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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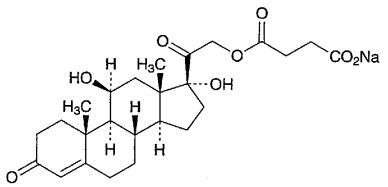

$C_{25}H_{33}NaO_8$: 484.51

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

dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Dissolve 0.01 g of the dried matter obtained in (1) in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.1 g of the dried matter obtained in (1) add 2 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Filter the solution to remove the precipitate formed, mix the filtrate with 1 mL of dilute hydrochloric acid, filter if necessary, then adjust the solution to a pH of about 6 with diluted ammonia TS (1 in 10), and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Determine the infrared absorption spectrum of the dried matter obtained in (1) as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Succinate and Hydrocortisone Succinate Reference Standard in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(5) Hydrocortisone Sodium Succinate responds to the Qualitative Tests (1) for sodium salt.

Optical rotation $[\alpha]_D^{20}$: +135 – +145° (0.1 g, calculated on the dried basis, ethanol (95), 10 mL, 100 mm).

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

(2) Other steroids—Dissolve 0.025 g of Hydrocortisone Sodium Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 0.025 g of hydrocortisone in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 6 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 3 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution (1) is not more intense than the spot from the standard solution (1). Any spot other than the principal spot and the above spot obtained from the sample solution is not more than one, and is not more intense than the spot from the standard solution (2).

Loss on drying Not more than 2.0% (0.5 g, 105°C, 3 hours).

Assay Weigh accurately about 0.01 g of Hydrocortisone Sodium Succinate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Hydrocortisone Succinate Reference Standard, previously dried at 105°C for 3 hours, proceed in the same manner as directed for the sample solution, 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 240 nm as directed under the Ultraviolet-visible Spectrophotometry, respectively.

Amount (mg) of $C_{25}H_{33}NaO_8$
= amount (mg) of Hydrocortisone Succinate Reference Standard
$\times \frac{A_T}{A_S} \times 1.0475$

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

Hydrocortisone Succinate

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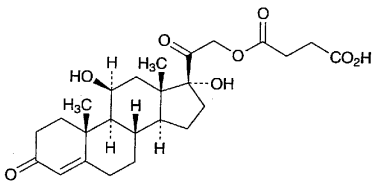

$C_{25}H_{34}O_8$: 462.53
11β,17,21-Trihydroxypregn-4-ene-3,20-dione 21-(hydrogen succinate) [*2203-97-6*]

Hydrocortisone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{34}O_8$.

Description Hydrocortisone Succinate occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), sparingly soluble in ethanol (95), and practically insoluble in water.

Identification (1) To 3 mg of Hydrocortisone Succinate 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 dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Succinate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Succinate and Hydrocortisone Succinate Reference Standard in methanol, respectively, then