Colistin Sulphate

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

Document History and Schedule for the Adoption Process

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Colistin Sulphate. Page 1954
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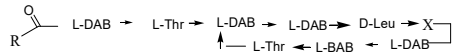
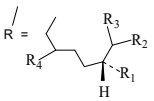
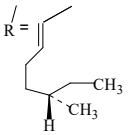




, X H2SO4

DAB = 2,4-diaminobutanoic acid

Polymyxin	X	R1	R2	R3	R4	Mol. Formula	M_r
E1	L-Leu	CH_3	CH_3	H	H	$C_{53}H_{100}N_{16}O_{13}$	1169
E2	L-Leu	CH_3	H	H	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E3	L-Leu	H	CH_3	H	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E4	L-Leu	H	H	H	H	$C_{51}H_{96}N_{16}O_{13}$	1141
E6	L-Leu	CH_3	CH_3	H	OH	$C_{53}H_{100}N_{16}O_{14}$	1185
E1-7MOA	L-Leu	H	CH_3	CH_3	H	$C_{53}H_{100}N_{16}O_{13}$	1169
E1-I	L-Ile	CH_3	CH_3	H	H	$C_{53}H_{100}N_{16}O_{13}$	1169
E1-Nva	L-Nva	CH_3	CH_3	H	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E2-I	L-Ile	CH_3	H	H	H	$C_{52}H_{98}N_{16}O_{13}$	1155
E2-Val	L-Val	CH_3	H	H	H	$C_{51}H_{96}N_{16}O_{13}$	1141



polymyxin	X	Mol. Formula	M_r
2,3-dehydro E1	L-Leu	$C_{53}H_{98}N_{16}O_{13}$	1167

Colistin Sulphate is a mixture of the sulphates of polypeptides produced by certain strains of *Bacillus polymyxa* var. *colistinus*.

Colistin Sulphate contains not less than 19000 IU per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white or almost white powder; hygroscopic.

Identification

Tests A, C and D may be omitted if test B and E are carried out. Test B may be omitted if tests A, C, D and E are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

NOTE — Carry out the test protected from light.

Mobile phase. A mixture of 75 volumes of *phenol* and 25 volumes of *water.*

Test solution. Dissolve 5 mg of the substance under examination in 1 ml of a mixture of equal volumes of *hydrochloric acid* and *water*, heat at 135° for 5 hours in a sealed tube. Evaporate to dryness on a water-bath and continue the heating until moistened the *blue litmus paper* does not turn red and dissolve the residue in 0.5 ml of *water*.

Reference solution (a). A 0.2 per cent w/v solution of *leucine IPRS* in *water.*

Reference solution (b). A 0.2 per cent w/v solution of *threonine IPRS* in *water*.

Reference solution (c). A 0.2 per cent w/v solution of *phenylalanine IPRS* in *water*.

Reference solution (d). A 0.2 per cent w/v solution of *serine IPRS* in *water.*

Apply to the plate 5 µl of each solution, as bands of 10 mm, then place the plate in the chromatographic tank so that it is not in contact with the mobile phase, and allow it to become impregnated with the vapour of the mobile phase for at least 12 hours. Allow the mobile phase to rise half of plate. After development, dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 5 minutes. The principal zone in the chromatogram obtained with the test solution show zones corresponds to that in the chromatograms obtained with reference solution (a) and (b), but shows no zones corresponding to those in the chromatograms obtained with reference solution (c) and (d); the zone chromatogram with the test solution also shows a zone with a very low *Rf* value of 2, 4-diaminobutyric acid.

B. In the test for Composition, the peaks due to *polymyxin E1* and *polymyxin E2* in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with reference solution (a).

C. Dissolve 5 mg in 3 ml of *water,* add 3 ml of *dilute sodium hydroxide solution*. Shake and add 0.5 ml of a 1.0 per cent w/v solution of *copper sulphate pentahydrate;* A violet colour is produced.

D. Dissolve 50 mg in 1 ml of *1 M hydrochloric acid*; add 0.5 ml of *0.01 M iodine*. The solution remains coloured.

E. It gives reaction (A) of sulphates (2.3.1).

Tests

pH (2.4.24**)**. 4.0 to 6.0, determined in 1.0 per cent w/v solution in *carbon dioxide-free water.*

Composition. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5 mg of the substance under examination in 8 ml of *water* and dilute to 10.0 ml with *acetonitrile*.

Reference solution (a). Dissolve 5 mg of *colistin for system suitability A IPRS* in 8 ml of *water* and dilute to 10.0 ml with *acetonitrile.*

Reference solution (b). Dilute a suitable volume of reference solution (a) with a mixture of 20 volumes of *acetonitrile* and 80 volumes of *water* to obtain a solution having known concentration of 0.35 per cent w/v of polymyxin E1 taking in to account the assigned content of polymyxin E1 in *colistin for system suitability A IPRS*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution prepared by dissolving 4.46 g of *anhydrous sodium sulphate* in 900 ml of *water*, adjusted to pH 2.4 with *dilute phosphoric acid* and dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Polymyxin E4	0.28	---
Polymyxin E2-Val	0.28	---
Polymyxin E6	0.39	---
Polymyxin E2-I	0.42	---
Polymyxin E2	0.5	---
Polymyxin impurity A[1]	0.53	---
Polymyxin E3	0.56	---

Polymyxin E1-Nva	0.59	---
Polymyxin E1-I	0.82	---
Polymyxin 2,3-dehydro E1	0.9	0.3
Polymyxin E1(Retention time about 21 minutes)	1.0	---
Polymyxin E1-7MOA	1.1	---
Polymyxin impurity B[2]	1.3	---

[1]unknown structure,
[2][N^4-Dab^5]polymyxin E1.

Inject reference solution (a) to identify the peaks due to polymyxin E4, polymyxin E2-Val, polymyxin E6, polymyxin E2-I, polymyxin E2, polymyxin impurity A, polymyxin E3, polymyxin E1-Nva, polymyxin E1-I, polymyxin 2,3-dehydro E1, polymyxin E1, polymyxin E1-7MOA and polymyxin impurity B.

Inject reference solution (a) and (b) The test is not valid unless the resolution between the peaks due to polymyxin E6 and polymyxin E2-I is not less than 2.0 and polymyxin 2,3-dehydro E1 and polymyxin E1 is not less than 3.0, the peak-to-valley ratio (Hp/Hv) is not less than 1.1, where Hp is the height above the baseline of the peak due to polymyxin impurity A and Hv is the height above the baseline of the lowest point of the curve separating this peak due to polymyxin E2 in the chromatogram obtained with reference solution (a) and the signal-to-noise ratio for the peak due to polymyxin E1 is not less than 10.0 in the chromatogram obtained with reference solution (b).

Inject the test solution. Run the chromatogram 1.5 times the retention time of polymyxin E1. The area of any peak corresponding to polymyxin E1-I is not more than 8.5 per cent, the area of any peak corresponding to polymyxin E3 is not more than 5.5 per cent, the area of any peak corresponding to polymyxin E1-7MOA is not more than 5.0 per cent, the area of any peak corresponding to polymyxin E6 and polymyxin E1-Nva, each of, is not more than 4.5 per cent, the sum of the areas of the peaks corresponding to polymyxin E4 and polymyxin E2-Val is not more than 3.0 per cent, the area of any peak corresponding to polymyxin E2-I is not more than 2.5 per cent, the area of any peak corresponding to polymyxin 2,3-dehydro E1 is not more than 1.5 per cent, and the sum of polymyxin E4, polymyxin E2-Val, polymyxin E6, polymyxin E2-I, polymyxin E2, polymyxin E3, polymyxin E1-Nva, polymyxin E1-I, polymyxin 2,3-dehydro E1, polymyxin E1 and polymyxin E1-7MOA peaks is not less than 86.0 per cent, ignore any peak with an area less than 0.35 per cent, calculated by area normalisation.

Related substances. Determine by liquid chromatography (2.4.14). as described under Composition.
In the chromatogram obtained with the test solution, the area of any peak corresponding to polymyxin impurity B is not more than 4.0 per cent, the area of any other secondary peak is not more than 2.5 per cent, not more than 4 such impurities exceed 1.0 per cent and the sum of the areas of all the secondary peaks is not more than 11.0 per cent. Ignore the peaks due to polymyxin E4, polymyxin E2-Val, polymyxin E6, polymyxin E2-I, polymyxin E2, polymyxin E3, polymyxin E1-Nva, polymyxin E1-I, polymyxin 2,3-dehydro E1 and polymyxin E1 and any peak with an area less than 0.35 per cent, calculated by area normalisation.

Sulphates. 16.0 per cent to 18.0 per cent, calculated on dried basis.

Dissolve 0.25 g in 100 ml of *water* and adjusted to pH 11 with *concentrated ammonia*. Add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *phthalein purple* as an indicator and titrate with *0.1 M sodium edetate*, add 50 ml *ethanol (95 per cent)*, when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears. Perform the blank determination and make any necessary correction.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of SO_4.

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 3.5 per cent, determined on 1.0 g by drying in vacuum at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics (2.2.10). Use *colistin sulphate for microbiological assay IPRS* as the reference.

Storage. Store protected from light and moisture.