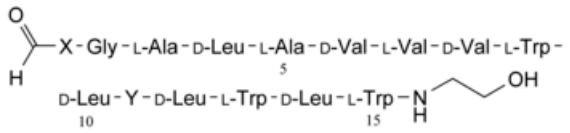
Edition: BP 2025 (Ph. Eur. 11.6 update)

Gramicidin

General Notices

(Ph. Eur. monograph 0907)



Gramicidin	X	Y	Mol. formula	M_r
A1	L-Val	L-Trp	$C_{99}H_{140}N_{20}O_{17}$	1882
A2	L-Ile	L-Trp	$C_{100}H_{142}N_{20}O_{17}$	1896
B1	L-Val	L-Phe	$C_{97}H_{139}N_{19}O_{17}$	1843
C1	L-Val	L-Tyr	$C_{97}H_{139}N_{19}O_{18}$	1859
C2	L-Ile	L-Tyr	$C_{98}H_{141}N_{19}O_{18}$	1873

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Gramicidin consists of a family of antimicrobial linear polypeptides, usually obtained by extraction from tyrothricin, the complex isolated from the fermentation broth of *Brevibacillus brevis* Dubos. The main component is gramicidin A1, together with gramicidins A2, B1, C1 and C2 in particular.

Content

Minimum 900 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder, slightly hygroscopic.

Solubility

Practically insoluble in water, soluble in methanol, sparingly soluble in ethanol (96 per cent).

mp

About 230 °C.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

A. Dissolve 0.100 g in *alcohol R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *alcohol R*. Examined between 240 nm and 320 nm (*2.2.25*), the solution shows 2 absorption maxima, at 282 nm and 290 nm, a shoulder at about 275 nm and an absorption minimum at 247 nm. The specific absorbance at the maximum at 282 nm is 105 to 125.
B. Thin-layer chromatography (*2.2.27*).

Test solution Dissolve 5 mg of the substance to be examined in 6.0 mL of *alcohol R*.

Reference solution (a) Dissolve 5 mg of *gramicidin CRS* in 6.0 mL of *alcohol R*.

Reference solution (b) Dissolve 5 mg of *tyrothricin CRS* in 6.0 mL of *alcohol R*.

Plate *TLC silica gel plate R*.

Mobile phase *methanol R*, *butanol R*, *water R*, *glacial acetic acid R*, *butyl acetate R* (3:9:15:24:49 *V/V/V/V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Dip the plate into *dimethylaminobenzaldehyde solution R2*. Heat at 90 °C until the spots appear.

System suitability The chromatogram obtained with reference solution (b) shows 2 clearly separated spots or 2 clearly separated groups of spots.

Results The principal spot or group of principal spots in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot or group of principal spots in the chromatogram obtained with reference solution (a) and to the spot or group of spots with the highest R_F value in the chromatogram obtained with reference solution (b).

C. Examine the chromatograms obtained in the test for composition.

Results The 3 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 3 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

Composition

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dissolve 25 mg of the substance to be examined in 10 mL of *methanol R* and dilute to 25 mL with the mobile phase.

Reference solution (a) Dissolve 25 mg of *gramicidin CRS* in 10 mL of *methanol R* and dilute to 25 mL with the mobile phase.

Reference solution (b) Dilute 1.0 mL of reference solution (a) to 50.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm,

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 50 °C.

Mobile phase *water R*, *methanol R* (29:71 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 282 nm.

Injection 20 µL.

Run time 2.5 times the retention time of gramicidin A1.

Relative retention With reference to gramicidin A1 (retention time = about 22 min): gramicidin C1 = about 0.7; gramicidin C2 = about 0.8; gramicidin A2 = about 1.2; gramicidin B1 = about 1.9.

System suitability Reference solution (a):

— *resolution*: minimum 1.5 between the peaks due to gramicidin A1 and gramicidin A2,

— the chromatogram obtained is concordant with the chromatogram supplied with *gramicidin CRS*.

Limits:

— *sum of gramicidins A1, A2, B1, C1 and C2*: minimum 95.0 per cent,

— *ratio of gramicidin A1 to the sum of gramicidins A1, A2, B1, C1 and C2*: minimum 0.60,

— *disregard limit*: the area of the peak due to gramicidin A1 in the chromatogram obtained with reference solution (b).

Related substances

Liquid chromatography (*2.2.29*) as described in the test for composition.

Limit:

— *any impurity*: maximum 2.0 per cent and not more than 1 peak is more than 1.0 per cent; disregard the peaks due to gramicidins A1, A2, B1, C1 and C2.

Loss on drying (*2.2.32*)

Maximum 3.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 1.0 g.

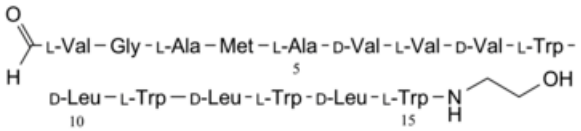
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*), using the turbidimetric method. Use *gramicidin CRS* as the reference substance.

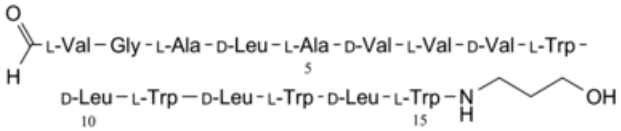
STORAGE

In an airtight container, protected from light.

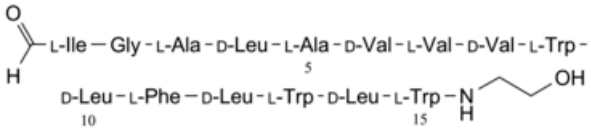
IMPURITIES



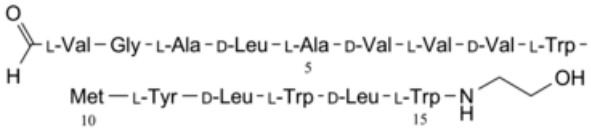
A. [4-methionine]gramicidin A1,



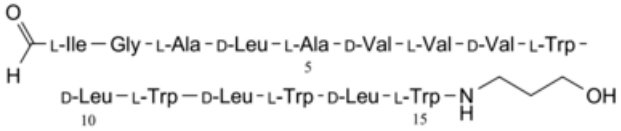
B. gramicidin A1 3-hydroxypropyl,



C. gramicidin B2,



D. [10-methionine]gramicidin C1,



E. gramicidin A2 3-hydroxypropyl.

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