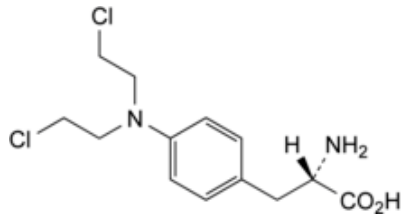
Edition: BP 2025 (Ph. Eur. 11.6 update)

Melphalan

General Notices

(Ph. Eur. monograph 1698)



$C_{13}H_{18}Cl_2N_2O_2$ 305.2 *148-82-3*

Action and use

Cytotoxic alkylating agent.

Preparations

Melphalan Tablets

Melphalan for Injection

Ph Eur

DEFINITION

4-[Bis(2-chloroethyl)amino]-L-phenylalanine.

Content

94.0 per cent to 102.0 per cent (anhydrous and diethylamine-free substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water, slightly soluble in methanol. It dissolves in dilute mineral acids.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison *Ph. Eur. reference spectrum of melphalan*.

TESTS

Appearance of solution

If intended for use in the manufacture of parenteral preparations, the solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.25 g in *dilute hydrochloric acid R* and dilute to 25 mL with the same acid.

Specific optical rotation (*2.2.7*)

-36.0 to -30.0 (anhydrous and diethylamine-free substance).

Dissolve 0.175 g at 45 °C for 10 min in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Use freshly prepared solutions and protect from light.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *methanol R1* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dilute 1.0 mL of test solution (a) to 10.0 mL with *methanol R1*.

Reference solution (a) Dissolve 50.0 mg of *melphalan hydrochloride CRS* in *methanol R1* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *methanol R1*.

Reference solution (b) Dilute 10.0 mL of test solution (a) to 100.0 mL with *methanol R1*.

Reference solution (c) Dilute 1.0 mL of reference solution (b) to 100.0 mL with *methanol R1*.

Reference solution (d) Dilute 5.0 mL of reference solution (b) to 100.0 mL with *methanol R1*.

Reference solution (e) In order to prepare impurity I *in situ*, dissolve 5 mg of *melphalan for system suitability CRS* (containing impurities B, D, G, H and J) in *methanol R1*, dilute to 5.0 mL with the same solvent and heat at 60 °C for 15 min.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

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

Mobile phase:

— *mobile phase A*: mixture of 5 volumes of *acetonitrile for chromatography R* and 95 volumes of *water R* containing 0.01 per cent *V/V* of *triethylamine R*, 0.05 per cent *m/m* of *ammonium acetate R* and 0.05 per cent *V/V* of *glacial acetic acid R*;

— *mobile phase B*: mixture of 40 volumes of *water R* containing 0.01 per cent *V/V* of *triethylamine R*, 0.05 per cent *m/m* of *ammonium acetate R* and 0.05 per cent *V/V* of *glacial acetic acid R*, and 60 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 0	0 → 100
20 - 25	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 260 nm.

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

Identification of impurities Use the chromatogram supplied with *melphalan for system suitability CRS* and the chromatogram obtained with reference solution (e) to identify the peaks due to impurities B, D, G, H, I and J.

Relative retention With reference to melphalan (retention time = about 10 min): impurity B = about 0.3; impurity D = about 0.6; impurity I = about 0.8; impurity J = about 1.04; impurity G = about 1.4; impurity H = about 1.5.

System suitability Reference solution (e):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity J and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to melphalan.

Limits:

— *impurity D*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (d) (3.0 per cent);

— *impurity G*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent);

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

— *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

— *impurity I*: 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 the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *total*: not more than 11 times the area of the principal peak in the chromatogram obtained with reference solution (d) (5.5 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Impurity K (diethylamine)

Gas chromatography (2.2.28).

Test solution Dissolve 0.125 g of substance to be examined in 0.15 mL of *hydrochloric acid R* and dilute to 5.0 mL with *dimethyl sulfoxide R*.

Reference solution Dilute 1 mL of *methanol R* and 0.125 g of *diethylamine R1* (impurity K) to 10.0 mL with *dimethyl sulfoxide R*. Dilute 1.0 mL of the solution to 100.0 mL with *dimethyl sulfoxide R*.

Column:

— *material*: glass;

— *size*: *l* = 1.6 m, Ø = 4 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* coated with potassium carbonate (149-177 µm).

Carrier gas *nitrogen for chromatography R*.

Flow rate 42.5 mL/min.

Temperature:

— *column*: 170 °C;

— *injection port*: 190 °C;

— *detector*: 250 °C.

Detection Flame ionisation.

Injection 1 µL.

Elution order Methanol, impurity K.

System suitability Reference solution:

— *resolution*: minimum 2.0 between the peaks due to methanol and impurity K.

Limit:

— *impurity K*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent).

Water (2.5.12)

Maximum 5.0 per cent, determined on 0.200 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 $C_{13}H_{18}Cl_2N_2O_2$ taking into account the assigned content of *melphalan hydrochloride CRS* and a conversion factor of 0.8933.

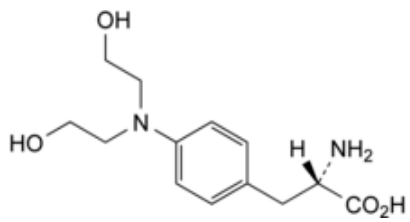
STORAGE

In an airtight container, protected from light.

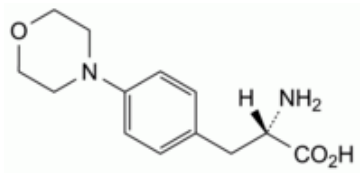
IMPURITIES

Specified impurities *B, D, G, H, I, J, K.*

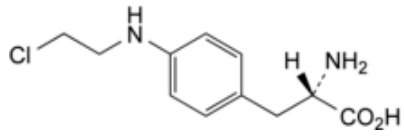
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical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



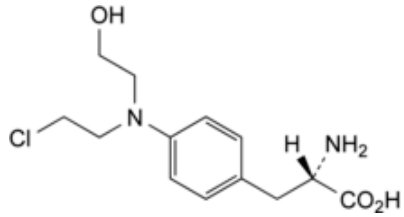
A. 4-[bis(2-hydroxyethyl)amino]-L-phenylalanine,



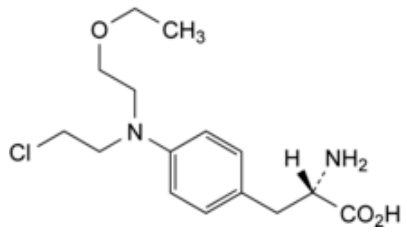
B. 4-morpholin-4-yl-L-phenylalanine,



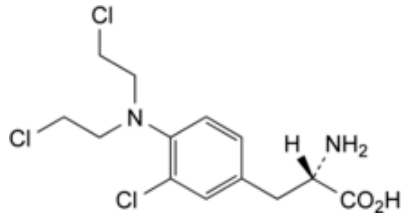
C. 4-[(2-chloroethyl)amino]-L-phenylalanine,



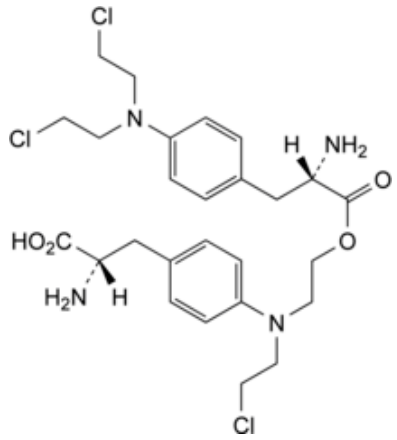
D. 4-[(2-chloroethyl)(2-hydroxyethyl)amino]-L-phenylalanine,



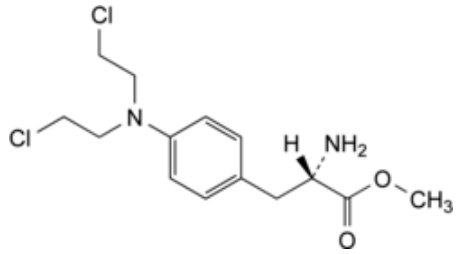
E. 4-[(2-chloroethyl)(2-ethoxyethyl)amino]-L-phenylalanine,



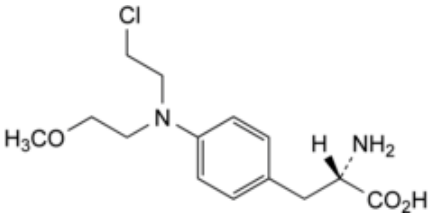
F. 4-[bis(2-chloroethyl)amino]-3-chloro-L-phenylalanine (3-chloromelphalan),



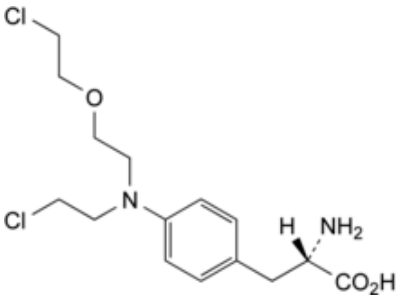
G. 4-[[2-[[4-[bis(2-chloroethyl)amino]-L-phenylalanyl]oxy]ethyl](2-chloroethyl)amino]-L-phenylalanine (melphalan dimer),



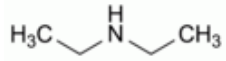
H. methyl 4-[bis(2-chloroethyl)amino]-L-phenylalaninate,



I. 4-[(2-chloroethyl)(2-methoxyethyl)amino]-L-phenylalanine,



J. 4-[[2-(2-chloroethoxy)ethyl](2-chloroethyl)amino]-L-phenylalanine,



K. *N*-ethylethanamine (diethylamine).

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