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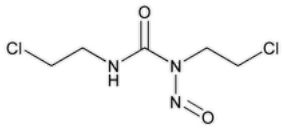
Carmustine



$C_5H_9Cl_2N_3O_2$ 214.05

Urea, *N*,*N*′-bis(2-chloroethyl)-*N*-nitroso-;

1,3-Bis(2-chloroethyl)-1-nitrosourea CAS RN®: 154-93-8; UNII: U68WG3173Y.

DEFINITION

Carmustine contains NLT 98.0% and NMT 102.0% of $C_5H_9Cl_2N_3O_2$, calculated on the anhydrous and solvent-free basis.

[**Caution—**Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197F**▲ (CN 1-May-2020)

Sample: Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

Standard: A similar preparation of USP Carmustine RS

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

[Note—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

Mobile phase: Acetonitrile and water (3:7)

Standard solution: 1.5 mg/mL of USP Carmustine RS in acetonitrile

Sample solution: 1.5 mg/mL of Carmustine in acetonitrile

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 200 nm

Refrigerated autosampler temperature: 4°–5°

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.9

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_5H_9Cl_2N_3O_2$ in the portion of Carmustine 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 Carmustine RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

Organic Impurities

- **Procedure 1: Limit of Ether-Insoluble Substances**

[Note—Perform in a well-ventilated fume hood.]

Analysis: Transfer 1.0 g of sample to a suitable container containing 10 mL of anhydrous ether, stir for 5 min, and immediately filter through a tared glass-filtering crucible of medium pore size. Wash the container with an additional 10 mL of ether, and filter through the same glass-filtering crucible. Dry the crucible at 105° for 1 h. Cool in a desiccator and weigh.

Acceptance criteria: The weight of the residue does not exceed 0.1%.

- **Procedure 2: Limit of Carmustine Related Compound A**

[Note—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

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

Carmustine standard solution: Use the *Standard solution*, prepared as directed in the *Assay*.

Standard stock solution: 0.75 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

Standard solution: 0.0075 mg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard stock solution*

System suitability solution 1: 0.75 µg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard solution*

System suitability solution 2: Transfer 5.0 mL of *Carmustine standard solution* and 10.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with acetonitrile to volume. Transfer 5.0 mL of this solution into a 50-mL volumetric flask, and dilute with acetonitrile to volume.

System suitability

Samples: *Carmustine standard solution*, *System suitability solution 1*, and *System suitability solution 2*

[Note—The relative retention times for carmustine related compound A and carmustine are 0.3 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 10 between carmustine related compound A and carmustine, *System suitability solution 2*

Tailing factor: NMT 1.9, *Carmustine standard solution*

Relative standard deviation: NMT 5%, *System suitability solution 1*

Analysis

[Note—Run the *Sample solution* at least 1.5 times the retention time of carmustine.]

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine taken:

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

r_U = peak response of carmustine related compound A from the *Sample solution*

r_S = peak response of carmustine related compound A from the *Standard solution*

C_S = concentration of carmustine related compound A in the *Standard solution* (mg/mL)

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

Calculate the percentage of each unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria

Carmustine related compound A: NMT 0.5%

Any unspecified impurity: NMT 0.1%

- **Procedure 3: Limit of 2-Chloroethylamine**

[Note—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

Standard solution 1 (0.2%): 1.2 mg/mL of 2-chloroethylamine monohydrochloride in methanol. [Note—1.2 mg/mL of 2-chloroethylamine monohydrochloride is equivalent to 0.8 mg/mL of 2-chloroethylamine.]

Standard solution 2 (0.1%): 0.4 mg/mL of USP Carmustine RS in methanol

Sample solution: 0.4 g/mL of Carmustine in methanol

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic plate (20-cm × 20-cm) coated with silica gel 60

Application volume: 1 µL

Developing solvent system 1: Ethyl acetate

Developing solvent system 2: Ethyl acetate and methanol (7:3)

Spray reagent 1: Diethylamine

Spray reagent 2: 0.1 N silver nitrate solution

Analysis

Samples: *Standard solution 1 (0.2%)*, *Standard solution 2 (0.1%)*, and *Sample solution*

Develop with *Developing solvent system 1* for 27 min, followed by air drying for 5 min. Develop again in *Developing solvent system 2* for 8 min, followed by air drying for 10 min. Spray the plate with *Spray reagent 1*, and heat the plate for 20 min in an oven at 100°. Allow the plates to cool to room temperature, and spray the plate with *Spray reagent 2*. Allow the plate to be exposed to UV light at 365 nm for 15 min. Examine the plate under UV light.

Acceptance criteria

2-Chloroethylamine: The spot for 2-chloroethylamine from the *Sample solution* is not more intense than the principal spot from *Standard solution 1 (0.2%)*.

Any unspecified impurity: Any spot if present in the chromatogram from the *Sample solution*, except the principal spot of carmustine and the spot of 2-chloroethylamine, is not more intense than the principal spot from *Standard solution 2 (0.1%)*.

- **Procedure 4: Limit of 2-Chloroethanol**

Standard solution: 0.02 mg/mL of 2-chloroethanol in acetonitrile

System suitability solution: 0.01 mg/mL of 2-chloroethanol in acetonitrile, diluted from the *Standard solution*

Sample solution: 10 mg/mL of Carmustine in acetonitrile. [Note—Prepare in low-actinic glassware, and keep refrigerated until use.]

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm column bonded with a 1-µm film of phase G16

Temperature

Injector: 90°

Detector: 260°

Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	80	14
80	30	200	3

Carrier gas: Helium

Flow rate: 7 mL/min

Injection size: 5 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 2-chloroethanol in the portion of Carmustine taken:

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

r_U = peak response of 2-chloroethanol from the *Sample solution*

r_S = peak response of 2-chloroethanol from the *Standard solution*

C_S = concentration of 2-chloroethanol in the *Standard solution* (mg/mL)

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

Acceptance criteria

2-Chloroethanol: NMT 0.1%

- **Procedure 5: Limit of Acetaldehyde**

Standard solution: 10 µg/mL of acetaldehyde in acetonitrile

Sample solution: 10 mg/mL of Carmustine in acetonitrile. [Note—Prepare in low-actinic glassware, and keep refrigerated until use.]

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 30-m × 0.53-mm column bonded with a 5-µm film of phase G1

Temperature

Injector: 70°

Detector: 260°

Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	210	3

Injector split ratio: 15:1

Carrier gas: Helium

Flow rate: 3 mL/min

Injection size: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetaldehyde in the portion of Carmustine taken:

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

r_U = peak response of acetaldehyde from the *Sample solution*

r_S = peak response of acetaldehyde from the *Standard solution*

C_S = concentration of acetaldehyde in the *Standard solution* (mg/mL)

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

Acceptance criteria

Acetaldehyde: NMT 0.1%

SPECIFIC TESTS

• **Water Determination, *Method I*⟨921⟩:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers at a temperature between 2° and 8°.

• **USP Reference Standards ⟨11⟩**

USP Carmustine RS

USP Carmustine Related Compound A RS

1,3-Bis(2-chloroethyl) urea.

$C_5H_{10}Cl_2N_2O$ 185.05

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

Topic/Question	Contact	Expert Committee
CARMUSTINE	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 35(3)
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