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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF EMTRICITABINE, BICTEGRAVIR AND TENOFOVIR IN BULK AND TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method with a high sensitivity was developed for simultaneous estimation of Emtricitabine, Bictegravir and Tenofovir in pharmaceutical dosage form. Chromatographic separation of Emtricitabine, Bictegravir and Tenofovir were successfully achieved on an Agilent C18 (150×4.6mm, 5µ) with an mobile phase composed of a mixture of 0.01N Na_2 HPO_4: acetonitrile (50:50, v/v) at a flow rate of 1.00 mL min−1. The drugs were quantified using a wavelength of 272 nm. The reversed-phase HPLC method has been validated as per International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The proposed method showed a good linearity in the concentration range of 25–150 µg/mL for Emtricitabine, 6.25-37.5 µg/mL for Bictegravir and 3.125-18.75 µg/mL for Tenofovir under optimized conditions. The statistical performance of the HPLC method was fully validated and the performance results of the proposed HPLC method were considerably satisfactory with reference to the RSD values of validation parameters like linearity, system precision, method precision, robustness, ruggedness etc.,. The validated method was successfully applied to quantify the Emtricitabine, Bictegravir and Tenofovir in tablet form.

KEYWORDS: Emtricitabine, Bictegravir and Tenofovir, RP-HPLC.

INTRODUCTION

Emtricitabine[1] is a nucleoside reverse transcriptase inhibitor (NRTI) indicated for the treatment of HIV infection in adults or combined with Emtricitabine alafenamide for the prevention of HIV-1 infection in high risk adolescents and adults. Emtricitabine is a cytidine analogue. The drug works by inhibiting HIV reverse transcriptase, preventing transcription of HIV RNA to DNA.

Bictegravir[2] is a recently approved investigational drug that has been used in trials studying the treatment of HIV-1 and HIV-2 infection. It has been approved for HIV-1 monotherapy combined with 2 other antiretrovirals in a single tablet.

Tenofovir[3] is an acyclic nucleotide diester analog of adenosine monophosphate. In the moststrict sense and due to the fact that it presents a phosphate group bound to the nitrogenous base, it is determined as an actual nucleotide analog.

Emtricitabine is described chemically 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)-1, 3 oxathiolan-5-yl] cytosine. It has a molecular formulaC_8H_{10} FN_3O_3S and has the following structural formula.

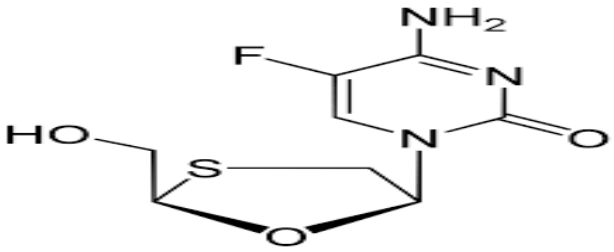

Fig. 1: Chemical structure of emtricitabine.

Bictegravir is described chemically 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. It has a molecular formula C_8H_{11} N_3O_3S and has the following structural formula

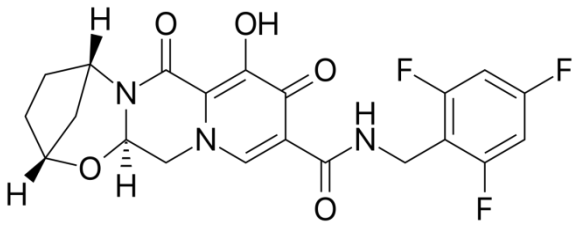

Fig. 2: Chemical structure of bictegravir.

Tenofovir is described chemically ({[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl]oxy}methyl) phosphonic acid. It has a molecular formula $C_9H_{14}N_5O_4P$ and has the following structural formula

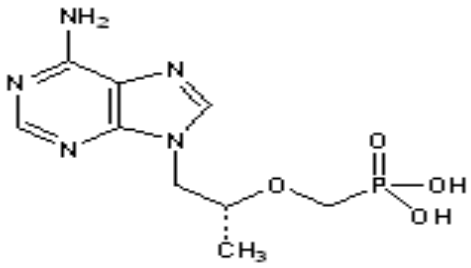

Fig. 3: Chemical structure of tenofovir.

According to the literature search, there are few high performance liquid chromatography (HPLC) methods for estimation of selected drugs in single or in combination with other drugs[4-8] were reported.

METHODS

Chemicas and Reagents

All the reagents used in the experimental work were of analytical grade. HPLCgrade water was prepared by Milli-Qreverse osmosis (Millipore, Bedford, USA) and meets European Pharma-copoeia requirements. ACN (Sigma–Aldrich, Merk and Rankem) were used for preparing the mobile phase. Mobile Phase was used as solvent. Working standards of Emtricitabine, Bictegravir and Tenofovir were provided by Glenmark Pharmaceuticals (Mahape, Navi Mumbai). Biktarvy® (containing 200mg of Emtricitabline, 50mg of Bictegravir and 25mg of Tenofovir) were purchased from local market.

Chramatographic conditions (Instrumentation and Analytical conditions)

Instruments

An Alliance 2695 (Waters, USA) chromatographic system was used, equipped with a Quaternary pump, and waters 2996 photo diode array detector, Agilent C18 (150×4.6mm,5µ), auto sampler thermostat and degasser. Chromatographic software Empower was used for data collection and processing Separations were performed using Agilent C18 (150×4.6mm, 5µ) packed with 5 µm particle size. A 1m long steel capillary with 0.25 mm internal diameter, was inserted between the injection system and

the entrance of the column, and injection volume was 10µL. Separations and simultaneous determination of Emtricitabine, Bictegravir and Tenofovir were performed using the mixture of Acetonitrile: Water (50:50 v/v) as a mobile phase. Mobile phase was filtered through a 0.45µm Millipore filter. The flow rate was 1.0 mL min−1 and the UV detection was performed at 272 nm**.**

Analytical procedure

Preparation of standard stock solutions

Accurately Weighed and transferred 50mg of Emtricitabine, 6.25mg & of Tenofovir and 12.5mg of Bictegravir working Standards into a 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (1000µg/ml Emtricitabine, & 125µg/ml Tenofovir and 250 µg/ml of Bictegravir).

Assay sample preparation

The label claim Emtricitabine200mg, Bictegravir50mg and Tenofovir25mg per unit formulation Assay was performed with the above formulation. Average % Assay for Emtricitabine, Bictegravir and Tenofovir. Obtained was 99.50%, 99.78% and 99.89% respectively.

Validation

Chromatographic separation was optimized in the aim to obtain a resolution above 1.5 between all components, with the respect of stationary and mobile phase compositions, flow rate, sample volume, detection wavelength and temperature.

The method was validated for linearity, precision (Repeatability and intermediate precision), specificity, limit of quantitation, limit of detection and robustness.

Linearity

Standard calibration curves were prepared with six calibrators over a concentration range of 25-150µg/ml for Emtricitabine, 6.25-37.5 µg/ml for Bictegravir and 3.125-18.75 µg/ml for Tenofovir. The data of peak area versus drug concentration were treated by linear least square regression analysis. The standard curves were evaluated for linearity.

Precision

The precision of the assay was studied with respect to both repeatability and

intermediated precision. Repeatability was calculated from six replicate injections of freshly prepared solution in the same equipment on the same day. Repeatability for Emtricitabine, Bictegravir and Tenofovir was realized with a 1.1, 0.7, and 1.0 solution. The experiment was repeated by assaying freshly prepared solution at the same concentration on 2 additionally consecutive days to determine intermediate precision. Precision was expressed by the % of the relative standard deviation (R.S.D.) of the analyte peaks.

Specificity: Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Limits of Detection and Quantization: Limits of detection (LOD) and limits of quantization (LOQ) were provided and calculation was made with the following equations:

LOD=3.3 σ /SLOQ=10 σ /S

When σ was the standard deviation of the response (Estimated from the standard deviation of *y*- intercepts or regression lines) and S was the slope of the standard curve.

Sensitivity

The sensitivity (*6x*) of an analytical method is defined by the minimum variation that requires to be applied to the magnitude measured in order to obtain a significant variation in the signal measured.

Robustness

Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate (±20%), organic content in mobile phase (± 2%). Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

RESULTS AND DISCUSSION

Firstly, HPLC conditions were optimized to obtain a desired peak with high purity and resolution. Therefore, the various parameters affecting the peak shape, retention time and resolution were investigated in detail. The separation efficiency of PhenomenexC18 (150x4.6 mm, 5µ) the same conditions, and the proposed column was chosen for the further optimization of parameters. During our preliminary experiments, the series of aqueous

mobile phases containing buffer solutions with the different pH values in combination with different organic modifiers including the different ratios of acetonitrile, methanol and triethylamine were tested for obtaining the optimum separation conditions. Acetonitrile and *Ortho*-Phosphoric acid were selected as the eluents. The chromatographic analysis time was shortened with high organic solvent content, and also, the buffer solutions in the mobile phase ensured stable chromatographic retention times preventing broad peaks. The effect of the mobile phase pH on the retention time and peak shape of the analyte was studied especially in the acidic region. The best retention time and peak shape were achieved with 0.01nna2hpo4 buffer. The best separation was achieved with the mobile phase consisting of 0.01NNA2HPO4: Acetonitrile (50:50, v/v). The calibration curves analysis were constructed by plotting the peak area aganist the concentration of the drugs.

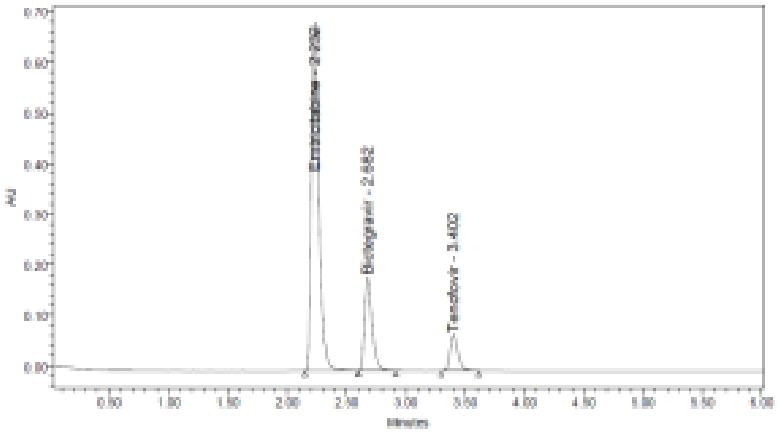

Fig. 4: Typical chromatogram.

Method validation

The method was validated for linearity, precision, accuracy, robustness, ruggedness, forced degradation and stability.

Linearity was prepared in the range of 25-150µg/ml for Emtricitabine and 6.25-37.5µg/ml for Bictegravir and 3.125-18.75 for Tenofovir solutions are analyzed through the high pressure liquid chromatographic technique. The peak area were plotted against concentration was subjected to linear plots shown in figures 5, 6&7.

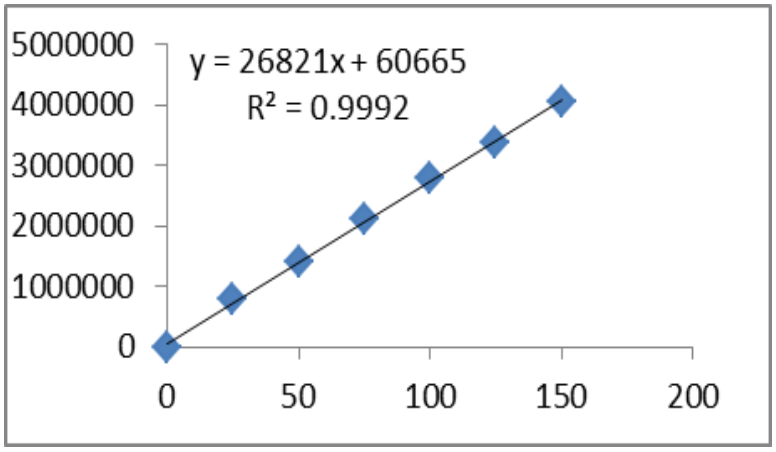

Fig. 5: Linearity plot for emtricitabine.

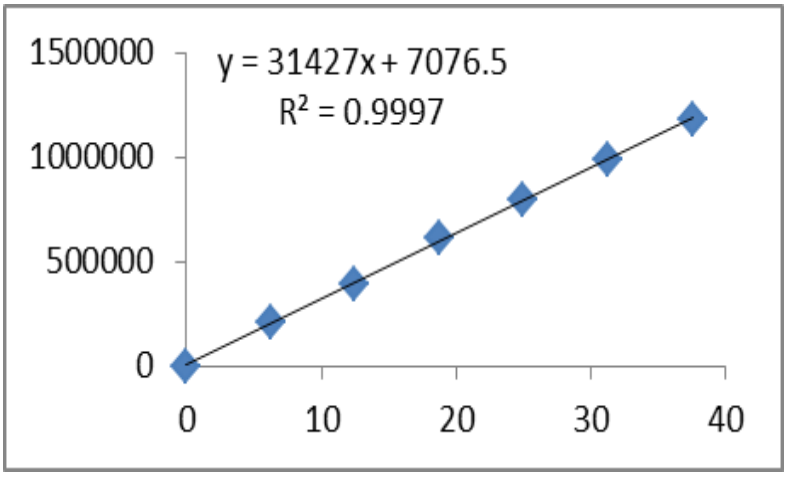

Fig. 6: Linearity plot for bictegravir.

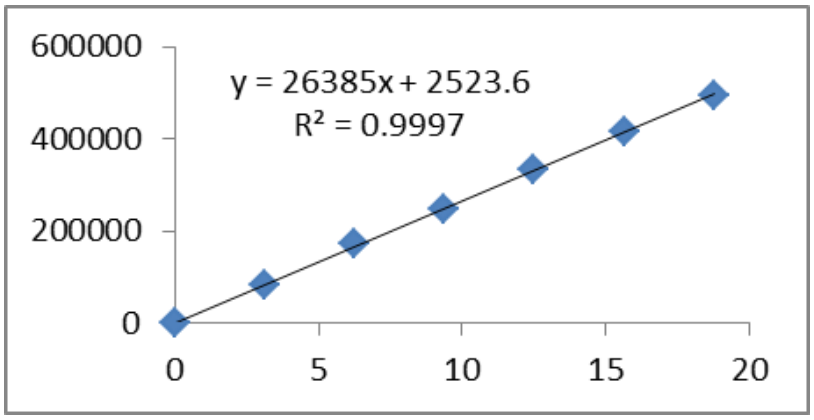

Fig. 7: Linearity plot for tenofovir.

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for three drugs and obtained as 1.1%, 0.7% and 1.0% respectively for Emtricitabine, bictegravir and Tenofovir. As the limit of Precision was less than "2" the

system precision was passed in this method.

Table 1: Recovery of emtricitabine drug.

% Level	Amount Spiked (µg /mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	50.0642	100.13	100.24%
	50	50.27307	100.55	
	50	49.89169	99.78	
100%	100	99.33034	99.33	
	100	100.7251	100.73	
	100	100.9009	100.90	
150%	150	149.0973	99.40	
	150	151.5272	101.02	
	150	150.5039	100.34	

Table 2: Recovery of Bictegravir drug.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	100.13	12.39038	99.12	100.30%
	100.55	12.61468	100.92	
	99.78	12.60695	100.86	
100%	99.33	24.90153	99.61	
	100.73	24.91722	99.67	
	100.90	24.95321	99.81	
150%	99.40	37.9134	101.10	
	101.02	37.81887	100.85	
	100.34	37.78199	100.75	

Table 3: Recovery of tenofovir drug.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	6.25	6.183131	98.93	100.09%
	6.25	6.292435	100.68	
	6.25	6.308884	100.94	
100%	12.5	12.54129	100.33	
	12.5	12.45715	99.66	
	12.5	12.47962	99.84	
150%	18.75	18.68194	99.64	
	18.75	18.798	100.85	
	18.75	18.847	100.75	

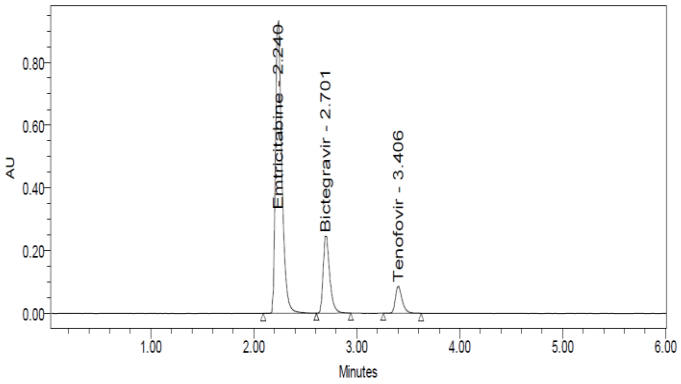

Fig. 8: Chromatogram for accuracy 50%.

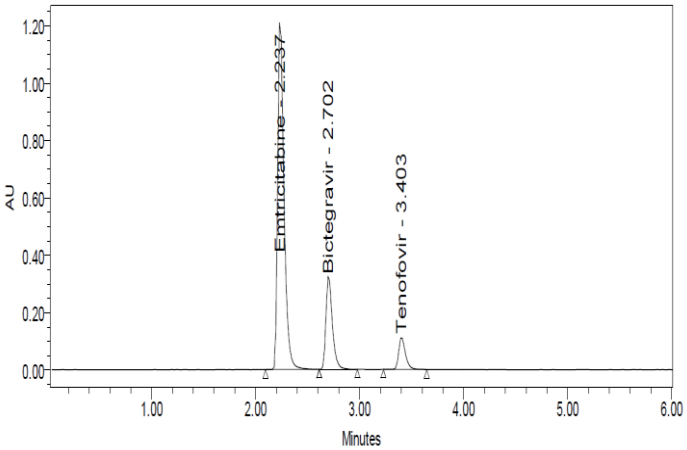

Fig. 9: Chromatogram for accuracy 100%.

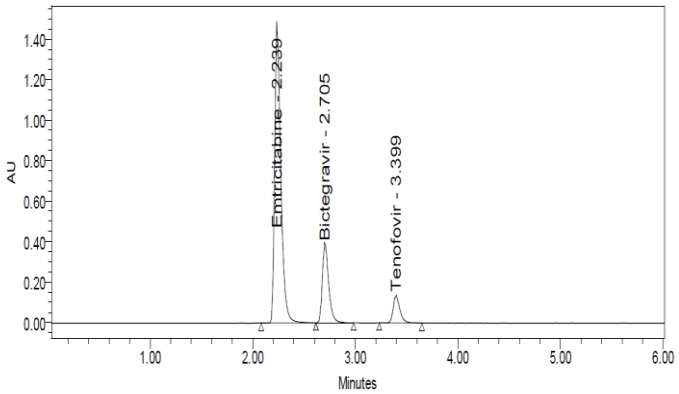

Fig. 10: Chromatogram for accuracy 150%.

The limit of detection (LOD) for Emtricitabine, Bictegravir and Tenofovir were found to be 0.99, 0.30 and 0.10 µg/mL. calculated from related equation (S/N = 3). The similar study claimed that a narrowworking range (LOQ) such as 3.00, 0.91 and 0.30 µ g /mL.

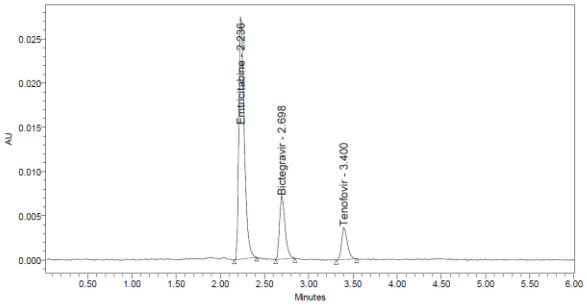

Fig. 9: Chromatogram for LOD.

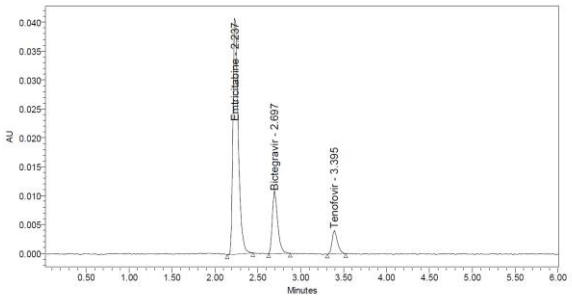

Fig. 9: Chromatogram for LOQ.

Forced 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.

Table 4: Degradation date of emtricitabine.

S. no.	Degradation Condition	% Area Recovery	% Drug Degraded
1	Acid	93.86	6.14
2	Alkali	95.07	4.93
3	Oxidation	96.02	3.98
4	Thermal	97.65	2.35
5	UV	98.24	1.76
6	Water	99.17	0.83

Table 5: Degradation date of bictegravir.

S. no.	Degradation Condition	% Area Recovery	% Drug Degraded
1	Acid	94.18	5.82
2	Alkali	95.21	4.79
3	Oxidation	96.01	3.99
4	Thermal	97.33	2.67
5	UV	98.30	1.70
6	Water	99.38	0.62

Table 6: Degradation date of tenofovir.

S. no.	Degradation Condition	% Area Recovery	% Drug Degraded
1	Acid	93.75	6.25
2	Alkali	95.22	4.78
3	Oxidation	95.93	4.07
4	Thermal	97.65	2.35
5	UV	98.55	1.45
6	Water	99.48	0.52

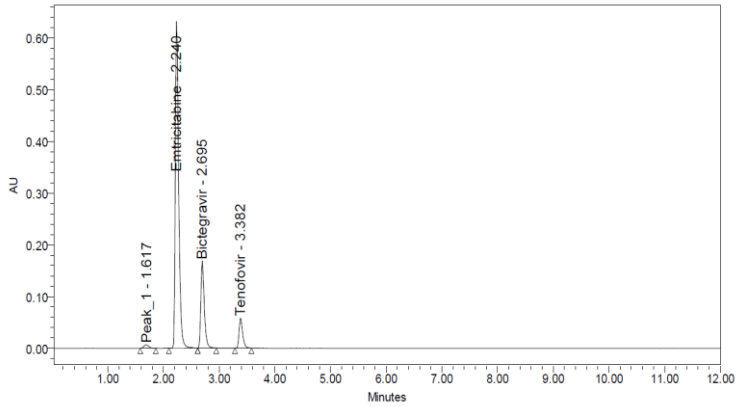

Fig.11: Chromatogramfor acid degradation emtricitabine, Bictegravir and Tenofovir.

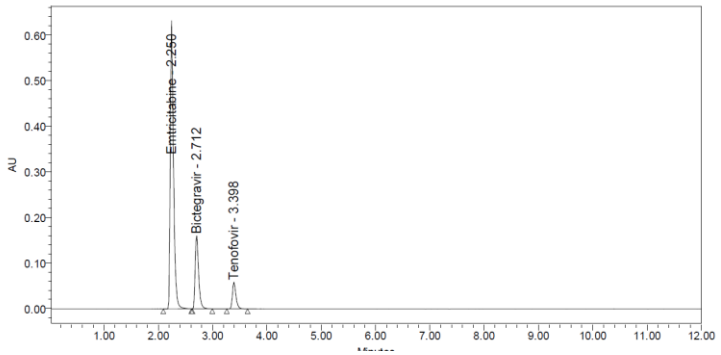

Fig.12: ChromatogramforBase Degradation Emtricitabine, Bictegravir and Tenofovir.

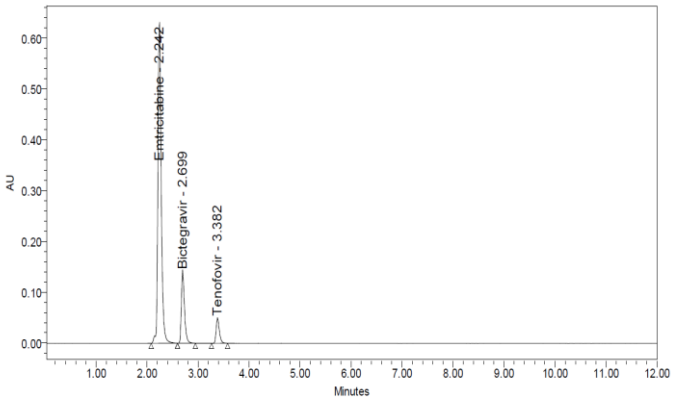

Fig. 13: Chromatogram for Peroxide Degradation Emtricitabine, Bictegravir and

Tenofovir.

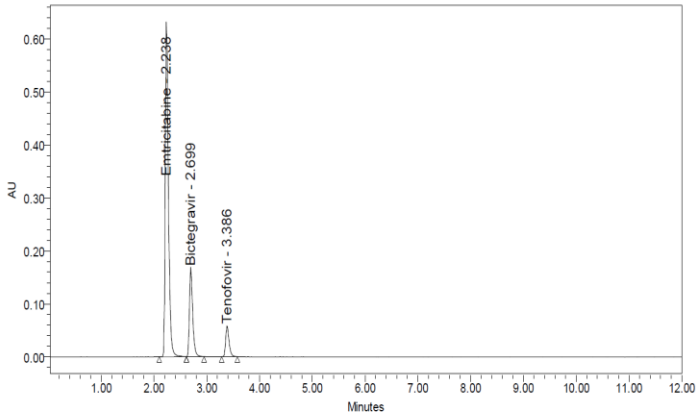

Fig. 14: Chromatogram for Thermal Degradation Emtricitabine, Bictegravir and Tenofovir.

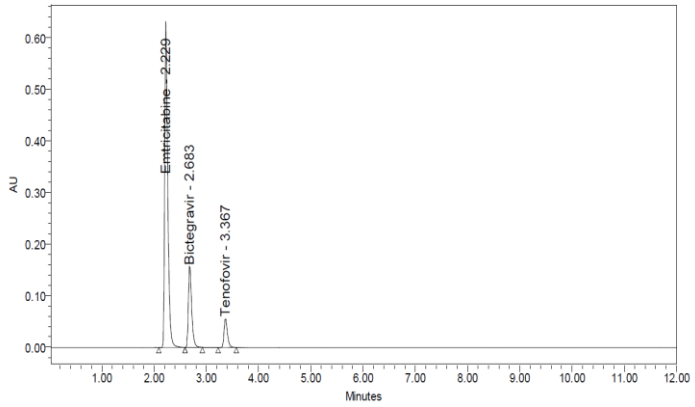

Fig. 15: Chromatogram for UV Degradation Emtricitabine, Bictegravir and Tenofovir.

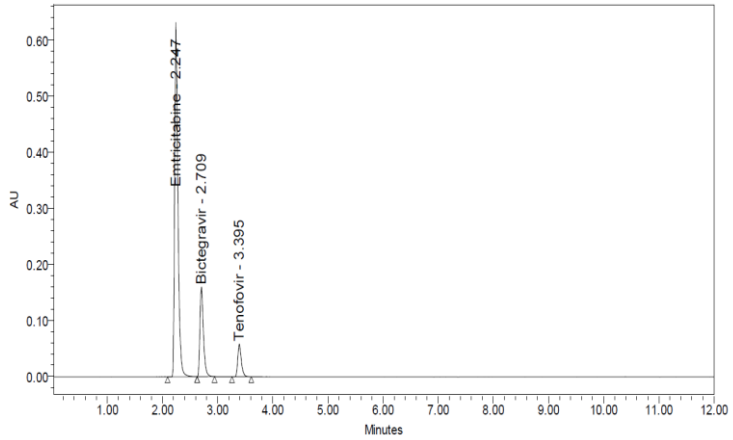

Fig. 16: Chromatogram for water degradation Emtricitabine, Bictegravir and Tenofovir.

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (23°C) and temperature plus (27°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit

Table 7: Robustness data for Emtricitabine, Bictegravir and Tenofovir.

S. no.	Condition	%RSD of Emtricitabine	%RSD of Bictegravir	%RSD of Tenofovir
1	Flow rate (-) 0.9ml/min	0.5	0.6	0.9
2	Flow rate (+)1.1ml/min	0.6	0.7	0.4
3	Mobile phase (-) 55B:45A	11	0.9	0.2
4	Mobile phase (+) 45B:55A	0.4	0.4	0.5
5	Temperature (-) 23°C	0.3	0.2	1.3
6	Temperature (+) 27°C	0.5	0.8	1.4

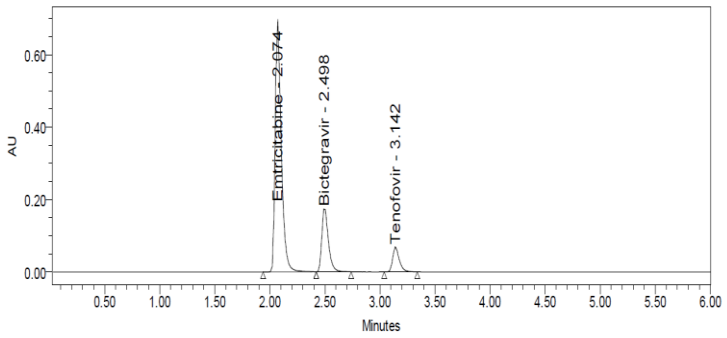

Fig. 17: Chromotagram for flow plus.

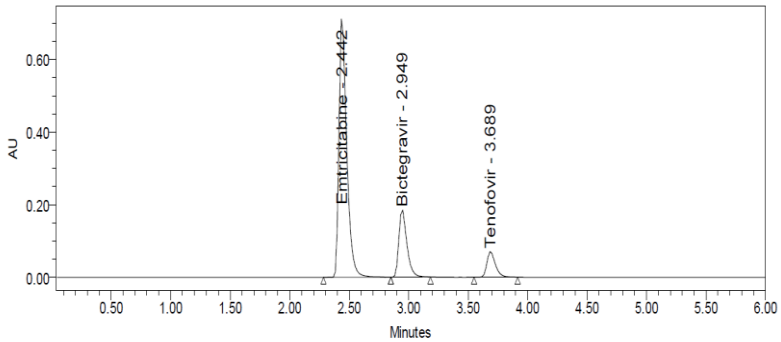

Fig. 18: Chromotagram for flow minus.

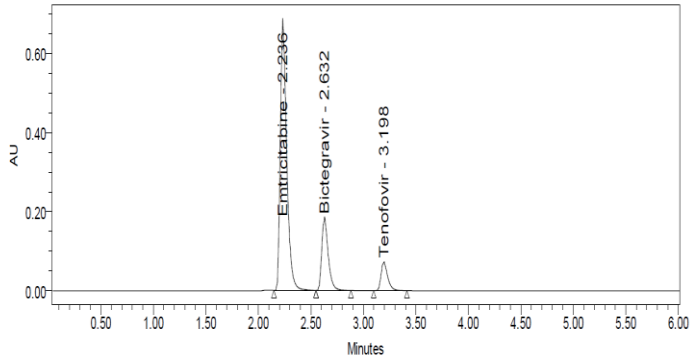

Fig. 19: Chromotagram for mobile phase minus.

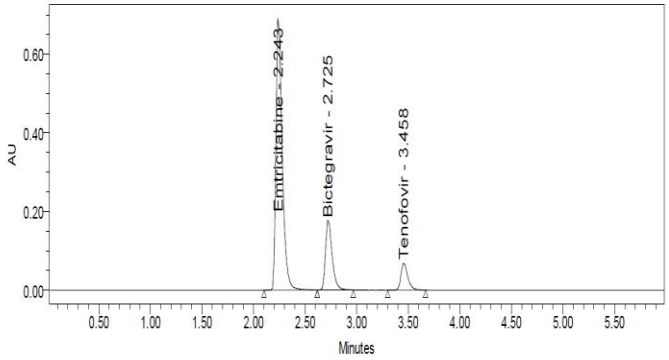

Fig. 20: Chromotagram for mobile phase.

CONCLUSION

A highly sensitive and effective validated reversed-phase HPLC method was successfully developed with a low LOD value for Emtricitabine, Bictegravir and Tenofovir assay. The Emtricitabine, Bictegravir and Tenofovir were subjected to forced degradation under several stress conditions. The satisfactory results were achieved from degradation studies, which revealed that the method was stability indicating. Besides This method was validated for linearity, accuracy, precision, robustness of Emtricitabine, Bictegravir and Tenofovir drug. The RSD values for all parameters were found to be less 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used as better analytical tool for pharmaceutical formulations of Emtricitabine, Bictegravir and Tenofovir drug.

REFERENCES

1. https://go.drugbank.com/drugs/DB00879
2. https://go.drugbank.com/drugs/DB11799
3. https://go.drugbank.com/drugs/DB14126

4. Bhushan P. Badgujar, MoreshwarP.Mahajan et al.., Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Tenofovir Alafenamide and Emrtricitabine in Bulk and Tablet Dosage Form, International Journal of ChemTech Research, 2017; 10(5): 731-739.
5. Dr. VVSS. Appala Raju et al.., Bioanalytical Method Development and Validation For Simultaneous Determination of Bictegravir, Emrtricitabine and Tenofovir In HumanPlasma By Lcms/Ms, WJPPS, 2018; 7(12).
6. ChinnalalaiahRunja , Pigili Ravi Kumar et al.., A Validated Stability Indicating RP-HPLC Method for the Determination of Emrtricitabine, Tenofovir Disoproxil Fumarate, Elvitegravir and Cobicistat in Pharmaceutical Dosage Form, J Chromatogr Sci, May-Jun, 2016; 54(5): 759-64.
7. Prashant S. Devrukhakar et al…, Prashant S. Devrukhakar, Roshan Borkar, Nalini Shastri, K. V. Surendranath, "A Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Emrtricitabine, Tenofovir, and a Efavirenz and Statistical Approach to Determine the Effect of Variables", International Scholarly Research Notices, 2013.
8. Vasanti M. Suvarna et al.., Development and Validation of Stability Indicating Rp-Hplc Method For Tenofovir Solid Lipid Nanoparticles, Int J Pharm Sci Res, 2017; 8(2): 658-66.