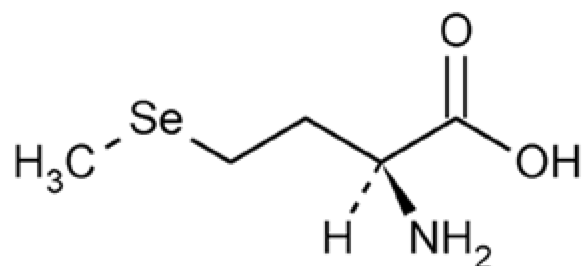
Selenomethionine



$C_5H_{11}NO_2Se$ 196.11

Butanoic acid, 2-amino-4-(methylseleno)-, (S)-.

(S)-2-Amino-4-(methylselenyl)butyric acid [1464-42-2].

Selenomethionine contains not less than 97.0 percent and not more than 103.0 percent of $C_5H_{11}NO_2Se$ and contains not less than 39.0 percent and not more than 41.0 percent of Se, calculated on the as-is basis.

Packaging and storage— Preserve in well-closed containers.

USP Reference standards 11 — USP L-Methionine RS.

USP Selenomethionine RS.

Identification, Infrared Absorption 197K .

Specific rotation 781S : between +17.0 and +19.5 .

Test solution: 10 mg per mL, in 1 N hydrochloric acid.

Heavy metals, Method I 231 : 0.002%.

Limit of sodium—

Standard stock solution— Transfer an accurately weighed quantity of about 254 mg of sodium chloride, previously dried at 105℃for 2 hours, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix to obtain a solution having a known concentration of about 10 µg of sodium per mL.

Standard solutions— Into separate 100-mL volumetric flasks, pipet 2.0, 5.0, and 10.0 mL of the Standard stock solution. To each flask add 2.0 mL of potassium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix to obtain Standard solutions having known concentrations of about 0.2, 0.5, and 1.0 µg of sodium per mL.

Test solution— Transfer about 1.0 g of Selenomethionine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask. Add 2.0 mL of potassium chloride solution (1 in 5) and 1.0 mL of hydrochloric acid, dilute with water to volume, and mix.

Procedure— Concomitantly determine the absorbances of the Standard solutions and the Test solution at the sodium emission line of 589 nm with a suitable atomic absorption spectrophotometer (see Spectrophotometry and Light-scattering 851) equipped with a sodium hollow-cathode lamp and an oxidizing air–acetylene flame. Plot the absorbances of the Standard solutions versus their concentrations, in µg per mL, of sodium, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration of sodium, in µg per mL, in the Test solution. Calculate the percentage of sodium in the portion of Selenomethionine taken by the formula:

$$0.1C/W$$

in which C is the concentration, in µg per mL, of sodium in the Test solution; and W is the weight, in g, of Selenomethionine taken for the Test solution: not more than 0.1% is found.

Chromatographic purity—

Developing solvent— Prepare a mixture of butanol, glacial acetic acid, and water (80:20:20).

Spray reagent— Prepare a solution containing 200 mg of ninhydrin in 100 mL of alcohol.

Standard solution— Dissolve about 50 mg of USP Selenomethionine RS, accurately weighed, in 2 mL of water, warming if necessary, dilute with methanol to 10.0 mL, and mix to obtain a solution having a known concentration of about 5 mg per mL.

Diluted standard solution— Quantitatively dilute a portion of the Standard solution with methanol to obtain a solution having a known concentration of about 50 µg per mL.

Test solution— Dissolve about 50 mg of Selenomethionine, accurately weighed, in 2 mL of water, warming if necessary, dilute with methanol to 10.0 mL, and mix.

Procedure— Separately apply 10-µL portions of the Test solution, the Standard solution, and the Diluted standard solution to a suitable thin-layer chromatographic plate (see Chromatography 621) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in the Developing solvent until the solvent front has moved about

three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with Spray reagent, and dry it at 110℃or 10 minutes. The RF value of the principal spot obtained from the chromatogram of the Test solution corresponds to that obtained from the chromatogram of the Standard solution, and no spot, other than the principal spot, in the chromatogram of the Test solution is larger or more intense than the principal spot obtained from the Diluted standard solution (1.0%).

Content of selenium— [Caution—Selenium is toxic; handle it with care.]

Selenium stock solution— Dissolve 1 g of metallic selenium, accurately weighed, in a minimum volume of nitric acid. Evaporate to dryness, add 2 mL of water, and evaporate to dryness. Repeat the addition of water and evaporation to dryness three times. Dissolve the residue in 3 N hydrochloric acid, transfer to a 1000-mL volumetric flask, dilute with 3 N hydrochloric acid to volume, and mix. This solution contains about 1000 µg of selenium per mL.

Standard solutions— To separate 100-mL volumetric flasks, transfer 2.0, 5.0, and 10.0 mL of the Selenium stock solution, dilute the contents of each flask with water to volume, and mix to obtain Standard solutions containing 20, 50, and 100 µg of selenium per mL, respectively.

Test solution— Transfer about 250 mg of Selenomethionine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure— Concomitantly determine the absorbances of the Standard solutions and the Test solution at the selenium emission line of 196 nm with a suitable atomic absorption spectrophotometer (see Spectrophotometry and Light-scattering 851) equipped with a selenium hollow-cathode lamp and an air–acetylene flame, using water as the blank. Plot the absorbances of the Standard solutions versus their concentrations, in µg per mL, of selenium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration of selenium, in µg per mL, in the Test solution. Calculate the percentage of Se in the portion of Selenomethionine taken by the formula:

$$0.2C/W$$

in which C is the concentration, in µg per mL, of Se in the Test solution; and W is the weight, in g, of Selenomethionine taken for the Test solution.

Assay—

Mobile phase— Prepare a filtered and degassed solution of 6.8 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.75 ±0.25. Make adjustments if necessary (see System Suitability under Chromatography 621).

System suitability preparation— Dissolve suitable quantities of USP l-Methionine RS and USP Selenomethionine RS in Mobile phase to obtain a solution containing about 0.8 mg per mL and 0.16 mg per mL, respectively.

Standard preparation— Dissolve an accurately weighed quantity of USP Selenomethionine RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.16 mg per mL.

Assay preparation— Transfer about 40 mg of Selenomethionine, accurately weighed, to a 250-mL volumetric flask, dissolve in Mobile phase with sonication, dilute with Mobile phase to volume, and mix. Filter through a 0.45-µm membrane.

Chromatographic system (see Chromatography 621)— The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1 with polar end-capping. The flow rate is about 1.0 mL per minute. Chromatograph the System suitability preparation, and record the peak responses as directed for Procedure: the relative retention times are about 0.8 for methionine and 1.0 for selenomethionine; the resolution, R, between methionine and selenomethionine is not less than 3.0; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_5H_{11}NO_2Se$ in the portion of Selenomethionine taken by the formula:

$$25C/W(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Selenomethionine RS in the Standard preparation; W is the weight, in g, of the portion of Selenomethionine taken to prepare the Assay preparation; and r_U and r_S are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.