Reference solution (c). Dissolve 5 mg of *amikacin CRS* and 5 mg of *amikacin impurity A CRS* in *water R* and dilute to 50 ml with the same solvent. Then prepare as prescribed for test solution (a).

Blank solution. Prepare as described for test solution (a) using 0.2 ml of *water R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 30 °C.

Mobile phase: mix 30 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 6.5 with a 22 g/l solution of *potassium hydroxide R*, and 70 volumes of *methanol R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 340 nm.

Injection: 20 µl of test solution (a) and reference solutions (a) and (c).

Run time: 4 times the retention time of amikacin.

System suitability: reference solution (c):

– *resolution*: minimum 3.5 between the peaks due to amikacin and impurity A (see Figure 1289.-1).

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent);

– *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *sum of impurities other than A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard any peak due to the blank.

Water (*2.5.12*): maximum 8.5 per cent, determined on 0.200 g.

Sulphated ash (*2.4.14*)): maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

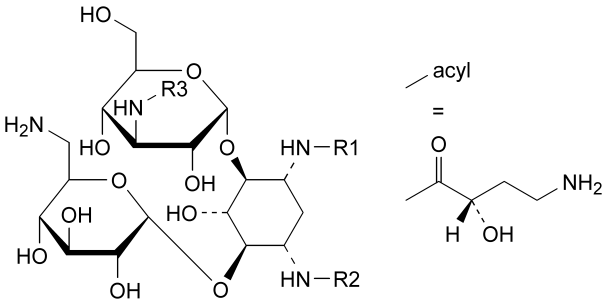
Injection: test solution (b) and reference solution (b).

System suitability:

– *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (b).

Calculate the percentage content of $C_{22}H_{43}N_5O_{13}$ from the declared content of *amikacin CRS*.

IMPURITIES



A. R1 = R3 = H, R2 = acyl: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

B. R1 = R2 = acyl, R3 = H: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1,3-*N*-bis[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

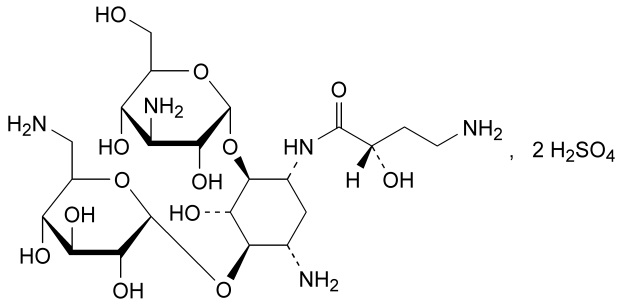
C. R1 = R2 = H, R3 = acyl: 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-*O*-[3-[[(2*S*)-4-amino-2-hydroxybutanoyl]amino]-3-deoxy-α-D-glucopyranosyl]-2-deoxy-D-streptamine,

D. R1 = R2 = R3 = H: kanamycin.

01/2008:1290
corrected 6.0

AMIKACIN SULPHATE

Amikacini sulfas



$C_{22}H_{47}N_5O_{21}S_2$ M_r 782
[39831-55-5]

DEFINITION

6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulphate.

Antimicrobial substance obtained from kanamycin A.

Semi-synthetic product derived from a fermentation product.

Content: 96.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *amikacin sulphate CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 25 mg of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 25 mg of *amikacin sulphate CRS* in *water R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 5 mg of *kanamycin monosulphate CRS* in 1 ml of the test solution and dilute to 10 ml with *water R*.

Plate: *TLC silica gel plate R*.

Mobile phase: the lower layer of a mixture of equal volumes of *concentrated ammonia R, methanol R* and *methylene chloride R*.

Application: 5 µl.

Development: over a path of 15 cm.

Drying: in air.

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

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.

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 (a).

C. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

pH (*2.2.3*): 2.0 to 4.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*): + 76 to + 84 (dried substance).

Dissolve 0.50 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Maintain the solutions at 10 °C.*

Test solution (a). Dissolve 0.100 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent. In a ground-glass-stoppered vial, add 0.2 ml of this solution to 2.0 ml of a 10 g/l solution of *2,4,6-trinitrobenzene sulphonic acid R*. Then add 3.0 ml of *pyridine R* and close the vial tightly. Shake vigorously for 30 s and heat on a water-bath at 75 °C for 2 h. Cool in cold water for 2 min and add 2 ml of *glacial acetic acid R*. Shake vigorously for 30 s.

Test solution (b). Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (a). Dissolve 10.0 mg of *amikacin impurity A CRS* in *water R* and dilute to 100.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

Reference solution (b). Dissolve 50.0 mg of *amikacin sulphate CRS* in *water R* and dilute to 50.0 ml with the same solvent. Then prepare as prescribed for test solution (a).

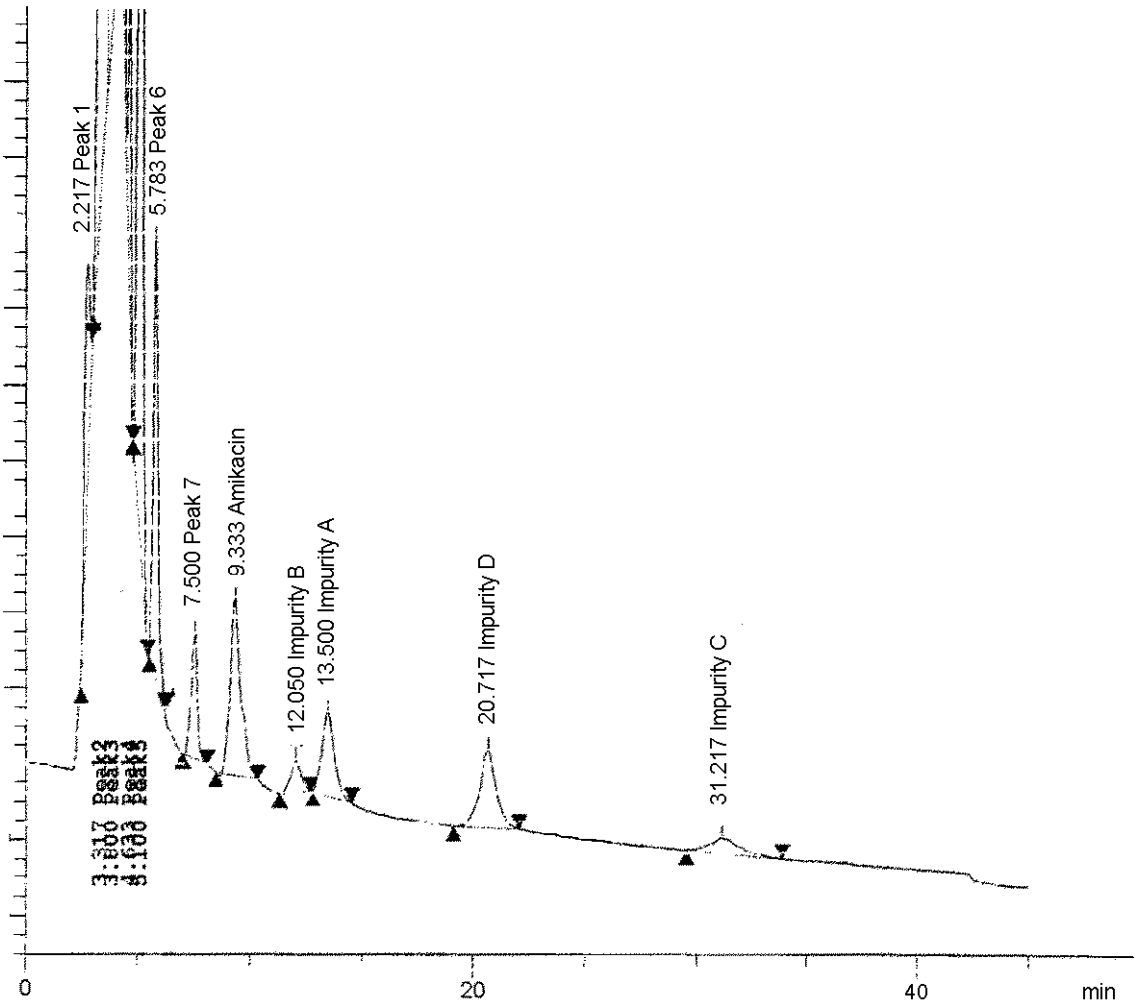


Figure 1290.-1. – *Chromatogram for the test for related substances of amikacin sulphate*

Reference solution (c). Dissolve 5 mg of *amikacin sulphate CRS* and 5 mg of *amikacin impurity A CRS* in *water R* and dilute to 50 ml with the same solvent. Then prepare as prescribed for test solution (a).

Blank solution. Prepare as described for test solution (a) using 0.2 ml of *water R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 30 °C.

Mobile phase: mix 30 volumes of a 2.7 g/l solution of *potassium dihydrogen phosphate R*, adjusted to pH 6.5 with a 22 g/l solution of *potassium hydroxide R*, and 70 volumes of *methanol R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 340 nm.

Injection: 20 µl of test solution (a) and reference solutions (a) and (c).

Run time: 4 times the retention time of amikacin.

System suitability: reference solution (c):

– *resolution*: minimum 3.5 between the peaks due to amikacin and impurity A (see Figure 1290.-1).

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

– *any other impurity*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *sum of impurities other than A*: not more than 1.5 times the area of the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); disregard any peak due to the blank and any peak eluting before the principal peak.

Sulphate: 23.3 per cent to 25.8 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *ethanol (96 per cent) R* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of sulphate (SO_4).

Loss on drying (*2.2.32*): maximum 13.0 per cent, determined on 0.500 g by drying in an oven at 105 °C at a pressure not exceeding 0.7 kPa for 3 h.

Pyrogens (*2.6.8*). If intended for use in the manufacture of parenteral dosage forms 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 5 ml of a solution containing 25 mg of the substance to be examined in *water for injections R*.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

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

System suitability:

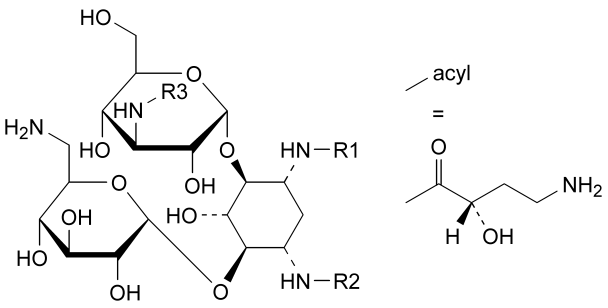
– *repeatability*: maximum relative standard deviation of 2.0 per cent after 6 injections of reference solution (b).

Calculate the percentage content of $C_{22}H_{47}N_5O_{21}S_2$ from the declared content of *amikacin sulphate CRS*.

STORAGE

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

IMPURITIES



A. R1 = R3 = H, R2 = acyl: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

B. R1 = R2 = acyl, R3 = H: 4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1,3-*N*-bis[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine,

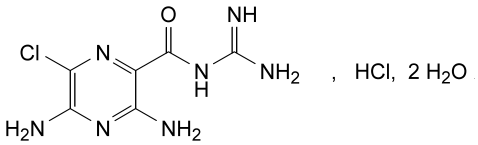
C. R1 = R2 = H, R3 = acyl: 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-*O*-[3-[[(2*S*)-4-amino-2-hydroxybutanoyl]amino]-3-deoxy-α-D-glucopyranosyl]-2-deoxy-D-streptamine,

D. R1 = R2 = R3 = H: kanamycin.

01/2008:0651

AMILORIDE HYDROCHLORIDE

Amiloridi hydrochloridum



$C_6H_9Cl_2N_7O,2H_2O$ M_r 302.1

[17440-83-4]

DEFINITION

3,5-Diamino-*N*-carbamimidoyl-6-chloropyrazine-2-carboxamide hydrochloride dihydrate.

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

CHARACTERS

Appearance: pale yellow or greenish-yellow powder.

Solubility: slightly soluble in water and in anhydrous ethanol.