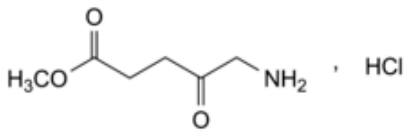
Edition: BP 2025 (Ph. Eur. 11.6 update)

Methylaminolevulinate Hydrochloride

General Notices

(Ph. Eur. monograph 3073)



$C_6H_{12}ClNO_3$ 181.6 *79416-27-6*

Ph Eur

DEFINITION

Methyl 5-amino-4-oxopentanoate hydrochloride.

Content

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

CHARACTERS

Appearance

White or slightly yellow powder, hygroscopic.

Solubility

Very soluble in water, slightly soluble in anhydrous ethanol, very slightly soluble in heptane.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison methylaminolevulinate hydrochloride CRS.

B. Dissolve 25 mg in 5 mL of water R. The solution gives reaction (a) of chlorides (2.3.1).

TESTS

Impurity A

Thin-layer chromatography (2.2.27).

Test solution Dissolve 50.0 mg of the substance to be examined in water R and dilute to 2.0 mL with the same solvent.

Reference solution Dissolve 22.0 mg of 1,3-diaminopropan-2-one dihydrochloride monohydrate R (impurity A) in water R and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with water R.

Plate TLC silica gel plate R.

Mobile phase acetic acid R, water R, acetone R, butanol R (10:20:35:35 *V/V/V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection Spray with ninhydrin solution R3 and heat at 105 °C for 5 min.

Retardation factors Impurity A = about 0.03; methylaminolevulinate = about 0.5.

Limit:

— *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with the reference solution (0.8 per cent).

Related substances

Liquid chromatography (2.2.29).

Carry out the test protected from light. Store the solutions at 2-8 °C.

Solvent mixture methanol R, water R (10:90 *V/V*).

Solution A Dissolve 0.23 g of ammonium acetate R1 in 950 mL of water R, adjust to pH 2.5 with phosphoric acid R and dilute to 1000 mL with water R.

Test solution (a) Dissolve 75.0 mg of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Test solution (b) Dissolve 75.0 mg of the substance to be examined in solution A and dilute to 25.0 mL with solution A.

Test solution (c) Dilute 1.0 mL of test solution (a) to 5.0 mL with the solvent mixture.

Reference solution (a) Dissolve 12.0 mg of methylaminolevulinate impurity B CRS in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 10.0 mL of the solution to 20.0 mL with the solvent mixture.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 100.0 mL with the solvent mixture.

Reference solution (c) Dissolve 12.0 mg of *methylaminolevulinate impurity C CRS* in 1 mL of *tetrahydrofuran R* and dilute to 100.0 mL with solution A. Dilute 5.0 mL of the solution to 200.0 mL with solution A.

Reference solution (d) Dissolve 30.0 mg of *methylaminolevulinate hydrochloride CRS* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Reference solution (e) Dissolve 50 mg of *benzoic acid R* (impurity I) and 50 mg of *hippuric acid R* (impurity H) in solution A and dilute to 100 mL with solution A. Dilute 1 mL of the solution to 10 mL with solution A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography compatible with 100 per cent aqueous mobile phases R* (5 µm);

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dissolve 0.77 g of *ammonium acetate R1* in 950 mL of *water for chromatography R*, adjust to pH 5.5 with *glacial acetic acid R* and dilute to 1000 mL with *water for chromatography R*;

— *mobile phase B*: *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	100	0
1 - 19	100 → 10	0 → 90

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 275 nm.

Autosampler Set at 5 °C.

Injection 30 µL of test solutions (a) and (b) and reference solutions (a), (b), (c) and (e).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peak due to impurity B; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (e) to identify the peaks due to impurities H and I.

Relative retention With reference to methylaminolevulinate (retention time = about 6 min): impurity B = about 0.5; impurity I = about 1.8; impurity H = about 1.9; impurity C = about 2.9.

System suitability:

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (b);

— *resolution*: minimum 1.5 between the peaks due to impurities I and H in the chromatogram obtained with reference solution (e).

Calculation of percentage contents:

— for impurity B, use test solution (a) and the concentration of impurity B in reference solution (a);

— for impurities other than B, use test solution (b) and the concentration of impurity C in reference solution (c).

Limits:

— *impurity B*: maximum 1.8 per cent;

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

— *total (excluding impurity B)*: maximum 0.3 per cent;

— *reporting threshold*: 0.05 per cent.

Water (*2.5.12*)

Maximum 0.5 per cent, determined on 1.00 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 (c) and reference solution (d).

Calculate the percentage content of $C_6H_{12}ClNO_3$ taking into account the assigned content of *methylaminolevulinate hydrochloride CRS*.

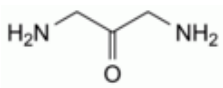
STORAGE

In an airtight container at a temperature of 2 °C to 8 °C.

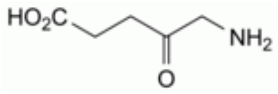
IMPURITIES

Specified impurities *A, B.*

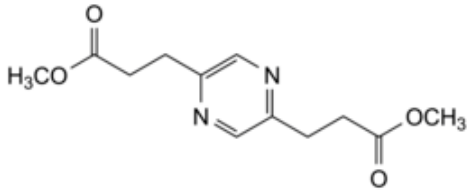
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical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



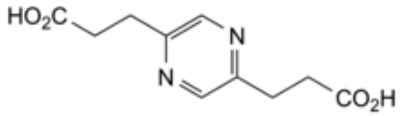
A. 1,3-diaminopropan-2-one,



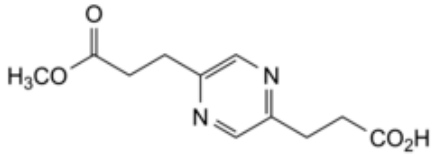
B. 5-amino-4-oxopentanoic acid (5-aminolevulinic acid),



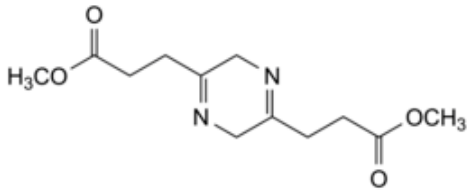
C. dimethyl 3,3′-(pyrazine-2,5-diyl)dipropanoate,



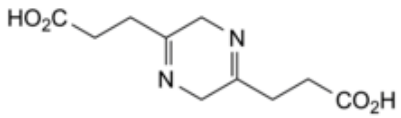
D. 3,3′-(pyrazine-2,5-diyl)dipropanoic acid,



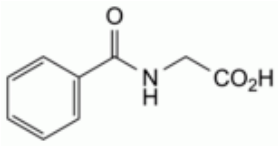
E. 3-[5-(3-methoxy-3-oxopropyl)pyrazin-2-yl]propanoic acid,



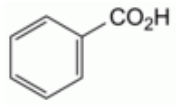
F. dimethyl 3,3′-(3,6-dihydropyrazine-2,5-diyl)dipropanoate,



G. 3,3′-(3,6-dihydropyrazine-2,5-diyl)dipropanoic acid,



H. benzamidoacetic acid (hippuric acid),



I. benzoic acid.

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