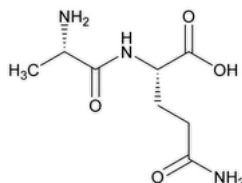


Status: Currently Official on 13-Feb-2025
 Official Date: Official as of 01-Jun-2023
 Document Type: Dietary Supplement Monographs
 DocId: GUID-0B064CAB-E5FC-479A-9ACF-6C7EEEC9133C_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M7103_03_01
 DOI Ref: xa7jv

© 2025 USPC
 Do not distribute

L-Alanyl-L-glutamine



$C_8H_{15}N_3O_4$ 217.23

L-2-(1-Oxo-2-amino-propylamino)-4-amino-4-oxobutanoic acid CAS RN®: 39537-23-0.

DEFINITION

L-Alanyl-L-glutamine contains NLT 98.0% and NMT 101.5% of L-alanyl-L-glutamine ($C_8H_{15}N_3O_4$), calculated on the anhydrous and solvent-free basis, and excluding alanine and glutamine.

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS** (197), *Infrared Spectroscopy*: **197A**
- **B.** It meets the requirements for [Optical Rotation](#), [Specific Rotation \(781S\)](#) in *Specific Tests*.

ASSAY

PROCEDURE

Sample: 300 mg

Blank: Mix 5 mL of formic acid with 50 mL of glacial acetic acid.

Titrimetric system

(See [Titrimetry \(541\)](#).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 5 mL of formic acid, add 50 mL of glacial acetic acid, and titrate with the *Titrant*. Perform a *Blank* determination, and make any necessary correction.

Calculate the percentage of L-alanyl-L-glutamine ($C_8H_{15}N_3O_4$) in the *Sample* taken:

$$\text{Result}_1 = [(V_S - V_B) \times N \times (F/W)] \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 217.2 mg/mEq

W = *Sample* weight (mg)

Calculate the percentage of L-alanyl-L-glutamine ($C_8H_{15}N_3O_4$) in the *Sample* taken, excluding alanine and glutamine:

$$\text{Result}_2 = [(\text{Result}_1 - a - b)] / [(100 - Ala - Gln)] \times 100$$

$$a = Ala \times (M_{r1}/M_{r2})$$

$$b = Gln \times (M_{r1}/M_{r3})$$

Ala = percentage of alanine from the *Related Compounds* test

Gln = percentage of glutamine from the *Related Compounds* test

M_{r1} = molecular weight of L-alanyl-L-glutamine, 217.2

M_{r2} = molecular weight of alanine, 89.1

M_{r3} = molecular weight of glutamine, 146.1

Acceptance criteria: 98.0%–101.5% on the anhydrous and solvent-free basis, and excluding alanine and glutamine

IMPURITIES

• [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

• [CHLORIDE AND SULFATE, Chloride \(221\)](#)

Sample: 0.89 g

Standard solution: 0.50 mL of 0.010 N hydrochloric acid

Acceptance criteria: NMT 200 µg/g

• [CHLORIDE AND SULFATE, Sulfate \(221\)](#)

Sample: 0.98 g

Standard solution: 0.20 mL of 0.010 N sulfuric acid

Acceptance criteria: NMT 200 µg/g

Change to read:

• [IRON \(241\), Procedures, Procedure 1](#)  (CN 1-JUN-2023) : NMT 10 µg/g

• [RESIDUAL SOLVENTS \(467\)](#)

Acceptance criteria

Isopropanol: NMT 0.5%

[NOTE—For the *Acceptance criteria* for any other residual solvents, see [Residual Solvents \(467\)](#).]

• **LIMIT OF AMMONIUM**

Standard stock solution: Dissolve 1.486 g of ammonium chloride in 500.0 mL of water.

Standard calibration solutions: Transfer 0.01, 0.1, 1.0, and 10.0 mL of *Standard stock solution* into separate 100-mL volumetric flasks, and dilute with water to volume. The final concentrations are 0.1, 1, 10, and 100 µg/mL of ammonium ions (NH_4^+), respectively.

Sample solution: Transfer 1.0 g of L-Alanyl-L-glutamine to a 150-mL beaker containing a plastic-coated stirring bar, add 100.0 mL of water, and stir until dissolved.

Electrode system: Use a gas-sensing, ammonia-specific indicating electrode with internal reference connected to a pH meter capable of measuring potentials with a minimum reproducibility of ± 0.1 mV (see [pH \(791\)](#)). Condition the electrode according to the manufacturer's instructions.

Analysis

Standard response line: Transfer 100 mL of water into a 150-mL beaker containing a plastic-coated stirring bar, insert the electrode into the water, stir, and measure the potential. Add 1 mL of 10 N sodium hydroxide solution, stir, and measure the potential after stabilization (about 3 min). The potential difference must be below 20 mV. Transfer 100.0 mL of each of *Standard calibration solutions* (0.1, 1, 10, 100 µg/mL of ammonium ions) into separate 150-mL beakers, and add 1 mL of 10 N sodium hydroxide. Insert the electrode into each solution, stir, and measure the potential after stabilization (about 3 min). Plot a curve (four calibration points) of the potential (mV) as function of ammonium ion concentrations (µg/mL).

Sample: *Sample solution*

Rinse the electrode, insert it into the *Sample solution*, add 1 mL of 10 N sodium hydroxide, and stir. Check the pH, which must be above 11; if not, adjust with 10 N sodium hydroxide. After 3 min, measure the potential, and determine the corresponding ammonium ion concentration from the calibration curve.

Calculate the content of ammonium in the portion of the *Sample* taken:

$$\text{Result} = (V \times C)/W$$

V = volume of the *Sample solution* (mL)

C = concentration of ammonium ions in the *Sample solution* determined from the *Standard response line* (µg/mL)

W = weight of L-Alanyl-L-glutamine taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 200 µg/g

• **RELATED COMPOUNDS**

Buffer solution: Dissolve 6.84 g of monobasic potassium phosphate in 1000 mL of water.

Mobile phase: Acetonitrile and *Buffer solution* (650:350)

System suitability solution: Transfer 25 mg of [USP L-Alanyl-L-glutamine RS](#) and 5 mg of [USP L-Alanyl-L-alanine RS](#) into a 25-mL volumetric flask, and dilute with water to volume. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

Standard solution 1: 0.025 mg/mL of [USP L-Alanine RS](#) in *Mobile phase*

Standard solution 2: 0.1 mg/mL of [USP Glutamine RS](#) in *Mobile phase*

Sample solution: 2.5 mg/mL of L-Alanyl-L-glutamine in *Mobile phase*

Chromatographic system