Lamivudine and tenofovir disoproxil tablets (Lamivudini et tenofoviri disoproxili compressi)

2025-01

Category. Antiretroviral (Nucleoside/Nucleotide reverse transcriptase inhibitor).

Storage. Lamivudine and tenofovir disoproxil tablets should be kept in a tightly closed container.

Additional information. Strength in the 20th Invitation to Manufacturers and Suppliers of Medicinal Products for HIV Infections and Related Diseases to Submit an Expression of Interest (EOI) for Product Evaluation to the WHO Prequalification Unit – Medicines Team: 300 mg Lamivudine and 300 mg Tenofovir disoproxil fumarate. 300 mg of tenofovir disoproxil fumarate is equivalent to approximately 245 mg of tenofovir disoproxil.

Requirements

Comply with the monograph for *Tablets*.

Definition. Lamivudine and tenofovir disoproxil tablets contain Lamivudine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of lamivudine ($C_8H_{11}N_3O_3S$) and tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$) stated on the label.

Manufacture. The manufacturing process and the product packaging are designed and controlled so as to minimize the moisture content of the tablets.

Identity tests

Either test A, or tests B and C may be performed.

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions and solutions given under "Assay", but using as the detector, a diode array detector in the range of 220 nm to 400 nm. The retention time and the UV spectra of the principal peaks in the chromatogram obtained with solution (1) correspond to the retention times and the UV spectra of the corresponding peaks due to lamivudine and tenofovir disoproxil in the chromatograms obtained with solution (2) and (3).

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions and solutions given under "Assay". The retention time of the principal peaks in the chromatogram obtained with solution (1) correspond to the retention times of the corresponding peaks due to lamivudine and tenofovir disoproxil in the chromatograms obtained with solution (2) and (3).

Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R6 as the coating substance and a freshly prepared mixture of ethyl acetate R, water R, anhydrous formic acidR and glacial acetic acid R (71:14:7:7 *V/V/V/V*) as the mobile phase. Apply separately to the plate 5 µL of each of the following 3 solutions in a mixture of methanol R and formic acid (~1080 g/L) TS (9:1 *V/V*). For solution (A), disperse a quantity of the powdered tablets, nominally equivalent to 10 mg of tenofovir disoproxil, in 2 mL, sonicate for 5 minutes and filter. For solution (B), use a solution containing 6 mg of lamivudine RS per mL. For solution (C), use a solution containing 6 mg of tenofovir disoproxil fumarate RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of air and examine the chromatogram under ultraviolet light (254 nm and 365 nm). The two principal spots in the chromatogram obtained with solution (A) correspond in position, appearance and intensity with the corresponding spots due to lamivudine and tenofovir disoproxil obtained with solution (B) and (C).

Dissolution. Carry out the test described under *5.5 Dissolution test for oral dosage forms*, using as the dissolution medium 900 mL of hydrochloric acid (~4 g/L) TS and rotating the paddle at 50 revolutions per minute. At 30 minutes, withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Dilute 5.0 mL of the solution to 25.0 mL using as a diluent a mixture of 95 volumes of 0.1% (v/v) of trifluoroacetic acid R in water R and 5 volumes of acetonitrile R.

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the chromatographic conditions and solutions as described under "Assay".

For each of the tablets tested, calculate the amount of lamivudine and tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$) in the medium from the results obtained, using the declared content of lamivudine ($C_8H_{11}N_3O_3S$) in lamivudine RS and the declared content of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$) in tenofovir disoproxil fumarate RS. Each mg of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$) corresponds to 0.817 mg of tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$)

Evaluate the results as described under *5.5 Dissolution test for oral dosage forms*, Acceptance criteria. The amount of lamivudine ($C_8H_{11}N_3O_3S$) and tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$) released is not less than 80% (Q) of the corresponding amounts declared on the label.

Tests for related substances. Perform the test in subdued light using low-actinic glassware. 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 end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

Use the following conditions for gradient elution:

mobile phase A: acetate buffer pH 4.2; and

mobile phase B: acetonitrile R.

Prepare the acetate buffer pH 4.2 by dissolving 9.64 g of ammonium acetate R in 900 mL of water R, adjust the pH to 4.2 with glacial acetic acid R and dilute to 1000 mL with water R.

Time (minutes)	Mobile phase A (% *V*/*V*)	Mobile phase B (% *V*/*V*)	Comments
0–2	100	0	Isocratic
2–17	100 to 95	0 to 5	Linear gradient
17–47	95 to 60	5 to 40	Linear gradient
47–62	60 to 25	40 to 75	Linear gradient
62–63	25 to 100	75 to 0	Return to initial composition
63–75	100	0	Re-equilibration

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm. Maintain the column temperature at 25 °C and the autosampler temperature between 2 °C and 6 °C.

Prepare the following solutions using water R as diluent.

For solution (1), transfer a quantity of the powdered tablets, nominally equivalent to 184 mg of tenofovir disoproxil, to a 250 mL volumetric flask. Add about 175 mL of diluent and sonicate at room temperature for about 30 minutes with intermittent shaking. Allow to cool to room temperature, dilute to volume and filter.

For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL.

For solution (3), dilute 10.0 mL of solution (2) to 100.0 mL.

Dissolve the content of a vial of tenofovir disoproxil fumarate for system suitability RS (containing tenofovir disoproxil fumarate and impurity H) in 0.5 mL of water R.

For solution (5)(used to generate of tenofovir disoproxil impurity A), dissolve 10 mg of tenofovir disoproxil fumarate RS in 10 mL. Heat the solution carefully in a boiling water-bath for 20 minutes. Cool to room temperature and dilute 1 mL of the solution to 10 mL.

For solution (6), use a solution containing 0.2 mg of fumaric acid R per mL.

For solution (7), dissolve 25 mg of cytosine R and 25 mg of uracil R and dilute to 50.0 mL. Dilute 1.0 mL of this solution to 100.0 mL.

For solution (8), dissolve or disperse a suitable amount of each of the excipients stated on the label in 10 mL, dilute to 100.0 mL and filter. Adjust the weight of the excipient so that its concentration resembles the concentration of the excipient in solution (1), provided this information is available. Inject 10 µL each of solutions (1), (2), (3), (4), (5), (6), (7) and (8).

Use the chromatogram obtained with solution (4) and the chromatogram supplied with tenofovir disoproxil fumarate for system suitability RS to identify the peak due to the tenofovir disoproxil impurity H in the chromatogram obtained with solution (1), if present.

Use the chromatogram obtained with solution (5) to identify the peak due to the tenofovir disoproxil impurity A in the chromatogram obtained with solution (1), if present.

Use the chromatogram obtained with solution (6) to identify the peak due to fumaric acid in the chromatogram obtained with solution (1). The peak due to fumaric acid is eluted at about 2.5 minutes and may appear either as a single or a split peak.

Use the chromatogram obtained with solution (7) to identify the peaks due to lamivudine impurities E (cytosine) and F (uracil) in

the chromatogram obtained with solution (1), if present.

Use the chromatogram obtained with solution (8) to identify the peaks due to excipients.

The impurities, if present, are eluted at the following relative retentions with reference to tenofovir disoproxil (retention time about 48 minutes):

Impurity	Relative retention	Impurity Classification
Lamivudine impurity E	0.09	Synthesis/Degradation
Lamivudine impurity F	0.11	Synthesis/Degradation
Lamivudine impurity A	0.17	Synthesis
Lamivudine impurity G	0.20	Synthesis/Degradation
Lamivudine impurity H	0.21	Synthesis/Degradation
Lamivudine impurity B	0.38	Synthesis
Lamivudine	0.39	-
Lamivudine impurity J	0.45	Degradant
Lamivudine impurity C	0.54	Synthesis
Tenofovir disoproxil impurity R	0.30	
Tenofovir disoproxil impurity N	0.33	Synthesis/Degradation
Tenofovir disoproxil impurity A	0.63	Synthesis/Degradation
Tenofovir disoproxil impurity F	0.73	Degradation
Tenofovir disoproxil impurity E	0.76	Synthesis/Degradation
Tenofovir disoproxil impurity B	0.80 and 0.81	Synthesis
Tenofovir disoproxil impurity L	0.87	Synthesis
Tenofovir disoproxil impurity C	0.88	Synthesis
Tenofovir disoproxil impurity D	0.90	Synthesis
Tenofovir disoproxil impurity M	0.94	Synthesis
Tenofovir disoproxil impurity P	0.96	Synthesis
Tenofovir disoproxil impurity O	0.97	Synthesis
Tenofovir disoproxil impurity I	0.98	Synthesis/Degradation
Tenofovir disoproxil impurity H	1.01	Synthesis
Tenofovir disoproxil impurity Q	1.10	Synthesis/Degradation
Tenofovir disoproxil impurity J	1.19	Synthesis/Degradation

Note: Tenofovir disoproxil impurities B and C may appear as single or split peaks. If they appear as split peaks, use the sum of the two peaks in the calculation of the concentration. ("Synthesis" stands for synthesis-related impurity; "Degradation" for degradation product.)

The test is not valid unless:

in the chromatogram obtained with solution (3), the signal-to-noise ratio of the peak due to tenofovir disoproxil is at least 20; and

in the chromatogram obtained with solution (4), the resolution between the peaks due to tenofovir disoproxil and tenofovir

disoproxil impurity H is at least 1.2.

*[**Note from the Secretariat.** It is intended to use the peak-to-valley ratio in the verification of the system suitability once the International Chemical Reference Substance on tenofovir disoproxil for system suitability has been established.]*

In the chromatogram obtained with solution (1):

the area of any peak corresponding to tenofovir disoproxil impurity A, when multiplied by a correction factor of 0.7, is not greater than five times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) (5.0%).

the area of any peak corresponding to either tenofovir disoproxil impurity F, I or J, is not greater than 0.75 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) (0.75%);

the area of any peak corresponding to tenofovir disoproxil impurity D is not greater than 3 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.3%);

the area of any peak corresponding to tenofovir disoproxil impurity N, when multiplied by a correction factor of 0.5, is not greater than two times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to tenofovir disoproxil impurity E or Q is not greater than two times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to lamivudine impurity E, when multiplied by a correction factor of 0.6, is not greater than two times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.2%);

the area of any peak corresponding to lamivudine impurity F or J, when multiplied by a correction factor of 2.2, is not greater than two times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.2%); and

the area of any peak corresponding to either lamivudine impurity G or H, is not greater than two times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.2%).

Determine the sum of the areas of any peaks corresponding to lamivudine impurities G and H and the corrected areas of any peaks corresponding to lamivudine impurities E, F and J. Calculate the percentage content of lamivudine related impurities using the area of the peak due to lamivudine in the chromatogram obtained with solution (2) as a reference. Disregard any peak with an area or a corrected area of less than 0.5 times the area of the peak due to lamivudine in the chromatogram obtained with solution (3) (0.05%).

Determine the sum of the areas of any peaks corresponding to tenofovir disoproxil impurities F, E, I, D, Q and J and the corrected areas of any peaks corresponding to tenofovir disoproxil impurities N and A. Calculate the percentage content of tenofovir disoproxil related impurities using the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) as a reference. Disregard any peak with an area or a corrected area of less than 0.5 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (3) (0.05%) and any peak due to fumaric acid.

The sum of both lamivudine and tenofovir disoproxil related impurities is not greater than 5.0%.

Assay. Perform the test in subdued light using low-actinic glassware. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (3.5 µm).

Use the following conditions for gradient elution:

mobile phase A: 0.1% (v/v) of trifluoroacetic acid R in water R;

mobile phase B: Acetonitrile R.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase (% v/v)	Comments
0 – 2.0	97	3	Isocratic
2.0 – 12.0	97 to 50	3 to 50	Linear gradient
12.0 – 15.0	50	50	Isocratic
15.0 – 16.0	50 to 97	50 to 3	Return to initial composition

16.0 – 20.0	97	3	Re-equilibration

Operate at a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm. Maintain the column temperature at 30 °C.

Use as a diluent a mixture of 95 volumes of 0.1% (v/v) of trifluoroacetic acid R in water R and 5 volumes of acetonitrile R.

Prepare the following solutions. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally containing 300.0 mg of lamivudine, to a 100 mL volumetric flask. Add about 30 mL of the diluent and sonicate for about 10 minutes with intermittent shaking. Then add 50 mL acetonitrile and sonicate for a further 30 minutes. Allow to cool to room temperature, dilute to volume with the diluent and filter. Dilute 5.0 mL of this solution to 100.0 mL with the diluent. For solution (2), dissolve 30.0 mg of lamivudine RS in diluent and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 10.0 mL with diluent. For solution (3), dissolve 30.0 mg of tenofovir disoproxil fumarate RSin diluent and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 10.0 mL with diluent.

Inject 20 µL each of solutions (1), (2) and (3).

Measure the areas of the peaks corresponding to lamivudine and tenofovir disoproxil obtained in the chromatograms of solutions (1), (2) and (3) and calculate the percentage content of lamivudine ($C_8H_{11}N_3O_3S$) and tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$) in the tablets using the declared content of lamivudine ($C_8H_{11}N_3O_3S$) in lamivudine RS and the declared content of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$)in tenofovir disoproxil fumarate RS. Each mg of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$) corresponds to 0.817 mg of tenofovir disoproxil ($C_{19}H_{30}N_5O_{10}P$).

Impurities. The impurities limited by the requirements of this monograph include those listed in the monographs on Lamivudine and Tenofovir disoproxil fumarate, excluding lamivudine impurity D and tenofovir disoproxil impurity G.