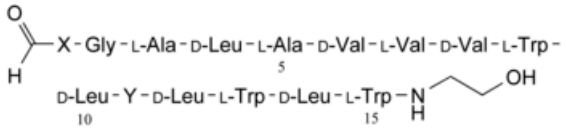
Edition: BP 2025 (Ph. Eur. 11.6 update)

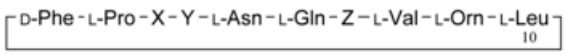
Tyrothricin

General Notices

(Ph. Eur. monograph 1662)



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



Tyrocidin	Mol. formula	M_r	X	Y	Z
A	$C_{66}H_{88}N_{13}O_{13}$	1271	L-Phe	D-Phe	L-Tyr
B	$C_{68}H_{89}N_{14}O_{13}$	1311	L-Trp	D-Phe	L-Tyr
C	$C_{70}H_{90}N_{15}O_{13}$	1350	L-Trp	D-Trp	L-Tyr
D	$C_{72}H_{91}N_{16}O_{12}$	1373	L-Trp	D-Trp	L-Trp
E	$C_{66}H_{88}N_{13}O_{12}$	1255	L-Phe	D-Phe	L-Phe

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Mixture of antimicrobial linear and cyclic polypeptides, isolated from the fermentation broth of *Brevibacillus brevis* Dubos. consists mainly of gramicidins and tyrocidins as described above; other related compounds may be present in smaller amounts.

Potency

180 IU/mg to 280 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white powder.

Solubility

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

IDENTIFICATION

First identification: B.

Second identification: A.

A. Thin-layer chromatography (2.2.27).

Test solution Dissolve 5 mg of the substance to be examined in 4.0 mL of *ethanol (96 per cent) R*.

Reference solution Dissolve 5 mg of *tyrothricin CRS* in 4.0 mL of *ethanol (96 per cent) R*.

Plate *TLC silica gel F_{254} plate R*.

Mobile phase *methanol R*, *butanol R*, *water R*, *acetic acid R*, *butyl acetate R* (2.5:7.5:12:20:40 *V/V/V/V/V*).

Application 1 µL.

Development Over 2/3 of the plate.

Drying In a current of warm air.

Detection A Examine in ultraviolet light at 254 nm.

Results A The principal spots or groups of principal spots in the chromatogram obtained with the test solution are simila in position and size to the principal spots or groups of principal spots in the chromatogram obtained with the reference solution. The upper group corresponds to gramicidins, the lower group to tyrocidins.

Detection B Spray with *dimethylaminobenzaldehyde solution R2*. Heat the plate in a current of warm air until the spots appear.

System suitability Reference solution:

— the chromatogram shows 2 clearly separated spots or groups of spots.

Results B The principal spots or groups of principal spots in the chromatogram obtained with the test solution are simila in position, colour and size to the principal spots or groups of principal spots in the chromatogram obtained with the reference solution. The upper group corresponds to gramicidins, the lower group to tyrocidins.

B. Composition (see Tests).

TESTS

Composition

Liquid chromatography (2.2.29): use the normalisation procedure. *Prepare the solutions immediately before use.*

Test solution Dissolve 5 mg of the substance to be examined in 2 mL of *methanol R* and dilute to 5.0 mL with the mobile phase.

Reference solution (a) Dissolve 5 mg of *tyrothricin CRS* in 2 mL of *methanol R* and dilute to 5.0 mL with the mobile phase.

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

Column:

— *size*: *l* = 0.25 m, *Ø* = 4.6 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 60 °C.

Mobile phase 0.79 g/L solution of *ammonium sulfate R*, *methanol R* (25:75 *V/V*).

Flow rate 1.2 mL/min.

Detection Spectrophotometer at 280 nm.

Injection 25 µL.

Run time 6 times the retention time of gramicidin A1. Use the chromatogram obtained with reference solution (a) and the chromatogram supplied with *tyrothricin CRS* to identify the peaks due to gramicidin A1, gramicidin A2 and the tyrocidins.

Relative retention With reference to gramicidin A1 (retention time = about 10 min): gramicidin C1 = about 0.8; gramicidin C2 = about 0.9; gramicidin A2 = about 1.1; tyrocidins = about 1.5 to 6.

System suitability Reference solution (a):

— *peak-to-valley ratio*: minimum 3.0, where H_p = height above the baseline of the peak due to gramicidin A2 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to gramicidin A1.

Limits:

— *sum of gramicidins*: 25 per cent to 50 per cent,

— *sum of tyrocidins*: 50 per cent to 70 per cent,

— *total*: minimum 85 per cent,

— *disregard limit*: the sum of the areas of the peaks due to gramicidins in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*)

Maximum 4.0 per cent, determined on 1.000 g by drying under high vacuum at 60 °C for 3 h.

Sulfated ash (*2.4.14*)

Maximum 1.5 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.

Test solution Prepare a solution of tyrothricin containing about the same amount of gramicidin as the corresponding solution of *gramicidin CRS* i.e. 5 times more concentrated.

STORAGE

In an airtight container, protected from light.