Purity (1) Chloroform—To 0.010 g of Colchicine add 2 mL of sodium hydroxide TS and 1 drop of aniline, and heat the mixture while shaking: no odor of phenyl isocyanide (toxic) is perceptible.

(2) Colchiceine—Dissolve 0.10 g of Colchicine in 10 mL of water, and to 5 mL of this solution add 2 drops of iron (III) chloride TS: no definite green color is produced.

(3) Other alkaloids—Dissolve 0.10 g of Colchicine in 20 mL of water, and to 2.0 mL of this solution add 0.5 mL of 2,4,6-trinitrophenol TS: the solution is clear.

Loss on drying Not more than 5.0% (1 g, 105°C, 3 hours).

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

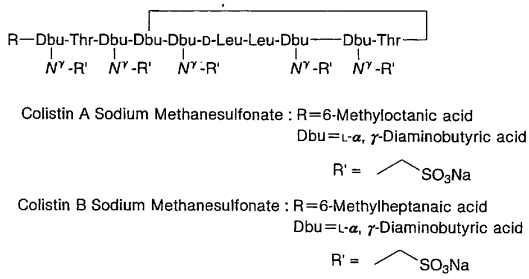
Assay Weigh accurately about 0.4 g of Colchicine, previously dried, dissolve in 25 mL of acetic anhydride, and titrate with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 19.972 mg of $C_{22}H_{25}NO_6$

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

Colistin Sodium Methanesulfonate

コリスチンメタンスルホン酸ナトリウム



[*8068-28-8*, Colistin Sodium Methanesulfonate]

Colistin Sodium Methanesulfonate, when dried, contains not less than 10,000 Units per mg. The potency of Colistin Sodium Methanesulfonate is expressed as mass of colistin A ($C_{53}H_{100}N_{16}O_{13}$: 1168.46).

Description Colistin Sodium Methanesulfonate occurs as a white to light yellowish white powder.

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

Identification (1) Dissolve 0.02 g of Colistin Sodium Methanesulfonate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, and add 5 drops of copper (II) sulfate TS while shaking: a blue-purple color develops.

(2) Dissolve 0.04 mg of Colistin Sodium Methanesulfonate in 1 mL of 1 mol/L hydrochloric acid TS, and add 0.5 mL of dilute iodine TS: the color of iodine disappears.

(3) Determine the infrared absorption spectrum of Colistin Sodium Methanesulfonate, 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 dried Colistin Sodium Methanesulfonate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Colistin Sodium Methanesulfonate responds to the Qualitative Test (1) for sodium salt.

pH Dissolve 0.1 g of Colistin Sodium Methanesulfonate in 10 mL of water, and allow to stand for 30 minutes: the pH of the solution is between 6.5 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 0.16 g of Colistin Sodium Methanesulfonate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test using Apparatus B (not more than 2 ppm).

(4) Free colistin—Dissolve 0.08 g of Colistin Sodium Methanesulfonate in 3 mL of water, add 0.05 mL of a solution of silicotungstic acid 26-water (1 in 10), and compare the solution with the reference suspension described under the Test Methods for Plastic Containers: the turbidity is not greater than that of the reference suspension (not more than 0.25%).

Loss on drying Not more than 3.0% (0.1 g, reduced pressure, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions.

(1) Test organism—*Escherichia coli* NIHJ

(2) Culture medium—To 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 20.0 g of agar add 1000 mL of water, then add a suitable amount of sodium hydroxide TS so that the pH of the medium is being 6.5 to 6.6 after sterilization, sterile, and use this as the seeded agar medium and the agar medium for base layer.

(3) Standard solution—Weigh accurately an amount of Colistin Sodium Methanesulfonate Reference Standard, previously dried, dissolve in phosphate buffer solution, pH 6.0 to make a solution containing 100,000 Units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(4) Sample solution—Weigh accurately an amount of Colistin Sodium Methanesulfonate, previously dried, dissolve in phosphate buffer solution, pH 6.0 to make a solution containing about 100,000 Units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration sample solution and the low concentra-

tion sample solution, respectively.

Containers and storage Containers—Tight containers.

Cortisone Acetate

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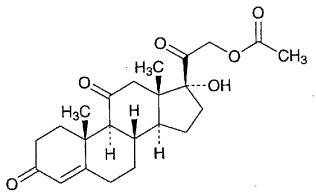

$C_{23}H_{30}O_6$: 402.48
17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate
[*50-04-4*]

Cortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of $C_{23}H_{30}O_6$.

Description Cortisone Acetate occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in chloroform, sparingly soluble in methanol, in acetone and in 1,4-dioxane, slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 240°C (with decomposition).

Identification **(1)** Add 2 mL of sulfuric acid to 2 mg of Cortisone Acetate, and allow to stand for a while: a yellowish green color is produced, and it gradually changes to yellow-orange. Examine the solution under ultraviolet light: the solution shows a light green fluorescence. Add carefully 10 mL of water to this solution: the color of the solution is discharged, and the solution remains clear.

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

(3) To 0.05 g of Cortisone 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 spectrum of Cortisone Acetate, 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 Cortisone Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Cortisone Acetate and Cortisone Acetate Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation $[\alpha]_D^{20}$: +208 – +217° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Other steroids—Dissolve 0.10 g of Cortisone Acetate in 10 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 solution 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 1,2-dichloroethane, methanol and water (470:30:1) 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 1.0% (0.5 g, 105°C, 3 hours).

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

Assay Dissolve about 0.01 g each of Cortisone Acetate and Cortisone Acetate Reference Standard, previously dried and accurately weighed, in 50 mL of methanol, add exactly 5 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 10 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 cortisone acetate to that of the internal standard.

$$\text{Amount (mg) of } C_{23}H_{30}O_6 = \text{amount (mg) of Cortisone Acetate Reference Standard} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

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

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

Flow rate: Adjust the flow rate so that the retention time of cortisone acetate is about 12 minutes.

System suitability—

System performance: Dissolve 0.01 g of cortisone acetate in 50 mL of methanol, add 5 mL of a solution of propyl parahydroxybenzoate in methanol (3 in 5000), and add methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, propylparahydroxybenzoate and cortisone acetate are eluted in this order with the resolution between these peaks being not less than 4.

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 cortisone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.