Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-May-2020
Document Type: USP Monographs
DocId: GUID-054C8526-37E9-4700-A2C3-3EA3E52E265A_5_en-US
DOI: https://doi.org/10.31003/USPNF_M51460_05_01
DOI Ref: p945h

Methotrexate for Injection

DEFINITION

Methotrexate for Injection is a sterile, freeze-dried preparation of methotrexate sodium with or without suitable added substances, buffers, and/or diluents. It contains NLT 95.0% and NMT 115.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

[**Caution**—Great care should be taken to prevent inhaling particles of methotrexate sodium and exposure to skin.]

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

Sample: 2.5 mg/mL of methotrexate in water from Methotrexate for Injection. 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

Add the following:

▲• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-Aug-2019)

ASSAY

Change to read:

• **Procedure**

Buffer: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37); adjusted, if necessary, with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0

Mobile phase: Acetonitrile and *Buffer* ▲(8:92)▲ (USP 1-Aug-2019)

Standard solution: 0.1 mg/mL of USP Methotrexate RS in *Mobile phase*

Sample solution: Nominally equivalent to 0.1 mg/mL of methotrexate from 1 container of Methotrexate for Injection in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 302 nm

Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-Aug-2019) packing L1

▲**Autosampler temperature:** 5 ± 3°▲ (USP 1-Aug-2019)

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: ▲*Standard solution*▲ (USP 1-Aug-2019)

Suitability requirements

▲**Tailing factor:** NMT 2.0▲ (USP 1-Aug-2019)

Relative standard deviation: NMT ▲1.0%▲ (USP 1-Aug-2019)

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 Methotrexate for 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* (mg/mL)

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

Acceptance criteria: 95.0%–115.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meets the requirements

IMPURITIES

Add the following:

▲• **Organic Impurities**

Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability stock solution: 0.05 mg/mL each of USP Methotrexate Related Compound B RS and USP Methotrexate Related Compound C RS prepared as follows. Transfer USP Methotrexate Related Compound B RS and USP Methotrexate Related Compound C RS into a suitable volumetric flask, add ammonium hydroxide equivalent to 2.5% of the final volume, and sonicate for 5 min. Add *Mobile phase* equivalent to 20% of the final volume and continue to sonicate until completely dissolved. Dilute with *Mobile phase* to volume.

System suitability solution: 2.5 µg/mL each of USP Methotrexate Related Compound B RS and USP Methotrexate Related Compound C RS in *Mobile phase* from *System suitability stock solution*

Sensitivity solution: 0.25 µg/mL of USP Methotrexate RS in *Mobile phase* from *Standard solution*

Sample solution: Nominally equivalent to 0.5 mg/mL of methotrexate from 1 container of Methotrexate for Injection in *Mobile phase*

System suitability

Samples: *System suitability solution* and *Sensitivity solution*

Suitability requirements

Resolution: NLT 1.2 between the methotrexate related compound B and methotrexate related compound C peaks, *System suitability solution*

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

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual impurity in the portion of Methotrexate for Injection taken:

$$\text{Result} = r_U/\{\Sigma[r_U \times (1/F)] + r_M\} \times (1/F) \times 100$$

r_U = peak area of each individual impurity from the *Sample solution*

F = relative response factor for each individual impurity (see *Table 1*)

r_M = peak area of methotrexate from the *Sample solution*

Table 1

Name	Relative Retention Time	Relative Response Factor
Methotrexate related compound B	0.36	0.72
Methotrexate related compound C	0.42	1.0
Methotrexate	1.0	—
Any other individual impurity	—	1.0

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Acceptance Criteria, NMT (%)
Each individual impurity	0.5
Total impurities	2.0

▲ (USP 1-Aug-2019)

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample: Constitute as directed in the labeling, except that water is used as the diluent.

Acceptance criteria: 7.0–9.0

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩*, Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*.

Change to read:

• **Bacterial Endotoxins Test** ⟨85⟩**:** ▲Meets the requirements▲ (USP 1-Aug-2019)

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

• **Other Requirements:** Meets the requirements in *Labeling* ⟨7⟩*, Labels and Labeling for Injectable Products*

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements* ⟨659⟩*, Injection Packaging*, protected from light.

Change to read:

• **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 1-Aug-2019)

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

Topic/Question	Contact	Expert Committee
METHOTREXATE FOR INJECTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 44(3)

Current DocID: GUID-054C8526-37E9-4700-A2C3-3EA3E52E265A_5_en-US

DOI: https://doi.org/10.31003/USPNF_M51460_05_01

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