evaporate the methanol to dryness, and repeat the test on the residues.

Optical rotation $[\alpha]_D^{20}$: +147 – +153° (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 nm).

Purity Other steroids—Dissolve 0.025 g of Hydrocortisone Succinate in exactly 10 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.025 g of hydrocortisone in exactly 10 mL of methanol. Pipet 1 mL of this solution, dilute with methanol to exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 3 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 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 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 2.0% (0.5 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.05 g each of Hydrocortisone Succinate and Hydrocortisone Succinate Reference Standard, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL 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 succinate to that of the internal standard, respectively.

Amount (mg) of $C_{25}H_{34}O_8$
= amount (mg) of Hydrocortisone Succinate Reference Standard
$\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of butyl parahydroxy benzoate in methanol (1 in 2500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of acetic acid-sodium acetate buffer solution, pH 4.0 and acetonitrile (3:2).
Flow rate: Adjust the flow rate so that the retention time of hydrocortisone succinate is about 5 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, hydrocortisone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone succinate to that of the internal standard is not more than 1.0%.

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

Hydrocotarnine Hydrochloride

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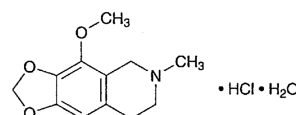

$C_{12}H_{15}NO_3.HCl.H_2O$: 275.73
5,6,7,8-Tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinoline monohydrochloride monohydrate [*5985-55-7*, anhydride]

Hydrocotarnine Hydrochloride, when dried, contains not less than 98.0% of $C_{12}H_{15}NO_3.HCl$: 257.72.

Description Hydrocotarnine Hydrochloride occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Hydrocotarnine Hydrochloride (1 in 10,000) 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 Hydrocotarnine Hydrochloride 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 Hydrocotarnine Hydrochloride (1 in 50) responds to the Qualitative Tests (2) for chloride.

pH Dissolve 1.0 g of Hydrocotarnine Hydrochloride in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Hydrocotarnine Hydrochloride in 10 mL of water: the solution is clear, and when perform the test with this solution as directed under the Ultraviolet-visible Spectrophotometry, using water as the blank, the absorbance at 400 nm is not more than 0.17.

(2) Heavy metals—Proceeds with 1.0 g of Hydrocotarnine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Hydrocotarnine Hydrochloride in 10 mL of diluted ethanol (99.5) (1 in 2), and use this solution as the sample solution.

Pipet 1 mL of the sample solution, add diluted ethanol (99.5) (1 in 2) 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 10 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 acetone, toluene, ethanol (99.5) and ammonia water (28) (20:20:3:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 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 7.0% (1 g, 105°C, 3 hours).

Residue on ignition Not more than 0.20% (1 g).

Assay Weigh accurately about 0.5 g of Hydrocotarnine Hydrochloride, previously dried. Dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming. Cool, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 25.772 mg of $C_{12}H_{15}NO_3.HCl$

Containers and storage Containers—Tight containers.

Hydroxocobalamin Acetate

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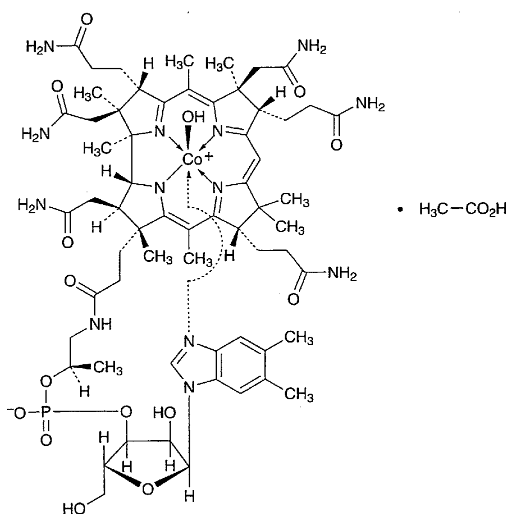

$C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$: 1406.41
Coα-[α-(5,6-Dimethylbenz-1*H*-imidazol-1-yl)]-*Coβ*-hydroxocobamide monoacetate
[*13422-51-0*, Hydroxocobalamin]

Hydroxocobalamin Acetate contains not less than 95.0% of $C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$, calculated on the dried basis.

Description Hydroxocobalamin Acetate occurs as dark red crystals or powder. It is odorless.

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

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Hydroxocobalamin Acetate in acetic acid-sodium acetate buffer solution, pH 4.5 (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) Mix 1 mg of Hydroxocobalamin Acetate with 0.05 g of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until the solution develops a light red. Then add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color develops immediately. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Add 0.5 mL of ethanol (99.5) and 1 mL of sulfuric acid to 0.02 g of Hydroxocobalamin Acetate, and heat the mixture: the odor of ethyl acetate is perceptible.

Purity Cyanocobalamin and colored impurities—Dissolve 0.050 g of Hydroxocobalamin Acetate in exactly 5 mL each of acetic acid-sodium acetate buffer solution, pH 5.0, in two tubes. To one tube add 0.15 mL of potassium thiocyanate TS, allow to stand for 30 minutes, and use this solution as the sample solution (1). To the other tube add 0.10 mL of potassium cyanide TS, allow to stand for 30 minutes, and use this solution as the sample solution (2). Separately, dissolve 3.0 mg of Cyanocobalamin Reference Standard in exactly 10 mL of acetic acid-sodium acetate buffer solution, pH 5.0, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Apply 20 μL each of the sample solution and the standard solution 25 mm in length along the starting line, 10 mm apart from each other, on a plate of silica gel for thin-layer chromatography. Develop the plate for 18 hours with 2-butanol saturated with water, while supporting the plate at an angle of about 15° to a horizontal plane, and air-dry the plate: the spot from the sample solution (1) corresponding to that from the standard solution is not more intense than the spot from the standard solution, and the spots other than the principal spot from the sample solution (2) are not more intense than the spot from the standard solution.

Loss on drying Not more than 12% (0.05 g, in vacuum at a pressure not exceeding 0.67 kP, phosphorus (V) oxide, 100°C, 6 hours).

Assay Weigh accurately about 0.02 g of Hydroxocobalamin Acetate, and dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 2 mL of this solution into a 50-mL volumetric flask, add 1 mL of a solution of potassium cyanide (1 in 1000), and allow to stand for 30 minutes at ordinary temperature. Add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.02 g of Cyanocobalamin Reference Standard after determining the loss on drying in the same manner as for Cyanocobalamin, and dissolve in