Leucine

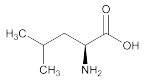
Type of Posting	Revision Bulletin (POSTPONEMENT)
Posting Date	29–Jul–2016
Official Date	01–Aug–2016
Expert Committee	Non-Botanical Dietary Supplements
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Non-Botanical Dietary Supplements Expert Committee has revised the Leucine monograph. The purpose for the revision is to postpone the revision to the *Related Compounds* section of this monograph recently published in *First Supplement to USP 39–NF 34,* because of comments received regarding the performance of the analytical procedure and potential impurity limit compliance issues. Additionally, the previous procedure that was removed from the *Related Compounds* section has been reinstated as the official procedure.

The Leucine Revision Bulletin supersedes the revision of the Leucine monograph published in *First Supplement to USP 39–NF 34*, which is scheduled to become official August 01, 2016. The Revision Bulletin will be incorporated in the First Supplement to *USP 40–NF 35*.

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

Leucine



$C_6H_{13}NO_2$ 131.17
L-Leucine [61-90-5].

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

IDENTIFICATION
• **A. INFRARED ABSORPTION ⟨197K⟩**

ASSAY
• **PROCEDURE**
Sample: 130 mg of Leucine
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 leucine ($C_6H_{13}NO_2$) in the portion of 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 0.4%
• **CHLORIDE AND SULFATE ⟨221⟩,** *Chloride*
Standard solution: 0.50 mL of 0.020 N hydrochloric acid
Sample: 0.73 g of Leucine
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 Leucine
Acceptance criteria: NMT 0.03%
• **IRON ⟨241⟩:** NMT 30 ppm

Delete the following:

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

Change to read:

• **RELATED COMPOUNDS**
■**Buffer solution:** 0.2 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.8.
Mobile phase: Acetonitrile and *Buffer solution* (2:98)
System suitability solution: 0.25 mg/mL each of USP L-Leucine RS and USP L-Isoleucine RS in *Mobile phase*
Standard solution: 0.025 mg/mL of USP L-Isoleucine RS in *Mobile phase*
Sample solution: 5.0 mg/mL of Leucine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3-µm packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for isoleucine and leucine are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between leucine and isoleucine
Relative standard deviation: NMT 2.0% each for leucine and isoleucine
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of isoleucine in the portion of Leucine taken:

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

r_U = peak response of isoleucine from the *Sample solution*
r_S = peak response of isoleucine from the *Standard solution*
C_S = concentration of USP L-Isoleucine RS in the *Standard solution* (mg/mL)
C_U = concentration of Leucine in the *Sample solution* (mg/mL)
Calculate the percentage of any unspecified impurity in the portion of Leucine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*
Acceptance criteria
Isoleucine: NMT 0.8%
Any unspecified impurity: NMT 0.2%
Total unspecified impurities: NMT 1.0%
•(Postponed until 1-August-2017)• (RB 1-Aug-2016)
■1S (*USP39*)
•**System suitability solution:** 0.4 mg/mL each of USP L-Leucine RS and USP L-Valine RS in 0.1 N hydrochloric acid
Standard solution: 0.05 mg/mL of USP L-Leucine 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 Leucine in 0.1 N hydrochloric acid
Chromatographic system
(See *Chromatography* ⟨621⟩*, 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: +14.9° to +17.3°
- **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 0.2%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.

Change to read:

- **USP Reference Standards** ⟨**11**⟩
  ■USP L-Isoleucine RS■1S *(USP39)*● (Postponed until 1-August-2017)● (RB 1-Aug-2016)
  USP L-Leucine RS
  ●USP L-Valine RS● (RB 1-Aug-2016)