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.

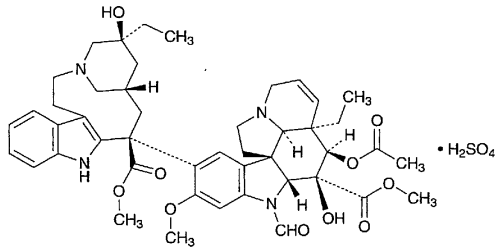
Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of vinblastine and the internal standard in this order with the resolution between these peaks being not less than 7.

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

of vincristine is about 19 minutes.

Selection of column: Dissolve 0.010 g each of Vincristine Sulfate and vinblastine sulfate in 100 mL of water. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of vincristine and vinblastine in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of vincristine from 20 μL of the standard solution is between 5 mm and 15 mm.

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

Loss on drying Not more than 12.0% (0.05 g, in vacuum, 105°C, 2 hours).

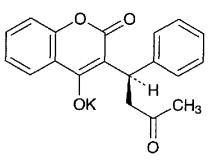
Assay Weigh accurately about 0.01 g of Vincristine Sulfate, dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 5 mL of this solution, add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Determine the absorbance A of this solution at the maximum wavelength at about 296 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of } C_{46}H_{56}N_4O_{10}.H_2SO_4 = \frac{A}{177} \times 5000$$

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

Warfarin Potassium

ワルファリンカリウム



and enantiomer

$C_{19}H_{15}KO_4$: 346.42
Monopotassium (*RS*)-2-oxo-3-(3-oxo-1-phenylbutyl)-chromen-4-olate [*2610-86-8*]

Warfarin Potassium, when dried, contains not less than 98.0% and not more than 102.0% of $C_{19}H_{15}KO_4$.

Description Warfarin Potassium occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

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

It is affected by light.

Identification (1) Dissolve 0.1 g of Warfarin Potassium in 25 mL of water, and add 3 drops of dilute hydrochloric acid. Collect the precipitate produced, wash with four 5-mL portions of water, and dry at 105°C for 1 hour: the residue melts between 157°C and 167°C.

(2) Determine the absorption spectrum of a solution of Warfarin Potassium in 0.02 mol/L potassium hydroxide TS (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, 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 Warfarin Potassium in 0.02 mol/L hydrochloric acid TS (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) The filtrate obtained in (1) responds to the Qualitative Tests for potassium salts.

pH Dissolve 1.0 g of Warfain Potassium in 100 mL of water: the pH of the solution is between 7.2 and 8.3.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Warfarin Potassium in 10 mL of water: the solution is clear and colorless.

(2) Alkaline colored substances—Dissolve 1.0 g of Warfarin Potassium in a solution of sodium hydroxide (1 in 20) to make exactly 10 mL, and determine the absorbance at 385 nm within 15 minutes as directed under the Ultraviolet-visible Spectrophotometry, using a solution of sodium hydroxide (1 in 20) as a blank: it does not exceed 0.20.

(3) Heavy metals—Dissolve 2.0 g of Warfarin Potassium in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) 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, 2 mL of dilute acetic acid and ethanol (95) to make 50 mL (not more than 10 ppm).

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

Loss on drying Not more than 10.0% (1 g, 105°C, 3 hours).

Residue on ignition 24.3 - 25.7% (after drying, 0.4 g, 700°C).

Assay Weigh accurately about 0.1 g of Warfarin Potassium, previously dried, and add 0.02 mol/L potassium hydroxide TS to make exactly 100 mL. Pipet 10 mL of this solution, and add 0.02 mol/L potassium hydroxide TS to make exactly 1000 mL. Determine the absorbance A of this solution at the maximum wavelength at about 308 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of } C_{19}H_{15}KO_4 = \frac{A}{405} \times 100{,}000$$

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

Warfarin Potassium Tablets

ワルファリンカリウム錠

Warfarin Potassium Tablets contain not less than 95% and not more than 105% of the labeled amount of warfarin potassium ($C_{19}H_{15}KO_4$: 346.42).

Method of preparation Prepare as directed under Tablets, with Warfarin Potassium.