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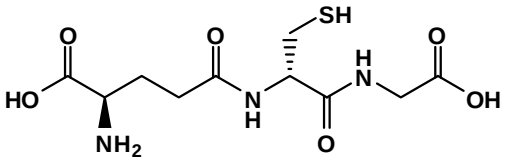
MOA OF Glutathione

Product name: L-Glutathione Reduced

CAS NO.: 70-18-8



$C_{10}H_{17}N_3O_6S$ 307.32

(2S)-2-Amino-4-[1-(carboxymethyl) carbamoyl-(2R)-2-sulfanylethylcarbamoyl] butanoic acid [70-18-8]
Glutathione, when dried, contains not less than98.0% and not more than 101.0% of C10H17N3O6S.

Description:

Glutathione occurs as a white crystalline powder. It is freely soluble in water, and practically insoluble in ethanol (99.5). Melting point: about 185℃ (with decomposition).

Identification:

Determine the infrared absorption spectrum of Glutathione, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation: <2.49> $[a]^{20}_{D}$: －15.5 – －17.5° (after drying, 2 g, water, 50 mL, 100 mm).

Purity:

(1) Clarity and color of solution—Dissolve 1.0 g of Glutathione in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Glutathione according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Glutathione according to Method 1, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Glutathione in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 mL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 4 with respect to glutathione is not more than 3/4 times the peak area of glutathione from the standard solution, and the total area of the peaks other than the peak of glutathione is not more than the peak area of glutathione from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 *μ*m in particle diameter)
.
Column temperature: A constant temperature of about 30℃.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate and 2.02 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 970 mL of this solution add 30 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of glutathione is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of glutathione beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL.Confirm that the peak area of glutathione obtained from 10 μL of this solution is equivalent to 8 to 12% of that obtained from 10μL of the standard solution.

System performance: Dissolve 50 mg of glutathione, 10 mg of D-phenylglycine and 50 mg of ascorbic acid in 100 mL of water. When the procedure is run with 10μL of this solution under the above operating conditions, ascorbic acid, glutathione and D-phenylglycine are eluted in this order, and the resolutions between the peaks of ascorbic acid and glutathione and between the peaks of glutathione and D-phenylglycine are not less than 5, respectively.

System repeatability: When the test is repeated 6 times with 10μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glutathione is not more than 1.5%.

Limit Test for Sulfates（Not more than 0.03%）
Test preparation　Unless otherwise specified, weigh a quantity of the substance being examined as prescribed under individual monographs, dissolve it in about 40 ml of water, neutralize the solution with hydrochloric acid and filter if necessary. Transfer the solution to a 50 ml Nessler cylinder, add 2 ml of dilute hydrochloric acid and mix well.
Reference preparation　Transfer a volume of potassium sulfate standard solution as prescribed under individual monographs to a 50 ml Nessler cylinder, dilute with water to about 40 ml, add 2 ml of dilute hydrochloric acid and mix well.
Procedure　To each of the Nessler cylinders described above add 5 ml of 25% barium chloride solution, dilute with water to 50 ml and mix well, allow to stand for 10 minutes and compare the opalescence produced by viewing down the vertical axis of the cylinder against a black background.
Potassium sulfate standard solution　Dissolve 0.181 g of potassium sulfate in water in a 1000ml volumetric flask, and dilute to the volume, mix well(each ml is equivalent to 100 μg of SO4).

Limit Test for Ammonium（Not more than 0.02%）

Unless otherwise specified, palce a quantity of the substance being examined as prescribed under individual monographs in a distillation flask, add 200 ml of ammonia-free distilled water and 1 g of magnesium oxide, heat to distill, introduce the distillate to a 50 ml Nessler cylinder containing 1 drop of dilute hydrochloric acid TS and 5 ml of ammonia-free distilled water.When the volume of the distillate is about 40 ml, stop distillation, add 2 ml of alkaline mercuric potassium iodide TS, mix well, allow to stand for 15 minutes. Compare the colour produced with that of a refernce solution containing 2 ml of ammonium chloride standard solution treated in the same manner.

Ammonium chloride standard solution　Place 31.5 mg of ammonium chloride, accurately weighed, in a 1000 ml volumetric flask, dissolve it in water and dilute to volume, mix well(each ml is equivalent to 10 *μg* of NH_4).

Limit Test for Iron （Not more than 10ppm）

Unless otherwise specified, dissolve a quantity of the substance being examined as described under individual monographs in water to 25 mL.Transfer the solution to a 50 mL Nessler cylinder, add 4 mL of dilute hydrochloric acid and 50 mg of ammonium thiocyanate solution and sufficient water to priduce 50mL, mix well. Compare the colpur produce with that of a reference preparation containing a volume of standard iron solution as prescribed under individual monographs and subjected to the same treatment.

Iron standard solution Dissolve 0.863g of ferric ammonium sulfate FeNH4(SO4)2·12H2O, accurately weighed, in water in a 1000 ml volumetric flask, add 2.5 ml of sulfuric acid, dilute with water to volume and mix well. This is the stock solution.

Transfer 10 ml of the stock solution, accurately measured, to a 100 ml volumetric flask immediately before use, add water to volume and mix well (each ml is equivalent to 10 μg of Fe).

Loss on drying: <2.41> Not more than 0.5% (1 g, 105℃, 3hours).

Residue on ignition: <2.44> Not more than 0.1% (1 g).

Assay: Weigh accurately about 0.5g of the substance to be examined, previously dried, dissolve in 50 mL of a solution of metaphosphoric acid (1 in 50), and titrate with 0.05mol/L iodine VS, add 1 mL of starch indicator when the endpoint approaches. Continue titration until the solution appears blue color. Carry out a blank titration in the same manner, and make any necessary correction. 1 mL of 0.05mol/L iodine is equivalent to 30.733 mg of C10H17N3O6S.

Calculate the percentage of glutathione (C10H17N3O6S) in the portion of glutathione taken:

Result = [(V-B) X N X F X 100] / W

V　=titrant volume of the sample (ml)

B　= titrant volume of the blank(ml)

N　= titrant normality (mEq/ml)

F　= equivalency factor, 307.33 mg/mEq

W　=weight of the Sample(mg)

Acceptance criteria: not less than 98.0%

Containers and storage　Containers—Tight containers.