ed under the Thin-layer Chromatography. Spot 10 μL 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 chloroform and methanol (97:3) 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, platinum crucible).

Assay Weigh accurately about 0.02 g of Fluocinonide and Fluocinonide Reference Standard, previously dried, dissolve each in 50 mL of acetonitrile, to each add exactly 8 mL of the internal standard solution and water to make 100 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 fluocinonide to that of the internal standard, respectively.

$$\text{Amount (mg) of fluocinonide } (C_{26}H_{32}F_2O_7) = \text{amount (mg) of Fluocinonide Reference Standard} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of propyl benzoate in acetonitrile (1 in 100).

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 for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of acetonitrile and water (1:1).

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

System suitability—

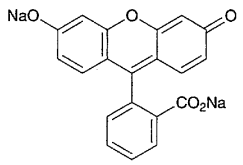
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fluocinonide and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

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

Containers and storage Containers—Well-closed containers.

Fluorescein Sodium

フルオレセインナトリウム



$C_{20}H_{10}Na_2O_5$: 376.27

Disodium 2-(6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate [*518-47-8*]

Fluorescein Sodium contains not less than 98.5% of $C_{20}H_{10}Na_2O_5$, calculated on the dried basis.

Description Fluorescein Sodium occurs as an orange powder. It is odorless, and tasteless.

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

It is hygroscopic.

Identification **(1)** To a solution of Fluorescein Sodium (1 in 100) having a strong green fluorescence, add a large quantity of water: the fluorescence remains. Acidify the solution with hydrochloric acid: the fluorescence disappears. Then render the solution alkaline with sodium hydroxide TS: the fluorescence reappears.

(2) Place 1 drop of a solution of Fluorescein Sodium (1 in 2000) on a piece of filter paper: a yellow spot develops. Expose the spot, while moist, to the vapor of bromine for 1 minute and then to ammonia vapor: the yellow color of the spot changes to red.

(3) Char 0.5 g of Fluorescein Sodium by ignition, cool, mix the residue with 20 mL of water, and filter: the filtrate responds to the Qualitative Tests for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1 g of Fluorescein Sodium in 10 mL of water: the solution is clear, and shows a red color.

(2) Chloride—Dissolve 0.15 g of Fluorescein Sodium in 20 mL of water, add 6 mL of dilute nitric acid and water to make 30 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.355%).

(3) Sulfate—Dissolve 0.20 g of Fluorescein Sodium in 30 mL of water, add 2.5 mL of dilute hydrochloric acid and water to make 40 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Zinc—Dissolve 0.10 g of Fluorescein Sodium in 10 mL of water, add 2 mL of hydrochloric acid, and filter. To the filtrate add 0.1 mL of potassium hexacyanoferrate (II) TS: no turbidity is produced immediately.

(5) Related substances—Dissolve 0.20 g of Fluorescein Sodium in 10 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under the Thin-layer Chromatography. Spot 5 μL of the

sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (30:15:1) to a distance of about 10 cm, and air-dry the plate: any colored spot other than the principal spot does not appear.

Loss on drying Not more than 10.0% (1 g, 105°C, constant mass).

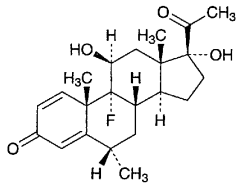
Assay Transfer about 0.5 g of Fluorescein Sodium, accurately weighed, to a separator. Dissolve in 20 mL of water, add 5 mL of dilute hydrochloric acid, and extract the solution with four 20-mL portions of a mixture of 2-methyl-1-propanol and chloroform (1:1). Wash each extract successively with the same 10 mL of water. Evaporate the combined extracts on a water bath with the aid of a current of air. Dissolve the residue in 10 mL of ethanol (99.5), evaporate the solution on a water bath to dryness, dry the residue at 105°C for 1 hour, and weigh as fluorescein ($C_{20}H_{12}O_5$: 332.31).

Amount (mg) of $C_{20}H_{10}Na_2O_5$
= amount (mg) of fluorescein ($C_{20}H_{12}O_5$) × 1.1323

Containers and storage Containers—Tight containers.

Fluorometholone

フルオロメトロン



$C_{22}H_{29}FO_4$: 376.46
9-Fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione [*426-13-1*]

Fluorometholone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{29}FO_4$.

Description Fluorometholone occurs as a white to light yellowish white, odorless, crystalline powder.

It is freely soluble in pyridine, slightly soluble in methanol, in ethanol (99.5) and in tetrahydrofuran, and practically insoluble in water and in diethyl ether.

Identification (1) Proceed with 7 mg of Fluorometholone 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 liquid responds to the Qualitative Tests (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fluorometholone 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 Fluorometholone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

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

Optical rotation $[\alpha]_D^{20}$: +52 – +60° (after drying, 0.1 g, pyridine, 10 mL, 100 mm).

Purity (1) Heavy metals—Proceed with 1.0 g of Fluorometholone according to Method 3, 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 0.020 g of Fluorometholone in 10 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add tetrahydrofuran 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 25 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 dichloromethane, acetone and methanol (45:5:1) to a distance of about 12 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% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

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

Assay Weigh accurately about 0.10 g each of Fluorometholone and Fluorometholone Reference Standard, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, and add diluted methanol (7 in 10) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and diluted methanol (7 in 10) to make 100 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 determine the ratios, Q_T and Q_S, of the peak area of fluorometholone to that of the internal standard.

Amount (mg) of fluorometholone ($C_{22}H_{29}FO_4$)
= amount (mg) of Fluorometholone Reference Standard
$\times \frac{Q_T}{Q_S}$

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).