Specific optical rotation (*2.2.7*): + 30.5 to + 32.5, determined on solution S and calculated with reference to the dried substance.

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

Test solution (a). Dissolve 0.10 g of the substance to be examined in 5 ml of *dilute ammonia R2* and dilute to 10 ml with *water R*.

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

Reference solution (a). Dissolve 10 mg of *glutamic acid CRS* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (b). Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c). Dissolve 10 mg of *glutamic acid CRS* and 10 mg of *aspartic acid CRS* in *water R* and dilute to 25 ml with the same solvent.

Apply to the plate 5 µl of each solution. Dry the plate in a current of air for 15 min. Develop over a path of 15 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, spray with *ninhydrin solution R* and heat at 100-105 °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) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Chlorides (*2.4.4*). Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution, to which 1 ml of *water R* is added instead of *dilute nitric acid R*, complies with the limit test for chlorides (200 ppm).

Sulphates (*2.4.13*). Dilute 5 ml of solution S to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*). 50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*). In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*). 2.0 g complies with limit test D for heavy metals (10 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 0.130 g in 50 ml of *carbon dioxide-free water R* with gentle heating. Cool. Using 0.1 ml of *bromothymol blue solution R1* as indicator, titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to blue.

1 ml of *0.1 M sodium hydroxide* is equivalent to 14.71 mg of $C_5H_9NO_4$.

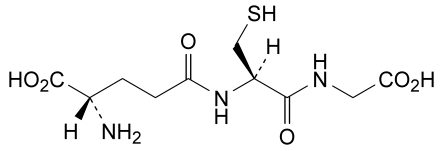
STORAGE

Protected from light.

01/2008:1670
corrected 6.0

GLUTATHIONE

Glutathionum



$C_{10}H_{17}N_3O_6S$ M_r 307.3
[70-18-8]

DEFINITION

L-γ-Glutamyl-L-cysteinylglycine.

Fermentation product.

Content: 98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, very slightly soluble in ethanol (96 per cent) and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *glutathione CRS*.

TESTS

Solution S. Dissolve 5.0 g in *distilled 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*).

Specific optical rotation (*2.2.7*): – 15.5 to – 17.5 (dried substance).

Dissolve 1.0 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances. Capillary electrophoresis (*2.2.47*).
Prepare the solutions immediately before use.

Internal standard solution (a). Dissolve 0.100 g of *phenylalanine R* in the electrolyte solution and dilute to 50.0 ml with the same solution.

Internal standard solution (b). Dilute 10.0 ml of internal standard solution (a) to 100.0 ml with the electrolyte solution.

Test solution (a). Dissolve 0.200 g of the substance to be examined in the electrolyte solution and dilute to 10.0 ml with the same solution.

Test solution (b). Dissolve 0.200 g of the substance to be examined in internal standard solution (b) and dilute to 10.0 ml with the same solution.

Reference solution (a). Dissolve 20 mg of the substance to be examined in internal standard solution (a) and dilute to 10.0 ml with the same solution.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the electrolyte solution.

Reference solution (c). Dissolve 0.200 g of the substance to be examined in 5 ml of the electrolyte solution. Add 1.0 ml of internal standard solution (a), 0.5 ml of a 2 mg/ml solution of *L-cysteine R* (impurity B) in the electrolyte solution, 0.5 ml of a 2 mg/ml solution of *oxidised L-glutathione R* (impurity C)

in the electrolyte solution and 0.5 ml of a 2 mg/ml solution of *L-γ-glutamyl-L-cysteine R* (impurity D) in the electrolyte solution. Dilute to 10.0 ml with the electrolyte solution.

Capillary:

– *material*: uncoated fused silica;

– *size*: length to the detector cell = 0.5 m; total length = 0.6 m; Ø = 75 µm.

Temperature: 25 °C.

Electrolyte solution. Dissolve 1.50 g of *anhydrous sodium dihydrogen phosphate R* in 230.0 ml of *water R* and adjust to pH 1.80 with *phosphoric acid R*. Dilute to 250.0 ml with *water R*. Check the pH and, if necessary, adjust with *phosphoric acid R* or *dilute sodium hydroxide solution R*.

Detection: spectrophotometer at 200 nm.

Preconditioning of a new capillary: rinse the new capillary before the first injection with *0.1 M hydrochloric acid* at 138 kPa for 20 min and with *water R* at 138 kPa for 10 min; for complete equilibration, condition the capillary with the electrolyte solution at 350 kPa for 40 min, and subsequently at a voltage of 20 kV for 60 min.

Preconditioning of the capillary: rinse the capillary with the electrolyte solution at 138 kPa for 40 min.

Between-run rinsing: rinse the capillary with *water R* at 138 kPa for 1 min, with *0.1 M sodium hydroxide* at 138 kPa for 2 min, with *water R* at 138 kPa for 1 min, with *0.1 M hydrochloric acid* at 138 kPa for 3 min and with the electrolyte solution at 138 kPa for 10 min.

Injection: test solutions (a) and (b), reference solutions (b) and (c) and the electrolyte solution (blank): under pressure (3.45 kPa) for 5 s.

Migration: apply a voltage of 20 kV.

Run time: 45 min.

Relative migration with reference to the internal standard (about 14 min): impurity A = about 0.77; impurity B = about 1.04; impurity E = about 1.2; impurity C = about 1.26; impurity D = about 1.3.

System suitability:

– *resolution*: minimum 1.5 between the peaks due to the internal standard and impurity B in the chromatogram obtained with reference solution (c); if necessary, increase the pH with *dilute sodium hydroxide solution R*;

– *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to glutathione in the chromatogram obtained with reference solution (c); if necessary, lower the pH with *phosphoric acid R*;

– check that in the electropherogram obtained with test solution (a) there is no peak with the same migration time as the internal standard (in such case correct the area of the phenylalanine peak).

Limits: test solution (b):

– *corrected areas*: divide all the peak areas by the corresponding migration times;

– *correction factors*: for the calculation of content, multiply the ratio of time-corrected peak areas of impurity and the internal standard by the corresponding correction factor: impurity B = 3.0; impurity D = 1.4;

– *impurities A, B, E*: for each impurity, not more than 0.5 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (0.5 per cent);

– *impurities C, D*: for each impurity, not more than the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (1.0 per cent);

– *any other impurity*: for each impurity, not more than 0.2 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (0.2 per cent);

– *total*: not more than 2.5 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (2.5 per cent);

– *disregard limit*: 0.05 times the ratio of the area of the peak due to glutathione to the area of the peak due to the internal standard in the electropherogram obtained with reference solution (b) (0.05 per cent).

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulphates (*2.4.13*): maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Ammonium (*2.4.1, Method B*): maximum 200 ppm, determined on 50 mg.

Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*): maximum 10 ppm.

In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers, add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the test.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

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 3 h.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

In a ground-glass-stoppered flask, dissolve 0.500 g of the substance to be examined and 2 g of *potassium iodide R* in 50 ml of *water R*. Cool the solution in iced water and add 10 ml of *hydrochloric acid R1* and 20.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand in the dark for 15 min. Titrate with *0.1 M sodium thiosulphate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

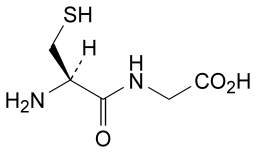
1 ml of *0.05 M iodine* is equivalent to 30.73 mg of $C_{10}H_{17}N_3O_6S$.

STORAGE

Protected from light.

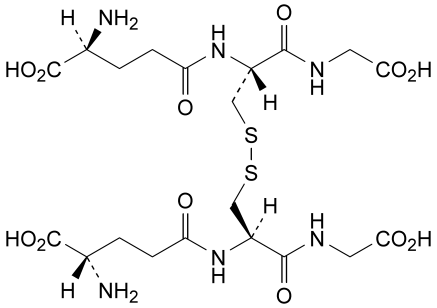
IMPURITIES

Specified impurities: A, B, C, D, E.

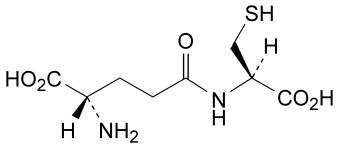


A. L-cysteinylglycine,

B. cysteine,



C. bis(L-γ-glutamyl-L-cysteinylglycine) disulfide (L-glutathione oxidised),



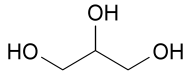
D. L-γ-glutamyl-L-cysteine,

E. unknown structure (product of degradation).

01/2008:0496

GLYCEROL

Glycerolum



$C_3H_8O_3$ M_r 92.1
[56-81-5]

DEFINITION

Propane-1,2,3-triol.

Content: 98.0 per cent *m/m* to 101.0 per cent *m/m* (anhydrous substance).

CHARACTERS

Aspect: syrupy liquid, unctuous to the touch, colourless or almost colourless, clear, very hygroscopic.

Solubility: miscible with water and with alcohol, slightly soluble in acetone, practically insoluble in fatty oils and in essential oils.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. It complies with the test for refractive index (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Preparation: to 5 ml add 1 ml of *water R* and mix carefully.
Comparison: *Ph. Eur. reference spectrum of glycerol (85 per cent).*

C. Mix 1 ml with 0.5 ml of *nitric acid R*. Superimpose 0.5 ml of *potassium dichromate solution R*. A blue ring develops at the interface of the liquids. Within 10 min, the blue colour does not diffuse into the lower layer.

D. Heat 1 ml with 2 g of *potassium hydrogen sulphate R* in an evaporating dish. Vapours (acrolein) are evolved which blacken filter paper impregnated with *alkaline potassium tetraiodomercurate solution R*.

TESTS

Solution S. Dilute 100.0 g to 200.0 ml with *carbon dioxide-free water R*.

Appearance of solution. Solution S is clear (*2.2.1*). Dilute 10 ml of solution S to 25 ml with *water R*. The solution is colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 50 ml of solution S add 0.5 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Refractive index (*2.2.6*): 1.470 to 1.475.

Aldehydes: maximum 10 ppm.

Place 7.5 ml of solution S in a ground-glass-stoppered flask and add 7.5 ml of *water R* and 1.0 ml of *decolorised pararosaniline solution R*. Close the flask and allow to stand for 1 h at a temperature of 25 ± 1 °C. The absorbance (*2.2.25*) of the solution measured at 552 nm is not greater than that of a standard prepared at the same time and in the same manner using 7.5 ml of *formaldehyde standard solution (5 ppm CH_2O) R* and 7.5 ml of *water R*. The test is not valid unless the standard is pink.

Esters. Add 10.0 ml of *0.1 M sodium hydroxide* to the final solution obtained in the test for acidity or alkalinity. Boil under a reflux condenser for 5 min. Cool. Add 0.5 ml of *phenolphthalein solution R* and titrate with *0.1 M hydrochloric acid*. Not less than 8.0 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

Impurity A and related substances. Gas chromatography (*2.2.28*).

Test solution. Dilute 10.0 ml of solution S to 100.0 ml with *water R*.

Reference solution (a). Dilute 10.0 g of *glycerol R1* to 20.0 ml with *water R*. Dilute 10.0 ml of the solution to 100.0 ml with *water R*.

Reference solution (b). Dissolve 1.000 g of *diethylene glycol R* in *water R* and dilute to 100.0 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 10.0 ml with reference solution (a). Dilute 1.0 ml of this solution to 20.0 ml with reference solution (a).

Reference solution (d). Mix 1.0 ml of the test solution and 5.0 ml of reference solution (b) and dilute to 100.0 ml with *water R*. Dilute 1.0 ml of this solution to 10.0 ml with *water R*.

Reference solution (e). Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water R*.

Column:

– *size*: *l* = 30 m, Ø = 0.53 mm,