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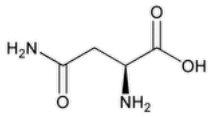
Asparagine



$C_4H_8N_2O_3 \cdot H_2O$ 150.13
$C_4H_8N_2O_3$ 132.12
L-Asparagine;
L-α-Aminosuccinamic acid, monohydrate CAS RN®: 5794-13-8.
Anhydrous CAS RN®: 70-47-3.

DEFINITION

Asparagine is anhydrous, or contains one molecule of water of hydration. It contains NLT 95.5% and NMT 102.0% of asparagine ($C_4H_8N_2O_3$), as L-asparagine, calculated on the dried basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K
[Note—Use USP Asparagine Anhydrous RS and USP Asparagine Monohydrate RS for the evaluation of the anhydrous and monohydrate forms of Asparagine, respectively.]

• **B. Chromatographic Identity**
Analysis: Examine the chromatograms obtained in the *Assay*.
Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

• **Procedure**
Mobile phase: Dissolve 13.61 g of potassium phosphate, monobasic and 2.16 g of sodium 1-octanesulfonate in about 900 mL of water. Adjust with phosphoric acid to a pH of 2.2, and dilute with water to 1 L. Add 5.0 mL of acetonitrile, and mix well.
Diluent: Water
System suitability solution: 1.5 mg/mL of USP Asparagine Anhydrous RS and 0.075 mg/mL of USP Aspartic Acid RS in *Diluent*
Standard solution: 1.5 mg/mL of USP Asparagine Anhydrous RS in *Diluent*
Sample solution: 1.5 mg/mL of Asparagine Anhydrous in *Diluent* or 1.7 mg/mL of Asparagine Monohydrate in *Diluent*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 25°
Flow rate: 0.7 mL/min
Injection volume: 20 µL
Run time: 20 min
System suitability
Samples: *System suitability solution* and *Standard solution*
[Note—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 5 between the asparagine and aspartic acid peaks, *System suitability solution*
Tailing factor: NMT 2.0 determined from the asparagine peak, *Standard solution*
Relative standard deviation: NMT 1% determined from the asparagine peak, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of asparagine ($C_4H_8N_2O_3$) in the portion of sample taken:

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

r_U = peak area of asparagine from the *Sample solution*

r_S = peak area of asparagine from the *Standard solution*

C_S = concentration of USP Asparagine Anhydrous RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 95.5%–102.0% on the dried basis

IMPURITIES

• **Residue on Ignition ⟨281⟩**

Sample: 1.0 g

Acceptance criteria: NMT 0.1%

Change to read:

• **▲Lead ⟨251⟩, *Procedures, Procedure 1*▲** (CN 1-Jun-2023)

Sample: 1 g

Control: 5 mL of *Diluted standard lead solution* (5 µg of lead)

Acceptance criteria: NMT 5 ppm

• **Organic Impurities**

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

Sensitivity solution: 0.005 mg/mL of USP Asparagine Related Compound A RS, USP Asparagine Anhydrous RS, and USP Aspartic Acid RS in *Diluent*

Standard solution: 0.01 mg/mL of USP Asparagine Related Compound A RS, USP Asparagine Anhydrous RS, and USP Aspartic Acid RS in *Diluent*

Sample solution: 2.0 mg/mL of Asparagine Anhydrous in *Diluent* or 2.3 mg/mL of Asparagine Monohydrate in *Diluent*

System suitability

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

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Resolution: NLT 5 between the asparagine and aspartic acid peaks, *System suitability solution*

Relative standard deviation: NMT 5.0% determined from the aspartic acid peak, *Standard solution*

Signal-to-noise ratio: NLT 10 determined from the aspartic acid peak, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of asparagine related compound A in the portion of sample taken:

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

r_U = peak area of asparagine related compound A from the *Sample solution*

r_S = peak area of asparagine related compound A from the *Standard solution*

C_S = concentration of USP Asparagine Related Compound A RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of aspartic acid in the portion of sample taken:

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

r_U = peak area of aspartic acid from the *Sample solution*

r_S = peak area of aspartic acid from the *Standard solution*

C_S = concentration of USP Aspartic Acid RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of each individual unidentified impurity in the portion of sample taken:

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

r_U = peak area of each individual unidentified impurity from the *Sample solution*

r_S = peak area of asparagine from the *Standard solution*

C_S = concentration of USP Asparagine Anhydrous RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Asparagine related compound A[a]	0.4	1.0
Asparagine	1.0	—
Aspartic acid	1.6	1.0
Each individual unidentified impurity	—	0.5
Total impurities	—	3.0

[a] 2,2'-(3,6-Dioxopiperazine-2,5-diyl)diacetamide.

SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 10 mg/mL, in 6 N hydrochloric acid
Acceptance criteria: +33.0° to +36.5°, measured at 20°

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

• **Loss on Drying** ⟨731⟩
Sample: Dry a sample at 130° for 3 h.
Acceptance criteria
Anhydrous: NMT 1.0%
Monohydrate: 11.5%–12.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at room temperature.

• **Labeling:** Label it to indicate whether it is anhydrous or the monohydrate.

• **USP Reference Standards** ⟨11⟩
USP Asparagine Anhydrous RS
USP Asparagine Monohydrate RS
USP Asparagine Related Compound A RS
2,2'-(3,6-Dioxopiperazine-2,5-diyl)diacetamide.
$C_8H_{12}N_4O_4$ 228.21
USP Aspartic Acid RS

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

Topic/Question	Contact	Expert Committee
ASPARAGINE	Documentary Standards Support	SE2020 Simple Excipients

Chromatographic Database Information: Chromatographic Database

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