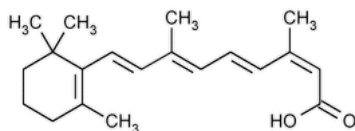


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Isotretinoin



$C_{20}H_{28}O_2$ 300.44

Retinoic acid, 13-*cis*-;

3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-*cis*-4-*trans*-6-*trans*-8-*trans*-nonatetraenoic acid CAS RN[®]: 4759-48-2; UNII: EH28UP18IF.

DEFINITION

Isotretinoin contains NLT 98.0% and NMT 102.0% of isotretinoin ($C_{20}H_{28}O_2$), calculated on the dried basis.

[CAUTION—Isotretinoin is teratogenic. Avoid inhalation and skin contact.]

[NOTE—Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedures.]

IDENTIFICATION

Change to read:

- **A.** [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy](#): ▲197A or ▲(USP 1-Dec-2024) 197M

Delete the following:

- ▲ **B.** [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Ultraviolet-Visible Spectroscopy](#): ▲(USP 1-Dec-2024)

Add the following:

- ▲ **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲(USP 1-Dec-2024)

ASSAY

Change to read:

PROCEDURE

▲ **Mobile phase:** [Methanol](#), [glacial acetic acid](#), and [water](#) (77: 0.5: 22.5)

Diluent A: 2 g/L of [butylated hydroxytoluene](#) in [tetrahydrofuran](#)

Diluent B: [Acetonitrile](#)

System suitability solution: Transfer 2 mg of [USP Isotretinoin RS](#) into a 100-mL clear glass volumetric flask. Dissolve in 10 mL of *Diluent A*, and dilute with *Diluent B* to volume. Expose the flask to ultraviolet light for about 60 min. Protect the solution from light to avoid further degradation of isotretinoin.

[NOTE—Use of a 366 nm UV lamp as the ultraviolet light source may be suitable.]

[NOTE—The *System suitability solution* will contain, in addition to isotretinoin, the degradation products of 2Z,4Z-retinoic acid and 2Z,6Z-retinoic acid. Other degradation products may be present.]

Standard stock solution: 1.0 mg/mL of [USP Isotretinoin RS](#) prepared as follows. Transfer an appropriate amount of [USP Isotretinoin RS](#) into a suitable volumetric flask. Dissolve in 10% of the flask volume of *Diluent A*, and dilute with *Diluent B* to volume. Use the solution immediately to prepare the *Standard solution*.

Standard solution: 0.1 mg/mL of [USP Isotretinoin RS](#) prepared from the *Standard stock solution* in *Diluent B*. Store the solution in a refrigerator and inject within 16 h after preparation.

Sample stock solution: 1.0 mg/mL of Isotretinoin prepared as follows. Transfer an appropriate amount of Isotretinoin into a suitable volumetric flask. Dissolve in 10% of the flask volume of *Diluent A*, and dilute with *Diluent B* to volume. Use the solution immediately to prepare the *Sample solution*.

Sample solution: 0.1 mg/mL of Isotretinoin prepared from the *Sample stock solution* in *Diluent B*. Store the solution in a refrigerator and inject within 16 h after preparation.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 355 nm

Column: 4.6-mm × 15-cm; 3-µm packing [L1](#)

Temperatures

Autosampler: 5°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 20 µL

Run time: NLT 1.5 times the retention time of isotretinoin

System suitability

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

[NOTE—The relative retention times for 2Z,4Z-retinoic acid, isotretinoin, and 2Z,6Z-retinoic acid are 0.98, 1.00, and 1.05, respectively.]

Suitability requirements

Peak-to-valley ratio: NLT 4.0 for 2Z,4Z-retinoic acid and isotretinoin, *System suitability solution*

Resolution: NLT 1.5 between isotretinoin and 2Z,6Z-retinoic acid, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of isotretinoin (C₂₀H₂₈O₂) in the portion of Isotretinoin taken:

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

r_U = peak response of isotretinoin from the *Sample solution*

r_S = peak response of isotretinoin from the *Standard solution*

C_S = concentration of [USP Isotretinoin RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Isotretinoin in the *Sample solution* (mg/mL)▲ (USP 1-Dec-2024)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

- [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

Delete the following:

- ▲ **LIMIT OF TRETINOIN**▲ (USP 1-Dec-2024)

Add the following:

- ▲ **ORGANIC IMPURITIES**

Diluent A, Diluent B, and System suitability solution: Prepare as directed in the Assay.

Solution A: 0.5% (v/v) [glacial acetic acid](#) in [water](#)

Solution B: [Acetonitrile](#), [methanol](#), and *Solution A* (37:30:33)

Solution C: [Methanol](#) and *Solution A* (77:23)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
20	100	0
25	0	100
60	0	100
61	100	0
80	100	0

Standard stock solution: 500 µg/mL each of [USP Isotretinoin RS](#) and [USP Tretinoin RS](#) prepared as follows. Transfer appropriate quantities of [USP Isotretinoin RS](#) and [USP Tretinoin RS](#) to a suitable volumetric flask. Dissolve in 10% of the flask volume of *Diluent A* and 10% with

Diluent B to volume. Store the solution at 8° and use within 14 h after preparation.