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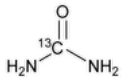
Urea C 13



($^{13}CH_4N_2O$) 61.05

Urea [^{13}C] CAS RN®: 58069-82-2.

DEFINITION

Urea C 13 contains NLT 98.0% and NMT 102.0% of urea C 13 ($^{13}CH_4N_2O$).

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 ($^{13}CH_4N_2O$) 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*

Calculate the percentage of carbon-13 enrichment in USP Urea C 13 RS and Urea C 13:

$$\text{Result} = [A/(A + B - C)] \times 100$$

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

B = average response of the m/z 189 fragment in urea C 13, *System suitability solution* or *Sample solution*

C = contribution due to natural abundance of carbon-13, *System suitability solution* or *Sample solution*

Oxygen-18 enrichment calculation

Step 1: Determine the measured mass of urea TMS in each injection of the *Control solution*:

$$\text{Result} = (\Sigma M_i \times r_i)/\Sigma r_i$$

M_i = mass of the fragment$_i$ for m/z 189, 190, 191, 192, and 193

r_i = response of the corresponding m/z fragment

Calculate the average measured mass ($M_{c,ave}$) of urea TMS derivative.

Determine the mass correction factor (Δ):

$$\text{Result} = M_{c,ave} - M_{c,theor}$$

$M_{c,ave}$ = average mass of urea TMS derivative

$M_{c,theor}$ = theoretical mass of urea TMS derivative, 189.385

Step 2: Determine the measured mass of urea C 13 TMS in each injection of the *Sample solution*:

$$\text{Result} = (\Sigma M_i \times r_i)/\Sigma r_i$$

M_i = mass of the fragment$_i$ for m/z 189, 190, 191, 192, and 193

r_i = response of the corresponding m/z fragment

Calculate the average measured mass ($M_{u,ave}$) of the urea C 13 TMS derivative characteristic ion from the triplicate injections of the *Sample solution*.

Step 3: Calculate the measured molecular weight (M_u) of urea C 13 in the *Sample solution*:

$$\text{Result} = M_{u,ave} - \Delta - M_{TMS}$$

$M_{u,ave}$ = average mass of urea C 13 TMS derivative in the *Sample solution* from *Step 2*

Δ = correction factor from *Step 1*

M_{TMS} = theoretical mass of the $Si_2C_5H_{13}$ fragment, 129.329

Step 4: Calculate the average mass of oxygen (O_U) in the *Sample solution*:

$$\text{Result} = M_U - M_T$$

M_U = average mass of urea C 13 in the *Sample solution* from *Step 3*

M_T = theoretical mass of the $^{13}CN_2H_4$ fragment, 45.044

Step 5: Calculate the percentage of enrichment of oxygen-18 in the Urea C 13 taken:

$$\text{Result} = [(O_U - A_1)/(A_2 - A_1)] \times 100$$

O_U = average mass of oxygen in the *Sample solution* from *Step 4*

A_1 = theoretical mass of the natural isotope of oxygen, 15.995

A_2 = theoretical mass of the oxygen-18 isotope, 17.999

Acceptance criteria

Carbon-13: NLT 99%

Oxygen-18: NMT 15%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers at room temperature.
- **USP Reference Standards** (11)

  USP Urea RS

  USP Urea C 13 RS

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

Topic/Question	Contact	Expert Committee
UREA C 13	Documentary Standards Support	SM42020 Small Molecules 4

Chromatographic Database Information: Chromatographic Database

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