

Add the following:

■ Midazolam Injection

» Midazolam Injection is a sterile solution of Midazolam Hydrochloride in Water for Injection or of Midazolam in Water for Injection prepared with the aid of Hydrochloric Acid. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of midazolam ($C_{18}H_{13}ClFN_3$). It may contain Sodium Chloride, Benzyl Alcohol, and/or a chelating agent.

Packaging and storage—Preserve in single-dose containers, preferably of Type 1 glass. Store at between 15° and 30°.

Labeling—Label to indicate the vehicle used and the names and concentrations of any added preservatives. Indicate if the product is preservative-free.

USP Reference standards (11)—USP Benzyl Alcohol RS. USP Endotoxin RS. USP Midazolam RS.

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

Bacterial endotoxins (85)—It contains not more than 8.33 USP Endotoxin Units per mg of midazolam.

Change to read:

pH (791): between 2.5 and 3.7. (RB 1-Dec-2009).

Change to read:

Benzyl alcohol content (if present)—

Phosphate buffer—Dissolve 3.4 g of monobasic sodium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase—Prepare a filtered and degassed mixture of Phosphate buffer and acetonitrile (65 : 35). Make adjustments if necessary (see System Suitability under Chromatography (621)).

System suitability solution—Dissolve accurately weighed quantities of USP Midazolam RS and USP Benzyl Alcohol RS in Mobile phase to obtain a solution containing about 0.05 mg of USP Midazolam RS per mL and 0.5 mg of USP Benzyl Alcohol RS per mL.

Standard solution—Dissolve an accurately weighed quantity of USP Benzyl Alcohol RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.5 mg per mL.

Test solution—Transfer an accurately measured volume of Injection to a suitable volumetric flask, dilute with Mobile phase to obtain a concentration of about 0.5 mg per mL of benzyl alcohol, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the resolution, R , between benzyl alcohol and midazolam is not less than 6.0; the tailing factor is not more than 2.0 for benzyl alcohol;

and the relative standard deviation for replicate injections calculated for benzyl alcohol is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 μ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the benzyl alcohol peak. Calculate the quantity, in mg per mL, of benzyl alcohol in the volume of Injection taken by the formula:

$$DC(r_U / r_S)$$

in which D is the dilution factor used in preparing the Test solution; C is the concentration, in mg per mL, of USP Benzyl Alcohol RS in the Standard solution; and r_U and r_S are the peak responses of benzyl alcohol obtained from the Test solution and the Standard solution, respectively. The content of benzyl alcohol meets the requirements for Added Substances under Injections (1). (RB1-Dec-2009)

Particulate matter (788): meets the requirements for small-volume injections.

Related compounds—[NOTE—Protect all prepared Standard and sample solutions from light.]

Phosphate buffer, Solution A, Solution B, Mobile phase, and Standard preparation—Proceed as directed in the Assay.

Standard solution—Dilute an aliquot of the Standard preparation quantitatively, and stepwise if necessary, with Solution A to obtain a solution having a known concentration of about 0.5 μ g per mL.

Control solution—Dilute an aliquot of the Standard solution quantitatively with Solution A to obtain a solution having a known concentration of about 0.1 μ g per mL.

Test solution—Prepare as directed for Assay preparation in the Assay.

Chromatographic system (see Chromatography (621))—Proceed as directed in the Assay except to chromatograph the Standard solution and the Control solution, and record the peak responses as directed for Procedure: the column efficiency, determined from the Standard solution, is not less than 5500 theoretical plates; the tailing factor for the midazolam peak in the Standard solution is not more than 2.5; the signal-to-noise ratio for the Control solution is not less than 10; and the relative standard deviation for replicate injections of the Standard solution is not more than 8.0%.

Procedure—Separately inject equal volumes (about 50 μ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(1/F)(C_S / C_T)(r_i / r_S)$$

in which F is the relative response factor and is equal to 0.51 for the peak eluting at a relative retention between 0.79 and 0.97 with respect to midazolam, and is equal to 1.0 for all other peaks; C_S is the concentration, in mg per mL, of midazolam in the Standard solution; C_T is the concentration, in mg per mL, of midazolam in the Test solution based on the label claim; r_i is the individual impurity peak response obtained from the Test solution; and r_S is the midazolam peak response obtained from the Standard solution. Not more than 0.5% of the largest known impurity is found; not more than 0.1% of the largest unknown impurity is found; and not more than 1.0% of total impurities is found. [NOTE—Disregard all solvent- and excipient-related peaks.]

Other requirements: meets the requirements for Sterility Tests (71) and Injections (1).

Assay—[NOTE—Protect all prepared Standard and sample solutions from light.]

Phosphate buffer—Dissolve 13.4 g of dibasic sodium phosphate heptahydrate in water, dilute with water to 2000 mL, and mix. Adjust with phosphoric acid to a pH of 5.0 \pm 0.1.

Solution A—Prepare a filtered and degassed mixture of Phosphate buffer, acetonitrile, and methanol (90 : 80 : 30).

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Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Phosphate buffer* (75 : 25).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Midazolam RS in about 2 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a solution having a known concentration of about 0.2 mg per mL.

Assay preparation—[NOTE—The midazolam present in the Injection converts from open-ring to the closed-ring form when diluted with *Solution A*. The midazolam potency is determined based on the peak area of the closed-ring form. It takes approximately 60 minutes at 40° or 2 to 3 hours at room temperature to complete the conversion. The *Standard preparation* is not subject to this conversion process.] Transfer an accurately measured volume of Injection to a suitable volumetric flask, and dilute with *Solution A* to obtain a solution containing about 0.2 mg of midazolam per mL, and mix. Transfer the resulting solution into suitable crimp top vials, seal tightly, and heat at about 40° for 60 minutes. Allow the *Assay preparation* to cool to room temperature before injection.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	<i>Solution A</i> (%)	<i>Solution B</i> (%)	Elution
0–15	100	0	isocratic
15–20	100→0	0→100	linear gradient
20–35	0	100	isocratic
35–37	0→100	100→0	linear gradient
37–45	100	0	re-equilibration

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5500 theoretical plates; the tailing factor is not more 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 preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the midazolam peaks. Calculate the percentage of the labeled amount of midazolam (C₁₈H₁₃ClFN₃) in the portion of Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which *C_S* is the concentration, in mg per mL, of midazolam in the *Standard preparation*; *C_U* is the concentration, in mg per mL, of midazolam in the *Assay preparation*, based on the label claim; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. ■2S (USP32)