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RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IMPURITIES FROM EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE TABLET

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ABSTRACT: Stability indicating RP-HPLC gradient method developed for simultaneous determination of impurities and degradation products from Emtricitabine and Tenofovir Disoproxil Fumarate in pharmaceutical tablet dosage form. The chromatographic separation was achieved by using column ACE C18 (250 x 4.6, 5μ). The mobile phase-A consists of 0.01M potassium dihydrogen phosphate buffer with pH 4.0 adjusted using diluted ortho-phosphoric acid and mobile phase-B as methanol. The flow rate was 1mL min-1 throughout the gradient program with detection wavelength of 270 nm for both components with its related impurities. The column temperature was 30°C and injection volume of 20μl. A well separation of degradation products from main peak and respective known impurities was found with resolution between adjacent peaks greater than 2.0. The method found linear from LOQ to 0.4% level for all impurities with respect to concentration for Emtricitabine(0.7 mg/mL) and Tenofovir Disoproxil Fumarate (1.06 mg/mL) The developed method was validated for specificity, linearity, precision, LOD, LOQ, accuracy and robustness as per ICH guideline Q2A (R1). The results were indicating that the method was selective and stability indicating for determination of impurities from Emtricitabine and Tenofovir Disoproxil Fumarate tablet dosage form.
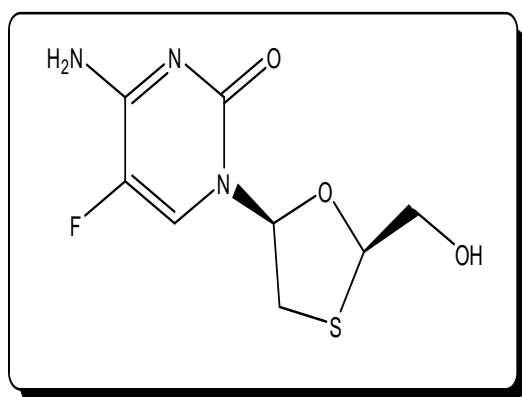
INTRODUCTION: Chemically Emtricitabine (EMT) is 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1, 3 - oxathiolan - 5-yl) cytosine [1] (**Fig.1**). Emtricitabine is an antiretroviral agentbelonging to the class of nucleotide reverse transcriptase inhibitors and used for the prevention of perinatal HIV-1and also active against Hepatitis B virus. Emtricitabine is the (-) enantiomer of a thioanalog of cytidine, which differs from other cytidine analogs it has a fluorine in the 5th position. Chemically Tenofovir Disoproxil Fumarate (TENO) is 9-[(R)-2-[[bis[(Isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate (1:1) (**Fig.1**).

It is an antiretroviral agent belonging to the class of nucleotide reverse transcriptase inhibitors [1]. Tenofovir Disoproxil Fumarate is converted intercellularly to the diphosphate.
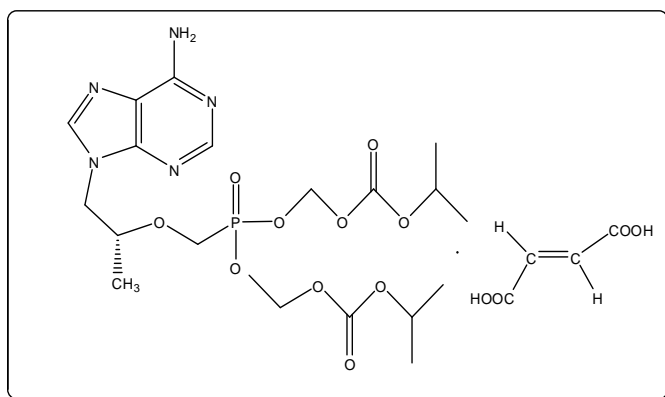
This diphosphate halts the DNA synthesis of HIV through competitive inhibition of reverse transcriptase and incorporation into viral DNA. The literature survey reveals that various analytical methods have been reported for estimation of Emtricitabine and Tenofovir Disoproxil Fumarate alone or different combination by HPLC [2-5], HPTLC [6] and UV [7-8].

There was no any method reported for estimation of impurities and degradation products from combination of Emtricitabine and Tenofovir Disoproxil Fumarate Tablet dosage form. Hence present work describes the RP-HPLC method for simultaneous estimation of related substances and degradation products from tablet dosage form.
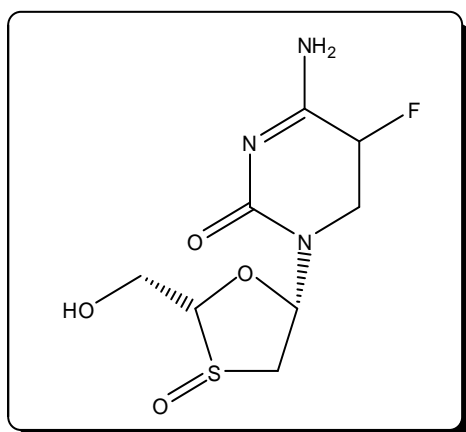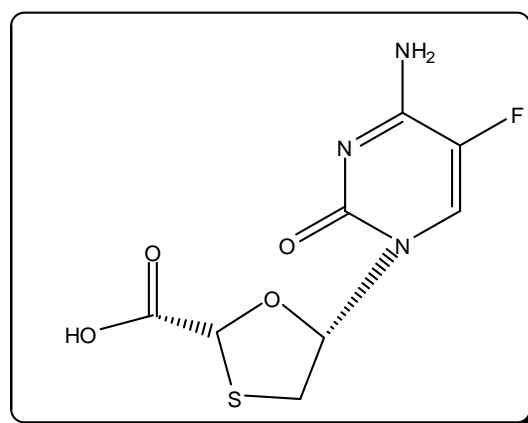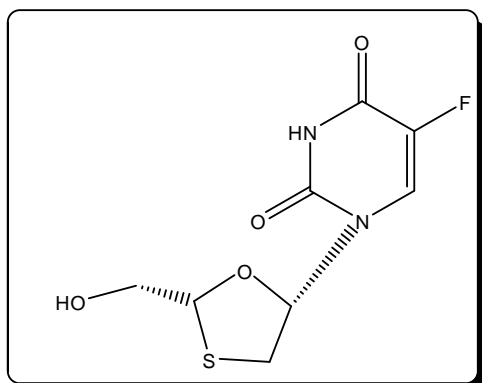
EMTRICITABINE

TENOFOVIR DISOPROXIL FUMARATE

EMT_S-OXIDE

EMT_CARBOXYLIC ACID

EMT_DES AMINO
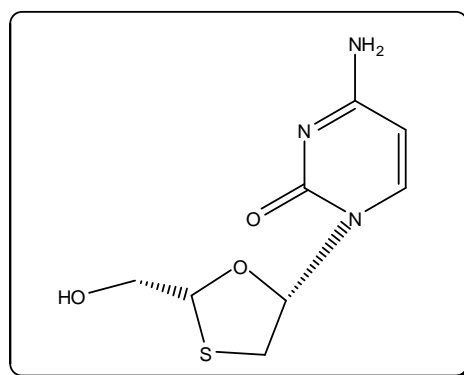
EMT_LAMIVUDINE
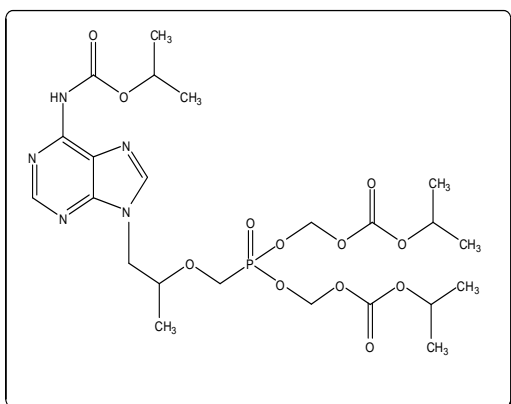
TENO_ CARBONYL

TENO_MIX DIMER
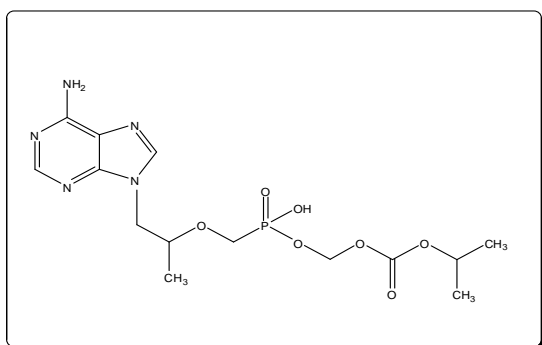
TENO_MONO ESTER
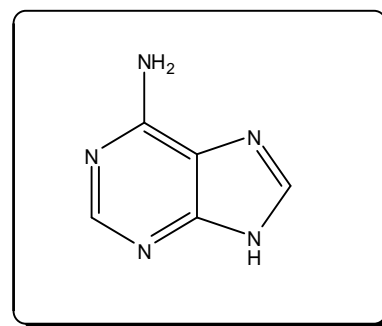
TENO_ADENINE
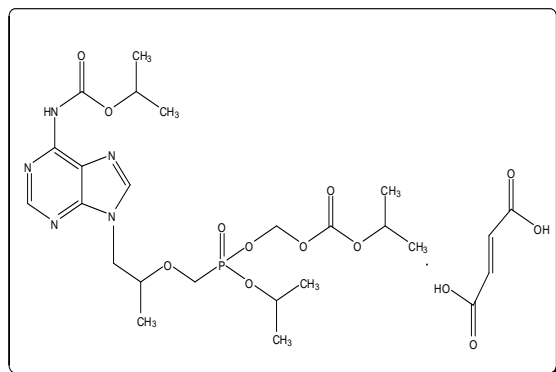
TENO_ISOPROPYL IMPURITY
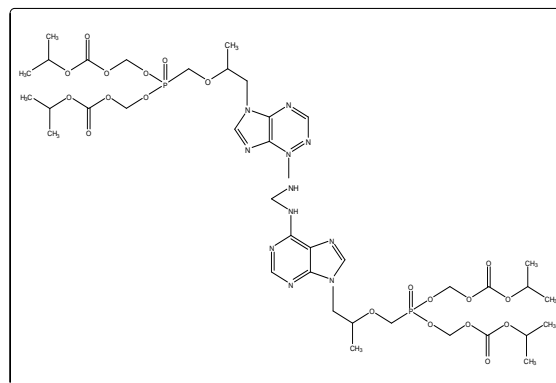
TENO_MONO POC
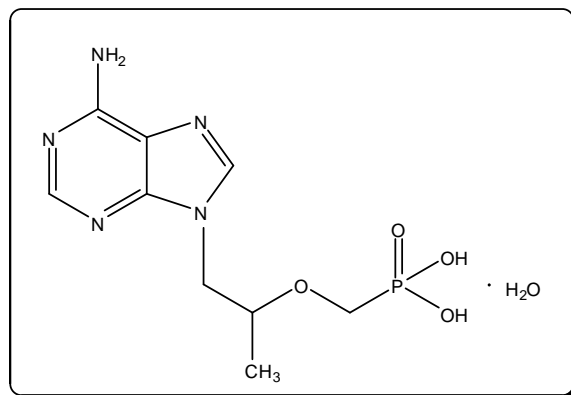
TENO_TENOFOVIR IMPURITY

FIG.1: STRUCTURES OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE AND ITS RELATED SUBSTANCES.

1. MATERIALS AND METHODS:
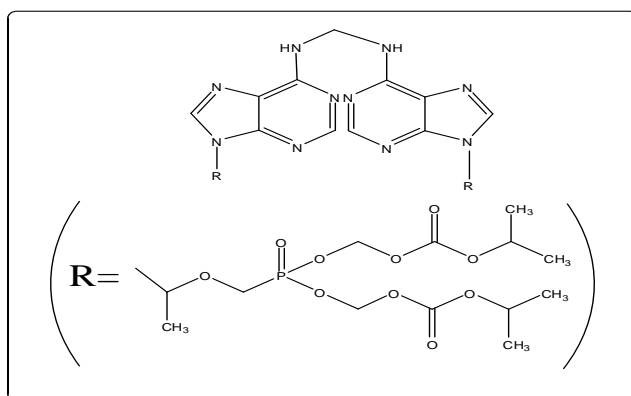## 1.1 Chemicals and reagents:
Emtricitabine and Tenofovir Disoproxil Fumarate and its related impurities obtained from Veeprho laboratories Ltd. Marketed formulation of Emtricitabine and Tenofovir Disoproxil Fumarate purchased from local market manufactured by Emcure pharmaceuticals ltd with brand name Tavin-EM. Analytical grade potassium dihydrogen phosphateobtained from Merck (Mumbai India), HPLC grade methanol obtained from Merck (Darmstadt, Germany), water from milli-Q purification system (Millipore, Bedford, USA) and

GR grade ortho-phosphoric acid obtained from (Merck, Mumbai).

1.2 Equipment and chromatographic conditions:
Waters HPLC system with photo diode array detector was used for method development and forced degradation studies. The HPLC system consists of 2695 separation module and 2998 photo diode array detector. The output signal was monitored and processed using Empower 2 software. The chromatographic separation was achieved by using column ACE C18 (250 mm×4.6 mm, 5.0μ).. The buffer used was 0.01M potassium dihydrogen phosphate buffer adjusted pH 4.0 using

diluted ortho phosphoric acid as mobile phase-A and methanol as mobile phase-B. The flow rate was 1.0 mL/ min throughout the gradient program and the detection wavelength was 270 nm for both components. The column temperature was maintained at 30°C and the injection volume was 20 µL. A mixture of water and methanol in the proportion of 90:10 (v/v) respectively used as a diluent. The gradient program was time(min)/ % mobile phase-B: 0/3, 5/3, 10/12, 15/20, 18/45, 28/60, 35/75, 38/75, 40/3 and 45/3.

1.3 Preparation of stock solutions:
Stock solution of Emtricitabine (0.7 mg/mL) and Tenofovir Disoproxil Fumarate (1.06 mg/mL) was prepared by dissolving appropriate amount of drug into diluent. A stock solution of impurities (0.1 mg/mL) was prepared individually in diluent. Working solutions were prepared from stock solutions respectively.

1.4 Preparation of sample solutions:
Ten tablets were powdered with mortar pastel and transferred powder equivalent to 70 mg of Emtricitabine into 100 mL volumetric flask added about 70 mL diluent and sonicated for 10 min makeup to volume with diluent and mix well kept solution on bench for 2 min and filtered above solution through 0.45µm Nylon syringe filter.

2. RESULTS AND DISCUSSION:
2.1 Method development and optimization:
The objective of present research work was to develop and validate reverse phase liquid chromatographic method to separate all impurities from each other and from Emtricitabine and

Tenofovir Disoproxil Fumarate. In order to get rapid separation with optimum resolution of impurities gradient method was adopted. During forced degradation study found that the no any unknown impurity co-eluting with known impurities and there are only two major degradents for Emtricitabine (S-Oxide impurity) and For Tenofovir Disoproxil Fumarate (Mono ester impurity).The chromatographic separation with resolution between adjacent impurities greater than 2.0 was achieved with gradient program as time (min) / % mobile phase-B: 0/3, 5/3, 10/12, 15/20, 18/45, 28/60, 35/75, 38/75, 40/3 and 45/3.The major degradents formed were conformed for UV spectra for respective degradent.

2.2 Method validation:
Method validation is nothing but the documentary evidence to prove that the developed method is reliable for normal usage and its intended application. The developed method was validated for specificity, linearity, precision, LOD, LOQ, accuracy and robustness as per ICH guideline Q2A (R1).

2.2.1 System suitability:
To ensure that the system was working correctly during the analysis the resolution, tailing factor, theoretical plates and %RSD were checked for evaluation of System suitability. The parameters such as tailing factor should be not more than 2.0, theoretical plate should be not less than 10,000 and %RSD for replicate injections of standard solution should not more than 5.0 were monitored. The results were summarized in **(Table 1)** as below,

TABLE 1: CHROMATOGRAPHIC DATA

Name	RRT	Resolution[c]	Tailing	RRF[d]	Plates
EMT_Carboxylic acid	0.430[a]	NA	1.01	0.86[d]	7867
TENO_Adenine	0.268[b]	2.65	1.41	2.85[e]	4030
EMT_S-oxide	0.623[a]	5.12	0.94	1.12[d]	8153
EMT_Lamivudine	0.714[a]	5.85	1.00	1.50[d]	7735
TENO_Teno Impurity	0.451[b]	9.78	1.04	2.70[e]	63551
Emtricitabine	1.000	14.15	1.14	1.00	116522
EMT_Des amino	1.057[a]	4.86	1.04	2.60[d]	135924
TENO_Monoester	0.717[b]	28.18	1.30	2.95[e]	305912
TENO_Mono POC	0.764[b]	10.12	1.03	1.75[e]	267505
TENO_Isopropyl	0.836[b]	12.55	1.03	1.80[e]	285525
Tenofovir Disoproxil	1.000	23.73	1.09	1.00	681738
TENO_Mix dimer	1.067[b]	9.05	1.18	2.10[e]	1086533
TENO_Carbonyl	1.119[b]	7.44	0.96	0.95[e]	1019176

[a] RRT were calculated against retention time of Emtricitabine
[b] RRT were calculated against retention time of Tenofovir Disoproxil
[c] Resolution between adjacent peaks
[d] RRF were calculated against response of Emtricitabine
[e] RRF were calculated against response of Tenofovir Disoproxil

2.2.2 Specificity and forced degradation:

The stress conditions used for the degradation study include acid hydrolysis (0.1 M HCl at bench top for 10 min), base hydrolysis (0.1 M NaoH at bench top for 10 min), peroxide (10% H_2O_2 at bench top for 5 min), water hydrolysis (70°C for 30 min) and heat (70°C for 12 Hr). The peak purity data found within acceptance limit and all peaks found homogeneous. The chromatograms for forced degradation study summarized in **(Fig.2).**

a) Typical chromatogram of Impurity spike in sample:
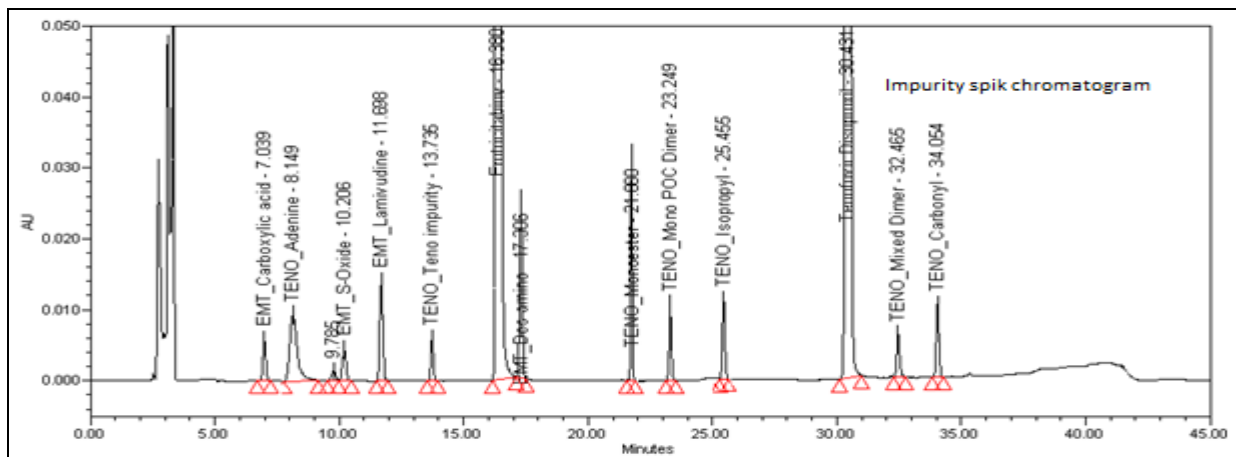
b) Typical chromatogram of peroxide degradation:
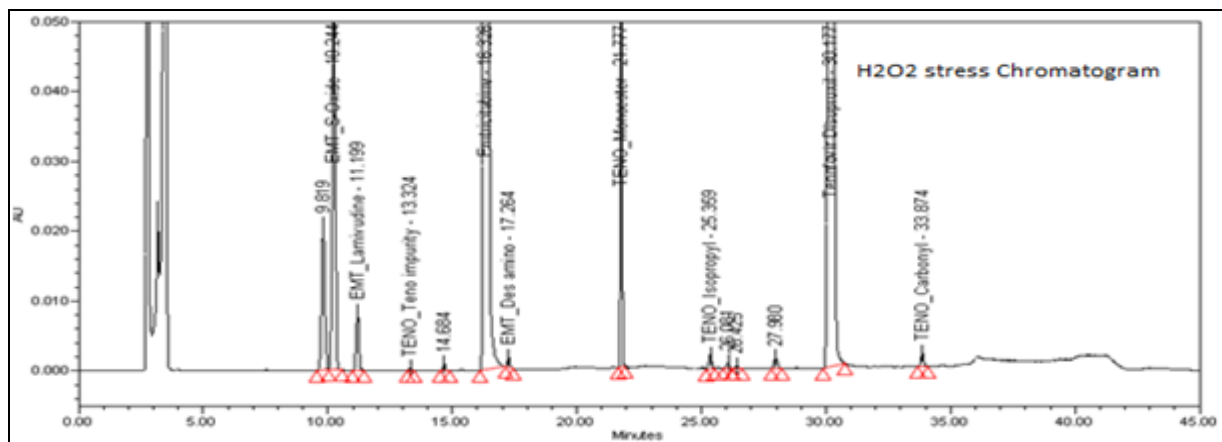
c) Typical chromatogram of Acid degradation:

d) Typical chromatogram of Water degradation:

FIG.2 (a-d): TYPICAL CHROMATOGRAMS OF IMPURITIES SPIK WITH SAMPLE, AND STRESS SAMPLE.

2.2.3 Precision:

The precision of the method was verified by injecting six individual preparations of impurity spike at 0.2% level with respect to target concentration of Emtricitabine (0.7 mg/mL) and Tenofovir Disoproxil Fumarate (1.06 mg/mL) in sample respectively.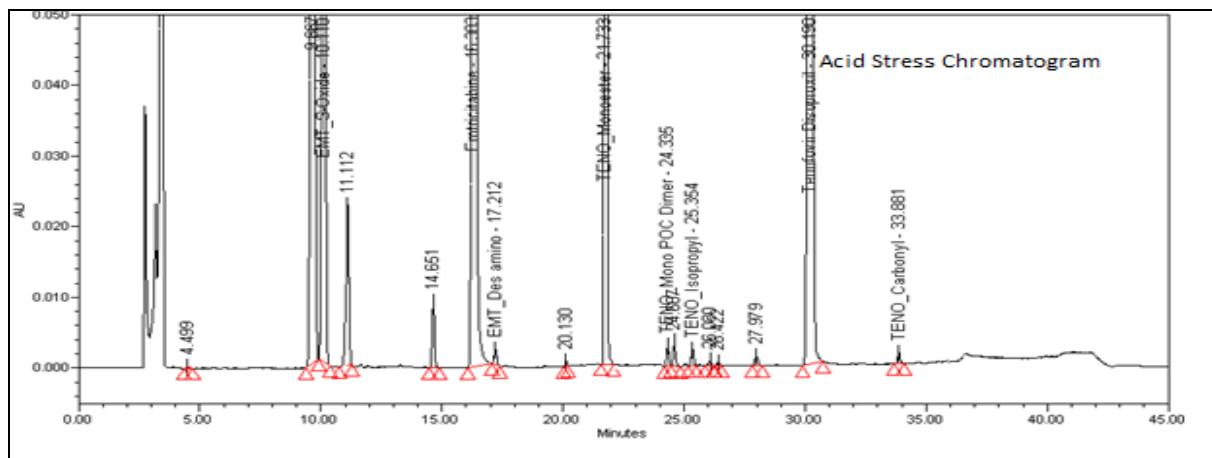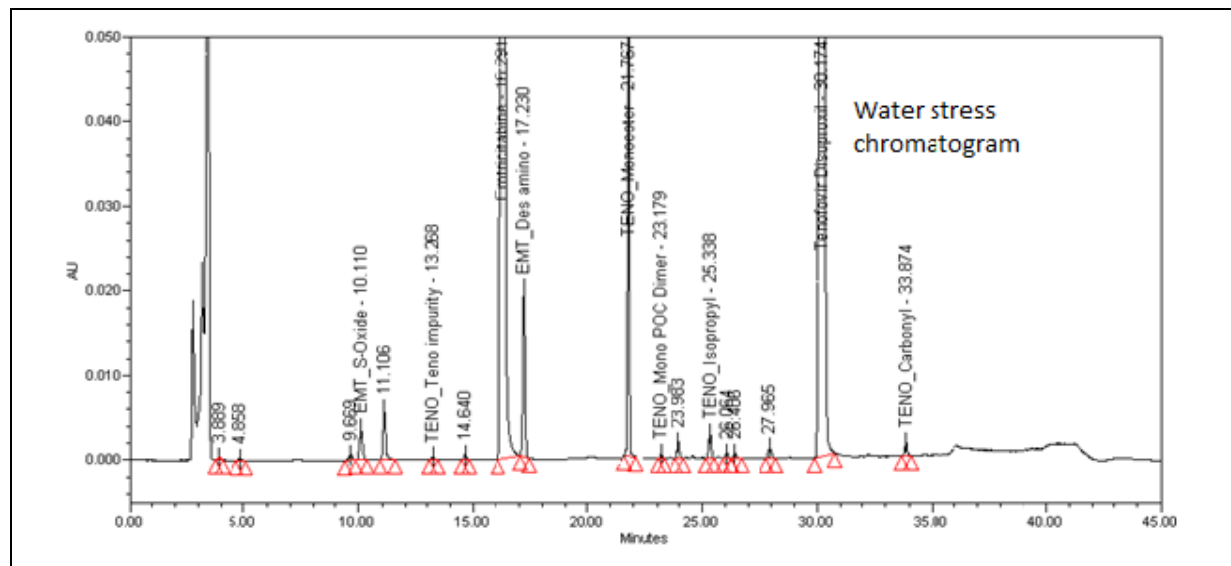 The % RSD for respective impurity in spike sample was found below5.0%. The results for precision and intermediate precision with respective impurity in spike sample was summarized in **(Table-2a, 2b)**

2.2.4 Limit of detection and quantification:

The LOD and LOQ values are determined by injecting solutions with different concentration and by using slope method. The results for LOQ and LOD values for respective impurity was summarized in **(Table-2a, 2b)**

2.2.5 Linearity:

 The series of solutions were prepared by diluting the impurity stock solution to different concentrations from the LOQ to 200% (i.e. LOQ to 0.4% with respect to Emtricitabine 0.7 mg/mL and Tenofovir Disoproxil Fumarate 1.06 mg/mL). The correlation coefficients, slopes, and y-intercepts of the calibration plots are reported. The results show there was an excellent correlation between the peak area and concentration. The results for linearity were summarized in **(Table-2a, 2b).**

2.2.6 Accuracy:

The accuracy was determined in triplicate by spiking respective impurities in sample at 0.1%, 0.2% and 0.4% level with respect to analyte concentration of Emtricitabine 0.7 mg/mL and Tenofovir Disoproxil Fumarate 1.06 mg/mL. The results for accuracy summarized in **(Table-2a, 2b)**.

2.2.7 Robustness:

To study the effect of change in method parameters the flow rate changed to 0.8 and 1.2 ml/min. The effect of pH was studied at pH 3.8 and 4.2. The effect of column temperature was studied at 25^0C and 35^0C. The resolution between adjacent peaks was evaluated. In all the deliberate varied chromatographic conditions the resolution found greater than 1.5.

2.2.8 Solution stability:

No changes in the area count of the respective impurities were observed during solution stability performed using the related substances method. The results from solution stability confirmed that both standard and test solutions were stable for up to 24 hrs.

TABLE 2a: LOQ, LOQ, RECOVERY, LINEARITY AND PRECISION DATA FOR EMTRICITABINE

Parameter	Emtricitabine	Imp-A	Imp-B	Imp-C	Imp-D
LOD (%	0.018	0.021	0.025	0.019	0.016
LOQ (%) 0.067	0.072	0.091	0.059	0.056	
Recovery @0.1%[a]	- ±0.37	101.1 ±0.41	99.5 ±0.56	100.3 ±1.30	99.6
Recovery @0.2% [a]	- ±1.09	100.4 ±1.01	101.3 ±0.84	101.8 ±0.86	98.6
Recovery @0.4% [a]	- ±0.20	100.3 ±0.60	99.9 ±0.09	101.2 ±0.18	100.7
Slope	35583.9	33085.9	42258.6	57589.5	93565.2
Correlation Coefficient	0.9998	0.9994	0.9984	0.9993	0.9971
Y-Intercept	-	1.472.40	1.94	1.15	0.86
Precision (%RSD)[b]	-	1.96	1.00	1.12	0.63
Intermediate Precision (%RSD)[b]	-	1.99	1.84	0.74	0.82

[a] Mean±%RSD for three determinations.
[b] % RSD for six determinations.
All Impurities are spiked with respect to target concentration of 0.7mg/ml for Emtricitabine and 1.06mg/ml for Tenofovir Disoproxil.
Imp-A: EMT_Carboxylic acid, Imp-B: EMT_S-Oxide, Imp-C: EMT_Lamivudine, Imp-D: EMT_Des amino

TABLE 2b: LOQ, LOQ, RECOVERY, LINEARITY AND PRECISION DATA FOR TENOFOVIR DISOPROXIL

Parameter	Tenofovir	Imp-1	Imp-2	Imp-3	Imp-4
LOD (%	0.017	0.026	0.012	0.011	0.022
LOQ (%)	0.062	0.087	0.041	0.038	0.078
Recovery @0.1%[a]	-	101.6 ±0.39	102.5 ±0.99	101.7 ±0.40	101.8 ±0.33
Recovery @0.2% [a]	-	101.0 ±0.34	101.5 ±0.91	103.9 ±1.84	101.8 ±0.26
Recovery @0.4% [a]	-	99.7 ±0.30	100.9 ±0.33	100.5 ±0.07	100.9 ±0.32
Slope	19714.5	63509.0	66426.3	70793.8	42304.1
Correlation Coefficient	0.9995	1.0000	0.9996	0.9999	0.9998
Y-Intercept	-1.25	-0.97	1.67	-0.18	-1.62
Precision (%RSD)[b]	-	0.57	0.73	0.35	1.01
Intermediate Precision (%RSD)b	-	0.84	1.16	0.56	1.17

TABLE 2B (Cont.): LOQ, LOQ, RECOVERY, LINEARITY AND PRECISION DATA FOR TENOFOVIR DISOPROXIL

Parameter	Imp-5	Imp-6	Imp-7
LOD (%	0.022	0.023	0.024
LOQ (%)	0.070	0.069	0.084
Recovery @0.1%[a]	99.0 ±1.31	98.6 ±0.59	99.6 ±0.99
Recovery @0.2% [a]	100.5 ±1.15	100.6 ±1.35	98.2 ±0.66
Recovery @0.4% [a]	100.7 ±0.22	99.5 ±0.32	101.3 ±0.71
Slope	41709.2	47093.3	21445.4
Correlation Coefficient	0.9999	0.9999	0.9997
Y-Intercept	-1.05	0.92	-1.02
Precision (%RSD)[b]	0.96	0.69	1.54
Intermediate Precision (%RSD)b	1.08	1.55	0.88

[a] Mean±%RSD for three determinations.

[b] % RSD for six determinations.

All Impurities are spiked with respect to target concentration of 0.7mg/ml for Emtricitabine and 1.06mg/ml for Tenofovir Disoproxil.

Imp-1: TENO_Adenine, Imp-2: TENO_Teno impurity, Imp-3: TENO_Monoester, Imp-4: TENO_Mono POC, Imp-5: TENO_Isopropyl, Imp-6: TENO_Mix dimer, Imp-7: TENO_Carbonyl

CONCLUSIONS: A rapid, specific, precise, accurate, linear and robust RP-HPLC method developed for determination of related substances from Emtricitabine and Tenofovir Disoproxil Fumarate in pharmaceutical tablet dosage form. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of Emtricitabine and Tenofovir Disoproxil Fumarate in pharmaceutical tablet dosage form.

REFERENCES:

1. www.rxlist.com, Emtricitabine, Tenofovir Disoproxil Fumarate, 2010, 6-10
2. Vanitha C, Vishnuvadhan P, Ramalingam R and Alagaraja M, Simultenious determination of efavirienz, lamivudine and Tenofovir Disoproxil fumarate in tablet dosage form by using RP-HPLC. Journal of pharmacy research, (2012), 5, suppl 6: 3185-3188.
3. Karunakaran A, Kamarajan K and Vetrichelvan T, Validated RP - HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Pure and in Tablet Dosage Form. Pharmacia Sinica., (2010), 1 (2), 52-60.
4. Srivani M, Narasihmarao R and Swapna J, Analytical method development and method validation for the simultaneous Estimation of Lamivudine and Stavudine in tablet dosage form by RP-HPLC, IJPBS, (2011), 1(4), 551-559
5. Jayaseelan S, Ganesh S, Rajasekhar M, Sekar V and Perumal P, A New Analytical Method Development and Validation for the Simultaneous Estimation of Lamivudine and Stavudine in Tablet Dosage Form by Rp-Hplc Method, Int J Pharm Tech Res, (2010), 2(2), 1539-1542.
6. Pradeep K, Dwivedi S.C and Ashok K, Validated HPTLC Method for the Determination of Tenofovir as Bulk Drug and in Pharmaceutical Dosage Form, Res J Chem Sci, (2011), 1(7), 33-37.
7. Behera A, Parida A, Meher A.K, Gowri D and Sankar S.K.M, Development and Validation of Spectrophotometric method for determination of Emtricitabine and Tenofovir Disoproxil Fumarate in bulk and Tablet dosage form. International Journal of PharmTech Research., (2011), 3(3), 1874–1882.
8. Anindita B, Aurobinda P, Amit K.M, Dannana G.S, Swapna K.M and Sudam C.S, Development and validation of spectrophotometric method for determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Tablet dosage Form, Int J Pharm Tech Res, (2011), 3(3), 1874-1882.

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