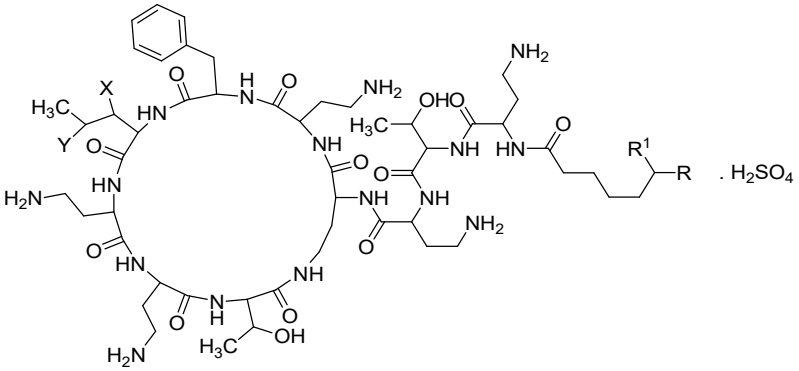
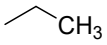
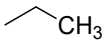
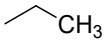
Polymyxin B Sulphate



Polymyxin	R	R^1	X	Y
B1		—CH_3	—H	—CH_3
B2	—CH_3	—CH_3	—H	—CH_3
B3		—H	—H	—CH_3
B1-I		—CH_3	—CH_3	—H

Polymyxin B1.
$C_{56}H_{98}N_{16}O_{13}$ Mol. Wt. 1203.5

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methyloctanamide.

Polymyxin B2.
$C_{55}H_{96}N_{16}O_{13}$ Mol. Wt. 1189.5

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methylheptanamide.

Polymyxin B3.
$C_{55}H_{96}N_{16}O_{13}$ Mol. Wt. 1189.5

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-isobutyl-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]octanamide.

Polymyxin B1-I.
$C_{56}H_{98}N_{16}O_{13}$ Mol. Wt. 1203.5

N-[(*S*)-4-Amino-1-{[(2*S*,3*R*)-1-{[(*S*)-4-amino-1-oxo-1-({(3*S*,6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-15-benzyl-3-[(*R*)-1-hydroxyethyl]-12-[(*S*)-sec-butyl]-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptaazacyclotricosan-21-yl}amino)butan-2-yl]amino}-3-hydroxy-1-oxobutan-2-yl]amino}-1-oxobutan-2-yl]-6-methyloctanamide.

Polymyxin B Sulphate is the sulphate salt of a kind of polymyxin, a substance produced by the growth of *Bacillus polymyxa* (Prazmowski) Migula (Fam. Bacillaceae), or a mixture of two or more such salts. It has a potency of not less than 6000 Polymyxin B units per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white to buff-coloured powder.

Identification

A. In the test for Composition of Polymyxins, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. Dissolve about 2 mg in 5 ml of *water,* add 5 ml of *2.5 M sodium hydroxide* and 5 drops of 1 per cent w/v solution of *cupric sulphate,* shake after addition of each drop; a reddish- violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a 0.5 per cent w/v solution.

Composition of Polymyxins. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.05 per cent w/v solution of *polymyxin B sluphate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 4.5 g of *sodium sulphate anhydrous* in 1000 ml of *water,* adjusted to pH 2.3 with *dilute orthophosphoric acid* and 20 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Name	Relative retention time
Polymyxin B2[a]	0.5
Polymyxin B3	0.6
Polymyxin B1-I	0.8
Polymyxin B1[a]	1.0

[a] These components are not reported individually. They are only reported in the sum of polymyxins.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to polymyxin B2 and polymyxin B3 is not less than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for the polymyxin B1 peak.

Inject the reference solution and the test solution. Run the chromatogram 1.4 times the retention time of the principal peak. The area of any peak corresponding to polymyxin B3 is not more than 0.06 times the area of the corresponding peak in the chromatogram obtained with the reference solution (6.0 per cent), the area of any peak corresponding to polymyxin B1-I is not more than 0.15 times the area of the corresponding peak in the chromatogram obtained with the reference solution (15.0 per cent). The sum of polymyxin B1, polymyxin B1-I, polymyxin B2, and polymyxin B3 is not less than 80 per cent.

Related substances. Determine by liquid chromatography (2.4.14), as described under Composition of Polymyxins.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3.0 per cent and the sum of all the secondary peaks is not more than 17.0 per cent, calculated by area normalisation. Ignore any peak with an area less than 0.007 times the area of polymyxin B1 peak in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 5.0 per cent, moistening the charred residue with 2 ml of *nitric acid* and 5 drops of *sulphuric acid*.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 0.1 g in a capillary-stoppered bottle by drying under vacuum at 60° for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10).

Polymyxin B sulphate intended for use in the manufacture of parenteral preparations complies with the following additional tests.

Sterility. Comply with the test for sterility (2.2.11).

Pyrogens. Complies with the test for pyrogens (2.2.8), using per kg of the rabbit's weight, 1.0 ml of a $20*10^3$ polymyxin B units per ml solution of the substance under examination in pyrogen free 0.9 per cent w/v solution of *sodium chloride.*

Storage. Store protected from light and moisture.

Labelling. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

2.4.26. Solubility. Freely soluble in *water;* slightly soluble in *ethanol.*