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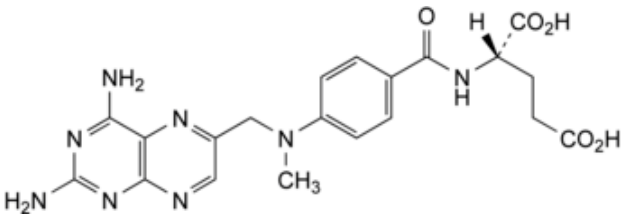
Edition: BP 2025 (Ph. Eur. 11.6 update)

Methotrexate

General Notices

(Ph. Eur. monograph 0560)



$C_{20}H_{22}N_8O_5$ 454.4 *59-05-2*

Action and use

Dihydrofolate reductase inhibitor; cytostatic.

Preparations

Methotrexate Injection

Methotrexate Oral Solution

Methotrexate Tablets

Ph Eur

DEFINITION

(2*S*)-2-[[4-[[(2,4-Diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid.

Content

97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

Yellow or orange, crystalline, hygroscopic powder.

Solubility

Practically insoluble in water, in ethanol (96 per cent) and in methylene chloride. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides and carbonates.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *methotrexate CRS*.

TESTS

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 40.0 mg of the substance to be examined in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 100.0 mL with mobile phase A.

Test solution (b) Dissolve 25.0 mg of the substance to be examined in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 50.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (a) Dissolve 25.0 mg of *methotrexate CRS* in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 50.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with mobile phase A. Dilute 5.0 mL of this solution to 50.0 mL with mobile phase A.

Reference solution (c) Dilute 5.0 mL of reference solution (b) to 25.0 mL with mobile phase A.

Reference solution (d) Dissolve 5 mg of the substance to be examined, 5 mg of *4-aminofolic acid R* (impurity B), 5 mg of *methotrexate impurity C CRS*, 5 mg of *methotrexate impurity D CRS* and 5 mg of *methotrexate impurity E CRS* in a mixture of 0.5 mL of *dilute ammonia R1* and 5 mL of mobile phase A and dilute to 100 mL with mobile phase A.

Reference solution (e) Dissolve 8 mg of *methotrexate for peak identification CRS* (containing impurities H and I) in a mixture of 0.1 mL of *dilute ammonia R1* and 1 mL of mobile phase A and dilute to 20 mL with mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 5 volumes of *acetonitrile R* and 95 volumes of a 3.4 g/L solution of *anhydrous sodium dihydrogen phosphate R* previously adjusted to pH 6.0 with a 42 g/L solution of *sodium hydroxide R*;

— *mobile phase B*: mix 50 volumes of *acetonitrile R* and 50 volumes of a 3.4 g/L solution of *anhydrous sodium dihydrogen phosphate R* previously adjusted to pH 6.0 with a 42 g/L solution of *sodium hydroxide R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 20	100 → 95	0 → 5
20 - 28	95 → 50	5 → 50
28 - 37	50	50

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c), (d) and (e).

Identification of impurities Use the chromatogram supplied with *methotrexate for peak identification CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities H and I; use the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B, C, D and E.

Relative retention With reference to methotrexate (retention time = about 18 min): impurity B = about 0.3; impurity C = about 0.4; impurity D = about 0.9; impurity E = about 1.4; impurity I = about 1.5; impurity H = about 1.6.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to impurities B and C and minimum 1.5 between the peaks due to impurity D and methotrexate, in the chromatogram obtained with reference solution (d); minimum 1.5 between the peaks due to impurities I and H in the chromatogram obtained with reference solution (e).

Limits:

— *correction factors*: multiply the peak area of the impurity by its correction factor: impurity E = 0.8; impurity I = 1.4;

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurities B, E*: for each impurity, not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent);

— *impurities H, I*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

— *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent);

— *sum of impurities other than B, C and E*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *disregard limit*: 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent).

Enantiomeric purity

Liquid chromatography (2.2.29).

Test solution Dissolve 20.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Reference solution (b) Dissolve 4.0 mg of *methotrexate for system suitability CRS* (containing impurity F) in the mobile phase and dilute to 20.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.15 m, Ø = 4.0 mm;

— *stationary phase*: *bovine albumin coated silica gel for chiral separation R* (7 µm).

Mobile phase Add 500 mL of a 7.1 g/L solution of *anhydrous disodium hydrogen phosphate R* to 600 mL of a 6.9 g/L solution of *sodium dihydrogen phosphate monohydrate R*, mix and adjust to pH 6.9 with *dilute sodium hydroxide solution R*. To 920 mL of this mixture add 80 mL of *propanol R*.

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 302 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *methotrexate for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity F.

Relative retention With reference to methotrexate (retention time = about 4 min): impurity F = about 1.6.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to methotrexate and impurity F.

Limit:

— *impurity F*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Water (2.5.12)

Maximum 13.0 per cent, determined on 0.10 g.

Sulfated ash (2.4.14)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (2.2.29) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{20}H_{22}N_8O_5$ taking into account the assigned content of *methotrexate CRS*.

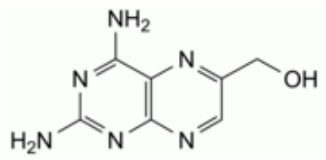
STORAGE

In an airtight container, protected from light.

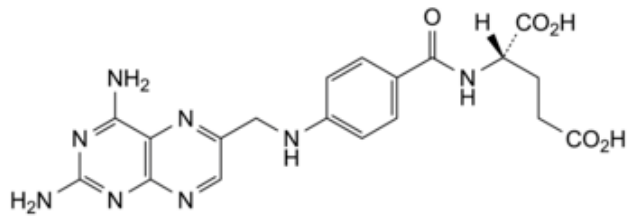
IMPURITIES

Specified impurities *B, C, E, F, H, I.*

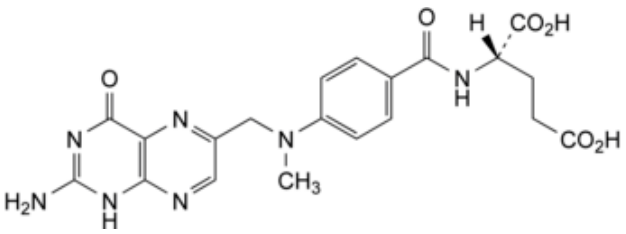
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A, D, G, J, K, L.*



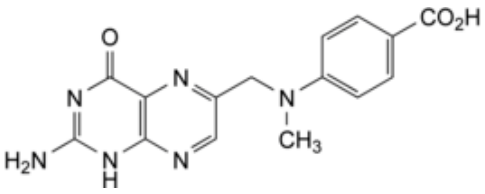
A. (2,4-diaminopteridin-6-yl)methanol,



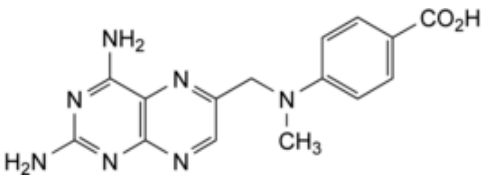
B. (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid (4-aminofolic acid, aminopterin),



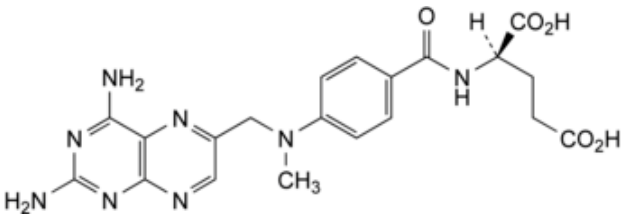
C. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid (*N*-methylfolic acid, methopterin),



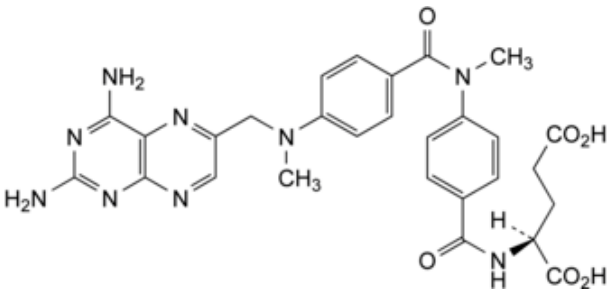
D. 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoic acid (N^{10}-methylpteroic acid),



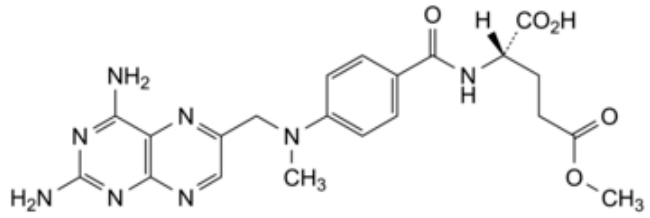
E. 4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid (4-amino-N^{10}-methylpteroic acid, APA),



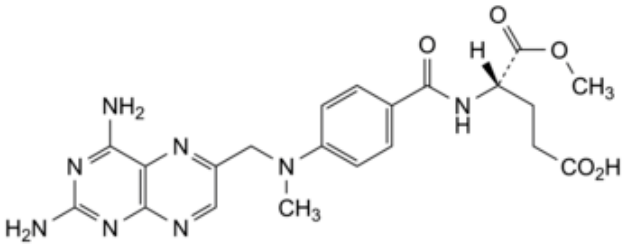
F. (2*R*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid ((*R*)-methotrexate),



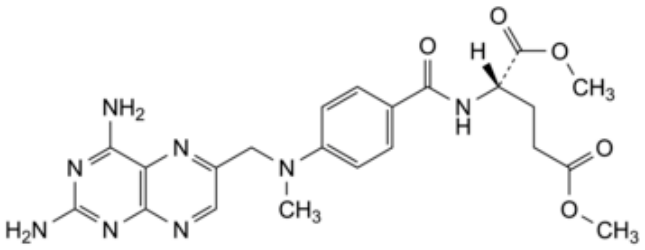
G. (2*S*)-2-[[4-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]methylamino]benzoyl]amino]pentanedioic acid,



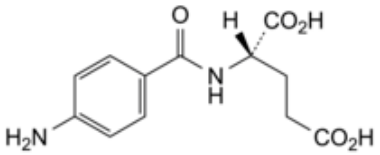
H. (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 5-methyl ester),



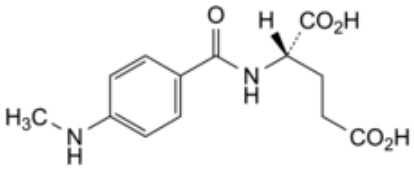
I. (4*S*)-4-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 1-methyl ester),



J. dimethyl (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioate (methotrexate dimethyl ester),



K. (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid,



L. (2*S*)-2-[[4-(methylamino)benzoyl]amino]pentanedioic acid.

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