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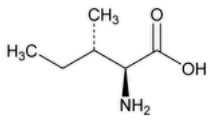
Isoleucine



$C_6H_{13}NO_2$ 131.17

L-Isoleucine CAS RN®: 73-32-5; UNII: 04Y7590D77.

DEFINITION

Isoleucine contains NLT 98.5% and NMT 101.5% of L-isoleucine ($C_6H_{13}NO_2$), calculated on the dried basis.

IDENTIFICATION

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

ASSAY

• **Procedure**

Sample: 130 mg of Isoleucine

Blank: Mix 3 mL of formic acid and 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 3 mL of formic acid and 50 mL of glacial acetic acid. Titrate with the *Titrant*. Perform the blank determination.

Calculate the percentage of L-isoleucine ($C_6H_{13}NO_2$) in the *Sample* taken:

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

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

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

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

F = equivalency factor, 131.2 mg/mEq

W = *Sample* weight (mg)

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

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.3%

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

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of Isoleucine

Acceptance criteria: NMT 0.05%

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

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of Isoleucine

Acceptance criteria: NMT 0.03%

Change to read:

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

• **Related Compounds**

System suitability solution: 0.4 mg/mL each of USP L-Isoleucine RS and USP L-Valine RS in 0.1 N hydrochloric acid

Standard solution: 0.05 mg/mL of USP L-Isoleucine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the *Sample solution*.]

Sample solution: 10 mg/mL of Isoleucine in 0.1 N hydrochloric acid

Chromatographic system

(See *Chromatography ⟨621⟩, General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

System suitability

Sample: *System suitability solution*

Suitability requirements: The chromatogram of the *System suitability solution* exhibits two clearly separated spots.

Analysis

Samples: *System suitability solution*, *Standard solution*, and *Sample solution*

After air-drying the plate, spray with *Spray reagent*, and heat between 100° and 105° for 15 min. Examine the plate under white light.

Acceptance criteria: Any secondary spot of the *Sample solution* is not larger or more intense than the principal spot of the *Standard solution*.

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- **OPTICAL ROTATION ⟨781S⟩, *Procedures, Specific Rotation***

Sample solution: 40 mg/mL in 6 N hydrochloric acid

Acceptance criteria: +38.9° to +41.8°

- **pH ⟨791⟩**

Sample solution: 10 mg/mL in water

Acceptance criteria: 5.5–7.0

- **LOSS ON DRYING ⟨731⟩**

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP L-Isoleucine RS

USP L-Valine RS

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

Topic/Question	Contact	Expert Committee
ISOLEUCINE	Fatkhulla K Tadjimukhamedov Associate Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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