LAMIVUDINE

Final text for addition to The International Pharmacopoeia

This monograph was adopted at the Fortieth WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2005 for addition to the 4th edition of the International Pharmacopoeia.

LAMIVUDINUM

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\begin{align*}
\text{Chemical name.} & \quad (-)-4\text{-amino}-1\{\text{(2R,5S)\text{-2-(hydroxymethyl)}\text{-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one; CAS Reg. NO. 134678-17-4.}} \\
\text{Description.} & \quad \text{A white or almost white powder.} \\
\text{Solubility.} & \quad \text{Soluble in water; sparingly soluble in methanol R; practically insoluble in acetone R.}
\end{align*}
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Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

Storage. Lamivudine should be kept in a well-closed container, protected from light.

Additional information. Lamivudine may exhibit polymorphism.

Requirements

Definition Lamivudine contains not less than 97.0% and not more than 103.0% of C₈H₁₁N₃O₃S, calculated with reference to the dried substance.

Manufacture. The production method is validated to demonstrate that the substance, if tested, would comply with a limit of not more than 0.3% for 2S,5R lamivudine enantiomer using a suitable chiral chromatographic method.

Identity test

- Either tests A, B and D or tests C and D may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of lamivudine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of lamivudine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120°C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B.

B. The absorption spectrum of the final solution prepared for the Assay, when observed between 210 nm and 300 nm, exhibits one maximum at about 280 nm; the specific absorbance (A 1% 1cm) is between 577 to 637.
C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from lamivudine RS or with the reference spectrum of lamivudine. If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and lamivudine RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from lamivudine RS.

D. Determine the specific optical rotation using a 10 mg/ml solution in methanol R and calculate with reference to the dried substance; \([\alpha]_D^{25^\circ} = -135^\circ \text{ to } -144^\circ\).

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, procedure 4. Determine the heavy metals content according to method A; not more than 20 µg/g.

**Sulfated ash.** Not more than 2.0 mg/g.

**Loss on Drying.** Dry for 3 hours at 105°C; it loses not more than 5 mg/g.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase, use a mixture of 5 volumes of methanol R and 95 volumes of buffer pH 3.8 (a 1.9 g/l solution of ammonium acetate R, previously adjusted to pH 3.8 with glacial acetic acid R).

Prepare the following solutions. For solution (1) prepare a 0.5 mg/ml solution of the test substance in the mobile phase. For solution (2) dilute 1.0 ml of solution (1) to 100 ml with mobile phase and then dilute 1.0 ml of this solution to 10 ml. For solution (3) dissolve 25 mg of salicylic acid R in 100 ml of mobile phase, dilute 1.0 ml of the resulting solution to 50 ml with the mobile phase and then further dilute 1.0 ml to 10 ml with the mobile phase. For solution (4) dissolve about 5 mg of lamivudine for system suitability RS (containing lamivudine and lamivudine impurities A, B and J) in the mobile phase, add 1 ml of solution (CU) prepared as described below and dilute to 10 ml with the mobile phase. For solution (CU) dissolve 5 mg of cytosine R and 5 mg of uracil R in the mobile phase, dilute to 50 ml with the mobile phase and dilute 1 ml of the resulting solution to 10 ml with the mobile phase.

**Note:** The means of identifying the impurity peaks is subject to confirmation.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 277 nm.

Maintain the temperature of the column at 35°C.

Inject separately 10 µl each of solutions (1), (2) (3) and (4). Record the chromatograms for about 3 times the retention time of lamivudine. The test is not valid unless in the chromatogram obtained

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1 Waters Hypersil BDS is suitable.
with solution (4) the resolution factor between the peaks due to lamivudine and impurity B is greater than 1.5.

Use the chromatogram supplied with lamivudine for system suitability RS and the chromatogram obtained with solutions (4) and (3) to identify the peaks due to impurities A, B, E, F, J and C. The impurity peaks are eluted at the following relative retention times with reference to lamivudine (retention time about 11 to 12 minutes): impurity E 0.31, impurity F 0.36, impurity A 0.40, impurity B 0.9, impurity J 1.5, impurity C (salicylic acid) 2.6.

In the chromatogram obtained with solution (1):
- the area of any peak corresponding to impurity A is not greater than 3 times the area of the peak in the chromatogram obtained with solution (2) (0.3%),
- the area of any peak corresponding to impurity B is not greater than twice the area of the peak in the chromatogram obtained with solution (2) (0.2%),
- the area of any peak corresponding to impurity C (salicylic acid) is not greater than that of the principal peak in the chromatogram obtained with solution (3) (0.1%),
- the area of any individual peak corresponding to impurity E, when multiplied by a correction factor of 0.6, is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%),
- the area of any individual peak corresponding to impurity F or J, when multiplied by a correction factor of 2.2, is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%),
- the area of any other peak, apart from the principal peak, is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%),
- the sum of the areas of all the peaks, apart from the principal peak, is not greater than 6 times the area of the peak obtained with solution (2) (0.6%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (2) (0.05%).

**Assay.** Transfer into a 500-ml volumetric flask about 0.05 g, accurately weighed, and dissolve in about 400 ml of water using an ultrasonic bath, if necessary. Cool to room temperature and dilute to volume with water and mix.

Dilute 5 ml of this solution to 50 ml with 0.1M H₂SO₄ and mix. For the blank, use a solution prepared by mixing 5 ml of water with 50 ml of 0.1M H₂SO₄.

Measure the absorbance of a 1-cm layer of the final solution at a maximum about 280 nm against a solvent cell containing the blank. Calculate the content of C₈H₁₁N₃O₃S using the absorptivity value of 60.7 (A₁%₁cm= 607).

**Impurities**

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.

A. 243.2 C₈H₉N₃O₄S
(2RS,5SR)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylic acid,

B. 229.3 C_{8}H_{11}N_{3}O_{3}S

4-amino-1-[(2RS,5RS)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1H)-one,

C. 138.1 C_{7}H_{6}O_{3}

2-hydroxybenzoic acid (salicylic acid),

D. 229.3 C_{8}H_{11}N_{3}O_{3}S

(+)-4-amino-1-[(2S,5R)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1H)-one (ent-lamivudine),

[see under Manufacture]

E. 111.1 C_{4}H_{3}N_{3}O

4-aminopyrimidin-2(1H)-one (cytosine),

F. 112.1 C_{4}H_{4}N_{2}O_{2}

pyrimidine-2,4(1H,3H)-dione (uracil),

G. 245.3 C_{8}H_{11}N_{3}O_{4}S

4-amino-1-[(2R,3S,5S)-2-(hydroxymethyl)-3-oxo-1,3\lambda^{4}-oxathiolan-5-yl]pyrimidin-2(1H)-one,
H. 245.3 C₈H₁₁N₃O₄S

4-amino-1-\{(2R,3R,5S)-2-(hydroxymethyl)-3-oxo-1,3\(\lambda^4\)-oxathiolan-5-yl\}pyrimidin-2(1H)-one,

I. 229.3 C₈H₁₁N₃O₃S

(+)-4-amino-1-\{(2S,4S)-2-(hydroxymethyl)-1,3-oxathiolan-4-yl\}pyrimidin-2(1H)-one,

J. 230.2 C₈H₁₀N₂O₄S

1-\{(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl\}pyrimidine-2,4(1H,3H)-dione.

**Reagents**

**Cytosine** 4-aminopyrimidin-2(1H)-one

A commercially available reagent of suitable grade.

**Salicylic acid R.** 2-hydroxybenzoic acid; C₇H₆O₃.

A commercially available reagent of suitable grade.

*Storage.* Keep protected from light.

**Uracil** pyrimidine-2,4(1H,3H)-dione

A commercially available reagent of suitable grade.