Dolutegravir, lamivudine and tenofovir disoproxil tablets (Dolutegraviri, lamivudini et tenofoviri disoproxili compressi)

2025-01

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

Storage. Dolutegravir, lamivudine and tenofovir disoproxil tablets should be kept in a tightly closed container.

Labelling. The designation of the container should state that the active ingredient, dolutegravir, is in the sodium form and that the quantity should be indicated in terms of the equivalent amount of dolutegravir. The quantities of the two other active ingredients should be indicated in terms of the amounts of lamivudine and tenofovir disoproxil fumarate.

Additional information. Strength in the current WHO Model List of Essential Medicines: 50 mg Dolutegravir, 300 mg Lamivudine and 300 mg Tenofovir disoproxil fumarate.

Requirements

Comply with the monograph for *Tablets*.

Definition. Dolutegravir, lamivudine and tenofovir disoproxil tablets contain Dolutegravir sodium, Lamivudine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of dolutegravir ($C_{20}H_{19}F_2N_3O_5$), lamivudine ($C_8H_{11}N_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$) 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. They ensure that, if tested, the tablets would comply with a water content limit of not more than 50 mg/g when determined as described under *2.8 Determination of water by the Karl Fischer method*, Method A, using 0.5 g of the powdered tablets.

Identity tests

Either test A or test B 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". The retention time of the three principal peaks in the chromatogram obtained with solution (1) correspond to the retention time of the corresponding peaks due to dolutegravir, lamivudine and tenofovir disoproxil in the chromatograms obtained with solutions (2), (3) and (4).

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 acid R 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 4 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 containing 12 mg of lamivudine, in 2 mL, sonicate for 5 minutes and filter. For solution (B), use a solution containing 1 mg of dolutegravir sodium RS per mL. For solution (C), use a solution containing 6 mg of lamivudine RS per mL. For solution (D), use a solution containing 6 mg of tenofovir disoproxil fumarate RS. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of air. Allow the plate to cool and examine the chromatogram under ultraviolet light (254 nm and 365 nm). The three principal spots in the chromatogram obtained with solution (A) correspond in position, appearance and intensity with the corresponding spots due to dolutegravir, lamivudine and tenofovir disoproxil obtained with solution (B), (C) and (D).

Dissolution. Carry out the test described under *5.5 Dissolution test for oral dosage forms*, using as the dissolution medium 900 mL of dissolution buffer, pH 6.8, 0.5% SDS TS and rotating the paddle at 75 revolutions per minute. At 30 minutes, withdraw a sample of 10 mL of the medium through an in-line filter (sample (A)). Add 10 mL of the dissolution medium, maintained at 37.0 °C, to each dissolution vessel and continue the dissolution for a further 30 minutes. At 60 minutes, withdraw again a sample of 10 mL of the dissolution medium through an in-line filter (sample (B)). Dilute 5.0 mL each of sample (A) and sample (B) to 25.0 mL with diluent (1), described under "Assay", and use the obtained solution as solution (1) and solution (2).

Measure the concentration of lamivudine and tenofovir disoproxil fumarate in solution (1) and the concentration of dolutegravir in solution (2). 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 total amount each of lamivudine, tenofovir disoproxil fumarate and dolutegravir in the medium from the results obtained, using the declared content of dolutegravir sodium ($C_{20}H_{18}F_2NaO_5$) in dolutegravir sodium RS, 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 dolutegravir sodium is equivalent to 0.950 mg of dolutegravir.

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 fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$) released after 30 minutes is not less than 80% (Q) of the corresponding amounts declared on the label and the amount of dolutegravir ($C_{20}H_{18}F_2N_3O_5$) released after 60 minutes is not less than 80% (Q) of the corresponding amount declared on the label.

Tests for related substances

A. **Lamivudine- and tenofovir disoproxil-related substances**

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 at 6 °C.

Prepare the following solutions using water R as diluent.

For solution (1), transfer a quantity of the powdered tablets, nominally containing 225 mg of Tenofovir disoproxil fumarate, 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.

For solution (4), dissolve the content of a vial of tenofovir disoproxil fumarate for system suitability RS in 0.5 mL of water R.

For solution (5), 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 a suitable amount of each of the excipients stated on the label in 10 mL of a suitable solvent and dilute to 100.0 mL with the diluent.

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 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 as a single or split peaks.

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
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
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

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 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 impurity F, tenofovir impurity I or tenofovir impurity 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 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 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 impurity E or impurity 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 impurities 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 impurity 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 impurities F, E, I , D, Q and J and the corrected areas of any peaks corresponding to tenofovir 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 the lamivudine and tenofovir disoproxil related impurities is not greater than 5.0%.

B. **Dolutegravir-related substances**

Perform the test in subdued light and without any prolonged interruptions, preferably 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 particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl and pentafluorophenyl groups (5 µm).

Use the following conditions for gradient elution:

-**Mobile phase A**: 0.186 g disodium edetate R in 1000 mL of water R, adjusted to pH 2.0 with phosphoric acid (~20 g/L) TS;
-**Mobile phase B**: 90 volumes of methanol R and 10 volumes of tetrahydrofuran R.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments

0–2	60	40	Isocratic
2–32	60 to 50	40 to 50	Linear gradient
32–56	50 to 20	50 to 80	Linear gradient
56–62	20	80	Isocratic
62–63	20 to 60	80 to 40	Return to initial composition
63–70	60	40	Re-equilibration

Operate at a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 320 nm. Maintain the column temperature at 45 °C.

Prepare the following solutions using as the diluent a mixture of 60 volumes of water R and 40 volumes of acetonitrile R.

For solution (1), transfer a quantity of the powdered tablets, nominally equivalent to 87.5 mg dolutegravir, to a 250 mL volumetric flask. Add about 180 mL diluent and sonicate for five minutes, cool to room temperature, dilute to volume and filter.

For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 10.0 mL of this solution to 50.0 mL.

For solution (3), dilute 2 mg of dolutegravir impurity D RS in 5 mL of acetonitrile R. Dilute 1 mL of this solution to 10 mL.

For solution (4), dissolve 5 mg of dolutegravir sodium RS in 1 mL acetonitrile R. Add 4.5 mL water R and 4.5 mL hydrochloric acid (~ 420 g/L) TS and boil the solution under reflux for 1 hour. Cool the solution to room temperature and dilute 1 mL of it to 10 mL with a mixture of 6 volumes of water R and 4 volumes of acetonitrile R.

For solution (5), mix 1 mL of solution (3) with 1 mL of solution (4).

Inject 30 µL each of solutions (1), (2), (3), (4) and (5).

Use the chromatogram obtained with solution (3) and the chromatogram supplied with dolutegravir impurity D RS to identify the peak due to the impurity D. Use the chromatogram obtained with solution (4) to identify the peak due to impurity H (the chromatogram usually shows two principal peaks: the peak due to dolutegravir and the peak due to impurity H).

The impurities, if present, are eluted at the following relative retentions with reference to dolutegravir (retention time about 27 minutes):

Impurity	Relative retention	Impurity Classification
Dolutegravir impurity C	0.67	Synthesis
Dolutegravir impurity F	0.70	Synthesis
Dolutegravir impurity D	0.76	Synthesis
Dolutegravir impurity H	0.79	Synthesis/Degradation
Dolutegravir impurity E	0.89	Synthesis
Dolutegravir impurity J	1.72	Synthesis
Dolutegravir impurity K	1.74	Synthesis
Dolutegravir impurity L	2.06	Synthesis

The test is not valid unless, in the chromatogram obtained with solution (5). the resolution between the peaks due to impurity D and impurity H is at least 1.5. Also, the test is not valid unless, in the chromatogram obtained with solution (2), the peak due to dolutegravir is obtained with a signal-to-noise ratio of at least 20.

In the chromatogram obtained with solution (1):

- the area of any dolutegravir impurity peak is not greater than the area of the peak due to dolutegravir in the chromatogram obtained with solution (2) (0.2%).

Assay. Perform the test in subdued light and without any prolonged interruptions, preferably 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.

Prepare the following diluents. For diluent (1), mix 95 volumes of 0.1% (v/v) of trifluoroacetic acid R in water R and 5 volumes of acetonitrile R. For diluent (2), mix 60 volumes of water R and 40 volumes of acetonitrile R.

Prepare the following solution. 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 diluent (1) and sonicate for about 10 minutes with intermittent shaking until the larger pieces have disintegrated. Add 50 mL acetonitrile and sonicate for about 30 minutes. Allow to cool to room temperature, dilute to volume with diluent (1) and filter. Dilute 5.0 mL of this solution to 100.0 mL with diluent (1). For solution (2), dissolve 26.3 mg of dolutegravir sodium RS in 30 mL diluent (2) by sonication for about 10 minutes. Allow to cool to room temperature and dilute to 100.0 mL with diluent (1). Dilute 5.0 mL of this solution to 50.0 mL with diluent (1). For solution (3), dissolve 30.0 mg of lamivudine RS in diluent (1) and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 10.0 mL with diluent (1). For solution (4), dissolve 30.0 mg of tenofovir disoproxil fumarate RS in diluent (1) and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of this solution to 10.0 mL with diluent (1).

Inject 20 µL each of solutions (1), (2), (3) and (4).

Measure the areas of the peaks corresponding to dolutegravir, lamivudine and tenofovir disoproxil obtained in the chromatograms of solutions (1), (2), (3) and (4) and calculate the percentage content of dolutegravir ($C_{20}H_{18}F_2N_3O_5$), lamivudine ($C_8H_{11}N_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$) in the tablets using the declared content of dolutegravir sodium ($C_{20}H_{18}F_2NaO_5$) in dolutegravir sodium RS, 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 dolutegravir sodium is equivalent to 0.950 mg of dolutegravir.

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