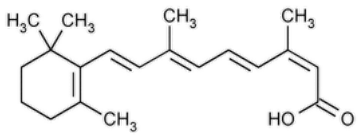
Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-Dec-2024
Document Type: USP Monographs
DocId: GUID-BD7E3647-B667-4075-8F3A-1B3F8E34A2F5_5_en-US
DOI: https://doi.org/10.31003/USPNF_M43635_05_01
DOI Ref: facoh

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 2*Z*,4*Z*-retinoic acid and 2*Z*,6*Z*-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 2*Z*,4*Z*-retinoic acid, isotretinoin, and 2*Z*,6*Z*-retinoic acid are 0.98, 1.00, and 1.05, respectively.]

Suitability requirements

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

Resolution: NLT 1.5 between isotretinoin and 2*Z*,6*Z*-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 ($C_{20}H_{28}O_2$) in the portion of Isotretinoin taken:

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

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

Standard solution: 0.5 µg/mL each of USP Isotretinoin RS and USP Tretinoin RS from the *Standard stock solution* in *Diluent B*. Store the solution at 8° and inject within 14 h after preparation.

Sensitivity solution: 0.25 µg/mL of USP Isotretinoin RS from the *Standard solution* in *Diluent B*. Store the solution at 8° and inject within 14 h after preparation.

Sample solution: 500 µg/mL of Isotretinoin prepared as follows. Transfer an appropriate amount of Isotretinoin to a suitable volumetric flask. Dissolve in 10% of the flask volume of *Diluent A*, and dilute with *Diluent B* to volume. Store the solution at 8° and inject within 14 h after preparation.

Chromatographic system

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

Mode: LC

Detector: UV 310 and 355 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Autosampler temperature: 8°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times in *Table 2* are provided as information that could aid in peak assignment.]

Table 2

Name	Relative Retention Time
2*Z*,4*Z*-Retinoic acid[a]	0.98
Isotretinoin	1.0
2*Z*,6*Z*-Retinoic acid[b]	1.05
Tretinoin	1.3

[a] (2*Z*,4*Z*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid.

[b] (2*Z*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid.

Suitability requirements

Peak-to-valley ratio: NLT 4.0 for 2*Z*,4*Z*-retinoic acid and isotretinoin at 355 nm, *System suitability solution*

Resolution: NLT 1.5 between isotretinoin and 2*Z*,6*Z*-retinoic acid at 355 nm, *System suitability solution*

Relative standard deviation: NMT 5.0% for isotretinoin and tretinoin at 355 nm, *Standard solution*

Signal-to-noise ratio: NLT 10 at 355 nm and NLT 5 at 310 nm for isotretinoin, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tretinoin in the portion of Isotretinoin taken:

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

r_U = peak response of tretinoin at 355 nm from the *Sample solution*

r_S = peak response of tretinoin at 355 nm from the *Standard solution*

C_S = concentration of USP Tretinoin RS in the *Standard solution* (µg/mL)

C_U = concentration of Isotretinoin in the *Sample solution* (µg/mL)

Calculate the percentage of any unspecified impurity, using the 355 nm wavelength, in the portion of Isotretinoin taken:

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

r_U = peak response of each impurity at 355 nm from the *Sample solution*

r_S = peak response of isotretinoin at 355 nm from the *Standard solution*

C_S = concentration of USP Isotretinoin RS in the *Standard solution* (µg/mL)

C_U = concentration of Isotretinoin in the *Sample solution* (µg/mL)

Additionally, calculate the percentage of any unspecified impurity having a relative retention time (RRT) within the range of 0.3–0.5, using the 310 nm wavelength, in the portion of Isotretinoin taken:

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

r_U = peak response of each impurity at 310 nm from the *Sample solution*

r_S = peak response of isotretinoin at 310 nm from the *Standard solution*

C_S = concentration of USP Isotretinoin RS in the *Standard solution* (µg/mL)

C_U = concentration of Isotretinoin in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Acceptance Criteria, NMT (%)
Tretinoin	1.0
Any unspecified impurity[a]	0.10
Total impurities[b]	0.5▲ (USP 1-Dec-2024)

[a] For the unspecified impurities having a relative retention time (RRT) within the range of 0.3–0.5, evaluated using both the 355 and 310 nm wavelengths, the higher of the two calculated results is assessed and included in the total.

[b] Total impurities excludes tretinoin.

SPECIFIC TESTS

• **Loss on Drying** ⟨731⟩

Analysis: Dry a sample in a vacuum at room temperature for 16 h.

Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in tight containers, under an atmosphere of an inert gas. ▲Store at room temperature and▲ (USP 1-Dec-2024) protect from light.

• **USP Reference Standards** ⟨11⟩

USP Isotretinoin RS

USP Tretinoin RS

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

Topic/Question	Contact	Expert Committee
ISOTRETINOIN	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 49(3)

Current DocID: GUID-BD7E3647-B667-4075-8F3A-1B3F8E34A2F5_5_en-US

DOI: https://doi.org/10.31003/USPNF_M43635_05_01

DOI ref: facoh