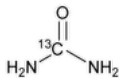


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Urea C 13



(¹³CH₄N₂O) 61.05

Urea [¹³C] CAS RN®: 58069-82-2.

DEFINITION

Urea C 13 contains NLT 98.0% and NMT 102.0% of urea C 13 (¹³CH₄N₂O).

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197A or 197K
- **B.** The intensity of the major peak with mass-to-charge (m/z) ratio of 190 in the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Isotopic Purity*.

ASSAY

PROCEDURE

Mobile phase: [Acetonitrile](#), [methanol](#), and [water](#) (89:10:1)

System suitability solution: 2.5 mg/mL of [USP Urea C 13 RS](#) and 0.003 mg/mL of biuret in *Mobile phase*

Standard solution: 2 mg/mL of [USP Urea C 13 RS](#) in *Mobile phase*

Sample solution: 2 mg/mL of Urea C 13 in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 25-cm; 5-μm packing [L8](#)

Flow rate: 0.8 mL/min

Injection volume: 20 μL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between urea and biuret, *System suitability solution*

Relative standard deviation: NMT 1%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of urea C 13 (¹³CH₄N₂O) in the portion of Urea C 13 taken:

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

r_U = peak response of urea C 13 from the *Sample solution*

r_S = peak response of urea C 13 from the *Standard solution*

C_S = concentration of [USP Urea C 13 RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Urea C 13 in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

LIMIT OF BIURET

Standard solution: 0.033 mg/mL of biuret in water

Sample solution: 33.3 mg/mL of Urea C 13 in water

Analysis: Treat 3 mL of the *Sample solution* and 3 mL of the *Standard solution* separately as follows. To each solution add 3 mL of [sodium hydroxide](#) solution (10 in 100) and 3 drops of copper sulfate solution (0.5 in 100), and allow to stand for 5 min.

Acceptance criteria: NMT 0.1%; any reddish-violet color in the *Sample solution* is not more intense than that in the *Standard solution*.

Change to read:

• **ISOTOPIC PURITY**

Control stock solution: 2.0 mg/mL of [USP Urea RS](#) in [dimethylformamide](#)

Control solution: Transfer 0.5 mL of the *Control stock solution* to a vial. Add 0.5 mL of [bis\(trimethylsilyl\)trifluoroacetamide with trimethylchlorosilane](#) (TMS) derivatizing agent. Cap the vial and shake about 10 times. Heat at 90°–110° on a heating block for about 60 min.

System suitability stock solution: 2.0 mg/mL of [USP Urea C 13 RS](#) in [dimethylformamide](#)

System suitability solution: Transfer 0.5 mL of the *System suitability stock solution* to a vial. Add 0.5 mL of TMS derivatizing agent. Cap the vial and shake about 10 times. Heat at 90°–110° on a heating block for about 60 min.

Sample stock solution: 2.0 mg/mL of Urea C 13 in [dimethylformamide](#)

Sample solution: Transfer 0.5 mL of the *Sample stock solution* to a vial. Add 0.5 mL of TMS derivatizing agent. Cap the vial and shake about 10 times. Heat at 90°–110° on a heating block for about 60 min.

Chromatographic system

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

Mode: GC

Detector: Mass spectrometer, positive ionization

Monitoring mode: Selected ion monitoring set up to monitor all ions individually in the m/z ratio range of 188–193

Solvent delay: Suitable time. [NOTE—Time may vary depending on the instrument and manufacturer's instructions.]

Column: 0.25-mm × 30-m capillary; coated with a 0.25-μm film of phase [G27](#)

Temperatures

Detector: 280°

Injection port: 280°

Column: See [Table 1](#).

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	10	250	0

Carrier gas: Helium

Flow rate: ▲1 mL/min▲ (ERR 1-Sep-2023)

Injection volume: 1 μL

Injection type: Split, split ratio 16:1

System suitability

Samples: *Control solution* and *System suitability solution*

Suitability requirements

Most abundant ion: Fragment at m/z 189, *Control solution*

Relative standard deviation: NMT 5%, *Control solution*

Most abundant ion: Fragment at m/z 190, *System suitability solution*

Relative standard deviation: NMT 5%, *System suitability solution*

Carbon-13: NLT 99% in [USP Urea C 13 RS](#), *System suitability solution*

Analysis

Samples: *Control solution*, *System suitability solution*, and *Sample solution*

Inject each solution in triplicate. [NOTE—Calculate the average responses of all the fragments between m/z 188 and m/z 193 from triplicate injections of each solution.]

Carbon-13 enrichment calculation

Calculate the contribution of carbon-13 (C) due to natural abundance to the response of derivatized Urea C 13:

$$C = (A \times D)/E$$

A = average response of the m/z 190 fragment in urea C 13, *System suitability solution* or *Sample solution*

D = average response of the m/z 188 fragment in urea, *Control solution*

E = average response of the m/z 189 fragment in urea, *Control solution*