Isotretinoin Capsules

» Isotretinoin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isotretinoin ($C_{20}H_{28}O_2$).

Caution—Isotretinoin is teratogenic. Avoid inhalation and skin contact.

Packaging and storage—Preserve in tight containers, protected from light. Store at controlled room temperature, in a dry place.

Add the following:

•**Labeling—**When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.• (RB 01-Dec-2008)

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

Change to read:

Dissolution ⟨711⟩**—***[•Caution—Carry out all the tests under subdued light and use low-actinic glassware.]*

TEST 1—• (RB 01-Dec-2008)

Medium—

STAGE 1: simulated gastric fluid with pepsin, prepared freshly and purged with nitrogen.

STAGE 2: 0.13 N sodium hydroxide, prepared by transferring 5 g of sodium hydroxide to a 1-L volumetric flask and dissolving in and diluting with water to volume. 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 minutes.

Standard solution—Transfer about 10 mg of USP Isotretinoin RS, accurately weighed, to a 200-mL low-actinic volumetric flask; add 25.0 mL of *Stage 1 Medium* and about 150 mL of *Stage 2 Medium;* sonicate until completely dissolved (about 20 minutes); 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 solution—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 minutes. 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 minutes, withdraw 20 mL of *Medium* (*Stage 1* and *Stage 2*), immediately pass the solution through a suitable 0.45-µm filter, discard the first 5 mL, and collect the solution in argon-charged, low-actinic glassware. Dilute, if necessary, using low-actinic glassware, with *Stage 2 Medium,* to obtain a theoretical concentration of about 0.0055 mg per mL of isotretinoin, assuming complete dissolution, based on the label claim.

Capsule shell correction—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 hour in a bath having a temperature of 37.0 ± 0.5°, stirring occasionally. Filter, and dilute as described for *Sample solution.*

Procedure—Determine the amount of $C_{20}H_{28}O_2$ dissolved by employing UV absorption at the wavelength of maximum absorbance at about 343 nm, in portions of the *Sample solution* in comparison with the *Standard solution,* correcting for the Capsule shell absorbance, and using *Medium* (*Stage 1* and *Stage 2*) as the blank. Calculate the percentage of $C_{20}H_{28}O_2$ dissolved by the formula:

$$\frac{(A_U - A_{CS}) \times C_S \times D_U \times 100}{A_S \times LC}$$

in which A_U, A_{CS}, and A_S are the absorbances obtained from the *Sample solution,* the *Capsule shell correction,* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution;* D_U is the dilution factor of the *Sample solution;* 100 is the conversion factor to percentage; and *LC* is the Capsule label claim, in mg.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{20}H_{28}O_2$ is dissolved in 60 minutes.

•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 minutes.

Buffer solution—Transfer 3.4 g of monobasic potassium phosphate to a 1-L volumetric flask, dissolve in and dilute with water to volume, and adjust with phosphoric acid to a pH of 2.10 ± 0.05.

Mobile phase—Prepare a filtered and degassed mixture of methanol and *Buffer solution* (81 : 19). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Transfer about 44 mg, accurately weighed, of USP Isotretinoin RS to a 100-mL low-actinic volumetric flask. Add 15 mL of 1-propanol, and sonicate for about 15 minutes. Add 50 mL of *Medium,* and sonicate for 10 minutes. Fill with *Medium* to volume. Transfer 5.0 mL to a 100-mL low-actinic 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 Capsule label claim, in mg.

Test solution—Pass a portion of the solution under test through a suitable 0.45-µm filter.

Chromatographic system—The liquid chromatograph is equipped with a 358-nm detector and a 4.6-mm × 5-cm column containing 5-µm packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 1000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of isotretinoin dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

in which r_U and r_S are the peak responses for the *Test solution* and the *Standard solution,* respectively; C_S is the concentration, in mg per mL, of the *Standard solution;* 900 is the volume, in mL, of *Me-*

dium; 100 is the percentage conversion factor; and *L* is the Capsule label claim, in mg.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{20}H_{28}O_2$ is dissolved in 90 minutes.

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 per L of pancreatin (Prepared by dissolving 12.37 g of boric acid and 14.91 g of potassium chloride in water and diluting 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, and mix until dissolved. Just before starting the test, dissolve a quantity of pancreatin to obtain a final concentration of 50 mg per L.); 900 mL.

Apparatus 2: 75 rpm, with sinkers.

Time: 90 minutes.

Standard solution—Transfer about 45 mg, accurately weighed, of USP Isotretinoin RS to a 100-mL volumetric flask. Add 60 mL of 0.1 N sodium hydroxide, and sonicate until dissolved. Dilute with 0.1 N sodium hydroxide to volume. Dilute this solution with *Medium* to obtain a final concentration of about *L*/1000 mg/mL, where *L* is the Capsule label claim, in mg.

Test solution—Pass a portion of the solution under test through a suitable 0.45-µm filter.

Mobile phase—Prepare a filtered and degassed mixture of 0.5% acetic acid in methanol and 0.5% acetic acid in water (71 : 29). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system—The liquid chromatograph is equipped with a 353-nm detector and a 4.6-mm × 25-cm column containing 10-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 1800 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of isotretinoin dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

in which r_U and r_S are the peak responses for the *Test solution* and the *Standard solution*, respectively; C_S is the concentration, in mg per mL, of the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the percentage conversion factor; and *L* is the Capsule label claim, in mg.

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{20}H_{28}O_2$ is dissolved in 90 minutes.• (RB 01-Dec-2008)

Uniformity of dosage units ⟨905⟩: meet the requirements.

Chromatographic purity—

Methylene chloride reagent and *Mobile phase*—Proceed as directed in the *Assay*.

Standard solution—Dissolve an accurately weighed quantity of USP Tretinoin RS in *Methylene chloride reagent* to obtain a solution having a known concentration of about 0.5 mg per mL. Dilute an accurately measured volume of this solution quantitatively, and stepwise if necessary, with hexanes to obtain a solution having a known concentration of about 1 µg per mL.

Test solution—Transfer 50.0 mL of the stock solution retained from the *Assay preparation* to a 200-mL volumetric flask, dilute with hexanes to volume, and mix to obtain a solution having a concentration of about 0.1 mg of isotretinoin per mL.

Chromatographic system—Proceed as directed in the *Assay*, using the *Standard preparation* prepared in the *Assay*.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and allow the *Test solution* to elute for not less than two times the retention time of isotretinoin. Record the chromatograms, and measure the peak responses: the peak response for any impurity is not more than that of the tretinoin response obtained from the *Standard solution* (1.0%); and the sum of all the peak responses, excluding that of isotretinoin, obtained from the *Test solution*, is not more than 1.5 times the tretinoin response obtained from the *Standard solution* (1.5%).

Assay—

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.

Mobile phase—Prepare a filtered and degassed mixture of hexanes, ethyl acetate, and glacial acetic acid (970 : 30 : 0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve accurately weighed quantities of USP Isotretinoin RS and USP Tretinoin RS in *Methylene chloride reagent* to obtain a solution having known concentrations of about 1 mg of each Reference Standard per mL. Transfer 1.0 mL of this solution, and dilute quantitatively with hexanes to 100.0 mL to obtain a solution having known concentrations of about 0.01 mg of each Reference Standard per mL.

Assay preparation—Weigh an accurately counted number of Capsules, equivalent to about 200 mg of isotretinoin, and calculate the average weight per Capsule. With a sharp blade, carefully open the Capsules, without loss of material, and 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. [NOTE—Reserve a portion of this stock solution for the *Chromatographic purity* test.] Transfer 5.0 mL of the stock solution to a 200-mL volumetric flask, dilute with hexanes to volume, and mix to obtain a solution having a concentration of 0.01 mg of isotretinoin per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm × 25-cm column containing packing L3. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times for isotretinoin and tretinoin are about 0.75 and 1.00, respectively; the resolution, *R*, between isotretinoin and tretinoin is not less than 3.0; the tailing factor for the isotretinoin peak is not greater than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 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 isotretinoin ($C_{20}H_{28}O_2$) in each of the Capsules taken by the formula:

$$20{,}000(C/N)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Isotretinoin RS in the *Standard preparation; N* is the number of Capsules taken; and r_U and r_S are the isotretinoin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.