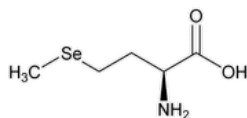


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Selenomethionine



$C_5H_{11}NO_2Se$ 196.11

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

(S)-2-Amino-4-(methylselenyl)butyric acid CAS RN®: 3211-76-5.

DEFINITION

Selenomethionine contains NLT 97.0% and NMT 103.0% of selenomethionine ($C_5H_{11}NO_2Se$) and contains NLT 39.0% and NMT 41.0% of selenium (Se), both calculated on the as-is basis.

IDENTIFICATION

- A. [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy](#): 197K

ASSAY

• PROCEDURE

Mobile phase: 6.8 g/L of [monobasic potassium phosphate](#) in [water](#). Adjust with [phosphoric acid](#) to a pH of 2.75 ± 0.25 .

System suitability solution: 0.16 mg/mL of [USP Selenomethionine RS](#) and 0.8 mg/mL of [USP L-Methionine RS](#) in *Mobile phase*

Standard solution: 0.16 mg/mL of [USP Selenomethionine RS](#) in *Mobile phase*

Sample solution: 0.16 mg/mL of Selenomethionine in *Mobile phase*. Sonicate and pass through a membrane filter of 0.45- μ m pore size.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm $\times$ 25-cm; packing L1 with polar end-capping

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for methionine and selenomethionine are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between the methionine and selenomethionine peaks

Tailing factor: NMT 2.0 for the selenomethionine peak

Relative standard deviation: NMT 2.0% for the selenomethionine peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of selenomethionine ($C_5H_{11}NO_2Se$) in the portion of Selenomethionine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of [USP Selenomethionine RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Selenomethionine in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the as-is basis

IMPURITIES

• LIMIT OF SODIUM

Standard stock solution: 10 µg/mL of sodium from [sodium chloride](#), previously dried at 105° for 2 h

Standard solutions: 0.2, 0.5, and 1.0 µg/mL of sodium prepared as follows. Pipet 2.0, 5.0, and 10.0 mL of *Standard stock solution* into separate 100-mL volumetric flasks. To each flask add 2.0 mL of [potassium chloride](#) solution (1 in 5) and 1.0 mL of [hydrochloric acid](#), and dilute with [water](#) to volume.

Sample solution: Transfer 100 mg of Selenomethionine to a 100-mL volumetric flask, add 2.0 mL of [potassium chloride](#) solution (1 in 5) and 1.0 mL of [hydrochloric acid](#), and dilute with [water](#) to volume.

Instrumental conditions

(See [Atomic Absorption Spectroscopy \(852\)](#).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 589 nm

Lamp: Sodium hollow-cathode

Flame: Air–acetylene

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the solutions. Plot the absorbances of the *Standard solutions* versus their concentrations (µg/mL of sodium), and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration of sodium, C_{Na} (µg/mL), in the *Sample solution*.

Calculate the percentage of sodium in the portion of Selenomethionine taken:

$$\text{Result} = (C_{Na}/C_{SA}) \times F \times 100$$

C_{Na} = concentration of sodium in the *Sample solution* (µg/mL), as determined from the regression line

C_{SA} = concentration of Selenomethionine in the *Sample solution* (mg/mL)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: NMT 0.1%

Change to read:

• CHROMATOGRAPHIC PURITY

Standard solution A: Transfer 50 mg of [USP Selenomethionine RS](#) to a 10-mL volumetric flask, add 2 mL of [water](#), warm to dissolve if necessary, and dilute with [methanol](#) to volume.

Standard solution B: Transfer 1.0 mL of *Standard solution A* to a 100-mL volumetric flask and dilute with [methanol](#) to volume.

Sample solution: Transfer 50 mg of Selenomethionine to a 10-mL volumetric flask, add 2 mL of [water](#), warm to dissolve if necessary, and dilute with [methanol](#) to volume.

Chromatographic system

(See ▲ [Chromatography \(621\), General Procedures, Thin-Layer Chromatography](#) ▲ (IRA 1-Mar-2022)).

Mode: TLC

Absorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: [Butanol](#), [glacial acetic acid](#), and [water](#) (4:1:1)

Spray reagent: 2 mg/mL of [ninhydrin](#) in [alcohol](#)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Proceed as directed in the chapter. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by spraying with *Spray reagent* and drying it at 110° for 10 min.

Acceptance criteria: NMT 1.0%. The R_f value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, and no spot, other than the principal spot of the *Sample solution*, is larger or more intense than the principal spot of *Standard solution B*.