tion corresponds in R_F value to that of *Standard solution 1;* and the *Test solution* shows not more than 3 additional spots, the intensity and size of which do not exceed those of the spot from *Standard solution 2.*

Assay—

Mobile phase—Prepare a suitable filtered solution of acetonitrile and water (60:40).

Internal standard solution—Prepare a solution of betamethasone dipropionate in methanol containing 0.6 mg per mL.

Standard preparation—Using an accurately weighed quantity of USP Betamethasone Benzoate RS , prepare a solution in methanol having a known concentration of about 0.6 mg per mL. Mix 5.0 mL of this solution and 10.0 mL of the *Internal standard solution* to obtain a *Standard preparation* having a known concentration of about 0.2 mg of betamethasone benzoate per mL.

Assay preparation—Transfer about 60 mg of Betamethasone Benzoate, accurately weighed, to a 100-mL volumetric flask. Dilute with methanol to volume, and mix. Mix 5.0 mL of this solution and 10.0 mL of the *Internal standard solution*.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains 5- µm packing L1. The flow rate is about 1 mL per minute. Chromatograph three replicate injections of the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%; and the resolution factor between betamethasone benzoate and the internal standard is not less than 3.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatograms, and measure the responses for the major peaks. The retention times are about 7 and 5 minutes for betamethasone dipropionate and betamethasone benzoate, respectively. Calculate the quantity, in mg, of $C_{29}H_{33}FO_6$ in the portion of Betamethasone Benzoate taken by the formula:

$$300C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone Benzoate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios of the betamethasone benzoate peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Benzoate Gel

» Betamethasone Benzoate Gel contains an amount of betamethasone benzoate ($C_{29}H_{33}FO_6$) equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of betamethasone benzoate ($C_{29}H_{33}FO_6$).

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15 ° and 30 °. Protect from freezing.

USP Reference standards ⟨11⟩**—**
USP Betamethasone Benzoate RS
USP Methyltestosterone RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* both relative to the internal standard, as obtained in the *Assay.*

Microbial enumeration tests ⟨61⟩ **and Tests for specified microorganisms** ⟨62⟩**—**It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Minimum fill ⟨755⟩**:** meets the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol, water, and acetonitrile (23:18:9). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Dissolve suitable quantities of USP Methyltestosterone RS in methanol to obtain a solution containing about 250 µg per mL.

Standard preparation—Dissolve an accurately weighed quantity of USP Betamethasone Benzoate RS in methanol, and dilute quantitatively, and stepwise if necessar y, with methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution,* dilute with methanol to volume, and mix.

Assay preparation—Transfer an accurately weighed portion of Gel, equivalent to about 0.5 mg of betamethasone benzoate, to a 125-mL separatory funnel, add 20 mL of water and 2 mL of saturated sodium acetate solution, shake to disperse the Gel, add 2.0 mL of *Internal standard solution,* and mix. Extract this solution with one 50-mL portion of chloroform followed by three 40-mL portions of chloroform. Discard the aqueous layer. Wash the chloroform extract with 10 mL of water, allow to stand for 10 minutes, then pass through chloroform-wetted glass fiber filter paper and anhydrous sodium sulfate into a suitable container. Evaporate to dr yness under vacuum at 30 °. Dissolve the residue in 10 mL of methanol.

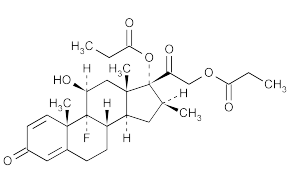
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 236-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. The column temperature is maintained at 30 °. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.33 for betamethasone benzoate and 1.0 for methyltestosterone; the resolution, *R,* between methyltestosterone and betamethasone benzoate is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betamethasone benzoate ($C_{29}H_{33}FO_6$) in the portion of Gel taken by the formula:

$$0.01C(R_U / R_S)$$

in which C is the concentration, in µg per mL, of USP Betamethasone Benzoate RS in the *Standard preparation;* and R_U and R_S are the ratios of the peak responses obtained for betamethasone benzoate and methyltestosterone from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Dipropionate



$C_{28}H_{37}FO_7$ 504.60

Pregna-1,4-diene-3,20-dione, 9-fluoro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11β,16β).

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5593-20-4].

» Betamethasone Dipropionate contains not less than 97.0 percent and not more than 103.0 percent of $C_{28}H_{37}FO_7$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩**—**
USP Beclomethasone Dipropionate RS
USP Betamethasone Dipropionate RS
USP Betamethasone Valerate RS

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution: 1 mg per mL, in chloroform.

Developing solvent system: a mixture of chloroform and acetone (7:1).

Specific rotation ⟨781S⟩**:** between +63° and +70°.

Test solution: 10 mg per mL, in dioxane.

Loss on drying ⟨731⟩**:** Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.2%, a platinum crucible being used.

Chromatographic purity—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Dissolve accurately weighed quantities of USP Betamethasone Dipropionate RS and USP Betamethasone Valerate RS in *Mobile phase* to obtain a solution having final concentrations of about 0.05 mg of each per mL.

Test solution—Transfer about 3 mg of Betamethasone Dipropionate, accurately weighed, to a suitable flask. Add 10 mL of *Mobile phase,* and shake until dissolved.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between betamethasone valerate and betamethasone dipropionate is not less than 4.0; and the column efficiency is not less than 8000 theoretical plates.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all the peak responses. Calculate the percentage of each impurity in the portion of Betamethasone Dipropionate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the peak response for each impurity; and r_s is the sum of the responses for all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

Assay—

Mobile phase—Prepare a suitable acetonitrile solution (about 1 in 2), degassed by ultrasonic vibration for 5 to 10 minutes, such that the retention time of betamethasone dipropionate is approximately 14 minutes and that of beclomethasone dipropionate is approximately 18 minutes. [NOTE—Do not leave the mobile phase in the column overnight, but flush the system after use with water for 15 minutes, followed by methanol for 15 minutes.]

Internal standard solution—Prepare a solution of USP Beclomethasone Dipropionate RS in a solution of acetic acid in methanol (1 in 1000) having a known concentration of about 0.9 mg per mL.

Standard preparation—Prepare a solution of USP Betamethasone Dipropionate RS in a solution of acetic acid in methanol (1 in 1000) having a known concentration of about 0.6 mg per mL. Transfer 5.0 mL of this solution to a suitable vial, and add 5.0 mL of *Internal standard solution* to obtain a solution having known concentrations of about 0.3 mg of betamethasone dipropionate and about 0.45 mg of beclomethasone dipropionate per mL.

Assay preparation—Accurately weigh about 60 mg of Betamethasone Dipropionate. Dilute quantitatively and stepwise with a solution of acetic acid in methanol (1 in 1000) to obtain a solution containing about 0.6 mg per mL. Transfer 5.0 mL of this solution to a suitable vial, and add 5.0 mL of *Internal standard solution.*

Procedure—Separately inject equal volumes (between 5 µL and 25 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* ⟨621⟩) operated at room temperature, by means of a suitable microsyringe or sampling valve, adjusting the specimen size and other operating parameters such that the peak obtained from the internal standard in the *Standard preparation* is about 0.6 full-scale. Typically, the apparatus is fitted with a 4-mm × 30-cm column that contains packing L1, and is equipped with a UV detector capable of monitoring absorption at 254 nm or 240 nm and a suitable recorder, and is capable of operating at a column pressure of up to 3500 psi. In a suitable chromatogram, the lowest and highest peak area ratios (R_S) of three successive injections of the *Standard preparation* agree within 2.0%. Determine the ratio of the peak heights, at equivalent retention times, obtained with the *Assay preparation* and the *Standard preparation,* and calculate the quantity, in mg, of $C_{28}H_{37}FO_7$ in the portion of Betamethasone Dipropionate taken by the formula:

$$200C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone Dipropionate RS in the *Standard preparation;* and R_U and R_S are the peak height ratios of betamethasone dipropionate to the internal standard obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Dipropionate Topical Aerosol

» Betamethasone Dipropionate Topical Aerosol is a solution, in suitable propellants in a pressurized container, of betamethasone dipropionate ($C_{28}H_{37}FO_7$) equivalent to not less than 90.0 percent and not more than 110.0 per cent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in tight, pressurized containers, and avoid exposure to excessive heat. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩**—**
USP Beclomethasone Dipropionate RS
USP Betamethasone Dipropionate RS

Thin-layer chromatographic identification test ⟨201⟩**—**

Test solution—Place the container in a dry ice–methanol bath for about 5 minutes. Open the can by means of a tube-cutter, and allow the propellant to evaporate under a gentle stream of nitrogen for about 1 hour. Transfer about 3 mL of the residue to a 50-mL centrifuge tube. Add 10 mL of a mixture of methanol and water (4:1), and shake vigorously. Centrifuge to clarify.

Standard solution: USP Betamethasone Dipropionate RS in methanol containing 3.2 mg per mL.

Application volume: 25 µL.

Developing solvent system: a mixture of toluene and ethyl acetate (1:1).