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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF EMTRICITABINE, BICTEGRAVIR AND TENOFOVIR ALAFENAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A Stability-indicating reversed phase high liquid chromatography (RP-HPLC) was developed which can separate and accurately quantitate Emtricitabine, Bictegravir and Tenofovir alafenamide. This method was successfully validated for the purpose of conducting stability studies in Quality Control (QC) laboratories. The Chromatographic separation was achieved on Inertsil ODS C_{18} (4.6 x 250mm, 5µm) as stationary phase with a mobile phase comprising of Methanol, Acetonitrile and Phosphate buffer of pH 3 adjusted with 5 % Orthophosphoric Acid (30:30:40 V/V). The mobile phase was sonicated for 10 minutes and filtered through 0.45µ membrane filter. The flow rate was 1 ml/min; column temperature of 40 ± 2^0 C and UV detection was performed at 260 nm. The retention times of Emtricitabine, Bictegravir and Tenofovir was found to be 1.125, 2.108 and 2.745 min respectively. The proposed RP-HPLC Method was

validated and all the parameters are given for Method Validation. The method was quantitatively evaluated in terms of linearity, precision, accuracy, selectivity and robustness as per standard guidelines. The method is simple, convenient and suitable for the analysis of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical formulations.

Keywords: Stability, RP-HPLC, Emtricitabine, Bictegravir, Tenofovir

1. INTRODUCTION:

Analytical Chemistry is often described as the area of chemistry responsible for characterizing the composition of matter **[1]**. Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter **[2]**. Emtricitabine is chemically 4-Amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one is a nucleoside reverse transcriptase inhibitor used for the treatment and prophylaxis of HIV **[3]**. Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA **[4]**. Bictegravir is (1S,11R,13R)-5-Hydroxy-3,6-dioxo-N-(2,4,6-trifluorobenzyl)-12-oxa-2,9-diazatetracyclo[11.2.1.0~2,11~.0~4,9~]hexadeca-4,7-diene-7-carboxamide and is a human immunodeficiency virus (HIV) integrase strand transfer inhibitor, the fourth in this class of agents that target the viral integrase used only in combination with other antiretroviral agents in the treatment of HIV infection and it has had limited use **[5]**. This drug is associated with a low rate of serum aminotransferase elevations during therapy, but has not been linked to instances of acute, clinically apparent liver injury. Tenofovir alafenamide is chemically Isopropyl (2S)-2-[[[(1R)-2-(6-aminopurin-9-yl)-1-methyl-ethoxy]methyl-phenoxy-phosphoryl]amino]propanoate and is used to treat chronic (long term) HBV in adults and children 12 years of age and older who have stable liver disease. Tenofovir is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs) **[6]**. It works by decreasing the amount of HIV and HBV in the blood. Tenofovir alafenamide is also a component of several FDA-approved fixed-dose combination) (FDC) tablets for treating HIV infection. The combination of Emtricitabine, Bictegravir and tenofovir alafenamide FDC tablet is also approved for HIV pre-exposure prophylaxis **[7]**.

Literature survey revealed that there are few analytical methods such as UV-VIS, HPLC, HPTLC, LC-MS for the determination of Emtricitabine, Bictegravir and Tenofovir alone or in combination **[8-12]**. Moreover reported methods were not much cost-

effective in terms of solvent consumption and total run time of the analysis and hence the present study was undertaken. The present investigation was carried out in the view of establishing a simple, rapid, and robust stability indicating RP-HPLC method for the simultaneous estimation of above drug combinations.

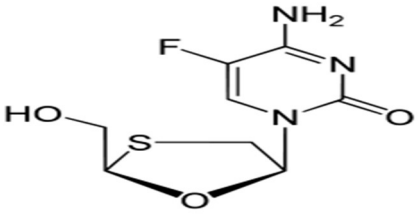

Figure 1: Chemical Structure of Bictegravir

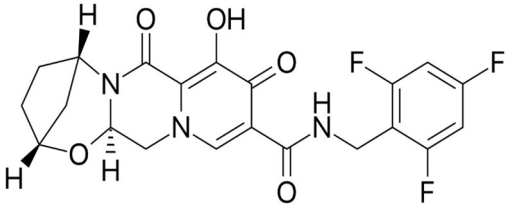

Figure 2: Chemical Structure of Emtricitabine

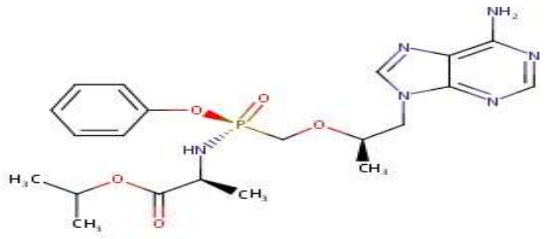

Figure 3: Chemical Structure of Tenofovir

2. METHODOLOGY

2.1 Chemicals and Reagents:

All chemicals and reagents used were analytical grade and included hydrochloric acid, monobasic phosphate, Orthophosphoric acid, methanol, Acetonitrile were purchased from Merck Ltd., Mumbai. Highly purified deionized water was obtained from Millipore, Milli-Q purification system. API of Emtricitabine, Bictegravir and Tenofovir were obtained as gift samples from Nutech Biosciences, Hyb. Double distilled water was used for preparing mobile phase solutions. The tablet of Emtricitabine, Bictegravir, Tenofovir (Commercial Name-Taffic) was obtained from a local market.

2.2 Instrumentation:

Isocratic high pressure Water's HPLC with an LC-Pump and variable wavelength programmed PDA Detector and operating software Chromaster with version Empower 3 was used. Chromatographic separation was carried out by using Inertsil ODS C_{18} (4.6 x 250mm, 5µm).

2.3 Chromatographic Condition:

The mobile phase consisting of Methanol, Acetonitrile and Phosphate buffer (pH 3) in

the ratio of 30:30:40 v/v was degassed and filtered through Millipore vacuum filter system containing 0.45 µm membrane filter. Separation was carried out by pumping the mobile phase at a flow rate of 1.0 ml/min at ambient temperature and effluence was monitored at 260 nm and UV Spectrum was shown in **Figure 4** and chromatogram was shown in **Figure 5**.

2.4 Preparation of Solutions:

2.4.1. Preparation of 0.025M Phosphate buffer:

1.7g of potassium dihydrogen ortho phosphate was weighed and taken in a 500 ml volumetric flask and adjust the P^H upto 3 with 5% and finally the solution was filtered by using 0.45 Micron membrane filter and keep for sonication up to a time period of 10 min.

2.4.2. ***Preparation of mobile phase:***

Accurately measured 300 ml (30%) of Methanol, 40 ml (40%) of above phosphate buffer and 300 ml of Acetonitrile HPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the diluent.

2.4.3. Preparation of Tenofovir, Emtricitabine and Bictegravir Standard solution:

Accurately weigh and transfer 12.5 mg of Tenofovir, 100 mg of Emtricitabine and 25 mg of Bictegravir working standard into a 50 ml clean dry volumetric flask add about 35 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent and chromatogram was shown in **Figure 6**.

2.4.4. Preparation of Tenofovir, Emtricitabine and Bictegravir Sample solution:

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 12.5 mg of Tenofovir AF, 100 mg of Emtricitabine and 25 mg of Bictegravir in (marketed formulation=467.8 mg of tablet Powder) sample into a 50 ml clean dry volumetric flask add about 35 ml of Diluent and sonicate it up to 30 min to dissolve it completely and make volume up to the mark with the same solvent and filter through 0.45 micron Injection filter. Further pipette 0.75 ml of Tenofovir, Emtricitabine and Bictegravir from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and chromatogram was shown in **Figure 7**.

2.5 Validation of Analytical Method:

2.5.1. **Linearity:**

The ability to check results which are directly proportional to the concentration of analyte in the given sample range is called as

Linearity. The Linearity was carried out by injecting five different concentrations and calibration curve was plotted and evaluated by its correlation coefficient and relationship was evaluated using the least square method with in Microsoft Excel. The linearity was obtained in the range of 50-250 µg/ml for Emtricitabine, 12.5-62.5 µg/ml for Bictegravir and 6.25-31.25 for Tenofovir respectively and results are tabulated in **Table 2, 3, 4** and calibration curves at **Figure 8, 9, 10**.

2.5.2 Accuracy:

The accuracy of an analytical method was determined by preparing solutions of different concentrations that is 80%,100% and 120% in which the amount of pure drug was varied for that is 8mg,10mg and 12mg for 80%,100% and 120% respectively and the amount of marketed formulation was kept constant. The solutions were prepared in triplicates and the accuracy was indicated by % recovery and results are tabulated in **Table 8, 9, 10**.

2.5.3 Precision:

The peak area which is obtained by determination of six replicates of a fixed amount of drug is called as precision. The Precision obtained was also determined in terms of intra and inter variation in the peak areas for a set of drug solutions in three days. The Peak Area variation was calculated in terms of %RSD and results are tabulated in **Table 6, 7**.

2.5.4 Specifity:

The Specifity of the proposed RP-HPLC method was determined by separation of two peaks with parameters like retention time, resolution and tailing factor

2.5.5 Robustness:

The variations in analytical parameters like flow rate and different pH is studied by Robustness and results are tabulated in **Table 13, 14, 15**.

2.5.6 Ruggedness:

As per the method drug solutions were prepared and injected under variable conditions and results are tabulated in **Table 16, 17, 18**.

2.5.7 Limit of Detection and Limit of Quantification:

As per ICH Guidelines several approaches are mentioned in order to determine the detection and quantification limits. LOD and LOQ were based on the standard deviation of the response and slopes using signal to noise ratio as per the guidelines and results are tabulated in **Table 11 and 12**.

2.6 Forced Degradation Studies

The forced degradation studies conducted on the sample using alkaline, acid, oxidative, thermal, photolytic and UV degradations. To these conditions the sample was exposed and the purity of the peak was studied, thus

showing the method can effectively separated the degradation products from the pure ingredient and chromatograms were represented from **Figure 17-21**.

3. RESULTS AND DISCUSSION:

System suitability parameters and precision were evaluated and were found to be within limits. Between concentration of component and the instrument response plot is drawn and was found to be linear in the concentration range of 50-250 µg/ml for Emtricitabine, 12.5-62.5 µg/ml for Bictegravir and 6.25-31.25 for Tenofovir respectively with good correlation coefficient. Precision and accuracy of the method are determined in %RSD and was found to be within limits. All the system suitability parameters were found to be within the standard limit.

4. CONCLUSION:

The proposed chromatographic method is accurate, precise, rapid, sensitive, selective and robust. The solvents used for the separation of drugs are economical and easily available and usage of same solvents for washing of the column provides an efficient way for the quantification of Emtricitabine, Bictegravir and Tenofovir in bulk drug and also in marketed dosage form. Stability indicated method for the separation of Emtricitabine, Bictegravir and Tenofovir in the respective dosage forms was established and validated as per guidelines of ICH. There was no merging between peaks of active ingredients and any other additive ingredient indicates the purity. Hence the above mentioned method can be used for routine analysis of both the drugs in bulk and pharmaceutical dosage forms and also can be used in Drug monitoring and in bioavailability studies.

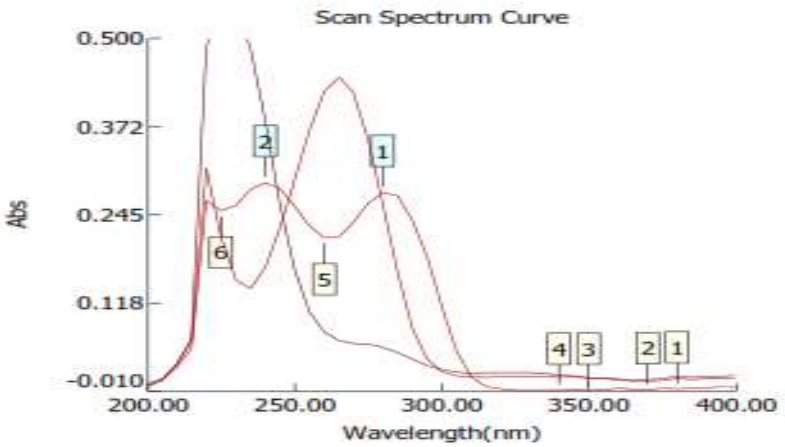

Figure 4: UV Spectrum of Emtricitabine, Bictegravir and Tenofovir

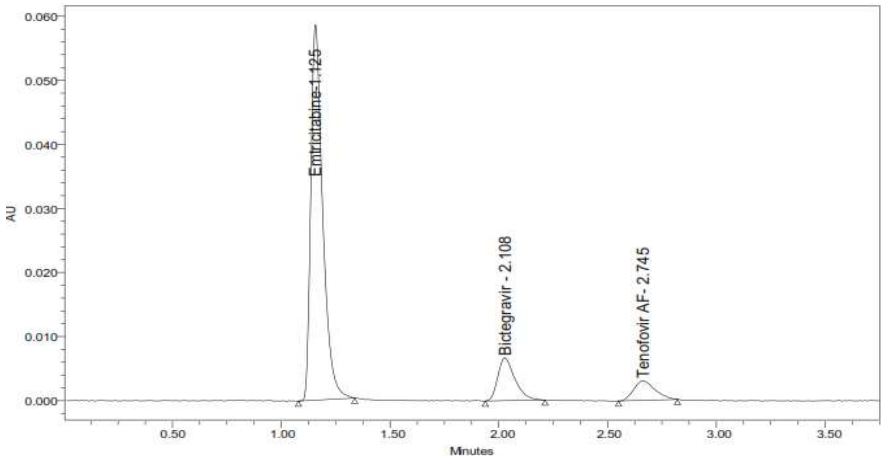

Figure 5: Optimized Chromatogram of Emtricitabine, Bictegravir and Tenofovir

Table 1: System Suitability Parameters of Emtricitabine, Bictegravir and Tenofovir

S. No.	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Emtricitabine	1.125	956581	25309	2.82	1.43	2212.79
2	Bictegravir	2.108	154741	38603	3.97	1.43	3283.92
3	Tenofovir	2.745	89833	16767	7.25	1.28	3577.83

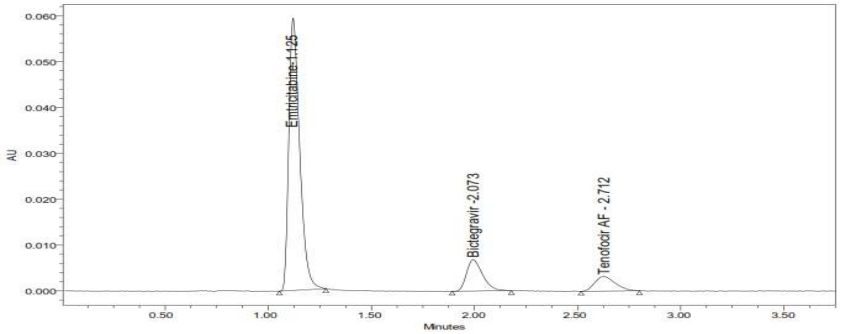

Figure 6: Standard Chromatogram for Emtricitabine, Bictegravir and Tenofovir

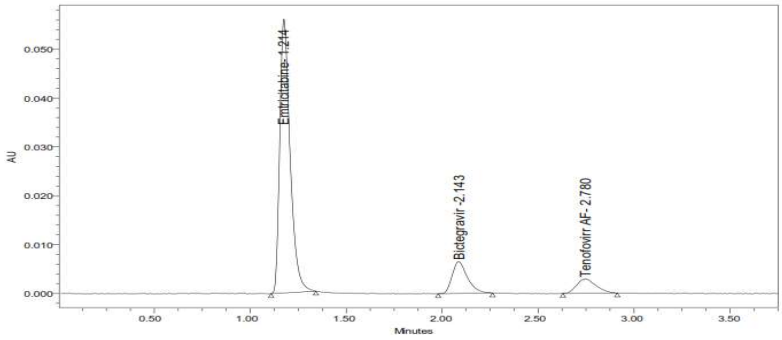

Figure 7: Sample Chromatogram for Emtricitabine, Bictegravir and Tenofovir

Table 2: Linearity Results for Emtricitabine

S. No	Linearity Level	Concentration	Area
1	I	50	522088
2	II	100	734633
3	III	150	950658
4	IV	200	1192066
5	V	250	1430452
Correlation Coefficient			0.999

Table 3: Linearity Results for Bictegravir

S. No	Linearity Level	Concentration	Area
1	I	12.5	65477
2	II	25	110790
3	III	37.5	153097
4	IV	50	193120
5	V	62.5	239955
Correlation Coefficient			0.999

Table 4: Linearity Results for Tenofovir

S. No	Linearity Level	Concentration	Area
1	I	6.25	47257
2	II	12.5	67723
3	III	18.75	89884
4	IV	25	109712
5	V	31.25	134068
Correlation Coefficient			0.999

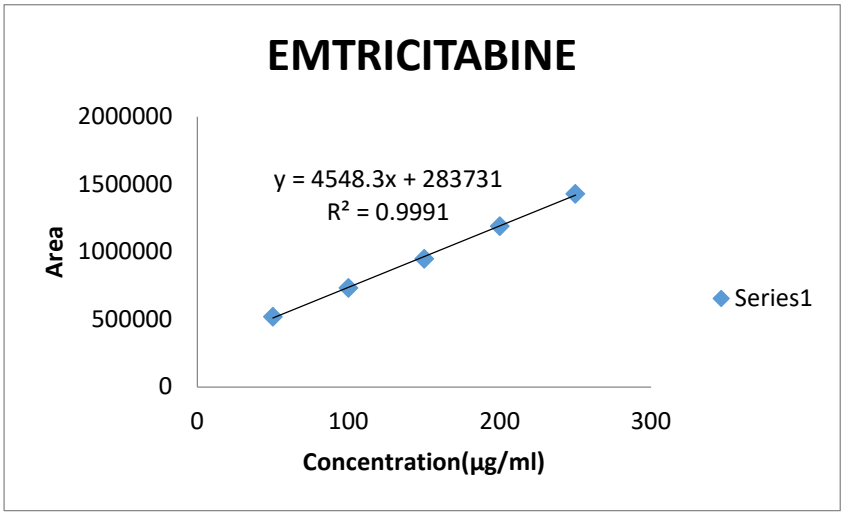

Figure 8: Calibration graph for Emtricitabine

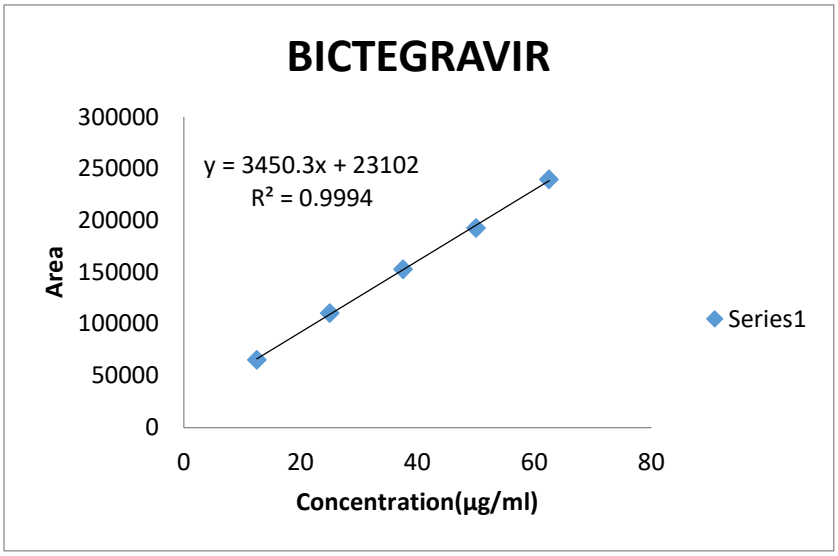

Figure 9: Calibration graph for Bictegravir

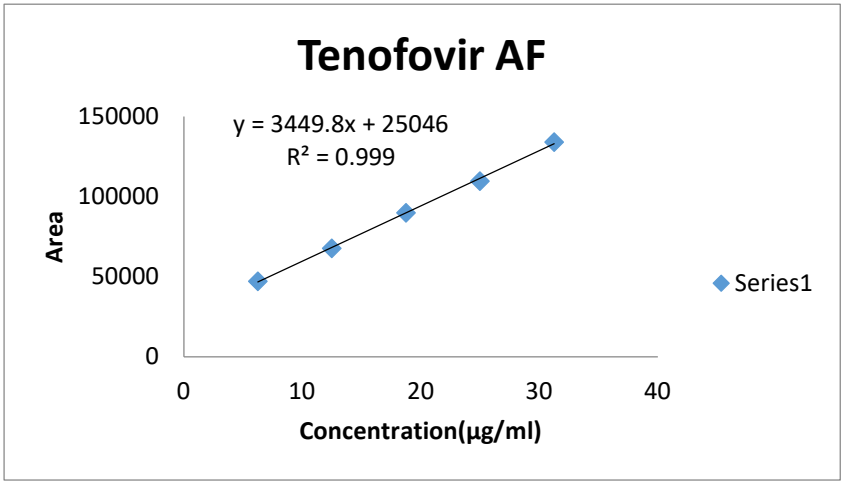

Figure 10: Calibration graph for Tenofovir AF

Table 5: Analytical performance parameters

Parameters	Emtricitabine	Bictegravir	Tenofovir
Slope (m)	4548.3	3450.3	3449.8
Intercept (c)	283731	23102	25046
Correlation coefficient (R^2)	0.999	0.999	0.999

Table 6: Results of Precision for Emtricitabine, Bictegravir and Tenofovir

Injection	Emtricitabine	Bictegravir	Tenofovir
Injection-1	957498.0	158363.0	89485.0
Injection-2	958373.0	158376.0	89474.0
Injection-3	958377.0	158237.0	89648.0
Injection-4	958374.0	158373.0	89467.0
Injection-5	959484.0	158932.0	89364.0
Injection-6	954484.0	158383.0	89464.0
Average	957765.0	158444.0	89483.7
Standard Deviation	1726.6	245.3	91.7
%RSD	0.2	0.2	0.1

Table 7: Results of Intermediate Precision for Emtricitabine, Bictegravir and Tenofovir

Injection	Emtricitabine	Bictegravir	Tenofovir
Injection-1	959473.0	158387.0	87983.0
Injection-2	958474.0	158327.0	87838.0
Injection-3	958373.0	158363.0	87537.0
Injection-4	958363.0	158736.0	87538.0
Injection-5	959373.0	158373.0	87373.0
Injection-6	958363.0	157368.0	87293.0
Average	958736.5	158259.0	87593.7
Standard Deviation	534.3	461.8	267.1
%RSD	0.1	0.3	0.3

Table 8: Accuracy (recovery) data for Emtricitabine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	476290	100	99.38	99.38	99.98
100%	957024	200	199.69	99.85	
150%	1448027	300	302.15	100.72	

Table 9: Accuracy (recovery) data for Bictegravir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	77719	25	25.06	100.25	99.78
100%	154381	50	49.78	99.57	
150%	231466	75	74.64	99.52	

Table 10: Accuracy (recovery) data for Tenofovir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	45290	12.5	12.58	100.63	99.99
100%	89720	25	24.92	99.67	
150%	134564	37.5	37.37	99.66	

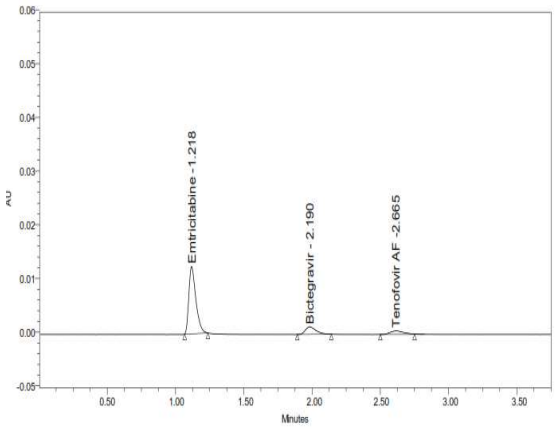

Figure 10: Chromatogram of LOD

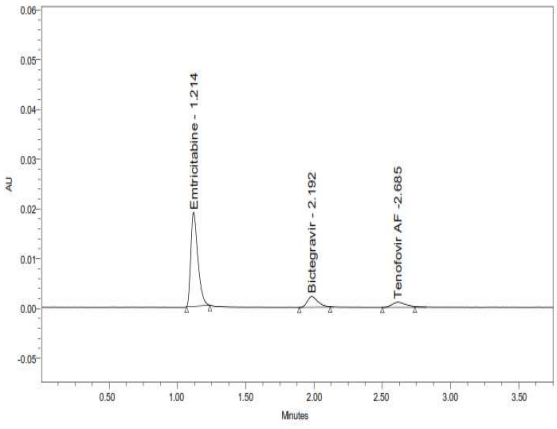

Figure 11: Chromatogram of LOQ

Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Emtricitabine	66	197	2.98
Bictegravir	66	198	3.00
Tenofovir	66	194	2.94

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Emtricitabine	**66**	**659**	**9.98**
Bictegravir	**66**	**660**	**10**
Tenofovir	**66**	**658**	**9.97**

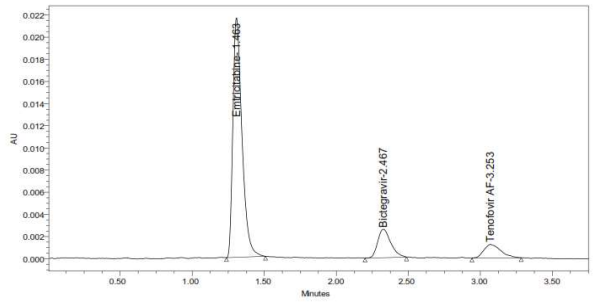

Figure 13: Chromatogram showing less flow

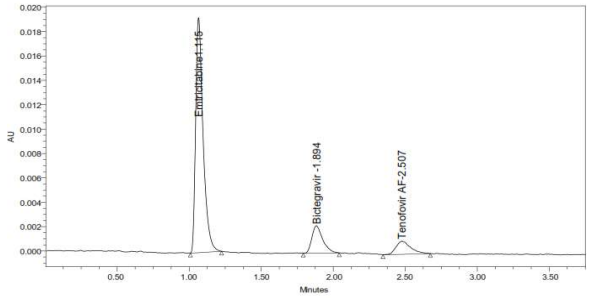

Figure 14: Chromatogram showing more flow

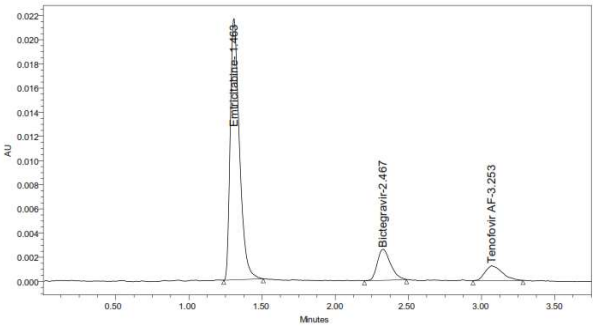

Figure 15: Chromatogram showing less organic composition

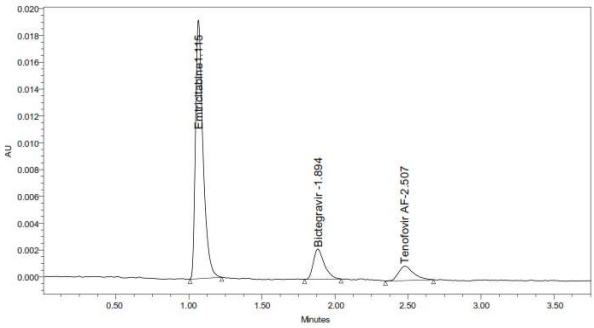

Figure 16: Chromatogram showing less organic composition

Table 13: System suitability results for Emtricitabine

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	**0.9**	**2263.65**	**1.40**
2	**1.0**	**2112**	**1.45**
3	**1.1**	**2151.29**	**1.44**

Table 14: System suitability results for Bictegravir

S. No.	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	**0.9**	**3331.30**	**1.29**	**3.87**
2	**1.0**	**3186.09**	**1.33**	**3.90**
3	**1.1**	**2971.64**	**1.41**	**3.89**

Table 15: System suitability results for Tenofovir

S. No.	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	**0.9**	**3035.38**	**1.40**	**7.38**
2	**1.0**	**3353.63**	**1.27**	**7.31**
3	**1.1**	**3465.98**	**1.41**	**7.11**

Table 16: System suitability results for Emtricitabine

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2445.83	1.40
2	*Actual	2112	1.45
3	10% more	2104.64	1.39

Table 17: System suitability results for Bictegravir

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	3594.68	1.29	4.68
2	*Actual	3186.09	1.33	3.90
3	10% more	2935.13	1.48	3.61

Table 18: System suitability results for Tenofovir

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	10% less	5094.60	1.32	9.15
2	*Actual	3353.63	1.27	7.31
3	10% more	3252.62	1.37	6.02

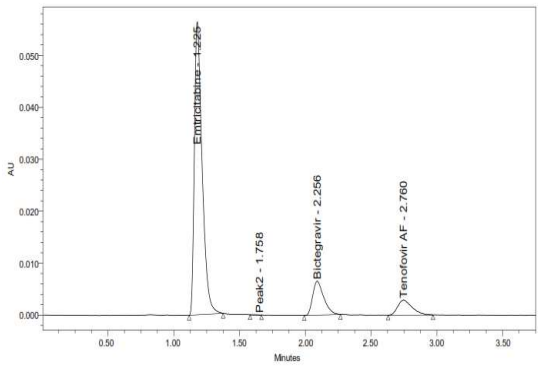

Figure 17: Chromatogram showing acid degradation

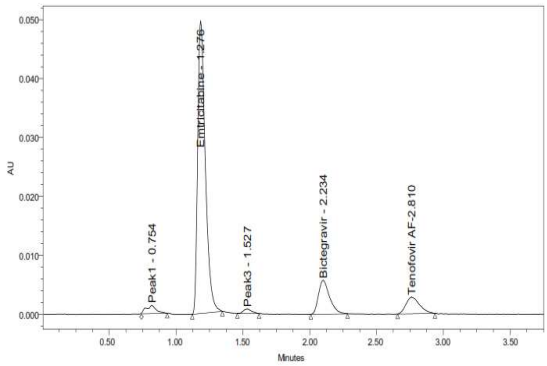

Figure 18: Chromatogram showing Base degradation

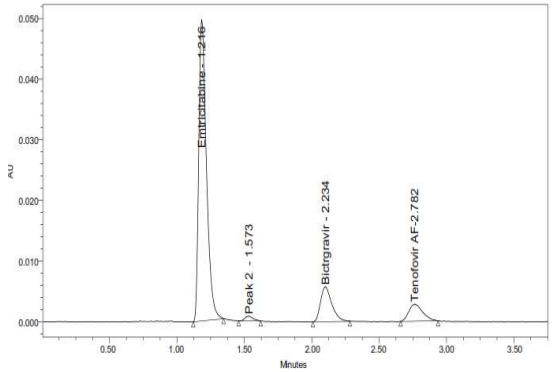

Figure 19: Chromatogram showing Peroxide degradation

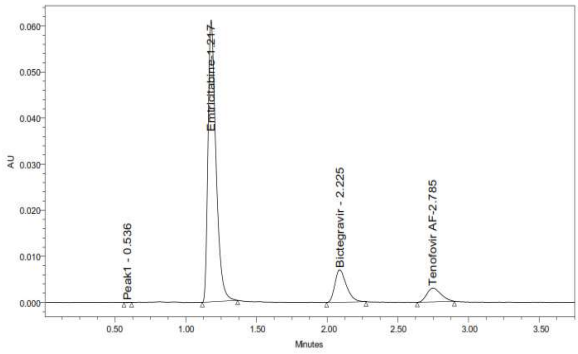

Figure 20: Chromatogram showing Photo degradation

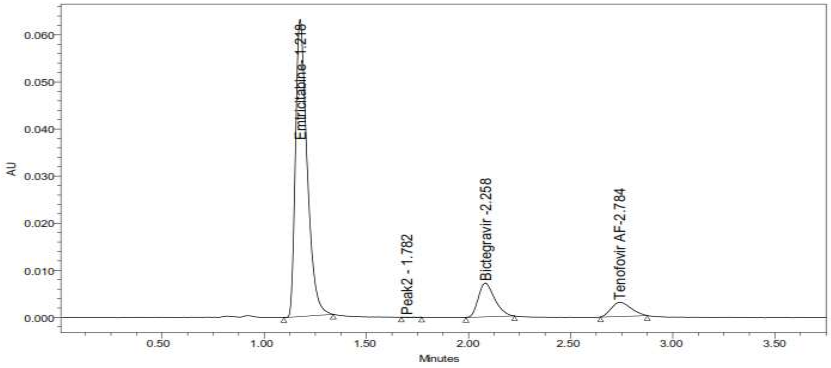

Figure 21: Chromatogram showing Thermal degradation

5. REFERENCES:

[1] B K Sharma, Instrumental Methods of Chemical Analysis, Goel publishing house, Meerut, 28th Ed, 2012: 286-385.

[2] Conner's K A, A Text Book of Pharmaceutical Analysis, CBS Publishers and Distributors Pvt.

[3] Ltd., New Delhi, 3rd ed, 2001, 3-6.

[4] Kumar, Ajay & Sunil, Jawla & Yadav, Gajanand. Recent analytical method developed by RP-HPLC. Global Journal of Pharmacology, 2013(7): 232-240.

[5] Kokkirala, Tej & Duvvuri, Suryakala. (2019). RP-HPLC method development and validation for the estimation of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical dosage form. Journal of Taibah University for Science, 2019; 13:1137-1146.

[6] https://www.drugbank.ca/drugs/DB00879

[7] https://www.drugbank.ca/drugs/DB11799

[8] https://www.drugbank.ca/drugs/DB09299

[9] http://static.medicine.iupui.edu/divisions/infdresearch/ GS1489.pdf

[10] Pavan Kumar Kasu, Battu Satyanarayana. Real-Time Fortitude of Emtricitabine, Dolutegravir And Tenofovir In Pharmaceutical Dosage By Rp-Hplc Method Through Stability Studies. Journal of Pharmaceutical Negative Results, 2022; 13(9):678–689.

[11] Gurumukhi VC, Bari SB. Quantification and validation of stability-indicating RP-HPLC method for efavirenz in bulk and tablet dosage form using quality by design (QbD): A shifting paradigm.

Journal of Chromatographic Science. 2022 Feb;60(2):143-56.

[12] 10.Stellbrink HJ, Lazzarin A, Woolley I, Llibre JM. The potential role of bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) single-tablet regimen in the expanding spectrum of fixed-dose combination therapy for HIV. HIV medicine. 2020;21:3–16

[13] Gurmeet S. Chhabra, Aayushi Rajora, Dinesh K. Mishra. Stability indicating RP-HPLC method for the determination of Tenofovir in pharmaceutical formulation. Research Journal of Pharmacy and Technology. 2021; 14(12):6335-6339.

[14] Akram NMD, Umamahesh M. A New Validated RP-HPLC Method for the Determination of Emtricitabine and Tenofovir AF in its Bulk and Pharmaceutical Dosage Forms. Journal of Chemical and Pharmaceutical Sciences. 2017; 10(4): 54–59.

[15] Abdelhay, M.H.; Gazy, A.A.; ShaalanRA.; Ashour HK. Selective RP-HPLC DAD Method for Determination of Tenofovir fumarate and Emtricitabine in BulkPowder and in Tablets. Acta Chromatographica. 2015; 27: 41–54.