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Midazolam Injection

DEFINITION

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 NLT 90.0% and NMT 110.0% of the labeled amount of midazolam ($C_{18}H_{13}ClFN_3$). It may contain Sodium Chloride, Benzyl Alcohol, and/or a chelating agent.

IDENTIFICATION

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

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

• **Procedure**

Buffer: 6.7 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of 5.0 ± 0.1.

Solution A: Prepare a filtered and degassed mixture of acetonitrile, methanol and *Buffer* (8:3:9).

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100
35	0	100
37	100	0
45	100	0

Standard solution: Dissolve USP Midazolam RS in about 2 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a 0.2-mg/mL solution.

Sample solution: [Note—The midazolam present in the Injection converts from the open-ring form 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 min at 40° or 2–3 h at room temperature to complete the conversion. The *Standard solution* is not subject to this conversion process.] Transfer a volume of Injection to a suitable volumetric flask, and dilute with *Solution A* to obtain a solution containing about 0.2 mg/mL of midazolam. Transfer the resulting solution into suitable crimp top vials, seal tightly, and heat at about 40° for 60 min. Allow this solution to cool to room temperature before injection.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5500 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of $C_{18}H_{13}ClFN_3$ in the portion of Injection taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

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

• **Procedure**

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: Use *Standard solution* in the *Assay*.

Standard solution: 0.5 µg/mL USP Midazolam RS in *Solution A* from *Standard stock solution*

Control solution: 0.1 µg/mL USP Midazolam RS in *Solution A* from *Standard solution*

System suitability

Samples: *Standard solution* and *Control solution*

Suitability requirements

Tailing factor: NMT 2.5 for midazolam peak, *Standard solution*

Column efficiency: NLT 5500 theoretical plates, *Standard solution*

Signal-to-noise ratio: NLT 10, *Control solution*

Relative standard deviation: NMT 8.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*

r_S = peak response of midazolam from the *Standard solution*

C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

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

F = relative response factor; 0.51 for the peak eluting at a relative retention between 0.79 and 0.97 with respect to midazolam; 1.0 for all other peaks

Acceptance criteria

Individual known impurity: NMT 0.5%

Individual unknown impurity: NMT 0.1%

Total impurities: NMT 1.0%

[Note—Disregard all solvent- and excipient-related peaks.]

SPECIFIC TESTS

• **Benzyl Alcohol Content** (if present)

Buffer: 3.4 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (7:13)

System suitability solution: 0.05 mg/mL of USP Midazolam RS and 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Standard solution: 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Sample solution: Transfer a measured volume of Injection to a suitable volumetric flask. Dilute with *Mobile phase* to obtain a concentration of about 0.5 mg/mL of benzyl alcohol, based on the labeled content of benzyl alcohol in the Injection.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; L1 packing

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between benzyl alcohol and midazolam

Tailing factor: NMT 2.0 for benzyl alcohol

Relative standard deviation: NMT 2.0% for benzyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the volume of Injection taken:

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

r_U = peak response of benzyl alcohol from the *Sample solution*

r_S = peak response of benzyl alcohol from the *Standard solution*

C_S = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: The content of benzyl alcohol meets the requirements in *Injections and Implanted Drug Products ⟨1⟩, Specific Tests, Vehicles and added substances*.

• **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections

• **Bacterial Endotoxins Test ⟨85⟩:** It contains NMT 8.33 USP Endotoxin Units/mg of midazolam.

• **pH ⟨791⟩:** 2.5–3.7

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Other Requirements:** It meets the requirements for *Injections and Implanted Drug Products ⟨1⟩*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in single-dose containers, preferably of Type 1 glass. Store 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 Midazolam RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
MIDAZOLAM INJECTION	Documentary Standards Support	SM52020 Small Molecules 5

Topic/Question	Contact	Expert Committee
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM52020 Small Molecules 5

Chromatographic Database Information: Chromatographic Database

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