calculated on the anhydrous basis. The potency of Kitasamycin Tartrate is expressed as mass (potency) of kitasamycin based on the amount of leucomycin $A_5$ ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of Kitasamycin Tartrate is equivalent to 0.530 mg of leucomycin $A_5$ ($C_{39}H_{65}NO_{14}$).

**Description** Kitasamycin Tartrate occurs as a white to light yellowish white powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Kitasamycin Tartrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Kitasamycin Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Dissolve 1 g of Kitasamycin Tartrate in 20 mL of water, add 3 mL of sodium hydroxide TS, add 20 mL of *n*-butyl acetate, shake well, and discard the *n*-butyl acetate layer. To the aqueous layer add 20 mL of *n*-butyl acetate, and shake well. The aqueous layer so obtained responds to the Qualitative Tests <*1.09*> (1) for tartrate.

**pH** <*2.54*> Dissolve 3.0 g of Kitasamycin Tartrate in 100 mL of water: the pH of the solution is between 3.0 and 5.0.

**Content ratio of the active principle** Dissolve 20 mg of Kitasamycin Tartrate in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 $\mu$L of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amounts of leucomycin $A_5$, leucomycin $A_4$ and leucomycin $A_1$ by the area percentage method: the amount of leucomycin $A_5$ is 40 – 70%, leucomycin $A_4$ is 5 – 25%, and leucomycin $A_1$ is 3 – 12%. The relative retention times of leucomycin $A_4$ and leucomycin $A_1$ with respect to leucomycin $A_5$ are 1.2 and 1.5, respectively.

*Operating conditions*—

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

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

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

Mobile phase: To a suitable amount of a solution of ammonium acetate (77 in 500) add diluted phosphoric acid (1 in 150) to adjust the pH to 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin $A_5$ is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin $A_5$.

*System suitability*—

System performance: Dissolve about 20 mg of Leucomycin $A_5$ Reference Standard and about 20 mg of Josamycin Reference Standard in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 $\mu$L of this solution under the above operating conditions, leucomycin $A_5$ and josamycin 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 5 $\mu$L of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin $A_5$ is not more than 1.0%.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Kitasamycin Tartrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**Water** <*2.48*> Not more than 3.0% (0.1 g, volumetric titration, direct titration).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

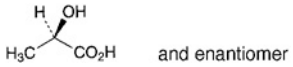
(iii) Standard solutions—Weigh accurately an amount of Leucomycin $A_5$ Reference Standard, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 $\mu$g (potency) and 7.5 $\mu$g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 $\mu$g (potency) and 7.5 $\mu$g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

# Lactic Acid

乳酸



and enantiomer

$C_3H_6O_3$: 90.08

(2*RS*)-2-Hydroxypropanoic acid [*50-21-5*]

Lactic Acid is a mixture of lactic acid and lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of $C_3H_6O_3$.

**Description** Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, unpleasant odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It is hygroscopic.

Specific gravity $d_{20}^{20}$: about 1.20

**Identification** A solution of Lactic Acid (1 in 50) changes blue litmus paper to red and responds to the Qualitative Tests <*1.09*> for lactate.

**Purity** **(1)** Chloride <*1.03*>—Perform the test with 1.0 g of Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

**(2)** Sulfate <*1.14*>—Perform the test with 2.0 g of Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

**(3)** Heavy metals <*1.07*>—To 2.0 g of Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

**(4)** Iron <*1.10*>—Prepare the test solution with 4.0 g of Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

**(5)** Sugars—To 1.0 g of Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling's TS for 5 minutes: no red precipitate is produced.

**(6)** Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

**(7)** Glycerin or mannitol—Shake 10 mL of Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

**(8)** Volatile fatty acids—Warm Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

**(9)** Cyanide—Transfer 1.0 g of Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) by shaking until a pale red color develops, add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, add dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, add 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluensulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make exactly 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

**(10)** Readily carbonizable substances—Superimpose slowly 5 mL of Lactic Acid, previously kept at 15°C, upon 5 mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

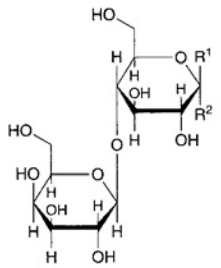
**Assay** Weigh accurately about 3 g of Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate <*2.50*> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 90.08 mg of $C_3H_6O_3$

**Containers and storage** Containers—Tight containers.

# Anhydrous Lactose

無水乳糖




α-Lactose : $R^1$=H, $R^2$=OH
β-Lactose : $R^1$=OH, $R^2$=H

$C_{12}H_{22}O_{11}$: 342.30

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranose (β-lactose)
β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose (α-lactose)
[*63-42-3*, Anhydrous Lactose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Anhydrous Lactose is β-lactose or a mixture of β-lactose and α-lactose.

The relative quantities of α-lactose and β-lactose in Anhydrous Lactose is indicated as the isomer ratio.

◆**Description** Anhydrous Lactose occurs as white crystals or powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

**Identification** Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with ◆the Reference Spectrum or◆ the spectrum of Anhydrous Lactose Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +54.4 – +55.9°. Weigh accurately about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C,

and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.04.

**(2)** Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose in 25 mL of freshly boiled and cooled water by heating, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless. To this solution add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

◆**(3)** Heavy metals <*1.07*>—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).◆

**(4)** Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 80°C, 2 hours).

**Water** <*2.48*> Not more than 1.0% (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

◆**Microbial limit** <*4.05*> The total viable aerobic microbial count is not more than 100 per g, and the total count of fungi and yeast is not more than 50 per g, and *Salmonella* and *Escherichia coli* should not be observed.◆

**Isomer ratio** Place 1 mg of Anhydrous Lactose in an 5-mL screw capped reaction vial for gas chromatography, add 0.45 mL of dimethylsulfoxide, stopper, and shake well. Add 1.8 mL of a mixture of pyridine and trimethylsilylimidazole (72:28), mix, and allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 $\mu$L of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine peak areas of $\alpha$-lactose and $\beta$-actose, $A_a$ and $A_b$, and calculate the content (%) of $\beta$-lactose in Anhydrous Lactose by the following equation.

Content (%) of $\beta$-lactose $= [A_b/(A_a + A_b)] \times 100$

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Sample injection port: about 275°C

Column: A column 4 mm in inside diameter and 0.9 m in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215°C.

Carrier gas: Helium

Flow rate: A constant flow rate of about 40 mL per minute.

*System suitability*—

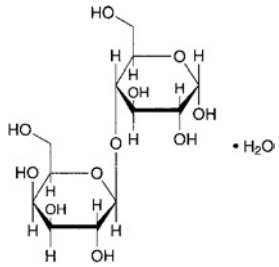
System performance: Prepare a solution with 1 mg of a mixture of $\alpha$-lactose and $\beta$-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 2 $\mu$L of this solution under the above operating conditions, and determine the retention times of the peaks of $\alpha$-lactose and $\beta$-lactose: a ratio of the retention time of $\alpha$-lactose to that of $\beta$-lactose is about 0.7 with the resolution between these peaks being not less than 3.0.

◆**Containers and storage** Containers—Well-closed containers.◆

# Lactose Hydrate

## Lactose

乳糖水和物



$C_{12}H_{22}O_{11}.H_2O$: 360.31

$\beta$-D-Galactopyranosyl-(1→4)-$\alpha$-D-glucopyranose monohydrate

[*64044-51-5*, Mixture of $\alpha$- and $\beta$-lactose monohydrate]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Lactose Hydrate is the monohydrate of $\beta$-D-galactopyranosyl-(1→4)-$\alpha$-D-glucopyranose.

◆It is a disaccharide obtained from milk, consist of one unit of glucose and one unit of galactose.◆

◆The label states the effect where it is the granulated powder.◆

◆**Description** Lactose Hydrate occurs as white, crystals, powder or granulated powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

**Identification** Determine the infrared absorption spectrum of Lactose Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with ◆the Reference Spectrum or◆ the spectrum of Lactose Hydrate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +54.4 – +55.9°. Weigh accurately about 10 g of Lactose Hydrate, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and deter-

mine the optical rotation of this solution in a 100-mm cell.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Lactose Hydrate in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.04.

**(2)** Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate in 25 mL of freshly boiled and cooled water by heating, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless. To this solution add 0.4 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

◆**(3)** Heavy metals <*1.07*>—Dissolve 4.0 g of Lactose Hydrate in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).◆

**(4)** Proteins and light absorbing substances—Dissolve 1.0 g of Lactose Hydrate in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

◆**Loss on drying** <*2.41*> Not more than 0.5%. For the granulated powder, not more than 1.0% (1 g, 80°C, 2 hours).◆

**Water** <*2.48*> 4.5 – 5.5%. ◆For the granulated powder, 4.0 – 5.5%◆ (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

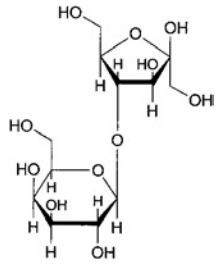
**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

◆**Microbial limit** <*4.05*> The total viable aerobic microbial count is not more than 100 per g, and the total count of fungi and yeast is not more than 50 per g. *Salmonella* and *Escherichia coli* should not be observed.◆

◆**Containers and storage** Containers—Well-closed containers.◆

# Lactulose

ラクツロース



$C_{12}H_{22}O_{11}$: 342.30

*β*-D-Galactopyranosyl-(1→4)-D-fructose [*4618-18-2*]

Lactulose is a solution of lactulose prepared by isomerizing lactose under the existing of alkaline and purified by ion-exchange resin.

It contains not less than 50.0% and not more than 56.0% of $C_{12}H_{22}O_{11}$.

**Description** Lactulose occurs as a clear, colorless or light yellow, viscous liquid. It is odorless, and has a sweet taste.

It is miscible with water and with formamide.

**Identification** **(1)** To 0.7 g of Lactulose add 10 mL of water, 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100), and heat in a water bath for 5 to 10 minutes: a blue color develops.

**(2)** Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 1 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes, and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add 5 mL of Fehling's TS, and boil for 5 minutes: a red precipitate is produced.

**pH** <*2.54*> To 2.0 g of Lactulose add water to make 15 mL: the pH of the solution is between 3.5 and 5.5.

**Specific gravity** <*2.56*> $d_{20}^{20}$: 1.320 – 1.360

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 5.0 g of Lactulose according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

**(2)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test (not more than 2 ppm).

**(3)** Glactose and lactose—Determine the heights of the peaks corresponding to D-galactose and lactose respectively, on the chromatogram obtained in Assay from the sample solution and the standard solution, and calculate the ratios of the peak heights of D-galactose and lactose to that of the internal standard from the sample solution, $Q_{Ta}$ and $Q_{Tb}$, and then from the standard solution, $Q_{Sa}$ and $Q_{Sb}$: it contains D-galactose of not more than 11%, and lactose of not more than 6%.

$$\text{Amount (mg) of D-galactose } (C_6H_{12}O_6) = W_S \times (Q_{Ta}/Q_{Sa})$$

$W_S$: Amount (mg) of D-galactose

$$\text{Amount (mg) of lactose } (C_{12}H_{22}O_{11}.H_2O) = W_S \times (Q_{Tb}/Q_{Sb})$$

$W_S$: Amount (mg) of lactose Hydrate

**Loss on drying** <*2.41*> Not more than 35% (0.5 g, in vacuum, 80°C, 5 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1 g of Lactulose, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of Lactulose Reference Standard, accurately about 80 mg of D-galactose and accurately about 40 mg of lactose monohydrate, add exactly 10 mL of the internal standard solution and water to make 50 mL, and

use this solution as the standard solution. Perform the test with 20 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 height of lactulose to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{12}H_{22}O_{11} = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Lactulose Reference Standard

*Internal standard solution*—A solution of D-mannitol (1 in 20).

*Operating conditions*—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 50 cm in length, packed with gel type strong acid ion-exchange resin for liquid chromatography (degree of crosslinkage: 6%) (11 μm in particle diameter).

Column temperature: A constant temperature of about 75°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time of lactulose is about 18 minutes.

*System suitability*—

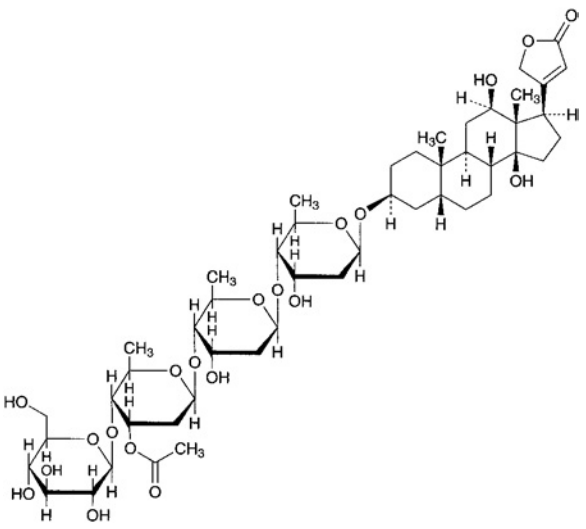
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lactulose and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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 ratios of the peak heights of lactulose, galactose and lactose to the height of the internal standard are not more than 2.0%, respectively.

**Containers and storage** Containers—Tight containers.

# Lanatoside C

ラナトシド C



$C_{49}H_{76}O_{20}$: 985.12

3β-[β-D-Glucopyranosyl-(1→4)-3-*O*-acetyl-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyloxy]-12β,14-dihydroxy-5β,14β-card-20(22)-enolide [*17575-22-3*]

Lanatoside C, when dried, contains not less than 90.0% and not more than 102.0% of $C_{49}H_{76}O_{20}$.

**Description** Lanatoside C occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

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

It is hygroscopic.

**Identification** Place 1 mg of Lanatoside C to a small test tube having an internal diameter of about 10 mm, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids, a brown ring is produced, and the color of the upper layer near the contact zone gradually changes to blue through purple. Finally the color of the entire acetic acid layer changes to blue-green through deep blue.

**Purity** Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C Reference Standard in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. 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 dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +32 – +35° (after drying, 0.5 g, methanol, 25 mL, 100 mm).

**Loss on drying** <2.41> Not more than 7.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.5% (0.1 g).

**Assay** Weigh accurately about 50 mg each of Lanatoside C and Lanatoside C Reference Standard, previously dried, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution into 25-mL light-resistant, volumetric flasks, and add 5 mL of 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, $A_T$ and $A_S$, of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

$$\text{Amount (mg) of } C_{49}H_{76}O_{20} = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Lanatoside C Reference Standard

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Lanatoside C Tablets

ラナトシド C 錠

Lanatoside C Tablets contain not less than 90% and not more than 110% of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$: 985.12).

**Method of preparation** Prepare as directed under Tablets, with Lanatoside C.

**Identification** **(1)** Shake a quantity of powdered Lanatoside C Tablets, equivalent to 1 mg of Lanatoside C according to the labeled amount, with 3 mL of diethyl ether, and filter. Wash the residue with two 3-mL portions of diethyl ether, and air-dry. To the remaining residue add 10 mL of a mixture of chloroform and methanol (9:1), shake, and filter. Wash the residue with two 5-mL portions of a mixture of chloroform and methanol (9:1), combine the filtrate and washings, and evaporate on a water bath to a smaller volume. Transfer the solution to a small test tube having an internal diameter of about 10 mm, further evaporate on a water bath to dryness, and proceed as directed in the Identification under Lanatoside C.

**(2)** Perform the test with the sample solution and the standard solution obtained in the Assay as directed under Thin-layer Chromatography <*2.03*>. Spot 25 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: the spots obtained from the sample solution and the standard solution show a black color, and have the same *R*f values.

**Uniformity of dosage unit** <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Warm 1 tablet of Lanatoside C Tablets with 5 mL of water until the tablet is disintegrated, add 30 mL of ethanol (95), disperse finely the particles with the aid of ultrasonic radiation, add ethanol (95) to make exactly *V* mL of a solution containing about 5 μg of lanatoside C ($C_{49}H_{76}O_{20}$) in each mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Lanatoside C Reference Standard, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 2 mL of this solution, add 10 mL of water, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and diluted ethanol (95) (17 in 20) into three brown glass-stoppered test tubes T, S and B, previously containing exactly 10 mL of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, add exactly 1 mL each of dilute hydrogen peroxide TS immediately, shake vigorously, and allow to stand at a constant temperature between 25° C and 30°C for 40 minutes. Determine the fluorescence intensities, $F_T$, $F_S$ and $F_B$, of the subsequent solutions from the sample solution and the standard solution and the diluted ethanol (95) (17 in 20) at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <*2.22*>, respectively.

$$\text{Amount (mg) of lanatoside C } (C_{49}H_{76}O_{20}) = W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (V/5000)$$

$W_S$: Amount (mg) of Lanatoside C Reference Standard

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Take 1 tablet of Lanatoside C Tablets, and perform the test with 500 mL of diluted hydrochloric acid (3 in 500) deaired by a suitable method as the test solution at 100 revolutions per minute as directed in the Paddle method. Take 20 mL of the dissolved solution at 60 minutes after starting the test, and filter through a membrane filter (not more than 0.8 μm). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Lanatoside C Reference Standard in vacuum over phosphorus (V) oxide at 60°C for 4 hours, weigh accurately a portion of it, equivalent to 100 times an amount of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$), dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add the test solution to make exactly 500 mL, warm at 37 ± 0.5°C for 60 minutes, and use this solution as the standard solution. Pipet 3 mL each of the sample solution, the standard solution and the test solution, and transfer to glass-stoppered brown test tubes T, S and B, respectively. To these solutions add exactly 10 mL each of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, and shake. Immediately add exactly 0.2 mL each of diluted hydrogen peroxide TS (1 in 100), shake well, and allow to stand at a constant temperature between 30°C and 37°C for 45 minutes. Determine immediately the fluorescence intensities, $F_T$, $F_S$ and $F_B$, of the sample solution and the standard solution at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <*2.22*>.

Dissolution rate of each of six Lanatoside C Tablets after 60 minutes should be not less than 65%.

Requirement of retest is not applied to Lanatoside C Tablets.

$$\text{Dissolution rate (\%) to labeled amount of lanatoside C } (C_{49}H_{76}O_{20}) = W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (1/C)$$

$W_S$: Amount (mg) of Lanatoside C Reference Standard.
$C$: Labeled amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$) in each tablet.

**Assay** Weigh accurately and powder not less than 20 Lanatoside C Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of lanatoside C ($C_{45}H_{76}O_{20}$), into a 100-mL light-resistant volumetric flask, add 50 mL of ethanol (95), and shake for 15 minutes. Then dilute with ethanol (95) to make exactly 100 mL. Filter this solution, discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 5 mg of Lanatoside C Reference Standard, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, dissolve in ethanol (95) to make exactly 100

mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into light-resistant, glass-stoppered test tubes, add 3 mL each of alkaline 2,4,6-trinitrophenol TS, shake well and allow these solutions to stand between 22°C and 28°C for 25 minutes. Determine the absorbances, $A_T$ and $A_S$, of the subsequent sample solution and the subsequent standard solution at 490 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by the same manner with 5 mL of ethanol (95), as the blank.

$$\text{Amount (mg) of lanatoside C } (C_{49}H_{76}O_{20}) = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Lanatoside C Reference Standard

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# 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.

$$\text{Iodine value} = [(a-b) \times 1.269]/W$$

$W$: amount (g) of sample.
$a$: 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 Rurificatus*

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.

$$\text{Iodine value} = [(a - b) \times 1.269]/W$$

*W*: amount (g) of sample.
*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 follow-

ing 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).

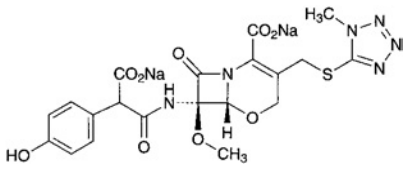
**(4)** Beef tallow—Dissolve 5 g of Lard in 20 mL of diethyl ether, stopper lightly with absorbent cotton, and allow to stand at 20°C for 18 hours. Collect the separated crystals, moisten them with ethanol (95), and examine under a microscope of 200 magnifications: the crystals are in the form of rhomboidal 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.

# Latamoxef Sodium

ラタモキセフナトリウム



$C_{20}H_{18}N_6Na_2O_9S$: 564.44

Disodium (6*R*,7R)-7-[2-carboxylato-2-(4-hydroxyphenyl)acetamino]-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [*64953-12-4*]

Latamoxef Sodium contains not less than 830 $\mu$g (potency) and not more than 940 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Latamoxef Sodium is expressed as mass (potency) of latamoxef ($C_{20}H_{20}N_6O_9S$: 520.47).

**Description** Latamoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

**Identification (1)** Determine the absorption spectrum of a solution of Latamoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Latamoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Determine the spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> ($^1$H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around $\delta$3.5 ppm and at around $\delta$4.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

**(4)** Latamoxef Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-32$ – $-40°$ (0.5 g calculated on the anhydrous basis, phosphate buffer solutiuon, pH 7.0, 50 mL, 100 mm).

**pH** <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and pale yellow.

**(2)** Heavy metals <*1.07*>—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is masses. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and burn the ethanol. After cooling, add 1 mL of sulfuric acid. Proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1*H*-tetrazole-5-thiol, having the relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef, obtained from the sample solution is not more than the peak area of latamoxef from the standard solution, and the peak area of decarboxylatamoxef, having the relative retention time of about 1.7 with respect to the first peak of the two peaks of latamoxef, is not more than 2 times that of latamoxef from the standard solution. For this calculation, use the peak area for 1-methyl-1*H*-tetrazole-5-thiol after multiplying by its response factor, 0.52.

*Operating conditions*—

Proceed as directed in the operating conditions in the Assay.

*System suitability*—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of latamoxef is not more than 2.0%.

**Water** <*2.48*> Not more than 5.0% (0.5 g, volumetric titration, back titration).

**Isomer ratio** Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the sample solution. Perform the test with 5 $\mu$L of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas, $A_a$ and $A_b$, of the

two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: $A_a/A_b$ is between 0.8 and 1.4.

*Operating conditions*—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.7 g of ammonium acetate in water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of the first eluted peak of latamoxef is about 8 minutes.

*System suitability*—

System performance: When the procedure is run with 5 $\mu$L of the sample solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 $\mu$L of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

**Assay** Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium Reference Standard, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of latamoxef to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of latamoxef } (C_{20}H_{20}N_6O_9S) = W_S \times (Q_T/Q_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Latamoxef Ammonium Reference Standard

*Internal standard solution*—A solution of *m*-cresol (3 in 200).

*Operating conditions*—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra *n*-butylammonium bromide in water to make exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of latamoxef is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, latamoxef and the internal standard 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 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Not exceeding 5°C.

# Lauromacrogol

## Polyoxyethylene Lauryl Alcohol Ether

ラウロマクロゴール

Lauromacrogol is a polyoxyethylene ether prepared by the polymerization of ethylene oxide with laury alcohol.

**Description** Lauromacrogol is a colorless or light yellow, clear liquid or a white, petrolatum-like or waxy solid. It has a characteristic odor, and a somewhat bitter and slightly irritative taste.

It is very soluble in ethanol (95), in diethyl ether and in carbon tetrachloride.

It is freely soluble or dispersed as fine oily drops in water.

**Identification** **(1)** Shake well 0.5 g of Lauromacrogol with 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, then shake with 5 mL of chloroform, and allow to stand: the chloroform layer becomes blue in color.

**(2)** Dissolve 0.35 g of Lauromacrogol in 10 mL of carbon tetrachloride, and perform the test as directed in the Solution method under Infrared Spectrophotometry <*2.25*> using a 0.1-mm fixed cell: it exhibits absorption at the wave numbers of about 1347 $cm^{-1}$, 1246 $cm^{-1}$ and 1110 $cm^{-1}$.

**Purity** **(1)** Acidity—Transfer 10.0 g of Lauromacrogol into a flask, and add 50 mL of neutralized ethanol. Heat on a water bath nearly to boil, shaking once or twice while heating. Cool, and add 5.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

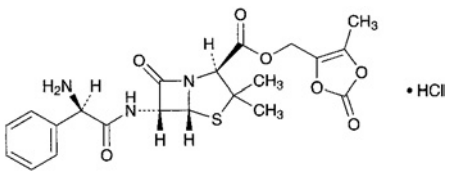
**(2)** Unsaturated compound—Shake 0.5 g of Lauromacrogol with 10 mL of water, and add 5 drops of bromine TS: the color of the solution does not disappear.

**Residue on ignition** <*2.44*> Not more than 0.2% (1 g).

**Containers and storage** Containers—Tight containers.

# Lenampicillin Hydrochloride

レナンピシリン塩酸塩



$C_{21}H_{23}N_3O_7S.HCl$: 497.95

5-Methyl-2-oxo[1,3]dioxol-4-ylmethyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-

oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [*80734-02-7*]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methyloxodioxolenylmethyl ester.

It contains not less than 653 $\mu$g (potency) and not more than 709 $\mu$g (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

**Description** Lenampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (95), and freely soluble in *N*,*N*-dimethylformamide.

**Identification** **(1)** Determine the infrared absorption spectrum of Lenampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Lenampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** To 1 mL of a solution of Lenampicillin Hydrochloride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: + 174 – + 194° (0.2 g calculated on the anhydrous de-residual solventization basis, ethanol (95), 20 mL, 100 mm).

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Lenampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloridein, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak height of ampicillin to that of the internal standard: the amount of ampicillin is not more than 1.0%.

$$\text{Amount (\%) of ampicillin } (C_{16}H_{19}N_3O_4S) = (W_S/W_T) \times (Q_T/Q_S) \times 2$$

$W_S$: Amount [mg (potency)] of Ampicillin Reference Standard

$W_T$: Amount (mg) of the sample

*Internal standard solution*—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, ampicillin and the internal standard 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 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.

**(4)** Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid ($C_{16}H_{21}N_3O_5S$: 367.42) is not more than 3.0%.

$$\text{Each mL of 0.01 mol/L sodium thiosulfate VS} = 0.45 \text{ mg of } C_{16}H_{21}N_3O_5S$$

**(5)** Residual solvent <*2.46*>—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add *N*,*N*-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 $\mu$L each of the sample solution, standard solution (1) and standard solution (2) as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the ratios, $Q_{Ta}$ and $Q_{Tb}$, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, $Q_{Sa1}$ and $Q_{Sb1}$, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, $Q_{Sa2}$ and $Q_{Sb2}$, of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

$$\text{Amount (\%) of 2-propanol} = (W_{Sa}/W_T) \times \{(2Q_{Ta} - 3Q_{Sa1} + Q_{Sa2})/(Q_{Sa2} - Q_{Sa1})\}$$

Amount (%) of ethyl acetate
$= (W_{Sb}/W_T) \times \{(2Q_{Tb} - 3Q_{Sb1} + Q_{Sb2})/(Q_{Sb2} - Q_{Sb1})\}$

$W_{Sa}$: Amount (g) of 2-propanol
$W_{Sb}$: Amount (g) of ethyl acetate
$W_T$: Amount (g) of the sample

*Internal standard solution*—A solution of cyclohexane in *N*,*N*-dimethylformamide (1 in 1000).
*Operating conditions*—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with tetrakishydroxypropylethylenediamine for gas chromatography at the ratio of 10 to 15%.
Column temperature: A constant temperature of about 80°C.
Injection port temperature: A constant temperature of about 160°C.
Carrier gas: Nitrogen
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1 minute.
*System suitability*—
System performance: When the procedure is run with 4 μL of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.
System repeatability: When the test is repeated 3 times with 4 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5.0%.

**Water** ⟨*2.48*⟩ Not more than 1.5% (1 g, volumetric titration, direct titration).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (1 g).

**Assay** Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride Reference Standard, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 10 mL, and use these solutions as the sample solution and 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, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of lenampicillin to that of the internal standard.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$)
$= W_S \times (Q_T/Q_S) \times 1000$

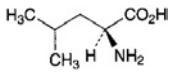
$W_S$: Amount [mg (potency)] of Lenampicillin Hydrochloride Reference Standard

*Internal standard solution*—A solution of ethyl aminobenzoate in the mobile phase (1 in 4000).
*Operating conditions*—
Detector: An ultraviolet absorption photometer (wavelength: 254 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 25°C.
Mobile phase: Dissolve 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.
Flow rate: Adjust the flow rate so that the retention time of lenampicillin is about 6 minutes.
*System suitability*—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, lenampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
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 ratios of the peak area of lenampicillin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# L-Leucine

L-ロイシン




$C_6H_{13}NO_2$: 131.17
(2*S*)-2-Amino-4-methylpentanoic acid [*61-90-5*]

L-Leucine, when dried, contains not less than 98.5% of $C_6H_{13}NO_2$.

**Description** L-Leucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.
It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).
It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Leucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +14.5 – +16.0° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** ⟨*2.54*⟩ Dissolve 1.0 g of L-Leucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.
**(2)** Chloride ⟨*1.03*⟩—Dissolve 0.5 g of L-Leucine in 40 mL of water and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).
**(3)** Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Leucine in 40 mL of water and 1 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 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

**(4)** Ammonium <*1.02*>—Perform the test with 0.25 g of L-Leucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

**(5)** Heavy metals <*1.07*>—Proceed with 1.0 g of L-Leucine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(6)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of L-Leucine according to Method 2, and perform the test (not more than 2 ppm).

**(7)** Related substances—Dissolve 0.10 g of L-Leucine by warming, after cooling, add water to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L 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) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

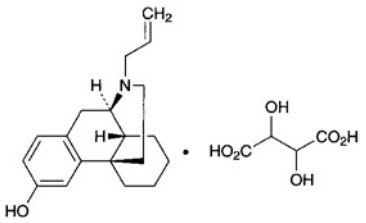
**Assay** Weigh accurately about 0.13 g of L-Leucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.12 mg of $C_6H_{13}NO_2$

**Containers and storage** Containers—Well-closed containers.

# Levallorphan Tartrate

レバロルファン酒石酸塩




$C_{19}H_{25}NO.C_4H_6O_6$: 433.49
17-Allylmorphinan-3-ol monotartrate [*71-82-9*]

Levallorphan Tartrate, when dried, contains not less than 98.5% of $C_{19}H_{25}NO.C_4H_6O_6$.

**Description** Levallorphan Tartrate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification (1)** Determine the absorption spectrum of a solution of Levallorphan Tartrate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Levallorphan Tartrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Levallorphan Tartrate (1 in 30) responds to the Qualitative Tests <*1.09*> (1) and (2) for tartrate.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-37.0$ – $-39.2°$ (after drying, 0.2 g, water, 10 mL, 100 mm).

**pH** <*2.54*> Dissolve 0.2 g of Levallorphan Tartrate in 20 mL of water: the pH of this solution is between 3.3 and 3.8.

**Melting point** <*2.60*> 174 – 178°C

**Purity (1)** Clarity and color of solution—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Levallorphan Tartrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 0.20 g of Levallorphan Tartrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 $\mu$L 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 and ammonia TS (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.10% (1 g).

**Assay** Weigh accurately about 0.5 g of Levallorphan Tartrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 43.35 mg of $C_{19}H_{25}NO.C_4H_6O_6$

**Containers and storage** Containers—Well-closed containers.

# Levallorphan Tartrate Injection

レバロルファン酒石酸塩注射液

Levallorphan Tartrate Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of levallorphan tartrate ($C_{19}H_{25}NO.C_4H_6O_6$: 433.49).

**Method of preparation** Prepare as directed under Injection, with Levallorphan Tartrate.

**Description** Levallorphan Tartrate Injection is a clear, colorless liquid.

pH: 3.0 – 4.5

**Identification** Take an exact volume of Levallorphan Tartrate Injection, equivalent to 3 mg of Levallorphan Tartrate according to the labeled amount, add 5 mL of water and 2 drops of dilute hydrochloric acid, and wash with five 15-mL portions of diethyl ether by a vigorous shaking. Take the water layer, evaporate the diethyl ether remained by warming on a water bath, and after cooling, add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 281 nm.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Take wxactly a volume of Levallorphan Tartrate Injection, equivalent to about 2 mg of levallorphan tartrate ($C_{19}H_{25}NO.C_4H_6O_6$), add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of levallorphan tartrate for assay, previously dried at 80°C for 4 hours on phosphorus (V) oxide under reduced pressure, and dissolve in water to make exactly 100 mL. Pipet 2 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 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following operating conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of levallorphan to that of the internal standard:

$$\text{Amount (mg) of } C_{19}H_{25}NO.C_4H_6O_6 = W_S \times (Q_T/Q_S) \times (1/50)$$

$W_S$: Amount (mg) of levallorphan tartrate for assay

*Internal standard solution*—Dissolve 0.04 g of isobutyl parahydroxybenzoate in 10 mL of ethanol (95), add water to make 100 mL, and to 10 mL of this solution add water to make 100 mL.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 280 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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 300 mL of this solution add 200 mL of acetonitrile.

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

*System suitability*—

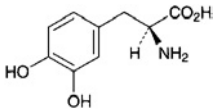
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the internal standard and levallorphan are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of levallorphan to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Levodopa

レボドパ




$C_9H_{11}NO_4$: 197.19

3-Hydroxy-L-tyrosine [*59-92-7*]

Levodopa, when dried, contains not less than 98.5% of $C_9H_{11}NO_4$.

**Description** Levodopa occurs as white or slightly grayish white crystals or crystalline powder. It is odorless.

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

It dissolves in dilute hydrochloric acid.

The pH of a saturated solution of Levodopa is between 5.0 and 6.5.

Melting point: about 275°C (with decomposition).

**Identification** **(1)** To 5 mL of a solution of Levodopa (1 in 1000) add 1 mL of ninhydrin TS, and heat for 3 minutes in a water bath: a purple color develops.

**(2)** To 2 mL of a solution of Levodopa (1 in 5000) add 10 mL of 4-aminoantipyrine TS, and shake: a red color develops.

**(3)** Dissolve 3 mg of Levodopa in 0.001 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Absorbance** <*2.24*> $E^{1\%}_{1\,cm}$ (280 nm): 136 – 146 (after drying, 30 mg, 0.001 mol/L hydrochloric acid TS, 1000 mL).

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: −11.5 – −13.0° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 nm).

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of

Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Dissolve 0.5 g of Levodopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.3 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

**(3)** Sulfate <*1.14*>—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

**(4)** Heavy metals <*1.07*>—Proceed with 1.0 g of Levodopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(5)** Arsenic <*1.11*>—Dissolve 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

**(6)** Related sulstances—Dissolve 0.10 g of Levodopa in 10 mL of sodium disulfite TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium disulfite TS to make exactly 25 mL. Pipet 1 mL of this solution, add sodium disulfite TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10:5:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

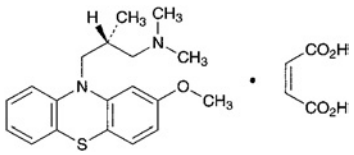
**Assay** Weigh accurately about 0.3 g of Levodopa, previously dried, dissolve in 3 mL of formic acid, add 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 19.72 mg of $C_9H_{11}NO_4$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Levomepromazine Maleate

レボメプロマジンマレイン酸塩



$C_{19}H_{24}N_2OS.C_4H_4O_4$: 444.54
(2*R*)-3-(2-Methoxy-10*H*-phenothiazin-10-yl)-*N*,*N*, 2-trimethylpropylamine monomaleate [*7104-38-3*]

Levomepromazine Maleate, when dried, contains not less than 98.0% of $C_{19}H_{24}N_2OS.C_4H_4O_4$.

**Description** Levomepromazine Maleate occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: 184 – 190°C (with decomposition).

**Identification (1)** Dissolve 5 mg of Levomepromazine Maleate in 5 mL of sulfuric acid: a red-purple color develops, which slowly becomes deep red-purple. To this solution add 1 drop of potassium dichromate TS: a brownish yellow-red color is produced.

**(2)** To 0.2 g of Levomepromazine Maleate add 5 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake well. Separate the diethyl ether layer, wash twice with 10-mL portions of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours: the residue melts <*2.60*> between 124°C and 128°C.

**(3)** To 0.5 g of Levomepromazine Maleate add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5-mL portions of chloroform, separate and evaporate the water layer to dryness. To the residue add 2 to 3 drops of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine all the diethyl ether extracts, evaporate the diethyl ether in a water bath at a temperature of about 35°C with the aid of a current of air: the residue melts <*2.60*> between 128°C and 136°C.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-13.5$ – $-16.5°$ (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

**Purity (1)** Clarity and color of solution—To 0.5 g of Levomepromazine Maleate add 10 mL of methanol, and dissolve by warming: the solution is clear, and colorless or pale yellow.

**(2)** Chloride <*1.03*>—Dissolve 0.5 g of Levomepromazine Maleate in 40 mL of methanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

**(3)** Heavy metals <*1.07*>—Proceed with 2.0 g of

Levomepromazine Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (2 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

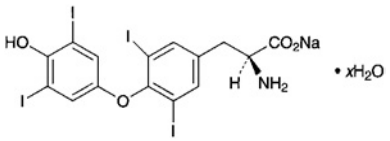
**Assay** Weigh accurately about 1 g of Levomepromazine Maleate, previously dried, and dissolve in a mixture of 40 mL of acetic acid (100) and 20 mL of acetone for nonaqueous titration. Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.45 mg of $C_{19}H_{24}N_2OS.C_4H_4O_4$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Levothyroxine Sodium Hydrate

レボチロキシンナトリウム水和物




$C_{15}H_{10}I_4NNaO_4.xH_2O$
Monosodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosinate hydrate [*25416-65-3*]

Levothyroxine Sodium Hydrate contains not less than 97.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85), calculated on the dried basis.

**Description** Levothyroxine Sodium Hydrate occurs as a pale yellowish white to light yellow-brown powder. It is odorless.

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

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

**Identification** **(1)** Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

**(2)** To 0.5 mg of Levothyroxine Sodium Hydrate add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes, and add 1.5 mL of ammonia solution (28): a yellowish red color is produced.

**(3)** Determine the absorption spectrum of a solution of Levothyroxine Sodium Hydrate in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Moisten Levothyroxine Sodium Hydrate with sulfuric acid, and ignite: the residue responds to the Qualitative Tests ⟨*1.09*⟩ (1) and (2) for sodium salt.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: −5 – −6° (0.3 g, calculated on the dried basis, a mixture of ethanol (95) and sodium hydroxide TS (2:1), 10 mL, 100 mm).

**Purity** **(1)** Clarity and color of solution—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2:1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

**(2)** Soluble halides—Dissolve 0.01 g of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. To the filtrate add water to make 10 mL, then add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of water and 1 drop of dilute nitric acid, and proceed as directed above.

**(3)** Related substances—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (14:1) to make exactly 50 mL, 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 *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ 7 – 11% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Assay** Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate, and proceed as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of a freshly prepared sodium bisulfate solution (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate ⟨*2.50*⟩ the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.6657 mg of $C_{15}H_{10}I_4NNaO_4$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Levothyroxine Sodium Tablets

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Levothyroxine Sodium Tablets contain not less than 90% and not more than 110% of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$: 798.85).

**Method of preparation** Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

**Identification (1)** Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28): a yellowish red color develops.

**(2)** To a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 10 mL of ethanol (95), shake, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of levothyroxine sodium for thin-layer chromatography in 100 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 20 $\mu$L 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 *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R*f value.

**Purity** Soluble halides—Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 25 mL of water, warm to 40°C, shake for 5 minutes, add 3 drops of dilute nitric acid, and filter. To the filtrate add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of water and 3 drops of dilute nitric acid, and proceed as directed above.

**Uniformity of dosage units** <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 20 $\mu$L of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratio of the peak area of levothyroxine to that of the internal standard. Calculate the mean value from the ratios of each peak area of 10 samples: the deviation (%) of the mean value and the ratio of each peak area should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of the mean value of the 30 samples used in the 2 tests and the ratio of each peak area: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

*Internal standard solution*—A solution of ethinylestradiol in a mixture of acetonitrile and diluted phosphoric acid (1 in 10) (9:1) (3 in 40,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel.

Column temperature: A constant temperature at about 25°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (1340:660:1).

Flow rate: Adjust the flow rate so that the retention time of levothyroxine is about 9 minutes.

Selection of column: To 5 mL of a solution of levothyroxine sodium in 0.01 mol/L sodium hydroxide TS (1 in 200,000) add 1 mL of the internal standard solution. Proceed with 20 $\mu$L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of levothyroxine and the internal standard in this order with the resolution between these peaks being not less than 2.0.

**Assay** Weigh accurately and powder not less than 20 Levothyroxine Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 3 mg of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), into a crucible, and add potassium carbonate amounting to twice the mass of the powder. In the case that the weighed powder is less than 4 g, add 8 g of potassium carbonate to the crucible. Mix well, and gently tap the crucible on the bench to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again by tapping. Heat the crucible strongly at a temperature between 675°C and 700°C for 25 minutes. Cool, add 30 mL of water, heat gently to boiling, and filter into a flask. To the residue add 30 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with hot water until the filtrate measures 300 mL. Add slowly 7 mL of freshly prepared bromine TS and diluted phosphoric acid (1 in 2) in the ratio of 3.5 mL to 1 g of the added potassium carbonate, and boil until starch-potassium iodide paper is no longer colored blue by the evolved gas. Wash the inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume of not less than 250 mL. Cool, add 5 mL of a solution of phenol (1 in

20), again rinse the inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate <*2.50*> immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.3329 mg of $C_{15}H_{10}I_4NNaO_4$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Lidocaine

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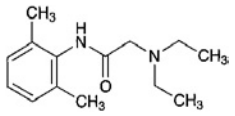

$C_{14}H_{22}N_2O$: 234.34
2-Diethylamino-*N*-(2,6-dimethylphenyl)acetamide
[*137-58-6*]

Lidocaine, when dried, contains not less than 99.0% of $C_{14}H_{22}N_2O$.

**Description** Lidocaine occurs as white to pale yellow crystals or crystalline powder.

It is very soluble in methanol and in ethanol (95), soluble in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification** **(1)** Dissolve 0.04 g of Lidocaine in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Lidocaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 66 – 69°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid, and add water to make 10 mL: the solution is clear and colorless to light yellow.

**(2)** Chloride <*1.03*>—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

**(3)** Sulfate <*1.14*>—Dissolve 0.5 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.096%).

**(4)** Heavy metals <*1.07*>—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(5)** Related substances—Dissolve 0.10 g of Lidocaine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 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 with fluorescent indicator 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, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Dissolve about 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS) until the color of the solution changes from purple to blue-green through blue. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.43 mg of $C_{14}H_{22}N_2O$

**Containers and storage** Containers—Tight containers.

# Lidocaine Injection

## Lidocaine Hydrochloride Injection

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Lidocaine Injection is an aqueous injection.

It contains not less than 95% and not more than 105 % of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O.HCl$: 270.80).

**Method of preparation** Prepare as directed under Injections, with Lidocaine and an equivalent amount of Hydrochloric Acid.

No preservative is added in the case of intravenous injections.

**Description** Lidocaine Injection is a colorless, clear liquid.
pH: 5.0 – 7.0

**Identification** To a volume of Lidocaine Injection, equivalent to 0.02 g of Lidocaine Hydrochloride ($C_{14}H_{22}N_2O.HCl$) according to the labeled amount, add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 10 mL

of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 265 nm.

**Extractable volume** <6.05> It meets the requirement.

**Pyrogen** <4.04> Perform the test with Lidocaine Injection stored in a container in a volume exceeding 10 mL and intended to intravenous injection: it meets the requirements of the Pyrogen Test.

**Assay** To an exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride ($C_{14}H_{22}N_2O.HCl$), add exactly 10 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 85 mg of lidocaine for assay, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS, and add exactly 10 mL of the internal standard solution, then add 0.001 mol/L hydrochloric acid TS to make 50 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of lidocaine to that of the internal standard.

$$\text{Amount (mg) of lidocaine hydrochloride } (C_{14}H_{22}N_2O.HCl) = W_S \times (Q_T/Q_S) \times 1.1556$$

$W_S$: Amount (mg) of lidocaine for assay

*Internal standard solution*—A solution of benzophenone in methanol (1 in 4000).

*Operating conditions*—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11:9).

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

*System suitability*—

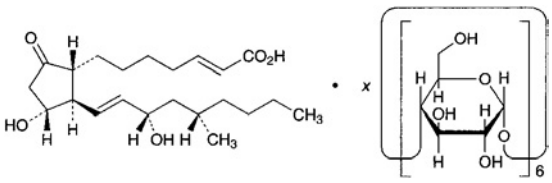
System performance: When proceed with 5 μL of the standard solution under the above operating conditions, lidocaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lidocaine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Limaprost Alfadex

リマプロスト　アルファデクス



$C_{22}H_{36}O_5 \cdot xC_{36}H_{60}O_{30}$

(2*E*)-7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*,5*S*)-3-hydroxy-5-methylnon-1-en-1-yl]-5-oxocyclopentyl}hept-2-enoic acid − α-cyclodextrin [*100459-01-6* (limaprost: alfadex = 1:1; clathrate compound)]

Limaprost Alfadex is a α-cyclodextrin clathrate compound of limaprost.

It contains not less than 2.8% and not more than 3.2% of limaprost ($C_{22}H_{36}O_5$: 380.52), calculated on the anhydrous basis.

**Description** Limaprost Alfadex occurs as a white powder.

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

It is hygroscopic.

**Identification** **(1)** Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and use the upper layer as the sample solution (1). Separately, to 20 mg of Limaprost Alfadex add 5 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution (2). Evaporate the solvent of the sample solutions (1) and (2) under reduced pressure, add 2 mL of sulfuric acid to each of the residue, and shake them for 5 minutes: the solution obtained from the sample solution (1) develops an orange-yellow color while the solution from the sample solution (2) does not develop any color.

**(2)** Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent of the upper layer under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), 5 mL of 1,3-dinitrobenzene TS, add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) while ice-cooling, and allow to stand in a dark place while ice-cooling for 20 minutes: a purple color develops.

**(3)** To 50 mg of Limaprost Alfadex add 1 mL of iodine TS, dissolve by heating in a water bath, and allow to stand: a dark blue precipitate is formed.

**(4)** Determine the absorption spectrum of a solution of Limaprost Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it does not exhibit a maximum between 200 nm and 400 nm. To 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +125 – 135° (0.1 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm)

**Purity** Related substances—Perform the test immediately after preparation of the sample solution. Dissolve 0.10 g of Limaprost Alfadex in 2 mL of water, add 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solution (1), add dilute ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 3 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following operating conditions, and determine each peak area by the automatic integration method: the area of the peak of 17-epi-isomer, having the relative retention time of about 1.1 with respect to limaprost, and the area of the peak of 11-deoxy substance, having the relative retention time of about 2.1 with respect to limaprost, are not larger than the peak area of limaprost from the standard solution (2), and the area of the peak other than the principal peak and other than the peaks mentioned above is not larger than 1/3 times the peak area of limaprost from the standard solution (2). The total area of the peaks other than limaprost from the samples solution is not larger than the peak area of limaprost from the standard solution (1).

*Operating conditions*—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of limaprost beginning after the solvent peak.

*System suitability*—

Test for required detectability: To exactly 1 mL of the standard solution (1) add dilute ethanol to make exactly 10 mL. Confirm that the peak area of limaprost obtained from 3 μL of this solution is equivalent to 8 to 12% of that of limaprost obtained from 3 μL of the standard solution (1).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution (1) under the above conditions, the relative standard deviation of the peak area of limaprost is not more than 2.0%.

**Water** ⟨*2.48*⟩ Not more than 6.0% (0.2 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.1 g of Limaprost Afladex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Limaprost Reference Standard, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 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 ratios, $Q_T$ and $Q_S$, of the peak area of limaprost to that of the internal standard.

Amount (mg) of limaprost ($C_{22}H_{36}O_5$) = $W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of Limaprost Reference Standard

*Internal standard solution*—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

*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 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile for liquid chromatography and 2-propanol for liquid chromatography (9:5:2)

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

*System suitability*—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the internal standard and limaprost are eluted in this order with the resolution between these peaks being not less than 7.

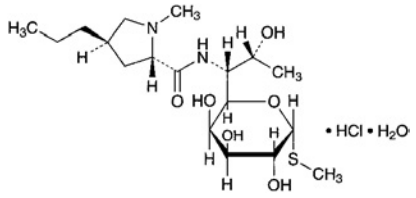
System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of limaprost to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding −10°C.

# Lincomycin Hydrochloride Hydrate

リンコマイシン塩酸塩水和物



$C_{18}H_{34}N_2O_6S.HCl.H_2O$: 461.01

Methyl 6,8-dideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside monohydrochloride monohydrate [*7179-49-9*]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.

It contains not less than 825 μg (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride Hydrate is expressed as mass (potency) of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

**Description** Lincomycin Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

**Identification** **(1)** Determine the infrared absorption spectrum of Lincomycin Hydrochloride Hydrate as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** A solution of Lincomycin Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +135 – +150° (0.5 g, water, 25 mL, 100 mm).

**pH** ⟨*2.54*⟩ Dissolve 0.10 g of Lincomycin Hydrochloride Hydrate in 1 mL of water: 3.0 – 5.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Lincomycin Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

**(3)** Lincomycin B—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 with respect to lincomycin, by the automatic integration method: the peak area of lincomycin B is not more than 5.0% of the sum of the peak areas of lincomycin and lincomycin B.

*Operating conditions—*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

*System suitability—*

Test for required detectability: Measure exactly 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of lincomycin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 20 μL of the sample solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Water** ⟨*2.48*⟩ 3.0 – 6.0% (0.5 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Lincomycin Hydrochloride Hydrate and Lincomycin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and 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 lincomycin.

Amount [μg (potency)] of lincomycin ($C_{18}H_{34}N_2O_6S$) $= W_S \times (A_T/A_S) \times 1000$

$W_S$: Amount [mg (potency)] of Lincomycin Hydrochloride Reference Standard

*Operating conditions—*

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

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 46°C.

Mobile phase: To 13.5 mL phosphoric acid add water to make 1000 mL, and adjust the pH to 6.0 with ammonia TS. To 780 mL of this solution add 150 mL of acetonitrile and 150 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of lincomycin is about 9 minutes.

*System suitability—*

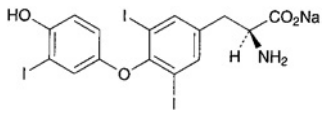
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetrical factor of the peak of lincomycin are not less than 4000 and not more than 1.3, respectively.

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 lincomycin is not more than 2.0%.

**Containers and storage** Containers—Tight containers.

# Liothyronine Sodium

リオチロニンナトリウム




$C_{15}H_{11}I_3NNaO_4$: 672.96

Monosodium *O*-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosinate [*55-06-1*]

Liothyronine Sodium contains not less than 95.0% of $C_{15}H_{11}I_3NNaO_4$, calculated on the dried basis.

**Description** Liothyronine Sodium occurs as a white to light brown powder. It is odorless.

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

It dissolves in sodium hydroxide TS and in ammonia TS.

**Identification** **(1)** To 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000) add 1 mL of ninhydrin TS, and warm in a water bath for 5 minutes: a purple color develops.

**(2)** Heat 0.02 g of Liothyronine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.

**(3)** Determine the absorption spectrum of a solution of Liothyronine Sodium in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Ignite 0.02 g of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to the residue, shake, and filter: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +18 – +22° (0.2 g, calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4:1), 10 mL, 100 mm).

**Purity** **(1)** Soluble halide—To 10 mg of Liothyronine Sodium add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. Add water to the filtrate to make 10 mL, and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.

Control solution: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 1 drop of dilute nitric acid and water to make 10 mL, and add 3 drops of silver nitrate TS.

**(2)** Iodine and iodide—Dissolve 0.10 g of Liothyronine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid, and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100) to the filtrate, mix for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh exactly 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. Pipet 1 mL of this solution, add 10 mL of dilute hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid, and mix. Filter the mixture into a Nessler tube, and perform the test with the filtrate in the same manner as for the sample.

**(3)** Related substances—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of diluted ammonia TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of this solution, add diluted ammonia TS (1 in 3) to make exactly 50 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. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 4.0% (0.2 g, 105°C, 2 hours).

**Assay** Weigh accurately about 25 mg of Liothyronine Sodium, and proceed as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water again. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.7477 mg of $C_{15}H_{11}I_3NNaO_4$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Liothyronine Sodium Tablets

リオチロニンナトリウム錠

Liothyronine Sodium Tablets contain not less than 90% and not more than 110% of the labeled amount of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$: 672.96).

**Method of preparation** Prepare as directed under Tablets, with Liothyronine Sodium.

**Identification** **(1)** To a glass-stoppered centrifuge tube add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium according to the labeled amount, add 30 mL of dilute sodium hydroxide TS, shake vigorously, and centrifuge. Transfer the supernatant liquid to a separator, add 10 mL of dilute hydrochloric acid, and extract with two 20-mL portions of ethyl acetate. Filter each extract successively through absorbent cotton previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of liothyronine sodium for thin-layer chromatography in methanol to make 50 mL, 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 *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R*f value.

**(2)** The colored solution obtained in the Assay is blue in color.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L so-

dium hydroxide VS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 200 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15 %. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

*Internal standard solution*—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

*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 octadecylsylanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted methanol (57 in 100).

Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

*System suitability*—

System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine 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 200 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

**Assay** Weigh accurately not less than 20 Liothyronine Sodium Tablets, and finely powder. Place an accurately weighed portion of the powder, equivalent to about 50 μg of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$), in an agate mortar, add 1 g of powdered potassium carbonate, and mix well. Transfer the mixture cautiously to a porcelain crucible, and compact the contents by gently tapping the crucible on a table. Add an additional 1.5 g of powdered potassium carbonate to the same agate mortar, mix well with any content adhering to the mortar, cautiously overlay the mixture on the top of the same porcelain crucible, and compact the charge again in the same manner. Ignite the combined mixture in the crucible between 675°C and 700°C for 30 minutes. Cool, add a few mL of water to the crucible, heat gently to boiling, and filter the contents of the crucible through a glass filter (G4) into a 20-mL volumetric flask. Wash the residue with water, and combine the washings with the filtrate. Cool, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 200 mL. Measure exactly 5 mL of the solution, and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. To 2 mL of this solution, exactly measured, add a solution of potassium carbonate (1 in 8) to make exactly 20 mL, and use the solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into glass-stoppered test tubes, add 3.0 mL of diluted sulfuric acid (4 in 25) and 2.0 mL of potassium permanganate TS, and heat on a water bath for 15 minutes. Cool, add 1.0 mL of diluted sodium nitrite TS (1 in 10), swirl to mix, and add 1.0 mL of a solution of ammonium amidosulfate (1 in 10). Allow to stand at room temperature for 10 minutes with occasional shaking. Then add 1.0 mL of potato starch TS and 1.0 mL of a freshly prepared, diluted potassium iodide TS (1 in 40), swirl to mix, and transfer each solution to a 20-mL volumetric flask. Rinse the test tube with water, collect the washings in the volumetric flask, add water to make 20 mL, and allow to stand for 10 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of potassium carbonate (1 in 8) in the same manner as the sample solution as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at the wavelength of maximum absorption at about 600 nm, respectively.

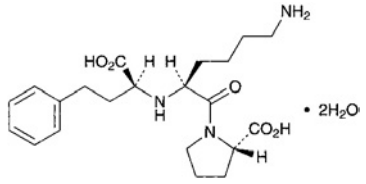
Amount (mg) of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$)
$= W_S \times (A_T/A_S) \times (1/2000) \times 1.3513$

$W_S$: Amount (mg) of potassium iodide for assay

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Lisinopril Hydrate

リシノプリル水和物




$C_{21}H_{31}N_3O_5 \cdot 2H_2O$: 441.52
(2*S*)-1-{(2*S*)-6-Amino-2-[(1*S*)-1-carboxy-3-phenylpropylamino]hexanoyl}pyrrolidine-2-carboxylic acid dihydrate
[*83915-83-7*]

Lisinopril Hydrate contains not less than 98.5% and not more than 101.0% of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49), calculated on the anhydrous basis.

**Description** Lisinopril Hydrate occurs as a white crystalline powder, having a slight characteristic odor.

It is soluble in water, sparingly soluble in methanol, and

practically insoluble in ethanol (99.5).

Melting point: about 160°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Lisinopril Hydrate in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Lisinopril Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $[\alpha]_D^{25}$: −43.0 – −47.0° (0.25 g calculated on the anhydrous basis, 0.25 ml/L zinc acetate buffer solution, pH 6.4, 25 mL, 100 mm).

**Purity** **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Lisinopril Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances—Dissolve about 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.2 with respect to lisinopril, is not larger than 1/5 times the peak area of lisinopril from the standard solution, the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution, and the total area of the peaks other than lisinopril is not larger than the peak area of lisinopril from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

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

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 10 | 90→50 | 10→50 |
| 10 – 25 | 50 | 50 |

Flow rate: About 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril beginning after the solvent peak.

*System suitability*—

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μL of this solution is equivalent to 3.5 to 6.5% of that with 15 μL of the standard solution.

System performance: To 10 mg of Lisinopril Hydrate and 2 mL of a solution of anhydrous caffeine (1 in 1000) add water to make 200 mL. When the procedure is run with 15 μL of this solution under the above operating conditions, lisinopril and caffeine 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 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

**Water** <2.48> Not less than 8.0% and not more than 9.5% (0.3 g, volumetric titration, back titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.55 mg of $C_{21}H_{31}N_3O_5$

**Containers and storage** **Containers—Well-closed containers.**

# Lisinopril Tablets

リシノプリル錠

Lisinopril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$: 405.49).

**Method of preparation** Prepare as directed under Tablets, with Lisinopril Hydrate.

**Identification** To an amount of powdered Lisinopril Tablets, equivalent to 10 mg of lisinopril, add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of Lisinopril 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 30 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 acetonitrile, acetic acid (100), water and ethyl acetate (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 120 °C: the principal spot with the sample solution and the spot with the standard solution show a red-purple color and their *R*f values are the same.

**Purity** Related substances Powder not less than 20 Lisinopril Tablets. Take a portion of the powder, equivalent

to about 25 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add exactly 25 mL of water, shake for 20 minutes, filter, and use the filtrate as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 2.0 with respect to lisinopril, is not more than 2/3 times the peak area of lisinopril from the standard solution.

*Operating conditions*—

Proceed as directed in the Purity (2) under Lisinopril Hydrate.

*System suitability*—

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 $\mu$L of this solution is equivalent to 3.5 to 6.5% of that with 15 $\mu$L of the standard solution.

System performance: Proceed as directed in the Purity (2) under Lisinopril Hydrate.

System repeatability: When the test is repeated 6 times with 15 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Lisinopril Tablets add exactly 5 mL each of the internal standard solution per every 1 mg of lisinopril according to the labeled amount, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Hereafter, proceed as directed in the Assay.

$$\text{Amount (mg) of lisinopril } (C_{21}H_{31}N_3O_5) = W_S \times (Q_T/Q_S) \times (C/10)$$

$W_S$: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

*Internal standard solution*—A solution of anhydrous caffeine (1 in 20,000)

**Dissolution** <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Lisinopril Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 60 minutes after starting the test for a 5-mg tablet, 90 minutes after starting the test for a 10-mg tablet and a 20-mg tablet, and filter through a membrane filter with pore size of not more than 0.5 $\mu$m. Discard the first 10 mL of the filtrate, pipet the subsequent $V$ mL, add water to make exactly $V'$ mL so that each mL contains about 5.6 $\mu$g of lisinopril ($C_{21}H_{31}N_3O_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of lisinopril for assay, separately determined the water content <2.48> in the same manner as Lisinopril Hydrate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 $\mu$L 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 lisinopril. The dissolution rate for a 5-mg tablet in 60 minutes is not less than 80%, for a 10-mg tablet in 90 minutes is not less than 80%, and for a 20-mg tablet in 90 minutes is not less than 75%.

$$\text{Dissolution rate (\%) with respect to the labeled amount of lisinopril } (C_{21}H_{31}N_3O_5) = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$$

$W_S$: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

*Operating conditions*—

Detector, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

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 $\mu$m in particle diameter).

Flow rate: Adjust the flow rate so that the retention time of lisinopril is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 50 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of lisinopril are not less than 1000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

**Assay** Weigh accurately the mass of not less than 20 Lisinopril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add exactly 25 mL of the internal standard solution, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of lisinopril for assay, separately determined the water content <2.48> in the same manner as Lisinopril Hydrate, add exactly 50 mL of the internal standard solution to dissolve, and use this solution as the standard solution. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of lisinopril to that of the internal standard.

$$\text{Amount (mg) of lisinopril } (C_{21}H_{31}N_3O_5) = W_S \times (Q_T/Q_S) \times (1/2)$$

$W_S$: Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of anhydrous caffeine (1 in 20,000)

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

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

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (19:1).

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

*System suitability—*

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lisinopril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

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 lisinopril to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

# Lithium Carbonate

炭酸リチウム

$Li_2CO_3$: 73.89

Lithium Carbonate, when dried, contains not less than 99.5% of $Li_2CO_3$.

**Description** Lithium Carbonate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid.

The pH of a solution of Lithium Carbonate (1 in 100) is between 10.9 and 11.5.

**Identification** **(1)** Perform the test as directed under Flame Coloration Test <*1.04*> (1) with Lithium Carbonate: a persistent red color appears.

**(2)** Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid, and add 4 mL of sodium hydroxide TS and 2 mL of disodium hydrogenphosphate TS: a white precipitate is produced. To the precipitate add 2 mL of hydrochloric acid: it dissolves.

**(3)** A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests <*1.09*> for carbonate.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

**(2)** Acetic acid-insoluble substances—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute acetic acid, filter the insoluble substances using filter paper for quantitative analysis, wash with five 10-mL portions of water, and ignite the insoluble substances together with the filter paper to incinerate: the mass of the residue is not more than 1.5 mg.

**(3)** Chloride <*1.03*>—To 0.40 g of Lithium Carbonate add 10 mL of water and 7 mL of dilute nitric acid, and dissolve by heating to boil. After cooling, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

**(4)** Sulfate <*1.14*>—To 0.40 g of Lithium Carbonate add 10 mL of water and 4 mL of dilute hydrochloric acid, and dissolve by heating to boil. After cooling, add 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

**(5)** Heavy metals <*1.07*>—To 4.0 g of Lithium Carbonate add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing, and dissolve. Evaporate the solution on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a slight red color, then add 2 mL of dilute acetic acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Evaporate 10 mL of hydrochloric acid on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to make 50 mL (not more than 5 ppm).

**(6)** Iron—Prepare the test solution with 1.0 g of Lithium Carbonate according to Method 2 using 11 mL of dilute hydrochloric acid, and perform the test according to Method B. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

**(7)** Aluminum—To 10 mL of solution A obtained in (6) add 10 mL of water and 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and shake. Add 1 mL of a solution of L-ascorbic acid (1 in 100), 2 mL of aluminon TS and water to make 50 mL, shake well, and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 0.1758 g of aluminum potassium sulfate 12-water in water to make 1000 mL. To 1.0 mL of this solution add 10 mL of solution B obtained in (6) and water to make 20 mL, add 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and proceed in the same manner.

**(8)** Barium—To 20 mL of solution A obtained in (6) add 6 mL of water, 0.5 mL of dilute hydrochloric acid, 3 mL of ethanol (95) and 2 mL of potassium sulfate TS, and allow to stand for 1 hour: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 17.8 mg of barium chloride dihydrate in water to make 1000 mL. To 6 mL of this solution add 20 mL of solution B, 0.5 mL of dilute hydrochloric acid and 3 mL of ethanol (95), and proceed in the same manner.

**(9)** Calcium—Weigh accurately about 5 g of Lithium Carbonate, add 50 mL of water and 15 mL of hydrochloric acid, and dissolve. Remove carbon dioxide from the solution by boiling, add 5 mL of ammonium oxalate TS, then make alkaline with ammonia TS, and allow to stand for 4 hours. Filter the produced precipitate through a glass filter (G4),

wash with warm water until the turbidity of the washing is not produced with calcium chloride TS within 1 minute. Transfer the precipitate and the glass filter into a beaker, add water until the glass filter is covered with water, then add 3 mL of sulfuric acid, heat between 70°C and 80°C, and titrate with 0.02 mol/L potassium permanganate VS until a pale red color persists for 30 seconds: the amount of calcium (Ca: 40.08) is not more than 0.05%.

Each mL of 0.02 mol/L potassium permanganate VS
= 2.004 mg of Ca

**(10)** Magnesium—To 3.0 mL of solution A obtained in (6) add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, then add 5 mL of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (6), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

**(11)** Potassium—Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL, and use this solution as the sample solution. To 5 mL of the sample solution add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetraphenylborate (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution add 1.0 mL of dilute acetic acid, shake, and proceed in the same manner.

**(12)** Sodium—Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly 25 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution (1). Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Measure exactly 25 mL of the sample stock solution, add exactly 20 mL of the standard solution, then add water to make exactly 100 mL, and use this solution as the sample solution (2). Determine emission intensities of sodium using a flame photometer with the sample solution (1) and the sample solution (2) under the following conditions. Adjust the wavelength dial to 589 nm, atomize the sample solution (2) into the flame, then adjust the sensitivity so that the emission intensity $L_S$ shows 100 adjustment, and determine emission intensity $L_T$ of the sample solution (1). Then, make the other conditions identical, change the wavelength dial to 580 nm, determine emission intensity $L_B$ of the sample solution (1): the amount of sodium, calculated from the following equation, is not more than 0.05%.

Amount (%) of sodium (Na)
$= \{(L_T - L_B)/(L_S - L_T)\} \times (W'/W) \times 100$

$W$: Amount (mg) of the sample in 25 mL of the sample stock solution
$W'$: Amount (mg) of sodium in 20 mL of the standard solution

**(13)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

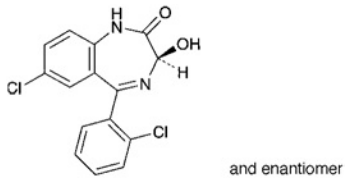
**Assay** Weigh accurately about 1 g of Lithium Carbonate, previously dried, add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate <*2.50*> the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.5 mol/L sulfuric acid VS
= 36.95 mg of $Li_2CO_3$

**Containers and storage** Containers—Well-closed containers.

# Lorazepam

ロラゼパム



and enantiomer

$C_{15}H_{10}Cl_2N_2O_2$: 321.16
(3*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one [*846-49-1*]

Lorazepam, when dried, contains not less than 98.5% of $C_{15}H_{10}Cl_2N_2O_2$.

**Description** Lorazepam occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

**Identification** **(1)** To 0.02 g of Lorazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, and cool: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

**(2)** Determine the absorption spectrum of a solution of Lorazepam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Lorazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(4)** Perform the test with Lorazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

**Absorbance** <*2.24*> $E^{1\%}_{1cm}$ (229 nm): 1080 – 1126 (after drying, 1 mg, ethanol (95), 200 mL).

**Purity** **(1)** Chloride <*1.03*>—To 1.0 g of Lorazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Lorazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Lorazepam according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.10 g of Lorazepam in 20 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 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 $\mu$L 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 chloroform, 1,4-dioxane and acetic acid (100) (91:5:4) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.3% (1 g).

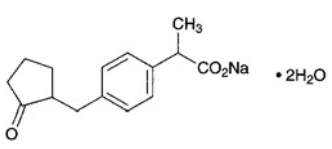
**Assay** Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone, and titrate <*2.50*> with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS
= 32.12 mg of $C_{15}H_{10}Cl_2N_2O_2$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Loxoprofen Sodium Hydrate

ロキソプロフェンナトリウム水和物




$C_{15}H_{17}NaO_3.2H_2O$: 304.31
Monosodium 2-{4-[(2-oxocyclopentyl)methyl]phenyl} propanoate dihydrate
[*80382-23-6*]

Loxoprofen Sodium Hydrate contains not less than 98.5% of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28), calculated on the anhydrous basis.

**Description** Loxoprofen Sodium Hydrate occurs as white to yellowish white crystals or crystalline powder.

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

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of Loxoprofen Sodium Hydrate in freshly boiled and cooled water (1 in 20) is between 6.5 and 8.5.

**Identification** **(1)** Determine the absorption spectrum of a solution of Loxoprofen Sodium Hydrate (1 in 55,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Loxoprofen Sodium Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless or pale yellow. The color is not darker than that of diluted Matching Fluid for Color A (1 in 2).

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Loxoprofen Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 $\mu$L 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 1,2-dichloroethane and acetic acid (100) (9:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** <*2.48*> 11.0 – 13.0% (0.2 g, direct titration).

**Assay** Weigh accurately about 60 mg of Loxoprofen Sodium Hydrate, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Loxoprofen Reference Standard, previously dried in a desiccator (in vacuum, 60°C) for 3 hours, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, proceed in the same manner as directed for the preparation of the sample solution, and use so obtained solution as the standard solution. Perform the test with 10 $\mu$L 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 loxoprofen to that of the internal standard.

Amount (mg) of loxoprofen sodium ($C_{15}H_{17}NaO_3$) = $W_S \times (Q_T/Q_S) \times 1.089$

$W_S$: Amount (mg) of Loxoprofen Reference Standard

*Internal Standard Solution*—A solution of ethyl benzoate in diluted methanol (3 in 5) (7 in 50,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 222 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 methanol, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

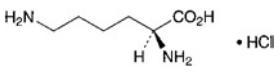
System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of loxoprofen to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# L-Lysine Hydrochloride

## Lysine Hydrochloride

L-リジン塩酸塩




$C_6H_{14}N_2O_2.HCl$: 182.65

(2*S*)-2,6-Diaminohexanoic acid monohydrochloride

[*657-27-2*]

L-Lysine Hydrochloride, when dried, contains not less than 98.5% of $C_6H_{14}N_2O_2.HCl$.

**Description** L-Lysine Hydrochloride occurs as a white powder. It is odorless, and has a slight, characteristic taste.

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

**Identification (1)** Determine the infrared absorption spectrum of L-Lysine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60°C, and repeat the test with the residue.

**(2)** A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +19.0 – +21.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** <2.54> Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <1.14>—Perform the test with 0.6 g of L-Lysine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

**(3)** Ammonium <1.02>—Perform the test with 0.25 g of L-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

**(4)** Heavy metals <1.07>—Proceed with 2.0 g of L-Lysine Hydrochloride 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).

**(5)** Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

**(6)** Related substances—Dissolve 0.10 g of L-Lysine Hydrochloride in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 20 mL, 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 and ammonia water (28) (67:33) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 9.132 mg of $C_6H_{14}N_2O_2HCl$

**Containers and storage** Containers—Tight containers.

# Lysozyme Hydrochloride

リゾチーム塩酸塩


$C_{616}H_{963}N_{193}O_{182}S_{10}.x$HCl
[*12650-88-3*, egg white lysozyme]

Lysozyme Hydrochloride is a hydrochloride of a basic polypeptide obtained from albumen of hen's egg, and has an activity to hydrolyze mucopolysaccharides.

It contains not less than 0.9 mg (potency) of lysozyme per mg, calculated on the dried basis.

**Description** Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

The pH of a solution of Lysozyme Hydrochloride (3 in 200) is between 3.0 and 5.0.

**Identification** **(1)** To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-purple color develops.

**(2)** Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** **(1)** Clarity of solution—To 5 mL of a solution of Lysozyme Hydrochloride (3 in 200) add, if necessary, dilute hydrochloric acid to adjust the pH to 3: the solution is clear.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Lysozyme Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <*2.41*> Not more than 8.0% (0.1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 2.0% (0.5 g).

**Nitrogen** Perform the test as directed under Nitrogen Determination <*1.08*>: the amount of nitrogen (N: 14.01) is between 16.8% and 18.6%, calculated on the dried basis.

**Assay** Weigh accurately an amount of Lysozyme Hydrochloride, equivalent to about 25 mg (potency), dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 2 mL of this solution, add phosphate buffer solution, pH 6.2 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Lysozyme Reference Standard (separately determine its loss on drying <*2.41*> in the same manner as Lysozyme Hydrochloride), equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, add phosphate buffer solution, pH 6.2 to them to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 $\mu$L of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance under Ultraviolet-visible Spectrophotometry <*2.24*>, $A_T$, of this solution at 640 nm, using water as the blank. Determine the absorbances, $A_{S1}$ and $A_{S2}$, of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis
$= (W_S/2W_T) \times \{(A_{S1} - A_T)/(A_{S1} - A_{S2}) + 1\}$

$W_S$: Amount (mg) of Lysozyme Reference Standard, calculated on the dried basis.

$W_T$: Amount (mg) of the sample, calculated on the dried basis.

**Containers and storage** Containers—Tight containers.

# Macrogol 400

## Polyethylene Glycol 400

マクロゴール **400**

Macrogol 400 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of $n$ ranges from 7 to 9.

**Description** Macrogol 400 occurs as a clear, colorless and viscous liquid. It has no odor or a slight, characteristic odor.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is soluble in diethyl ether.

It is slightly hygroscopic.

Congealing point: 4 – 8°C

Specific gravity $d_{20}^{20}$: 1.110 – 1.140

**Identification** Dissolve 0.05 g of Macrogol 400 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** <*2.54*> Dissolve 1.0 g of Macrogol 400 in 20 mL of water: the pH of this solution is between 4.0 and 7.0.

**Purity** **(1)** Acidity—Dissolve 5.0 g of Macrogol 400 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

**(2)** Ethylene glycol and diethylene glycol—Dissolve 4.0 g of Macrogol 400 in water to make exactly 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg each of ethylene glycol and diethylene glycol, dissolve in water to make exactly 100 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. Determine the peak heights, $H_{Ta}$ and $H_{Sa}$, of ethylene glycol of each solution, and the peak heights, $H_{Tb}$ and $H_{Sb}$, of diethylene glycol, and calculate the amount of ethylene glycol and diethylene glycol: the sum of the contents of ethylene glycol and diethylene glycol is not more than 0.25%.

$$\text{Amount (mg) of ethylene glycol} = W_{Sa} \times (H_{Ta}/H_{Sa}) \times (1/10)$$

$$\text{Amount (mg) of diethylene glycol} = W_{Sb} \times (H_{Tb}/H_{Sb}) \times (1/10)$$

$W_{Sa}$: Amount (mg) of ethylene glycol for gas chromatography
$W_{Sb}$: Amount (mg) of diethylene glycol for gas chromatography

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A colum about 3 mm in inside diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography, 150 to 180 μm in particle diameter, coated with D-sorbitol at the ratio of 12%.

Column temperature: A constant temperature of about 165 °C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of diethylene glycol is about 8 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column clearly dividing peaks of ethylene glycol and diethylene glycol in this order.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diethylene glycol obtained from 2 μL of the standard solution composes about 80% of the full scale.

**Average molecular mass** Add 42 g of phthalic anhydride to 300 mL of freshly distilled pyridine, exactly measured, in a 1-L light-resistant glass-stoppered bottle. Shake the bottle vigorously to dissolved the solid, and allow to stand for 16 hours or more. Pipet 25 mL of this solution into an about 200-mL glass-stoppered pressure bottle. Add about 1.5 g of Macrogol 400, accurately weighed, stopper the bottle, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (W \times 4000)/(a - b)$$

$W$: Amount (g) of sample.
$a$: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination.
$b$: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample.

Average molecular mass is between 380 and 420.

**Water** <2.48> Not more than 1.0% (2 g, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Containers and storage** Containers—Tight containers.

# Macrogol 1500

## Polyethylene Glycol 1500

マクロゴール 1500

Macrogol 1500 is a mixture containing equal amounts of lower and higher polymers of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of $n$ is 5 or 6 for the lower polymers and from 28 to 36 for the higher.

**Description** Macrogol 1500 occurs as a white, smooth petrolatum-like solid. It is odorless or has a faint, characteristic odor.

It is very soluble in water, in pyridine and in diphenyl ether, freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Congealing point: 37 – 41°C

**Identification** Dissolve 0.05 g of Macrogol 1500 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** <2.54> Dissolve 1.0 g of Macrogol 1500 in 20 mL of water: the pH of the solution is between 4.0 and 7.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 5.0 g of Macrogol 1500 in 50 mL of water: the solution is clear and colorless.

**(2)** Acidity—Dissolve 5.0 g of Macrogol 1500 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

**(3)** Ethylene glycol and diethylene glycol—Place 50.0 g of Macrogol 1500 in a distilling flask, add 75 mL of diphenyl ether, warm to dissolve if necessary, distil slowly under a reduced pressure of 0.13 to 0.27 kPa and take 25 mL of the distillate in a 100-mL container with 1-mL graduation. To the distillate add exactly 20 mL of water, shake vigorously, cool in ice water, congeal the diphenyl ether, and filtrate into a 25-mL volumetric flask. Wash the residue with 5.0 mL of ice-cold water, combine the washings with the filtrate, warm to room temperature, and add water to make 25 mL. Transfer this solution to a glass-stoppered flask, shake with 25.0 mL of freshly distilled acetonitrile, and use this solution as the sample solution. Separately, to 62.5 mg of diethylene glycol

add a mixture of water and freshly distilled acetonitrile (1:1) to make exactly 25 mL, and use this solution as the standard solution. Take exactly 10 mL each of the sample solution and the standard solution, and add to each exactly 15 mL of cerium (IV) diammonium nitrate TS. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*> within 2 to 5 minutes: the absorbance of the solution obtained from the sample solution at the wavelength of maximum absorption at about 450 nm is not larger than the absorbance of the solution obtained from the standard solution.

**Water** <*2.48*> Not more than 1.0% (2 g, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Containers and storage** Containers—Tight containers.

# Macrogol 4000

## Polyethylene Glycol 4000

マクロゴール **4000**

Macrogol 4000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of $n$ ranges from 59 to 84.

**Description** Macrogol 4000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in methanol and in pyridine, and practically insoluble in ethanol (99.5) and in diethyl ether.

Congealing point: 53 – 57°C

**Identification** Dissolve 0.05 g of Macrogol 4000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** <*2.54*> Dissolve 1.0 g of Macrogol 4000 in 20 mL of water: the pH of this solution is between 4.0 and 7.5.

**Purity** **(1)** Clarity and color of solution—A solution of 5.0 g of Macrogol 4000 in 50 mL of water is clear and colorless.

**(2)** Acidity—Dissolve 5.0 g of Macrogol 4000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthelein TS: the color of the solution is red.

**Average molecular mass** Weigh accurately about 12.5 g of Macrogol 4000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <*2.50*> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (W \times 4000)/(a - b)$$

$W$: Amount (g) of sample.
$a$: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination.
$b$: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample.

Average molecular mass is between 2600 and 3800.

**Water** <*2.48*> Not more than 1.0% (2 g, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.2% (1 g).

**Containers and storage** Containers—Well-closed containers.

# Macrogol 6000

## Polyethylene Glycol 6000

マクロゴール **6000**

Macrogol 6000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of $n$ ranges from 165 to 210.

**Description** Macrogol 6000 is a white, paraffin-like solid, occurring as flakes or powder. It is ordorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in pyridine, and practically insoluble in methanol, in ethanol (95), in ethanol (99.5) and in diethyl ether.

Congealing point: 56 – 61°C

**Identification** Dissolve 0.05 g of Macrogol 6000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** <*2.54*> Dissolve 1.0 g of Macrogol 6000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 5.0 g of Macrogol 6000 in 50 mL of water: the solution is clear and colorless.

**(2)** Acidity—Dissolve 5.0 g of Macrogol 6000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

**Average molecular mass** Weigh accurately about 12.5 g of Macrogol 6000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of

freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at 98 ± 2°C, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at 98 ± 2°C for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination in the same manner.

$$\text{Average molecular mass} = (W \times 4000)/(a - b)$$

$W$: Amount (g) of sample.
$a$: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination.
$b$: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample.

Average molecular mass is between 7300 and 9300.

**Water** <2.48> Not more than 1.0% (2 g, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Containers and storage** Containers—Well-closed containers.

# Macrogol 20000

## Polyethylene Glycol 20000

マクロゴール 20000

Macrogol 20000 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of $n$ lies between 340 and 570.

**Description** Macrogol 20000 occurs as white, paraffin-like flakes or powder. It is ordorless or has a faint, characteristic odor.

It is freely soluble in water and in pyridine, and practically insoluble in methanol, in ethanol (95), in anhydrous diethyl ether, in petroleum benzine and in macrogol 400.

Congealing point: 56 – 64°C

**Identification** Dissolve 0.05 g of Macrogol 20000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

**pH** <2.54> Dissolve 1.0 g of Macrogol 20000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 5.0 g of Macrogol 20000 in 50 mL of water: the solution is clear and colorless.

**(2)** Acidity—Dissolve 5.0 g of Macrogol 20000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol / L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

**Average molecular mass** Weigh accurately about 15 g of Macrogol 20000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of 98 ± 2°C, to the same depth as the mixture in the bottle. Maintain the temperature of the bath at 98 ± 2°C for 60 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\text{Average molecular mass} = (W \times 4000)/(a - b)$$

$W$: Amount (g) of sample.
$a$: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination.
$b$: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample.

Average molecular mass is between 15000 and 25000.

**Water** <2.48> Not more than 1.0% (2 g, direct titration).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Containers and storage** Containers—Well-closed containers.

# Macrogol Ointment

## Polyethylene Glycol Ointment

マクロゴール軟膏

**Method of preparation**

| | |
|---|---|
| Macrogol 4000 | 500 g |
| Macrogol 400 | 500 g |
| To make | 1000 g |

Melt Macrogol 4000 and Macrogol 400 by warming on a water bath at 65°C, and mix well until it congeals. Less than 100 g of Macrogol 4000 or Macrogol 400 may be replaced by an equal amount of Macrogol 400 or Macrogol 4000 to prepare 1000 g of a proper soft ointment.

**Description** Macrogol Ointment is white in color. It has a faint, characteristic odor.

**Identification** Dissolve 0.05 g of Macrogol Ointment in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, filter if necessary, and add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10) to the filtrate: a yellow-green precipitate is formed.

**Containers and storage** Containers—Tight containers.

# Magnesium Carbonate

炭酸マグネシウム

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate.

Magnesium Carbonate contains not less than 40.0% and not more then 44.0% of magnesium oxide (MgO: 40.30).

''Heavy magnesium carbonate'' may be used as commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0-mL graduation line in the Precipitation test.

**Description** Magnesium Carbonate occurs as white, friable masses or powder. It is odorless.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in 1-propanol.

It dissolves in dilute hydrochloric acid with effervescence.

Its saturated solution is alkaline.

**Identification** **(1)** Dissolve 1 g of Magnesium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, neutralize with sodium hydroxide TS, and filter, if necessary: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt.

**(2)** Magnesium Carbonate responds to the Qualitative Tests <*1.09*> (1) for carbonate.

**Purity** **(1)** Soluble salts—To 2.0 g of Magnesium Carbonate add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make exactly 100 mL. Evaporate 50 mL of the solution on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue does not exceed 10.0 mg.

**(2)** Heavy metals <*1.07*>—Moisten 1.0 g of Magnesium Carbonate with 4 mL of water, dissolve by addition of 10 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate, and add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 30 ppm).

**(3)** Iron—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 200 ppm).

**(4)** Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid, and perform the test (not more than 5 ppm).

**(5)** Calcium oxide—Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6 mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2″-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <*2.50*> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes form red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

**(6)** Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

**Precipitation test** Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation in ml).

**Assay** Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction. From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

Each mg of calcium oxide (CaO)
= 0.36 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

**Containers and storage** Containers—Well-closed containers.

# Magnesium Oxide

酸化マグネシウム

MgO: 40.30

Magnesium Oxide, when ignited, contains not less than 96.0% of MgO.

When 5 g of Magnesium Oxide has a volume not more than 30 mL, it may be labeled heavy magnesium oxide.

**Description** Magnesium Oxide occurs as a white powder or granules. It is odorless.

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

It dissolves in dilute hydrochloric acid.

It absorbs moisture and carbon dioxide in air.

**Identification** A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for magnesium salt.

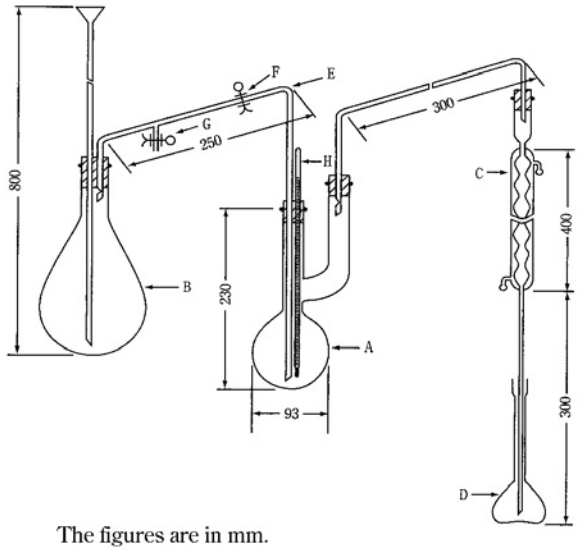
**Purity** **(1)** Alkali and soluble salts—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat on a water bath for 5 minutes, and filter immediately. After cooling, to 50 mL of the filtrate add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 10 mg.

**(2)** Carbonate—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool, and add 5 mL of acetic acid (31): almost no effervescence occurs.

**(3)** Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid, and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, neutralize with ammonia TS, and add 2 mL of dilute acetic acid, 4.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

**(4)** Iron ⟨*1.10*⟩—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

**(5)** Calcium oxide—Weigh accurately about 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2′,2″-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate ⟨*2.50*⟩ with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.




The figures are in mm.

A: Distilling flask of about 300-mL capacity.
B: Steam generator of about 1000-mL capacity, containing a few boiling tips to prevent bumping
C: Condenser
D: Receiver: 200-mL volumetric flask
E: Steam-introducing tube having an internal diameter of about 8 mm
F, G: Rubber tube with a clamp
H: Thermometer

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The mass of calcium oxide (CaO: 56.08) is not more than 1.5%.

**(6)** Arsenic ⟨*1.11*⟩—Dissolve 0.20 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 10 ppm).

**(7)** Acid-insoluble substances—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid dropwise, while shaking, and boil for 5 minutes. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the ignited residue does not more than 2.0 mg.

**(8)** Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the

rubber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under the Oxygen Flask Combustion Method. No corrective solution is used in this procedure.

Amount (mg) of fluoride (F: 19.00) in the test solution
= amount (mg) of fluoride in 5 mL of the standard solution
× $(A_T/A_S)$ × $(200/V)$

The content of fluoride (F) is not more than 0.08%.

**Loss on ignition** ⟨*2.43*⟩ Not more than 10% (0.25 g, 900°C, constant mass).

**Assay** Ignite Magnesium Oxide to constant mass at 900°C, weigh accurately about 0.2 g of the residue, dissolve in 10 mL of water and 4.0 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed, deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

Each mg of calcium oxide (CaO)
= 0.36 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

**Containers and storage** Containers—Tight containers.

# Magnesium Silicate

ケイ酸マグネシウム

Magnesium Silicate contains not less than 45.0% of silicon dioxide ($SiO_2$: 60.08) and not less than 20.0% of magnesium oxide (MgO: 40.30), and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

**Description** Magnesium Silicate occurs as a white, fine powder. It is odorless and tasteless.

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

**Identification** **(1)** Mix 0.5 g of Magnesium Silicate with 10 mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for magnesium salt.

**(2)** Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Magnesium Silicate, and fuse again: an infusible matter appears in the bead, which changes to an opaque bead with a web-like structure upon cooling.

**Purity** **(1)** Soluble salts—Add 150 mL of water to 10.0 g of Magnesium Silicate, heat on a water bath for 60 minutes with occasional shaking, then cool, dilute with water to 150 mL, and centrifuge. Dilute 75 mL of the resultant transparent liquid with water to 100 mL, and use this solution as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 0.02 g.

**(2)** Alkalinity—To 20 mL of the sample solution obtained in (1) add 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

**(3)** Chloride ⟨*1.03*⟩—Take 10 mL of the sample solution obtained in (1), add 6 mL of dilute nitric acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

**(4)** Sulfate ⟨*1.14*⟩—To the residue obtained in (1) add about 3 mL of dilute hydrochloric acid, and heat on a water bath for 10 minutes. Add 30 mL of water, filter, wash the residue on the filter with water, combine the washings with the filtrate, and dilute to 50 mL with water. To 4 mL of the solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

**(5)** Heavy metals ⟨*1.07*⟩—To 1.0 g of Magnesium Silicate add 20 mL of water and 3 mL of hydrochloric acid, and boil for 2 minutes. Filter, and wash the residue on the filter with two 5-mL portions of water. Evaporate the combined filtrate and washings on a water bath to dryness, add 2 mL of dilute acetic acid to the residue, warm until solution is complete, filter, if necessary, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

**(6)** Arsenic ⟨*1.11*⟩—To 0.4 g of Magnesium Silicate add 5 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 5 ppm).

**Loss on ignition** ⟨*2.43*⟩ Not more than 34% (0.5 g, 850°C, 3 hours).

**Acid-consuming capacity** ⟨*6.04*⟩ Place about 0.2 g of Magnesium Silicate, accurately weighed, in a glass-stoppered flask, add exactly 30 mL of 0.1 mol/L hydrochloric acid VS and 20 mL of water, shake at 37 ± 2°C for 1 hour, and cool. Pipet 25 mL of the supernatant liquid, and titrate ⟨*2.50*⟩ the excess hydrochloric acid, while stirring well, with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5.

1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed in the preceding Loss on ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid VS.

**Assay** **(1)** Silicon dioxide—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water bath to dryness, add 25 mL of water to the residue, and heat on a water bath for 15 minutes with occasional stirring. Filter the supernatant liquid through filter paper for assay, add 25 mL of hot water to the residue, stir, and decant the supernatant liquid on the filter paper to filter. Wash the residue in the same manner with two 25-mL portions of hot water, transfer the residue onto the filter paper, and wash with hot water until the last washing does not respond to the Qualitative Tests <*1.09*> (1) for sulfate. Place the residue and the filter paper in a platinum crucible, incinerate with strong heating, and ignite between 775°C and 825°C for 30 minutes, then cool, and weigh the residue as *a* (g). Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as *b* (g).

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \{(a - b)/W\} \times 100$$

*W*: Mass (g) of the sample

**(2)** Magnesium oxide—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to a 50-mL conical flask, add 10 mL of 0.5 mol/L sulfuric acid VS, and heat on a water bath for 15 minutes. Cool, transfer to a 100-mL volumetric flask, wash the conical flask with water, add the washings to the volumetric flask, dilute with water to 100 mL, and filter. Pipet 50 mL of the filtrate, shake with 50 mL of water and 5 mL of diluted 2,2′,2″-nitrilotrisethanol (1 in 2), add 2.0 mL of ammonia TS and 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

$$\text{Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS} = 2.015 \text{ mg of MgO}$$

**(3)** Ratio of percentage (%) of magnesium oxide (MgO) to silicon dioxide ($SiO_2$)—Calculate the quotient from the percentages obtained in (1) and (2).

**Containers and storage** Containers—Well-closed containers.

# Magnesium Stearate

ステアリン酸マグネシウム

Magnesium Stearate consists chiefly magnesium salts of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

It contains, when dried, not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31).

**Description** Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

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

**Identification** **(1)** Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make exactly 50 mL, mix, and use this solution as the sample solution: the sample solution responds to the Qualitative Tests <*1.09*> for magnesium.

**(2)** The retention times of the peaks corresponding to stearic acid and palmitic acid in the chromatogram of the sample solution correspond to those of methyl stearate and methyl palmitate in the chromatogram of the system suitability solution, as obtained in the Purity (5).

**Purity** **(1)** Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, and filter after cooling. To 10 mL of the filtrate add 0.05 mL of bromothymol blue TS, and add exactly 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS: the color of the solution changes.

**(2)** Chloride <*1.03*>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 1.40 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.10%).

**(3)** Sulfate <*1.14*>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 10.2 mL of 0.01 mol/L sulfuric acid VS (not more than 1.0%).

**(4)** Heavy metals <*1.07*>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 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 (not more than 20 ppm).

**(5)** Relative content of stearic acid and palmitic acid—Transfer exactly 0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for about 10 minutes to dissolve the solids. Add 4.0 mL of heptane through the condenser, and reflux for about 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Transfer the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, to another flask.

Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, mix, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas chromatography <*2.02*> according to the following conditions, and determine the area, $A$, of the methyl stearate peak and the total of the areas, $B$, of all of fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

$$\text{Content (\%) of stearic acid} = (A/B) \times 100$$

Similarly, calculate the percentage of palmitic acid in Magnesium Stearate. The methyl stearate peak, and the total of the methyl stearate and methyl palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively, in the chromatogram.

*Operating conditions*—

Detector: A hydrogen flame-ionization detector maintained at a constant temperature of about 260°C.

Sample injection port: A splitless injection system maintained at a constant temperature of about 220°C.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain this temperature for 5 minutes.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of methyl stearate is about 32 minutes.

Split ratio: Splitless

Time span of measurement: About 1.5 time as long as the retention time of methyl stearate beginning after the solvent peak.

*System suitability*—

Test for required detection: Place exactly 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography, each previously dried in a desiccator (silica gel) for 4 hours, in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. To exactly 1 mL of the solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μL of this solution is equivalent to 5 to 15% of that from the solution for system suitability test.

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, methyl palmitate and methyl stearate are eluted in this order, with the relative retention time of methyl palmitate to methyl stearate being about 0.86, and with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 6.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

**Loss on drying** <*2.41*> Not more than 6.0% (2 g, 105°C, constant mass).

**Microbial limit** <*4.05*> The total viable aerobic microbial count is not more than 1000 per g, and the total count of fungi and yeasts is not more than 500 per g. *Salmonella* and *Escherichia coli* should not be observed.

**Assay** Transfer about 0.5 g of previously dried Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of 1-butanol and ethanol (99.5) (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution, pH 10, 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45°C to 50°C to make the solution clear, and after cooling, titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to purple in color. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.431 mg of Mg

**Containers and storage** Containers—Tight containers.

# Magnesium Sulfate Hydrate

硫酸マグネシウム水和物

$MgSO_4.7H_2O$: 246.47

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0% of magnesium sulfate ($MgSO_4$: 120.37).

**Description** Magnesium Sulfate Hydrate occurs as colorless or white crystals. It has a cooling, saline, bitter taste.

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

It dissolves in dilute hydrochloric acid.

**Identification** A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests <*1.09*> for magnesium salt and for sulfate.

**pH** <*2.54*> Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

**(3)** Heavy metals <*1.07*>—Proceed with 2.0 g of Magnesium 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)** Zinc—Dissolve 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water, and add 1 mL of acetic acid and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.

**(5)** Calcium—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of standard calcium solution and 5.0 mL of dilute hydrochloric acid, add water to make exactly 100 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, and determine the absorbances, $A_T$ and $A_S$, of both solutions: $A_T$ is not bigger than $A_S - A_T$ (not more than 0.02%).

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air
Lamp: Calcium hollow-cathod lamp
Wavelength: 422.7 nm

**(6)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

**Loss on ignition** <*2.43*> 45.0 – 52.0% (1 g, after drying at 105°C for 2 hours, ignite at 450°C for 3 hours).

**Assay** Weigh accurately about 0.6 g of Magnesium Sulfate Hydrate, previously ignited at 450°C for 3 hours after drying at 105°C for 2 hours, and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 6.018 mg of $MgSO_4$

**Containers and storage** Containers—Well-closed containers.

# Magnesium Sulfate Injection

硫酸マグネシウム注射液

Magnesium Sulfate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of magnesium sulfate hydrate ($MgSO_4.7H_2O$: 246.47).

**Method of preparation** Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

**Description** Magnesium Sulfate Injection is a clear, colorless liquid.

**Identification** Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate according to the labeled amount, and add water to make 20 mL: the solution responds to the Qualitative Tests <*1.09*> for magnesium salt and for sulfate.

**pH** <*2.54*> 5.5 – 7.0 When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform the test.

**Bacterial endotoxins** <*4.01*> Less than 0.09 EU/mg.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Measure exactly a volume of Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate hydrate ($MgSO_4.7H_2O$), and add water to make 75 mL. Then add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and proceed as directed in the Assay under Magnesium Sulfate Hydrate.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4.7H_2O$

**Containers and storage** Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

# Magnesium Sulfate Mixture

硫酸マグネシウム水

Magnesium Sulfate Mixture contains not less than 13.5 w/v% and not more than 16.5 w/v% of magnesium sulfate hydrate ($MgSO_4.7H_2O$: 246.47).

**Method of preparation**

| | |
|---|---|
| Magnesium Sulfate Hydrate | 150 g |
| Bitter Tincture | 20 mL |
| Dilute Hydrochloric Acid | 5 mL |
| Purified Water | a sufficient quantity |
| | To make 1000 mL |

Prepare before use, with the above ingredients.

**Description** Magnesium Sulfate Mixture is a light yellowish clear liquid. It has a bitter and acid taste.

**Identification (1)** Magnesium Sulfate Mixture responds to the Qualitative Tests <*1.09*> for magnesium salt.

**(2)** Magnesium Sulfate Mixture responds to the Qualitative Tests <*1.09*> (2) for chloride.

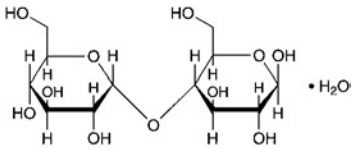
**Assay** Pipet 10 mL of Magnesium Sulfate Mixture, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add 50 mL of water and 5 mL of pH 10.7 ammonia-ammonium chloride buffer solution, and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4.7H_2O$

**Containers and storage** Containers—Tight containers.

# Maltose Hydrate

マルトース水和物



$C_{12}H_{22}O_{11}.H_2O$: 360.31

α-D-Glucopyranosyl-(1→4)-β-D-glucopyranose monohydrate [*6363-53-7*]

Maltose Hydrate, when dried, contains not less than 98.0% of $C_{12}H_{22}O_{11}.H_2O$.

**Description** Maltose Hydrate occurs as white crystals or crystalline powder.

It has a sweet taste.

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

**Identification** **(1)** Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS, and heat for 5 minutes on a water bath: an orange color develops.

**(2)** Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is formed.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +126 – +131° Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

**pH** ⟨*2.54*⟩ The pH of a solution of Maltose Hydrate (1 in 10) is between 4.5 and 6.5.

**Purity** **(1)** Clarity and color of solution—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60 °C in a water bath to dissolve, and after cooling, add water to make 50 mL: the solution is clear, and has no more color than the following control solution.

Control solution: Add water to a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 3.0 mL of Ferric Chloride Stock CS and 2.0 mL of Cupric Sulfate Stock CS to make 10.0 mL. To 1.0 mL of this solution add water to make 50 mL.

**(2)** Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

**(3)** Sulfate ⟨*1.14*⟩—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

**(4)** Heavy metals ⟨*1.07*⟩—Proceed with 5.0 g of Maltose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

**(5)** Arsenic ⟨*1.11*⟩—Dissolve 1.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, then heat to concentrate to 5 mL, and use this solution as the test solution after cooling. Perform the test (not more than 1.3 ppm).

**(6)** Dextrin, soluble starch and sulfite—Dissolve 1.0 g of Maltose Hydrate in 10 mL of water, and add 1 drop of iodine TS: a yellow color appears, and the color changes to a blue by adding 1 drop of starch TS.

**(7)** Nitrogen—Weigh accurately about 2 g of Maltose Hydrate, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩ using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01%.

**(8)** Related substances—Dissolve 0.5 g of Maltose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of the peaks which appear before the peak of maltose from the sample solution is not larger than 1.5 times of the peak area of maltose from the standard solution, and the total area of the peaks which appear after the peak of maltose from the sample solution is not larger than 1/2 time of the peak area of maltose from the standard solution.

*Operating conditions*—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the sensitivity so that the peak height of maltose obtained from 20 μL of the standard solution is about 30 mm.

Time span of measurement: About 2 times as long as the retention time of maltose.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose Reference Standard, previously dried, dissolve in exactly 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following operating conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of maltose to that of the internal standard.

$$\text{Amount (mg) of } C_{12}H_{22}O_{11}.H_2O = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Maltose Reference Standard

*Internal standard solution*—A solution of ethylene glycol (1 in 50).

*Operating conditions*—

Detector: A differential refractometer.

Column: A stainless steel column about 8 mm in inside diameter and about 55 cm in length, packed with gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linking: 8 %) (10 μm in particle diameter).

Column temperature: A constant temperature of about

50°C.

Mobile phase: Water

Flow rate: Adjust the flow rate so that the retention time of maltose is about 18 minutes.

Selection of column: Dissolve 0.25 g of maltose, 0.25 g of glucose and 0.4 g of ethylene glycol in water to make 100 mL. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of maltose, glucose and ethylene glycol in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

**Containers and storage** Containers—Tight containers.

# Freeze-dried Mamushi Antivenom, Equine

乾燥まむしウマ抗毒素

Freeze-dried Mamushi Antivenom, Equine, is a preparation for injection which is dissolved before use.

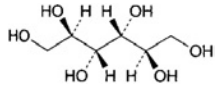
It contains *Agkistrodon Halys* antivenom in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Mamushi Antivenom, Equine, in the Minimum Requirements for Biological Products.

**Description** Freeze-dried Mamushi Antivenom, Equine, becomes a colorless or light yellow-brown, clear liquid, or a slightly white-turbid liquid on addition of solvent.

# D-Mannitol

D-マンニトール



$C_6H_{14}O_6$: 182.17
D-Mannitol [*69-65-8*]

D-Mannitol, when dried, contains not less than 98.0% of $C_6H_{14}O_6$.

**Description** D-Mannitol occurs as white crystals or powder. It is odorless, and has a sweet taste with a cold sensation.

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

It dissolves in sodium hydroxide TS.

**Identification (1)** To 5 drops of a saturated solution of D-Mannitol add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

**(2)** Determine the infrared absorption spectrum of D-Mannitol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 1 g of D-Mannitol in 3 mL of warm water, then allow to stand at 5°C for 24 hours or until crystals appear, and filter. Wash the crystals so obtained with a few amount of cold water, dry at 105°C for 4 hours, and perform the test with the crystals.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: + 137 – + 145° Weigh accurately about 1.0 g of D-Mannitol, previously dried, dissolve in 80 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 20), and add diluted sulfuric acid (1 in 35) to make exactly 100 mL. Measure the optical rotation of this solution in a 100-mm cell.

**Melting point** <*2.60*> 166 – 169°C

**Purity (1)** Clarity and color of solution—Dissolve 2.0 g of D-Mannitol in 10 mL of water by warming: the solution is clear and colorless.

**(2)** Acidity—Dissolve 5.0 g of D-Mannitol in 50 mL of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

**(3)** Chloride <*1.03*>—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

**(4)** Sulfate <*1.14*>—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

**(5)** Heavy metals <*1.07*>—Proceed with 5.0 g of D-Mannitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

**(6)** Nickel—Dissolve 0.5 g of D-Mannitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

**(7)** Arsenic <*1.11*>—Prepare the test solution with 1.5 g of D-Mannitol according to Method 1, and perform the test (not more than 1.3 ppm).

**(8)** Sugars—To 5.0 g of D-Mannitol add 15 mL of water and 4.0 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS), and add water to make 50 mL. Pipet 10 mL of this solution into a flask, boil gently with 10 mL of water and 40 mL of Fehling's TS for 3 minutes, and allow to stand to precipitate copper (I) oxide. Filter the supernatant liquid through a glass filter (G4), wash the precipitate with hot water until the last washing no longer shows an alkaline reaction, and filter the washings through the glass filter described above. Dissolve the precipitate in 20 mL of iron (III) sulfate TS in the flask, filter through the glass filter described above, and wash the filter with water. Combine the washings and the filtrate, heat to 80°C, and titrate <*2.50*> with 0.02 mol/L potassium permanganate: the consumed volume is not more than 1.0 mL.

**Loss on drying** <*2.41*> Not more than 0.30% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of D-Mannitol, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly

50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

**Containers and storage** Containers—Tight containers.

# D-Mannitol Injection

## D-Mannite Injection

D-マンニトール注射液

D-Mannitol Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of D-mannitol ($C_6H_{14}O_6$: 182.17).

**Method of preparation** Prepare as directed under Injections, with D-Mannitol. No preservative is added.

**Description** D-Mannitol Injection is a clear, colorless liquid. It has a sweet taste.

It may precipitate crystals.

**Identification** Concentrate D-Mannitol Injection on a water bath to make a saturated solution. Proceed with 5 drops of this solution as directed in the Identification (1) under D-Mannitol.

**pH** <*2.54*> 4.5 – 7.0

**Bacterial endotoxins** <*4.01*> Less than 0.50 EU/mL.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Measure exactly a volume of D-Mannitol Injection, equivalent to about 5 g of D-Mannitol ($C_6H_{14}O_6$), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under D-Mannitol.

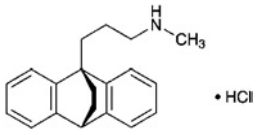
Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

**Containers and storage** Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

# Maprotiline Hydrochloride

マプロチリン塩酸塩




$C_{20}H_{23}N.HCl$: 313.86

3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-*N*-methylpropylamine monohydrochloride [*10347-81-6*]

Maprotiline Hydrochloride, when dried, contains not less than 99.0% of $C_{20}H_{23}N.HCl$.

**Description** Maprotiline Hydrochloride occurs as a white crystalline powder.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.

Melting point: about 244°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Maprotiline Hydrochloride in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Maprotiline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample with ethanol (99.5), filter, dry the crystals so obtained, and perform the test with the crystals.

**(3)** To 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> for chloride.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Maprotiline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances—Dissolve 0.10 g of Maprotiline Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 180 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 31.39 mg of $C_{20}H_{23}N.HCl$

**Containers and storage** Containers—Well-closed containers.

# Freeze-dried Live Attenuated Measles Vaccine

乾燥弱毒生麻しんワクチン

Freeze-dried Live Attenuated Measles Vaccine is a preparation for injection which is dissolved before use.

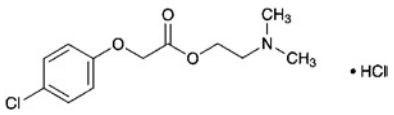
It contains live attenuated measles virus.

It conforms to the requirements of Freeze-dried Live Attenuated Measles Vaccine in the Minimum Requirements for Biological Products.

**Description** Freeze-dried Live Attenuated Measles Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

# Meclofenoxate Hydrochloride

メクロフェノキサート塩酸塩



$C_{12}H_{16}ClNO_3.HCl$: 294.17
2-(Dimethylamino)ethyl (4-chlorophenoxy)acetate monohydrochloride [*3685-84-5*]

Meclofenoxate Hydrochloride contains not less than 98.0% of $C_{12}H_{16}ClNO_3.HCl$, calculated on the anhydrous basis.

**Description** Meclofenoxate Hydrochloride occurs as white crystals or crystalline powder. It has a faint, characteristic odor and a bitter taste.

It is freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Meclofenoxate Hydrochloride (1 in 20) is between 3.5 and 4.5.

**Identification** **(1)** To 0.01 g of Meclofenoxate Hydrochloride add 2 mL of ethanol (95), dissolve by warming if necessary, cool, add 2 drops of a saturated solution of hydroxylammonium chloride in ethanol (95) and 2 drops of a saturated solution of potassium hydroxide in ethanol (95), and heat in a water bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid, and add 3 drops of iron (III) chloride TS: a red-purple to dark purple color develops.

**(2)** Dissolve 0.05 g of Meclofenoxate Hydrochloride in 5 mL of water, and add 2 drops of Reinecke salt TS: a light red precipitate is formed.

**(3)** Determine the absorption spectrum of a solution of Meclofenoxate Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** A solution of Meclofenoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

**Melting point** <*2.60*> 139 – 143°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Meclofenoxate Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <*1.14*>—Perform the test with 1.0 g of Meclofenoxate Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

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

**(4)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Meclofenoxate Hydrochloride according to method 3, and perform the test (not more than 2 ppm).

**(5)** Organic acids—To 2.0 g of Meclofenoxate Hydrochloride add 50 mL of diethyl ether, shake for 10 minutes, filter through a glass filter (G3), wash the residue with two 5-mL portions of diethyl ether, and combine the washings with the filtrate. To this solution add 50 mL of neutralized ethanol and 5 drops of phenolphthalein TS, and neutralize with 0.1 mol/L sodium hydroxide VS: the volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.54 mL.

**Water** <*2.48*> Not more than 0.50% (1 g, dirct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

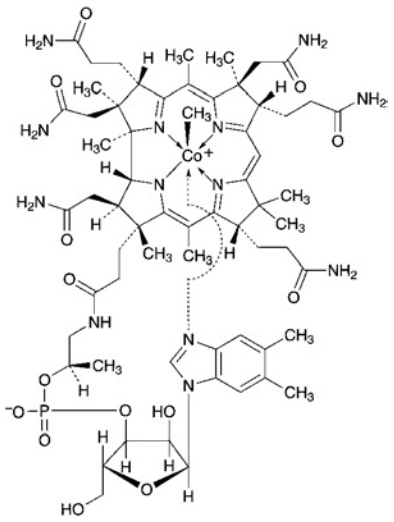
**Assay** Weigh accurately about 0.4 g of Meclofenoxate Hydrochloride, dissolve in 70 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)]. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.42 mg of $C_{12}H_{16}ClNO_3.HCl$

**Containers and storage** Containers—Tight containers.

# Mecobalamin

メコバラミン



$C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38
*Co*α-[α-(5,6-Dimethyl-1*H*-benzoimidazol-1-yl)]-*Co*β-methylcobamide [*13422-55-4*]

Mecobalamin contains not less than 98.0% of $C_{63}H_{91}CoN_{13}O_{14}P$, calculated on the anhydrous basis.

**Description** Mecobalamin occurs as dark red crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It is affected by light.

**Identification** **(1)** Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Mecobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution, pH 7.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Mix 1 mg of Mecobalamin with 0.05 g of potassium bisulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

**Purity** **(1)** Clarity and color of solution—Dissolve 20 mg of Mecobalamin in 10 mL of water: the solution is clear and red color.

**(2)** Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak area of mecobalamin and others of the sample solution by the automatic integration method: each area of the peaks other than mecobalamin is not larger than 0.5% of the peak area of mecobalamin, and the total area of the peaks other than mecobalamin is not larger than 2.0%.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of mecobalamin.

*System suitability*—

Test for required detection: To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add the mobile phase to make exactly 10 mL. Confirm that the peak area of mecobalamin obtained from 10 μL of this solution is equivalent to 7 to 13% of that of mecobalamin obtained from 10 μL of the test solution for system suitability.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the test solution for system suitability under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 3.0%.

**Water** <*2.48*> Not more than 12% (0.1 g, direct titration).

**Assay** Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin Reference Standard (separately, determine the water <*2.48*> in the same manner as mecobalamin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL of 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 mecobalamin in each solution.

$$\text{Amount (mg) of } C_{63}H_{91}CoN_{13}O_{14}P = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Mecobalamin Reference Standard, calculated on the anhydrous basis

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 266 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: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

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

*System suitability*—

System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And when the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of mecobalamin is not less than 6000.

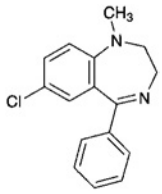
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Medazepam

メダゼパム




$C_{16}H_{15}ClN_2$: 270.76

7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine [*2898-12-6*]

Medazepam, when dried, contains not less than 98.5% of $C_{16}H_{15}ClN_2$.

**Description** Medazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It gradually changes in color by light.

**Identification** **(1)** Dissolve 0.01 g of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

**(2)** Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Perform the test with Medazepam as directed under Flame Coloration Test <*1.04*> (2): a green color is produced.

**Melting point** <*2.60*> 101 – 104°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Medazepam in 10 mL of methanol: the solution is clear and light yellow to yellow in color.

**(2)** Chloride <*1.03*>—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Medazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(4)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Medazepam according to Method 3, and perform the test (not more than 2 ppm).

**(5)** Related substances—Dissolve 0.25 g of Medazepam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28) (60:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.08 mg of $C_{16}H_{15}ClN_2$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Medicinal Carbon

薬用炭

**Description** Medicinal Carbon occurs as a black, odorless and tasteless powder.

**Identification** Place 0.5 g of Medicinal Carbon in a test tube, and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

**Purity** **(1)** Acidity or alkalinity—Boil 3.0 g of Medicinal

Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water, and filter: the filtrate is colorless and neutral.

**(2)** Chloride <*1.03*>—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

**(3)** Sulfate <*1.14*>—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

**(4)** Sulfide—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead (II) acetate paper does not become brown when held in the evolved gas within 5 minutes.

**(5)** Cyanogen compounds—Place a mixture of 5 g of Medicinal Carbon, 2 g of L-tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distil to 25 mL. Dilute the distillate with water to 50 mL. To 25 mL of the diluted distillate add 1 mL of a solution of iron (II) sulfate heptahydrate (1 in 20), heat the mixture almost to boiling, cool, and filter. To the filtrate add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

**(6)** Acid soluble substances—To about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water, and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite the residue strongly: the mass of the residue is not more than 3.0%.

**(7)** Heavy metals <*1.07*>—Proceed with 0.5 g of Medicinal Carbon according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

**(8)** Zinc—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water, and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL, add 1 drop of sodium sulfide TS, and allow to stand for 3 minutes: the liquid produces no turbidity.

**(9)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Medicinal Carbon according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** <*2.41*> Not more than 15.0% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 4.0% (1 g).

**Adsorptive power** **(1)** Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate. Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

**(2)** Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <*2.50*> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS. The difference between the two titrations is not less than 1.2 mL.

**Containers and storage** Containers—Well-closed containers.

# Medicinal Soap

薬用石ケン

Medicinal Soap is sodium salts of fatty acids.

**Description** Medicinal Soap occurs as white to light yellow powder or granules. It has a characteristic odor free from rancidity.

Medicinal Soap is sparingly soluble in water, and slightly soluble in ethanol (95).

A solution of Medicinal Soap (1 in 100) is alkaline.

**Fatty acid** Dissolve 25 g of Medicinal Soap in 300 mL of hot water, add 60 mL of dilute sulfuric acid slowly, and warm in a water bath for 20 minutes. After cooling, filter off the precipitate, and wash with warm water until the washing no longer shows acidity to methyl orange TS. Transfer the precipitate to a small beaker, and heat on a water bath to complete separation of water and transparent fatty acids. Filter the fatty acid into a small beaker while warm, dry at 100°C for 20 minutes, and perform the test with this material as directed under Fats and Fatty Oils <*1.13*>. The congealing point of the fatty acid is between 18°C and 28°C. The acid value is 185 – 205. The iodine value is 82 – 92.

**Purity** **(1)** Acidity or alkalinity—Dissolve 5.0 g of Medicinal Soap in 85 mL of neutralized ethanol by warming on a water bath, filter while hot through absorbent cotton, and wash the filter and the residue with three 5-mL portions of hot neutralized ethanol. Combine the filtrate and the washings, add hot neutralized ethanol to make exactly 100 mL, and perform the following tests quickly using this as the sample solution at 70°C.

(i) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS to 40 mL of the sample solution: a red color develops.

(ii) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS to 40 mL of the sample solution:

no red color develops.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Medicinal Soap according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Ethanol-insoluble substances—Weigh accurately about 2 g of Medicinal Soap, dissolve by warming in 100 mL of neutralized ethanol, filter the solution through a glass filter (G4), wash the residue with hot neutralized ethanol, and dry at 105°C for 4 hours: the mass of the residue is not more than 1.0%.

**(4)** Water-insoluble substances—Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

**(5)** Alkali carbonates—To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

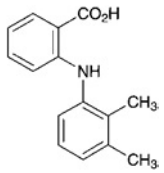
**Loss on drying** Not more than 5.0% in the case of the powder, and not more than 10.0% in the case of the granules.

Weigh accurately about 0.5 g of Medicinal Soap in a tared beaker, add 10 g of sea sand (No. 1), previously dried at 105°C for 1 hour, and again weigh the beaker. Add 10 mL of ethanol (95), evaporate on a water bath to dryness with thorough stirring, and dry at 105°C for 3 hours.

**Containers and storage** Containers—Well-closed containers.

# Mefenamic Acid

メフェナム酸




$C_{15}H_{15}NO_2$: 241.29
2-(2,3-Dimethylphenylamino)benzoic acid [*61-68-7*]

Mefenamic Acid, when dried, contains not less than 99.0% of $C_{15}H_{15}NO_2$.

**Description** Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.

It is sparingly soluble in diethyl ether, slightly soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 225°C (with decomposition).

**Identification (1)** Dissolve 0.01 g of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of *p*-nitrobenzene diazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS, and mix thoroughly: an orange-red color is produced.

**(2)** Dissolve 0.01 g of Mefenamic Acid in 2 mL of sulfuric acid, and heat: the solution shows a yellow color and a green fluorescence.

**(3)** Dissolve 7 mg of Mefenamic Acid in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity (1)** Chloride <*1.03*>—To 1.0 g of Mefenamic Acid add 20 mL of sodium hydroxide TS, and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make 100 mL, and mix well. Remove the produced precipitate by filtration, discard the first 10 mL of the filtrate, and to subsequent 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of nitric acid and water to make 50 mL (not more than 0.071%).

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Mefenamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Mefenamic Acid according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.10 g of Mefenamic Acid, in 5 mL of a mixture of chloroform and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (3:1) to make exactly 200 mL, pipet 10 mL of this solution, add a mixture of chloroform and methanol (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 25 $\mu$L 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 2-methyl-1-propanol and ammonia solution (28) (3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

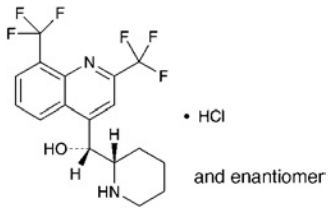
**Assay** Weigh accurately about 0.5 g of Mefenamic Acid, previously dried, and dissolve in 100 mL of ethanol (95), previously neutralized to phenol red TS with 0.1 mol/L sodium hydroxide VS, by warming gently. Cool, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.13 mg of $C_{15}H_{15}NO_2$

**Containers and storage** Containers—Well-closed containers.

# Mefloquine Hydrochloride

メフロキン塩酸塩




$C_{17}H_{16}F_6N_2O.HCl$: 414.77
(1*RS*)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol monohydrochloride
[*51773-92-3*]

Mefloquine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{17}H_{16}F_6N_2O.HCl$.

**Description** Mefloquine Hydrochloride occurs as white crystals or a white crystalline powder.

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

It dissolves in sulfuric acid.

A solution of Mefloquine Hydrochloride in methanol (1 in 20) shows no optical rotation.

Melting point: about 260°C (with decomposition).

**Identification** **(1)** Dissolve 2 mg of Mefloquine Hydrochloride in 1 mL of sulfuric acid: the solution shows a blue fluorescence under ultraviolet light (main wavelength: 365 nm).

**(2)** Determine the absorption spectrum of a solution of Mefloquine Hydrochloride in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Mefloquine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(4)** To 5 mL of a solution of Mefloquine Hydrochloride (1 in 1000) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is formed, and the separated precipitate dissolves on the addition of an excess amount of ammonia TS.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mefloquine Hydrochloride according to Method 2 using a quartz crucible, 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*>—To 1.0 g of Mefloquine Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, gradually heat, and incinerate by ignition at 800°C. If a carbonized residue still retains, moisten the residue with a little amount of nitric acid, and ignite again to incinerate. After cooling, to the residue add 3 mL of hydrochloric acid, warm on a water bath to dissolve, and perform the test using this solution as the test solution (not more than 2 ppm).

**(3)** Related substances—Dissolve 50 mg of Mefloquine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than mefloquine and the peak eluted first from the sample solution is not larger than the peak area of mefloquine from the standard solution, and the total area of the peaks other than the peak of mefloquine and the peak eluted first from the sample solution is not larger than 2.5 times the peak area of mefloquine from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

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

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 14) (24:1).

Flow rate: Adjust the flow rate so that the retention time of mefloquine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of mefloquine.

*System suitability*—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of mefloquine obtained with 10 $\mu$L of this solution is equivalent to 40 to 60% of that obtained with 10 $\mu$L of the standard solution.

System performance: Dissolve 10 mg of mefloquine hydrochloride and 5 mg of diprophylline in 50 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, diprophylline and mefloquine 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 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mefloquine is not more than 2.0%.

**(4)** Residual solvent—The test is specified separately.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g, platinum crucible).

**Assay** Weigh accurately about 0.5 g of Mefloquine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and ti-

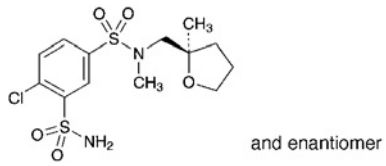
trate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.48 mg of $C_{17}H_{16}F_6N_2O.HCl$

**Containers and storage** Containers—Well-closed containers.

# Mefruside

メフルシド




and enantiomer

$C_{13}H_{19}ClN_2O_5S_2$: 382.88
4-Chloro-*N*-methyl-*N*-[(2*RS*)-2-methyltetrahydrofuran-2-ylmethyl]-3-sulfamoylbenzenesulfonamide [*7195-27-9*]

Mefruside, when dried, contains not less than 98.5% of $C_{13}H_{19}ClN_2O_5S_2$.

**Description** Mefruside occurs as a white crystalline powder.

It is very soluble in dimethylformamide, freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

A solution of Mefruside in dimethylformamide (1 in 10) has no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of Mefruside in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Mefruside, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Perform the test with Mefruside as directed under Flame Coloration Test (2): a green color appears.

**Melting point** <2.60> 149 – 152°C

**Purity** **(1)** Heavy metals <1.07>—Dissolve 1.0 g of Mefruside in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. 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 30 mL of acetone, 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 1.0 g of Mefruside according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.20 g of Mefruside in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Mefruside, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 13 mL of water to 80 mL of *N,N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 38.29 mg of $C_{13}H_{19}ClN_2O_5S_2$

**Containers and storage** Containers—Well-closed containers.

# Mefruside Tablets

メフルシド錠

Mefruside Tablets contain not less than 95% and not more than 105% of the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$: 382.88).

**Method of preparation** Prepare as directed under Tablets, with Mefruside.

**Identification** **(1)** Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.3 g of Mefruside according to the labeled amount, shake with 15 mL of heated methanol for 20 minutes, and filter. Add 25 mL of water to the filtrate, and allow to stand while ice-cooling for 30 minutes. Filter the white precipitate formed, wash with water, and dry at 105°C for 2 hours: the precipitate melts <2.60> between 149°C and 152°C.

**(2)** Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.01 g of Mefruside according to the labeled amount, shake with 70 mL of methanol strongly for 15 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 274 nm and 278 nm, and between 283 nm and 287 nm.

**Dissolution** <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Mefruside Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Take 20 mL or more of

the dissolved solution 45 minutes after starting the test, and filter through a filter paper for quantitative analysis (5C). Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 70 mg of mefruside for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add water 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 the standard solution at 285 nm in a layer of 5 cm in length as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Mefruside Tablets in 45 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)
$= W_S \times (A_T/A_S) \times (1/C) \times 36$

$W_S$: Amount (mg) of mefruside for assay.
$C$: Labeled amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) in 1 tablet.

**Assay** Weigh accurately not less than 20 Mefruside Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 65 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$), shake with 70 mL of methanol for 15 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly 10 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 65 mg of mefruside for assay, previously dried at 105°C for 2 hours, 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. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

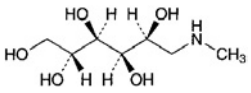
Amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of mefruside for assay

**Containers and storage** Containers—Tight containers.

# Meglumine

メグルミン



$C_7H_{17}NO_5$: 195.21
1-Deoxy-1-methylamino-D-glucitol [*6284-40-8*]

Meglumine, when dried, contains not less than 99.0% of $C_7H_{17}NO_5$.

**Description** Meglumine occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

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

The pH of a solution of Meglumine (1 in 10) is between 11.0 and 12.0.

**Identification** **(1)** To 1 mL of a solution of Meglumine (1 in 10) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color develops.

**(2)** To 2 mL of a solution of Meglumine (1 in 10) add 1 drop of methyl red TS, and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boric acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color develops.

**(3)** Dissolve 0.5 g of Meglumine in 1 mL of diluted hydrochloric acid (1 in 3), and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall of the container with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5), and dry at 105°C for 1 hour: the residue thus obtained melts <*2.60*> between 149°C and 152°C.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-16.0$ – $-17.0°$ (after drying, 1 g, water, 10 mL, 100 mm).

**Melting point** <*2.60*> 128 – 131°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Meglumine in 10 mL of water: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 10 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

**(3)** Sulfate <*1.14*>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

**(4)** Heavy metals <*1.07*>—Proceed with 2.0 g of Meglumine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(5)** Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Meglumine according to Method 3, and perform the test (not more than 1 ppm).

**(6)** Reducing substances—To 5 mL of a solution of Meglumine (1 in 20) add 5 mL of Fehling's TS, and boil for 2 minutes: no red-brown precipitate is produced.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Meglumine, previously dried, dissolve in 25 mL of water, and titrate <*2.50*> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
$= 19.52$ mg of $C_7H_{17}NO_5$

**Containers and storage** Containers—Tight containers.

# Meglumine Amidotrizoate Injection

アミドトリゾ酸メグルミン注射液

Meglumine Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 46.9 w/v% and not more than 51.8 w/v% of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

**Method of preparation**

| | |
|---|---|
| Amidotrizoic Acid (anhydrous) | 493.2 g |
| Meglumine | 156.8 g |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Meglumine Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

**Identification** **(1)** To 2 mL of Meglumine Amidotrizoate Injection add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

**(2)** To 1 mL of Meglumine Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

**Optical rotation** ⟨*2.49*⟩ $\alpha_D^{20}$: −3.63 – −4.20° (100 mm).

**pH** ⟨*2.54*⟩ 6.0 – 7.7

**Purity** **(1)** Primary aromatic amines—Mix 0.40 mL of Meglumine Amidotrizoate Injection with 6 mL of water, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

**(2)** Iodine and iodide—To 0.50 mL of Meglumine Amidotrizoate Injection add water to make 20 mL, shake with 5 mL of dilute nitric acid, filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Pyrogen** ⟨*4.04*⟩ Prepare a solution with isotonic sodium chloride solution so as to contain 0.40 mL of Meglumine Amidotrizoate Injection per 1 mL, and perform the test: it meets the requirements.

**Assay** To an exactly measured 1 mL of Meglumine Amidotrizoate Injection add water to make exactly 200 mL, pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (previously determine the loss on drying ⟨*2.41*⟩ in the same manner as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of amidotrizoic acid to that of the internal standard.

$$\text{Amount (mg) of amidotrizoic acid } (C_{11}H_9I_3N_2O_4) = W_S \times (Q_T/Q_S) \times 2$$

$W_S$: Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

*Internal standard solution*—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 25°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid is about 5 minutes.

*System suitability*—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, amidotrizoic 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Meglumine Iotalamate Injection

イオタラム酸メグルミン注射液

Meglumine Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of iotalamic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

**Method of preparation**

| | |
|---|---|
| **(1)** | |
| Iotalamic Acid | 227.59 g |
| Meglumine | 72.41 g |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |
| **(2)** | |
| Iotalamic Acid | 455 g |
| Meglumine | 145 g |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Injections, with the above ingredients (1) or (2).

**Description** Meglumine Iotalamate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

**Identification** **(1)** To 1 mL of Meglumine Iotalamate Injection add 1 mL of potassium naphthoquinone sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

**(2)** To a volume of Meglumine Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid while shaking: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 4 hours. Proceed with the precipitate so obtained as directed in the Identification (2) under Iotalamic Acid.

**Optical rotation** ⟨*2.49*⟩

Method of preparation (1) $\alpha_D^{20}$: −1.67 – −1.93° (100 mm).

Method of preparation (2) $\alpha_D^{20}$: −3.35 – −3.86° (100 mm).

**pH** ⟨*2.54*⟩ 6.5 – 7.7

**Purity** **(1)** Primary aromatic amines—To a volume of Meglumine Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

**(2)** Iodine and iodide—Take a volume of Meglumine Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, and proceed as directed in the Purity (2) under Sodium Iotalamate Injection.

**Bacterial endotoxins** ⟨*4.01*⟩ Less than 0.90 EU/mL.

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Assay** To an exactly measured volume of Meglumine Iotalamate Injection, equivalent to about 4 g of iotalamic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 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 iotalamic acid to that of the internal standard.

$$\text{Amount (mg) of iotalamic acid } (C_{11}H_9I_3N_2O_4) = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of iotalamic acid for assay

*Internal standard solution*—A solution of L-tryptophan in the mobile phase (3 in 2500).

*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 20°C.

Mobile phase: Dissolve 3.9 g of phosphoric acid and 2.8 mL of triethylamine in water to make 2000 mL. To this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of iotalamic acid is about 6 minutes.

*System suitability*—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, iotalamic acid and the internal standard 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 ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Meglumine Sodium Amidotrizoate Injection

アミドトリゾ酸ナトリウムメグルミン注射液

Meglumine Sodium Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$: 613.91).

**Method of preparation**

| | | |
|---|---|---|
| **(1)** | | |
| Amidotrizoic Acid (anhydrous) | | 522.77 g |
| Sodium Hydroxide | | 25.16 g |
| Meglumine | | 43.43 g |
| Water for Injection | a sufficient quantity | |
| | To make | 1000 mL |
| **(2)** | | |
| Amidotrizoic Acid (anhydrous) | | 471.78 g |
| Sodium Hydroxide | | 5.03 g |
| Meglumine | | 125.46 g |
| Water for Injection | a sufficient quantity | |
| | To make | 1000 mL |
| **(3)** | | |
| Amidotrizoic Acid (anhydrous) | | 597.30 g |
| Sodium Hydroxide | | 6.29 g |
| Meglumine | | 159.24 g |
| Water for Injection | a sufficient quantity | |
| | To make | 1000 mL |

Prepare as directed under Injections, with the above ingredients (1), (2) or (3).

**Description** Meglumine Sodium Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

**Identification** **(1)** To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 1 g of Amidotrizoic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

**(2)** To 1 mL of Meglumine Sodium Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

**(3)** Meglumine Sodium Amidotrizoate Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

**Optical rotation** <*2.49*>

Method of preparation (1) $\alpha_D^{20}$: −1.01 – −1.17° (100 mm).
Method of preparation (2) $\alpha_D^{20}$: −2.91 – −3.36° (100 mm).
Method of preparation (3) $\alpha_D^{20}$: −3.69 – −4.27° (100 mm).

**pH** <*2.54*> 6.0 – 7.7

**Purity** **(1)** Primary aromatic amines—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.20 g of Amidotrizoic Acid according to the labeled amount, add 6 mL of water, mix, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

**(2)** Iodine and iodide—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.25 g of Amidotrizoic Acid according to the labeled amount, add water to make 20 mL, add 5 mL of dilute nitric acid, shake well, and filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

**Extractable volume** <*6.05*> It meets the requirement.

**Pyrogen** <*4.04*> Dilute Maglumine Sodium Amidotrizoate Injection with isotonic sodium chloride solution so as to contain 0.20 g of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$) per mL according to the labelled amount, and perform the test: it meets the requirements.

**Assay** To an exactly measured volume of Meglumine Sodium Amidotrizoate Injection, equivalent to about 0.5 g of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (previously determine the loss on drying <*2.41*> in the same manner as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of amidotrizoic acid to that of the internal standard.

$$\text{Amount (mg) of amidotrizoic acid } (C_{11}H_9I_3N_2O_4) = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

*Internal standard solution*—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 25°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid is about 5 minutes.

*System suitability—*

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, amidotrizoic 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Meglumine Sodium Iodamide Injection

ヨーダミドナトリウムメグルミン注射液

Meglumine Sodium Iodamide Injection is an aqueous solution for injection.

It contains not less than 59.7 w/v% and not more than 65.9 w/v% of iodamide ($C_{12}H_{11}I_3N_2O_4$: 627.94).

**Method of preparation**

| | |
|---|---|
| Iodamide | 627.9 g |
| Sodium Hydroxide | 6.0 g |
| Meglumine | 165.9 g |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Meglumine Sodium Iodamide Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

**Identification** **(1)** To 2 mL of Meglumine Sodium Iodamide Injection add 25 mL of water, and add 3 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is formed. Filter the precipitate by suction through a glass filter (G3), and wash with two 10-mL portions of water. Transfer the precipitate to a suitable flask, add 100 mL of water, dissolve by heating, and gently boil until the volume becomes about 30 mL. After cooling, collect the separated crystals by filtration, dry at 105°C for 1 hour, and proceed as directed in the Identification (1) and (2) under Iodamide.

**(2)** Determine the infrared absorption spectrum of the dried crystals obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3390 $cm^{-1}$, 1369 $cm^{-1}$, 1296 $cm^{-1}$, 1210 $cm^{-1}$ and 1194 $cm^{-1}$.

**(3)** To 1 mL of Meglumine Sodium Iodamide Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color is produced.

**(4)** Meglumine Sodium Iodamide Injection responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

**Optical rotation** ⟨*2.49*⟩ $\alpha_D^{20}$: −3.84 – −4.42° (100 mm).

**pH** ⟨*2.54*⟩ 6.5 – 7.5

**Purity** **(1)** Primary aromatic amines—Mix 0.30 mL of Meglumine Sodium Iodamide Injection and 6 mL of water, then add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and proceed as directed in the Purity (2) under Iodamide: the absorbance is not more than 0.22.

**(2)** Iodine and iodide—To 0.40 mL of Meglumine Sodium Iodamide Injection add water to make 20 mL, then add 5 mL of dilute nitric acid, shake well, filter by suction through a glass filter (G3). To the filtrate add 5 mL of chloroform, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of a strong hydrogen peroxide solution, and shake vigorously: the chloroform layer has no more color than the control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. To a 0.10-mL portion of this solution add 20 mL of water, 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of strong hydrogen peroxide solution, and shake vigorously.

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Pyrogen** ⟨*4.04*⟩ Dilute Meglumine Sodium Iodamide Injection with isotonic sodium chloride solution so as to contain 0.30 mL of Meglumine Sodium Iodamide Injection per mL according to the labeled amount, and perform the test: it meets the requirements.

**Assay** To an exactly measured 8 mL of Meglumine Sodium Iodamide Injection add sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution into a saponification flask, add 30 mL of sodium hydroxide TS and 1 g of zinc powder, and proceed as directed in the Assay under Iodamide.

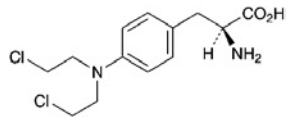
Each mL of 0.1 mol/L silver nitrate VS
= 20.93 mg of $C_{12}H_{11}I_3N_2O_4$

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Melphalan

メルファラン



$C_{13}H_{18}Cl_2N_2O_2$: 305.20
4-Bis(2-chloroethyl)amino-L-phenylalanine [*148-82-3*]

Melphalan contains not less than 93.0% of $C_{13}H_{18}Cl_2N_2O_2$, calculated on the dried basis.

**Description** Melphalan occurs as a white, to light yellowish white, crystalline powder.

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

It dissolves in dilute hydrochloric acid and in dilute sodium hydroxide TS.

It is gradually colored by light.

Optical rotation $[\alpha]_D^{20}$: about $-32°$ (0.5 g, calculated on the dried basis, methanol, 100 mL, 100 mm).

**Identification** **(1)** To 0.02 g of Melphalan add 50 mL of methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20), and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

**(2)** Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS, and heat on a water bath for 10 minutes. After cooling, add dilute nitric acid to acidify, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for chloride.

**(3)** Determine the absorption spectrum of a solution of Melphalan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and conpare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** **(1)** Ionisable chloride—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of diluted nitric acid (1 in 40), stir for 2 minutes, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Melphalan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Melphalan according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** <*2.41*> Not more than 7.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.3% (1 g).

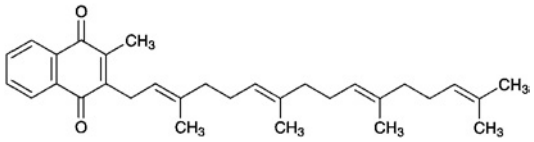
**Assay** Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5), and heat under a reflux condenser on a water bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration). Make any necessary correction by using the results obtained in the Purity (1).

Each mL of 0.1 mol/L silver nitrate VS
= 15.26 mg of $C_{13}H_{18}Cl_2N_2O_2$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Menatetrenone

メナテトレノン



$C_{31}H_{40}O_2$: 444.65
2-Methyl-3-[(2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-1,4-naphthoquinone [*863-61-6*]

Menatetrenone contains not less than 98.0% of $C_{31}H_{40}O_2$, calculated on the dehydrated basis.

**Description** Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by light.

Melting point: about 37°C

**Identification** **(1)** Dissolve 0.1 g of Menatetrenone in 5 mL of ethanol (99.5) by warming, cool, and add 1 mL of a solution of potassium hydroxide in ethanol (95) (1 in 10): a blue color develops, and upon standing it changes from blue-purple to red-brown through red-purple.

**(2)** Determine the infrared absorption spectrum of Menatetrenone, after melting by warming if necessary, as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Menatetrenone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Menatetrenone according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Menadione—To 0.20 g of Menatetrenone add 5 mL of diluted ethanol (99.5) (1 in 2), shake well, and filter. To 0.5 mL of the filtrate add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazorone in ethanol (99.5) (1 in 20) and 1 drop of ammonia water (28), and allow to stand for 2 hours: no blue-purple color develops.

**(3)** cis Isomer—Dissolve 0.10 g of Menatetrenone in 10 mL of hexane, and use this solution as the sample solution. Pipet 1 mL of this solution, add hexane to make exactly 50 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 with fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of hexane and dibutyl ether (17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot corresponding to relative *R*f value 1.1 regarding to the principal spot from the sample solution is not more intense than the spot from the standard solution.

**(4)** Related substances—Conduct this procedure without exposure to daylight, using a light-resistant vessel. Dissolve 0.10 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (99.5) 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. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of menatetrenone from the sample solution is not larger than the peak area of menatetrenone from the standard solution.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of menatetrenone beginning after the solvent peak.

*System suitability*—

Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20 μL of this solution is equivalent to 7 to 13% of that of menatetrenone obtained from 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

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 menatetrenone is not more than 1.0%.

**Water** ⟨*2.48*⟩ Not more than 0.5% (0.5 g, volumetric titration, direct titration).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Conduct this procedure without exposure to daylight, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone Reference Standard (separately, determine the water ⟨*2.48*⟩ in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 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 menatetrenone to that of the internal standard.

$$\text{Amount (mg) of } C_{31}H_{40}O_2 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Menatetrenone Reference Standard, calculated on the dehydrated basis

*Internal standard solution*—A solution of phytonadione in 2-propanol (1 in 20,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 270 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: Methanol

Flow rate: Adjust the flow rate so that the retention time of menatetrenone is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

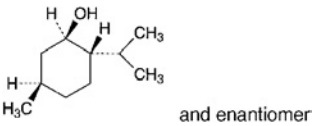
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# *dl*-Menthol

*dl*-メントール



and enantiomer

$C_{10}H_{20}O$: 156.27

(1*RS*,2*SR*,5*RS*)-5-Methyl-2-(1-methylethyl)cyclohexanol [*89-78-1*]

*dl*-Menthol contains not less than 98.0% of $C_{10}H_{20}O$.

**Description** *dl*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

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

It sublimes gradually at room temperature.

**Identification** **(1)** Triturate *dl*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

**(2)** Shake 1 g of dl-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer possesses no aroma of menthol is separated.

**Congealing point** <2.42> 27 – 28°C

**Optical rotation** <2.49> $[\alpha]_D^{20}$: −2.0 – +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

**Purity** **(1)** Non-volatile residue—Volatilize 2.0 g of *dl*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

**(2)** Thymol—Add 0.20 g of dl-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

**(3)** Nitromethane or nitroethane—To 0.5 g of *dl*-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, then add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N*,*N*-diethyl-*N'*-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

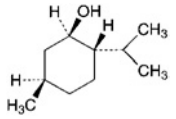
**Assay** Weigh accurately about 2 g of *dl*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash down the condenser with 20 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of $C_{10}H_{20}O$

**Containers and storage** Containers—Tight containers.
Storage—In a cold place.

# *l*-Menthol

*l*-メントール




$C_{10}H_{20}O$: 156.27
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol
[*2216-51-5*]

*l*-Menthol contains not less than 98.0% of $C_{10}H_{20}O$.

**Description** *l*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

*l*-Menthol is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

*l*-Menthol sublimes gradually at room temperature.

**Identification** **(1)** Triturate *l*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

**(2)** Shake 1 g of *l*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer which possesses no aroma of menthol is separated.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: −45.0 – −51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

**Melting point** <2.60> 42 – 44°C

**Purity** **(1)** Non-volatile residue—Volatilize 2.0 g of *l*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

**(2)** Thymol—Add 0.20 g of *l*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

**(3)** Nitromethane or nitroethane—To 0.5 g of *l*-Menthol placed in a flask add 2 mL of sodium hydroxide solution (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add another 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N*,*N*-diethyl-*N'*-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

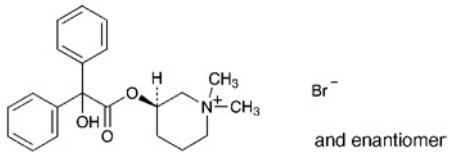
**Assay** Weigh accurately about 2 g of *l*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash the condenser with 20 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of $C_{10}H_{20}O$

**Containers and storage** Containers—Tight containers.
Storage—In a cold place.

# Mepenzolate Bromide

メペンゾラート臭化物




$C_{21}H_{26}BrNO_3$: 420.34
(3*RS*)-3-[(Hydroxy)(diphenyl)acetoxy]-1,1-dimethylpiperidinium bromide [*76-90-4*]

Mepenzolate Bromide, when dried, contains not less than 98.5% of mepenzolate bromide ($C_{21}H_{26}BrNO_3$).

**Description** Mepenzolate Bromide is white to pale yellow crystals or crystalline powder. It is odorless, and has a bitter

taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in hot water, slightly soluble in water and in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 230°C (with decomposition).

**Identification** **(1)** To 0.03 g of Mepenzolate Bromide add 10 drops of sulfuric acid: a red color develops.

**(2)** Dissolve 0.01 g of Mepenzolate Bromide in 20 mL of water and 5 mL of dilute hydrochloric acid, and to 5 mL of this solution add 1 mL of Dragendorff's TS: an orange precipitate is produced.

**(3)** Determine the absorption spectrum of a solution of Mepenzolate Bromide in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of water and 3 mL of nitric acid by heating. This solution responds to the Qualitative Tests <*1.09*> for Bromide.

**Purity** **(1)** Heavy Metals <*1.07*>—Proceed with 1.0 g of Mepenzolate Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not less than 20 ppm).

**(2)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Mepenzolate Bromide according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.40 g of Mepenzolate Bromide in exactly measured 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 40 mg of benzophenone in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, 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 $\mu$L each of the sample solution, standard solutions (1) and (2) on a plate of silica gel with fluorecent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol, water and acetic acid (100) (3:3:2:1) to a distance of about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

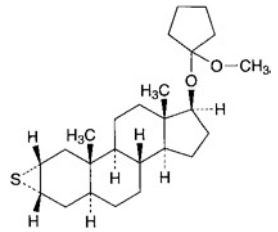
**Assay** Weigh accurately about 0.35 g of Mepenzolate Bromide, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.03 mg of $C_{21}H_{26}BrNO_3$

**Containers and storage** Containers—Tight containers.

# Mepitiostane

メピチオスタン



$C_{25}H_{40}O_2S$: 404.65

2$\alpha$,3$\alpha$-Epithio-17$\beta$-(1-methoxycyclopentyloxy)-5$\alpha$-androstane [*21362-69-6*]

Mepitiostane contains not less than 96.0% and not more than 102.0% of $C_{25}H_{40}O_2S$, calculated on the anhydrous basis.

**Description** Mepitiostane occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

**Identification** **(1)** Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

**(2)** Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt <*2.60*> between 144°C and 149°C.

**(3)** Determine the infrared absorption spectrum of Mepitiostane as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +20 – +23° (0.1 g, chloroform, 10 mL, 100 mm).

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of

Mepitiostane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 20 mg of Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution. Separately, dissolve 10 mg of Epitiostanol Reference Standard in a mixture of acetone and triethylamine (1000:1) to make exactly 10 mL. Pipet 1 mL and 3 mL of this solution, to each add a mixture of acetone and triethylamine (1000:1) to make exactly 25 mL, 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 5 μL each 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 hexane and acetone (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, heat between 120°C and 130°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution showing the same *R*f value as the standard solutions are not more intense than the spot from the standard solution (2), and the remaining spots other than the principal spot are not more intense than the spot from the standard solution (1).

**Water** <*2.48*> Not more than 0.7% (0.3 g, back titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL of this solution, add 10 mL of ethanol (99.5), mix with exactly 2 mL each of 0.01 mol/L hydrochloric acid TS and the internal standard solution, add ethanol (99.5) to make 20 mL, allow to stand at ordinary temperature for 30 minutes, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Epitiostanol Reference Standard, dissolve in exactly 2 mL of the internal standard solution, add ethanol (99.5) 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of epitiostanol to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{25}H_{40}O_2S = W_S \times (Q_T/Q_S) \times 5 \times 1.3202$$

$W_S$: Amount (mg) of Epitiostanol Reference Standard, calculated on the anhydrous basis

*Internal standard solution*—A solution of *n*-octylbenzene in ethanol (99.5) (1 in 300).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (20:3).

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

*System suitability*—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, epitiostanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

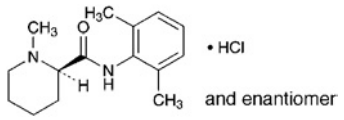
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epitiostanol to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

# Mepivacaine Hydrochloride

メピバカイン塩酸塩




$C_{15}H_{22}N_2O.HCl$: 282.81

(2*RS*)-*N*-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide monohydrochloride [*1722-62-9*]

Mepivacaine Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{22}N_2O.HCl$.

**Description** Mepivacaine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water and in methanol, soluble in acetic acid (100), sparingly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

A solution of Mepivacaine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 256°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Mepivacaine Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Mepivacaine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Mepivacaine Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

**pH** <*2.54*> Dissolve 0.2 g of Mepivacaine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Mepivacaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <*1.14*>—Perform the test with 0.5 g of Mepivacaine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

**(3)** Heavy metals <*1.07*>—Proceed with 2.0 g of Mepivacaine Hydrochloride 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)** Related substances—Dissolve 0.10 g of Mepivacaine Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 4 mL of this solution, add methanol to make exactly 50 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 $\mu$L 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, methanol and ammonia solution (28) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly bismuth nitrate-potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Mepivacaine Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100) and add 70 mL of acetic anhydride. Titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.28 mg of $C_{15}H_{22}N_2O.HCl$

**Containers and storage** Containers—Tight containers.

# Mepivacaine Hydrochloride Injection

メピバカイン塩酸塩注射液

Mepivacaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$: 282.81).

**Method of preparation** Prepare as directed under Injections, with Mepivacaine Hydrochloride.

**Description** Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

pH: 4.5 – 6.8

**Identification** To a volume of Mepivacaine Hydrochloride Injection, equivalent to 0.02 g of Mepivacaine Hydrochloride according to the labeled amount, add 1 mL of sodium hydrochloride TS, and extract with 20 mL of hexane. To 8 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, shake vigorously, and determine the absorption spectrum of the water layer separated as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 261 nm and 265 nm, and between 270 nm and 273 nm.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** To an exactly measured volume of Mepivacaine Hydrochloride Injection, equivalent to about 40 mg of Mepivacaine Hydrochloride according to the labeled amount, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloride TS to make 20 mL, and use this solution as the standard solution. Perform the test with 5 $\mu$L 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 mepivacaine to that of the internal standard.

Amount (mg) of $C_{15}H_{22}N_2O.HCl$
$= W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of mepivacaine hydrochloride for assay

*Internal standard solution*—A solution of benzophenone in methanol (1 in 4000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11:9).

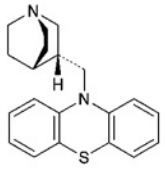
Flow rate: Adjust the flow rate so that the retention time of mepivacaine is about 6 minutes.

Selection of column: Proceed with 5 $\mu$L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of mepivacaine and benzophenone in this order with the resolution between these peaks being not less than 6.

**Containers and storage** Containers—Hermetic containers.

# Mequitazine

メキタジン



and enantiomer

$C_{20}H_{22}N_2S$: 322.47
10-[(3*RS*)-1-Azabicyclo[2.2.2]oct-3-ylmethyl]-10*H*-phenothiazine [*29216-28-2*]

Mequitazine, when dried, contains not less than 98.5% of $C_{20}H_{22}N_2S$.

**Description** Mequitazine occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.

It is gradually colored by light.

A solution of Mequitazine in methanol (1 in 50) shows no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of Mequitazine in ethanol (95) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Mequitazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 146 – 150°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mequitazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.05 g of Mequitazine in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, then 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 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 with a mixture of ethyl acetate, methanol and diethylamine (7:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 3 and they are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

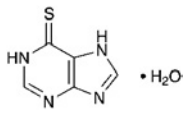
**Assay** Weigh accurately about 0.25 g of Mequitazine, dissolve in 50 mL of acetic acid (100), titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.25 mg of $C_{20}H_{22}N_2S$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Mercaptopurine Hydrate

メルカプトプリン水和物



$C_5H_4N_4S.H_2O$: 170.19
1,7-Dihydro-6*H*-purine-6-thione monohydrate
[*6112-76-1*]

Mercaptopurine Hydrate contains not less than 98.0% of mercaptopurine ($C_5H_4N_4S$: 152.18), calculated on the anhydrous basis.

**Description** Mercaptopurine Hydrate occurs as light yellow to yellow crystals or crystalline powder. It is odorless.

It is practically insoluble in water, in acetone and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

**Identification** **(1)** Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100), and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice bath, and adjust the pH with acetic acid (31) to about 5. Collect the separated crystals by filtration, recrystallize from water, and dry at 120°C for 30 minutes: the crystals melt <*2.60*> between 218°C and 222°C (with decomposition).

**(2)** Determine the absorption spectrum of a solution of Mercaptopurine Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** **(1)** Clarity of solution—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

**(2)** Sulfate <*1.14*>—Dissolve 0.05 g of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS, and allow to stand for 5 minutes: no turbidity is produced.

**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Mercaptopurine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(4)** Hypoxanthine—Dissolve 50 mg of Mercaptopurine Hydrate in exactly 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the sample solution. Separately, dissolve 5.0 mg of hypoxanthine in a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 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 $\mu$L 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 methanol, chloroform, *n*-butyl formate and ammonia solution (28) (8:6:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution is not observed at the same place as that from the standard solution, or if a spot is observed at the same place, it is not larger than that from the standard solution.

**(5)** Phosphorus—Take 0.20 g of Mercaptopurine Hydrate in a crucible, add 2 mL of diluted sulfuric acid (3 in 7), then heat gently, slowly adding dropwise several 0.5-mL portions of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool, and dissolve the residue in 10 mL of water. Transfer the solution to a 25-mL volumetric flask, wash the crucible with two 4-mL portions of water, combine the washings with the solution in the volumetric flask, and use this solution as the sample solution. Separately, dissolve 0.4396 g of potassium dihydrogenphosphate in water to make exactly 200 mL. To 2.0 mL of this solution add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 16 mL of water, and use this solution as the standard solution. To the sample solution and the standard solution add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of hexaammonium heptamolybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 25 mL, and allow to stand for 5 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank: the absorbance of the subsequent solution of the sample solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

**Water** <*2.48*> 10.0 – 12.0% (0.2 g, back titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.25 g of Mercaptopurine Hydrate, dissolve in 90 mL of *N,N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination with a mixture of 90 mL of *N,N*-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 15.22 mg of $C_5H_4N_4S$

**Containers and storage** Containers—Well-closed containers.

# Mercurochrome

## Merbromin

マーキュロクロム

Mercurochrome is a sodium salt of a mixture of brominated and mercurized fluoresceins.

When dried, it contains not less than 18.0% and not more than 22.4% of bromine (Br: 79.90), and not less than 22.4% and not more than 26.7% of mercury (Hg: 200.59).

**Description** Mercurochrome occurs as blue-green to greenish red-brown scales or granules. It is odorless.

It is freely soluble in water, but sometimes leaves a small amount of insoluble matter. It is practically insoluble in ethanol (95) and in diethyl ether.

**Identification** **(1)** A solution of Mercurochrome (1 in 2000) shows a red color and a yellow-green fluorescence.

**(2)** To 5 mL of a solution of Mercurochrome (1 in 250) add 3 drops of dilute sulfuric acid: a reddish orange precipitate is produced.

**(3)** Heat 0.1 g of Mercurochrome with small crystals of iodine in a test tube: red crystals are sublimed on the upper part of the tube. If yellow crystals are produced, scratch with a glass rod: the color of the crystals changes to red.

**(4)** Place 0.1 g of Mercurochrome in a porcelain crucible, add 1 mL of a solution of sodium hydroxide (1 in 6), evaporate to dryness with stirring, and ignite. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and shake with 3 drops of chlorine TS and 2 mL of chloroform: a yellowish brown color develops in the chloroform layer.

**Purity** **(1)** Dyestuff—Dissolve 0.40 g of Mercurochrome in 20 mL of water, add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

**(2)** Soluble halides—Dissolve 5.0 g of Mercurochrome in 80 mL of water, add 10 mL of dilute nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes protected from direct sunlight: no turbidity is produced, or even if produced, it is not more than that of the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, and proceed as directed above.

**(3)** Soluble mercury salts—To 5 mL of the filtrate obtained in (1) add 5 mL of water, and use this solution as the sample solution. Dissolve 40 mg of mercury (II) chloride, weighed accurately, in water to make 1000 mL, and add 3 mL of dilute sulfuric acid to 20 mL of this solution. To 5 mL of the solution add 5 mL of water, and use this as the control solution. Add 1 drop each of sodium sulfide TS to these solutions, and compare: the sample solution has no more color than the control solution.

**(4)** Insoluble mercury compounds—Dissolve 2.5 g of Mercurochrome in 50 mL of water, allow to stand for 24

hours, centrifuge, and wash the precipitate with small portions of water until the last washing becomes colorless. Transfer the precipitate to a glass-stoppered flask, add exactly 5 mL of 0.05 mol/L iodine VS, allow to stand for 1 hour with frequent agitation, add 4.3 mL of 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS: a blue color develops.

**Loss on drying** ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 5 hours).

**Assay (1)** Mercury—Weigh accurately about 0.6 g of Mercurochrome, previously powdered and dried, transfer to an iodine flask, dissolve in 50 mL of water, add 8 mL of acetic acid (31), 20 mL of chloroform and exactly 30 mL of 0.05 mol/L iodine VS, stopper tightly, and allow to stand for 1 hour with frequent, vigorous shaking. Titrate ⟨*2.50*⟩ the excess iodine with 0.1 mol/L sodium thiosulfate VS with vigorous shaking (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

**(2)** Bromine—Weigh accurately about 0.5 g of Mercurochrome, previously powdered and dried, in a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, and titrate ⟨*2.50*⟩ the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 7.990 mg of Br

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Mercurochrome Solution

## Merbromin Solution

マーキュロクロム液

Mercurochrome Solution contains not less than 0.42 w/v% and not more than 0.56 w/v% of mercury (Hg: 200.59).

**Method of preparation**

| | |
|---|---|
| Mercurochrome | 20 g |
| Purified Water | a sufficient quantity |
| | To make 1000 mL |

Prepare by mixing the above ingredients.

**Description** Mercurochrome Solution is a dark red liquid.

**Identification (1)** To 1 mL of Mercurochrome Solution add 40 mL of water: the resulting solution shows a red color and a yellow-green fluorescence.

**(2)** Dilute 1 mL of Mercurochrome Solution with 4 mL of water, and add 3 drops of dilute sulfuric acid: a red-orange precipitate is produced.

**(3)** Evaporate 5 mL of Mercurochrome Solution to dryness, and proceed with the residue as directed in the Identification (3) under Mercurochrome.

**(4)** To 5 mL of Mercurochrome Solution add 1 mL of a solution of sodium hydroxide (1 in 6), and proceed as directed in the Identification (4) under Mercurochrome.

**Purity** Dyestuff—To 20 mL of Mercurochrome Solution add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

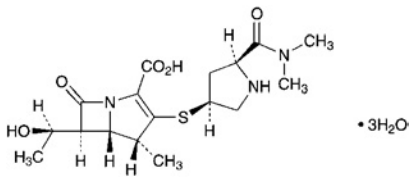
**Assay** Transfer exactly measured 30 mL of Mercurochrome Solution to an iodine flask, dilute with 20 mL of water, add 8 mL of acetic acid (31) and 20 mL of chloroform, and proceed as directed in the Assay (1) under Mercurochrome.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Meropenem Hydrate

メロペネム水和物



$C_{17}H_{25}N_3O_5S.3H_2O$: 437.51
(4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-(Dimethylcarbamoyl)pyrrolidin-3-ylsulfanyl]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate [*119478-56-7*]

Meropenem Hydrate contains not less than 980 $\mu$g (potency) and not more than 1010 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Meropenem Hydrate is expressed as mass (potency) of meropenem ($C_{17}H_{25}N_3O_5S$: 383.46).

**Description** Meropenem Hydrate occurs as a white to light yellow crystalline powder.

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

**Identification (1)** Dissolve 0.01 g of Meropenem Hydrate in 2 mL of water, add 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

**(2)** Determine the absorption spectra of solutions of Meropenem Hydrate and Meropenem Reference Standard (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectra of Meropenem Hydrate and Meropenem Reference Standard as directed in the potassium bromide disk method under In-

frared Spectrophotometry <2.25>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: −17 – −21° (0.22 g calculated as the anhydrous basis, water, 50 mL, 100 mm).

**pH** <2.54> Dissolve 0.2 g of Meropenem Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

**Purity** **(1)** Clarity and color of solution—Being specified separately.

**(2)** Heavy metals <1.07>—Proceed with 2.0 g of Meropenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Being specified separately.

**Water** <2.48> Not less than 11.4% and not more than 13.4% (0.35 g, volumetric titration, direct titration).

**Residue on ignition** <2.44> Being specified separately.

**Bacterial endotoxins** <4.01> Less than 0.12 EU/mg (potency).

**Assay** Weigh accurately an amount of Meropenem Hydrate and Meropenem Reference Standard, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add triethylamine-phosphate buffer solution, pH 5.0 to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μL of the sample solution and the 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 meropenem to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of meropenem } (C_{17}H_{25}N_3O_5S) = W_S \times (Q_T/Q_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Meropenem Reference Standard

*Internal standard solution*—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 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 25°C.

Mobile phase: A mixture of triethylamine-phosphate buffer solution, pH 5.0 and methanol (5:1).

Flow rate: Adjust the flow rate so that the retention time of meropenem is about 7 minutes.

*System suitability*—

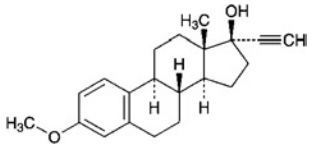
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, meropenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of meropenem to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Tight containers.

# Mestranol

メストラノール




$C_{21}H_{26}O_2$: 310.43

3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol

[*72-33-3*]

Mestranol, when dried, contains not less than 97.0% and not more than 102.0% of $C_{21}H_{26}O_2$.

**Description** Mestranol occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification** **(1)** Dissolve 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2:1): a red-purple color develops with a yellow-green fluorescence.

**(2)** Determine the absorption spectrum of a solution of Mestranol in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mestranol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Mestranol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Mestranol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +2 – +8° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

**Melting point** <2.60> 148 – 154°C

**Purity** **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Mestranol according to Method 2, 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 1.0 g of Mestranol according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.10 g of Mestranol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 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 chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 10 mg each of Mestranol and Mestranol Reference Standard, previously dried, dissolve in ethanol (99.5) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 279 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

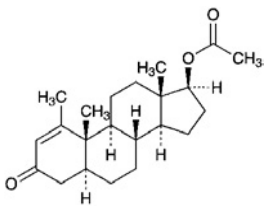
$$\text{Amount (mg) of } C_{21}H_{26}O_2 = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Mestranol Reference Standard

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Metenolone Acetate

メテノロン酢酸エステル



$C_{22}H_{32}O_3$: 344.49
1-Methyl-3-oxo-5$\alpha$-androst-1-en-17$\beta$-yl acetate [*434-05-9*]

Metenolone Acetate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$.

**Description** Metenolone Acetate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in ethanol (95) and in methanol, sparingly soluble in diethyl ether and in sesame oil, slightly soluble in hexane and in petroleum ether, and practically insoluble in water.

**Identification** **(1)** Dissolve 1 mg of Metenolone Acetate in 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1), and heat for 30 minutes in a water bath: a red-brown color develops.

**(2)** To 0.01 g of Metenolone Acetate add 0.5 mL of dilute sodium hydroxide-ethanol TS, and heat for 1 minute on a water bath. After cooling, add 0.5 mL of diluted sulfuric acid (1 in 2), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

**(3)** Dissolve 0.05 g of Metenolone Acetate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), and boil for 2 hours under a reflux condenser. After cooling, add this solution gradually to 50 mL of cold water, and stir for 15 minutes. Filter the precipitate so obtained by suction through a glass filter (G4), wash with 10 mL of water, and dry at 105°C for 1 hour: it melts ⟨*2.60*⟩ between 157°C and 161°C.

**(4)** Determine the infrared absorption spectrum of Metenolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +39 – +42° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

**Melting point** ⟨*2.60*⟩ 141 – 144°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.50 g of Metenolone Acetate in 10 mL of 1,4-dioxane: the solution is clear and colorless to pale yellow.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Metenolone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 35 mg of Metenolone Acetate in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, dilute with chloroform to exactly 250 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 $\mu$L 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 cyclohexane and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

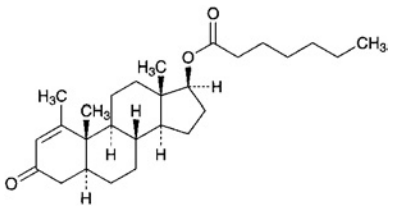
**Assay** Weigh accurately about 10 mg of Metenolone Acetate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance $A$ of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Amount (mg) of } C_{22}H_{32}O_3 = (A/391) \times 10{,}000$$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Metenolone Enanthate

メテノロンエナント酸エステル



$C_{27}H_{42}O_3$: 414.62
1-Methyl-3-oxo-5$\alpha$-androst-1-en-17$\beta$-yl heptanoate
[*303-42-4*]

Metenolone Enanthate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{27}H_{42}O_3$.

**Description** Metenolone Enanthate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in ethanol (95), in acetone, in 1,4-dioxane and in chloroform, freely soluble in methanol, in ethyl acetate, in diethyl ether, in cyclohexane, in petroleum ether and in toluene, soluble in sesame oil, and practically insoluble in water.

**Identification** **(1)** Heat 1 mg of Metenolone Enanthate with 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1) on a water bath for 30 minutes: a red-brown color develops.

**(2)** Dissolve 0.05 g of Metenolone Enanthate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), boil under a reflux condenser for 2 hours, cool, add slowly this solution to 50 mL of cold water, and stir for 15 minutes. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the washings become neutral, and dry at 105°C for 1 hour: it melts <*2.60*> between 156°C and 162°C.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +39 – +43° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

**Melting point** <*2.60*> 67 – 72°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Metenolone Enanthate in 10 mL of 1,4-dioxane: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Metenolone Enanthate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 20 mg of Metenolone Enanthate in exactly 10 mL of chloroform, 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 $\mu$L 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 and ethyl acetate (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

**Loss on drying** <*2.41*> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.1 g of Metenolone Enanthate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute with methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute again with methanol to make exactly 100 mL. Determine the absorbance $A$ of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{27}H_{42}O_3 = (A/325) \times 100{,}000$$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Metenolone Enanthate Injection

メテノロンエナント酸エステル注射液

Metenolone Enanthate Injection is an oily solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of metenolone enanthate ($C_{27}H_{42}O_3$: 414.62).

**Method of preparation** Prepare as directed under Injections, with Metenolone Enanthate.

**Description** Metenolone Enanthate Injection is a clear, pale yellow, oily liquid.

**Identification** **(1)** Measure a volume of Metenolone Enanthate Injection, equivalent to 0.1 g of Metenolone Enanthate according to the labeled amount, add 20 mL of petroleum ether, and extract with three 20-mL portions of diluted acetic acid (100) (5 in 7). Combine the extracts, wash with 20 mL of petroleum ether, add 300 mL of cold water while cooling in an ice bath, and stir sufficiently. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the last washing becomes neutral, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 6 hours. With this sample, proceed as directed in the Identification (1) under Metenolone Enanthate.

**(2)** Measure a volume of Metenolone Enanthate Injection, equivalent to 0.01 g of Metenolone Enanthate according to the labeled amount, dissolve in 10 mL of chloroform, and use this solution as the sample solution. Separately dissolve 0.01 g of metenolone enanthate in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 $\mu$L 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 toluene to a distance of about 15 cm, and air-dry the plate. Again develop this plate with a mixture of cyclohexane and ethyl acetate (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same $R$f value.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** To an exactly measured volume of Metenolone Enanthate Injection, equivalent to about 0.1 g of metenolone enanthate ($C_{27}H_{42}O_3$), add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 0.1 g of metenolone enanthate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 3 mL each of the sample solution and standard solution, and treat each solution as follows: add 10 mL of isoniazid TS, exactly measured, add methanol to make exactly 20 mL, and allow to stand for 60 minutes. Determine the absorbances, $A_T$ and $A_S$, of the solutions from the sample solution and standard solution, respectively, at 384 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution obtained by proceeding with 3 mL of chloroform as the blank.

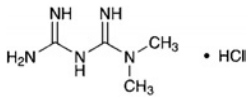
Amount (mg) of metenolone enanthate ($C_{27}H_{42}O_3$)
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of metenolone enanthate for assay

**Containers and storage** Containers—Hermetic containers.
Storage—Light-resistant.

# Metformin Hydrochloride

メトホルミン塩酸塩



$C_4H_{11}N_5.HCl$: 165.62
1,1-Dimethylbiguanide monohydrochloride
[*1115-70-4*]

Metformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_4H_{11}N_5.HCl$.

**Description** Metformin Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and slightly soluble in ethanol (99.5).

Melting point: about 221°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Metformin Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Metformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Metformin Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Metformin Hydrochloride 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).

**(2)** Related substances—Dissolve 2.5 g of Metformin Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 0.10 g of 1-cyanoguanidine add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 $\mu$L each of the sample solution and standard solutions (1), (2) and (3) on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water and acetic acid (100) (30:20:5:3) to a distance of about 10 cm, air-dry the plate, then dry at 105°C for 10 minutes. Spray evenly sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS on the plate: the spot other than the principal spot is not more intense than the spot with the standard solution (1), the number of them showing more intense than the spot with the standard solution (2) is not more than two, and the spot appeared at the position corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.1 g of Metformin Hydrochloride, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <*2.50*> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
$= 4.141$ mg of $C_4H_{11}N_5.HCl$

**Containers and storage** Containers—Tight containers.

# Metformin Hydrochloride Tablets

メトホルミン塩酸塩錠

Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5.HCl$: 165.62).

**Method of preparation** Prepare as directed under Tablets, with Metformin Hydrochloride.

**Identification** Shake an amount of powdered Metformin Hydrochloride Tablets, equivalent to 250 mg of Metformin Hydrochloride according to the labeled amount, with 25 mL of 2-propanol, and filter. Evaporate the filtrate under reduced pressure in a water bath at 40°C, and determine the infrared absorption spectrum of the residue as directed in the

potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3370 $cm^{-1}$, 3160 $cm^{-1}$, 1627 $cm^{-1}$, 1569 $cm^{-1}$ and 1419 $cm^{-1}$.

**Uniformity of dosage unit** ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

**Dissolution** Being specified separately.

**Assay** Weigh accurately the mass of not less than 20 Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of metformin hydrochloride ($C_4H_{11}N_5$.HCl), add 70 mL of a mixture of water and acetonitrile (3:2), shake for 10 minutes, add the mixture of water and acetonitrile (3:2) to make exactly 100 mL, and filter through a membrane filter with a pore size of not more than 0.45 $\mu$m. Discard the first 10 mL of the filtrate, pipet 3 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the mixture of water and acetonitrile (3:2) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of metformin to that of the internal standard.

Amount (mg) of metformin hydrochloride ($C_4H_{11}N_5$.HCl)
$= W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of metformin hydrochloride for assay

*Internal standard solution*—Dissolve 0.3 g of isobutyl parahydroxybenzoate in 100 mL of the mixture of water and acetonitrile (3:2).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 235 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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 0.8 g of sodium lauryl sulfate in 620 mL of diluted phosphoric acid (1 in 2500), and add 380 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of metformin is about 10 minutes.

*System suitability*—

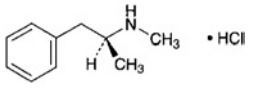
System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, metformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metformin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

# Methamphetamine Hydrochloride

メタンフェタミン塩酸塩



$C_{10}H_{15}N$.HCl: 185.69
(2*S*)-*N*-Methyl-1-phenylpropan-2-amine monohydrochloride [*51-57-0*]

Methamphetamine Hydrochloride, when dried, contains not less than 98.5% of $C_{10}H_{15}N$.HCl.

**Description** Methamphetamine Hydrochloride occurs as colorless crystals or a white, crystalline powder. It is odorless.

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

The pH of a solution of Methamphetamine Hydrochloride (1 in 10) is between 5.0 and 6.0.

**Identification** **(1)** To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of hydrogen hexachloroplatinate (IV) TS: an orange-yellow, crystalline precipitate is produced.

**(2)** To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of iodine TS: a brown precipitate is produced.

**(3)** To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of 2,4,6-trinitrophenol TS: a yellow, crystalline precipitate is produced.

**(4)** A solution of Methamphetamine Hydrochloride (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +16 – +19° (after drying, 0.2 g, water, 10 mL, 100 mm).

**Melting point** ⟨*2.60*⟩ 171 – 175°C

**Purity** **(1)** Acidity or alkalinity—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS, and use this solution as the sample solution.

(i) To 20 mL of the sample solution add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color develops.

(ii) To 20 mL of the sample solution add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color develops.

**(2)** Sulfate ⟨*1.14*⟩—Dissolve 0.05 g of Methamphetamine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: the solution remains unchanged.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Methamphetamine Hydrochloride, previously dried, and dissolve in 50 mL of a

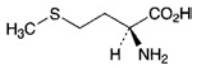
mixture of acetic anhydride and acetic acid (100) (7:3). Titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.57 mg of $C_{10}H_{15}N.HCl$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# L-Methionine

L-メチオニン



$C_5H_{11}NO_2S$: 149.21
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid
[*63-68-3*]

L-Methionine, when dried, contains not less than 98.5% of $C_5H_{11}NO_2S$.

**Description** L-Methionine occurs as white crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in formic acid, soluble in water, and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Methionine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨2.49⟩ $[\alpha]_D^{20}$: +21.0 – +25.0° (after drying, 0.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

**pH** ⟨2.54⟩ Dissolve 0.5 g of L-Methionine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

**(2)** Chloride ⟨1.03⟩—Dissolve 0.5 g of L-Methionine in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 40 mL. Perform the test using this solution as the test solution.

Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 40 mL. In this test, to the test solution and the control solution add 10 mL each of silver nitrate TS (not more than 0.021%).

**(3)** Sulfate ⟨1.14⟩—Perform the test with 0.6 g of L-Methionine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

**(4)** Ammonium ⟨1.02⟩—Perform the test with 0.25 g of L-Methionine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

**(5)** Heavy metals ⟨1.07⟩—Dissolve 1.0 g of L-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve by warming, cool, and add water to make 50 mL. 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 20 ppm).

**(6)** Arsenic ⟨1.11⟩—Transfer 1.0 g of L-Methionine to a 100-mL decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, add 2-mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

**(7)** Related substances—Dissolve 0.10 g of L-Methionine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, 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. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨2.41⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨2.44⟩ Not more than 0.1% (1 g).

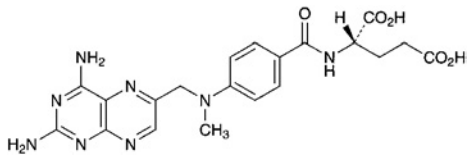
**Assay** Weigh accurately about 0.15 g of L-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.92 mg of $C_5H_{11}NO_2S$

**Containers and storage** Containers—Tight containers.

# Methotrexate

メトトレキサート



$C_{20}H_{22}N_8O_5$: 454.44
*N*-{4-[(2,4-Diaminopteridin-6-ylmethyl)(methyl)amino]benzoyl}-L-glutamic acid
[*59-05-2*]

Methotrexate is a mixture of 4-amino-10-methylfolic

acid and closely related compounds.

It contains not less than 94.0% and not more than 102.0% of $C_{20}H_{22}N_8O_5$, calculated on the anhydrous basis.

**Description** Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

**Identification** **(1)** Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Methotrexate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Methotrexate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Water** <*2.48*> Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 25 mg each of Methotrexate and Methotrexate Reference Standard, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 $\mu$L each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and measure the peak areas, $A_T$ and $A_S$, of methotrexate in each solution.

$$\text{Amount (mg) of } C_{20}H_{22}N_8O_5 = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Methotrexate Reference Standard, calculated on the anhydrous basis

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 302 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 (10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of methotrexate is about 8 minutes.

*System suitability*—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

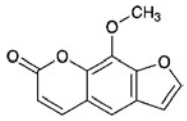
System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Methoxsalen

メトキサレン



$C_{12}H_8O_4$: 216.19

9-Methoxy-7*H*-furo[3,2-*g*]chromen-7-one

[*298-81-7*]

Methoxsalen contains not less than 98.0% and not more than 102.0% of $C_{12}H_8O_4$, calculated on the anhydrous basis.

**Description** Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless.

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

**Identification** **(1)** To 0.01 g of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

**(2)** To 0.01 g of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

**(3)** Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** <*2.60*> 145 – 149°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Methoxsalen according to Method 4, 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 1.0 g of Methoxsalen according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform, and use this solution as the sample

solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 mL. Pipet 1 mL of this solution, add chloroform to make exactly 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 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 chloroform, hexane and ethyl acetate (40:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** <2.48> Not more than 0.5% (1 g, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen Reference Standard, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of } C_{12}H_8O_4 = W_S \times (A_T/A_S)$$

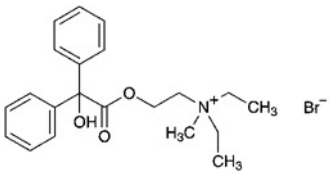
$W_S$: Amount (mg) of Methoxsalen Reference Standard, calculated on the anhydrous basis

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Methylbenactyzium Bromide

メチルベナクチジウム臭化物




$C_{21}H_{28}BrNO_3$: 422.36

*N*,*N*-Diethyl-2-[(hydroxyl)(diphenyl)acetoxy]-*N*-methylethylaminium bromide [*3166-62-9*]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of $C_{21}H_{28}BrNO_3$.

**Description** Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 50) is between 5.0 and 6.0.

**Identification** **(1)** Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution, pH 7.0, 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

**(2)** To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 105°C for 1 hour: the crystals melt <2.60> between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

**(3)** Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests <1.09> (1) for bromide.

**Melting point** <2.60> 168 – 172°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Methylbenactyzium Bromide in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <1.14>—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

**(3)** Heavy metals <1.07>—Proceed with 2.0 g of Methylbenactyzium Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** <2.41> Not more than 0.5% (2 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 42.24 \text{ mg of } C_{21}H_{28}BrNO_3$$

**Containers and storage** Containers—Tight containers.

# Methylcellulose

メチルセルロース

Cellulose, methyl ether
[*9004-67-5*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Methylcellulose is a methyl ether of cellulose.

It contains not less than 26.0% and not more than 33.0% of methoxy group (-$OCH_3$: 31.03), calculated on the dried basis.

The viscosity of Methylcellulose is shown in millipas-

cal second (mPa·s).

◆**Description** Methylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

Methylcellulose swells, when water is added, and forms a clear or slightly turbid, viscous liquid.◆

**Identification** **(1)** Disperse evenly 1.0 g of Methylcellulose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

**(2)** Add 1.0 g of Methylcellulose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 5°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

**(3)** To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of diluted sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water.Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color first, then changes to purple color within 100 minutes.

**(4)** Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

**(5)** Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

**Viscosity** ⟨*2.53*⟩ Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 5°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20±0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20±0.1°C as directed in Method II (2) under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

*Operating conditions*—

Apparatus: Brookfield type viscometer LV model

Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

| Labeled viscosity (mPa·s) | Rotor No. | Retation frequency /min | Conversion factor |
|---|---|---|---|
| Not less than 600 and less than 1400 | 3 | 60 | 20 |
| 〃 1400 〃 3500 | 3 | 12 | 100 |
| 〃 3500 〃 9500 | 4 | 60 | 100 |
| 〃 9500 〃 99,500 | 4 | 6 | 1000 |
| 〃 99,500 | 4 | 3 | 2000 |

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

**pH** ⟨*2.54*⟩ Allow the sample solution obtained in the Viscosity to stand at 20±2°C for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

**Purity** Heavy metals—Put 1.0 g of Methylcellulose in a 100-mL Kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until to use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 1 hour).

**Residue on ignition** ⟨*2.44*⟩ Not more than 1.5% (1 g).

**Assay** (i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20

mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Methylcellulose, transfer to the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2°C. In the case when the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid in a reaction bottle, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using micro-syringe, weigh accurately, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 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 ratios, $Q_T$ and $Q_S$, of the peak area of iodomethane to that of the internal standard.

$$\text{Content (\%) of methoxy group (-CH}_3\text{O)} = (Q_T/Q_S) \times (W_S/W) \times 21.86$$

$W_S$: Amount (mg) of iodomethane in the standard solution
$W$: Amount (mg) of sample, calculated on the dried basis

*Internal standard solution*—A solution of *n*-octane in *o*-xylene (3 in 100).

*Operating conditions*—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

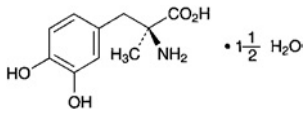
*System suitability*—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order, with complete separation of these peaks.

◆**Containers and storage** Containers—Well-closed containers.◆

# Methyldopa Hydrate

メチルドパ水和物




$C_{10}H_{13}NO_4.1½H_2O$: 238.24

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate [*41372-08-1*]

Methyldopa Hydrate contains not less than 98.0% of methyldopa ($C_{10}H_{13}NO_4$: 211.21), calculated on the anhydrous basis.

**Description** Methyldopa Hydrate occurs as a white to pale grayish white, crystalline powder.

It is slightly soluble in water, in methanol and in acetic acid (100), very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

**Identification** **(1)** To 0.01 g of Methyldopa Hydrate add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

**(2)** Determine the absorption spectrum of a solution of Methyldopa Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Methyldopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Methyldopa Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: −25 – −28° (calculated on the anhydrous basis, 1 g, aluminum (III) chloride TS, 20 mL, 100 mm).

**Purity** **(1)** Acidity—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.

**(2)** Chloride <1.03>—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

**(3)** Heavy metals <1.07>—Proceed with 2.0 g of Methyldopa Hydrate according to Method 2, 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 Methyldopa Hydrate in 5 mL of dilute hydrochloric acid, and perform the test (not more than 2 ppm).

**(5)** 3-*O*-Methylmethyldopa—Dissolve 0.10 g of Methyl-

dopa Hydrate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-*O*-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 $\mu$L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

**Water** <*2.48*> 10.0 – 13.0% (0.2 g, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Methyldopa Hydrate, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 to 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of $C_{10}H_{13}NO_4$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Methyldopa Tablets

メチルドパ錠

Methyldopa Tablets contain not less than 90% and not more than 110% of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$: 211.21).

**Method of preparation** Prepare as directed under Tablets, with Methyldopa Hydrate.

**Identification** **(1)** To a quantity of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa Hydrate according to the labeled amount, add 10 mL of water, and heat in a water bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the supernatant solution to a filter paper, and dry with warm air. Place 1 drop of ninhydrin TS over the spot, and heat for 5 minutes at 100°C: a purple color develops.

**(2)** To 0.5 mL of the supernatant liquid obtained in the Identification (1) add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS, and shake well: a deep purple color develops.

**(3)** To 0.7 mL of the supernatant liquid obtained in the Identification (1) add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 283 nm.

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Methyldopa Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Take 30 mL or more of the dissolved solution 60 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.8 $\mu$m. Discard the first 10 mL of the filtrate, pipet the subsequent $V$ mL, add water to make exactly $V'$ mL so that each mL contains about 25 $\mu$g of methyldopa ($C_{10}H_{13}NO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of methyldopa for assay (its loss on drying <*2.41*> is determined, separately, at 125°C for 2 hours), and dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water 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 the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Methyldopa Tablets in 60 minutes is not less than 75%.

Dissolution rate (%) with respect to
the labeled amount of methyldopa ($C_{10}H_{13}NO_4$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45$$

$W_S$: Amount (mg) of methyldopa for assay, calculated on the dried basis

$C$: Labeled amount (mg) of methyldopa ($C_{10}H_{13}NO_4$) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa ($C_{10}H_{13}NO_4$), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa Reference Standard (previously dry at 125°C for 2 hours, and determine the loss on drying <*2.41*>), dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 520 nm, respectively.

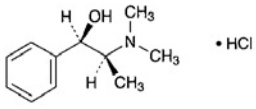
Amount (mg) of methyldopa ($C_{10}H_{13}NO_4$)
$= W_S \times (A_T/A_S)$

$W_S$: amount (mg) of Methyldopa Reference Standard, calculated on the dried basis

**Containers and storage** Containers—Well-closed containers.

# *dl*-Methylephedrine Hydrochloride

*dl*-メチルエフェドリン塩酸塩




and enantiomer

$C_{11}H_{17}NO.HCl$: 215.72
(1*RS*,2*SR*)-2-Dimethylamino-1-phenylpropan-1-ol monohydrochloride [*18760-80-0*]

*dl*-Methylephedrine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{11}H_{17}NO.HCl$.

**Description** *dl*-Methylephedrine Hydrochloride occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (99.5), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride.

A solution of *dl*-Methylephedrine Hydrochloride (1 in 20) shows no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of *dl*-Methylephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of *dl*-Methylephedrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of *dl*-Methylephedrine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

**pH** <*2.54*> The pH of a solution prepared by dissolving 1.0 g of *dl*-Methylephedrine Hydrochloride in 20 mL of water is between 4.5 and 6.0.

**Melting point** <*2.60*> 207 – 211°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of *dl*-Methylephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of *dl*-Methylephedrine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 each peak area by the automatic integration method: the total area of the peaks other than the peak of methylephedrine is not more than the peak area of methylephedrine from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 257 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 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of methylephedrine beginning after the solvent peak.

*System suitability*—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of methylephedrine obtained from 20 μL of this solution is equivalent to 7 to 13% of that of methylephedrine obtained from 20 μL of the standard solution.

System performance: Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, methylephedrine 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylephedrine is not more than 2.0%.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of *dl*-Methylephedrine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.57 mg of $C_{11}H_{17}NO.HCl$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# 10% *dl*-Methylephedrine Hydrochloride Powder

## *dl*-Methylephedrine Hydrochloride Powder

*dl*-メチルエフェドリン塩酸塩散 10%

10% *dl*-Methylephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$: 215.72).

**Method of preparation**

| | |
|---|---|
| *dl*-Methylephedrine Hydrochloride | 100 g |
| Starch, Lactose Hydrate or their mixture | a sufficient quantity |
| To make | 1000 g |

Prepare as directed under Powders, with the above ingredients.

**Identification** Determine the absorption spectrum of a solution of 10% *dl*-Methylephedrine Hydrochloride Powder (1 in 200) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 264 nm.

**Assay** Weigh accurately about 0.5 g of 10% *dl*-Methylephedrine Hydrochloride Powder, add exactly 4 mL of the internal standard solution and 25 mL of water, shake vigorously for 20 minutes to dissolve, add water to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, if necessary, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 4 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 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 ratios of the peak area, $Q_T$ and $Q_S$, of methylephedrine to that of the internal standard.

Amount (mg) of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$)
$= W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of *dl*-methylephedrine hydrochloride for assay

*Internal standard solution*—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 10,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 257 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 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

*System suitability*—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, methylephedrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

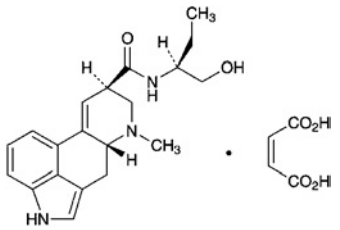
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylephedrine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Methylergometrine Maleate

メチルエルゴメトリンマレイン酸塩




$C_{20}H_{25}N_3O_2.C_4H_4O_4$: 455.50

(8*S*)-*N*-[(1*S*)-1-(Hydroxymethyl)propyl]-6-methyl-9,10-didehydrolergoline-8-carboxamide monomaleate

[*7054-07-1*]

Methylergometrine Maleate, when dried, contains not less than 95.0% and not more than 105.0% of $C_{20}H_{25}N_3O_2.C_4H_4O_4$.

**Description** Methylergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.

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

It gradually changes to yellow by light.

Melting point: about 190°C (with decomposition).

**Identification** **(1)** A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.

**(2)** The solution obtained in the Assay develops a deep blue in color. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylergometrine Maleate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** To 5 mL of a solution of Methylergometrine Maleate

(1 in 500) add 1 drop of potassium permanganate TS: the red color of the test solution fades immediately.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +44 – +50° (after drying, 0.1 g, water, 20 mL, 100 mm).

**Purity** Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately 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, and immediately develop the plate with a mixture of chloroform, methanol and water (75:25:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 2.0% (0.2 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Assay** Weigh accurately about 10 mg each of Methylergometrine Maleate and Methylergometrine Maleate Reference Standard, previously dried, add water to make exactly 250 mL, and use these solutions as the sample solution and the standard solution. Pipet 2 mL each of the sample solution and the standard solution separately into brown glassstoppered test tubes, add exactly 4 mL each of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while ice cooling, warm for 10 minutes at 45°C, and allow to stand for 20 minutes at room temperature. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with 2.0 mL of water in the same manner, as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 545 nm, respectively.

$$\text{Amount (mg) of } C_{20}H_{25}N_3O_2.C_4H_4O_4 = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Methylergometrine Maleate Reference Standard

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Methylergometrine Maleate Tablets

メチルエルゴメトリンマレイン酸塩錠

Methylergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$: 455.50).

**Method of preparation** Prepare as directed under Tablets, with Methylergometrine maleate.

**Identification** **(1)** The sample solution obtained in the Assay shows a blue fluorescence.

**(2)** The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

**Uniformity of dosage unit** ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirements of the Content uniformity test.

Transfer 1 tablet of Methylergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, add 10 mL of water, shake for 10 minutes vigorously, and disintegrate the tablet. Add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform, and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly $V$ mL of a solution containing about 5 μg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate Reference Standard, previously dried in a desiccator ( in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform, shake vigorously for 10 minutes, and centrifuge for 5 minutes. Discard the water layer, and use the chloroform layer as the standard solution. Pipet 20 mL each of the sample solution and the standard solution separately into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake for 5 minutes vigorously. Centrifuge these solutions for 5 minutes, take the water layers, and allow them to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

$$\text{Amount (mg) of methylergometrine maleate } (C_{20}H_{25}N_3O_2.C_4H_4O_4) = W_S \times (A_T/A_S) \times (V/250)$$

$W_S$: Amount (mg) of Methylergometrine Maleate Reference Standard

**Dissolution** ⟨*6.10*⟩ Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Methylergometrine Maleate Tablets at 100 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with a pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, to exactly $V$ mL of the subsequent filtrate add water to make exactly $V'$ mL so that each mL contains about 0.13 μg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate Reference Standard, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide), and dissolve in water to make exactly 100 mL. Pipet 5

mL of this solution, add water to make exactly 100 mL, then pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine immediately the intensities of the fluorescence, $F_T$ and $F_S$, of the sample solution and the standard solution at 338 nm as the excitation wavelength and at 427 nm as the fluorescence wavelength as directed under Fluorometry <*2.22*>.

The dissolution rate of Methylergometrine Maleate Tablets in 30 minutes should be not less than 70%.

Dissolution rate (%) with respect to the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
$= W_S \times (F_T/F_S) \times (V'/V) \times (1/C) \times (9/20)$

$W_S$: Amount (mg) of Methylergometrine Maleate Reference Standard

C: Labeled amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 Methylergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 mg of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$), transfer to a brown separator, add 15 mL of sodium hydrogen carbonate solution (1 in 20), and extract with four 20-mL portions of chloroform. Filter each portion of the chloroform extracts through a pledget of absorbent cotton, previously moistened with chloroform, into another dried, brown separator, combine all the extracts, and use this extract as the sample solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate Reference Standard, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water, and add water to make exactly 100 mL. Pipet 3 mL of this solution, and transfer to a brown separator, proceed in the same manner as the preparation of the sample solution, and use this extract as the standard solution. To each total volume of the sample solution and the standard solution add exactly 25 mL each of dilute *p*-dimethylaminobenzadehyde-ferric chloride TS, and after vigorous shaking for 5 minutes, allow to stand for 30 minutes. Draw off the water layer, centrifuge, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using dilute 4-dimethylaminobenzaldehyde-ferric chloride TS as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

Amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2.C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (3/100)$

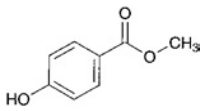
$W_S$: Amount (mg) of Methylergometrine Maleate Reference Standard

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル



$C_8H_8O_3$: 152.15
Methyl 4-hydroxybenzoate [*98-76-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Methyl Parahydroxybenzoate, when dried, contains not less than 98.0% and not more than 102.0% of $C_8H_8O_3$.

◆**Description** Methyl Parahydroxybenzoate, occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.◆

**Identification** **(1)** The melting point <*2.60*> of Methyl Parahydroxybenzoate is between 125°C and 128°C.

◆**(2)** Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of cobalt (II) chloride colorimetric stock solution, 12.0 mL of iron (III) chloride colorimetric stock solution and 2.0 mL of cupper (II) sulfate colorimetric stock solution add water to make 1000 mL.

**(2)** Acidity—Dissolve 0.20 g of Methyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

◆**(3)** Heavy metals <*1.07*>—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, 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 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

**(4)** Related substances—Dissolve 0.10 g of Methyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, 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

methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1.0 g of Methyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 152.1 mg of $C_8H_8O_3$

◆**Containers and storage** Containers—Well-closed containers.◆

# Compound Methyl Salicylate Spirit

複方サリチル酸メチル精

**Method of preparation**

| | |
|---|---|
| Methyl Salicylate | 40 mL |
| Capsicum Tincture | 100 mL |
| *d*- or *dl*-Camphor | 50 g |
| Ethanol | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Medicated Spirits, with the above ingredients.

**Description** Compound Methyl Salicylate Spirit is a reddish yellow liquid, having a characteristic odor and a burning taste.

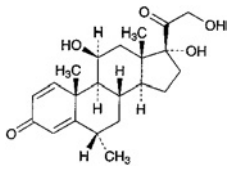
**Identification (1)** Shake 1 mL of Compound Methyl Salicylate Spirit with 5 mL of dilute ethanol, and add 1 drop of iron (III) chloride TS: a purple color is produced (methyl salicylate).

**(2)** Shake thoroughly 0.5 mL of Compound Methyl Salicylate Spirit with 10 mL of chloroform, and use this solution as the sample solution. Dissolve 0.04 g of methyl salicylate in 10 mL of chloroform, 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 the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and chloroform (4:1) to a distance of about 10 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *R*f value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

**Containers and storage** Containers—Tight containers.

# Methylprednisolone

メチルプレドニゾロン



$C_{22}H_{30}O_5$: 374.47
11*β*,17,21-Trihydroxy-6*α*-methylpregna-1,4-diene-3,20-dione [*83-43-2*]

Methylprednisolone, when dried, contains not less than 96.0% and not more than 104.0% of $C_{22}H_{30}O_5$.

**Description** Methylprednisolone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in 1,4-dioxane, slightly soluble in ethanol (95) and in chloroform, and practically insoluble in water and in diethyl ether.

Melting point: 232 – 240°C (with decomposition).

**Identification (1)** Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color develops with no fluorescence. Then add 10 mL of water to this solution: the color fades, and a gray, flocculent precipitate is produced.

**(2)** Dissolve 0.01 g of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

**(3)** Determine the absorption spectrum of a solution of Methylprednisolone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +79 – +86° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

**Purity** Related substances—Dissolve 50 mg of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 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 dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Then heat at 105°C for 10 minutes, cool, and spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (0.2 g).

**Assay** Weigh accurately about 10 mg of Methylpredniso-

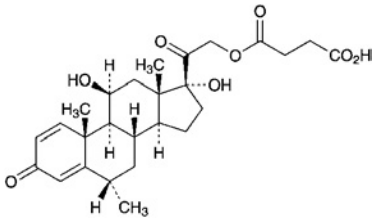
lone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance $A$ at the wavelength of maximum absorption at about 243 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{22}H_{30}O_5 = (A/400) \times 1000$$

**Containers and storage** Containers—Tight containers.

# Methylprednisolone Succinate

メチルプレドニゾロンコハク酸エステル



$C_{26}H_{34}O_8$: 474.54
11$\beta$,17,21-Trihydroxy-6$\alpha$-methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate) [*2921-57-5*]

Methylprednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{26}H_{34}O_8$.

**Description** Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

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

Melting point: about 235°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Methylprednisolone Succinate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylprednisolone Succinate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Methylprednisolone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Methylprednisolone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in ethanol (95), evaporating to dryness, and drying.

**Optical rotation** <*2.49*> $[\alpha]_D^{25}$: +99 – +103° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Methylprednisolone Succinate according to Method 4, 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 2.0 g of Methylprednisolone Succinate according to Method 3, and perform the test (not more than 1 ppm).

**(3)** Related substances—Dissolve 15 mg of Methylprednisolone Succinate in 5 mL of methanol, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than the peak of methylprednisolone succinate is not more than 1/2 of the peak area of methylprednisolone succinate from the standard solution, and the total area of the peaks other than the peak of methylprednisolone succinate is not more than the peak area of methylprednisolone succinate from the standard solution.

*Operating conditions—*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of methylprednisolone succinate.

*System suitability—*

Test for required detectability: Pipet 1 mL of the standard solution, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of methylprednisolone succinate obtained from 5 $\mu$L of this solution is equivalent to 7 to 13% of that obtained from 5 $\mu$L of the standard solution.

System performance: Proceed as directed in the System suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylprednisolone succinate is not more than 1.0%.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

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

**Assay** Weigh accurately about 15 mg each of Methylprednisolone Succinate and Methylprednisolone Succinate Reference Standard, previously dried, dissolve separately in 5 mL of methanol, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of methylprednisolone succinate to that of the internal standard.

$$\text{Amount (mg) of } C_{26}H_{34}O_8 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Methylprednisolone Succinate

Reference Standard

*Internal standard solution*—A solution of ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) (3 in 20,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 25°C.

Mobile phase: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of 0.05 mol/L disodium hydrogen phosphate TS to make a solution having pH 5.5. To 640 mL of this solution add 360 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylprednisolone succinate is about 6 minutes.

*System suitability*—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, methylprednisolone succinate 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylprednisolone succinate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Methylrosanilinium Chloride

## Crystal Violet

メチルロザニリン塩化物

$C_{25}H_{30}ClN_3$: 407.98

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with pentamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride ($C_{25}H_{30}ClN_3$)], calculated on the dried basis.

**Description** Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

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

**Identification** **(1)** To 1 mL of sulfuric acid add 1 mg of Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

**(2)** Dissolve 0.02 g of Methylrosanilinium Chloride in 10 mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.

**(3)** To 5 mL of the sample solution obtained in (2) add 0.5 g of zinc powder, and shake: the solution is decolorized. Place 1 drop of this solution on filter paper, and apply 1 drop of ammonia TS adjacent to it: a blue color is produced at the zone of contact of the both solutions.

**Purity** **(1)** Ethanol-insoluble substances—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105°C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water bath, and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol (95) until the last washing does not show a purple color, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0%.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Methylrosanilinium Chloride according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(3)** Zinc—To 0.10 g of Methylrosanilinium Chloride add 0.1 mL of sulfuric acid, and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again, and filter. To the filtrate add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

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

**Loss on drying** <*2.41*> Not more than 7.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 1.5% (0.5 g).

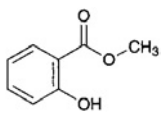
**Assay** Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighed, to a wide-mouthed, conical flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve, and add exactly 50 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Heat to boil, and boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate <*2.50*> the excess titanium (III) chloride with 0.05 mol/L ammonium iron (III) sulfate VS until a faint, red color is produced (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 20.40 mg of $C_{25}H_{30}ClN_3$

**Containers and storage** Containers—Tight containers.

# Methyl Salicylate

サリチル酸メチル




$C_8H_8O_3$: 152.15

Methyl 2-hydroxybenzoate [*119-36-8*]

Methyl Salicylate contains not less than 98.0% of $C_8H_8O_3$.

**Description** Methyl Salicylate is a colorless to pale yellow liquid. It has a strong, characteristic odor.

It is miscible with ethanol (95) and with diethyl ether.

It is very slightly soluble in water.

Specific gravity $d_{20}^{20}$: 1.182 – 1.192

Boiling point: 219 – 224°C

**Identification** Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute, and add 1 drop of iron (III) chloride TS: a purple color develops.

**Purity** **(1)** Acidity—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS, and titrate <*2.50*> with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

**(2)** Heavy metals <*1.07*>—Shake 10.0 mL of Methyl Salicylate thoroughly with 10 mL of water, add 1 drop of hydrochloric acid, and saturate with hydrogen sulfide by passing it through the mixture: neither the oily layer nor the aqueous layer shows a dark color.

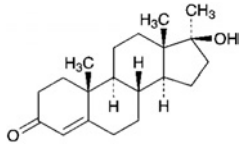
**Assay** Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and heat on a water bath for 2 hours under a reflux condenser. Cool, and titrate <*2.50*> the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS
= 76.07 mg of $C_8H_8O_3$

**Containers and storage** Containers—Tight containers.

# Methyltestosterone

メチルテストステロン




$C_{20}H_{30}O_2$: 302.45

17$\beta$-Hydroxy-17$\alpha$-methylandrost-4-en-3-one [*58-18-4*]

Methyltestosterone, when dried, contains not less than 98.0% and not more than 102.0% of $C_{20}H_{30}O_2$.

**Description** Methyltestosterone occurs as white to pale yellow, crystals or crystalline powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Methyltestosterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyltestosterone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Methyltestosterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.55*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Methyltestosterone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +79 – +85° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

**Melting point** <*2.60*> 163 – 168°C

**Purity** Related substances—Dissolve 40 mg of Methyltestosterone in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 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 $\mu$L 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 chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 10 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of methyltestosterone to that of the internal standard.

$$\text{Amount (mg) of } C_{20}H_{30}O_2 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Methyltestosterone Reference Standard

*Internal standard solution*—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 241 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about

35°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone 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 internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

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 ratios of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Methyltestosterone Tablets

メチルテストステロン錠

Methyltestosterone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$: 302.45).

**Method of preparation** Prepare as directed under Tablets, with Methyltestosterone.

**Identification** To a portion of powdered Methyltestosterone Tablets, equivalent to 10 mg of Methyltestosterone according to the labeled amount, add 50 mL of acetone, shake for 30 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone, and use this solution as the sample solution. Separately, dissolve 10 mg of Methyltestosterone Reference Standard 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 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 chloroform and ethanol (95) (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spot from the sample solution and the standard solution show the same *R*f value.

**Uniformity of dosage units** <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyltestosterone Tablets add 5 mL of water to disintegrate, add 50 mL of methanol, and shake for 30 minutes. Add methanol to make exactly 100 mL, and centrifuge. Measure exactly $V$ mL of the supernatant liquid, add methanol to make exactly $V'$ mL of a solution containing about 10 μg of methyltestosterone ($C_{20}H_{30}O_2$) per ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methyltestosterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, and dissolve in 5 mL of water and 50 mL of methanol, then add 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. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at the wavelength of maximum absorption at about 241 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <*2.25*>.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2) = W_S \times (A_T/A_S) \times (V'/V) \times (1/10)$$

$W_S$: Amount (mg) of Methyltestosterone Reference Standard

**Assay** Weigh accurately the mass of not less than 20 Methyltestosterone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of methyltestosterone ($C_{20}H_{30}O_2$), add about 70 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, filter through a membrane filter (not exceeding 0.45 μm in pore size), and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Methyltestosterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 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 determine the ratios, $Q_T$ and $Q_S$, of the peak area of methyltestosterone to that of the internal standard.

$$\text{Amount (mg) of methyltestosterone } (C_{20}H_{30}O_2) = W_S \times (Q_T/Q_S) \times (5/4)$$

$W_S$: Amount (mg) of Methyltestosterone Reference Standard

*Internal standard solution*—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 241 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 35°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

*System suitability*—

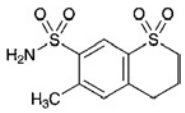
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

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 methyltestosterone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Meticrane

メチクラン



$C_{10}H_{13}NO_4S_2$: 275.34
6-Methylthiochromane-7-sulfonamide 1,1-dioxide
[*1084-65-7*]

Meticrane, when dried, contains not less than 98.0% of $C_{10}H_{13}NO_4S_2$.

**Description** Meticrane occurs as white, crystals or crystalline powder. It is odorless and has a slight bitter taste.

It is freely soluble in dimethylformamide, slightly soluble in acetonitrile and in methanol, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 234°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Meticrane in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Meticrane, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Ammonium <*1.02*>—Perform the test with 0.10 g of Meticrane. Prepare the control solution with 3.0 mL of Standard Ammonium Solution (not more than 0.03%).

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Meticrane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Meticrane according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.05 g of Meticrane in 50 mL of acetonitrile. To 5 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

*Operating conditions 1*—

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 $\mu$m in particle diameter).

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

Mobile phase: A mixture of water and acetonitrile (17:3).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of meticrane beginning after the solvent peak.

*System suitability 1*—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 $\mu$L of this solution is equivalent to 7 to 13% of that of meticrane obtained from 10 $\mu$L of the standard solution.

System performance: Dissolve 0.01 g each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions 1, caffeine and meticrane are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

*Operating conditions 2*—

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 2 minutes.

Time span of measurement: About 10 times as long as the retention time of meticrane beginning after the solvent peak.

*System suitability 2*—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 $\mu$L of this solution is equivalent to 7 to 13% of that of meticrane obtained from 10 $\mu$L of the standard solution.

System performance: Dissolve 0.02 g each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions 2, meticrane and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

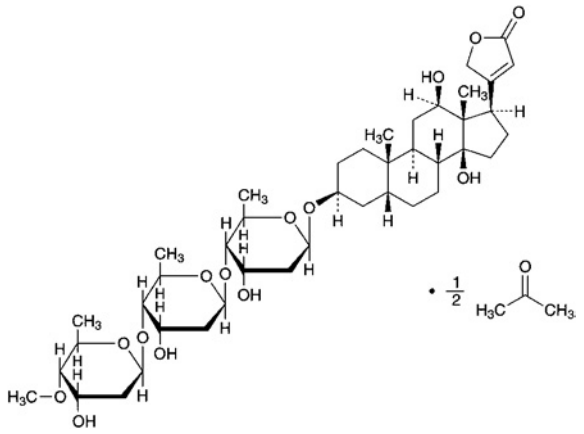
**Assay** Weigh accurately about 0.5 g of Meticrane, previously dried, dissolve in 50 mL of dimethylformamide, add 5 mL of water, and titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 27.54 mg of $C_{10}H_{13}NO_4S_2$

**Containers and storage** Containers—Well-closed containers.

# Metildigoxin

メチルジゴキシン



$C_{42}H_{66}O_{14}.\frac{1}{2}C_3H_6O$: 824.00

3$\beta$-[2,6-Dideoxy-4-*O*-methyl-$\beta$-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-$\beta$-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-$\beta$-D-*ribo*-hexopyranosyloxy]-12$\beta$,14-dihydroxy-5$\beta$-card-20(22)-enolide—acetone (2/1)
[*30685-43-9*, acetone-free]

Metildigoxin contains not less than 96.0% and not more than 103.0% of $C_{42}H_{66}O_{14}.\frac{1}{2}C_3H_6O$, calculated on the anhydrous basis.

**Description** Metildigoxin occurs as a white to light yellowish white, crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

**Identification** **(1)** Dissolve 2 mg of Metildigoxin in 2 mL of acetic acid (100), shake well with 1 drop of iron (III) chloride TS, and add gently 2 mL of sulfuric acid to divide into two layers: a brown color develops at the interface, and a deep blue color gradually develops in the acetic acid layer.

**(2)** Dissolve 2 mg of Metildigoxin in 2 mL of 1,3-dinitrobenzene TS, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and shake: a purple color gradually develops, and changes to blue-purple.

**(3)** Determine the absorption spectrum of a solution of Metildigoxin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Metildigoxin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Determine the infrared absorption spectrum of Metildigoxin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Metildigoxin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

**Optical rotation** <*2.49*> $[\alpha]_{546.1}^{20}$: +22.0 – +25.5° (1 g, calculated on the anhydrous basis, pyridine, 10 mL, 100 mm).

**Purity** **(1)** Arsenic <*1.11*>—Prepare the test solution with 0.5 g of Metildigoxin according to Method 3, and perform the test (not more than 4 ppm).

**(2)** Related substances—Dissolve 10 mg of Metildigoxin in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 $\mu$L 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-butanone and chloroform (3:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Acetone** Weigh accurately about 0.1 g of Metildigoxin, dissolve in exactly 2 mL of the internal standard solution, add *N,N*-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of acetone in a 50-mL volumetric flask containing about 10 mL of *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, then add *N,N*-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 $\mu$L each of the sample solution and the standard solution as directed under Gas Chromatography <*2.02*>, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of acetone to that of the internal standard: the amount of acetone is between 2.0% and 5.0%.

$$\text{Amount (\%) of acetone} = (W_S/W_T) \times (Q_T/Q_S)$$

$W_S$: Amount (g) of acetone
$W_T$: amount (g) of the sample

*Internal standard solution*—A solution of *t*-butanol in *N,N*-dimethylformamide (1 in 2000).

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 2 mm in inside diameter and 1 to 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 $\mu$m in particle diameter).

Column temperature: A constant temperature between 170°C and 230°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of acetone is about 2 minutes.

Selection of column: Proceed with 1 $\mu$L of the standard so-

lution under the above operating conditions, and calculate the resolution. Use a column giving elution of acetone and *t*-butanol in this order with the resolution between these peaks being not less than 2.0.

**Water** ⟨*2.48*⟩ Not more than 3.0% (0.3 g, direct titration).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

**Assay** Weigh accurately 0.1 g each of Metildigoxin and Metildigoxin Reference Standard, and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL each of the solutions, add methanol to each to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and the standard solution, add 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS to each, shake well, add methanol to make exactly 25 mL, and allow to stand at 20 ± 0.5°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a solution prepared by mixing 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS and adding methanol to make exactly 25 mL as the blank. Determine the maximum absorbances, $A_T$ and $A_S$, of the subsequent solutions obtained from the sample solution and the standard solution, respectively, by measuring every 5 minutes, at 495 nm.

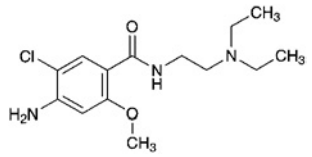
$$\text{Amount (mg) of } C_{42}H_{66}O_{14}.\tfrac{1}{2}C_3H_6O = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Metildigoxin Reference Standard, calculated on the anhydrous basis

**Containers and storage** Containers—Tight containers.

# Metoclopramide

メトクロプラミド



$C_{14}H_{22}ClN_3O_2$: 299.80
4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide [*364-62-5*]

Metoclopramide, when dried, contains not less than 99.0% of $C_{14}H_{22}ClN_3O_2$.

**Description** Metoclopramide occurs as white crystals or a crystalline powder, and is odorless.

It is freely soluble in acetic acid (100), soluble in methanol and in chloroform, sparingly soluble in acetic anhydride, in ethanol (95) and in acetone, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

**Identification** **(1)** Dissolve 0.01 g of Metoclopramide 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.

**(2)** Dissolve 0.01 g of Metoclopramide in 5 mL of dilute hydrochloric acid and 20 mL of water, and to 5 mL of this solution add 1 mL of Dragendorff's TS: a reddish orange precipitate is produced.

**(3)** Dissolve 0.1 g of Metoclopramide in 1 mL of 1 mol/L hydrochloric acid TS, and dilute with water to make 100 mL. To 1 mL of the solution add water to make 100 mL, determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** ⟨*2.60*⟩ 146 – 149°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Metoclopramide in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Metoclopramide as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Dissolve 1.0 g of Metoclopramide in 5 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.10 g of Metoclopramide in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of this solution, exactly measured, with methanol to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia solution (28) (19:1) to a distance of about 10 cm. Dry the plate, first in air and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Dissolve about 0.4 g of Metoclopramide, previously dried and accurately weighed, in 50 mL of acetic acid (100), add 5 mL of acetic anhydride, and warm for 5 minutes. Allow to cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform the blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 29.98 \text{ mg of } C_{14}H_{22}ClN_3O_2$$

**Containers and storage** Containers—Well-closed containers.

# Metoclopramide Tablets

メトクロプラミド錠

Metoclopramide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of

metoclopramide ($C_{14}H_{22}ClN_3O_2$: 299.80).

**Method of preparation** Prepare as directed under Tablets, with Metoclopramide.

**Identification** **(1)** To a quantity of powdered Metoclopramide Tablets, equivalent to 50 mg of Metoclopramide according to the labeled amount, add 15 mL of 0.5 mol/L hydrochloric acid TS, and heat in a water bath at 70°C for 15 minutes while frequent shaking. After cooling, centrifuge for 10 minutes, and to 5 mL of the supernatant liquid add 1 mL of 4-dimethylaminobenzaldehyde-hydrochloric acid TS: a yellow color develops.

**(2)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 270 nm and 274 nm, and between 306 nm and 310 nm.

**Uniformity of dosage units** <*6.02*> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Metoclopramide Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, disperse the particles with the aid of ultrasonic waves, then add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly $V$ mL of a solution so that each mL contains about 12 $\mu$g of metoclopramide ($C_{14}H_{22}ClN_3O_2$), and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of metoclopramide } (C_{14}H_{22}ClN_3O_2) = W_S \times (A_T/A_S) \times (V/1000)$$

$W_S$: Amount (mg) of metoclopramide for assay

**Dissolution** <*6.10*> Being specified separately.

**Assay** Weigh accurately not less than 20 Metoclopramide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of metoclopramide ($C_{14}H_{22}ClN_3O_2$), add 300 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

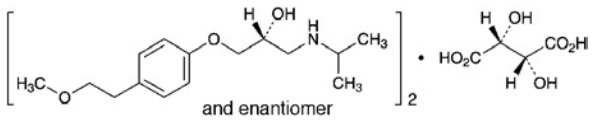
$$\text{Amount (mg) of metoclopramide } (C_{14}H_{22}ClN_3O_2) = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of metoclopramide for assay

**Containers and storage** Containers—Tight containers.

# Metoprolol Tartrate

メトプロロール酒石酸塩




$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$: 684.81
(2*RS*)-1-[4-(2-Methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol hemi-(2*R*,3*R*)-tartrate
[*56392-17-7*]

Metoprolol Tartrate, when dried, contains not less than 99.0% and not more than 101.0% of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$.

**Description** Metoprolol Tartrate occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

Optical rotation $[\alpha]_D^{20}$: +7.0 – +10.0° (after drying, 1 g, water, 50 mL, 100 mm).

**Identification** **(1)** Determine the absorption spectrum of a solution of Metoprolol Tartrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Metoprolol Tartrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample from a solution in acetone (23 in 1000), filter and dry the crystals, and perform the test with the crystals.

**(3)** A solution of Metoprolol Tartrate (1 in 5) responds to the Qualitative Tests <*1.09*> (1) for tartrate.

**pH** <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Metoprolol Tartrate in 10 mL of water is between 6.0 and 7.0.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Metoprolol Tartrate 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).

**(2)** Related substances—Dissolve 0.10 g of Metoprolol Tartrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 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 $\mu$L each of the sample solution and standard solution

on a plate of silica gel for thin-layer chromatography. After saturating the plate with the atmosphere by allowing to stand in a developing vessel, which contains the developing solvent and a glass vessel containing ammonia water (28), develop with the developing solvent, a mixture of ethyl acetate and methanol (4:1), to a distance of about 12 cm, and air-dry the plate. Allow to stand the plate in an iodine vapors until the spot with the standard solution appears obviously: the spot other than the principal spot and other than the spot on the original point is not more than three spots, and they are not more intense than the spot with the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Metoprolol Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.24 mg of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$

**Containers and storage** Containers—Well-closed containers.

# Metoprolol Tartrate Tablets

メトプロロール酒石酸塩錠

Metoprolol Tartrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$: 684.81).

**Method of preparation** Prepare as directed under Tablets, with Metoprolol Tartrate.

**Identification** To an amount of powdered Metoprolol Tartrate Tablets, equivalent to 10 mg of Metoprolol Tartrate according to the labeled amount, add 100 mL of ethanol (95), shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

**Uniformity of dosage units** ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Metoprolol Tartrate Tablets add 1 mL of water for every 10 mg of Metoprolol Tartrate, shake for 20 minutes, then add 75 mL of ethanol (95), shake for 15 minutes, add ethanol (95) to make exactly 100 mL, and centrifuge. Pipet $V$ mL of the supernatant liquid, add ethanol (95) to make exactly $V'$ so that each mL contains about 0.1 mg of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 5 mL of water, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using ethanol (95) as the blank.

Amount (mg) of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/5)$

$W_S$: Amount (mg) of metoprolol tartrate for assay

**Dissolution** ⟨*6.10*⟩ Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Metoprolol Tartrate Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet the subsequent $V$ mL, add water to make exactly $V'$ mL so that each mL contains about 22 μg of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 200 mL. Pipet 8 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 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 metoprolol. The dissolution rate in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

$W_S$: Amount (mg) of metoprolol tartrate for assay
C: Labeled amount (mg) of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$) in 1 tablet

*Operating conditions*—
Proceed as directed in the Assay.
*System suitability*—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metoprolol are not less than 2000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metoprolol is not more than 2.0%.

**Assay** Weigh accurately the mass of not less than 20 Metoprolol Tartrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.12 g of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2.C_4H_6O_6$), add 60 mL of a mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) and exactly 10 mL of the internal standard solution, shake for 15 minutes, and add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.12 g of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 60 mL of the mixture of ethanol (99.5) and

1 mol/L hydrochloric acid TS (100:1), add exactly 10 mL of the internal standard solution, then add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 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 determine the ratios, $Q_T$ and $Q_S$, of the peak area of metoprolol to that of the internal standard.

Amount (mg) of metoprolol tartrate $((C_{15}H_{25}NO_3)_2.C_4H_6O_6) = W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of metoprolol tartrate for assay

*Internal standard solution*—A solution of ethyl parahydroxybenzoate in the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) (1 in 500).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 274 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 25°C.

Mobile phase: Dissolve 14.0 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust to pH 3.2 with diluted perchloric acid (17 in 2000). To 750 mL of this solution add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of metoprolol is about 8 minutes.

*System suitability*—

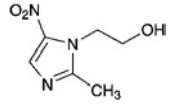
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, metoprolol and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

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 ratio of the peak area of metoprolol to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

# Metronidazole

メトロニダゾール




$C_6H_9N_3O_3$: 171.15

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethanol [*443-48-1*]

Metronidazole, when dried, contains not less than 99.0% and not more than 101.0% of $C_6H_9N_3O_3$.

**Description** Metronidazole occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and slightly soluble in water.

It dissolves in dilute hydrochloric acid.

It is colored to yellow-brown by light.

**Identification (1)** Determine the absorption spectrum of a solution of Metronidazole in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Metronidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** ⟨2.60⟩ 159 – 163°C

**Purity (1)** Heavy metals ⟨1.07⟩—Proceed with 1.0 g of Metronidazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** 2-Methyl-5-nitroimidazol—Dissolve 0.10 g of Metronidazole in acetone to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in acetone to make exactly 20 mL, then pipet 5 mL of this solution, add acetone to make exactly 100 mL, 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. Immediately develop the plate with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

**Loss on drying** ⟨2.41⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

**Residue on ignition** ⟨2.44⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Metronidazole, previously dried, and dissolve in 30 mL of acetic acid (100). Titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of *p*-naphtholbenzein TS) until the color of the solution changes from orange-yellow to green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.12 mg of $C_6H_9N_3O_3$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Metronidazole Tablets

メトロニダゾール錠

Metronidazole Tablets contain not less than 93.0%

and not more than 107.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$: 171.15).

**Method of preparation** Prepare as directed under Tablets, with Metronidazole.

**Identification** **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 275 nm and 279 nm.

**(2)** Shake vigorously a quantity of powdered Metronidazole Tablets, equivalent to 0.20 g of Metronidazole according to the labeled amount, with 20 mL of acetone for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.10 g of metronidazole 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 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate immediately with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the principal spots obtained from the sample solution and the standard solution is the same.

**Uniformity of dosage units** <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metronidazole Tablets add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 25 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter the solution through a membrane filter with pore size of 0.45 $\mu$m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

$$\text{Amount (mg) of metronidazole } (C_6H_9N_3O_3) = W_S \times (A_T/A_S) \times 10$$

$W_S$: Amount (mg) of metronidazole for assay

**Assay** Weigh accurately the mass of not less than 20 Metronidazole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole ($C_6H_9N_3O_3$), add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 10 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 $\mu$m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of metronidazole for assay, previously dried in vacuum on silica gel for 24 hours, dissolve in the mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L 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 metronidazole.

$$\text{Amount (mg) of metronidazole } (C_6H_9N_3O_3) = W_S \times (A_T/A_S) \times 10$$

$W_S$: Amount (mg) of metronidazole for assay

*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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of metronidazole is about 5 minutes.

*System suitability*—

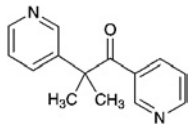
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Metyrapone

メチラポン




$C_{14}H_{14}N_2O$: 226.27

2-Methyl-1,2-di(pyridin-3-yl)propan-1-one [*54-36-4*]

Metyrapone, when dried, contains not less than 98.0% of $C_{14}H_{14}N_2O$.

**Description** Metyrapone occurs as a white to pale yellow, crystalline powder. It has a characteristic odor and a bitter taste.

It is very soluble in methanol, in ethanol (95), in acetic anhydride, in chloroform, in diethyl ether and in nitrobenzene, and sparingly soluble in water.

It dissolves in 0.5 mol/L sulfuric acid TS.

**Identification** **(1)** Mix 5 mg of Metyrapone with 0.01 g of 1-chloro-2,4-dinitrobenzene, melt by gently heating for 5 to 6 seconds, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

**(2)** Determine the absorption spectrum of a solution of Metyrapone in 0.5 mol/L sulfuric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** <*2.60*> 50 – 54°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of

Metyrapone in 5 mL of methanol: the solution is clear and colorless to pale yellow.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Metyrapone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Metyrapone, according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.25 g of Metyrapone in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 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 these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 $\mu$L 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 chloroform and methanol (15:1) to a distance of about 10 cm, and air-dry the plate for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

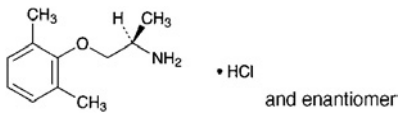
**Assay** Weigh accurately about 0.2 g of Metyrapone, previously dried, dissolve in 10 mL of nitrobenzene and 40 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.31 mg of $C_{14}H_{14}N_2O$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Mexiletine Hydrochloride

メキシレチン塩酸塩




$C_{11}H_{17}NO.HCl$: 215.72
(1*RS*)-2-(2,6-Dimethylphenoxy)-1-methylethylamine monohydrochloride [*5370-01-4*]

Mexiletine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{11}H_{17}NO.HCl$.

**Description** Mexiletine Hydrochloride occurs as a white powder.

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

A solution of Mexiletine Hydrochloride (1 in 20) shows no optical rotation.

**Identification (1)** Determine the absorption spectrum of a solution of Mexiletine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mexiletine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Mexiletine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Mexiletine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample from ethanol (95), filter, dry the crystals, and repeat the test on the crystals.

**(3)** A solution of Mexiletine Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

**pH** <*2.54*> Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the pH of this solution is between 3.8 and 5.8.

**Melting point** <*2.60*> 200 – 204°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy Metals <*1.07*>—Proceed with 2.0 g of Mexiletine Hydrochloride 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).

**(3)** Related substances—Dissolve 20 mg of Mexiletine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each peak area of the peaks other than the peak of mexiletine from the sample solution is not larger than the peak area of mexiletine from the standard solution.

*Operating conditions*—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of mexiletine obtained from 20 $\mu$L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 3 times as long as the retention time of mexiletine beginning after peaks of the solvent.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride Reference

Standard, each previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 $\mu$L 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 mexiletine to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{11}H_{17}NO.HCl = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Mexiletine Hydrochloride Reference Standard

*Internal standard solution*—A solution of phenetylamine hydrochloride in the mobile phase (3 in 5000).

*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 octylsilanized silica gel for liquid chromatography (about 7 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of sodium dihydrogenphosphate dihydrate in 600 mL of water, and add 420 mL of acetonitrile.

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

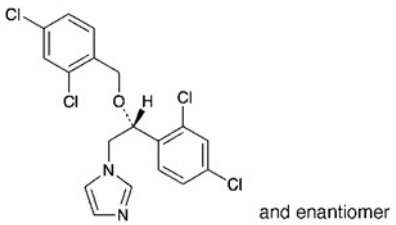
Selection of column: Proceed with 20 $\mu$L of the standard solution under the above conditions, and calculate the resolution. Use a column giving elution of the internal standard and mexiletine in this order with the resolution between these peaks being not less than 9.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Miconazole

ミコナゾール




$C_{18}H_{14}Cl_4N_2O$: 416.13

1-[(2*RS*)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole [*22916-47-8*]

Miconazole, when dried, contains not less than 98.5% of $C_{18}H_{14}Cl_4N_2O$.

**Description** Miconazole occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), soluble in diethyl ether, and practically insoluble in water.

A solution of Miconazole in methanol (1 in 20) shows no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of Miconazole in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Miconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 84 – 87°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Miconazole 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).

**(2)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Miconazole according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.10 g of Miconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 $\mu$L 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, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 60%, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

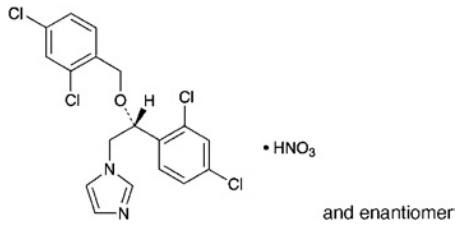
**Assay** Weigh accurately about 0.3 g of Miconazole, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of *p*-naphtholbenzein TS) until the color of the solution changes from light yellow-brown to light yellow-green. Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 41.61 \text{ mg of } C_{18}H_{14}Cl_4N_2O$$

**Containers and storage** Containers—Tight containers.

# Miconazole Nitrate

ミコナゾール硝酸塩




$C_{18}H_{14}Cl_4N_2O.HNO_3$: 479.14
1-[(2*RS*)-2-(2,4-Dichlorobenzyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole mononitrate
[*22832-87-7*]

Miconazole Nitrate, when dried, contains not less than 98.5% of $C_{18}H_{14}Cl_4N_2O.HNO_3$.

**Description** Miconazole Nitrate occurs as a white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetone and in acetic acid (100), and very slightly soluble in water and in diethyl ether.

Melting point: about 180°C (with decomposition).

**Identification** **(1)** To 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

**(2)** Determine the absorption spectrum of a solution of Miconazole Nitrate in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

**(4)** A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests <*1.09*> for nitrate.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL (not more than 0.09%).

**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Miconazole Nitrate 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).

**(4)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3, and perform the test (not more than 2 ppm).

**(5)** Related substances—Dissolve 0.10 g of Miconazole Nitrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 $\mu$L 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 *n*-hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

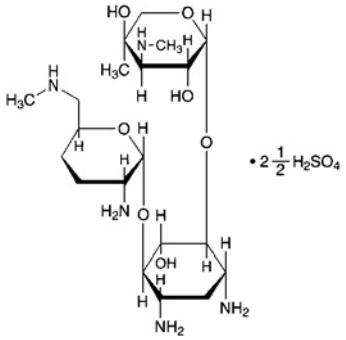
**Assay** Weigh accurately about 0.35 g of Miconazole Nitrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.91 mg of $C_{18}H_{14}Cl_4N_2O.HNO_3$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Micronomicin Sulfate

ミクロノマイシン硫酸塩




$C_{20}H_{41}N_5O_7.2½H_2SO_4$: 708.77
2-Amino-2,3,4,6-tetradeoxy-6-methylamino-α-D-*erythro*-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine hemipentasulfate
[*52093-21-7*, Micronomicin]

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora sagamiensis*.

It contains not less than 590 $\mu$g (potency) and not more than 660 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micronomicin ($C_{20}H_{41}N_5O_7$: 463.57).

**Description** Micronomicin Sulfate occurs as a white to light

yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

**Identification** **(1)** Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their *R*f values are the same.

**(2)** To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +110 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**pH** <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Micronomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.5 g of Micronomicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Micronomicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 0.40 g of Micronomicin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-buthanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Water** <*2.48*> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

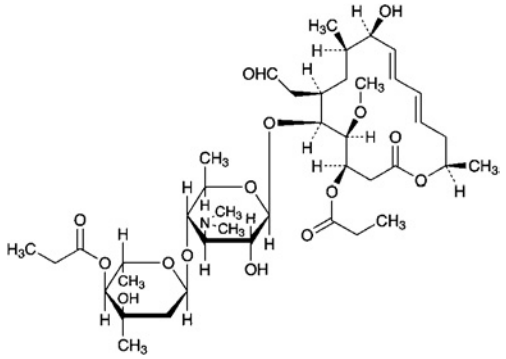
(iii) Standard solutions—Weigh accurately an amount of Micronomicin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 $\mu$g (potency) and 0.5 $\mu$g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Micronomicin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 $\mu$g (potency) and 0.5 $\mu$g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

# Midecamycin

ミデカマイシン



$C_{41}H_{67}NO_{15}$: 813.97

(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[2,6-Dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-*β*-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide

[*35457-80-8*]

Midecamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces mycarofaciens*.

It contains not less than 950 $\mu$g (potency) and not more than 1020 $\mu$g (potency) per mg, calculated on the dried basis. The potency of Midecamycin is expressed as mass (potency) of midecamycin ($C_{41}H_{67}NO_{15}$).

**Description** Midecamycin occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95), and very slightly soluble in water.

**Identification** **(1)** Determine the absorption spectrum of a

solution of Midecamycin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Midecamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Midecamycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 153 – 158°C

**Purity** Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**Loss on drying** <2.41> Not more than 2.0% (1.0 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

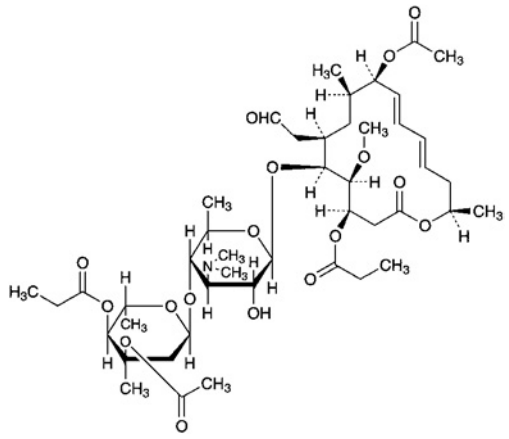
(iii) Standard solutions—Weigh accurately an amount of Midecamycin Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions – Weigh accurately an amount of Midecamycin, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

# Midecamycin Acetate

ミデカマイシン酢酸エステル



$C_{45}H_{71}NO_{17}$: 898.04

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-9-Acetoxy-5-[3-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide [*55881-07-7*]

Midecamycin Acetate is a derivative of midecamycin.

It contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin Acetate is expressed as mass of midecamycin acetate ($C_{45}H_{71}NO_{17}$).

**Description** Midecamycin Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

**Identification (1)** Determine the absorption spectrum of a solution of Midecamycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Acetate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Midecamycin Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or spectrum of dried Midecamycin Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <2.41> Not more than 2.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Midecamycin Acetate Reference Standard, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin Acetate, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

# Migrenin

ミグレニン

Migrenin is composed of 90 parts of antipyrine, 9 parts of caffeine, and 1 part of citric acid in mass.

Migrenin, when dried, contains not less than 87.0% and not more than 93.0% of antipyrine ($C_{11}H_{12}N_2O$: 188.23) and not less than 8.6% and not more than 9.5% of caffeine ($C_8H_{10}N_4O_2$: 194.19).

**Description** Migrenin occurs as a white powder or crystalline powder. It is odorless and has a bitter taste.

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

The pH of a solution of Migrenin (1 in 10) is between 3.0 and 4.0.

It is affected by moisture and light.

**Identification** **(1)** To 5 mL of a solution of Migrenin (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

**(2)** To 5 mL of a solution of Migrenin (1 in 50) add 1 drop of hydrochloric acid and 0.2 mL of formaldehyde solution, heat in a water bath for 30 minutes, add an excess of ammonia TS, and filter. Acidify the filtrate with hydrochloric acid, shake with 3 mL of chloroform, and separate the chloroform layer. Evaporate the chloroform solution on a water bath, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid to the residue, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the residue over a vessel containing 3 drops of ammonia TS: a red-purple color develops, disappearing on the addition of 2 to 3 drops of sodium hydroxide TS.

**(3)** A solution of Migrenin (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for citrate.

**Melting point** ⟨*2.60*⟩ 104 – 110°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Migrenin in 40 mL of water: the solution is clear and colorless to pale yellow.

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

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** **(1)** Antipyrine—Weigh accurately about 0.25 g of Migrenin, previously dried in an iodine flask, dissolve in 25 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Add 15 mL of chloroform to dissolve the precipitate so obtained, and titrate ⟨*2.50*⟩ the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS
= 9.411 mg of $C_{11}H_{12}N_2O$

**(2)** Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine Reference Standard, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of caffeine to that of the internal standard.

$$\text{Amount (mg) of caffeine } (C_8H_{10}N_4O_2) = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Caffeine Reference Standard

*Internal standard solution*—A solution of ethenzamide in chloroform (1 in 50).

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with siliceous earth for gas chromatography (180 to 250 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethenzamide is about 4 minutes.

*System suitability*—

System performance: Dissolve 0.9 g of antipyrine and 0.09

g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μL of this solution under the above operating conditions, caffeine and antipyrine are eluted in this order with the resolution between these peaks being not less than 1.5.

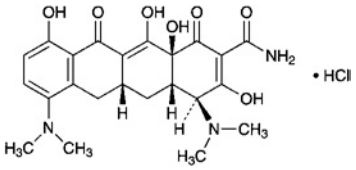
System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of caffeine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Minocycline Hydrochloride

ミノサイクリン塩酸塩



$C_{23}H_{27}N_3O_7$.HCl: 493.94

(4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride [*13614-98-7*]

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline.

It contains not less than 890 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Minocycline Hydrochloride is expressed as mass (potency) of minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

**Description** Minocycline Hydrochloride occurs as a yellow crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (95).

**Identification** **(1)** Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Minocycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Minocycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

**pH** <*2.54*> Dissolve 1.0 g of Minocycline Hydrochloride in 100 mL of water: the pH of the solution is between 3.5 and 4.5.

**Purity** **(1)** A solution of Minocycline Hydrochloride (1 in 100) is clear, and when the test is performed within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.06.

**(2)** Heavy metals <*1.07*>—Proceed with 0.5 g of Minocycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

**(3)** Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test immediately after the preparation of the sample solution with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2 %, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

*Operating conditions*—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition.

Time span of measurement: About 2.5 times as long as the retention time of minocycline beginning after the solvent peak.

*System suitability*—

Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of minocycline obtained from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

**Water** <*2.48*> Not less than 4.3% and not more than 8.0% (0.3 g, volumetric titration, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.5% (1 g).

**Assay** Weigh accurately an amount of Minocycline Hydrochloride and Minocycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μL each of the sample solution and the standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the peak areas, $A_T$ and $A_S$, of minocycline of these solutions.

Amount [$\mu$g (potency)] of minocycline ($C_{23}H_{27}N_3O_7$) $= W_S \times (A_T/A_S) \times 1000$

$W_S$: Amount [mg (potency)] of Minocycline Hydrochloride Reference Standard

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 280 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of a solution of ammonium oxalate monohydrate (7 in 250), *N,N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) to 6.2 with tetrabutylammonium hydroxide TS.

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

*System suitability—*

System performance: Dissolve 0.05 g (potency) of Minocycline Hydrochloride Reference Standard in 25 mL of water. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 $\mu$L of this solution under the above operating conditions, epiminocycline and minocycline are eluted in this order with the resolution between these peaks being not less than 2.0.

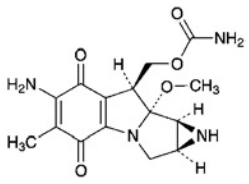
System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of peak areas of minocycline is not more than 2.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Mitomycin C

マイトマイシン C



$C_{15}H_{18}N_4O_5$: 334.33

(1a*S*,8*S*,8a*R*,8b*S*)-6-Amino-4,7-dioxo-8a-methoxy-5-methyl-1,1a,2,8,8a,8b-hexahydroazirino[2′,3′:3,4]pyrrolo[1,2-*a*]indol-8-ylmethyl carbamate [*50-07-7*]

Mitomycin C is a substance having antitumor activity produced by the growth of *Streptomyces caespitosus*.

It contains not less than 970 $\mu$g (potency) and not more than 1030 $\mu$g (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C ($C_{15}H_{18}N_4O_5$).

**Description** Mitomycin C occurs as blue-purple, crystals or crystalline powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Mitomycin C (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mitomycin C Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Mitomycin C as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Mitomycin C Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the sample solution is not more than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not more than 3 times the peak area of mitomycin C from the standard solution.

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 254 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 $\mu$m in particle diameter).

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

Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.

Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 10 | 100 | 0 |
| 10 – 30 | 100 → 0 | 0 → 100 |
| 30 – 45 | 0 | 100 |

Flow rate: About 1.0 mL/min

Time span of measurement: About 2 times as long as the

retention time of mitomycin C beginning after the solvent peak.

*System suitability*—

Test for required detection: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of methanol. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

**Loss on drying** ⟨*2.41*⟩ Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Assay** Weigh accurately an amount of Mitomycin C and Mitomycin C Reference Standard, equivalent to about 25 mg (potency), dissolve each in *N*,*N*-dimethylacetamide to make exactly 50 mL, and use these solutions as the sample solution and 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 mitomycin C.

$$\text{Amount [}\mu\text{g (potency)] of } C_{15}H_{18}N_4O_5 = W_S \times (A_T/A_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Mitomycin C Reference Standard

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 365 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with phenylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 40 mL of 0.5 mol/L ammonium acetate TS add 5 mL of diluted acetic acid (100) (1 in 20) and water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of mitomycin C is about 7 minutes.

*System suitability*—

System performance: Dissolve about 25 mg of Mitomycin C Reference Standard and about 0.375 g of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of *N*,*N*-dimethylacetamide. When the procedure is run with 10 μL of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde 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 mitomycin C is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Morphine and Atropine Injection

モルヒネ·アトロピン注射液

Morphine and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.91 w/v% and not more than 1.09 w/v% of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

**Method of preparation**

| | |
|---|---|
| Morphine Hydrochloride Hydrate | 10 g |
| Atropine Sulfate Hydrate | 0.3 g |
| Water for Injection | a significant quantity |
| To make | 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Morphine and Atropine Injection is a clear, colorless liquid.

It is gradually colored by light.

pH: 2.5 – 5.0

**Identification** To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of morphine hydrochloride in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of atropine sulfate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained 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 methanol and ammonia solution (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the two spots obtained from the sample solution show the same color tone and the same *R*f value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Assay (1)** Morphine hydrochloride hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL 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 morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= W_S \times (Q_T/Q_S) \times 1.1679$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of etilefrine hydrochloride (1 in 500).

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

*System suitability*—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, morphine and the internal standard 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 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

**(2)** Atropine sulfate hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate Reference Standard (separately determine its loss on drying <2.41> in the same manner as directed under Atropine Sulfate Hydrate), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 areas of atropine to that of the internal standard.

Amount (mg) of atropine sulfate hydrate [$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$]
$= W_S \times (Q_T/Q_S) \times (1/25) \times 1.027$

$W_S$: Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

*Internal standard solution*—A solution of etilefrine hydrochloride (1 in 12,500).

*Operating conditions*—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Flow rate: Adjust the flow rate so that the retention time of morphine is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 20 $\mu$L of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is not less than 3.

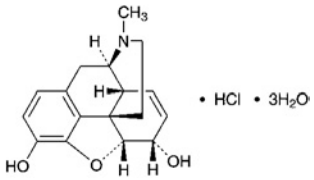
System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Morphine Hydrochloride Hydrate

モルヒネ塩酸塩水和物




$C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84

(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate [*6055-06-7*]

Morphine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl$: 321.80), calculated on the anhydrous basis.

**Description** Morphine Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

It is colored by light.

**Identification** **(1)** Determine the absorption spectrum of a solution of Morphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Morphine Hydrochloride in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Morphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at

the same wave numbers.

**(3)** A solution of Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: −111 – −116° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**pH** <2.54> The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water is between 4.0 and 6.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <1.14>—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

**(3)** Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

**(4)** Related substances—Dissolve 0.1 g of Morphine Hydrochloride Hydrate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Water** <2.48> 13 – 15% (0.1 g, direct titration).

**Residue on ignition** <2.44> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.5 g of Morphine Hydrochloride Hydrate, dissolve in 3.0 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), mix, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.18 mg of $C_{17}H_{19}NO_3.HCl$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Morphine Hydrochloride Injection

モルヒネ塩酸塩注射液

Morphine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84).

**Method of preparation** Prepare as directed under Injections, with Morphine Hydrochloride Hydrate.

**Description** Morphine Hydrochloride Injection is a clear, colorless liquid.

It is affected by light.

pH: 2.5 – 5.0

**Identification** Take a volume of Morphine Hydrochloride Injection, equivalent to 0.04 g of Morphine Hydrochloride Hydrate according to the labeled amount, add water to make 20 mL, and use this solution as the sample solution. To 5 mL of the sample solution add water to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm. And to 5 mL of the sample solution add dilute sodium hydroxide TS to make 100 mL, and determine the absorption spectrum: it exhibits a maximum between 296 nm and 300 nm.

**Extractable volume** <6.05> It meets the requirement.

**Assay** Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), and add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 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 morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride ($C_{17}H_{19}NO_3.HCl.3H_2O$)
$= W_S \times (Q_T/Q_S) \times 4 \times 1.1679$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of etilefrine hydrochloride (1 in 500).

*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: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that retention time of morphine is about 10 minutes.

*System suitability*—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard 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 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Morphine Hydrochloride Tablets

モルヒネ塩酸塩錠

Morphine Hydrochloride Tablets contain not less than 93% and not more than 107% of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$: 375.84).

**Method of preparation** Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

**Identification** Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

**Assay** Take not less than 20 Morphine Hydrochloride Tablets, weigh accurately, and powder. Weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3.HCl.3H_2O$), add exactly 10 mL of the internal standard solution, extract the mixture with ultrasonic waves for 10 minutes, and add water to make 50 mL. Filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 morphine to that of the internal standard.

$$\text{Amount (mg) of morphine hydrochloride } (C_{17}H_{19}NO_3.HCl.3H_2O) = W_S \times (Q_T/Q_S) \times 1.1679$$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of etilefrine hydrochloride (1 in 500).

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

*System suitability*—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, morphine and the internal standard 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 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Freeze-dried Live Attenuated Mumps Vaccine

乾燥弱毒生おたふくかぜワクチン

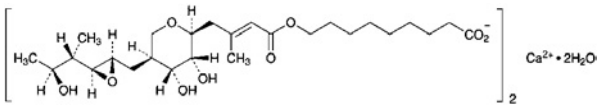
Freeze-dried Live Attenuated Mumps Vaccine is a dried preparation containing live attenuated mumps virus.

It conforms to the requirements of Freeze-dried Live Attenuated Mumps Vaccine in the Minimum Requirements of Biologic Products.

**Description** Freeze-dried Live Attenuated Mumps Vaccine becomes a clear, colorless, yellowish or reddish liquid on addition of solvent.

# Mupirocin Calcium Hydrate

ムピロシンカルシウム水和物



$C_{52}H_{86}CaO_{18}.2H_2O$: 1075.34

Monocalcium bis[9-((2*E*)-4-{(2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-dihydroxy-3,4,5,6-tetrahydro-2*H*-pyran-2-yl}-3-methylbut-2-enoyloxy)nonanoate] dihydrate [*115074-43-6*]

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Pseudomonas fluorescens*.

It contains not less than 895 $\mu$g (potency) and not more than 970 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Mupirocin Calcium Hydrate is expressed as mass (potency) of mupirocin ($C_{26}H_{44}O_9$: 500.62).

**Description** Mupirocin Calcium Hydrate occurs as a white powder and has a bitter taste.

It is freely soluble in methanol and slightly soluble in water and in ethanol (95).

**Identification** **(1)** To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of *N,N'*-dicyclohexylcarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchorate hexahydrate-ethanol TS to the solution, and shake: a dark purple color develops.

**(2)** Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 219 nm and 224 nm.

**(3)** Determine the infrared absorption spectrum of Mupirocin Calcium Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1708 $cm^{-1}$, 1648 $cm^{-1}$, 1558 $cm^{-1}$, 1231 $cm^{-1}$, 1151$cm^{-1}$ and 894 $cm^{-1}$.

**(4)** A solution of Mupirocin Calcium Hydrate (3 in 1000) responds to the Qualitative Tests <*1.09*> (3) for calcium salt.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-16$ – $-20°$ (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

**Purity** **(1)** Related substances—Dissolve 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make 10 mL, and use this solution as the sample solution (1). Pipet 2 mL of this solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the sample solution (2). Preserve these sample solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 $\mu$L of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas of each peak of the sample solution (1) and the sample solution (2) by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of principal related substance (appeared at about 0.7 of the relative retention time to mupirocin) is not more than 4.0%, and the total amount of related substances (the total area of the peaks other than of the solvent and mupirocin) is not more than 6.0%.

$$\text{Amount (\%) of principal related substance} = \frac{A_i}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \dfrac{A \times 100}{A + A_m}}$$

$$\text{Total amount (\%) of related substances} = \frac{A}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \dfrac{A \times 100}{A + A_m}}$$

$A$: Total peak areas other than of the solvent and mupirocin from the sample solution (1)

$A_i$: Peak area of the relative retention time of about 0.7 to mupirocin from the sample solution (1)

$A_m$: A value of 50 times of peak area of mupirocin from the sample solution (2)

$P$: Potency per mg obtained from the assay.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of mupirocin beginning after the solvent peak.

*System suitability*—

Test for required detection: Pipet 1 mL of the sample solution (2), and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 $\mu$L of this solution is equivalent to 4 to 6% of that obtained from 20 $\mu$L of the sample solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the sample solution (2) under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 2.0%.

**(2)** Inorganic salt from manufacturing process—Being specified separately.

**Water** <*2.48*> Not less than 3.0% and not more than 4.5% (0.5 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Mupirocin Calcium Hydrate and Mupirocin Lithium Reference Standard, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Preserve these solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 $\mu$L of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the peak areas, $A_T$ and $A_S$, of mupirocin of each solution.

$$\text{Amount [}\mu\text{g (potency)] of mupirocin } (C_{26}H_{44}O_9) = W_S \times (A_T/A_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Mupirocin Lithium Reference Standard

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 240 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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100), and add water to make 1000 mL. To 300 mL of this solution add 100 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of

mupirocin is about 12.5 minutes.

*System suitability*—

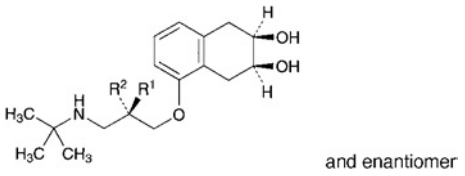
System performance: Dissolve about 20 mg of Mupirocin Lithium Reference Standard and about 5 mg of ethyl parahydroxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, mupirocin and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.

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 mupirocin is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Nadolol

ナドロール




and enantiomer

$C_{17}H_{27}NO_4$: 309.40

$R^1$ = OH, $R^2$ = H

(2*RS*,3*SR*)-5-{(2*SR*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropyloxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol

$R^1$ = H, $R^2$ = OH

(2*RS*,3*SR*)-5-{(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropyloxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol [*42200-33-9*]

Nadolol, when dried, contains not less than 98.0% of $C_{17}H_{27}NO_4$.

**Description** Nadolol occurs as a white to yellow-brownish white crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in water and in chloroform.

A solution of Nadolol in methanol (1 in 100) shows no optical rotation.

Melting point: about 137°C

**Identification** **(1)** Determine the absorption spectrum of a solution of Nadolol in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Nadolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1585 $cm^{-1}$, 1460 $cm^{-1}$, 1092 $cm^{-1}$, 935 $cm^{-1}$ and 770 $cm^{-1}$.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nadolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 0.5 g of Nadolol in 10 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 100 μL each of the sample solution and a mixture of methanol and chloroform (1:1) as a control solution with 25 mm each of width at an interval of about 10 mm on the starting line of a plate 0.25 mm in thickness of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform and diluted ammonia TS (1 in 3) (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm), and confirm the positions of the principal spot and the spots other than the principal spot from the sample solution. Scratch and collect the silica gel of the positions of the plate corresponding to the principal spot and the spots other than the principal spot. To the silica gel collected from the principal spot add exactly 30 mL of ethanol (95), and to the silica gel from the spots other than the principal spot add exactly 10 mL of ethanol (95). After shaking them for 60 minutes, centrifuge, and determine the absorbances of these supernatant liquids at 278 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Separately, proceed in the same manner with each position of the silica gel from the control solution corresponding to the principal spot and the spots other than the principal spot of the sample solution, and perform a blank determination to make correction. Amount of the related substances calculated by the following equation is not more than 2.0%.

$$\text{Amount (\%) of related substances} = \{A_b/(A_b + 3A_a)\} \times 100$$

$A_a$: Corrected absorbance of the principle spot.

$A_b$: Corrected absorbance of the spots other than the principle spot.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Isomer ratio** Prepare a paste with 0.01 g of Nadolol as directed in the paste method under Infrared Spectrophotometry <*2.25*> so that its transmittance at an absorption band at a wave number of about 1585 $cm^{-1}$ is 25 to 30%, and determine the infrared absorption spectrum between 1600 $cm^{-1}$ and 1100 $cm^{-1}$. Determine the absorbances, $A_{1265}$ and $A_{1250}$, from the transmittances, $T_{1265}$ and $T_{1250}$, at wave numbers of about 1265 $cm^{-1}$ (racemic substance A) and 1250 $cm^{-1}$ (racemic substance B), respectively: the ratio $A_{1265}/A_{1250}$ is between 0.72 and 1.08.

**Assay** Weigh accurately about 0.28 g of Nadolol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green-blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

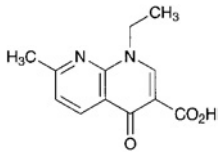
Each mL of 0.1 mol/L perchloric acid VS
= 30.94 mg of $C_{17}H_{27}NO_4$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Nalidixic Acid

ナリジクス酸



$C_{12}H_{12}N_2O_3$: 232.24
1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid [*389-08-2*]

Nalidixic Acid, when dried, contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$.

**Description** Nalidixic Acid occurs as white to light yellow crystals or crystalline powder.

It is sparingly soluble in *N*,*N*-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS.

**Identification** **(1)** Determine the absorption spectrum of a solution of Nalidixic Acid in 0.01 mol/L sodium hydroxide TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Nalidixic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 225 – 231°C

**Purity** **(1)** Chloride <*1.03*>—To 2.0 g of Nalidixic Acid add 50 mL of water, warm at 70°C for 5 minutes, cool quickly, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.012%).

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nalidixic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 20 mg of Nalidixic Acid in 20 mL of 0.01 mol/L sodium hydroxide TS. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 1000 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 each peak area by the automatic integration method: the area of the peak other than nalidixic acid with the sample solution is not larger than the peak area of nalidixic acid with the standard solution, and the total area of the peaks other than the peak of nalidixic acid with the sample solution is not larger than 2.5 times the peak area of nalidixic acid with the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 260 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 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nalidixic acid is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of nalidixic acid beginning after the solvent peak.

*System suitability*—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of nalidixic acid obtained with 10 μL of this solution is equivalent to 40 to 60% of that with 10 μL of the standard solution.

System performance: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid are eluted in this order with the resolution between these peaks being not less than 13.

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 nalidixic acid is not more than 2.0%.

**Loss on drying** <*2.41*> Not more than 0.20% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.2% (1 g).

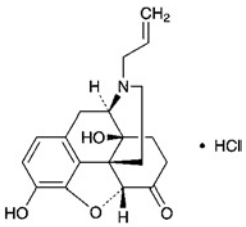
**Assay** Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration). Separately, to 50 mL of *N*,*N*-dimethylformamide add 13 mL of a mixture of water and methanol (89:11), perform a blank determination with the solution, and make any necessary correction.

Each mL of 0.1 mol/L tetramethyl ammonium hydroxide VS
= 23.22 mg of $C_{12}H_{12}N_2O_3$

**Containers and storage** Containers—Tight containers.

# Naloxone Hydrochloride

ナロキソン塩酸塩



$C_{19}H_{21}NO_4$.HCl: 363.84
(5*R*,14*S*)-17-Allyl-4,5-epoxy-3,14-dihydroxymorphinan-6-one monohydrochloride [*357-08-4*]

Naloxone Hydrochloride contains not less than 98.5% of $C_{19}H_{21}NO_4$.HCl, calculated on the dried basis.

**Description** Naloxone Hydrochloride occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.

It is hygroscopic.

It is gradually colored by light.

**Identification** **(1)** Determine the absorption spectrum of a solution of Naloxone Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Naloxone Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Naloxone Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

**Optical rotation** <*2.49*> $[\alpha]_D^{25}$: $-170$ – $-181°$ (0.25 g calculated on the dried basis, water, 10 mL, 100 mm).

**pH** <*2.54*> Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

**Purity** Related substances—Conduct this procedure as rapidly as possible without exposure to light, using light-resistant containers. Dissolve 0.08 g of Naloxone Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia-saturated 1-butanol TS and methanol (20:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyanoferrate (III) TS on the plate: the number of the spot other than the principal spot from the sample solution is not more than 1 and it is not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 2.0% [0.1 g, 105°C, 5 hours. Use a desiccator (phosphorus (V) oxide) for cooling].

**Residue on ignition** <*2.44*> Not more than 0.2% (0.1 g).

**Assay** Weigh accurately about 0.3 g of Naloxone Hydrochloride, dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.38 mg of $C_{19}H_{21}NO_4$.HCl

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Naphazoline and Chlorpheniramine Solution

ナファゾリン·クロルフェニラミン液

Naphazoline and Chlorpheniramine Solution contains not less than 0.045 w/v% and not more than 0.055 w/v% of naphazoline nitrate ($C_{14}H_{14}N_2$.$HNO_3$: 273.29), and not less than 0.09 w/v% and not more than 0.11 w/v% of chlorpheniramine maleate ($C_{16}H_{19}ClN_2$.$C_4H_4O_4$: 390.86).

**Method of preparation**

| | |
|---|---|
| Naphazoline Nitrate | 0.5 g |
| Chlorpheniramine Maleate | 1 g |
| Chlorobutanol | 2 g |
| Glycerin | 50 mL |
| Purified Water | a sufficient quantity |
| | To make 1000 mL |

Dissolve, and mix the above ingredients.

**Description** Naphazoline and Chlorpheniramine Solution is a clear, colorless liquid.

**Identification** **(1)** To 20 mL of Naphazoline and Chlorpheniramine Solution add 2 mL of a solution of potassium hydroxide (7 in 10) and 5 mL of pyridine, and heat at 100°C for 5 minutes: a red color is produced (chlorobutanol).

**(2)** Place 10 mL of Naphazoline and Chlorpheniramine Solution in a glass-stoppered test tube, add 10 mL of ethanol (95), 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (glycerin).

**(3)** To 20 mL of Naphazoline and Chlorpheniramine Solution add 5 mL of sodium hydroxide TS, extract with 10 mL of diethyl ether, and separate the diethyl ether layer. Take 5 mL of this solution, distil off the solvent, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.01 g each of naphazoline nitrate and Chlorpheniramine Maleate Reference Standard in 10 mL and 5 mL of methanol, respectively, and use these solutions

as standard solutions (1) and (2). 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 with fluorescent indicator 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 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 the spots from standard solutions (1) and (2). Spray evenly Dragendorff's TS on the plate: the spots from standard solutions (1) and (2) and the corresponding spot from the sample solutions reveal an orange color.

**Assay** Pipet 4 mL of Naphazoline and Chlorpheniramine Solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of naphazoline nitrate for assay, dried at 105°C for 2 hours, and about 0.1 g of Chlorpheniramine Maleate Reference Standard, dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, $Q_{Ta}$ and $Q_{Tb}$, of the peak height of naphazoline nitrate and chlorpheniramine maleate to that of the internal standard of the sample solution, and the ratios, $Q_{Sa}$ and $Q_{Sb}$, of the peak height of naphazoline nitrate and chlorpheniramine maleate to that of the internal standard of the standard solution.

Amount (mg) of naphazoline nitrate ($C_{14}H_{14}N_2.HNO_3$)
$= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/25)$

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/25)$

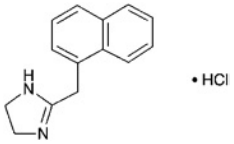
$W_{Sa}$: Amount (mg) of naphazoline nitrate for assay
$W_{Sb}$: Amount (mg) of Chlorpheniramine Maleate Reference Standard

*Internal standard solution*—A solution of ethenzamide in methanol (1 in 1000).
*Operating conditions*—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column, about 4 mm in inside diameter and 25 to 30 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 acetonitrile and a solution of sodium laurylsulfate (1 in 500) in diluted phosphoric acid (1 in 1000) (1:1).
Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine maleate is about 10 minutes.
Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of the internal standard, naphazoline nitrate and chlorpheniramine maleate in this order.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Naphazoline Hydrochloride

ナファゾリン塩酸塩



$C_{14}H_{14}N_2.HCl$: 246.74
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole monohydrochloride [*550-99-2*]

Naphazoline Hydrochloride, when dried, contains not less than 98.5% of $C_{14}H_{14}N_2.HCl$.

**Description** Naphazoline Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.
It is freely soluble in water, soluble in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.
Melting point: 255 – 260°C (with decomposition).

**Identification** **(1)** To 10 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.
**(2)** To 30 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Evaporate the combined diethyl ether extracts to dryness with the aid of a current of air. Dry the residue at 80°C for 1 hour: the residue melts ⟨*2.60*⟩ between 117°C and 120°C.
**(3)** Dissolve 0.02 g of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water, and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.
**(4)** A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

**pH** ⟨*2.54*⟩ Dissolve 0.10 g of Naphazoline Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Naphazoline Hydrochloride in 10 mL of water: the solution is clear and colorless.
**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Naphazoline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Naphazoline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

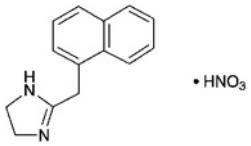
Each mL of 0.1 mol/L perchloric acid VS

= 24.67 mg of $C_{14}H_{14}N_2.HCl$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Naphazoline Nitrate

ナファゾリン硝酸塩



$C_{14}H_{14}N_2.HNO_3$: 273.29
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole mononitrate [*5144-52-5*]

Naphazoline Nitrate, when dried, contains not less than 98.5% of $C_{14}H_{14}N_2.HNO_3$.

**Description** Naphazoline Nitrate occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in water, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification** **(1)** To 10 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

**(2)** To 20 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness with the aid of a current of air, and dry the residue at 80°C for 1 hour: the residue so obtained melts ⟨*2.60*⟩ between 117°C and 120°C.

**(3)** A solution of Naphazoline Nitrate (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for nitrate.

**pH** ⟨*2.54*⟩ Dissolve 0.1 g of Naphazoline Nitrate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

**Melting point** ⟨*2.60*⟩ 167 – 170°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Naphazoline Nitrate in 50 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Naphazoline Nitrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

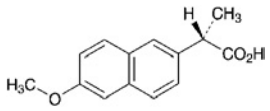
**Assay** Weigh accurately about 0.4 g of Naphazoline Nitrate, previously dried, dissolve in 10 mL of acetic acid (100) and 40 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.33 mg of $C_{14}H_{14}N_2.HNO_3$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Naproxen

ナプロキセン



$C_{14}H_{14}O_3$: 230.26
(2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid [*22204-53-1*]

Naproxen, when dried, contains not less than 98.5% of $C_{14}H_{14}O_3$.

**Description** Naproxen occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in methanol, in ethanol (99.5) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

**Identification** **(1)** Dissolve 0.01 g of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100), and shake: a yellow to yellow-brown color develops. To this solution add 5 mL of chloroform, and shake: a light red-purple color develops in the chloroform layer.

**(2)** To 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300) add 4 mL of hydroxylamine perchlorate-dehydrated ethanol TS and 1 mL of *N*,*N*′-dicyclohexylcarbodiimide-dehydrated ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-dehydrated ethanol TS, and shake: a red-purple color develops.

**(3)** Determine the absorption spectrum of a solution of Naproxen in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Determine the infrared absorption spectrum of Naproxen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{25}$: +63.0 – +68.5° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

**Melting point** ⟨*2.60*⟩ 154 – 158°C

**Purity** **(1)** Clarity of solution—Dissolve 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance at 400 nm is not more than 0.070.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Naprox-

en according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Naproxen according to Method 3, and perform the test (not more than 1 ppm).

**(4)** Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of chloroform and ethanol (99.5) (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 50 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 $\mu$L 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, dichloromethane, tetrahydrofuran and acetic acid (100) (50:30:17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5g of Naproxen, previously dried, add 100 mL of diluted methanol (4 in 5), dissolve by gentle warming if necessary, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

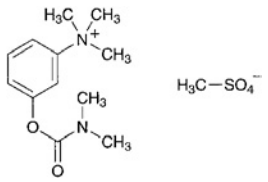
Each mL of 0.1 mol/L sodium hydroxide VS
= 23.03 mg of $C_{14}H_{14}O_3$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Neostigmine Methylsulfate

ネオスチグミンメチル硫酸塩



$C_{13}H_{22}N_2O_6S$: 334.39
3-(Dimethylcarbamoyloxy)-*N*,*N*,*N*-trimethylanilinium methyl sulfate [*51-60-5*]

Neostigmine Methylsulfate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{13}H_{22}N_2O_6S$.

**Description** Neostigmine Methylsulfate occurs as a white, crystalline powder.

It is very soluble in water, and freely soluble in acetonitrile and in ethanol (95).

**Identification (1)** Determine the absorption spectrum of a solution of Neostigmine Methylsulfate (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of Neostigmine Methylsulfate Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Neostigmine Methylsulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Neostigmine Methylsulfate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** <*2.54*> Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.0 and 5.0.

**Melting point** <*2.60*> 145 – 149°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

**(3)** Dimethylaminophenol—Dissolve 0.10 g of Neostigmine Methylsulfate in 5 mL of water, add 1 mL of sodium hydroxide TS, and while cooling with ice, add 1 mL of diazobenzenesulfonic acid TS: no color develops.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Neostigmine Methylsulfate and Neostigmine Methylsulfate Reference Standard, previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 $\mu$L 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 neostigmine in each solution.

Amount (mg) of $C_{13}H_{22}N_2O_6S$
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of Neostigmine Methylsulfate Reference Standard

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 259 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen-

phosphate dihydrate in 1000 mL of water, adjust to pH 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of neostigmine is about 9 minutes.

*System suitability—*

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylaminophenol in 50 mL of the mobile phase. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, dimethylaminophenol and neostigmine 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 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of neostigmine methylsulfate is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Neostigmine Methylsulfate Injection

ネオスチグミンメチル硫酸塩注射液

Neostigmine Methylsulfate Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$: 334.39).

**Method of preparation** Prepare as directed under Injections, with Neostigmine Methylsulfate.

**Description** Neostigmine Methylsulfate Injection is a clear, colorless liquid.

It is slowly affected by light.

pH: 5.0 – 6.5

**Identification** Take a volume of Neostigmine Methylsulfate Injection equivalent to 5 mg of neostigmine methylsulfate according to the labeled amount, add water to make 10 mL if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 257 nm and 261 nm.

**Extractable volume** <*6.05*> It meets the requirement.

**Bacterial endotoxins** <*4.01*> Less than 5 EU/mg.

**Assay** Use Neostigmine Methylsulfate Injection as the sample solution. Separately, weigh accurately about 25 mg of Neostigmine Methylsulfate Reference Standard, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Neostigmine Methylsulfate.

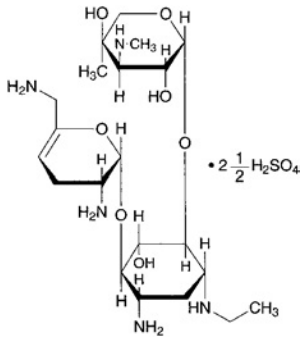
Amount (mg) of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$) $= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of Neostigmine Methylsulfate Reference Standard

**Containers and storage** Containers—Hermetic containers. Storage—Light-resistant.

# Netilmicin Sulfate

ネチルマイシン硫酸塩



$C_{21}H_{41}N_5O_7.2\frac{1}{2}H_2SO_4$: 720.78

3-Deoxy-4-*C*-methyl-3-methylamino-$\beta$-L-arabinopyranosyl-(1→6)-[2,6-diamino-2,3,4,6-tetradeoxy-$\alpha$-D-*glycero*-hex-4-enopyranosyl-(1→4)]-2-deoxy-1-*N*-ethyl-D-streptamine hemipentasulfate

[*56391-57-2*]

Netilmicin Sulfate is the sulfate of a derivative of sisomicin.

It contains not less than 595 $\mu$g (potency) and not more than 720 $\mu$g (potency) per mg, calculated on the dried basis. The potency of Netilmicin Sulfate is expressed as mass (potency) of netilmicin ($C_{21}H_{41}N_5O_7$: 475.58).

**Description** Netilmicin Sulfate occurs as a white to light yellowish white powder.

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

It is hygroscopic.

**Identification** **(1)** Dissolve 30 mg of Netilmicin Sulfate in 3 mL of water, and add 0.2 mL of bromine TS: the solution is immediately decolorized.

**(2)** Dissolve 15 mg each of Netilmicin Sulfate and Netilmicin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 5 $\mu$L 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, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butabol TS on the plate, and heat at 100°C for 5 minutes: the principal spots from the sample solution and the standard solution exhibit a red-purple to red-brown color and show the same *R*f value.

**(3)** A solution of Netilmicin Sulfate (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for sulfate.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +88 – +96° (0.1 g calculated on the dried basis, water, 10 mL, 100 mm).

**pH** ⟨*2.54*⟩ Dissolve 0.5 g of Netilmicin Sulfate in 5 mL of water: the pH of this solution is between 3.5 and 5.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Netilmicin Sulfate in 5 mL of water: the solution is clear and colorless to light yellow.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Netilmicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 50 mg of Netilmicin Sulfate, calculated on the dried basis, in water to make 5 mL, and use this solution as the sample solution. Pipet 0.5 mL, 1 mL, and 1.5 mL of the sample solution, add water to each to make exactly 50 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. 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 (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butabol TS on the plate, and heat at 100°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (3), and the total amount of the intensity of the spots other than the principal spot from the sample solution is not more than 6%.

**Loss on drying** ⟨*2.41*⟩ Not more than 15.0% (0.15 g, in vacuum not exceeding 0.67 kPa, 110°C, 3 hours). Sampling should be carried out in a manner to avoid moisture absorption.

**Residue on ignition** ⟨*2.44*⟩ Not more than 1.0% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium − Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Netilmicin Sulfate Reference Standard equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

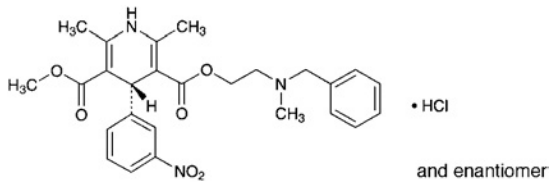
(iv) Sample solutions—Weigh accurately an amount of Netilmicin Sulfate equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, not exceeding 5°C, under nitrogen or argon atmosphere.

# Nicardipine Hydrochloride

ニカルジピン塩酸塩




$C_{26}H_{29}N_3O_6$.HCl: 515.99

2-[Benzyl(methyl)amino]ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride [*54527-84-3*]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of $C_{26}H_{29}N_3O_6$.HCl.

**Description** Nicardipine Hydrochloride occurs as a pale greenish yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water, in acetonitrile and in acetic anhydride.

A solution of Nicardipine Hydrochloride in methanol (1 in 20) shows no optical rotation.

It is gradually affected by light.

**Identification** **(1)** Determine the absorption spectrum of a solution of Nicardipine Hydrochloride in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Nicardipine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Dissolve 0.02 g of Nicardipine Hydrochloride in 10 mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

**Melting point** ⟨*2.60*⟩ 167 – 171°C

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Nicardipine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Nicardipine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, then take exactly 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solu-

tion 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 each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of nicardipine from the sample solution is not larger than the peak area of nicardipine from the standard solution, and the total area of each peak other than the peak of nicardipine from the sample solution is not more than twice the peak area of nicardipine from the standard solution.

*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: A mixture of a solution of perchloric acid (43 in 50,000) and acetonitrile (3:2).

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

Time span of measurement: About 4 times as long as the retention time of nicardipine beginning after the solvent peak.

*System suitability*—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that of nicardipine obtained from 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Nicardipine Hydrochloride and nifedipine in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, nicardipine and nifedipine 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 areas of nicardipine is not more than 3%.

**Loss on drying** <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.9 g of Nicardipine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.60 mg of $C_{26}H_{29}N_3O_6$.HCl

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Nicardipine Hydrochloride Injection

ニカルジピン塩酸塩注射液

Nicardipine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6$.HCl: 515.99).

**Method of preparation** Prepare as directed under Injections, with Nicardipine Hydrochloride.

**Description** Nicardipine Hydrochloride Injection occurs as a clear pale yellow liquid.

It is gradually changed by light.

**Identification** To a volume of Nicardipine Hydrochloride Injection, equivalent to 1 mg of Nicardipine Hydrochloride according to the labeled amount, add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm, and between 351 nm and 355 nm.

**pH** <2.54> 3.0 – 4.5

**Purity** Related substances—Conduct the procedure without exposure to day-light using light-resistant vessels. To a volume of Nicardipine Hydrochloride Injection, equivalent to 5 mg of Nicardipine Hydrochloride according to the labeled amount, add the mobile phase to make 10 mL, and use this solution as the sample solution. To exactly 2 mL of the sample solution add 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 of these solutions by the automatic integration method: the areas of the peaks other than nicardipine from the sample solution are not more than the peak area of nicardipine from the standard solution, and the total of the areas of the peaks other than nicardipine from the sample solution is not more than 2 times of the peak area of nicardipine from the standard solution.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of nicardipine beginning after the solvent peak.

*System suitability*—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating

conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

**Bacterial endotoxins** ⟨*4.01*⟩ Less than 8.33 EU/mg.

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Assay** Conduct the procedure without exposure to day-light using light-resistant vessels. To an exact volume of Nicardipine Hydrochloride Injection, equivalent to about 2 mg of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6$.HCl), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nicardipine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 $\mu$L 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 nicardipine to that of the internal standard.

Amount (mg) of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6$.HCl)
$= W_S \times (Q_T/Q_S) \times (1/25)$

$W_S$: Amount (mg) of nicardipine hydrochloride for assay

*Internal standard solution*—A solution of di-*n*-butyl phthalate in methanol (1 in 625).

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL. To 320 mL of this solution add 680 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 8 minutes.

*System suitability*—

System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, nicardipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

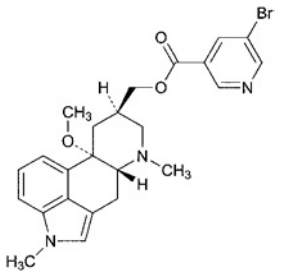
System repeatability: When the test is repeated 5 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

# Nicergoline

ニセルゴリン




$C_{24}H_{26}BrN_3O_3$: 484.39

[(8*R*,10*S*)-10-Methoxy-1,6-dimethylergolin-8-yl]methyl 5-bromopyridine-3-carboxylate

[*27848-84-6*]

Nicergoline, when dried, contains not less than 98.5% and not more than 101.0% of $C_{24}H_{26}BrN_3O_3$.

**Description** Nicergoline occurs as white to light yellow, crystals or crystalline powder.

It is soluble in acetonitrile, in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

It is gradually colored to light brown by light.

Melting point: about 136°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Nicergoline in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Nicergoline as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +5.2 – +6.2° (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Nicergoline according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances—Dissolve 25 mg of Nicergoline in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 with respect to nicergoline, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and other than the peak mentioned above is not larger than 2.5 times the peak area of nicergoline from

the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total area of the peaks other than the peak of nicergoline is not larger than 7.5 times the peak area of nicergoline from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 288 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 25°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

*System suitability*—

Test for required detectability: To 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, 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 nicergoline is not more than 4.0%.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (2 g, in vacuum, 60°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.22 mg of $C_{24}H_{26}BrN_3O_3$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Nicergoline Powder

ニセルゴリン散

Nicergoline Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

**Method of preparation** Prepare as directed under Powders, with Nicergoline.

**Identification** Vigorously shake for 10 minutes a quantity of Nicergoline Powder, equivalent to 10 mg of Nicergoline according to the labeled amount, with 20 mL of diluted ethanol (4 in 5), and centrifuge for 10 minutes. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

**Purity** Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline after the solvent peak.

*System suitability*—

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μL of this solution is equivalent to 3 to 7% of that with 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

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

**Uniformity of dosage unit** ⟨*6.02*⟩ The Nicergoline Powder in single-unit container meets the requirement of the Mass variation test.

**Particle size** ⟨*6.03*⟩ It meets the requirements of Powders.

**Dissolution** ⟨*6.10*⟩ Perform the test according to the following method: It meets the requirement.

Weigh accurately a quantity of Nicergoline Powder, equivalent to about 5 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), and perform the test at 50 revolutions per minute according to the Paddle method using 900 mL of 2nd fluid for dissolution test

as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a laminated polyester fiver filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of nicergoline for assay, previously dried in vacuum at 60 °C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, and add 2nd fluid for dissolution test to make exactly 100 mL. Pipet 10 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 225 nm, $A_{T1}$ and $A_{S1}$, and at 250 nm, $A_{T2}$ and $A_{S2}$, of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$)

$$= (W_S/W_T) \times \{(A_{T1} - A_{T2})/(A_{S1} - A_{S2})\} \times (1/C) \times 9$$

$W_S$: Amount (mg) of nicergoline for assay
$W_T$: Amount (g) of sample
$C$: Labeled amount (mg) of nicergoline ($C_{24}H_{26}BrN_3O_3$) in 1 g

**Assay** Weigh accurately a quantity of Nicergoline Powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L 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 nicergoline.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3) = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of nicergoline for assay

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 288 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 $\mu$m in particle diameter).

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

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

*System suitability—*

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Nicergoline Tablets

ニセルゴリン錠

Nicergoline Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$: 484.39).

**Method of preparation** Prepare as directed under Tablets, with Nicergoline.

**Identification** Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of Nicergoline according to the labeled amount, add 20 mL of ethanol (99.5), shake vigorously for 10 minutes, and filter through a 0.45-$\mu$m pore-size membrane filter. To 2 mL of the filtrate add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

**Purity** Related substances—Perform the test with 20 $\mu$L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

*Operating conditions—*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

*System suitability—*

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 $\mu$L of this solution is equivalent to 3 to 7% of that with 20 $\mu$L of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave, and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet exactly 4 mL of the supernatant liquid, add diluted ethanol (4 in 5) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, $A_{T1}$ and $A_{S1}$, and at 340 nm, $A_{T2}$ and $A_{S2}$, of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3) = W_S \times \{(A_{T1} - A_{T2})/(A_{S1} - A_{S2})\} \times (1/2)$$

$W_S$: Amount (mg) of nicergoline for assay

**Dissolution** Being specified separately.

**Assay** Weigh accurately the mass of not less than 20 Nicergoline Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L 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 nicergoline.

$$\text{Amount (mg) of nicergoline } (C_{24}H_{26}BrN_3O_3) = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of nicergoline for assay

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 288 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 $\mu$m in particle diameter).

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

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

*System suitability*—

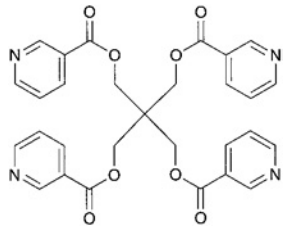
System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Niceritrol

ニセリトロール



$C_{29}H_{24}N_4O_8$: 556.52
Pentaerythritol tetranicotinate
[*5868-05-3*]

Niceritrol, when dried, contains not less than 99.0% of $C_{29}H_{24}N_4O_8$.

**Description** Niceritrol occurs as a white to pale yellowish white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, soluble in *N,N*-dimethylformamide, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

**Identification** **(1)** Determine the absorption spectrum of a solution of Niceritrol in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Niceritrol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 162 – 165°C

**Purity** **(1)** Chloride <*1.03*>—To 2.0 g of Niceritrol add 50 mL of water, and warm at 70°C for 20 minutes, while shaking occasionally. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Niceritrol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Niceritrol according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

**(4)** Pyridine—Dissolve 0.5 g of Niceritrol in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N,N*-

dimethylformamide to make exactly 100 mL, then pipet 0.5 mL of this solution, add *N,N*-dimethylformamide to make exactly 10 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. Determine each peak area of pyridine in both solutions: the peak area of pyridine from the sample solution is not larger than the peak area of pyridine from the standard solution.

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 10% on acid-treated and silanized siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 2 minutes.

*System suitability*—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical steps of the peak of pyridine is not less than 1500 steps.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pyridine is not more than 3.0%.

**(5)** Free acids—Transfer about 1 g of Niceritrol, weighed accurately, to a separator, dissolve in 20 mL of chloroform, and extract with 20 mL and then 10 mL of water while shaking well. Combine the whole extracts, and titrate ⟨*2.50*⟩ with 0.01 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, make any necessary correction, and calculate the amount of free acid by the following equation: it is not more than 0.1%.

Each mL of 0.01 mol/L sodium hydroxide VS
= 1.231 mg of $C_6H_5NO_2$

**(6)** Related substances—Dissolve 0.10 g of Niceritrol in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet exactly 2 mL of this solution, add chloroform to make exactly 20 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

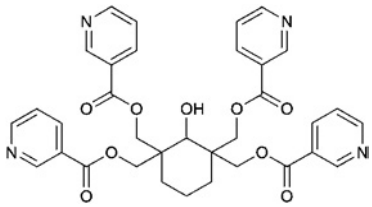
**Assay** Weigh accurately about 1 g of Niceritrol, previously dried, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, boil gently for 20 minutes under a reflux condenser with a carbon dioxide absorber (soda lime). After cooling, titrate ⟨*2.50*⟩ immediately the excess sodium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 69.57 mg of $C_{29}H_{24}N_4O_8$

**Containers and storage** Containers—Well-closed containers.

# Nicomol

ニコモール



$C_{34}H_{32}N_4O_9$: 640.64

(2-Hydroxycyclohexane-1,1,3,3-tetrayl)tetramethyl tetranicotinate [*27959-26-8*]

Nicomol, when dried, contains not less than 98.0% of $C_{34}H_{32}N_4O_9$.

**Description** Nicomol occurs as a white, crystalline powder. It is odorless and tasteless.

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

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

**Identification** **(1)** Mix 0.01 g of Nicomol with 0.02 g of 1-chloro-2,4-dinitrobenzene, add 2 mL of dilute ethanol, heat in a water bath for 5 minutes, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

**(2)** Dissolve 0.1 g of Nicomol in 5 mL of dilute hydrochloric acid, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

**(3)** Determine the absorption spectrum of a solution of Nicomol in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Determine the infrared absorption spectrum of Nicomol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** ⟨*2.60*⟩ 181 – 185°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Nicomol in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

**(2)** Acidity—To 1.0 g of Nicomol add 50 mL of freshly boiled and cooled water, shake for 5 minutes, filter, and to 25

mL of the filtrate add 0.60 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

**(3)** Chloride <*1.03*>—Dissolve 0.6 g of Nicomol in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 15 mL of dilute nitric acid and water to make 50 mL (not more than 0.024%).

**(4)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nicomol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(5)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Nicomol according to Method 3, and perform the test (not more than 2 ppm).

**(6)** Related substances—Dissolve 0.20 g of Nicomol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 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 $\mu$L 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 dichloromethane, ethanol (95), acetonitrile and ethyl acetate (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 1.5 g of Nicomol, previously dried, add exactly 40 mL of 0.5 mol/L sodium hydroxide VS, and boil gently under a reflux condenser connected to a carbon dioxide absorption tube (soda lime) for 10 minutes. After cooling, titrate <*2.50*> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 80.08 mg of $C_{34}H_{32}N_4O_9$

**Containers and storage** Containers—Tight containers.

# Nicomol Tablets

ニコモール錠

Nicomol Tablets contain not less than 95% and not more than 105% of the labeled amount of nicomol ($C_{34}H_{32}N_4O_9$: 640.64).

**Method of preparation** Prepare as directed under Tablets, with Nicomol.

**Identification** To a portion of powdered Nicomol Tablets, equivalent to 0.5 g of Nicomol according to the labeled amount, add 20 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) and (2) under Nicomol.

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Nicomol Tablets at 75 revolutions per minute according to the Paddle method using 900 mL of the 1st fluid for dissolution test. Take 20 mL or more of the dissolved solution 60 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 $\mu$m. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent, add the 1st fluid for dissolution test to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in the 1st fluid for dissolution test to make exactly 100 mL, then pipet 2 mL of this solution, add the 1st fluid for dissolution test 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 at 262 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Nicomol Tablets in 60 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of nicomol ($C_{34}H_{32}N_4O_9$)
$= W_S \times (A_T/A_S) \times (1/C) \times 225$

$W_S$: Amount (mg) of nicomol for assay
$C$: Labeled amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$) in 1 tablet

**Assay** Weigh accurately not less than 20 Nicomol Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of nicomol ($C_{34}H_{32}N_4O_9$), add 100 mL of 1 mol/L hydrochloric acid TS, shake well, add water to make exactly 500 mL, and filter. Discard the first 50 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 50 mL of 1 mol/L hydrochloric acid TS and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in 50 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add 20 mL of 1 mol/L hydrochloric acid TS and water 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 the standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

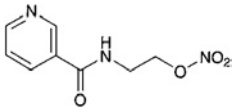
Amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$)
$= W_S \times (A_T/A_S) \times (25/2)$

$W_S$: Amount (mg) of nicomol for assay

**Containers and storage** Containers—Tight containers.

# Nicorandil

ニコランジル



$C_8H_9N_3O_4$: 211.17
*N*-[2-(Nitrooxy)ethyl]pyridine-3-carboxamide [*65141-46-0*]

Nicorandil contains not less than 98.5% and not more than 101.0% of $C_8H_9N_3O_4$, calculated on the anhydrous basis.

**Description** Nicorandil occurs as white crystals.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), soluble in acetic anhydride, and sparingly soluble in water.

Melting point: about 92°C (with decomposition).

**Identification** Determine the infrared absorption spectrum of Nicorandil as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Sulfate <*1.14*>—Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010%).

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nicorandil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 20 mg of Nicorandil in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 $\mu$L of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of *N*-(2-hydroxyethyl)isonicotinamide nitric ester, having the relative retention time of about 0.86 with respect to nicorandil, is not more than 0.5% of the peak area of nicorandil, the area of all other peaks is less than 0.1%, and the sum area of the peaks other than nicorandil and *N*-(2-hydroxyethyl)isonicotinamide nitric ester is not more than 0.25% of the total peak area.

*Operating conditions*—

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

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran, triethylamine and trifluoroacetic acid (982:10:5:3).

Flow rate: Adjust the flow rate so that the retention time of nicorandil is about 18 minutes.

Time span of measurement: About 3 times as long as the retention time of nicorandil beginning after the solvent peak.

*System suitability*—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 500 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicorandil obtained with 10 $\mu$L of this solution is equivalent to 2 to 8% of that with 10 $\mu$L of the solution for system suitability test.

System performance: Dissolve 10 mg of *N*-(2-hydroxyethyl)isonicotinamide nitric ester in the mobile phase to make 100 mL. To 1 mL of this solution add 10 mL of the sample solution. When the procedure is run with this solution under the above operating conditions, *N*-(2-hydroxyethyl)isonicotinamide nitric ester and nicorandil are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicorandil is not more than 1.5%.

**Water** <*2.48*> Not more than 0.1% (2 g, volumetric titration, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Nicorandil, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

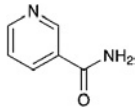
Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of $C_8H_9N_3O_4$

**Containers and storage** Containers—Tight containers.

Storage—At a temperature between 2°C and 8°C.

# Nicotinamide

ニコチン酸アミド



$C_6H_6N_2O$: 122.12
Pyridine-3-carboxamide [*98-92-0*]

Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of $C_6H_6N_2O$.

**Description** Nicotinamide occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

Nicotinamide is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

**Identification** **(1)** Mix 5 mg of Nicotinamide with 0.01 g of 1-chloro-2,4-dinitrobenzene, heat gently for 5 to 6 seconds, and fuse the mixture. Cool, and add 4 mL of potassi-

um hydroxide-ethanol TS: a red color is produced.

**(2)** To 0.02 g of Nicotinamide add 5 mL of sodium hydroxide TS, and boil carefully: the gas evolved turns moistened red litmus paper blue.

**(3)** Dissolve 0.02 g of Nicotinamide in water to make 1000 mL. Determine the absorption spectrum of the solultion as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinamide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**pH** <*2.54*> Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

**Melting point** <*2.60*> 128 – 131°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Nicotinamide in 20 mL of water: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Take 0.5 g of Nicotinamide, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

**(3)** Sulfate <*1.14*>—Take 1.0 g of Nicotinamide, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

**(4)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nicotinamide according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(5)** Readily carbonizable substances <*1.15*>—Take 0.20 g of Nicotinamide, and perform the test. The solution has no more color than Matching Fluid A.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Nicotinamide and Nicotinamide Reference Standard, both previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make them exactly 100 mL. Pipet 8 mL each of these solutions, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of nicotinamide to that of the internal standard.

Amount (g) of nicotinamide ($C_6H_6N_2O$) = $W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of dried Nicotinamide Reference Standard

*Internal standard solution*—A solution of nicotinic acid (1 in 1250).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicotinamide is about 7 minutes.

*System suitability*—

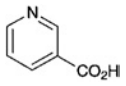
System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, nicotinic acid and nicotinamide are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Nicotinic Acid

ニコチン酸




$C_6H_5NO_2$: 123.11

Pyridine-3-carboxylic acid [*59-67-6*]

Nicotinic Acid, when dried, contains not less than 99.5% of $C_6H_5NO_2$.

**Description** Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is sparingly soluble in water, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

**Identification** **(1)** Triturate 5 mg of Nicotinic Acid with 0.01 g of 1-chloro-2,4-dinitrobenzene, and fuse the mixture by gentle heating for 5 to 6 seconds. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is produced.

**(2)** Dissolve 0.02 g of Nicotinic Acid in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinic Acid Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**pH** <*2.54*> Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

**Melting point** <*2.60*> 234 – 238°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Perform the test with 0.5 g of Nico-

tinic Acid. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

**(3)** Sulfate <*1.14*>—Dissolve 1.0 g of Nicotinic Acid in 3 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS and 3 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.019%).

**(4)** Nitro compounds—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS, and add water to make 20 mL: the solution has no more color than Matching Fluid A.

**(5)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nicotinic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 1 hour).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.31 mg of $C_6H_5NO_2$

**Containers and storage** Containers—Well-closed containers.

# Nicotinic Acid Injection

ニコチン酸注射液

Nicotinic Acid Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 110% of the labeled amount of nicotinic acid ($C_6H_5NO_2$: 123.11).

**Method of preparation** Prepare as directed under Injections, with Nicotinic Acid. It may contain Sodium Carbonate or Sodium Hydroxide as a solubilizer.

**Description** Nicotinic Acid Injection is a clear, colorless liquid.

pH: 5.0 – 7.0

**Identification** **(1)** To a volume of Nicotinic Acid Injection, equivalent to 0.1 g of Nicotinic Acid according to the labeled amount, add 0.3 mL of dilute hydrochloric acid, and evaporate on a water bath to 2 mL. After cooling, collect the crystals formed, wash with small portions of ice-cold water until the last washing shows no turbidity on the addition of silver nitrate TS, and dry at 105°C for 1 hour: the crystals melt <*2.60*> between 234°C and 238°C. With the crystals, proceed as directed in the Identification (1) under Nicotinic Acid.

**(2)** Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, determine the absorbances of this solution, $A_1$ and $A_2$, at each wavelength of maximum and minimum absorption, respectively: the ratio $A_2/A_1$ is between 0.35 and 0.39.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid ($C_6H_5NO_2$), and add the mobile phase to make exactly 100 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 sample solution. Separately, weigh accurately about 0.1 g of Nicotinic Acid Reference Standard, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 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 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 nicotinic acid to that of the internal standard.

Amount (mg) of nicotinic acid ($C_6H_5NO_2$)
$= W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of Nicotinic Acid Reference Standard

*Internal standard solution*—A solution of caffeine in the mobile phase (1 in 1000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 260 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 35°C.

Mobile phase: Dissolve 1.1 g of sodium 1-octane sulfonate in a mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and methanol (4:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 9 minutes.

*System suitability*—

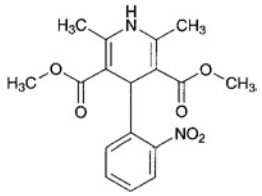
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, nicotinic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

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 ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Nifedipine

ニフェジピン



$C_{17}H_{18}N_2O_6$: 346.33
Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [*21829-25-4*]

Nifedipine contains not less than 98.0% and not more than 102.0% of $C_{17}H_{18}N_2O_6$, calculated on the dried basis.

**Description** Nifedipine occurs as a yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetone and in dichloromethane, sparingly soluble in methanol, in ethanol (95) and in acetic acid (100), slightly soluble in diethyl ether, and practically insoluble in water.

It is affected by light.

**Identification** **(1)** Dissolve 0.05 g of Nifedipine in 5 mL of ethanol (95), and add 5 mL of hydrochloric acid and 2 g of zinc powder. Allow to stand for 5 minutes, and filter. Perform the test with the filtrate as directed under Qualitative Tests <*1.09*> for primary aromatic amines: a red-purple color develops.

**(2)** Determine the absorption spectrum of a solution of Nifedipine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Nifedipine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 172 – 175°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.

**(2)** Chloride <*1.03*>—To 2.5 g of Nifedipine add 12 mL of dilute acetic acid and 13 mL of water, and heat to boil. After cooling, filter, and discard the first 10 mL of the filtrate. To 5 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

**(3)** Sulfate <*1.14*>—To 4 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054%).

**(4)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nifedipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(5)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Nifedipine according to Method 3, and perform the test (not more than 2 ppm).

**(6)** Basic substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (100) (5:3), and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.

**(7)** Dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylate—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.15 g of Nifedipine in dichloromethane to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-dicarboxylate for thin-layer chromatography in exactly 10 mL of dichloromethane. Measure exactly 1 mL of this solution, add dichloromethane to make exactly 20 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 $\mu$L 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 cyclohexane and ethyl acetate (3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (0.5 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** The procedure should be performed under protection from direct sunlight in light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine, and dissolve in methanol to make exactly 200 mL. Measure exactly 5 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance $A$ of this solution at the wavelength of maximum absorption at about 350 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of } C_{17}H_{18}N_2O_6 = (A/142.3) \times 40{,}000$$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Nilvadipine

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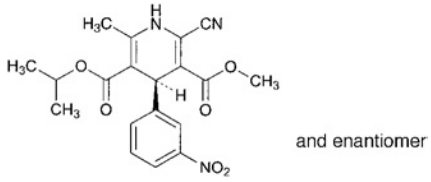


and enantiomer

$C_{19}H_{19}N_3O_6$: 385.37

3-Methyl 5-(1-methylethyl) (4*RS*)-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

[*75530-68-6*]

Nilvadipine contains not less than 98.0% and not more than 102.0% of $C_{19}H_{19}N_3O_6$.

**Description** Nilvadipine occurs as a yellow crystalline powder.

It is freely soluble in acetonitrile, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Nilvadipine in acetonitrile (1 in 20) shows no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of Nilvadipine in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nilvadipine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Nilvadipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Nilvadipine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 167 – 171°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nilvadipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances—Dissolve 20 mg of Nilvadipine in 20 mL of acetonitrile, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.

*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 25°C.

Mobile phase: A mixture of phosphate buffer solution, pH 7.4, methanol and acetonitrile (32:27:18).

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

Time span of measurement: About 2.5 times as long as the retention time of nilvadipine beginning after the solvent peak.

*System suitability*—

Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μL of this solution is equivalent to 7 to 13% of that obtained from 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nilvadipine is not less than 3300 and not more than 1.3, respectively.

System repeatability: Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

**Loss on drying** <*2.41*> Not more than 0.1% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 25 mg each of Nilvadipine and Nilvadipine Reference Standard, dissolve in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 20 mL of the internal standard solution, 20 mL of water and methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under the Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of nilvadipine to that of the internal standard.

$$\text{Amount (mg) of } C_{19}H_{19}N_3O_6 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Nilvadipine Reference Standard

*Internal standard solution*—A solution of acenaphthene in methanol (1 in 200).

*Operating conditions*—

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

Column: A stainless steel column 4 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 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydoxide TS, adjust the pH to 7.0 with diluted phos-

phoric acid (1 in 10), and add 900 mL of acetonitrile.

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

*System suitability*—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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 ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

# Nilvadipine Tablets

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Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine ($C_{19}H_{19}N_3O_6$: 385.37).

**Method of preparation** Prepare as directed under Tablets, with Nilvadipine.

**Identification** To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of Nilvadipine according to the labeled amount, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 nm.

**Uniformity of dosage units** ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nilvadipine Tablets add $V$ mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine ($C_{19}H_{19}N_3O_6$) according to the labeled amount, add exactly $V$ mL of the internal standard solution, and disperse the particles with the aid of ultrasonic waves. Centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine Reference Standard, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of nilvadipine } (C_{19}H_{19}N_3O_6) = W_S \times (Q_T/Q_S) \times (V/100)$$

$W_S$: Amount (mg) of Nilvadipine Reference Standard

*Internal standard solution*—A solution of acenaphthene in acetonitrile (1 in 500).

**Dissolution** ⟨*6.10*⟩ Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Nilvadipine Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of Nilvadipine Reference Standard, equivalent to 10 times the labeled amount of Nilvadipine Tablets, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of water, 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 nilvadipine: the dissolution rate in 30 minutes is not less than 85%.

$$\text{Dissolution rate (\%) with respect to the labeled amount of nilvadipine } (C_{19}H_{19}N_3O_6) = W_S \times (A_T/A_S) \times (1/C) \times 9$$

$W_S$: Amount (mg) of Nilvadipine Reference Standard

$C$: Labeled amount (mg) of nilvadipine ($C_{19}H_{19}N_3O_6$) in 1 tablet

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4 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 25°C.

Mobile phase: A mixture of phosphate buffer solution, pH 7.4, methanol and acetonitrile (7:7:6)

Flow rate: Adjust the flow rate so that the retention time of nilvadipineis about 5 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 nilvadipine are not less than 2000 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 nilvadipine is not more than 1.5%.

**Assay** Weigh accurately not less than 20 Nilvadipine Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 5 mg of nilvadipine ($C_{19}H_{19}N_3O_6$), add 10 mL of a mixture of acetonitrile and water (7:3) and exactly 25 mL of the internal standard solution, shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine Reference Standard, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of

acetonitrile and water (7:3) to make 50 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, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of nilvadipine to that of the internal standard.

$$\text{Amount (mg) of nilvadipine } (C_{19}H_{19}N_3O_6) = W_S \times (Q_T/Q_S) \times (1/4)$$

$W_S$: Amount (mg) of Nilvadipine Reference Standard

*Internal standard solution*—A solution of acenaphthene in acetonitrile (1 in 500).

*Operating conditions*—

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

Column: A stainless steel column 4 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 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydoxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

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

*System suitability*—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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 ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

# Nitrazepam

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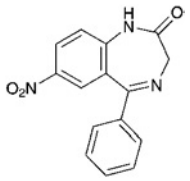

$C_{15}H_{11}N_3O_3$: 281.27

7-Nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one

[*146-22-5*]

Nitrazepam, when dried, contains not less than 99.0% of $C_{15}H_{11}N_3O_3$.

**Description** Nitrazepam occurs as white to yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in acetone and in chloroform, slightly soluble in methanol, in ethanol (95) and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 227°C (with decomposition).

**Identification** **(1)** To 3 mL of a solution of Nitrazepam in methanol (1 in 500) add 0.1 mL of sodium hydroxide TS: a yellow color is produced.

**(2)** To 0.02 g of Nitrazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines.

**(3)** Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS, and heat on a water bath: a purple color is produced.

**(4)** Determine the absorption spectrum of a solution of Nitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow to light yellow in color.

**(2)** Heavy metals <1.07>—Proceed with 1.0 g of Nitrazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <1.11>—Prepare the test solution with 1.0 g of Nitrazepam according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.25 g of Nitrazepam in a 10 mL of mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, pipet 2 mL of this solution, add a mixture of methanol and chloroform (1:1) to make exactly 50 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and ethyl acetate (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Nitrazepam, previously dried, and dissolve in 40 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L perchloric acid VS} = 28.13 \text{ mg of } C_{15}H_{11}N_3O_3$$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Nitrendipine

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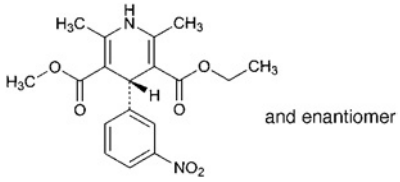



$C_{18}H_{20}N_2O_6$: 360.36
3-Ethyl 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[*39562-70-4*]

Nitrendipine, when dried, contains not less than 98.5% and not more than 101.0% of $C_{18}H_{20}N_2O_6$.

**Description** Nitrendipine occurs as a yellow crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to brownish yellow by light.

A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of Nitrendipine in methanol (1 in 80,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Nitrendipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 157 – 161°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Nitrendipine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances—Conduct this procedure rapidly using light-resistant vessels. Dissolve 40 mg of Nitrendipine in 5 mL of acetonitrile, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately 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 each peak area by the automatic integration method, and calculate the amount of related substances by the following equation: the amount of a related substance, having the relative retention time of about 0.8 with respect to nitrendipine, is not more than 1.0%, a related substance, having the relative retention time of about 1.3, is not more than 0.25%, and other related substances are not more than 0.2%, respectively. The total amount of the substances other than nitrendipine is not more than 2.0%.

$$\text{Amount (\%) of related substance} = A_T/A_S$$

$A_T$: Each peak area other than nitrendipine obtained from the sample solution
$A_S$: Peak area of nitrendipine obtained from the standard solution

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 25°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5).

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

Time span of measurement: About 2.5 times as long as the retention time of nitrendipine beginning after the solvent peak.

*System suitability*—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of nitrendipine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: Dissolve 10 mg of Nitrendipine and 3 mg of propyl parahydroxybenzoate in 5 mL of acetonitrile, and add the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and nitrendipine 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 peak area of nitrendipine is not more than 2.0%.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Nitrendipine, previously dried, dissolve in 60 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 100), add 50 mL of water, and titrate <*2.50*> with 0.1 mol/L serium (IV) tetraammonium sulfate VS until the red-orange color of the solution vanishes (indicator: 3 drops of 1,10-phenanthroline TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L serium (IV) tetraammonium sulfate VS
= 18.02 mg of $C_{18}H_{20}N_2O_6$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Nitrendipine Tablets

ニトレンジピン錠

Nitrendipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$: 360.36).

**Method of preparation** Prepare as directed under Tablets, with Nitrendipine.

**Identification** Shake a quantity of powdered Nitrendipine Tablets, equivalent to 5 mg of Nitrendipine according to the labeled amount, with 70 mL of methanol, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, and between 350 nm and 354 nm.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Nitrendipine Tablets add 15 mL of diluted acetonitrile (4 in 5), stir until the tablet is completely disintegrated, and further stir for 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 20 mL, and centrifuge. Pipet $V$ mL of the supernatant liquid, equivalent to about 1 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (4 in 5) to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\text{Amount (mg) of nitrendipine } (C_{18}H_{20}N_2O_6) = W_S \times (Q_T/Q_S) \times (1/V) \times (1/5)$$

$W_S$: Amount (mg) of nitrendipine for assay

*Internal standard solution*—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

**Dissolution** <6.10> Perform the test according to the following method: it meets the requirement.

Conduct this procedure using light-resistant vessels. Perform the test with 1 tablet of Nitrendipine Tablets at 100 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium (a solution of polysorbate 80 (3 in 5000) for a 5-mg tablet, and a solution of polysorbate 80 (3 in 2000) for a 10-mg tablet). Withdraw 20 mL or more of the dissolution medium 45 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent $V$ mL, add the dissolution medium to make exactly $V'$ mL so that each mL contains about 5.6 μg of nitrendipine ($C_{18}H_{20}N_2O_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nitrendipine for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium 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, $A_T$ and $A_S$, of nitrendipine. The dissolution rate in 45 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

$W_S$: Amount (mg) of nitrendipine for assay

$C$: Labeled amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$) in 1 tablet

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 356 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 25°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5)

Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 9 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 nitrendipine are not less than 5000 and not more than 2.0, 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 nitrendipine is not more than 2.0%.

**Assay** Conduct this procedure using light-resistant vessels. To 20 tablets of Nitrendipine Tablets add 150 mL of diluted acetonitrile (4 in 5), stir until the tablets completely disintegrate, and stir for further 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 200 mL, and centrifuge. Pipet a volume of the supernatant liquid, equivalent to about 2 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 10 mL of the internal standard solution and diluted acetonitrile (4 in 5) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nitrendipine for assay, previously dried at 105°C for 2 hours, and dissolve in diluted acetonitrile (4:5) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution and diluted acetonitrile (4:5) 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 determine the ratios, $Q_T$ and $Q_S$, of the peak area of nitrendipine to that of the internal standard.

$$\text{Amount (mg) of nitrendipine } (C_{18}H_{20}N_2O_6) = W_S \times (Q_T/Q_S) \times (1/50)$$

$W_S$: Amount (mg) of nitrendipine for assay

*Internal standard solution*—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 25° C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5)

Flow rate: Adjust the flow rate so that the retention time of nitrendipine 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 internal standard and nitrendipine 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 peak area of nitrendipine is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Nitrogen

窒素

$N_2$: 28.01

Nitrogen contains not less than 99.5 vol% of $N_2$.

**Description** Nitrogen is a colorless gas and is odorless.

Nitrogen (1 mL) dissolves in 65 mL of water and in 9 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

Nitrogen (1000 mL) at 0°C and at a pressure of 101.3 kPa weighs about 1.251 g.

It is inert and does not support combustion.

**Identification** The flame of a burning wood splinter is extinguished immediately in an atmosphere of Nitrogen.

**Purity** Carbon dioxide—Maintain the containers of Nitrogen at a temperature between 18°C and 22°C for more than 6 hours before the test, and correct the volume to be at 20°C and 101.3 kPa.

Pass 1000 mL of Nitrogen into 50 mL of barium hydroxide TS in a Nessler tube during 15 minutes through a delivery tube with an orifice approximately 1 mm in diameter, keeping the end of the tube at a distance of 2 mm from the bottom of the Nessler tube: any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

**Assay** Collect the sample as directed under Purity. Introduce 1.0 mL of Nitrogen into a gas-measuring tube or syringe for gas chromatography from a metal cylinder with a pressure-reducing valve, through a directly connected polyvinyl chloride tube. Perform the test with this solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak area $A_T$ of oxygen. Separately, introduce 1.0 mL of oxygen into the gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard gas mixture. Proceed with 1.0 mL of this mixture in the same manner under Nitrogen, and measure the peak area $A_S$ of oxygen.

$$\text{Amount (vol\%) of } N_2 = 100 - (A_T/A_S)$$

*Operating conditions*—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with zeolite for gas chromatography (250 to 350 μm in particle diameter).

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

Carrier gas: Hydrogen or helium

Flow rate: Adjust the flow rate so that the retention time of oxygen is about 3 minutes.

Selection of column: Introduce 1.0 mL of oxygen into the gas mixer, add Nitrogen to make 100 mL, and mix thoroughly. Proceed with 1.0 mL of this mixture under the above operating conditions. Use a column giving well-resolved peaks of oxygen and nitrogen in this order.

System repeatability: Repeat the test 5 times according to the above conditions with the standard gas mixture. Relative standard deviation of peak area of oxygen is not more than 2.0%.

**Containers and storage** Containers—Metal cylinders.

Storage—Not exceeding 40°C.

# Nitroglycerin Tablets

ニトログリセリン錠

Nitroglycerin Tablets contain not less than 80% and not more than 120% of the labeled amount of nitroglycerin ($C_3H_5N_3O_9$: 227.09).

**Method of preparation** Prepare as directed under Tablets, with nitroglycerin.

**Identification** **(1)** Weigh a quantity of powdered Nitroglycerin Tablets, equivalent to 6 mg of nitroglycerin ($C_3H_5N_3O_9$) according to the labeled amount, shake thoroughly with 12 mL of diethyl ether, filter, and use the filtrate as the sample solution. Evaporate 5 mL of the sample solution, dissolve the residue in 1 to 2 drops of sulfuric acid, and add 1 drop of diphenylamine TS: a deep blue color develops.

**(2)** Evaporate 5 mL of the sample solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame, and concentrate to about 0.1 mL. Cool, heat the residue with 0.02 g of potassium hydrogen sulfate: the odor of acrolein is perceptible.

**Purity** Free nitrate ion—Transfer an accurately measured quantity of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin ($C_3H_5N_3O_9$) according to the labeled amount, to a separator, add 40 mL of isopropylether and 40 mL of water, shake for 10 minutes, and allow the layers to

separate. Collect the aqueous layer, add 40 mL of isopropylether, shake for 10 minutes, collect the aqueous layer, filter, and use the filtrate as the sample solution. Separately, transfer 10 mL of Standard Nitric Acid Solution to a separator, add 30 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the sample solution, shake for 10 minutes, continue the procedure in the same manner as the sample solution, and use the solution so obtained as the standard solution. Transfer 20 mL each of the sample solution and the standard solution to Nessler tubes, respectively, shake well with 30 mL of water and 0.06 g of Griess-Romijin's nitric acid reagent, allow to stand for 30 minutes, and observe the tubes horizontally: the sample solution has no more color than the standard solution.

**Uniformity fo dosage units** ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirements of the Content uniformity test.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, and add exactly $V$ mL of acetic acid (100) to provide a solution containing about 30 $\mu$g of nitroglycerin ($C_3H_5N_3O_9$) per ml. Shake vigorously for 1 hour, and after disintegrating the tablet, centrifuge, and use the supernatant liquid as the sample solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet with 0.05 mL of acetic acid (100), and grind down it with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly $V$ mL of a solution containing about 30 $\mu$g of nitroglycerin ($C_3H_5N_3O_9$) per ml. Shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 5 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, add 2 mL each of salicylic acid TS shake, allow to stand for 15 minutes, and add 10 mL each of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

$$\text{Amount (mg) of nitroglycerin } (C_3H_5N_3O_9) = W_S \times (A_T/A_S) \times (V/2000) \times 0.7487$$

WS: Amount (mg) of potassium nitrate

Calculate the average content from the contents of 10 tablets: it meets the requirements of the test when each content deviates from the average content by not more than 25%. When there is 1 tablet showing a deviation exceeding 25% and not exceeding 30%, determine the content of an additional 20 tablets in the same manner. Calculate the 30 deviations from the new average of all 30 tablets: it meets the requirements of the test when 1 tablet may deviate from the average content by between 25% and 30%, but no tablet deviates by more than 30%.

**Disintegration** ⟨*6.09*⟩ It meets the requirement, provided that the time limit of the test is 2 minutes, and the use of the disks is omitted.

**Assay** Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin ($C_3H_5N_3O_9$), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 10 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, to each solution add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes, and add 10 mL of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

$$\text{Amount (mg) of nitroglycerin } (C_3H_5N_3O_9) = W_S \times (A_T/A_S) \times (1/20) \times 0.7487$$

$W_S$: Amount (mg) of potassium nitrate

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant, and not exceeding 20°C.

# Nitrous Oxide

亜酸化窒素

$N_2O$: 44.01

Nitrous Oxide contains not less than 97.0 vol% of $N_2O$.

**Description** Nitrous Oxide is a colorless gas at room temperature and at atmospheric pressure, and is odorless.

1 mL of Nitrous Oxide dissolves in 1.5 mL of water and in 0.4 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa. It is soluble in diethyl ether and in fatty oils.

1000 mL of Nitrous Oxide at 0°C and at a pressure of 101.3 kPa weighs about 1.96 g.

**Identification** **(1)** A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

**(2)** Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography ⟨*2.02*⟩ according to the conditions of the Assay: the retention time of the main peak from Nitrous Oxide coincides with that of nitrous oxide.

**Purity** Maintain the containers of Nitrous Oxide between 18°C and 22°C for more than 6 hours before the test, and

correct the volume at 20°C and at a pressure of 101.3 kPa.

**(1)** Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 100 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

**(2)** Carbon dioxide—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

**(3)** Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 2000 mL of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

**(4)** Potassium permanganate-reducing substance—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

**(5)** Chloride <*1.03*>—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

**(6)** Carbon monoxide—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatography from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test with this according to the Gas Chromatography <*2.02*> under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

*Operating conditions*—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 $\mu$m zeolite for gas chromatography (0.5 nm in pore size).

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

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

Selection of column: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of oxygen, nitrogen and carbon monoxide in this order.

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the selection of column is about 10 cm.

**Assay** Withdraw Nitrous Oxide as directed in the Purity.

Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the peak area $A_T$ of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard mixed gas. Proceed with 1.0 mL of this mixture as directed in the case of Nitrous Oxide, and determine the peak area $A_S$ of nitrogen in the same manner.

$$\text{Amount (vol\%) of } N_2O = 100 - 3 \times (A_T/A_S)$$

*Operating conditions*—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with silica gel for gas chromatography (300 to 500 $\mu$m in particle diameter).

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

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

Selection of column: To 3.0 mL of nitrogen in a gas mixer add Nitrous Oxide to make 100 mL, and mix well. Proceed with 1.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and nitrous oxide in this order.

System repeatability: Repeat the test five times with the standard mixed gas under the above operating conditions: the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

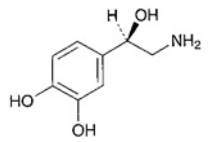
**Containers and storage** Containers—Metal cylinders.

Storage—Not exceeding 40°C.

# Noradrenaline

## Norepinephrine

ノルアドレナリン




and enantiomer

$C_8H_{11}NO_3$: 169.18

4-[(1*RS*)-2-Amino-1-hydroxyethyl]benzene-1,2-diol

[*51-41-2*]

Noradrenaline, when dried, contains not less than

98.0% of *dl*-norepinephrine ($C_8H_{11}NO_3$).

**Description** Noradrenaline occurs as a white to light brown or slightly reddish brown, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

It gradually changes to brown by air and by light.

**Identification** **(1)** Determine the absorption spectrum of a solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Noradrenaline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid TS, and add water to make 100 mL: the solution is clear and colorless.

**(2)** Arterenone—Dissolve 50 mg of Noradrenaline in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it is not more than 0.1.

**(3)** Adrenaline—Dissolve 10.0 mg of Noradrenaline in 2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of this solution, add water to make 10 mL, then mix with 0.3 mL of a solution of sodium nitrite (1 in 100), and allow to stand for 1 minute: the solution has no more color than the following control solution.

Control solution: Dissolve 2.0 mg of Adrenaline Bitartrate Reference Standard and 90 mg of Noradrenaline Bitartrate Reference Standard in water to make exactly 10 mL. Measure exactly 1 mL of this solution, add 1.0 mL of diluted acetic acid (100) (1 in 2) and water to make 10 mL, and proceed in the same manner.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, in vacuum, silica gel, 18 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Noradrenaline, previously dried, dissolve in 50 mL of acetic acid for nonaqueous titration by warming, if necessary, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.92 mg of $C_8H_{11}NO_3$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

# Noradrenaline Injection

## Noradrenaline Hydrochloride Injection
## Norepinephrine Hydrochloride Injection
## Norepinephrine Injection

ノルアドレナリン注射液

Noradrenaline Injection is an aqueous solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of *dl*-noradrenaline ($C_8H_{11}NO_3$: 169.18).

**Method of preparation** Dissolve Noradrenaline in 0.01 mol/L hydrochloric acid TS, and prepare as directed under Injections.

**Description** Norepinephrine Injection is a clear, colorless liquid.

It gradually becomes a pale red color by light and by air.

pH: 2.3 – 5.0

**Identification** Transfer a volume of Noradrenaline Injection, equivalent to 1 mg of Noradrenaline according to the labeled amount, to each of two test tubes A and B, and add 1 mL of water to each tube. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5, to A, and 10 mL of phosphate buffer solution, pH 6.5, to B. To each of these solutions add 1.0 mL of iodine TS, allow to stand for 5 minutes, and add 2.0 mL of sodium thiosulfate TS: no color or a pale red color develops in test tube A, and a deep red-purple color develops in test tube B.

**Purity** **(1)** Arterenone—Measure a volume of Noradrenaline Injection, equivalent to 10 mg of Noradrenaline according to the labeled amount, add water to make exactly 20 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.10.

**(2)** Adrenaline—Measure a volume of Noradrenaline Injection, equivalent to 5 mg of Noradrenaline according to the labeled amount, add 1 mL of diluted acetic acid (100) (1 in 2) and water to make exactly 10 mL, and proceed as directed in the Purity (3) under Noradrenaline.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Pipet a volume of Noradrenaline Injection, equivalent to about 5 mg of *dl*-noradrenaline ($C_8H_{11}NO_3$), add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Noradrenaline Bitartrate Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Piper 5 mL each of the sample solution and the standard solution, add 0.2 mL each of starch TS, then add iodine TS dropwise with swirling until a persistent blue color is produced. Add 2 mL of iodine TS, and shake. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution, pH 6.5, and shake. Immediately after allowing to stand for 3 minutes, add sodium thiosulfate TS drop-

wise until a red-purple color develops, then add water to make exactly 50 mL. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 515 nm within 5 minutes as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

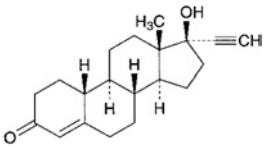
Amount (mg) of *dl*-noradrenaline ($C_8H_{11}NO_3$)
$= W_S \times (A_T/A_S) \times 0.5016$

$W_S$: Amount (mg) of Noradrenaline Bitartrate Reference Standard

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

# Norethisterone

ノルエチステロン




$C_{20}H_{26}O_2$: 298.42
17-Hydroxy-19-nor-17$\alpha$-pregn-4-en-20-yn-3-one [*68-22-4*]

Norethisterone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{20}H_{26}O_2$.

**Description** Norethisterone occurs as a white to pale yellowish white, crystalline powder. It is odorless.
It is soluble in chloroform, sparingly soluble in ethanol (95) and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.
It is affected by light.

**Identification** **(1)** To 2 mg of Norethisterone add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color develops and a yellow-brown precipitate is formed.
**(2)** To 25 mg of Norethisterone add 3.5 mL of a solution of 0.05 g of hydroxylammonium chloride and 0.05 g of anhydrous sodium acetate trihydrate in 25 mL of methanol. Heat under a reflux condenser on a water bath for 5 hours, cool, and add 15 mL of water. Collect the precipitate formed, wash with 1 to 2 mL of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 5 hours: the crystals melt <*2.60*> between 112°C and 118°C.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-23$ – $-27°$ (after drying, 0.25 g, chloroform, 25 mL, 200 mm).

**Melting point** <*2.60*> 203 – 209°C

**Loss on drying** <*2.41*> Not more than 0.5% (0.5 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

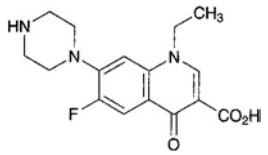
**Assay** Weigh accurately about 0.2 g of Norethisterone, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.84 mg of $C_{20}H_{26}O_2$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Norfloxacin

ノルフロキサシン




$C_{16}H_{18}FN_3O_3$: 319.33
1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid [*70458-96-7*]

Norfloxacin, when dried, contains not less than 99.0% of $C_{16}H_{18}FN_3O_3$.

**Description** Norfloxacin occurs as a white to pale yellow crystalline powder.
It is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) and in acetone, very slightly soluble in methanol, and practically insoluble in water.
It dissolves in dilute hydrochloric acid TS and in sodium hydroxide TS.
It is hygroscopic.
It is gradually colored by light.

**Identification** **(1)** Dissolve 0.01 g of Norfloxacin in a solution of sodium hydroxide (1 in 250) to make 100 mL. To 5 mL of this solution add a solution of sodium hydroxide (1 in 250) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.44*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
**(2)** Dissolve a suitable amount of Norfloxacin in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. Determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Sulfate <*1.14*>—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually diluted hydrochloric acid (1 in 3) to this solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.50 mL of 0.005 mol/L sulfuric acid

VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add diluted hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromophenol blue TS and water to make 50 mL (not more than 0.024%).

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Norfloxacin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Norfloxacin according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of a mixture of methanol and acetone (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetone (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetone (1:1) to make exactly 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 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5 – 7 μm in particle diameter). Develop with a mixture of methanol, chloroform, toluene, diethylamine and water (20:20:10:7:4) to a distance of about 9 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

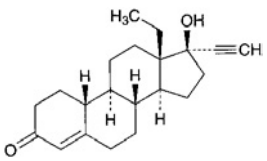
**Assay** Weigh accurately about 0.5 g of Norfloxacin, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.93 mg of $C_{16}H_{18}FN_3O_3$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Norgestrel

ノルゲストレル




$C_{21}H_{28}O_2$: 312.45
13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one [*6533-00-2*]

Norgestrel, when dried, contains not less than 98.0% of $C_{21}H_{28}O_2$.

**Description** Norgestrel occurs as white crystals or crystalline powder.

It is soluble in tetrahydrofuran and in chloroform, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

**Identification** **(1)** Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a red-orange fluorescence.

**(2)** Determine the infrared absorption spectrum of Norgestrel, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** ⟨*2.60*⟩ 206 – 212°C

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 30 mg of Norgestrel in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (0.5 g).

**Assay** Weigh accurately about 0.2 g of Norgestrel, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.25 mg of $C_{21}H_{28}O_2$

**Containers and storage** Containers—Well-closed containers.

# Norgestrel and Ethinylestradiol Tablets

ノルゲストレル·エチニルエストラジオール錠

Norgestrel and Ethinylestradiol Tablets contain not less than 90% and not more than 110% of the labeled amount of norgestrel ($C_{21}H_{28}O_2$: 312.45) and ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

**Method of preparation** Prepare as directed under Tablets, with Norgestrel and Ethinylestradiol.

**Identification (1)** Weigh a quantity of Norgestrel and Ethinylestradiol Tablets, equivalent to 10 mg of Norgestrel according to the labeled amount, previously powdered, add 10 mL of chloroform, shake for 10 minutes, and filter. To 2 mL of the filtrate add 6 mL of sodium hydroxide TS, shake vigorously, and centrifuge. Take 1 mL of the chloroform layer, evaporate on a water bath to dryness, dissolve the residue in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. Examine under ultraviolet light (main wavelength: 365 nm): this solution shows a red-orange fluorescence (norgestrel).

**(2)** Take 1 mL of the filtrate obtained in (1), evaporate on a water bath to dryness, add 1 mL of boric acid-methanol buffer solution to the residue, shake, and cool in ice. Add 1 mL of ice-cold diazo TS, shake, add 1 mL of sodium hydroxide TS, and shake: a red-orange color develops (ethinylestradiol).

**(3)** Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 10 mg of Norgestrel Reference Standard and 1 mg of Ethinylestradiol Reference Standard, respectively, in 10 mL of chloroform, and use these 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 20 $\mu$L 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 1,2-dichloroethane, methanol and water (368:32:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of *p*-toluenesulfonate in ethanol (95) (1 in 5) on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365nm): two spots from the sample solution show the similar color tone and *R*f value to each spot from the standard solutions (1) and (2).

**Uniformity of dosage units** <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 $\mu$m, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel Reference Standard and of Ethinylestradiol Reference Standard, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 $Q_{Tb}$, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, $Q_{Sa}$ and $Q_{Sb}$, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

Amount (mg) of norgestrel ($C_{21}H_{28}O_2$)
$= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/100)$

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
$= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/100)$

$W_{Sa}$: Amount (mg) of Norgestrel Reference Standard
$W_{Sb}$: Amount (mg) of Ethinylestradiol Reference Standard

*Internal standard solution*—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

*Operating conditions*—

Proceed as directed in the operating conditions in the Assay.

*System suitability*—

Proceed as directed in the system suitability in the Assay.

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 50 mL or more of the dissolved solution 45 minutes after starting the test, and membrane filter through a membrane filter with a pore size not exceeding 0.8 $\mu$m. Discard the first 10 mL of the filtrate, transfer exactly 30 mL of the subsequent into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 $\mu$m in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent on a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel Reference Standard and about 2.5 mg of Ethinylestradiol Reference Standard dissolve in diluted methanol (7 in 10) to make exactly 100 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 $\mu$L 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 $A_{Tb}$, of norgestrel and ethinylestradiol from the sample solution, and the peak areas, $A_{Sa}$ and $A_{Sb}$, of norgestrel and ethinylestradiol from the standard solution.

The dissolution rate of Norgestrel and Ethinylestradiol Tablets in 45 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of norgestrel ($C_{21}H_{28}O_2$)
$= W_{Sa} \times (A_{Ta}/A_{Sa}) \times (1/C_a) \times (9/5)$

Dissolution rate (%) with respect to the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$)

$= W_{Sb} \times (A_{Tb}/A_{Sb}) \times (1/C_b) \times (9/5)$

$W_{Sa}$: Amount (mg) of Norgestrel Reference Standard
$W_{Sb}$: Amount (mg) of Ethinylestradiol Reference Standard
$C_a$: Labeled amount (mg) of norgestrel ($C_{21}H_{28}O_2$) in 1 tablet
$C_b$: Labeled amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$) in 1 tablet

*Operating conditions*—
Proceed as directed in the operating conditions in the Assay.
*System suitability*—
Proceed as directed in the system suitability in the Assay.

**Assay** Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel ($C_{21}H_{28}O_2$), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 $\mu$m, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel Reference Standard and about 5 mg of Ethinylestradiol Reference Standard, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 $Q_{Tb}$, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, $Q_{Sa}$ and $Q_{Sb}$, of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

Amount (mg) of norgestrel ($C_{21}H_{28}O_2$)
$= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/50)$

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
$= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/50)$

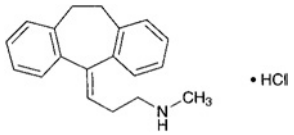
$W_{Sa}$: Amount (mg) of Norgestrel Reference Standard
$W_{Sb}$: Amount (mg) of Ethinylestradiol Reference Standard

*Internal standard solution*—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).
*Operating conditions*—
Detector: Norgestrel—An ultraviolet absorption photometer (wavelength: 241 nm).
Ethinylestradiol—A fluorophotometer (excitation wavelength: 281 nm, fluorescence wavelength: 305 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 (10 $\mu$m in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of acetonitrile and water (11:9).
Flow rate: Adjust the flow rate so that the retention time of norgestrel is about 10 minutes.
*System suitability*—
System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, ethinylestradiol, norgestrel and the internal standard are eluted in this order, and the resolution between the peaks of norgestrel and the internal standard is not less than 8.
System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

**Containers and storage** Containers—Tight containers.

# Nortriptyline Hydrochloride

ノルトリプチリン塩酸塩




$C_{19}H_{21}N.HCl$: 299.84
3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptен-5-ylidene)-*N*-methylpropylamine monohydrochloride
[*894-71-3*]

Nortriptyline Hydrochloride, when dried, contains not less than 98.5% of $C_{19}H_{21}N.HCl$.

**Description** Nortriptyline Hydrochloride occurs as a white to yellowish white, crystalline powder. It is odorless, or has a faint, characteristic odor.
It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.
The pH of a solution of Nortriptyline Hydrochloride (1 in 100) is about 5.5.
Melting point: 215 – 220°C

**Identification** **(1)** To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 mL of bromine TS: the color of the test solution disappears.
**(2)** To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 to 2 drops of a solution of quinhydrone in methanol (1 in 40): a red color gradually develops.
**(3)** Determine the absorption spectrum of a solution of Nortriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
**(4)** Determine the infrared absorption spectrum of Nortriptyline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
**(5)** A solution of Nortriptyline Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very light yellow.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Nortriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Nortriptyline Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 4 $\mu$L 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 cyclohexane, methanol and diethylamine (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.98 mg of $C_{19}H_{21}N.HCl$

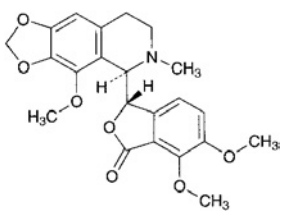
**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Noscapine

## Narcotine

ノスカピン



$C_{22}H_{23}NO_7$: 413.42
(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one [*128-62-1*]

Noscapine, when dried, contains not less than 98.5% of $C_{22}H_{23}NO_7$.

**Description** Noscapine occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is very soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

**Identification (1)** Determine the absorption spectrum of a solution of Noscapine in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Noscapine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +42 – +48° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

**Melting point** <*2.60*> 174 – 177°C

**Purity (1)** Chloride <*1.03*>—Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 0.4 mL of 0.01 mol/L hydrochloric acid add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.02%).

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Noscapine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Morphine—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge, and collect the aqueous layer: the solution so obtained has no more color than a pale red.

**(4)** Related substances—Dissolve 0.7 g of Noscapine in 50 mL of acetone, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 100 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 $\mu$L 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, toluene, ethanol (99.5) and ammonia solution (28) (60:60:9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (2 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and

make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.34 mg of $C_{22}H_{23}NO_7$

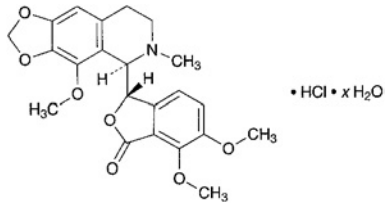
**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Noscapine Hydrochloride Hydrate

## Narcotine Hydrochloride

ノスカピン塩酸塩水和物



$C_{22}H_{23}NO_7.HCl.xH_2O$

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one monohydrochloride hydrate [*912-60-7*, anhydride]

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0% of noscapine hydrochloride $C_{22}H_{23}NO_7.HCl$: 449.88.

**Description** Noscapine Hydrochloride Hydrate occurs as colorless or white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in acetic acid (100), and in acetic anhydride, soluble in ethanol (95), and practically insoluble in diethyl ether.

**Identification** **(1)** To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

**(2)** To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of a solution of ammonium vanadate (V) in sulfuric acid (1 in 200): an orange color is produced.

**(3)** Dissolve 0.02 g of Noscapine Hydrochloride Hydrate in 1 mL of water, and add 3 drops of sodium acetate TS: a white, flocculent precipitate is produced.

**(4)** Dissolve 1 mg of Noscapine Hydrochloride Hydrate in 1 mL of diluted sulfuric acid (1 in 35), shake with 5 drops of a solution of disodium chlomotropate dihydrate (1 in 50), and add 2 mL of sulfuric acid dropwise: a purple color is produced.

**(5)** Dissolve 0.1 g of Noscapine Hydrochloride Hydrate in 10 mL of water, make the solution alkaline with ammonia TS, and shake with 10 mL of chloroform. Separate the chloroform layer, wash with 5 mL of water, and filter. Distil most of the filtrate on a water bath, add 1 mL of ethanol (99.5), and evaporate to dryness. Dry the residue at 105°C for 4 hours: the residue so obtained melts <*2.60*> between 174°C and 177°C.

**(6)** Make a solution of Noscapine Hydrochloride Hydrate (1 in 50) alkaline with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

**Purity** Morphine—Dissolve 10 mg of Noscapine Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

**Loss on drying** <*2.41*> Not more than 9.0% (0.5 g, 120°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.5% (1 g).

**Assay** Weigh accurately about 0.5 g of Noscapine Hydrochloride Hydrate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.99 mg of $C_{22}H_{23}NO_7.HCl$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Nystatin

ナイスタチン

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of *Streptomyces noursei*.

It contains not less than 4600 units (potency) per mg, calculated on the dried basis. The potency of Nystatin is expressed as the unit of nystatin ($C_{47}H_{75}NO_{17}$: 926.09), and one unit corresponds to 0.27 $\mu$g of nystatin ($C_{47}H_{75}NO_{17}$).

**Description** Nystatin occurs as a white to light yellow-brown powder.

It is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

**Identification** **(1)** Dissolve 1 mg of Nystatin in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes, and cool. To this solution add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color develops.

**(2)** To 10 mg of Nystatin add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1), heat at not exceeding 50°C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nys-

tatin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** Heavy metals <*1.07*>—Proceed with 1.0 g of Nystatin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <*2.41*> Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

**(i)** Test organism—*Saccharomyces cerevisiae* ATCC 9763

**(ii)** Culture medium—Use the medium 2) Medium for test organism [12] under (1) Agar media for seed and base layer.

**(iii)** Standard solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin Reference Standard equivalent to about 60,000 units, previously dried at 40°C for 2 hours in vacuum (not more than 0.67 kPa), dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

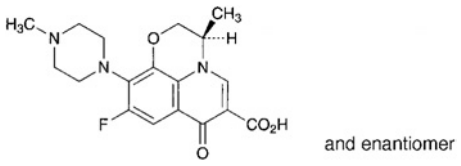
**(iv)** Sample solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin equivalent to about 60,000 units, dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

# Ofloxacin

オフロキサシン



and enantiomer

$C_{18}H_{20}FN_3O_4$: 361.37

(3*RS*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-[1,4]benzooxazine-6-carboxylic acid [*82419-36-1*]

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin ($C_{18}H_{20}FN_3O_4$).

**Description** Ofloxacin occurs as pale yellowish white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A soluton of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

It is changed in color by light.

Melting point: about 265°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Ofloxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Ofloxacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Related substances—Conduct this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ofloxacin obtained from the sample solution is not more than 0.4 times the peak area of ofloxacin from the standard solution, and the total area of the peaks other than ofloxacin from the sample solution is not more than the peak area from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 294 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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ofloxacin is about 20 minutes.

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin beginning after the solvent peak.

*System suitability*—

Test for required detectability: Measure 1 mL of the standard solution, and add a mixture of water and acetonitrile

(6:1) to make exactly 20 mL. Confirm that the peak area of ofloxacin obtained from 10 μL of this solution is equivalent to 4 to 6% of that from 10 μL of the standard solution.

System performance: To 0.5 mL of the sample solution add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6:1) (1 in 20,000) and a mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin 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 ofloxacin is not more than 2.0%.

**Loss on drying** ⟨*2.41*⟩ Not less than 0.2% (1 g, 105°C, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{18}H_{20}FN_3O_4$

**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 diethyl ether and with carbon disulfide.

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 the flow rate so that the retention time of methyl behenate is about 18 minutes.

Detection sensitivity: Adjust the detection sensitivity 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.

# 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 Hy-

drate, 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 $\mu$L 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 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 Alkaloids Hydrochlorides

アヘンアルカロイド塩酸塩

Opium Alkaloids Hydrochlorides consist of the hydrochlorides of some of the main alkaloids obtained from opium.

It contains not less than 47.0% and not more than 52.0% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 35.0% and not more than 41.0% of other opium alkaloids.

**Description** Opium Alkaloids Hydrochlorides occur as a white to light brown powder.

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

It is colored by light.

**Identification** **(1)** Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 60 mg of Morphine Hydrochloride Hydrate, 40 mg of Noscapine Hydrochloride Hydrate, 10 mg of Codein Phosphate Hydrate and 10 mg of Papaverine Hydrochloride in 10 mL each of diluted ethanol (1 in 2), and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 $\mu$L each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): each spot from the sample solution is the same in color tone and *R*f value with the corresponding spot from the standard solutions (1), (2), (3) and (4) (morphine, noscapine, codeine and papaverine).

**(2)** A solution of Opium Alkaloids Hydrochlorides (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

**pH** <*2.54*> Dissolve 1.0 g of Opium Alkaloids Hydrochlorides in 50 mL of water: the pH of the solution is between 3.0 and 4.0.

**Purity** **(1)** Clarity and color of solution − Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance <*2.24*> at 420 nm is not more than 0.20.

**(2)** Meconic acid—Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 2 mL of water, and pour into a polyethylene column 1 cm in inside diameter, packed with about 0.36 g of aminopropylsilanized silica gel for pretreatment (55 – 105 $\mu$m in particle diameter) and previously washed through with 5 mL of water. Then, wash the column with 5 mL of water, 5 mL of methanol and 10 mL of 0.1 mol/L hydrochloric acid in this order, then elute with 2 mL of 1 mol /L hydrochloric acid, and use the eluate as the test solution. To the test solution add 2 mL of dilute sodium hydroxide TS and 1 drop of iron (III) chloride TS: no red color develops.

**Loss on drying** <*2.41*> Not more than 6.0% (0.5 g, 120°C, 8 hours).

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

**Assay** Weigh accurately about 0.1 g of Opium Alkaloids Hydrochlorides, and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of morphine hydrochloride for assay, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L 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 morphine, codeine, papaverine, thebaine, narceine and noscapine, $A_{T1}$, $A_{T2}$, $A_{T3}$, $A_{T4}$, $A_{T5}$ and $A_{T6}$, from the sample solution, and the peak area of morphine, $A_S$, from the standard solution.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)
$= W_S \times (A_{T1}/A_S) \times 0.8867$

Amount (mg) of other opium alkaloids
$= W_S$
$\times \{(A_{T2} + 0.29A_{T3} + 0.20A_{T4} + 0.19A_{T5} + A_{T6})/A_S\}$
$\times 0.8867$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

The relative retention time of codine, papaverine, thebaine, narceine and noscapine with respect to morphine obtained under the following operating conditions are as follows.

| Component | Relative retention time |
|---|---|
| codeine | 1.1 |
| papaverine | 1.9 |
| thebaine | 2.5 |
| narceine | 2.8 |
| noscapine | 3.6 |

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

*System suitability—*

System performance: Dissolve 60 mg of Morphine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate, 10 mg of Papaverine Hydrochloride and 40 mg of Noscapine Hydrochloride Hydrate in water to make 50 mL. When the procedure is run with 20 $\mu$L of this solution under the above operating conditions, morphine, codeine, papaverine and noscapine are eluted in this order with the complete separation between these peaks and with the resolution between the peaks of morphine and codeine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Opium Alkaloids Hydrochlorides Injection

アヘンアルカロイド塩酸塩注射液

Opium Alkaloids Hydrochlorides Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34).

**Method of preparation**

| | |
|---|---|
| Opium Alkaloids Hydrochlorides | 20 g |
| Water for Injection | a sufficient quantity |
| To make | 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Opium Alkaloids Hydrochlorides Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

**Identification** To 1 mL of Opium Alkaloids Hydrochlorides Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution, and proceed as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

**Extractable volume** ⟨*6.05*⟩ It meets the requirements.

**Assay** Pipet 2 mL of Opium Alkaloids Hydrochlorides Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay (1) under Opium Alkaloids Hydrochlorides.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)

$$= W_S \times (Q_T/Q_S) \times 0.8867$$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of etilefrine hydrochloride (1 in 500).

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Opium Alkaloids and Atropine Injection

アヘンアルカロイド·アトロピン注射液

Opium Alkaloids and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate Hydrate [$(C_{17}H_{23}NO_3)_2 . H_2SO_4 . H_2O$: 694.84].

**Method of preparation**

| | |
|---|---|
| Opium Alkaloids Hydrochlorides | 20 g |
| Atropine Sulfate Hydrate | 0.3 g |
| Water for Injection | a sufficient quantity |
| To make | 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Opium Alkaloids and Atropine Injection is a colorless or light brown, clear liquid.

It is affected by light.

pH: 2.5 – 3.5

**Identification** **(1)** To 1 mL of Opium Alkaloids and Atropine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

**(2)** To 2 mL of Opium Alkaloids and Atropine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Atropine Sulfate Reference Standard in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 $\mu$L 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 and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.2 *R*f value among the several spots from the sample solution and an

orange colored spot from the standard solution show the same color tone, and have the same *R*f value (atropine).

**Extractable volume** ⟨*6.05*⟩ It meets the requirements.

**Assay (1)** Morphine—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 morphine to that of the internal standard.

Amount (mg) of morphine ($C_{17}H_{19}NO_3$)
$= W_S \times (Q_T/Q_S) \times 0.8867$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of ethylefrine hydrochloride (1 in 500).

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

*System suitability*—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, morphine and the internal standard 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 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

**(2)** Atropine sulfate hydrate—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 2 mL of the internal standard solution, and add 10 mL of diluted dilute hydrochloric acid (1 in 10). Shake this solution with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate Reference Standard (determine previously loss on drying ⟨*2.41*⟩ in the same manner as directed under Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use this solution as the standard solution. Perform the test with 2 $\mu$L each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of atropine to that of the internal standard.

Amount (mg) of atropine sulfate hydrate
[$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$]
$= W_S \times (Q_T/Q_S) \times (1/50) \times 1.027$

$W_S$: Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

*Internal standard solution*—A solution of homatropine hydrobromide (1 in 4000).

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 $\mu$m siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.

*System suitability*—

System performance: When the procedure is run with 2 $\mu$L of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Opium Alkaloids and Scopolamine Injection

アヘンアルカロイド·スコポラミン注射液

Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 1.80 w/v% and not more than 2.20 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34) and not less than 0.054 w/v% and not more than 0.066 w/v% of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31).

**Method of preparation**

| | |
|---|---|
| Opium Alkaloids Hydrochlorides | 40 g |
| Scopolamine Hydrobromide Hydrate | 0.6 g |
| Water for Injection | a sufficient quantity |
| To make | 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Opium Alkaloids and Scopolamine Injection is a clear, colorless to light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

**Identification** **(1)** To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

**(2)** To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide Reference Standard in 100 mL of water. To 2 mL of this solution add 2 mL of ammonia TS, proceed with this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 $\mu$L 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 and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (scopolamine).

**Extractable volume** <*6.05*> It meets the requirements.

**Assay** **(1)** Morphine—Pipet 1 mL of Opium Alkaloids and Scopolamine Injection, add 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 morphine to that of the internal standard.

$$\text{Amount (mg) of morphine } (C_{17}H_{19}NO_3) = W_S \times (Q_T/Q_S) \times 0.8867$$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of etilefrin hydrochloride (1 in 500).

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

*System suitability*—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, morphine and the internal standard 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 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

**(2)** Scopolamine hydrobromide hydrate—Pipet 2 mL of Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoporamine Hydrobromide Reference Standard (determine previously its loss on drying <*2.41*> in the same manner as directed under Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use thus obtained solution as the standard solution. Perform the test with 2 $\mu$L each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of scopolamine to that of the internal standard.

$$\text{Amount (mg) of scopolamine hydrobromide hydrate } (C_{17}H_{21}NO_4.HBr.3H_2O) = W_S \times (Q_T/Q_S) \times (1/50) \times 1.1406$$

$W_S$: Amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

*Internal standard solution*—A solution of homatropine hydrobromide (1 in 4000).

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

*System suitability—*

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Weak Opium Alkaloids and Scopolamine Injection

弱アヘンアルカロイド·スコポラミン注射液

Weak Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($C_{17}H_{19}NO_3$: 285.34) and not less than 0.027 w/v% and not more than 0.033 w/v% of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31).

**Method of preparation**

| | |
|---|---|
| Opium Alkaloids Hydrochlorides | 20 g |
| Scopolamine Hydrobromide Hydrate | 0.3 g |
| Water for Injection | a sufficient quantity |
| To make | 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Weak Opium Alkaloids and Scopolamine Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

**Identification (1)** To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

**(2)** To 2 mL of Weak Opium Alkaloids and Scopolamine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide Reference Standard in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained 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 and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 *R*f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same *R*f value (scopolamine).

**Extractable volume** <*6.05*> It meets the requirements.

**Assay (1)** Morphine—Pipet 2 mL of Weak Opium Alkaloids and Scopolamine Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 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 morphine to that of the internal standard.

$$\text{Amount (mg) of morphine } (C_{17}H_{19}NO_3) = W_S \times (Q_T \times Q_S) \times 0.8867$$

$W_S$: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

*Internal standard solution*—A solution of etilefrin hydrochloride (1 in 500).

*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: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

*System suitability—*

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard 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 20 μL of the standard solution under the above operating

conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

**(2)** Scopolamine hydrobromide hydrate—Pipet 4 mL of Weak Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scoporamine Hydrobromide Reference Standard (separately determine its loss on drying <*2.41*> in the same manner as directed under Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 $\mu$L each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of scopolamine to that of the internal standard.

Amount (mg) of scopolamine hydrobromide hydrate ($C_{17}H_{21}NO_4.HBr.3H_2O$)
$= W_S \times (Q_T/Q_S) \times (1/50) \times 1.1406$

$W_S$: Amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

*Internal standard solution*—A solution of homatropine hydrobromide (1 in 4000).

*Operating conditions*—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 $\mu$m siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

*System suitability*—

System performance: When the procedure is run with 2 $\mu$L of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 2 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# 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*> $\alpha_D^{20}$: +85 – +99° (100 mm).

**Specific gravity** <*1.13*> $d_{20}^{20}$: 0.842 – 0.848

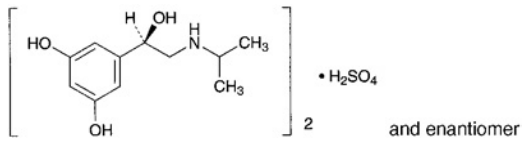
**Purity** Heavy metals <*1.07*>—Proceed with 1.0 mL of Orange Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Orciprenaline Sulfate

オルシプレナリン硫酸塩




$(C_{11}H_{17}NO_3)_2.H_2SO_4$: 520.59

5-{(1*RS*)-1-Hydroxy-2-[(1-methylethyl)amino]ethyl}benzene-1,3-diol hemisulfate [*5874-97-5*]

Orciprenaline Sulfate contains not less than 98.5% of $(C_{11}H_{17}NO_3)_2.H_2SO_4$, calculated on the dried basis.

**Description** Orciprenaline Sulfate occurs as white crystals or crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Orciprenaline Sulfate (1 in 20) shows no optical rotation.

Melting point: about 220°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Or-

ciprenaline Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

**pH** <2.54>Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3 mL of Matching Fluid T add 1 mL of diluted hydrochloric acid (1 in 40).

**(2)** Heavy metals <1.07>—Proceed with 2.0 g of Orciprenaline Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Orciprenalone—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 328 nm is not more than 0.075.

**Loss on drying** <2.41> Not more than 1.5% (1 g, in vacuum, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming on a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

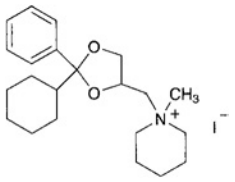
Each mL of 0.1 mol/L perchloric acid VS
= 52.06 mg of $(C_{11}H_{17}NO_3)_2.H_2SO_4$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Oxapium Iodide

オキサピウムヨウ化物



$C_{22}H_{34}INO_2$: 471.42

1-(2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-ylmethyl)-1-methylpiperidinium iodide [6577-41-9]

Oxapium Iodide, when dried, contains not less than 98.5% of $C_{22}H_{34}INO_2$.

**Description** Oxapium Iodide occurs as a white, crystalline powder.

It is soluble in acetonitrile, in methanol and in ethanol (95), slightly soluble in water, in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Oxapium Iodide in methanol (1 in 100) does not show optical rotation.

**Identification (1)** Determine the infrared absorption spectrum of Oxapium Iodide, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** Dissolve 0.1 g of Oxapium Iodide in 10 mL of methanol, and add 2 mL of dilute nitric acid and 2 mL of silver nitrate TS: a greenish yellow precipitate is formed.

**Melting point** <2.60> 198 – 203°C

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Oxapium Iodide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 0.05 g of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 20°C to 30°C.

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine add water to make 1000 mL. To 50 mL of this solution add 500 mL of acetonitril, 10 mL of dilute acetic acid and 440 mL of water.

Flow rate: Adjust the flow rate so that the retention time of oxapium is about 4 minutes.

Selection of column: Dissolve 0.05 g of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of oxapium and benzophenone in this order with the resolution between these peaks being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 μL of the standard solution composes 5 to 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of oxapium beginning after the peak of iodide ion.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.7 g of Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic an-

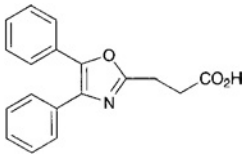
hydride and acetic acid (100) (9:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration, platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.14 mg of $C_{22}H_{34}INO_2$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Oxaprozin

オキサプロジン



$C_{18}H_{15}NO_3$: 293.32
3-(4,5-Diphenyloxazol-2-yl)propanoic acid [*21256-18-8*]

Oxaprozin, when dried, contains not less than 98.5% of $C_{18}H_{15}NO_3$.

**Description** Oxaprozin occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually affected by light.

**Identification** Determine the infrared absorption spectrum of Oxaprozin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** <2.24> $E^{1\%}_{1\,cm}$ (285 nm): 455 – 495 (after drying, 10 mg, methanol, 1000 mL).

**Melting point** <2.60> 161 – 165°C

**Purity** **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Oxaprozin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Arsenic <1.11>—Prepare the test solution with 2.0 g of Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

**(3)** Related substances—Dissolve 0.10 g of Oxaprozin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL, 3 mL and 1 mL of this solution, add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (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 (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of the spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

**Loss on drying** <2.41> Not more than 0.3% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.3% (1 g).

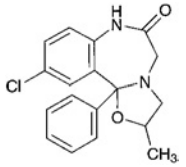
**Assay** Weigh accurately about 0.5 g of Oxaprozin, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.33 mg of $C_{18}H_{15}NO_3$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Oxazolam

オキサゾラム



$C_{18}H_{17}ClN_2O_2$: 328.79
10-Chloro-2-methyl-11b-phenyl-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one [*24143-17-7*]

Oxazolam, when dried, contains not less than 99.0% of $C_{18}H_{17}ClN_2O_2$.

**Description** Oxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), soluble in 1,4-dioxane and in dichloromethane, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It gradually changes in color by light.

Melting point: about 187°C (with decomposition).

**Identification** **(1)** Dissolve 0.01 g of Oxazolam in 10 mL of ethanol (95) by heating, and add 1 drop of hydrochloric acid: a light yellow color develops, and the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

**(2)** Dissolve 0.01 g of Oxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

**(3)** Place 2 g of Oxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS,

and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4), and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 20 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry in vacuum at 60°C for 1 hour: the crystals melt ⟨*2.60*⟩ between 96°C and 100°C.

**(4)** Determine the absorption spectrum of a solution of Oxazolam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(5)** Proceed with Oxazolam as directed under Flame Coloration Test ⟨*1.04*⟩ (2), and perform the test: a green color appears.

**Absorbance** ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (246 nm): 410 – 430 (after drying, 1 mg, ethanol (95), 100 mL).

**Purity** **(1)** Chloride ⟨*1.03*⟩—To 1.0 g of Oxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Oxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Place 1.0 g of Oxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate monohydrate solution, heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.05 g of Oxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.65 g of Oxazolam, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and 1,4-dioxane (1:1). Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

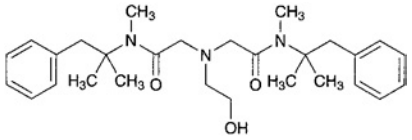
Each mL of 0.1 mol/L perchloric acid VS
= 32.88 mg of $C_{18}H_{17}ClN_2O_2$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Oxethazaine

## Oxetacaine

オキセサゼイン



$C_{28}H_{41}N_3O_3$: 467.64
2,2′-(2-Hydroxyethylimino)bis[*N*-(1,1-dimethyl-2-phenylethyl)-*N*-methylacetamide] [*126-27-2*]

Oxethazaine, when dried, contains not less than 98.5% of $C_{28}H_{41}N_3O_3$.

**Description** Oxethazaine occurs as a white to pale yellowish white, crystalline powder.

**Identification** **(1)** Determine the absorption spectrum of a solution of Oxethazaine in ethanol (95) (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Oxethazaine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibits similar intensities of absorption at the same wave numbers.

**Melting point** ⟨*2.60*⟩ 101 – 104°C

**Purity** **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Oxethazaine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 0.40 g of Oxethazaine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropylether, tetrahydrofuran, methanol and ammonia solution (28) (24:10:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**(4)** 2-Aminoethanol—To 1.0 g of Oxethazaine add methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenzene in methanol (1 in 25), shake well, and heat at 60°C for 20 minutes: the solution has no more color than the following control solution.

Control solution: To 0.10 g of 2-aminoethanol add methanol to make exactly 200 mL, pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Proceed as directed above.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

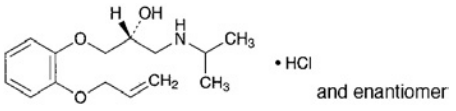
**Assay** Weigh accurately about 0.9 g of Oxethazaine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.76 mg of $C_{28}H_{41}N_3O_3$

**Containers and storage** Containers—Tight containers.

# Oxprenolol Hydrochloride

オクスプレノロール塩酸塩




$C_{15}H_{23}NO_3.HCl$: 301.81
(2*RS*)-1-[2-(Allyloxy)phenoxy]-3-(1-methylethyl)aminopropan-2-ol monohydrochloride
[*6452-73-9*]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{23}NO_3.HCl$.

**Description** Oxprenolol Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

**Identification (1)** To 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer, and a blue-purple color develops in the water layer.

**(2)** To 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

**(3)** Determine the infrared absorption spectrum of Oxprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(4)** A solution of Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

**pH** ⟨*2.54*⟩ Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

**Melting point** ⟨*2.60*⟩ 107 – 110°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Oxprenolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample solution, and add 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. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 $\mu$L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate in a developing chamber saturated with ammonia vapor with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

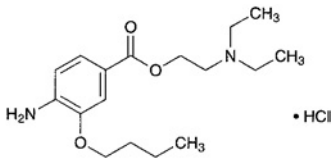
Each mL of 0.1 mol/L perchloric acid VS
= 30.18 mg of $C_{15}H_{23}NO_3.HCl$

**Containers and storage** Containers—Tight containers.

# Oxybuprocaine Hydrochloride

## Benoxinate Hydrochloride

オキシブプロカイン塩酸塩



$C_{17}H_{28}N_2O_3$.HCl: 344.88
2-(Diethylamino)ethyl 4-amino-3-butyloxybenzoate monohydrochloride [*5987-82-6*]

Oxybuprocaine Hydrochloride, when dried, contains not less than 99.0% of $C_{17}H_{28}N_2O_3$.HCl.

**Description** Oxybuprocaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a saline taste. It exhibits anesthetic properties when placed on the tongue.

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

The pH of a solution of Oxybuprocaine Hydrochloride (1 in 10) is between 5.0 and 6.0.

It is gradually colored by light.

**Identification (1)** Dissolve 0.01 g of Oxybuprocaine Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests ⟨*1.09*⟩ for primary aromatic amines.

**(2)** Dissolve 0.1 g of Oxybuprocaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 5 hours: the crystals melt ⟨*2.60*⟩ between 103°C and 106°C.

**(3)** Determine the absorption spectrum of a solution of Oxybuprocaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** A solution of Oxybuprocaine Hydrochloride (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

**Melting point** ⟨*2.60*⟩ 158 – 162°C

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Oxybuprocaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

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

**(3)** Related substances—Dissolve 0.25 g of Oxybuprocaine Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 50 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 chloroform, ethanol (95) and formic acid (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

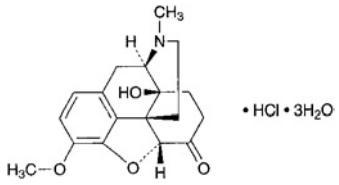
Each mL of 0.1 mol/L perchloric acid VS
= 34.49 mg of $C_{17}H_{28}N_2O_3$.HCl

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Oxycodone Hydrochloride Hydrate

オキシコドン塩酸塩水和物



$C_{18}H_{21}NO_4$.HCl.3$H_2O$: 405.87
(5*R*)-4,5-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one monohydrochloride trihydrate [*124-90-3,* anhydride]

Oxycodone Hydrochloride Hydrate contains not less than 98.0% of $C_{18}H_{21}NO_4$.HCl (mol. wt.: 351.83), calculated on the anhydrous basis.

**Description** Oxycodone Hydrochloride Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water is between 3.8 and 5.8.

It is affected by light.

**Identification (1)** Determine the absorption spectrum of a solution of Oxycodone Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spec-

trum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Oxycodone Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Oxycodone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-140$ – $-149°$ (0.5 g, calculated on the anhydrous basis, water, 25 mL, 100 mm).

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

**(2)** Morphine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthole TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. To this solution add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, add 10 mL of chloroform, shake, centrifuge, and separate the water layer: the color of the solution is not more intense than a pale red.

**(3)** Codeine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and warm: no blue color is produced. Add 1 drop of nitric acid: no red color develops.

**(4)** Thebaine—Dissolve 0.10 g of Oxycodone Hydrochloride Hydrate in 2 mL of diluted hydrochloric acid (1 in 10), and heat the solution in a water bath for 25 minutes. After cooling, add 0.5 mL of 4-aminoantipyrine hydrochloride TS and 0.5 mL of a solution of potassium hexacyanoferrate (III) (1 in 100), and shake. Then shake the solution with 2 mL of ammonia TS and 3 mL of chloroform: no red color develops in the chloroform layer.

**Water** <*2.48*> 12 – 15% (0.2 g, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.5 g of Oxycodone Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.18 mg of $C_{18}H_{21}NO_4.HCl$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Compound Oxycodone Injection

## Compound Hycodenone Injection

複方オキシコドン注射液

Compound Oxycodone Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$: 405.87), and not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$: 275.73).

**Method of preparation**

| | |
|---|---|
| Oxycodone Hydrochloride Hydrate | 8 g |
| Hydrocotarnine Hydrochloride Hydrate | 2 g |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Compound Oxycodone Injection is a clear, colorless to pale yellow liquid.

It is affected by light.

pH: 2.5 – 4.0

**Identification** **(1)** To 1 mL of Compound Oxycodone Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

**(2)** Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

**(3)** Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

**Extractable volume** <*6.05*> It meets the requirement

**Assay** Pipet 2 mL of Compound Oxycodone Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 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 10 $\mu$L 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 $Q_{Tb}$, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, $Q_{Sa}$ and $Q_{Sb}$, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$)
$= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times 1.1536 \times (1/25)$

Amount (mg) of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$)
$= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times 1.0699 \times (1/25)$

$W_{Sa}$: Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

$W_{Sb}$: Amount (mg) of hydrocotarnine hydrochloride for assay

*Internal standard solution*—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

*Operating conditions*—

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

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogen phosphate TS add 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarnine in this order, with complete separation of these peaks.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Compound Oxycodone and Atropine Injection

## Hycoato Injection

複方オキシコドン·アトロピン注射液

Compound Oxycodone and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$: 405.87), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$: 275.73), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

**Method of preparation**

| | |
|---|---|
| Oxycodone Hydrochloride Hydrate | 8 g |
| Hydrocotarnine Hydrochloride Hydrate | 2 g |
| Atropine Sulfate Hydrate | 0.3 g |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Compound Oxycodone and Atropine Injection is a colorless or pale yellow, clear liquid.

It is affected by light.

pH: 2.5 – 4.0

**Identification (1)** To 1 mL of Compound Oxycodone and Atropin Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

**(2)** Evaporate 1 mL of Compound Oxycodone and Atropin Injection on a water bath, and dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

**(3)** Evaporate 1 mL of Compound Oxycodone and Atropin Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

**(4)** To 1 mL of Compound Oxycodone and Atropine Injection add 0.5 mL of 2,4-dinitrophenylhydrazine-ethanol TS, and allow to stand for 1 hour. Centrifuge, and add acetone to the supernatant liquid until no more precipitate is produced. Allow to stand for 20 minutes, and centrifuge. To the supernatant liquid add potassium hydroxide TS until the liquid is light purple. Shake the liquid with 5 mL of dichloromethane, and separate the dichloromethane layer. Take 0.5 mL of the dichloromethane layer, and evaporate to dryness on a water bath. Add 5 drops of fuming nitric acid to the residue, and evaporate to dryness on a water bath. Cool, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced (atropine).

**Extractable volume** <*6.05*> It meets the requirement.

**Assay (1)** Oxycodone hydrochloride hydrate and hydrocotarnine hydrochloride hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 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 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 $Q_{Sb}$, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, $Q_{Sa}$ and $Q_{Sb}$, of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4.HCl.3H_2O$)
$= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times 1.1536 \times (1/25)$

Amount (mg) of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3.HCl.H_2O$)
$= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times 1.0699 \times (1/25)$

$W_{Sa}$: Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

$W_{Sb}$: Amount (mg) of hydrocotarnine hydrochloride for assay

*Internal standard solution*—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

*Operating conditions*—

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

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L sodium dihydrogenphosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone hydrochloride is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarine in this order with complete separation of these peaks.

**(2)** Atropine sulfate hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane layer through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate Reference Standard (separately determine its loss on drying <*2.41*> in the same manner as directed under Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use so obtained 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 ratios, $Q_T$ and $Q_S$, of the peak area of atropine to that of the internal standards.

Amount (mg) of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$
$= W_S \times (Q_T/Q_S) \times (1/50) \times 1.027$

$W_S$: Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

*Internal standard solution*—A solution of homatropine hydrobromide (1 in 4000).

*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 180- to 250-μm siliceous earth for gas chromatography coated with 1 to 3% of 50% phenyl-methylsilicone polymer.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and atropine in this order with the resolution between these peaks being not less than 3.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Oxydol

オキシドール

Oxydol contains not less than 2.5 w/v% and not more than 3.5 w/v% of hydrogen peroxide ($H_2O_2$: 34.01). It contains suitable stabilizers.

**Description** Oxydol occurs as a clear, colorless liquid. It is odorless or has an odor resembling that of ozone.

It gradually decomposes upon standing or upon vigorous agitation.

It rapidly decomposes when in contact with oxidizing substances as well as reducing substances.

It, when alkalized, decomposes with effervescence.

It is affected by light.

pH: 3.0 – 5.0

Specific gravity $d_{20}^{20}$: about 1.01

**Identification** 1 mL of Oxydol responds to the Qualitative Tests <*1.09*> for peroxide.

**Purity** **(1)** Acidity—To 25.0 mL of Oxydol add 2 drops of phenolphthalein TS and 2.5 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

**(2)** Heavy metals <*1.07*>—To 5.0 mL of Oxydol add 20 mL of water and 2 mL of ammonia TS, evaporate on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid by heating, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2 mL of dilute acetic acid, 2.5 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

**(3)** Arsenic <*1.11*>—To 1.0 mL of Oxydol add 1 mL of ammonia TS, evaporate on a water bath to dryness, take the residue, prepare the test solution according to Method 1, and perform the test (not more than 2 ppm).

**(4)** Organic stabilizer—Extract 100 mL of Oxydol with 50-mL, 25-mL and 25-mL portions of a mixture of chloroform and diethyl ether (3:2) successively, combine the extracts in a tared vessel, and evaporate the combined extract on a water bath. Dry the residue over silica gel to constant mass: the mass of the residue is not more than 50 mg.

**(5)** Nonvolatile residue—Evaporate 20.0 mL of Oxydol on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 20 mg.

**Assay** Pipet 1.0 mL of Oxydol, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate <*2.50*> with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS
$= 1.701$ mg of $H_2O_2$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, and not exceeding 30°C.

# Oxygen

酸素

$O_2$: 32.00

Oxygen contains not less than 99.5 v/v% of $O_2$.

**Description** Oxygen is a colorless gas, and is odorless.

1 mL of Oxygen dissolves in 32 mL of water, and in 7 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Oxygen at 0°C and at a pressure of 101.3 kPa weighs about 1.429 g.

**Identification** **(1)** Put a glowing splinter of wood into Oxygen: it bursts into flame immediately.

**(2)** Transfer 1 mL each of Oxygen and oxygen directly from metal cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <*2.02*> according to the conditions of Purity (5): the retention time of principal peak from Oxygen coincides with that of oxygen.

**Purity** Keep the containers of Oxygen between 18°C and 22°C for not less than 6 hours before carrying out the following tests, and calculate the volume to be used with reference to the gas at 20°C and at 101.3 kPa.

**(1)** Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 1000 mL of Oxygen through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

**(2)** Carbon dioxide—Pass 1000 mL of Oxygen through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that of the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

**(3)** Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, mix, and use these as solution A and solution B, respectively. Pass 2000 mL of Oxygen through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

**(4)** Chloride <*1.03*>—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, mix, and use these as solution A an solution B, respectively. Pass 1000 mL of Oxygen through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

**(5)** Nitrogen—Introduce 1.0 mL of Oxygen into a gas-measuring tube or syringe for gas chromatography from a metal hermetic container under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the peak area $A_T$ of nitrogen. Introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, and allow to mix thoroughly. Perform the test in the same manner with 1.0 mL of this mixture as directed above, and determine the peak area $A_S$ of nitrogen: $A_T$ is not larger than $A_S$.

*Operating conditions*—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 250- to 355-$\mu$m zeolite for gas chromatography (0.5 mm).

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

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 5 minutes.

Selection of column: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL, and mix well. Proceed with 1.0 mL of the mixture under the above operating conditions. Use a column giving well-resolved peaks of Oxygen and nitrogen in this order.

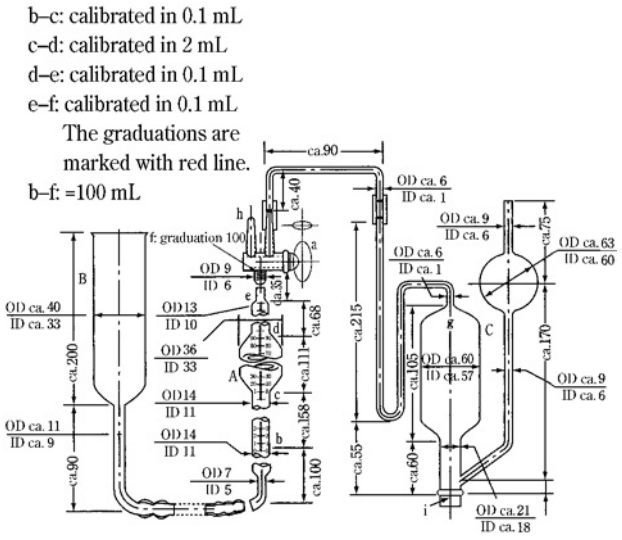
**Assay** (i) Apparatus—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock a, b – c, d – e and e – f are graduated in 0.1 mL, and c – d is graduated in 2 mL. A is properly connected with a leveling tube B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball g of the gas pipette C a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS, and stopper with a rubber stopper i. Connect C with A using the thick rubber tube.

(ii) Procedure—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h, and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C, and transfer the Oxygen to g by lifting B. Close a, and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward, and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant, and designate this as $V$ (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least four times, and measure the volume of residual gas. Calculate $V$ and the volume of Oxygen used as the sample with reference to the gas at 20°C and at 101.3 kPa.

Volume (mL) of oxygen ($O_2$)
= calculated volume of the sample (mL)
− calculated volume of $V$ (mL)

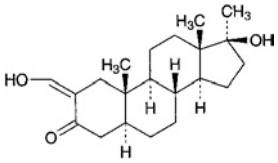
**Containers and storage** Containers—Metal cylinders.

Storage—Not exceeding 40°C.




# Oxymetholone

オキシメトロン



$C_{21}H_{32}O_3$: 332.48
17$\beta$-Hydroxy-2-hydroxymethylene-17$\alpha$-methyl-5$\alpha$-androstan-3-one [*434-07-1*]

Oxymetholone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{32}O_3$.

**Description** Oxymetholone occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored and decomposed by light.

**Identification** **(1)** Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95), and add 1 drop of iron (III) chloride TS: a purple color develops.

**(2)** Dissolve 0.01 g of Oxymetholone in methanol to make 50 mL. To 5 mL of the solution add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Oxymetholone as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +34 – +38° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

**Melting point** <*2.60*> 175 – 182°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear, and shows a colorless to pale yellow color.

**(2)** Related subslances—Dissolve 50 mg of Oxymetholone in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 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 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99.5) (49:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 100°C for 3 to 5 minutes: any spot other than the principal spot and starting point obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

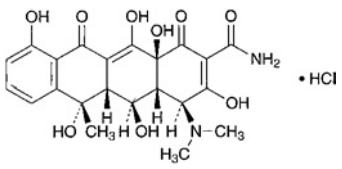
**Assay** Weigh accurately about 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance $A$ of this solution at the wavelength of maximum absorption at about 315 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.

$$\text{Amount (mg) of } C_{21}H_{32}O_3 = (A/541) \times 50{,}000$$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Oxytetracycline Hydrochloride

オキシテトラサイクリン塩酸塩



$C_{22}H_{24}N_2O_9$.HCl: 496.89
(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-Dimethylamino-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride [*2058-46-0*]

Oxytetracycline Hydrochloride is the hydrochloride

of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces rimosus*.

It contains not less than 880 $\mu$g (potency) and not more than 945 $\mu$g (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline ($C_{22}H_{24}N_2O_9$: 460.43).

**Description** Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of Oxytetracycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-188$ – $-200°$ (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Oxytetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

**(2)** Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 4-epioxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epioxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of $\beta$-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as $\beta$-apooxytetracycline stock solution. Pipet 1 mL of 4-epioxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of $\beta$-apooxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 4-epioxytetracycline and tetracycline obtained from the sample solution are not more than each of the peak area obtained from the standard solution, and the total area of the peaks, $\alpha$-apooxytetracycline having the relative retention time of about 2.1 with respect to oxytetracycline, $\beta$-apooxytetracycline and the peaks, which appear between $\alpha$-apooxytetracycline and $\beta$-apooxytetracycline, is not more than the peak area of $\beta$-apooxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the principal peak, obtained from the sample solution is not more than 4 times the peak area of 4-epioxytetracycline from the standard solution.

*Operating conditions*—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 $\mu$m in particle diameter).

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

Mobile phase A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of *t*-butanol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 100 g of *t*-butanol and water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 20 | 70 → 10 | 30 → 90 |
| 20 – 35 | 10 → 20 | 90 → 80 |

Flow rate: 1.0 mL/min

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline beginning after the solvent peak.

*System suitability*—

Test for required detectability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epioxytetracycline obtained from 20 $\mu$L of this solution is equivalent to 14 to 26% of that from 20 $\mu$L of the standard solution.

System performance: Dissolve 8 mg of $\alpha$-apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as $\alpha$-apooxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epioxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of $\beta$-apooxytetracycline stock solution and 6 mL of $\alpha$-apooxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 $\mu$L of this solution under the above operating conditions, 4-epioxytetracycline, oxytetracycline, tetracycline, $\alpha$-apooxytetracycline and $\beta$-apooxytetracycline are eluted in this order with the resolutions between the peaks, 4-epioxytetracycline and oxytetracycline, oxytetracycline and tetracycline, and $\alpha$-apooxytetracycline and $\beta$-apooxytetracycline being not less than 4, not less than 5 and not less than 4, respectively,

and the symmetry coefficient of the peak of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 $\mu$L of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-epioxytetracycline is not more than 2.0%.

**Loss on drying** ⟨*2.41*⟩ Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.5% (1 g).

**Assay** Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL each of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 $\mu$L 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 oxytetracycline.

Amount [$\mu$g (potency)] of oxytetracycline ($C_{22}H_{24}N_2O_9$) $= W_S \times (A_T/A_S) \times 1000$

$W_S$: Amount [mg (potency)] of Oxytetracycline Hydrochloride Reference Standard

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust the flow rate so that the retention time of oxytetracycline is about 7 minutes.

*System suitability—*

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.

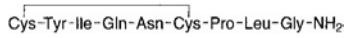
System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Oxytocin

オキシトシン




$C_{43}H_{66}N_{12}O_{12}S_2$: 1007.19
[*50-56-6*]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.

It contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the dehydrated and de-acetic acid basis.

**Description** Oxytocin occurs as a white powder.

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

It dissolves in hydrochloric acid TS.

The pH of a solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0.

It is hygroscopic.

**Identification** Determine the absorption spectrum of a solution of Oxytocin (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Constituent amino acids** Put about 1 mg of Oxytocin in a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with Nitrogen, seal the tube under reduced pressure, and heat at 110 to 115°C for 16 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, add 2 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 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 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the respective molar ratios with respect to leucine: 0.95 – 1.05 for aspartic acid, 0.95 – 1.05 for glutamic acid, 0.95 – 1.05 for proline, 0.95 – 1.05 for glycine, 0.80 – 1.10 for isoleucine, 0.80 – 1.05 for tyrosine and 0.80 – 1.05 for cystine, and not more than 0.01 each for others.

*Operating conditions—*

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B and C according to the following table.

| Mobile phase | A | B | C |
|---|---|---|---|
| Citric acid monohydrate | 19.80 g | 22.00 g | 6.10 g |
| Trisodium citrate dihydrate | 6.19 g | 7.74 g | 26.67 g |
| Sodium chloride | 5.66 g | 7.07 g | 54.35 g |
| Ethanol (99.5) | 260.0 mL | 20.0 mL | — |
| Benzyl alcohol | — | — | 5.0 mL |
| Thiodiglycol | 5.0 mL | 5.0 mL | — |
| Lauromacrogol solution (1 in 4) | 4.0 mL | 4.0 mL | 4.0 mL |
| Capryric acid | 0.1 mL | 0.1 mL | 0.1 mL |
| Water | a sufficient amount | a sufficient amount | a sufficient amount |
| Total amount | 2000 mL | 1000 mL | 1000 mL |
| pH | 3.3 | 3.2 | 4.9 |

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) | Mobile phase C (vol%) |
|---|---|---|---|
| 0 – 9 | 100 | 0 | 0 |
| 9 – 25 | 0 | 100 | 0 |
| 25 – 61 | 0 | 100 → 0 | 0 → 100 |
| 61 – 80 | 0 | 0 | 100 |

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 minutes while passing Nitrogen, and use this solution as Solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for more than 30 minutes while passing Nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

*System suitability—*

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

**Purity (1)** Acetic acid—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 200 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 acetic acid to that of the internal standard: the amount of acetic acid is not less than 6.0% and not more than 10.0%.

$$\text{Amount (\%) of acetic acid } (C_2H_4O_2) = (W_S/W_T) \times (Q_T/Q_S) \times (1/10)$$

$W_S$: Amount (mg) of acetic acid (100)

$W_T$: Amount (mg) of the sample

*Internal standard solution*—A solution of propionic acid in the mobile phase (1 in 10,000).

*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 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 3 minutes.

*System suitability—*

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.

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 acetic acid to that of the internal standard is not more than 2.0%.

**(2)** Related substances—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount

of each peak other than Oxytocin is not more than 1.5%, and the total of them is not more than 5.0%.

*Operating conditions*—

Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of oxytocin.

*System suitability*—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 $\mu$L of this solution is equivalent to 5 to 15% of that from 50 $\mu$L of the solution for system suitability test.

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 $\mu$L of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 50 $\mu$L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

**Water** *⟨2.48⟩* Not more than 5.0% (50 mg, coulometric titration).

**Assay** Weigh accurately an amount of Oxytocin, equivalent to about 13,000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 1 bottle of the Oxytocin Reference Standard in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 $\mu$L 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 oxytocin.

Units per mg of Oxytocin, calculated on the dehydrated and de-acetic acid basis

$$= (W_S/W_T) \times (A_T/A_S) \times 100$$

$W_S$: Units per mL of the standard solution

$W_T$: Amount (mg) of sample, calculated on the dehydrated and de-acetic acid basis

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 30 | 70→40 | 30→60 |
| 30 – 30.1 | 40→70 | 60→30 |
| 30.1 – 45 | 70 | 30 |

Flow rate: About 1.0 mL per minute.

*System suitability*—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 20 mL of the mobile phase A. When the procedure is run with 25 $\mu$L of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—At 2 to 8°C.

# Oxytocin Injection

オキシトシン注射液

Oxytocin Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled oxytocin Units.

**Method of preparation** Prepare as directed under Injections, with Oxytocin.

**Description** Oxytocin Injection is a colorless, clear liquid.

**pH** *⟨2.54⟩* 2.5 – 4.5

**Bacterial endotoxins** *⟨4.01⟩* Less than 10 EU/oxytocin Unit.

**Extractable volume** *⟨6.05⟩* It meets the requirement

**Foreign insoluble matter** *⟨6.06⟩* Perform the test according to the Method 1: it meets the requirement.

**Insoluble particulate matter** *⟨6.07⟩* Perform the test according to the Method 1: it meets the requirement.

**Sterility** *⟨4.06⟩* Perform the test according to the Membrane filtration method: it meets the requirement.

**Assay** Measure exactly a portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the sample solution. Separately, dissolve 1 bottle of Oxytocin Reference Standard in the mobile phase A to make exactly 20 mL. Pipet a suitable volume of this solution, dilute with the diluent to make a known concentration solution so that each

mL contains about 1 Unit, and use this solution as the standard solution. Perform the test with exactly 100 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 oxytocin.

$$\text{Units per mL of Oxytocin Injection} = W_S \times (A_T/A_S) \times (b/a)$$

$W_S$: Units per mL of the standard solution

$a$: Volume (mL) of sample

$b$: Total volume of the sample solution prepared by diluting with the diluent

Diluent: Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (99.5) in water to make 1000 mL.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 30 | 70→40 | 30→60 |
| 30 – 30.1 | 40→70 | 60→30 |
| 30.1 – 45 | 70 | 30 |

Flow rate: About 1.0 mL per minute.

*System suitability*—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 100 mL of the mobile phase A. When the procedure is run with 100 μL of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

**Containers and storage** Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

# Pancreatin

パンクレアチン

Pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog, and has amylolytic, proteolytic and lipolytic activities.

It contains not less than 2800 starch saccharifying activity units, not less than 28,000 proteolytic activity units, and not less than 960 lipolytic activity units per g.

It is usually diluted with suitable excipients.

**Description** Pancreatin occurs as a white to light yellow powder. It has a characteristic odor.

**Purity (1)** Rancidity—Pancreatin has no unpleasant or rancid odor and is tasteless.

**(2)** Fat—Add 20 mL of diethyl ether to 1.0 g of Pancreatin, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue does not exceed 20 mg.

**Loss on drying** <2.41> Not more than 4.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

**Residue on ignition** <2.44> Not more than 5% (1 g).

**Assay (1)** Starch digestive activity <4.03>

(i) Substrate solution—Use potato starch TS for amylolytic activity test, prepared by adding 10 mL of phosphate buffer solution for pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL. Pipet 10 mL of this solution, and add ice-cold water to make exactly 100 mL.

(iii) Procedure—Proceed as directed in (i) Measurement of starch saccharifying activity of (1) Assay for starch digestive activity under Digestion Test..

**(2)** Protein digestive activity <4.03>

(i) Substrate solution—Use the substrate solution 2 described in (2) Assay for protein digestive activity under Digestion Test after adjusting the pH to 8.5.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 200 mL.

(iii) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test, using trichloroacetic acid TS B as the precipitation reagent.

**(3)** Fat digestive activity <4.03>

(i) Emulsifier—Prepare with 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II as directed in (3) Assay for fat digestive activity under Digestion Test.

(ii) Substrate solution—Use the substrate solution described in (3) Assay for fat digestive activity under the Digestion Test.

(iii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and

add ice-cold water to make exactly 100 mL.

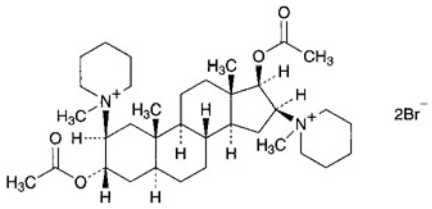
(iv) Procedure—Proceed as directed in (3) Assay for fat digestive activity under Digestion Test, using phosphate buffer solution, pH 8.0, as the buffer solution.

**Containers and storage** Containers—Tight containers.

Storage—Not exceeding 30°C.

# Pancuronium Bromide

パンクロニウム臭化物



$C_{35}H_{60}Br_2N_2O_4$: 732.67

1,1′-(3α,17β-Diacetoxy-5α-androstan-2β,16β-diyl)bis(1-methylpiperidinium) dibromide [*15500-66-0*]

Pancuronium Bromide contains not less than 98.0% and not more than 102.0% of $C_{35}H_{60}Br_2N_2O_4$, calculated on the dehydrated basis.

**Description** Pancuronium Bromide occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95) and in acetic anhydride.

It is hygroscopic.

**Identification** **(1)** Determine the infrared absorption spectrum of Pancuronium Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** A solution of Pancuronium Bromide (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for bromide.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +38 – +42° (0.75 g calculated on the dehydrated basis, water, 25 mL, 100 mm).

**pH** <*2.54*> The pH of a solution of Pancuronium Bromide (1 in 100) is between 4.5 and 6.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Pancuronium Bromide in 10 mL of water: the solution is clear and colorless.

**(2)** Related substances—Dissolve 50 mg of Pancuronium Bromide in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, weigh exactly 5 mg of dacuronium bromide for thin-layer chromatography, add ethanol (95) to make exactly 25 mL, 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 standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, acetonitrile and a solution of sodium iodide (1 in 5) (17:2:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of sodium nitrite in methanol (1 in 100) on the plate, allow to stand for 2 minutes, and spray evenly potassium bismuth iodide TS on the plate: a spot from the sample solution, corresponding to that from the standard solution (2), has no more color than that from the standard solution (2), and the spots other than the principal spot and the above mentioned spot from the sample solution have no more color than the spot from the standard solution (1).

**Water** <*2.48*> Not more than 8.0% (0.3 g, volumetric titration, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pancuronium Bromide, dissolve in 50 mL of acetic anhydride by warming, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

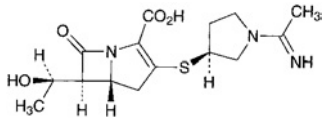
Each mL of 0.1 mol/L perchloric acid VS
= 36.63 mg of $C_{35}H_{60}Br_2N_2O_4$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Panipenem

パニペネム



$C_{15}H_{21}N_3O_4S$: 339.41

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-[(3*S*)-1-(1-iminoethyl)pyrrolidin-3-ylsulfanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid [*87726-17-8*]

Panipenem contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous and desolvent basis. The potency of Panipenem is expressed as mass (potency) of panipenem ($C_{15}H_{21}N_3O_4S$).

**Description** Panipenem occurs as a white to light yellow, crystalline powder or mass.

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

It is hygroscopic.

It deliquesces in the presence of moisture.

**Identification** **(1)** Dissolve 0.02 g of Panipenem in 2 mL of water, add 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

**(2)** Determine the absorption spectrum of a solution of Panipenem in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a

maximum between 296 nm and 300 nm.

**(3)** Determine the infrared absorption spectrum of Panipenem as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1760 $cm^{-1}$, 1676 $cm^{-1}$, 1632 $cm^{-1}$, 1588 $cm^{-1}$, 1384 $cm^{-1}$ and 1249 $cm^{-1}$.

**Absorbance** <2.24> $E^{1\%}_{1\,cm}$ (298 nm): 280 – 310 (50 mg calculated on the anhydrous and desolvent basis, 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 2500 mL).

**Optical rotation** <2.49> $[\alpha]^{20}_{D}$: +55 – +65° (0.1 g, calculated on the anhydrous and desolvent basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

**pH** <2.54> Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of the solution is between 4.5 and 6.5.

**Purity** **(1)** Clarity and color of solution—Being specified separately.

**(2)** Heavy metals <1.07>—Proceed with 1.0 g of Panipenem according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Residual solvents <2.46>—Weigh accurately about 0.2 g of Panipenem, transfer to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution and 2 mL of water to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, pipet 15 mL of ethanol (99.5) and 3 mL of acetone, add water to make exactly 200 mL. Pipet 1 mL and 2 mL of this solution, and add water to them to make exactly 20 mL. Transfer exactly 2 mL each of these solutions to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution, seal tightly a rubber stopper with aluminum cap, and use these solutions as the standard solution (1) and the standard solution (2). Shake gently in a water bath at a constant room temperature, and allow to stand for 30 minutes. Perform the test with 1 mL of the sample gas in each container as directed under Gas Chromatography <2.02> according to the following condition. Calculate the ratios, $Q_{Ta}$ and $Q_{Tb}$, of the peak area of ethanol and acetone to that of the internal standard from the sample solution, the ratios, $Q_{Sa1}$ and $Q_{Sb1}$, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (1), and the ratios, $Q_{Sa2}$ and $Q_{Sb2}$, of the peak area of ethanol and acetone to that of the internal standard from the standard solution (2). Calculate the amount of the ethanol and acetone by the following formula: ethanol is not more than 5.0% and acetone is not more than 1.0%.

$$\text{Amount (\%) of ethanol in Panipenem} = 15 \times 0.79 \times \{(Q_{Ta} + Q_{Sa2} - 2Q_{Sa1})/2(Q_{Sa2} - Q_{Sa1})\} \times (1/1000) \times (100/W)$$

$W$: Amount (g) of Panipenem

$$\text{Amount (\%) of acetone in Panipenem} = 3 \times 0.79 \times \{(Q_{Tb} + Q_{Sb2} - 2Q_{Sb1})/2(Q_{Sb2} - Q_{Sb1})\} \times (1/1000) \times (100/W)$$

$W$: amount (g) of Panipenem

0.79: Specific gravity ($d^{20}_{20}$) of ethanol (99.5) and acetone

*Internal standard solution*—A solution of 1-propanol (1 in 400).

*Operating conditions*—

Detector: Hydrogen flame-ionization detector

Column: A glass column 1 mm in inside diameter and 40 m in length, coated with porous polymer bead for gas chromatography.

Column temperature: A constant temperature of about 140°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 1-propanol is about 6 minutes.

*System suitability*—

System performance: When the procedure is run with 1 mL of the gas of the standard solution (2) under the above operating conditions, ethanol, acetone and the internal standard are eluted in this order with the resolution between ethanol and acetone being not less than 4.

System repeatability: When the test is repeated 6 times with 1 mL of the gas of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 5.0%.

**(4)** Related substances—Being specified separately.

**Water** <2.48> Weigh accurately about 0.5 g of Panipenem, transfer to a 15-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with 1 $\mu$L of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following condition, and calculate the ratios, $Q_T$, $Q_{S1}$ and $Q_{S2}$ of the peak area of water to that of the internal standard. Calculate the amount of water by the following formula: water is not more than 5.0%.

$$\text{Amount of water (\%)} = (W_S/W_T) \times \{(Q_T + Q_{S2} - 2Q_{S1})/2(Q_{S2} - Q_{S1})\} \times (1/100) \times 100$$

$W_S$: Amount (g) of weighed water

$W_T$: Amount (g) of Panipenem

*Internal standard solution*—A solution of acetonitrile in methanol (1 in 100).

*Operating conditions*—

Detector: A thermal conductivity detector

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 125°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of acetonitrile is about 8 minutes.

*System suitability*—

System performance: When the procedure is run with 1 $\mu$L of the standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted

in this order with the resolution between water and internal standard being not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of water to that of the internal standard is not more than 5.0%.

**Residue on ignition** Being specified separately.

**Bacterial endotoxins** ⟨*4.01*⟩ Less than 0.15 EU/mg (potency).

**Assay** Weigh accurately an amount of Panipenem and Panipenem Reference Standard, equivalent to about 0.1 g (potency), dissolve separately in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test within 30 minutes after preparation of the solutions with 10 μL 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 panipenem to that of the internal standard.

Amount [μg (potency)] of panipenem ($C_{15}H_{21}N_3O_4S$)
$= W_S \times (Q_T/Q_S) \times 1000$

$W_S$: Amount [mg (potency)] of Panipenem Reference Standard

*Internal standard solution*—A solution of sodium *p*-styrenesulfonate in 0.02 mol / L 3-(*N*-morpholino) propanesulfonic acid buffer solution, pH 7.0 (1 in 1000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

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

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

Mobile phase: A mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 8.0 and acetonitrile (50:1).

Flow rate: Adjust the flow rate so that the retention time of the internal standard 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, panipenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

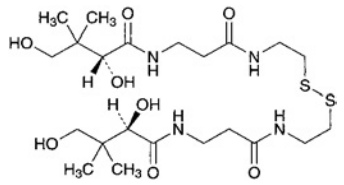
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of panipenem to that of the internal standard is not more than 2.0%.

**Containers and storage** Containers—Tight containers.

Storage—Not exceeding −10°C.

# Pantethine

パンテチン



$C_{22}H_{42}N_4O_8S_2$: 554.72

Bis(2-{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoylamino}ethyl) disulfide

[*16816-67-4*]

Pantethine is an aqueous solution containing 80% of pantethine.

Pantethine contains not less than 98.0% of pantethine ($C_{22}H_{42}N_4O_8S_2$), calculated on the anhydrous basis.

**Description** Pantethine is a clear, colorless to pale yellow viscous liquid.

It is miscible with water, with methanol and with ethanol (95).

It is decomposed by light.

**Identification** **(1)** To 0.7 g of Pantethine add 5 mL of sodium hydroxide TS, shake, and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color develops.

**(2)** To 0.7 g of Pantethine add 3 mL of water, shake, add 0.1 g of zinc powder and 2 mL of acetic acid (100), and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

**(3)** To 1.0 g of Pantethine add 500 mL of water, and shake. To 5 mL of this solution add 3 mL of 1 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylammonium chloride in sodium hydroxide TS (3 in 140), and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS, and add 1 mol/L hydrochloric acid TS dropwise until the solution has no color, and then add 1 mL of iron (III) chloride TS: a red-purple color develops.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +15.0 – +18.0° (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Pantethine 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).

**(2)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Pantethine according to Method 3, and perform the test (not more than 1 ppm).

**(3)** Related substances—Dissolve 0.6 g of Pantethine in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, 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 2-butanone saturated with water to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand for about 10 minutes in iodide vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**(4)** Mercapto compounds—To 1.5 g of Pantethine add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red color is not developed.

**Water** ⟨*2.48*⟩ 18 – 22% (0.2 g, volumetric titration, direct titration).

**Residue on Ignition** ⟨*2.44*⟩ Not more than 0.1% (2 g).

**Assay** Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle, and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately, and warm at 40 to 50°C for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

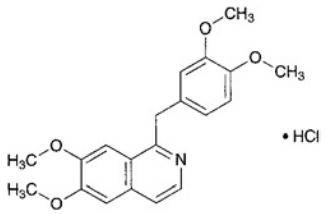
Each mL of 0.05 mol/L bromine VS
= 5.547 mg of $C_{22}H_{42}N_4O_8S_2$

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 10°C.

# Papaverine Hydrochloride

パパベリン塩酸塩




$C_{20}H_{21}NO_4$.HCl: 375.85
6,7-Dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline monohydrochloride [*61-25-6*]

Papaverine Hydrochloride, when dried, contains not less than 98.5% of $C_{20}H_{21}NO_4$.HCl.

**Description** Papaverine Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Papaverine Hydrochloride (1 in 50) is between 3.0 and 4.0.

**Identification (1)** To 1 mg of Papaverine Hydrochloride add 1 drops of formaldehyde-sulfuric acid TS: a colorless to light yellow-green color is produced, and it gradually changes to deep red, then to brown.

**(2)** Dissolve 0.02 g of Papaverine Hydrochloride in 1 mL of water, and add 3 drops of sodium acetate TS: a white precipitate is produced.

**(3)** Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

**(4)** Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath, and dry the residue at 105°C for 3 hours: the residue so obtained melts ⟨*2.60*⟩ between 145°C and 148°C.

**(5)** Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

**Purity (1)** Clarity and color of solution—Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Morphine—Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrate (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

**(3)** Readily carbonizable substances ⟨*1.15*⟩—Perform the test with 0.12 g of Papaverine Hydrochloride: the solution has no more color than Matching Fluid S or P.

**Loss on drying** ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.5 g of Papaverine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.59 mg of $C_{20}H_{21}NO_4$.HCl

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Papaverine Hydrochloride Injection

パパベリン塩酸塩注射液

Papaverine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of papaverine hydrochloride ($C_{20}H_{21}NO_4$.HCl: 375.85).

**Method of preparation** Prepare as directed under Injections, with Papaverine Hydrochloride.

**Description** Papaverine Hydrochloride Injection is a clear, colorless liquid.

pH: 3.0 – 5.0

**Identification** **(1)** To 1 mL of Papaverine Hydrochloride Injection add 3 drops of sodium acetate TS: a white precipitate is produced.

**(2)** Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of Papaverine hydrochloride according to the labeled amount, with water to 10 mL, render the solution alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C for 3 hours: the residue so obtained melts ⟨*2.60*⟩ between 145°C and 148°C.

**(3)** Proceed with 1 mg each of the residue obtained in (2) as directed in the Identification (1) and (3) under Papaverine Hydrochloride.

**(4)** Alkalify 2 mL of Papaverine Hydrochloride Injection with ammonia TS, filter the precipitate off, and acidity the filtrate with dilute nitric acid: the solution responds to Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Assay** Dilute an exactly measured volume of Papaverine Hydrochloride Injection, equivalent to about 0.2 g of papaverine hydrochloride ($C_{20}H_{21}NO_4$.HCl), with water to 10 mL, render the solution alkaline with ammonia TS, and extract with 20-mL, 15-mL, 10-mL and 10-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and re-extract the washings with two 5-mL portions of chloroform. Combine all the chloroform extracts, and distil the chloroform on a water bath. Dissolve the residue in 30 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.79 mg of $C_{20}H_{21}NO_4$.HCl

**Containers and storage** Containers—Hermetic containers.
Storage—Light-resistant.

# Paraffin

パラフィン

Paraffin is a mixture of solid hydrocarbons obtained from petroleum.

**Description** Paraffin occurs as a colorless or white, more or less transparent, crystalline mass. It is odorless and tasteless.

Paraffin is sparingly soluble in diethyl ether and practically insoluble in water, in ethanol (95) and in ethanol (99.5).

Specific gravity $d_{20}^{20}$: about 0.92 (proceed as directed in the Specific gravity (2) under Fats and Fatty Oils ⟨*1.13*⟩).

**Identification** **(1)** Heat Paraffin strongly in a porcelain dish, and ignite: it burns with a bright flame and the odor of paraffin vapor is perceptible.

**(2)** Heat 0.5 g of Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

**Melting point** ⟨*2.60*⟩ 50 – 75°C (Method 2).

**Purity** **(1)** Acidity or alkalinity—Boil 10.0 g of Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS in a water bath for 5 minutes, and shake vigorously: a red color is not produced. Add 0.20 mL of 0.02 mol/L sodium hydroxide VS to this solution, and shake: a red color is produced.

**(2)** Heavy metals ⟨*1.07*⟩—Ignite 2.0 g of Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, 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 10 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Paraffin according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Sulfur compounds—To 4.0 g of Paraffin add 2 mL of ethanol (99.5), further add 2 drops of a clear saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and heat for 10 minutes at 70°C with occasional shaking: no dark brown color develops in the aqueous layer.

**(5)** Readily carbonizable substances—Melt 5.0 g of Paraffin placed in a Nessler tube at a temperature near the melting point. Add 5 mL of sulfuric acid for readily carbonizable substances, and warm at 70°C for 5 minutes in a water bath. Remove the tube from the water bath, immediately shake vigorously and vertically for 3 seconds, and warm for 1 minute in a water bath at 70°C. Repeat this procedure five times: the color of the sulfuric acid layer is not darker than that of the following control solution.

Control solution: Add 1.5 mL of Cobaltous Chloride Colorimetric Stock Solution, 0.5 mL of Cupric Sulfate Colorimetric Stock Solution and 5 mL of liquid paraffin to 3.0 mL of Ferric Chloride Colorimetric Stock Solution, and shake vigorously.

**Containers and storage** Containers—Well-closed containers.

# Liquid Paraffin

流動パラフィン

Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petrolatum.

Tocopherols of a suitable form may by added at a concentration not exceeding 0.001% as a stabilizer.

**Description** Liquid Paraffin is a colorless, transparent, oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C.

**Identification** **(1)** Heat Liquid Paraffin strongly in a por-

celain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

**(2)** Heat 0.5 of Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

**Specific gravity** ⟨*2.56*⟩ $d_{20}^{20}$: 0.860 – 0.890

**Viscosity** ⟨*2.53*⟩ Not less than 37 $mm^2/s$ (Method 1, 37.8°C).

**Purity** **(1)** Odor—Transfer a suitable amount of Liquid Paraffin to a small beaker, and heat on a water bath: a foreign odor is not perceptible.

**(2)** Acidity or alkalinity—Shake vigorously 10 mL of Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide: a red color develops.

**(3)** Heavy metals ⟨*1.07*⟩—Ignite 2.0 g of Liquid Paraffin in a crucible, first moderately until charred, then between 450 °C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, 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 10 ppm).

**(4)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Liquid Paraffin, according to Method 3 except that after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), add 1.5 mL of hydrogen peroxide (30), fire to burn, and perform the test (not more than 2 ppm).

**(5)** Solid paraffin—Transfer 50 mL of Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 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.

**(6)** Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

**(7)** Polycyclic aromatic hydrocarbons—Take 25 mL of Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution obtained as the sample solution. Transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to another 50-mL separator, shake vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

**(8)** Readily carbonizable substances—Transfer 5 mL of Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the Liquid Paraffin layer remains unchanged in color, and the sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Ferric Chloride Colorimetric Stock Solution with 1.5 mL of Cobaltous Chloride Colorimetric Stock Solution and 0.50 mL of Cupric Sulfate Colorimetric Stock Solution.

**Containers and storage** Containers—Tight containers.

# Light Liquid Paraffin

軽質流動パラフィン

Light Liquid Paraffin is a mixture of hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

**Description** Light Liquid Paraffin is a clear, colorless oily liquid, nearly free from fluorescence. It is odorless and tasteless.

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

Boiling point: above 300°C

**Identification** **(1)** Heat Light Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

**(2)** Heat 0.5 of Light Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

**Specific gravity** ⟨*2.56*⟩ $d_{20}^{20}$: 0.830 – 0.870

**Viscosity** ⟨*2.53*⟩ Less than 37 $mm^2/s$ (Method 1, 37.8°C).

**Purity** **(1)** Odor—Transfer a suitable amount of Light Liquid Paraffin to a small beaker, and heat on a water bath: no foreign odor is perceptible.

**(2)** Acidity or alkalinity—Shake vigorously 10 mL of Light Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide: a red color develops.

**(3)** Heavy metals ⟨*1.07*⟩—Ignite 2.0 g of Light Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of

hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, 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 10 ppm).

**(4)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Light Liquid Paraffin according to Method 3, and perform the test (not more than 2 ppm).

**(5)** Solid paraffin—Transfer 50 mL of Light Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 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.

**(6)** Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Light Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

**(7)** Polycyclic aromatic hydrocarbons—Take 25 mL of Light Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as the sample solution. Separately, transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to a 50-mL separator, add 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

**(8)** Readily carbonizable substances—Transfer 5 mL of Light Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the liquid paraffin layer remains unchanged in color, and sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Ferric Chloride Colorimetric Stock Solution with 1.5 mL of Cobaltous Chloride Colorimetric Stock Solution and 0.50 mL of Cupric Sulfate Colorimetric Stock Solution.

**Containers and storage** Containers—Tight containers.

# Paraformaldehyde

パラホルムアルデヒド

$(CH_2O)_n$
Poly(oxymethylene) [*30525-89-4*]

Paraformaldehyde contains not less than 95.0% of $CH_2O$: 30.03.

**Description** Paraformaldehyde occurs as a white powder. It has a slight odor of formaldehyde, but a very strong irritating odor is perceptible when it is heated.

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

It dissolves in hot water, in hot dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

It sublimes at about 100°C.

**Identification** **(1)** Dissolve 0.1 g of Paraformaldehyde in 5 mL of ammonia TS, add 5 mL of silver nitrate TS, shake, and add 3 mL of a solution of sodium hydroxide (1 in 10): a mirror of metallic silver is immediately formed on the sides of the container.

**(2)** Add a solution of 0.04 g of salicylic acid in 5 mL of sulfuric acid to 0.02 g of Paraformaldehyde, and warm slowly: a persistent, dark red color is produced.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.20 g of Paraformaldehyde in 10 mL of ammonia TS: the solution is clear and colorless.

**(2)** Acidity or alkalinity—To 0.5 g of Paraformaldehyde add 10 mL of water, shake vigorously for 1 minute, and filter: the filtrate is neutral.

**(3)** Chloride <*1.03*>—Dissolve 1.5 g of Paraformaldehyde in 75 mL of water and 7.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 7.5 mL of sodium carbonate TS, a volume of diluted nitric acid (3 in 10) required for neutralization of the sample, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.006%).

**(4)** Sulfate <*1.14*>—Dissolve 1.5 g of Paraformaldehyde in 45 mL of water and 4.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at abut 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize the diluted hydrochloric acid (3 in 5), and boil for 5 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 4.5 mL of sodium carbonate TS add an equal volume of diluted hydrochloric acid (3 in 5) for the neutralization of the sample and 15 mL of water, and boil for 5 minutes. After cooling, add 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid and water to make

50 mL (not more than 0.011%).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Dissolve about 50 mg of Paraformaldehyde, accurately weighed, in 10 mL of potassium hydroxide TS in an iodine flask. Add 40 mL of water and an exactly measured 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand for 5 minutes. Then add 5 mL of dilute hydrochloric acid, stopper immediately, allow to stand for 15 minutes, and titrate ⟨*2.50*⟩ the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of $CH_2O$

**Containers and storage** Containers—Tight containers.

# Dental Paraformaldehyde Paste

歯科用パラホルムパスタ

**Method of preparation**

| | |
|---|---|
| Paraformaldehyde, finely powdered | 35 g |
| Procaine Hydrochloride, finely powdered | 35 g |
| Hydrous Lanolin | a sufficient quantity |
| | To make 100 g |

Prepare as directed under Ointments, with the above ingredients.

**Description** Dental Paraformaldehyde Paste is yellowish white in color. It has a characteristic odor.

**Identification** **(1)** To 0.15 g of Dental Paraformaldehyde Paste add 20 mL of diethyl ether and 20 mL of 0.5 mol/L sodium hydroxide TS, shake well, separate the water layer, and dilute with water to make 100 mL. To 1 mL of this solution add 10 mL of acetylacetone TS, and heat on a water bath for 10 minutes: a yellow color is produced (paraformaldehyde).

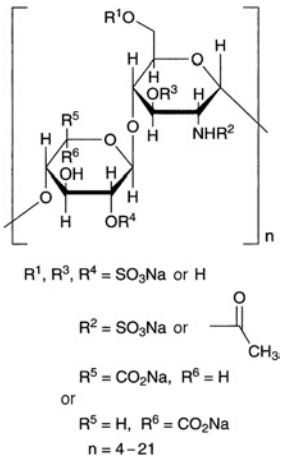
**(2)** To the diethyl ether layer obtained in (1) add 5 mL of dilute hydrochloric acid and 20 mL of water, shake well, and separate the water layer: the solution responds to Qualitative Tests ⟨*1.09*⟩ for primary aromatic amines (procaine hydrochloride).

**(3)** To 0.15 g of Dental Paraformaldehyde Paste add 25 mL of diethyl ether and 25 mL of water, shake, separate the water layer, filter, and use the filtrate as the sample solution. Seperately, dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as 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 ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots from the sample solution and standard solution show the same *R*f value.

**Containers and storage** Containers—Tight containers.

# Parnaparin Sodium

パルナパリンナトリウム




Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and with copper (II) acetate, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6400.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per milligram calculated with reference of the dried substance.

**Description** Parnaparin Sodium occurs as a white or light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

**Identification** **(1)** Mix 0.1 mL of a solution of Parnaparin Sodium (1 in 20) and 10 mL of a solution of tritoluidine blue O (1 in 100,000), and shake the mixture: the blue color of solution immediately changes to purple.

**(2)** A solution of Parnaparin Sodium (1 in 20) responds to Qualitative Tests ⟨*1.09*⟩ for sodium salt.

**pH** ⟨*2.54*⟩ Dissolve 0.1 g of Parnaparin Sodium in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Parnaparin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Parnaparin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 8.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours)

**Molecular mass** Calculate the molecular mass of Parnaparin Sodium by the following methods: The mass-average molecular mass ranges between 4500 and 6400.

(i) Creation of calibration curve Weigh 20 mg of low-molecular mass heparin for calibration of molecular mass, and dissolve it in 2.0 mL of the mobile phase as the standard

solution. Perform the test with 50 μL of the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak height, $H_{UV}$, in chromatogram obtained by the ultraviolet absorption photometer, and determine the peak height, HRI, in chromatogram obtained by the differential refractometer. Calculate the ratio of $H_{UV}$ to $H_{RI}$, $H_{RI}/H_{UV}$, at each peak. Assume the molecular mass in the 4th peak from the low molecular mass in chromatogram obtained by the ultraviolet absorption photometer as 2400, and make the calculation of the standard coefficient from dividing 2400 by the $H_{RI}/H_{UV}$ at the corresponding peak. Make the calculation to multiply the $H_{RI}/H_{UV}$ at each peak by the standard coefficient, and determine the molecular mass of each peak by the calculation. Prepare the calculation curve by plotting the logarithm of molecular masses at each peak on the vertical axis and the retention time on the chromatogram obtained by the differential refractometer on the horizontal axis.

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 234 nm) and a differential refractometer.

Column: Connect two stainless steel columns which are 7.5 mm in inside diameter and 30 cm in length, and are packed with porous silica gel for liquid chromatography; one column, the molecular mass of limited size exclusion is about 500,000; the other, the molecular mass of limited size exclusion is about 100,000. Connect a pump, the about 500,000-molecular mass of limited size exclusion column, the about 100,000-molecular mass of limited size exclusion column, the ultraviolet absorption photometer and the differential refractometer in this order.

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

Mobile phase: Dissolve 28.4 g of sodium sulfate anhydride in 1000 mL of water, and 5.0 with 0.05 mol/L sulfuric acid TS.

Flow rate: 0.5 mL/min

*System suitability—*

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, confirm that more than ten peaks in chromatogram obtained as directed under either the Ultraviolet-visible Spectrophotometry, or the Differential Refractometry are observed.

System repeatability: When the tests repeated 6 times with 50 μL of the standard solution under the above operating conditions, relative standard deviation of the 4th peak height in chromatogram ($H_{UV}$ and $H_{RI}$) is not more than 3.0%.

(ii) Determination of molecular mass Dissolve the 20 mg of Parnaparin Sodium with 2.0 mL of mobile phase, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Divide the main peak observed between 30 min and 45 min to 30 sec-interval fractions, and determine the strength of differential refractometer of each 30 sec-interval fraction. Determine the molecular mass of each fraction using the calibration curve and the retention time of each fraction. Determine the mean of molecular mass in the entire peak using the strength of differential refractometer and the molecular mass in every fractions.

Mean molecular mass of parnaparin sodium

$$= \Sigma(n_i \cdot M_i)/\Sigma n_j$$

$n_i$: The differential refractometer strength of fraction i in the main peak of chromatogram

$M_i$: Molecular mass of fraction i in main peak

$\Sigma n_i$: Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

*Operating conditions—*

Detector: A differential refractometer.

Column, column temperature, mobile phase, and flow rate: Proceed as directed in (i) Creation of calibration curve.

*System suitability—*

Proceed as directed in (i) Creation of calibration curve.

**Distribution of molecular mass** The molecular mass of Parnaparin Sodium is calculated as directed in the determination of molecular mass and the distribution of molecular mass is determined by the following equation: the molecular mass of not less than 80% parnaparin sodium is between 1500 and 10,000.

Distribution of molecular mass (%)

$$= (\Sigma n_j/\Sigma n_i) \times 100$$

$n_i$: The differential refractometer strength of fraction i in the main peak of chromatogram

$\Sigma n_j$: Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

**The degree of sulfate ester** Dissolve 0.5 g of Parnaparin Sodium with 10 mL water. Treat the solution with 5 mL of a strongly basic ion exchange resin, and subsequently with 10 mL of a strongly acidic ion exchange resin. Dilute the solution with water to 50 mL, and titrate <2.50> with 0.1 mol/L Sodium hydroxide VS (potentiometric titration). Determine the degree of sulfate ester of Parnaparin Sodium from the equivalence point by the following equation; it is between 2.0 and 2.4.

The degree of sulfate ester

= the first equivalence point (mL)/[the second equivalence point (mL) – first equivalence point (mL)]

**Total nitrogen** Weigh accurately about 0.10 g of Parnaparin Sodium which is dried, and perform the test as directed under Nitrogen Determination <1.08>: it contains not less than 1.9% and not more than 2.3% of nitrogen (N:14.01).

**Anti-factor IIa activity** When the potency of anti-factor IIa activity is determined according to the following method, it contains not less than 35 and not more than 60 low-molecular-mass-heparin unit per milligram calculated with reference to the dried substance.

(i) Standard solution Dissolve low-molecular weight heparin standard with isotonic sodium chloride solution to make solutions which contain 0.1, 0.2 and 0.3 low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it with isotonic sodium chloride solution to adjust the solution which contains 4 μg parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of the sample solution and the standard solution, separately. To

each tube add 0.10 mL of human normal plasma and mix it, and incubate at 37 ± 1°C accurately for 1 min. Next, to each test tube add 0.10 mL of activated thromboplastin-time assay solution, which is pre-warmed at 37 ± 1°C, and after the mixing incubate accurately for 5 min at 37 ± 1°C. Then, to each tube add 0.10 mL of sodium calcium solution (277 in 100,000) which is pre-warmed at 37 ± 1°C, mix it, start a stop watch simultaneously, and permit to stand at the same temperature. Determine the time for the first appearance of fibrin clot.

(iv) Calculation Determine the low-molecular-mass-heparin unit (anti-factor IIa activity) of the sample solution from calibration curve obtained plots of clotting times for each standard solution; calculate the low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium as following equation.

The low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium

= the low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL of sample solution × ($b/a$)

$a$: Amount (mg) of Parnaparin Sodium taken
$b$: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution.

**The ratio of anti-factor Xa activity to anti-factor IIa activity**
Divide the anti-factor Xa activity, obtained in the Assay, by the anti-factor IIa activity which has been obtained from the test according to the method of anti-factor IIa activity; the ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

**Assay**
(i) Standard solution Dissolve low-molecular-mass-heparin for calculation of molecular mass in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units, (anti-factor Xa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it in isotonic sodium chloride solution to make a solution which contains 7 $\mu$g parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of either the sample solution or the standard solution, separately. Subsequently to the every tubes add 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III solution, and 0.10 mL of normal human plasma, and mix them. To another plastic tube transfer 0.20 mL of these solutions, separately, and incubate for accurate 3 minutes at 37 ± 1°C. Next, to each tube add 0.10 mL of facter Xa TS and mix it, permit to stand 37 ± 1°C accurately for 30 seconds, and immediately add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 min at 37 ± 1°C. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin solution, and 0.10 mL of normal human plasma to every tubes, and mix well. To another plastic tube transfer 0.2 mL of the solution, separately, and add both 0.30 mL of water and 0.30 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of both the sample solution and the standard solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a solution obtained from this solution as the blank.

(iv) Calculation method Determine the low-molecular-mass unit (anti-factor Xa activity) of the sample solution using the calibration curve prepared from the absorbance of the standard solutions and their logarithmic concentrations, and calculate the low-molecular-mass unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium.

Low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium

= the low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mL of the sample solution × ($b/a$)

$a$: Amount (mg) of Parnaparin Sodium taken
$b$: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution.

**Container and Storage**
Container—Well-closed 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

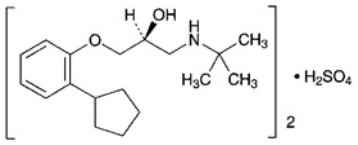
**Unsaponifiable matters** ⟨*1.13*⟩ Not more than 1.5%.

**Iodine value** ⟨*1.13*⟩ 84 – 103

**Containers and storage** Containers—Tight containers.

# Penbutolol Sulfate

ペンブトロール硫酸塩



$(C_{18}H_{29}NO_2)_2.H_2SO_4$: 680.94
(2*S*)-3-(2-Cyclopentylphenoxy)-1-(1,1-dimethylethyl)aminopropan-2-ol hemisulfate
[*38363-32-5*]

Penbutolol Sulfate, when dried, contains not less than 98.5% of $(C_{18}H_{29}NO_2)_2.H_2SO_4$.

**Description** Penbutolol Sulfate occurs as a white crystalline powder.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in acetic anhydride and in diethyl ether.

**Identification** **(1)** Determine the absorption spectrum of a solution of Penbutolol Sulfate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Penbutolol Sulfate, previously dried, as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Dissolve 0.1 g of Penbutolol Sulfate in 25 mL of water by warming, and cool: this solution responds to Qualitative Tests ⟨*1.09*⟩ for sulfate.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: −23 – −25° (after drying, 0.2 g, methanol, 20 mL, 100 mm).

**Melting point** ⟨*2.60*⟩ 213 – 217°C

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Penbutolol Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.8 g of Penbutolol Sulfate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, ethanol (95) and ammonia solution (28) (85:12:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (1 g).

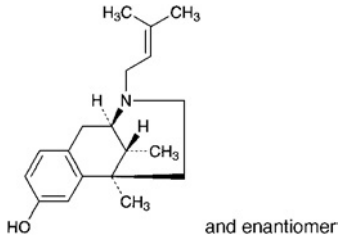
**Assay** Weigh accurately about 0.8 g of Penbutolol Sulfate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 68.09 mg of $(C_{18}H_{29}NO_2)_2.H_2SO_4$

**Containers and storage** Containers—Well-closed containers.

# Pentazocine

ペンタゾシン



and enantiomer

$C_{19}H_{27}NO$: 285.42
(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzoazocin-8-ol
[*359-83-1*]

Pentazocine, when dried, contains not less than 99.0% of $C_{19}H_{27}NO$.

**Description** Pentazocine occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in diethyl ether and practically insoluble in water.

**Identification** **(1)** To 1 mg of Pentazocine add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is produced, and it changes to grayish brown immediately.

**(2)** Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and heat in a water bath for 2 minutes: the color of the solution changes from light yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

**(3)** Determine the absorption spectrum of a solution of Pentazocine in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Absorbance** <2.24> $E_{1\,cm}^{1\%}$ (278 nm): 67.5 – 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).

**Melting point** <2.60> 150 – 158°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Pentazocine in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

**(2)** Heavy metals <1.07>—Proceed with 1.0 g of Pentazocine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <1.11>—Prepare the test solution with 1.0 g of Pentazocine according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.20 g of Pentazocine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 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 chloroform, methanol and isopropylamine (94:3:3) to a distance of about 13 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

**Residue on ignition** <2.44> Not more than 0.2% (1 g).

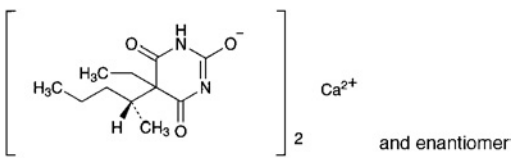
**Assay** Weigh accurately about 0.5 g of Pentazocine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.54 mg of $C_{19}H_{27}NO$

**Containers and storage** Containers—Well-closed containers.

# Pentobarbital Calcium

ペントバルビタールカルシウム




$C_{22}H_{34}CaN_4O_6$: 490.61
Monocalcium bis[5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidin-2-olate]
[*76-74-4*, Pentobarbital]

Pentobarbital Calcium contains not less than 98.0% and not more than 102.0% of $C_{22}H_{34}CaN_4O_6$, calculated on the dried basis.

**Description** Pentobarbital Calcium occurs as a white powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

A solution of Pentobarbital Calcium (1 in 100) shows no optical rotation.

**Identification** **(1)** Determine the infrared absorption spectrum of Pentobarbital Calcium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** To 1 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, shake with 5 mL of dilute hydrochloric acid and 10 mL of water, allow to cool, and filter. To the filtrate add 1 drop of methyl red TS, and add ammonia TS until a slight yellow color develops: the solution responds to Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

**Purity** **(1)** Chloride <1.03>—To 1.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 2.5 mL of dilute nitric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 15 mL add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 1.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

**(2)** Heavy metals <1.07>—To 2.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, cool, add water to make 80 mL, shake well, and filter. Discard the first 10 mL of the filtrate, to the subsequent 40 mL add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, then add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

**(3)** Related substances – Dissolve 10 mg of Pentobarbital Calcium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 calculate the areas of each peak by the automatic integration method: the area of any peak other than the peak of pentobarbital from the sample solution is not bigger than 3/10 of the peak area of pentobarbital from the standard so-

lution, and the total of these peak area is not bigger than the peak area of pentobarbital from the standard solution.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of pentobarbital beginning after the solvent peak.

*System suitability*—

Test for required detection: Pipet 2 mL of the standard solution, add water to make exactly 20 mL, and confirm that the peak area of pentobarbital obtained from 20 $\mu$L of this solution is equivalent to 5 to 15% of that of pentobarbital obtained from 20 $\mu$L of the standard solution.

System performance: Proceed as directed in the system performance in the Assay.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pentobarbital is not more than 5%.

**Loss on drying** ⟨*2.41*⟩ Not more than 7.0% (1 g, 105°C, 5 hours).

**Assay** Weigh accurately about 20 mg of Pentobarbital Calcium, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Pentobarbital Reference Standard, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 pentobarbital to that of the internal standard.

$$\text{Amount (mg) of } C_{22}H_{34}CaN_4O_6 = W_S \times (Q_T/Q_S) \times 1.0841$$

$W_S$: Amount (mg) of Pentobarbital Reference Standard

*Internal standard solution*—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitorile, and add water to make 100 mL.

*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 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.36 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitorile.

Flow rate: Adjust the flow rate so that the retention time of pentobarbital is about 7 minutes.

*System suitability*—

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, pentobarbital and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

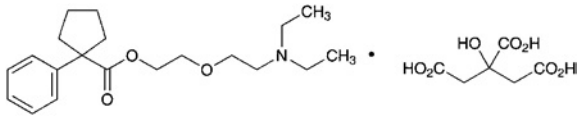
System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Well-closed containers.

# Pentoxyverine Citrate

## Carbetapentane Citrate
## Carbetapentene Citrate

ペントキシベリンクエン酸塩



$C_{20}H_{31}NO_3.C_6H_8O_7$: 525.59

2-[2-(Diethylamino)ethoxy]ethyl 1-phenylcyclopentanecarboxylate monocitrate

[*23142-01-0*]

Pentoxyverine Citrate, when dried, contains not less than 98.5% of $C_{20}H_{31}NO_3.C_6H_8O_7$.

**Description** Pentoxyverine Citrate occurs as a white, crystalline powder.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

**Identification** **(1)** Dissolve 0.1 g of Pentoxyverine Citrate in 10 mL of water, and add 10 mL of Reinecke salt TS: a light red precipitate is formed.

**(2)** Determine the infrared absorption spectrum of Pentoxyverine Citrate, previously dried, as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Pentoxyverine Citrate (1 in 10) responds to Qualitative Tests ⟨*1.09*⟩ (1) and (2) for citrate.

**Melting point** ⟨*2.60*⟩ 92 – 95°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Pentoxyverine Citrate in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Pentoxyverine Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Pentoxyverine Citrate according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.20 g of Pentoxyverine Citrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, 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 each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28) (25:10:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

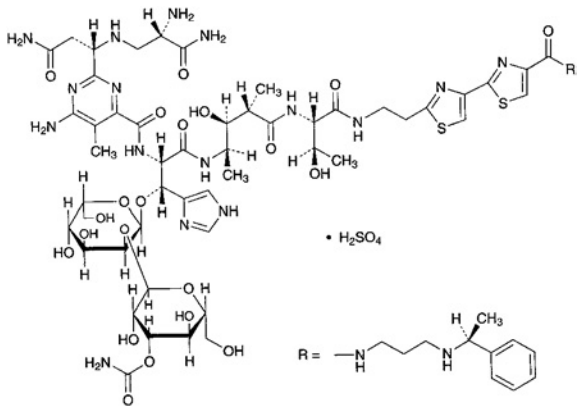
**Assay** Weigh accurately about 0.5 g of Pentoxyverine Citrate, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.56 mg of $C_{20}H_{31}NO_3.C_6H_8O_7$

**Containers and storage** Containers—Well-closed containers.

# Peplomycin Sulfate

ペプロマイシン硫酸塩



$C_{61}H_{88}N_{18}O_{21}S_2.H_2SO_4$: 1571.67
$N^1$-{3-[(1*S*)-(1-Phenylethyl)amino]propyl} bleomycinamide monosulfate [*70384-29-1*]

Peplomycin Sulfate is the sulfate of a substance having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 865 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is expressed as mass (potency) of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

**Description** Peplomycin Sulfate occurs as a white to light yellowish white powder.

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

It is hygroscopic.

**Identification (1)** To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate Reference Standard in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that from the standard soution.

*Operating conditions*—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

**(3)** A solution of Peplomycin Sulfate (1 in 200) responds to Qualitative Tests <1.09> (1) and (2) for sulfate.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: −2 – −5° (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution, pH 5.3, 10 mL, 100 mm).

**pH** <2.54> The pH of a solution obtained by dissolving 0.10 g of Peplomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

**Purity (1)** Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

**(2)** Copper—Dissolve exactly 75 mg of Peplomycin Sulfate in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to 5.0 mL of Standard Copper Stock Solution add diluted nitric acid (1 in 100) to make exactly 100 mL. To 3.0 mL of this solution add diluted nitric acid (1 in 100) to make exactly 100 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 200 ppm).

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: Copper hollow cathode lamp
Wavelength: 324.8 nm

**(3)** Related substances—Dissolve about 10 mg of Peplomycin Sulfate in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chro-

matography <2.01> according to the following conditions. Determine the areas of the peaks, appeared after the peak of copper sulfate, by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than peplomycin is not more than 7.0%.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 (7 μm in particle diameter).

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

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 60 | 100 → 0 | 0 → 100 |
| 60 – 75 | 0 | 100 |

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin beginning after the peak of copper sulfate.

*System suitability*—

Test for required detectability: Measure exactly 1 mL of the sample solution, add water to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of peplomycin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of peplomycin is not more than 2.0%.

**Loss on drying** <2.41> Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar media for seed and base layer, and for transferring test organism

| | |
|---|---|
| Glycerin | 10.0 g |
| Peptone | 10.0 g |
| Meat extract | 10.0 g |
| Sodium chloride | 3.0 g |
| Agar | 15.0 g |
| Water | 1000 mL |

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iii) Liquid medium for suspending test organism

| | |
|---|---|
| Glycerin | 10.0 g |
| Peptone | 10.0 g |
| Meat extract | 10.0 g |
| Sodium chloride | 3.0 g |
| Water | 1000 mL |

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iv) Preparation of agar medium of seeded layer—Inoculate the test organism onto the slant of the agar medium for transferring test organism, and incubate the slant at 27°C for 40 to 48 hours. Inoculate the subcultured test organism into 100 mL of the liquid medium for suspending test organism, incubate at 25 to 27°C for 5 days while shaking, and use this suspension as the suspension of the test organism. Keep the suspension of the test organism at a temperature of not exceeding 5°C and use within 14 days. Add 0.5 mL of the suspension of the test organism in 100 mL of the Agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the agar medium of seeded layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics with the exception of the amounts of the agar medium for base layer and the agar medium of seeded layer to put in the Petri dish, which are 5.0 mL and 8.0 mL, respectively.

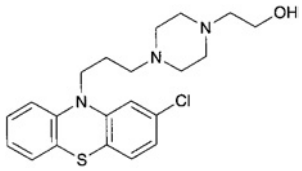
(vi) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C, and use within 15 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Peplomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μg (potency) and 2 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers

# Perphenazine

ペルフェナジン




$C_{21}H_{26}ClN_3OS$: 403.97

2-{4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl}ethanol [*58-39-9*]

Perphenazine, when dried, contains not less than 98.5% of $C_{21}H_{26}ClN_3OS$.

**Description** Perphenazine occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in ethanol (95), soluble in acetic acid (100), sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

**Identification** **(1)** Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is produced.

**(2)** Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and allow to stand for 4 hours. Collect the crystals, wash with a small volume of methanol, and dry at 105°C for 1 hour: the crystals so obtained melt <*2.60*> between 237°C and 244°C (with decomposition).

**(3)** Determine the absorption spectrum of a solution of Perphenazine in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Perphenazine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 10 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Perphenazine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Perform the test with Perphenazine as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

**Melting point** <*2.60*> 95 – 100°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Perphenazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Perform the test in the current of nitrogen in light-resistant containers under the protection from sunlight. Dissolve 0.10 g of Perphenazine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solution 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 1-butanol and 1 mol/L ammonia TS (5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the sample solution is not more intense than that from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 65°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Perphenazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.20 mg of $C_{21}H_{26}ClN_3OS$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Perphenazine Tablets

ペルフェナジン錠

Perphenazine Tablets contain not less than 90% and not more than 110% of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$: 403.97).

**Method of preparation** Prepare as directed under Tablets, with Perphenazine.

**Identification** **(1)** Shake well a quantity of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine according to the labeled amount, with 10 mL of methanol, and filter. Evaporate 2 mL of the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

**(2)** Add 5 mL of the filtrate obtained in the Identification (1) to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and proceed as directed in the Identification (2) under Perphenazine.

**(3)** Determine the absorption spectrum of the filtrate obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate, and determine the absorption spectrum: it exhibits a maximum between 256 nm and 260 nm.

**Uniformity of dosage units** <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 Perphenazine Tablet by shaking with 5 mL of water, shake well with 70 mL of methanol, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet $x$ mL of the supernatant liquid, add methanol to make exactly $V$ mL of a solution containing about 4 $\mu$g of perphenazine ($C_{21}H_{26}ClN_3OS$) in each ml, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of perphenazine for assay, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of perphenazine } (C_{21}H_{26}ClN_3OS) = W_S \times (A_T/A_S) \times (V/25) \times (1/x)$$

$W_S$: Amount (mg) of perphenazine for assay

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Perphenazine Tablets at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Take 30 mL or more of the dissolved solution 90 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.8 $\mu$m. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine Reference Standard, previously dried in vacuum with phosphorus (V) oxide at 65°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add 2nd fluid for dissolution test to make exactly 250 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Perphenazine Tablets in 90 minutes is not less than 70%.

$$\text{Dissolution rate (\%) with respect to the labeled amount of perphenazine } (C_{21}H_{26}ClN_3OS) = W_S \times (A_T/A_S) \times (1/C) \times 36$$

$W_S$: Amount (mg) of Perphenazine Reference Standard
$C$: Labeled amount (mg) of perphenazine ($C_{21}H_{26}ClN_3OS$) in 1 tablet

**Assay** Weigh accurately and powder not less than 20 Perphenazine Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of perphenazine ($C_{21}H_{26}ClN_3OS$), add 70 mL of methanol, shake well, and add methanol to make exactly 100 mL. Filter the solution, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 10 mg of perphenazine for assay, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, and dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

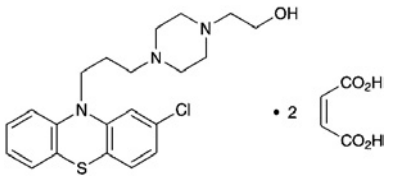
$$\text{Amount (mg) of perphenazine } (C_{21}H_{26}ClN_3OS) = W_S \times (A_T/A_S) \times (2/5)$$

$W_S$: Amount (mg) of perphenazine for assay

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Perphenazine Maleate

ペルフェナジンマレイン酸塩




$C_{21}H_{26}ClN_3OS.2C_4H_4O_4$: 636.11
2-{4-[3-(2-Chlorophenothiazin-10-yl)propyl]piperazin-1-yl}ethanol dimaleate [*58-39-9*, Perphenazine]

Perphenazine Maleate, when dried, contains not less than 98.0% of $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$.

**Description** Perphenazine Maleate occurs as a white to light yellow powder. It is odorless.

It is sparingly soluble in acetic acid (100), slightly soluble in water and in ethanol (95), and practically insoluble in chloroform.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 175°C (with decomposition).

**Identification** **(1)** Dissolve 8 mg of Perphenazine Maleate in 5 mL of sulfuric acid: a red color is produced, which becomes deep red-purple on warming.

**(2)** Dissolve 0.3 g of Perphenazine Maleate in 3 mL of dilute hydrochloric acid, add 2 mL of water and 3 mL of ammonia solution (28), shake, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (5)]. Evaporate the combined chloroform extracts on a water bath to dryness, dissolve the residue in 20 mL of methanol, and pour into 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25). Allow to stand for 4 hours, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <*2.60*> between 237°C and 244°C (with decomposition).

**(3)** Determine the absorption spectrum of a solution of Perphenazine Maleate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 30 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Perform the test with Perphenazine Maleate as directed under Flame Coloration Test <*1.04*> (2): a green color ap-

pears.

**(5)** Evaporate the aqueous layer reserved in (2) to dryness. To the residue add 1 mL of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate in a water bath at about 35°C with the aid of a current of air: the residue melts <2.60> between 128°C and 136°C.

**Purity** **(1)** Heavy metals <1.07>—Proceed with 2.0 g of perphenazine maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(2)** Arsenic <1.11>—Prepare the test solution with 1.0 g of Perphenazine Maleate according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Perphenazine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.81 mg of $C_{21}H_{26}ClN_3OS.2C_4H_4O_4$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Perphenazine Maleate Tablets

ペルフェナジンマレイン酸塩錠

Perphenazine Maleate Tablets contain not less than 93% and not more than 107% of the labeled amount of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$: 636.11).

**Method of preparation** Prepare as directed under Tablets, with Perphenazine Maleate.

**Identification** **(1)** Shake a quantity of powdered Perphenazine Maleate Tablets, equivalent to 0.04 g of Perphenazine Maleate according to the labeled amount, with 3 mL of dilute hydrochloric acid and 30 mL of water, centrifuge, filter the supernatant solution, add 3 mL of ammonia solution (28) to the filtrate, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (4).] Wash the combined chloroform extracts with two 5-mL portions of water, and separate the chloroform layer. Evaporate 6 mL of the chloroform solution on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Perphenazine Maleate.

**(2)** Evaporate 20 mL of the chloroform solution obtained in (1) on a water bath to dryness, dissolve the residue in 20 mL of methanol, and filter, if necessary. Warm the filtrate, add 5 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), allow to stand for 4 hours, and proceed as directed in the Identification (2) under Perphenazine Maleate.

**(3)** To 2 mL of the filtrate obtained in the Assay add water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 253 nm and 257 nm and between 303 nm and 313 nm.

**(4)** Filter, if necessary, the aqueous layer reserved in (1), evaporate the filtrate to make about 5 mL, add 2 mL of dilute sulfuric acid, and extract with two 10-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate on a water bath to dryness, dissolve the residue in 5 mL of sulfuric acid TS, and add 1 to 2 drops of potassium permanganate TS: the red color of potassium permanganate TS fades immediately.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Perphenazine Maleate Tablets by shaking with 15 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously with 50 mL of methanol, add water to make exactly 100 mL, and centrifuge. Pipet $x$ mL of the supernatant liquid, add water to make exactly $V$ mL of a solution containing about 6 $\mu$g of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) in each ml, and use this solution as the sample solution. Separately, weigh accurately 30 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 0.1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS, 10 mL of methanol and water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Amount (mg) of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/50)$

$W_S$: Amount (mg) of perphenazine maleate for assay

**Assay** Weigh accurately and powder not less than 20 Perphenazine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$), shake well with 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in a mixture of 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, 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. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Amount (mg) of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$)
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of perphenazine maleate for assay

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Adsorbed Purified Pertussis Vaccine

沈降精製百日せきワクチン

Adsorbed Purified Pertussis Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing the protective antigen of *Bordetella pertussis* to make the antigen insoluble.

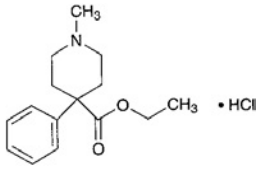
It conforms to the requirements of Adsorbed Purified Pertussis Vaccine in the Minimum Requirements for Biological Products.

**Description** Adsorbed Purified Pertussis Vaccine forms a homogeneous, white turbidity on shaking.

# Pethidine Hydrochloride

## Operidine

ペチジン塩酸塩



$C_{15}H_{21}NO_2$.HCl: 283.79
Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate monohydrochloride [*50-13-5*]

Pethidine Hydrochloride, when dried, contains not less than 98.0% of $C_{15}H_{21}NO_2$.HCl.

**Description** Pethidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

**Identification** **(1)** Determine the absorption spectrum of a solution of Pethidine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pethidine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Pethidine Hydrochloride (1 in 50) responds to Qualitative Tests <*1.09*> (2) for chloride.

**Melting point** <*2.60*> 187 – 189°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Pethidine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <*1.14*>—Perform the test with 0.20 g of Pethidine Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

**(3)** Related substances—Dissolve 0.05 g of Pethidine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase 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. Determine each peak area obtained from both solutions by the automatic integration method: the total area of the peaks other than that of pethidine from the sample solution is not larger than the peak area of perthidine from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 257 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 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of pethidine beginning after the solvent peak.

*System suitability*—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 μL of this solution is equivalent to 5 to 15% of that of pethidine obtained from 20 μL of the standard solution.

System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution according to the above operating conditions, pethidine and isoamyl 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pethidine is not more than 2.0%.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 0.5 g of Pethidine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.38 mg of $C_{15}H_{21}NO_2$.HCl

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Pethidine Hydrochloride Injection

## Operidine Injection

ペチジン塩酸塩注射液

Pethidine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of pethidine hydrochloride ($C_{15}H_{21}NO_2$.HCl: 283.79).

**Method of preparation** Prepare as directed under Injections, with Pethidine Hydrochloride.

**Description** Pethidine Hydrochloride Injection is a clear, colorless liquid.
It is affected by light.
pH 4.0 – 6.0

**Identification** Take a volume of Pethidine Hydrochloride Injection equivalent to 0.1 g of Pethidine Hydrochloride according to the labeled amount, and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride ($C_{15}H_{21}NO_2$.HCl) according to the labeled amount, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pethidine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 $\mu$L 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 pethidine to that of the internal standard.

Amount (mg) of pethidine hydrochloride ($C_{15}H_{21}NO_2$.HCl)
$= W_S \times (Q_T/Q_S)$

$W_S$: Amount (mg) of pethidine hydrochloride for assay

*Internal standard solution*—A solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 12,500).
*Operating conditions*—
Detector: An ultraviolet absorption photometer (wavelength: 257 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 $\mu$m in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.
*System suitability*—
System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, pethidine and the internal standard 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 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pethidine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

# Hydrophilic Petrolatum

親水ワセリン

**Method of preparation**

| | |
|---|---|
| White Beeswax | 80 g |
| Stearyl Alcohol or Cetanol | 30 g |
| Cholesterol | 30 g |
| White Petrolatum | a sufficient quantity |
| | To make 1000 g |

Melt and mix Stearyl Alcohol or Cetanol, White Beeswax and White Petrolatum on a water bath. Add Cholesterol, and melt completely by stirring. Stop warming, and stir until the mixture congeals.

**Description** Hydrophilic Petrolatum is white in color. It has a slight, characteristic odor.
When mixed with an equal volume of water, it retains the consistency of ointment.

**Containers and storage** Containers—Tight containers.

# White Petrolatum

白色ワセリン

White Petrolatum is a decolorized and purified mixture of hydrocarbons obtained from petroleum.

**Description** White Petrolatum is a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless.
It is practically insoluble in water, in ethanol (95) and in ethanol (99.5).

It dissolves in diethyl ether making a clear liquid or producing slight insoluble substances.

It becomes a clear liquid when warmed.

**Melting point** ⟨*2.60*⟩ 38 – 60°C (Method 3).

**Purity** **(1)** Color—Melt White Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: Add 3.4 mL of water to 1.6 mL of Ferric Chloride Colorimetric Stock Solution.

**(2)** Acidity or alkalinity—To 35.0 g of White Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the White Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

**(3)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of White Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(4)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of White Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

**(5)** Sulfur compound—To 4.0 g of White Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

**(6)** Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of White Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

**(7)** Fats and fatty oils or resins—To 10.0 g of White Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.05% (2 g).

**Containers and storage** Containers—Tight containers.

# Yellow Petrolatum

黄色ワセリン

Yellow Petrolatum is a purified mixture of hydrocarbons obtained from petroleum.

**Description** Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass, It is odorless and tasteless.

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

It dissolves in diethyl ether, in petroleum benzine and in turpentine oil, making a clear liquid or producing slight insoluble substances.

It becomes a yellow, clear liquid with slight fluorescence when warmed.

**Melting point** ⟨*2.60*⟩ 38 – 60°C (Method 3).

**Purity** **(1)** Color—Melt Yellow Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: To 3.8 mL of Ferric Chloride Stock CS add 1.2 mL of Cobaltous Chloride Stock CS.

**(2)** Acidity or alkalinity—To 35.0 g of Yellow Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the Yellow Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

**(3)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Yellow Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(4)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Yellow Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

**(5)** Sulfur compound—To 4.0 g of Yellow Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

**(6)** Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of Yellow Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

**(7)** Fats and fatty oils or resins—To 10.0 g of Yellow Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.05% (2 g).

**Containers and storage** Containers—Tight containers.

# Petroleum Benzin

石油ベンジン

Petroleum Benzin is a mixture of low-boiling point

hydrocarbons from petroleum.

**Description** Petroleum Benzin occurs as a colorless, clear, volatile liquid. It shows no fluorescence. It has a chracteristic odor.

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

It is practically insoluble in water.

It is very flammable.

Specific gravity $d_{20}^{20}$: 0.65 – 0.71

**Purity** **(1)** Acid—Shake vigorously 10 mL of Petroleum Benzin with 5 mL of water for 2 minutes, and allow to stand: the separated aqueous layer does not change moistened blue litmus paper to red.

**(2)** Sulfur compounds and reducing substances—To 10 mL of Petroleum Benzin add 2.5 mL of ammonia-ethanol TS and 2 to 3 drops of silver nitrate TS, and warm the mixture at about 50°C for 5 minutes, protected from light: no brown color develops.

**(3)** Fatty oil and sulfur compounds—Drop and evaporate 10 mL of Petroleum Benzin in small portions on odorless filter paper spread on a previously warmed glass plate: no spot or no foreign odor is perceptible.

**(4)** Benzene—Warm 5 drops of Petroleum Benzin with 2 mL of sulfuric acid and 0.5 mL of nitric acid for about 10 minutes, allow to stand for 30 minutes, transfer the mixture to a porcelain dish, and dilute with water: no odor of nitrobenzene is perceptible.

**(5)** Residue on evaporation—Evaporate 140 mL of Petroleum Benzin on a water bath to dryness, and heat the residue at 105°C to constant mass: the mass is not more than 1 mg.

**(6)** Readily carbonizable substances—Shake vigorously 5 mL of Petroleum Benzin with 5 mL of sulfuric acid for readily carbonizable substances for 5 minutes in a Nessler tube, and allow to stand: the sulfuric acid layer has no more color than Matching Fluid A.

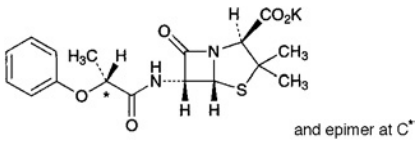
**Distilling range** ⟨*2.57*⟩ 50 – 80°C, not less than 90 vol%.

**Containers and storage** Containers—Tight containers.

Storage—Remote from fire, and not exceeding 30°C.

# Phenethicillin Potassium

フェネチシリンカリウム




$C_{17}H_{19}KN_2O_5S$: 402.51

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*RS*)-2-phenoxypropanoylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*132-93-4*]

Phenethicillin Potassium contains not less than 1400 units and not more than 1480 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$). One unit of Phenethicillin Potassium is equivalent to 0.68 μg of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$).

**Description** Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Phenethicillin Potassium (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Phenethicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Phenethicillin Potassium responds to Qualitative Tests ⟨*1.09*⟩ (1) for potassium salt.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +217 – +244° (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

**L-α-Phenethicillin potassium** Dissolve about 50 mg of Phenethicillin Potassium in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, $A_D$ and $A_L$, of D-α-phenethicillin and L-α-phenethicillin by the automatic integration method: $A_L/(A_D + A_L)$ is between 0.50 and 0.70.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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: Adjust the pH of a mixture of a solution of diammonium hydrogen phosphate (1 in 150) and acetonitrile (41:10) to 7.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of L-α-phenethicillin is about 25 minutes.

*System suitability*—

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, D-α-phenethicillin and L-α-phenethicillin 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 sample solution under the above operating conditions, the relative standard deviation of the peak area of L-α-phenethicillin is not more than 2.0%.

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Phenethicillin Potassium 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).

**(2)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 50 mg of Phenethicillin

Potassium in 50 mL of the mobile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 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 each peak area by the automatic integration method: the total of the peak areas other than D-α-phenethicillin and L-α-phenethicillin obtained from the sample solution is not more than 5 times the total of the peak areas of D-α-phenethicillin and L-α-phenethicillin from the standard solution.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the L-α-Phenethicillin potassium.

Time span of measurement: About 1.5 times as long as the retention time of L-α-phenethicillin.

*System suitability*—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L-α-phenethicillin obtained from 10 μL of this solution is equivalent to 14 to 26% of that from 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the L-α-Phenethicillin potassium.

**Loss on drying** <*2.41*> Not more than 1.0% (0.1 g, in vacuum, 60°C, 3 hours).

**Assay** Weigh accurately an amount of Phenethicillin Potassium and dried L-Phenethicillin Potassium Reference Standard, equivalent to about 40,000 units, dissolve each in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use these solutions as the sample solution and standard solution, respectively. Pipet 2 mL each of these solutions in 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS to them, and allow to stand for exactly 15 minutes. To them add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, and allow them to stand for exactly 15 minutes. Add 0.2 – 0.5 mL of starch TS, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the same manner as above without allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, $V_T$ and $V_S$, of 0.005 mol/L iodine VS consumed in the sample solution and standard solution.

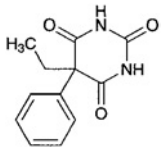
$$\text{Amount (unit) of } C_{17}H_{19}KN_2O_5S = W_S \times (V_T/V_S)$$

$W_S$: Amount (unit) of L-Phenethicillin Potassium Reference Standard

**Containers and storage** Containers—Well-closed containers.

# Phenobarbital

フェノバルビタール



$C_{12}H_{12}N_2O_3$: 232.24

5-Ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione

[*50-06-6*]

Phenobarbital, when dried, contains not less than 99.0% of $C_{12}H_{12}N_2O_3$.

**Description** Phenobarbital occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in *N,N*-dimethylformamide, freely soluble in ethanol (95), in acetone and in pyridine, soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

**Identification** **(1)** Boil 0.2 g of Phenobarbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

**(2)** Dissolve 0.1 g of Phenobarbital in 5 mL of diluted pyridine (1 in 10), shake the solution with 0.3 mL of copper (II) sulfate TS, and allow to stand for 5 minutes: a light red-purple precipitate is produced. Shake the mixture with 5 mL of chloroform: the chloroform layer remains colorless. Dissolve 0.1 g of Phenobarbital in a mixture of 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7, and 5 mL of diluted pyridine (1 in 10), then add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS: a light red-purple precipitate is produced in the water layer. Shake again: the chloroform layer remains colorless.

**(3)** Shake 0.4 g of Phenobarbital with 0.1 g of anhydrous sodium carbonate and 4 mL of water, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat on a water bath for 30 minutes under a reflux condenser, and allow to stand for 1 hour. Filter the crystals, wash with 7 mL of sodium hydroxide TS, then with a small amount of water, recrystallize from a mixture of ethanol (95) and chloroform (1:1), and dry at 105°C for 30 minutes: the crystals melt <*2.60*> between 181°C and 185°C.

**(4)** Dissolve 0.1 g of Phenobarbital in 2 mL of sulfuric acid, shake the solution with 5 to 6 mg of potassium nitrate, and allow to stand for 10 minutes: a yellow to yellow-brown color develops.

**Melting point** <*2.60*> 175 – 179°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Phenobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Dissolve 0.30 g of Phenobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take

0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

**(3)** Sulfate <*1.14*>—Dissolve 0.40 g of Phenobarbital in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone, and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

**(4)** Heavy metals <*1.07*>—Proceed with 1.0 g of Phenobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

**(5)** Phenylbarbituric acid—Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95) for 3 minutes: the solution is clear.

**(6)** Readily carbonizable substances <*1.15*>—Perform the test with 0.5 g of Phenobarbital. The solution has not more color than Matching Fluid A.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination using a mixture of 50 mL of *N,N*-dimethylformamide and 22 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 23.22 mg of $C_{12}H_{12}N_2O_3$

**Containers and storage** Containers—Well-closed containers.

# 10% Phenobarbital Powder

## Phenobarbital Powder

フェノバルビタール散 10%

10% Phenobarbital Powder contains not less than 9.3% and not more than 10.7% of phenobarbital ($C_{12}H_{12}N_2O_3$: 232.24).

**Method of preparation**

| | |
|---|---|
| Phenobarbital | 100 g |
| Starch, Lactose Hydrate or their mixture | a sufficient quantity |
| | To make 1000 g |

Prepare as directed under Powders, with the above ingredients.

**Identification** Shake thoroughly 5 g of 10% Phenobarbital Powder with 20 mL of hexane, and filter. Collect the residue, and dry on a water bath, then extract with four 30-mL portions of chloroform. Filter the combined chloroform extracts, and evaporate the filtrate to dryness. Dry the residue at 105°C for 1 hour: the residue so obtained melts <*2.60*> between 174°C and 179°C. With the residue, proceed as directed in the Identification (1) and (2) under Phenobarbital.

**Assay** Weigh accurately about 10 g of 10% Phenobarbital Powder, transfer to a glass-stoppered flask, and add exactly 100 mL of a mixture of chloroform and ethanol (95) (10:1). Stopper tightly, shake, and allow to stand for 30 minutes. Transfer the mixture to a glass-stoppered centrifuge tube, and centrifuge. Measure exactly 50 mL of the supernatant liquid, evaporate on a water bath to dryness, dissolve the residue in 50 mL of *N,N*-dimethylformamide, and proceed as directed in the Assay under Phenobarbital.

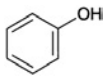
Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 23.22 mg of $C_{12}H_{12}N_2O_3$

**Containers and storage** Containers—Well-closed containers.

# Phenol

## Carbolic Acid

フェノール




$C_6H_6O$: 94.11
Phenol [*108-95-2*]

Phenol contains not less than 98.0% of $C_6H_6O$.

**Description** Phenol occurs as colorless to slightly red crystals or crystalline masses. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and soluble in water.

Phenol (10 g) is liquefied by addition of 1 mL of water.

The color changes gradually through red to dark red by light or air.

It cauterizes the skin, turning it white.

Congealing point: about 40°C

**Identification** **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Phenol (1 in 100): a blue-purple color develops.

**(2)** Add bromine TS dropwise to 5 mL of a solution of Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

**Purity** **(1)** Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

**(2)** Residue on evaporation—Weigh accurately about 5 g of Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

**Assay** Dissolve about 1.5 g of Phenol, accurately weighed, in water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05

mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at once stopper the flask, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of $C_6H_6O$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Phenol for Disinfection

## Carbolic Acid for Disinfection

消毒用フェノール

Phenol for Disinfection contains not less than 95.0% of phenol ($C_6H_6O$: 94.11).

**Description** Phenol for Disinfection occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor.

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

Phenol for Disinfection (10 g) is liquefied by addition of 1 mL of water.

It cauterizes the skin, turning it white.

Congealing point: about 30°C

**Identification** **(1)** To 10 mL of a solution of Phenol for Disinfection (1 in 100) add 1 drop of iron (III) chloride TS: a blue-purple color is produced.

**(2)** To 5 mL of a solution of Phenol for Disinfection (1 in 10,000) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

**Purity** **(1)** Clarity of solution—Dissolve 1.0 g of Phenol for Disinfection in 15 mL of water: the solution is clear.

**(2)** Residue on evaporation—Weigh accurately about 5 g of Phenol for Disinfection, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.10% of the mass of the sample.

**Assay** Dissolve about 1 g of Phenol for Disinfection, accurately weighed, in water to make exactly 1000 mL. Pipet 25 mL of the solution into an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper immediately, shake well, 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.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of $C_6H_6O$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Liquefied Phenol

## Liquefied Carbolic Acid

液状フェノール

Liquefied Phenol is Phenol maintained in a liquid condition by the presence of 10% of Water or Purified Water.

It contains not less than 88.0% of phenol ($C_6H_6O$: 94.11)

**Description** Liquefied Phenol is a colorless or slightly reddish liquid. It has a characteristic odor.

It is miscible with ethanol (95), with diethyl ether and with glycerin.

A mixture of equal volumes of Liquefied Phenol and glycerin is miscible with water.

The color changes gradually to dark red on exposure to light or air.

It cauterizes the skin, turning it white.

Specific gravity $d_{20}^{20}$: about 1.065

**Identification** **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Liquefied Phenol (1 in 100): a blue-purple color develops.

**(2)** Add bromine TS dropwise to 5 mL of a solution of Liquefied Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

**Boiling point** <*2.57*> Not more than 182°C.

**Purity** **(1)** Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Liquefied Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

**(2)** Residue on evaporation—Weigh accurately about 5 g of Liquefied Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

**Assay** Dissolve about 1.7 g of Liquefied Phenol, accurately weighed, in a water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at one stopper the flask tightly, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of $C_6H_6O$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Dental Phenol with Camphor

歯科用フェノール·カンフル

**Method of preparation**

| | |
|---|---|
| Phenol | 35 g |
| *d*- or *dl*-Camphor | 65 g |
| To make | 100 g |

Melt Phenol by warming, add *d*-Camphor or *dl*- Camphor, and mix.

**Description** Dental Phenol with Camphor is a colorless or light red liquid. It has a characteristic odor.

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Phenol and Zinc Oxide Liniment

フェノール·亜鉛華リニメント

**Method of preparation**

| | |
|---|---|
| Liquefied Phenol | 22 mL |
| Powdered Tragacanth | 20 g |
| Carmellose Sodium | 30 g |
| Glycerin | 30 mL |
| Zinc Oxide | 100 g |
| Purified Water | a sufficient quantity |
| To make | 1000 g |

Mix Liquefied Phenol, Glycerin and Purified Water, add Powdered Tragacanth in small portions by stirring, and allow the mixture to stand overnight. To the mixture add Carmellose Sodium in small portions by stirring to make a pasty mass, add Zinc Oxide in small portions, and prepare the liniment as directed under Liniments. Less than 5 g of Powdered Tragacanth or Carmellose Sodium can be replaced by each other to make 50 g in total.

**Description** Phenol and Zinc Oxide Liniment is a white, pasty mass. It has a slight odor of phenol.

**Identification** **(1)** Shake well 1 g of Phenol and Zinc Oxide Liniment with 10 mL of diethyl ether, and filter. To the filtrate add 10 mL of dilute sodium hydroxide TS, shake well, and separate the water layer. To 1 mL of the water layer add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and add 3 mL of sodium hydroxide TS: a yellow color develops (phenol).

**(2)** Place 1 g of Phenol and Zinc Oxide Liniment in a porcelain crucible, heat gradually raising the temperature until the content is charred, and then ignite it strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is produced (zinc oxide).

**(3)** Shake 0.5 g of Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, separate the chloroform layer, and use this solution as the sample solution. Separately, dissolve 0.01 g of phenol in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L 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 solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots obtained from the sample solution and the standard solution show the same *R*f value.

**Containers and storage** Containers—Tight containers.

# Phenolated Water

フェノール水

Phenolated Water contains not less than 1.8 w/v% and not more than 2.3 w/v% of phenol ($C_6H_6O$: 94.11).

**Method of preparation**

| | |
|---|---|
| Liquefied Phenol | 22 mL |
| Water or Purified Water | a sufficient quantity |
| To make | 1000 mL |

Mix the above ingredients.

**Description** Phenolated Water is a colorless, clear liquid, having the odor of phenol.

**Identification** **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water: a blue-purple color develops.

**(2)** To 5 mL of a solution of Phenolated Water (1 in 200) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

**Assay** Take exactly 2 mL of Phenolated Water into an iodine flask, add 25 mL of water, then add exactly 40 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes, and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper tightly at once, shake well, 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.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of $C_6H_6O$

**Containers and storage** Containers—Tight containers.

# Phenolated Water for Disinfection

消毒用フェノール水

Phenolated Water for Disinfection contains not less than 2.8 w/v% and not more than 3.3 w/v% of phenol ($C_6H_6O$: 94.11).

**Method of preparation**

| | |
|---|---|
| Phenol for Disinfection | 31 g |
| Water or Purified Water | a sufficient quantity |
| | To make 1000 mL |

Mix the above ingredients.

**Description** Phenolated Water for Disinfection is a clear, colorless liquid, having the odor of phenol.

**Identification** **(1)** Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water for Disinfection: a blue-purple color develops.

**(2)** Proceed with 5 mL of a solution of Phenolated Water for Disinfection (1 in 200) as directed in the Identification (2) under Phenol for Disinfection.

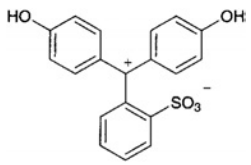
**Assay** Take exactly 5 mL of Phenolated Water for Disinfection, add water to make exactly 100 mL, then pipet 25 mL of the solution into an iodine flask, and proceed as directed in the Assay under Phenol for Disinfection.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of $C_6H_6O$

**Containers and storage** Containers—Tight containers.

# Phenolsulfonphthalein

フェノールスルホンフタレイン




$C_{19}H_{14}O_5S$: 354.38
2-[Bis(4-hydroxyphenyl)methyliumyl]benzenesulfonate
[*143-74-8*]

Phenolsulfonphthalein, when dried, contains not less than 98.0% of $C_{19}H_{14}O_5S$.

**Description** Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

**Identification** **(1)** Dissolve 5 mg of Phenolsulfonphthalein in 2 to 3 drops of sodium hydroxide TS, add 2 mL of 0.05 mol/L bromine VS and 1 mL of dilute sulfuric acid, shake well, and allow to stand for 5 minutes. Render the solution alkaline with sodium hydroxide TS: a deep blue-purple color develops.

**(2)** Dissolve 0.01 g of Phenolsulfonphthalein in diluted sodium carbonate TS (1 in 10) to make 200 mL. To 5 mL of this solution add diluted sodium carbonate TS (1 in 10) to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** **(1)** Insoluble substances—To about 1 g of Phenolsulfonphthalein, accurately weighed, add 20 mL of a solution of sodium hydrogen carbonate (1 in 40). Allow the mixture to stand for 1 hour with frequent shaking, dilute with water to 100 mL, and allow to stand for 24 hours. Collect the insoluble substances using a tared glass filter (G4), wash with 25 mL of a solution of sodium hydrogen carbonate (1 in 100) and with five 5-mL portions of water, and dry at 105°C for 1 hour: the mass of the residue is not more than 0.2%.

**(2)** Related substances—Dissolve 0.10 g of Phenolsulfonphthalein in 5 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Pipet 0.5 mL of this solution, add dilute sodium hydroxide TS to make exactly 100 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of *t*-amyl alcohol, acetic acid (100) and water (4:1:1) to a distance of about 15 cm, and air-dry the plate. After allowing the plate to stand in an ammonia vapor, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, silica gel, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.15 g of Phenolsulfonphthalein, previously dried, transfer to an iodine flask, dissolve in 30 mL of a solution of sodium hydroxide (1 in 250), and add water to make 200 mL. Add exactly measured 50 mL of 0.05 mol/L bromine VS, add 10 mL of hydrochloric acid to the solution quickly, and stopper immediately. Allow the mixture to stand for 5 minutes with occasional shaking, add 7 mL of potassium iodide TS, stopper again immediately, and shake gently for 1 minute. Titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 4.430 mg of $C_{19}H_{14}O_5S$

**Containers and storage** Containers—Well-closed containers.

# Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Phenolsulfonphthalein Injection is an aqueous solution for injection.

It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$: 354.38).

**Method of preparation**

| | |
|---|---|
| Phenolsulfonphthalein | 6 g |
| Sodium Chloride | 9 g |
| Sodium Bicarbonate | 1.43 g |
| (or Sodium Hydroxide) | (0.68 g) |
| Water for Injection | a sufficient quantity |
| | To make 1000 mL |

Prepare as directed under Injections, with the above ingredients.

**Description** Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

**Identification** To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

**pH** ⟨*2.54*⟩ 6.0 – 7.6

**Extractable volume** ⟨*6.05*⟩ It meets the requirement.

**Sensitivity** To 1.0 mL of Phenolsulfonphthalein Injection add 5 mL of water. To 0.20 mL of this solution add 50 mL of freshly boiled and cooled water and 0.40 mL of 0.01 mol/L sodium hydroxide VS: a deep red-purple color develops, and it changes to light yellow on the addition of 0.40 mL of 0.005 mol/L sulfuric acid VS.

**Assay** Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of phenolsulfonphthalein for assay, previously dried in a desiccator (silica gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 559 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

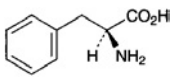
Amount (mg) of phenolsulfonphthalein ($C_{19}H_{14}O_5S$)
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of phenolsulfonphthalein for assay

**Containers and storage** Containers—Hermetic containers.

# L-Phenylalanine

L-フェニルアラニン




$C_9H_{11}NO_2$: 165.19
(2*S*)-2-Amino-3-phenylpropanoic acid [*63-91-2*]

L-Phenylalanine, when dried, contains not less than 98.5% of $C_9H_{11}NO_2$.

**Description** L-Phenylalanine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

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

It dissolves in dilute hydrochloric acid.

**Identification** Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: $-33.0$ – $-35.5°$ (after drying, 0.5 g, water, 25 mL, 100 mm).

**pH** ⟨*2.54*⟩ Dissolve 0.20 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

**(2)** Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

**(3)** Sulfate ⟨*1.14*⟩—Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

**(4)** Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Phenylalanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

**(5)** Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of L-Phenylalanine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. 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 20 ppm).

**(6)** Arsenic ⟨*1.11*⟩—Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution (not more than 2 ppm).

**(7)** Related substances—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, 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) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.30% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

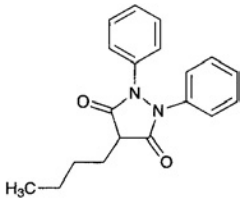
**Assay** Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$= 16.52$ mg of $C_9H_{11}NO_2$

**Containers and storage** Containers—Tight containers.

# Phenylbutazone

フェニルブタゾン



$C_{19}H_{20}N_2O_2$: 308.37
4-Butyl-1,2-diphenylpyrazolidine-3,5-dione [*50-33-9*]

Phenylbutazone, when dried, contains not less than 99.0% of $C_{19}H_{20}N_2O_2$.

**Description** Phenylbutazone occurs as a white to slightly yellowish white, crystalline powder. It is odorless, and is at first tasteless but leaves a slightly bitter aftertaste.

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

It dissolves in sodium hydroxide TS.

**Identification** **(1)** To 0.1 g of Phenylbutazone add 1 mL of acetic acid (100) and 1 mL of hydrochloric acid, and heat on a water bath under a reflux condenser for 30 minutes. Add 10 mL of water, and cool with ice water. Filter, and to the filtrate add 3 to 4 drops of sodium nitrite TS. To 1 mL of this solution add 1 mL of 2-naphthol TS and 3 mL of chloroform, and shake: a deep red color develops in the chloroform layer.

**(2)** Dissolve 1 mg of Phenylbutazone in 10 mL of dilute sodium hydroxide TS, and dilute with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Melting point** ⟨*2.60*⟩ 104 – 107°C

**Purity** **(1)** Clarity of solution—Dissolve 1.0 g of Phenylbutazone in 20 mL of sodium hydroxide solution (2 in 25), and allow to stand at 25 ± 1°C for 3 hours: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it is not more than 0.05.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Phenylbutazone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of phenylbutazone, according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Readily carbonizable substances—Dissolve 1.0 g of Phenylbutazone in 20 mL of sulfuric acid, and allow to stand at 25 ± 1°C for exactly 30 minutes: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it is not more than 0.10.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

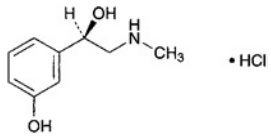
**Assay** Weigh accurately about 0.5 g of Phenylbutazone, previously dried, dissolve in 25 mL of acetone, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS until the solution shows a blue color which persists for 15 seconds (indicator: 5 drops of bromothymol blue TS). Perform a blank determination with a mixture of 25 mL of acetone and 16 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 30.84 mg of $C_{19}H_{20}N_2O_2$

**Containers and storage** Containers—Tight containers.

# Phenylephrine Hydrochloride

フェニレフリン塩酸塩



$C_9H_{13}NO_2$.HCl: 203.67
(1*R*)-1-(3-Hydroxyphenyl)-2-methylaminoethanol monohydrochloride [*61-76-7*]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_9H_{13}NO_2$.HCl.

**Description** Phenylephrine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

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

The pH of a solution of Phenylephrine Hydrochloride (1 in 100) is between 4.5 and 5.5.

**Identification** **(1)** To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is produced. To the solution so obtained add 1 mL of diethyl ether, and shake vigorously: no blue color develops in the diethyl ether layer.

**(2)** To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of iron (III) chloride TS: a persistent purple color is produced.

**(3)** Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS, and rub the inner side of the test tube with a glass rod: a precipitate is produced. Collect the precipitate, wash with a few drops of ice-cold water, and dry at 105°C for 2 hours: it melts ⟨*2.60*⟩ between 170°C and 177°C.

**(4)** A solution of Phenylephrine Hydrochloride (1 in 100) responds to Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: −42.0 – −47.5° (after drying, 0.5 g, water, 10 mL, 100 mm).

**Melting point** ⟨*2.60*⟩ 140 – 145°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of

Phenylephrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

**(2)** Sulfate <*1.14*>—Take 0.5 g of Phenylephrine Hydrochloride, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

**(3)** Ketone—Dissolve 0.20 g of Phenylephrine Hydrochloride in 1 mL of water, and add 2 drops of sodium pentacyanonitrosylferrate (III) TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution: Prepare as directed above without Phenylephrine Hydrochloride.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.2% (1 g).

**Assay** Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloric acid, and immediately stopper tightly. Shake the mixture, and allow to stand for 15 minutes. To this solution add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes, and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

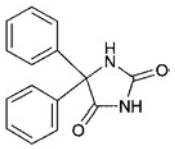
Each mL of 0.05 mol/L bromine VS
= 3.395 mg of $C_9H_{13}NO_2.HCl$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Phenytoin

## Diphenylhydantoin

フェニトイン



$C_{15}H_{12}N_2O_2$: 252.27
5,5-Diphenylimidazolidine-2,4-dione [*57-41-0*]

Phenytoin, when dried, contains not less than 99.0% of $C_{15}H_{12}N_2O_2$.

**Description** Phenytoin occurs as a white, crystalline powder or granules. It is odorless and tasteless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 296°C (with decomposition).

**Identification (1)** Dissolve 0.02 g of Phenytoin in 2 mL of ammonia TS, and add 5 mL of silver nitrate TS: a white precipitate is produced.

**(2)** Boil a mixture of 0.01 g of Phenytoin, 1 mL of ammonia TS and 1 mL of water, and add dropwise 2 mL of a mixture prepared from 50 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

**(3)** Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide, and fuse: the gas evolved turns moistened red litmus paper blue.

**(4)** Add 3 mL of chlorinated lime TS to 0.1 g of phenytoin, shake for 5 minutes, and dissolve the oily precipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid dropwise, then add 4 mL of water. Filter the white precipitate thus obtained, wash with water, and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of diluted ethanol (9 in 10), and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95), and dry: the melting point <*2.60*> is between 165°C and 169°C.

**Purity (1)** Clarity and color of solution—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool, and mix the solution with 5 mL of acetone: the solution is clear and colorless.

**(2)** Acidity or alkalinity—Shake 2.0 g of phenytoin with 40 mL of water for 1 minute, filter, and perform the following tests using this filtrate as the sample solution.

(i) To 10 mL of the sample solution add 2 drops of phenolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii) To 10 mL of the sample solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

**(3)** Chloride <*1.03*>—Dissolve 0.30 g of Phenytoin in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 30 mL of acetone, 0.60 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of dilute nitric acid, and add water to 50 mL (not more than 0.071%).

**(4)** Heavy metals <*1.07*>—Proceed with 1.0 g of Phenytoin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <*2.41*> Not more than 0.5% (2 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately, and titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until a light red color, which persists for 1 minute, develops.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.23 mg of $C_{15}H_{12}N_2O_2$

**Containers and storage** Containers—Well-closed containers.

# Phenytoin Powder

## Diphenylhydantoin Powder

フェニトイン散

Phenytoin Powder contains not less than 95% and not more than 105% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

**Method of preparation** Prepare as directed under Powders, with Phenytoin.

**Identification** Weigh a portion of Phenytoin Powder, equivalent to 0.3 g of Phenytoin according to the labeled amount, stir well with two 100-mL portions of diethyl ether, and extract. Combine the diethyl ether extracts, and filter. Evaporate the filtrate on a water bath to dryness, and proceed with the residue as directed in the Identification under Phenytoin.

**Assay** Weigh accurately Phenytoin Powder, equivalent to about 0.5 g of phenytoin ($C_{15}H_{12}N_2O_2$), add exactly 100 mL of ethanol (95), stir for 30 minutes, and centrifuge. Pipet 50 mL of the supernatant liquid, add 0.5 mL of thymolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops, then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 12.5 mL of silver nitrate TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until a light red color persists for 1 minute.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.23 mg of $C_{15}H_{12}N_2O_2$

**Containers and storage** Containers—Well-closed containers.

# Phenytoin Tablets

## Diphenylhydantoin Tablets

フェニトイン錠

Phenytoin Tablets contain not less than 95% and not more than 105% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

**Method of preparation** Prepare as directed under Tablets, with Phenytoin.

**Identification** Proceed with the residue obtained in the Assay as directed in the Identification under Phenytoin.

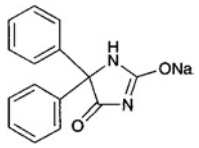
**Assay** Weigh accurately and powder not less than 20 Phenytoin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of phenytoin ($C_{15}H_{12}N_2O_2$), transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin ($C_{15}H_{12}N_2O_2$).

**Containers and storage** Containers—Well-closed containers.

# Phenytoin Sodium for Injection

## Diphenylhydantoin Sodium for Injection

注射用フェニトインナトリウム




$C_{15}H_{11}N_2NaO_2$: 274.25
Monosodium 5,5-diphenyl-4-oxoimidazolidin-2-olate
[*630-93-3*]

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$), and contains not less than 92.5% and not more than 107.5% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

**Method of preparation** Prepare as directed under Injections.

**Description** Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless.

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

The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12.

It is hygroscopic.

A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air, and a crystalline precipitate of phenytoin is produced.

**Identification** **(1)** With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

**(2)** Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <*1.09*> (1) for sodium salt.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <*2.41*> Not more than 2.5% (1 g, 105°C, 4 hours).

**Assay** Weigh accurately the content of not less than 10 preparations of Phenytoin Sodium for Injection, transfer about 0.3 g of the content, previously dried and accurately weighed, to a separator, dissolve in 50 mL of water, add 10

mL of dilute hydrochloric acid, and extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate on a water bath. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

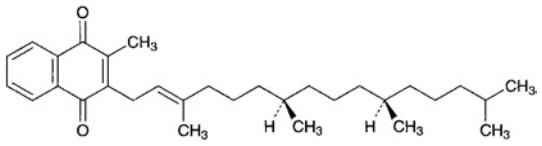
Amount (mg) of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$)
= amount (mg) of phenytoin ($C_{15}H_{12}N_2O_2$) × 1.0871

**Containers and storage** Containers—Hermetic containers.

# Phytonadione

## Phytomenadione
## Vitamin $K_1$

フィトナジオン



$C_{31}H_{46}O_2$: 450.70
2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]-1,4-naphthoquinone [*84-80-0*]

Phytonadione contains not less than 97.0% and not more than 102.0% of $C_{31}H_{46}O_2$.

**Description** Phytonadione is a clear yellow to orange-yellow, viscous liquid.

It is miscible with isooctane.

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

It decomposes gradually and changes to a red-brown by light.

Specific gravity $d_{20}^{20}$: about 0.967

**Identification (1)** Determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Phytonadione as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Refractive index** <*2.45*> $n_D^{20}$: 1.525 – 1.529

**Purity (1)** Ratio of absorbances—Determine the absorbances, $A_1$, $A_2$ and $A_3$, of a solution of Phytonadione in isooctane (1 in 100,000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the ratio $A_2/A_1$ is between 0.69 and 0.73, and the ratio $A_2/A_3$ is between 0.74 and 0.78. Determine the absorbances, $A_4$ and $A_5$, of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio $A_4/A_5$ is between 0.28 and 0.34.

**(2)** Heavy metals <*1.07*>—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Menadione—Dissolve 20 mg of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1:1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

**Isomer ratio** Conduct this procedure rapidly and without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatograph <*2.01*> according to the following conditions, and determine the peak areas of *Z*-isomer and E-isomer, $A_{TZ}$ and $A_{TE}$: $A_{TZ}/(A_{TZ}+A_{TE})$ is between 0.05 and 0.18.

*Operating conditions*—

Proceed as directed in the operating conditions in the Assay.

*System suitability*—

System performance: When the procedure is run with 50 μL of the sample solution under the above operating conditions, *Z*-isomer and *E*-isomer 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 50 μL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the peaks of *Z*-isomer and *E*-isomer is not more than 2.0%.

**Assay** Conduct this procedure rapidly and without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione Reference Standard, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solution add exactly 7 mL of the internal standard solution and the mobile phase to make 25 mL, and use these as the sample solution and standard solution, respectively. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard.

$$\text{Amount (mg) of } C_{31}H_{46}O_2 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Phytonadione Reference Standard

*Internal standard solution*—A solution of cholesterol benzoate in the mobile phase (1 in 400).

*Operating conditions*—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with porous silica gel for

liquid chromatography (5 $\mu$m in particle diameter).

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

Mobile phase: A mixture of hexane and *n*-amyl alcohol (4000 : 3).

Flow rate: Adjust the flow rate so that the retention time of the peak of *E*-isomer of phytonadione is about 25 minutes.

*System suitability—*

System performance: When the procedure is run with 50 $\mu$L of the standard solution under the above operating conditions, the internal standard, *Z*-isomer and *E*-isomer are eluted in this order with the resolution between the peaks of *Z*-isomer and *E*-isomer being not less than 1.5.

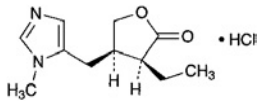
System repeatability: When the test is repeated 6 times with 50 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, at a cold place.

# Pilocarpine Hydrochloride

ピロカルピン塩酸塩



$C_{11}H_{16}N_2O_2$.HCl: 244.72

(3*S*,4*R*)-3-Ethyl-4-(1-methyl-1*H*-imidazol-5-ylmethyl)-4,5-dihydrofuran-2(3*H*)-one monohydrochloride

[*54-71-7*]

Pilocarpine Hydrochloride, when dried, contains not less than 99.0% of $C_{11}H_{16}N_2O_2$.HCl.

**Description** Pilocarpine Hydrochloride occurs as colorless crystals or white powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in ethanol (95), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Pilocarpine Hydrochloride (1 in 10) is between 3.5 and 4.5.

It is hygroscopic.

It is affected by light.

**Identification** **(1)** Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300), and shake the mixture vigorously: a violet color develops in the chloroform layer while no color or a light yellow color is produced in the aqueous layer.

**(2)** To 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20) add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

**Melting point** <*2.60*> 200 – 203°C

**Purity** **(1)** Sulfate—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water, and use this solution as the sample solution. To 5.0 mL of the sample solution add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

**(2)** Nitrate—To 2.0 mL of the sample solution obtained in (1) add 2 mL of iron (II) sulfate TS, and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color develops at the zone of contact.

**(3)** Related substances—Dissolve 0.3 g of Pilocarpine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 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 $\mu$L 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 chloroform, methanol and ammonia TS (85:14:2) to a distance of about 13 cm, and dry the plate at 105°C for 10 minutes. Cool, and spray evenly bismuth potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**(4)** Readily carbonizable substances <*1.15*>—Take 0.25 g of Pilocarpine Hydrochloride, and perform the test: the solution has no more color than Matching Fluid B.

**Loss on drying** <*2.41*> Not more than 3.0% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.5% (0.1 g).

**Assay** Weigh accurately about 0.5 g of Pilocarpine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.47 mg of $C_{11}H_{16}N_2O_2$.HCl

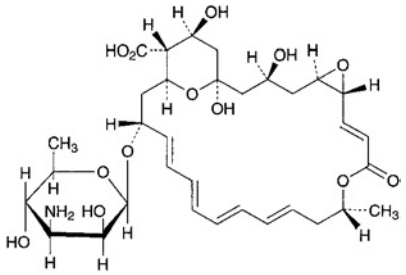
**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Pimaricin

## Natamycin

ピマリシン



$C_{33}H_{47}NO_{13}$: 665.73

(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy-$\beta$-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-

pentaene-25-carboxylic acid [*7681-93-8*]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces natalensis*.

It contains not less than 900 $\mu$g (potency) and not more than 1020 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin ($C_{33}H_{47}NO_{13}$).

**Description** Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

**Identification** **(1)** To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

**(2)** Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pimaricin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +243 – +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pimaricin according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(2)** Related substances—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 $\mu$L of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the total area of the peaks other than pimaricin by the automatic integration method: not more than 4.0%.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 303 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 $\mu$m in particle diameter).

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

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol and tetrahydrofuran (47:44:2).

Flow rate: Adjust the flow rate so that the retention time of pimaricin is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pimaricin.

*System suitability*—

Test for required detectability: Measure exactly 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the peak area of pimaricin obtained from 10 $\mu$L of this solution is equivalent to 7 to 13% of that from 10 $\mu$L of the solution for system suitability test.

System performance: When the procedure is run with 10 $\mu$L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pimaricin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of pimaricin is not more than 2.0%.

**Water** <*2.48*> Between 6.0% and 9.0% (0.2 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Pimaricin and Pimaricin Reference Standard, equivalent to about 25 mg (potency), and dissolve each in methanol to make exactly 100 mL. Pipet 2 mL each of these solution, add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances at 295.5 nm, $A_{T1}$ and $A_{S1}$, at 303 nm, $A_{T2}$ and $A_{S2}$, and at 311 nm, $A_{T3}$ and $A_{S3}$, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount [}\mu\text{g (potency)] of } C_{33}H_{47}NO_{13} = W_S \times \frac{A_{T2} - \dfrac{A_{T1} + A_{T3}}{2}}{A_{S2} - \dfrac{A_{S1} + A_{S3}}{2}} \times 1000$$

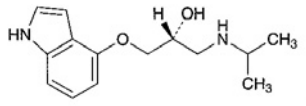
$W_S$: Amount [mg (potency)] of Pimaricin Reference Standard

**Containers and storage** Containers—Tight containers.

Storage—Light resistant.

# Pindolol

ピンドロール




and enantiomer

$C_{14}H_{20}N_2O_2$: 248.32

(2*RS*)-1-(1*H*-Indol-4-yloxy)-3-(1-methylethyl)aminopropan-2-ol [*13523-86-9*]

Pindolol, when dried, contains not less than 98.5% of $C_{14}H_{20}N_2O_2$.

**Description** Pindolol occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in dilute sulfuric acid and in acetic acid (100).

**Identification** **(1)** To 1 mL of a solution of Pindolol in methanol (1 in 10,000) add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, is produced.

**(2)** Dissolve 0.05 g of Pindolol in 1 mL of dilute sulfuric acid, and add 1 mL of Reinecke salt TS: a light red precipitate is produced.

**(3)** Determine the absorption spectrum of a solution of Pindolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Determine the infrared absorption spectrum of Pindolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** <*2.24*> $E^{1\%}_{1\,cm}$ (264 nm): 333 – 350 (10 mg, methanol, 500 mL).

**Melting point** <*2.60*> 169 – 173°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100), and observe immediately: the solution is clear, and has no more color than the following control solution.

Control solution: Measure accurately 4 mL of Matching Fluid A, add exactly 6 mL of water, and mix.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pindolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Pindolol according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.10 g of Pindolol in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, 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 chloroform, acetone and isopropylamine (5:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

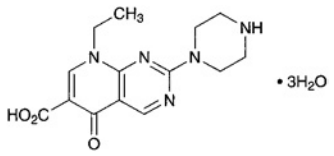
**Assay** Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate <*2.50*> with 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS
= 24.83 mg of $C_{14}H_{20}N_2O_2$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Pipemidic Acid Hydrate

ピペミド酸水和物



$C_{14}H_{17}N_5O_3.3H_2O$: 357.36
8-Ethyl-5-oxo-2-(piperazin-1-yl)-5,8-dihydropyrido[2,3-*d*]pyrimidine-6-carboxylic acid trihydrate
[*51940-44-4*, anhydride]

Pipemidic Acid Hydrate, when dried, contains not less than 98.5% and not more than 101.0% of pipemidic acid ($C_{14}H_{17}N_5O_3$: 303.32).

**Description** Pipemidic Acid Hydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Melting point: about 250°C (with decomposition).

**Identification** **(1)** Dissolve 0.1 g of Pipemidic Acid Hydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pipemidic Acid Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute nitric acid, and filter through a glass filter (G3). To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

**(2)** Sulfate <*1.14*>—Dissolve 1.0 g of Pipemidic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute hydrochloric acid, and filter through a glass filter (G3). To 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

**(3)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pipemidic Acid Hydrate according to Method 2, 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 Pipemidic Acid Hydrate according to Method 3, and perform the test (not more than 2 ppm).

**(5)** Related substances—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of diluted acetic acid (100) (1 in 20), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetic acid (100) (1 in 20) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L 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 chloroform, methanol, formic acid and triethylamine (25:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> 14.5 – 16.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Pipemidic Acid Hydrate, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

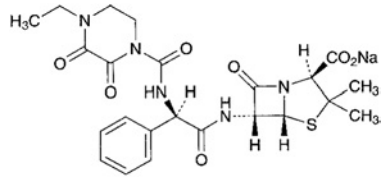
Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Piperacillin Sodium

ピペラシリンナトリウム



$C_{23}H_{26}N_5NaO_7S$: 539.54

Monosodium (2*S*,5*R*,6*R*)-6-{(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetylamino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [*59703-84-3*]

Piperacillin Sodium contains not less than 863 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Sodium is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

**Description** Piperacillin Sodium occurs as a white powder or mass.

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

**Identification (1)** Determine the infrared absorption spectrum of Piperacillin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** Piperacillin Sodium responds to Qualitative Tests <*1.09*> (1) for sodium salt.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +175 – +190° (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

**pH** <*2.54*> Dissolve 1.0 g of Piperacillin Sodium in 4 mL of water: the pH of the solution is between 5.0 and 7.0.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g of Piperacillin Sodium in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

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

**(4)** Related substances—Dissolve 0.1 g of Piperacillin Sodium in 50 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of the peak of ampicillin appeared at the retention time of about 7 minutes from the sample solution is not larger than 1/2 of that of piperacillin from the standard solution, the total area of related compounds 1 appeared at the retention times of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. The peak areas of ampicillin, related compounds 1 and related compound 2 are used after multiplying by their relative response factor, 1.39, 1.32 and 1.11, respectively.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and 0.2 mol/L potassium dihydrogenphosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogenphosphate (25:24:1).

Flowing of the mobile phase: Control the gradient by mix-

ing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 7 | 100 | 0 |
| 7 – 13 | 100→83 | 0→17 |
| 13 – 41 | 83 | 17 |
| 41 – 56 | 83→20 | 17→80 |
| 56 – 60 | 20 | 80 |

Flow rate: 1.0 mL per minute. The retention time of piperacillin is about 33 minutes.

Time span of measurement: About 1.8 times as long as the retention time of piperacillin beginning after the solvent peak.

*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 piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of piperacillin is not more than 2.0%.

**Water** ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Piperacillin Sodium, equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Piperacillin Reference Standard, equivalent to about 0.1 g (potency), 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, 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak height of piperacillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of piperacillin } (C_{23}H_{27}N_5O_7S) = W_S \times (Q_T/Q_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Piperacillin Reference Standard

*Internal standard solution*—A solution of acetanilide in the mobile phase (1 in 5000).

*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 25°C.

Mobile phase: To 60.1 g of acetic acid (100) and 101.0 g of triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

*System suitability—*

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and piperacillin 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Piperacillin Sodium for Injection

注射用ピペラシリンナトリウム

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

**Method of preparation** Prepare as directed under Injections, with Piperacillin Sodium.

**Description** Piperacillin Sodium for Injection is a white powder or masses.

**Identification** Proceed as directed in the Identification under Piperacillin Sodium.

**pH** ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of Piperacillin Sodium, in 4 mL of water is 5.0 – 7.0.

**Purity** **(1)** Clarity and color of solution—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of Piperacillin Sodium according to the labeled amount, in 17 mL of water: the solution is clear and colorless.

**(2)** Related substances—Proceed as directed in the Purity (4) under Piperacillin Sodium.

**Water** ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

**Bacterial endotoxins** ⟨*4.01*⟩ Less than 0.04 EU/mg (potency).

**Uniformity of dosage units** ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

**Foreign insoluble matter** ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

**Insoluble particulate matter** ⟨*6.07*⟩ Perform the test according to Method 1: it meets the requirement.

**Sterility** ⟨*4.06*⟩ Perform the test according to the Membrane

filtration method: it meets the requirement.

**Assay** Weigh accurately the mass of the contents of not less than 10 Piperacillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of Piperacillin Sodium according to the labeled amount, dissolve in water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Piperacillin Reference Standard, and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Piperacillin Sodium.

Amount [mg (potency)] of piperacillin ($C_{23}H_{27}N_5O_7S$)
$= W_S \times (Q_T/Q_S)$

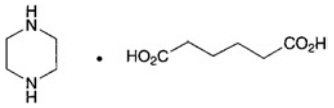
$W_S$: Amount [mg (potency)] of Piperacillin Reference Standard

*Internal standard solution*—A solution of acetonitrile in the mobile phase (1 in 5000).

**Containers and storage** Containers—Hermetic containers. Polyethylene or polypropylene containers for aqueous injections may be used.

# Piperazine Adipate

ピペラジンアジピン酸塩



$C_4H_{10}N_2.C_6H_{10}O_4$: 232.28
Piperazine hexanedioate [*142-88-1*]

Piperazine Adipate, when dried, contains not less than 98.5% of $C_4H_{10}N_2.C_6H_{10}O_4$.

**Description** Piperazine Adipate occurs as a white, crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in water and in acetic acid (100), and practically insoluble in ethanol (95), in acetone and in diethyl ether.

Melting point: about 250°C (with decomposition).

**Identification** **(1)** Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness on a water bath, and dry the residue at 105°C for 1 hour: the melting point <*2.60*> is between 152°C and 155°C.

**(2)** To 3 mL of a solution of Piperazine Adipate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

**(3)** Determine the infrared absorption spectrum of Piperazine Adipate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** <*2.54*> The pH of a solution of Piperazine Adipate (1 in 20) is between 5.0 and 6.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Piperazine Adipate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid for nonaqueous titration and 40 mL of acetone for nonaqueous titration, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$= 11.61$ mg of $C_4H_{10}N_2.C_6H_{10}O_4$

**Containers and storage** Containers—Well-closed containers.

# Piperazine Phosphate Hydrate

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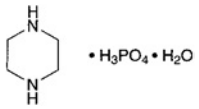


$C_4H_{10}N_2.H_3PO_4.H_2O$: 202.15
Piperazine monophosphate monohydrate [*18534-18-4*]

Piperazine Phosphate Hydrate contains not less than 98.5% of piperazine phosphate ($C_4H_{10}N_2.H_3PO_4$: 184.13), calculated on the anhydrous basis.

**Description** Piperazine Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in formic acid, sparingly soluble in water, very slightly soluble in acetic acid (100), and practically insoluble in methanol, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

Melting point: about 222°C (with decomposition).

**Identification** **(1)** To 3 mL of a solution of Piperazine Phosphate Hydrate (1 in 100) add 3 drops of Reinecke salt TS:a light red precipitate is formed.

**(2)** Determine the infrared absorption spectrum of Piperazine Phosphate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Piperazine Phosphate Hydrate (1 in 100) responds to Qualitative Tests <*1.09*> (1) and (3) for phos-

phate.

**pH** ⟨*2.54*⟩ Dissolve 1.0 g of Piperazine Phosphate Hydrate in 100 mL of water: the pH of the solution is between 6.0 and 6.5.

**Purity** **(1)** Chloride ⟨*1.03*⟩—To 0.5 g of Piperazine Phosphate Hydrate 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 0.25 mL of 0.01 mol/L hydrochloric acid (not more than 0.018%).

**(2)** Heavy metals ⟨*1.07*⟩—To 2.0 g of Piperazine Phosphate Hydrate add 5 mL of dilute hydrochloric acid, 30 mL of water and 2 mL of dilute acetic acid, and dissolve. Add sodium hydroxide TS, adjust the pH of the solution to 3.3, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Dissolve 2.0 g of Piperazine Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and use this solution as the test solution. Perform the test (not more than 1 ppm).

**(4)** Related substances—Dissolve 50 mg of Piperazine Phosphate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 $\mu$L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, ammonia solution (28) and ethanol (99.5) (8:3:3:2) to a distance of about 13 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnamaldehyde TS, and allow to stand for 15 minutes: the spots other than the principal spot and the spot on the starting line from the sample solution are not more intense than the spot from the standard solution.

**Water** ⟨*2.48*⟩ 8.0 – 9.5% (0.3 g, direct titration).

**Assay** Weigh accurately about 0.15 g of Piperazine Phosphate Hydrate, dissolve in 10 mL of formic acid, add 60 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.207 mg of $C_4H_{10}N_2.H_3PO_4$

**Containers and storage** Containers—Well-closed containers.

# Piperazine Phosphate Tablets

ピペラジンリン酸塩錠

Piperazine Phosphate Tablets contain not less than 95% and not more than 105% of the labeled amount of piperazine phosphate hydrate ($C_4H_{10}N_2.H_3PO_4.H_2O$: 202.15).

**Method of preparation** Prepare as directed under Tablets, with Piperazine Phosphate Hydrate.

**Identification** Take a quantity of Piperazine Phosphate Tablets equivalent to 0.1 g of Piperazine Phosphate Hydrate according to the labeled amount, previously powdered, add 10 mL of water, shake while warming for 10 minutes, allow to cool, and filter. To 3 mL of the filtrate add 3 drops of Reinecke salt TS: a light red precipitate is formed.

**Disintegration** ⟨*6.09*⟩ It meets the requirement. The time limit of the test is 10 minutes.

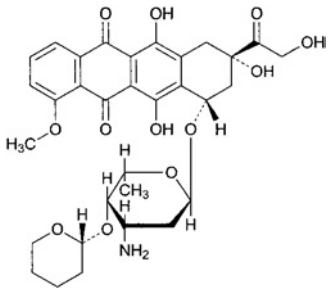
**Assay** Weigh accurately not less than 20 Piperazine Phosphate Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of piperazine phosphate hydrate ($C_4H_{10}N_2.H_3PO_4.H_2O$) according to the labeled amount. Add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. To the residue add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Repeat twice the same procedure with 5 mL each of acetic acid (100), combine all the supernatant liquids, add 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.107 mg of $C_4H_{10}N_2.H_3PO_4.H_2O$

**Containers and storage** Containers—Tight containers.

# Pirarubicin

ピラルビシン



$C_{32}H_{37}NO_{12}$: 627.64
(2*S*,4*S*)-4-{3-Amino-2,3,6-trideoxy-4-*O*-[(2*R*)-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyloxy}-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione [*72496-41-4*]

Pirarubicin is a derivative of daunorubicin.

It contains not less than 950 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin ($C_{32}H_{37}NO_{12}$).

**Description** Pirarubicin occurs as a red-orange crystalline powder.

It is soluble in chloroform, very slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

**Identification** **(1)** Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Deter-

mine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pirarubicin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Dissolve 5 mg each of Pirarubicin and Pirarubicin Reference Standard in 5 mL of chloroform, and use these solutions as the sample solution and 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 chloroform and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the necked eye: the principal spot obtained from the sample solution and the spot from the standard solution show a red-orange color and the same *R*f value.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +195 – +215° (10 mg, chloroform, 10 mL, 100 mm).

**Purity** **(1)** Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.

**(2)** Heavy metals <1.07>—Proceed with 1.0 g of Pirarubicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase 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 each peak area by the automatic integration method: the peak area of doxorubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, obtained from the sample solution are not more than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, from the sample solution is not more than 5 times the peak area of pirarubicin from the standard solution. For these calculations, use the peak area for doxorubicin after multiplying by the relative response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their relative response factors, 1.09, respectively.

*Operating conditions—*

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of pirarubicin.

*System suitability—*

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

**Water** <2.48> Not more than 2.0% (0.1 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Pirarubicin and Pirarubicin Reference Standard, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 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 ratios, $Q_T$ and $Q_S$, of the peak area of pirarubicin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of } C_{32}H_{37}NO_{12} = W_S \times (Q_T/Q_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Pirarubicin Reference Standard

*Internal standard solution*—A solution of 2-naphthol in the mobile phase (1 in 1000).

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 254 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 25°C.

Mobile phase: A mixture of 0.05 mol/L ammonium formate buffer solution, pH 4.0 and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

*System suitability—*

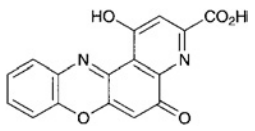
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

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 ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Pirenoxine

ピレノキシン




$C_{16}H_8N_2O_5$: 308.25

1-Hydroxy-5-oxo-5*H*-pyrido[3,2-*a*]phenoxazine-3-carboxylic acid [*1043-21-6*]

Pirenoxine, when dried, contains not less than 98.0% of $C_{16}H_8N_2O_5$.

**Description** Pirenoxine occurs as a yellow-brown powder. It is odorless, and has a slightly bitter taste.

It is very slightly soluble in dimethylsulfoxide, and practically insoluble in water, in acetonitrile, in ethanol (95), in tetrahydrofuran and in diethyl ether.

Melting point: about 250°C (with decomposition).

**Identification** **(1)** Dissolve 2 mg of Pirenoxine in 10 mL of phosphate buffer solution, pH 6.5, add 5 mL of a solution of L-ascorbic acid (1 in 50), and shake vigorously: a dark purple precipitate is formed.

**(2)** Determine the absorption spectrum of a solution of Pirenoxine in phosphate buffer solution, pH 6.5 (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Pirenoxine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pirenoxine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 10 mg of Pirenoxine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase 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. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than pirenoxine is not larger than the peak area of pirenoxine from the standard solution.

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 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 35°C.

Mobile phase: Dissolve 1.39 g of tetra *n*-butylammonium chloride and 4.5 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust the pH to 6.5 with phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of pirenoxine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pirenoxine.

*System suitability—*

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 30 mL. Confirm that the peak area of pirenoxine obtained from 5 μL of this solution is equivalent to 5 to 8% of that of pirenoxine obtained from 5 μL of the standard solution.

System performance: Dissolve 3 mg of Pirenoxine and 16 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, pirenoxine and methyl 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenoxine is not more than 1.0%.

**Loss on drying** <*2.41*> Not more than 1.5% (0.5 g, in vacuum, 80°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

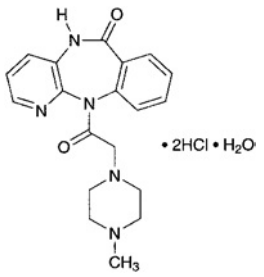
**Assay** Weigh accurately about 0.1 g of Pirenoxine, previously dried, dissolve in 140 mL of dimethylsulfoxide by heating on a water bath. After cooling, add 30 mL of water, and titrate <*2.50*> immediately with 0.02 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium hydroxide VS
= 6.165 mg of $C_{16}H_8N_2O_5$

**Containers and storage** Containers—Tight containers.

# Pirenzepine Hydrochloride Hydrate

ピレンゼピン塩酸塩水和物




$C_{19}H_{21}N_5O_2.2HCl.H_2O$: 442.34

11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one dihydrochloride monohydrate [*29868-97-1*, anhydride]

Pirenzepine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of pirenzepine hydrochloride ($C_{19}H_{21}N_5O_2.2HCl$: 424.32), calculated on the anhydrous basis.

**Description** Pirenzepine Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder.

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

The pH of a solution by dissolving 1 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is between 1.0 and 2.0.

Melting point: about 245°C (with decomposition).

It is gradually colored by light.

**Identification** **(1)** Determine the absorption spectrum of a

solution of Pirenzepine Hydrochloride Hydrate (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pirenzepine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Pirenzepine Hydrochloride Hydrate (1 in 50) responds to Qualitative Tests <*1.09*> for chloride.

**Purity** **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is clear and not more color than that of the following control solution.

Control solution: To 1.2 mL of Matching fluid for color F add 8.8 mL of diluted hydrochloric acid (1 in 40).

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pirenzepine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 0.3 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water. To 1 mL of this solution add 5 mL of methanol and the mobile phase A to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add 5 mL of methanol and the mobile phase A to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pirenzepine is not more than 3/10 times the peak area of pirenzepine from the standard solution, and the total area of the peaks other than pirenzepine is not more than 3/5 times the peak area of pirenzepine from the standard solution.

*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 $\mu$m in particle diameter).

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

Mobile phase A: Dissolve 2 g of sodium lauryl sulfate in 900 mL of water, adjust the pH to 3.2 with acetic acid (100), and add water to make 1000 mL.

Mobile phase B: Methanol

Mobile phase C: Acetonitrile

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) | Mobile phase C (vol%) |
|---|---|---|---|
| 0 – 15 | 55 → 25 | 30 | 15 → 45 |
| 15 – | 25 | 30 | 45 |

Flow rate: Adjust the flow rate so that the retention time of pirenzepine is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of pirenzepine beginning after the solvent peak.

*System suitability*—

Test for required detectability: Pipet 1 mL of the standard solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Confirm that the peak area of pirenzepine obtained from 10 $\mu$L of this solution is equivalent to 7 to 13% of that from 10 $\mu$L of the standard solution.

System performance: Dissolve 0.1 g of phenylpiperazine hydrochloride in 10 mL of methanol. Mix 1 mL of this solution and 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make 10 mL. When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, pirenzepine and phenylpiperazine 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 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenzepine is not more than 2.0%.

**Water** <*2.48*> Not less than 3.5% and not more than 5.0% (0.3 g, volumetric titration, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pirenzepine Hydrochloride Hydrate, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

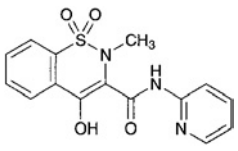
Each mL of 0.1 mol/L perchloric acid VS
= 14.14 mg of $C_{19}H_{21}N_5O_2.2HCl$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Piroxicam

ピロキシカム



$C_{15}H_{13}N_3O_4S$: 331.35

4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide [*36322-90-4*]

Piroxicam contains not less than 98.5% and not

more than 101.0% of $C_{15}H_{13}N_3O_4S$, calculated on the dried basis.

**Description** Piroxicam occurs as a white to pale yellow crystalline powder.

It is sparingly soluble in acetic anhydride, slightly soluble in acetonitrile, in methanol and in ethanol (99.5), very slightly soluble in acetic acid (100), and practically insoluble in water.

Melting point: about 200°C (with decomposition).

**Identification** **(1)** Dissolve 0.1 g of Piroxicam in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 200 mL. To 1 mL of this solution add the mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Piroxicam as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with dichloromethane, evaporate the solvent, dry the residue on a water bath, and perform the test.

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Piroxicam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 75 mg of Piroxicam in 50 mL of acetonitrile for liquid chromatography, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile for liquid chromatography to make exactly 10 mL. Pipet 1 mL of this solution, add acetonitrile for liquid chromatography to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than piroxicam is not larger than the peak area of piroxicam with the standard solution, and the total area of the peaks other than piroxicam is not larger than 2 times the peak area of piroxicam with the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 $\mu$m in particle diameter).

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

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile for liquid chromatography (3:2).

Flow rate: Adjust the flow rate so that the retention time of piroxicam is about 10 minutes.

Time span of measurement: About 5 times as long as the retention time of piroxicam beginning after the solvent peak.

*System suitability*—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile for liquid chromatography to make exactly 20 mL. Confirm that the peak area of piroxicam obtained with 20 $\mu$L of this solution is equivalent to 17.5 to 32.5% of that with 20 $\mu$L of the standard solution.

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piroxicam are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piroxicam is not more than 2.0%.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.2% (1 g).

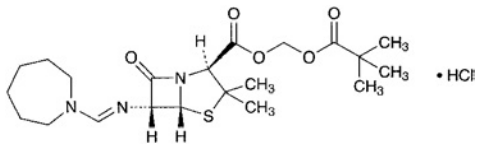
**Assay** Weigh accurately about 0.25 g of Piroxicam, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.14 mg of $C_{15}H_{13}N_3O_4S$

**Containers and storage** Containers—Tight containers.

# Pivmecillinam Hydrochloride

ピブメシリナム塩酸塩



$C_{21}H_{33}N_3O_5S.HCl$: 476.03

2,2-Dimethylpropanoyloxymethyl (2*S*,5*R*,6*R*)-6-[(azepan-1-ylmethylene)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [*32887-03-9*]

Pivmecillinam Hydrochloride contains not less than 630 $\mu$g (potency) and not more than 710 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43).

**Description** Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

**Identification** **(1)** Determine the infrared absorption spectrum of Pivmecillinam Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the

Reference Spectrum or the spectrum of Pivmecillinam Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(2)** Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +200 – +220° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

**Purity** **(1)** Heavy metals <1.07>—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Arsenic <1.11>—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 50 mg of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride Reference Standard in 4.0 mL of water, 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 standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μL of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot is not observable.

**Water** <2.48> Not more than 1.0% (0.25 g, coulometric titration).

**Assay** Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. 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 determine the ratios, $Q_T$ and $Q_S$, of the peak area of pivmecillinam to that of the internal standard.

Amount [μg (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$) $= W_S \times (Q_T/Q_S) \times 1000$

$W_S$: Amount [mg (potency)] of Pivmecillinam Hydrochloride Reference Standard

*Internal standard solution*—A solution of diphenyl in the mobile phase (1 in 12,500).

*Operating conditions*—

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

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pivmecillinam is about 6.5 minutes.

*System suitability*—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pivmecillinam and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

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 ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Live Oral Poliomyelitis Vaccine

経口生ポリオワクチン

Live Oral Poliomyelitis Vaccine contains live attenuated poliovirus of type I, II and III.

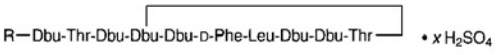
Monovalent or bivalent product may be prepared, if necessary.

Live Oral Poliomyelitis Vaccine conforms to the requirements of Live Oral Poliomyelitis Vaccine in the Minimum Requirements for Biological Products.

**Description** Live Oral Poliomyelitis Vaccine is a light yellow-red to light red, clear liquid.

# Polymixin B Sulfate

ポリミキシン B 硫酸塩




Polymyxin $B_1$ : R = 6-Methyloctanoic acid
Dbu = L-α, γ-Diaminobutyric acid

Polymyxin $B_2$ : R = 6-Methylheptanoic acid
Dbu = L-α, γ-Diaminobutyric acid

Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa*.

It contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B Sulfate is expressed as mass unit of polymixin B ($C_{55\text{-}56}H_{96\text{-}98}N_{16}O_{13}$). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate ($C_{55\text{-}56}H_{96\text{-}98}N_{16}O_{13}.1\text{-}2H_2SO_4$).

**Description** Polymixin B Sulfate occurs as a white to yellow-brown powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification** **(1)** To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

**(2)** Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate Reference Standard separately into two glass stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the sample solution and standard solution (1). Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine separately in 10 mL of water, and use these solutions as the standard solutions (2), (3), (4) and (5), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 3 μL each of the sample solution, the standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography, and expose the plate to a saturated vapor of the developing solvent for 15 hours. Develop the plate with a mixture of phenol and water (3:1) to a distance of about 13 cm while without exposure to light, and dry the plate at 110°C for 5 minutes. Spray evenly ninhydrin-acetic acid TS on the plate, and heat at 110°C for 5 minutes: *R*f value of each spot obtained from the sample solution is the same with *R*f value of the corresponding spots from the standard solution (1). Each of the spots from the sample solution appears at the position corresponding to each of the spots from the standard solutions (2), (3) and (4), but not appears at the position corresponding to the spot from the standard solution (5).

**(3)** A solution of Polymixin B Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> for sulfate.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: −78 – −90° (0.5 g calculated on the dried basis, water, 25 mL, 100 mm).

**pH** <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Polymixin B Sulfate in 50 mL of water is between 5.0 and 7.0.

**Phenylalanine** Weigh accurately about 0.375 g of Polymixin B Sulfate, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine absorbances, $A_1$, $A_2$, $A_3$, $A_4$ and $A_5$, of this solution at 252 nm, at 258 nm, at 264 nm, at 280 nm and at 300 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and calculate the amount of phenylalanine by the following equation: the amount of phenylalanine calculated on the dried basis is not less than 9.0% and not more than 12.0%.

$$\text{Amount (\%) of phenylalanine} = \{(A_2 - 0.5A_1 + 0.5A_3 - 1.8A_4 + 0.8A_5)/W_T\} \times 9.4787$$

$W_T$: Amount (g) of the sample, calculated on the dried basis

**Purity** Heavy metals <*1.07*>—Proceed with 1.0 g of Polymixin B Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**Loss on drying** <*2.41*> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.75% (1 g).

**Assay** Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Agar media for seed and base layer

| | |
|---|---|
| Peptone | 10.0 g |
| Meat extract | 3.0 g |
| Sodium chloride | 30.0 g |
| Agar | 20.0 g |
| Water | 1000 mL |

Mix all the ingredients, and sterilize. Adjust the pH <*2.54*> of the solution so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Polymixin B Sulfate Reference Standard, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Polymixin B Sulfate, equivalent to about 200,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Polyoxyl 40 Stearate

ステアリン酸ポリオキシル 40

Polyoxyl 40 Stearate is the monostearate of condensation polymers of ethylene oxide represented by the formula $H(OCH_2CH_2)_nOCOC_{17}H_{35}$, in which $n$ is approximately 40.

**Description** Polyoxyl 40 Stearate occurs as a white to light yellow, waxy solid or powder. It is odorless or has a faint fat-like odor.

It is soluble in water, in ethanol (95) and in diethyl ether.

**Congealing point** ⟨*2.42*⟩ 39.0 – 44.0°C

**Congealing point of the fatty acid** ⟨*1.13*⟩ Not below 53°C.

**Acid value** ⟨*1.13*⟩ Not more than 1.

**Saponification value** ⟨*1.13*⟩ 25 – 35

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Polyoxyl 40 Stearate in 20 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Polyoxyl 40 Stearate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.67 g of Polyoxyl 40 Stearate, according to Method 3, and perform the test (not more than 3 ppm).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g)

**Containers and storage** Containers—Tight containers.

# Polysorbate 80

ポリソルベート 80

Polysorbate 80 is a polyoxyethylene ether of anhydrous sorbitol, partially esterified with oleic acid.

**Description** Polysorbate 80 is a colorless or orange-yellow, viscous liquid, having a faint, characteristic odor and a warm, slightly bitter taste.

It is miscible with methanol, with ethanol (95), with warm ethanol (95), with pyridine and with chloroform.

It is freely soluble in water and slightly soluble in diethyl ether.

The pH of a solution of Polysorbate 80 (1 in 20) is between 5.5 and 7.5.

**Identification** **(1)** To 5 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of sodium hydroxide TS, boil for 5 minutes, cool, and acidify with dilute hydrochloric acid: the solution is opalescent.

**(2)** To 5 mL of a solution of polysorbate 80 (1 in 20) add 2 to 3 drops of bromine TS: the color of the test solution is discharged.

**(3)** Mix 6 mL of Polysorbate 80 with 4 mL of water at an ordinary, or lower than ordinary, temperature: a jelly-like mass is produced.

**(4)** To 10 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of ammonium thiocyanate-cobaltous nitrate TS, shake well, add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

**Viscosity** ⟨*2.53*⟩ 345 – 445 $mm^2/s$ (Method 1, 25°C).

**Specific gravity** ⟨*1.13*⟩ $d_{20}^{20}$: 1.065 – 1.095

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

**Saponification value** ⟨*1.13*⟩ 45 – 55

**Iodine value** ⟨*1.13*⟩ 19 – 24 Use chloroform instead of cyclohexane, and titrate without using an indicator, until the yellow color of iodine disappears.

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Polysorbate 80 according to Method 2, 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 1.0 g of Polysorbate 80 according to Method 3, and perform the test (not more than 2 ppm).

**Water** ⟨*2.48*⟩ Not more than 3.0% (1 g, back titration).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (2 g).

**Containers and storage** Containers—Tight containers.

# Potash Soap

カリ石ケン

Potash Soap contains not less than 40.0% as fatty acids.

**Method of preparation**

| | |
|---|---|
| Fixed oil | 470 mL |
| Potassium Hydroxide | a sufficient quantity |
| Water or Purified Water | a sufficient quantity |
| | To make 1000 g |

Dissolve Potassium Hydroxide, in required quantity for saponification, in Water or Purified Water, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol if necessary, stir thoroughly, heat in a water bath, and continue the saponification. After complete saponification, add Water or Purified Water to make 1000 g.

**Description** Potash Soap occurs as a yellow-brown, transparent, unctuous, soft mass, having a characteristic odor.

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

**Purity** Silicic acid and alkalinity—Dissolve 10 g of Potash Soap in 30 mL of ethanol (95), and add 0.50 mL of 1 mol/L hydrochloric acid VS: no turbidity is produced. Add 1 drop of phenolphthalein TS to this solution: no red color develops.

**Assay** Weigh accurately about 5 g of Potash Soap, dissolve in 100 mL of hot water, and transfer to a separator. Acidify the mixture with dilute sulfuric acid, and cool. Extract the solution with 50-mL, 40-mL, and 30-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10-mL portions of water until the washing contains no acid. Trans-

fer the diethyl ether solution to a tared flask, evaporate diethyl ether on a water bath at a temperature as low as possible. Dry the residue at 80°C to constant mass, and weigh as fatty acids.

**Containers and storage** Containers—Tight containers.

# Potassium Bromide

臭化カリウム

KBr: 119.00

Potassium Bromide, when dried, contains not less than 99.0% of KBr.

**Description** Potassium Bromide occurs as colorless or white crystals, granules or crystalline powder. It is odorless.

It is freely soluble in water and in glycerin, soluble in hot ethanol (95), and slightly soluble in ethanol (95).

**Identification** A solution of Potassium Bromide (1 in 10) responds to Qualitative Tests <*1.09*> for potassium salt and for bromide.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.

**(2)** Alkalinity—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling, and cool: no color develops.

**(3)** Chloride—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.

**(4)** Sulfate <*1.14*>—Proceed with 2.0 g of Potassium Bromide, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

**(5)** Iodide—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple to purple color develops in the chloroform layer.

**(6)** Bromate—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water, and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

**(7)** Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Bromide 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).

**(8)** Barium—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

**(9)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 110°C, 4 hours).

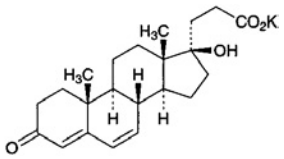
**Assay** Weigh accurately about 0.4 g of Potassium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and exactly measured 50 mL of 0.1 mol/L silver nitrate VS, and titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 11.90 mg of KBr

**Containers and storage** Containers—Tight containers.

# Potassium Canrenoate

カンレノ酸カリウム




$C_{22}H_{29}KO_4$: 396.56
Monopotassium 17-hydroxy-3-oxo-17$\alpha$-pregna-4,6-diene-21-carboxylate [*2181-04-6*]

Potassium Canrenoate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{22}H_{29}KO_4$.

**Description** Potassium Canrenoate occurs as a pale yellowish white to pale yellow-brown, crystalline powder.

It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

**Identification** **(1)** Dissolve 2 mg of Potassium Canrenoate in 2 drops of sulfuric acid: an orange color develops. Observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Add 1 drop of acetic anhydride to this solution: the color of the solution changes to red.

**(2)** Determine the absorption spectrum of a solution of Potassium Canrenoate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Potassium Canrenoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(4)** The solution of Potassium Canrenoate (1 in 10) responds to Qualitative Tests <*1.09*> (1) for potassium salt.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-71$ – $-76°$ (after drying, 0.2 g, methanol, 20 mL, 100 mm).

**pH** <*2.54*> Dissolve 1.0 g of Potassium Canrenoate in 20 mL of water: the pH of this solution is between 8.4 and 9.4.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Potassium Canrenoate in 5 mL of water: the solution is clear,

and shows a pale yellow to light yellow color.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Canrenoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Canrenoate according to Method 3, and perform the test (not more than 2 ppm).

**(4)** Canrenone—Place 0.40 g of Potassium Canrenoate in a glass-stoppered centrifuge tube, cool in ice-water to a temperature not higher than 5°C, add 6 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, being cooled to a temperature not higher than 5°C to dissolve, and add 8 mL of water being cooled to a temperature not higher than 5°C. Add exactly 10 mL of chloroform, allow to stand for 3 minutes at a temperature not higher than 5°C, shake vigorously for 2 minutes, and centrifuge. Drain off the water layer, collect 5 mL of the chloroform layer, transfer to a glass-stoppered centrifuge tube containing 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, cooled to a temperature not higher than 5°C, and 4 mL of water cooled to a temperature not higher than 5°C, shake for 1 minute, and centrifuge. Drain off the water layer, pipet 2 mL of the chloroform layer, and add chloroform to make exactly 10 mL. Determine the absorbance of this solution at 283 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it is not more than 0.67.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.2 g of Potassium Canrenoate, previously dried, dissolve in 75 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Use a solution of saturated potassium chloride-acetic acid (100) as the internal liquid.). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.66 mg of $C_{22}H_{29}KO_4$

**Containers and storage** Containers—Tight containers.

# Potassium Carbonate

炭酸カリウム

$K_2CO_3$: 138.21

Potassium Carbonate, when dried, contains not less than 99.0% of $K_2CO_3$.

**Description** Potassium Carbonate occurs as white granules or powder. It is odorless.

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

A solution of Potassium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

**Identification** A solution of Potassium Carbonate (1 in 10) responds to Qualitative Tests <*1.09*> for potassium salt and for carbonate.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Dissolve 1.0 g of Potassium Carbonate in 2 mL of water and 6 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 6 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution to dryness, and dilute with water to 50 mL (not more than 20 ppm).

**(3)** Sodium—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water, and perform the test as directed under Flame Coloration Test <*1.04*> (1): no persisting yellow color is produced.

**(4)** Arsenic <*1.11*>—Prepare the test solution with 0.5 g of Potassium Carbonate, according to Method 1, and perform the test (not more than 4 ppm).

**Loss on drying** <*2.41*> Not more than 1.0% (3 g, 180°C, 4 hours).

**Assay** Dissolve about 1.5 g of Potassium Carbonate, previously dried and accurately weighed, in 25 mL of water, titrate with 0.5 mol/L sulfuric acid VS until the blue color of the solution changes to yellow-green, boil cautiously, then cool, and titrate <*2.50*> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 69.10 mg of $K_2CO_3$

**Containers and storage** Containers—Tight containers.

# Potassium Chloride

塩化カリウム

KCl: 74.55

Potassium Chloride, when dried, contains not less than 99% of KCl.

**Description** Potassium Chloride occurs as colorless or white crystals or crystalline powder. It is odorless, and has a saline taste.

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

A solution of Potassium Chloride (1 in 10) is neutral.

**Identification** A solution of Potassium Chloride (1 in 50) responds to Qualitative Tests <*1.09*> for potassium salt and for chloride.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

**(2)** Acidity and alkalinity—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: no red color develops. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

**(3)** Bromide—Dissolve 1.0 g of Potassium Chloride in

water to make 100 mL. To 5 mL of the solution add 3 drops of dilute hydrochloric acid and 1 mL of chloroform, and add 3 drops of sodium toluensulfonchloramide TS dropwise while shaking: no yellow to yellow-red color develops in the chloroform layer.

**(4)** Iodide—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes, and shake again: no red-purple to purple color develops in the chloroform layer.

**(5)** Heavy metals <*1.07*>—Proceed with 4.0 g of Potassium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

**(6)** Calcium and magnesium—Dissolve 0.20 g of Potassium Chloride in 20 mL of water, add 2 mL of ammonia TS, 2 mL of ammonium oxalate TS and 2 mL of disodium hydrogenphosphate TS, and then allow to stand for 5 minutes: no turbidity is produced.

**(7)** Sodium—Dissolve 1.0 g of Potassium Chloride in 20 mL of water, and perform the Flame Coloration Test <*1.04*> (1): no persistent, yellow color develops.

**(8)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 1, and perform the test (not more than 2 ppm).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 130°C, 2 hours).

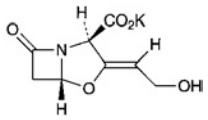
**Assay** Weigh accurately about 0.2 g of Potassium Chloride, previously dried, dissolve in 50 mL of water, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS = 7.455 mg of KCl

**Containers and storage** Containers—Tight containers.

# Potassium Clavulanate

クラブラン酸カリウム




$C_8H_8KNO_5$: 237.25
Monopotassium (2*R*,5*R*)-3-[(1*Z*)-2-hydroxyethylidene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*61177-45-5*]

Potassium Clavulanate is the potassium salt of a substance having $\beta$-lactamase inhibiting activity produced by the growth of *Streptomyces clavuligerus*.

It contains not less than 810 $\mu$g (potency) and not more than 860 $\mu$g (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavularic acid ($C_8H_9NO_5$: 199.16).

**Description** Potassium Clavulanate occurs as a white to light yellowish white, crystalline powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (95).

It is hygroscopic.

**Identification (1)** To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Potassium Clavulanate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Potassium Clavulanate responds to Qualitative Tests <*1.09*> (1) for potassium salt.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +53 – +63° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

**Purity (1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Clavulanate according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Clavulanate according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than clavulanic acid from the sample solution is not more than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not more than 2 times of the peak area of clavulanic acid from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

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

Mobile phase A: Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.

Mobile phase B: A mixture of the mobile phase A and methanol (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

| Time after injection of sample (min) | Mobile phase A (vol%) | Mobile phase B (vol%) |
|---|---|---|
| 0 – 4 | 100 | 0 |
| 4 – 15 | 100 → 0 | 0 → 100 |
| 15 – 25 | 0 | 100 |

Flow rate: 1.0 mL per minute.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

*System suitability*—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of clavulanic acid obtained from 20 $\mu$L of this solution is equivalent to 7 to 13% of that from 20 $\mu$L of the standard solution.

System performance: Dissolve 10 mg each of Potassium Clavulanate and Amoxycillin in 100 mL of the mobile phase A. When the procedure is run with 20 $\mu$L of this solution under the above operating conditions, clavulanic acid and amoxycillin are eluted in this order with the resolution between these peaks being not less than 8 and the number of theoretical plates of the peak of clavulanic acid is not less than 2500.

System repeatability: When the test is repeated 3 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clavulanic acid is not more than 2.0%.

**Water** ⟨*2.48*⟩ Not more than 1.5% (5 g, volumetric titration, direct titration).

**Assay** Weigh accurately an amount of Potassium Clavulanate and Lithium Clavulanate Reference Standard, equivalent to about 12.5 mg (potency), dissolve each in 30 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 $\mu$L 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 clavularic acid to that of the internal standard.

Amount [$\mu$g (potency)] of clavularic acid ($C_8H_9NO_5$) $= W_S \times (Q_T/Q_S) \times 1000$

$W_S$: Amount [mg (potency)] of Lithium Clavulanate Reference Standard

*Internal standard solution*—Dissolve 0.3 g of sulfanilamide in 30 mL of methanol, and add water to make 100 mL.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of sodium acetate trihydrate in 900 mL of water, adjust to pH 4.5 with diluted acetic acid (31) (2 in 5), and add 30 mL of methanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of clavularic acid is about 6 minutes.

*System suitability*—

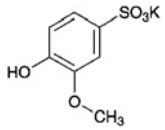
System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, clavularic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clavularic acid to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Potassium Guaiacolsulfonate

グアヤコールスルホン酸カリウム




$C_7H_7KO_5S$: 242.29

Monopotassium 4-hydroxy-3-methoxybenzenesulfonate [*1321-14-8*]

Potassium Guaiacolsulfonate contains not less than 98.5% of $C_7H_7KO_5S$, calculated on the anhydrous basis.

**Description** Potassium Guaiacolsulfonate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in water and in formic acid, soluble in methanol, and practically insoluble in ethanol (95), in acetic anhydride and in diethyl ether.

**Identification** **(1)** To 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) add 2 drops of iron (III) chloride TS: a blue-purple color develops.

**(2)** Dissolve 0.25 g of Potassium Guaiacolsulfonate in water to make 500 mL, and to 10 mL of this solution add phosphate buffer solution, pH 7.0, to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests ⟨*1.09*⟩ for potassium salt.

**pH** ⟨*2.54*⟩ Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the solution is clear and colorless.

**(2)** Sulfate ⟨*1.14*⟩—Perform the test with 0.8 g of Potassium Guaiacolsulfonate . Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than

0.030%).

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

**(4)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Potassium Guaiacolsulfonate according to Method 1, and perform the test (not more than 2 ppm).

**(5)** Related substances—Dissolve 0.20 g of Potassium Guaiacolsulfonate in 200 mL of mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area obtained from these solutions by the automatic integration method: the total area of peaks other than the peak of potassium guaiacolsulfonate from the sample solution is not larger than the peak area of potassium guaiacolsulfonate from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 to 25 cm in length, packed with dimethylaminopropylsilanized silica gel, (5 to 10 $\mu$m in particle diameter).

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

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate VS and methanol (20:1).

Flow rate: Adjust the flow rate so that the retention time of potassium guaiacolsulfonate is about 10 minutes.

Selection of column: Weigh 50 mg each of potassium guaiacolsulfonate and guaiacol, and dissolve in 50 mL of the mobile phase. Proceed with 5 $\mu$L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of guaiacol and potassium guaiacolsulfonate in this order with the resolution of these peaks being not less than 4.

Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiacolsulfonate from 5 $\mu$L of the standard solution is not less than 10 mm.

Time span of measurement: About twice as long as the retention time of potassium guaiacolsulfonate.

**Water** <*2.48*> 3.0 – 4.5% (0.3 g, direct titration).

**Assay** Weigh accurately about 0.3 g of Potassium Guaiacolsulfonate, dissolve in 2.0 mL of formic acid, add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.23 mg of $C_7H_7KO_5S$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Potassium Hydroxide

水酸化カリウム

KOH: 56.11

Potassium Hydroxide contains not less than 85.0% of KOH.

**Description** Potassium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks and in other forms. It is hard and brittle, and shows a crystalline fracture.

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

It rapidly absorbs carbon dioxide in air.

It deliquesces in the presence of moisture.

**Identification** **(1)** A solution of Potassium Hydroxide (1 in 500) is alkaline.

**(2)** A solution of Potassium Hydroxide (1 in 25) responds to Qualitative Tests <*1.09*> for potassium salt.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Hydroxide in 20 mL of water: the solution is clear and colorless.

**(2)** Chloride <*1.03*>—Dissolve 2.0 g of Potassium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 8 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

**(3)** Heavy metals <*1.07*>—Dissolve 1.0 g of Potassium Hydroxide in 5 mL of water, add 7 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 30 ppm).

**(4)** Sodium—Dissolve 0.10 g of Potassium Hydroxide in 10 mL of dilute hydrochloric acid, and perform the test as directed under Flame Coloration Test <*1.04*> (1): no persistent yellow color develops.

**(5)** Potassium carbonate—The amount of potassium carbonate ($K_2CO_3$: 138.21) is not more than 2.0% when calculated by the following equation using $B$ (mL) obtained in the Assay.

$$\text{Amount of potassium carbonate (mg)} = 138.21 \times B$$

**Assay** Weigh accurately about 1.5 g of Potassium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate <*2.50*> with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount $A$ (mL) of 0.5 mol/L sulfuric acid VS consumed, then add 2 drops of methyl orange TS, and titrate <*2.50*> again with 0.5 mol/L sulfuric acid VS until the solution changes to a persistent light red color. Record the

amount $B$ (mL) of 0.5 mol/L sulfuric acid VS consumed.

Calculate the amount KOH from the amount, $A$ (mL) − $B$ (mL).

Each mL of 0.5 mol/L sulfuric acid VS
= 56.11 mg of KOH

**Containers and storage** Containers—Tight containers.

# Potassium Iodide

ヨウ化カリウム

KI: 166.00

Potassium Iodide, when dried, contains not less than 99.0% of KI.

**Description** Potassium Iodide occurs as colorless or white crystals, or a white crystalline powder.

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

It is slightly deliquescent in moist air.

**Identification** A solution of Potassium Iodide (1 in 20) responds to Qualitative Tests <*1.09*> for potassium salt and for iodide.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.

**(2)** Alkalinity—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 0.50 mL of 0.005 mol/L sulfuric acid and 1 drop of phenolphthalein TS: no color develops.

**(3)** Chloride, bromide and thiosulfate—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 to 3 minutes, and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color develops. The solution has no more turbidity than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, and 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

**(4)** Nitrate, nitrite and ammonium—Place 1.0 g of Potassium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on it. Heat the test tube carefully on a water bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.

**(5)** Cyanide—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, then add 4 mL of hydrochloric acid: no green color develops.

**(6)** Iodate—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

**(7)** Heavy metals <*1.07*>—Proceed with 2.0 g of Potassium Iodide 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).

**(8)** Barium—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

**(9)** Sodium—Dissolve 1.0 g of Potassium Iodide in 10 mL of water, and perform the Flame Coloration Test (1) <*1.04*>: a yellow color develops, but does not persist.

**(10)** Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Potassium Iodide according to Method 1, and perform the test (not more than 5 ppm).

**Loss on drying** <*2.41*> Not more than 1.0% (2 g, 105°C, 4 hours).

**Assay** Weigh accurately about 0.5 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate <*2.50*> with 0.05 mol/L potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 16.60 mg of KI

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Potassium Permanganate

過マンガン酸カリウム

$KMnO_4$: 158.03

Potassium Permanganate, when dried, contains not less than 99.0% of $KMnO_4$.

**Description** Potassium Permanganate occurs as dark purple crystals and has a metallic luster.

It is soluble in water.

A solution of Potassium Permanganate (1 in 1000) has a slightly sweet, astringent taste.

**Identification** A solution of Potassium Permanganate (1 in 100) responds to Qualitative Tests <*1.09*> for permanganate.

**Purity** **(1)** Water-insoluble substances—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared glass filter (G4), wash with water until the last washing shows no color, and dry at 105°C for 2 hours: the mass of the residue is not more than 4 mg.

**(2)** Arsenic <*1.11*>—Dissolve 0.40 g of Potassium Permanganate in 10 mL of water, add 1 mL of sulfuric acid, add hydrogen peroxide (30) dropwise until the solution remains colorless, and evaporate on a sand bath nearly to dryness. Dissolve the residue in 5 mL of water, and perform the test with this solution as the test solution: the color produced is not more intense than the following standard color.

Standard color: To 10 mL of water add 1 mL of sulfuric acid and the same volume of hydrogen peroxide (30) as used for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and carry out

the test with this solution in the same manner as the test solution (not more than 5 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, silica gel, 18 hours).

**Assay** Weigh accurately about 0.6 g of Potassium Permanganate, previously dried, dissolve in water to make exactly 200 mL, and use this solution as the sample solution. Pipet 25 mL of 0.05 mol/L oxalic acid VS into a 500-mL conical flask, add 200 mL of diluted sulfuric acid (1 in 20), and keep at a temperature between 30°C and 35°C. Transfer the sample solution to a buret. Add quickly 23 mL of the sample solution from the buret to the flask while shaking gently, and then allow the flask to stand until the red color disappears. Warm the mixture to a temperature between 55°C and 60°C, and continue the titration ⟨*2.50*⟩ slowly until the red color persists for 30 seconds.

Each mL of 0.05 mol/L oxalic acid VS
= 3.161 mg of $KMnO_4$

**Containers and storage** Containers—Tight containers.

# Potassium Sulfate

硫酸カリウム

$K_2SO_4$: 174.26

Potassium Sulfate, when dried, contains not less than 99.0% of $K_2SO_4$.

**Description** Potassium Sulfate occurs as colorless crystals or a white, crystalline powder. It has a slightly saline, somewhat bitter taste.

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

**Identification** A solution of Potassium Sulfate (1 in 20) responds to Qualitative Tests ⟨*1.09*⟩ for potassium salt and for sulfate.

**Purity** **(1)** Clarity and color of solution, and acid or alkali—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water: the solution is clear, colorless and neutral.

**(2)** Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

**(3)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Potassium 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)** Sodium—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test ⟨*1.04*⟩ (1): no persistent yellow color develops.

**(5)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 1.0% (1 g, 110°C, 4 hours).

**Assay** Weigh accurately about 0.5 g of Potassium Sulfate, previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of $K_2SO_4$
= amount (mg) of barium sulfate ($BaSO_4$) × 0.7466

**Containers and storage** Containers—Well-closed containers.

# Potato Starch

*Amylum Solani*

バレイショデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Potato Starch consists of starch granules derived from the tuber of *Solanum tuberosum* Linné (*Solanaceae*).

◆**Description** Potato Starch occurs as a white powder.

It is practically insoluble in water and in ethanol (99.5).◆

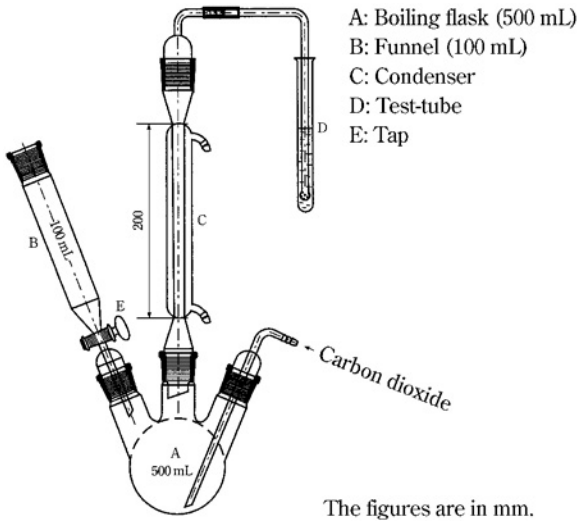
**Identification** **(1)** Under a microscope, Potato Starch, preserved in a mixture of water and glycerin (1:1), appears as unevenly ovoid or pyriform simple grains usually 30 – 100 μm, often more than 100 μm in diameter, or spherical simple grains 10 – 35 μm in diameter, rarely 2- to 4-compound grains; ovoid or pyriform simple grains with eccentric hilum, spherical simple grains with non-centric or slightly eccentric hilum; striation distinct in all grains; a black cross, its intersection point on hilum, is observed when grains are put between two nicol prisms fixed at right angle to each other.

**(2)** To 1 g of Potato Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

**(3)** To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed, and the color disappears by heating.

**pH** ⟨*2.54*⟩ Put 5.0 g of Potato Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 5.0 and 8.0.

**Purity** **(1)** Iron—To 1.5 g of Potato Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (2 in 10) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test so-




lution is not darker than that of the control solution (not more than 10 ppm).

**(2)** Oxidizing substances—To 4.0 g of Potato Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

**(3)** Sulfur dioxide—

(i) Apparatus Use as shown in the figure.

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Potato Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = (V/W) \times 1000 \times 3.203$$

$W$: Amount (g) of the sample

$V$: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

**Loss on drying** <2.41> Not more than 20.0% (1 g, 130°C, 90 minutes).

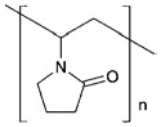
**Residue on ignition** <2.44> Not more than 0.6% (1 g).

◆**Containers and storage** Containers—Well-closed containers.◆

# Povidone

## Polyvidone
## Polyvinylpyrrolidone

ポビドン



$(C_6H_9NO)_n$

Poly[(2-oxopyrrolidin-1-yl)ethylene] [*9003-39-8*]

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone. It contains not less than 11.5% and not more than 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis.

It has a nominal K-value of not less than 25 and not more than 90. The nominal K-value is shown on the label.

**Description** Povidone occurs as a white to slightly yellowish fine powder. It is odorless or has a faint, characteristic odor.

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

It is hygroscopic.

**Identification** Determine the infrared absorption spectrum of Povidone, previously dried at 105°C for 6 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Povidone Reference Standard previously dried at 105°C for 6 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

**pH** <2.54> Dissolve 1.0 g of Povidone in 20 mL of water: the pH of this solution is between 3.0 and 5.0 for Povidone having the nominal K-value of 30 or less, and between 4.0 and 7.0 for Povidone having the nominal K-value exceeding 30.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Povidone in 20 mL of water: the solution is clear and colorless to pale yellow, or pale red.

**(2)** Heavy metals <1.07>—Proceed with 2.0 g of Povidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Aldehydes—Weigh accurately about 1.0 g of Povidone and dissolve in 0.05 mol/L pyrophosphate buffer solu-

tion, pH 9.0 to make exactly 100 mL. Stopper, heat at 60°C for 60 minutes, allow to cool to room temperature, and use this solution as the sample solution. Separately, dissolve 0.100 g of freshly distilled acetaldehyde in water previously cooled to 4°C to make exactly 100 mL. Allow to stand at 4°C for about 20 hours, pipet 1 mL of this solution, add 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL, and use this solution as the standard solution. Measure 0.5 mL each of the sample solution, standard solution and water (for blank test), transfer to separate cells, add 2.5 mL of 0.05 mol/L pyrophosphate buffer solution, pH 9.0, and 0.2 mL of $\beta$-nicotinamide adenine dinucleotide TS to each of these cells, mix and stopper tightly. Allow to stand for 2 to 3 minutes at 22 ± 2°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the control solution. Determine the absorbances, $A_{T1}$, $A_{S1}$ and $A_{B1}$ of the subsequent solutions of the sample solution, the standard solution and water at 340 nm. Add 0.05 mL of aldehyde dehydrogenase solution to each of the cells, mix and stopper tightly. Allow to stand for 5 minutes at 22 ± 2°C. Determine the absorbances, $A_{T2}$, $A_{S2}$ and $A_{B2}$ of these solutions in the same manner as above: the content of aldehydes is not more than 500 ppm (expressed as acetaldehyde).

$$\text{Content (ppm) of aldehydes expressed as acetaldehyde} = \frac{1000}{W} \times \frac{(A_{T2}-A_{T1})-(A_{B2}-A_{B1})}{(A_{S2}-A_{S1})-(A_{B2}-A_{B1})}$$

$W$: Amount (g) of povidone, calculated on the anhydrous basis.

**(4)** 1-Vinyl-2-pyrrolidone—Weigh accurately about 0.25 g of Povidone, dissolve in diluted methanol (1 in 5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 $\mu$L 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 1-vinyl-2-pyrrolidone in each solution: the content of 1-vinyl-2-pyrrolidone is not more than 10 ppm.

$$\text{Content (ppm) of 1-vinyl-2-pyrrolidone} = (2.5/W) \times (A_T/A_S)$$

$W$: Amount (g) of Povidone, calculated on the anhydrous basis.

*Operating conditions—*

Detector: An ultraviolet spectrophotometer (detection wavelength: 254 nm).

Column: Stainless steel columns about 4 mm in inside diameter and about 25 mm in length, and about 4 mm in inside diameter and about 250 mm in length, packed with octylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter), and use them as a guard column and a separation column, respectively.

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

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 10 minutes.

Selection of column: Dissolve 0.01 g of 1-vinyl-2-pyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution add diluted methanol (1 in 5) to make 100 mL. Proceed with 50 $\mu$L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of 1-vinyl-2-pyrrolidone and vinyl acetate in this order with the resolution between these peaks being not less than 2.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone obtained from 50 $\mu$L of the standard solution is between 10 mm and 15 mm.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of obtained peak areas of 1-vinyl-2-pyrrolidone is not more than 2%.

Washing of the guard column: After each test with the sample solution, wash away the polymeric material of Povidone from the guard column by passing the mobile phase through the column backwards for about 30 minutes at the same flow rate as applied in the test.

**(5)** Peroxides—Weigh exactly an amount of Povidone, equivalent to 4.0 g calculated on the anhydrous basis, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. To 25 mL of the sample solution add 2 mL of titanium (III) chloride-sulfuric acid TS, and mix. Allow to stand for 30 minutes, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared by adding 2 mL of 13 % sulfuric acid to 25 mL of the sample solution as a blank: the absorbance of the subsequent solution of the sample solution at 405 nm is not more than 0.35 (not more than 400 ppm, expressed as hydrogen peroxide).

**(6)** Hydrazine—Transfer 2.5 g of Povidone to a 50-mL centrifuge tube, add 25 mL of water, and stir to dissolve. Add 500 $\mu$L of a solution of salicylaldehyde in methanol (1 in 20), stir and warm at 60°C for 15 minutes in a water bath. Allow to cool, add 2.0 mL of toluene, stopper tightly, shake vigorously for 2 minutes, centrifuge, and use the upper layer of the mixture as the sample solution. Separately, dissolve 0.09 g of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 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 $\mu$L each of the sample solution and standard solution on a plate coated with a 0.25-mm layer of dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and water (2:1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the *R*f value of the fluorescent spot from the standard solution is about 0.3, and the fluorescence of the spot from the sample solution corresponding to the spot from the standard solution is not more intense than that of the spot from the standard solution (not more than 1 ppm).

**Water** <*2.48*> Not more than 5.0% (0.5 g, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**K-value** Weigh accurately an amount of Povidone, equivalent to 1.00 g calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL, allow to stand for 60

minutes, and use this solution as the sample solution. Perform the test with the sample solution and with water at 25°C as directed in Method 1 under Viscosity Determination ⟨*2.53*⟩, and calculate the K-value by the following formula.

$$K = \frac{1.5 \log \eta_{rel} - 1}{0.15 + 0.003\,c} + \frac{\sqrt{300\,c \log \eta_{rel} + (c + 1.5\,c \log \eta_{rel})^2}}{0.15\,c + 0.003\,c^2}$$

$c$: Mass (g) of Povidone in 100 mL of the solution, calculated on the anhydrous basis.
$\eta_{rel}$: Kinematic viscosity of the sample solution relative to that of water.

The K-value of Povidone is not less than 90% and not more than 108% of the nominal K-value.

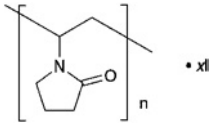
**Assay** Weigh accurately about 0.1 g of Povidone, and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium slfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing to flow down the inside wall of the flask. Heat the flask gradually over a free flame until the solution has a clear, yellow-green color and the inside wall of the flask is free from a carbonaceous material, and then heat for further 45 minutes. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (2 in 5) through the funnel, rinse cautiously the funnel with 10 ml of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to get 80 to 100 mL of the distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate ⟨*2.50*⟩ the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.025 mol/L sulfuric acid VS
= 0.700 mg of N

**Containers and storage** Containers—Tight containers.

# Povidone-Iodine

ポビドンヨード




$(C_6H_9NO)_n.xI$
Poly[(2-oxopyrrolidin-1-yl)ethylene] iodine [*25655-41-8*]

Povidone-Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer.

It contains not less than 9.0% and not more than 12.0% of available iodine (I: 126.90), and not less than 9.5% and not more than 11.5% of nitrogen (N: 14.01), calculated on the dried basis.

**Description** Povidone-Iodine occurs as a dark red-brown powder. It has a faint, characteristic odor.

It is freely soluble in water and in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Povidone-Iodine in 100 mL of water is between 1.5 and 3.5.

**Identification** **(1)** To 10 mL of diluted starch TS (1 in 10) add 1 drop of a solution of Povidone-Iodine (1 in 10): a deep blue color develops.

**(2)** To 1 mL of a solution of Povidone-Iodine (1 in 100) add 1 mL of sodium thiosulfate TS, and add 1 mL of ammonium thiocyanate-cobaltous nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.30 g of Povidone-Iodine in 100 mL of water: the solution is clear and brown.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Povidone-Iodine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

**(4)** Iodide ion—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 100 mL of water, and add sodium hydrogensulfite TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate ⟨*2.50*⟩ the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS until the solution develops a red-brown color, and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 12.69 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%) of available iodine from the total amount (%) of iodine: it is not more than 6.6%.

**Loss on drying** ⟨*2.41*⟩ Not more than 8.0% (1 g, 100°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.05% (5 g).

**Assay** **(1)** Available iodine—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 30 mL of water, and titrate ⟨*2.50*⟩ with 0.02 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

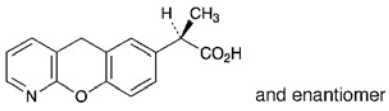
Each mL of 0.02 mol/L sodium thiosulfate VS
= 2.538 mg of I

**(2)** Nitrogen—Weigh accurately about 20 mg of Povidone-Iodine, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩.

**Containers and storage** Containers—Tight containers.

# Pranoprofen

プラノプロフェン




$C_{15}H_{13}NO_3$: 255.27
(2*RS*)-2-(10*H*-9-Oxa-1-azaanthracen-6-yl)propanoic acid
[*52549-17-4*]

Pranoprofen, when dried, contains not less than 98.5% of $C_{15}H_{13}NO_3$.

**Description** Pranoprofen occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) and in acetic anhydride, very slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Pranoprofen in *N,N*-dimethylformamide (1 in 30) shows no optical rotation.

**Identification** **(1)** Dissolve 0.02 g of Pranoprofen in 1 mol/L hydrochloric acid TS to make 100 mL, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pranoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 186 – 190°C

**Purity** **(1)** Chloride < *1.03* > —Dissolve 0.5 g of Pranoprofen in 40 mL of methanol, and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.30 mL of 0.01 mol/L hydrochloric acid add 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pranoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of the Standard Lead Solution (not more than 10 ppm).

**(3)** Related Substances—Dissolve 50 mg of Pranoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase 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. Determine each peak area from both solutions by the automatic integration method: the each area of the peaks other than the peak of pranoprofen from the sample solution is not larger than the peak area of pranoprofen from the standard solution, and the total peak area of them is not larger than twice of the peak area of pranoprofen from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 275 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 25°C.

Mobile phase: Dissolve 7.02 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution add 1 volume of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pranoprofen is about 10 minutes.

Selection of column: Dissolve 4 mg each of Pranoprofen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pranoprofen and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranoprofen from 10 μL of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About three times as long as the retention time of pranoprofen.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

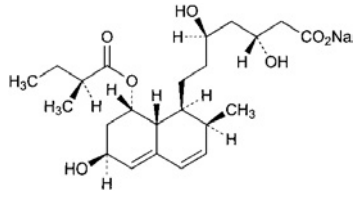
**Assay** Weigh accurately about 0.4 g of Pranoprofen, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.53 mg of $C_{15}H_{13}NO_3$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Pravastatin Sodium

プラバスタチンナトリウム




$C_{23}H_{35}NaO_7$: 446.51
Monosodium (3*R*,5*R*)-3,5-dihydroxy-7-{(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-

8-[(2*S*)-2-methylbutanoyloxy]-
1,2,6,7,8,8a-hexahydronaphthalen-1-yl} heptanoate
[*81131-70-6*]

Pravastatin Sodium contains not less than 98.5% and not more than 101.0% of $C_{23}H_{35}NaO_7$, calculated on the anhydrous basis and corrected on the amount of residual solvent.

**Description** Pravastatin Sodium occurs as a white to yellowish white, powder or crystalline powder.

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

It is hygroscopic.

**Identification** **(1)** Determine the absorption spectrum of a solution of Pravastatin Sodium (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 2970 $cm^{-1}$, 2880 $cm^{-1}$, 1727 $cm^{-1}$ and 1578 $cm^{-1}$.

**(3)** Dissolve 50 mg of Pravastatin Sodium in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 24 mg of Pravastatin 1,1,3,3-tetramethylbutylammonium Reference Standard 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 $\mu$L 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 acetic acid (100) (80:16:1) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the color tone and the *R*f value of the principal spot with the sample solution are not different with them of the spot with the standard solution.

**(4)** A solution of Pravastatin Sodium (1 in 10) responds to Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

**Optical rotation** ⟨*2.49*⟩: +153 – +159° (0.1 g calculated on the anhydrous basis and corrected on the amount of residual solvent, water, 20 mL, 100 mm).

**pH** ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Pravastatin Sodium 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).

**(2)** Related substances—Dissolve 0.10 g of Pravastatin Sodium in 100 mL of a mixture of water and methanol (11:9), and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water and methanol (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pravastatin is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin is not larger than the peak area of pravastatin from the standard solution. Keep the sample solution and the standard solution at not over than15°C.

*Operating conditions*—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of pravastatin beginning after the solvent peak.

*System suitability*—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and methanol (11:9) to make exactly 50 mL. Confirm that the peak area of pravastatin obtained with 10 $\mu$L of this solution is equivalent to 7 to 13% of that with 10 $\mu$L of the standard solution.

System performance: Dissolve 5 mg of Pravastatin Sodium in 50 mL of the mixture of water and methanol (11:9). When the procedure is run with 10 $\mu$L of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.0%.

(3) Residual solvent—Being specified separately.

**Water** ⟨*2.48*⟩ Not more than 4.0% (0.5 g, volumetric titration, direct titration).

**Assay** Weigh accurately about 0.1 g of Pravastatin Sodium, and dissolve in a mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11:9) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-tetramethylbutylamine Reference Standard (previously determine the water with 0.5 g by direct titration in volumetric titration) dissolve in the mixture of water and methanol (11:9) to make exactly 25 mL. Proceed with exactly 10 mL of this solution in the same manner for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with 10 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of pravastatin to that of the internal standard.

$$\text{Amount (mg) of } C_{23}H_{35}NaO_7 = W_S \times (Q_T/Q_S) \times 4 \times 1.0518$$

$W_S$: Amount (mg) of Pravastatin 1,1,3,3-tetramethylbutylamine Reference Standard, calculated on the anhydrous basis

*Internal standard solution*—A solution of ethyl parahydroxybenzoate in the mixture of water and methanol (11:9) (3 in 4000).

*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 25°C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (550:450:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 21 minutes.

*System suitability*—

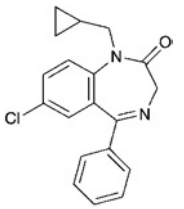
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 10.

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 ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Prazepam

プラゼパム




$C_{19}H_{17}ClN_2O$: 324.80

7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one [*2955-38-6*]

Prazepam, when dried, contains not less than 98.5% of $C_{19}H_{17}ClN_2O$.

**Description** Prazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in acetic anhydride, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

**Identification** **(1)** Dissolve 0.01 g of Prazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

**(2)** Dissolve 0.01 g of Prazepam in 1000 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Prazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(4)** Perform the Flame Coloration Tests <*1.04*> (2) with Prazepam: a green color appears.

**Melting point** <*2.60*> 145 – 148°C

**Purity** **(1)** Chloride <*1.03*>—To 1.0 g of Prazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

**(2)** Sulfate <*1.14*>—To 20 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

**(3)** Heavy metals <*1.07*>—Proceed with 2.0 g of Prazepam according to Method 2, 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 Prazepam according to Method 3, and perform the test (not more than 2 ppm).

**(5)** Related substances—Dissolve 0.40 g of Prazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL, 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 chloroform and acetone (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.20% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Prazepam, previously dried, dissolve in 60 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.48 mg of $C_{19}H_{17}ClN_2O$

**Containers and storage** Containers—Tight containers.

# Prazepam Tablets

プラゼパム錠

Prazepam Tablets contain not less than 93% and not more than 107% of the labeled amount of prazepam ($C_{19}H_{17}ClN_2O$: 324.80).

**Method of preparation** Prepare as directed under Tablets, with Prazepam.

**Identification** **(1)** To a quantity of powdered Prazepam Tablets, equivalent to 0.05 g of Prazepam according to the labeled amount, add 25 mL of acetone, shake well, and filter. Take 5 mL of the filtrate, evaporate on a water bath to dryness, and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

**(2)** To a quantity of powdered Prazepam Tablets, equivalent to 0.02 g of Prazepam according to the labeled amount, add 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and filter. To 5 mL of the filtrate add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 50 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm, and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Proceed with 1 tablet of Prazepam Tablets according to the Basket method, using 900 mL of 0.1 mol/L hydrochloric acid TS as the test solution at 100 rotations per minute. 30 minutes after starting the test, separate 20 mL or more of the dissolved solution, and filter with a membrane filter with pore size not more than 0.8 $\mu$m. Discard the first 10 mL of the filtrate, measure exactly the subsequent $V$ mL of the filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly $V'$ mL so that each mL of this solution might contain about 5 $\mu$g of prazepam ($C_{19}H_{17}ClN_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of prazepam for assay, previously dried at 105°C for 2 hours, add 200 mL of 0.1 mol/L hydrochloric acid TS and dissolve with shaking, or by ultrasonication if necessary, add 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Prazepam Tablets during 30 minutes is not less than 80%.

Dissolution rate (%) of prazepam ($C_{19}H_{17}ClN_2O$) to the labeled amount
$= W_S \times (A_T/A_S) \times (V'/V) \times (90/C)$

$W_S$: Amount (mg) of prazepam for assay.
$C$: Labeled amount (mg) of prazepam ($C_{19}H_{17}ClN_2O$) in each tablet.

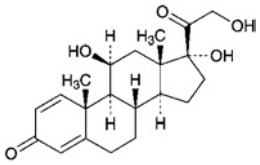
**Assay** Weigh accurately not less than 20 Prazepam Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 50 mg of prazepam ($C_{19}H_{17}ClN_2O$), add 30 mL of acetone, shake well, centrifuge, and separate the supernatant. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 6.496 mg of $C_{19}H_{17}ClN_2O$

**Containers and storage** Containers—Tight containers.

# Prednisolone

プレドニゾロン




$C_{21}H_{28}O_5$: 360.44
11$\beta$,17,21-Trihydroxypregna-1,4-diene-3,20-dione
[*50-24-8*]

Prednisolone, when dried, contains not less than 97.0% and not more than 102.0% of $C_{21}H_{28}O_5$.

**Description** Prednisolone occurs as a white, crystalline powder.

It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate and in chloroform, and very slightly soluble in water.

Melting point: about 235°C (with decomposition).

**Identification** **(1)** To 2 mg of Prednisolone add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

**(2)** Determine the infrared absorption spectrum of Prednisolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Prednisolone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Prednisolone and Prednisolone Reference Standard in ethyl acetate, respectively, then evaporate the ethyl acetate to dryness, and repeat the test on the residues.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +113 – +119° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

**Purity** **(1)** Selenium—To 0.10 g of Prednisolone add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light yel-

low clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 6 mL of nitric acid, then add water to make exactly 50 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, and determine constant absorbances, $A_T$ and $A_S$, obtained on a recorder after rapid increasing of the absorption: $A_T$ is smaller than $A_S$ (not more than 30 ppm).

Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: A selenium hollow cathode lamp

Wavelength: 196.0 nm

Temperature of sample atomizer: When an electric furnace is used, about 1000°C.

Carrier gas: Nitrogen or argon

**(2)** Related substances—Dissolve 20 mg of Prednisolone in exactly 2 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of hydrocortisone and 10 mg of prednisolone acetate each in a mixture of methanol and chloroform (1:1) to make exactly 100 mL, and use these solutions as the standard solution (1) and standard solution (2). 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 (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene and diethylamine (55:45:2) to a distance of about 15 cm, and air-dry the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the sample solution corresponding to those from the standard solutions (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), and no spots other than the principal spot, hydrocortisone and prednisolone acetate appear from the sample solution.

**Loss on drying** <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (0.5 g).

**Assay** Dissolve about 25 mg each of Prednisolone and Prednisolone Reference Standard, previously dried and accurately weighed, in 50 mL of methanol, add exactly 25 mL of the internal standard solution to each, and add methanol to make 100 mL. To 1 mL each of these solutions add the mobile phase to make 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of these solutions 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 prednisolone to that of the internal standard.

$$\text{Amount (mg) of } C_{21}H_{28}O_5 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Prednisolne Reference Standard

*Internal standard solution*—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 247 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with fluorosilanized 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 methanol (13:7).

Flow rate: Adjust the flow rate so that the retention time of prednisolone is about 15 minutes.

*System suitability*—

System performance: Dissolve 25 mg of Prednisolone and 25 mg of hydrocortisone in 100 mL of methanol. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrocortisone and prednisolone 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 ratios of the peak area of prednisolone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Prednisolone Tablets

プレドニゾロン錠

Prednisolone Tablets contain not less than 90% and not more than 110% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

**Method of preparation** Prepare as directed under Tablets, with Prednisolone.

**Identification** **(1)** Weigh a quantity of powdered Prednisolone Tablets, equivalent to 0.05 g of Prednisolone according to the labeled amount, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour, and proceed as directed in the Identification (1) under Prednisolone.

**(2)** Determine the infrared absorption spectra of the residue obtained in (1) and Prednisolone Reference Standard, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the Reference Standard in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

**Uniformity of dosage units** <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Prednisolone Tablets to a volumetric flask, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet $x$ mL of the supernatant liquid, and add methanol to make exactly $V$ mL to provide a solution that contains about 10 μg of prednisolone ($C_{21}H_{28}O_5$) per ml, and use this solution as the sample solution. Separately, weigh accurately

about 10 mg of Prednisolone Reference Standard, previously dried at 105°C for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, and add 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. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of prednisolone } (C_{21}H_{18}O_5) = W_S \times (A_T/A_S) \times (V/100) \times (1/x)$$

$W_S$: Amount (mg) of Prednisolone Reference Standard

**Dissolution** <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Predonisolone Tablets at 100 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Twenty minutes after the start of the test, take 20 mL or more of the dissolved solution, and filter through a membrane filter with pore size of 0.8 $\mu$m or less. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 10 mg of Prednisolone Reference Standard, previously dried at 105°C for 3 hours, and dissolve in ethanol (95) 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, $A_T$ and $A_S$, of the sample solution and standard solution at the maximum wavelength at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Prednisolone Tablets after 20 minutes should be not less than 70%.

$$\text{Dissolution rate (\%) with respect to the labeled amount of prednisolone } (C_{21}H_{28}O_5) = W_S \times (A_T/A_S) \times (45/C)$$

$W_S$: Amount (mg) of Prednisolone Reference Standard.
$C$: Labeled amount (mg) of prednisolone ($C_{21}H_{28}O_5$) in 1 tablet.

**Assay** Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone ($C_{21}H_{28}O_5$), add 1 mL of water, and shake gently. Add exactly 5 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1 mL of this solution add the mobile phase to make 10 mL, and filter through a membrane filter with pore size of 0.45 $\mu$m. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Reference Standard, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, add exactly 25 mL of the internal standard solution, and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone with these solutions.

$$\text{Amount (mg) of prednisolone } (C_{21}H_{28}O_5) = W_S \times (Q_T/Q_S) \times (1/5)$$

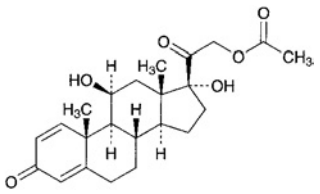
$W_S$: Amount (mg) of Prednisolone Reference Standard

*Internal standard solution*—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

**Containers and storage** Containers—Tight containers.

# Prednisolone Acetate

プレドニゾロン酢酸エステル



$C_{23}H_{30}O_6$: 402.48
11$\beta$,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-acetate [*52-21-1*]

Prednisolone Acetate, when dried, contains not less than 96.0% and not more than 102.0% of $C_{23}H_{30}O_6$.

**Description** Prednisolone Acetate occurs as a white, crystalline powder.

It is slightly soluble in methanol, in ethanol (95), in ethanol (99.5), and in chloroform, and practically insoluble in water.

Melting point: about 235°C (with decomposition).

**Identification** **(1)** To 2 mg of Prednisolone Acetate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

**(2)** Determine the infrared absorption spectra of Prednisolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum in a range between 4000 $cm^{-1}$ and 650 $cm^{-1}$ with the Infrared Reference Spectrum or the spectrum of previously dried Prednisolone Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the Reference Standard in ethanol (99.5), respectively, evaporate to dryness, and repeat the test on the residues.

**Optical rotation** <2.49> $[\alpha]_D^{20}$: +128 – +137° (after drying, 70 mg, methanol, 20 mL, 100 mm).

**Purity** Related substanes—Dissolve 0.20 g of Prednisolone Acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Separately, dissolve 20 mg each of prednisolone, cortisone acetate and hydrocortisone acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1). Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 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 5 $\mu$L 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 dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (wavelength: 254 mm): the spots from the sample solution corresponding to those from the standard solution are

not more intense than the spots from the standard solution, and any spot from the sample solution other than the principal spot and the spots from prednisolone, cortisone acetate and hydrocortisone acetate does not appear.

**Loss on drying** ⟨*2.41*⟩ Not more than 1.0% (0.5 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

**Assay** Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate Reference Standard, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the text with 10 $\mu$L 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 height of prednisolone acetate to that of the internal standard.

$$\text{Amount (mg) of } C_{23}H_{30}O_6 = W_S \times (Q_T/Q_S)$$

$W_S$: Amount (mg) of Prednisolone Acetate Reference Standard

*Internal standard solution*—A solution of butyl parahydroxybenzoate in methanol (3 in 1000).

*Operating conditions*—

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

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

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

Flow rate: Adjust the flow rate so that the retention time of prednisolone acetate is about 10 minutes.

*System suitability*—

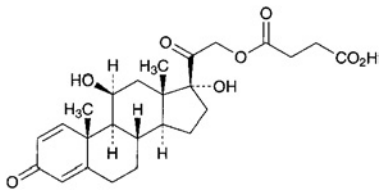
System performance: When the procedure is run with 10 $\mu$L of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

# Prednisolone Succinate

プレドニゾロンコハク酸エステル



$C_{25}H_{32}O_8$: 460.52

11$\beta$,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(hydrogen succinate) [*2920-86-7*]

Prednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{32}O_8$.

**Description** Prednisolone Succinate occurs as a white, fine, crystalline powder. It is odorless.

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

Melting point: about 205°C (with decomposition).

**Identification** **(1)** To 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

**(2)** Determine the infrared absorption spectrum of Prednisolone Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Prednisolone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: +114 – +120° (after drying, 67 mg, methanol, 10 mL, 100 mm).

**Purity** Related substances—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1 mL of the solution, add methanol to make exactly 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 5 $\mu$L 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 ethanol (95) (2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 10 mg each of Prednisolone

Succinate and Prednisolone Succinate Reference Standard, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

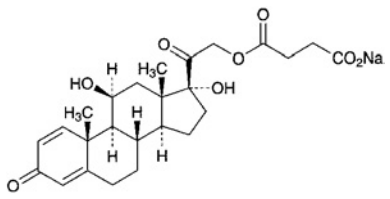
$$\text{Amount (mg) of } C_{25}H_{32}O_8 = W_S \times (A_T/A_S)$$

$W_S$: Amount (mg) of Prednisolone Succinate Reference Standard

**Containers and storage** Containers—Tight containers.

# Prednisolone Sodium Succinate for Injection

注射用プレドニゾロンコハク酸エステルナトリウム



$C_{25}H_{31}NaO_8$: 482.50
Monosodium 11$\beta$,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-succinate [*1715-33-9*]

Prednisolone Sodium Succinate for Injection is a preparation for injection which is dissolved before used.

It contains not less than 72.4% and not more than 83.2% of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$), and the equivalent of not less than 90% and not more than 110% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

The amount should be stated as the amount of prednisolone ($C_{21}H_{28}O_5$).

**Method of preparation** Prepare as directed under Injections, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide.

It contains a suitable buffer agent.

**Description** Prednisolone Sodium Succinate for Injection occurs as a white powder or porous, friable mass.

It is freely soluble in water.

It is hygroscopic.

**Identification (1)** To 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

**(2)** Dissolve 0.01 g of Prednisolone Sodium Succinate for Injection in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

**(3)** Dissolve 0.1 g of Prednisolone Sodium Succinate for Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and filter. Add 1 mL of dilute hydrochloric acid to the filtrate, shake, and filter if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6, and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

**(4)** Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

**pH** <2.54> Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

**Purity** Clarity and color of solution—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

**Loss on drying** <2.41> Not more than 2.0% (0.15 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

**Assay** Take a quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.1 g of prednisolone ($C_{21}H_{28}O_5$), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate Reference Standard, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 $\mu$L of the sample solution and standard solution as directed under Liquid Chromatography according <2.01> to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of prednisolone succinate to that of the internal standard.

$$\text{Amount (mg) of prednisolone sodium succinate } (C_{25}H_{31}NaO_8) = W_S \times (Q_T/Q_S) \times 5 \times 1.0477$$

$$\text{Amount (mg) of prednisolone } (C_{21}H_{28}O_5) = W_S \times (Q_T/Q_S) \times 5 \times 0.7827$$

$W_S$: Amount (mg) of Prednisolone Succinate Reference Standard

*Internal standard solution*—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.32 g of tetra *n*-butylammonium bromide, 3.22 g of disodium hydrogen phosphate dodacahy-

drate and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of prednisolone succinate is about 15 minutes.

*System suitability—*

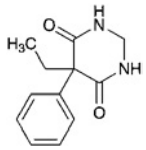
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

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 ratios of the peak area of prednisolone succinate to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Primidone

プリミドン




$C_{12}H_{14}N_2O_2$: 218.25

5-Ethyl-5-phenyl-2,3-dihyropyrimidine-4,6(1*H*,5*H*)-dione

[*125-33-7*]

Primidone, when dried, contains not less than 98.5% of $C_{12}H_{14}N_2O_2$.

**Description** Primidone occurs as a white, crystalline powder or granules. It is odorless and has a slightly bitter taste.

It is soluble in *N,N*-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

**Identification** **(1)** Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.

**(2)** Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate, and heat: the gas evolved changes moistened red litmus paper to blue.

**Melting point** ⟨*2.60*⟩ 279 – 284°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Primidone in 10 mL of *N,N*-dimethylformamide: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Primidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** 2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethyl silyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 μL of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard: $Q_T$ is not more than $Q_S$.

*Internal standard solution*—A solution of stearylalcohol in pyridine (1 in 2000).

*Operating conditions—*

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliceous earth for gas chromatography (125 to 150 μm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of stearylalcohol is about 10 minutes.

*System suitability—*

System performance: When the procedure is run with 2 μL of the standard solution under the above operating condition, 2-ethyl-2-phenylmalonediamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.2% (1 g).

**Assay** Weigh accurately about 20 mg each of Primidone and Primidone Reference Standard, previously dried, dissolve each in 20 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 25 mL, and use these solutions as the sample solution and standard solution, respectively. Determine the absorbance, $A_1$, of the sample solution and the standard solution at the wavelength of maximum absorption at about 257 nm, and the absorbances, $A_2$ and $A_3$, at the wavelength of minimum absorption at about 254 nm and at about 261 nm, as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using ethanol (95) as the blank.

$$\text{Amount (mg) of } C_{12}H_{14}N_2O_2 = W_S \times \{(2A_1 - A_2 - A_3)_T / (2A_1 - A_2 - A_3)_S\}$$

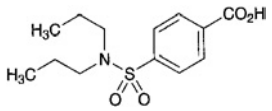
$W_S$: Amount (mg) of Primidone Reference Standard

where, $(2A_1 - A_2 - A_3)_T$ is the value from the sample solution, and $(2A_1 - A_2 - A_3)_S$ is from the standard solution.

**Containers and storage** Containers—Tight containers.

# Probenecid

プロベネシド



$C_{13}H_{19}NO_4S$: 285.36
4-(Dipropylaminosulfonyl)benzoic acid [*57-66-9*]

Probenecid, when dried, contains not less than 98.0% of $C_{13}H_{19}NO_4S$.

**Description** Probenecid occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste, followed by unpleasant bitter.

Probenecid is sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

Melting point: 198 – 200°C

**Identification** **(1)** Heat Probenecid strongly: the odor of sulfur dioxide is perceptible.

**(2)** Determine the absorption spectrum of a solution of Probenecid in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probenecid Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**Purity** **(1)** Acidity—To 2.0 g of Probenecid add 100 mL of water, heat on a water bath with occasional shaking for 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

**(2)** Chloride <*1.03*>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of nitric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

**(3)** Sulfate <*1.14*>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of hydrochloric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

**(4)** Heavy metals <*1.07*>—Proceed with 2.0 g of Probenecid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(5)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Probenecid according to Method 3, and perform the test (not more than 2 ppm).

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Probenecid, previously dried, and dissolve in 50 mL of neutralized ethanol. Titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 28.54 mg of $C_{13}H_{19}NO_4S$

**Containers and storage** Containers—Well-closed containers.

# Probenecid Tablets

プロベネシド錠

Probenecid Tablets contain not less than 95% and not more than 105% of the labeled amount of probenecid ($C_{13}H_{19}NO_4S$: 285.36).

**Method of preparation** Prepare as directed under Tablets, with Probenecid.

**Identification** **(1)** Weigh a quantity of powdered Probenecid Tablets, equivalent to 0.5 g of Probenecid according to the labeled amount, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake, and filter. Evaporate the filtrate on a water bath to about 20 mL. After cooling, collect produced crystals, recrystallize with 50 mL of dilute ethanol, and dry at 105°C for 4 hours: it melts <*2.60*> between 198°C and 200°C. With the crystals so obtained, proceed as directed in the Identification (1) under Probenecid.

**(2)** Determine the absorption spectrum of a solution of the dried crystals obtained in (1) in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 224 nm and 226 nm and between 247 nm and 249 nm, and a minimum between 234 nm and 236 nm.

**Dissolution** <*6.10*> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Probenecid Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 30 mL or more of the dissolved solution 30 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.8 $\mu$m. Discard the first 10 mL of the filtrate, pipet the subsequent $V$ mL, add 2nd fluid for dissolution test to make exactly $V'$ mL so that each mL contains about 14 $\mu$g of probenecid ($C_{13}H_{19}NO_4S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Probenecid Reference Standard, previously dried at 105°C for 4 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 1 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 244 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Probenecid Tablets in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the

labeled amount of probenecid ($C_{13}H_{19}NO_4S$)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

$W_S$: Amount (mg) of Probenecid Reference Standard.
$C$: Labeled amount (mg) of probenecid ($C_{13}H_{19}NO_4S$) in 1 tablet.

**Assay** Weigh accurately, and powder not less than 20 Probenecid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.15 g of probenecid ($C_{13}H_{19}NO_4S$), add 200 mL of ethanol (95) and 5 mL of 1 mol/L hydrochloric acid TS, and heat on a water-bath at 70°C for 30 minutes with occasional shaking. After cooling, add ethanol (95) to make exactly 250 mL, and filter. Discard the first 20 mL of the filtrate. To 5 mL of the subsequent filtrate, exactly measured, add 5 mL of 0.1 mol/L hydrochloric acid TS, dilute with ethanol (95) to exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of Probenecid Reference Standard, previously dried at 105°C for 4 hours, dissolve in 5 mL of 1 mol/L hydrochloric acid TS, and add ethanol (95) to make exactly 250 mL. Pipet 5 mL of the solution, add 5 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 248 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared by mixing 5 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make exactly 250 mL as the blank.

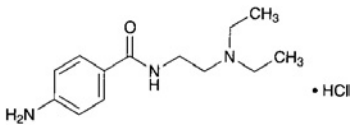
Amount (mg) of probenecid ($C_{13}H_{19}NO_4S$)
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of Probenecid Reference Standard

**Containers and storage** Containers—Well-closed containers.

# Procainamide Hydrochloride

プロカインアミド塩酸塩



$C_{13}H_{21}N_3O.HCl$: 271.79
4-Amino-*N*-(2-diethylaminoethyl)benzamide monohydrochloride [*614-39-1*]

Procainamide Hydrochloride, when dried, contains not less than 98.0% of $C_{13}H_{21}N_3O.HCl$.

**Description** Procainamide Hydrochloride occurs as a white to light yellow, crystalline powder. It is odorless.

It is very soluble in water, freely soluble in methanol, in acetic acid (100) and in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is hygroscopic.

**Identification (1)** Dissolve 1 g of Procainamide Hydrochloride in 10 mL of water, add 10 mL of sodium hydroxide TS, and extract with two 10-mL portions of a mixture of diethyl ether and chloroform (1:1). Combine the extracts, add calcium chloride for drying, and dry the extracts for 30 minutes. Decant the solution into a small flask, add 5 mL of pyridine, and slowly add dropwise 1 mL of benzoyl chloride. Heat the mixture on a water bath for 30 minutes, add 20 mL of a mixture of diethyl ether and chloroform (1:1), shake, and pour the mixture into 100 mL of sodium hydroxide TS, then shake. Separate the organic solvent layer, wash it with 20 mL of water, cool to 10°C, and allow the crystals to separate. Collect the separated crystals, recrystallize from 10 mL of dilute ethanol, and dry at 105°C for 1 hour: the crystals so obtained melt <*2.60*> between 180°C and 187°C.

**(2)** Dissolve 0.01 g of Procainamide Hydrochloride 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.

**(3)** A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chloride.

**pH** <*2.54*> Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

**Melting point** <*2.60*> 165 – 169°C

**Purity (1)** Clarity of solution—Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the solution is clear.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Procainamide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.20 g of Procainamide Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then spot 10 $\mu$L each of a solution of ammonia solution (28) in methanol (11 in 50) on each of the above spots. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (700:300:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.30% (2 g, 105°C, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (2 g).

**Assay** Weigh accurately about 0.5 g of Procainamide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.18 mg of $C_{13}H_{21}N_3O.HCl$

**Containers and storage** Containers—Tight containers.

# Procainamide Hydrochloride Injection

プロカインアミド塩酸塩注射液

Procainamide Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl: 271.79).

**Method of preparation** Prepare as directed under Injections, with Procainamide Hydrochloride.

**Description** Procainamide Hydrochloride Injection is a clear, colorless or light yellow liquid.

pH: 4.0 – 6.0

**Identification (1)** Proceed with a volume of Procainamide Hydrochloride Injection, equivalent to 1 g of Procainamide Hydrochloride according to the labeled amount, as directed in the Identification (1) under Procainamide Hydrochloride.

**(2)** Dilute a volume of Procainamide Hydrochloride Injection, equivalent to 0.01 g of Procainamide Hydrochloride according to the labeled amount, with 1 mL of dilute hydrochloric acid and water to 5 mL: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

**(3)** Procainamide Hydrochloride Injection responds to the Qualitative Tests <*1.09*> (2) for chloride.

**Assay** Dilute an accurately measured volume of Procainamide Hydrochloride Injection, equivalent to about 0.5 g of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl), with 5 mL of hydrochloric acid and water to 50 mL, cool to 15°C, and titrate <*2.50*> with 0.1 mol/L sodium nitrite VS (potentiometric titration method or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.18 mg of $C_{13}H_{21}N_3O$/HCl

**Containers and storage** Containers—Hermetic containers.

# Procainamide Hydrochloride Tablets

プロカインアミド塩酸塩錠

Procainamide Hydrochloride Tablets contain not less than 95% and not more than 105% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl: 271.79).

**Method of preparation** Prepare as directed under Tablets, with Procainamide Hydrochloride.

**Identification (1)** Shake a quantity of powdered Procainamide Hydrochloride Tablets, equivalent to 1.5 g of Procainamide Hydrochloride according to the labeled amount, with 30 mL of water, filter, and use the filtrate as the sample solution. To 20 mL of the sample solution add 10 mL of sodium hydroxide TS, and proceed as directed in the Identification (1) under Procainamide Hydrochloride.

**(2)** To 0.2 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Procainamide Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 30 mL or more of the dissolved solution 30 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.8 $\mu$m. Discard the first 10 mL of the filtrate, pipet the subsequent $V$ mL, add 2nd fluid for dissolution test to make exactly $V'$ mL so that each mL contains about 7 $\mu$g of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test 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 at 278 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Procainamide Hydrochloride Tablets in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 4.5$

$W_S$: Amount (mg) of procainamide hydrochloride for assay.
$C$: Labeled amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl) in 1 tablet.

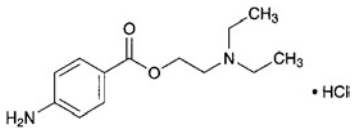
**Assay** Weigh accurately and powder not less than 20 Procainamide Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of procainamide hydrochloride ($C_{13}H_{21}N_3O$.HCl), stir well with 25 mL of 1 mol/L hydrochloric acid TS, centrifuge, and separate the supernatant liquid. Wash the residue with four 10-mL portions of 1 mol/L hydrochloric acid VS in the same manner. Add 10 mL of a solution of potassium bromide (3 in 10) to the combined supernatant liquid, cool to below 15°C, and titrate <*2.50*> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.18 mg of $C_{13}H_{21}N_3O$.HCl

**Containers and storage** Containers—Tight containers.

# Procaine Hydrochloride

プロカイン塩酸塩



$C_{13}H_{20}N_2O_2$.HCl: 272.77
2-(Diethylamino)ethyl 4-aminobenzoate monohydrochloride
[*51-05-8*]

Procaine Hydrochloride, when dried, contains not less than 99.0% of $C_{13}H_{20}N_2O_2$.HCl.

**Description** Procaine Hydrochloride occurs as white crystals or crystalline powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Procaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Procaine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Procaine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

**pH** <*2.54*> The pH of a solution prepared by dissoluing 1.0 g of Procaine Hydrochloride in 20 mL of water is between 5.0 and 6.0.

**Melting point** <*2.60*> 155 – 158°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Procaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

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

**(3)** Related substances—To 1.0 g of Procaine Hydrochloride add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and water to make exactly 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 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 dibutyl ether, *n*-hexane and acetic acid (100) (20:4:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate more at 105°C for 10 minutes, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. The principal spot from the sample solution stays at the origin.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, silica gel, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.4 g of Procaine Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15°C, and titrate <*2.50*> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.28 mg of $C_{13}H_{20}N_2O_2$.HCl

**Containers and storage** Containers—Well-closed containers.

# Procaine Hydrochloride Injection

プロカイン塩酸塩注射液

Procaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl: 272.77).

**Method of preparation** Prepare as directed under Injections, with Procaine Hydrochloride.

**Description** Procaine Hydrochloride Injection is a clear, colorless liquid.

**Identification** **(1)** To a volume of Procaine Hydrochloride Injection, equivalent to 0.01 g of Procaine Hydrochloride according to the labeled amount, add water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 219 nm and 223 nm, and between 289 nm and 293 nm.

**(2)** Procaine Hydrochloride Injection responds to the Qualitative Tests <*1.09*> (2) for chloride.

**pH** <*2.54*> 3.3 – 6.0

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** To an exactly measured volume of Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride ($C_{13}H_{20}N_2O_2$.HCl), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of procaine hydrochloride for assay, previously dried in a desiccator (silica gel) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solu-

tion. 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, and calculate the ratios, $Q_T$ and $Q_S$, of the peak area of procaine hydrochloride to that of the internal standard.

$$\text{Amount (mg) of procaine hydrochloride } (C_{13}H_{20}N_2O_2.HCl) = W_S \times (Q_T/Q_S) \times (2/5)$$

$W_S$: Amount (mg) of procaine hydrochloride for assay

*Internal standard solution*—A solution of caffeine in the mobile phase (1 in 1000).

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of procaine is about 10 minutes.

*System suitability*—

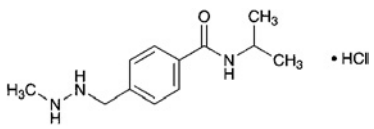
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, procaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

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 ratios of the peak area of procaine to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Hermetic containers.

# Procarbazine Hydrochloride

プロカルバジン塩酸塩



$C_{12}H_{19}N_3O.HCl$: 257.76

*N*-(1-Methylethyl)-4-[(2-methylhydrazino)methyl]benzamide monohydrochloride [*366-70-1*]

Procarbazine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{12}H_{19}N_3O.HCl$.

**Description** Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.

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

It dissolves in dilute hydrochloric acid.

Melting point: about 223°C (with decomposition).

**Identification (1)** Dissolve 0.01 g of Procarbazine Hydrochloride in 1 mL of diluted copper (II) sulfate TS (1 in 10), and add 4 drops of sodium hydroxide TS: a green precipitate is formed immediately, and the color changes from green through yellow to orange.

**(2)** Determine the absorption spectrum of a solution of Procarbazine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(3)** Determine the infrared absorption spectrum of Procarbazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(4)** A solution of Procarbazine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

**pH** <2.54> Dissolve 0.10 g of Procarbazine Hydrochloride in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

**Purity (1)** Heavy metals <1.07>—Proceed with 1.0 g of Procarbazine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Immerse slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200), allow to stand for 1 minute, lift the plate from the solution, dry it in cold wind for 10 minutes, then dry in warm wind for 5 minutes, and then dry at 60°C for 5 minutes. After cooling, spot 5 μL each of the sample solution and standard solution on the plate. Develop the plate with a mixture of methanol and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 1 spot other than the principal spot and the spot of the starting point from the sample solution appears, and is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.15 g of Procarbazine Hydrochloride, previously dried, place in a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, and cool to room temperature. To this solution add 5 mL of chloroform, and titrate <2.50>, while shaking, with 0.05 mol/L potassium iodate VS until the purple color of the

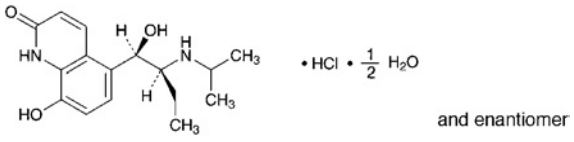
chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the purple color disappeared.

Each mL of 0.05 mol/L potassium iodate VS
= 8.592 mg of $C_{12}H_{19}N_3O.HCl$

**Containers and storage** Containers—Tight containers.

# Procaterol Hydrochloride Hydrate

プロカテロール塩酸塩水和物




$C_{16}H_{22}N_2O_3.HCl.\frac{1}{2}H_2O$: 335.83
8-Hydroxy-5-{(1*RS*,2*SR*)-1-hydroxy-2-[(1-methylethyl)amino]butyl]} quinolin-2(1*H*)-one monohydrochloride hemihydrate [*62929-91-3*, anhydride]

Procaterol Hydrochloride Hydrate contains not less than 98.5% of procaterol hydrochloride ($C_{16}H_{22}N_2O_3.HCl$: 326.82), calculated on the anhydrous basis.

**Description** Procaterol Hydrochloride Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is soluble in water, in formic acid and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Procaterol Hydrochloride Hydrate (1 in 100) is between 4.0 and 5.0.

It is gradually colored by light.

Melting point: about 195°C (with decomposition).

The solution of Procaterol Hydrochloride Hydrate (1 in 20) shows no optical rotation.

**Identification** **(1)** Determine the absorption spectrum of a solution of Procaterol Hydrochloride Hydrate (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Procaterol Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Procaterol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Procaterol Hydrochloride Hydrate in 30 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3.0 mL of Ferric Chloride Stock CS add water to make 50 mL.

**(2)** Heavy metals <*1.07*>—Proceed with 2.0 g of Procaterol Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

**(3)** Related substances—Dissolve 0.10 g of Procaterol Hydrochloride Hydrate in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample 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 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than procaterol from the sample solution is not larger than the peak area of procaterol from the standard solution.

*Operating conditions*—

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

Column: A stainless steel column about 4 mm in inside diameter and about 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 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water. To 760 mL of this solution add 230 mL of methanol and 10 mL of glacial acetic acid.

Flow rate: Adjust the flow rate so that the retention time of procaterol is about 15 minutes.

Selection of column: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution add diluted methanol (1 in 2) to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of procaterol and threoprocaterol in this order with the resolution of these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μL of the standard solution is not less than 10 mm.

Time span of measurement: 2.5 times as long as the retention time of procaterol beginning after the solvent peak.

**Water** <*2.48*> 2.5 – 3.3% (0.5 g, direct titration).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming, and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water bath for 30 minutes, cool, add 60 mL of acetic anhydride, and titrate <*2.50*> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

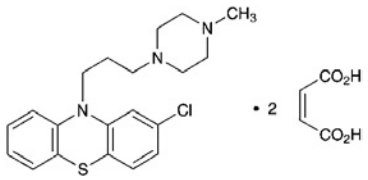
Each mL of 0.1 mol/L perchloric acid VS
= 32.68 mg of $C_{16}H_{22}N_2O_3.HCl$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Prochlorperazine Maleate

プロクロルペラジンマレイン酸塩



$C_{20}H_{24}ClN_3S.2C_4H_4O_4$: 606.09
2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10*H*-phenothiazine dimaleate [*84-02-6*]

Prochlorperazine Maleate, when dried, contains not less than 98.0% of $C_{20}H_{24}ClN_3S.2C_4H_4O_4$.

**Description** Prochlorperazine Maleate occurs as a white to light yellow powder. It is odorless, and has a slightly bitter taste.

It is slightly soluble in acetic acid (100), very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It gradually acquires a red tint by light.

Melting point: 195 – 203°C (with decomposition).

**Identification** **(1)** Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color develops, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder add 1 drop of potassium dichromate TS: a green-brown color develops, which changes to brown on standing.

**(2)** Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water, and filter through glass filter (G4). Wash the residue with three 10-mL portions of water, and dry at 105°C for 1 hour: it melts <*2.60*> between 195°C and 198°C (with decomposition).

**(3)** Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10), and extract with three 3-mL portions of diethyl ether [reserve the aqueous layer, and use for test (4)]. Evaporate the combined diethyl ether extracts on a water bath to dryness, dissolve the residue in 10 mL of methanol by warming, and pour into 30 mL of a solution of 2,4,6-trinitrophenol in methanol (1 in 75), previously warmed to 50°C. Allow to stand for 1 hour, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <*2.60*> between 252°C and 258°C (with decomposition).

**(4)** To the aqueous layer reserved in (3) add boiling chips, and heat on a water bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water bath for 10 minutes, and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300), and heat on a water bath for 15 minutes: a red-purple color is produced.

**Purity** Heavy metals <*1.07*>—Proceed with 1.0 g of Prochlorperazine Maleate 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).

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Prochlorperazine Maleate, previously dried, dissolve in 60 mL of acetic acid (100) while stirring and warming. Cool, and titrate <*2.50*> with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 15.15 mg of $C_{20}H_{24}ClN_3S.2C_4H_4O_4$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Prochlorperazine Maleate Tablets

プロクロルペラジンマレイン酸塩錠

Prochlorperazine Maleate Tablets contain not less than 95% and not more than 105% of the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$: 606.09).

**Method of preparation** Prepare as directed under Tablets, with Prochlorperazine Maleate.

**Identification** **(1)** Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 5 mg of Prochlorperazine Maleate according to the labeled amount, add 15 mL of acetic acid (100), shake, and filter. To 5 mL of the filtrate add 3 mL of sulfuric acid, and shake: a light red color develops. To this solution add 1 drop of potassium dichromate TS: a green-brown color is produced and changes to brown on standing.

**(2)** Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.08 g of Prochlorperazine Maleate according to the labeled amount, add 15 mL of methanol and 1 mL of dimethylamine, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.08 g of Prochlorperazine Maleate Reference Standard in 15 mL of a mixture of methanol and dimethylamine (15:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 $\mu$L 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 and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and standard solution show a red-purple color, and has the same *R*f value.

**(3)** To a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.04 g of Prochlorperazine Maleate according to the labeled amount, add 10 mL of 1 mol/L hydrochloric acid TS and 20 mL of diethyl ether, shake, and centrifuge. Transfer the diethyl ether layer to a separator, wash with 5 mL of 0.05 mol/L sulfuric acid TS, and evaporate on a water bath to dryness. Dissolve the residue in

5 mL of sulfuric acid TS, filter, if necessary, and add 1 to 2 drops of potassium permanganate TS: the red color of the test solution is discharged immediately.

**Assay** Weigh accurately and powder not less than 20 Prochlorperazine Maleate Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 16 mg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$), transfer to a glass-stoppered centrifuge tube, add exactly 25 mL of a mixture of *N,N*-dimethylformamide and dimethylamine (100:1), stopper tightly, shake vigorously for 15 minutes, and centrifuge. Use the supernatant liquid as the sample solution. Separately, weigh accurately about 64 mg of Prochlorperazine Maleate Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in a mixture of *N,N*-dimethylformamide and dimethylamine (100:1) to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and the standard solution into glass-stoppered centrifuge tubes, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0, and 20 mL of cyclohexane, stopper tightly, and centrifuge after shaking vigorously for 5 minutes. Pipet 10 mL each of the cyclohexane layer of these solutions into glass-stoppered centrifuge tubes, add exactly 20 mL of palladium (II) chloride TS and 5 mL of *N,N*-dimethylformamide, stopper tightly, and centrifuge after shaking vigorously for 15 minutes. Determine the absorbances, $A_T$ and $A_S$, of the water layers obtained from the sample solution and the standard solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using palladium (II) chloride TS as the blank.

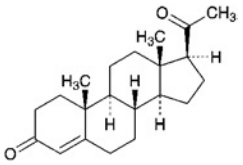
Amount (mg) of prochlorperazine maleate ($C_{20}H_{24}ClN_3S.2C_4H_4O_4$)
$= W_S \times (A_T/A_S) \times (1/4)$

$W_S$: Amount (mg) of Prochlorperazine Maleate Reference Standard

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Progesterone

プロゲステロン



$C_{21}H_{30}O_2$: 314.46
Pregn-4-ene-3,20-dione [*57-83-0*]

Progesterone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{30}O_2$.

**Description** Progesterone occurs as white crystals or crystalline powder. It is odorless.

It is soluble in methanol, in ethanol (95), in ethanol (99.5) and in 1,4-dioxane, sparingly soluble in diethyl ether, and practically insoluble in water.

**Identification** **(1)** To 0.05 g of progesterone add a solution of 0.05 g of hydroxylammonium chloride and 0.05 g of anhydrous sodium acetate in 5 mL of ethanol (95). Boil for 2 hours under a reflux condenser, evaporate the ethanol to 3 mL, and add 10 mL of water. Filter by suction, and wash the precipitate on the filter with a small amount of water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour: the dried crystals melt <*2.60*> between 235°C and 240°C.

**(2)** Determine the infrared absorption spectrum of Progesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Progesterone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone Reference Standard in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: +174 – +182° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

**Melting point** <*2.60*> 128 – 133°C or 120 – 122°C

**Purity** Related substances—Dissolve 80 mg of Progesterone in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (0.5 g).

**Assay** Weigh accurately about 10 mg of Progesterone, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. To 5 mL of this solution, exactly measured, add ethanol (99.5) to make exactly 50 mL, and determine the absorbance $A$ at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of $C_{21}H_{30}O_2 = (A/540) \times 10{,}000$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

# Progesterone Injection

プロゲステロン注射液

Progesterone Injection is an oily solution for injec-

tion.

It contains not less than 90% and not more than 110% of the labeled amount of progesterone ($C_{21}H_{30}O_2$: 314.46).

**Method of preparation** Prepare as directed under Injections, with Progesterone.

**Description** Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

**Identification** Transfer a volume of Progesterone Injection, equivalent to 0.02 g of Progesterone according to the labeled amount, to a separator. Add 40 mL of hexane, and mix thoroughly, then extract with three 20-mL portions of diluted ethanol (99.5) (9 in 10). Evaporate the combined extracts on a water bath to dryness. Add 75 mg of 2,4-dinitrophenylhydrazine and 30 mL of ethanol (95) to the residue, and boil for 15 minutes under a reflux condenser. Add 1 mL of hydrochloric acid, and heat for 15 minutes. Cool, and collect the precipitate on a glass filter (G4). Wash the precipitate with five 10-mL portions of hexane and three 5-mL portions of ethanol (95). Then wash with diluted hydrochloric acid (1 in 20) until the washings become colorless, and dry at 105°C for 3 hours: the residue melts <*2.60*> between 269°C and 275°C.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Measure exactly a volume of Progesterone Injection, equivalent to about 50 mg of progesterone ($C_{21}H_{30}O_2$), and dissolve in chloroform to make exactly 100 mL. To exactly measured 3 mL of this solution add chloroform to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Progesterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 5 mL each of the sample solution and standard solution, add exactly measured 10 mL of isoniazid TS and methanol to make exactly 20 mL, respectively. Allow to stand for 45 minutes, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution, prepared with 5 mL of chloroform in the same manner, as the blank. Determine the absorbances, $A_T$ and $A_S$, of the subsequent solutions of the sample solution and the standard solution at 380 nm.

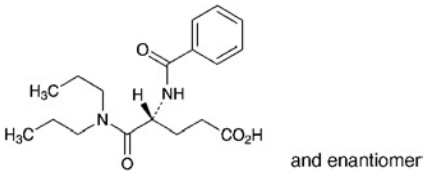
Amount (mg) of progesterone ($C_{21}H_{30}O_2$)
$= W_S \times (A_T/A_S)$

$W_S$: Amount (mg) of Progesterone Reference Standard

**Containers and storage** Containers—Hermetic containers.
Storage—Light-resistant.

# Proglumide

プログルミド




$C_{18}H_{26}N_2O_4$: 334.41
(4*RS*)-4-Benzoylamino-*N,N*-dipropylglutaramic acid
[*6620-60-6*]

Proglumide, when dried, contains not less than 98.5% of $C_{18}H_{26}N_2O_4$.

**Description** Proglumide occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

**Identification** **(1)** Put 0.5 g of Proglumide in a round bottom tube, add 5 mL of hydrochloric acid, seal the tube, and heat the tube carefully at 120°C for 3 hours. After cooling, open the tube, filter the content to collect crystals separated out, wash the crystals with 50 mL of water, and dry at 100°C for 1 hour: the melting point <*2.60*> of the crystals is between 121°C and 124°C.

**(2)** Determine the infrared absorption spectrum of Proglumide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Absorbance** <*2.24*> $E^{1\%}_{1\,cm}$ (225 nm): 384 – 414 (after drying, 4 mg, methanol, 250 mL)

**Melting point** <*2.60*> 148 – 150°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Proglumide according to Method 2, 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*>—To 1.0 g of Proglumide add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide (30), burn the ethanol, and prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

**(3)** Related substances—Dissolve 0.10 g of Proglumide in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl

acetate, acetic acid (100) and methanol (50:18:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.10% (1 g, reduced pressure, phosphorus (V) oxide, 60°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

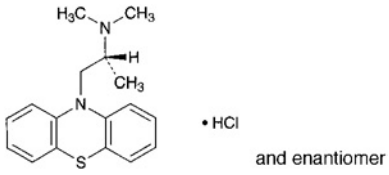
**Assay** Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.44 mg of $C_{18}H_{26}N_2O_4$

**Containers and storage** Containers—Well-closed containers.

# Promethazine Hydrochloride

プロメタジン塩酸塩




$C_{17}H_{20}N_2S.HCl$: 320.88
(2*RS*)-*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-ylamine monohydrochloride [*58-33-3*]

Promethazine Hydrochloride, when dried, contains not less than 98.0% of $C_{17}H_{20}N_2S.HCl$.

**Description** Promethazine Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

A solution of Promethazine Hydrochloride (1 in 25) shows on optical rotation.

Melting point: about 223°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Promethazine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Promethazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** Dissolve 0.5 g of Promethazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. To 5 mL of the filtrate add dilute nitric acid to make acidic: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

**pH** ⟨*2.54*⟩ The pH of a solution of Promethazine Hydrochloride (1 in 10) is between 4.0 and 5.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water, protecting from direct sunlight: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Promethazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Perform the test under the protection from sunlight. Dissolve 0.10 g of Promethazine Hydrochloride in exactly 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of isopromethazine hydrochloride for thin-layer chromatography in ethanol (95) to make exactly 100 mL, 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and diethylamine (19:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution corresponding to the spots from the standard solution (2) are not more intense than the spot from the standard solution (2), and any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution (1).

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

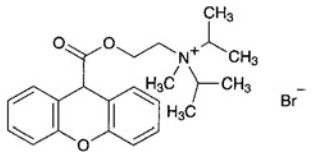
**Assay** Weigh accurately about 0.5 g of Promethazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.09 mg of $C_{17}H_{20}N_2S.HCl$

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Propantheline Bromide

プロパンテリン臭化物




$C_{23}H_{30}BrNO_3$: 448.39
*N*-Methyl-*N*,*N*-bis(1-methylethyl)-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethylaminium bromide [*50-34-0*]

Propantheline Bromide, when dried, contains not less than 98.0% and not more than 102.0% of $C_{23}H_{30}BrNO_3$.

**Description** Propantheline Bromide occurs as a white to yellowish white, crystalline powder. It is odorless and has a very bitter taste.

It is very soluble in water, in ethanol (95), in acetic acid (100) and in chloroform, soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Propantheline Bromide (1 in 50) is between 5.0 and 6.0.

Melting point: about 161°C (with decomposition, after drying).

**Identification** **(1)** To 5 mL of a solution of Propantheline Bromide (1 in 20) add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60°C, and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates, and wash with water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour: the crystals melt ⟨*2.60*⟩ between 217°C and 222°C.

**(2)** Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

**(3)** To 5 mL of a solution of Propantheline Bromide (1 in 10) add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests ⟨*1.09*⟩ (1) for bromide.

**Purity** Xanthene-9-carboxylic acid and xanthone—Dissolve 10 mg of Propantheline Bromide in exactly 2 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 1.0 mg of xanthene-9-carboxylic acid and 1.0 mg of xanthone in exactly 40 mL of chloroform, and use this solution as the standard solution. Perform the test immediately 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 with fluorescent indicator for thin-layer chromatography, and air-dry the plate for 10 minutes. Develop the plate with a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (2 g, 105°C, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

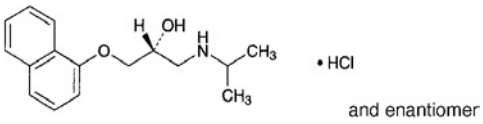
**Assay** Weigh accurately about 1 g of Propantheline Bromide, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.84 g of $C_{23}H_{30}BrNO_3$

**Containers and storage** Containers—Well-closed containers.

# Propranolol Hydrochloride

プロプラノロール塩酸塩




$C_{16}H_{21}NO_2$.HCl: 295.80
(2*RS*)-1-(1-Methylethyl)amino-3-(naphthalen-1-yloxy)propan-2-ol monohydrochloride [*318-98-9*]

Propranolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{16}H_{21}NO_2$.HCl.

**Description** Propranolol Hydrochloride occurs as a white, crystalline powder.

It is freely soluble in methanol, soluble in water and in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Propranolol Hydrochloride in methanol (1 in 40) shows no optical rotation.

It is gradualy colored to yellowish white to light brown by light.

**Identification** **(1)** Determine the absorption spectrum of a solution of Propranolol Hydrochloride in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths

**(2)** Determine the infrared absorption spectrum of Propranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

**pH** ⟨*2.54*⟩ The pH of a solution prepared by dissolving 0.5 g of Propranolol Hydrochloride in 50 mL of water is 5.0 – 6.0.

**Melting point** ⟨*2.60*⟩ 163 – 166°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Propranolol Hydrochloride in 20 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Propranolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 20 mg of Propranolol Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 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 each peak area by the automatic integration method: the area of the peak other than propranolol is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol is not larger than 2 times the peak area of propranolol from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 292 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 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of propranolol is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of propranolol.

*System suitability*—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 $\mu$L of this solution is equivalent to 17 to 33% of that with 20 $\mu$L of the standard solution.

System performance: When the procedure is run with 20 $\mu$L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propranolol is not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propranolol is not more than 2.0%.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.5 g of Propranolol Hydrochloride, previously dried, dissolove in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.58 mg of $C_{16}H_{21}NO_2$.HCl

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Propranolol Hydrochloride Tablets

プロプラノロール塩酸塩錠

Propranolol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl: 295.80).

**Method of preparation** Prepare as directed under Tablets, with Propranolol Hydrochloride.

**Identification** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 288 nm and 292 nm, and between 317 nm and 321 nm.

**Uniformity of dosage units** ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propranolol Hydrochloride Tablets add 20 mL of water, and shake until the tablet is completely disintegrated. Add 50 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add methanol to make exactly $V'$ mL so that each mL contains about 20 $\mu$g of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol 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 the standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/25)$

$W_S$: Amount (mg) of propranolol hydrochloride for assay

**Dissolution** ⟨*6.10*⟩ Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Propranolol Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 $\mu$m. Discard the first 10 mL of the filtrate, pipet $V$ mL of the subsequent filtrate, add water to make exactly $V'$ mL so that each mL contains about 10 $\mu$g of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water 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 at 290 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩. The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl)
$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

$W_S$: Amount (mg) of propranolol hydrochloride for assay
$C$: Labeled amount (mg) of propranolol hydrochloride

($C_{16}H_{21}NO_2$.HCl) in 1 tablet

**Assay** Weigh accurately the mass of not less than 20 Propranolol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl), add 60 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Filter, discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol 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 the standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of propranolol hydrochloride ($C_{16}H_{21}NO_2$.HCl)
$= W_S \times (A_T/A_S) \times (2/5)$

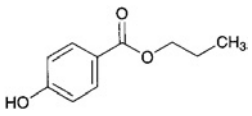
$W_S$: Amount (mg) of propranolol hydrochloride for assay

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Propyl Parahydroxybenzoate

パラオキシ安息香酸プロピル



$C_{10}H_{12}O_3$: 180.20
Propyl 4-hydroxybenzoate [*94-13-3*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Propyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of $C_{10}H_{12}O_3$.

◆**Description** Propyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and very slightly soluble in water.◆

**Identification** **(1)** The melting point <*2.60*> of Propyl Parahydroxybenzoate is between 96°C and 99°C.

◆**(2)** Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of cobalt (II) chloride colorimetric stock solution, 12.0 mL of iron (III) chloride colorimetric stock solution and 2.0 mL of cupper (II) sulfate colorimetric stock solution add water to make 1000 mL.

**(2)** Acidity—Dissolve 0.20 g of Propyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

◆**(3)** Heavy metals <*1.07*>—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, 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 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

**(4)** Related substances—Dissolve 0.10 g of Propyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, 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 methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

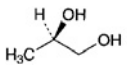
**Assay** Weigh accurately about 1.0 g of Propyl Parahydroxybenzoate add exactly 20 mL of 1 mol/L VS, heat at about 70 °C for 1 hour, and immediately cool in ice. Titrate <*2.50*> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
$= 180.2$ mg of $C_{10}H_{12}O_3$

◆**Containers and storage** Containers—Well-closed containers.◆

# Propylene Glycol

プロピレングリコール



and enantiomer

$C_3H_8O_2$: 76.09
(2*RS*)-Propane-1,2-diol [*57-55-6*]

**Description** Propylene Glycol is a clear, colorless, viscous liquid. It is odorless, and has a slightly bitter taste.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is freely soluble in diethyl ether.

It is hygroscopic.

**Identification** **(1)** Mix 2 to 3 drops of Propylene Glycol with 0.7 g of triphenylchloromethane, add 1 mL of pyridine,

and heat under a reflux condenser on a water bath for 1 hour. After cooling, dissolve the mixture in 20 mL of acetone by warming, shake with 0.02 g of activated charcoal, and filter. Concentrate the filtrate to about 10 mL, and cool. Collect the separated crystals, and dry in a desiccator (silica gel) for 4 hours: the crystals melt ⟨*2.60*⟩ between 174°C and 178°C.

**(2)** Heat gently 1 mL of Propylene Glycol with 0.5 g of potassium hydrogen sulfate: a characteristic odor is evolved.

**Specific gravity** ⟨*2.56*⟩ $d_{20}^{20}$: 1.035 – 1.040

**Purity** **(1)** Acidity—Mix 10.0 mL of Propylene Glycol with 50 mL of freshly boiled and cooled water, and add 5 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: the solution has a red color.

**(2)** Chloride ⟨*1.03*⟩—Perform the test with 2.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

**(3)** Sulfate ⟨*1.14*⟩—Perform the test with 10.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

**(4)** Heavy metals ⟨*1.07*⟩—Perform the test with 5.0 g of Propylene Glycol according to Method 1. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

**(5)** Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Propylene Glycol according to Method 1, and perform the test (not more than 2 ppm).

**(6)** Glycerin—Heat 1.0 g of Propylene Glycol with 0.5 g of potassium hydrogen sulfate and evaporate to dryness: no odor of acrolein is perceptible.

**Water** ⟨*2.48*⟩ Not more than 0.5% (2 g, direct titration).

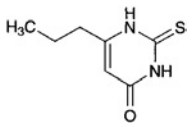
**Residue on ignition** ⟨*2.44*⟩ Weigh accurately about 20 g of Propylene Glycol in a tared crucible, and heat to boiling. Stop heating, and immediately ignite to burn. Cool, moisten the residue with 0.2 mL of sulfuric acid, and heat strongly with care to constant mass: the mass of the residue is not more than 0.005%.

**Distilling range** ⟨*2.57*⟩ 184 – 189°C, not less than 95 vol%.

**Containers and storage** Containers—Tight containers.

# Propylthiouracil

プロピルチオウラシル



$C_7H_{10}N_2OS$: 170.23
6-Propyl-2-thiouracil [*51-52-5*]

Propylthiouracil, when dried, contains not less than 98.0% of $C_7H_{10}N_2OS$.

**Description** Propylthiouracil occurs as a white powder. It is odorless, and has a bitter taste.

It is sparingly soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

**Identification** **(1)** Shake well 0.02 g of Propylthiouracil with 7 mL of bromine TS for 1 minute, and heat until the color of bromine TS disappears. Cool, filter, and add 10 mL of barium hydroxide TS to the filtrate: a white precipitate is produced. The color of the precipitate does not turn purple within 1 minute.

**(2)** To 5 mL of a hot saturated solution of Propylthiouracil add 2 mL of a solution of sodium pentacyanoammine ferroate (II) *n*-hydrate (1 in 100): a green color develops.

**Melting point** ⟨*2.60*⟩ 218 – 221°C

**Purity** **(1)** Sulfate ⟨*1.14*⟩—Triturate Propylthiouracil finely in a mortar. To 0.75 g of the powder add 25 mL of water, heat for 10 minutes on a water bath, cool, filter, and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.077%).

**(2)** Thiourea—Dissolve 0.30 g of Propylthiouracil in 50 mL of water by heating under a reflux condenser for 5 minutes, cool, and filter. To 10 mL of the filtrate add 3 mL of ammonia TS, shake well, and add 2 mL of silver nitrate TS: the solution has no more color than the following control solution.

Control solution: Weigh exactly 60 mg of thiourea, and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and proceed with 10 mL of this solution in the same manner.

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Propylthiouracil, previously dried, and add 30 mL of water. Add 30 mL of 0.1 mol/L sodium hydroxide VS from a burette, heat to boil, and dissolve by stirring. Wash down the solid adhering to the wall of the flask with a small amount of water, and add 50 mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently for 5 minutes, add 1 to 2 mL of bromothymol blue TS, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS until a persistent blue-green color develops. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of $C_7H_{10}N_2OS$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Propylthiouracil Tablets

プロピルチオウラシル錠

Propylthiouracil Tablets contain not less than 93% and not more than 107% of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$: 170.23).

**Method of preparation** Prepare as directed under Tablets,

with Propylthiouracil.

**Identification** To a quantity of powdered Propylthiouracil Tablets, equivalent to 0.3 g of Propylthiouracil according to the labeled amount, add 5 mL of ammonia TS, allow to stand for 5 minutes with occasional shaking, add 10 mL of water, and centrifuge. To the supernatant liquid add acetic acid (31), collect the precipitate produced, recrystallize from water, and dry at 105°C for 1 hour: it melts <*2.60*> between 218°C and 221°C. Proceed with the residue as directed in the Identification under Propylthiouracil.

**Dissolution** <*6.10*> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Propylthiouracil Tablets at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 $\mu$m. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propylthiouracil for assay, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, $A_T$ and $A_S$, of the sample solution and standard solution at 274 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. The dissolution rate of Propylthiouracil Tablets in 30 minutes should be not less than 80%.

Dissolution rate (%) with respect to the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$)
$= W_S \times (A_T/A_S) \times (1/C) \times 90$

$W_S$: Amount (mg) of propylthiouracil for assay.
$C$: Labeled amount (mg) of propylthiouracil ($C_7H_{10}N_2OS$) in 1 tablet.

**Assay** Weigh accurately and powder not less than 20 Propylthiouracil Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of propylthiouracil ($C_7H_{10}N_2OS$), transfer to a Soxhlet extractor, and extract with 100 mL of acetone for 4 hours. Evaporate the acetone extract by warming on a water bath to dryness. To the residue add 30 mL of water, and proceed as directed in the Assay under Propylthiouracil.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of $C_7H_{10}N_2OS$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Protamine Sulfate

プロタミン硫酸塩

Protamine Sulfate is the sulfate of protamine prepared from the mature spermary of fish belonging to the family *Salmonidae* and others.

**Description** Protamine Sulfate occurs as a white to light grayish yellow powder.

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

The pH of a solution of Protamine Sulfate (1 in 100) is between 4.0 and 7.0.

**Identification** **(1)** Dissolve 1 mg of Protamine Sulfate in 2 mL of water, add 5 drops of a solution prepared by dissolving 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10) and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.

**(2)** Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.

**(3)** An aqueous solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> for sulfate.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.

**(2)** Nitrogen—Weigh accurately about 10 mg of Protamine Sulfate, previously dried at 105°C to constant mass, and perform the test as directed under Nitrogen Determination <*1.08*>: not more than 0.255 mg of nitrogen (N: 14.01) is found for each mg of Protamine Sulfate.

**Potency as antiheparin** (i) Sample solution—Dissolve 20.0 mg of Protamine Sulfate in isotonic sodium chloride solution to make exactly 20 mL.

(ii) Heparin sodium standard solution—Dissolve 10.0 mg of Heparin Sodium Reference Standard in isotonic sodium chloride solution to make a standard solution containing exactly 0.7 mg per ml.

(iii) Sulfated whole blood—Place 250 mL of fresh bovine blood in a wide-mouthed stoppered polyethylene bottle containing 50 mL of a solution of sodium sulfate decahydrate (9 in 50), and store between 1°C and 4°C. Remove any clotted substance before use.

(iv) Thrombokinase extract—To 1.5 g of acetone-dried cattle brain add 60 mL of water, extract at 50°C for 10 to 15 minutes, and centrifuge for 2 minutes at 1500 revolutions per minute. To the supernatant add cresol to make 0.3% as a preservative, and store between 1°C and 4°C. The potency of this solution will be maintained for several days.

(v) Procedure—To one of 10 clean, glass-stoppered test tubes, 13 mm in inside diameter and 150 mm in length, transfer 1.30 mL of isotonic sodium chloride solution and 0.20 mL of thrombokinase extract, then add exactly 1 mL of sulfated whole blood, stopper the tube, mix the contents by inverting once, and note the time on a stop watch. When the solid clot which is formed at the bottom of the tube does not fall on inverting the tube, designate this time as the control clotting time. Adjust appropriately the volume of thrombokinase extract so that the control clotting time is between 2 and 3 minutes. To the nine remaining tubes add 0.50 mL of the sample solution and the same volume of thrombokinase extract as was used in the previous measurement of the control clotting time, pipet into the tubes 0.43 mL, 0.45 mL, 0.47 mL, 0.49 mL, 0.50 mL, 0.51 mL, 0.53 mL, 0.55 mL and 0.57 mL of the heparin sodium standard solution, respectively,

and make the volume in each tube up to 1.50 mL by adding isotonic sodium chloride solution. Add finally 1.0 mL of sulfated whole blood, stopper, mix the contents by inverting once, and determine the clotting times with a stop watch. The estimated ratio, $v/V$, is between 0.85 and 1.15, where $v$ is the volume of the heparin sodium standard solution and $V$ is the volume of the sample solution in that tube in which the clotting time is most nearly the same as the control clotting time.

**Containers and storage** Containers—Tight containers.

# Protamine Sulfate Injection

プロタミン硫酸塩注射液

Protamine Sulfate Injection is an aqueous solution for injection.

The amount of Protamine Sulfate should be labeled.

**Method of preparation** Prepare as directed under Injections, with Protamine Sulfate.

**Description** Protamine Sulfate Injection is a colorless liquid. It is odorless or has the odor of preservatives.

**Identification** **(1)** Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate according to the labeled amount, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

**(2)** Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

**(3)** Protamine Sulfate Injection responds to the Qualitative Tests <*1.09*> for sulfate.

**pH** <*2.54*> 5.0 – 7.0

**Purity** Nitrogen—Transfer an exactly measured volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate according to the labeled amount, to a Kjeldahl flask, and evaporate on a water bath with the aid of a current of air to dryness. Perform the test as directed under Nitrogen Determination <*1.08*>: 0.225 to 0.255 mg of nitrogen (N: 14.01) is found for each mg of the labeled amount of Protamine Sulfate.

**Extractable volume** <*6.05*> It meets the requirement.

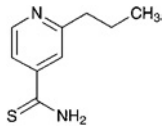
**Potency as antiheparin** Proceed as directed in the Potency as antiheparin under Protamine Sulfate, but use the following sample solution.

Sample solution: Dilute an exactly measured volume of Protamine Sulfate Injection, equivalent to 20.0 mg of Protamine Sulfate according to the labeled amount, with isotonic sodium chloride solution to make exactly 20 mL.

**Containers and storage** Containers—Hermetic containers.

# Prothionamide

プロチオナミド




$C_9H_{12}N_2S$: 180.27
2-Propylpyridine-4-carbothioamide [*14222-60-7*]

Prothionamide, when dried, contains not less than 98.0% of $C_9H_{12}N_2S$.

**Description** Prothionamide occurs as yellow crystals or crystalline powder. It has a slight, characteristic odor.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

**Identification** **(1)** Mix 0.05 g of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this mixture to a test tube, and heat for several seconds over a small flame until the mixture is fused. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange-red color develops.

**(2)** Place 0.5 g of Prothionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and mix well. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt <*2.60*> between 198°C and 203°C (with decomposition).

**Melting point** <*2.60*> 142 – 145°C

**Purity** **(1)** Clarity and color of solution—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear, and shows a yellow color.

**(2)** Acidity—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water bath with agitation, and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature, and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Prothionamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(4)** Arsenic <*1.11*>—Prepare the test solution with 0.6 g of Prothionamide according to Method 3, and perform the test. To the test solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite to burn (not more

than 3.3 ppm).

**Loss on drying** ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 3 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Prothionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination.

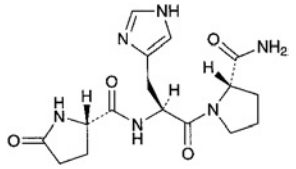
Each mL of 0.1 mol/L perchloric acid VS
= 18.03 mg of $C_9H_{12}N_2S$

**Containers and storage** Containers—Well-closed containers.

Storage—Light-resistant.

# Protirelin

プロチレリン



$C_{16}H_{22}N_6O_4$: 362.38

5-Oxo-L-prolyl-L-histidyl-L-prolinamide [*24305-27-9*]

Protirelin contains not less than 98.5% of $C_{16}H_{22}N_6O_4$, calculated on the dehydrated basis.

**Description** Protirelin occurs as a white powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100).

It is hygroscopic.

**Identification** **(1)** Take 0.01 g of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube, and heat carefully at 110°C for 5 hours. After cooling, open the seal, transfer the contents into a beaker, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 0.08 g of L-glutamic acid, 0.12 g of L-histidine hydrochloride monohydrate and 0.06 g of L-proline in 20 mL of water, 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, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the three spots obtained from the sample solution show the same color and the same *R*f value as each corresponding spots obtained from the standard solution.

**(2)** Determine the infrared absorption spectrum of Protirelin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Optical rotation** ⟨*2.49*⟩ $[\alpha]_D^{20}$: −66.0 – −69.0° (0.1 g calculated on the dehydrated basis, water, 20 mL, 100 mm).

**pH** ⟨*2.54*⟩ Dissolve 0.20 g of Protirelin in 10 mL of water: the pH of this solution is between 7.5 and 8.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Protirelin in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Protirelin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Related substances—Dissolve 0.20 g of Protirelin in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 200 mL, 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 (1) of silica gel for thin-layer chromatography, and spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of 1-butanol, water, pyridine and acetic acid (100) (4:2:1:1) to a distance of about 12 cm, and dry the plates at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plates. Successively spray evenly a solution of sodium carbonate decahydrate (1 in 10) on it: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and heat at 80°C for 5 minutes: no colored spot appears.

**Water** ⟨*2.48*⟩ Not more than 5.0% (0.1 g, direct titration).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.3% (0.2 g).

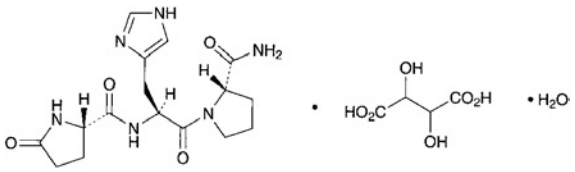
**Assay** Weigh accurately about 70 mg of Protirelin dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 7.248 mg of $C_{16}H_{22}N_6O_4$

**Containers and storage** Containers—Tight containers.

# Protirelin Tartrate Hydrate

プロチレリン酒石酸塩水和物



$C_{16}H_{22}N_6O_4.C_4H_6O_6.H_2O$: 530.49
5-Oxo-L-prolyl-L-histidyl-L-prolinamide monotartrate monohydrate [*24305-27-9*, Protirelin]

Protirelin Tartrate Hydrate, calculated on the anhydrous basis, contains not less than 98.5% of protirelin tartrate ($C_{16}H_{22}N_6O_4.C_4H_6O_6$: 512.48).

**Description** Protirelin Tartrate Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: about 187°C (with decomposition).

**Identification** **(1)** To 1 mL of a solution of Protirelin Tartrate Hydrate (1 in 1000) add 2 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color develops.

**(2)** Dissolve 0.03 g of Protirelin Tartrate Hydrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

**(3)** To 0.20 g of Protirelin Tartrate Hydrate add 5.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride (monohydrate) and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 $\mu$L 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, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry at 80°C for 5 minutes: the three spots obtained from the sample solution show, respectively, the same color and the same *R*f value as the corresponding spot from the standard solution.

**(4)** A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests <*1.09*> for tartrate.

**Optical rotation** <*2.49*> $[\alpha]_D^{20}$: $-50.0$ – $-53.0°$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

**pH** <*2.54*> Dissolve 1.0 g of Protirelin Tartrate Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

**Purity** **(1)** Clarity and color of solution—Dissolve 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Protirelin Tartrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(3)** Arsenic <*1.11*>—Take 1.0 g of Protirelin Tartrate Hydrate in a porcelain crucible. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol, and heat gradually to incinerate. If a carbonized material still remains in this method, moisten with a small quantity of nitric acid, and ignite to incinerate. After cooling, add 10 mL of dilute hydrochloric acid, heat on a water bath to dissolve the residue, use this solution as the test solution, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.60 g of Protirelin Tartrate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L each of the sample solution and standard solution on a plate (1) of silica gel for thin-layer chromatography. Spot 5 $\mu$L of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform, methanol and ammonia solution (28) (6:4:1) to a distance of about 10 cm, and dry at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plate. Then, spray evenly a solution of sodium carbonate decahydrate (1 in 10) on the plate: the spots other than the principal spot from the sample solution are not more intense than those from the standard solution in color. On the other hand, spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and dry at 80°C for 5 minutes: no colored spot is obtained.

**Water** <*2.48*> Not more than 4.5% (0.2 g, direct titration).

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

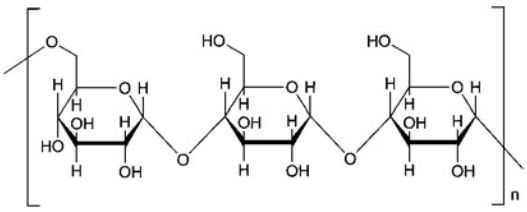
**Assay** Weigh accurately about 0.5 g of Protirelin Tartrate Hydrate, dissolve in 80 mL of acetic acid (100) by warming, cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.25 mg of $C_{16}H_{22}N_6O_4.C_4H_6O_6$

**Containers and storage** Containers—Well-closed containers.

# Pullulan

プルラン



$(C_{18}H_{30}O_{15})_n$
Poly[6)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→] [*9057-02-7*]

Pullulan is a neutral simple polysaccharide produced by the growth of *Aureobasidium pullulans*. It has a chain structure of repeated α-1,6 binding of maltotriose composed of three glucoses in α-1,4 binding.

**Description** Pullulan occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

**Identification** **(1)** Dissolve 10 g of Pullulan in 100 mL of water with stirring by adding in small portions: a viscous solution is produced.

**(2)** Mix 10 mL of the viscous solution obtained in (1) with 0.1 mL of pullulanase TS, and allow to stand: the solution loses its viscosity.

**(3)** To 10 mL of a solution of Pullulan (1 in 50) add 2 mL of macrogol 600: a white precipitate is formed immediately.

**Viscosity** ⟨*2.53*⟩ Take exactly 10.0 g of Pullulan, previously dried, dissolve in water to make exactly 100 g, and perform the test at 30±0.1°C as directed in Method 1: the kinematic viscosity is between 100 and 180 $mm^2/s$.

**pH** ⟨*2.54*⟩ Dissolve 1.0 g of Pullulan in 10 mL of freshly boiled and cooled water: the pH is between 4.5 and 6.5.

**Purity** **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 4.0 g of Pullulan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

**(2)** Nitrogen—Weigh accurately about 3 g of Pullulan, previously dried, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩: the amount of nitrogen (N: 14.01) is not more than 0.05%. Use 12 mL of sulfuric acid for the decomposition, and add 40 mL of a solution of sodium hydroxide (2 in 5).

**(3)** Monosaccharide and oligosaccharides—Dissolve 0.8 g of Pullulan, previously dried, in 100 mL of water, and designate this solution as the sample stock solution. To 1 mL of the sample stock solution add 0.1 mL of potassium chloride saturated solution, and shake vigorously with 3 mL of methanol. Centrifuge, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of the sample stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 0.2 mL each of the sample solution, the standard solution and water, transfer them gently to each test tube containing 5 mL of a solution of anthrone in diluted sulfuric acid (3 in 4) (1 in 500) and cooling in ice water, stir immediately, then heat at 90°C for 10 minutes, and cool immediately. Perform the test with these solutions so obtained as directed under Ultraviolet-visible Spectrophtometry ⟨*2.24*⟩ using water as a blank, and determine the absorbances at 620 nm, $A_T$, $A_S$ and $A_B$: the amount of monosaccharide and oligosaccharides is not more than 10.0%.

Amount (%) of monosaccharide and oligosaccharides
$= \{(A_T - A_B)/(A_S - A_B)\} \times 8.2$

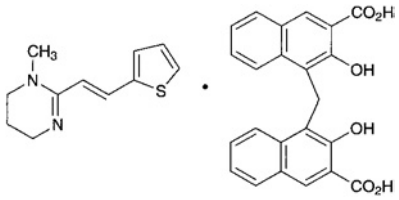
**Loss on drying** ⟨*2.41*⟩ Not more than 6.0% (1 g, in vacuum, 90°C, 6 hours).

**Residue on ignition** ⟨*2.44*⟩ Not more than 0.3% (2 g).

**Containers and storage** Containers—Well-closed containers.

# Pyrantel Pamoate

ピランテルパモ酸塩



$C_{11}H_{14}N_2S.C_{23}H_{16}O_6$: 594.68
1-Methyl-2-[(1*E*)-2-(thien-2-yl)vinyl]-1,4,5,6-tetrahydropyrimidine mono[4,4′-methylenebis(3-hydroxy-2-naphthoate)] (1/1) [*22204-24-6*]

Pyrantel Pamoate, when dried, contains not less than 98.0% of $C_{11}H_{14}N_2S.C_{23}H_{16}O_6$.

**Description** Pyrantel Pamoate occurs as a light yellow to yellow, crystalline powder. It is odorless and tasteless.

It is sparingly soluble in *N,N*-dimethylformamide, very slightly soluble in methanol and in ethanol (95), and practically insoluble in water, in ethyl acetate and in diethyl ether.

Melting point: 256 – 264°C (with decomposition).

**Identification** **(1)** To 0.05 g of Pyrantel Pamoate add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1:1), and shake vigorously: a yellow precipitate is produced. Filter the solution, and use the filtrate as the sample solution. Use the precipitate for the test (2). To 0.5 mL of the sample solution add 1 mL of a solution of 2,3-indolinedione in sulfuric acid (1 in 1000): a red color develops.

**(2)** Collect the precipitate obtained in the test (1), wash with methanol, and dry at 105°C for 1 hour. To 0.01 g of the dried precipitate add 10 mL of methanol, shake well, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color develops.

**(3)** Dissolve 0.1 g of Pyrantel Pamoate in 50 mL of *N,N*-dimethylformamide, and add methanol to make 200 mL. To 2 mL of the solution add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-

visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** Determine the infrared absorption spectrum of Pyrantel Pamoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Purity** **(1)** Chloride <*1.03*>—To 1.0 g of Pyrantel Pamoate add 10 mL of dilute nitric acid and 40 mL of water, and heat on a water bath with shaking for 5 minutes. After cooling, add water to make 50 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

**(2)** Sulfate <*1.14*>—To 0.75 g of Pyrantel Pamoate add 5 mL of dilute hydrochloric acid and water to make 100 mL, and heat on a water bath for 5 minutes with shaking. After cooling, add water to make 100 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.144%).

**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pyrantel Pamoate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(4)** Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Pyrantel Pamoate according to Method 3, and perform the test (not more than 2 ppm).

**(5)** Related substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in 10 mL of *N,N*-dimethylformamide, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 $\mu$L 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, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the spot of pyrantel and the spot of pamoic acid from the sample solution are not more intense than the spot of pyrantel (*R*f value: about 0.3) from the standard solution.

**Loss on drying** <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

**Residue on ignition** <*2.44*> Not more than 0.3% (1 g).

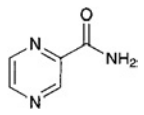
**Assay** Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes, and extract. Extract further with two 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, add 30 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.47 mg of $C_{11}H_{14}N_2S.C_{23}H_{16}O_6$

**Containers and storage** Containers—Tight containers.

# Pyrazinamide

ピラジナミド



$C_5H_5N_3O$: 123.11
Pyrazine-2-carboxamide [*98-96-4*]

Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of $C_5H_5N_3O$.

**Description** Pyrazinamide occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5) and in acetic anhydride.

**Identification** **(1)** Determine the absorption spectrum of a solution of Pyrazinamide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pyrazinamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <*2.60*> 188 – 193°C

**Purity** **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pyrazinamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

**(2)** Related substances—Dissolve 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 $\mu$L 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) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.1 g of Pyrazinamide,

previously dried, dissolve in 50 mL of acetic anhydride, and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 12.31 mg of $C_5H_5N_3O$

**Containers and storage** Containers—Well-closed containers.

# Pyridostigmine Bromide

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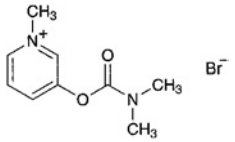


$C_9H_{13}BrN_2O_2$: 261.12
3-Dimethylcarbamoyloxy-1-methylpyridinium bromide
[*101-26-8*]

Pyridostigmine Bromide, when dried, contains not less than 98.5% of $C_9H_{13}BrN_2O_2$.

**Description** Pyridostigmine Bromide occurs as a white, crystalline powder. It is odorless or has a slightly characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Pyridostigmine Bromide (1 in 10) is between 4.0 and 6.0.

It is deliquescent.

**Identification** **(1)** Dissolve 0.02 g of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: a light red precipitate is produced.

**(2)** To 0.1 g of Pyridostigmine Bromide add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

**(3)** Determine the absorption spectrum of a solution of Pyridostigmine Bromide in 0.1 mol/L hydrochloric acid TS (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(4)** A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests ⟨1.09⟩ for Bromide.

**Melting point** ⟨2.60⟩ 153 – 157°C

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Pyridostigmine Bromide in 10 mL of water: the solution is clear and colorless.

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

**(3)** Arsenic ⟨1.11⟩—Prepare the test solution with 1.0 g of Pyridostigmine Bromide according to Method 1, and perform the test (not more than 2 ppm).

**(4)** Related substances—Dissolve 0.10 g of Pyridostigmine Bromide in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 25 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonium chloride TS (5:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in color.

**Loss on drying** ⟨2.41⟩ Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 5 hours).

**Residue on ignition** ⟨2.44⟩ Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.3 g of Pyridostigmine Bromide, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate ⟨2.50⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

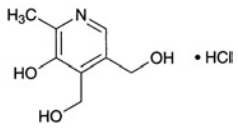
Each mL of 0.1 mol/L perchloric acid VS
= 26.11 mg of $C_9H_{13}BrN_2O_2$

**Containers and storage** Containers—Hermetic containers.

# Pyridoxine Hydrochloride

## Vitamin $B_6$

ピリドキシン塩酸塩



$C_8H_{11}NO_3$.HCl: 205.64
4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol monohydrochloride [*58-56-0*]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of $C_8H_{11}NO_3$.HCl.

**Description** Pyridoxine Hydrochloride occurs as a white to pale yellow, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

It is gradually affected by light.

Melting point: about 206°C (with decomposition).

**Identification** **(1)** Determine the absorption spectrum of a solution of Pyridoxine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a

solution of Pyridoxine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pyridoxine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyridoxine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**(3)** A solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> for chloride.

**pH** <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Pyridoxine Hydrochloride in 50 mL of water is between 2.5 and 3.5.

**Purity** **(1)** Clarity and color of solution—Dissolve 1.0 g of Pyridoxine hydrochloride in 20 mL of water: the solution is clear and colorless.

**(2)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pyridoxine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

**(3)** Related substances—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 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 2 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (99.5) (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate, and air-dry: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <*2.41*> Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

**Residue on ignition** <*2.44*> Not more than 0.1% (1 g).

**Assay** Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.56 mg of $C_8H_{11}NO_3$.HCl

**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.

# Pyridoxine Hydrochloride Injection

## Vitamin $B_6$ Injection

ピリドキシン塩酸塩注射液

Pyridoxine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl: 205.64).

**Method of preparation** Prepare as directed under Injections, with Pyridoxine Hydrochloride.

**Description** Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.

It is gradually affected by light.

pH: 3.0 – 6.0

**Identification** **(1)** To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.05 g of Pyridoxine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible spectrophotometry <*2.24*>: it exhibits a maximum between 288 nm and 292 nm.

**(2)** To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.01 g of Pyridoxine Hydrochloride according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.01 g of Pyridoxine Hydrochloride Reference Standard in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 $\mu$L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (99.5) (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate: the spots obtained from the sample solution and the standard solution are blue in color and have the same *R*f value.

**Bacterial endotoxins** <*4.01*> Less than 3.0 EU/mg.

**Extractable volume** <*6.05*> It meets the requirement.

**Assay** Measure exactly a volume of Pyridoxine Hydrochloride Injection, equivalent to about 20 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl), dilute with water, if necessary, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 1

mL each of the sample solution and standard solution, add 2.0 mL of barbital buffer solution, 9.0 mL of 2-propanol and 2.0 mL of a freshly prepared solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000), shake well, add 2-propanol to make exactly 25 mL, and allow to stand for 90 minutes. Determine the absorbances, $A_T$ and $A_S$, of the subsequent sample solution and subsequent standard solution, respectively, at 650 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner with 1 mL of water, as the blank.

Amount (mg) of pyridoxine hydrochloride ($C_8N_{11}NO_3$.HCl)
$= W_S \times (A_T/A_S) \times (1/5)$

$W_S$: Amount (mg) of Pyridoxine Hydrochloride Reference Standard

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

# Pyroxylin

ピロキシリン

Pyroxylin is a nitric acid ester of cellulose. It is usually moistened with 2-propanol or some other solvent.

**Description** Pyroxylin occurs as a white cotton-like substance or white flakes.

It is freely soluble in acetone, and very slightly soluble in diethyl ether.

Upon heating or exposure to light, it is decomposed with the evolution of nitrous acid vapors.

**Identification** Ignite Pyroxylin: it burns very rapidly with a luminous flame.

**Purity** **(1)** Clarity of solution—Dissolve 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, in 25 mL of a mixture of diethyl ether and ethanol (95) (3:1): the solution is clear.

**(2)** Acidity—Shake 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, with 20 mL of water for 10 minutes: the filtrate is neutral.

**(3)** Water-soluble substances—Evaporate 10 mL of the filtrate obtained in (2) on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.5 mg.

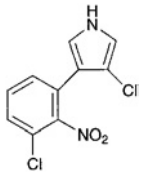
**(4)** Residue on ignition—Weigh accurately about 2 g of Pyroxylin, previously dried at 80°C for 2 hours, and moisten with 10 mL of a solution of castor oil in acetone (1 in 20) to gelatinize the sample. Ignite the contents to carbonize the sample, heat strongly at about 500°C for 2 hours, and allow to cool over silica gel: the amount of the residue is not more than 0.30%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant, packed loosely, remote from fire, and preferably in a cold place.

# Pyrrolnitrin

ピロールニトリン



$C_{10}H_6Cl_2N_2O_2$: 257.07

3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole [*1018-71-9*]

Pyrrolnitrin contains not less than 970 $\mu$g (potency) and not more than 1020 $\mu$g (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin ($C_{10}H_6Cl_2N_2O_2$).

**Description** Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

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

**Identification** **(1)** Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

**(2)** Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

**Melting point** <2.60> 124 – 128°C

**Purity** Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 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 $\mu$L 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 xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

**Loss on drying** <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

**Residue on ignition** <2.44> Not more than 0.1% (1 g).

**Assay** Conduct this procedure using light-resistant vessels.

Weigh accurately an amount of Pyrrolnitrin and Pyrrolnitrin Reference Standard, equivalent to about 50 mg (potency), dissolve in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the ratios, $Q_T$ and $Q_S$, of the peak area of pyrrolnitrin to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of } C_{10}H_6Cl_2N_2O_2 = W_S \times (Q_T/Q_S) \times 1000$$

$W_S$: Amount [mg (potency)] of Pyrrolnitrin Reference Standard

*Internal standard solution*—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).

*Operating conditions*—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of pyrrolnitrin is about 9 minutes.

*System suitability*—

System performance: When the procedure is run with 5 $\mu$L of the standard solution under the above operating conditions, pyrrolnitrin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

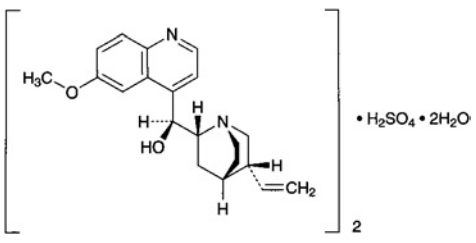
System repeatability: When the test is repeated 6 times with 5 $\mu$L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

# Quinidine Sulfate Hydrate

キニジン硫酸塩水和物




$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$: 782.94

(9*S*)-6′-Methoxycinchonan-9-ol hemisulfate monohydrate [*6591-63-5*]

Quinidine Sulfate Hydrate, when dried, contains not less than 98.5% of quinidine sulfate $[(C_{20}H_{24}N_2O_2)_2.H_2SO_4$: 746.91].

**Description** Quinidine Sulfate Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is freely soluble in ethanol (95) and in boiling water, sparingly soluble in water, and practically insoluble in diethyl ether. Quinidine Sulfate Hydrate, previously dried, is freely soluble in chloroform.

It darkens gradually by light.

Optical rotation $[\alpha]_D^{20}$: +275 – +287° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

**Identification** **(1)** Dissolve 0.01 g of Quinidine Sulfate Hydrate in 10 mL of water and 2 to 3 drops of dilute sulfuric acid: a blue fluorescence is produced.

**(2)** To 5 mL of an aqueous solution of Quinidine Sulfate Hydrate (1 in 1000) add 1 to 2 drops of bromine TS, then add 1 mL of ammonia TS: a green color develops.

**(3)** To 5 mL of an aqueous solution of Quinidine Sulfate Hydrate (1 in 100) add 1 mL of silver nitrate TS, stir with a glass rod, and allow to stand for a short interval: a white precipitate is produced, and it dissolves on addition of nitric acid.

**(4)** Dissolve 0.4 g of Quinidine Sulfate Hydrate in 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <*1.09*> for sulfate.

**pH** <*2.54*> Dissolve 1.0 g of Quinidine Sulfate Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

**Purity** **(1)** Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinidine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at about 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), and dry at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

**(2)** Related substances—Dissolve 20 mg of Quinidine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 $\mu$L each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate their amount by the area percentage method: the amount of dihydroquinidine sulfate is not more than 15.0%, and those of quinine sulfate and dihydroquinine sulfate are not more than 1.0%. The total area of the peaks other than the principal peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

*Operating conditions*—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 $\mu$m in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane-