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, and dilute with dimethylformamide to volume. Transfer 1.0 mL of this solution to a suitable size screw-capped vial, and evaporate the solution with the aid of nitrogen at 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, and securely close. Heat the vial on a water bath at 50° for 15 min, and cool to room temperature.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 2-mm × 1.8-m; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB
Temperatures
Column: 165°
Injection port: 170°
Detector: 250°
Carrier gas: Dry helium
Flow rate: 30 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the analyte and internal standard peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms to obtain NLT 40% of maximum recorder response, and measure the peak response of each component.
Calculate the percentage of the labeled amount of clioquinol (C_9H_5ClINO) taken:

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

R_U = peak response ratio of clioquinol to the internal standard from the *Sample solution*
R_S = peak response ratio of clioquinol to the internal standard from the *Standard solution*
C_S = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of clioquinol in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

- **HYDROCORTISONE**

Mobile phase: Acetonitrile, methanol, and water (1: 1: 2.75)
Standard stock solution: 1 mg/mL of USP Hydrocortisone RS in alcohol
Standard solution: *Standard stock solution* and alcohol (1:9)
Sample solution: Transfer nominally 10 mg of hydrocortisone from Ointment to a 50-mL centrifuge tube. Add 30 mL of alcohol, and heat on a steam bath just to boiling. Shake for 15 min, and centrifuge. 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.
System suitability stock solution: 0.5 mg/mL of methylparaben in alcohol
System suitability solution: Transfer 2 mL of *System suitability stock solution* and 20 mL of *Standard stock solution* into a 200-mL volumetric flask, and dilute with alcohol to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Columns
Guard: Packing L2
Analytical: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for methylparaben and hydrocortisone are 0.6 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 2.0 between the hydrocortisone and methylparaben peaks
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of hydrocortisone ($C_{21}H_{30}O_5$) taken:

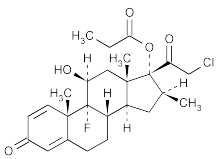
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
- **MINIMUM FILL ⟨755⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Clioquinol RS
USP Hydrocortisone RS

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)-, (11β,16β)-;
21-Chloro-9-fluoro-11β,17-dihydroxy-16β-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

Delete the following:

• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm • (Official 1-Jan-2018)

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 NLT 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. Transfer 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)