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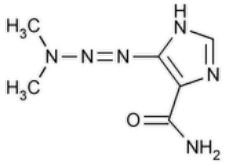
Dacarbazine



$C_6H_{10}N_6O$ 182.18

1*H*-Imidazole-4-carboxamide, 5-(3,3-dimethyltriaz-1-enyl)-;

5-(3,3-Dimethyltriaz-1-enyl)-1*H*-imidazole-4-carboxamide CAS RN®: 4342-03-04.

DEFINITION

Dacarbazine contains NLT 97.0% and NMT 102.0% of dacarbazine ($C_6H_{10}N_6O$).

[**Caution—**Great care should be taken in handling Dacarbazine, as it is a potent cytotoxic agent.]

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K
- **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**

Protect solutions containing dacarbazine from light and store at 2°–8°.

Solution A: 4.1 g/L of sodium acetate in water. Adjust with phosphoric acid to a pH of 7.0.

Solution B: 4.1 g/L of sodium acetate in water. Adjust with phosphoric acid to a pH of 5.5. Mix acetonitrile and this solution (25:75).

Mobile phase: See *Table 1*. Return to original conditions, and equilibrate the system for 10 min.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	5	95
23	5	95
25	100	0
35	100	0

Diluent: 0.1 mg/mL of citric acid in water

Standard solution: 0.1 mg/mL of USP Dacarbazine RS in *Diluent*

Sample solution: 0.1 mg/mL of Dacarbazine in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Autosampler temperature: 2°–8°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of dacarbazine ($C_6H_{10}N_6O$) in the portion of Dacarbazine 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 Dacarbazine RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 97.0%–102.0%

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

Change to read:

• **Organic Impurities**

Protect solutions containing dacarbazine and its related compounds from light and store at 2°–8°.

Solution A, Solution B, Mobile phase, and **Chromatographic system:** Proceed as directed in the *Assay*.

Diluent: 4.0 mg/mL of citric acid in water

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

Standard solution: 4.0 µg/mL of USP Dacarbazine RS prepared as follows. Transfer a suitable amount of *Standard stock solution* into a volumetric flask and dilute with water.

Dacarbazine related compound A standard solution: 0.04 mg/mL of USP Dacarbazine Related Compound A RS in water. Sonicate to ensure complete dissolution.

Dacarbazine related compound B standard solution: 0.04 mg/mL of USP Dacarbazine Related Compound B RS in water. Sonicate to ensure complete dissolution.

Sensitivity solution: 2.0 µg/mL of USP Dacarbazine RS in water from *Standard solution*

Sample solution: 4.0 mg/mL of Dacarbazine in *Diluent*. Sonicate to ensure complete dissolution.

System suitability

Samples: *Dacarbazine related compound A standard solution*, *Dacarbazine related compound B standard solution*, and *Sensitivity solution*

Suitability requirements

Relative standard deviation 1: NMT 5.0%, *Dacarbazine related compound A standard solution*

Relative standard deviation 2: NMT 5.0%, *Dacarbazine related compound B standard solution*

Signal-to-noise ratio: ▲NLT▲ (ERR 1-Dec-2021) 10, *Sensitivity solution*

Analysis

Samples: *Standard solution*, *Dacarbazine related compound A standard solution*, *Dacarbazine related compound B standard solution*, and *Sample solution*

Calculate the percentage of 5-aminoimidazole-4-carboxamide (free base of USP Dacarbazine Related Compound A RS) in the portion of Dacarbazine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of 5-aminoimidazole-4-carboxamide from the *Sample solution*

r_S = peak response of dacarbazine related compound A from the *Dacarbazine related compound A standard solution*

C_S = concentration of USP Dacarbazine Related Compound A RS in the *Dacarbazine related compound A standard solution* (mg/mL)

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

M_{r1} = molecular weight of 5-aminoimidazole-4-carboxamide, 126.12

M_{r2} = molecular weight of USP Dacarbazine Related Compound A RS, 162.58

Calculate the percentage of 2-azahypoxanthine (anhydrous) (anhydrous form of USP Dacarbazine Related Compound B RS) in the portion of Dacarbazine taken:

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

r_U = peak response of 2-azahypoxanthine (anhydrous) from the *Sample solution*

r_S = peak response of dacarbazine related compound B from the *Dacarbazine related compound B standard solution*

C_S = concentration of USP Dacarbazine Related Compound B RS in the *Dacarbazine related compound B standard solution* (mg/mL)

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

Calculate the percentage of each individual unspecified impurity in the portion of Dacarbazine taken:

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

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

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

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

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

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Aminoimidazole-4-carboxamide[a]	0.28	1.0
2-Azahypoxanthine (anhydrous)[b]	0.45	1.0
Dacarbazine	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

[a] It is the free base of USP Dacarbazine Related Compound A RS ($C_4H_6N_4O$).

[b] It is the anhydrous form of USP Dacarbazine Related Compound B RS ($C_4H_3N_5O$).

• **Limit of Dimethylamine**

System suitability stock solution A: 20 mg/mL of dimethylamine in water prepared as follows. Transfer USP Dimethylamine Solution RS into a suitable volumetric flask and dilute with water to volume.

System suitability stock solution B: 20 mg/mL of triethylamine in water

System suitability solution: *System suitability stock solution A* and *System suitability stock solution B* (1:1). Transfer 5 µL of this solution into a 20-mL headspace vial by syringe through the cap septum.

Standard solution: 10 mg/mL of dimethylamine in water prepared as follows. Transfer USP Dimethylamine Solution RS into a suitable volumetric flask and dilute with water to volume.

Standard: Transfer 5 µL of *Standard solution* into a 20-mL headspace vial by syringe through the cap septum (0.05 mg).

Sample: Transfer 100 mg of Dacarbazine into a 20-mL headspace vial, cap and seal. Add 5 µL of water by syringe through the cap septum.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 30-m x 0.53-mm; 1.0-µm coating of phase G50

Temperatures

Injection port: 210°

Detector: 300°

Column: See *Table 3*.

Table 3

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	0	35	8
35	40	200	1

Headspace operating parameters: [Note—Headspace operating parameters can be modified in order to optimize the performance.]

Temperatures

Oven: 100°

Loop: 110°

Transfer line: 120°

Times

Vial equilibration: 10 min

Pressurization: 0.5 min

Loop fill: 0.25 min

Loop equilibration: 0.05 min

Injection: 0.5 min

Loop size: 1.0 mL

Carrier gas: Helium

Flow rate: 2 mL/min

Injection type: Split, split ratio 25:1

System suitability

Samples: *Standard* and *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between the dimethylamine and triethylamine peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard*

Relative standard deviation: NMT 15.0%, *Standard*

Analysis

Samples: *Standard* and *Sample*

Calculate the percentage of dimethylamine in the portion of Dacarbazine taken:

$$\text{Result} = (r_U/r_S) \times (W_S/W_U) \times 100$$

r_U = peak response of dimethylamine from the *Sample*

r_S = peak response of dimethylamine from the *Standard*

W_S = weight of dimethylamine in the *Standard* (mg)

W_U = weight of Dacarbazine in the *Sample* (mg)

Acceptance criteria: NMT 0.05%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, in a refrigerator.
- **USP Reference Standards** ⟨11⟩

USP Dacarbazine RS

USP Dacarbazine Related Compound A RS

5-Aminoimidazole-4-carboxamide hydrochloride.

$C_4H_6N_4O \cdot HCl$ 162.58

USP Dacarbazine Related Compound B RS

2-Azahypoxanthine monohydrate.

$C_4H_3N_5O \cdot H_2O$ 155.12

USP Dimethylamine Solution RS

40% of dimethylamine in water

$(CH_3)_2NH$ 45.09

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

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

Chromatographic Database Information: Chromatographic Database

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