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

Lymecycline Capsules

General Notices

Action and use

Tetracycline antibacterial.

DEFINITION

Lymecycline Capsules contain Lymecycline.

The capsules comply with the requirements stated under Capsules and with the following requirements.

Content of lymecycline, $C_{29}H_{38}N_4O_{10}$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

The contents of the capsules comply with the following tests.

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Shake a quantity of the contents of the capsules containing 5 mg of Lymecycline with 10 mL of *methanol*, centrifuge and use the supernatant liquid.
(2) 0.05% w/v of *lymecycline BPCRS* in *methanol*.
(3) 0.05% w/v of each of *tetracycline hydrochloride BPCRS*, *chlortetracycline hydrochloride BPCRS* and *doxycycline hyclate BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel H*. Adjust the pH of a 10% w/v solution of *disodium edetate* to 8.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate in an oven at 110° for 1 hour.
(b) Use the mobile phase as described below.
(c) Apply 1 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry it in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows three clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. To 0.5 mg add 2 mL of *sulfuric acid*. A purplish-red colour is produced.
C. Dissolve 50 mg in 5 mL of *water*, add 50 mg of *ninhydrin*, boil and add 15 mL of *water*. A bluish-violet colour is produced.
D. Dissolve 0.2 g in 5 mL of *water*, add 0.3 mL of *orthophosphoric acid* and distil. To 1 mL of the distillate add 10 mL of *chromotropic acid–sulfuric acid solution*. A violet colour is produced.

TESTS

Dissolution

Carry out the procedure protected from light. Comply with the requirements for Monographs of the British Pharmacopoeia in the *dissolution test for tablets and capsules*, Appendix XII B1.

TEST CONDITIONS

(a) Use Apparatus 2, rotating the paddle at 75 revolutions per minute.
(b) Use 900 mL of 0.1M *hydrochloric acid*, at a temperature of 37°, as the medium.

PROCEDURE

(1) After 60 minutes withdraw a 20 mL sample of the medium and filter. To 5 mL of this solution add 50 mL of *water* and 5 mL of 5M *sodium hydroxide*, mix with shaking and add sufficient *water* to produce 100 mL. Exactly 6 minutes after adding the sodium hydroxide measure the *absorbance* of the resulting solution at the maximum at 380 nm, Appendix II B using a solution prepared by diluting 5 mL of 5M *sodium hydroxide* to 100 mL with *water* in the reference cell.
(2) Add 50 mL of *water* and 5 mL of 5M *sodium hydroxide* to 2 mL of a 0.09% w/v solution of *tetracycline hydrochloride BPCRS* in *0.1M hydrochloric acid*, mix with shaking and add sufficient *water* to produce 100 mL. Exactly 6 minutes after adding the sodium hydroxide measure the *absorbance* of the resulting solution using a solution prepared by diluting 5 mL of 5M *sodium hydroxide* to 100 mL with *water* in the reference cell.

DETERMINATION OF CONTENT

Calculate the total content of lymecycline, $C_{29}H_{38}N_4O_{10}$, in the medium from the absorbances obtained and using the declared content of $C_{22}H_{24}N_20_8$,HCl in *tetracycline hydrochloride BPCRS*. Each mg of $C_{22}H_{24}N_20_8$,HCl is equivalent to 0.9241 mg of $C_{22}H_{24}N_20_8$ (tetracycline). Multiply the content of tetracycline by 1.356 to obtain the content of lymecycline.

Free tetracycline

Not more than 2.5%, when determined by the following method. To a quantity of the contents of the capsules containing 0.5 g of Lymecycline add 50 mL of *butyl acetate* and allow to stand at 25° for 1 hour. Filter (Whatman GF/C is suitable) and extract the filtrate with two 25-mL quantities of *0.1M hydrochloric acid*. Combine the extracts, add sufficient *0.1M hydrochloric acid* to produce 50 mL and dilute 10 mL of this solution to 100 mL with *0.1M hydrochloric acid*. The *absorbance* of the resulting solution at the maximum at 355 nm, Appendix II B, using 0.1M *hydrochloric acid* in the reference cell, is not greater than 0.64.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing 0.125 g of Lymecycline in 5 mL of *water*. Add 1 mL of a 4% w/v solution of *sodium metabisulfite* and allow to stand in the dark at 20 to 25° for 16 to 24 hours, without stirring. Add 50 mL of 0.05M *hydrochloric acid*, shake to dissolve the precipitate and dilute to 100 mL with *water* (contains approximately 0.1% w/v of tetracycline).
(2) 0.1% w/v of *tetracycline hydrochloride BPCRS* in 0.01M *hydrochloric acid*.
(3) 0.025% w/v of *4-epitetracycline hydrochloride EPCRS* (impurity A) in 0.01M *hydrochloric acid*.
(4) 0.01% w/v of *anhydrotetracycline hydrochloride EPCRS* (impurity C) in 0.01M *hydrochloric acid*.
(5) 0.02% w/v of *4-epianhydrotetracycline hydrochloride EPCRS* (impurity D) in 0.01M *hydrochloric acid*.

(6) Mix 1 volume of solution (2), 2 volumes of solution (3) and 5 volumes of solution (5) and dilute to 25 volumes with 0.01M *hydrochloric acid*.

(7) Mix 40 volumes of solution (3), 20 volumes of solution (4) and 5 volumes of solution (5) and dilute to 200 volumes with 0.01M *hydrochloric acid*.

(8) Dilute 1 volume of solution (7) to 50 volumes with 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *styrene-divinylbenzene copolymer* (8 µm) with a pore size of 10 nm (PLRP-S 100A is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 1.0 mL per minute.

(d) Use a column temperature of 60°.

(e) Use a detection wavelength of 254 nm.

(f) Inject 20 µL of solutions (1), (6) and (7).

(g) Allow the chromatography to proceed for 5 times the retention time of the principal peak in the chromatogram obtained with solution (1).

When the chromatograms are recorded under the prescribed conditions, the retention time of tetracycline is about 8 minutes. The retention times relative to tetracycline are: impurity E, about 0.50; impurity A, about 0.6; impurity F, about 0.68; impurity B (eluting on the tail of the principal peak), about 1.2; impurity D, about 1.45; impurity G, about 1.45; impurity C, about 2.95.

MOBILE PHASE

Weigh 80.0 g of *2-methyl-2-propanol* and transfer to a 1000 mL volumetric flask with the aid of 200 mL of *water*. Add 100 mL of a 3.5% w/v solution of *dipotassium hydrogen orthophosphate* adjusted to pH 8.0 with *dilute orthophosphoric acid*, 200 mL of a 1% w/v solution of *tetrabutylammonium hydrogen sulfate* adjusted to pH 8.0 with *dilute sodium hydroxide solution* and 10 mL of a 4% w/v solution of *sodium edetate* adjusted to pH 8.0 with *dilute sodium hydroxide solution*; dilute to 1000 mL with *water*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (6):

the *resolution* between the peaks due to impurity A (first peak) and tetracycline (second peak) is at least 3.0;

the *resolution* between the peaks due to tetracycline and impurity D (third peak) is at least 5.0;

the *symmetry factor* of the peak due to tetracycline is not more than 1.25.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.6 times the area of the corresponding peak in the chromatogram obtained with solution (7) (8%);

the area of any peak corresponding to impurity C is not greater than twice the area of the corresponding peak in the chromatogram obtained with solution (7) (2%);

the area of any peaks corresponding to impurities E and F are not greater than 0.4 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (2% each);

the area of any peak with a retention time relative to tetracycline of about 1.6 is not greater than 0.14 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (0.7%);

the area of any peak corresponding to impurity B is not greater than 0.1 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (0.5%);

the sum of the areas of any peaks corresponding to impurities D and G is not greater than the area of the peak corresponding to impurity D in the chromatogram obtained with solution (7) (0.5%);

the area of any other *secondary peak* is not greater than 0.1 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (0.5%);

the sum of the areas of all the *secondary peaks* is not greater than 2.2 times the area of the peak corresponding to impurity A in the chromatogram obtained with solution (7) (11%).

Disregard any peak with an area less than the area of the peak corresponding to impurity A in the chromatogram obtained with solution (8) (0.1%).

Water

The contents of the capsules contain not more than 7.0% w/w of water, Appendix IX C. Use 0.1 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions prepared immediately before use.

(1) Dissolve a quantity of the mixed contents of 20 capsules containing 0.125 g of Lymecycline in 5 mL of *water*. Add 1 mL of a 4% w/v solution of *sodium metabisulfite* and allow to stand in the dark at 20 to 25° for 16 to 24 hours, without stirring. Add 50 mL of 0.05M *hydrochloric acid*, shake to dissolve the precipitate and dilute to 100 mL with *water* (contains approximately 0.1% w/v of tetracycline).
(2) 0.1% w/v of *tetracycline hydrochloride BPCRS* in 0.01M *hydrochloric acid*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

Inject solution (2) six times. The Assay is not valid unless the relative standard deviation of the area of the principal peak is at most 1.0%.

DETERMINATION OF CONTENT

Calculate the content of $C_{29}H_{38}N_4O_{10}$ in the capsules using the declared content of $C_{22}H_{24}N_20_8$,HCl in *tetracycline hydrochloride BPCRS*. Each mg of $C_{22}H_{24}N_20_8$,HCl is equivalent to 0.9241 mg of $C_{22}H_{24}N_20_8$ (tetracycline). Multiply the content of tetracycline by 1.356 to obtain the content of lymecycline.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A to G listed under Lymecycline.

204 mg of Lymecycline is equivalent to approximately 150 mg of tetracycline.