



## EMTRICITABINE AND TENOFOVIR TABLETS

### Draft proposal for *The International Pharmacopoeia* (September 2010)

#### **REVISED DRAFT FOR COMMENT**

This document was provided by a quality control expert and was discussed at the recent WHO consultation on specifications for medicines and quality control laboratory issues. Previous comments received have been incorporated into this revised draft. Should you have any comments, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; e-mail: [kopps@who.int](mailto:kopps@who.int) with a copy to Ms C. Mendy [mendyc@who.int](mailto:mendyc@who.int) by 15 October 2010.

**During the past few years we have moved more towards an electronic system for sending out our draft monographs for comment, for convenience and in order to speed up the process. If you do not already receive our documents electronically, please let us have your e-mail address (to [bonnyw@who.int](mailto:bonnyw@who.int)) and we will add it to our electronic mailing list.**

---

© World Health Organization 2010

All rights reserved.

This draft is intended for a restricted audience only, i.e. the individuals and organizations having received this draft. The draft may not be reviewed, abstracted, quoted, reproduced, transmitted, distributed, translated or adapted, in part or in whole, in any form or by any means outside these individuals and organizations (including the organizations' concerned staff and member organizations) without the permission of WHO. The draft should not be displayed on any web site.

Please send any request for permission to:

Dr Sabine Kopp, Quality Assurance Programme, Medicines Quality Assurance Programme, Quality & Safety: Medicines (QSM), Department of Essential Medicines and Pharmaceutical Policies (EMP), World Health Organization, CH-1211 Geneva 27, Switzerland. Fax: (41-22) 791 4730; e-mail: [kopps@who.int](mailto:kopps@who.int).

The designations employed and the presentation of the material in this draft do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this draft. However, the printed material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This draft does not necessarily represent the decisions or the stated policy of the World Health Organization.

**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT**  
**QAS/10.351**  
*International Pharmacopoeia monograph on*  
**EMTRICITABINE AND TENOFOVIR TABLETS**

	<b>Date</b>
Preparation of first draft by laboratory	August 2009
First draft mailed out for comments	March 2010
Any comments received reviewed in Consultation on Specifications for Medicines and Quality Control Laboratory Issues	10-12 May 2010
Revised draft discussed during tele-/videoconference on specifications for medicines	25 August 2010
Revised draft mailed out for comments	September 2010
Collation of comments received	October 2010
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for possible adoption	18-22 October 2010
Any further action as necessary	...

**Draft proposal for *The International Pharmacopoeia*  
(September 2010)**

***EMTRICITABINI ET TENOFOVIRI COMPRESSI*  
EMTRICITABINE AND TENOFOVIR TABLETS**

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

**Storage.** Emtricitabine and tenofovir tablets should be kept in a tightly closed container.

**Additional information.** Strength in the current WHO Model list of essential medicines: 200 mg Emtricitabine and 300 mg Tenofovir disoproxil fumarate.

**Requirements**

Comply with the monograph for “Tablets”.

**Definition.** Emtricitabine and tenofovir tablets contain Emtricitabine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amounts of emtricitabine ( $C_8H_{10}FN_3O_3S$ ) and 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 60 mg/g when determined as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the powdered tablets.

**Identity tests**

- Either tests A and B or test C 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 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5  $\mu$ l of each of the following three solutions in methanol R. For solution (A) disperse a quantity of powdered tablets to obtain a concentration of 5 mg of Emtricitabine per ml, filter and use the filtrate. For solution (B) use 5 mg of emtricitabine RS. For solution (C) use 7.5 mg of tenofovir disoproxil fumarate RS per ml. After removing the plate from the chromatographic chamber,

allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

One of the two principal spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B and the other one corresponds with that obtained with solution C.

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Stain the plate with iodine vapour and examine the chromatogram in daylight.

One of the two principal spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B and the other one corresponds with that obtained with solution C.

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

B.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 50 volumes of heptane R, 30 volumes of glacial acetic acid R and 20 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions in ethanol R. For solution (A) disperse a quantity of powdered tablets to obtain a concentration of 10 mg of Tenofovir disoproxil fumarate per ml, filter and use the filtrate. For solution (B) use 2 mg of fumaric acid R per ml. Develop the plate in an unsaturated tank over a path of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

One of the spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test B.1 but using silica gel R5 as the coating substance. Spray lightly with a 16 g/l solution of potassium permanganate R and examine the chromatogram in daylight.

One of the spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

C. See the test described under Assay. The retention times of the principal peaks in the chromatogram obtained with the test solution are similar to those due to emtricitabine, tenofovir disoproxil and to fumarate in the chromatogram obtained with the reference solution.

**Dissolution**

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of hydrochloric acid (~0.4 g/l) TS, and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium and filter. Allow the filtered sample to cool to room temperature and dilute if necessary [solution (1)]. Prepare solution (2) using 0.22 mg of emtricitabine RS and 0.33 mg of tenofovir disoproxil fumarate RS per ml of dissolution medium. Determine the content of emtricitabine ( $C_8H_{10}FN_3O_3S$ ) and tenofovir disoproxil fumarate ( $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ ) as described under Assay using solution (1) and solution (2).

For each of the six tablets tested, calculate the total amount of emtricitabine ( $C_8H_{10}FN_3O_3S$ ) and tenofovir disoproxil fumarate ( $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ ) in the medium from the results obtained. For both substances, the amount in solution for each tablet is not less than 80% of the amount stated on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and no tablet contains less than 60%.

**Tenofovir monoester.** Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay.

After preparation, keep the solutions at about 6°C, or use an injector with cooling.

Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powder containing about 100 mg of Tenofovir disoproxil fumarate, accurately weighed, in 100 ml of the diluent and filter. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 5 µg of Tenofovir disoproxil fumarate per ml. For solution (3) heat carefully 1 mg of tenofovir disoproxil fumarate RS per ml of water R in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35°C.

Inject 20 µl of solution (3). The peak due to tenofovir monoester elutes at a relative retention of about 0.9 with reference to tenofovir disoproxil (retention time about 18 minutes).

Inject separately 20 µl each of solutions (1) and (2). The test is not valid unless in the chromatogram obtained with solution (1), three principal peaks are shown.

In the chromatogram obtained with solution (1), the area of any peak due to tenofovir monoester, is not greater than seven times the area of the principal peak obtained with solution (2) (3.5%).

**Assay**

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)<sup>1</sup>.

The mobile phases for gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

Mobile phase A: 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 95 volumes of water R.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 25 volumes of water R.

<b>Time (min)</b>	<b>Mobile phase A (% v/v)</b>	<b>Mobile phase B (% v/v)</b>	<b>Comments</b>
0 – 9	93	7	Isocratic
9 – 15	93 to 0	7 to 100	Linear gradient
15 – 19	0	100	Isocratic
19 – 19.1	0 to 93	100 to 7	Return to initial composition
19.1-30	93	7	Re-equilibration

After preparation, keep the solutions at about 6 °C, or use an injector with cooling.

Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Tenofovir disoproxil fumarate, accurately weighed in 100 ml of the diluent and filter. For solution (2) use 0.1 mg of tenofovir disoproxil fumarate RS and 66.7 µg of emtricitabine RS per ml of diluent. If necessary, adapt the concentration of solution (2) according to the ratio of Emtricitabine and Tenofovir disoproxil fumarate in the tablets. For solution (3) use 0.02 mg of fumaric acid R per ml of water R.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35°C.

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

The test is not valid unless in the chromatograms obtained with solutions (1) and (2), three principal peaks are shown and the resolution factor between those peaks is at least 5.

<sup>1</sup> Hypersil BDS column.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of emtricitabine ( $C_8H_{10}FN_3O_3S$ ) and tenofovir disoproxil fumarate ( $C_{19}H_{30}N_5O_{10}P, C_4H_4O_4$ ) in the tablets.

*[Note from Secretariat: a test for related substances is not available, due to the overlapping of the impurities from both APIs and the non-availability of reference substances for the impurities that would be required to allow the identification of the peaks.]*

\*\*\*

**New reagents to be added to Ph.Int.**

**Hydrochloric acid (~0.4 g/l) TS.**

\*\*\*

Revised draft for comment