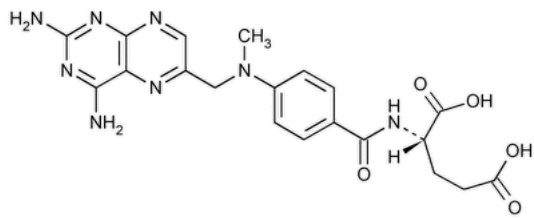


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Methotrexate



$C_{20}H_{22}N_8O_5$ 454.45
L-Glutamic acid, N-[4-[(2,4-diamino-6-pteridiny)l)methyl]methylamino]benzoyl];
L-(+)-N-(p-[(2,4-Diamino-6-pteridiny)l)methyl]methylamino)benzoyl)glutamic acid;
(S)-2-(4-[(2,4-Diaminopteridin-6-yl)methyl](methyl)amino)benzamido)pentanedioic acid;
4-Amino-10-methylfolic acid CAS RN®: 59-05-2; UNII: YL5FZ2Y5U1.

DEFINITION
Methotrexate contains NLT 98.0% and NMT 102.0% of methotrexate ($C_{20}H_{22}N_8O_5$), calculated on the anhydrous basis.
[CAUTION—Great care should be taken to prevent inhaling particles of methotrexate and exposing the skin to it.]

IDENTIFICATION
• **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197A or 197K. Do not dry specimens.
• **B. SPECTROSCOPIC IDENTIFICATION TESTS (197), Ultraviolet-Visible Spectroscopy:** 197U
Sample solution: 10 µg/mL of Methotrexate in [0.1 N hydrochloric acid VS](#)
Acceptance criteria: Meets the requirements

ASSAY
Change to read:
• **PROCEDURE**
Buffer: 3.4 g/L of [anhydrous monobasic sodium phosphate](#) in [water](#). Adjust with ▲1 N [sodium hydroxide](#)▲ (ERR 1-Oct-2024) to a pH of 5.8.
Solution A: [Acetonitrile](#) and *Buffer* (1:19)
Solution B: [Acetonitrile](#) and *Buffer* (1:1)
Mobile phase: See [Table 1](#).

Table 1

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

Standard stock solution: 1.0 mg/mL of [USP Methotrexate RS](#) prepared as follows. Transfer a known amount of [USP Methotrexate RS](#) to a suitable volumetric flask, dissolve in [dimethyl sulfoxide](#) equivalent to 5% of the final volume, and dilute with *Solution A* to volume.
Standard solution: 0.2 mg/mL of [USP Methotrexate RS](#) from the *Standard stock solution*, in *Solution A*
Sample stock solution: 1.0 mg/mL of Methotrexate prepared as follows. Transfer a known amount of Methotrexate to a suitable volumetric flask, dissolve in [dimethyl sulfoxide](#) equivalent to 5% of the final volume, and dilute with *Solution A* to volume.
Sample solution: 0.2 mg/mL of Methotrexate from the *Sample stock solution*, in *Solution A*

Chromatographic system(See [Chromatography \(621\)](#), [System Suitability](#).)**Mode:** LC**Detector:** UV 280 nm**Column:** 4.6-mm × 25-cm; 5-μm packing [L1](#)**Column temperature:** 15°**Flow rate:** 1.0 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.6**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of methotrexate ($C_{20}H_{22}N_8O_5$) in the portion of Methotrexate taken:

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

 r_U = peak response of methotrexate 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* (mg/mL) C_U = concentration of Methotrexate in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis**IMPURITIES**• **RESIDUE ON IGNITION (281):** NMT 0.1%• **ORGANIC IMPURITIES, PROCEDURE 1: RELATED COMPOUNDS****Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.**Standard stock solution A:** Use the *Standard solution* from the Assay.**Standard solution A:** 0.1 μg/mL of [USP Methotrexate RS](#) in *Solution A*, from *Standard stock solution A***Standard stock solution B:** 0.1 mg/mL of [USP Methotrexate Related Compound B RS](#), 0.2 mg/mL of [USP Methotrexate Related Compound C RS](#), and 0.1 mg/mL of [USP Methotrexate Related Compound E RS](#) prepared as follows. Transfer known quantities of [USP Methotrexate Related Compound B RS](#), [USP Methotrexate Related Compound C RS](#), and [USP Methotrexate Related Compound E RS](#) to a suitable volumetric flask. Dissolve in [dimethyl sulfoxide](#) equivalent to 5% of the final volume. Add 75% of the flask volume of *Solution A* and sonicate to dissolve, if needed. Dilute with *Solution A* to volume.**Standard solution B:** 0.4 μg/mL of [USP Methotrexate Related Compound C RS](#), 0.2 μg/mL of [USP Methotrexate Related Compound B RS](#), and 0.2 μg/mL of [USP Methotrexate Related Compound E RS](#) from *Standard stock solution B*, in *Solution A***System suitability solution:** 0.1 mg/mL of [USP Methotrexate System Suitability Mixture RS](#), 0.2 μg/mL of [USP Methotrexate Related Compound B RS](#), 0.4 μg/mL of [USP Methotrexate Related Compound C RS](#), and 0.2 μg/mL of [USP Methotrexate Related Compound E RS](#) prepared as follows. Transfer a known quantity of [USP Methotrexate System Suitability Mixture RS](#) to a suitable volumetric flask, and dissolve in [dimethyl sulfoxide](#) equivalent to about 2% of the final volume. Add *Standard stock solution B* equivalent to 0.2% of the final volume, and dilute with *Solution A* to volume.**System suitability****Samples:** *Standard solution A* and *System suitability solution***Suitability requirements****Resolution:** NLT 1.7 between methotrexate related compound B and methotrexate related compound C; NLT 10.0 between methotrexate related compound C and methotrexate; and NLT 5.0 between methotrexate related compound I and methotrexate related compound H, *System suitability solution***Relative standard deviation:** NMT 5.0%, *Standard solution A***Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

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

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

 r_U = peak response of methotrexate related compound B or methotrexate related compound C from the *Sample solution*