Minimum fill ⟨755⟩: meets the requirements.

Assay for clioquinol—

Internal standard solution, *Standard solution*, *Standard preparation,* and *Chromatographic system*—Prepare as directed in the *Assay for clioquinol* under *Clioquinol and Hydrocortisone Cream*.

Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 150 mg of clioquinol, to a 125-mL separator. Add 75 mL of *n*-hexane, insert the stopper in the separator, and mix until the specimen is completely dispersed. Extract with 25 mL of dimethylformamide, collecting the extract in a 50-mL volumetric flask. Repeat the extraction with two 10-mL portions of dimethylformamide, collecting the extracts in the 50-mL volumetric flask, dilute with dimethylformamide to volume, and mix. T ransfer 1.0 mL of this solution to a suitable size screw-capped vial, and evaporate the solution with the aid of nitrogen at about 60 ° to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), and pipet 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution* into the glass vial, fitted with a polytef-lined septum, securely close, and mix. Heat the vial on a water bath at 50 ° for 15 minutes, and cool to room temperature.

Procedure—Proceed as directed for *Procedure* in the *Assay for clioquinol* under *Clioquinol and Hydrocortisone Cream*. Calculate the quantity, in mg, of C_9H_5ClINO in the portion of Ointment taken by the formula:

$$150C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Clioquinol 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.

Assay for hydrocortisone—

Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Prepare as directed in the *Assay for hydrocortisone* under *Clioquinol and Hydrocortisone Cream*.

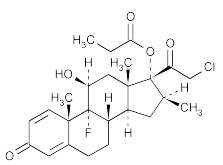
Assay preparation—Transfer an accurately weighed quantity of Ointment, equivalent to about 10 mg of hydrocortisone, to a 50-mL centrifuge tube. Add 30 mL of alcohol and heat on a steam bath just to boiling. Shake for 15 minutes, and centrifuge. Quantitatively transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.

Procedure—Proceed as directed for *Procedure* in the *Assay for hydrocortisone* under *Clioquinol and Hydrocortisone Cream*. Calculate the quantity, in mg, of $C_{21}H_{30}O_5$ in the portion of Ointment taken by the formula:

$$0.1C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Hydrocortisone RS in the *Standard preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Clobetasol Propionate



$C_{25}H_{32}ClFO_5$ 466.97

Pregna-1,4-diene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (11*β*,16*β*)-; 21-Chloro-9-fluoro-11*β*,17-dihydroxy-16*β*-methylpregna-1,4-diene-3,20-dione 17-propionate [25122-46-7; 25122-41-2].

DEFINITION

Clobetasol Propionate contains NLT 97.0% and NMT 102.0% of $C_{25}H_{32}ClFO_5$, calculated on the dried basis.

IDENTIFICATION

• **Infrared Absorption** ⟨197M⟩

ASSAY

• **Procedure**

Solution A: 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile, methanol, and *Solution A* (19:4:17)

Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol

Standard solution: Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate.

System suitability solution: 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

Sample solution: Transfer 4 mg of Clobetasol Propionate to a 100-mL volumetric flask, add 40.0 mL of *Internal standard solution*, and dilute with methanol to volume.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

Column efficiency: NLT 5000 theoretical plates for the clobetasol peak

Tailing factor: NMT 2.0 for the clobetasol peak

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of $C_{25}H_{32}ClFO_5$ in the portion of Clobetasol Propionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

R_S = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities

• **Residue on Ignition** ⟨281⟩**:** NMT 0.1%, using a platinum crucible

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm

Organic Impurities

• **PROCEDURE**

Solution A, Mobile phase, System suitability solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sample solution: 0.1 mg/mL of Clobetasol Propionate in *Mobile phase*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Clobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area for each impurity

r_T = sum of the areas of all of the peaks

Acceptance criteria

Any individual impurity: NMT 1.0%

Total impurities: NMT 2.5%

SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** ⟨**741**⟩**:** Approximately 196°

• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +98° to +104° at 20°

Sample solution: 10 mg/mL in dioxane

• **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 3 h: it loses NMT 2.0% of its weight.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Clobetasol Propionate RS

USP Clobetasol Propionate Related Compound A RS

9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(*R*)-spiro-2′-[4′-chloro-5′-ethylfuran-3′(2′*H*)-one].

$C_{25}H_{30}ClFO_4$ 448.96

Clobetasol Propionate Cream

DEFINITION

Clobetasol Propionate Cream is Clobetasol Propionate in a suitable cream base. It contains NL T 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ($C_{25}H_{32}ClFO_5$).

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩

Standard solution: 0.6 mg/mL of USP Clobetasol Propionate RS in chloroform

Test solution: Transfer a portion of Cream equivalent to 0.75 mg of clobetasol propionate to a 25-mL, plastic-stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 60° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Cool to room temperature, add 3.5 mL of water, and mix. Centrifuge at 3500 rpm for 10 min. T ransfer 5 mL of the supernatant to a 100-mL separator, add 1 g of sodium chloride and 10 mL of water, and mix. Extract with 5 mL of chloroform by shaking for 1 min, collect the lower layer, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of chloroform.

Developing solvent system: Chloroform, acetone, and alcohol (100:10:5)

Acceptance criteria: The R_F value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

ASSAY

• **PROCEDURE**

Buffer: 0.05 M monobasic sodium phosphate. Adjust with 50% sodium hydroxide solution to a pH of 5.5.

Mobile phase: Acetonitrile, methanol, and *Buffer* (95:20:85)

Internal standard solution: 0.2 mg/mL of beclomethasone dipropionate in methanol

System suitability solution: 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

Standard solution: 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.

Sample solution: Nominally 0.04 mg/mL of clobetasol propionate. In a suitable flask, dissolve a portion of Cream equivalent to 1.0 mg of clobetasol propionate in 10.0 mL of the *Internal standard solution* and 15.0 mL of methanol, and shake vigorously to disperse the Cream. Centrifuge at about 3500 rpm for 10 min, and pass a portion of the supernatant through a filter of 0.45- µm pore size.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

Column efficiency: NLT 5000 theoretical plates for the clobetasol propionate peak

Tailing factor: NMT 2.0 for the clobetasol propionate peak

Relative standard deviation: NMT 2.0% for the clobetasol propionate peak

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of clobetasol propionate ($C_{25}H_{32}ClFO_5$) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

R_S = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

C_S = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS

• **MINIMUM FILL** ⟨**755**⟩**:** Meets the requirements

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.