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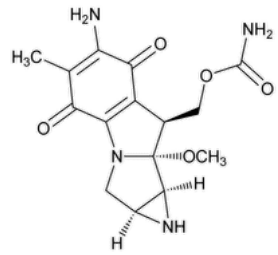
Mitomycin



$C_{15}H_{18}N_4O_5$ 334.33

Azirino[2',3':3,4]pyrrolo[1,2-*a*]indole-4,7-dione, 6-amino-8-[[(aminocarbonyl)oxy]methyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-, [1a*S*-(1aα,8β,8aα,8bα)]-;
(1a*S*,8*S*,8a*R*,8b*S*)-(6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-8-yl)methyl carbamate; Mitomycin C CAS RN®: 50-07-7; UNII: 50SG953SK6.

DEFINITION

Mitomycin has a potency of NLT 970 µg/mg of mitomycin ($C_{15}H_{18}N_4O_5$).

IDENTIFICATION

Change to read:

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

Analysis: Do not dry the sample and standard.

Acceptance criteria: Meets the requirements

• **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**

Mobile phase: Dissolve 1.54 g of ammonium acetate in 250 mL of methanol. Add 5.0 mL of 0.83 N acetic acid and water to make 1000 mL.

System suitability solution: 0.5 mg/mL of USP Mitomycin RS and 7.5 mg/mL of 3-ethoxy-4-hydroxybenzaldehyde in *N,N*-dimethylacetamide

Standard solution: 0.5 mg/mL of USP Mitomycin RS in *N,N*-dimethylacetamide

Sample solution: 0.5 mg/mL of Mitomycin in *N,N*-dimethylacetamide

Chromatographic system

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

Mode: LC

Detector: UV 365 nm

Column: 3.9-mm × 30-cm; 10-µm packing L11

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

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

[Note—The relative retention times for mitomycin and 3-ethoxy-4-hydroxybenzaldehyde are 1.0 and 1.4, respectively.]

Suitability requirements

Resolution: NLT 1.8 between mitomycin and 3-ethoxy-4-hydroxybenzaldehyde, *System suitability solution*

Tailing factor: NMT 1.3, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of mitomycin ($C_{15}H_{18}N_4O_5$) in the portion of Mitomycin taken:

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

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

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

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

P = potency of mitomycin in USP Mitomycin RS (µg/mg)

Acceptance criteria: NLT 970 µg/mg

IMPURITIES

• **Organic Impurities**

Solution A: 0.77 g/L of ammonium acetate

Solution B: Methanol and *Solution A* (20:80)

Solution C: Methanol and *Solution A* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	0	100
45	0	100
50	100	0

Standard solution: 0.025 mg/mL of USP Mitomycin RS in methanol

Sensitivity solution: 2.5 µg/mL of USP Mitomycin RS in methanol

Sample solution: 5 mg/mL of Mitomycin in methanol

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

Suitability requirements

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Mitomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

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

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

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

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

P = potency of mitomycin in USP Mitomycin RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

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

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Albomitomycin C[a]	0.6	1.0	0.5
Mitomycin	1.0	—	—
Mitomycin B[b]	1.2	1.0	0.5
Cinnamamide	1.3	2.9	0.5
Mitomycin A[c]	1.6	1.0	0.5
Any individual unspecified impurity	—	1.0	0.5
Total impurities	—	—	2.0

[a] {(1*S*,2*S*,4a*S*,8a*R*,9*S*,9a*R*)-7-Amino-9a-methoxy-6-methyl-5,8-dioxo-1,2,3,5,8,8a,9,9a-octahydro-1,2,4a-metheno-(epinitrilo)pyrrolo[1,2-*a*]indol-9-yl}methyl carbamate.

[b] {(1a*S*,8*S*,8a*R*,8b*S*)-8a-Hydroxy-6-methoxy-1,5-dimethyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-8-yl}methyl carbamate.

[c] {(1a*S*,8*S*,8a*R*,8b*S*)-6,8a-Dimethoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-8-yl}methyl carbamate.

SPECIFIC TESTS

- **Crystallinity ⟨695⟩:** Meets the requirements
- **pH ⟨791⟩**
  Sample: 5-mg/mL suspension in water
  Acceptance criteria: 6.0–7.5
- **Water Determination, *Method I* ⟨921⟩:** NMT 2.5%
- **Sterility Tests ⟨71⟩:** Where the label states that Mitomycin is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Mitomycin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 10.0 USP Endotoxin Units/mg of mitomycin.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.
- **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP Reference Standards ⟨11⟩**
  USP Mitomycin RS

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

Topic/Question	Contact	Expert Committee
MITOMYCIN	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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