of methanol by warming, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.05 g of Hydrocortisone Butyrate add 2 mL of potassium hydrox-ide-ethanol TS, and heat on a water bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl butyrate is perceptible.

(4) Determine the infrared absorption spectrum of Hydrocortisone Butyrate, 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.

Optical rotation $[\alpha]_D^{25}$: +48 – +52° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Purity (1) Heavy metals—Proceed with 1.0 g of Hydrocortisone Butyrate 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).

(2) Other steroids—Dissolve 0.025 g of Hydrocortisone Butyrate in 5 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 2 mL of this solution, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add tetrahydrofuran 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 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 1,2-dichloroethane, methanol and water (470:30:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more than two in number, and not more intense than those from the standard solution in color.

Loss on drying Not more than 1.0% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.05 g of Hydrocortisone Butyrate, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, and add ethanol (99.5) to make exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 241 nm as directed under the Ultraviolet-visible Spectrophotometry.

$$\text{Amount (mg) of } C_{25}H_{36}O_6 = \frac{A}{375} \times 25{,}000$$

Containers and storage Containers—Tight containers.

Hydrocortisone Sodium Phosphate

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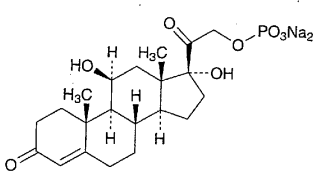

$C_{21}H_{29}Na_2O_8P$: 486.40

Disodium 11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-phosphate [*6000-74-4*]

Hydrocortisone Sodium Phosphate contains not less than 96.0% and not more than 102.0% of $C_{21}H_{29}Na_2O_8P$, calculated on the dried basis.

Description Hydrocortisone Sodium Phosphate occurs as a white to light yellow powder. It is odorless.

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

It is hygroscopic.

Identification (1) To 2 mg of Hydrocortisone Sodium Phosphate add 2 mL of sulfuric acid: a yellowish green fluorescence is exhibited initially, then gradually changes through orange-yellow to dark red. Examine the solution under ultraviolet light (main wavelength: 254 nm): an intense, light green fluorescence is exhibited. To this solution add carefully 10 mL of water: the color changes from yellow to orange-yellow with a light green fluorescence and a yellow-brown, flocculent floating substance is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Sodium Phosphate as directed in the paste method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Hydrocortisone Sodium Phosphate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate Reference Standard in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(3) Moisten 1.0 g of Hydrocortisone Sodium Phosphate with a small quantity of sulfuric acid, and incinerate by gradual heating. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests for sodium salt and for phosphate.

Optical rotation $[\alpha]_D^{20}$: +121 – +129° (1 g, calculated on the dried basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

pH Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 100 mL of water: the pH of this solution is between 7.5 and 9.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride—Dissolve 0.30 g of Hydrocortisone Sodium Phosphate in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 100 mL. To 5 mL of this solution add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.600%).

(3) Heavy metals—Proceed with 0.5 g of Hydrocortisone Sodium Phosphate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(4) Arsenic—Prepare the test solution with 1.0 g of

Hydrocortisone Sodium Phosphate according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.25 g of Hydrocortisone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution into separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, 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 with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of each solution from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the amount of free phosphoric acid is not more than 1.0%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = \frac{A_T}{A_S} \times \frac{1}{W} \times 257.8$$

W: Amount (mg) of Hydrocortisone Sodium Phosphate, calculated on the dried basis.

(6) Free hydrocortisone—Dissolve 0.025 g of Hydrocortisone Sodium Phosphate in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh 0.025 g of Hydrocortisone Reference Standard, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 200 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 the peak areas, A_T and A_S, of hydrocortisone from each solution: A_T is not larger than A_S.

Operating conditions—

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

Detection sensitivity: Adjust the detection sensitivity so that the peak height of hydrocortisone from 20 μL of the standard solution composes about 10% of the full scale.

Loss on drying Not more than 5.0% (1 g, in vacuum, 80°C, 5 hours).

Assay Weigh accurately about 0.02 g each of Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate Reference Standard (determine its loss on drying before using), dissolve each in 50 mL of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. 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 hydrocortisone phosphate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{21}H_{29}Na_2O_8P = \text{amount (mg) of Hydrocortisone Sodium Phosphate Reference Standard, calculated on the dried basis} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 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 (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 2.6 and methanol (1:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone phosphate is about 10 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 hydrocortisone phosphate and isopropyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 8.

Containers and storage Containers—Tight containers.

Hydrocortisone Sodium Succinate

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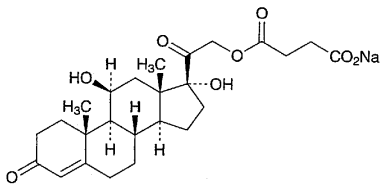

$C_{25}H_{33}NaO_8$: 484.51

Monosodium 11β,17,21-trihydroxypregn-4-ene-3,20-dione 21-succinate [*125-04-2*]

Hydrocortisone Sodium Succinate, calculated on the dried basis, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{33}NaO_8$.

Description Hydrocortisone Sodium Succinate occurs as white powder or masses. It is odorless.

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

It is hygroscopic.

It is gradually colored by light.

Identification **(1)** Dissolve 0.2 g of Hydrocortisone Sodium Succinate in 20 mL of water, and add 0.5 mL of dilute hydrochloric acid with stirring: a white precipitate is formed. Collect the precipitate, wash it with two 10-mL portions of water, and dry at 105°C for 3 hours. To 3 mg of this dried matter add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to