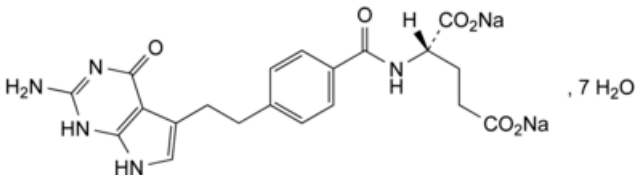
Edition: BP 2025 (Ph. Eur. 11.6 update)

Pemetrexed Disodium Heptahydrate

General Notices

(Ph. Eur. monograph 2637)



$C_{20}H_{19}N_5Na_2O_6,7H_2O$ 597.5 *357166-29-1*

Action and use

Thymidylate synthetase inhibitor; cytostatic.

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DEFINITION

Disodium (2*S*)-2-[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]pentanedioate heptahydrate.

Content

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

CHARACTERS

Appearance

White or almost white powder.

Solubility

Freely soluble in water, very slightly soluble in anhydrous ethanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (2.2.24).

Comparison *pemetrexed disodium heptahydrate CRS*.

B. It gives reaction (a) of sodium (2.3.1).
C. Enantiomeric purity (see Tests).
D. Water (see Tests).

TESTS

Solution S

Dissolve 0.56 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Appearance of solution

Solution S is not more opalescent than reference suspension II (2.2.1) and not more intensely coloured than reference solution GY_4 or Y_4 (2.2.2, *Method II*).

pH (2.2.3)

7.5 to 8.4 for solution S.

Enantiomeric purity

Liquid chromatography (2.2.29). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h.*

Solution A Dissolve 8 g of *β-cyclodextrin R* in 900 mL of *water for chromatography R*. Add 15 mL of *triethylamine R* then 6 mL of *phosphoric acid R* and adjust to pH 6.0 with *phosphoric acid R*. Dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 12.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 6 mg of *pemetrexed for system suitability CRS* (containing impurity E) in *water R* and dilute to 25 mL with the same solvent.

Reference solution (b) Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 3.0 mL of this solution to 10.0 mL with *water R*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

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

— *temperature*: 40 °C.

Mobile phase *acetonitrile for chromatography R*, solution A (5:95 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 230 nm.

Injection 50 µL.

Run time 1.5 times the retention time of pemetrexed.

Relative retention With reference to pemetrexed (retention time = about 30 min): impurity E = about 0.94.

System suitability:

— *symmetry factor*: maximum 2.0 for the principal peak in the chromatogram obtained with reference solution (b);

— *peak-to-valley ratio*: minimum 5.0, where H_p = height above the baseline of the peak due to impurity E and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to pemetrexed in the chromatogram obtained with reference solution (a).

Calculation of percentage content:

— for impurity E, use the concentration of pemetrexed disodium heptahydrate in reference solution (b).

Limit:

— *impurity E*: maximum 0.3 per cent.

Column rinse: the following program is given for information only.

Use a gradient column rinse before column storage or after 30 sample injections to avoid build-up on the column. If a drifting baseline is observed, allow additional time for equilibration with the mobile phase. If a blank chromatogram exhibits broad humps, perform a gradient column rinse.

Rinsing solution A *water for chromatography R*.

Rinsing solution B *acetonitrile for chromatography R*.

Time (min)	Mobile phase (per cent *V/V*)	Rinsing solution A (per cent *V/V*)	Rinsing solution B (per cent *V/V*)
0 - 4	100 → 0	0 → 50	0 → 50
4 - 9	0	50	50
9 - 14	0	50 → 10	50 → 90
14 - 54	0	10	90
54 - 69	0	10 → 95	90 → 5
69 - 100	0	95	5

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h*.

Solution A 1.45 g/L solution of *ammonium formate R* in *water for chromatography R*, adjusted to pH 3.5 with *formic acid R*.

Test solution Dissolve 20.0 mg of the substance to be examined in *water R* and dilute to 100.0 mL with the same solvent.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with, *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

Reference solution (b) In order to prepare impurities B and C *in situ*, dissolve 30 mg of the substance to be examined in 10 mL of a 4.0 g/L solution of *sodium hydroxide R*, heat at 70 °C for 40 min and allow to cool. Dilute 1 mL of the solution to 10 mL with *water R*.

Reference solution (c) Dissolve the contents of a vial of *pemetrexed impurity mixture CRS* (impurities A and D) in 1 mL of *water R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (3.5 µm).

Mobile phase:

— *mobile phase A*: *acetonitrile R*, solution A (5:95 *V/V*);

— *mobile phase B*: *acetonitrile R*, solution A (30:70 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 3	100	0
3 - 45	100 → 0	0 → 100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 250 nm.

Injection 20 µL.

Identification of impurities Use the chromatogram supplied with *pemetrexed impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and D; use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B and C.

Relative retention With reference to pemetrexed (retention time = about 26 min): impurity A = about 0.82; impurity B = about 0.87; impurity C = about 0.88; impurity D = about 0.90.

System suitability:

— *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity B and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity C in the chromatogram obtained with reference solution (b);

— *signal-to-noise ratio*: minimum 30 for the principal peak in the chromatogram obtained with reference solution (a).

Calculation of percentage contents:

— *correction factor*: multiply the peak area of impurity D by 1.5;

— for each impurity, use the concentration of pemetrexed disodium heptahydrate in reference solution (a).

Limits:

— *impurities A, D*: for each impurity, maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.6 per cent;

— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

19.5 per cent to 22.1 per cent, determined on 0.050 g.

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h*.

Acetate buffer Mix 1.7 mL of *glacial acetic acid R* and 900 mL of *water for chromatography R*, adjust to pH 5.3 with a 760 g/L solution of *sodium hydroxide R* in *water for chromatography R* and dilute to 1000 mL with *water for chromatography R*.

Test solution Dissolve 30.0 mg of the substance to be examined in *water R* and dilute to 200.0 mL with the same solvent.

Reference solution Dissolve 30.0 mg of *pemetrexed disodium heptahydrate CRS* in *water R* and dilute to 200.0 mL with the same solvent.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (3.5 µm);

— *temperature*: 30 °C.

Mobile phase *acetonitrile R*, acetate buffer (11:89 *V/V*).

Flow rate 2.0 mL/min.

Detection Spectrophotometer at 285 nm.

Injection 20 µL.

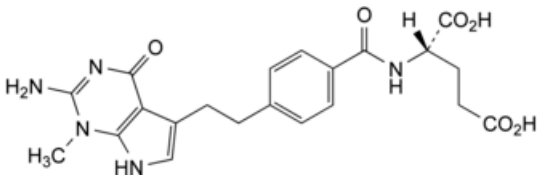
Run time Twice the retention time of pemetrexed (retention time = about 3 min).

Calculate the percentage content of $C_{20}H_{19}N_5Na_2O_6$ taking into account the assigned content of *pemetrexed disodium heptahydrate CRS*.

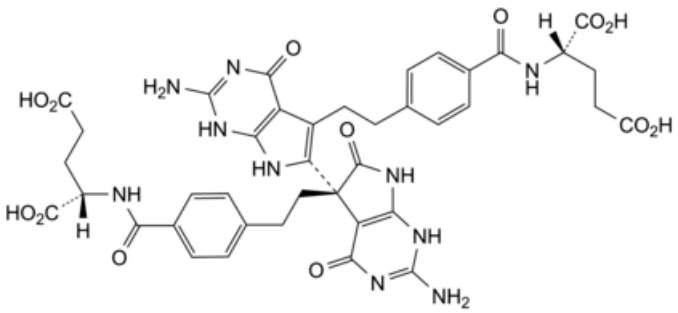
IMPURITIES

Specified impurities *A, D, E*.

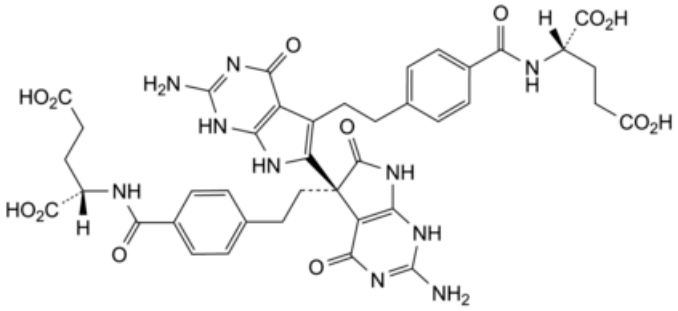
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical 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) *B, C*.



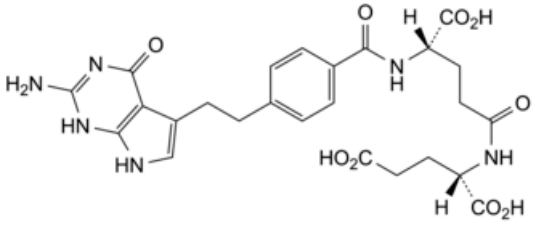
A. (2*S*)-2-[4-[2-(2-amino-1-methyl-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]pentanedioic acid,



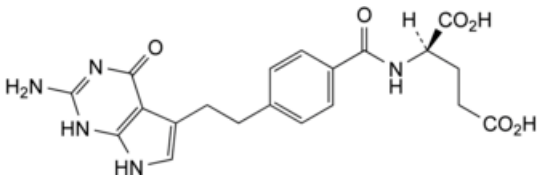
B. (2*S*,2′*S*)-2,2′-[(4^5*R*)-4^2,5^2-diamino-4^4,4^6,5^4-trioxo-4^1,4^4,4^6,4^7,5^4,5^7-hexahydro-5^1*H*-4(5,5),5(6,5)-bis(pyrrolo[2,3-*d*]pyrimidina)-1,8(1)-dibenzenaoctaphane-1^4,8^4-dicarboxamido]dipentanedioic acid,



C. (2*S*,2′*S*)-2,2′-[(4^5*S*)-4^2,5^2-diamino-4^4,4^6, 5^4-trioxo-4^1,4^4,4^6,4^7,5^4,5^7-hexahydro-5^1*H*-4(5,5),5(6,5)-bis(pyrrolo[2,3-*d*]pyrimidina)-1,8(1)-dibenzenaoctaphane-1^4,8^4-dicarboxamido]dipentanedioic acid,



D. (2*S*)-2-[(4*S*)-4-[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]-4-carboxybutanamido]pentanedioic acid,



E. (2*R*)-2-[4-[2-(2-amino-4-oxo-4,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzamido]pentanedioic acid.

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