



**DRAFT MONOGRAPH FOR *THE INTERNATIONAL  
PHARMACOPOEIA***

**EFAVIRENZ, EMTRICITABINE AND TENOFOVIR TABLETS**

**(August 2010)**

***DRAFT FOR COMMENT***

This document was provided by a quality control expert and was discussed during a video-teleconference on specifications for medicines held on 25 August 2010. Should you have any comments thereon, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mails: [kopps@who.int](mailto:kopps@who.int) with a copy to Ms C. Mendy [mendyc@who.int](mailto:mendyc@who.int) by 26 October 2010.

**In order to speed up the process for receiving draft monographs and for sending comments, 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.**

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**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/10.391**

*International Pharmacopoeia monograph on*

**EFAVIRENZ, EMTRICITABINE AND TENOFOVIR TABLETS**

|  | <b>Date</b>        |
|--|--------------------|
| Preparation of first draft by laboratory   | August 2010        |
| Comments discussed during video-/teleconference on specifications for medicines        | 25 August 2010     |
| Draft monograph mailed out for comment   | September 2010     |
| Collation of comments  | October 2010       |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | 18-22 October 2010 |
| Further action as necessary  |                    |

## DRAFT MONOGRAPH FOR THE INTERNATIONAL PHARMACOPOEIA

### EFAVIRENZ, EMTRICITABINE AND TENOFOVIR TABLETS

**Category.** Antiretroviral (Non-nucleoside/Nucleoside/Nucleotide Reverse Transcriptase Inhibitors).

**Storage.** Efavirenz, emtricitabine and tenofovir tablets should be kept in a tightly closed container.

**Additional information.** Strength in the current WHO Model list of essential medicines: 600 mg Efavirenz, 200 mg Emtricitabine and 300 mg Tenofovir disoproxil fumarate.

#### Requirements

Comply with the monograph for “Tablets”.

**Definition.** Efavirenz, emtricitabine and tenofovir tablets contain Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of efavirenz ( $C_{14}H_9ClF_3NO_2$ ), 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 four solutions in methanol R. For solution (A) disperse a quantity of the 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 per ml. For solution (C) use 7.5 mg of tenofovir disoproxil fumarate RS per ml. For solution (D) use 15 mg of efavirenz 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).

The three principal spots obtained with solution A correspond in position, appearance and intensity with those obtained with solutions B, C and D.

- 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.

The three principal spots obtained with solution A correspond in position, appearance and intensity with those obtained with solutions B, C and D.

- 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 the 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 due to efavirenz, emtricitabine, tenofovir disoproxil and fumarate in the chromatogram obtained with the test solution are similar to those 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, 1000 ml of a 2% solution of sodium dodecyl sulfate R, and rotating the paddle at 100 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Allow the filtered sample to cool to room temperature and dilute if necessary [solution (1)]. Prepare solution (2) using 0.60 mg of efavirenz RS, 0.20 mg of emtricitabine RS and 0.30 mg of tenofovir disoproxil fumarate RS per ml of dissolution medium. Determine the content of efavirenz ( $C_{14}H_9ClF_3NO_2$ ), 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 efavirenz ( $C_{14}H_9ClF_3NO_2$ ), 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. 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). The test is not valid unless the resolution between these peaks is at least 5.

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

In the chromatogram obtained with solution (1), the area of any peak due to tenofovir monoester, is not greater than 7 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.

| Time (min) | Mobile phase A (% v/v) | Mobile phase B (% v/v) | Comments                      |
|------------|------------------------|------------------------|-------------------------------|
| 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

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

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 0.2 mg of efavirenz RS, 0.1 mg of tenofovir disoproxil fumarate RS and 66.7 µg of emtricitabine RS per ml of diluent. For solution (c) use 0.02 mg of fumaric acid R per ml of water R.

If necessary adapt the concentration of solution (2) according to the ratio of Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate in the tablets.

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), four principal peaks, that elute at the following retention times, are shown: fumarate (about 2.5 minutes), emtricitabine (about 9 minutes), tenofovir disoproxil (about 18 minutes) and efavirenz (about 22 minutes).

Calculate the content of efavirenz ( $C_{14}H_9ClF_3NO_2$ ), 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.

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