Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates based on Olopatadine peak
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{23}NO_3 \cdot HCl$ in the portion of Olopatadine Hydrochloride taken:

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

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of USP Olopatadine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of Olopatadine Hydrochloride in the *Sample solution* (mg/mL)
Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

Inorganic Impurities
- **Residue on Ignition** ⟨**281**⟩**:** NMT 0.1%
- **Heavy Metals,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

Organic Impurities
- **Procedure**

[Note—Protect solutions from light.]
Mobile phase: Proceed as directed in the *Assay*.
Blank solution: *Mobile phase*
System suitability solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*
Sample solution: 0.2 mg/mL of Olopatadine Hydrochloride in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
Run time: At least 2.5 times the retention time of the major peak
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for olopatadine and olopatadine related compound B are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 2.0 between olopatadine and olopatadine related compound B
Column efficiency: NLT 2000 theoretical plates, olopatadine peak
Tailing factor: NMT 2.0, olopatadine peak
Relative standard deviation: NMT 2.0%, olopatadine peak
Analysis
Sample: *Sample solution*
Calculate the percentage of each impurity in the portion of Olopatadine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
F = relative response factor for each individual impurity (see *Impurity Table 1*)
[Note—Disregard any peaks corresponding to those of the *Blank solution*.]
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 0.25%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
α-Hydroxy Olopatadine[a]	0.4	1.0	0.2
Olopatadine *E*-isomer[b]	0.7	1.3	0.1
Olopatadine	1.0	—	—
Any other individual impurity	—	1.0	0.1

[a] (*Z*)-2-{11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-yl}-2-hydroxyacetic acid.
[b] 11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid.

SPECIFIC TESTS

- **pH** ⟨**791**⟩**:** Between 2.0 and 4.0, in a solution (1 in 100)
- **Loss on Drying** ⟨**731**⟩**:** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers and store at room temperature.
- **USP Reference Standards** ⟨**11**⟩
USP Olopatadine Hydrochloride RS
USP Olopatadine Related Compound B RS
(*Z*)-3-{2-(Carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}-*N*,*N*-dimethylpropan-1-amine oxide.
$C_{21}H_{23}NO_4$ 353.41

Olopatadine Hydrochloride Ophthalmic Solution

DEFINITION

Olopatadine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Olopatadine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of olopatadine ($C_{21}H_{23}NO_3$). It may contain suitable antimicrobial agents.

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

[Note—Protect solutions from light.]
Buffer: Dissolve 13.6 g of monobasic potassium phosphate in 1 L of water, add 1 mL of triethylamine, and mix. Adjust with phosphoric acid to a pH of 3.0.
Mobile phase: Acetonitrile and *Buffer* (7:18)
Standard solution: 0.1 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*
Sample solution: Equivalent to 0.1 mg/mL of olopatadine in *Mobile phase*, from Olopatadine Hydrochloride Ophthalmic Solution

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 2000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{21}H_{23}NO_3$ in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of the *Sample solution*
r_S = peak response of the *Standard solution*
C_S = concentration of olopatadine hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of olopatadine, 337.41
M_{r2} = molecular weight of olopatadine hydrochloride, 373.87

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

[NOTE—Protect solutions from light.]

• **PROCEDURE 1: LIMIT OF EARLY ELUTING IMPURITIES**
Mobile phase: Proceed as directed in the *Assay*.
Blank solution: *Mobile phase*
System suitability solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS and 0.02 mg/mL of USP Olopatadine Related Compound B RS in *Mobile phase*
Standard solution: 0.2 mg/mL of USP Olopatadine Hydrochloride RS in *Mobile phase*
Sample solution: Equivalent to 0.2 mg/mL of olopatadine in *Mobile phase*, from Olopatadine Hydrochloride Ophthalmic Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
Run time: At least 1.6 times the retention time of the major peak
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between olopatadine and olopatadine related compound B, *System suitability solution*
Column efficiency: NLT 2000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = peak response of olopatadine from the *Standard solution*
C_S = concentration of olopatadine hydrochloride in the *Standard solution* (mg/mL)
C_U = nominal concentration of olopatadine in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of olopatadine, 337.41
M_{r2} = molecular weight of olopatadine hydrochloride, 373.87
F = relative response factor for each individual impurity (see *Impurity Table 1*)

[NOTE—Disregard any peaks corresponding to those of the *Blank solution* and any peaks with relative retention time, measured with respect to olopatadine, greater than 1.5.]

Acceptance criteria
Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Olopatadine *E*-isomer[a]	0.7	1.3	0.5
Olopatadine	1.0	—	—
Olopatadine related compound B	1.2	1.0	2
Olopatadine carbaldehyde[b]	1.3	4.5	0.5
Any unspecified impurity	—	1.0	0.5

[a] 11-[(*E*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid.
[b] (*Z*)-11-[3-(dimethylamino)propylidene]-6,11-dihydrodibenzo[*b,e*]oxepine-2-carbaldehyde.

• **PROCEDURE 2: LIMIT OF LATE ELUTING IMPURITIES**
Buffer: Proceed as directed in the *Assay*.
Mobile phase: Acetonitrile and *Buffer* (1:1)
Blank solution: *Mobile phase*
System suitability solution: 0.02 mg/mL of USP Olopatadine Hydrochloride RS and 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*
Standard solution: 0.01 mg/mL of USP Olopatadine Related Compound C RS in *Mobile phase*
Sample solution: Use the *Sample solution* from the test for *Limit of Early Eluting Impurities*.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 299 nm
Column: 4.6-mm × 15-cm; 5-µm packing L7
Flow rate: 1 mL/min
Injection size: 30 µL
Run time: At least 3 times the retention time of the olopatadine related compound C peak
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for olopatadine and olopatadine related compound C are 0.3 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 7.0 between olopatadine and olopatadine related compound C, *System suitability solution*
Column efficiency: NLT 2000 theoretical plates, *Standard solution*
Tailing factor: NMT 2.0, *Standard solution*
Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

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

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of olopatadine related compound C from the *Standard solution*

C_S = concentration of USP Olopatadine Related Compound C RS in the *Standard solution* (mg/mL)

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

[NOTE—Disregard any peaks corresponding to those of the *Blank solution* and any peaks with a relative retention time, measured with respect to olopatadine related compound C, less than 0.7.]

Acceptance criteria

Individual impurities: NMT 1% of olopatadine related compound C is found; and NMT 0.5% of any other individual impurity is found.

Total impurities: NMT 3%. [NOTE—Total impurities are the sum of olopatadine related compound B, olopatadine related compound C, Olopatadine *E*-isomer, Olopatadine carbaldehyde, and all other impurities found in the tests for *Limit of Early Eluting Impurities* and *Limit of Late Eluting Impurities*.]

SPECIFIC TESTS

- **STERILITY TESTS ⟨71⟩:** Meets the requirements
- **pH ⟨791⟩:** Between 5.0 and 8.0
- **OSMOLALITY AND OSMOLARITY ⟨785⟩:** Between 260 and 320 mOsmol/kg

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store between 4° and 25°.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Olopatadine Hydrochloride RS

USP Olopatadine Hydrochloride Related Compound B RS
(*Z*)-3-{2-(Carboxymethyl)dibenzo[*b,e*]oxepin-11(6*H*)-ylidene}-*N,N*-dimethylpropan-1-amine oxide.
$C_{21}H_{23}NO_4$ 353.41

USP Olopatadine Hydrochloride Related Compound C RS
11-Oxo-6,11-dihydrodibenzo[*b,e*]oxepin-2-yl acetic acid.
$C_{16}H_{12}O_4$ 268.26

Oleovitamin A and D

DEFINITION

Oleovitamin A and D is a solution of vitamin A and vitamin D in fish liver oil or in an edible vegetable oil. The vitamin D is present as ergocalciferol or cholecalciferol obtained by the activation of ergosterol or 7-dehydrocholesterol, or from natural sources. Oleovitamin A and D contains NLT 90.0% of the labeled amounts of vitamins A and D.

ASSAY

- **VITAMIN A**

Analysis: Proceed as directed in *Vitamin A Assay* ⟨571⟩.

Acceptance criteria: NLT 90.0% of the labeled amount

- **VITAMIN D**

Analysis: Weigh a quantity of Oleovitamin A and D expected to contain the equivalent of 125–250 µg of vitamin D, but NMT 7.5 mg of vitamin A, and proceed as directed for *Chemical Method* in *Vitamin D Assay* ⟨581⟩. If the assay specimen contains less than the equivalent of 2.5 µg/g of vitamin D, or if the ratio of vitamin A to vitamin D exceeds 300:1, proceed as directed for *Biological Method* in *Vitamin D Assay* ⟨581⟩.

Acceptance criteria: NLT 90.0% of the labeled amount

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and air, preferably under an atmosphere of an inert gas. Store in a dry place.
- **LABELING:** Label it to indicate the content of vitamin A in terms of retinol per g. The vitamin A content may also be expressed in USP Vitamin A Units per g. Label it to show whether it contains ergocalciferol, cholecalciferol, or vitamin D from a natural source. Label it to indicate also the vitamin D content, in µg, of ergocalciferol or cholecalciferol per g. Its vitamin D content may be expressed also in USP Vitamin D Units per g.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Cholecalciferol RS

USP Ergocalciferol RS

Oleovitamin A and D Capsules

DEFINITION

Oleovitamin A and D Capsules contain NLT 90.0% of the labeled amounts of vitamins A and D. The oil in Oleovitamin A and D Capsules is a solution of vitamin A and vitamin D in fish liver oil or in an edible vegetable oil. The vitamin D is present as ergocalciferol or cholecalciferol obtained by the activation of ergosterol or 7-dehydrocholesterol or from natural sources.

ASSAY

- **VITAMIN A ASSAY ⟨571⟩**

Sample Transfer NLT 5 Capsules to a saponification flask, add 10–20 mL of water, and heat for 10 min. Crush any remaining solids with the blunt end of a glass rod.

Analysis: Proceed as directed in *Chemical Method* for *Procedure*, in the second paragraph, beginning with "Reflux in an all-borosilicate glass apparatus".

Acceptance criteria: NLT 90.0% of the labeled amount

- **VITAMIN D ASSAY ⟨581⟩**

Sample: Transfer NLT 5 Capsules to a saponification flask, add 10–20 mL of water, and heat for 10 min. Crush any remaining solids with the blunt end of a glass rod.

Analysis: Proceed as directed in *Chemical Method*. The *Sample Preparation* shall contain NMT the equivalent of 7.5 mg of vitamin A or NLT 125 µg of vitamin D.

Proceed as directed in *Biological Method* if the ratio of vitamin A to vitamin D exceeds 300:1.

Acceptance criteria: NLT 90.0% of the labeled amount

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a dry place.
- **LABELING:** Label the Capsules to indicate the content, in mg, of vitamin A in each Capsule. The vitamin A content in each Capsule may be expressed also in USP Vitamin A Units. Label the Capsules to show whether they contain ergocalciferol, cholecalciferol, or vitamin D from a natural source. Label the Capsules to indicate also the vitamin D content, in µg, in each Capsule. The vitamin D content may be expressed also in USP Vitamin D Units in each Capsule.