Methotrexate Injection

**Type of Posting**  
Revision Bulletin

**Posting Date**  
26–Apr–2019

**Official Date**  
01–May–2019

**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 (C₂₉H₂₈N₄O₈).

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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>34</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>35</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**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 directed in the Assay. 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 (C₂₉H₂₈N₄O₈) in the portion of Injection taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_U}{C_D} \right) \times 100
\]

\[
r_U = \text{peak response from the Sample solution}
\]

\[
r_S = \text{peak response from the Standard solution}
\]

\[
C_U = \text{nominal concentration of methotrexate in the Sample solution (µg/mL)}
\]

**Acceptance criteria:** 90.0%–110.0%

IMPURITIES

**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 µg/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 the labeled amount of methotrexate related compound B and methotrexate related compound C in the portion of Injection taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_U}{C_D} \right) \times (M_U/M_D) \times 100
\]

\[
r_U = \text{peak response of each corresponding impurity from the Sample solution}
\]

\[
r_S = \text{peak response of each corresponding Reference Standard from the Standard solution}
\]

\[
C_U = \text{concentration of each corresponding Reference Standard in the Standard solution (µg/mL)}
\]

\[
C_D = \text{nominal concentration of methotrexate in the Sample solution (µg/mL)}
\]

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

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_U}{C_D} \right) \times (M_U/M_D) \times 100
\]
2 Methotrexate

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

(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:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100
\]

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

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

### Table 2 (continued)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate related compound B</td>
<td>0.67</td>
<td>0.3</td>
</tr>
<tr>
<td>Methotrexate related compound C</td>
<td>0.73</td>
<td>☑️ 4.0 (RB 1-May-2019)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

*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.
  - USP Methotrexate Related Compound C RS (S)-2-4-[(2-Amino-4-oxo-1,4-dihydropteridin-6-yl)methyl](methyl)amino)benzamido)pentanedioic acid.
  - USP Methotrexate Related Compound E RS 4-[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino)benzoic acid, hemihydrochloride.
  - USP Methotrexate Related Compound E RS 4-[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino)benzoic acid, hemihydrochloride.
  - USP Methotrexate Related Compound E RS 4-[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino)benzoic acid, hemihydrochloride.