Isotretinoin Capsules

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Reason for Revision	Compliance

In accordance with the Rules and Procedures of the Council of Experts, the Chemical Medicines Monographs 3 Expert Committee has revised the Isotretinoin Capsules monograph. The purpose for the revision is to add *Dissolution Test* 6 for a generic product approved by FDA.

The Isotretinoin Capsules Revision Bulletin supersedes the currently official Isotretinoin Capsules monograph. The Revision Bulletin will be incorporated in the *Second Supplement* to *USP 39–NF34*.

Should you have any questions, please contact Feiwen Mao (301–816–8320 or fm@usp.org.)

Isotretinoin Capsules

DEFINITION

Isotretinoin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$).

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

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

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**

Protect the *System suitability solution*, *Standard solution*, *Sample stock solution*, and *Sample solution* from direct light.

Diluent: Heat 0.1 N sodium hydroxide to about 60°–70°. Cool to room temperature, purge with helium or nitrogen, and store in a plastic container.

Solution A: 0.5% Acetic acid in methanol

Solution B: 0.5% Acetic acid in water

Mobile phase: *Solution A* and *Solution B* (71:29)

System suitability solution: 0.04 mg/mL of USP Isotretinoin RS and 0.02 mg/mL of USP Tretinoin RS in *Diluent*

Standard solution: 0.04 mg/mL of USP Isotretinoin RS in *Diluent*

Sample stock solution: 0.4 mg/mL of isotretinoin in *Diluent* prepared as follows. Transfer NLT 10 Capsules to a suitable volumetric flask. Add *Diluent* to the volumetric flask to fill about 50% of the volume, sonicate for 1 h with occasional shaking to disperse all of the contents, and dilute with *Diluent* to volume.

Sample solution: Nominally 0.04 mg/mL of isotretinoin in *Diluent* from the *Sample stock solution*. Pass the solution through a suitable membrane filter of 0.45-µm or finer pore size.

Chromatographic system

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

Mode: LC

Detector: UV 353 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

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

[NOTE—The relative retention times for isotretinoin and tretinoin are 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the isotretinoin and tretinoin peaks, *System suitability solution*

Column efficiency: NLT 2000 for the isotretinoin peak, *System suitability solution*

Tailing factor: NMT 2.0 for the isotretinoin peak, *System suitability solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) in the portion of Capsules taken:

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

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of isotretinoin in the *Standard solution* (mg/mL)

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

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

[**CAUTION—**Carry out all the tests under subdued light and use low-actinic glassware.]

Test 1

Medium

Stage 1: Simulated gastric fluid with pepsin, prepared fresh and purged with nitrogen

Stage 2: 0.13 N sodium hydroxide (5 g/L of sodium hydroxide in water). Prepare fresh, and purge with nitrogen.

Apparatus (see *Disintegration* ⟨**701**⟩)**:** No disks; the apparatus is adjusted so that the bottom of the basket-rack assembly descends to 1.0 ± 0.1 cm from the inside bottom surface of the vessel on the downward stroke; the 10-mesh stainless steel cloth in the basket-rack assembly is replaced with a 40-mesh stainless steel cloth; a 10-mesh stainless-steel cloth is fitted to the top of the basket-rack assembly.

Time: 60 min

Standard solution: Transfer 10 mg of USP Isotretinoin RS to a 200-mL volumetric flask. Add 25.0 mL of *Stage 1 Medium* and about 150 mL of *Stage 2 Medium*, sonicate until completely dissolved (about 20 min), and dilute with *Stage 2 Medium* to volume. Pass 20 mL of this solution through a suitable filter, discarding the first 5 mL. Dilute 5.0 mL of the filtrate with *Stage 2 Medium* to 50 mL.

Sample solutions: Perform a dissolution test on each of 6 Capsules. Place 1 Capsule in one of the tubes in each of six basket-rack assemblies. Place each basket in a 1-L beaker containing 100 mL of *Stage 1 Medium* in a bath having a temperature of 37.0 ± 0.5°. Allow to stand for 30 min. Carefully add 800 mL of *Stage 2 Medium* to each beaker. With the disintegration apparatus operating, connect each basket-rack assembly to the drive rod in a timed sequence. After 60 min, withdraw 20 mL of *Medium* (*Stage 1* and *Stage 2*), and immediately pass the solution through a suitable membrane filter of 0.45-µm pore size. Discard the first 5 mL, and collect the solution in argon-charged glassware. Dilute, if necessary, with *Stage 2 Medium* to obtain a theoretical concentration of 5.5 µg/mL of isotretinoin, assuming complete dissolution, based on the label claim.

Capsule shell correction solution: Empty the contents of 3 Capsules. Wash the Capsule shells in several 20-mL aliquots of chloroform. Allow the Capsule shells to air dry. Place the Capsule shells in a 1-L flask containing 100 mL of *Stage 1 Medium* and 800 mL of *Stage 2 Medium*. Allow the flask to stand for about 1 h in a bath with a temperature of 37.0 ± 0.5°, stirring occasionally. Filter, and dilute as described for the *Sample solution*.

Analysis

Detector: UV 343 nm

Blank: *Medium* (*Stage 1* and *Stage 2*)

Samples: *Standard solution*, *Sample solutions*, and *Capsule shell correction solution*

Determine the amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved, correcting for the Capsule shell absorbance.

Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = [(A_U - A_C)/A_S] \times (C_S/L) \times D \times 100$$

A_U = absorbance of the *Sample solution*
A_C = absorbance of the *Capsule shell correction solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
D = dilution factor for the *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.05 M phosphate buffer, pH 7.8, containing 0.5% (w/v) solid *N,N*-dimethyldodecylamine *N*-oxide; 900 mL

Apparatus 1: 20-mesh basket; 100 rpm

Time: 90 min

Buffer solution: 3.4 mg/mL of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.10 ± 0.05.

Mobile phase: Methanol and *Buffer solution* (81:19)

Standard solution: Transfer about 44 mg of USP Isotretinoin RS to a 100-mL volumetric flask. Add 15 mL of 1-propanol, and sonicate for about 15 min. Add 50 mL of *Medium*, and sonicate for 10 min. Dilute with *Medium* to volume. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. Dilute this solution with *Medium* to obtain a final concentration of about (L/1000) mg/mL, where L is the label claim, in mg/Capsule.

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 358 nm
Column: 4.6-mm × 5-cm; 5-µm packing L1
Flow rate: 2.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

Medium: Borate buffer, pH 8.0, containing 0.5% cetrimide and 50 mg/L of pancreatin. Dissolve 12.37 g of boric acid and 14.91 g of potassium chloride in water, and dilute with water to 1 L. To 250 mL of this solution add 19.5 mL of 0.2 M sodium hydroxide solution, and dilute with water to 1 L. Adjust with 0.2 M sodium hydroxide to a pH of 8.00 ± 0.05, if necessary. Add 5 g of cetrimide. Just before starting the test, dissolve a quantity of pancreatin to obtain a final concentration of 50 mg/L; 900 mL.

Apparatus 2: 75 rpm, with sinkers

Time: 90 min

Mobile phase: 0.5% Acetic acid in methanol and 0.5% acetic acid in water (71:29)

Standard solution: 0.45 mg/mL of USP Isotretinoin RS in 0.1 N sodium hydroxide. Dilute this solution with *Medium* to obtain a final concentration of (L/1000) mg/mL, where L is the label claim, in mg/Capsule.

Sample solution: Pass a portion of the solution under test through a suitable membrane filter of 0.45-µm pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 353 nm
Column: 4.6-mm × 25-cm; 10-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1800 theoretical plates
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL

Tolerances: NLT 70% (Q) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.

Test 4: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 4*.

Medium: 50 mM monobasic potassium phosphate at pH 7.4 containing 70 mg/L of pancreatin and 4.5% (v/v) of lauryl dimethyl amine oxide as a 30% solution prepared as follows. Dissolve 6.8 g of monobasic potassium phosphate in 920 mL of water. Adjust by the addition of approximately 35 mL of 1 N sodium hydroxide to a pH of 7.4 ± 0.1. Add 70 mg of pancreatin and 45 mL of 30% solution of lauryl dimethyl amine oxide, and stir gently to mix. The pancreatin should be added on the day of use; 900 mL. [NOTE—Not all of the pancreatin visibly dissolves.]

Apparatus 2: 75 rpm, with spiral coated sinker. [NOTE—A suitable sinker is available as catalog number CAPWHT-02 from www.qla-llc.com.]

Time: 90 min

Mobile phase: Methanol, water, and glacial acetic acid (80: 20: 0.5)

Standard stock solution 1: 0.28 mg/mL of USP Isotretinoin RS prepared as follows. Transfer USP Isotretinoin RS to a suitable volumetric flask, and add metha-

nol equivalent to 10% of the final volume. Sonicate to dissolve, and dilute with *Medium* to volume.
Standard solution: 0.028 mg/mL of USP Isotretinoin RS in *Medium*, from *Standard stock solution 1*
Standard stock solution 2: 8.8 µg/mL of USP Tretinoin RS prepared as follows. Transfer USP Tretinoin RS to a suitable volumetric flask, and add methanol equivalent to 20% of the final volume. Sonicate to dissolve, and dilute with *Medium* to volume to obtain a 0.22-mg/mL solution. Transfer 2 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.
System suitability solution: 45 µg/mL of USP Isotretinoin RS and 0.88 µg/mL of USP Tretinoin RS in *Medium* from *Standard stock solution 1* and *Standard stock solution 2*
Sample solution: Pass a portion of the solution under test through a suitable PVDF membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 353 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Temperatures
Autosampler: 4°
Column: 40°
Flow rate: 1.5 mL/min
Injection volume: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times of isotretinoin and tretinoin are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 3.0 between the isotretinoin and tretinoin peaks, *System suitability solution*
Tailing factor: 0.8–1.3 for the isotretinoin peak, *System suitability solution*
Relative standard deviation: NMT 2.0%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
Tolerances: NLT 75% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.
Test 5: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 5*.
Medium: pH 7.7 phosphate buffer containing 50 mg/L of pancreatin and 1.5% *N*,*N*-dimethyldodecylamine-*N*-oxide prepared as follows. Prepare a solution of 27.2 g/L of monobasic potassium phosphate in water (*Solution A*) and a solution of 8 g/L of sodium hydroxide pellets in water (*Solution B*). Transfer 250 mL of *Solution A* and 215 mL of *Solution B* to a 1000-mL volumetric flask, and dilute with water to volume. Adjust with *Solution B* to a pH of 7.7. Add 50 mg of pancreatin and 15 g of *N*,*N*-dimethyldodecylamine-*N*-oxide to this solution (the pancreatin should be added on the day of use); degassed; 900 mL.
Apparatus 2: 75 rpm, with sinkers
Time: 60 min
Mobile phase: Acetonitrile, water, and glacial acetic acid (85: 15: 0.5)
Standard solution: 0.45 mg/mL of USP Isotretinoin RS in methanol. Dilute this solution with *Medium* to obtain a final concentration of (*L*/900) mg/mL, where *L* is the label claim, in mg/Capsule.
Sample solution: Pass a portion of the solution under test through a suitable PVDF membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 353 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Flow rate: 1.5 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Isotretinoin RS in the *Standard solution* (mg/mL)
L = label claim (mg/Capsule)
V = volume of *Medium*, 900 mL
Tolerances: NLT 80% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.
•**Test 6:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 6*.
Protect the solutions from light and perform the test under subdued light.
Medium: 1% Cetrimide (w/v) in pH 6.8 phosphate buffer containing an amount of pancreatin equivalent to 1750 USP Units of protease activity per L; degassed; 900 mL
Apparatus 2: 100 rpm
Time: 120 min
Standard stock solutions: Prepare three solutions containing 0.08, 0.2, and 0.32 mg/mL of USP Isotretinoin RS in methanol. Sonicate if necessary.
Standard solutions: Prepare three solutions containing 0.8, 2.0, and 3.2 µg/mL of USP Isotretinoin RS in *Medium* from the *Standard stock solutions*. Pass a portion of the solutions through a suitable PVDF membrane filter of 0.45-µm pore size, and discard the first few mL of the filtrate.
Sample stock solution: Pass a portion of the solution under test through a suitable polyethylene full flow filter of 35-µm pore size, and then through a suitable PVDF membrane filter of 0.45-µm pore size. Discard the first few mL of the filtrate.
Sample solution: Transfer the *Sample stock solution* to a suitable volumetric flask and dilute with *Medium* to volume according to *Table 1*.

Table 1

Tablet Strength (mg)	Sample Stock Solution (mL)	Final Volume (mL)	Dilution Factor
10	6.0	25.0	4.2
20	3.0	25.0	8.3
30	4.0	50.0	12.5
40	3.0	50.0	16.7

Instrumental conditions
Mode: UV
Analytical wavelength: At maximum absorbance about 348 nm
Blank: *Medium*
Analysis
Samples: *Standard solutions* and *Sample solution*
Calibration curve: Construct a calibration curve of the concentration of USP Isotretinoin RS (µg/mL) versus absorbance from the *Standard solutions*. The linear regression coefficient is NLT 0.99.
Determine the concentration of isotretinoin in the *Sample solution* from the *Calibration curve*, and calculate the percentage of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) dissolved:

$$\text{Result} = C_U \times V \times (D/L) \times (1/F) \times 100$$

C_U = nominal concentration of isotretinoin in the *Sample solution* (µg/mL)
V = volume of *Medium*, 900 mL
D = dilution factor for the *Sample solution* (see *Table 1*)
L = label claim (mg/Capsule)
F = conversion factor, 1000 µg/mg

Tolerances: NLT 80% (*Q*) of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$) is dissolved.● (RB 1-Feb-2016)

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

IMPURITIES

- **ORGANIC IMPURITIES**

Methylene chloride reagent: Transfer 50 g of sodium bicarbonate to 1000 mL of methylene chloride, shake, and allow to stand overnight. At the time of use, filter suitable portions of this solution, and add 10 mg of butylated hydroxytoluene per mL of solution.
Mobile phase: Hexanes, ethyl acetate, and glacial acetic acid (970: 30: 0.1)
System suitability stock solution: 1 mg/mL each of USP Isotretinoin RS and USP Tretinoin RS in *Methylene chloride reagent*
System suitability solution: 0.01 mg/mL each of USP Isotretinoin RS and USP Tretinoin RS in hexanes from *System suitability stock solution*
Standard stock solution: 0.5 mg/mL of USP Tretinoin RS in *Methylene chloride reagent*
Standard solution: 1.0 µg/mL of USP Tretinoin RS from *Standard stock solution* in hexanes
Sample stock solution: Take a number of Capsules equivalent to about 200 mg of isotretinoin, and with a sharp blade carefully open the Capsules without loss of material. Transfer the contents by pipetting 5 mL of *Methylene chloride reagent* over each Capsule, and rinsing with hexanes. Collect the washings in a 500-mL volumetric flask, dilute with hexanes to volume, and mix.
Sample solution: Nominally 0.1 mg/mL of isotretinoin in hexanes. Transfer 50.0 mL of *Sample stock solution* to a 200-mL volumetric flask, and dilute with hexanes to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 365 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 1 mL/min
Injection volume
System suitability: 20 µL
Analysis: 50 µL
Run time: NLT 2 times the retention time of isotretinoin
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for isotretinoin and tretinoin are about 0.75 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 3.0 between isotretinoin and tretinoin
Tailing factor: NMT 2.5 for the isotretinoin peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms, and measure the peak responses.
Acceptance criteria: The peak response for any impurity is NMT that of the tretinoin response from the *Standard solution* (1.0%); and the sum of all the peak responses, excluding that of isotretinoin, from the *Sample solution*, is NMT 1.5 times the tretinoin response from the *Standard solution* (1.5%).

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature in a dry place.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Isotretinoin RS
USP Tretinoin RS