Assay Weigh accurately about 0.01 g of Vinblastine Sulfate, and dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 5 mL of this solution, and add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Determine the absorbance A of this solution at the maximum wavelength at about 269 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of } C_{46}H_{58}N_4O_9.H_2SO_4 = \frac{A}{184} \times 5000$$

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

Vinblastine Sulfate for Injection

注射用硫酸ビンブラスチン

Vinblastine Sulfate for Injection is a preparation for injection which is dissolved before use. When dried, it contains not less than 90.0% and not more than 110.0% of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9.H_2SO_4$: 909.05).

Method of preparation Prepare as directed under Injection, with Vinblastine Sulfate.

Description Vinblastine Sulfate for Injection occurs as white to pale yellow, light masses or powder.

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

It is hygroscopic.

Identification **(1)** Proceed as directed in the Identification (1) and (4) under Vinblastine Sulfate.

(2) Determine the absorption spectrum of a solution of Vinblastine Sulfate for Injection (1 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 267 nm and 271 nm.

(3) Dissolve 0.02 g of Vinblastine Sulfate for Injection in 10 mL of sodium chloride TS, adjust the pH to between 9 and 10 with ammonia TS, and extract with two 5-mL portions of chloroform. Wash the combined chloroform extracts with a small quantity of sodium chloride TS, add a small quantity of anhydrous sodium sulfate, and allow to stand for several minutes. Filter through a pledget of absorbent cotton, evaporate the filtrate under reduced pressure to dryness, and dissolve the residue in a small quantity of chloroform. Perform the test with this solution as directed in the solution method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3600 cm^{-1}, 3480 cm^{-1}, 1738 cm^{-1} and 1611 cm^{-1}.

pH Dissolve 0.01 g of Vinblastine Sulfate for Injection in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Purity **(1)** Clarity and color of solution—Proceed as directed in the Purity (1) under Vinblastine Sulfate.

(2) Related substances—Dissolve 0.010 g of Vinblastine Sulfate for Injection 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 25 mL, and use this solution as the standard solution. Perform the test with 20 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 these solutions by the automatic integration method: the total area of the peaks other than the principal peak of the sample solution is not larger than the peak area of vinblastine from the standard solution, and the area of any peak other than the principal peak of the sample solution is not larger than 1/4 of the peak area of vinblastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: To 7 mL of diethylamine add water to make 500 mL, and adjust with phosphoric acid to a pH of 7.5. To 380 mL of this solution add 620 mL of a mixture of methanol and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of vinblastine is about 20 minutes.

Selection of column: Proceed with 20 μL of the standard solution obtained in the Assay under the above operating conditions, and calculate the resolution. Use a column giving elution of vinblastine and the internal standard in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of vinblastine from 10 μL of the standard solution is between 5 mm and 15 mm.

Time span of measurement: About 1.7 times as long as the retention time of vinblastine after the solvent peak.

Loss on drying Not more than 15.0% (0.01 g, in vacuum, phosphorus (V) oxide, 25°C, 2 hours).

Assay Weigh accurately the contents of not less than 10 samples of Vinblastine Sulfate for Injection. Weigh accurately about 0.01 g of the contents, previously dried, and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Vinblastine Sulfate Reference Standard (determine its loss on drying before using in the same manner as Vinblastine Sulfate), and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of vinblastine to that of the internal standard, respectively.

$$\text{Amount (mg) of vinblastine sulfate } (C_{46}H_{58}N_4O_9.H_2SO_4) = \text{amount (mg) of Vinblastine Sulfate Reference Standard, calculated on the dried basis} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dibenzyl in

methanol (2 in 625).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: To 7 mL of diethylamine add water to make 500 mL, and adjust with phosphoric acid to a pH of 7.5. To 380 mL of this solution add 620 mL of a mixture of methanol and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of vinblastine is about 20 minutes.

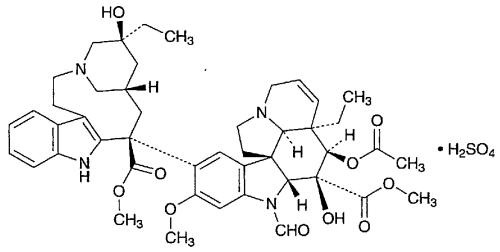
Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of vinblastine and the internal standard in this order with the resolution between these peaks being not less than 7.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, and in a cold place.

Vincristine Sulfate

硫酸ビンクリスチン



$C_{46}H_{56}N_4O_{10}.H_2SO_4$: 923.04

Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [*2068-78-2*]

Vincristine Sulfate contains not less than 95.0% and not more than 105.0% of $C_{46}H_{56}N_4O_{10}.H_2SO_4$, calculated on the dried basis.

Description Vincristine Sulfate occurs as a white to light yellowish white powder.

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

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: +28.5 – +35.5° (0.20 g, calculated on the dried basis, water, 10 mL, 100 mm).

Identification **(1)** Dissolve 5 mg of Vincristine Sulfate in 2 mL of cerium (IV) tetraammonium sulfate-phosphoric acid TS: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Vincristine Sulfate (1 in 50,000) 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) Dissolve 0.02 g of Vincristine Sulfate in 10 mL of sodium chloride TS, adjust the pH to between 9 and 10 with ammonia TS, and extract with two 5-mL portions of chloroform. Wash the combined chloroform extracts with a small quantity of sodium chloride TS, add a small quantity of anhydrous sodium sulfate, and allow to stand for several minutes. Filter through a pledget of absorbent cotton, evaporate the filtrate to dryness under reduced pressure, and dissolve the residue in a small quantity of chloroform. Determine the infrared absorption spectrum of the solution as directed in the solution method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Vincristine Sulfate (1 in 100) responds to the Qualitative Tests for sulfate.

pH Dissolve 0.010 g of Vincristine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.025 g of Vincristine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 0.025 g of Vincristine 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 20 mL, and use this solution as the standard solution. Perform the test with 20 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 these solutions by the automatic integration method: the total area of the peaks other than the principal peak of the sample solution is not larger than the peak area of vincristine from the standard solution, and the area of any peak other than the principal peak of the sample solution is not larger than 2/5 of the peak area of vincristine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

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

Column temperature: Room temperature.

Mobile phase: Use methanol as the mobile phase A, and a mixture of water and diethylamine (197:3) adjusted with phosphoric acid to a pH of 7.5 as the mobile phase B. Run a mixture of the mobile phase A and the mobile phase B (31:19) for 24 minutes after injection of the sample, and run a mixture of the mobile phase A and the mobile phase B for subsequent 30 minutes, increasing the composition ratio of the mobile phase A by 1% per minute. For subsequent 4 minutes, run a mixture of the mobile phase A and the mobile phase B, decreasing the composition ratio of the mobile phase A by 7.5% per minute, then continue running a mixture of the mobile phase A and the mobile phase B (31:19).

Flow rate: Adjust the flow rate so that the retention time