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

Glycine

General Notices

(Ph. Eur. monograph 0614)

$C_2H_5NO_2$ 75.1 *56-40-6*

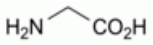
Action and use

Amino acid used for bladder irrigation during surgery.

Preparation

Glycine Irrigation Solution

Ph Eur



DEFINITION

2-Aminoacetic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder.

Solubility

Freely soluble in water, very slightly soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison glycine CRS.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of ethanol (60 per cent V/V) R, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (2.2.27).

Test solution Dissolve 10 mg of the substance to be examined in water R and dilute to 10.0 mL with the same solvent.

Reference solution Dissolve 10 mg of glycine CRS in water R and dilute to 10.0 mL with the same solvent.

Plate cellulose for chromatography R as the coating substance.

Mobile phase glacial acetic acid R, water R, butanol R (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying At 80 °C for 30 min.

Detection Spray with ninhydrin solution R and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve 50 mg in 5 mL of water R, add 1 mL of strong sodium hypochlorite solution R and boil for 2 min. Add 1 mL of hydrochloric acid R and boil for 4-5 min. Add 2 mL of hydrochloric acid R and 1 mL of a 20 g/L solution of resorcinol R, boil for 1 min and cool. Add 10 mL of water R and mix. To 5 mL of the solution add 6 mL of dilute sodium hydroxide solution R. The solution is violet with greenish-yellow fluorescence. After a few minutes, the colour becomes orange and then yellow and an intense fluorescence remains.

TESTS

Solution S

Dissolve 5.0 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 not more intensely coloured than reference solution Y_7 (2.2.2, Method II).

pH (2.2.3)

5.9 to 6.4.

Dilute 10 mL of solution S to 20 mL with carbon dioxide-free water R.

Related substances

Liquid chromatography (2.2.29).

Solvent mixture To 500 mL of the mobile phase add 1.5 mL of phosphoric acid R.

Test solution Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 20.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 80 mg of *iminodiacetic acid R* (impurity A) and 80 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution (c) Dissolve 50.0 mg of *glycine anhydride R* (impurity B), 50.0 mg of *diglycine R* (impurity H) and 50.0 mg of *triglycine R* (impurity I) in the solvent mixture and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 100.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R1* (5 µm);

— *temperature*: 25 °C.

Mobile phase Dissolve 1.4 g of *sodium pentanesulfonate R* in 900 mL of *water for chromatography R*, adjust to pH 2.2 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL.

Run time 4 times the retention time of glycine.

Identification of impurities Use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B, H and I.

Relative retention With reference to glycine (retention time = about 5.5 min): impurity A = about 0.7; impurity B = about 0.75; impurity H = about 1.7; impurity I = about 2.0.

System suitability Reference solution (b):

— *resolution*: minimum 5.0 between the peaks due to impurity A and glycine.

Calculation of percentage contents:

— for impurities B, H and I, use the concentration of the corresponding impurity in reference solution (c);

— for impurities other than B, H and I, use the concentration of glycine in reference solution (a).

Limits:

— *impurities B, H, I*: for each impurity, maximum 0.10 per cent;

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

— *total*: maximum 0.2 per cent;

— *reporting threshold*: 0.05 per cent.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *water R* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions (a), (b) and (d) into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:

— for any ninhydrin-positive substance detected at 570 nm, use the concentration of glycine in reference solution (a);

— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:

— *any ninhydrin-positive substance*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 1.0 per cent;

— *reporting threshold*: 0.05 per cent.

Chlorides (*2.4.4*)

Maximum 75 ppm.

Dissolve 0.67 g in *water R* and dilute to 15 mL with the same solvent.

Ammonium

Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:

— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 2 h.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 70.0 mg in 5 mL of *anhydrous formic acid R*. Add 50 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 7.51 mg of $C_2H_5NO_2$.

IMPURITIES

Specified impurities *B, H, I*.

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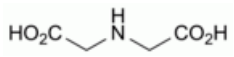
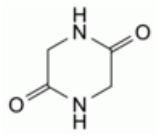
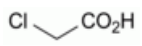
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical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



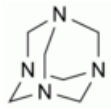
A. 2,2′-iminodiacetic acid,



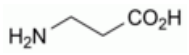
B. piperazine-2,5-dione (glycine anhydride),



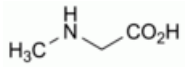
C. 2-chloroacetic acid,



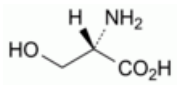
D. 1,3,5,7-tetraazatricyclo[3.3.1.1^{3,7}]decane,



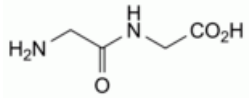
E. 3-aminopropanoic acid (β-alanine),



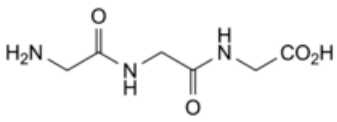
F. 2-(methylamino)acetic acid (sarcosine),



G. (2*S*)-2-amino-3-hydroxypropanoic acid (serine),



H. 2-[(2-aminoacetyl)amino]acetic acid (diglycine),



I. 2-[[2-[(2-aminoacetyl)amino]acetyl]amino]acetic acid (triglycine).

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