Assay **(1)** Procedure (i) Total flavin content—Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, and dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add 5 mL of zinc chloride TS, and heat in a water bath for 30 minutes. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of Riboflavin Reference Standard, previously dried at 105°C for 2 hours, dissolve in 200 mL of diluted acetic acid (31) (1 in 100) by warming, cool, add water to make exactly 500 mL. Measure exactly 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 450 nm as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank.

$$\text{Total amount (mg) of flavin} = \text{amount (mg) of Riboflavin Reference Standard} \times \frac{A_T}{A_S} \times \frac{4}{5}$$

(ii) Peak area ratio of flavin adenine dinucleotide—Dissolve 0.1 g of Flavin Adenine Dinucleotide Sodium in 200 mL of water,and use this solution as the sample solution. Perform the test with 5 μL of this solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak area, A, of flavin adenine dinucleotide, and the total area, S, of the peaks other than flavin adenine dinucleotide by the automatic integration method.

$$\text{Peak area ratio of flavin adenine dinucleotide} = \frac{1.08 \times A}{1.08 \times A + S}$$

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 450 nm).

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

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (1 in 500) and methanol (4:1).

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

Selection of column: Dissolve about 0.02 g each of Flavin Adenine Dinucleotide Sodium and riboflavin trisodium phosphate 12-water in 100 mL of water. Proceed with this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of flavin adenine dinucleotide and riboflavin phosphate in this order with the resolution of these peaks being not less than 2.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of flavin adenine dinucleotide obtained from 5 μL of the sample solution is between 60% and 100% of the full scale.

Time span of measurement: About 4.5 times as long as the retention time of flavin adenine dinucleotide.

(2) Calculation equation

$$\text{Amount (mg) of } C_{27}H_{31}N_9Na_2O_{15}P_2 = f_T \times f_R \times \frac{829.52}{376.37}$$

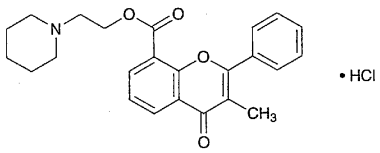
f_T: Total amount (mg) of flavin in Flavin Adenine Dinucleotide Sodium obtained from the procedure (i).

f_R: Peak area ratio of flavin adenine dinucleotide in Flavin Adenine Dinucleotide Sodium obtained from the procedure (ii).

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

Flavoxate Hydrochloride

塩酸フラボキサート



$C_{24}H_{25}NO_4.HCl$: 427.92

2-(Piperidine-1-yl)ethyl 3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxylate monohydrochloride

[*3717-88-2*]

Flavoxate Hydrochloride, when dried, contains not less than 99.0% of $C_{24}H_{25}NO_4.HCl$.

Description Flavoxate Hydrochloride occurs as white crystals or crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Flavoxate Hydrochloride in 0.01 mol/L hydrochloric acid TS (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.

(2) Determine the infrared absorption spectrum of Flavoxate Hydrochloride, previously dried, as directed in the potassium bromide disk 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.

(3) A solution of Flavoxate Hydrochloride (1 in 100) responds to the Qualitative Tests for chloride.

Purity **(1)** Heavy metals—Proceed with 2.0 g of Flavoxate 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—Prepare the test solution with 2.0 g of Flavoxate Hydrochloride according to Method 4, and perform the test using Apparatus B (not more than 1 ppm).

(3) Related substances—Dissolve 0.080 g of Flavoxate Hydrochloride 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 20 mL, then pipet 1

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 the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the 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 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 Not more than 1.0% (1 g, reduced pressure, silica gel, 2 hours).

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

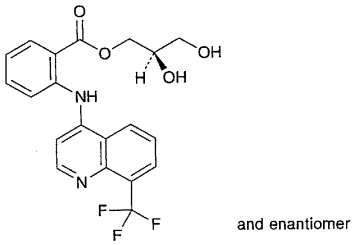
Assay Weigh accurately about 0.6 g of Flavoxate Hydrochloride, previously dried, add 10 mL of acetic acid (100) and 40 mL of acetonitrile to dissolve, add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.79 mg of $C_{24}H_{25}NO_4.HCl$

Containers and storage Containers—Tight containers.

Floctafenine

フロクタフェニン



and enantiomer

$C_{20}H_{17}F_3N_2O_4$: 406.36
(*RS*)-2,3-Dihydroxypropyl 2-[8-(trifluoromethyl)-quinoline-4-ylamino]benzoate [*23779-99-9*]

Floctafenine, when dried, contains not less than 98.5% of $C_{20}H_{17}F_3N_2O_4$.

Description Floctafenine occurs as a white to pale yellowish white crystalline powder.

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

A solution of Floctafenine in 0.1 mol/L hydrochloric acid TS (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Floctafenine in 0.1 mol/L hydrochloric acid TS (1 in 100,000) 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.

(2) Determine the infrared absorption spectrum of Floctafenine, previously dried, as directed in the potassium bromide disk 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.

Melting point 176 - 180°C

Purity (1) Heavy metals—Proceed with 2.0 g of Floctafenine 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—Prepare the test solution with 1.0 g of Floctafenine according to Method 4, and perform the test using Apparatus B (not more than 2 ppm).

(3) Related substances—Dissolve 0.020 g of Floctafenine 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 100 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 from both solutions by the automatic integration method: the total area of the peaks other than the peak of floctafenine from the sample solution is not larger than the peak area of floctafenine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 224 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 25°C.

Mobile phase: Adjust the pH of a mixture of methanol, water and phosphoric acid (240:160:1) to 3.5 with sodium hydroxide TS.

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

Selection of column: Dissolve 0.01 g of floctafenine and 0.05 g of ethyl parahydroxybenzoate in 250 mL of the mobile phase. Proceed with 20 μL of this solution according to the above operating conditions, and calculate the resolution. Use a column giving elution of floctafenine and ethyl parahydroxybenzoate 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 floctafenine from 20 μL of the standard solution is between 5 % and 15% of the full scale.

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

Loss on drying Not more than 1.0% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 0.6 g of Floctafenine, previously dried, dissolve in 30 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.