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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMERATE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

An accurate, precise and simple stability indicating Chromatographic method for development and validation for the simultaneous estimation of Emtricitabine and tenofovir Disoproxil Fumerate in its pharmaceutical dosage form by HPLC was carried out by using ACE C18 (150 mmx4.6 mm i.d, 5 µ) column with gradient program, mobile phase A: 0.01M Citrate buffer pH 4.0 with Ammonia & mobile phase B: ACN , Flow rate 1.2 ml/min., Injection volume 20 µl, UV detection was performed by using wavelength at 270 nm. The method was linear over the concentration range of 20 µg/mL to 70 µg/mL for Emtricitabine (r^2- 0.9997) and 25 µg/mL to 100 µg/mL for Tenofovir disoproxil fumerate (r^2- 0.9996) with limits of detection and quantification of 0.112 µg/ml & 0.346 µg/ml for Emtricitabine and 0.192 µg/ml & 0.585 µg/ml for tenofovir Disoproxil Fumerate. Forced degradation study was carried out according to ICH guidelines in Acid Degradation, Base Degradation, Oxidative Degradation, Thermal Degradation conditions and the method was specific. A study to establish the stability of standard and test preparations on bench top was conducted and the solutions were stable for 24 hours.

KEYWORDS: Emtricitabine, Tenofovir Disoproxil Fumerate, RP-HPLC, ICH Guidelines.

INTRODUCTION

Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a synthetic nucleoside analogue of cytidine. 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. 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**.**

MATERIALS AND METHODS

Emtricitabine and Tenofovir disoproxil fumerate API was obtained as a gift sample from MYLAN, Bollaram, Hyderabad.

Table. 1: Instruments Used.

S. No.	Name of Instruments
1	Waters HPLC 2695 with UV detector
2	ACE C18 column 150 X 4.6, 5µ
3	Thermo scientific Finn Pipette
4	Inolab WTW series pH meter
5	Afroset Electronic Balance FX -400
6	Elma S 300H Ultra sonicator
7	Spectra lab water bath shaker
8	Hermle Z 323 centrifuge
9	Empower software version pro e 2

Table. 2: Chemicals Used.

S. No.	Name	Grade	Make
1	Citric acid monohydrate	Analytical Reagent	Merck
2	Ammonia	HPLC	Merck
3	ACN	HPLC	Merck
4	Hydrochloric acid	HPLC	Merck
5	Water	HPLC	Milli Q (Purificationsystem)

Selection of Wavelength: The sensitivity of method that uses UV detector depends upon the proper selection of wavelength is that gives maximum absorbance and good response for the given candidate drug. In setting up the conditions for development of the assay method, the choice of the detection wavelength was based on the scanned absorption spectrum for Emtricitabine and Tenofovir disproxil fumerate. The UV-spectrum of Emtricitabine and Tenofovir disproxil fumerate was separately scanned in the wavelength range of 200-400 nm against blank. After correlation of the both spectrums 270nm wavelength was selected for the analysis.[1-6]

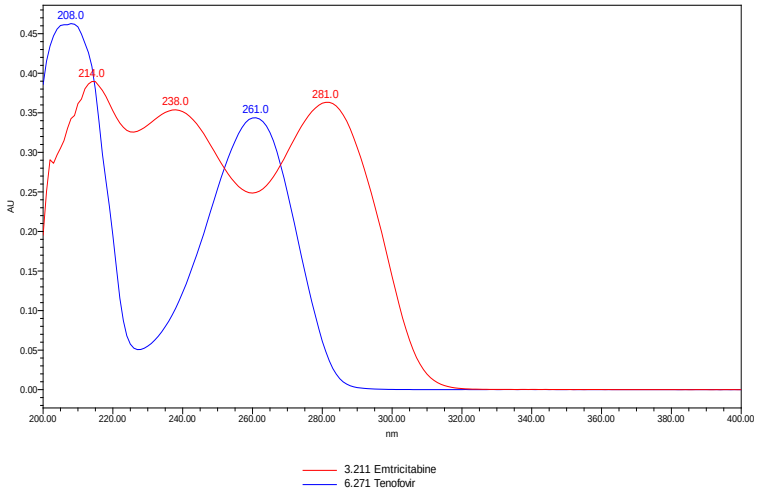

Figure. 1: Overlay UV spectrum of EMT and TDF.

Optimized Chromatographic Conditions

Column : ACE C18 (150 mmx4.6 mm i.d, 5 µ)

Flow rate : 1.2 mL/min

Selected wave length : 270nm

Program : Gradient

Detector : UV Detector/ PDA

Mobile phase A : 0.01M Citrate buffer pH 4.0 with Ammonia

Mobile phase B : ACN

Injection volume : 20 µL

Temperature : 30ºC

Time (min)	Mobile phase-A	Mobile phase-B
0	70	30
4	20	80
7	20	80
8	70	30
12	70	30

Preparation of 0.01M Hydrochloric Acid (Diluent): About 8.5mL of concentrated hydrochloric acid was diluted to 1000 mL with water in a volumetric flask and mixed well. 100 mL of this solution was further diluted to 1000 mL with water and mixed.

Preparation of 0.01M Citrate Buffer (Mobile Phase A): Accurately 2 gm of citric acid monohydrate was weighed and transferred in to a 1000 mL standard flask and diluted to 1000 mL with water. The pH was adjusted to 4.0 with ammonia, filtered and degassed.

Preparation of Mobile Phase B

ACN was used as mobile phase B.

Preparation of Standard Stock Solution: Accurately 40 mg of Emtricitabine and 60mg of Tenofovir disoproxil fumerate working standard were transfered into a 100mL volumetric flask. 50 mL diluent was added and sonicated to dissolve and made up to volume with diluent.

Preparation of Standard Solution: About 5 mL of the standard stock solution was pipetted out into a 50mL volumetric flask and diluted up to the volume with diluent and filtered through 0.45 PVDF filter.

Test Preparation: Accurately weighed amount of the powder equivalent to 200mg of Emtricitabine and 300 mg of Tenofovir disoproxil fumerate were transfered into a 500mL volumetric flask. 400mL of diluent was added and sonicated for 45min with intermediate shaking. It is allowed to cool at room temperature, diluted to volume with diluent and mixed well. A portion of the above solution was centrifuged in a centrifuge tube with cap at 5000 rpm for 10 min. About 5.0 mL of the above solution was pipetted into 50mL volumetric flask and diluted to volume with diluent and mixed well. The solution was filtered through 0.45µ PVDF (Millipore make).

Method Validation

System suitability[7-9]

About 20µL of blank, standard solution (six times) was injected, chromatogram was recorded and peak response was measured.

The tailing factor for the Emtricitabine and Tenofovir disoproxil fumerate peak should be not more than 2.0 from the chromatogram of standard solution.

The Relative standard deviation of Emtricitabine and Tenofovir disoproxil fumerate peak area from six replicate injections of standard solution should be not more than 2.0%.

Table. 3: Data for System Suitability.

System suitability parameters	Observed values		Acceptance criteria
	Emtricitabine	Tenofovir DF	
The USP plate count from standard chromatogram	13438	48847	NLT 3000
The Tailing factor from standard chromatogram	1.06	1.05	NMT 2
% RSD from 6 replicate injections of standard solution	0.14	0.15	NMT 2

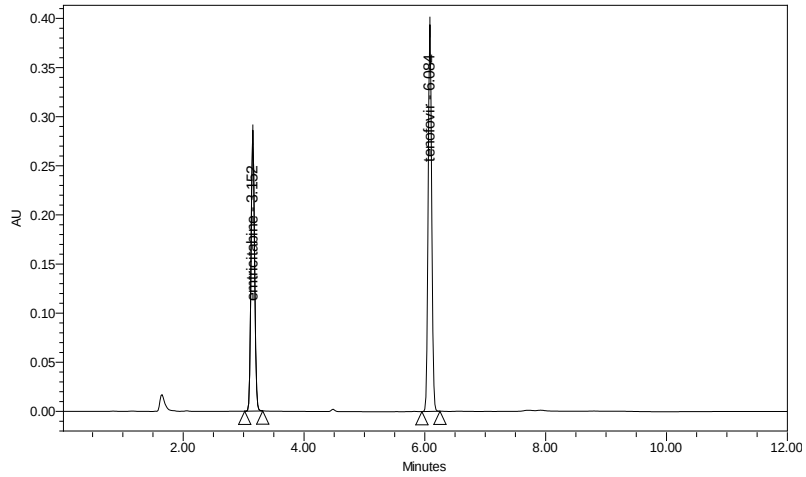

Figure. 2: System Suitability Chromatogram for Standard.

System Precision

Procedure

System precision was performed by injecting six replicate injections of Emtricitabine and Tenofovir disoproxil fumerate (40 ppm of Emtricitabine and 60ppm of Tenofovir disoproxil fumerate) working standard.

Table. 4: Data for System precision for Emtricitabine.

Injection Number	Emtricitabine Peak area
1	1276471
2	1255856
3	1254000
4	1252648
5	1256997
6	1255857
Average	1256313.167
SD	1643.222373
%RSD	0.14

For Tenofovir disoproxil fumerate

Injection number	Tenofovir DF Peak area
1	1609813
2	1610262
3	1606962
4	1604478
5	1610537
6	1606525
Average	1608096.167
SD	2467.470236
%RSD	0.15

Precision of Test Method

Table 5: Data for Method precision for Emtricitabine.

S. No.	Area	% Assay
1.	1146433.0	99.1
2.	1163281.5	100.7
3.	1136656.5	98.3
4.	1148538.5	99.2
5.	1136979.0	98.5
6.	1151405.0	99.5
	Mean	99.2
	% RSD	0.9

For Tenofovir disoproxil fumerate

S. No.	Area	% Assay
1.	1608364.5	99.6
2.	1600735.0	99.2
3.	1596586.5	98.9
4.	1602959.0	99.2
5.	1576688.5	97.9
6.	1599553.0	99.0
	Mean	99.0
	% RSD	0.6

Intermediate Precision

The ruggedness of method was demonstrated by conducting the precision study on different HPLC system and different column of same make on different day. Assay was performed for six individual test preparations of 200/300mg strengths as per test method.

Table. 6: Data for Intermediate Precision (Column to Column, System to System variation).

System suitability parameters	Observed value		Acceptance criteria
	Column 1 & system 1	Column 2 & system 2	
The Tailing factor for Emtricitabine peak	1.06	1.02	NMT 2.0
The Tailing factor for Tenofovir DF peak	1.05	1.01	NMT 2.0
% Relative standard deviation for Emtricitabine peak	0.14	0.24	NMT 2.0
% Relative standard deviation for Tenofovir DF peak	0.15	0.14	NMT2.0

*(n = 6)

Table. 7: Data for Intermediate Precision for EMT and TDF.

S. No.	% Assay			
	Emtricitabine		Tenofovir DF	
	Column–1 and system 1	Column–2 and system-2	Column–1 and system-1	Column–2 and system-2
1	99.1	100.6	99.6	100.3
2	100.7	98.4	99.2	98.2
3	98.3	100.5	98.9	100.2
4	99.2	100.8	99.2	100.6
5	98.5	98.5	97.9	98.3
6	99.5	98.7	99.0	98.3
Average	99.2	99.6	99.0	99.3
% RSD	0.9	1.2	0.6	1.2

Linearity

Linearity of detector response was established by plotting a graph between concentrations versus peak area. A series of solutions Emtricitabine and Tenofovir disoproxil fumerate standard was prepared in the concentration ranging from 20 µg/mL to 70 µg/mL for Emtricitabine and 25 µg/mL to 100 µg/mL for Tenofovir disoproxil fumerate and analyzed as per test method. A graph was plotted with concentration in µg/mL on X- axis versus response (area) on Y-axis and determined the correlation coefficient.[10-12]

Table. 8: Data for Linearity for Emtricitabine.

S. No	% Level	Concentration (µg/mL)	Peak Area
1	40	20.032	462372
2	60	28.048	713211
3	80	35.064	948758
4	100	48.079	1165361
5	120	57.095	1405071
6	160	70.127	1892006

For Tenofovir disoproxil fumerate

S. No	% Level	Concentration (µg/mL)	Peak Area
1	40	25.968	639745
2	60	40.952	978324
3	80	57.936	1306936
4	100	71.92	1592068
5	120	86.904	1917759
6	160	100.872	2572312

Table. 9: Data for Linearity Parameters for EMT and TDF.

S. No	Parameter	Results	
		EMT	TDF
1	Linearity Range	20- 70 µg/mL	25- 100 µg/mL
2	Co-efficient of correlation(r^2)	0.9997	0.9996
3	Slope	29483	26635
4	Intercept	1845.6	14314.9
5	Residual sum of squares	413931014.4	903880385.9
6	Standard Error for slope	262.72	259.68
7	Standard Error for y intercept	10669	15766
8	LOD(µg/mL)	0.112	0.192
9	LOQ(µg/mL)	0.346	0.585

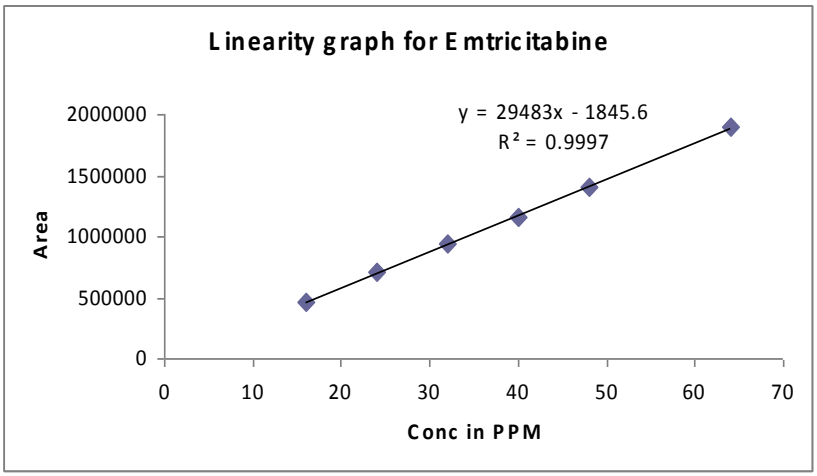

Figure. 3: Calibration Plot for Emtricitabine.

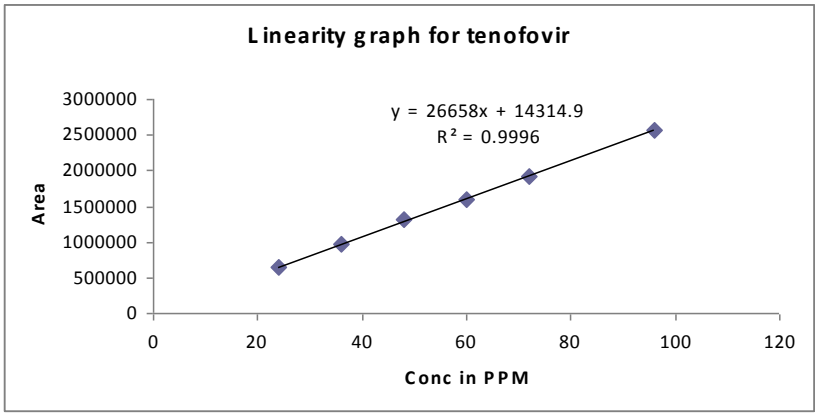

Figure. 4: Calibration Plot for Tenofovir disoproxil fumerate.

Accuracy: The accuracy was confirmed by recovery studies by adding known amount of placebo to the pure API of Emtricitabine and Tenofovir disoproxil fumerate from about 50% to 150% of the initial assay concentration. Sample solutions was prepared in triplicate for each level and analyzed as per test method.

Table 10: Data for Accuracy.

For Emtricitabine

Sample No.	% Spike level	mg added	mg found	% Recovery	Mean % Recovery	% RSD
1	50	99.3	99.95	100.7	101.1	0.7
2	50	99.8	100.36	100.6		
3	50	99.6	101.46	101.9		
1	100	197.61	199.89	101.2	100.6	0.6
2	100	197.51	198.55	100.5		
3	100	197.11	197.33	100.1		
1	150	296.71	296.01	99.8	99.8	0.4
2	150	297.11	295.32	99.4		
3	150	297.31	297.73	100.1		

For Tenofovir disoproxil fumerate

Sample No.	% Spike level	mg added	mg found	% Recovery	Mean % Recovery	% RSD
1	50	149.35	148.97	99.7	99.7	0.8
2	50	149.15	147.58	98.9		
3	50	149.15	149.76	100.4		
1	100	296.71	293.58	98.9	99.4	0.8
2	100	297.21	294.18	99.0		
3	100	296.81	298.11	100.4		
1	150	446.16	443.26	99.4	99.6	0.3
2	150	445.96	443.22	99.4		
3	150	445.96	446.11	100.0		

Interference from Degradation Products

A study was conducted to demonstrate the effective separation of degradants from Emtricitabine and Tenofovir disoproxil fumerate peak in Assay method. Separate portions of drug product and placebo were exposed to the following stress conditions to induce degradation.[12-15]

1) Treated with 0.1N HCL solution.

2) Treated with 0.1 NaOH solution.

3) Treated with 3% H_2O_2 solution for about 5 minutes on bench top.

4) Exposed to heat for about 24 hrs at 105ºC.

The chromatograms of the stressed samples were evaluated for peak purity of Emtricitabine and Tenofovir disoproxil fumerate.

5) Exposed to humidity at 25ºC/90%RH for about 48 hrs.

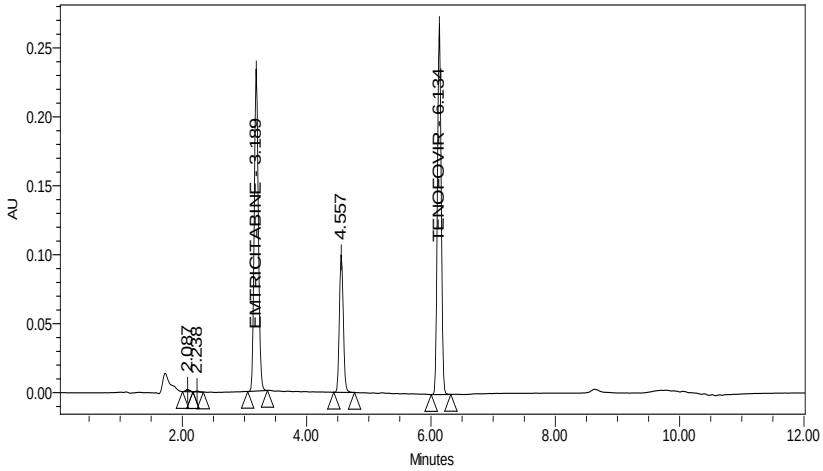

Figure. 5: Chromatogram for Acid Degradation Sample.

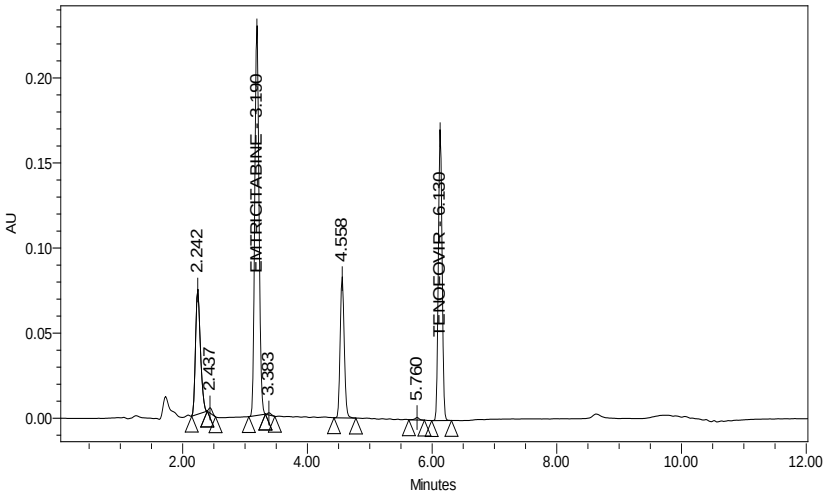

Figure. 6: Chromatogram for Base Degradation Sample.

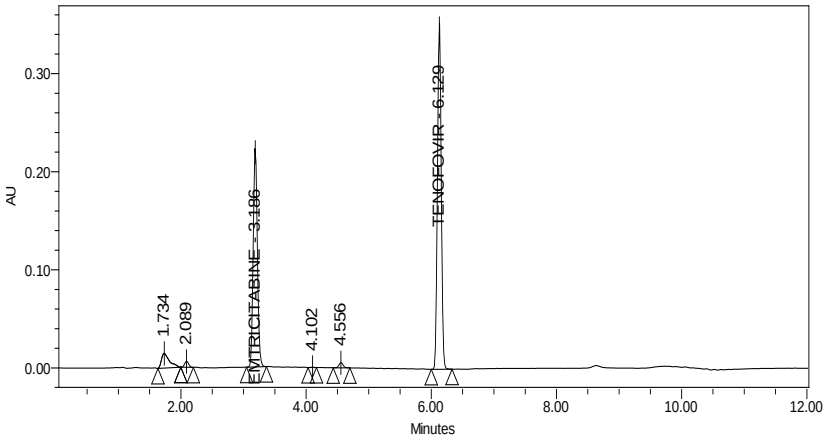

Figure. 7: Chromatogram for Peroxide Degradation Sample.

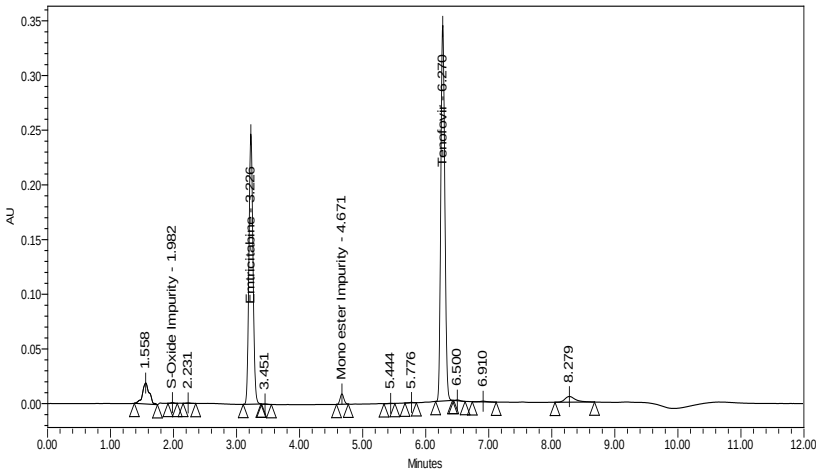

Figure. 8: Chromatogram for Thermal Degradation Sample.

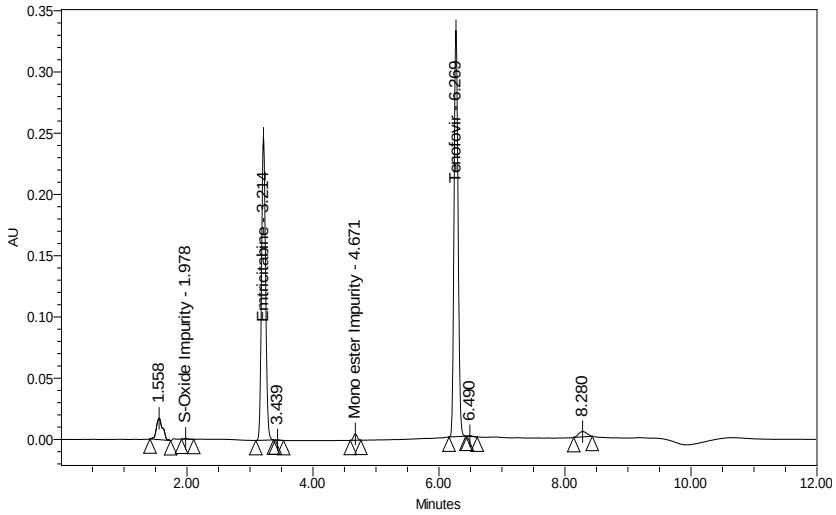

Figure. 9: Chromatogram for Humidity Degradation Sample.

Table 11: Data for Emtricitabine Degradation.

Name	Retention Time	Area	% Area
Acid	3.189	1073283	40.36
Base	3.190	1068103	39.51
Oxidation	3.186	1031224	38.08
Thermal	3.226	1098375	38.18
Humidity	3.253	1042165	38.12

Table. 12: Data for TDF Degradation.

Name	Retention Time	Area	% Area
Acid	6.134	1138997	42.83
Base	6.130	723577	26.77
Oxidation	6.129	1490223	55.02
Thermal	6.270	1518599	52.79
Humidity	6.213	1235235	46.23

Stability of Solutions

Bench Top Stability of Standard Preparation and Test Preparations

A study to establish the stability of standard and test preparations on bench top was conducted for 24 hours. The assay of standard and test preparations was estimated against freshly prepared standard at initial and after 24 hours.

Table. 13: Data for Bench Top Stability for Standard and Test Preparations For Emtricitabine.

Time (Hrs)	% Assay of standard preparation	Difference from initial	% Assay of test preparation		Difference from initial	
			Test - 1	Test - 2	Test - 1	Test – 2
Initial	98	NA	100.7	98.3	NA	NA
24	96.8	1.2	100.5	98.2	0.2	0.1

For Tenofovir disoproxil fumerate

Time (Hrs)	% Assay of standard preparation	Difference from initial	% Assay of test preparation		Difference from initial	
			Test - 1	Test - 2	Test - 1	Test – 2
Initial	97.8	NA	99.2	98.2	NA	NA
24	96.8	1.0	100.2	99.6	1.0	1.4

NA- Not Applicable

Acceptance Criteria

The % assay results of standard and test solutions should not deviate by ±2.0 from that of initial.

Bench Top Stability of Mobile Phase

A study to establish stability of mobile phase on bench top was conducted for 5 days.

The system suitability parameters were evaluated as per the test method by using the same mobile phase on different days (initial, after 1 day, 3 days and 5 days).

Table 14: Data for Mobile Phase Stability on Bench top for Emtricitabine.

System Suitability Parameters	Observed value				Acceptance Criteria (%RSD)
	At Initial	After 1 day	At Day-3	At Day-5	
The Tailing factor for Emtricitabine peak in Standard solution	1.06	1.07	1.06	1.04	NMT 2.0
%RSD of peak area of Emtricitabine	0.14	0.14	0.08	0.03	NMT 2.0
USP plate count of Emtricitabine peak	13438	12204	13411	11847	NLT 3000

For Tenofovir disoproxil fumerate

System Suitability Parameters	Observed value				Acceptance Criteria (%RSD)
	At Initial	After 1 day	At Day-3	At Day-5	
The Tailing factor for Tenofovir DFpeak	1.05	1.04	1.02	1.10	NMT 2.0
%RSD of peak area of Tenofovir DF	0.15	0.27	0.07	0.03	NMT 2.0
USP plate count of Tenofovir DF peak	48847	44742	45416	44478	NLT 3000

Robustness

Effect of Variation in Flow Rate

A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at the flow rate of 0.9 mL/min and 1.1 mL/min.

Table. 15: Data for Effect of Variation in Flow Rate.

System Suitability Parameters	Observed value		Acceptance Criteria
	0.9mL/min	1.1 mL/min	
The tailing factor for Emticitabine peak	1.07	1.06	NMT 2.0
The tailing factor for Tenofovir DF peak	1.06	1.03	NMT 2.0
% Relative standard deviation for Emtricitabine peak	0.26	0.09	NMT 2.0
% Relative standard deviation for Tenofovir DF	0.24	0.06	NMT2.0

Effect of Variation in Column Temperature

A study was conducted to determine the effect of variation in column oven temperature. The system suitability parameters were evaluated at 25°C and 35°C column oven temperatures.

Table. 16: Data for Effect of Variation in Column Temperature.

System Suitability Parameters	Observed value		Acceptance Criteria
	25°C	35°C	
The tailing factor for Emticitabine peak	1.07	1.08	NMT 2.0
The tailing factor for Tenofovir DFpeak	1.07	1.07	NMT 2.0
% Relative standard deviation for Emtricitabine peak	0.11	0.05	NMT 2.0
% Relative standard deviation for Tenofovir DF	0.11	0.06	NMT2.0

Effect of Variation in pH of Buffer in Mobile Phase

A study to establish the Effect of variation in pH of buffer in mobile phase was conducted. Mobile phase was prepared with buffer having different pH between 4.3 and 4.7. System suitability parameters were evaluated by using the above mobile phases.[16-18]

Table. 17: Data for Effect of Variation in pH of Buffer in Mobile Phase.

System Suitability Parameters	Observed value		Acceptance Criteria
	pH 4.3	pH 4.7	
The tailing factor for Emtricitabine peak	1.07	1.07	NMT 2.0
The tailing factor for Tenofovir DF peak	1.04	1.05	NMT 2.0
% Relative standard deviation for Emtricitabine peak	0.14	0.13	NMT 2.0
% Relative standard deviation for Tenofovir DF peak	0.27	0.11	NMT2.0

Filter Interference

A study to establish the suitability of filters was conducted by using two different filters namely, 0.45µm PVDF filter (Mfg. by: M/s. Millipore) and Nylon 0.45 µm filters (Mfg. by: M/s. Millipore). Test solutions prepared in duplicate was centrifuged and filtered through the above different filters and analyzed as per test method. The difference in % Assay between centrifuged and filtered test solutions was determined.

The standard solution prepared as per test method was filtered through the above different filters and analyzed along with unfiltered standard solution.[13,14]

The similarity factor of Emtricitabine and Tenofovir disoproxil Fumerate in filtered standard solutions against unfiltered standard solution was calculated.

Filter validation

Table. 18: Description for Filters.

Filter Description	Filters	
	Nylon	PVDF
Manufacturers Name	Millipore	Millipore
Size	0.45µm	0.45µm

Table. 19: Results of Filter Interference for standard.

Sample	Peak Area		Similarity Factor
	Emtricitabine	Tenofovir DF	
Centrifuged	1122852	1612386	NA
0.45µm PVDF	1120847	1607107	1.00
0.45µm NYLON	1119476	1604829	1.00

Table. 20: Results of Filter Interference for Test.

Sample Name	% Assay			Difference between Centrifuged and filtered sample	
	Centrifuged	0.45 µm PVDF filter	0.45 µm Nylon filter	0.45 µm PVDF filter	0.45 µm Nylon filter
Emtricitabine	97.3	97.1	97.3	0.2	0.0
Tenofovir	97.5	96.9	97.5	0.6	0.0

Acceptance Criteria: 1) The % assay of filtered test solutions should not deviate by ± 2.0 from that of centrifuged test solutions

RESULTS AND DISCUSSIONS

A wavelength of 270nm was selected as a detection wavelength for the estimation of Emtricitabine and Tenofovir disoproxil fumerate in RP-HPLC system. A simple, precise and accurate RP-HPLC method was developed for the analysis of Emtricitabine and Tenofovir disoproxil fumerate in tablet dosage form using the mobile phase A consisting of 0.01M citrate buffer (pH 4.0) and mobile phase B is 100% ACN. The flow rate was found to be optimized at 1.2 mL/min. It reduced the usage of mobile phase. The system suitability parameters like retention time, resolution, efficiency, capacity factor, tailing factor and % RSD was found to be within the limits for the optimized chromatogram.

Method precision: The method precision study was conducted for the Emtricitabine and Tenofovir disoproxil fumerate sample solutions. %RSD was found to be 0.9 for Emtricitabine and 0.6 for Tenofovir disoproxil fumerate. Assay was performed to determine the purity of the Emtricitabine and Tenofovir disoproxil fumerate solutions. The mean percentage purity

was found to be 99.2% for Emtricitabine and 99.0% for Tenofovir disoproxil fumerate respectively.

Intermediate precision: The intermediate precision (Ruggedness) was performed with two different columns and different instrument but there is no change in retention time, system suitability parameter and % content.

Linearity: It is evident that the responses for Emtricitabine and Tenofovir disoproxil fumerate was found to be linear in the studied concentration ranges from 20µg/mL to 70µg/mL and 25µg/mL to 100µg/mL respectively and the correlation coefficient was found to be r^2=0.9997 and r^2=0.9996 for Emtricitabine and Tenofovir disoproxil fumerate respectively.

Accuracy: The accuracy studies was carried out at 3 levels (50%, 100% and 150%) to ensure the accuracy of the method by adding known concentration of API to Placebo. The average percentage recovery was found to be in the range of 100% Emtricitabine, 99.5% for Tenofovir disoproxil fumerate respectively. Nearly 100% recovery showed that the method was free from the interference of the excipients used in the formulation.

Specificity: The specificity was performed under stress conditions like acid, base, peroxide, thermal and humidity was performed and observed the degradation of drugs. It was found to be within the limits. From the above chromatograms of forced degradation studies, and their purity plots, it can be inferred that peaks of the degradants was not interfere with the retention time of the main peak of Emtricitabine and Tenofovir disoproxil fumerate.

Bench top stability: Bench top stability for standard, test and mobile phase was performed. The difference in percentage assay of standard and test preparations between initial and after 24 hours is found to be within the limit. From the above study, it is concluded that the standard and test preparations are stable for 24 hours on bench top. The mobile phase was found to be clear and no haziness was observed during the stability period, it is concluded that the mobile phase is stable for 5 days on bench top.

Robustness: The Robustness was performed by making deliberate changes in flow rate, column temperature and pH of the buffer solution (mobile phase A). It shows that there is no change in the retention time even after making deliberate change in the analytical procedure. Then the method was found to be robust.

Filter validation: The filter validation was performed by using two different filters namely, 0.45µm PVDF filter (Mfg. by: M/s. Millipore) and Nylon 0.45 µm filters (Mfg. by: M/s. Millipore). The similarity factor of Emtricitabine and Tenofovir disoproxil Fumerate in filtered standard solutions against centrifuged standard solution is found to be within the limit. From the above study, it is concluded that both the (PVDF and Nylon) filters are suitable for standard and test solution filtration.

SUMMARY AND CONCLUSIONS

All the above parameters have shown that the developed method for the estimation of emtricitabine and tenofovir disoproxil fumerate in bulk and tablet dosage form by rp-hplc was successfully developed by using RP-HPLC and the method was validated as per the ICH(Q2B)guidelines and the results have proved that the method is selective, precise, accurate, stable and linear. Hence it was concluded that the developed method was found to be applicable for routine quantitative analysis for the estimation of emtricitabine and tenofovir disoproxil fumerate in bulk and tablet dosage form.

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