r_U = peak area of *R*-methotrexate from the *Sample solution*
r_S = peak area of Methotrexate from the *Diluted sample solution*
Acceptance criteria: NMT 3.0%

SPECIFIC TESTS
- **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
  USP Methotrexate System Suitability Mixture RS
  It contains Methotrexate, Methotrexate Dimethylester Hydrochloride
  (*S*)-Dimethyl-2-(4-{[(2,4-diaminopteridin-6-yl)methyl](methyl)amino}benzamido) pentanedioate hydrochloride.
  $C_{22}H_{26}N_8O_5 \cdot HCl$ 518.95
  and a small amount of Methotrexate related compound I
  (*S*)-4-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)-amino}benzamido)5-methoxy-5-oxopentanoic acid.
  $C_{21}H_{24}N_8O_5$ 468.47
  and Methotrexate related compound H
  (*S*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)-amino}benzamido)5-methoxy-5-oxopentanoic acid.
  $C_{21}H_{24}N_8O_5$ 468.47
  USP *R*-Methotrexate RS
  (*R*)-2-(4-{[(2,4-Diaminopteridin-6-yl)methyl](methyl)-amino}benzamido)pentanedioic acid.
  $C_{20}H_{22}N_8O_5$ 454.44

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 having 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 filter through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.
  Acceptance criteria: Meets the requirements

ASSAY
- **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* (10:90)
  System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*
  Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*
  Sample solution: Equivalent to 100 µg/mL of methotrexate from Injection in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 302 nm
  Column: 4.6-mm × 25-cm; packing L1
  Flow rate: 1.2 mL/min
  Injection volume: 10 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]
  Suitability requirements
  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 the labeled amount 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%

SPECIFIC TESTS
- **pH** ⟨**791**⟩**:** 7.0–9.0
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** NMT 0.4 USP Endotoxin Unit/mg of methotrexate sodium
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections* ⟨1⟩

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS
  USP Methotrexate RS

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
- **A. INFRARED ABSORPTION** ⟨**197K**⟩
  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 filter through a solvent-resistant membrane filter of 0.45-µm pore size. Air-dry the filtered precipitate.
  Acceptance criteria: Meets the requirements

ASSAY
- **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* (10:90)
System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*
Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*
Sample solution: Equivalent to 100 µg/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; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]
Suitability requirements
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 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* (µg/mL)
C_U = nominal concentration of methotrexate in the *Sample solution* (µg/mL)
Acceptance criteria: 95.0%–115.0%

PERFORMANCE TESTS
• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

SPECIFIC TESTS
• **pH ⟨791⟩**
Sample: Constituted 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* ⟨1⟩, *Constituted Solutions*.
• **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 0.4 USP Endotoxin Unit/mg of methotrexate sodium
• **STERILITY TESTS ⟨71⟩:** Meets the requirements
• **OTHER REQUIREMENTS:** Meets the requirements in *Injections* ⟨1⟩, *Labeling*

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve as described in *Injections* ⟨1⟩, *Containers for Sterile Solids*, protected from light.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Endotoxin RS
USP Methotrexate RS

Methotrexate Tablets

DEFINITION
Methotrexate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$).

IDENTIFICATION
• **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
Standard solution: 25 µg/mL of USP Methotrexate RS in dilute hydrochloric acid (1 in 100)
Sample solution: Dissolve 1 Tablet in 100 mL of dilute hydrochloric acid (1 in 100), and filter the solution.
Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

ASSAY
• **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* (10:90)
System suitability solution: 0.1 mg/mL each of USP Methotrexate RS and folic acid in *Mobile phase*
Standard solution: 100 µg/mL of USP Methotrexate RS in *Mobile phase*
Sample solution: Equivalent to 100 µg/mL of methotrexate from powdered Tablets (NLT 20 Tablets) in *Mobile phase*. Dissolve the methotrexate using a mechanical shaker or ultrasonic bath.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 302 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1.2 mL/min
Injection volume: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for folic acid and methotrexate are 0.35 and 1.0, respectively.]
Suitability requirements
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 the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Tablets 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%

PERFORMANCE TESTS
• **DISSOLUTION ⟨711⟩**
Medium: 0.1 N hydrochloric acid; 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Standard solution: USP Methotrexate RS in *Medium*
Detector: UV 306 nm (maximum absorbance)
Analysis: Determine the amount of $C_{20}H_{22}N_8O_5$ dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a *Standard solution*.
Tolerances: NLT 75% (*Q*) of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$) is dissolved.
• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in well-closed containers. A unit-of-use container contains a quantity of Tablets sufficient to provide one week's therapy as indicated in the labeling.