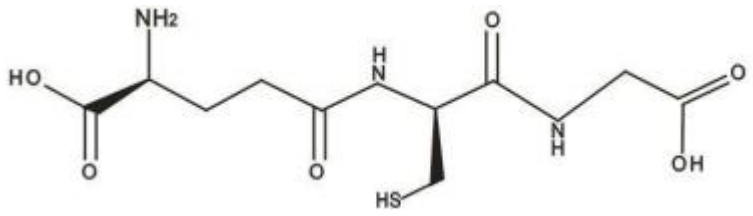
ZhenYiBio Technology Inc
No.29 Kejiliulu,Gaoxin District,Xi'an City,Shaanxi Province,China
TEL:+86-029-81294858 EMAIL:alexxue@zhenyibio.com

MOA of Glutathione

Product name: L-Glutathione Reduced

CAS NO.: 70-18-8



C10H17N3O6S 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, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation: [a]20D: －15.5 – －17.5° (after drying, 2 g, water, 50 mL, 100 mm).

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

Chloride（Not more than 200ppm）

Standard Preparation—Add 200ul sodium chloride to 25 ml of water, then add 10 mL of 6.5% nitric acid and 1ml of silver nitrate TS and sufficient water to make 50ml,mix..

Test Preparation—Dissolve 1g of the substance in 25 ml of water, then add 10 mL of 6.5% nitric acid and 1ml of silver nitrate TS and sufficient water to make 50ml,mix..

Procedure—To each of the two tubes containing the standard Preparation, the test preparation. Allow to stand for 5 minutes protected from direct sunlight, and view downward over a black background, the color of the solution from the Test Preparation is not darker than that of the solution from the Standard Preparation.

Sodium chloride standard solution —Place 0.165g sodium chloride in water in a 1000ml volumetric flask, dissolve it in water and dilute to volume, mix well(each ml is equivalent to 100μg of Cl)

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Sulfates（Not more than 300ppm）

Test preparation—Unless otherwise specified, weigh accurately 0.5g 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 tube, add 2 ml of dilute hydrochloric acid and mix well.

Standard preparation—Transfer a volume of potassium sulfate standard solution as prescribed under individual monographs to a 50 ml Nessler tube, dilute with water to about 40 ml, add 2 ml of dilute hydrochloric acid and mix well.

Procedure—To each of the two tubes containing the standard Preparation, the test preparation, 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 Nessler tube 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).

Heavy metals—(Not more than 10 ppm)

Standard Preparation—Add 200ul of lead nitrate solution and 2 mL pH 3.5 Acetate Buffer, dilute with water to 25 ml ,then add 2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix.

Test Preparation—Dissolve 2g of the substance in 25 ml of water, then add 2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix.

Procedure—To each of the two tubes containing the standard Preparation, the test preparation. Allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the Test Preparation is not darker than that of the solution from the Standard Preparation.

Lead nitrate standard solution: Dissolve 0.1599 g of lead nitrate in a 1000ml volumetric flask, add 5 ml of 65%-68% nitric acid and 50ml water, dissolve it and dilute to volume, mix well(each ml is equivalent to 100μg of Pb)

Arsenic （Not more than 2 ppm）

Prepare the test solution with 1.0 g of Glutathione according to arsenic stain method, and perform the test.

Ammonium（Not more than 200ppm）：

Standard solution : 10ug of ammonium from ammonium chloride standard solution.

Sample solution : 50mg of Glutathione.

Analysis—Transfer the Sample solution and the Standard solution to separate 25 ml jars fitted with caps, and dissolve in 1ml of water. Add 0.3g of magnesium oxide. Close immediately after placing a piece of silver-manganese paper 5-mm square, wetted with a few drops of water, under the caps. Swirl, avoiding projections of liquid, and allow to stand at 40° for 30min..

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 NH4).

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 50mL Nessler tube, add 4 mL of dilute hydrochloric acid and 50 mg of ammonium thiocyanate solution and sufficient water to priduce 50mL, mix well. Compare the colour 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).

Related substances—(Total related substances maximum 2.0%，L-Glutathione oxidized maximum 1.5%)

Mobile phase: 6.8g/L of potassium dihydrogen phosphate with 2.02g/L of sodium 1-heptane sulfonate. Adjust with phosphoric acid to a pH of 3.0. Mix 970mL of this solution with 30 mL of methanol.

System suitability solution: 0.1mg/mL of glutathione reference standard in Mobile phase.

Standard solution: 0.01mg/mL of glutathione reference standard in mobile phase

Sample solution: 50mg of glutathione in 100mL of mobile phase.

Chromatographic system

Mode: LC

Detector: UV 210nm

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 μm in particle diameter)

Column temperature: 30℃

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

Injection size: 10uL

System suitability

Sample: System suitability solution

Suitability requirements

Resolution: NLT 5.0 between the ascorbic acid and glutathione peaks: and NLT 5.0 between the glutathione and L-phenylalanine peaks

Relative standard deviation: NMT1.5% for replicate injections

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of any impurity in the portion of glutathione taken:

$$Result=(R_U/R_S)\times(C_S/C_U)\times 100$$

R_U =peak response of any peak from the Sample solution other than glutathione

R_S =peak response of the glutathione peak form the standard solution

C_S =concentration of glutathione reference standard in the standard solution(mg/mL)

C_U =concentration of glutathione in the sample solution(mg/mL)

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

Residue on ignition: (Not more than 0.1%).

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Ignite a suitable crucible (for example, silica, platinum, quartz, or porcelain) at 600 ± 50° for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant), and weigh it accurately. Weigh accurately 1 to 2 g of the substance, or the amount specified in the individual monograph, in the crucible.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. Cool; then, unless otherwise directed in the individual monograph, moisten the residue with a small amount (usually 1 mL) of sulfuric acid; heat gently until white fumes are no longer evolved; and ignite at 600 ± 50°, unless another temperature is specified in the individual monograph, until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately, and calculate the percentage of residue.

Assay（between 98.0%~101.0%）

Sample: 500mg of glutathione previously dried

Mode: Direct titration

Endpoint detection: Visual

Blank: 50mL of metaphosphoric acid (1 in 50)

Analysis: Dissolve the Sample in 50mL of metaphosphoric acid (1 in 50) and titrate with the Titrant.

Calculate the percentage of glutathione ($C_{10}H_{17}N_3O_6S$) in the portion of Glutathione taken:

$$Result = [(V-B) \times N \times F \times 100] / W$$

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: 98.0%-101.0% on the dried basis