Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Trenbolone Acetate taken:

$$\text{Result} = (100 \times r_U)/[(r_S + S) \times F]$$

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

r_S = peak response of Trenbolone Acetate from *Sample solution*

S = sum of the peak responses of each impurity, each divided by their respective response factor

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total specified and unspecified impurities: NMT 2.0%

Reporting level for impurities: NMT 0.10%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Trenbolone related compound B[a]	0.4	1.04	1.0
Trenbolone related compound C[b]	0.8	1.10	0.5
Trenbolone acetate	1.0	—	—
Related compound A[c]	1.2	1.0	0.5
Any unspecified impurity	—	1.00[d]	0.5

[a] Trenbolone.

[b] Trenbolone acetate 17α-isomer.

[c] Conjugated dihydrotrenbolone acetate, or 11,12-dihydrotrenbolone acetate.

[d] Unless determined otherwise.

SPECIFIC TESTS

- **ABSORBANCE**

  Sample solution: 100 mg/mL in dehydrated alcohol

  Spectrometric conditions

  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

  Analytical wavelength: 440 nm

  Cell: 2 cm

  Blank: Dehydrated alcohol

  Analysis

  Samples: *Sample solution* and *Blank*

  Acceptance criteria: NMT 0.3

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +39° to +43°

  Sample solution: 5 mg/mL in methanol

- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample in a vacuum at 60° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

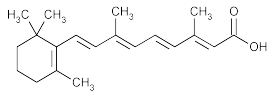
- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a refrigerator.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

  USP Trenbolone Acetate RS

  USP Trenbolone Acetate System Suitability Mixture RS

  Mixture containing trenbolone and conjugated dihydrotrenbolone acetate in a matrix of trenbolone acetate.

Tretinoin



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

Retinoic acid;

all-*trans*-Retinoic acid [302-79-4].

DEFINITION

Tretinoin contains NLT 97.0% and NMT 103.0% of tretinoin ($C_{20}H_{28}O_2$), calculated on the dried basis.

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

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨**197M**⟩
- **B. ULTRAVIOLET ABSORPTION** ⟨**197U**⟩

  Analytical wavelength: 352 nm

  Medium: Dilute 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to 1000 mL.

  Sample solution: 4 µg/mL in *Medium*

  Acceptance criteria: Absorptivities do not differ by more than 3.0%, calculated on the dried basis.

ASSAY

- **PROCEDURE**

  Sample: 240 mg of Tretinoin

  Titrimetric system

  Mode: Direct titration

  Titrant: 0.1 N sodium methoxide VS

  Endpoint detection: Visual

  Analysis: Dissolve the *Sample* in 50 mL of dimethylformamide, and add 3 drops of a 1-in-100 solution of thymol blue in dimethylformamide. Titrate with *Titrant* to a greenish endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 30.04 mg of tretinoin ($C_{20}H_{28}O_2$).

  Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1%

Delete the following:

- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** 20 ppm (Official 1-Jan-2018)
- **LIMIT OF ISOTRETINOIN**

  Mobile phase: Isooctane, isopropyl alcohol, and glacial acetic acid (99.65: 0.25: 0.1)

  System suitability stock solution: 250 µg/mL of USP Tretinoin RS in isooctane prepared as follows. Dissolve a quantity of USP Tretinoin RS in a minimum amount of methylene chloride, and add a suitable amount of isooctane to the known concentration.

  Standard stock solution: 250 µg/mL of USP Isotretinoin RS in isooctane prepared as follows. Dissolve a quantity of USP Isotretinoin RS in a minimum amount of methylene chloride, and add a suitable amount of isooctane to the known concentration.

  System suitability solution: Transfer 5 mL of *Standard stock solution* into a 100-mL volumetric flask, and add *System suitability stock solution* to volume.

  Standard solution: 12.5 µg/mL of USP Isotretinoin RS in isooctane from *Standard stock solution*

  Sample solution: Transfer 25 mg of Tretinoin into a 100-mL volumetric flask. Dissolve in a minimum amount of methylene chloride, and add isooctane to volume.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 352 nm
Column: 4.0-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for isotretinoin and tretinoin are about 0.84 and 1.00, respectively.]
Suitability requirements
Resolution: NLT 2.0 between isotretinoin and tretinoin
Relative standard deviation: NMT 2.0% for the isotretinoin peak
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of isotretinoin in the portion of Tretinoin 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* (µg/mL)
C_U = concentration of Tretinoin in the *Sample solution* (µg/mL)
Acceptance criteria: NMT 5.0%

SPECIFIC TESTS
- **LOSS ON DRYING** ⟨**731**⟩
Analysis: Dry a sample under vacuum at room temperature for 16 h.
Acceptance criteria: NMT 0.5%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably under an atmosphere of an inert gas, protected from light.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Isotretinoin RS
USP Tretinoin RS

Tretinoin Cream

DEFINITION
Tretinoin Cream contains NLT 90.0% and NMT 120.0% of the labeled amount of tretinoin ($C_{20}H_{28}O_2$).

IDENTIFICATION
- **A.** 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**
Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedure. Use stabilized tetrahydrofuran in the preparation of the *Standard solution* and the *Sample solution*.
Diluted phosphoric acid: Dilute 10 mL of phosphoric acid with water to 100 mL.
Buffer: 1.38 g/L of monobasic sodium phosphate in water. Adjust with *Diluted phosphoric acid* to a pH of 3.0.
Diluent A: Water and *Diluted phosphoric acid* (9:1)
Diluent B: Tetrahydrofuran and *Diluent A* (3:2)
Mobile phase: Tetrahydrofuran and *Buffer* (42:58)
Standard stock solution: 0.4 mg/mL of USP Tretinoin RS in tetrahydrofuran
Standard solution: 4 µg/mL of USP Tretinoin RS in *Diluent B* from *Standard stock solution*
Sample stock solution: Nominally 20 µg/mL of tretinoin in tetrahydrofuran prepared as follows. Transfer a quantity of Cream, equivalent to 1.0 mg of tretinoin, into a 50-mL volumetric flask, and add 20.0 mL of tetrahydrofuran. Shake the flask to disperse the cream, dilute with tetrahydrofuran to volume, mix, and filter if necessary.
Sample solution: 4 µg/mL of tretinoin in *Diluent B* from *Sample stock solution*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 3.9-mm × 15-cm; 4-µm packing L1
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of tretinoin ($C_{20}H_{28}O_2$) in the portion of Cream 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 Tretinoin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of tretinoin in the *Sample solution* (mg/mL)
Acceptance criteria: 90.0%–120.0%

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 Tretinoin RS

Tretinoin Gel

DEFINITION
Tretinoin Gel contains NLT 90.0% and NMT 130.0% of the labeled amount of $C_{20}H_{28}O_2$.

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

ASSAY
- **PROCEDURE**
[NOTE—Avoid exposure to strong light, and use low-actinic glassware in the performance of the following procedure.]
Mobile phase: Prepare a mixture of acetonitrile and water (17:3). Add 5 mL of glacial acetic acid to each L of the mixture, mix well, filter, and degas.