Methotrexate

\[
\text{C}_{20}\text{H}_{22}\text{N}_{8}\text{O}_{5} \quad 454.44
\]

\(\text{L-}\)-Glutamic acid, \(\text{N-}\{[\{2,4\text{-diamino-6-pter-}
\text{idinyl}\text{methyl}\text{methylamino}\text{benzoyl}\}}\};
\]

\(\text{L-}\text{(+-)}\text{N-}\{[\{2,4\text{-Diamino-6-pter-}
\text{idinyl}\text{methyl}\text{methylamino}\text{benzoyl}]\text{glutamic acid} \quad [59-05-2].
\]

**DEFINITION**

Methotrexate is a mixture of 4-amino-10-methylfolic acid and Methotrexate Related Compound C RS, and USP RS, USP Methotrexate Related Compound B RS, USP.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K): Do not dry specimens.
- **B. ULTRAVIOLET ABSORPTION** (197U):
  - Sample solution: 10 \(\mu\text{g/mL}\) in 0.1 N hydrochloric acid

**ASSAY**

**PROCEDURE**

- **Solution A**: 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary to 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0
- **Mobile phase**: Acetonitrile and Solution A (1:9)
- **System suitability solution**: 0.1 mg/mL each of USP Methotrexate RS and folic acid in Mobile phase
- **Standard solution**: 100 \(\mu\text{g/mL}\) of USP Methotrexate RS in Mobile phase
- **Sample solution**: 100 \(\mu\text{g/mL}\) of Methotrexate in Mobile phase

**Chromatographic system**

- **Mode**: LC
- **Detector**: UV 302 nm
- **Column**: 4.6-mm \(\times\) 25-cm; packing L1
- **Flow rate**: 1.2 mL/min
- **Injection size**: 10 \(\mu\text{L}\)

**System suitability**

- **Sample**: System suitability solution
  - **Resolution**: NLT 8.0 between the folic acid and methotrexate peaks
  - **Relative standard deviation**: NMT 2.5% for the methotrexate peak

**Analysis**

- **Samples**: Standard solution and Sample solution
  - Calculate the percentage of \(\text{C}_{20}\text{H}_{22}\text{N}_{8}\text{O}_{5}\) in the portion of Methotrexate taken:

  \[
  \text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100
  \]

  - \(r_u = \text{peak response from the Sample solution}\)
  - \(r_s = \text{peak response from the Standard solution}\)
  - \(C_s = \text{concentration of USP Methotrexate RS in the Standard solution (\(\mu\text{g/mL}\))}\)
  - \(C_u = \text{concentration of Methotrexate in the Sample solution (mg/mL)}\)

  - Calculate the percentage of 4-\(\{[\{2,4\text{-diaminopteridin-6-}
  \text{y}l\text{methyl}\text{methylamino}\}\text{benzoic acid in the portion of Methotrexate taken:}\)

  \[
  \text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_1/M_2) \times 100
  \]

  - \(r_u = \text{peak response from the Sample solution}\)
  - \(r_s = \text{peak response from the Standard solution}\)
  - \(C_s = \text{concentration of USP Methotrexate Related Compound E RS in the Standard solution (mg/mL)}\)
  - \(C_u = \text{concentration of Methotrexate in the Sample solution (mg/mL)}\)
  - \(M_1 = \text{molecular weight of 4-\{[\{2,4\text{-diaminopteridin-6-}
  \text{yl}methyl\text{methylamino}\}\}\text{benzoic acid, 325.33}\)
  - \(M_2 = \text{molecular weight of USP Methotrexate Related Compound E RS, 343.56}\)

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

Calculate the percentage of MTX-1-monomethylester, MTX-5-monomethylester, and any unspecified impurity in the portion of Methotrexate taken:

Result = \( \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100 \)

\( r_U \) = peak response from the Sample solution
\( r_S \) = peak response of methotrexate from the Standard solution
\( C_S \) = concentration of Methotrexate in the Standard solution (mg/mL)
\( C_U \) = concentration of Methotrexate in the Sample solution (mg/mL)

Acceptance criteria

Individual impurities: See Impurity Table 1. [NOTE—Disregard any unknown peak with an area less than the area of the methotrexate peak from the Sensitivity solution.]

### Impurity Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate related compound C</td>
<td>0.52</td>
<td>0.5</td>
</tr>
<tr>
<td>Methotrexate related compound B</td>
<td>0.59</td>
<td>0.3</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>4-[[2,4-Diaminopteridin-6-yl]methyl][methylamino]benzoic acid</td>
<td>2.16</td>
<td>0.3</td>
</tr>
</tbody>
</table>

### Impurity Table 1 (continued)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX-1-monomethylester</td>
<td>8.54</td>
<td>0.2</td>
</tr>
<tr>
<td>MTX-5-monomethylester</td>
<td>9.08</td>
<td>0.2</td>
</tr>
<tr>
<td>Any unspecified impurity</td>
<td>—</td>
<td>0.10</td>
</tr>
<tr>
<td>Total unspecified impurities</td>
<td>—</td>
<td>0.5</td>
</tr>
<tr>
<td>*(3)-2-(4-[[2,4-diaminopteridin-6-yl]methyl][methylamino]benzamido)-5-methoxy-5-oxopentanoic acid.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>*(3)-4-(4-[[2,4-diaminopteridin-6-yl]methyl][methylamino]benzamido)-5-methoxy-5-oxopentanoic acid.</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**SPECIFIC TESTS**
- **OPTICAL ROTATION, Specific Rotation (781S):** +19° to +24°, a 2-dm polarimeter tube being used
- **Sample solution:** 10 mg/mL in 0.05 M sodium carbonate

**WATER DETERMINATION, Method I (921):** NMT 12.0%

**ADDITIONAL REQUIREMENTS**
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
  USP Methotrexate RS
  USP Methotrexate Related Compound B RS
  USP Methotrexate Related Compound C RS
  USP Methotrexate Related Compound E RS