terone, and 1.0 for betamethasone acetate. Calculate the quantity, in mg, of betamethasone acetate ($C_{24}H_{31}FO_6$) in each mL of the Injectable Suspension taken by the formula:

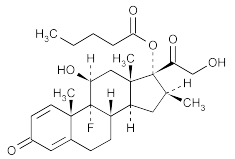
$$0.1C / V(R_U / R_S)$$

in which C is the concentration, in µg per mL, of USP Betamethasone Acetate RS in the *Standard preparation; V* is the volume, in mL, of Injectable Suspension taken; and R_U and R_S are the peak response ratios obtained for betamethasone acetate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of betamethasone ($C_{22}H_{29}FO_5$) equivalent to the quantity of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$), in each mL of the Injectable Suspension taken by the formula:

$$(392.46/516.41)(0.1C/V)(R_U / R_S)$$

in which 392.46 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively; C is the concentration, in µg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation; V* is the volume, in mL, of Injectable Suspension taken; and R_U and R_S are the peak response ratios obtained for betamethasone phosphate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively.

Betamethasone Valerate



$C_{27}H_{37}FO_6$ 476.59

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-17-[(1-oxopentyl)oxy]-, (11β,16β)-.

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-valerate [2152-44-5].

» Betamethasone Valerate contains not less than 97.0 percent and not more than 103.0 per cent of $C_{27}H_{37}FO_6$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Beclomethasone 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 alcohol.

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

Procedure—Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105 ° for 15 minutes.

Specific rotation ⟨781S⟩: between +75° and +82°.

Test solution: 10 mg per mL, in dioxane.

Loss on drying ⟨731⟩**—**Dry it at 105 ° for 3 hours: it loses not more than 0.5% 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, water, and glacial acetic acid (550:450:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Test solution—Transfer about 4 mg of Betamethasone V alerate, 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 *Test solution*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between betamethasone valerate and any impurity is not less than 1.5; and the column efficiency is not less than 9000 theoretical plates.

Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the per centage of each impurity in the portion of Betamethasone V alerate 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 all the peak responses: 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 filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 40 mg of beclomethasone dipropionate to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix.

Standard preparation—Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone V alerate RS per mL.

Assay preparation—Transfer about 60 mg of Betamethasone Valerate, accurately weighed, to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution, *R,* between betamethasone valerate and beclomethasone dipropionate is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.

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 $C_{27}H_{37}FO_6$ in the portion of Betamethasone V alerate taken by the formula:

$$300C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* and R_U

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and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Valerate Cream

» Betamethasone Valerate Cream contains an amount of betamethasone valerate ($C_{27}H_{37}FO_6$) equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$), in a suitable cream base.

Packaging and storage—Preserve in collapsible tubes or in tight containers.

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

Identification—Transfer an amount of Cream, equivalent to about 2 mg of betamethasone, to a separator, add 20 mL of water and 2 mL of dilute hydrochloric acid (1 in 120), and mix. Extract with four 50-mL portions of chloroform, and combine the extracts. Filter through a cotton pledget, previously layered over with anhydrous sodium sulfate. Evaporate the filtrates on a steam bath under a stream of dry nitrogen to dryness. Dissolve the residue in alcohol to obtain a solution containing about 1 mg per mL. Proceed as directed in *Identification* test *B* under *Betamethasone Valerate*, beginning with "Apply 10 µL of this solution."

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, *Internal standard solution*, *Standard preparation,* and *Chromatographic system*—Proceed as directed in the *Assay* under *Betamethasone Valerate*.

Assay preparation—Transfer an accurately weighed portion of Cream, equivalent to about 2.5 mg of betamethasone, to a 50-mL centrifuge tube. Add 10.0 mL of the *Internal standard solution* and 5.0 mL of a 1 in 1000 solution of glacial acetic acid in methanol. Insert the stopper into the tube, and place in a water bath held at 60 ° until the specimen melts. Remove from the bath, and shake vigorously until the specimen resolidifies. Repeat the heating and shaking two more times. Place the tube in an ice-methanol bath for 20 minutes, then centrifuge to separate the phases. Decant the clear supernatant into a suitable stoppered flask, and allow to warm to room temperature.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Valerate*. Calculate the quantity, in mg, of $C_{22}H_{29}FO_5$ in the portion of Cream taken by the formula:

$$(392.46 / 476.59)(15C)(R_U / R_S)$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Betamethasone Valerate Lotion

» Betamethasone Valerate Lotion contains an amount of Betamethasone Valerate ($C_{27}H_{37}FO_6$) equivalent to not less than 95.0 per cent and not more than 115.0 per cent of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$).

Packaging and storage—Preserve in tight, light-resistant containers, and store at controlled room temperature.

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

Identification—Mix an amount of Lotion, equivalent to about 5 mg of betamethasone, with a mixture of methanol and chloroform (2:1) to make 10 mL. Apply 20 µL of this solution and 20 µL of a Standard solution of USP Betamethasone Valerate RS in a mixture of methanol and chloroform (2:1) containing 0.6 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and ethyl acetate (1:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. View the spots under UV light: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

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.

pH ⟨791⟩: between 4.0 and 6.0.

Assay—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Betamethasone Valerate*.

Internal standard solution—Transfer about 50 mg of beclomethasone dipropionate to a 25-mL volumetric flask, add chloroform to volume, and mix.

Standard preparation—Transfer about 40 mg of USP Betamethasone Valerate RS, accurately weighed, to a 25-mL volumetric flask, add chloroform to volume, and mix. Pipet 2 mL of this solution into a 50-mL centrifuge tube, add 10 mL of 0.1 N hydrochloric acid, then add 2.0 mL of *Internal standard solution*. Insert the stopper into the tube, shake vigorously for about 2 minutes, and centrifuge to separate the phases. Using a syringe, transfer the lower, chloroform phase to a small stoppered vial. Evaporate the chloroform on a steam bath, at low heat, with the aid of a stream of nitrogen. Add 4.0 mL of a 1 in 1000 solution of glacial acetic acid in methanol, and swirl to dissolve the residue.

Assay preparation—Transfer an accurately weighed portion of Lotion, equivalent to about 2.5 mg of betamethasone, to a stoppered, 50-mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake to disperse the specimen. Add 2.0 mL of chloroform and 2.0 mL of *Internal standard solution*, insert the stopper, and proceed as directed for *Standard preparation*, beginning with "shake vigorously for about 2 minutes."

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Valerate*. Calculate the quantity, in mg, of betamethasone ($C_{22}H_{29}FO_5$) in the portion of Lotion taken by the formula:

$$(392.46 / 476.59)(4C)(R_U / R_S)$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation;* and R_U and R_S are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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