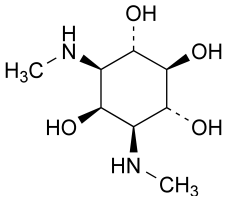
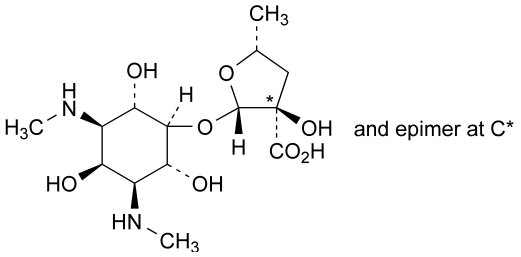
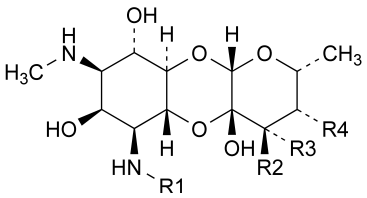
identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, C, F, G.*



A. 1,3-dideoxy-1,3-bis(methylamino)-*myo*-inositol (actinamine),



B. (2*S*,3*RS*,5*R*)-3-hydroxy-5-methyl-2-[[(1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,4,6-trihydroxy-3,5-bis(methylamino)cyclohexyl]oxy]tetrahydrofuran-3-carboxylic acid (actinospectinoic acid),

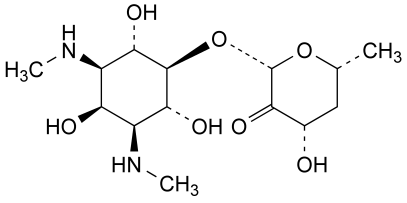


C. R1 = CH_3, R2 = R4 = H, R3 = OH: (2*R*,4*S*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4]benzodioxine-4,4a,7,9-tetrol ((4*S*)-dihydrospectinomycin),

D. R1 = CH_3, R2 = H, R3 = R4 = OH: (2*R*,3*R*,4*S*,4a*S*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-2-methyl-6,8-bis(methylamino)decahydro-2*H*-pyrano[2,3-*b*][1,4]benzodioxine-3,4,4a,7,9-pentol (dihydroxyspectinomycin),

E. R1 = R4 = H, R2 + R3 = O: (2*R*,4a*R*,5a*R*,6*S*,7*R*,8*R*,9*S*,9a*R*,10a*S*)-6-amino-4a,7,9-trihydroxy-2-methyl-8-(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one (*N*-desmethylspectinomycin),

G. R1 = CH_3, R2 + R3 = O, R4 = OH: (2*R*,3*S*,4a*R*,5a*R*,6*S*,7*S*,8*R*,9*S*,9a*R*,10a*S*)-3,4a,7,9-tetrahydroxy-2-methyl-6,8-bis(methylamino)decahydro-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one (tetrahydroxyspectinomycin),

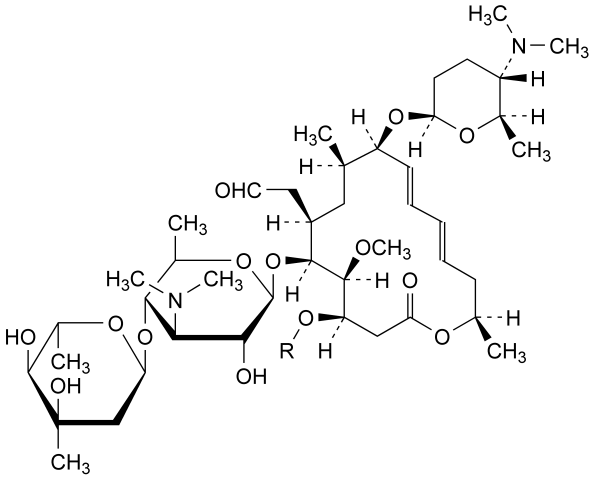


F. (2*S*,4*S*,6*R*)-4-hydroxy-6-methyl-2-[[(1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-2,4,6-trihydroxy-3,5-bis(methylamino)cyclohexyl]oxy]dihydro-2*H*-pyran-3(4*H*)-one (triol spectinomycin).

01/2008:0293

SPIRAMYCIN

Spiramycinum



Compound	R	Molec Formula	M_r
Spiramycin I	H	$C_{43}H_{74}N_2O_{14}$	843.1
Spiramycin II	CO-CH_3	$C_{45}H_{76}N_2O_{15}$	885.1
Spiramycin III	CO-CH_2-CH_3	$C_{46}H_{78}N_2O_{15}$	899.1

[8025-81-8]

DEFINITION

Macrolide antibiotic produced by the growth of certain strains of *Streptomyces ambofaciens* or obtained by any other means. The main component is (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*erythro*-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-2-one (spiramycin I; M_r 843). Spiramycin II (4-*O*-acetylspiramycin I) and spiramycin III (4-*O*-propanoylspiramycin I) are also present.

Potency: minimum 4100 IU/mg (dried substance).

CHARACTERS

Appearance: white or slightly yellowish powder, slightly hygroscopic.

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

IDENTIFICATION

A. Dissolve 0.10 g in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Examined between 220 nm and 350 nm (*2.2.25*), the solution shows an absorption maximum at 232 nm. The specific absorbance at the absorption maximum is about 340.

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

Test solution. Dissolve 40 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 40 mg of *spiramycin CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 40 mg of *erythromycin A CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel G plate R.*

Mobile phase: the upper layer of a mixture of 4 volumes of *2-propanol R*, 8 volumes of a 150 g/l solution of *ammonium acetate R* previously adjusted to pH 9.6 with *strong sodium hydroxide solution R*, and 9 volumes of *ethyl acetate R*.

Application: 5 µl.

Development: over 3/4 of the plate.

Drying: in air.

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

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 reference solution (a). If in the chromatogram obtained with the test solution 1 or 2 spots occur with R_F values slightly higher than that of the principal spot, these spots are similar in position and colour to the secondary spots in the chromatogram obtained with reference solution (a) and differ from the spots in the chromatogram obtained with reference solution (b).

C. Dissolve 0.5 g in 10 ml of *0.05 M sulphuric acid* and add 25 ml of *water R*. Adjust to about pH 8 with *0.1 M sodium hydroxide* and dilute to 50 ml with *water R*. To 5 ml of this solution add 2 ml of a mixture of 1 volume of *water R* and 2 volumes of *sulphuric acid R*. A brown colour develops.

TESTS

pH (*2.2.3*): 8.5 to 10.5.

Dissolve 0.5 g in 5 ml of *methanol R* and dilute to 100 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): – 80 to – 85 (dried substance).

Dissolve 1.00 g in a 10 per cent *V/V* solution of *dilute acetic acid R* and dilute to 50.0 ml with the same acid solution.

Composition. Liquid chromatography (*2.2.29*) as described in the test for related substances.

Injection: test solution and reference solution (a).

Calculate the percentage content using the declared content of spiramycins I, II and III in *spiramycin CRS*.

Composition of spiramycins (dried substance):

– *spiramycin I*: minimum 80.0 per cent,
– *spiramycin II*: maximum 5.0 per cent,
– *spiramycin III*: maximum 10.0 per cent,
– *sum of spiramycins I, II and III*: minimum 90.0 per cent.

Related substances. Liquid chromatography (*2.2.29*).

Prepare the solutions immediately before use.

Test solution. Dissolve 25.0 mg of the substance to be examined in a mixture of 3 volumes of *methanol R* and 7 volumes of *water R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (a). Dissolve 25.0 mg of *spiramycin CRS* in a mixture of 3 volumes of *methanol R* and 7 volumes of *water R* and dilute to 25.0 ml with the same mixture of solvents.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 100.0 ml with a mixture of 3 volumes of *methanol R* and 7 volumes of *water R*.

Reference solution (c). Dissolve 5 mg of *spiramycin CRS* in 15.0 ml of *buffer solution pH 2.2 R* and dilute to 25.0 ml with *water R*, then heat in a water-bath at 60 °C for 30 min.

Blank solution: *methanol R, water R* (3:7 *V/V*).

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm) (polar embedded octadecylsilyl methylsilica gel), with a pore size of 12.5 nm and a carbon loading of 15 per cent,
– *temperature*: 70 °C.

Mobile phase: mix 5 volumes of a 34.8 g/l solution of *dipotassium hydrogen phosphate R* previously adjusted to pH 6.5 with a 27.2 g/l solution of *potassium dihydrogen phosphate R*, 40 volumes of *acetonitrile R* and 55 volumes of *water R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 232 nm.

Injection: 20 µl of the blank solution, the test solution and reference solutions (b) and (c).

Run time: 3 times the retention time of spiramycin I.

Identification of spiramycins: use the chromatogram supplied with *spiramycin CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to spiramycins I, II and III.

Relative retention with reference to spiramycin I (retention time = 20 min to 30 min): spiramycin II = about 1.4; spiramycin III = about 2.0; impurity F = about 0.41; impurity A = about 0.45; impurity D = about 0.50; impurity G = 0.66; impurity B = about 0.73; impurity H = about 0.87; impurity E = about 2.5.

If necessary adjust the composition of the mobile phase by changing the amount of acetonitrile.

System suitability: reference solution (c):

– *resolution*: minimum 10.0 between the peaks due to impurity A and spiramycin I.

Limits:

– *impurities A, B, C, D, E, F, G, H*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent),
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (10.0 per cent),
– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard any peak due to the blank and the peaks due to spiramycins I, II and III.

Heavy metals (*2.4.8*): maximum 20 ppm.

1.0 g complies with limit test F. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 3.5 per cent, determined on 0.500 g by drying at 80 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 6 h.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

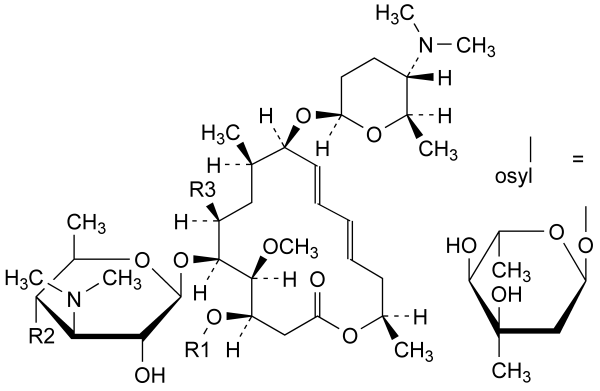
Carry out the microbiological assay of antibiotics (*2.7.2*).

STORAGE

In an airtight container.

IMPURITIES

Specified impurities: A, B, C, D, E, F, G, H.



A. R1 = H, R2 = OH, R3 = CH_2-CHO: (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-β-D-*erythro*-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-2-one (neospiramycin I),

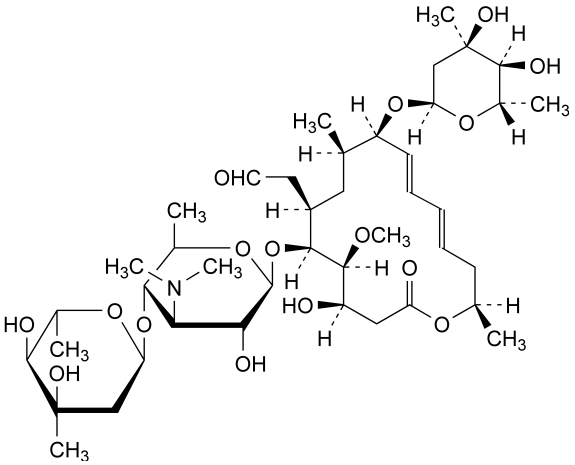
B. R1 = H, R2 = osyl, R3 = CH_2-CH_2OH: (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-4-hydroxy-7-(2-hydroxyethyl)-5-methoxy-9,16-dimethyl-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-β-D-*erythro*-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-2-one (spiramycin IV),

C. R1 = H, R2 = osyl, R3 = C(=CH_2)-CHO: (4*R*,5*S*,6*S*,7*S*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-7-(1-formylethenyl)-4-hydroxy-5-methoxy-9,16-dimethyl-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-β-D-*erythro*-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-2-one (17-methylenespiramycin I),

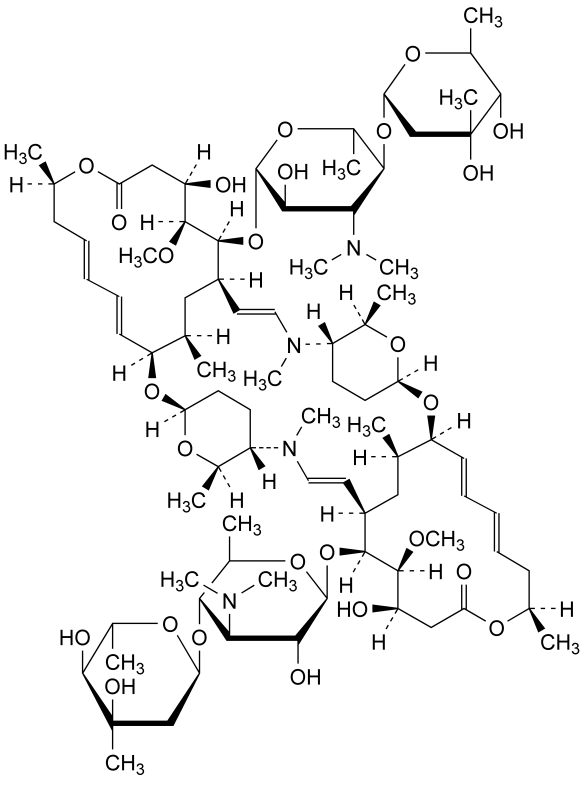
E. R1 = H, R2 = osyl, R3 = CH_2-CH_3: (4*R*,5*S*,6*S*,7*S*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-7-ethyl-4-hydroxy-5-methoxy-9,16-dimethyl-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-β-D-erythro-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-2-one (18-deoxy-18-dihydrospiramycin I or DSPM),

G. R1 = CO-CH_3, R2 = OH, R3 = CH_2-CHO: (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-β-D-*erythro*-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-4-yl acetate (neospiramycin II),

H. R1 = CO-C_2H_5, R2 = OH, R3 = CH_2-CHO: (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-10-[[2,3,4,6-tetradeoxy-4-(dimethylamino)-β-D-*erythro*-hexopyranosyl]oxy]oxacyclohexadeca-11,13-dien-4-yl propanoatate (neospiramycin III),



D. (4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-6-[[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-10-[(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-4-hydroxy-5-methoxy-9,16-dimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-2-one (spiramycin V),



F. spiramycin dimer.