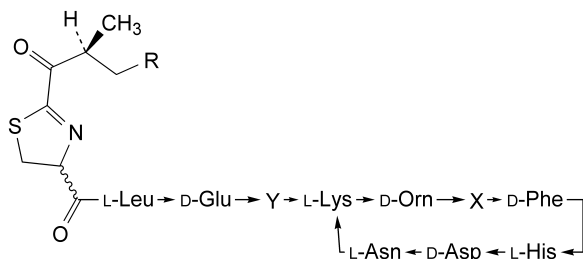


Figure 0465.-2. – Chromatogram of the test for impurity E in bacitracin obtained with reference solution (d) at 300 nm



E. X = Y = L-Ile, R = CH₃: bacitracin F,

F. X = Y = L-Ile, R = H: bacitracin H1,

G. X = L-Val, Y = L-Ile, R = CH₃: bacitracin H2,

H. X = L-Ile, Y = L-Val, R = CH₃: bacitracin H3,

I. X = L-Val, Y = L-Ile, R = H: bacitracin I1,

J. X = L-Ile, Y = L-Val, R = H: bacitracin I2,

K. X = Y = L-Val, R = CH₃: bacitracin I3.

CHARACTERS

Appearance: white or light yellowish-grey powder, hygroscopic.

Solubility: slightly soluble in water and in alcohol.

IDENTIFICATION

First identification: B, C.

Second identification: A, C.

A. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in 0.5 ml of *dilute hydrochloric acid R* and dilute to 1.0 ml with *water R*.

Reference solution. Dissolve 10 mg of *bacitracin zinc CRS* in 0.5 ml of *dilute hydrochloric acid R* and dilute to 1.0 ml with *water R*.

Plate: TLC silica gel plate R.

Mobile phase: glacial acetic acid R, water R, butanol R (1:2:4 V/V/V).

Application: 10 µl.

Development: over half of the plate.

Drying: at 100-105 °C.

Detection: spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results: the spots in the chromatogram obtained with the test solution are similar in position, size and colour to the spots in the chromatogram obtained with the reference solution.

B. It complies with the test for composition (see Tests).

C. Ignite about 0.15 g, allow to cool and dissolve the residue in 1 ml of *dilute hydrochloric acid R*. Add 4 ml of *water R*. The solution gives the reaction of zinc (2.3.1).

01/2008:0466

BACITRACIN ZINC

Bacitracinum zincum

DEFINITION

Zinc complex of bacitracin, which consists of a mixture of antimicrobial polypeptides produced by certain strains of *Bacillus licheniformis* or *Bacillus subtilis*, the main components being bacitracins A, B1, B2 and B3.

Content: minimum 60 IU/mg (dried substance).

TESTS

pH (2.2.3): 6.0 to 7.5.

Shake 1.0 g for about 1 min with 10 ml of *carbon dioxide-free water R* and filter.

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

Test solution. Dissolve 0.100 g of the substance to be examined in 50.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*.

Reference solution (a). Dissolve 20.0 mg of *bacitracin zinc CRS* in 10.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*.

Reference solution (b). Dilute 5.0 ml of the test solution to 100.0 ml with *water R*.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 10.0 ml with *water R*.

Reference solution (d). Dissolve 50.0 mg of the substance to be examined in 25.0 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*. Heat in a boiling water-bath for 30 min. Cool to room temperature.

Blank solution. A 40 g/l solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide R*.

Column:

- size: $l = 0.25$ m, $\varnothing = 4.6$ mm,
- stationary phase: *end-capped octadecylsilyl silica gel for chromatography R* (5 μ m).

Mobile phase: add 520 volumes of *methanol R1*, 40 volumes of *acetonitrile R* and 300 volumes of *water R* to 100 volumes of a 34.8 g/l solution of *dipotassium hydrogen phosphate R*, adjusted to pH 6.0 with a 27.2 g/l solution of *potassium dihydrogen phosphate R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 100 μ l; inject the blank, the test solution and reference solutions (a) and (c).

Run time: 3 times the retention time of bacitracin A.

Relative retention with reference to bacitracin A (retention time = 15 min to 25 min): bacitracin B1 = about 0.6; bacitracin B3 = about 0.8; impurity E = about 2.5.

If necessary, adjust the composition of the mobile phase by changing the amount of organic modifier whilst keeping the ratio constant between methanol and acetonitrile.

System suitability: reference solution (a):

- **peak-to-valley ratio:** minimum of 1.2, where H_p = height above the baseline of the peak due to bacitracin B1 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B2.

Limits:

- **bacitracin A:** minimum 40.0 per cent,
- **sum of bacitracins A, B1, B2 and B3:** minimum 70.0 per cent.
- **disregard limit:** the area of the peak due to bacitracin A in the chromatogram obtained with reference solution (c) (0.5 per cent); disregard any peak observed in the blank run.

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

See Figure 0466-1.

Limit:

- **sum of the areas of all peaks eluting before the peak due to bacitracin B1:** maximum 20.0 per cent.

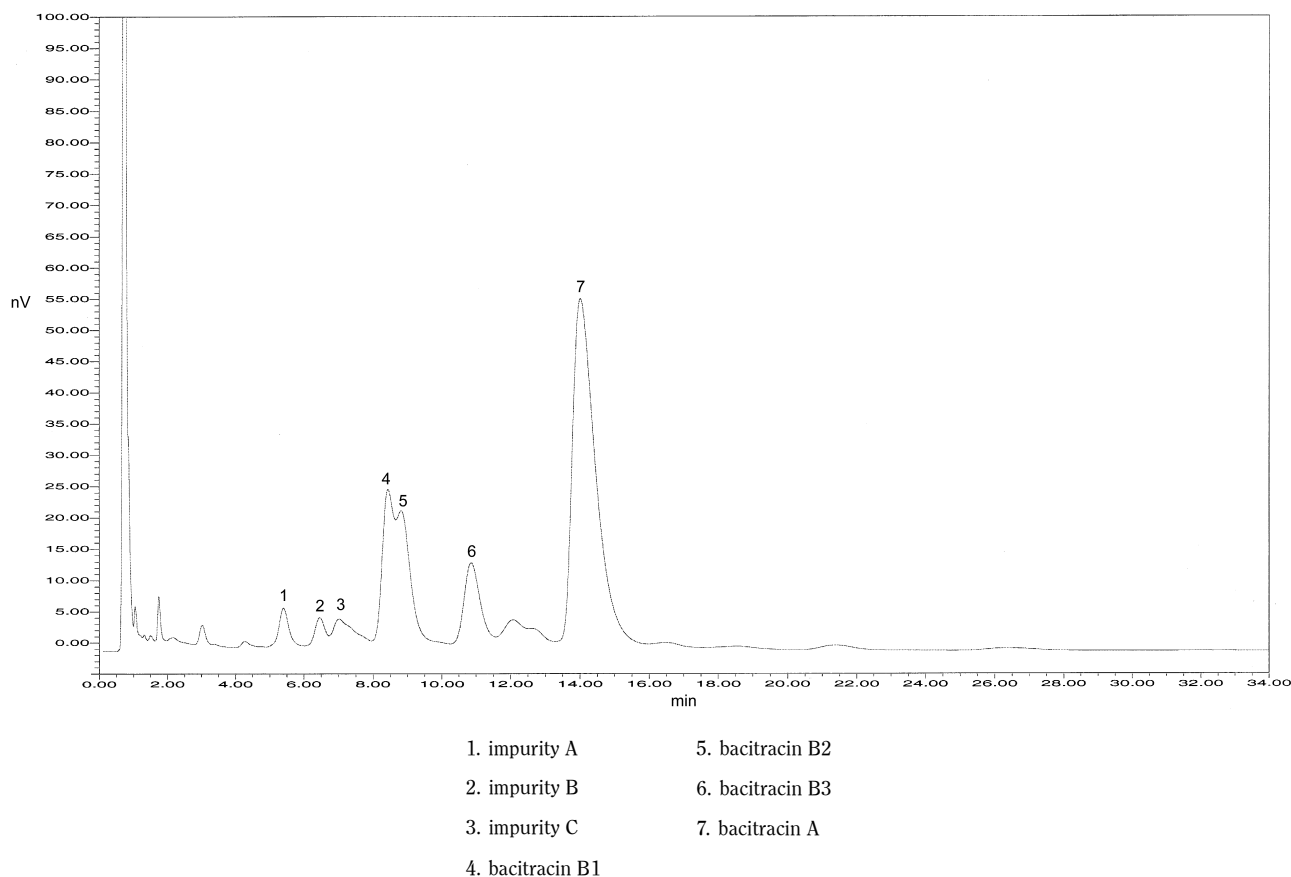


Figure 0466-1. – Chromatogram of the test for composition in bacitracin zinc obtained with the test solution at 254 nm

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

See Figure 0466-2.

Detection: spectrophotometer at 254 nm; spectrophotometer at 300 nm for reference solution (d).

Injection: test solution and reference solutions (b) and (d).

Limit:

- **impurity E:** not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent).

Zinc: 4.0 per cent to 6.0 per cent (dried substance).

Dissolve 0.200 g in a mixture of 2.5 ml of *dilute acetic acid R* and 2.5 ml of water. Add 50 ml of *water R*, 50 mg of *xylene orange triturate R* and sufficient *hexamethylenetetramine R* to produce a red colour. Add 2 g of *hexamethylenetetramine R* in excess. Titrate with 0.01 M *sodium edetate* until a yellow colour is obtained.

1 ml of 0.01 M *sodium edetate* is equivalent to 0.654 mg of Zn.

Loss on drying (2.2.32): maximum 5.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa for 3 h.

Sterility (2.6.1). If intended for administration by spraying into internal body cavities without a further appropriate sterilisation procedure, it complies with the test for sterility.

Pyrogens (2.6.8). If intended for administration by spraying into internal body cavities without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass

1 ml of the supernatant liquid obtained by centrifuging a suspension containing 11 mg per millilitre in a 9 g/l solution of *sodium chloride R*.

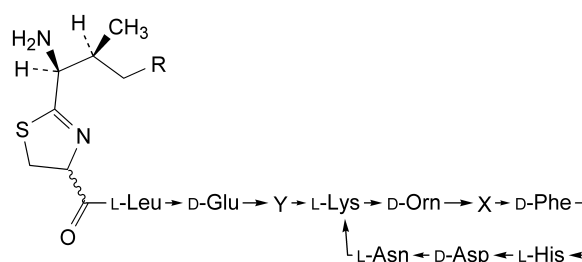
ASSAY

Suspend 50.0 mg in 5 ml of *water R*, add 0.5 ml of *dilute hydrochloric acid R* and dilute to 100.0 ml with *water R*. Allow the solution to stand for 30 min. Carry out the microbiological assay of antibiotics (2.7.2).

STORAGE

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES



A. X = L-Val, Y = L-Ile, R = H: bacitracin C1,

B. X = L-Ile, Y = L-Val, R = H: bacitracin C2,

C. X = Y = L-Val, R = CH₃: bacitracin C3,

D. X = Y = L-Val, R = H: bacitracin E,

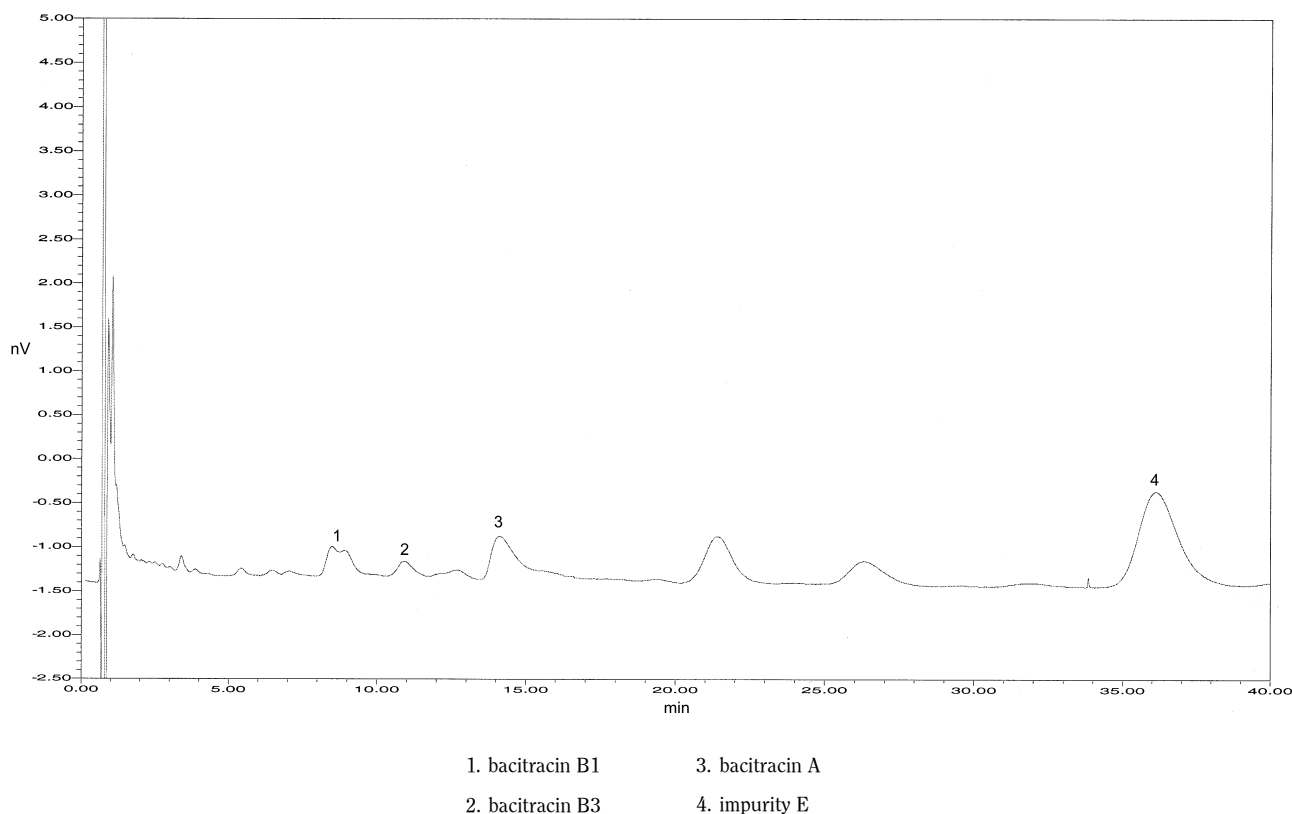
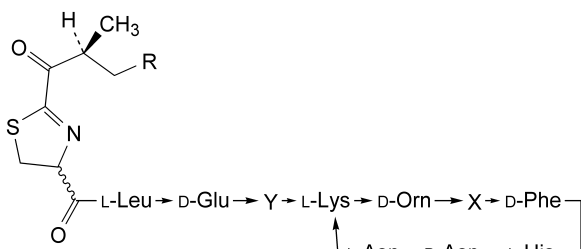


Figure 0466-2. — Chromatogram of the test for impurity E in bacitracin zinc obtained with reference solution (d) at 300 nm



E. X = Y = L-Ile, R = CH₃: bacitracin F,

F. X = Y = L-Ile, R = H: bacitracin H1,

G. X = L-Val, Y = L-Ile, R = CH₃: bacitracin H2,

H. X = L-Ile, Y = L-Val, R = CH₃: bacitracin H3,

I. X = L-Val, Y = L-Ile, R = H: bacitracin I1,

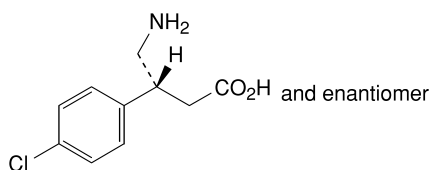
J. X = L-Ile, Y = L-Val, R = H: bacitracin I2,

K. X = Y = L-Val, R = CH₃: bacitracin I3.

01/2008:0653

BACLOFEN

Baclofenum



C₁₀H₁₂ClNO₂
[1134-47-0]

M_r 213.7

DEFINITION

(3*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid.

Content: 98.0 per cent to 101.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: slightly soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in acetone. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

It shows polymorphism (5.9).

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (2.2.25).

Test solution. Dissolve 70 mg in *water R* and dilute to 100.0 ml with the same solvent.

Spectral range: 220-320 nm.

Absorption maxima: at 259 nm, 266 nm and 275 nm.

Resolution (2.2.25): minimum 1.5 for the absorbance ratio.

Specific absorbance at the absorption maxima:

- at 259 nm: 9.8 to 10.8;
- at 266 nm: 11.5 to 12.7;
- at 275 nm: 8.4 to 9.3.

B. Infrared absorption spectrophotometry (2.2.24).

Preparation: discs prepared using 3 mg of substance and 300 mg of *potassium bromide R*.

Comparison: *baclofen CRS*.

If the spectra obtained in the solid state show differences, dissolve 0.1 g of each of the substances separately in 1 ml of *dilute sodium hydroxide solution R* and add 10 ml of *ethanol (96 per cent) R* and 1 ml of *dilute acetic acid R*. Allow to stand for 1 h. Filter, wash the precipitate with *ethanol (96 per cent) R* and dry *in vacuo*. Prepare new discs and record the spectra.

C. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution. Dissolve 10 mg of *baclofen CRS* in the mobile phase and dilute to 10 ml with the mobile phase.

Plate: *TLC silica gel G plate R*.

Mobile phase: *anhydrous formic acid R*, *water R*, *methanol R*, *chloroform R*, *ethyl acetate R* (5:5:20:30:40 V/V/V/V/V).

Application: 5 µl.

Development: over a path of 12 cm.

Drying: allow the solvents to evaporate.

Detection: spray with *ninhydrin solution R3* until the plate is slightly wet. Place in an oven maintained at 100 °C for 10 min. Examine in daylight.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution. The solution is not more opalescent than reference suspension II (2.2.1) and not more intensely coloured than reference solution BY₅ (2.2.2, *Method II*).

Dissolve 0.50 g in 1 M *sodium hydroxide* and dilute to 25 ml with the same solvent.

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 25.0 mg of *baclofen impurity A CRS* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (d). Dilute 2.0 ml of the test solution and 2.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (10 µm).

Mobile phase: dissolve 1.822 g of *sodium hexanesulphonate R* in 1 litre of a mixture of 560 volumes of *water R*, 440 volumes of *methanol R* and 5 volumes of *glacial acetic acid R*.