## Lamivudine tablets (Lamivudini compressi)

**Category.** Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

**Additional information.** Strengths in the current WHO Model list of essential medicines: 150 mg. Strength in the current WHO Model list of essential medicines for children: 150 mg.

The tablets may be uncoated or coated.

Requirements

Comply with the monograph for *Tablets*.

**Definition.** Lamivudine tablets contain Lamivudine. They contain not less than 90.0% and not more than 110.0% of the amount of lamivudine ($C_8H_{11}N_3O_3S$) stated on the label.

### Identity tests

-Either tests A and C, or tests B and C, or test D alone 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 Chromatography*, 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 10 µl of each of the following 2 solutions. For solution (A) shake a quantity of the powdered tablets containing about 50 mg of Lamivudine with 50 mL of methanol R, filter and use the filtrate. For solution (B) use 1.0 mg of lamivudine RS per mL of methanol. After removing the plate from the chromatographic chamber allow it to dry in a current of cool air and 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 Chromatography*, Thin-layer chromatography using the conditions described above under test A.1, but using silica gel R5 as the coating substance. 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. See the test described below under “Assay”, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that obtained with solution (2).

C. The *absorption spectrum (1.6)* of the final solution prepared for “Assay”, Method B, when observed between 210 nm and 300 nm, exhibits one maximum at about 280 nm.

D. To a quantity of the powdered tablets containing 50 mg of Lamivudine add 20 mL of methanol R, shake to dissolve and filter. Evaporate the filtrate in a stream of nitrogen and, using the test residue thus obtained, 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 test residue and the residue obtained by dissolving lamivudine RS in methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from lamivudine RS.

### Related substances

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given below under Assay method A.

Prepare the following solutions. For solution (1) to a quantity of the powdered tablets containing about 50 mg of Lamivudine, add 60 mL of mobile phase and sonicate for about 5 minutes. Dilute to 100 mL with the mobile phase. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. For solution (2) dilute 1.0 mL of solution (1) to 100 mL with the mobile phase and then dilute 1.0 mL of this solution to 10 mL.

For solution (3) dissolve about 5 mg of lamivudine for system suitability RS 1 (containing lamivudine and lamivudine impurities A and B) in the mobile phase, add 1 mL of solution (CUS) prepared as described below and dilute to 10 mL with the mobile phase. For solution (CUS) dissolve 25 mg of cytosine R, 25 mg of uracil R and 25 mg of salicylic acid 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.

Inject separately 20 µl each of solutions (1), (2) and (3). Record the chromatograms for about 3 times the retention time of lamivudine in solution (2). Use the chromatogram obtained with solution (3) to identify the peaks due to impurities E, F and C. The impurity peaks are eluted at the following relative retention with reference to lamivudine (retention time about 11 to 12 minutes): impurity E about 0.31; impurity F about 0.36; impurity A about 0.40; impurity B about 0.9; impurity C (salicylic acid) about 2.6. The test is not valid unless in the chromatogram obtained with solution (3) the resolution factor between the peaks due to lamivudine and impurity B is at least 1.5.

In the chromatogram obtained with solution (1) the area of any peak, other than the principal peak, is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); the area of not more than one such peak is greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than 6 times the area of the principal 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

-Either method A or B may be applied.

A. Carry out the test as described under *1.14.1 Chromatography*, 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 in the mobile phase. For solution (1) weigh and powder 20 tablets. To a quantity of the powder containing about 50 mg of Lamivudine, accurately weighed, add 60 mL of mobile phase and sonicate for about 5 minutes. Dilute to 100 mL with mobile phase. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. Dilute 10 mL of the filtrate to 25 mL with mobile phase. For solution (2) use 0.2 mg of lamivudine RS per mL. For solution (3) dissolve about 5 mg of lamivudine for system suitability RS 1 (containing lamivudine and lamivudine impurities A and B) in the mobile phase, add 1 mL of solution (CUS) prepared as described below and dilute to 10 mL with the mobile phase. For solution (CUS) dissolve 25 mg of cytosine R, 25 mg of uracil R and 25 mg of salicylic acid 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.

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 20 µl of solution (3). Record the chromatograms for about 3 times the retention time of lamivudine in solution (2). In the chromatogram obtained with solution (3) the impurity peaks are eluted at the following relative retention with reference to lamivudine (retention time about 11 to 12 minutes): impurity E (cytosine) about 0.31; impurity F (uracil) about 0.36; impurity A about 0.40; impurity B about 0.9; impurity C (salicylic acid) about 2.6. The assay is not valid unless in the chromatogram obtained with solution (3) the resolution factor between the peaks due to lamivudine and impurity B is at least 1.5.

Inject alternately 20 µl each of solutions (1) and (2).

Measure the areas of the peaks responses of lamivudine obtained in the chromatograms of solutions (1) and (2). Calculate the percentage content of lamivudine ($C_8H_{11}N_3O_3S$) in the tablets.

B. Weigh and powder 20 tablets. Transfer a quantity of the powder containing about 50 mg of Lamivudine, accurately weighed, to a 500 mL volumetric flask. Add about 400 mL of water R and sonicate for about 5 minutes. Make up to volume with water R. Filter a portion of this solution through a 0.45 µm filter, discarding the first few mL of the filtrate. Dilute 5 mL of this solution to 50 mL with sulfuric acid (0.1 mol/l) VS. Measure the *absorbance (1.6)* of this solution in a 1 cm layer at the maximum about 280 nm against a solvent cell containing the blank. For the blank use a solution prepared by diluting 5 mL of water R with 50 mL of sulfuric acid (0.1 mol/l) VS.

Calculate the percentage content of lamivudine ($C_8H_{11}N_3O_3S$) in the tablets using the absorptivity value of 60.7 ( $A^{1\%}_{1cm}$ = 607).

### Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph for *Lamivudine*.