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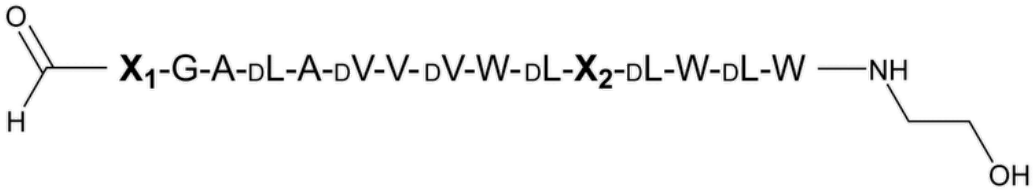
Gramicidin



Gramicidin	X_1	X_2	Molecular formula	Molecular weight
A1	V	W	$C_{99}H_{140}N_{20}O_{17}$	1882.33
A2	I	W	$C_{100}H_{142}N_{20}O_{17}$	1896.36
B1	V	F	$C_{97}H_{139}N_{19}O_{17}$	1843.30
C1	V	Y	$C_{97}H_{139}N_{19}O_{18}$	1859.29
C2	I	Y	$C_{98}H_{141}N_{19}O_{18}$	1873.32

Gramicidin

CAS RN®: 1405-97-6; UNII: 5IE62321P4.

DEFINITION

Gramicidin is an antibacterial substance produced by the growth of *Bacillus brevis* Dubos (Fam. Bacillaceae). It may be obtained from tyrothricin. It has a potency of NLT 900 µg of gramicidin per mg, calculated on the dried basis.

IDENTIFICATION

Delete the following:

▲• **Spectroscopic Identification Tests, ⟨197⟩***Ultraviolet-Visible Spectroscopy*▲ (USP 1-Dec-2023)

Add the following:

▲• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K▲ (USP 1-Dec-2023)

Add the following:

▲• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Composition*.▲ (USP 1-Dec-2023)

ASSAY

Change to read:

• **Antibiotics—Microbial Assays ⟨81⟩:** ▲Determine the median concentration (S3), starting with the concentration suggested in ⟨81⟩, *Table 7*, to obtain the optimum concentration–response relationship.▲ (USP 1-Dec-2023)

Acceptance criteria: It has a potency of NLT 900 µg of gramicidin per mg calculated on the dried basis.

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

Add the following:

▲• **Organic Impurities**

Mobile phase: Methanol and water (71:29)

Sensitivity solution: 2 µg/mL of USP Gramicidin RS in *Mobile phase*

Standard solution: 1 mg/mL of USP Gramicidin RS in *Mobile phase*

Sample solution: 1 mg/mL of Gramicidin in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 282 nm

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

Column temperature: 50°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution*

[NOTE—The relative retention times in *Table 1* are provided as information that could aid in peak assignment.]

Table 1

Name	Relative Retention Time
Gramicidin C1[a]	0.7
Gramicidin C2[b]	0.8
Gramicidin A1[c]	1.0
Gramicidin A2[d]	1.2
Gramicidin B1[e]	1.9

[a] *N*-Formyl-L-valylglycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-tyrosyl-D-leucyl-L-tryptophyl-D-leucyl-*N*-(2-hydroxyethyl)-L-tryptophanamide.

[b] *N*-Formyl-L-isoleucylglycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-tyrosyl-D-leucyl-L-tryptophyl-D-leucyl-*N*-(2-hydroxyethyl)-L-tryptophanamide.

[c] *N*-Formyl-L-valylglycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-*N*-(2-hydroxyethyl)-L-tryptophanamide.

[d] *N*-Formyl-L-isoleucylglycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-L-tryptophyl-D-leucyl-*N*-(2-hydroxyethyl)-L-tryptophanamide.

[e] *N*-Formyl-L-valylglycyl-L-alanyl-D-leucyl-L-alanyl-D-valyl-L-valyl-D-valyl-L-tryptophyl-D-leucyl-L-phenylalanyl-D-leucyl-L-tryptophyl-D-leucyl-*N*-(2-hydroxyethyl)-L-tryptophanamide.

Suitability requirements

Resolution: NLT 1.5 between gramicidin A1 and gramicidin A2, *Standard solution*

Signal-to-noise ratio: NLT 10 for the gramicidin A1 peak, *Sensitivity solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity peak (the peak other than gramicidins A1, A2, B1, C1, and C2):

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

r_U = peak response of the individual impurity

r_T = total response of all the peaks

Acceptance criteria: Disregard any impurity with a peak area less than that of gramicidin A1 in the *Sensitivity solution*. NMT 1 impurity exceeds 1.0%.

Any individual impurity: NMT 2.0▲ (USP 1-Dec-2023)

SPECIFIC TESTS

- **Melting Range or Temperature ⟨741⟩, *Procedures, Procedure for Class Ia*:** NLT 229°, determined after drying
- **Crystallinity ⟨695⟩:** Meets the requirements
- **Loss on Drying ⟨731⟩**

Sample: About 100 mg

Analysis: Dry the *Sample* in a capillary-stoppered bottle in vacuum at 60° for 3 h.

Acceptance criteria: NMT 3.0%

Add the following:

▲• **Composition**

Mobile phase, Sensitivity solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Organic Impurities*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each of the components (gramicidins A1, A2, B1, C1, and C2):

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

r_U = peak response of the individual component

r_T = total response of all the peaks

Acceptance criteria: See *Table 2*.

Table 2

Name	Acceptance Criteria
Ratio of gramicidin A1 to the sum of gramicidins A1, A2, B1, C1, and C2	NLT 0.60
Sum of gramicidins A1, A2, B1, C1, and C2	NLT 95.0%▲ (USP 1-Dec-2023)

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards ⟨11⟩**

USP Gramicidin RS

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

Topic/Question	Contact	Expert Committee
GRAMICIDIN	Julie Zhang Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

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