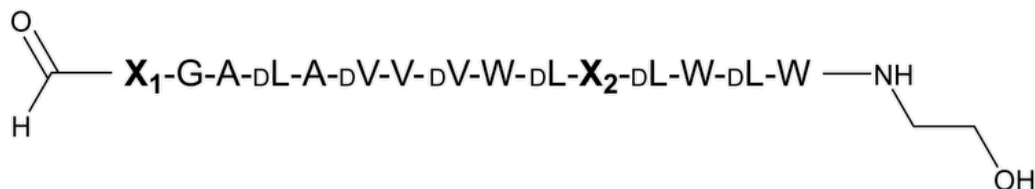


Status: Currently Official on 15-Feb-2025
 Official Date: Official as of 01-Dec-2023
 Document Type: USP Monographs
 DocId: GUID-5E45E63C-C24E-447F-864E-DF717D37781F_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M35850_03_01
 DOI Ref: 8a734

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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):

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)