Methotrexate Injection

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Expert Committee	Chemical Medicines Monographs 3
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 3 Expert Committee has revised the Methotrexate Injection monograph. The purpose for the revision is to widen the acceptance criteria for methotrexate related compound C in the test for *Organic Impurities* from NMT 3.0% to NMT 4.0% to be consistent with the FDA-approved specification.

The Methotrexate Injection Revision Bulletin supersedes the currently official monograph.

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

Methotrexate Injection

DEFINITION

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

Sample: Dilute, if necessary, a volume of Injection, equivalent to about 25 mg of methotrexate, with water to obtain a solution with a concentration of about 2.5 mg/mL. Adjust with 0.1 N hydrochloric acid to a pH of 4.0. Place the slurry in a 50-mL centrifuge tube, and centrifuge. Decant the supernatant, add 25 mL of acetone, shake, and pass through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.

Acceptance criteria: Meets the requirements

• **B.** 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**

Buffer: 3.4 mg/mL of anhydrous monobasic sodium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 6.0.

Solution A: Acetonitrile and *Buffer* (5:95)

Solution B: Acetonitrile and *Buffer* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0
30	50	50
34	50	50
35	100	0
40	100	0

Standard solution: 0.2 mg/mL of USP Methotrexate RS in *Solution A* prepared as follows. Add a sufficient amount of USP Methotrexate RS to a suitable volumetric flask and add dimethyl sulfoxide equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume.

Sample solution: Nominally 0.2 mg/mL of methotrexate from Injection prepared as follows. Transfer a sufficient amount of Injection to an appropriate volumetric flask. Add about 5% of the flask volume of dimethyl sulfoxide and sonicate for 2 min at ambient temperature, then add 30% of the flask volume of *Solution A* and sonicate. Dilute with *Solution A* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 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 methotrexate ($C_{20}H_{22}N_8O_5$) 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 Methotrexate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

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

Standard solution: 0.2 µg/mL each of USP Methotrexate RS, USP Methotrexate Related Compound B RS, USP Methotrexate Related Compound C RS, and USP Methotrexate Related Compound E RS in *Solution A* prepared as follows. Add a sufficient amount of each Reference Standard to a suitable volumetric flask and add dimethyl sulfoxide equivalent to 5% of the flask volume. Sonicate to achieve dissolution, then dilute with *Solution A* to volume. Sonicate if necessary to aid dissolution.

Sample solution: Nominally 0.2 mg/mL of methotrexate from Injection prepared as directed in the *Assay*

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between methotrexate related compound B and methotrexate related compound C

Relative standard deviation: NMT 5.0% each for methotrexate, methotrexate related compound B, methotrexate related compound C, and methotrexate related compound E

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methotrexate related compound B and methotrexate related compound C in the portion of Injection taken:

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

r_U = peak response of each corresponding impurity from the *Sample solution*

r_S = peak response of each corresponding Reference Standard from the *Standard solution*

C_S = concentration of each corresponding Reference Standard in the *Standard solution* (µg/mL)

C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Calculate the percentage of methotrexate related compound E free acid in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Methotrexate Related Compound E RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of methotrexate related compound E free acid, 325.33
M_{r2} = molecular weight of USP Methotrexate Related Compound E RS, 343.56

[NOTE—USP Methotrexate Related Compound E RS is 4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino} benzoic acid, hemihydrochloride.]

Calculate the percentage of any individual unspecified degradation product in the portion of Injection taken:

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

r_U = peak response of each unspecified degradation product from the *Sample solution*
r_S = peak response of methotrexate from the *Standard solution*
C_S = concentration of USP Methotrexate RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)

Acceptance criteria: See *Table 2*. The reporting threshold is 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methotrexate related compound B	0.67	0.3
Methotrexate related compound C	0.73	▲4.0▲ (RB 1-May-2019)
Methotrexate	1.0	—
Methotrexate related compound E free acid[a]	1.41	0.3
Any individual unspecified degradation product	—	0.2
Total unspecified degradation products	—	1.0

[a] 4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino}benzoic acid.

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 7.0–9.0
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 0.4 USP Endotoxin Units/mg of methotrexate sodium
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩

USP Methotrexate RS
USP Methotrexate Related Compound B RS
(*S*)-2-{4-[(2,4-Diaminopteridin-6-yl)methylamino] benzamido}pentanedioic acid.
$C_{19}H_{20}N_8O_5$ 440.41
USP Methotrexate Related Compound C RS
(*S*)-2-(4-{[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl) methyl](methyl)amino}benzamido)pentanedioic acid.
$C_{20}H_{21}N_7O_6$ 455.42
USP Methotrexate Related Compound E RS
4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino} benzoic acid, hemihydrochloride.
$C_{15}H_{15}N_7O_2 \cdot ½HCl$ 343.56 (anhydrous)
$C_{15}H_{15}N_7O_2$ 325.33 (free acid)

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