World Health Organization

TENOFOVIR DISOPROXIL FUMARATE TABLETS

(TENOFOVIRI DISOPROXILI FUMARATI COMPRESSI)

Draft proposal for inclusion in *The International Pharmacopoeia*

(26 August 2022)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (email: schmidth@who.int), with a copy to Ms Sinéad Jones (email: jonessi@who.int) by **21 October 2022**.

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the "*Working documents in public consultation*" link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/22.911

TENOFOVIR DISOPROXIL FUMARATE TABLETS

Description	Date
First draft prepared.	May 2022
Laboratory investigations to verify the suitability of the analytical provisions	May – July 2022
First draft sent out for public consultation	August – September 2022
Presentation at the 57th Meeting of the Expert Committee on Specifications for Pharmaceutical Preparations.	9-13 October 2023
Further follow-up action as required.	

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

Storage. Tenofovir disoproxil tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model List of Essential Medicines: 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. Tenofovir disoproxil tablets contain Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$. $C_4H_4O_4$) 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.

A. 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 peak in the chromatogram obtained with

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solution (1) corresponds to the retention time of the corresponding peak due to tenofovir disoproxil in the chromatograms obtained with solution (2).

B. 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 2 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 tenofovir disoproxil fumarate, in 2 mL, sonicate for 5 minutes and filter. For solution (B), 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 principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the corresponding spots due to tenofovir disoproxil obtained with solution (B).

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 (0.1 mol/L) VS 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.

Measure the absorbance (1.6) of a 1 cm layer of the resulting solution, suitably diluted if necessary, at the maximum of about 260 nm. For each of the tablets tested, calculate the total amount of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$. $C_4H_4O_4$) in the medium using the absorptivity value of 22.3 ($A_{1\,cm}^{1\%}$ = 223) for tenofovir disoproxil fumarate.

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Evaluate the results as described under *5.5 Dissolution test for oral dosage forms*, Acceptance criteria. The amount of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$. $C_4H_4O_4$) released is not less than 80% (Q) of the amount declared on the label.

Tests for 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 end-capped particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).[1]

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

[1] An Inertsil ODS-3v column was found suitable.

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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), use a solution containing 0.5 mg of tenofovir disoproxil for system suitability (containing tenofovir disoproxil and the impurity H) per mL.

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 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) and (7).

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.

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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 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

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.)

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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%); and
- 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%).

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- The sum of the areas of all impurity peaks, including the corrected areas of any peaks corresponding to tenofovir impurities N and A is not greater than 5 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with solution (2) (5.0%). 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.

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 (25 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 (5 µm).[2]

As the mobile phase use a mixture of a sodium dihydrogen phosphate buffer pH 2.3 and acetonitrile for chromatography R (60:40 v/v).

Prepare the sodium dihydrogen phosphate buffer pH 2.3 by dissolving 6.9 g of sodium dihydrogen phosphate R in 900 ml of water R, adding 1.0 mL of triethylamine R, adjusting the pH to 2.3 with phosphoric acid (~105 g/l) TS, and diluting to 1000 ml with water R.

Operate at 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 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.

[2] An Inertsil ODS-3v column was found suitable.

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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 tenofovir disoproxil fumarate, to a 100 mL volumetric flask. Add about 30 mL of diluent 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 and filter. Dilute 5.0 mL of this solution to 100.0 mL with diluent. For solution (2), dissolve 30.0 mg of tenofovir disoproxil fumarate 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.

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

Measure the areas of the peaks corresponding to tenofovir disoproxil obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$. $C_4H_4O_4$) in the tablets using the declared content of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P$. $C_4H_4O_4$) in tenofovir disoproxil fumarate RS.

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

Reference substances invoked

Tenofovir disoproxil for system suitability RS (containing tenofovir disoproxil and the impurity H)

International Chemical Reference Substance to be established.

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Tenofovir disoproxil fumarate RS

Established International Chemical Reference Substance.

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