tone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 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 1,2-dichloroethane, diethyl ether and ammonia solution (28) (200:100:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): number of the spots other than the principal spot from the sample solution is not more than 2, and they are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, 105°C, 4 hours).

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

Assay Weigh accurately about 0.5 g of Flunitrazepam, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.329 mg of $C_{16}H_{12}FN_3O_3$

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

Fluocinolone Acetonide

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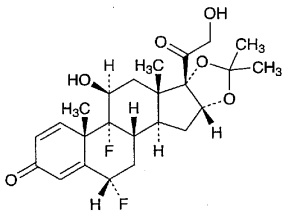

$C_{24}H_{30}F_2O_6$: 452.49
6α,9-Difluoro-11β,21-dihydroxy-16α,17-isopropylidenedioxypregna-1,4-diene-3,20-dione
[*67-73-2*]

Fluocinolone Acetonide, when dried, contains not less than 97.0% and not more than 102.0% of $C_{24}H_{30}F_2O_6$.

Description Fluocinolone Acetonide occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in acetone, soluble in ethanol (95) and ethanol (99.5), sparingly soluble in methanol and in chloroform, slightly soluble in acetonitrile, very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: 266 - 274°C (with decomposition).

Identification (1) To 2 mg of Fluocinolone Acetonide add 2 mL of sulfuric acid: a yellow color is produced.

(2) Dissolve 0.01 g of Fluocinolone Acetonide in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Proceed as directed under the Oxygen Flask Combustion Method with 0.01 g of Fluocinolone Acetonide, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. When the process is completed, shake well, and force the combustion gas into the absorbing liquid: this liquid responds to the Qualitative Tests for fluoride.

(4) Determine the infrared absorption spectrum of Fluocinolone Acetonide, 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 Fluocinolone Acetonide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Fluocinolone Acetonide and Fluocinolone Acetonide Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation $[\alpha]_D^{20}$: +98 - +108° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity Other steroids—Dissolve 0.015 g of Fluocinolone Acetonide in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 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 each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of fluocinolone acetonide from the sample solution is not larger than the peak area of fluocinolone acetonide from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water-saturated chloroform, methanol and acetic acid (100) (200:3:2).

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

Time span of measurement: About twice as long as the retention time of fluocinolone acetonide after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of fluocinolone acetonide obtained from 20 μL of this solution is equivalent to 4 to 6% of that of fluocinolone acetonide obtained from 20 μL of the standard solution.

System performance: Dissolve 0.015 g each of Fluocinolone Acetonide and triamcinolone acetonide in 25 mL of the mobile phase. To 5 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, triamcinolone acetonide and fluocinolone acetonide are eluted in

this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of fluocinolone acetonide is not more than 1.0%.

Loss on drying Not more than 1.0% (0.2 g, in vacuum, 105°C, 3 hours).

Residue on ignition Not more than 0.1% (0.2 g, platinum crucible).

Assay Dissolve about 0.02 g each of Fluocinolone Acetonide and Fluocinolone Acetonide Reference Standard, previously dried and accurately weighed, in 40 mL each of methanol, add exactly 10 mL each of the internal standard solution, then add water to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of these solutions 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 fluocinolone acetonide to that of the internal standard, respectively.

Amount (mg) of fluocinolone acetonide ($C_{24}H_{30}F_2O_6$) = amount (mg) of Fluocinolone Acetonide Reference Standard $\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of ethyl parahydroxybenzoate (1 in 2500).

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of fluocinolone acetonide is about 20 minutes.

System suitability—

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinolone acetonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fluocinonide

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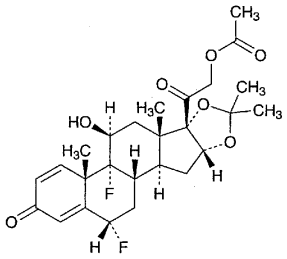

$C_{26}H_{32}F_2O_7$: 494.52

6α,9-Difluoro-11β,21-dihydroxy-16α,17-isopropylidenedioxypregna-1,4-diene-3,20-dione 21-acetate [*356-12-7*]

Fluocinonide, when dried, contains not less than 97.0% and not more than 103.0% of $C_{26}H_{32}F_2O_7$.

Description Fluocinonide occurs as white crystals or crystalline powder.

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

Identification **(1)** To 0.01 g of Fluocinonide add 4 mL of water and 1 mL of Fehling's TS, and heat: a red precipitate is formed.

(2) Prepare the test solution with 0.01 g of Fluocinonide as directed under the Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests for fluoride.

(3) Determine the absorption spectrum of a solution of Fluocinonide in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluocinonide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Fluocinonide and Fluocinonide Reference Standard, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare both spectra: both the sample and the Reference Standard exhibit similar intensities of absorption at the same wave numbers. If any difference appears in the absorption spectra, dissolve the sample and the Reference Standard in ethyl acetate, respectively, evaporate the ethyl acetate, and perform the test with the residue in the same manner.

Optical rotation $[\alpha]_D^{20}$: +81 – +89° (after drying, 0.2 g, chloroform, 20 mL, 100 mm).

Purity Other steroids—Dissolve 0.010 g of Fluocinonide in 2 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100, and use this solution as the standard solution. Perform the test with these solutions as direct-