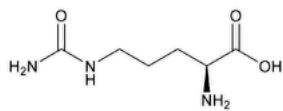


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# Citrulline



$C_6H_{13}N_3O_3$  175.19  
(S)-2-Amino-5-ureidopentanoic acid;  
L-Citrulline CAS RN®: 372-75-8.

**DEFINITION**  
Citrulline contains NLT 98.0% and NMT 102.0% of L-citrulline ( $C_6H_{13}N_3O_3$ ), calculated on the dried basis.

- IDENTIFICATION**
- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197K
  - **B.** It meets the requirements in the test for *Optical Rotation*.
  - **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

*Change to read:*

- **PROCEDURE**  
**Solution A:** 10 mM [octanesulfonic acid sodium salt](#) in water. Adjust with dilute [phosphoric acid](#) (1 in 10) to a pH of 2.5.  
**Solution B:** [Acetonitrile](#)  
**Mobile phase:** See [Table 1](#).

Table 1

| Time (min) | Solution A (%) | Solution B (%) |
|------------|----------------|----------------|
| 0          | 85             | 15             |
| 4          | 85             | 15             |
| 15         | 70             | 30             |
| 20         | 85             | 15             |
| 25         | 85             | 15             |

**System suitability solution:** 0.1 mg/mL of [USP L-Citrulline RS](#), ▲ 0.03 mg/mL of [USP N-Acetylorithine RS](#), ▲ (USP 1-May-2022) and 0.05 mg/mL of [USP N-Acetyl-L-leucine RS](#) in water

**Standard solution:** 0.1 mg/mL of [USP L-Citrulline RS](#) in water

**Sample solution:** 0.1 mg/mL of Citrulline in water

**Chromatographic system**  
(See [Chromatography \(621\), System Suitability](#).)

**Mode:** LC  
**Detector:** UV 200 nm  
**Column:** 4.6-mm × ▲25-cm▲ (USP 1-May-2022); 5-µm packing [L1](#)  
**Flow rate:** 1 mL/min  
**Injection volume:** 20 µL

**System suitability**

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

[NOTE—The relative retention times for L-citrulline, ▲*N*-acetylorcithine,▲ (USP 1-May-2022) and *N*-acetyl-L-leucine are 1.0, ▲1.1,▲ (USP 1-May-2022) and 2.4, respectively.]

**Suitability requirements**

**Resolution:** ▲NLT 2 between L-citrulline and *N*-acetylorcithine, *System suitability solution*▲ (USP 1-May-2022)

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of L-citrulline ( $C_6H_{13}N_3O_3$ ) in the portion of Citrulline taken:

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

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of [USP L-Citrulline RS](#) in the *Standard solution* (mg/mL)

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

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

• [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

• [CHLORIDE AND SULFATE \(221\)](#), *Chloride*

**Standard solution:** 0.10 mL of 0.020 N [hydrochloric acid](#)

**Sample:** 0.36 g of Citrulline

**Acceptance criteria:** NMT 0.02%

• [CHLORIDE AND SULFATE \(221\)](#), *Sulfate*

**Standard solution:** 0.10 mL of 0.020 N [sulfuric acid](#)

**Sample:** 0.48 g of Citrulline

**Acceptance criteria:** NMT 0.02%

**Change to read:**

• **RELATED COMPOUNDS**

**Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard solution:** ▲0.5 µg/mL▲ (USP 1-May-2022) of ▲[USP L-Citrulline RS](#)▲ (USP 1-May-2022) in water

▲***N*-Acetylorcithine standard solution:** 2.5 µg/mL of [USP N-Acetylorcithine RS](#) in water▲ (USP 1-May-2022)

**Sample solution:** 0.5 mg/mL of Citrulline in water

**Analysis**

**Samples:** *Standard solution*, ▲*N*-Acetylorcithine standard solution,▲ (USP 1-May-2022) and *Sample solution*

▲Calculate the percentage of *N*-acetylorcithine in the portion of Citrulline taken:

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

$r_U$  = peak response of *N*-acetylorcithine from the *Sample solution*

$r_S$  = peak response of *N*-acetylorcithine from the *N*-Acetylorcithine standard solution

$C_S$  = concentration of [USP N-Acetylorcithine RS](#) in the *N*-Acetylorcithine standard solution (mg/mL)

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

▲ (USP 1-May-2022)

Calculate the percentage of each ▲unspecified▲ (USP 1-May-2022) impurity in the portion of Citrulline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times \text{▲ (USP 1-May-2022)} \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of citrulline from the *Standard solution*