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

Mobile phase: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of palmatin and berberine in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated five times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of berberine is not more than 1.5%.

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

Berberine Tannate

タンニン酸ベルベリン

Berberine Tannate is a compound of berberine and tannic acid. It contains not less than 27.0% and not more than 33.0% of berberine ($C_{20}H_{19}NO_5$: 353.37), calculated on the anhydrous basis.

Description Berberine Tannate occurs as a yellow to light yellow-brown powder. It is odorless or has a faint, characteristic odor, and is tasteless.

It is practically insoluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification **(1)** To 0.1 g of Berberine Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. Cool, filter, and to 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced, and on allowing to stand, a bluish black precipitate is formed.

(2) Dissolve 0.01 g of Berberine Tannate in 10 mL of methanol and 0.4 mL of 1 mol/L hydrochloric acid TS, and add water to make 200 mL. To 8 mL of the solution add water to make 25 mL. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, 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 Berberine Tannate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Berberine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Acid—To 0.10 g of Berberine Tannate add 30 mL of water, and filter after shaking well. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the color of the solution changes from yellow to orange to red.

(2) Chloride—Shake 1.0 g of Berberine Tannate with 38 mL of water and 12 mL of dilute nitric acid for 5 minutes, and filter. Discard the first 5 mL of the filtrate, to 25 mL of the subsequent filtrate add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS by adding 6 mL of dilute nitric acid, 10 to 15 drops of bromophenol blue TS and water to make 50 mL (not more than 0.035%).

(3) Sulfate—Shake 1.0 g of Berberine Tannate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048%).

(4) Heavy metals—Proceed with 1.0 g of Berberine Tannate 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).

(5) Related substances—Dissolve 0.010 g of Berberine Tannate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 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 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total of the peak areas other than berberine of the sample solution is not larger than the peak area of berberine of the standard solution.

Operating conditions—

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

Selection of column: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of palmatin and berberine in this order with complete separation of these peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of berberine obtained from 10 μL of the standard solution is about 10% of the full scale.

Time span of measurement: About 2 times as long as the retention time of berberine, after the solvent peak.

Water Not more than 6.0% (0.7 g, direct titration).

Residue on ignition Not more than 1.0% (1 g).

Assay Weigh accurately about 0.03 g of Berberine Tannate, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Berberine Chloride Reference Standard (separately, determined the water content), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas,

A_T and A_S, of berberine in each solution.

Amount (mg) of $C_{20}H_{19}NO_5$
= amount (mg) of Berberine Chloride Reference Standard, calculated on the dehydrated basis
$\times \frac{A_T}{A_S} \times \frac{353.37}{371.82}$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 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 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

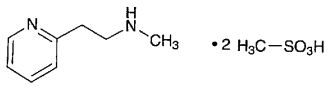
Selection of column: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of palmatin and berberine in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated five times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of berberine is not more than 1.5%.

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

Betahistine Mesilate

メシル酸ベタヒスチン



$C_8H_{12}N_2.2CH_4O_3S$: 328.41
N-Methyl-*N*-[2-(pyridin-2-yl)ethyl]amine dimethanesulfonate [*5638-76-6*, Betahistine]

Betahistine Mesilate, when dried, contains not less than 98.0% of $C_8H_{12}N_2.2CH_4O_3S$.

Description Betahistine Mesilate occurs as white crystals or crystalline powder. It is odorless, or has a faint, characteristic odor and a bitter taste.

It is very soluble in water, freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To 5 mL of a solution of Betahistine Mesilate (1 in 10) add 15 mL of sodium hydroxide TS and 20 mL of chloroform, and shake. Separate the chloroform layer, wash with 10 mL of water, and use the chloroform layer as the sample solution. Take 5 mL of the sample solution, evaporate the solvent under reduced pressure by warming, dissolve the residue in 1 mL of water, add 1 mL of acetaldehyde and 0.5 mL of sodium pentacyanonitrosylferrate (III) TS, and shake: a blue to blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Betahistine Mesilate in 0.1 mol/L methanesulfonic acid TS (1 in 20,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 0.03 g of Betahistine Mesilate add 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, mix well, and heat gradually. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and to the filtrate add 1 mL of barium chloride TS: a white precipitate is formed.

Melting point 110 - 114°C (after drying).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Betahistine Mesilate in 10 mL of water: the solution is clear and colorless.

(2) Chloride—Perform the test with 1.0 g of Betahistine Mesilate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals—Proceed with 1.0 g of Betahistine Mesilate 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).

(4) Arsenic—Prepare the test solution with 1.0 g of Betahistine Mesilate according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Betahistine Mesilate 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 the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: 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 Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 70°C, 24 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.2 g of Betahistine Mesilate, previously dried, dissolve in 1 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate 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
= 16.421 mg of $C_8H_{12}N_2.2CH_4O_3S$

Containers and storage Containers—Tight containers.