CONTROLLED COPY

MASTER COPY

Mylan	FINISHED PRODUCT (REGULATORY AND SHELF LIFE) STANDARD TEST PROCEDURE	Mylan, Nashik

Name of the Product: Emtricitabine and Tenofovir Disoproxil Fumarate Tablets (200 mg/300 mg)	
Product code: ETF000T66X1A	**Supersedes:** FPPETF506R-00
STP No.: FPPETF506R-01	**Effective Date:** 23 SEP 2021
Reference/s: Ph. Int. + IH	**Market:** PERU

	Department	Name	Sign & Date
Prepared by	QA	Ram Phad	22/10/20
Reviewed by	QA	Mukesh Malviya	24/10/20
Approved by	QC	Ann S. Undeglikar.	26.10.2020
	QA	Subhash Phad	11/11/20

1.0 DESCRIPTION:

Procedure:

Put 10 tablets on butter paper observe the size, shape and color. Compare the sample appearance to specification description. The tablet should be free of foreign matter or surface defects, including sticking, discoloration or disfiguration.

2.0 IDENTIFICATION (By HPLC):

Procedure:

Proceed as directed under the test Assay by HPLC. Compare the retention time of the Emtricitabine and Tenofovir Disoproxil peaks from the test chromatogram with that from the standard chromatogram. The retention time of the Emtricitabine and Tenofovir Disoproxil peaks in the chromatogram of the test preparation corresponds to that in the chromatogram of the standard preparation.

3.0 DISSOLUTION (By HPLC):

Reagents:

1.	Ammonium acetate	:	AR grade or equivalent
2.	Glacial acetic acid	:	AR grade or equivalent
3.	Acetonitrile	:	HPLC grade or equivalent
4.	Hydrochloric acid	:	AR grade or equivalent
5.	Water	:	Milli Q or HPLC grade

Dissolution parameters:

Medium	:	0.01 N hydrochloric acid (degassed)
Apparatus	:	USP – II (Paddle)
Volume	:	900 mL
RPM	:	50 rpm
Temperature	:	37°C ± 0.5°C
Time Point	:	As specified in the specification.

Dissolution Medium (0.01N hydrochloric acid):

Dilute 8.5 mL of concentrated Hydrochloric acid to 1000 mL with water in a suitable container. Further dilute 100 mL of the above solution to 1000 mL with water, mix well and degas.

Buffer:

Transfer an accurately weighed amount of about 0.77 g of Ammonium Acetate into a suitable beaker. Add 1000 mL of water and dissolve. Adjust the pH of the solution to 6.0 ± 0.05 with glacial acetic acid. Filter through 0.45 µm Nylon 66 membrane filter (Millipore make) and degas.

Mobile phase A: Buffer

Note: Mobile phase A is stable for 5 days on bench top.

Mobile phase B: Degassed acetonitrile.

Chromatographic condition:

Column	:	Hypersil BDS, C18, 150 mm × 4.6 mm, 5 µm or equivalent
Flow rate	:	1.0 mL /minute.
Wavelength	:	260 nm
Column temperature	:	Ambient
Injection Volume	:	10 µL

Gradient programme:

Time (in minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Curve type #
0	90	10	6
6	20	80	6
7	90	10	6
10	90	10	6

As per Waters Empower software

Standard preparation:

1. Weigh accurately and transfer about 56 mg of Emtricitabine RS / working standard and 85 mg of Tenofovir Disoproxil Fumarate RS / working standard into a 250 mL volumetric flask. Add about 150mL of dissolution medium.
2. Sonicate to dissolve, dilute to volume with dissolution medium and mix well.
3. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) or 0.45 µm PVDF membrane filter.

Note: Standard preparation is stable for 24 hours on bench top.

Test preparation:

1. Transfer 900 mL of the dissolution medium into each of the six dissolution vessels.
2. After reaching the set temperature, drop one tablet into each of the dissolution vessels and immediately start the run.
3. After the specified time, withdraw 10 mL of the sample from each of the dissolution vessel.
4. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) or 10 µ full flow filter of auto sampler or 0.45 µm PVDF membrane filter.

Note: Test preparation is stable for 24 hours on bench top.

Procedure:

Inject dissolution medium as blank (for 2 times), standard preparation (for 5 times) and test preparation into the chromatograph. Record the chromatograms and measure the peaks' responses (Refer Typical Chromatograms).

Note: The elution pattern of the peaks are Emtricitabine followed by Tenofovir Disoproxil respectively.

System suitability:

From standard preparation:

1. The tailing factor for both Emtricitabine and Tenofovir Disoproxil peaks should be not more than 2.0.

2. The relative standard deviation for the peak areas of Emtricitabine and Tenofovir Disoproxil for replicate injections should be not more than 2.0%.

Calculation:

$$\text{Amount of Emtricitabine dissolved as \% of Labeled amount} = \frac{A_{TE}}{A_{SE}} \times \frac{W_{SE}}{250} \times \frac{900}{LC_E} \times \frac{P_E}{100} \times 100$$

$$\text{Amount of Tenofovir Disoproxil Fumarate dissolved as \% of Labeled amount} = \frac{A_{TT}}{A_{ST}} \times \frac{W_{ST}}{250} \times \frac{900}{LC_T} \times \frac{P_T}{100} \times 100$$

Where,

A_{TE} = Peak area of Emtricitabine from test preparation.

A_{SE} = Average peak area of Emtricitabine from standard preparation.

W_{SE} = Weight of Emtricitabine standard taken, in mg, for standard preparation.

LC_E = Label claim, as Emtricitabine, in mg.

P_E = Potency of Emtricitabine standard in % (on as is basis).

A_{TT} = Peak area of Tenofovir Disoproxil from test preparation.

A_{ST} = Average peak area of Tenofovir Disoproxil from standard preparation.

W_{ST} = Weight of Tenofovir Disoproxil Fumarate standard taken, in mg, for standard preparation.

LC_T = Label claim, as Tenofovir Disoproxil Fumarate, in mg.

P_T = Potency of Tenofovir Disoproxil Fumarate standard in % (on as is basis).

4.0 RELATED SUBSTANCES (By HPLC):

Reagents:

1. Potassium dihydrogen phosphate	:	AR grade or equivalent
2. Orthophosphoric acid	:	HPLC grade or equivalent
3. Methanol	:	HPLC grade or equivalent
4. Decaethylene glycol Mono dodecyl ether	:	Sigma make or equivalent
5. Water	:	Milli-Q / HPLC grade or equivalent

Diluted orthophosphoric acid solution:

Dilute 2 mL of orthophosphoric acid to 100 mL with water.

Buffer:

Weigh accurately and transfer about 1.36 g of Potassium dihydrogen phosphate (KH_2PO_4) into a suitable container. Add 1000 mL of water and dissolve. Adjust the pH of this solution to 3.0 ± 0.05 with diluted orthophosphoric acid and mix. Filter through 0.45 µm Nylon 66 membrane filter (Millipore make) and degas.

Mobile phase A: Buffer.

Mobile phase B: Mix methanol and buffer in the ratio of 800:200 v/v respectively and degas.

Note: Mobile phase is stable for 3 days on bench top.

5% Decaethylene glycol Mono dodecyl ether solution preparation (5% DGME solution):

Weight 5 g of Decaethylene glycol Mono dodecyl ether into a 100 mL volumetric flask and add about 80 mL of water, sonicate to dissolve and dilute to volume with water and mix well.

Diluent: Mix 5% DGME, Methanol and water in the ratio of 20:10:70 v/v/v respectively.

Chromatographic conditions:

Column	:	Symmetry shield RP18, 250 x 4.6 mm, 5.0 µm or equivalent
Flow rate	:	1.3 mL /minute

Detector	:	UV at 270 nm
Column temperature	:	30°C ± 2°C
Sample tray temperature	:	10°C ± 2°C
Injection Volume	:	20 µL

Needle wash solution:

Mix methanol and water in the ratio of 90:10 v/v respectively.

Note: Perform needle wash prior to the analysis and throughout the analysis.

Gradient programme:

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Curve type #
0.00	100	0	6
10	100	0	6
40	20	80	6
65	20	80	6
67	100	0	6
75	100	0	6

As per Waters Empower software

Impurity stock solution:

Transfer an accurately weighed amount of about 0.5 mg of Tenofovir impurity of Tenofovir Disoproxil Fumarate, 1.7 mg of S-Oxide impurity of Emtricitabine (Emtricitabine sulfoxide impurity of Emtricitabine), 1.0 mg of Des amino impurity of Emtricitabine (Emtricitabine 5-Flurouracil analog of Emtricitabine) and 1.0 mg of lamivudine impurity of Emtricitabine into a 10 mL volumetric flask, add about 8 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix well.

System suitability solution:

1. Transfer an accurately weighed amount of about 16.5 mg of Emtricitabine RS/working standard and 25 mg of Tenofovir Disoproxil Fumarate RS/working standard into a 25 mL volumetric flask.

2. To the same flask add 1.0 mL of impurity stock solution and about 8 mL of diluent sonicate to dissolve, dilute to volume with diluent and mix well.
3. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make).

Standard preparation:

1. Weigh accurately and transfer about 66 mg of Emtricitabine RS/working standard and 100 mg of Tenofovir disoproxil Fumarate RS/working standard into a 100 mL volumetric flask.
2. Add about 70 mL of diluent, sonicate to dissolve and cool to temperature. Add few drops (which should not be more than 0.5 mL) of methanol and dilute to volume with diluent and mix well.
3. Pipette 5.0 mL of the above solution into a 100 mL volumetric flask, dilute to volume with diluent and mix well.
4. Further pipette 3.0 mL of the above solution into a 50 mL volumetric flask, dilute to volume with diluent and mix well.
5. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make).

Note: Standard preparation is stable for 48 hours on bench top.

Test Preparation:

1. Weigh accurately not less than 20 tablets and determine the average weight.
2. Crush not less than 20 tablets into a fine powder in a mortar using pestle.
3. Transfer an accurately weighed amount of the tablets' powder equivalent to about 66 mg of Emtricitabine or 100 mg of Tenofovir Disoproxil Fumarate into a 100 mL volumetric flask.
4. Add about 70 mL of diluent, sonicate for 20 minutes with intermediate shaking, cool to room temperature.
5. Add few drops (which should not be more than 0.5 mL) of methanol and dilute to volume with diluent and mix well.
6. Centrifuge a portion of the above solution, in a centrifuge tube with cap, at 5000 RPM, for about 10 minutes.
7. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make).

Note:

1. Maintain the sonicator temperature below 25°C.
2. Prepare the solution immediately prior to use.

Placebo Preparation:

1. Weigh accurately and transfer about 179 mg of placebo into a 100 mL volumetric flask.
2. Add about 70 mL of diluent, sonicate for 20 minutes with intermediate shaking, cool to room temperature.
3. Add few drops (which should not be more than 0.5 mL) of methanol and dilute to volume with diluent and mix well.
4. Centrifuge a portion of the above solution, in a centrifuge tube with cap, at 5000 RPM, for about 10 minutes.
5. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make).

Procedure:

Inject diluent as blank (for 3 times), system suitability solution, Placebo preparation, standard solution (for 2 times) and test preparation into the chromatograph. Record the chromatograms and measure the peaks' responses (Refer Typical Chromatograms).

Note:

1. Disregard the peaks due to the blank and placebo in the test preparation.
2. Substract the average of last two reproducible blank peak area from the standard peak area if any interference is observed.
3. Substract the blank peak area or placebo peak area from respective impurity peak in the test chromatogram.
4. Disregard the peak due to Fumaric acid (RRT of Fumaric acid with respect to Tenofovir Disoproxil peak is about 0.150) in the test chromatogram.
5. In system suitability solution chromatogram integrate peak due to Fumaric acid, Lamivudine, Tenofovir impurity, S-oxide impurity, Des amino impurity, Emtricitabine and Tenofovir Disoproxil.
6. The relative retention time (RRT), relative response factor (RRF), Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the impurities are as follows:

Relative retention times of impurities with respect to Emtricitabine peak:

Name of the Impurity	RRT (about)	RRF	LOD (%)	LOQ (%)
Fluro Cytosine impurity (Flucytosine)	0.17	-	-	-
Lamivudine@	0.39	1.51	0.0011	0.0046
S-Oxide impurity (Emtricitabine sulfoxide impurity)	0.50	0.97	0.0037	0.0128
Emtricitabine acid impurity	0.84	-	-	-
Des amino impurity (Emtricitabine 5-Flurouracil analog)@	1.08	1.22	0.0028	0.0039
Salicylic acid impurity	1.91	-	-	-
Emtricitabine menthyl ester impurity	3.60	-	-	-
Emtricitabine (RT about 17.9 minutes)	1.00	-	-	-

@ Monitor impurity upto 0.2%, if crosses 0.2%, it should be further investigated.

Relative retention times of impurities with respect to Tenofovir Disoproxil peak:

Name of the Impurity	RRT (about)	RRF	LOD (%)	LOQ (%)
Adenine impurity @	0.09	5.21	0.0002	0.0014
Tenofovir impurity @	0.20	2.43	0.0011	0.0038
Mono ester impurity (Tenofovir monosoproxil)	0.73	1.66	0.0009	0.0048
Isopropyl impurity *	0.93	1.21	0.0034	0.0062
Tenofovir disoproxil dimer impurity	1.40	1.92	0.0017	0.0071
Tenofovir Disoproxil (RT about 37 minutes)	1.00	-	-	-

Name of the Impurity	RRT (about)	RRF	LOD (%)	LOQ (%)
Mono POC dimer impurity	1.10	1.89	0.002	0.006
Mix dimer impurity	1.23	1.55	0.007	0.013

* Monitor impurity upto 0.3%, if crosses 0.3%, it should be further investigated.

@ Monitor impurity upto 0.2%, if crosses 0.2%, it should be further investigated.

System suitability:

From system suitability solution:

1. The resolution between Tenofovir impurity and S-Oxide impurity peaks should be not less than 2.0.
2. The resolution between Emtricitabine and Des amino impurity peaks should be not less than 2.0
3. The resolution between the peak of Fumaric acid and Lamivudine impurity should be not less than 2.5.

From standard preparation:

4. The tailing factor for both Emtricitabine and Tenofovir Disoproxil peaks should be not more than 2.0.
5. The ratio of peak areas of Emtricitabine and Tenofovir Disoproxil should be not less than 0.90 and should be not more than 1.10.

Calculation:

From Emtricitabine:

$$\text{Known impurity (\% w/w)} = \frac{A_{TE}}{A_{SE}} \times \frac{W_{SE}}{100} \times \frac{5}{100} \times \frac{3}{50} \times \frac{100}{W_T} \times \frac{P_E}{100} \times \frac{A_{WT}}{LC_E} \times \frac{1}{RRF} \times 100$$

Where,

A_{TE} = Peak area of respective Emtricitabine impurity from test preparation.

A_{SE} = Average peak area of Emtricitabine from standard preparation.

W_{SE} = Weight of Emtricitabine standard taken, in mg, for standard preparation.

	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

W_T = Weight of tablets' powder taken, in mg, for test preparation

A_{WT} = Average weight of the tablet, in mg.

P_E = Potency of Emtricitabine standard in %, on as is basis.

LC_E = Label claim as Emtricitabine, in mg per tablet.

RRF = Relative response factor.

From Tenofovir Disoproxil Fumarate:

$$\text{Known impurity (\% w/w)} = \frac{A_{TT}}{A_{ST}} \times \frac{W_{ST}}{100} \times \frac{5}{100} \times \frac{3}{50} \times \frac{100}{W_T} \times \frac{P_T}{100} \times \frac{A_{WT}}{LC_T} \times \frac{1}{RRF} \times 100$$

Where,

A_{TT} = Peak area of respective Tenofovir disoproxil Fumarate impurity from test preparation.

A_{ST} = Average peak area of Tenofovir disoproxil from standard preparation.

W_{ST} = Weight of Tenofovir disoproxil Fumarate standard taken, in mg, for standard preparation.

W_T = Weight of tablets' powder taken, in mg, for test preparation

A_{WT} = Average weight of the tablet, in mg.

P_T = Potency of Tenofovir disoproxil Fumarate standard in %, on as is basis.

LC_T = Label claim as Tenofovir disoproxil Fumarate, in mg per tablet.

RRF = Relative response factor.

$$\text{Unknown impurity (\% w/w)} = \frac{A_{TU}}{A_{ST}} \times \frac{W_{ST}}{100} \times \frac{5}{100} \times \frac{3}{50} \times \frac{100}{W_T} \times \frac{P_T}{100} \times \frac{A_{WT}}{LC_T} \times 100$$

Where,

A_{TU} = Peak area of Unknown impurity from test preparation.

A_{ST} = Average peak area of Tenofovir Disoproxil from standard preparation.

W_{ST} = Weight of Tenofovir disoproxil Fumarate standard taken, in mg, for standard preparation.

W_T = Weight of tablets' powder taken, in mg, for test preparation

A_{WT} = Average weight of the tablet, in mg.

P_T = Potency of Tenofovir Disoproxil Fumarate standard in %, on as is basis.

LC_T = Label claim as Tenofovir Disoproxil Fumarate, in mg per tablet.

Total impurities (% w/w) = Sum of all known and unknown impurities.

5.0 ASSAY (By HPLC):

Reagents:

1.	Ammonium acetate	:	AR grade or equivalent.
2.	Acetonitrile	:	HPLC grade or equivalent.
3.	Glacial acetic acid	:	AR grade or equivalent.
4.	Hydrochloric acid	:	AR grade or equivalent
5.	Water	:	Milli Q or HPLC grade or equivalent

Mobile phase A:

Transfer about 0.77 g of Ammonium Acetate, accurately weighed into a suitable beaker. Add 1000 mL of water, dissolve and mix well. Adjust the pH of the solution to 6.0 ± 0.05 with glacial acetic acid. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) and degas.

Note: Mobile phase A is stable for 5 days on bench top.

Mobile phase B: Acetonitrile.

Diluent (0.01N hydrochloric acid):

Dilute 8.5 mL of concentrated Hydrochloric acid to 1000 mL with water in a suitable container and mix well. Further dilute 100 mL of this solution to 1000 mL with water and mix.

Chromatographic condition:

Column	:	Hypersil BDS, C18, 150mm × 4.6mm, 5 µm or equivalent
Flow rate	:	1.0 mL /minute.
Wavelength	:	260 nm

Column temperature : Ambient

Injection Volume : 20 µL

Gradient Programme:

Time (in minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Curve type #
0	90	10	6
6	20	80	6
7	90	10	6
10	90	10	6

As per Waters Empower software

Impurity stock solution:

1. Transfer an accurately weighed amount of about 1 mg of Mono ester impurity of Tenofovir Disoproxil Fumarate into a 10 mL volumetric flask.
2. Add about 8 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix.

System suitability solution:

3. Transfer an accurately weighed amount of about 20 mg of Emtricitabine RS / working standard and 30 mg of Tenofovir Disoproxil Fumarate RS / working standard into a 50 mL volumetric flask. Dissolve in and dilute to volume with diluent.
4. Pipette 5.0 mL of the above solution into a 50 mL volumetric flask and add 1.0 mL of the above impurity stock solution and dilute to volume with diluent and mix well.

Standard preparation:

1. Weigh accurately and transfer about 60 mg of Tenofovir Disoproxil Fumarate RS / working standard and 40 mg of Emtricitabine RS / working standard into a 100 mL volumetric flask.
2. Add about 50 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix well.
3. Pipette 5.0 mL of the above solution into a 50 mL volumetric flask. Dilute to volume with diluent and mix well.

4. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) or 0.45 µm PVDF membrane filter.

Note: The standard preparation is stable for 24 hours on bench top.

Test preparation:

1. Weigh accurately 20 tablets and determine the average weight.
2. Crush the above weighed 20 tablets, into a fine powder in a mortar using pestle.
3. Transfer an accurately weighed amount of the above powder equivalent to about 200 mg of Emtricitabine or 300mg of Tenofovir Disoproxil Fumarate into a 500 mL volumetric flask.
4. Add about 400 mL of diluent, disperse the material completely and sonicate for 30 minutes with intermediate shaking.
5. Allow to cool to room temperature, dilute to volume with diluent and mix well.
6. Centrifuge a portion of the above solution, in a centrifuge tube with cap, at 5000 RPM, for about 10 minutes.
7. Pipette 5.0 mL of the clear centrifugate into a 50 mL volumetric flask and dilute to volume with diluent and mix well.
8. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) or 0.45 µm PVDF membrane filter.

Note: The test preparation is stable for 24 hours on bench top.

Procedure:

Inject diluent as blank (for 2 times), system suitability solution, standard preparation (for 5 times) and test preparation (for 2 times) into the chromatograph. Record the chromatograms and measure the peaks' responses (Refer Typical Chromatograms).

Note:

1. Disregard the peaks due to blank and Fumaric acid (RRT of Fumaric acid with respect to Tenofovir Disoproxil peak is about 0.24) in the test chromatogram.
2. The elution pattern of the peaks are Emtricitabine, Monoester impurity and Tenofovir Disoproxil respectively.

System suitability:

From system suitability solution:

1. Resolution between peaks due to Emtricitabine and Monoester impurity of Tenofovir Disoproxil Fumarate should be not less than 2.0.

From standard preparation:

2. The tailing factor for both Emtricitabine and Tenofovir Disoproxil peaks should be not more than 2.0.
3. The relative standard deviation for replicate injections of both the analyte peaks should be not more than 2.0%.

Calculation:

$$\text{Assay of Emtricitabine (mg per tablet)} = \frac{A_{TE}}{A_{SE}} \times \frac{W_{SE}}{100} \times \frac{5}{50} \times \frac{500}{W_T} \times \frac{50}{5} \times \frac{P_E}{100} \times A_{WT}$$

$$\text{Assay of Emtricitabine (\% of label claim)} = \frac{\text{Reported assay value in mg (Rounded off value)}}{LC_E} \times 100$$

$$\text{Assay of Tenofovir Disoproxil Fumarate (mg per tablet)} = \frac{A_{TT}}{A_{ST}} \times \frac{W_{ST}}{100} \times \frac{5}{50} \times \frac{500}{W_T} \times \frac{50}{5} \times \frac{P_T}{100} \times A_{WT}$$

$$\text{Assay of Tenofovir Disoproxil Fumarate (\% of label claim)} = \frac{\text{Reported assay value in mg (Rounded off value)}}{LC_T} \times 100$$

Where,

A_{TE} = Average peak area of Emtricitabine from test preparation.

A_{SE} = Average peak area of Emtricitabine from standard preparation.

W_{SE} = Weight of Emtricitabine standard taken, in mg, for standard preparation.

LC_E = Label claim, as Emtricitabine, in mg.

W_T = Weight of the tablets' powder taken, in mg, for test preparation

A_{WT} = Average weight of the tablet, in mg

P_E = Potency of Emtricitabine standard in % (on as is basis).

A_{TT} = Average peak area of Tenofovir Disoproxil from test preparation.

A_{ST} = Average peak area of Tenofovir Disoproxil from standard preparation.

W_{ST} = Weight of Tenofovir Disoproxil Fumarate standard taken, in mg, for standard preparation.

LC_T = Label claim, as Tenofovir Disoproxil Fumarate, in mg.

P_T = Potency of Tenofovir Disoproxil Fumarate standard in % (on as is basis).

6.0 WATER (By KF):

Procedure:

Crush not less than 4 tablets to get enough quantity of the tablets' powder for analysis.

Transfer 35 to 40 mL of methanol to the titration vessel, and titrate with the reagent to the electrometric end-point to consume any moisture that may be present (disregard the volume consumed, since it does not enter into the calculations). Quickly add about 500 mg of the tablets' powder and titrate with the reagent to the electrometric end-point. Calculate the water content of the sample by using following formula:

$$\text{Water content (\% w/w)} = \frac{\text{Titre value, in mL} \times \text{KF factor}}{\text{Weight of sample (mg)}} \times 100$$

ADDITIONAL TESTS:

7.0 IDENTIFICATION (BY THIN-LAYER CHROMATOGRAPHY):

Reagents:

Methanol	:	HPLC Grade
Dichloro methane	:	HPLC Grade
n-Butyl Alcohol	:	HPLC Grade

Developing solvent:

Mix n-Butyl Alcohol, Dichloro methane and Methanol in the ratio of 3:6:1v/v/v respectively.

Diluent: Methanol

Standard Solution -1:

Accurately weigh and transfer about 27 mg of Emtricitabine RS / working standard to a 200 mL volumetric flask. Add about 60 mL of diluent. Sonicate to dissolve and make up the volume with diluent and mix.

Standard Solution -2:

1. Accurately weigh and transfer about 20 mg of Tenofovir Disoproxil Fumarate RS / working standard to a 100 mL volumetric flask.
2. Add about 60 mL of diluent. Sonicate to dissolve and make up the volume with diluent and mix.

Test Solution:

1. Accurately weigh and transfer the sample equivalent to 50 mg of Tenofovir Disoproxil Fumarate into a 250 mL volumetric flask, add about 150 mL of diluent, sonicate for 20 minutes with intermediate shaking in cold water.
2. Cool the solution at room temperature make volume up to the mark with diluent and mix.
3. Filter the solution through 0.45 µm nylon filter.

Procedure:

1. Apply separately 10µL each of standard solution-1, standard solution-2 and sample solution to the thin-layer chromatographic plate coated with 0.25 mm layer chromatographic silica gel.
2. Dry the spots in a current of warm air and develop the plate in a paper-lined chromatographic chamber equilibrated with Developing solvent for about 1 hour prior to use.
3. Allow the chromatogram to develop until the solvent front has moved about 15 cm from the point of spotting.
4. Remove the plate from the chamber, mark the solvent front, and dry the point in a current of warm air. Make an observation within 10-20 minutes at 254 nm in UV chamber.
5. The principle spots obtained from the sample solution for Emtricitabine should correspond in appearance to that obtained from standard solution-1 and for Tenofovir should correspond in appearance to that obtained from standard solution-2.

8.0 COLOR IDENTIFICATIONS:

A. For FD & C Blue # 2 Aluminum Lake:

1. Take not less than 25 tablets for performing the test.
2. Scratch the coating part of the individual tablets by using cutter in a glass beaker. Segregate the coating material and tablet powder and use coating material for sample preparation.
3. Transfer coating material in to a suitable beaker containing 50 mL of water. Shake gently to dissolve only the coating material.
4. To the same beaker, add about 750 mg of sodium hydrogen tartrate. Warm gently to dissolve the contents.

Note: Do not allow the solution to boil.

5. Centrifuge this solution in a centrifuge tube for about 10 minutes at 5000 rpm.
6. Filter the supernatant solution through 0.45 µm PVDF or Nylon 66 membrane filter (Millipore make). Use the filtrate as test solution.
7. Using a suitable UV-VIS spectrophotometer, scan the above test solution in the range, 700-500 nm against water as blank. Record the wavelength at which maximum absorbance is obtained.

B. For Titanium Dioxide:

Procedure:

1. Take not less than 10 tablets and grind to a fine powder in a mortar with pestle.
2. To about 4.0 g of the tablets' powder, add 3 mL of concentrated sulfuric acid, and heat gently.
3. Heat till the white fumes no longer appear. Ignite at 600 ± 50°C in a suitable crucible till the black mass is removed completely.
4. To the residue add 5 mL of sulphuric acid and digest for 15 minutes. Cool, and dilute to 50 mL with water.
5. Filter, and to 5 mL of the clear filtrate add a few drops of hydrogen peroxide (30%): a yellow-red to orange-red color develops immediately.

9.0 UNIFORMITY OF DOSAGE UNITS: (By Content Uniformity)

Procedure:

Proceed as directed in the General test procedure, "Uniformity of Dosage Units – Ph.Eur" GTP No.: GTP017#.

Reagents:

1.	Ammonium acetate	:	AR grade or equivalent.
2.	Acetonitrile	:	HPLC grade or equivalent.
3.	Glacial acetic acid	:	AR grade or equivalent.
4.	Water	:	Milli Q or HPLC grade or equivalent

Mobile phase A:

Transfer about 0.77 g of Ammonium Acetate, accurately weighed into a suitable beaker. Add 1000 mL of water, dissolve and mix well. Adjust the pH of the solution to 6.0 ± 0.05 with glacial acetic acid. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) and degas.

Note: Mobile phase A is stable for 5 days on bench top.

Mobile phase B: Acetonitrile

Chromatographic condition:

Column	:	Hypersil BDS, C18, 150mm × 4.6mm, 5 µm or equivalent
Flow rate	:	1.0 mL /minute.
Wavelength	:	260 nm
Column temperature	:	Ambient
Injection Volume	:	20 µL

Gradient Programme:

Time (in minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Curve type #
0	90	10	6
6	20	80	6
7	90	10	6
10	90	10	6

As per Waters Empower software

Diluent (0.01N hydrochloric acid):

Dilute 8.5 mL of concentrated Hydrochloric acid to 1000 mL with water in a suitable container. Further dilute 100 mL of the above solution to 1000 mL with water, mix well and degas.

Impurity stock solution:

1. Transfer an accurately weighed amount of about 1 mg of Mono ester impurity of Tenofovir Disoproxil Fumarate into a 10 mL volumetric flask.
2. Add about 8 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix.

System suitability solution:

1. Transfer an accurately weighed amount of about 20 mg of Emtricitabine RS / working standard and 30 mg of Tenofovir Disoproxil Fumarate RS / working standard into a 50 mL volumetric flask. Dissolve in and dilute to volume with diluent.

2. Pipette 5.0 mL of the above solution into a 50 mL volumetric flask and add 1.0 mL of the above impurity stock solution and dilute to volume with diluent and mix well.

Standard preparation:

1. Transfer about 60 mg of Tenofovir Disoproxil Fumarate RS / working standard and 40 mg of Emtricitabine RS / working standard accurately weighed, into a 100 mL volumetric flask.
2. Add about 50 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix well.
3. Pipette 5.0 mL of the above solution into a 50 mL volumetric flask. Dilute to volume with diluent and mix well.
4. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) or 0.45 µm PVDF membrane filter.

Note: Standard preparation is stable for 24 hours on bench top.

Test preparation:

1. Transfer one tablet into a 500 mL volumetric flask.
2. Add about 400 mL of diluent, disperse the material completely and sonicate for 30 minutes with intermediate shaking.
3. Allow to cool to room temperature, dilute to volume with diluent and mix well.
4. Centrifuge a portion of the above solution, in a centrifuge tube with cap, at 5000 RPM, for about 10 minutes.
5. Pipette 5.0 mL of the clear centrifugate into a 50 mL volumetric flask and dilute to volume with diluent and mix well.
6. Filter the solution through 0.45 µm nylon 66 membrane filter (Millipore make) or 0.45 µm PVDF membrane filter.

Note: Test preparation is stable for 24 hours on bench top.

Procedure:

Inject diluent as blank (for 2 times), system suitability solution, standard preparation (for 5 times) and test preparation into the chromatograph. Record the chromatograms and measure the peaks' responses (Refer Typical Chromatograms).

Note:

1. Disregard the peaks due to blank and Fumaric acid (RRT of Fumaric acid with respect to Tenofovir Disoproxil peak is about 0.24) in the test chromatogram.
2. The elution pattern of the peaks are Emtricitabine, Monoester impurity and Tenofovir Disoproxil respectively.
3. RRT of Monoester impurity is about 1.20 with respect to Emtricitabine peak.

System suitability:

From system suitability solution:

1. Resolution between the peaks due to Emtricitabine and Monoester impurity of Tenofovir Disoproxil Fumarate should be not less than 2.0.

From standard preparation:

2. The tailing factor for both Emtricitabine and Tenofovir Disoproxil peaks should be not more than 2.0.
3. The relative standard deviation for both the analyte peaks from replicate injections should be not more than 2.0%.

Calculation:

$$\text{Content Uniformity of Emtricitabine (\% w/w of Label Claim)} = \frac{A_{TE}}{A_{SE}} \times \frac{W_{SE}}{100} \times \frac{5}{50} \times \frac{500}{LC_E} \times \frac{50}{5} \times \frac{P_E}{100} \times 100$$

$$\text{Content Uniformity of Tenofovir Disoproxil Fumarate (\% w/w of Label Claim)} = \frac{A_{TT}}{A_{ST}} \times \frac{W_{ST}}{100} \times \frac{5}{50} \times \frac{500}{LC_T} \times \frac{50}{5} \times \frac{P_T}{100} \times 100$$

Where,

A_{TE} = Peak area of Emtricitabine from test preparation.

A_{SE} = Average peak area of Emtricitabine from standard preparation.

W_{SE} = Weight of Emtricitabine standard taken, in mg, for standard preparation.

LC_E = Label claim, as Emtricitabine, in mg.

P_E = Potency of Emtricitabine standard in % (on as is basis).

A_{TT} = Peak area of Tenofovir Disoproxil from test preparation.

A_{ST} = Average peak area of Tenofovir Disoproxil from standard preparation.

W_{ST} = Weight of Tenofovir Disoproxil Fumarate standard taken, in mg, for standard preparation.

LC_T = Label claim, as Tenofovir Disoproxil Fumarate, in mg.

P_T = Potency of Tenofovir Disoproxil Fumarate standard in % (on as is basis).

10.0 MICROBIOLOGICAL TEST:

Procedure:

Proceed as directed under the General Test Procedure "Microbiological testing of Non Sterile Products (Microbial Enumeration tests and Tests for Specified Microorganisms)" GTP No.: GTP004#.

11.0 AVERAGE WEIGHT:

Procedure:

Perform the test on 20 tablets. Measure the average weight by using following formula.

$$\text{Average weight (mg)} = \frac{\text{Weight of 20 tablets (g)}}{20} \times 1000$$

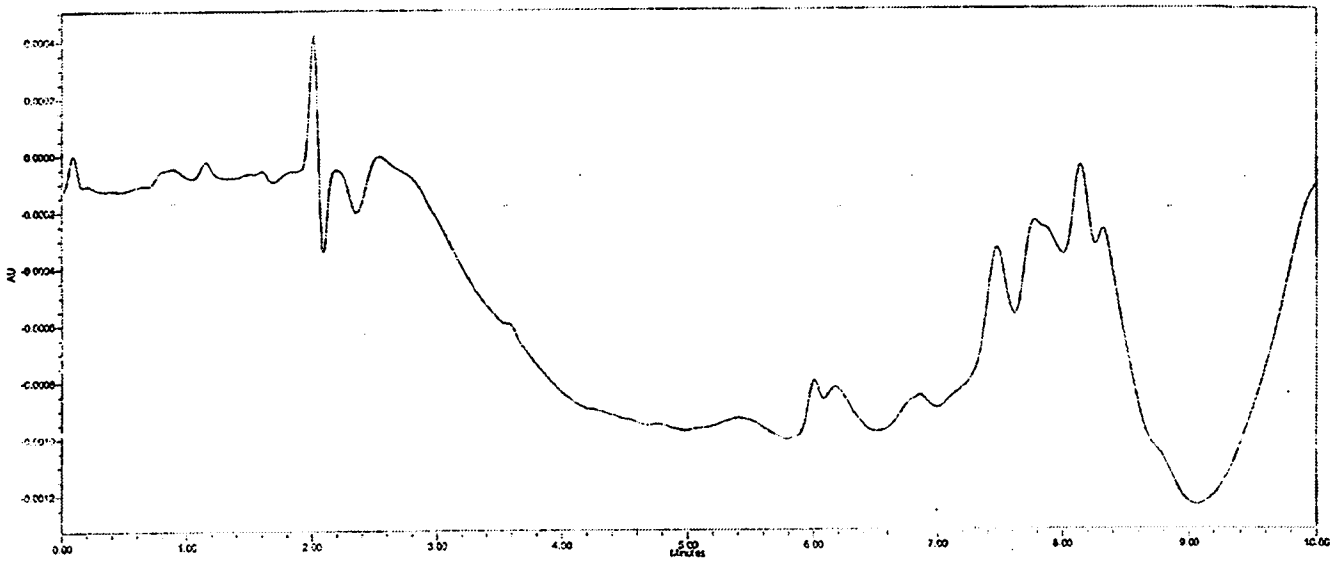
Note: Use the value obtained wherever applicable.

Reference Document(s): FPPETF008R-08

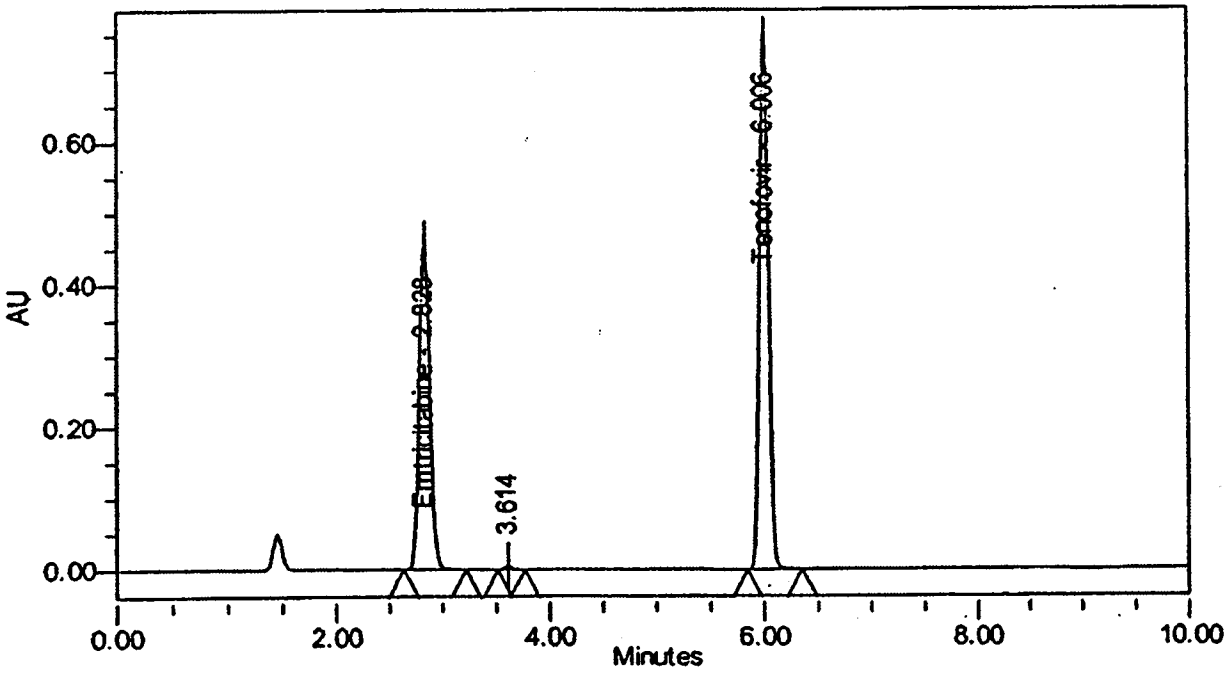
Follow the current version of the GTP.

Typical Chromatograms for Dissolution (By HPLC):

Blank Chromatogram:

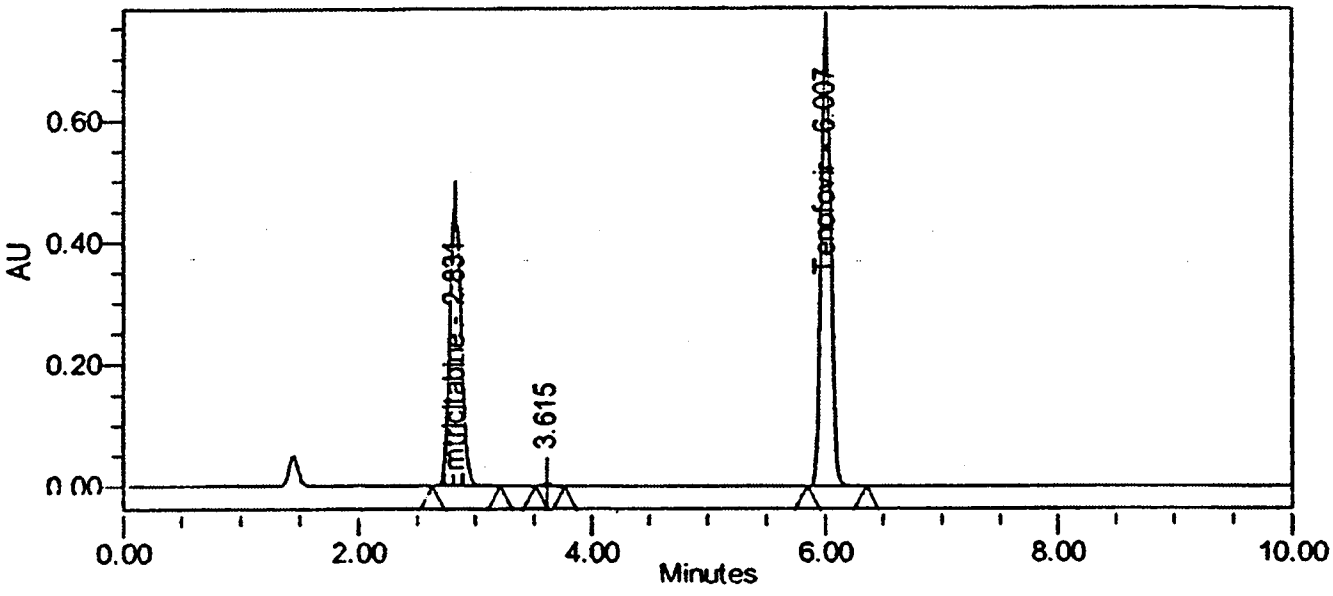


Standard preparation Chromatogram:



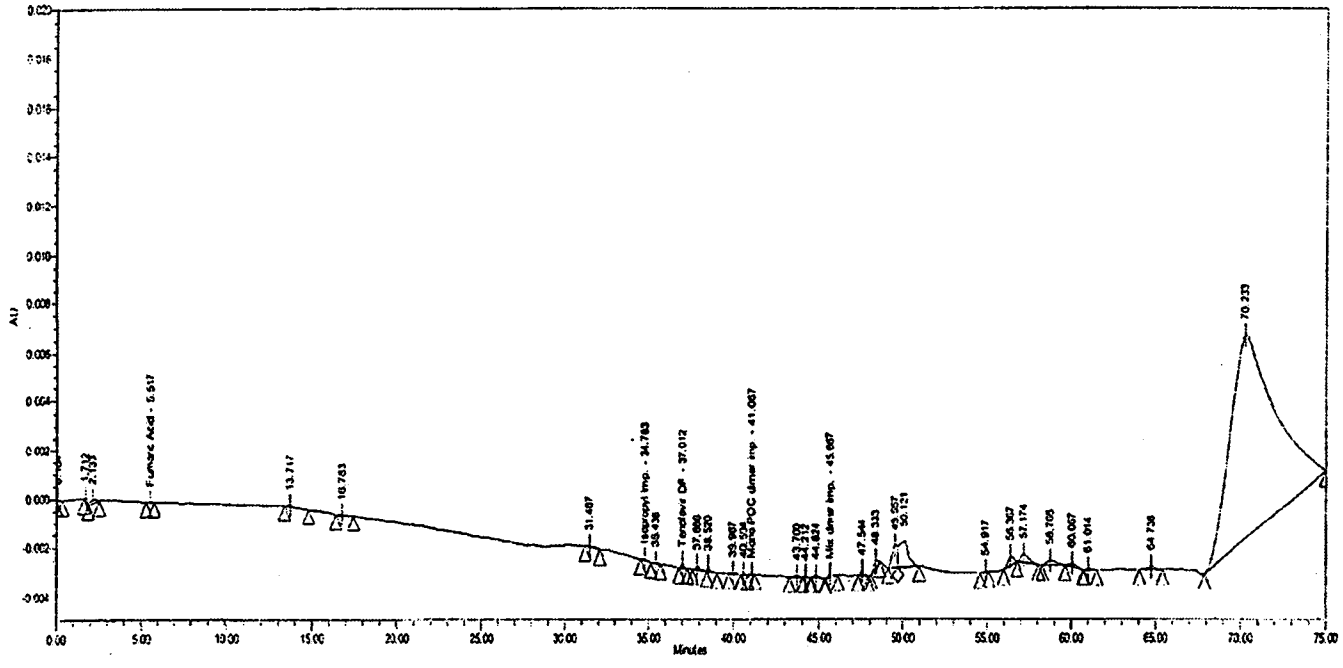
	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

Test preparation Chromatogram:



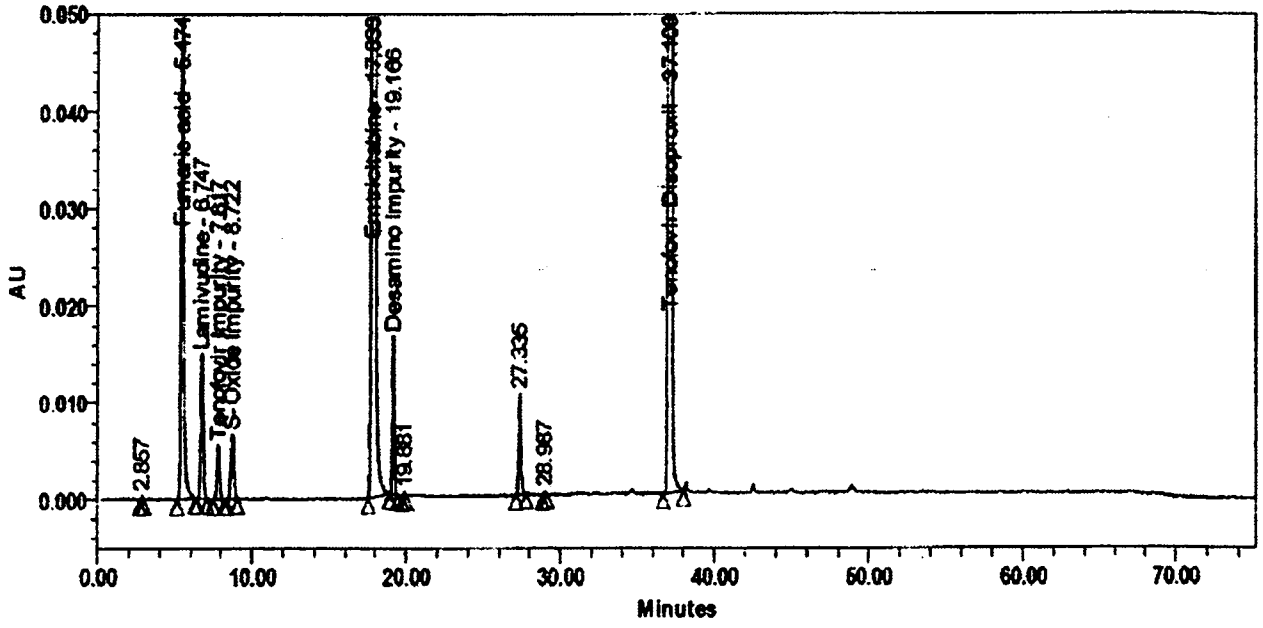
Typical Chromatograms for Related Substances (By HPLC):

Blank Chromatogram:

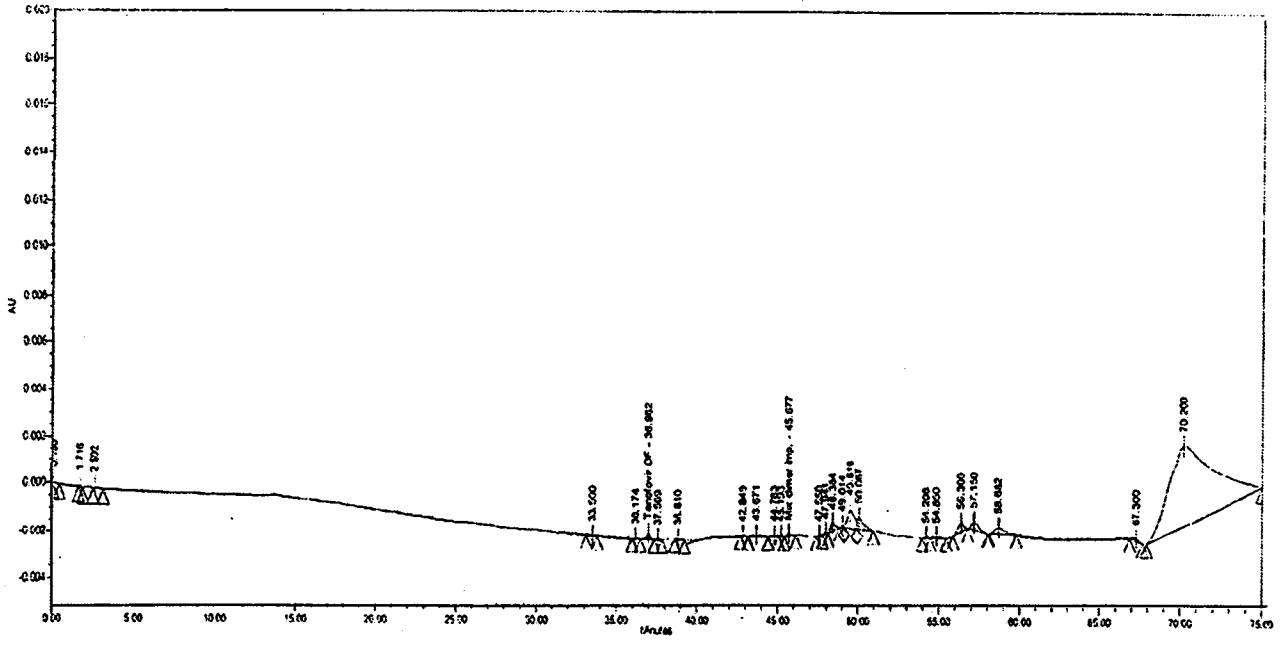


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

System suitability solution Chromatogram:

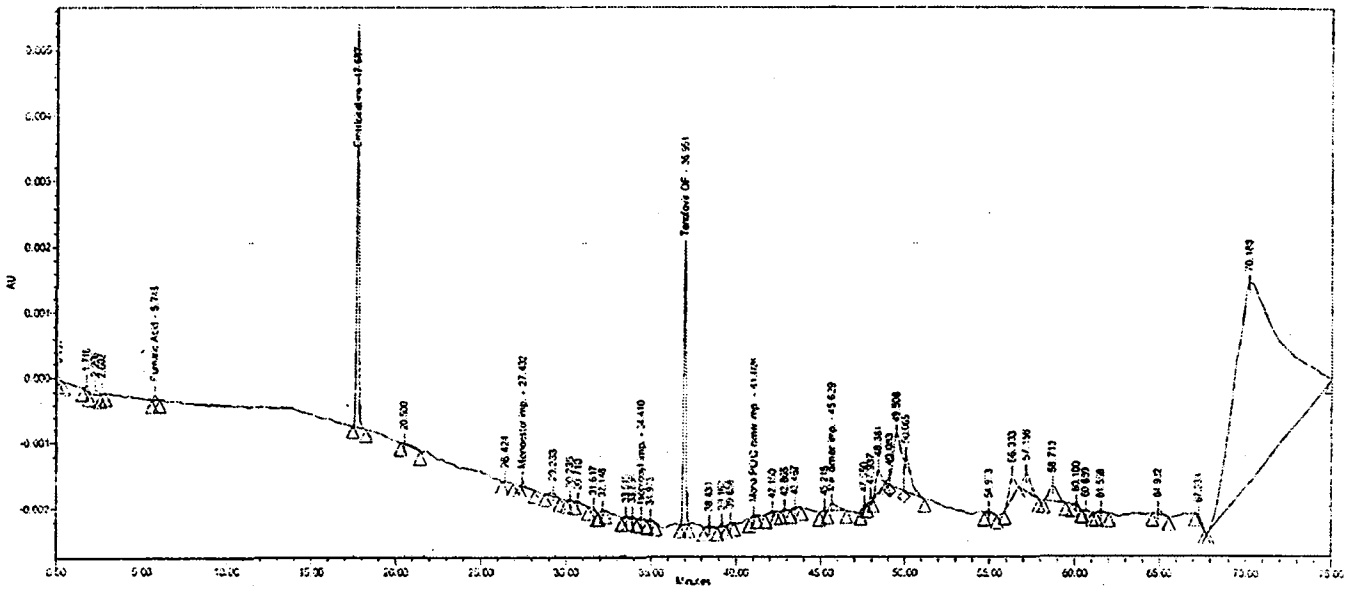


Placebo preparation Chromatogram:

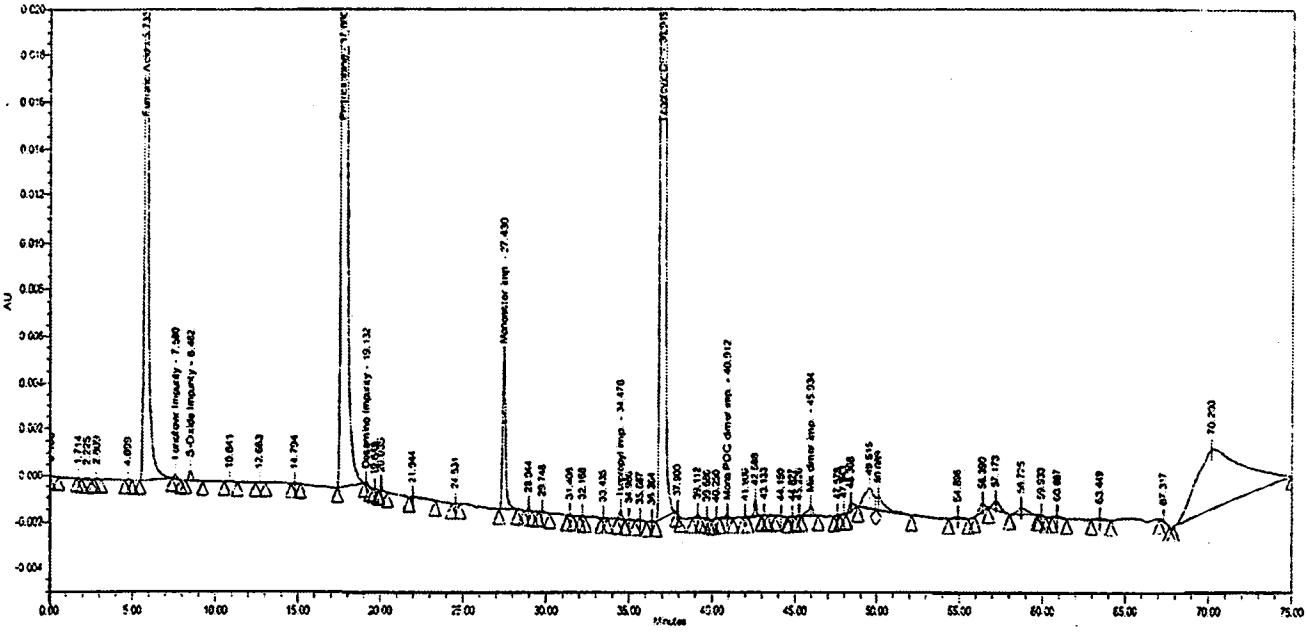


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	KMP 22/10/20	24/10/20

Standard solution Chromatogram:



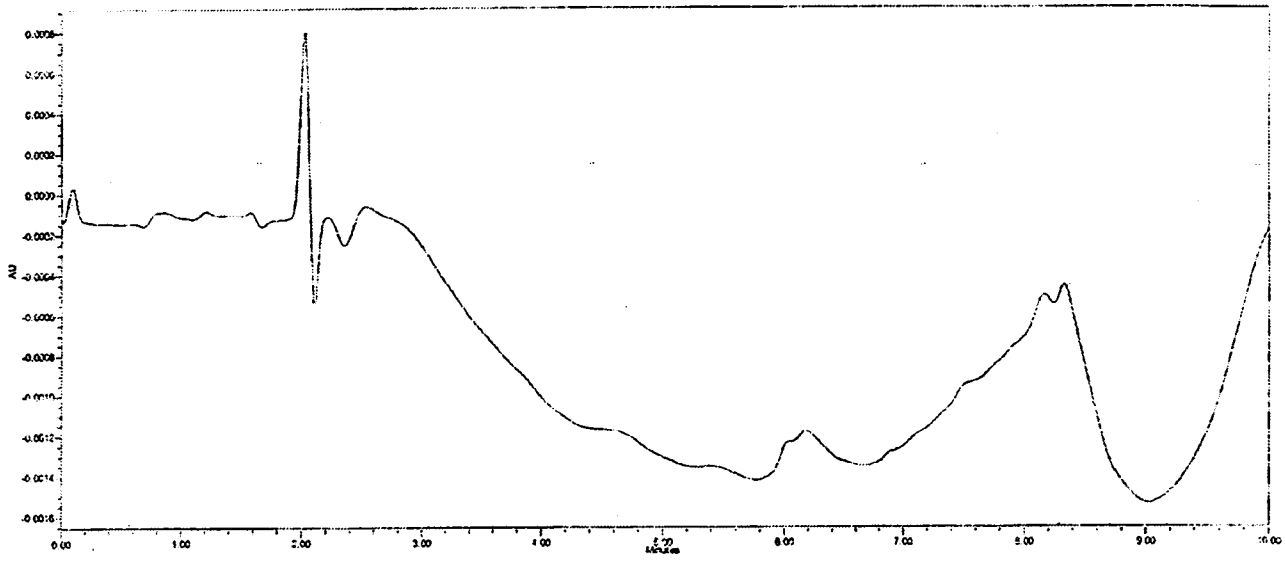
Test preparation Chromatogram:



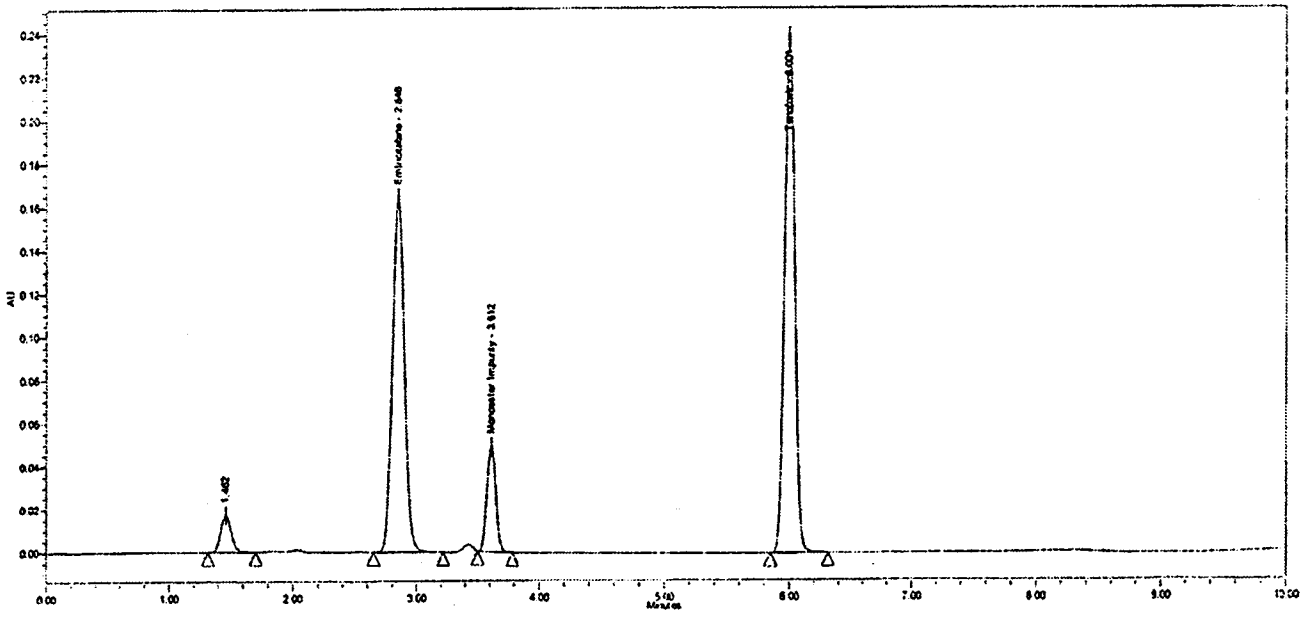
	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

Typical Chromatograms for ASSAY (By HPLC) & Uniformity Of Dosage Units (By Content Uniformity):

Blank Chromatogram:

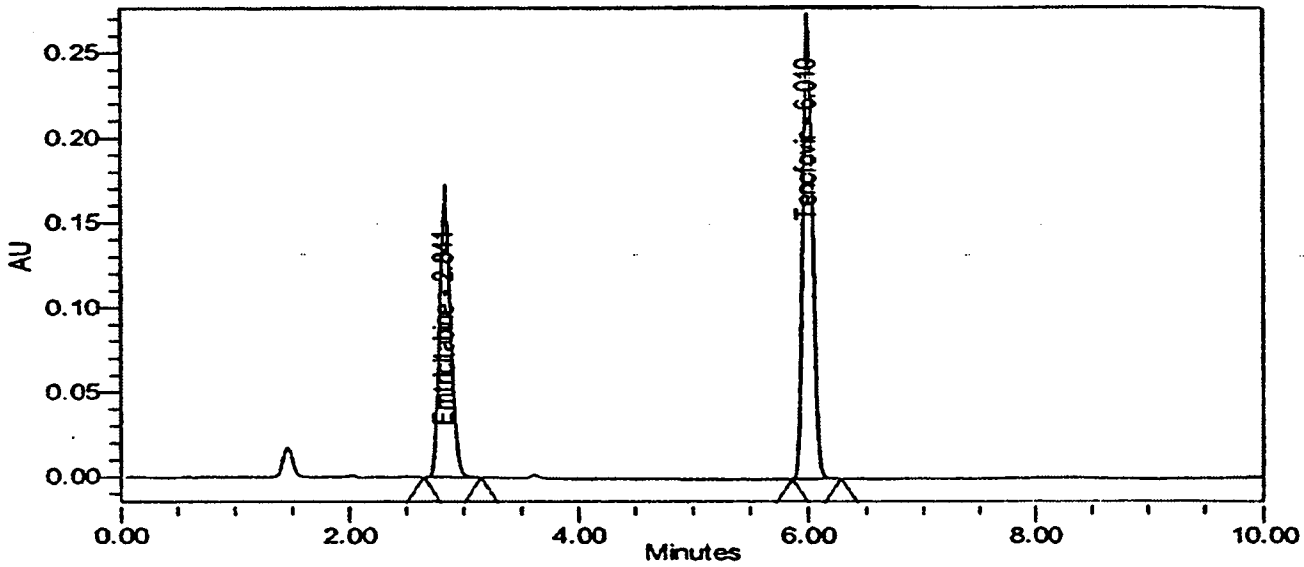


System suitability solution Chromatogram:

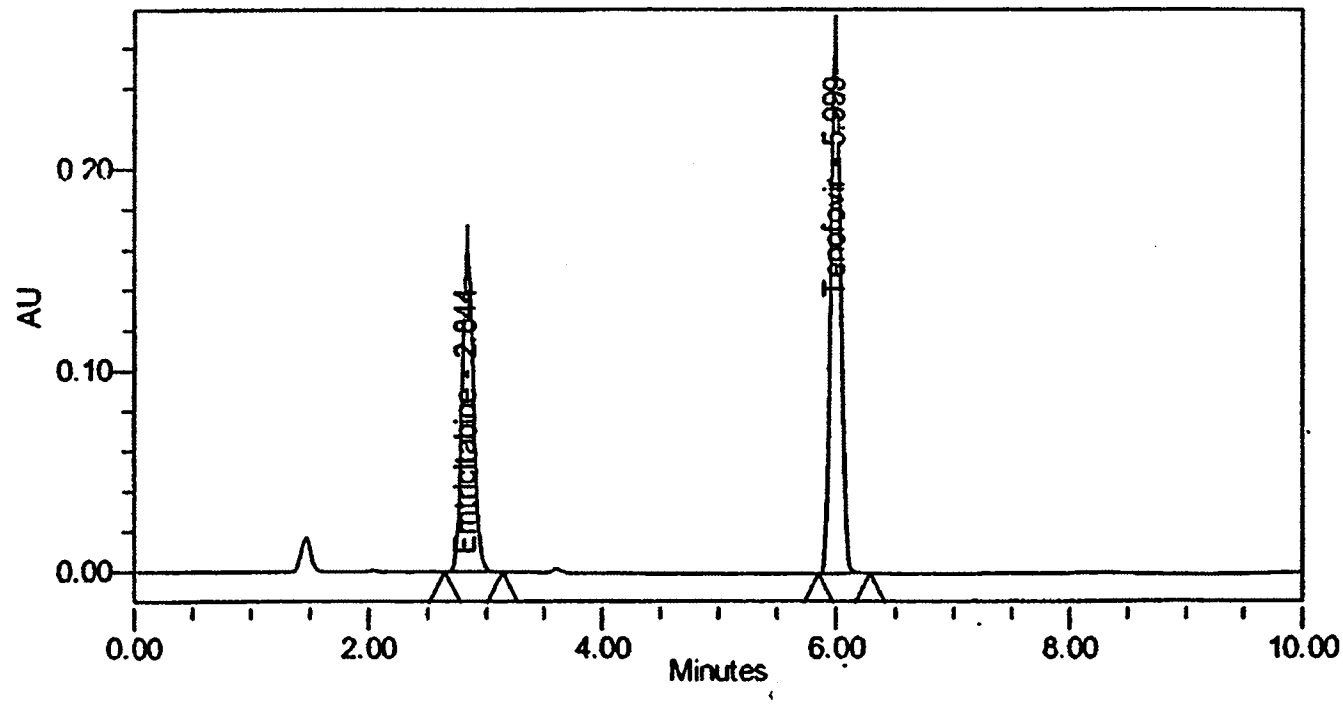


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

Standard preparation Chromatogram:



Test preparation Chromatogram:



	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

Mylan	FINISHED PRODUCT (REGULATORY AND SHELF LIFE) STANDARD TEST PROCEDURE	Mylan, Nashik
Name of the Product: Emtricitabine and Tenofovir Disoproxil Fumarate Tablets (200 mg/300 mg)		**STP No.:** FPPETF506R-01

Change History:

Effective Date	STP No.	Supersedes	Change Details
02 OCT 2017	FPPETF506R-00	Nil	New STP (Reference Change Control PR No. 1280662)
23 SEP 2021	FPPETF506R-01	FPPETF506R-00	As per Change Proposal PR No. 2275670 1. Format has been revised as per revised SOP. 2. Under the test procedure of 'Related Substance (By HPLC)' following changes have been made; 2.1 Needle wash solution and its Note has been incorporated. 2.2 Under 'Impurity stock solution, Lamivudine impurity has been newly incorporated and alternate name of 'S-Oxide impurity of Emtricitabine' and 'Des amino impurity of Emtricitabine' has been mentioned. 2.3 Under 'Note' section, the note, 'In system suitability solution chromatogram integrate peak due to Fumaric acid, Lamivudine, Tenofovir impurity, S-oxide impurity, Des amino impurity, Emtricitabine and Tenofovir Disoproxil' has been incorporated. 2.4 Under RRT, RRF table of Emtricitabine peak, 'Fluro cytosine impurity (Flucytosine)', 'Emtricitabine acid impurity', 'Salicylic acid impurity' and 'Emtricitabine menthyl ester impurity' has been newly incorporated and RRF value of S-oxide impurity has been changed from '0.56' to '0.97' and alternate name mentioned accordingly. 2.5 Under RRT, RRF table of Tenofovir Disoproxil peak, the RRF values of Mono POC dimer impurity and Mix dimer impurity has been changed from '1.33' to '1.89' and '0.66' to '1.55'. 2.6 Under System suitability, 'From system suitability solution', sentence 'The resolution

	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	24/10/20

Effective Date	STP No.	Supersedes	Change Details
			between the peak of Fumaric acid and Lamivudine impurity should be not less than 2.5' has been incorporated. 2.7 Typical chromatogram of System suitability solution has been changed. 3. Under the test procedure of 'Color Identifications for FD & C Blue # 2 Aluminum Lake' following changes have been made: 3.1 'Take about 25 tablets for performing the test' has been revised to 'Take not less than 25 tablets for performing the test'. 3.2 'Transfer 5 tablets in to a suitable beaker containing 50 mL of water. Shake gently to dissolve only the coating material' has been revised to 'Scratch the coating part of the individual tablets by using cutter in a glass beaker. Segregate the coating material and tablet powder and use coating material for sample preparation'. 3.3 'Remove the tablet from water once the coating material gets dissolved. Repeat the above procedure for dissolving the coating material with the remaining tablets' has been revised to 'Transfer coating material in to a suitable beaker containing 50 mL of water. Shake gently to dissolve only the coating material'.