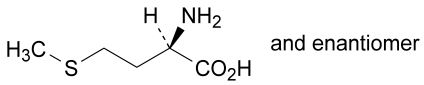
01/2008:0624
corrected 6.0

DL-METHIONINE

DL-Methioninum



$C_5H_{11}NO_2S$ M_r 149.2
[59-51-8]

DEFINITION

DL-Methionine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (2*RS*)-2-amino-4-(methylsulphanyl)butanoic acid, calculated with reference to the dried substance.

CHARACTERS

Almost white, crystalline powder or small flakes, sparingly soluble in water, very slightly soluble in alcohol. It dissolves in dilute acids and in dilute solutions of the alkali hydroxides.

It melts at about 270 °C (instantaneous method).

IDENTIFICATION

First identification: A, C.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *DL-methionine CRS*. Dry the substances at 105 °C.

B. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dissolve 2.50 g in *1 M hydrochloric acid* and dilute to 50.0 ml with the same acid. The angle of optical rotation (*2.2.7*) is – 0.05° to + 0.05°.

D. Dissolve 0.1 g of the substance to be examined and 0.1 g of *glycine R* in 4.5 ml of *dilute sodium hydroxide solution R*. Add 1 ml of a 25 g/l solution of *sodium nitroprusside R*. Heat to 40 °C for 10 min. Allow to cool and add 2 ml of a mixture of 1 volume of *phosphoric acid R* and 9 volumes of *hydrochloric acid R*. A deep-red colour develops.

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 5.4 to 6.1.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a). Dissolve 0.2 g in *water R* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a). Dissolve 20 mg of *DL-methionine CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b). Dilute 1 ml of reference solution (a) to 10 ml with *water R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air and spray with *ninhydrin solution R*. Heat the plate at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Chlorides. Dissolve 0.25 g in 35 ml of *water R*. Add 5 ml of *dilute nitric acid R* and 10 ml of *silver nitrate solution R2*. Allow to stand protected from light for 5 min. Any opalescence in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10 ml of *chloride standard solution (5 ppm Cl) R* and 25 ml of *water R* (200 ppm). Examine the tubes laterally against a black background.

Sulphates (*2.4.13*). Dissolve 1.0 g in 20 ml of *distilled water R*, heating to 60 °C. Cool to 10 °C and filter. 15 ml of the solution complies with the limit test for sulphates (200 ppm).

Heavy metals (*2.4.8*). 1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.140 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Immediately after dissolution, titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 14.92 mg of $C_5H_{11}NO_2S$.

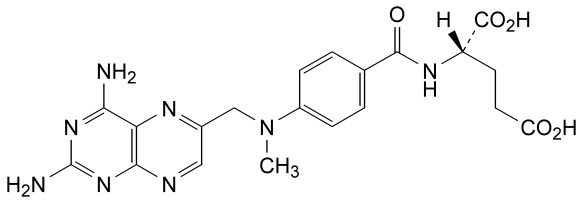
STORAGE

Store protected from light.

01/2008:0560

METHOTREXATE

Methotrexatum



$C_{20}H_{22}N_8O_5$ M_r 454.4
[59-05-2]

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. 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.

Reference solution (a). Dissolve 40.0 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 100.0 ml with mobile phase A.

Reference solution (b). Dilute 5.0 ml of the test solution 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*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

- *mobile phase A*: mix 5 volumes of *acetonitrile for chromatography 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 for chromatography 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
37 - 38	50 → 100	50 → 0
38 - 45	100	0

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 20 µl of the test solution 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.

Relative retention with reference to methotrexate (retention time = about 18 min): impurity B = about 0.3; impurity C = about 0.4; 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); if the resolution between impurity D and methotrexate does not comply, increase the flow rate to meet the requirement.

Limits:

- *correction factors*: for the calculation of content, multiply the peak areas of the following impurities by the corresponding 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 *(RS)-methotrexate R* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.15 m, Ø = 4.0 mm;
- *stationary phase*: *bovine albumin R* bound to *silica gel for chromatography R* (7 µm) with a pore size of 30 nm.

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.

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).

Heavy metals (*2.4.8*): maximum 50 ppm.

1.0 g complies with test C. Prepare the reference solution using 5 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 13.0 per cent, determined on 0.10 g.

Sulphated 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 and reference solution (a).

Calculate the percentage content of $C_{20}H_{22}N_8O_5$ from the declared content of *methotrexate CRS*.

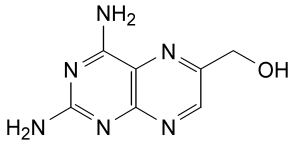
STORAGE

In an airtight container, protected from light.

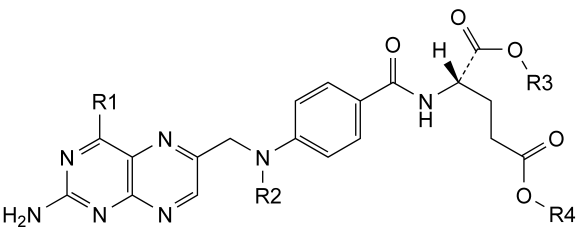
IMPURITIES

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,

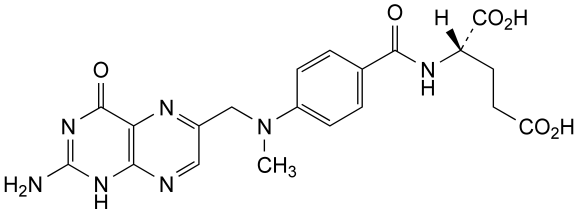


B. R1 = NH_2, R2 = R3 = R4 = H: (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]amino]benzoyl]amino]pentanedioic acid (4-aminofolic acid, aminopteridine),

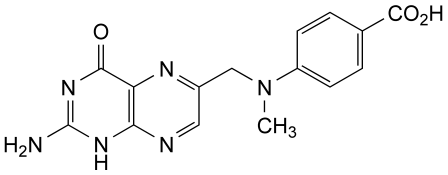
H. R1 = NH_2, R2 = R4 = CH_3, R3 = H: (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 5-methyl ester),

I. R1 = NH_2, R2 = R3 = CH_3, R4 = H: (4*S*)-4-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]-5-methoxy-5-oxopentanoic acid (methotrexate 1-methyl ester),

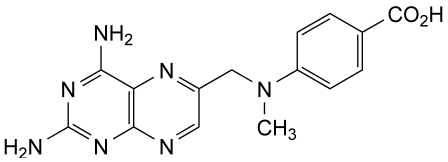
J. R1 = NH_2, R2 = R3 = R4 = CH_3: dimethyl (2*S*)-2-[[4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioate (methotrexate dimethyl ester),



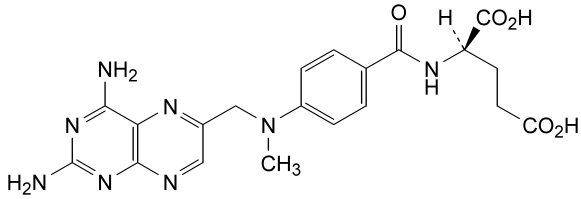
C. (2*S*)-2-[[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoyl]amino]pentanedioic acid (*N*-methylfolic acid, methopteridine),



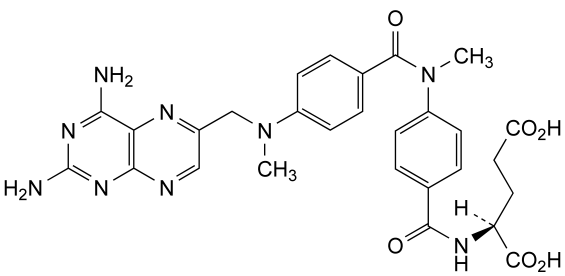
D. 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino]benzoic acid (N^{10}-methylpteroic acid),



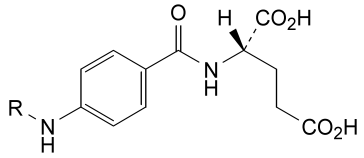
E. 4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid (4-amino-N^{10}-methylpteroic acid, APA),



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,



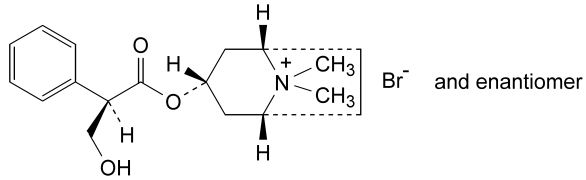
K. R = H: (2*S*)-2-[(4-aminobenzoyl)amino]pentanedioic acid,

L. R = CH_3: (2*S*)-2-[[4-(methylamino)benzoyl]amino]pentanedioic acid.

01/2008:0511
corrected 6.0

METHYLATROPINE BROMIDE

Methylatropini bromidum



$C_{18}H_{26}BrNO_3$ M_r 384.3
[2870-71-5]

DEFINITION

Methylatropine bromide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (1*R*,3*r*,5*S*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, freely soluble in water, sparingly soluble in alcohol.

It melts at about 219 °C, with decomposition.

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D, E.

A. It complies with the test for optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *methylatropine bromide CRS*.

C. To 5 ml of solution S (see Tests) add 2 ml of *dilute sodium hydroxide solution R*. No precipitate is formed.

D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

E. It gives reaction (a) of bromides (*2.3.1*).

TESTS

Solution S. Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_9 (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*; the solution is colourless. Add 0.5 ml of *0.01 M sodium hydroxide*; the solution is red.

Optical rotation (*2.2.7*). Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent. The angle of optical rotation, measured in a 2 dm tube, is – 0.25° to + 0.05°.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

Test solution. Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of *water R* and 9 volumes of *methanol R* and dilute to 5 ml with the same mixture of solvents.

Reference solution. Dilute 0.5 ml of the test solution to 100 ml with a mixture of 1 volume of *water R* and 9 volumes of *methanol R*.

Apply to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *methanol R*, 15 volumes of *anhydrous formic acid R*, 15 volumes of *water R* and 60 volumes of *ethyl acetate R*. Dry the plate at 100 °C to 105 °C until the solvent has evaporated, allow to cool and spray with *dilute potassium iodobismuthate solution R* until the spots appear. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Apomethylatropine. Dissolve 0.10 g in *0.01 M hydrochloric acid* and dilute to 100.0 ml with the same acid. Measure the absorbances (*2.2.25*) at the maxima at 252 nm and 257 nm. The ratio of the absorbance at 257 nm to that at 252 nm is at least 1.19.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 0.300 g in 50 ml of *anhydrous acetic acid R*, warming slightly if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 38.43 mg of $C_{18}H_{26}BrNO_3$.

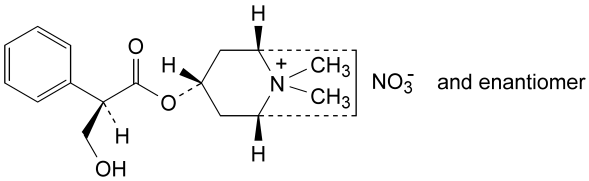
STORAGE

Store protected from light.

01/2008:0512
corrected 6.0

METHYLATROPINE NITRATE

Methylatropini nitras



$C_{18}H_{26}N_2O_6$ M_r 366.4
[52-88-0]