

Leucine

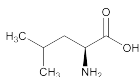
Type of Posting	Revision Bulletin
Posting Date	28–July–2017
Official Date	01–Aug–2017
Expert Committee	Non-Botanical Dietary Supplements
Reason for Revision	Test procedure omission

In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Non-Botanical Dietary Supplements Expert Committee has revised the Leucine 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 Leucine 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 Leucine Revision Bulletin supersedes the revision of the Leucine monograph published in *First Supplement to USP 40–NF 35*, which is 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 hdt@usp.org).

Leucine



C₆H₁₃NO₂ 131.17
L-Leucine [61-90-5].

DEFINITION

Leucine contains NLT 98.5% and NMT 101.5% of L-leucine (C₆H₁₃NO₂), 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 (C₆H₁₃NO₂) in the portion of the *Sample* taken:

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

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**

• (RB 1-Aug-2017)

- **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), *General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μ L

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)

• (RB 1-Aug-2017)

• USP L-Leucine RS

• USP L-Valine RS (RB 1-Aug-2016)