System suitability
Samples: *Standard solution* and *System suitability solution*
[NOTE—The relative retention times for trenbolone acetate related compound A and trenbolone acetate are 1.2 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between trenbolone acetate and trenbolone acetate related compound A, *System suitability solution*
Column efficiency: NLT 8000 theoretical plates for the trenbolone acetate peak, *System suitability solution*
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

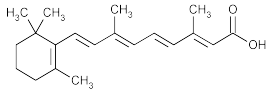
Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Trenbolone related compound B[a]	0.4	1.04	1.0
Trenbolone related compound C[b]	0.8	1.10	0.5
Trenbolone acetate	1.0	—	—
Related compound A[c]	1.2	1.0	0.5
Any unspecified impurity	—	1.00[d]	0.5

[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 *Spectrophotometry and Light-Scattering* ⟨851⟩.)
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].

» Tretinoin contains not less than 97.0 percent and not more than 103.0 percent of $C_{20}H_{28}O_2$, calculated on the dried basis.

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
NOTE—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⟩—
Solution: 4 µg per mL.
Medium: acidified isopropyl alcohol (prepared by diluting 1 mL of 0.01 N hydrochloric acid with isopropyl alcohol to 1000 mL).
Absorptivities at 352 nm, calculated on the dried basis, do not differ by more than 3.0%.

Loss on drying ⟨731⟩**—**Dry it in vacuum at room temperature for 16 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

Limit of isotretinoin—
Mobile phase—Prepare a suitable filtered and degassed mixture of isooctane, isopropyl alcohol, and glacial acetic acid (99.65:0.25:0.1), making adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).
System suitability solution—Dissolve a quantity of USP Tretinoin RS in a minimum amount of methylene chloride, add a suitable amount of isooctane to obtain a solution having a tretinoin concentration of about 250 µg per mL, and mix.
Standard solution—Dissolve an accurately weighed quantity of USP Isotretinoin RS in a minimum quantity of methylene chloride, and add isooctane to obtain a solution having a known concentration of about 250 µg per mL.
System suitability preparation—Pipet 5 mL of *Standard solution* into a 100-mL volumetric flask, add *System suitability solution* to volume, and mix.
Standard preparation—Pipet 5 mL of *Standard solution* into a 100-mL volumetric flask, add isooctane to volume, and mix.
Test preparation—Transfer about 25 mg of Tretinoin, accurately weighed, to a 100-mL volumetric flask, dissolve in a minimum quantity of methylene chloride, add isooctane to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 352-nm detector and a 4.0-mm × 25-cm column containing packing L3. The flow

rate is about 1 mL per minute. Chromatograph about 20 µL of *System suitability preparation*, and record the peak responses. The relative retention times for isotretinoin and tretinoin are about 0.84 and 1.00, respectively. The relative standard deviation of the isotretinoin peak response in replicate injections is not more than 2.0%, and the resolution, *R,* of isotretinoin and tretinoin is not less than 2.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of isotretinoin taken by the formula:

$$10(C / W)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Isotretinoin RS in the *Standard preparation*, *W* is the weight, in mg, of Tretinoin taken, and r_U and r_S are the peak responses of the isotretinoin peaks obtained from the *Test preparation* and the *Standard preparation*, respectively. The content of isotretinoin is not more than 5.0%.

Assay—Dissolve about 240 mg of Tretinoin, accurately weighed, in 50 mL of dimethylformamide, add 3 drops of a 1 in 100 solution of thymol blue in dimethylformamide, and titrate with 0.1 N sodium methoxide VS 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 $C_{20}H_{28}O_2$.

Tretinoin Cream

» Tretinoin Cream contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of tretinoin.

Packaging and storage—Preserve in collapsible tubes or in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Tretinoin RS

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Minimum fill ⟨*755*⟩: meets the requirements.

Assay—[NOTE—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 preparation* and the *Assay preparation*.]

Dilute phosphoric acid—Dilute 10 mL of phosphoric acid with water to 100 mL.

Phosphate buffer—Dissolve 1.38 g of monobasic sodium phosphate in 1000 mL of water, adjust with *Dilute phosphoric acid* to a pH of 3.0, and mix.

Diluting solution—Prepare a mixture of water and *Dilute phosphoric acid* (9:1).

Mobile phase—[NOTE—*Phosphate buffer* and tetrahydrofuran may be filtered and degassed separately *before* mixing.] Prepare a filtered and degassed mixture of *Phosphate buffer* and tetrahydrofuran (58:42). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Tretinoin RS in tetrahydrofuran to obtain a solution having a known concentration of about 0.4 mg per mL. Dilute a known volume of this solution, quantitatively and stepwise if necessary, with a mixture of tetrahydrofuran and *Diluting solution* (3:2) to obtain a solution having a known concentration of about 4 µg per mL.

Assay preparation—Transfer an accurately weighed quantity of Cream, equivalent to 1.0 mg of tretinoin, to 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. Transfer 5.0 mL of this solution to a 25-mL volumetric flask, dilute with a mixture of tetrahydrofuran and *Diluting solution* (3:2) to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 3.9-mm × 15-cm column that contains 4-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of tretinoin ($C_{20}H_{28}O_2$) in the portion of Cream taken by the formula:

$$0.250C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Tretinoin RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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.

Standard solution: 0.02 mg/mL of USP Tretinoin RS in methanol. [NOTE—Sonicate if needed.]

Sample solution: Transfer Tretinoin Gel, equivalent to 2 mg of tretinoin, to a 100-mL volumetric flask. Add about 70 mL of methanol, sonicate with intermittent shaking for 30 min, and dilute with methanol to volume. Centrifuge a portion of this solution at 3000 rpm for 10 min. Use the clear supernatant.

Chromatographic system

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