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RP-HPLC METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDIES FOR SIMULTANEOUS ESTIMATION OF EMTRICITABINE AND TENOFOVIR IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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Abstract: *A simple, Accurate, precise method was developed for the simultaneous estimation of the Emtricitabine and Tenofovir in Tablet dosage form. Chromatogram was run through Kromasil 250mm, C_{18}, 4.6 mm,5µm. Mobile phase containing Acetonitrile and Phosphate Buffer in the ratio of 60:40 was pumped through column at a flow rate of 1ml/min. Buffer used in this method was Phosphate buffer adjusted to pH 2.5 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength for Emtricitabine and Tenofovir was 270nm. Retention time of Emtricitabine and Tenofovir were found to be 2.269 min, 3.102 min respectively. %RSD of the Emtricitabine and Tenofovir were and found to be 0.89 and 1.1 respectively. %Assay was Obtained as 100.15%, 101.03% for Emtricitabine and Tenofovir respectively. LOD, LOQ values are obtained from regression equations of Emtricitabine (0.63ppm, 1.80ppm), Tenofovir (1.83ppm, 5.46ppm). Regression equation of Emtricitabine is y = 7087.6x + 36238, and of Tenofovir is y = 3268x + 15885.*

Key Words: *Emtricitabine and Tenofovir RP-HPLC*

DRUG PROFILE

Emtricitabine:

Emtricitabine (FTC), with trade name Emtriva (formerly Coviracil), is nucleoside reverse transcriptase inhibitor (NRTI) for the treatment HIV infection in adults and children.

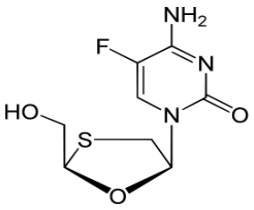

Figure 1.1: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.

Table 1.1

Synonym:	Coviracil
Application:	A HIV-1 reverse transcriptase (HIV-1 RT) inhibitor
CAS Number:	143491-57-0
Purity:	≥98%
Molecular Weight:	247.25
Molecular Formula:	$C_8H_{10}FN_3O_3S$
Appearance:	Crystalline
PhysicalState:	Solid
Solubility:	Soluble in DMSO (≥ 50 mg/ml), water (≥ 50 mg/ml), and ethanol (≥ 17 mg/ml).
Storage:	Store at -20° C
Pka	2.65

Description: Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA.

Pharmacodynamics: Emtricitabine a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply. Emtricitabine is always used with other anti-HIV medicines to treat people with HIV infection. Emtricitabine may lower the amount of HIV in the blood (viral load). Emtricitabine may also help to increase the number of T cells called CD4 cells. Lowering the amount of HIV in the blood lowers the chance of death or infections that happen when your immune system is weak (opportunistic infections). People taking emtricitabine may still get opportunistic infections or other conditions that happen with HIV infection.

Mechanism of action: Emtricitabine is a synthetic nucleoside analogue of cytidine, works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA.. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which is responsible for the inhibition of HIV-1 reverse transcriptase. It competes with the natural substrate deoxycytidine 5'-triphosphate and incorporates into nascent viral DNA, resulting in early chain termination. Therefore emtricitabine inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate deoxycytidine 5'-triphosphate and by its incorporation into viral DNA. By inhibiting HIV-1 reverse transcriptase, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness.

Absorption: Rapidly absorbed (mean absolute bioavailability of 93% for capsules, and 75% for solution). Food does not effect absorption.

Protein binding: Very low (less than 4%)

Metabolism: Minimally transformed (13%), most appears unchanged in urine

(86%). The biotransformation of emtricitabine includes oxidation of the thiol moiety to form the 3′-sulfoxide diastereomers (~ 9% of dose) and conjugation with glucuronic acid to form 2′-O-glucuronide (~ 4% of dose). In vitro studies indicate emtricitabine is not an inhibitor or cytochrome P450 enzymes.

Route of elimination: The renal clearance of emtricitabine is greater than the estimated creatinine clearance, suggesting elimination by both glomerular filtration and active tubular secretion.

Half life: 10 hours.

1.1.2 Tenofovir:

Tenofovir disoproxil fumarate, marketed by under the trade name Viread (as the TDF), belongs to a class known as nucleotide analogue (NRTIs), which block , a crucial viral enzyme in and infections . It is one of the , the most important medications which prevents further replication of the Human Immuno defeciency Virus(HIV).

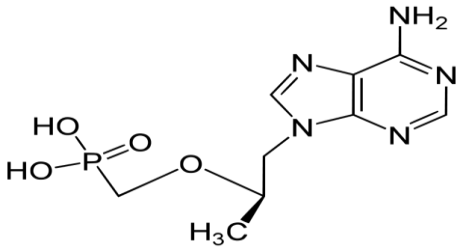

Figure 1.2: ({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid.

Table1.1

Synonym:	Viread
Application:	A selective HIV reverse transcriptase (HIV-1 RT) inhibitor
CAS Number:	147127-20-6
Purity:	≥98%
Molecular Weight:	287.21
Molecular Formula:	$C_9H_{14}N_5O_4P$
Appearance:	Powder
Physical State:	Solid
Solubility:	Soluble in DMSO (10 mM), 1.1 eq.NaOH (100 mM), water (2 mg/ml at 25 °C), and ethanol (<1 mg/ml at 25 °C).
Storage:	Store at -20° C
Melting Point:	480.00 °C (Predicted)
Boiling Point:	~616.1 °C at 760 mmHg
Density:	1.8 g/cm3 (Predicted)
Refractive Index:	n20D 1.74 (Predicted)
pK Values:	pKa: 1.61 (Predicted), pKb: 4.22 (Predicted)

Description: Tenofovir disoproxil fumarate (a prodrug of tenofovir), marketed by Gilead Sciences under the trade name Viread®, belongs to a class

of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. [Wikipedia] In vivo tenofovir disoproxil fumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate.

Pharmacodynamics: Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is currently in late-stage clinical trials for the treatment of hepatitis B. Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ.

Mechanism of action: Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Specifically, the drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. However, unlike the natural deoxynucleotides substrates, NRTIs and NTRTIs (nucleoside/tide reverse transcriptase inhibitors) lack a 3'-hydroxyl group on the deoxyribose moiety. As a result, following incorporation of an NRTI or an NtRTI, the next incoming deoxynucleotide cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain. Thus, when an NRTI or NtRTI is incorporated, viral DNA synthesis is halted, a process known as chain termination. All NRTIs and NtRTIs are classified as competitive substrate inhibitors.

Absorption: Tenofovir disoproxil fumarate is the water soluble diester prodrug of the active ingredient tenofoir. The oral bioavailability in fasted patients is approximately 25%. When a single oral dose (300 mg) is given to HIV-1 infected subjects in the fasted state, the maximum serum concentration was achieved in 1.0 ± 0.4 hours (Tmax). Cmax and AUC values are 0.30 ± 0.09 μg/mL and 2.29 ± 0.69 μg·hr/mL. Administration of food (high fat meal containing 40 to 50% fat) increases the oral bioavailability, with an increase in the AUC of approximately 40%. Cmax is lower in the oral powder, compared to the tablet formulation. However, the mean AUC is similar between the two formulations.

Protein binding: Very low: < 0.7% to human plasma proteins and < 7.2% to serum proteins

Metabolism: The cytochrome P450 enzyme system is not involved with the metabolism of tenofovir disoproxil or tenofovir.

Route of elimination: When tenofovir is given IV, 70-80% of the dose is recovered in the urine as unchanged drug within 72 hours of administration. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion. There may be competition for elimination with other compounds that are also renally eliminated.

Half life: When a single oral dose is given, the terminal elimination half-life is approximately 17 hours.

MATERIALS AND METHODS

Materials:

Emtricitabine, and Tenofovir,(active pharmaceutical ingredients). Emtricitabine and Tenofovir Formulation, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydro furan, tri ethyl amine, ortho-phosphoric acid etc.

Instrument:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Emtricitabine and Tenofovir and solutions.

Methods:

Buffer: (0.1%OPA)

1ML of Ortho phosphoric acid solution in a 1000ml of Volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Standard Preparation:

Accurately Weighed and transferred 20mg of emtricitabine and 30mg of tenofovir working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

METHOD VALIDATION:

The developed method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The developed method was validated with respect to parameters such as linearity, LOD, LOQ, precision, accuracy and specificity. Forced degradation studies were done according to ICH Harmonized Tripartite Guideline, Stability Testing of New Drug Substances and Products: Q1A (R2).

A. System suitability: The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their retention time, theoretical plates number (N) and tailing factor (T).

B. Specificity:

It is the ability to assess unequivocally the analyte in the presence of impurities, degradants and matrix. To determine this, 20 µl of blank, standard and sample solutions were injected separately in triplicate and respective chromatograms were recorded under the optimized conditions.

C. Limit of detection and limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the values of Signal to Noise (S/N) ratio for two drugs. For LOD, S/N ratio should be 3:1 and for LOQ, S/N ratio should be 10:1

D. Linearity:

The calibration curves were obtained with concentrations of the standard solutions of 2.5-15 µg/ml, 10-60 µg/ml and 2.5-15 µg/ml for VOX, SOF and VEL respectively. Linearity is evaluated by regression analysis, which was calculated by the least square regression method.

E. Accuracy:

To check the degree of accuracy, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150% levels.

F. Precision:

The precision of the analytical method was evaluated by the determination of the repeatability of the method (intra day precision) and intermediate precision (inter day precision) of the sample solutions. Repeatability was calculated by assaying six samples prepared on the same day. Intermediate precision was calculated by assaying 3 days. The relative standard deviation of the area of peaks was calculated.

G. Robustness:

Robustness was determined by analysis of samples under slight variations in chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 ml/min to 1.1 ml/min. The ratio of the organic phase (acetonitrile) was changed by +2% and -2%. The effect of retention time and peak parameters were studied.

STRESS TESTING STUDIES:

To demonstrate the stability-indicating of the method, forced degradation studies were performed under different stress conditions like acidic, basic, thermal, peroxide, UV and neutral environment. The extent of degradation and the interference of formed degradant peaks with analyte were investigated.

Optimized chromatogram

Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used	: Kromosil-250 (250mm x 4.6 mm, 5µm)
Buffer used	: Disodium hydrogen phosphate adjusted to pH 2.5
Mobile phase	: Acetonitrile : Phosphate buffer (60:40).
Flow rate	: 1ml/min
Diluent	: Drug dissolved in water and Acetonitrile(50:50)
Wavelength	: 270nm
Temperature	: 30◦C
Injection Volume	: 10µl
Run time	: 6min

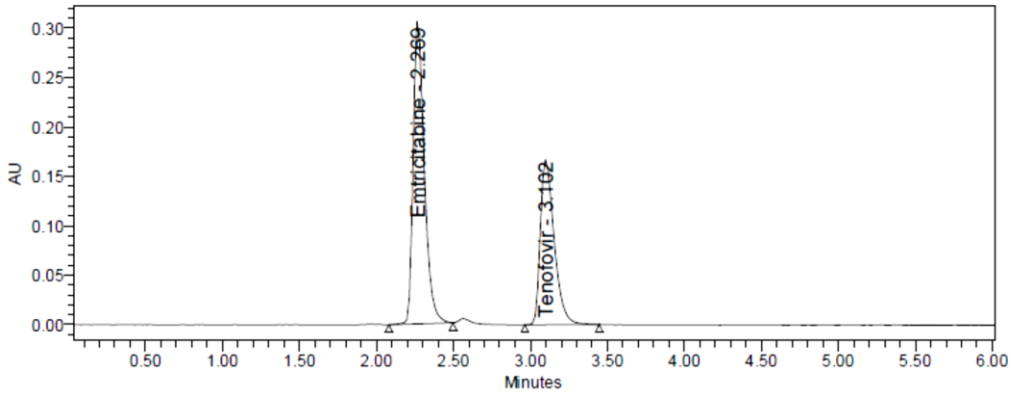

Figure1.3 : optimized chromatogram of Emtricitabine and Tenofovir

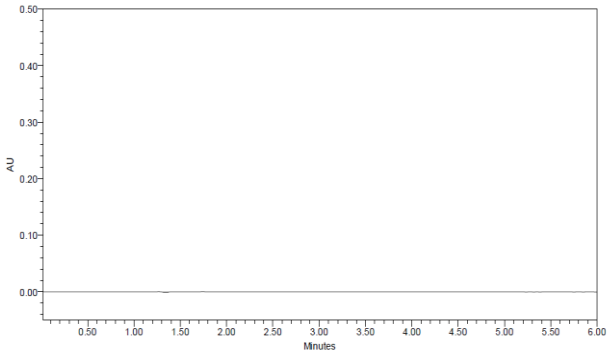

Figure1.4: Blank chromatogram

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Table 1.2: System suitability studies of Emtricitabine and Tenofovir

Property	Emtricitabine	Tenofovir
Retention time (t_R)	2.269± 0.3 min	3.102±0.3min
Theoretical plates (N)	4829 ± 163.48	5601± 163.48
Tailing factor (T)	1.35± 0.117	1.47± 0.117
Resolution	>2	>2

Linearity: Six Linear concentrations of Emtricitabine (50ppm-300ppm), Tenofovir(75ppm-450ppm) are prepared and injected. Regression equation of the the, Emtricitabine and Tenofovir are found to be, y = 7087.6x + 36238and y = 3268x + 15885 Regression co-efficient was 0.999.

Table 1.3: Linearity table of Emtricitabine and Tenofovir

S.No	Con Emtricitabine(µg/ml)	Response	Conc Tenofovir(µg/ml)	Response
1	0	0	0	0
2	50	420004	75	279486
3	100	754086	150	515591
4	150	1108693	225	749108
5	200	1461307	300	992111
6	250	1799177	375	1220702
7	300	2152341	450	1501269

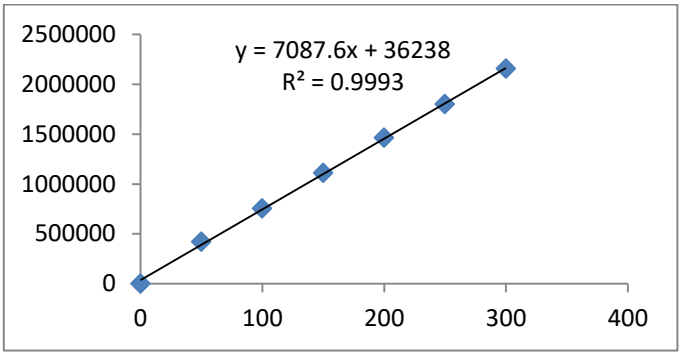

Figure 1.5: Linearity plot of Emtricitabine

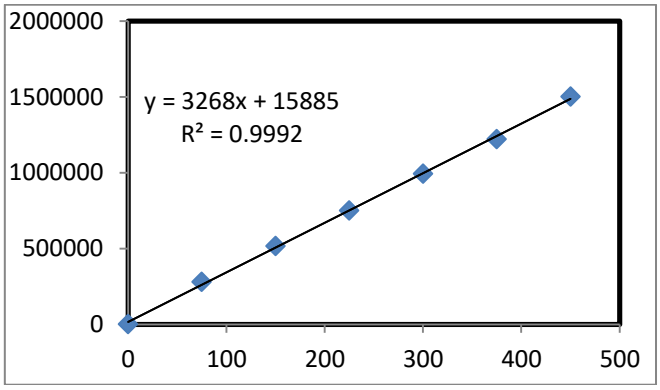

Figure 1.6: Linearity plot of Tenofovir

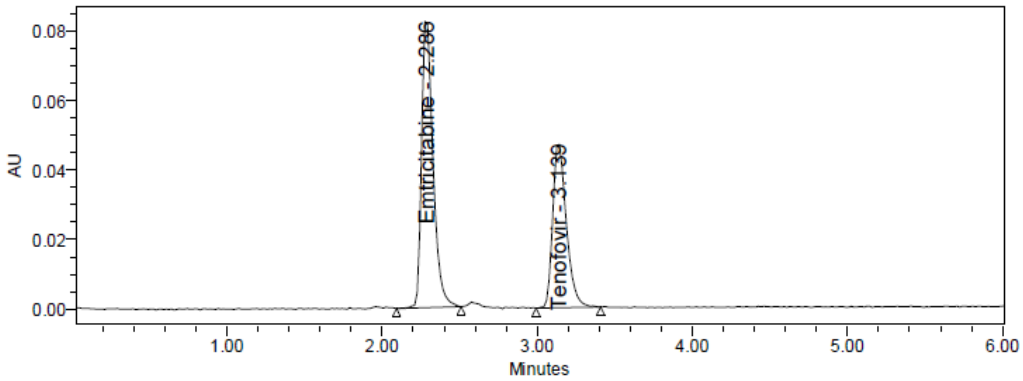

Figure 1.7: Linearity 50% Chromatogram of Emtricitabine and Tenofovir

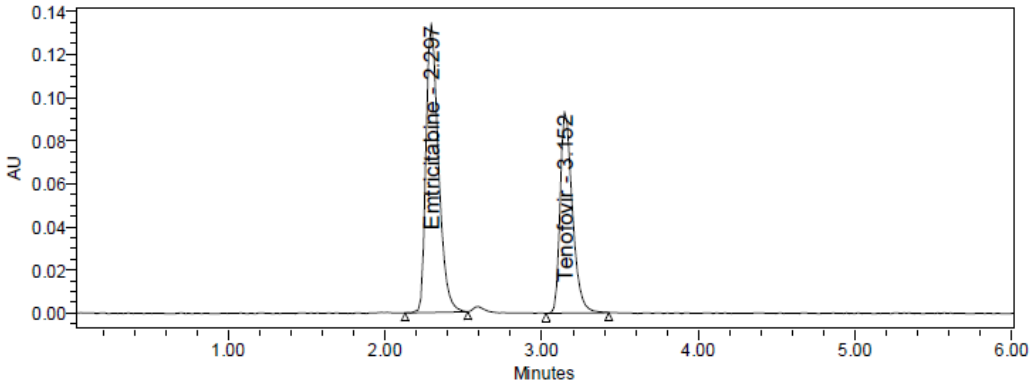

Figure 1.8: Linearity 100% Chromatogram of Emtricitabine and Tenofovir

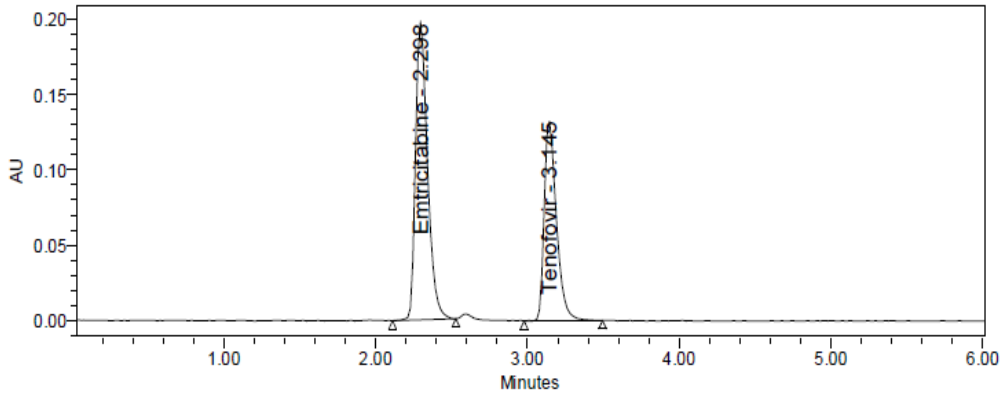

Figure 1.9: Linearity 150% Chromatogram of Emtricitabine and Tenofovir

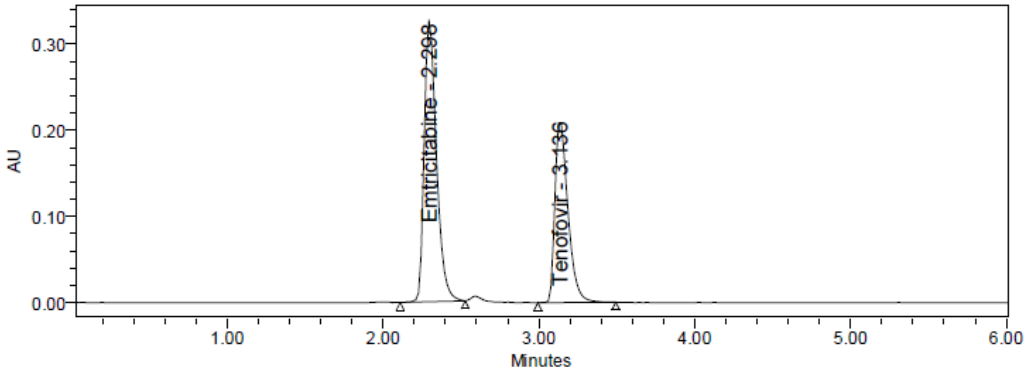

Figure 1.10: Linearity 200% Chromatogram of Emtricitabine and Tenofovir

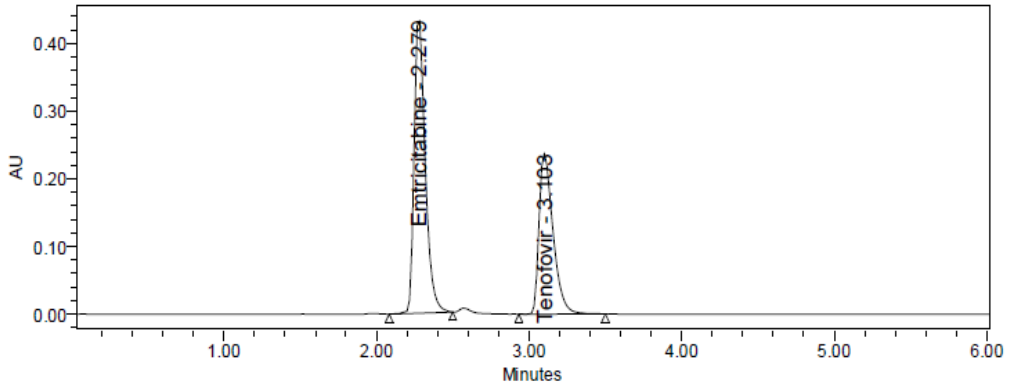

Figure 1.11: Linearity 250% Chromatogram of Emtricitabine and Tenofovir

Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Emtricitabine and Tenofovir were found to be 0.89% and 1.1% respectively.

Table:1.4: Repeatability results for Emtricitabine and Tenofovir .

Sr. No.	Emtricitabine	Tenofovir
1	1491523	1002069
2	1484619	1001854
3	1495280	1005479
4	1472931	1002187
5	1463068	1001224
6	1467674	996319
Mean	1479183	1001522
Std. Dev.	13207	2955.3
%RSD	0.89	1.1

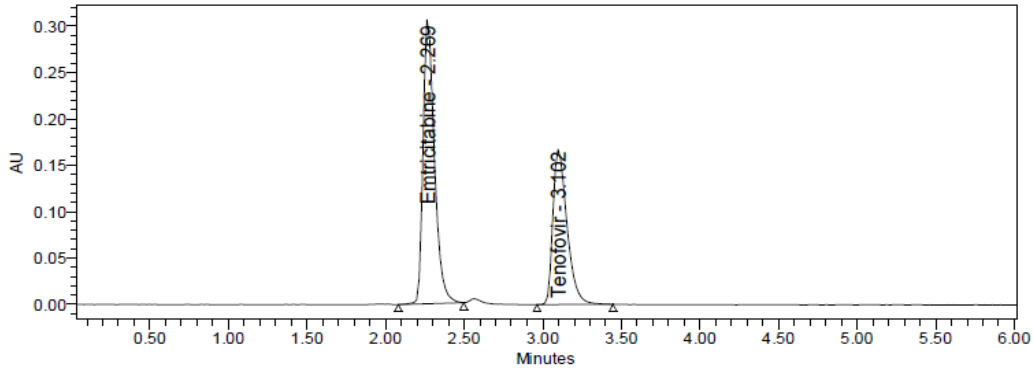

Figure 1.12: Repeatability Chromatogram of Emtricitabine and Tenofovir

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Emtricitabine and Tenofovir were 0.39% and 0.3%

Table1.5: Inter day precision results for Emtricitabine Tenofovir

Sr. No.	Emtricitabine	Tenofovir
1	1478501	977802
2	1478136	982759
3	1467842	975006
4	1466595	974116
5	1466095	978197
6	1469510	978972
Mean	1471113	977809
Std. Dev.	5706	3082
%RSD	0.39	0.3

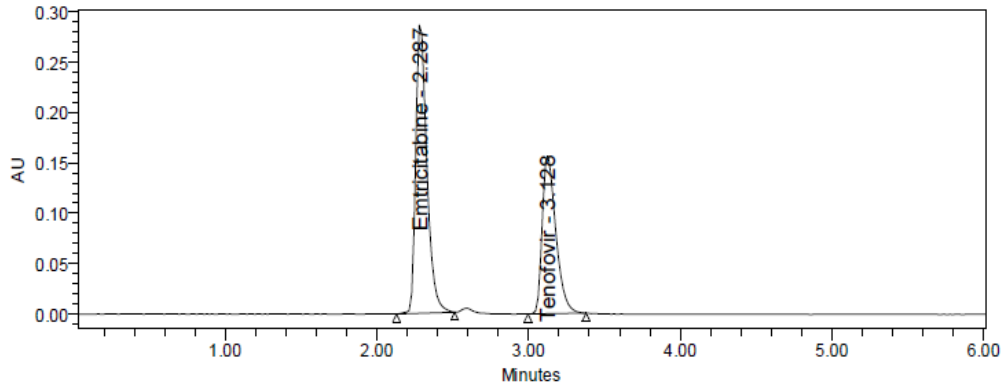

Figure1.13: Inter Day precision Chromatogram of Emtricitabine and Tenofovir.

Accuracy: Three concentrations were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table

Table1.6: Accuracy results of Emtricitabine and Tenofovir

Sample	Amount added (µg/ml)	Recovery (%)	% RSD
Emtricitabine	100	101.22	0.33
	200	100.60	1.18
	300	101.84	0.83
Tenofovir	150	100.28	0.58
	300	100.30	0.35
	450	99.70	0.70

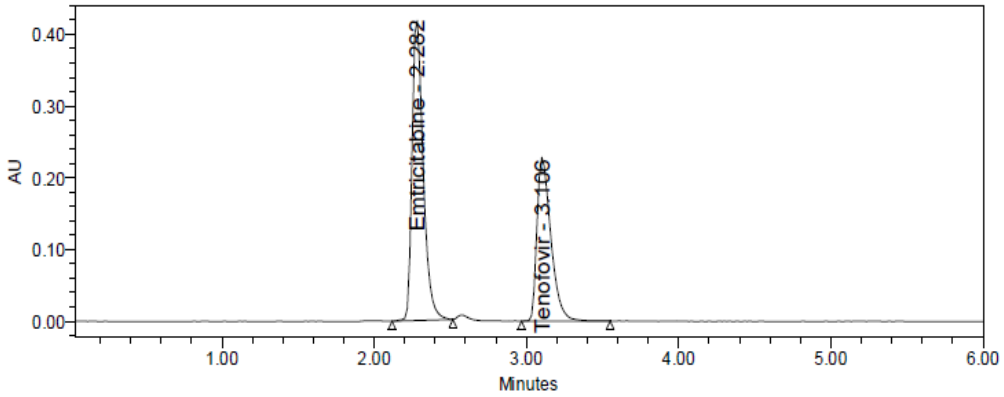

Figure1.14: Accuracy 50% Chromatogram of Emtricitabine and Tenofovir

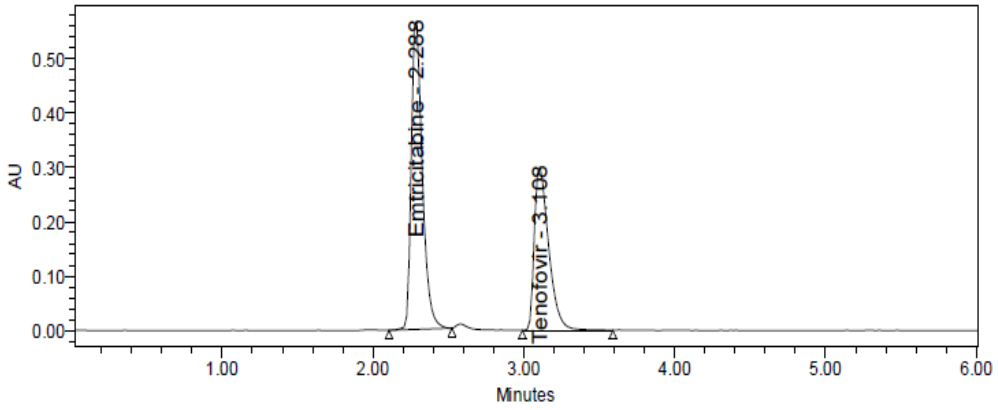

Figure1.15: Accuracy 100% Chromatogram of Emtricitabine and Tenofovir

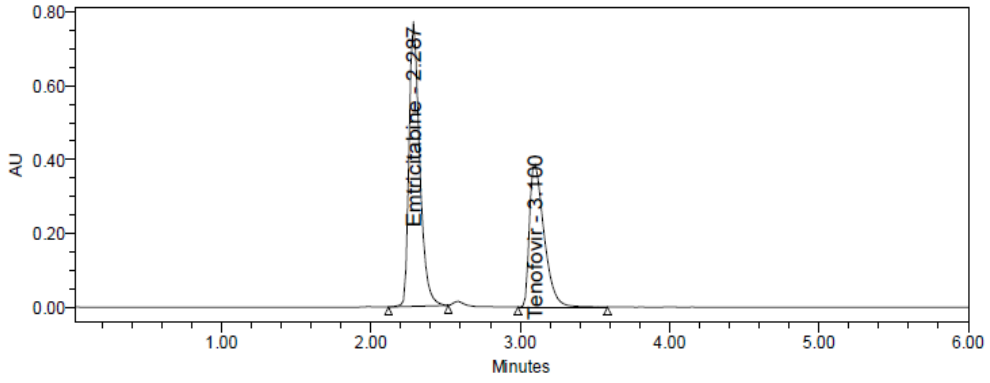

Figure 1.16:Accuracy 150% Chromatogram of Emtricitabine and Tenofovir

1. **LOD:** Limit of detection was calculated by intercept method and LOD for Emtricitabine and Tenofovir were found to be 0.63ppm, 1.80ppm respectively.

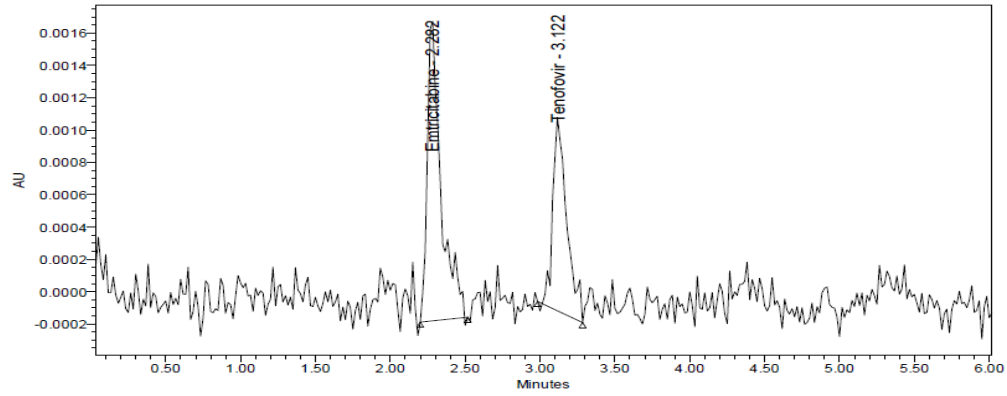

Figure1.17: LOD Chromatogram of Emtricitabine and Tenofovir .

LOQ: Limit of Quantification was calculated by intercept method and LOQ for Emtricitabine and Tenofovir were found to be 1.83ppm, 5.46ppm respectively.

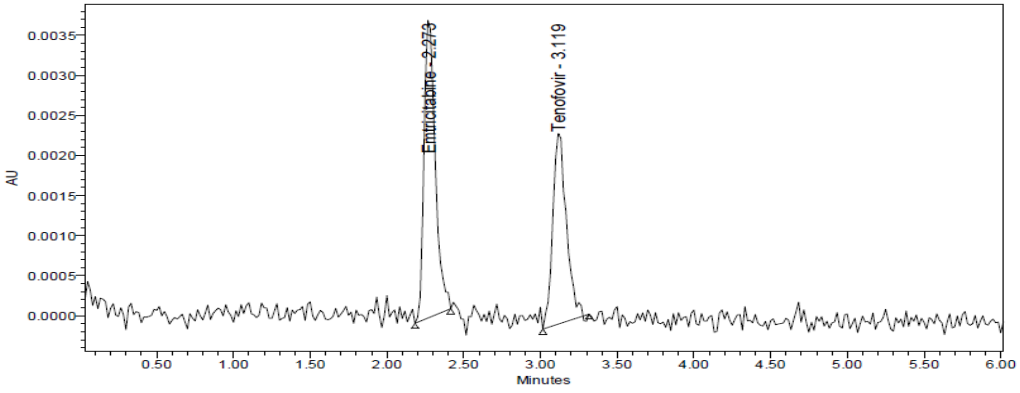

Figure 1.18:LOQ Chromatogram of of Emtricitabine and Tenofovir

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Table 1.7: Robustness data of Emtricitabine and Tenofovir .

S.NO	Robustness condition	Emtricitabine %RSD	Tenofovir %RSD
1	Flow minus	0.1	0.1
2	Flow Plus	1.9	1.9
3	Mobile phase minus	0.1	0.4
4	Mobile phase Plus	0.1	0.1

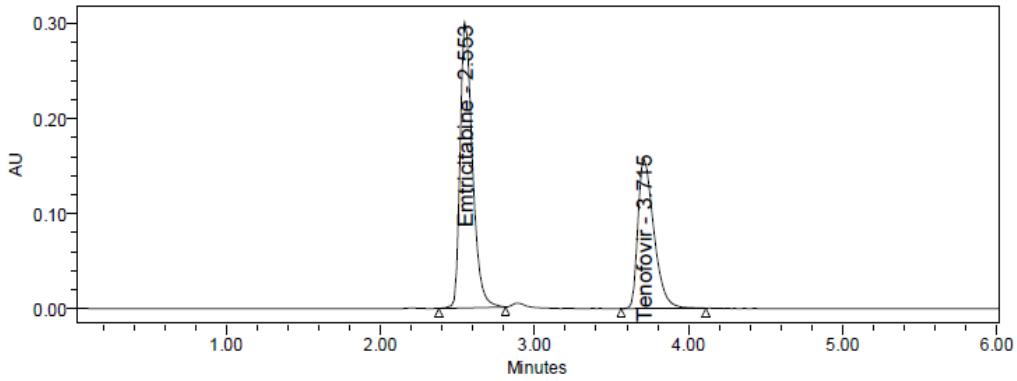

Figure 1.19: Flow minus Chromatogram of Emtricitabine and Tenofovir

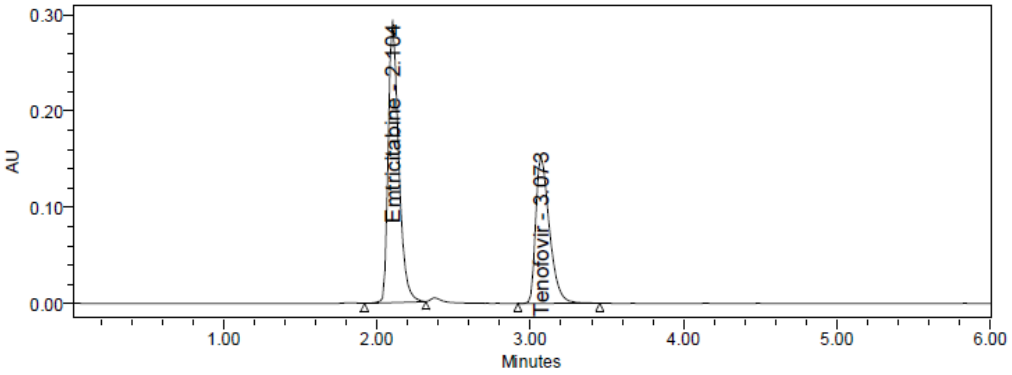

Figure1.20: Flow plus Chromatogram of Emtricitabine and Tenofovir

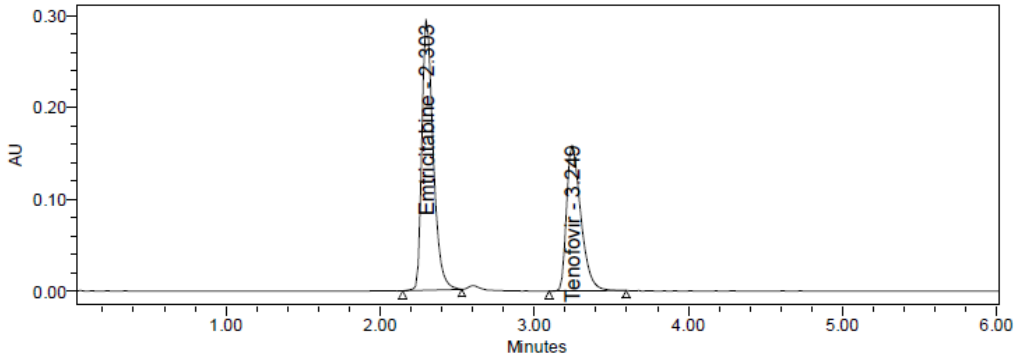

Figure 1.21:Mobile phase minus Chromatogram of Emtricitabine and Tenofovir

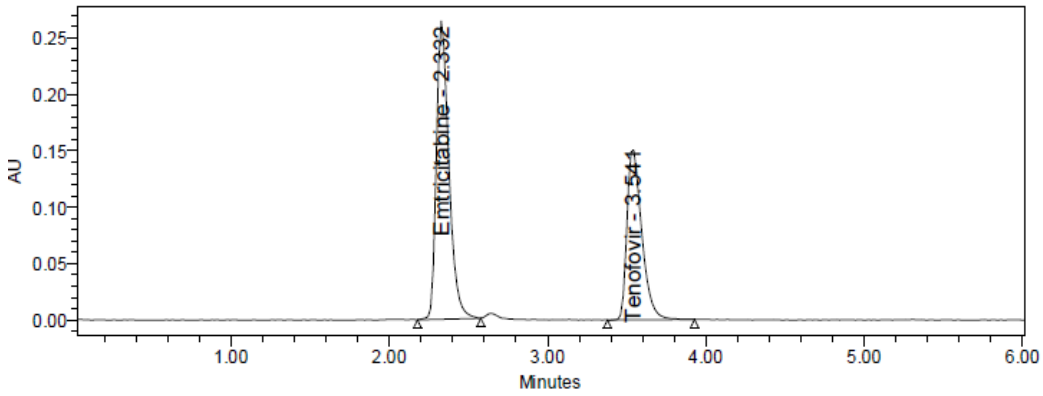

Figure 1.22:Mobile phase Plus Chromatogram of Emtricitabine and Tenofovir

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.15% and 101.03% for Emtricitabine and Tenofovir respectively.

Table1.8: Assay of Tablet

S. No.	Emtricitabine %Assay	Tenofovir %Assay
1	100.99	100.34
2	100.52	99.82
3	101.24	101.58
4	99.73	101.24
5	99.06	101.42
6	99.37	101.81
AVG	100.15	101.03
STDEV	0.8942	0.7797
%RSD	0.89	0.77

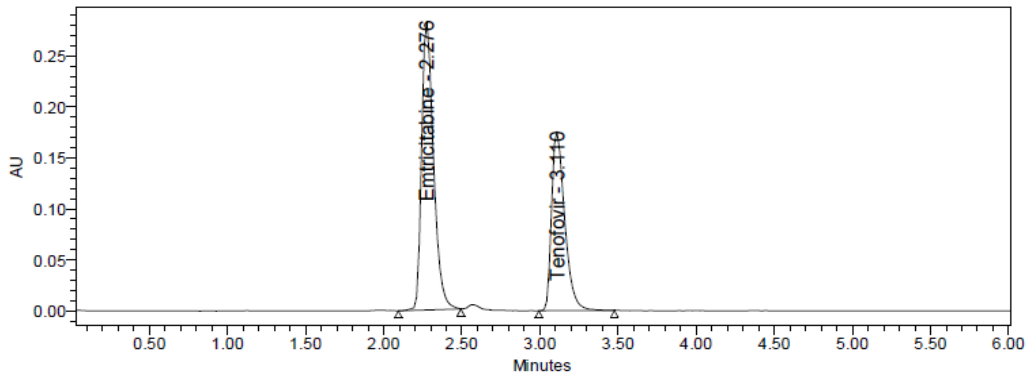

Figure1.23: Assay of Tablet

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Degradation procedure:

Oxidation:

To 1 ml of stock solution of Emtricitabine, Tenofovir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 200µg/ml & 300µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1ml of stock s solution Emtricitabine, Tenofovir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 200µg/ml & 300µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Emtricitabine, Tenofovir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 200µg/ml & 300µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 200µg/ml & 300µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 2000µg/ml & 3000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 200µg/ml & 300µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60^o. For HPLC study, the resultant solution was diluted to 200µg/ml & 300µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Degradation chromatograms

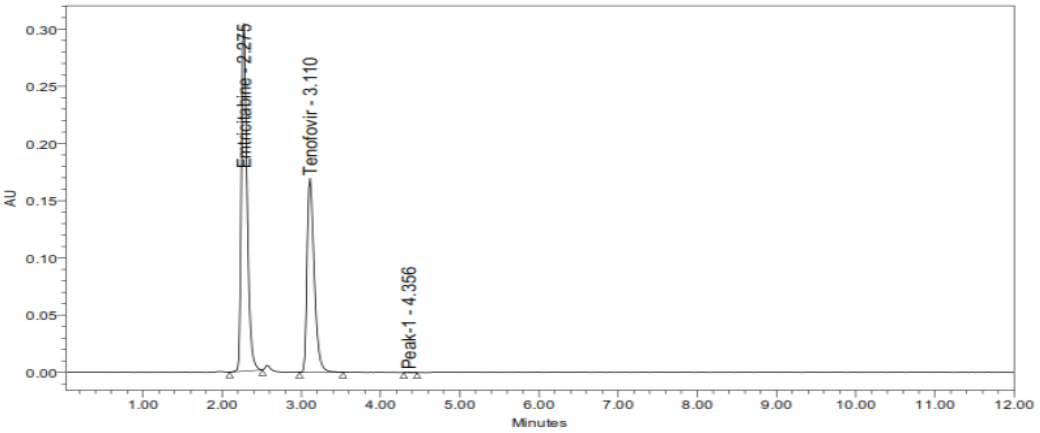

Figure1.24: Acid degradation chromatogram

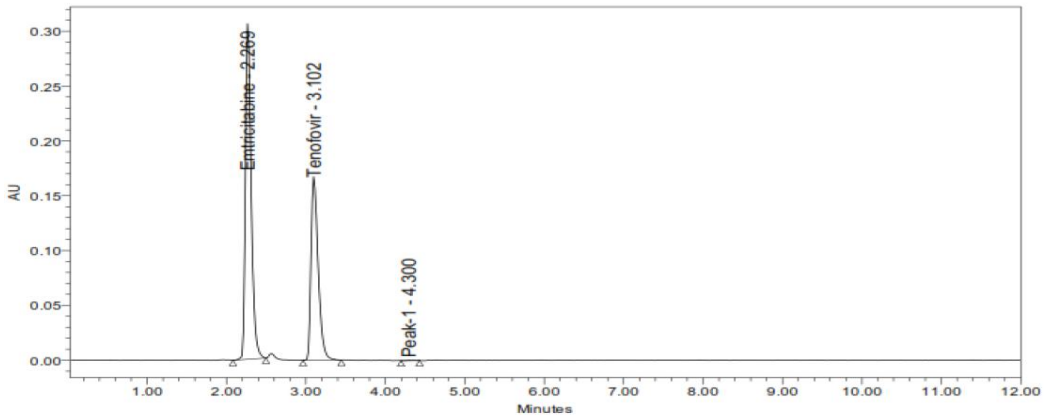

Figure1.25: Base degradation chromatogram

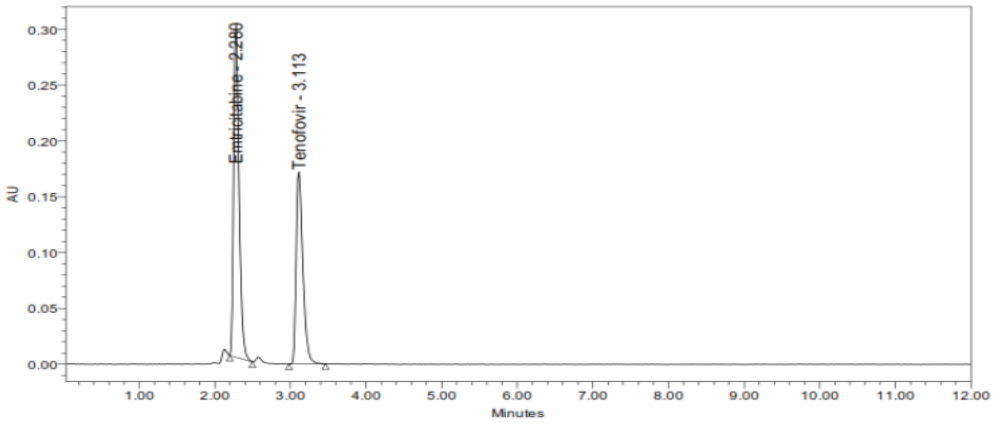

Figure1.26: Peroxide degradation chromatogram

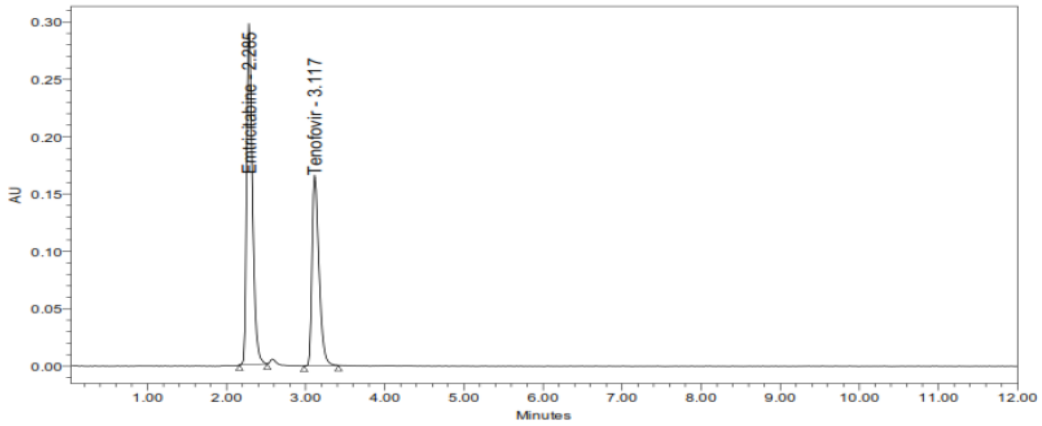

Figure 1.26:Thermal degradation chromatogram

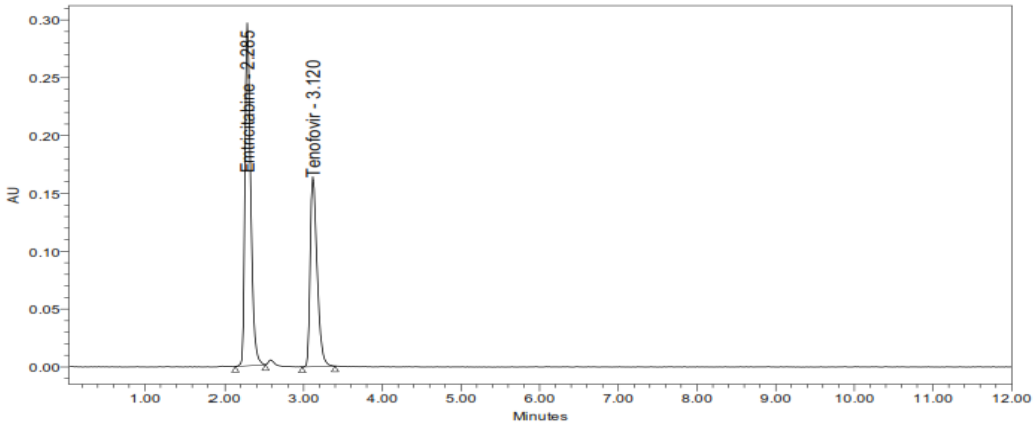

Figure1.27: UV degradation chromatogram

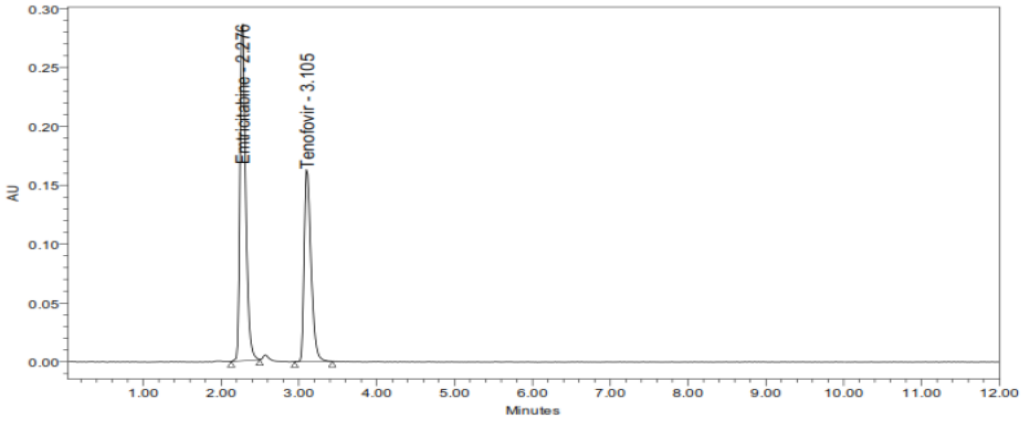

Figure1.28: Neutral degradation chromatogram

Table1.9: Degradation Data of Emtricitabine

S.NO	Degradation Condition	AREA	% ASAAY	AMOUNT DEGRADED %
1	Acid	1425235	96.50	3.50
2	Alkali	1444619	97.81	2.19
3	Oxidation	1457925	98.71	1.29
4	Thermal	1465896	99.25	0.75
5	UV	1474927	99.86	0.14
6	Water	1476395	99.96	0.04

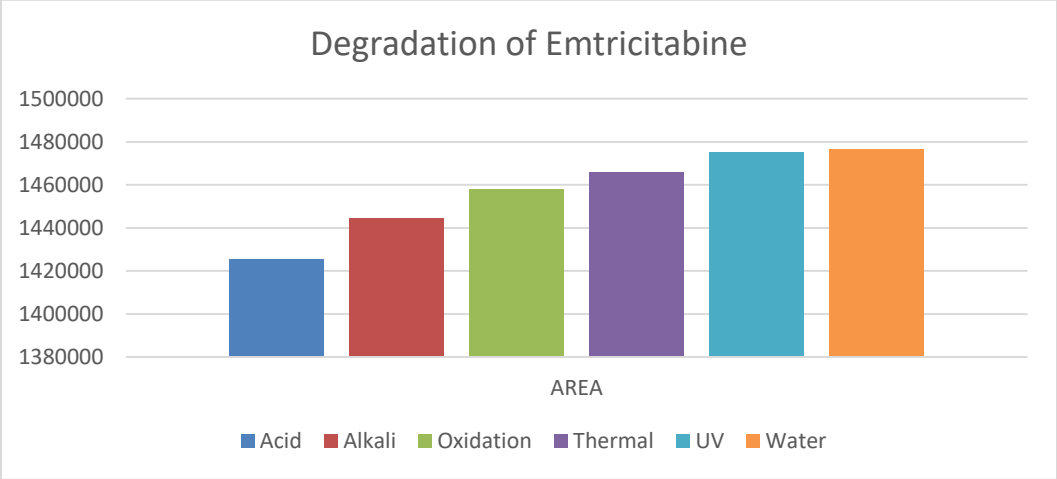

FIGURE 1.30: Showing degradation pattern

Table1.10: Degradation Data of Tenofovir

S.NO	Degradation Condition	AREA	%ASSAY	AMMOUNT DEGRADED %
1	Acid	962369	96.73	3.27
2	Alkali	972916	97.79	2.21
3	Oxidation	980400	98.54	1.46
4	Thermal	992141	99.72	0.28
5	UV	993702	99.88	0.12
6	Water	994727	99.98	0.02

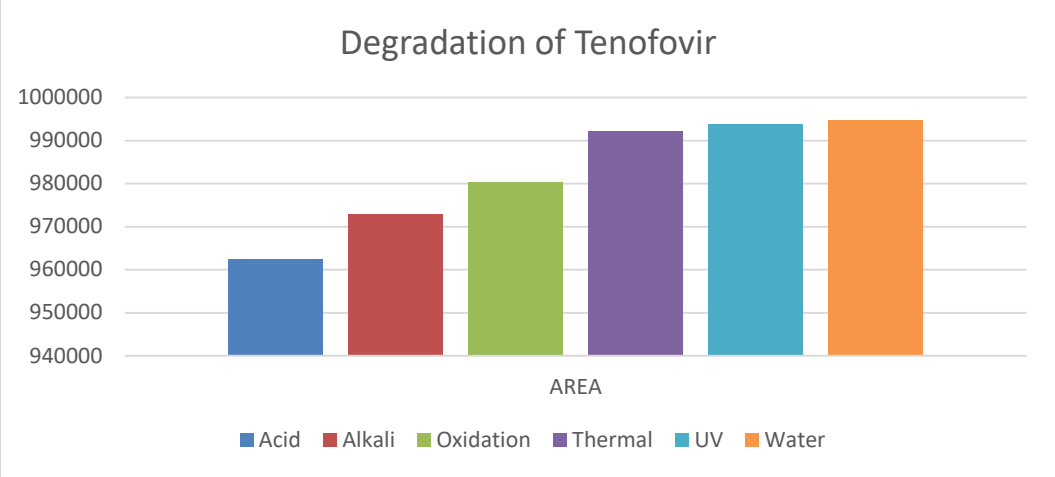

FIGURE 1.31: Showing degradation pattern

SUMMARY

Table 1.11:

Parameters	Emtricitabine	Tenofovir
Calibration range (mcg / ml)	50-300ppm	75-450ppm
Optimized wavelength	270nm	270nm
Retention time	2.269min	3.102min
Regression equation (Y*)	y = 7087.6x + 36268	y = 3268x + 15885
Correlation coefficient(r^2)	0.9993	0.9992
Precision (% RSD*)	0.89	1.1
% assay	100.15	101.3%
Limit of Detection (mcg / ml)	0.63ppm	1.80ppm
Limit of Quantitation (mcg / ml)	1.83ppm	5.46ppm

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Emtricitabine and Tenofovir in Tablet dosage form. Retention time of Emtricitabine and Tenofovir were found to be 2.269 min, 3.102 min respectively. %RSD of the Emtricitabine and Tenofovir were and found to be 0.89 and 1.1 respectively. %Assay was Obtained as 100.15%, 101.3% for Emtricitabine and Tenofovir respectively. LOD, LOQ values are obtained from regression equations of Emtricitabine (0.63ppm, 1.80ppm), Tenofovir(1.83ppm, 5.46ppm). Regression equation of Emtricitabine is y = 7087.6x + 36238, and of Tenofovir is y = 3268x + 15885. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

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