

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Acetate: 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 Hydrocortisone Acetate in 1 mL 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 Acetate add 2 mL of potassium hydroxide-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 acetate is perceptible.

(4) Determine the infrared absorption spectra of Hydrocortisone Acetate and Hydrocortisone Acetate Reference Standard, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry: both the sample and the Reference Standard exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and Reference Standard in ethanol (95), respectively, evaporate to dryness, and repeat the test on the residues.

Optical rotation $[\alpha]_D^{20}$: +158 – +165° (after drying, 0.05 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Other steroids—Dissolve 0.040 g of Hydrocortisone Acetate in 25 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μ L 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 dichloromethane, diethyl ether, methanol and water (160:30:8:1) to a distance of about 12 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 intense than the spot from the standard solution.

Loss on drying Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition Not more than 0.1% (0.5 g).

Assay Dissolve about 0.02 g each of Hydrocortisone Acetate and Hydrocortisone Acetate Reference Standard, previously dried and accurately weighed, in methanol, add exactly 10 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following operating conditions, and calculate the ratios, Q_T and Q_S , of the peak area of hydrocortisone acetate to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{23}H_{32}O_6 \\ = \text{amount (mg) of Hydrocortisone Acetate} \end{aligned}$$

Reference Standard

$$\times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of benzyl parahydroxy benzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of water and acetonitrile (13:7).

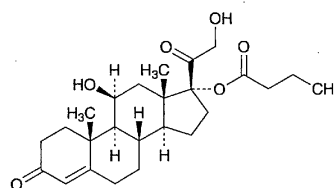
Flow rate: Adjust the flow rate so that the retention time of hydrocortisone acetate is about 8 minutes.

Selection of column: Proceed with 20 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of hydrocortisone acetate and the internal standard in this order with the resolution between these peaks being not less than 4.

Containers and storage Containers—Tight containers.

Hydrocortisone Butyrate

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$C_{25}H_{36}O_6$: 432.55

11 β ,17,21-Trihydroxypregn-4-ene-3,20-dione 17-butyrate
[13609-67-1]

Hydrocortisone Butyrate, when dried, contains not less than 96.0% and not more than 104.0% of $C_{25}H_{36}O_6$.

Description Hydrocortisone Butyrate occurs as a white powder. It is odorless.

It is freely soluble in tetrahydrofuran, in chloroform and in 1,2-dichloroethane, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 200°C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Butyrate: 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 (main wavelength: 254 nm). 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 Hydrocortisone Butyrate in 1 mL

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 hydroxide-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 μ L 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).

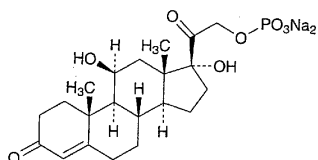
Assay Weigh accurately about 0.05 g of Hydrocortisone Butyrate, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, and add ethanol (99.5) to make exactly 50 mL. Determine the absorbance *A* of this solution at the wavelength of maximum absorption at 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

リン酸ヒドロコルチゾンナトリウム



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

Disodium 11 β ,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