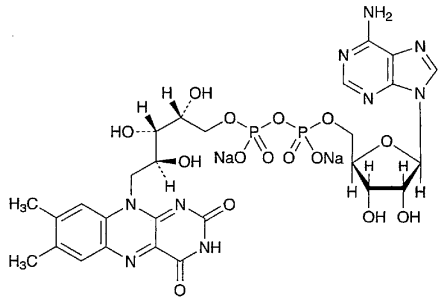
Flavin Adenine Dinucleotide Sodium

フラビンアデニンジヌクレオチドナトリウム



$C_{27}H_{31}N_9Na_2O_{15}P_2$: 829.51
Disodium 1-(6-amino-9*H*-purin-9-yl)-1-deoxy-β-D-ribofuranosin-5-yl (2*R*,3*S*,4*S*)-5-(3,4-dihydro-7,8-dimethyl-2,4-dioxobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypentyl diphosphate [*84366-81-4*]

Flavin Adenine Dinucleotide Sodium contains not less than 93.0% of $C_{27}H_{31}N_9Na_2O_{15}P_2$, calculated on the anhydrous basis.

Description Flavin Adenine Dinucleotide Sodium occurs as an orange-yellow to light yellow-brown powder. It is odorless or has a slight, characteristic odor, and has a slightly bitter taste.

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

It is hygroscopic.

It is decomposed by light.

Identification (1) A solution of Flavin Adenine Dinucleotide Sodium (1 in 100,000) is light yellow-green in color, and shows a strong yellow-green fluorescence. To 5 mL of the solution add 0.02 g of hydrosulfite sodium: the color and the fluorescence of the solution disappear, and gradually reappear when the solution is shaken in air. Add dilute hydrochloric acid or sodium hydroxide TS dropwise: the fluorescence of the solution disappears.

(2) Determine the infrared absorption spectrum of Flavin Adenine Dinucleotide Sodium 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) To 0.1 g of Flavin Adenine Dinucleotide Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. To the residue add 10 mL of diluted nitric acid (1 in 50), boil for 5 minutes, and after cooling, neutralize with ammonia TS, then filter the solution if necessary: the solution responds to the Qualitative Tests for sodium salt and the Qualitative Tests (1) and (3) for phosphate.

Optical rotation $[\alpha]_D^{20}$: $-21.0 - -25.5°$ (0.3 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH Dissolve 1.0 g of Flavin Adenine Dinucleotide Sodium 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 0.20 g of Flavin Adenine Dinucleotide Sodium in 10 mL of water: the solution is clear and orange-yellow in color.

(2) Free phosphoric acid—Weigh accurately about 0.02 g of Flavin Adenine Dinucleotide Sodium, dissolve in 10 mL of water, and use this solution as the sample solution. Separately, measure exactly 2 mL of Standard Phosphoric Acid Solution, add 10 mL of water, and use this solution as the standard solution. To each of the sample solution and standard solution add 2 mL of diluted perchloric acid (100 in 117), then add 1 mL of hexaammonium heptamolybdate TS and 2 mL of 2,4-diaminophenol hydrochloride TS, respectively, shake, add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry, using a solution prepared in the same manner with 2 mL of water, as the blank, and determine the absorbances, A_T and A_S, of the subsequent solutions of the sample solution and the standard solution at 730 nm, respectively: the amount of free phosphoric acid is less than 0.25%.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = \frac{A_T}{A_S} \times \frac{1}{W} \times 5.16$$

W: Amount (mg) of flavin adenine dinucleotide sodium, calculated on the anhydrous basis.

(3) Heavy metals—Proceed with 1.0 g of Flavin Adenine Dinucleotide 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).

(4) Arsenic—Prepare the test solution with 2.0 g of Flavin Adenine Dinucleotide Sodium according to Method 3, and perform the test using Apparatus B (not more than 1 ppm).

(5) Related substances—Dissolve 0.10 g of Flavin Adenine Dinucleotide Sodium in 200 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions. Determine the peak area, A, of flavin adenine dinucleotide and the area, S, of peaks other than the peak of flavin adenine dinucleotide by the automatic integration method: $S/(A+S)$ is not more than 0.10.

Operating conditions—

Column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions of Procedure (ii) in the Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Detection sensitivity: Adjust the detection sensitivity so that the peak height of flavin adenine dinucleotide obtained from 20 μL of the sample solution is between 60% and 100% of the full scale.

Water Take 50 mL of a mixture of methanol for Karl Fischer method and ethyleneglycol for Karl Fischer method (1:1) into a dry titration flask, and titrate with Karl Fischer TS until end point. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, transfer quickly to the titration flask, add an excess and constant volume of Karl Fischer TS, dissolve by stirring for 10 minutes, and perform the test: the water content is not more than 10.0%.

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

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

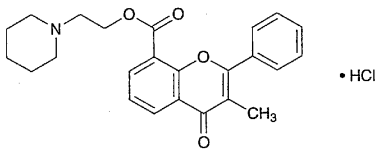
f_T: Total amount (mg) of flavin in Flavin Adenine Dinucleotide Sodium obtained from the procedure (i).

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