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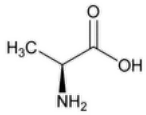
Alanine



$C_3H_7NO_2$ 89.09

L-Alanine CAS RN®: 56-41-7; UNII: OF5P57N2ZX.

DEFINITION

Alanine contains NLT 98.5% and NMT 101.5% of L-alanine ($C_3H_7NO_2$), calculated on the dried basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**

ASSAY

• **Procedure**

Sample: 80 mg of Alanine

Titrimetric system

(See *Titrimetry ⟨541⟩*.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Blank: 3 mL of formic acid in 50 mL of glacial acetic acid

Analysis: Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with *Titrant*.

Calculate the percentage of L-alanine ($C_3H_7NO_2$) in the portion of Alanine taken:

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

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, 89.09 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.15%

• **Chloride and Sulfate, *Chloride*⟨221⟩**

Standard solution: 0.70 mL of 0.020 N hydrochloric acid

Sample: 1.0 g of Alanine

Acceptance criteria: NMT 0.05%

• **Chloride and Sulfate, *Sulfate*⟨221⟩**

Standard solution: 0.30 mL of 0.020 N sulfuric acid

Sample: 1.0 g of Alanine

Acceptance criteria: NMT 0.03%

Change to read:

• **▲Iron ⟨241⟩, *Procedures, Procedure 1*▲ (CN 1-Jun-2023) :** NMT 30 ppm

• **Related Compounds**

Mobile phase: 0.008 N sulfuric acid solution

System suitability solution: A mixture of 0.05 mg/mL of USP Fumaric Acid RS, 0.05 mg/mL of USP Maleic Acid RS, and 3 mg/mL of USP Malic Acid RS in water

Maleic acid standard solution: 0.05 mg/mL of USP Maleic Acid RS in water

Malic acid standard solution: 3 mg/mL of USP Malic Acid RS in water

Fumaric acid standard solution: 0.05 mg/mL of USP Fumaric Acid RS in water

Sample solution: 100 mg/mL of Alanine in water

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.8-mm × 30-cm; 9-µm packing L17

Column temperature: 30°

Flow rate: 0.6 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between maleic acid and malic acid

Relative standard deviation: NMT 5.0% for each of fumaric acid, maleic acid, and malic acid

Analysis

Samples: Standard solutions and *Sample solution*

Calculate the percentage of each specified acid in the portion of Alanine taken:

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

r_U = peak response of maleic acid, malic acid, or fumaric acid from the *Sample solution*

r_S = peak response of maleic acid, malic acid, or fumaric acid from the corresponding Standard solution

C_S = concentration of USP Maleic Acid RS, USP Malic Acid RS, or USP Fumaric Acid RS in the corresponding Standard solution (mg/mL)

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

Calculate the percentage of any unspecified impurity in the portion of Alanine taken:

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

r_U = peak response of any unspecified impurity from the *Sample solution*

r_S = peak response of fumaric acid from the *Fumaric acid standard solution*

C_S = concentration of USP Fumaric Acid RS in the *Fumaric acid standard solution* (mg/mL)

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

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.5	0.05
Malic acid	0.6	0.05
Fumaric acid	1.0	0.05
Alanine	Not observed	—
Any unspecified impurity	—	0.05

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Total unspecified impurities	—	0.20

SPECIFIC TESTS

- **Optical Rotation,** *Specific Rotation*⟨781S⟩
  Sample solution: 100 mg/mL in 6 N hydrochloric acid
  Acceptance criteria: +13.7° to +15.1°
- **pH** ⟨791⟩**:** 5.5–7.0, in a solution (1 in 20)
- **Loss on Drying** ⟨731⟩
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP L-Alanine RS
  USP Fumaric Acid RS
  USP Maleic Acid RS
  USP Malic Acid RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ALANINE	Fatkhulla K Tadjimukhamedov Associate Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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