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VALIDATED METHOD FOR THE SIMULTANEOUS DETERMINATION OF EMTRICITABINE, BICTEGRAVIR AND TENOFOVIR ALAFENAMIDE IN PHARMACEUTICAL DOSAGE FORM USING UPLC

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Keywords:

Emtricitabine, Bictegravir, Tenofovir alafenamide, UPLC, Method validation

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ABSTRACT: The proposed study aimed to develop and validate a method for the simultaneous determination of emtricitabine, bictegravir, and tenofovir alafenamide in pharmaceutical dosage form using ultra-ultra dosage performance liquid chromatography (UPLC). The drugs were separated using HSS C18 (100 x 1.8mm, 1.7µ) column with a mobile phase composition consisting of 0.1% orthophosphoric acid (pH 2.2) and acetonitrile in the ratio 70:30%v/v at a flow rate of 0.3ml/min. The column temperature was maintained at 30°C, and a detection wavelength of 260nm was used. The retention times were found to be 0.62mins for emtricitabine, 0.89 min for bictegravir and 1.75 min for tenofovir alafenamide. The developed method was validated in accordance with the international conference on harmonization (ICH) guidelines and found to be accurate, precise, specific, and robust. The method obeyed Beer's law in the 50 – 300µg/ml concentration range for emtricitabine, 12.5 – 75µg/ml for bictegravir and 6.25 – 37.5µg/ml for tenofovir alafenamide, with a correlation coefficient of 0.999. The developed method can be used for the routine quantitative analysis in quality control for the determination of emtricitabine, bictegravir, and tenofovir alafenamide in the pharmaceutical dosage form.

INTRODUCTION: Emtricitabine [1-3] **Fig. 1A**, chemically designated as 4-amino-5-fluoro-1-[(2*R*, 5*S*) – 2 - (hydroxymethyl) - 1, 3 – oxathiolan – 5 - yl] pyrimidin-2-one is an antiviral drug. It has a molecular formula of $C_8H_{10}FN_3O_3S$ with a molecular weight of 247.25 g/mol and has a pKa value of 2.65. It is a white to off-white powder and soluble in water. It is used to treat HIV infection, which inhibits the activity of the human immunodeficiency virus (HIV) reverse transcriptase enzyme resulting in deoxyribonucleic acid (DNA) chain termination.

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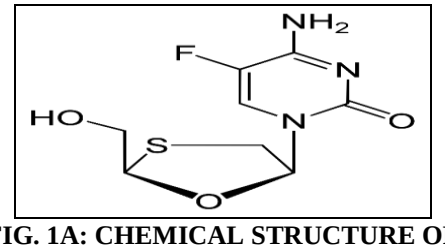

FIG. 1A: CHEMICAL STRUCTURE OF EMTRICITABINE

Bictegravir [4-6] **Fig. 1B**, chemical name 2,5-Methanopyrido [1',2':4,5] pyrazino [2,1-b] [1,3] oxazepine-10-carboxamide, 2,3,4,5,7,9,13,13a-octahydro – 8 – hydroxyl - 7, 9 – dioxo – N - [(2,4,6-trifluorophenyl) methyl]-, sodium salt (1:1), (2R,5S,13aR)- is an antiviral drug. It has a molecular formula of $C_{21}H_{17}F_3N_3NaO_5$ with molecular weight of 471.4g/mol has a pKa value of 9.81. It is an off-white to yellow solid having solubility in water.

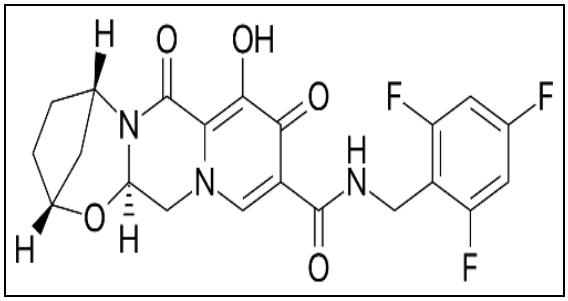

FIG. 1B: CHEMICAL STRUCTURE OF BICTEGRAVIR

It is used to treat HIV infection by acting as an HIV – I integrase strand transfer inhibitor, which results in the inhibition of HIV replication into the human genome. Tenofovir alafenamide [7-9] **Fig. 1C** with chemical nameL-alanine, N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)-1methylethoxy] methyl] phenoxyphosphinyl]-, 1-methylethyl ester, (2E)-2-butenedioate (2:1) is an antiviral drug. It has an empirical formula of $C_{21}H_{29}O_5N_6P•½(C_4H_4O_4)$ and a formula weight of 534.50g/mol. Tenofovir alafenamide is a white to off-white or tan powder and has solubility in water. It is used to treat chronic hepatitis B infection by stopping or slowing the growth of the virus.

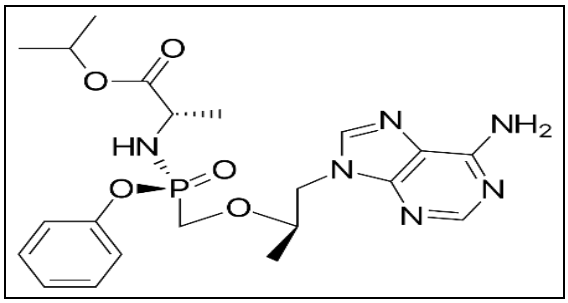

FIG. 1C: CHEMICAL STRUCTURE OF TENOFOVIR ALAFENAMIDE

The literature review reveals that there are very few methods developed for the simultaneous estimation of emtricitabine, bictegravir, and tenofovir alafenamide in their bulk and pharmaceutical dosage forms, such as reversed-phase high-performance liquid chromatography (RP-HPLC) [10-15], UPLC [16, 17] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [18]. The objective of the present study was to develop and validate a UPLC method for the simultaneous determination of emtricitabine, bictegravir, and tenofovir alafenamide in the pharmaceutical dosage form.

MATERIAL AND METHODS:

Reagents and Chemicals: The emtricitabine, bictegravir, and tenofovir alafenamide working standards were received as gift samples from Hetero Drugs Pvt. Ltd., Hyderabad, India. The tablets (Biktarvy) were purchased from a local pharmacy. All the solvents used were of HPLC grade and purchased from Merck, Mumbai, India. All the chemicals used for developing the method were of analytical reagent (AR) grade and purchased from Sigma Aldrich, India.

Instrumentation and Chromatographic Conditions: Water ACQUITY UPLC[19-21]system equipped with Binary solvent manager, a sample manager with HSS C18 (100 × 1.8mm, 1.7µ) column maintained at 30°C, a solvent tray, and ultra violet (UV) detector with detection wavelength set at 260nm was used for the simultaneous determination of emtricitabine, bictegravir and tenofovir alafenamide in pharmaceutical dosage form. All the parameters of UPLC were controlled by empowering software. Electronic balance, digital pH meter, and ultrasonic bath sonicator were other instruments used. The mobile phase used in this method was 0.1% orthophosphoric acid (pH 2.2) and acetonitrile in the ratio 70:30%v/v on isocratic mode at a flow rate of 0.3ml/min.

Preparation of Standard and Sample Solutions: Accurately weighed and transferred 100mg of Emtricitabine, 25mg of Bictegravir, and 12.5mg of Tenofovir working Standards into a 50 ml clean dry volumetric flasks, added 10ml of diluent, sonicated for 10 minutes and made up to final volume with diluents (2000µg/ml Emtricitabine, 500µg/ml Bictegravir and 250µg/ml of Tenofovir). 1ml from the above stock solutions was taken into a 10ml volumetric flask and made up to 10ml (200µg/ml Emtricitabine, 50µg/ml Bictegravir and 25µg/ml of Tenofovir) Accurately weighed equivalent weight

of the combination powder (Biktarvy tablets) sample transfer into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further, the volume was made up with diluent and filtered by milli-Q filters (2000µg/ml Emtricitabine, 500µg/ml Bictegravir and 250 µg/ml of Tenofovir). 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (200µg/ml Emtricitabine, 50µg/ml Bictegravir and 25µg/ml of Tenofovir).

Method Validation [22]:

System Suitability: For determining the system suitability, a standard solution was injected five times into the UPLC and calculated the system suitability parameters such as % relative standard deviation, plate count, tailing factor and resolution.

Linearity: Serial dilutions of standard drugs in the concentration range of 50 – 300µg/ml for emtricitabine, 12.5 – 75µg/ml for bictegravir, and 6.25 – 37.5µg/ml for tenofovir alafenamidewere prepared and injected into the UPLC. Linearity graphs were plotted between concentration and peak areas.

Accuracy: The solutions were prepared in three different concentration levels of 50%, 100%, and 150%, injected into UPLC, and % recoveries were calculated.

Precision: Intra and Inter-day precision studies determined the method's precision. The standard solution was injected six times on the same day (intra-day) and on different days (inter-day), and the % RSD was calculated.

Specificity: The specificity of the method was determined by injecting the placebo solution and comparing it with the standard solution for the interference with drug peaks.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ are determined by using standard deviation (SD) and slope of the calibration curve. The limiting values are calculated as per the following equations:

$$LOD = (3.3 \times SD)/ Slope \text{ and}$$

$$LOQ = (10 \times SD)/ Slope.$$

Robustness: The robustness of the method was determined by varying the optimum chromatographic conditions such as mobile phase ratio (±10%), flow rate (±0.1mL/min), and column oven temperature (±5ºC). The system suitability parameters were calculated and recorded.

Solution Stability: To prove the stability of the drugs, the standard solution, and the spiked solution were kept at room temperature for 24 h.

Forced Degradation Studies [23]: To test the stability of drugs under stress conditions, the standard solution and the spiked solution were subjected to the forced degradation conditions such as acidic condition (2N Hydrochloric acid, 30 min at 60°C), alkaline condition (2N sodium hydroxide, 30mins at 60°C), oxidative condition (20% hydrogen peroxide, 30 min at 60°C), thermal condition (105°C for6h) and photolytic condition (placing the beaker in UV Chamber for 7days or 200 Watt-hours/min photostability chamber).

RESULTS AND DISCUSSION: To develop the UPLC method for the simultaneous estimation of emtricitabine, bictegravir, and tenofovir alafenamide, initially many mobile phase compositions were tried to elute the drugs. Finally, a mobile phase consisting of 0.1% orthophosphoric acid (pH 2.2) and acetonitrile in the 70:30%v/v on isocratic mode at a flow rate of 0.3ml/min was selected as optimum conditions based on the peak parameters. A standard solution containing a concentration 200µg/ml of emtricitabine, 50µg/ml of bictegravir and 25µg/ml of tenofovir alafenamide were prepared and scanned in the range of 200-400nm for detecting the maximum absorption wavelength and was found to be 260nm based on the overlain UV spectrum as shown in **Fig. 2.**

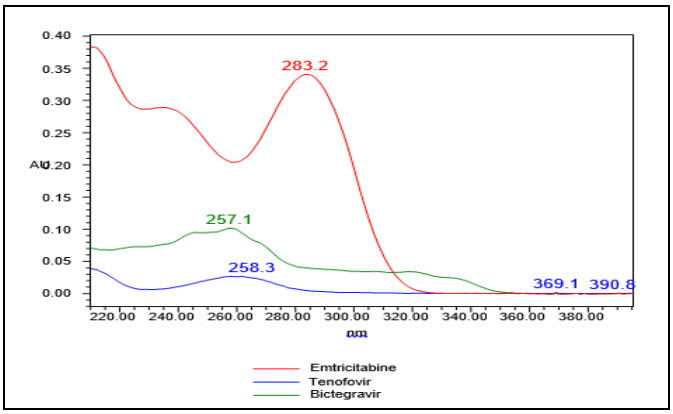

FIG. 2: OVERLAY UV SPECTRUM OF EMTRICITABINE, TENOFOVIR AND BICTEGRAVIR

Based on the system suitability parameters, HSS C18 (100 × 1.8mm, 1.7µ) column was selected. The retention time for the drugs were found to be 0.62 min for emtricitabine, 0.89 min for bictegravir and 1.75 min for tenofovir alafenamide.

The system suitability parameter results were summarized in **Table 1**, and the standard and sample chromatograms for the drugs were shown in **Fig. 3** and **4.**

TABLE 1: SYSTEM SUITABILITY AND VALIDATION PARAMETER RESULTS

Parameters Emtricitabine Bictegravir Tenofovir alafenamide			
Specificity Specific Specific Specific			
Precision (%RSD)	0.6	0.5	1.1
Accuracy (% recovery)	99.98% - 101.01%	99.35% - 100.22%	99.99% - 101.06%
Linearity range (µg/mL)	50 – 300	99.35% - 100.22%	6.25 – 37.5
Correlation coefficient, r	0.9999	0.9996	0.9999
Limit of Detection (µg/mL)	3.66	0.54	0.16
Limit of Quantitation (µg/mL)	3.66	1.63	0.49
Intermediate precision (%RSD)	0.4	0.5	1.5
Robustness	Robust	Robust Robust	
Stability	Stable	Stable Stable	
USP Plate count	2430	2322	3584
USP tailing factor	1.60	1.66	1.09
Resolution		3.3	7.7

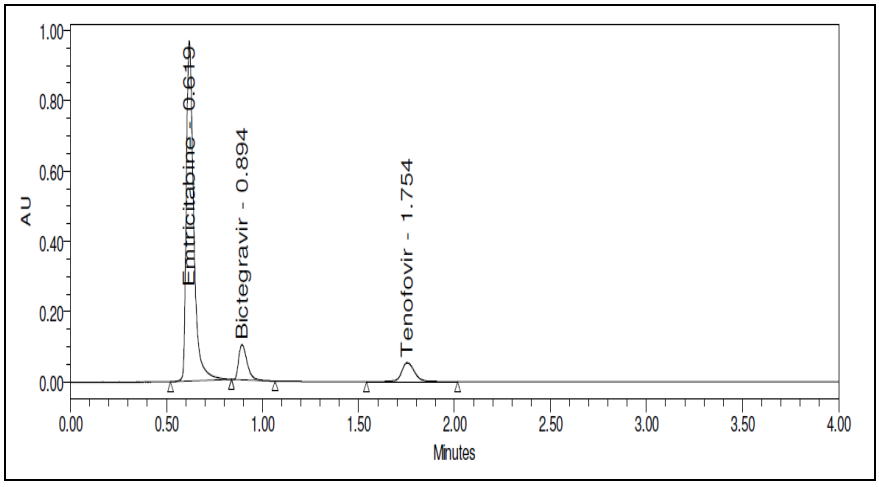

FIG. 3: STANDARD CHROMATOGRAM

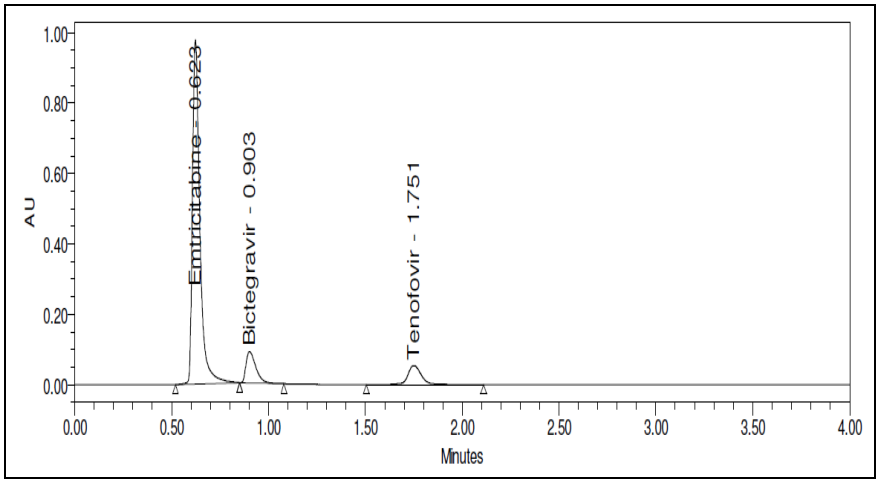

FIG. 4: SAMPLE CHROMATOGRAM

The drugs obeyed beer's law in the 50–300µg/ml concentration range for emtricitabine, 12.5–75µg/ml for bictegravir and 6.25–37.5µg/ml for tenofovir alafenamide, the correlation coefficient was found to be within limits. The linearity plots were shown in **Fig. 5A, 5B,** and **5C.**

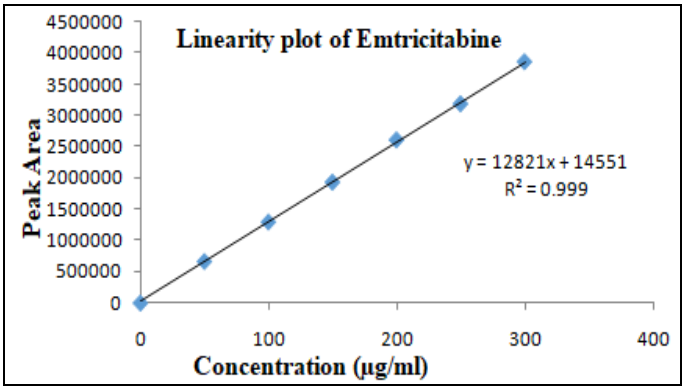

FIG. 5A: LINEARITY PLOT OF EMTRICITABINE

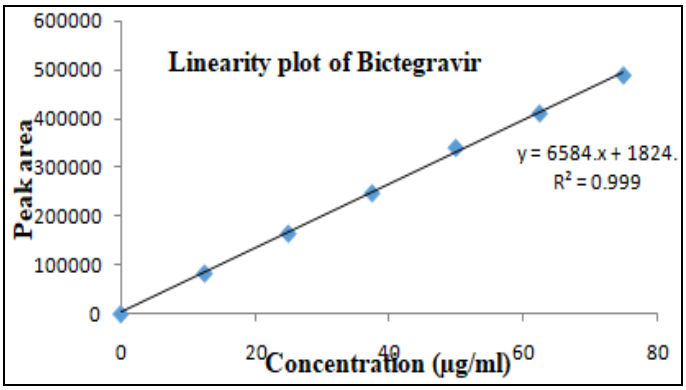

FIG. 5B: LINEARITY PLOT OF BICTEGRAVIR

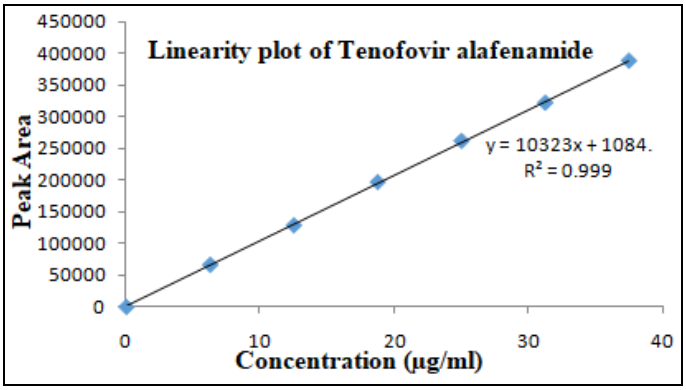

FIG. 5C: LINEARITY PLOT OF TENOFOVIR ALAFENAMIDE

The % recovery for emtricitabine was 99.98% - 101.01%, bictegravir was found to be 99.35% - 100.22%, and tenofovir alafenamide was found to be 99.99% - 101.06%indicating that the method was accurate. The % relative standard deviation (%RSD) for intra-day precision and intermediate precision was found to be 0.6 and 0.4 respectively for emtricitabine, 0.5 and 0.5 respectively for bictegravir and 1.1 and 1.5 respectively for tenofovir alafenamide, indicating that the method was precise. To determine the specificity of the method, the placebo solution was prepared and observed for the interfering peaks with the drug peaks. As there are no peaks interfering with drug peaks, the method was found to be specific. The placebo chromatogram was shown in **Fig. 6.**

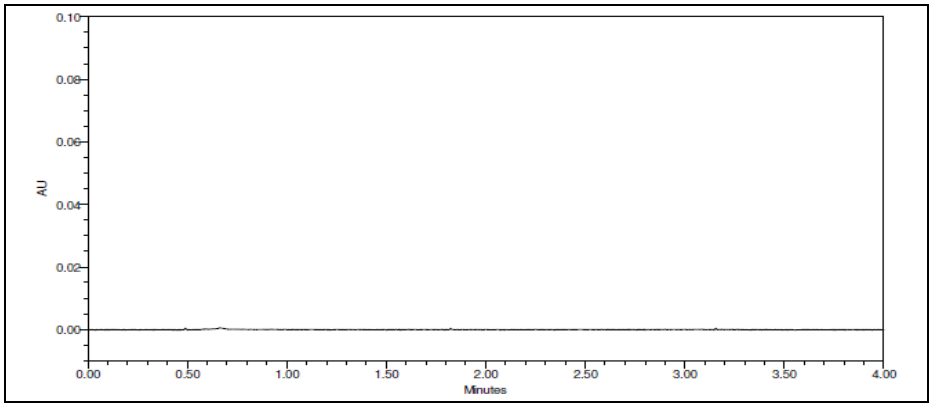

FIG. 6: PLACEBO CHROMATOGRAM

The LOD and LOQ was found to be 3.66 µg/ml and 11.11 µg/ml respectively for emtricitabine, 0.54 µg/ml and 1.63 µg/ml respectively for bictegravir and 0.16µg/ml and 0.49µg/ml respectively for tenofovir alafenamide. The method was found to be robust and stable when stored for 24 h as the results fall within the acceptance criteria. It was found that the amount recovered from both fresh and stored solutions was highly similar. From the forced degradation studies, it was known that the drugs were found to be stable when exposed to different stress conditions, as the net

degradation was found to be within limits. The results were summarized and shown in Table 2 and the chromatograms were presented in **FIG. 7A, 7B, 7C, 7D,** and **7E.**

TABLE 2: FORCED DEGRADATION STUDIES RESULTS FOR EMTRICITABINE, BICTEGRAVIR AND TENOFOVIR

Drug	Parameters	Stress Condition				
		Acidic	Basic	Oxidative	Photolytic	Thermal
Emtricitabine	% Assay	93.97	95.42	94.57	98.09	96.72
	% Degradation	6.03	4.58	5.43	1.91	3.28
Bictegravir	% Assay	93.89	96.08	95.23	98.55	97.35
	% Degradation	6.11	3.92	4.77	1.45	2.65
Tenofovir	% Assay	93.98	95.89	94.82	98.30	97.51
	% Degradation	6.02	4.42	5.18	1.70	2.49

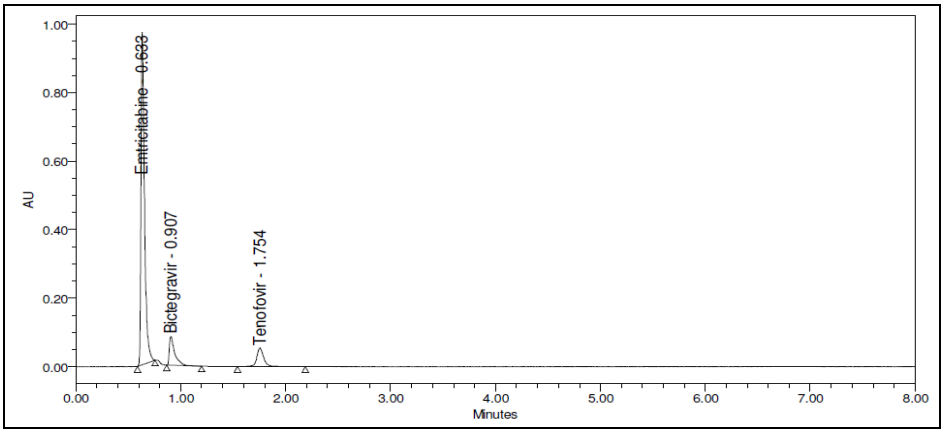

FIG. 7A: ACID DEGRADATION CHROMATOGRAM

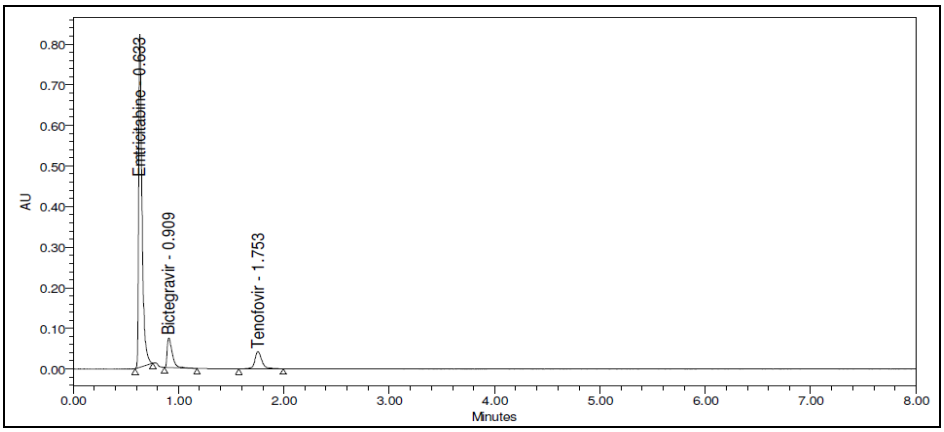

FIG. 7B: BASE DEGRADATION CHROMATOGRAM

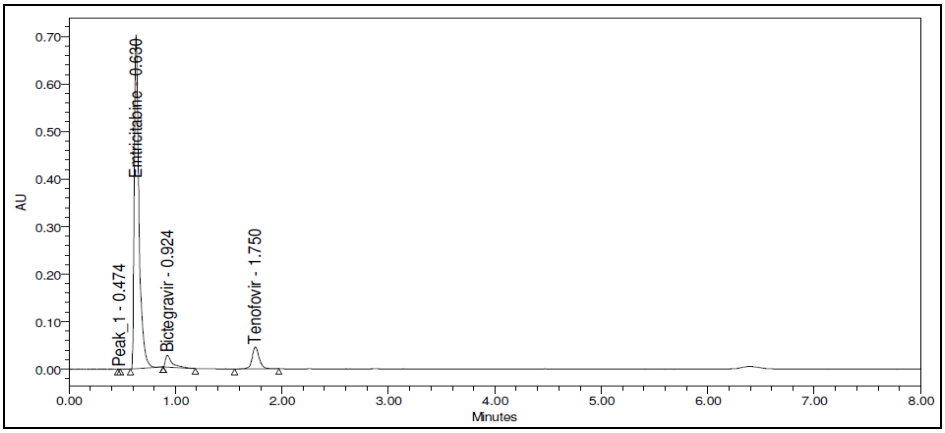

FIG. 7C: OXIDATIVE DEGRADATION CHROMATOGRAM

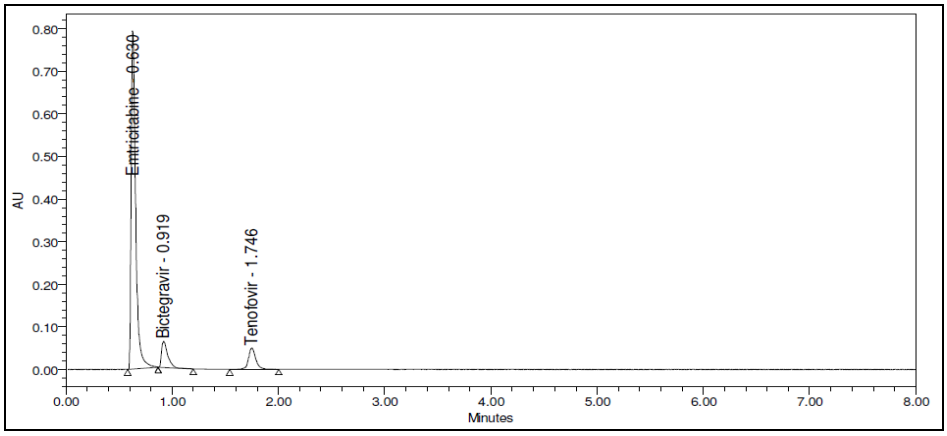

FIG. 7D: PHOTOLYTIC DEGRADATION CHROMATOGRAM

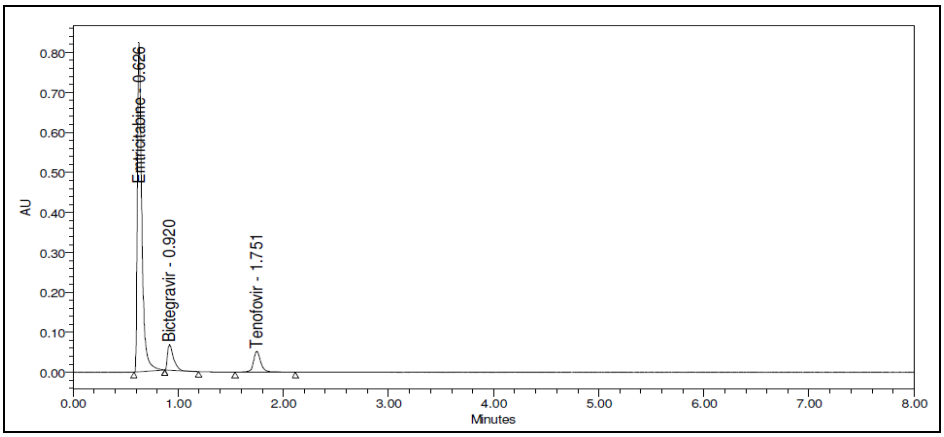

FIG. 7E: THERMAL DEGRADATION CHROMATOGRAM

CONCLUSION: A specific, accurate method was developed to simultaneously estimate emtricitabine, bictegravir and tenofovir alafenamide in pharmaceutical dosage form using UPLC. The method was validated by using various validation parameters, and the method was found to be linear, precise, accurate, specific and robust. The run time was 2 min, enabling rapid quantitation of many samples in routine and quality control analysis of capsule formulations.

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CONFLICTS OF INTERESTS: The authors declare that they have no conflict of interest.

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