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Add the following:

▲Calcipotriene and Betamethasone Dipropionate Ointment

DEFINITION

Calcipotriene and Betamethasone Dipropionate Ointment contains NLT 90.0% and NMT 114.0% of the labeled amount of calcipotriene ($C_{27}H_{40}O_3$) and NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone dipropionate ($C_{28}H_{37}FO_7$) in a suitable ointment base.

IDENTIFICATION

• **A.** The retention time of the betamethasone dipropionate peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Betamethasone Dipropionate*.

• **B.** The retention time of the calcipotriene peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Calcipotriene*.

• **C.** The UV spectrum of the betamethasone dipropionate peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Betamethasone Dipropionate*.

• **D.** The UV spectrum of the calcipotriene peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Calcipotriene*.

ASSAY

• **Betamethasone Dipropionate**

[Note—Protect solutions containing betamethasone dipropionate and beclomethasone dipropionate from light.]

Mobile phase: Acetonitrile and water (50:55)

Diluent: Acetonitrile and water (60:25)

Internal standard solution: 0.4 mg/mL of USP Beclomethasone Dipropionate RS in *Diluent*

Standard stock solution: 0.87 mg/mL of USP Betamethasone Dipropionate RS in *Diluent*

Standard solution: 0.012 mg/mL of USP Betamethasone Dipropionate RS from the *Standard stock solution* prepared as follows. Dilute 1.0 mL of the *Standard stock solution* with 2.0 mL of *Internal standard solution* followed by 22.0 mL of *Diluent* and 50.0 mL of *Mobile phase*.

Sample solution: Nominally 0.012 mg/mL of betamethasone dipropionate from Ointment prepared as follows. Suspend 0.675 g of Ointment in 25 mL of *n*-heptane by heating on a steam bath until the Ointment melts. Add 1.0 mL of *Internal standard solution* and 11.5 mL of *Diluent* and shake for 15 min. Add 25 mL of *Mobile phase* and clear by centrifugation, if necessary, using glass centrifuge tubes. Use the clear lower layer.

Chromatographic system

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

Mode: LC

Detector: UV 240 nm. For *Identification C*, use a diode array detector in the range of 200–400 nm.

Column: 4-mm × 7.5-cm; 4-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of betamethasone dipropionate

System suitability

Samples: *Standard solution* and *Sample solution*

[Note—The relative retention times for calcipotriene, betamethasone dipropionate, and beclomethasone dipropionate are about 0.78, 1.0, and 1.3, respectively.]

Suitability requirements

Resolution: NLT 1.0 between calcipotriene and betamethasone dipropionate; NLT 2.5 between betamethasone dipropionate and beclomethasone dipropionate, *Sample solution*

Tailing factor: 0.8–1.4 for betamethasone dipropionate, *Sample solution*

Relative standard deviation: NMT 2.0% for the peak height ratio of betamethasone dipropionate to beclomethasone dipropionate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone dipropionate ($C_{28}H_{37}FO_7$) in the portion of Ointment taken:

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

R_U = peak height ratio of betamethasone dipropionate to beclomethasone dipropionate from the *Sample solution*

R_S = peak height ratio of betamethasone dipropionate to beclomethasone dipropionate from the *Standard solution*

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

C_U = nominal concentration of betamethasone dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

- **Calcipotriene**

[Note—Protect solutions containing calcipotriene from light.]

Solution A: 1.32 g/L of dibasic ammonium phosphate in water

Buffer: *Solution A*, adjusted with 1 M phosphoric acid TS to a pH of 6.0

Mobile phase: Acetonitrile, methanol, and *Buffer* (20:50:30)

Diluent: Methanol and *Solution A* (70:30)

Internal standard solution: 0.1 mg/mL of USP Methyltestosterone RS in *Diluent*

System suitability solution: 0.0025 mg/mL each of USP Calcipotriene RS and USP Calcipotriene Related Compound C RS in acetonitrile, methanol, and *Solution A* (20:50:30)

Standard stock solution: 0.05 mg/mL of USP Calcipotriene RS in *Diluent*

Standard solution: 0.0025 mg/mL of USP Calcipotriene RS from the *Standard stock solution* prepared as follows. Dilute 1.0 mL of the *Standard stock solution* with 1.0 mL of *Internal standard solution* followed by 18.0 mL of *Diluent*. Transfer a portion to a glass test tube with a screw cap and heat at 50° for 15–18 h. Cool to room temperature in a water bath. Prepare this solution simultaneously with the *Sample solution*.

Sample solution: Nominally 0.0025 mg/mL of calcipotriene from Ointment prepared as follows. Suspend 1.0 g of Ointment in 10 mL of *n*-heptane by heating on a steam bath until the Ointment melts. Add 1.0 mL of *Internal standard solution* and 19.0 mL of *Diluent* and shake for 30 min. If necessary, clear the lower layer by centrifugation using glass centrifuge tubes. Transfer a portion of the lower layer to a glass test tube with a screw cap and heat at 50° for 15–18 h. Cool to room temperature in a water bath. Prepare this solution simultaneously with the *Standard solution*.

Chromatographic system

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

Mode: LC

Detector: UV 264 nm. For *Identification D*, use a diode array detector in the range of 200–400 nm.

Column: 4-mm × 12.5-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

Run time: NLT 1.2 times the retention time of calcipotriene

System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

[Note—The relative retention times for calcipotriene related compound C and calcipotriene are about 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between calcipotriene and calcipotriene related compound C, *System suitability solution*

Tailing factor: 0.8–1.4 for calcipotriene, *Sample solution*

Relative standard deviation: NMT 2.0% for the peak height ratio of calcipotriene to methyltestosterone, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—The relative retention times for betamethasone 17-propionate, betamethasone 21-propionate, methyl testosterone, betamethasone dipropionate, and calcipotriene (plus pre-calcipotriene) are about 0.28, 0.28, 0.38, 0.52, and 1.0, respectively. The controlled isomerization of calcipotriene is carried out at 50° producing a mixture of calcipotriene and the isomer (pre-calcipotriene), which is eluted as a single peak.]

Calculate the percentage of the labeled amount of calcipotriene ($C_{27}H_{40}O_3$) in the portion of Ointment taken:

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

R_U = peak height ratio of calcipotriene (plus pre-calcipotriene) to methyltestosterone from the *Sample solution*

R_S = peak height ratio of calcipotriene (plus pre-calcipotriene) to methyltestosterone from the *Standard solution*

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

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

Acceptance criteria: 90.0%–114.0%

IMPURITIES

• **Organic Impurities: Betamethasone Dipropionate**

[Note—Protect solutions containing betamethasone dipropionate from light.]

Buffer: 8.9 g/L of sodium phosphate dibasic dihydrate in water. Adjust with 1 M phosphoric acid TS to a pH of 7.0 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (50:55)

Sample solution: Suspend 1.0 g of Ointment in 10 mL of *n*-heptane by heating on a steam bath until the Ointment melts. Add 8.0 mL of *Mobile phase* and shake vigorously for 15 min. Clear the lower layer by centrifugation in glass centrifugation tubes and use the clear lower layer.

Chromatographic system

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

Mode: LC

Detector: UV 240 nm. [Note—It is recommended to use a diode array detector in the range of 200–320 nm. It makes it easier to identify the betamethasone dipropionate related impurities in the presence of calcipotriene, calcipotriene related impurities, and any excipients present.]

Column: 4-mm × 12.5-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

Run time: NLT 3.5 times the retention time of betamethasone dipropionate

System suitability

Sample: *Sample solution*

[Note—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.0 between betamethasone dipropionate and calcipotriene

Relative standard deviation: NMT 5.0% for betamethasone dipropionate

Analysis

Sample: *Sample solution*

Calculate the percentage of each betamethasone dipropionate related degradation product in the portion of Ointment taken:

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

r_U = peak response of each betamethasone dipropionate related degradation product

r_T = sum of the peak responses of betamethasone dipropionate and all betamethasone dipropionate related degradation products

Acceptance criteria: See *Table 1*. The reporting threshold is 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone 17-propionate[a]	0.29	0.8
Betamethasone 21-propionate[b]	0.38	0.8
Calcipotriene related compound C and pre-calcipotriene[c,d]	0.67	—
Calcipotriene[d]	0.75	—
Betamethasone dipropionate	1.00	—
Any unspecified betamethasone dipropionate related degradation product	—	0.5
Total betamethasone dipropionate related degradation products	—	2.5

[a] 9-Fluoro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17-yl propionate.

[b] 9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-21-yl propionate.

[c] (5*E*,6*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5(10),6,22-triene-1α,3β,24-triol.

[d] Included for identification purposes only.

• **Organic Impurities: Calcipotriene**

[Note—Protect solutions containing calcipotriene from light.]

Solution A: 1.32 g/L of dibasic ammonium phosphate in water

Buffer: *Solution A*, adjusted with 1 M phosphoric acid TS to a pH of 6.0 ± 0.1

Mobile phase: Acetonitrile, methanol, and *Buffer* (20:50:30)

Diluent: Acetonitrile, methanol, and *Solution A* (20:50:30)

System suitability stock solution: 0.05 mg/mL each of USP Calcipotriene RS and USP Calcipotriene Related Compound C RS in *Diluent*

System suitability solution: 1 µg/mL each of USP Calcipotriene RS and USP Calcipotriene Related Compound C RS from the *System suitability stock solution* prepared as follows. To 10.0 mL of the *System suitability stock solution* add 340 mL of *Diluent* and 150 mL of water.

Sample solution: Suspend 2.0 g of Ointment in 8 mL of *n*-heptane by heating on a steam bath until the Ointment melts. Add 8.0 mL of *Diluent* and shake vigorously for 15 min. Mix 3.5 mL of the lower phase with 1.5 mL of water, and clear by centrifugation using glass centrifuge tubes, if necessary. Filter through a suitable glass paper filter.

Chromatographic system

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

Mode: LC

Detector: UV 264 nm. [Note—It is recommended to use a diode array detector in the range of 200–320 nm. It makes it easier to identify the calcipotriene related impurities in the presence of betamethasone dipropionate, betamethasone dipropionate related impurities, and any excipients present.]

Column: 4-mm × 7.5-cm and 4-mm × 12.5-cm in series; 4-µm packing L1

Flow rate: 1 mL/min

Injection volume: 500 µL

Run time: NLT 2 times the retention time of calcipotriene

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.8 between calcipotriene related compound C and calcipotriene

Relative standard deviation: NMT 5.0% each for calcipotriene related compound C and calcipotriene

Analysis

Sample: *Sample solution*

Calculate the percentage of each calcipotriene related degradation product in the portion of Ointment taken:

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

r_U = peak response of each calcipotriene related degradation product

r_T = sum of the peak responses of calcipotriene and all calcipotriene related degradation products

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone dipropionate related impurity[a]	0.72	—
Betamethasone dipropionate related impurity[a]	0.76	—
Calcipotriene related compound C	0.92	1.5
Calcipotriene	1.0	—
Betamethasone dipropionate related impurity[a]	1.1	—
24-*epi*-calcipotriol[b]	1.24	1.6

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any unspecified calcipotriene related degradation product	—	0.7
Total calcipotriene related degradation products	—	3.0

[a] Included for identification purposes only.

[b] (5*Z*,7*E*,22*E*,24*R*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

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 is NMT 10^2 cfu/g. The total combined molds and yeasts count is NMT 10^1 cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP Reference Standards ⟨11⟩**

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

USP Calcipotriene RS

USP Calcipotriene Related Compound C RS

(5*E*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

$C_{27}H_{40}O_3$ 412.61

USP Methyltestosterone RS▲ (USP 1-Aug-2024)

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CALCIPOTRIENE AND BETAMETHASONE DIPROPIONATE OINTMENT	Documentary Standards Support	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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