Loss on drying

(vi) Residue on ignition※

(vii) Purity determination by mass balance method: Regarding purity evaluation in mass balance method, calculate the purity (%) on the dried or anhydrous basis setting related substances, residue on ignition, residual solvent, and other contaminants as deductions.

(viii) Assay (if possible, establish an absolute quantification method such as titration, etc.)

[Note] Quality evaluation items attached with※ marks are items whose adoption should be considered taking into account the material's use as a reference standard.

5.2. Quality evaluation items for reference standards used for other than quantitative assays

Shown below are examples of quality evaluation items whose adoption should be considered taking into account the material's use as a reference standard.

(i) Description: color and shape

(ii) Identification: establish a test method to identify or confirm the chemical structure.

i) Ultraviolet-visible absorption spectrum

ii) Infrared absorption spectrum

iii) Nuclear magnetic resonance spectrum (^{1}H)

iv) Mass spectrum

v) X-ray powder diffraction image (when the crystal form is specified)

vi) Retention time or *R*f value in chromatography

(iii) Purity

i) Related substances (total amount)

ii) Residual solvents

iii) Other contaminants

(iv) Water/Loss on drying

(v) Purity determination by mass balance method

(vi) Tests related to the uses of the materials

i) A reference standard for system suitability used for peak identification needs identification of relative retention time of the peak under the same conditions to the test method that is specified in the Monographs.

ii) A reference standard for system suitability needs identification of the resolution under the same conditions to the test method that is specified in the Monographs.

6. Reference materials specified in the JP

Materials that correspond to reference materials are described in the Reagents, Test Solutions <*9.41*> in the JP. Such materials are shown as follows:

- Materials described as reagents for assays
- Materials used for identification that described as reagents for thin-layer chromatography (some materials do not correspond to reference materials)
- Materials specified as reagents for purity
- Materials described as specific related substances in the item of Related substances in Purity in the Monographs
- Reference materials specified by JIS

In the JP (except ''Crude Drugs''), ''reagents for assays'' is specified as reference materials for assays of active ingredients in drug products, and some active ingredients with quality above a certain level are specified as reagents that are used as reference materials for identification of the active ingredients in drug products by thin-layer chromatography. However, these reference materials specified as reagents are not officially provided. Assays should be performed using reference standards, and it is considered appropriate that the ''reagents for assays'' described as reagents in the Reagents, Test Solutions <*9.41*> should be established as reference standards. Regarding new assay reagents that will be established for drug products except ''Crude Drugs,'' there is a need to consider gradual establishment of these ''reagents for assays'' as reference standards.

On the other hand, it is difficult to establish reference standards for indicator ingredients of ''Crude Drugs''; therefore, reference materials for assays of indicator ingredients are specified as reagents and quantitative NMR is included in specifications of the reagents so that a specification for assay with metrological traceability is established.

7. Precautions for the use of JP reference standards

7.1. JP reference standards are reference standards whose uses are specified in the Monographs and General Tests in the JP. Their detailed uses are described in the Monographs and their adequate qualities as reference standards are guaranteed when they are used for the described uses. Accordingly, their qualities for other uses are not guaranteed.

7.2. When a JP reference standard is used for a quantitative tests specified in the Monographs, if a correction coefficient is indicated on documents such as package in-

sert, multiply the standard amount by the correction coefficient to calculate the corrected amount for the use. If a correction coefficient is not indicated, do not correct the amount for use assuming that the purity of the reference standard is 100.0%.

7.3. If there is a description of ''amount of the reference standard, calculated on the dried basis'' or ''amount of the reference standard, calculated on the anhydrous basis'' in a calculation formula for an assay method in the Monographs, extra measurement of the loss on drying or the water content of the reference standard is required. If, however, the value of loss on drying or water content is indicated on document of the reference standard such as package insert, use of the indicated value is permitted.

7.4. JP reference standards have no established expiration date, therefore, obtain needed amount of a reference standard when it is needed, and then store the reference standard under specified conditions and use up it as quick as possible.

7.5. The quality of reference standards stored after unsealing of the packages are not guaranteed.

7.6. Normally, one packing unit of a reference standard contains the amount that enables several times of repeated test. However, some packages of reference standards whose raw material supply is scarce contain the amounts that enable only one-time test.

7.7. Information of JP reference standards necessary for the uses specified in the Monographs or General Tests is described on documents such as package inserts. However, their test results are not disclosed and certificates of analysis are not issued.

G10 Others

Basic Concepts for Quality Assurance of Drug Substances and Drug Products

Introduction

Quality of drug substances and products are generally assured through production and examination under appropriate Good Manufacturing Practice (GMP) control reflecting knowledge obtained from designing and developmental stages and manufacturing stage on management of raw materials and other materials, management of manufacturing process, specifications, etc. As shown in the General Notices, JP-listed drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the *Japanese Pharmacopoeia*. In addition to these, adherence to GMP, management of raw materials and other materials, and management of manufacturing process are fundamental factors required to assure the quality of JP-listed drugs in actual production.

The present chapter summarizes general concepts concerning measures for quality assurance of drug substances and products mainly aimed at chemicals, including chemically synthesized antibiotics and semisynthetic antibiotics, synthetic peptides, oligonucleotides, and biotechnological/biological products. Although radiopharmaceuticals, crude drugs, herbal products, and crude products of animal or plant origin are excluded from the subjects of the concepts, these concepts are useful for the management of any type of drugs.

Basic Concept

In recent years, the mainstream concept for quality of drugs is that their quality is assured by management of manufacturing process, including management of raw material and other materials, and quality tests of final products that are conducted mutually complementary.

1. Management of manufacturing process

1.1. Considerations of manufacturing process

Adequate design of manufacturing processes and knowledge of their capacity are important to establish manufacturing processes yielding drug substances or drug products that meet specifications and to manage the manufacturing processes, product quality, etc. appropriately and consistently.

From this standpoint, the limits for management of manufacturing processes should be based on information obtained from the entire process spanning the period from the early development through commercial scale production. The appropriateness of the limits also needs to be confirmed by evaluation, verification, review, and other examinations of manufacturing processes. In addition, the General Notices describes exception: for example, when an assurance that the level of an impurity is controlled within an allowable standard or an assurance that an impurity is effectively removed to below an accepted level by effective manufacturing process management is obtained, the purity test for the final product may be omitted, and in some cases, purity tests may be excluded from the specifications for the final product.

In-process tests are tests that may be performed during the manufacture of either the drug substance or drug product, rather than tests for the final product. In-process tests are performed for quality verification during manufacturing processes that are likely to influence product quality, or for confirmation of proper functioning of the manufacturing process. When proper management of manufacturing and quality is conducted, performing of in-process tests may allow omission of tests for the final product.

Usually in-process tests are properly designed according to the degree of the process's influence on quality, however, the use of internal action limits by the manufacturer to assess the consistency of the process at less critical steps is also important. Data obtained during development of the drug and during evaluation and verification to provide the basis for provisional action limits to be set for the manufacturing process, and these limits should be further refined based on additional manufacturing experience and data obtained after product approval.

1.2. Considerations of raw materials and other materials (starting materials, excipients, packaging materials, etc.)

The raw materials and other materials used in the production of drug substances (or drug products) should meet quality standards, appropriate for their intended use, and if necessary, appropriate setting of specifications is required. Especially, biological raw/source materials may require careful evaluation to establish the presence or the absence of deleterious endogenous or adventitious agents. Procedures that make use of affinity chromatography (for example, employing monoclonal antibodies), should be accompanied by appropriate measures to ensure that such process-related impurities or potential contaminants arising from their production and use do not compromise the quality and safety of the drug substance or drug product.

The quality of the excipients used in the drug product formulation (and in some cases, in the production of drug sub-

stance), as well as the primary packaging materials, should be controlled with specifications established based on the characteristics of the drug. If specifications for a material are described by the JP, as a rule, at least the JP criteria should be satisfied. Concerning excipients not listed in the JP, appropriate specifications should be established individually.

2. Quality tests of products (specifications)

''A specification'' is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. Specifications of the JP are defined sets of quality attributes needed for determination of whether the use of a drug substance or a drug product is appropriate for the purpose. ''Conformance to the specifications of the JP monograph'' means that the JP listed drug substances and drug products, when tested according to the procedures described in general tests and drug monographs, will meet the all acceptance criteria except criteria of ''Description'' and ''Containers and storage (for drug products)'' in the JP monographs.

However, specifications of monographs for drug substance and drug product are one part of a total control strategy for assurance of the quality and consistency of the substances/products. Other control strategies include complete characterization of the drug in developmental stage (specifications are established based on the characterization), and management of manufacturing process and products' quality, such as evaluation, verification, and review of manufacturing process, and management of raw materials and other materials, that is to say, adherence to the GMP.

3. Periodic or Skip Testing

Periodic or skip testing is the performance of specified tests at release on preselected batches and/or at predetermined intervals, rather than on a batch-to-batch basis with the understanding that those batches not being tested still must meet all acceptance criteria established for that product. When this concept is applied, it is necessary to show its appropriateness and be approved previously by regulatory authority. This concept may be applicable to, for example, residual solvents and microbiological testing for solid oral dosage forms. It is recognized that only limited data may be available at the time of submission of an application. Implementation of this test should therefore generally be considered post-approval. When tested, any failure to meet acceptance criteria established for the periodic test should be handled by proper notification of the appropriate regulatory authorities. If these data demonstrate a need to restore routine testing, then batch-by-batch release testing should be reinstated.

4. Real time release testing and parametric release

Determination of the suitability for release can be based on the result of real time release testing instead of final product testing when approved by the regulatory authority. Real-time release testing is a type of tests to evaluate the quality of in-process or final products based on process data (including results of in-process testing and data on process parameters). Parametric release can be considered a type of real time release. One example of parametric release is to determine the suitability for release of terminally sterilized drug products based on the data on sterilizing process instead of the results of sterility testing. In this case, the release of each batch is based on satisfactory results from monitoring specific parameters, e.g., temperature, pressure, and time during the terminal sterilization phase(s) of drug product manufacturing. Parametric release based on above parameters is more reliable in predicting sterility assurance than determination of suitability for release based on sterility testing using limited number of end-products.

Basic Concept of Quality Risk Management

Introduction

Quality Risk Management (QRM) is a crucial constituent of Pharmaceutical Quality System (PQS). PQS is a kind of the Quality System to control pharmaceutical quality in industries. Quality System is a basic concept of International Standards such as ISO 9001, ISO 14001, and ISO 27001. With its framework of maintenance and continuous improvement of business operation based on PDCA cycle (Plan →Do→Check→Act), PQS has been incorporated in ICH Q10 guideline as the basic philosophy. QRM is applicable to secure quality of every pharmaceuticals including drug substances, drug (medicinal) products, and biological and biotechnological products. Cooperating with a control strategy reflecting latest knowledge and understandings on products and manufacturing process, QRM contributes to realization and maintenance with consistent quality by responding flexibly and securely to risk regarding qualities.

Risks associated with the quality of pharmaceutical products are evaluated in the process of listing in Japanese Pharmacopoeia and the results are reflected in specifications of the individual monograph. However, the pharmaceuticals specified in the same monograph may each have different quality risk derived from difference in their manufacturing methods. Therefore, appropriate assessment and management is required for such risk to manufacturing quality in the course of actual drug development and manufacturing. Further, quality risk of pharmaceuticals should be re-evaluated on a regular basis during their lifecycle, i.e. from their initial development through commercialization to the end of manufacturing and sales, and it is required to take appropriate measures based on the results.

About a relationship between QRM and Japanese Pharmacopoeia, it may be said additionally as follows. In addition to conduction of the standard tests of Japanese Pharmacopoeia, it is important to plan and carry out measures to properly control elusive risk, which derived from alterations of manufacturing and quality management such as changes of raw materials and resources, in order to properly hold the pharmaceutical quality. Besides, depending on the results of risk re-evaluation, it may become necessary to revise specification tests specified by Japanese Pharmacopoeia.

1. Significance of QRM

It is commonly understood that risk is defined as the combination of the probability of occurrence of harm and the severity of that harm. However, achieving a shared understanding of the application of risk management among diverse stakeholders is difficult because of a large gap between stakeholders in type and size of risk recognized. In relation to pharmaceuticals, although there are a variety of stakeholders, including patients and medical practitioners as well as government and industry, the protection of the patient by applying QRM should be considered of prime importance.

The manufacturing and use of a drug (medicinal) product, including its components, necessarily entail some degree of risk. The risk to its quality is just one component of the

overall risk. The product quality should be maintained throughout the product lifecycle such that the attributes that are important to the quality of the drug (medicinal) product remain consistent with those used in the clinical studies. An effective QRM approach can further ensure the high quality of the drug (medicinal) product to the patient by providing a proactive means to identify and control potential quality issues during development and manufacturing. Additionally, use of QRM can improve the quality of measures and the speed of decision making if a quality problem arises. Effective QRM can provide regulators with greater assurance of a company's ability to deal with potential risks and can beneficially affect the extent and level of direct regulatory oversight.

As for QRM, it is neither always appropriate nor always necessary to use a formal risk management process. The use of informal risk management processes can also be considered acceptable. Appropriate use of QRM can facilitate but does not obviate industry's obligation to comply with regulatory requirements and does not replace appropriate communications between industry and regulators.

2. Scope of Application

QRM can be applied to every aspects of pharmaceutical quality. These aspects include development, manufacturing, distribution, and the inspection and submission/review processes throughout the lifecycle of drug substances, drug (medicinal) products, and biological and biotechnological products (including the use of raw materials, solvents, excipients, packaging and labeling materials in drug (medicinal) products, biological and biotechnological products).

3. Principle of QRM

Two primary principles of QRM are:

- Evaluation of the risk to quality should be based on scientific knowledge and ultimately link to the protection of the patient.
- Level of effort, formality and documentation of the QRM process should be commensurate with the level of risk.

4. General QRM Process

QRM is a systematic process for the assessment, control, communication and review of risks to the quality of the drug (medicinal) product across the product lifecycle. A model for QRM is outlined in the diagram (Figure 1). The emphasis on each component of the framework might differ from case to case but a robust process will incorporate consideration of all the elements at a level of detail that is commensurate with the specific risk. Decision nodes are not shown in the diagram because decisions can occur at any point in the process. These decisions might be to return to the previous step and seek further information, to adjust the risk models or even to terminate the risk management process based upon information that supports such a decision.

4.1. Initiation of QRM Process

QRM should include systematic processes designed to coordinate, facilitate and improve science-based decision making with respect to risk. Possible steps used to initiate and plan a QRM process might include the following:

- Define the problem and/or risk question, including pertinent assumptions identifying the potential for risk;
- Assemble background information and/or data on the potential hazard, harm or human health impact relevant to the risk assessment;
- Identify a leader and necessary resources;
- Specify a timeline, deliverables and appropriate level of decision making for the risk management process.

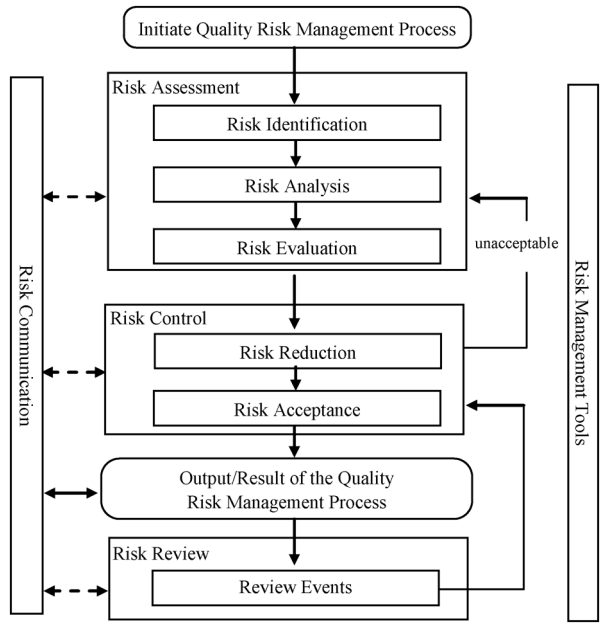


Fig. 1 Overview of a typical quality risk management process

In the process described above, persons in charge (decision makers) should take responsibility for coordinating quality risk management across various functions and departments of their organization; and assure that a QRM process is defined, deployed and reviewed and that adequate resources are available.

4.2. Risk Assessment

Risk assessment consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards. The step includes ''risk identification'', ''risk analysis'' and ''risk evaluation'',

As an assist to define risk clearly for purposes of risk assessment, the following three basic questions are often helpful.

1. What might go wrong?
2. What is the likelihood (probability) it will go wrong?
3. What are the consequences (severity)?

Risk identification is a systematic use of information to identify hazards referring to the risk question or problem description. Information can include historical data, theoretical analysis, informed opinions, and the concerns of stakeholders. Risk identification addresses the ''What might go wrong?'' question, including identifying the possible consequences. This provides the basis for further steps in the quality risk management process.

Risk analysis is the estimation of the risk associated with the identified hazards. It is the qualitative or quantitative process of linking the likelihood of occurrence and severity of harms. In some risk management tools, the ability to detect the harm (detectability) also factors in the estimation of risk.

Risk evaluation compares the identified and analyzed risk against given risk criteria. Risk evaluations consider the strength of evidence for all three of the fundamental questions.

The output of a risk assessment is either a quantitative estimate of risk or a qualitative description of a range of risk. When risk is expressed quantitatively, a numerical probability is used. Alternatively, risk can be expressed using qualitative descriptors, such as ''high'', ''medium'', or ''low'', which should be defined in as much detail as possible. Some-

times a ''risk score'' is used to further define descriptors in risk ranking. In quantitative risk assessments, a risk estimate provides the likelihood of a specific consequence, given a set of risk-generating circumstances. Thus, quantitative risk estimation is useful for one particular consequence at a time. Alternatively, some risk management tools use a relative risk measure to combine multiple levels of severity and probability into an overall estimate of relative risk. The intermediate steps within a scoring process can sometimes employ quantitative risk estimation.

4.3. Risk Control

Risk control includes decision making to reduce and/or accept risks. The purpose of risk control is to reduce the risk to an acceptable level. The amount of effort used for risk control should be proportional to the significance of the risk. Decision makers might use different processes, including benefit-cost analysis, for understanding the optimal level of risk control.

Risk control might focus on the following questions:

- Is the risk above an acceptable level?
- What can be done to reduce or eliminate risks?
- What is the appropriate balance among benefits, risks and resources?
- Are new risks introduced as a result of the identified risks being controlled?

Risk reduction focuses on processes for mitigation or avoidance of quality risk when it exceeds a specified (acceptable) level (see Fig. 1). Risk reduction might include actions taken to mitigate the severity and probability of harm. Processes that improve the detectability of hazards and quality risks might also be used as part of a risk control strategy. The implementation of risk reduction measures can introduce new risks into the system or increase the significance of other existing risks. Hence, it might be appropriate to revisit the risk assessment to identify and evaluate any possible change in risk after implementing a risk reduction process.

Risk acceptance is a decision to accept risk. Risk acceptance can be a formal decision to accept the residual risk or it can be a passive decision in which residual risks are not specified. For some types of harms, even the best quality risk management practices might not entirely eliminate risk. In these circumstances, it might be agreed that an appropriate quality risk management strategy has been applied and that quality risk is reduced to a specified (acceptable) level. This (specified) acceptable level will depend on many parameters and should be decided on a case-by-case basis.

4.4. Risk Communication

Risk communication is the sharing of information about risk and risk management between the decision makers and others. Parties can communicate at any stage of the risk management process (see Fig. 1: dashed arrows). The output/result of the quality risk management process should be appropriately communicated and documented (see Fig. 1: solid arrows). Communications might include those among interested parties; e.g., regulators and industry, industry and the patient, within a company, industry or regulatory authority, etc. The included information might relate to the existence, nature, form, probability, severity, acceptability, control, treatment, detectability or other aspects of risks to quality. Communication need not be carried out for each and every risk acceptance. Between the industry and regulatory authorities, communication concerning quality risk management decisions might be effected through existing channels as specified in regulations and guidances.

4.5. Risk Review

Risk management should be an ongoing part of the quality management process. A mechanism to review or monitor events should be implemented.

The output/results of the risk management process should be reviewed to take into account new knowledge and experience. Once a quality risk management process has been initiated, that process should continue to be utilized for events that might impact the original quality risk management decision, whether these events are planned (e.g., results of product review, inspections, audits, change control) or unplanned (e.g., root cause from failure investigations, recall). The frequency of any review should be based upon the level of risk. Risk review might include reconsideration of risk acceptance decisions (section 4.3).

5. Summary

The degree of rigor and formality of quality risk management should reflect available knowledge and be commensurate with the complexity and/ or criticality of the issue to be addressed.

Quality risk management is a process that supports science-based and practical decisions when integrated into quality systems. Appropriate use of QRM, however, does not obviate industry's obligation to comply with regulatory requirements.

6. Definitions

Decision Maker(s): Person(s) with the competence and authority to make appropriate and timely quality risk management decisions.

Detectability: The ability to discover or determine the existence, presence, or fact of a hazard.

Harm: Damage to health, including the damage that can occur from loss of product quality or availability.

Hazard: The potential source of harm (ISO/IEC Guide 51).

Product Lifecycle: All phases in the life of the product from the initial development through marketing until the product's discontinuation.

Quality: The degree to which a set of inherent properties of a product, system or process fulfills requirements (see ICH Q6A definition specifically for ''quality'' of drug substance and drug (medicinal) products.)

Quality Risk Management: A systematic process for the assessment, control, communication and review of risks to the quality of the drug (medicinal) product across the product lifecycle.

Quality System: The sum of all aspects of a system that implements quality policy and ensures that quality objectives are met.

Requirements: The explicit or implicit needs or expectations of the patients or their surrogates (e.g., health-care professionals, regulators and legislators). In this document, ''requirements'' refers not only to statutory, legislative, or regulatory requirements, but also to such needs and expectations.

Risk: The combination of the probability of occurrence of harm and the severity of that harm (ISO/IEC Guide 51).

Risk Acceptance: The decision to accept risk (ISO Guide 73).

Risk Analysis: The estimation of the risk associated with the identified hazards.

Risk Assessment: A systematic process of organizing information to support a risk decision to be made within a risk management process. It consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.

Risk Communication: The sharing of information about risk and risk management between the decision maker and other stakeholders.

Risk Control: Actions implementing risk management decisions (ISO Guide 73).

Risk Evaluation: The comparison of the estimated risk to given risk criteria using a quantitative or qualitative scale to determine the significance of the risk.

Risk Identification: The systematic use of information to identify potential sources of harm (hazards) referring to the risk question or problem description.

Risk Management: The systematic application of quality management policies, procedures, and practices to the tasks of assessing, controlling, communicating and reviewing risk.

Risk Reduction: Actions taken to lessen the probability of occurrence of harm and the severity of that harm.

Risk Review: Review or monitoring of output/results of the risk management process considering (if appropriate) new knowledge and experience about the risk.

Severity: A measure of the possible consequences of a hazard.

Stakeholder: Any individual, group or organization that can affect, be affected by, or perceive itself to be affected by a risk. Decision makers might also be stakeholders. For the purposes of this guideline, the primary stakeholders are the patient, healthcare professional, regulatory authority, and industry.

International Harmonization Implemented in the Japanese Pharmacopoeia Seventeenth Edition

Items for which harmonization has been agreed among the European Pharmacopoeia, the United States Pharmacopeia and the Japanese Pharmacopoeia are implemented in the Japanese Pharmacopoeia Seventeenth Edition (JP 17). They are shown in the tables below.

The column headed Harmonized items shows the harmonized items written in the Pharmacopoeial Harmonization Agreement Document, and the column headed JP 17 shows the items as they appear in JP 17. In the Remarks column, notes on any differences between JP 17 and the agreement are shown as occasion demands.

The date on which the agreement has been signed is shown on the top pf each table. In the case where the harmonized items have been revised and/or corrected, this is indicated in parenthesis.

Aug. 2005 (Rev. 2)

Harmonized items	JP 17	Remarks
Residue on Ignition/Sulphated Ash Test	**2.44 Residue on Ignition Test**	
(Introduction)	(Introduction)	JP's particular description: Explanation on this test. Explanation of the description in monograph, etc.
Procedure	1. Procedure	

June 2014

Harmonized items	JP 17	Remarks
Thermal Analysis	**2.52 Thermal Analysis**	
(Introduction)	(Introduction)	JP's particular description: Explanation of the case where thermogravimetry is used as an alternative method of loss on drying or water determination.
Thermogravimetry	1. Thermogravimetry	
Instrument	1.1. Instrument	
Temperature calibration	1.2. Temperature calibration	
Calibration of the electrobalance	1.3. Calibration of the electrobalance	JP's particular description: Setting of Calcium Oxalate Monohydrate RS for Calibration of Apparatus.
Method	1.4. Method	
Differential scanning calorimetry	2. Differential scanning calorimetry	
Instrument	2.1. Instrument	
Calibration of the instrument	2.2. Calibration of the instrument	
Temperature calibration	2.2.1. Temperature calibration	
Heat-quantity calibration	2.2.2. Heat-quantity calibration	
Operating procedure	2.3. Operating procedure	
Applications	2.4. Applications	
Phase changes	2.4.1. Phase changes	
Changes in chemical composition	2.4.2. Changes in chemical composition	
Application to phase diagrams	2.4.3. Application to phase diagrams	
Determination of purity	2.4.4. Determination of purity	
Figure 1 Thermogram	Fig. 1 Thermogram	
Figure 2 Thermal diagrams according to purity	Fig. 2 Thermal diagrams according to purity	
Table 1	Table 1	

Oct. 2007

Harmonized items	JP 17	Remarks
Characterisation of Crystalline and Partially Crystalline Solids by X-ray Powder Diffraction (XRPD)	**2.58 X-Ray Powder Diffraction Method**	
(Introduction)	(Introduction)	JP's particular description: Explanation on this method.
Principle	1. Principle	
Instrument	2. Instrument	
Instrument set-up	2.1 Instrument set-up	

X-ray radiation	2.2 X-ray radiation	
Radiation protection	2.3 Radiation protection	
Specimen preparation and mounting	3. Specimen preparation and mounting	
Specimen preparation	3.1 Specimen preparation	
Specimen mounting		''*Specimen mounting*'' is not stipulated.
Effect of specimen displacement		
Effect of specimen thickness and transparency		
Control of the instrument performance	4. Control of the instrument performance	
Qualitative phase analysis (Identification of phases)	5. Qualitative phase analysis (Identification of phases)	
Quantitative phase analysis	6. Quantitative phase analysis	
Polymorphic samples	6.1 Polymorphic samples	
Methods using a standard	6.2 Methods using a standard	
Estimate of the amorphous and crystalline fractions	7. Estimate of the amorphous and crystalline fractions	
Single crystal structure	8. Single crystal structure	
Figure 1 Diffraction of X-rays by a crystal according to Bragg's law	Fig. 1 Diffraction of X-rays by a crystal according to Bragg's law	
Figure 2 X-ray powder diffraction patterns collected for 5 different solid phases of a substance (the intensities are normalized)	Fig. 2 X-ray powder diffraction patterns collected for 5 different solid phases of a substance (the intensities are normalized)	
Figure 3 Geometric arrangement of the Bragg-Brentano parafocusing geometry	Fig. 3 Geometric arrangement of the Bragg-Brentano parafocusing geometry	

Nov. 2013 (Rev. 3)

Harmonized items	JP 17	Remarks
Bulk Density and Tapped Density of Powders	**3.01 Determination of Bulk and Tapped Densities**	
	(Introduction)	JP's particular description: Explanation of the test method.
Bulk density	1. Bulk density	
Method 1: Measurement in a graduated cylinder	1.1. Method 1: Measurement in a graduated cylinder	
Procedure	1.1.1. Procedure	
Method 2: Measurement in a volumeter	1.2. Method 2: Measurement in a volumeter	
Apparatus	1.2.1. Apparatus	
Procedure	1.2.2. Procedure	
Method 3: Measurement in a vessel	1.3. Method 3: Measurement in a vessel	
Apparatus	1.3.1. Apparatus	
Procedure	1.3.2. Procedure	
Tapped density	2. Tapped density	
Method 1	2.1. Method 1	
Apparatus	2.1.1. Apparatus	
Procedure	2.1.2. Procedure	

Method 2	2.2. Method 2	
Procedure	2.2.1. Procedure	
Method 3	2.3. Method 3	
Procedure	2.3.1. Procedure	
Measures of powder compressibility	3. Measures of powder compressibility	
Figure 1 Volumeter	Fig. 1 Volumeter	
Figure 2 Measuring vessel (left) and cap (right)	Fig. 2 Measuring vessel (left) and cap (right)	
Figure 3	Fig. 3 Tapping apparatus	

Nov. 2003

Harmonized items	JP 17	Remarks
Specific Surface Area	**3.02 Specific Surface Area by Gas Adsorption**	
(Introduction)	(Introduction)	JP's particular description: Explanation on this test.
	1. Measurememts	
Multi-point measurement	1.1. Multi-point measurement	
Single-point measurement	1.2. Single-point measurement	
Sample preparation Outgassing Adsorbate Quantity of sample	2. Sample preparation	
Measurements	3. Methods	
Method 1: The dynamic flow method	3.1. Method 1: The dynamic flow method	
Method 2: The volumetric method	3.2. Method 2: The volumetric method	
Reference materilals	4. Reference materilals	
Figure 1 Schematic diagram of the dynamic flow method apparatus	Fig. 1 Schematic diagram of the dynamic flow method apparatus	
Figure 2 Schematic diagram of the volumetric method apparatus	Fig. 2 Schematic diagram of the volumetric method apparatus	

May 2007

Harmonized items	JP 17	Remarks
Gas Pycnometric Density of Solids	**3.03 Powder Particle Density Determination**	
(Introduction)	(Introduction)	JP's particular description: Objects to be measured by this method.
Apparatus	1. Apparatus	
	2. Calibration of apparatus	The range of temperature during the measurement is described in the Procedure.
Method	3. Procedure	
Expression of the results		
Figure 1 Schematic diagram of a gas pycnometer	Fig. 1 Schematic diagram of a gas pycnometer	

June 2004 (Method 1)/May 2007 (Rev. 1) (Method 2)

Harmonized items	JP 17	Remarks
Paticle Size Determination	**3.04 Particle Size Determination**	
	(Introduction)	JP's particular description: Explanation on this test.
Optical microscopy	**1. Method 1. Optical microscopy**	JP's particular description: Explanation on this test.
Apparatus	1.1. Apparatus	
Adjustment	1.1.1. Adjustment	
Illumination	1.1.1.1. Illumination	
Visual characterization	1.1.1.2. Visual characterization	JP's particular description: Explanation on the method of particle size measurement.
Photographic characterization	1.1.1.3. Photographic characterization	
Preparation of the mount	1.2. Preparation of the mount	
	1.3. Characterization	
Crystallinity characterization	1.3.1. Crystallinity characterization	
Limit test of particle size by microscopy	1.3.2. Limit test of particle size by microscopy	
Particle size characterization	1.3.3. Particle size characterization	
Particle shape characterization	1.3.4. Particle shape characterization	
General observations	1.3.5. General observations	
Figure 1 Commonly used measurements of particle size	Fig. 1 Commonly used measurements of particle size	
Figure 2 Commonly used descriptions of particle shape	Fig. 2 Commonly used descriptions of particle shape	
Analytical sieving	**2. Method 2. Analytical sieving method**	JP's particular description: Explanation on this method.
Principles of analytical sieving	Principles of analytical sieving	
	2.1. Procedure	
Test sieves	2.1.1. Test sieves	
Test specimen	2.1.2. Test specimen	
Agitation methods	2.1.3. Agitation methods	
Endpoint determination	2.1.4. Endpoint determination	
Sieving methods	2.2. Sieving methods	
1) Methanical agitation dry sieving method	2.2.1. Methanical agitation (Dry sieving method)	
2) Air entrainment methods air jet and sonic sifter sieving	2.2.2. Air entrainment methods (Air jet and sonic shifter sieving)	
Interpretation	2.3. Interpretation	
Figure 1 Commonly used measurements of particle size	Fig. 1 Commonly used measurements of particle size	
Figure 2 Commonly used descriptions of particle shape	Fig. 2 Commonly used descriptions of particle shape	
Table 1 Size of standard sieve series in range of interest	Table 1 Size of standard sieve series in range of interest	

Oct. 2009

Harmonized items	JP 17	Remarks
Water-Solid Interactions	**3.05 Water-Solid Interactions: Determinations of Sorption-Desorption Isotherms and the Water Activity**	
	(Introduction)	JP's particular description: Explanation on the relevant methods.
Introduction	not specified	
Physical states of sorbed water	not specified	
Rates of water uptake	not specified	
Determination of Sorption-Desorption Isotherms	1. Determination of Sorption-Desorption Isotherms	
Principle	1.1. Principle	
Methods	1.2. Methods	
Report and interpretation of the data	1.3. Report and interpretation of the data	
Determination of the water activity	2. Determination of the water activity	
Principle	2.1. Principle	
Method	2.2. Method	
Figure 1 Example of an apparatus for the determination of the water sorption (other designs are possible)	Fig. 1 Example of an apparatus for the determination of the water sorption (other designs are possible)	
Table 1 Standard saturated salt solutions	Table 1 Standard saturated salt solutions	

June 2011 (Rev. 2)

Harmonized items	JP 17	Remarks
Bacterial Endotoxins Test	**4.01 Bacterial Endotoxins Test**	
(Introduction)	(Introduction)	
Apparatus	1. Apparatus	
	2. Preparation of solutions	
Preparation of standard endotoxin stock solution	2.1. Standard endotoxin stock solution	
Preparation of standard endotoxin solution	2.2. Standard endotoxin solution	
Preparation of sample solutions	2.3. Sample solutions	
Determination of maximum valid dilution	3. Determination of maximum valid dilution	
Gel-clot technique	4. Gel-clot techniques	
(1) Preparatory testing	4.1. Preparatory testing	
(i) Test for confirmation of labeled lysate sensitivity	4.1.1. Test for confirmation of labeled lysate reagent sensitivity	
(ii) Test for interfering factors	4.1.2. Test for interfering factors	
(2) Limit test	4.2. Limit test	
(i) Procedure	4.2.1. Procedure	
(ii) Interpretation	4.2.2. Interpretation	
(3) Quantitative test	4.3. Quantitative test	
(i) Procedure	4.3.1. Procedure	
(ii) Calculation and interpretation	4.3.2. Calculation and interpretation	

Photometric quantitative techniques	5. Photometric quantitative techniques	
(1) Turbidimetric technique	5.1. Turbidimetric technique	
(2) Chromogenic technique	5.2. Chromogenic technique	
(3) Preparatory testing	5.3. Preparatory testing	
(i) Assurance of criteria for the standard curve	5.3.1. Test for assurance of criteria for the standard curve	
(ii) Test for interfering factors	5.3.2. Test for interfering factors	
(4) Test	5.4. Quantitative test	
(i) Procedure	5.4.1. Procedure	
(ii) Calculation	5.4.2. Calculation of endotoxin concentration	
(iii) Interpretation	5.4.3. Interpretation	
Reagents, test solutions		These reagents and test solutions are being defined in ''9.41 Reagents, Test Solutions''.
Amoebocyte lysate		
Lysate TS		
Water for bacterial endotoxins test (BET)		
Table 1	Table 1	
Table 2	Table 2	
Table 3	Table 3	
Table 4	Table 4	

June 2009 (Rev. 1 – Corr. 1) (I. Microbial Enumeration Tests)/Jun. 2008 (Rev. 1) (II. Test for Specified Micro-organisms)

Harmonized items	JP 17	Remarks
Microbial Limit Test	**4.05 Microbiological Examination of Non-Sterile Produds**	
Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests	**I. Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests**	
1 Introduction	(Introduction)	
2 General procedures	1. General procedures	
3 Enumeration methods	2. Enumeration methods	
4 Growth promotion test, suitability of the counting method and negative controls	3. Growth promotion test, suitability of the counting method and negative controls	
4-1 General considerations		
4-2 Preparation of test strains	3.1. Preparation of test strains	
4-3 Negative control	3.2. Negative control	
4-4 Growth promotion of the media	3.3. Growth promotion of the media	
4-5 Suitability of the counting method in the presence of product	3.4. Suitability of the counting method in the presence of product	
4-6 Results and interpretation	3.5. Results and interpretation	
5 Testing of products	4. Testing of products	
5-1 Amount used for the test	4.1. Amount used for the test	
5-2 Examination of the product	4.2. Examination of the product	
5-3 Interpretation of the results	4.3. Interpretation of the results	

Table 1 Preparation and use of test micro-organisms	Table I-1 Preparation and use of test micro-organisms	
Table 2 Common neutralising agents for interfering substances	Table I-2 Common neutralizing agents for interfering substances	
Table 3 Most-probable-number values of micro-organisms	Table I-3 Most-probable-number values of micro-organisms	
Microbiological Examination of Non-Sterile Products: Tests for Specified Micro-organisms	**II. Microbiological Examination of Non-Sterile Products: Tests for Specified Micro-organisms**	
1 Introduction	(Introduction)	
2 General procedures	1. General procedures	
3 Growth promoting and inhibitory properties of the media, suitability of the test and negative controls	2. Growth promoting and inhibitory properties of the media, suitability of the test and negative controls	
3-1 Preparation of test strains	2.1. Preparation of test strains	
3-2 Negative control	2.2. Negative control	
3-3 Growth promotion and inhibitory properties of the media	2.3. Growth promotion and inhibitory properties of the media	
3-4 Suitability of the test method	2.4. Suitability of the test method	
4 Testing of products	3. Testing of products	
4-1 Bile-tolerant gram-negative bacteria	3.1. Bile-tolerant gram-negative bacteria	
4-2 *Escherichia coli*	3.2. *Escherichia coli*	
4-3 *Salmonella*	3.3. *Salmonella*	
4-4 *Pseudomonas aeruginosa*	3.4. *Pseudomonas aeruginosa*	
4-5 *Staphylococcus aureus*	3.5. *Staphylococcus aureus*	
4-6 *Clostridia*	3.6. *Clostridia*	
4-7 *Candida albicans*	3.7. *Candida albicans*	
5 Recommended solutions and culture media	4. Recommended solutions and culture media	
Table II-1 Growth promoting, inhibitory and indicative properties of media	Table II-1 Growth promoting, inhibitory and indicative properties of media	
Table II-2 Interpretation of results	Table II-2 Interpretation of results	

June 2009 (Rev. 1 – Corr. 3)

Harmonized items	JP 17	Remarks
Sterility	**4.06 Sterility Test**	
(Introduction)	(Introduction)	
Precautions against microbial contamination	1. Precautions against microbial contamination	
Culture media and incubation temperatures	2. Culture media and incubation temperatures	
Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test.		
Fluid thioglycollate medium	(i) Fluid thioglycollate medium	
Soya-bean casein digest medium	(ii) Soya-bean casein digest medium	

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined	3. Suitability of the culture medium	
Sterility	3.1. Sterility	
Growth promotion test of aerobes, anaerobes and fungi	3.2. Growth promotion test of aerobes, anaerobes and fungi	
Method suitability test	4. Method suitability test	
Membrane filtration	(i) Membrane filtration	
Direct inoculation	(ii) Direct inoculation	
Test for sterility of the product to be examined	5. Test for sterility of the product to be examined	
The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined		
Membrane filtration	5.1. Membrane filtration	
Aqueous solutions	(i) Aqueous solutions	
Soluble solids	(ii) Soluble solids	
Oils and oily solutions	(iii) Oils and oily solutions	
Ointments and creams	(iv) Ointments and creams	
Direct inoculation of the culture medium	5.2. Direct inoculation of the culture medium	
Oily liquids	(i) Oily liquids	
Ointments and creams	(ii) Ointments and creams	
Catgut and other surgical sutures for veterinary use		The items not included in JP.
Observation and interpretation of results	6. Observation and interpretation of results	
Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility	7. Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility	
Minimum number of items to be tested	8. Minimum number of items to be tested	
Table 1. Strains of the test micro-organisms suitable for use in the growth promotion test and the method suitability test	Table 1. Strains of the test micro-organisms suitable for use in the growth promotion test and the method suitability test	
Table 2. Minimum quantity to be used for each medium	Table 2. Minimum quantity to be used for each medium	
Table 3. Minimum number of items to be tested	Table 3. Minimum number of items to be tested	JP's particular description: A 100 mL or more of the labeled amount of preparation is defined as the large volume preparation.

Nov. 2010 (Rev. 1)

Harmonized items	JP 17	Remarks
Uniformity of Dosage Units	**6.02 Uniformity of Dosage Units**	
(Introduction)	(Introduction)	JP's particular description: Additional explanation on Liquids. Additional explanation for the part not containing drug substance.

Content uniformity	1. Content uniformity	
Solid dosage forms	(i) Solid dosage forms	
Liquid or Semi-Solid dosage forms	(ii) Liquid or semi-solid dosage forms	
Calculation of acceptance value	1.1. Calculation of acceptance value	
Mass variation	2. Mass variation	JP's particular description: Assuming that the concentration of drug substance is uniform in each lot.
Uncoated or film-coated tablets	(i) Uncoated or film-coated tablets	
Hard capsules	(ii) Hard capsules	
Soft capsules	(iii) Soft capsules	
Solid dosage forms other than tablets and capsules	(iv) Solid dosage forms other than tablets and capsules	
Liquid dosage forms	(v) Liquid dosage forms	The phrase ''in conditions of normal use. If necessary, compute the equivalent volume after determining the density.'' is deleted.
Calculation of acceptance value	2.1. Calculation of acceptance value	
Criteria	3. Criteria	
Solid, Semi-Solid and Liquid dosage forms	(i) Solid, semi-solid and liquid dosage forms	
Table 1 Application of content uniformity (CU) and mass variation (MV) test for dosage forms	Table 1 Application of content uniformity (CU) and mass variation (MV) test for dosage forms	JP's particular description: Addition of ''(divided forms, lyophilized forms)'' and ''(true solution)''.
Table 2	Table 2	

June 2004 (Rev. 1)

Harmonized items	JP 17	Remarks
Test for Extractable Volume of Parenteral Preparations	**6.05 Test for Extractable Volume of Parenteral Preparations**	
(Introduction)	(Introduction)	JP's particular description: Explanation on this Test
Single-dose containers	1. Single-dose containers	
Multi-dose containers	2. Multi-dose containers	
Cartridges and prefilled syringes	3. Cartridges and pre-filled syringes	
Parenteral infusions	4. Parenteral infusions	

June 2004 (Rev. 1)

Harmonized items	JP 17	Remarks
Particulate Matter in Injectables	**6.07 Insoluble Particulate Matter Test for Injections**	
(Introduction)	(Introduction)	
Method 1. Light obscuration particle count test	1. Method 1. Light obscuration particle count test	
	1.1. Apparatus	JP's particular description: Description of evaluation frequency.
	1.1.1. Calibration	JP's particular description
	1.1.1.1. Manual method	JP's particular description
	1.1.1.2. Electronic method	JP's particular description
	1.1.1.3. Automated method	JP's particular description
	1.1.2. Sample volume accuracy	JP's particular description
	1.1.3. Sample flow rate	JP's particular description

	1.1.4. Sensor	JP's particular description
	1.1.4.1. Sensor resolution (Particle size resolution of apparatus)	JP's particular description
	1.1.4.2. Particle counting accuracy	JP's particular description
	1.1.4.3. Threshold accuracy	JP's particular description
General precautions	1.2. General precautions	
Method	1.3. Method	
Evaluation	1.4. Evaluation	JP's particular description: Being classified into ''equal to or more than 100 mL'' and ''less than 100 mL''.
Method 2. Microscopic particle count test	2. Method 2. Microscopic particle count test	
	2.1. Apparatus	
General precautions	2.2. General precautions	
Method	2.3. Method	
Evaluation	2.4. Evaluation	JP's particular description: Being classified into ''equal to or more than 100 mL'' and ''less than 100 mL''.
	3. Reagents	JP's particular description
1. Circular diameter graticule	Fig. 1 Circular diameter graticule	

Oct. 2007 (Rev. 1)

Harmonized items	JP 17	Remarks
Disintegration	**6.09 Disintegration Test**	JP's particular description: This test is applied to Granules, Dry Syrups and Pills.
Apparatus	1. Apparatus	
Basket-rack assembly	(i) Basket-rack assembly	JP's particular description: The apparatus may be varied somewhat provided the specifications.
Disks	(ii) Disks	
	(iii) Auxiliary tube	JP's particular description
Procedure	2. Procedure	
	2.1. Immediate-release preparations	JP's particular description: Setting of a test for Granules, Dry Syrups and Pills. Allowing use of water as the test fluid. Setting of each intervals of the immersion. Setting of a definition of complete disintegration.
	2.2. Enteric coated preparations	Setting of a procedure for Granules. JP's particular description: Setting of a test for delayed-release preparations.
Figure 1 Disintegration apparatus	Fig. 1 Disintegration apparatus	
	Fig. 2 Auxiliary tube	JP's particular description.

June 2010 (Rev. 3)

Harmonized items	JP 17	Remarks
Dissolution	**6.10 Dissolution Test**	JP's particular description: The test also aims at preventing significant bioinequivalence.

Apparatus	1. Apparatus	
Apparatus 1 (Basket apparatus)	1.1. Apparatus for Basket Method (Apparatus 1)	
Apparatus 2 (Paddle apparatus)	1.2. Apparatus for Paddle Method (Apparatus 2)	JP's particular description: The sinker is allowed to use in the case only when specified in the monograph.
Apparatus 3 (Reciprocating cylinder)	not specified	
Apparatus 4 (Flow-through cell)	1.3. Apparatus for Flow-Through Cell Method (Apparatus 3)	
Apparatus suitability	2. Apparatus Suitability	
Procedure	3. Procedure	
Apparatus 1 or 2	3.1. Basket Method or Paddle Method	
Immediate-release dosage forms	3.1.1. Immediate-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	
Extended-release dosage forms	3.1.2. Extended-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	
Delayed-release dosage forms	3.1.3. Delayed-release Dosage Forms	
Procedure	(i) Procedure	Non-harmonized item: Alternative usage of Method A or B.
Method A	not specified	
Method B	not specified	
	(ii) Dissolution Medium	JP's particular description.
Time	(iii) Time	JP's particular description: Time is specified each for the 1st and 2nd fluids.
Apparatus 3	not specified	
Immediate-release dosage forms		
Procedure		
Dissolution medium		
Time		
Extended-release dosage forms		
Procedure		
Dissolution medium		
Time		
Delayed-release dosage forms		
Procedure		
Time		
Apparatus 4	3.2. Flow-Through Cell Method	
Immediate-release dosage forms	3.2.1. Immediate-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	

Extended-release dosage forms	3.2.2. Extended-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	
Delayed-release dosage forms	not specified	
Procedure	not specified	
Time	not specified	
Interpretation	4. Interpretation	JP's particular description: Follow Interpretation 1 when the value Q is specified in the individual monograph, otherwise follow Interpretation 2.
Immediate-release dosage forms	4.1. Immediate-release Dosage Forms	JP's particular description: Setting of Interpretation 2.
	4.1.1. Interpretation 1	
	4.1.2. Interpretation 2	
Extended-release dosage forms	4.2. Extended-release Dosage Forms	JP's particular description: Setting of Interpretation 2.
	4.2.1. Interpretation 1	
	4.2.2. Interpretation 2	
Delayed-release dosage forms	4.3. Delayed-release Dosage Forms	Non-harmonized items: Different dissolution medium. Deletion of disharmonized part on the value Q. JP's particular description: Setting of Interpretation 2.
	4.3.1. Interpretation 1	JP's particular description: The value Q is specified in the individual monograph.
	4.3.2. Interpretation 2	
Acceptance Table 1	Acceptance Table 1	
Acceptance Table 2	Acceptance Table 2	
Acceptance Table 3	Acceptance Table 3	
Acceptance Table 4	Acceptance Table 4	
Figure 1 Apparatus1 Basket stirring element	Fig. 1 Apparatus 1, Basket stirring element	
Figure 2 Paddle stirring element	Fig. 2 Apparatus 2, Paddle stirring element	
Figure 2a Alternative sinker	Fig. 2a Alternative sinker	
Figure 3 Apparatus 3	not specified	
Figure 4 Apparatus 4 (top) large cell for tablets and capsules (bottom) tablet holder for the large cell	Fig. 3 Apparatus 3 (top) large cell for tablets and capsules (bottom) tablet holder for the large cell	
Figure 5 Apparatus 4 (top) small cell for tablets and capsules (bottom) tablet holder for the small cell	Fig. 4 Apparatus 3 (top) small cell for tablets and capsules (bottom) tablet holder for the small cell	

June 2013

Harmonized items	JP 17	Remarks
Isomalt	**Isomalt Hydrate**	
Definition	limits of content	
Identification	Identification (1) (2)	JP's particular description: Color reaction, Standard solution.
Conductivity	Conductivity	
Reducing sugars	Purity (4) Reducing sugars	
Related substances	Purity (3) Related substances	JP's particular description: Test for required detectability, System repeatability.
Nickel	Purity (2) Nickel	
Water	Water	
Assay	Assay	JP's particular description: Standard solution, System repeatability.
Labelling	origin	

June 2014 (Rev. 2, Corr. 2)

Harmonized items	JP 17	Remarks
Ethanol	**Ethanol**	
Definition	limits of content	Setting of specific gravity at 15°C.
Identification A	not specified as Identification	Setting of Specific gravity as the specification.
Identification B	Identification	
Appearance	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Relative density	Specific gravity	Setting of specific gravity at 15°C.
Absorbance	Purity (4) Other impurities (absorbance)	
Volatile impurities	Purity (3) Volatile impurities	
Residue on evaporation	Purity (5) Residue on evaporation	
Storage	Containers and storage	

June 2014 (Rev. 2, Corr. 2)

Harmonized items	JP 17	Remarks
Ethanol, Anhydrous	**Anhydrous Ethanol**	
Definition	limits of content	Setting of specific gravity at 15°C.
Identification A	not specified as Identification	Setting of Specific gravity as the specification.
Identification B	Identification	
Appearance	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Relative density	Specific gravity	Setting of specific gravity at 15°C.
Absorbance	Purity (4) Other impurities (absorbance)	
Volatile impurities	Purity (3) Volatile impurities	
Residue on evaporation	Purity (5) Residue on evaporation	
Storage	Containers and storage	

Nov. 2005

Harmonized items	JP 17	Remarks
Calcium Disodium Edetate	**Calcium Sodium Edetate Hydrate**	
Definition	limits of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (3)	
pH	pH	
Purity (1) Chloride	Purity (2) Chloride	
Purity (2) Disodium edetate	Purity (4) Disodium edetate	
Water	Water	
Assay	Assay	

Nov. 2013 (Rev. 3)

Harmonized items	JP 17	Remarks
Sodium Chloride	**Sodium Chloride**	
Definition	limits of the content	
Identification A	Identification (1)	
Identification B	Identification (2)	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Bromides	Purity (5) Bromides	
Ferrocyanides	Purity (7) Ferrocyanides	
Iodides	Purity (6) Iodides	
Nitrites	not specified	
Phosphates	Purity (4) Phosphates	
Sulphates	Purity (3) Sulfates	
Aluminium	not specified	
Barium	Purity (10) Barium	
Iron	Purity (9) Iron	
Magnesium and alkaline-earth metals	Purity (11) Magnesium and alkaline-earth materials	
Potassium	not specified	
Loss on drying	Loss on drying	
Assay	Assay	

Nov. 2011 (Rev. 1)

Harmonized items	JP 17	Remarks
Carmellose	**Carmellose**	
Definition	origin	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Purity (1) Chloride	Purity (1) Chloride	
Purity (2) Sulfate	Purity (2) Sulfate	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	

July 2003 (Rev. 1)

Harmonized items	JP 17	Remarks
Carboxymethylcellulose Calcium	**Carmellose Calcium**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
Identification D	Identification (4)	
Alkalinity	Purity (1) Alkalinity	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Limit of chloride	Purity (2) Chloride	
Limit of sulfate	Purity (3) Sulfate	

Oct. 2001

Harmonized items	JP 17	Remarks
Croscarmellose Sodium	**Croscarmellose Sodium**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Settling volume	Precipitation test	
Degree of substitution	Degree of substitution	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Packaging and storage	Containers and storate	

June 2010 (Rev. 2)

Harmonized items	JP 17	Remarks
Citric Acid, Anhydrous	**Anhydrous Citric Acid**	
Definition	limits of content	
Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Oxalic acid	Purity (3) Oxalic acid	
Sulphates	Purity (2) Sulfates	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Assay	Assay	

June 2010 (Rev. 2)

Harmonized items	JP 17	Remarks
Citric Acid Monohydrate	**Citric Acid Hydrate**	
Definition	limits of content	

Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Oxalic acid	Purity (3) Oxalic acid	
Sulphates	Purity (2) Sulfates	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Assay	Assay	

Nov. 2010

Harmonized items	JP 17	Remarks
Crospovidone	**Crospovidone**	
Definition	limits of content	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Particle size	
Peroxides	Purity (4) Peroxides	
Water-soluble substances	Purity (2) Water-soluble substances	
Impurity A	Purity (3) 1-Vinyl-2-pyrrolidone	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Assay	Assay	
Storage	Containers and storage	

June 2013 (Rev. 1)

Harmonized items	JP 17	Remarks
Saccharin	**Saccharin**	
Definition	limits of content	
Identification	Identification	
Color and clarity of solution	Purity (1)	
Loss on drying	Loss on drying	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Residue on ignition	Residue on ignition	
Limit of benzoate and salicylate	Purity (3) Benzoate and salicylate	
Assay	Assay	

Feb. 2004 (Rev. 1)

Harmonized items	JP 17	Remarks
Saccharin Sodium	**Saccharin Sodium Hydrate**	
Definition	limits of content	
Identification B, C	Identification (2)	
Acidity or alkalinity	Purity (2) Acid or alkali	
Limit of benzoate and salicylate	Purity (4) Benzoate and salicylate	

Readily carbonisable substances	Purity (6) Readily carbonizable substances	
Water	Water	
Assay	Assay	

Nov. 2014 (Rev. 1, Corr. 1)

Harmonized items	JP 17	Remarks
Stearic Acid	**Stearic Acid**	
Definition	limits of content	
Identification A	not specified as Identification	
Identification B	not specified as Identification	Being specified as Acid value
Identification C	not specified as Identification	
Acidity	Purity (1) Acidity	
Iodine value	Iodine value	
Freezing point	Congealing point	JP's particular description: Apparatus
Assay	Assay	JP's particular description: Split ratio, Time span of measurement, Test for required detectability.
Labelling (type of stearic acid)	origin	

June 2013 (Corr. 3)

Harmonized items	JP 17	Remarks
Magnesium Stearate	**Magnesium Stearate**	
Definition	limits of content	
Identification A	Identification	
Identification B		Not specified because of a part of ''Relative content of stearic acid and palmitic acid''.
Acidity or alkalinity	Purity (1) Acidity or alkalinity	
Loss on drying	Loss on drying	
Limit of chloride	Purity (2) Chloride	
Limit of sulfate	Purity (3) Sulfate	
Limit of cadmium	not specified	
Limit of lead	not specified	
Limit of nickel	not specified	
Relative content of stearic acid and palmitic acid	Relative content of stearic acid and palmitic acid	JP's particular description: Time span of measurement, Test for required detectability.
Assay	Assay	

June 2012 (Rev. 1, Corr.1)

Harmonized items	JP 17	Remarks
Cellacefate	**Cellacefate**	
Definition	Content of the acetyl and carboxybenzoyl groups	
Identification	Identification	
Identification B	Identification	
Viscosity	Viscosity	
Water	Water	

Residue on ignition	Residue on ignition	
Limit of free acid	Purity (2) Free acids	
Phthalyl content	Assay (1) Carboxybenzoyl group	
Content of acetyl	Assay (2) Acetyl group	

June 2014 (Corr. 2)

Harmonized items	JP 17	Remarks
Gelatin (Gelling Grade)	**Gelatin**	
Definition	origin	In JP, gelatin prepared by enzymatic degradation is not included in the origin.
Identification A	Identification (1)	
Identification B	Identification (2)	
pH	pH	
Conductivity	Conductivity	
Sulphur dioxide	Purity (7) Sulfur dioxide	
Peroxides	Purity (6) Peroxides	
Gel strength (Bloom value)	Gel strength (Bloom value)	
Iron	Purity (2) Iron	
Chromium	Purity (3) Chromium	
Zinc	Purity (4) Zinc	
Loss on drying	Loss on drying	
Microbial contamination	Microbial limit	
Storage	Containers and storage	
Labelling	origin	

May 2005 (Rev. 1)

Harmonized items	JP 17	Remarks
Microcrystalline Cellulose	**Microcrystalline Cellulose**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (3)	
pH	pH	
Water-soluble substances	Purity (2) Water- soluble substances	
Ether-soluble substances	Purity(3) Diethyl ether-soluble substances	
Conductivity	Conductivity	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Bulk density	Bulk density	

May 2005 (Rev. 1)

Harmonized items	JP 17	Remarks
Powdered Cellulose	**Powdered Cellulose**	
Definition	origin	
Labeling	Labeling of mean degree of polymerization	
Identification A	Identification (1)	

Identification B	Identification (3)	
pH	pH	
Water-soluble substances	Purity (2) Water-soluble substances	
Ether-soluble substances	Purity (3) Diethyl ether- soluble substances	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	

June 2008 (Rev. 1)

Harmonized items	JP 17	Remarks
Talc	**Talc**	
Definition	origin, content of magnesium	
Identification	Identification	
Acidity and alkalinity	Purity (1) Acidity or alkalinity	
Aluminium	Purity (5) Aluminum	
Calcium	Purity (7) Calcium	
Iron	Purity (4) Iron	
Lead	Purity (6) Lead	
Magnesium	Assay	
Loss on ignition	Loss on ignition	

June 2011 (Rev. 2)

Harmonized items	JP 17	Remarks
Wheat Starch	**Wheat Starch**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Total protein	Purity (5) Total protein	Non-harmonized item: Decomposition accelerator
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Microbial contamination	not specified	

Nov. 2013 (Rev. 1)

Harmonized items	JP 17	Remarks
Rice Starch	**Rice Starch**	
Definition	Origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Oxidising substances	Purity (2) Oxidizing substances	

Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Microbial contamination	not specified	

June 2012 (Rev. 3)

Harmonized items	JP 17	Remarks
Corn Starch	**Corn Starch**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Limit of iron	Purity (1) Iron	
Limit of oxidizing substances	Purity (2) Oxidizing substances	
Limit of sulfur dioxide	Purity (3) Sulfur dioxide	
Microbial limits	not specified	

June 2011 (Rev. 2)

Harmonized items	JP 17	Remarks
Potato Starch	**Potato Starch**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Microbial contamination	not specified	

June 2013 (Rev. 3)

Harmonized items	JP 17	Remarks
Sodium Starch Glycolate	**Sodium Starch Glycolate**	
Definition	origin, limit of sodium	
Identification A	Identification (1)	
Identification B	Identification (3)	
pH	pH	
Loss on drying	Loss on drying	
Limit of iron	Purity (2) Iron	
Limit of sodium chloride	Purity (4) Sodium chloride	
Limit of sodium glycolate	Purity (3) Sodium glycolate	

Assay	Assay	
Microbial Contamination	Microbial limits	

Nov. 2010 (Rev. 4)

Harmonized items	JP 17	Remarks
Anhydrous Lactose	**Anhydrous Lactose**	
Definition	origin	
Identification	Identification	
Clarity and color of solution	Purity (1) Clarity and color of solution	No use of the reference suspension I
Specific rotation	Optical rotation	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Water	Water	
Protein and light-absorbing impurities	Purity (4) Protein and light absorbing substances	
Content of alpha and beta anomers	Isomer ratio	JP's particular description: System repeatability.
Microbial contamination	Microbial limit	JP's particular description: TYMC, *Salmonella*.

June 2008 (Rev. 2)

Harmonized items	JP 17	Remarks
Lactose Monohydrate	**Lactose Hydrate**	
Definition	origin	
Clarity and color of solution	Purity (1) Clarity and color of solution	
Identification	Identification	
Specific rotation	Optical rotation	
Acidity or alkalinity	Purity (2) Acid and alkali	
Residue on ignition	Residue on ignition	
Water	Water	
Protein and light-absorbing impurities	Purity (4) Protein and light absorbing substances	

Oct. 2007

Harmonized items	JP 17	Remarks
Sucrose	**Sucrose**	
Definition	origin	
Appearance of solution	Purity (2) Clarity of solution	
Conductivity	Conductivity	
Specific optical rotation	Optical rotation	
Dextrins	Dextrins	
Reducing sugars	Purity (4) Reducing sugars	
Sulphite	Purity (3) Sulfite	
Loss on drying	Loss on drying	
Bacterial endotoxins	Bacterial endotoxins	
Labelling	origin	

Nov. 2011 (Rev. 1, Corr.1)

Harmonized items	JP 17	Remarks
Ethyl Parahydroxybenzoate	**Ethyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

June 2010 (Rev. 1)

Harmonized items	JP 17	Remarks
Butyl Parahydroxybenzoate	**Butyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

Nov. 2011 (Rev. 1, Corr.1)

Harmonized items	JP 17	Remarks
Propyl Parahydroxybenzoate	**Propyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

Nov. 2011 (Rev. 1, Corr.1)

Harmonized items	JP 17	Remarks
Methyl Parahydroxybenzoate	**Methyl Parahydroxybenzoate**	
Definition	limits of content	

Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

June 2013

Harmonized items	JP 17	Remarks
Hydroxypropylcellulose	**Hydroxypropylcellulose**	
Definition	origin, limits of hydroxypropoxy group	
Identification A	Identification (1)	
Identification B	Identification (2)	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Silica	Purity (2) Silicon dioxide	
Assay for hydroxypropoxy groups	Assay	

June 2014 (Rev. 1, Corr. 1)

Harmonized items	JP 17	Remarks
Hypromellose	**Hypromellose**	
Definition	limits of content of methoxy group and hydroxypropoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

June 2006

Harmonized items	JP 17	Remarks
Hypromellose Phthalate	**Hypromellose Phthalate**	
Definition	origin, limit of carboxybenzoyl	
Packaging and storage	Containers and storage	

Viscosity	Viscosity	
Water	Water	
Residue on ignition	Residue on ignition	
Chloride	Purity (1) Chloride	
Limit of free phthalic acid	Purity (3) Phthalic acid	JP's particular description: System performance.
Phthalyl content	Assay	

June 2011 (Rev. 2, Corr.1)

Harmonized items	JP 17	Remarks
Benzyl Alcohol	**Benzyl Alcohol**	
Definition	limits of content	
Refractive index	Refractive index	
Acidity	Purity (2) Acidity	
Benzaldehyde and other related substances	Purity (3) Benzaldehyde and other related substances	
Peroxide value	Purity (4) Peroxide value	
Residue on evaporation	Purity (5) Residue on evaporation	
Assay	Assay	

Nov. 2015 (Rev. 1, Corr. 1)

Harmonized items	JP 17	Remarks
Povidone	**Povidone**	
Definition	origin, limits of content	
Labelling	origin	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
pH	pH	
Purity (1) Aldehydes	Purity (3) Aldehydes	
Purity (2) 1-Vinyl-2-pyrrolidone	Purity (4) 1-Vinyl-2-pyrrolidone	
Purity (3) Peroxides	Purity (5) Peroxides	
Purity (4) Hydrazine	Purity (6) Hydrazine	
Purity (5) Formic acid	Purity (7) Formic acid	
Purity (6) 2-Pyrrolidone	Purity (8) 2-Pyrrolidone	
Water	Water	
Residue on ignition	Residue on ignition	
K-value	K-value	
Assay	Assay	

Nov. 2014 (Corr. 2)

Harmonized items	JP 17	Remarks
Polysorbate 80	**Polysorbate 80**	
Definition	origin	
Identification (Composition of fatty acids)	Identification	
Acid value	Acid value	JP's particular description: Applying Fats and Fatty Oils Test 1.13, using ethanol (95) as the solvent.

Hydroxyl value	Hydroxyl value	
Peroxide value	Purity (3) Peroxide value	
Saponification value	Saponification value	
Composition of fatty acids	Composition of fatty acid	
Ethylene oxide and dioxan	Purity (2) Ethylene oxide and 1,4-dioxane	
Water	Water	
Total ash	Residue on ignition	
Storage	Containers and storage	

June 2014 (Corr. 1)

Harmonized items	JP 17	Remarks
Mannitol	**D-Mannitol**	
Definition	limits of content	
Identification by IR	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Conductivity	Conductivity	
Melting point	Melting point	
Reducing sugars	Purity (5) Glucose	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability System repeatability
Nickel	Purity (3) Nickel	
Loss on drying	Loss on drying	
Microbial contamination	not specified	
Bacterial endotoxins	not specified	
Assay	Assay	JP's particular description: System repeatability
Labelling	not specified	

July 2015 (Rev. 2, Corr. 2)

Harmonized items	JP 17	Remarks
Methylcellulose	**Methylcellulose**	
Definition	limits of content of methoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

June 2011 (Rev. 1)

Harmonized items	JP 17	Remarks
Anhydrous Dibasic Calcium Phosphate	**Anhydrous Dibasic Calcium Phosphate**	
Definition	limits of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Acid-insoluble substances	Purity (1) Acid-insoluble substances	
Chloride	Purity (2) Chloride	
Sulfate	Purity (3) Sulfates	
Carbonate	Purity (4) Carbonate	
Barium	Purity (6) Barium	
Loss on ignition	Loss on ignition	
Assay	Assay	

June 2011 (Rev. 1)

Harmonized items	JP 17	Remarks
Dibasic Calcium Phosphate	**Dibasic Calcium Phosphate Hydrate**	
Definition	limits of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Acid-insoluble substances	Purity (1) Acid-insoluble substances	
Chloride	Purity (2) Chloride	
Sulfate	Purity (3) Sulfates	
Carbonate	Purity (4) Carbonate	
Barium	Purity (6) Barium	
Loss on ignition	Loss on ignition	
Assay	Assay	

May 2007

Harmonized items	JP 17	Remarks
Powder Fineness	**General Information** **Powder Fineness**	

June 2004

Harmonized items	JP 17	Remarks
Powder Flow	**General Information** **Powder Flow**	
(Introduction)	(Introduction)	
Angel of repose	1. Angel of repose	
Basic methods for angel of repose	1.1 Basic methods for angel of repose	
Variations in angel of repose methods	1.2 Variations in angel of repose methods	
Angel of repose general scale of flowability	1.3 Angel of repose general scale of flowability	
Experimental considerations for angle of repose	1.4 Experimental considerations for angle of repose	
Recommended procedure for angle of repose	1.5 Recommended procedure for angle of repose	

Compressibility index and Hausner ratio	2. Compressibility index and Hausner ratio	
Basic methods for compressibility index and Hausner ratio	2.1 Basic methods for compressibility index and Hausner ratio	
Experimental considerations for the compressibility index and Hausner ratio	2.2 Experimental considerations for the compressibility index and Hausner ratio	
Recommended procedure for compressibility index and Hausner ratio	2.3 Recommended procedure for compressibility index and Hausner ratio	
Flow through an orifice	3. Flow through an orifice	
Basic methods for flow through an orifice	3.1 Basic methods for flow through an orifice	
Variations in methods for flow through an orifice	3.2 Variations in methods for flow through an orifice	
General scale of flowability for flow through an orifice	3.3 General scale of flowability for flow through an orifice	
Experimental considerations for flow through an orifice	3.4 Experimental considerations for flow through an orifice	
Recommended procedure for flow through an orifice	3.5 Recommended procedure for flow through an orifice	
Shear cell methods	4. Shear cell methods	
Basic methods for shear cell	4.1 Basic methods for shear cell	
Recommendations for shear cell	4.2 Recommendations for shear cell	
Table 1 Flow properties and corresponding angle of repose	Table 1 Flow properties and corresponding angle of repose	
Table 2 Scale of flowability	Table 2 Scale of flowability	

Nov. 2008

Harmonized items	JP 17	Remarks
	General Information	
Particle-size Analysis by Laser Light Diffraction	**Laser Diffraction Measurement of Particle Size**	
Introduction	Introduction	
Principle	1. Principle	
Instrument	2. Instrument	
Development of the method	3. Development of the method	
Sampling	3.1. Sampling	
Evaluation of the dispersion procedure	3.2. Evaluation of the dispersion procedure	
Optimisation of the liquid dispersion	3.3. Optimization of the liquid dispersion	
Optimisation of the gas dispersion	3.4. Optimization of the gas dispersion	
Determination of the concentration range	3.5. Determination of the concentration range	
Determination of the measuring time	3.6. Determination of the measuring time	
Selection of an appropriate optical model	3.7. Selection of an appropriate optical model	
Validation	3.8. Validation	
Measurement	4. Measurement	
Precautions	4.1. Precautions	

Measurement of the light scattering of dispersed sample(s)	4.2. Measurement of the light scattering of dispersed sample(s)	
Conversion of scattering pattern into particle-size distribution	4.3. Conversion of scattering pattern into particle-size distribution	
Replicates	4.4. Replicates	
Reporting of results	5. Reporting of results	
Control of the instrument performance	6. Control of the instrument performance	
Calibration	6.1. Calibration	
Qualification of the system	6.2. Qualification of the system	
Figure 1 Example of a set-up of laser light diffraction instrument	Fig. 1 Example of a set-up of laser light diffraction instrument	
Note	*Note* 1	
	Note 2	The first sentence of the harmonized text is described as Note 2.

Sep. 2002

Harmonized items	JP 17	Remarks
	General Information	
Amino Acid Analysis	**Amino Acid Analysis**	
Apparatus	Apparatus	
General precautions	General precautions	
Reference standard material	Reference standard material	
Calibration of instrumentation	Calibration of instrumentation	
Repeatability	Repeatability	
Sample preparation	Sample preparation	
Internal standards	Internal standards	
Protein hydrolysis	Protein hydrolysis	
Method 1	Method 1	
Hydrolysis solution	Hydrolysis solution	
Procedure	Procedure	
Liquid phase hydrolysis	Liquid phase hydrolysis	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 2	Method 2	
Hydrolysis solution	Hydrolysis solution	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 3	Method 3	
Hydrolysis solution	Hydrolysis solution	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 4	Method 4	
Oxidation solution	Oxidation solution	
Procedure	Procedure	
Method 5	Method 5	
Hydrolysis solution	Hydrolysis solution	
Liquid phase hydrolysis	Liquid phase hydrolysis	
Method 6	Method 6	
Hydrolysis solution	Hydrolysis solution	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 7	Method 7	

Reducing solution	Reducing solution	
Procedure	Procedure	
Method 8	Method 8	
Stock solutions	Stock solutions	
Reducing solution	Reducing solution	
Procedure	Procedure	
Method 9	Method 9	
Stock solutions	Stock solutions	
Carboxymethylation solution	Carboxymethylation solution	
Buffer solution	Buffer solution	
Procedure	Procedure	
Method 10	Method 10	
Reducing solution	Reducing solution	
Procedure	Procedure	
Method 11	Method 11	
Reducing solutions	Reducing solutions	
Procedure	Procedure	
Methodologies of amino acid analysis general principles	Methodologies of amino acid analysis general principles	
Method 1-Postcolumn ninhydrin detection general principle	Method 1-Postcolumn ninhydrin detection general principle	
Method 2-Postcolumn OPA fluorometric detection general principle	Method 2-Postcolumn OPA fluorometric detection general principle	
Method 3-Precolumn PITC derivatization general principle	Method 3-Precolumn PITC derivatization general principle	
Method 4-Precolumn AQC derivatization general principle	Method 4-Precolumn AQC derivatization general principle	
Method 5-Precolumn OPA derivatization general principle	Method 5-Precolumn OPA derivatization general principle	
Method 6-Precolumn DABS-Cl derivatization general principle	Method 6-Precolumn DABS-Cl derivatization general principle	
Method 7-Precolumn FMOC-Cl derivatization general principle	Method 7-Precolumn FMOC-Cl derivatization general principle	
Method 8-Precolumn NBD-F derivatization general principle	Method 8-Precolumn NBD-F derivatization general principle	
Data calculation and analysis	Data calculation and analysis	
Calculations	Calculations	
Amino acid mole percent	Amino acid mole percent	
Unknown protein samples	Unknown protein samples	
Known protein samples	Known protein samples	

June 2014 (Rev. 1)

Harmonized items	JP 17	Remarks
	General Information	
Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	**SDS-Polyacrylamide Gel Electrophoresis**	
Characteristics of polyacrylamide gels	1. Characteristics of polyacrylamide gels	
Denaturing polyacrylamide gel electrophoresis	2. Denaturing polyacrylamide gel electrophoresis	

Reducing conditions	2.1. Reducing conditions	
Non-reducing conditions	2.2. Non-reducing conditions	
Characteristics of discontinuous buffer system gel electrophoresis	3. Characteristics of discontinuous buffer system gel electrophoresis	
Preparing vertical discontinuous buffer SDS-polyacrylamide gels	4. Preparing vertical discontinuous buffer SDS polyacrylamide gels	
Assembling of the gel moulding cassette	4.1. Assembling the gel moulding cassette	
Preparation of the gel	4.2. Preparation of the gel	
Preparation of the resolving gel	4.2.1. Preparation of the resolving gel	
Preparation of the stacking gel	4.2.2. Preparation of the stacking gel	
Preparation of the sample	4.3. Preparation of the sample	
Mounting the gel in the electrophoresis apparatus and electrophoretic separation	4.4. Mounting the gel in the electrophoresis apparatus and electrophoretic separation	
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) — Gradient concentration gels	4.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) — Gradient concentration gels	
Detection of protein in gels	4.6. Detection of proteins in gels	
Coomassie staining	4.6.1. Coomassie staining	
Silver staining	4.6.2. Silver staining	
Recording of the results	4.7. Recording of the results	
Molecular-mass determination	4.8. Molecular-mass determination	
Validation of the test	4.9. Validation of the test	
Quantification of impurities	4.10. Quantification of impurities	
Reagents	5. Reagents	
30 per cent acrylamide/bisacrylamide (29:1) solution	(i) 30% acrylamide/bisacrylamide (29:1) solution	
1.5 M tris-hydrochloride buffer solution pH 8.8	(x) 1.5 M tris-hydrochloride buffer solution (pH 8.8)	
SDS-PAGE sample buffer (concentrated)	(iii) SDS-PAGE sample buffer (concentrated)	
SDS-PAGE sample buffer for reducing conditions (concentrated)	(iv) SDS-PAGE sample buffer for reducing conditions (concentrated)	
SDS-PAGE running buffer	(ii) SDS-PAGE running buffer	
Coomassie staining solution	(v) Coomassie staining solution	
Destaining solution	(ix) Destaining solution	
Fixing solution	(vii) Fixing solution	
Silver nitrate reagent	(viii) Silver nitrate reagent	
Developer solution	(vi) Developer solution	
Blocking solution	(xi) Blocking solution	
Table 1 Preparation of resolving gel	Table 1 Preparation of resolving gel	
Table 2 Preparation of stacking gel	Table 2 Preparation of stacking gel	

June 2010 (Corr. 2)

Harmonized items	JP 17	Remarks
	General Information	
Capillary Electrophoresis	**Capillary Electrophoresis**	
General principles	(General principles)	
Apparatus	Apparatus	

Capillary zone electrophoresis	1. Capillary Zone Electrophoresis	
Optimisation	Optimization	
Instrumental parameters	Instrumental parameters	
Voltage	(1) Voltage	
Polarity	(2) Polarity	
Temperature	(3) Temperature	
Capillary	(4) Capillary	
Electrolytic solution parameters	Electrolytic solution parameters	
Buffer type and concentration	(1) Buffer type and concentration	
Buffer pH	(2) Buffer pH	
Organic solvents	(3) Organic solvents	
Additives for chiral separations	(4) Additives for chiral separations	
Capillary gel electrophoresis	2. Capillary Gel Electrophoresis	
Characteristics of gels	Characteristics of Gels	
Capillary isoelectric focusing	3. Capillary Isoelectric Focusing	
Loading step	(1) Loading step	
loading in one step	(i) loading in one step	
sequential loading	(ii) sequential loading	
Focusing step	(2) Focusing step	
Mobilisation step	(3) Mobilization step	
Optimisation	Optimization	
Voltage	(1) Voltage	
Capillary	(2) Capillary	
Solutions	(3) Solutions	
Micellar electrokinetic chromatography (MEKC)	4. Micellar Electrokinetic Chromatography (MEKC)	
Optimisation	Optimization	
Instrumental parameters	Instrumental parameters	
Voltage	(1) Voltage	
Temperature	(2) Temperature	
Capillary	(3) Capillary	
Electrolytic solution parameters	Electrolytic solution parameters	
Surfactant type and concentration	(1) Surfactant type and concentration	
Buffer pH	(2) Buffer pH	
Organic solvents	(3) Organic solvents	
Additives for chiral separations	(4) Additives for chiral separations	
Other additives	(5) Other additives	
Quantification	Quantification	
Calculations	Calculations	
System Suitability	System Suitability	
Apparent number of theoretical plates	Apparent Number of Theoretical Plates	
Resolution	Resolution	
Symmetry factor	Symmetry Factor	
Signal-to-noise ratio	Signal-to-noise Ratio	

Sep. 2002

Harmonized items	JP 17	Remarks
	General Information	
Total Protein Assay	**Total Protein Assay**	
Method 1	Method 1 (UV method)	
Standard solution	Standard Solution	
Test solution	Test Solution	
Procedure	Procedure	
Light-scattering	Light-Scattering	
Calculations	Calculations	
Method 2	Method 2 (Lowry method)	JP's particular description: Explanatory footnote ''Example: the Minimum Requirements for Biological Products and individual monograph of JP'' is added.
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Reagents and solutions	Reagents and Solutions	
Copper sulfate reagent	Copper Sulfate Reagent	
SDS Solution	5% SDS TS	
Sodium hydroxide solution	Sodium Hydroxide Solution	
Alkaline copper reagent	Alkaline Copper Reagent	
Diluted folin-ciocalteu's phenol reagent	Diluted Folin's TS	
Procedure	Procedure	
Calculations	Calculations	
Interfering substances	Interfering Substances	
Sodium deoxycholate reagent	Sodium Deoxycholate Reagent	
Trichloroacetic acid reagent	Trichloroacetic Acid Reagent	
Procedure	Procedure	
Method 3	Method 3 (Bradford method)	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Coomassie reagent	Coomassie Reagent	
Procedure	Procedure	
Calculations	Calculations	
Method 4	Method 4 (Bicinchonic acid method)	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Reagents	Reagents and Solutions	
BCA Reagent	BCA Reagent	
Copper sulfate reagent	Copper Sulfate Reagent	
Copper-BCA reagent	Copper-BCA Reagent	
Procedure	Procedure	
Calculations	Calculations	
Method 5	Method 5 (Biuret method)	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	

Biuret reagent	Biuret Reagent	
Procedure	Procedure	
Calculations	Calculations	
Interfering substances	Interfering Substances	
Comments	Comments	
Method 6	Method 6 (Fluorometric method)	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Reagents	Reagents and Solutions	
Borate buffer	Borate Buffer	
Stock OPA reagent	Stock OPA Reagent	
OPA reagent	OPA Reagent	
Procedure	Procedure	
Calculations	Calculations	
Method 7	Method 7 (Nitrogen method)	
Procedure A	Procedure A	
Procedure B	Procedure B	
Calculations	Calculations	

Sep. 2002

Harmonized items	JP 17	Remarks
	General Information	
Isoelectric Focusing	**Isoelectric Focusing**	
General principles	(General principles)	
Theoretical aspects	Theoretical Aspects	
Practical aspects	Practical Aspects	
Apparatus	Apparatus	
Isoelectric focusing in polyacrylamide gels: detailed procedure	Isoelectric Focusing in Polyacrylamide Gels: Detailed Procedure	
Preparation of the gels	Preparation of the Gels	
1) Mould	Mould	
2) 7.5 per cent polyacrylamide gel	7.5% Polyacrylamide gel	
3) Preparation of the mould	Preparation of the mould	
Method	Method	
Variations to the detailed procedure (subject to validation)	Variations to the Detailed Procedure (Subject to Validation)	
Validation of iso-electric focusing procedures	Validation of Iso-Electric Focusing Procedures	
Specified variation to the general method	Specified Variations to the General Method	
Point to consider	Points to Consider	
Reagents	Reagents and Solutions	
Fixing solution for isoelectric focusing in polyacrylamide gel	Fixing solution for isoelectric focusing in polyacrylamide gel	
	Coomassie staining TS	JP's particular description
	Destaining solution	JP's particular description

Sep. 2002

Harmonized items	JP 17	Remarks
	General Information	
Peptide Mapping	**Peptide Mapping**	
Purpose and scope	Purpose and scope	
The peptide map	1. The peptide map	
Isolation and purification	2. Isolation and purification	
Selective cleavage of peptide bonds	3. Selective cleavage of peptide bonds	
Pretreatment of sample	3.1. Pretreatment of sample	
Pretreatment of the cleavage agent	3.2. Pretreatment of the cleavage agent	
Pretreatment of the protein	3.3. Pretreatment of the protein	
Establishment of optimal digestion conditions	3.4. Establishment of optimal digestion conditions	
pH	(i) pH	
Temperature	(ii) Temperature	
Time	(iii) Time	
Amount of cleavage agent	(iv) Amount of cleavage agent	
Chromatographic separation	4. Chromatographic separation	
Chromatographic column	4.1. Chromatographic column	
solvent	4.2. Solvent	
Mobile phase	4.3. Mobile phase	
Gradient selection	4.4. Gradient selection	
Isocratic selection	4.5. Isocratic selection	
Other parameters	4.6. Other parameters	
Validation	4.7. Validation	
Analysis and identification of peptides	5. Analysis and identification of peptides	
Table 1 Examples of cleavage agents	Table 1 Examples of cleavage agents	
Table 2 Techniques used for the separation of peptides	Table 2 Techniques used for the separation of peptides	

Nov. 2005

Harmonized items	JP 17	Remarks
	General Information	
Microbiological Examination of Non-Sterile Products:	**Microbial Attributes of Non-Sterile Pharmaceutical Products**	JP's particular description: 1. Definitions 2. Scope 3. Sampling plan and frequency of testing 4. Microbial control program
Acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use	5. Microbial acceptance criteria for non-sterile pharmaceutical products	JP's particular description: Explanation of the microbial acceptance criteria JP's particular description: 6. Acceptance criteria for crude drugs and crude drug preparations

Table 2. Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use Table 1. Acceptance criteria for microbiological quality of non-sterile dosage forms	Table 1. Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use Table 2. Acceptance criteria for microbiological quality of non-sterile dosage forms Table 3. Acceptance criteria for crude drugs and crude drug preparations	 JP's particular description

Feb. 2004

Harmonized items	JP 17	Remarks
	General Information	
Tablet Friability	**Tablet Friability Test**	

Appendix

Atomic Weight Table (2010)

In 1961 it was decided that the atomic weights of the elements would be based on values relative to the mass number of 12 (no fractions) for carbon (^{12}C). Ever since, there has been a marked improvement in the quality and quantity of data on the nuclide masses and isotope ratios of the elements using physical methods such as mass spectrometry. The Commission on Isotope Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC) collected and examined newly measured data and publishes a new atomic weight table every two years (in the odd years). Based on this table, in April of each year the Atomic Weight Subcommittee of the Chemical Society of Japan also publishes an atomic weight table. The numbers of the following Atomic Weight Table (2010) is based on the numbers published by the IUPAC in 2007[1]. For a more detailed explanation, the user is referred to a report[2] and a review[3] published by the CIAAW.

The atomic weight values of each of the elements shown in the atomic weight tables are, as stated in the preface to the table, for elements that originate on Earth and are present in substances that exist naturally. Atomic weights are, with the exception of single nuclide elements (elements consisting of one stable nuclide), not natural constants like the speed of light, but rather change depending on a variety of factors, such as the method of treatment or the origin of the substance containing that element. This is because the atomic weight is dependent on the relative frequency (isotope ratio) of the stable nuclides comprising each of the respective elements. Due to advancements in measurement techniques, the isotopic frequencies of each of the elements are not necessarily constant, and fluctuate due to a variety of processes that occur on the Earth. We have come to learn that this is reflected in the atomic weights. The result of this is that differences have arisen in the accuracy of the atomic weights between elements. The figures in parentheses that follow the atomic weight values in the atomic weight tables represent the uncertainty with respect to the last digit in the atomic weight. For example, in the case of hydrogen, 1.00794(7) means 1.00794 ± 0.00007.

The atomic weight of a single nuclide element is the most accurate and the precision is also high. This is because it is not necessary to consider the isotope ratio since single nuclide elements do not possess a multiple number of stable isotopes. The atomic weights of such elements are determined based on the mass[4] of each nuclide determined by physical techniques, taking into consideration the uncertainty with constant criteria.

Among the elements, the majority of samples gathered on Earth exhibit a constant isotope composition, however, some specific samples have isotope compositions that are different to these. These kinds of elements are indicated by a ''g'', which means the value in the atomic weight table cannot be used as is, depending on the sample, as the atomic weight of these elements. In relation to this, oxygen for example exists in a number of forms on Earth, such as in air, salt water, fresh water, and in rocks, and because the isotope compositions fluctuate among these substances, oxygen is not an element for which only one value can be used. Thus, an ''r'' is attached to an element for which a precise atomic weight cannot be given, no matter how much progress is made in techniques for measuring the isotope composition. On the other hand, it is also possible, depending on the element, to use an isotope that has undergone artificial fractionation as a reagent. Typical elements that are representative include hydrogen, lithium, boron, and uranium. This type of element is identified by an ''m'', and particularly in cases where the atomic weight is a problem, it is necessary to be careful by referring to the label of the reagent.

1) IUPAC Inorganic Chemistry Division, CIAAW: Standard Atomic Weights Revised. *Chem. Int.*, **29**, 18 (2007).
2) IUPAC Inorganic Chemistry Division, CIAAW: Atomic Weights of the Elements 2007. *Pure Appl. Chem.*, **81**, 2131 (2009).
3) J. R. De Laeter *et al.*: Atomic Weights of the Elements: Review 2000. *Pure Appl. Chem.*, **75**, 683 (2003).
4) G. Audi *et al.*: The A_{ME} 2003 Atomic Mass Evaluation (II). Tables, graphs and references. *Nucl. Phys. A*, **729**, 337 (2003).

Standard Atomic Weights 2010

(Scaled to $A_r(^{12}C) = 12$, where ^{12}C is a neutral atom in its nuclear and electronic ground state)

The atomic weights of many elements are not invariant but depend on the origin and treatment of the material. The standard values of $A_r(E)$ and the uncertainties (in parentheses, following the last significant figure to which they are attributed) apply to elements of natural terrestrial origin. The footnotes to this table elaborate the types of variation which may occur for individual elements and which may be larger than the listed uncertainties of values of $A_r(E)$. Names of elements with atomic number 112 to 118 are provisional.

Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Hydrogen	H	1	1.00794 (7)	g m r
Helium	He	2	4.002602 (2)	g r
Lithium	Li	3	[6.941 (2)]†	g m r
Beryllium	Be	4	9.012182 (3)	
Boron	B	5	10.811 (7)	g m r
Carbon	C	6	12.0107 (8)	g r
Nitrogen	N	7	14.0067 (2)	g r
Oxygen	O	8	15.9994 (3)	g r
Fluorine	F	9	18.9984032 (5)	
Neon	Ne	10	20.1797 (6)	g m
Sodium	Na	11	22.98976928 (2)	
Magnesium	Mg	12	24.3050 (6)	
Aluminium	Al	13	26.9815386 (8)	
Silicon	Si	14	28.0855 (3)	r
Phosphorus	P	15	30.973762 (2)	
Sulfur	S	16	32.065 (5)	g r
Chlorine	Cl	17	35.453 (2)	g m r
Argon	Ar	18	39.948 (1)	g r
Potassium	K	19	39.0983 (1)	
Calcium	Ca	20	40.078 (4)	g
Scandium	Sc	21	44.955912 (6)	
Titanium	Ti	22	47.867 (1)	
Vanadium	V	23	50.9415 (1)	
Chromium	Cr	24	51.9961 (6)	
Manganese	Mn	25	54.938045 (5)	
Iron	Fe	26	55.845 (2)	
Cobalt	Co	27	58.933195 (5)	
Nickel	Ni	28	58.6934 (4)	r
Copper	Cu	29	63.546 (3)	r
Zinc	Zn	30	65.38 (2)	r
Gallium	Ga	31	69.723 (1)	
Germanium	Ge	32	72.64 (1)	
Arsenic	As	33	74.92160 (2)	
Selenium	Se	34	78.96 (3)	r
Bromine	Br	35	79.904 (1)	
Krypton	Kr	36	83.798 (2)	g m
Rubidium	Rb	37	85.4678 (3)	g
Strontium	Sr	38	87.62 (1)	g r
Yttrium	Y	39	88.90585 (2)	
Zirconium	Zr	40	91.224 (2)	g
Niobium	Nb	41	92.90638 (2)	
Molybdenum	Mo	42	95.96 (2)	g r
Technetium*	Tc	43		
Ruthenium	Ru	44	101.07 (2)	g
Rhodium	Rh	45	102.90550 (2)	
Palladium	Pd	46	106.42 (1)	g
Silver	Ag	47	107.8682 (2)	g
Cadmium	Cd	48	112.411 (8)	g
Indium	In	49	114.818 (3)	
Tin	Sn	50	118.710 (7)	g
Antimony	Sb	51	121.760 (1)	g
Tellurium	Te	52	127.60 (3)	g
Iodine	I	53	126.90447 (3)	
Xenon	Xe	54	131.293 (6)	g m
Caesium (Cesium)	Cs	55	132.9054519 (2)	
Barium	Ba	56	137.327 (7)	
Lanthanum	La	57	138.90547 (7)	g
Cerium	Ce	58	140.116 (1)	g
Praseodymium	Pr	59	140.90765 (2)	
Neodymium	Nd	60	144.242 (3)	g
Promethium*	Pm	61		
Samarium	Sm	62	150.36 (2)	g
Europium	Eu	63	151.964 (1)	g
Gadolinium	Gd	64	157.25 (3)	g
Terbium	Tb	65	158.92535 (2)	
Dysprosium	Dy	66	162.500 (1)	g
Holmium	Ho	67	164.93032 (2)	
Erbium	Er	68	167.259 (3)	g
Thulium	Tm	69	168.93421 (2)	
Ytterbium	Yb	70	173.054 (5)	g
Lutetium	Lu	71	174.9668 (1)	g
Hafnium	Hf	72	178.49 (2)	
Tantalum	Ta	73	180.94788 (2)	
Tungsten	W	74	183.84 (1)	
Rhenium	Re	75	186.207 (1)	
Osmium	Os	76	190.23 (3)	g
Iridium	Ir	77	192.217 (3)	
Platinum	Pt	78	195.084 (9)	
Gold	Au	79	196.966569 (4)	
Mercury	Hg	80	200.59 (2)	
Thallium	Tl	81	204.3833 (2)	
Lead	Pb	82	207.2 (1)	g r
Bismuth*	Bi	83	208.98040 (1)	
Polonium*	Po	84		
Astatine*	At	85		
Radon*	Rn	86		
Francium*	Fr	87		
Radium*	Ra	88		
Actinium*	Ac	89		
Thorium*	Th	90	232.03806 (2)	g
Protactinium*	Pa	91	231.03588 (2)	
Uranium*	U	92	238.02891 (3)	g m
Neptunium*	Np	93		
Plutonium*	Pu	94		
Americium*	Am	95		
Curium*	Cm	96		
Berkelium*	Bk	97		
Californium*	Cf	98		
Einsteinium*	Es	99		
Fermium*	Fm	100		
Mendelevium*	Md	101		
Nobelium*	No	102		
Lawrencium*	Lr	103		
Rutherfordium*	Rf	104		
Dubnium*	Db	105		
Seaborgium*	Sg	106		
Bohrium*	Bh	107		
Hassium*	Hs	108		
Meitnerium*	Mt	109		
Darmstadtium*	Ds	110		
Roentgenium	Rg	111		
Copernicium*	Cn	112		

Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Ununtrium*	Uut	113		
Ununquadium*	Uuq	114		
Ununpentium*	Uup	115		
Ununhexium*	Uuh	116		
Ununoctium*	Uuo	118		

* : Element has no stable isotopes.

† : Commercially available Li materials have atomic weights that range between 6.939 and 6.996 ; if a more accurate value is required, it must be determined for the specific material.

g : Geological specimens are known in which the element has an isotopic composition outside the limits for normal material. The difference between the atomic weight of the element in such specimens and that given in the table may exceed the stated uncertainty.

m : Modified isotopic compositions may be found in commercially available material because it has been subjected to an undisclosed or inadvertent isotopic fractionation. Substantial deviations in atomic weight of the element from that given in the table can occur.

r : Range in isotopic composition of normal terrestrial material prevents a more precise A_r(E) being given ; the tabulated A_r(E) value should be applicable to any normal material.

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