Isoleucine

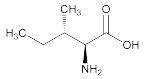
Type of Posting	Revision Bulletin
Posting Date	28–July–2017
Official Date	01–Aug–2017
Expert Committee	Non-Botanical Dietary Supplements
Reason for Revision	Test procedure omission

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Non-Botanical Dietary Supplements Expert Committee has revised the Isoleucine monograph. The purpose for the revision is to remove the currently postponed HPLC procedure for the *Related compounds* test in this monograph. The omission of this procedure would not affect the Isoleucine monograph because it has never been implemented. The TLC is still the official procedure for the *Related compounds* test. It will be replaced by a new, improved HPLC procedure in the future revision of the monograph

The Isoleucine Revision Bulletin supersedes the revision of the Isoleucine monograph published in *First Supplement* to *USP 40–NF 35*, which was scheduled to become official August 1, 2017. The Revision Bulletin will be incorporated in the *First Supplement* to *USP 41–NF 36*.

Should you have any questions, please contact Huy Dinh, Senior Scientific Liaison (301–816–8594 or htd@usp.org).

Isoleucine



$C_6H_{13}NO_2$ 131.17
L-Isoleucine [73-32-5].

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. INFRARED ABSORPTION ⟨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%
• **IRON ⟨241⟩:** NMT 30 ppm

Delete the following:

•• **HEAVY METALS,** *Method I* ⟨231⟩**:** NMT 15 ppm
● (Official 1-Jan-2018)

Change to read:

• **RELATED COMPOUNDS**
●
● (RB 1-Aug-2017)

•**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%● (RB 1-Aug-2016)

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.

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**
USP L-Isoleucine RS
●
● (RB 1-Aug-2017)
•USP L-Valine RS● (RB 1-Aug-2016)