

sponses. Calculate the percentage of isotretinoin dissolved by the formula:

$$\frac{r_u \times C_s \times 900 \times 100}{r_s \times L}$$

in 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 per centage 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.

Uniformity of dosage units (905): meet the requirements.

Chromatographic purity—

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)).

System suitability solution—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.

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—Weigh a number of Capsules, equivalent to about 200 mg of isotretinoin. 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. Transfer 50.0 mL of this solution 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 (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 *System suitability solution* [NOTE—The injection volume is about 20 µL], 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 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—[NOTE—Protect the *System suitability solution*, the *Standard preparation*, the *Assay stock preparation*, and the *Assay preparation* from direct light.]

Diluent—Heat 0.1 N sodium hydroxide to about 60 ° to 70 °. Cool it to room temperature and purge with helium or nitrogen. Store it in a plastic container.

Solvent A—Prepare a solution of 0.5% acetic acid in methanol.

Solvent B—Prepare a solution of 0.5% acetic acid in water.

Mobile phase—Prepare a mixture of *Solvent A* and *Solvent B* (71:29). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve suitable quantities of USP Isotretinoin RS and USP Tretinoin RS in *Diluent* to obtain a solution having known concentrations of about 0.04 mg per mL of isotretinoin and 0.02 mg per mL of tretinoin.

Standard preparation—Dissolve an accurately weighed quantity of USP Isotretinoin RS in *Diluent*, and dilute with *Diluent*, stepwise if necessary, to obtain a solution having a known concentration of about 0.04 mg per mL.

Assay stock preparation—Transfer not fewer than 10 Capsules to a suitable volumetric flask. Add *Diluent* to the volumetric flask to fill about 50% of the volume, sonicate for 1 hour with occasional shaking to disperse all the contents, and make up the volume with *Diluent* to obtain a solution having a known concentration of about 0.4 mg per mL of isotretinoin.

Assay preparation—Transfer 5 mL of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass the solution through a suitable 0.45-µm or finer porosity membrane filter.

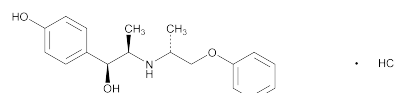
Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 353-nm detector and a 4.6-mm × 25-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the relative retention times for isotretinoin and tretinoin are about 1.0 and 1.3, respectively; the resolution, R , between isotretinoin and tretinoin is not less than 2.0; the tailing factor for the isotretinoin peak is not greater than 2.0; and the column efficiency determined from the isotretinoin peak is not less than 2000. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: 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 areas 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:

$$10(CV/N)(r_u / r_s)$$

in which C is the concentration, in mg per mL, of isotretinoin in the *Standard preparation*; V is the volume, in mL, of the volumetric flask used to prepare the *Assay stock preparation*; N is the number of Capsules taken; and r_u and r_s are the isotretinoin peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Isoxsuprine Hydrochloride



$C_{18}H_{23}NO_3 \cdot HCl$ 337.84
Benzenemethanol, 4-hydroxy- α -[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]-, hydrochloride, stereoisomer.

p-Hydroxy- α -[1-[(1-methyl-2-phenoxyethyl)amino]ethyl]benzyl alcohol hydrochloride.
(\pm)-(αR^*)-*p*-Hydroxy- α -[1-(1 S^*)-1-[(1 S^*)-1-methyl-2-phenoxyethyl]amino]ethyl]benzyl alcohol hydrochloride [579-56-6; 34331-89-0].

» Isoxsuprine Hydrochloride contains not less than 97.0 percent and not more than 103.0 percent of $C_{18}H_{23}NO_3 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards (11)—
USP Isoxsuprine Hydrochloride RS

Identification—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: 50 μ g per mL.

Medium: water.

C: To 1 mL of a solution (1 in 100), obtained by heating as necessary, add 3 mL of a 1 in 15 solution of sodium nitrite in 2 N sulfuric acid. Add ammonium hydroxide dropwise: a yellow precipitate is formed and it dissolves upon the addition of sodium hydroxide solution (1 in 5).

D: To 1 mL of a solution (1 in 100) add 1 mL of phosphomolybdic acid solution (1 in 100): a pale yellow to white precipitate is formed.

pH (791): between 4.5 and 6.0, in a solution (1 in 100).

Loss on drying (731)—Dry it at 105° for 1 hour: it loses not more than 0.5% of its weight.

Residue on ignition (281): not more than 0.2%.

Heavy metals, Method II (231): 0.002%.

Related compounds—To 10 mg, accurately weighed in a suitable vial, add 1 mL of *N*-trimethylsilylimidazole, and heat at 65° for 10 minutes. Add 5 mL of isooctane, wash with one 3-mL portion of water, and allow the layers to separate. Inject a 2- μ L portion of the isooctane solution into a gas chromatograph equipped with a 0.3-cm \times 2.0-m glass column packed with packing STA containing 3% liquid phase G2 and a flame-ionization detector. The column temperature is maintained at 215°, and the injection port and detector are maintained at 250°. The carrier gas is nitrogen, flowing at the rate of 25 mL per minute. Adjust the instrument to provide full-scale response for the major component. Inject a second 2- μ L portion of the isooctane solution with the attenuator adjusted to an 8-fold increase in sensitivity, and record the chromatogram from 0.5 to 1.5 relative to the retention time of the major peak. Measure the area of all minor peaks, and correct for differences in sensitivity settings. Calculate the percentage of related compounds present taken by the formula:

$$100A / B$$

in which *A* is the sum of the corrected area peaks for all minor peaks, and *B* is the sum of the corrected area peaks for the major and minor peaks. Not more than 2.0% is found.

Assay—Transfer about 50 mg of Isoxsuprine Hydrochloride, accurately weighed, to a 1000-mL volumetric flask, add water to volume, and mix. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Isoxsuprine Hydrochloride RS in the same medium having a known concentration of about 50 μ g per mL in 1-cm cells at the wavelengths of maximum absorbance at about 269 and 300 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{18}H_{23}NO_3 \cdot HCl$ in the Isoxsuprine Hydrochloride taken by the formula:

$$C(A_{U269} - A_{U300}) / (A_{S269} - A_{S300})$$

in which *C* is the concentration, in μ g per mL, of USP Isoxsuprine Hydrochloride RS in the Standard solution; and the par-

enthetic expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the assay solution (*U*) and the Standard solution (*S*), respectively.

Isoxsuprine Hydrochloride Injection

» Isoxsuprine Hydrochloride Injection is a sterile solution of Isoxsuprine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{18}H_{23}NO_3 \cdot HCl$.

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

USP Reference standards (11)—

USP Endotoxin RS

USP Isoxsuprine Hydrochloride RS

Identification—To a 60-mL separator transfer 10 mL of pH 9.0 buffer (prepared by mixing equal volumes of 0.1 M monobasic potassium phosphate and 0.1 N sodium hydroxide and, using a pH meter, adjusting to a pH of 9.0 by adding, as necessary, more of either solution) add 1 mL of Injection, and mix. Add 2 mL of chloroform, shake vigorously for 1 minute, filter the chloroform extract through a pledget of cotton, and mix the filtrate with 500 mg of potassium bromide. Evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask: the IR absorption spectrum of a potassium bromide dispersion of the isoxsuprine so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Isoxsuprine Hydrochloride RS that has been treated in the same manner.

Bacterial endotoxins (85)—It contains not more than 35.70 USP Endotoxin Units per mg of isoxsuprine hydrochloride.

pH (791): between 4.9 and 6.0.

Other requirements—It meets the requirements under *Injections* (1).

Assay—

pH 4.0 Citrate buffer—Mix equal volumes of 0.5 M citric acid and 0.5 M sodium citrate, and adjust, by the addition of either solution as necessary, the pH of the solution to 4.0 \pm 0.2.

Mixed solvent—Shake 40 mL of ether, 160 mL of isooctane, and 10 mL of water in a separator, remove and discard the water phase, and pass the solvent phase through a large pledget of cotton to remove excess water.

Standard preparation—Transfer about 40 mg of USP Isoxsuprine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 2 N sulfuric acid to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with 2 N sulfuric acid to volume, and mix. The concentration of USP Isoxsuprine Hydrochloride RS in the *Standard preparation* is about 80 μ g per mL.

Chromatographic column—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* (621)), packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 2 g of *Solid Support* and 1 mL of *pH 4.0 Citrate buffer*, and the upper segment is a mixture prepared as directed under *Assay preparation*.

Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 4 mg of isoxsuprine hydrochloride, to a 100-mL beaker, add 1 mL of dimethyl sulfoxide, and allow to stand for about 10 minutes, with occasional swirling. Add 1 mL of *pH 4.0 Citrate buffer* and 3 g of *Solid Support*, mix as directed under *Chromatographic column*, and transfer to the column. Pass 75 mL of *Mixed solvent* through the column, and discard the eluate. Elute the column with a solution prepared by mixing 0.2 mL of bis(2-ethylhexyl)phosphoric acid with 75 mL of *Mixed solvent*, and collect the eluate in a 125-mL