Edition: BP 2025 (Ph. Eur. 11.6 update)

Colistin Tablets

General Notices

Action and use

Antibacterial.

DEFINITION

Colistin Tablets contain Colistin Sulfate.

The tablets comply with the requirements stated under Tablets and with the following requirements

IDENTIFICATION

To a quantity of the powdered tablets containing 200,000 IU add 10 mL of *water*, shake and filter. Use the filtrate for the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, protected from light using the following solutions.
(1) Add 0.5 mL of *hydrochloric acid* to 0.5 mL of the filtrate, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water bath, continue to heat until any residual hydrogen chloride has been removed, dissolve the residue in 0.5 mL of *water* and centrifuge, if necessary.
(2) 0.25% w/v of L-*leucine* in *water*.
(3) 0.25% w/v of L-*threonine* in *water*.
(4) 0.25% w/v of L-*phenylalanine* in *water*.
(5) 0.25% w/v of L-*serine* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.
(b) Use the mobile phase described below.
(c) Apply 5 µL of each solution, as 10-mm bands. Place the plate in the tank so that it is not in contact with the mobile phase and expose it to the vapour of the mobile phase.
(d) After exposure of the plate to the mobile phase vapour for at least 12 hours, develop to 12 cm.
(e) Remove the plate, heat it at 100° to 105°, spray with *ninhydrin solution R1* and heat at 110° for 5 minutes.

MOBILE PHASE

25 volumes of *water* and 75 volumes of *phenol*.

CONFIRMATION

The bands in the chromatogram obtained with solution (1) correspond to those in the chromatograms obtained with solutions (2) and (3) and do not correspond to those in the chromatograms obtained with solutions (4) and (5). The chromatogram obtained with solution (1) also shows a band with a very low Rf value (2,4-diaminobutanoic acid).

B. Heat 0.5 mL of the filtrate with 0.5 mL of *chromotropic acid–sulfuric acid solution* at 100° for 30 minutes. No purple colour is produced (distinction from colistin sulfomethate).
C. The filtrate yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Composition

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation* procedure.

(1) Shake a quantity of the powdered tablets containing 600,000 IU with 40 mL of *water*, add sufficient *acetonitrile* to produce 50 mL and filter (Whatman GF/C filter followed by 0.45-µm nylon filter is suitable).
(2) Dissolve 5 mg of *colistin for system suitability EPCRS* in 8 mL of *water* and add sufficient *acetonitrile* to produce 10 mL.
(3) Dilute 1 volume of solution (2) to 100 volumes with a solution containing 20 volumes of *acetonitrile* and 80 volumes of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3.0 µm) (YMC Pack-pro is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 50º.
(e) Use a detection wavelength of 215 nm.
(f) Inject 20 µL of each solution.
(g) For solution (1) allow the chromatography to proceed for 1.5 times the retention time of polymyxin E1.

MOBILE PHASE

22 volumes of *acetonitrile R1* and 78 volumes of a solution prepared by dissolving 4.46 g of *anhydrous sodium sulfate* in 900 mL of *water*, adjusting the pH to 2.4 with *dilute orthophosphoric acid* and adding sufficient *water* to produce 1000 mL.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to polymyxin E1 (retention time, about 21 minutes) are: polymyxin E4, about 0.3; polymyxin E2-Val, about 0.3; polymyxin E6, about 0.39; polymyxin E2-I, about 0.42; polymyxin E2, about 0.50; impurity A, about 0.53; polymyxin E3, about 0.56; polymyxin E1-Nva, about 0.6, polymyxin E1-I, about 0.8, polymyxin 2,3-dehydro E1, about 0.9; polymyxin E1-7MOA, about 1.1; impurity B, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2):

the *resolution* between the peaks due to polymyxin E6 and polymyxin E2-I is at least 2.0;

the *resolution* between the peaks due to polymyxin 2,3-dehydro E1 and polymyxin E1 is at least 3.0;

the *peak-to-valley ratio* is at least 1.1, where H_p is the height above the baseline of the peak due to impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to polymyxin E2;

and in the chromatogram obtained with solution (3), the *signal to noise ratio* for the peak due to polymyxin E2 is not less than 10.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to polymyxins E1, E2, E3, E4, E6, E1-I, E1-Nva, E2-I, E2-Val, 2,3-dehydro E1 and impurities A and B using the chromatogram obtained with solution (2). Multiply the peak area of polymyxin 2,3-dehydro E1 by 0.3.

In the chromatogram obtained with solution (1), integrate all peaks present with an area greater than 0.35% to determine the total peak area. Calculate the percentage content of each of the impurities by *normalisation*:

polymyxin E1-I, maximum 8.5%;

polymyxin E3, maximum 5.5%;

polymyxin E1-7MOA, maximum 5.0%;

polymyxin E6, maximum 4.5%;

polymyxin E1-Nva, maximum 4.5%;

the sum of polymyxin E4 and polymyxin E2-Val, maximum 3.0%;

polymyxin E2-I, maximum 2.5%;

polymyxin 2,3-dehydro E1, maximum 1.5%;

the sum of polymyxins E1, E2, E3, E4, E6, E1-I, E1-Nva, E2-I, E2-Val and 2,3-dehydro E1, minimum 86.0%.

Related substances

Carry out the method for *liquid chromatography*, as described in the test for Composition.

LIMITS

In the chromatogram obtained with solution (1), integrate all peaks present with an area greater than 0.35% to determine the total peak area. Calculate the percentage content of each of the impurities by *normalisation*:

the area of any peak due to impurity B is not greater than 4.0%;

the area of any secondary peak is not greater than 2.5%;

the area of no more than 4 secondary peaks is greater than 1.0%;

the sum of the areas of all secondary peaks is not greater than 11.0%;

Disregard any peak related to polymyxins E1, E2, E3, E4, E6, E1-I, E1-NVa, E2-I, E2-Val 2,3-dehydro E1 and any peak with an area less than 0.35%.

ASSAY

Weigh and powder 20 tablets. Dissolve a suitable quantity of the powder in *phosphate buffer pH 6.0* and carry out the *microbiological assay of antibiotics*, Appendix XIV A. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated number of IU.

STORAGE

Colistin Tablets should be protected from light.

LABELLING

The strength is stated as the number of IU (Units) in each tablet.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Colistin Sulfate*.