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NOVEL STRESS INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR ALAFENAMIDE

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Abstract:

Objective: *The present study aimed to develop stress indicating reverse phase high-performance liquid chromatography (RP-HPLC) method for the estimation of Bictegravir, Emtricitabine and Tenofovir alafenamide in pharmaceutical dosage form and validated in accordance with ICH guidelines.*

Method: *The optimized conditions for the developed RP-HPLC method are Kromasil C18 (250 X 4.6mm, 5µ) column maintained at 30ºC with a mobile phase consisting of 0.01N Buffer (KH_2PO_4) and Acetonitrile in the ratio 60:40%v/v on isocratic mode at flow rate 1.0ml/min. The sample was detected at 272nm.*

Results: *The retention time of Bictegravir, Emtricitabine and Tenofovir alafenamide was found to be 2.9, 2.2 and 3.3min respectively. The developed method was validated for accuracy, precision, specificity, ruggedness, robustness and solution stability. The method obeyed Beer's law in the concentration range of 12.5µg/ml-75µg/ml for Bictegravir, 50µg/ml-300µg/ml for Emtricitabine and 6.25µg/ml -37.5µg/ml for Tenofovir alafenamide with a correlation coefficient of 0.999 for BIC, FTC and TAF respectively.*

Forced degradation studies were conducted by exposing the drug solution to various stress conditions such as acidic, basic, peroxide, neutral, photolytic and thermal conditions. The net degradation was found to be within the limits, indicating that the drug is stable in stressed conditions.

Conclusion: *The developed method for the estimation of Bictegravir, Emtricitabine and Tenofovir alafenamide can be utilized for the routine analysis of pharmaceutical dosage form.*

Keywords: *Stress indicating, Method development, Bictegravir, Validation, RP-HPLC.*

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INTRODUCTION:

Bictegravir[1-5](fig1) The chemical name of bictegravir sodium is 2,5Methanopyrido [1', 2':4,5]pyrazino[2,1b][1,3]oxazepine10carboxamide,2, 3,4,5,7,9,13,13aoctahydro8hydroxy7,9dioxoN[(2,4,6t rifluorophenyl)methyl],sodiumsalt(1:1),(2R,5S,13aR) Bictegravir sodium has a molecular formula of $C_{21}H_{17}F_3N_3NaO_5$ and a molecular weight of 471.4. Bictegravir sodium is an off-white to yellow solid with a solubility of 0.1 mg/ml in water at 20 °C.Bictegravir is a HIV-1 integrase strand transfer inhibitor (INSTI). The drug acts by inhibiting strand transfer of viral DNA into the host genome and thereby preventing HIV-1 replication. It is a novel INSTI that can be dosed without a boosting agent.

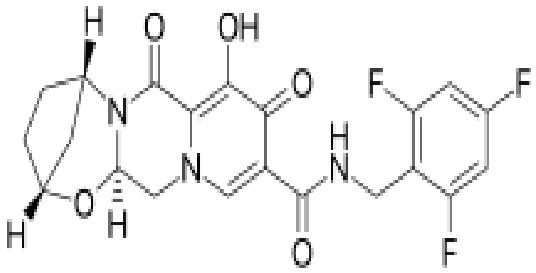

Fig. 1: Chemical structure of Bictegravir

Emtricitabine (fig2) The chemical name of FTC is 4-amino-5- fluoro-1-(2R-hydroxymethyl-1,3-oxathiolane-5S-yl)-(1H)-pyrimidinone-2-one. FTC is the (-) enantiomer of a thio analog of cytidine, which differs from other cytidine analogs in that it has a fluorine in the 5 positions. FTC has a molecular formula of C8H10FN3O3S and a molecular weight of 247.2. Emtricitabine is a white to off-white powder with a solubility of approximately 112 mg/ml in the water at 25°C. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) used in the treatment of HIV infection. The drug acts by inhibiting HIV reverse trancriptase, preventing transcription of HIV RNA to DNA. It belongs to category anti-viral.

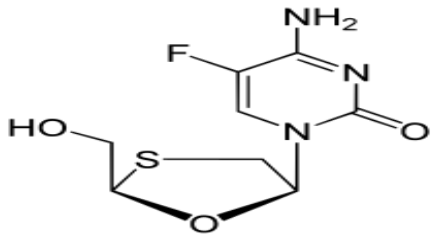

Fig. 2: Chemical structure of Emtricitabine

Tenofovir alafenamide (fig2) The chemical name of tenofovir alafenamide fumarate drug substance is L-alanine, N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)- 1-methylethoxy] methyl] phenoxy phosphinyl]-,1-methylethyl ester, (2E)-2-butenedioate (2:1). Tenofovir alafenamide fumarate is a white to off-white or tan powder with a solubility of 4.7 mg/ml in the water at 20°C. Tenofovir alafenamide is a nucleoside reverse transcriptase inhibitor (NRTI), a phosphonomidate prodrug of the nucleotide analog Tenofovir. TAF was designed to circulate systematically achieving higher metabolite concentration in peripheral blood mononuclear cells.

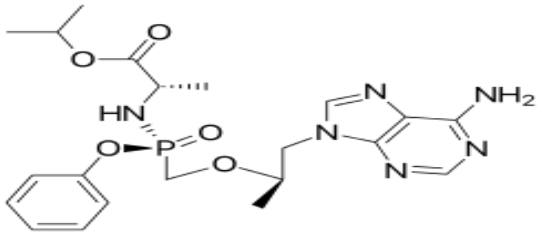

Fig. 3: Chemical structure of Tenofovir alafenamide

Biktarvy® (bictegravir, emtricitabine, and tenofovir alafenamide) is a fixed-dose combination tablet containing bictegravir (BIC), emtricitabine (FTC), and tenofovir alafenamide (TAF) for oral administration.Each tablet contains 50 mg of BIC (equivalent to 52.5 mg of bictegravir sodium), 200 mg of FTC, and 25 mg of TAF (equivalent to 28 mg of tenofovir alafenamide fumarate) and the following inactive ingredients: croscarmellose sodium, magnesium stearate, and microcrystalline cellulose. The tablets are film-coated[6-10]. with a coating material containing iron oxide black, iron oxide red, polyethylene glycol, polyvinyl alcohol, talc, and titanium dioxide. Biktarvy is indicated as a complete regimen for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults who have no antiretroviral treatment history or to replace the current antiretroviral regimen in those who are virologically-suppressed (HIV-1 RNA less than 50 copies per ml) on a stable antiretroviral regimen for at least 3 months with no history of treatment failure and no known substitutions associated with resistance to the individual components of Biktarvy®. The tablets are purplish brown, capsule-shaped, film-coated, and debossed with “GSI” on one side and “9883” on the other side.

The literature reviw reveals that here are very few analytical methods that have been reported for the determination of Bictegravir,emtricitabine, and tenofovir alafenamideusing LC coupled with tandem mass spectrometry method (LC-MS/MS) in pharmaceutical formulations at the time of commencement of research work.

As there was no method developed using RP-HPLC, the present study aimed to develop and validate a HPLC stability-indicating method for estimation of

Bictegravir, emtricitabine, and tenofovir alafenamide in pharmaceutical dosage form.

MATERIALS AND METHODS:

Reagents and chemicals

Bictegravir, Emtricitabine and Tenofovir alafenamide working standards were procured from spectrum pharma research solutions,Hyderabad as gift sample. The Biktarvy tablets were purchased from a local pharmacy.All the chemicals used were of AR grade purchased from Merck,Mumbai.All the solvents used were of HPLC grade.

Chromatographic conditions and instruments

The Waters HPLC 2695 system equipped with a quarternary solvent manager with PDA detector and Kromasil C18 (250×4.6mm,5µ) column was used for the determination of Bictegravir, Emtricitabine and Tenofovir alafenamide.The optimized conditions included 0.01N Buffer(KH_2PO_4) and Acetonitrile(60:40%v/v) as mobile phase run on an isocratic mode at a flow rate of 1.0ml/min.The column was maintained at 30ºC and detection was done at 272nm.Other pieces of equipments used in the method was pH meter, ultrasonic bath sonicator, and weighing balance.

Preparation of Diluent

A Mixture of water and acetonitrile in the ratio 50:50%v/v was used as diluent.

Preparation of Mobile phase

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36g of potassium dihydrogen Orthophosphate in a 1000ml of volumetric flask and 900ml milli-Q water were added and degassed with the help of sonicator and finally the volume was made up to the mark with water then pH was adjusted to 3.8 with OPA solution. A Mixture of Buffer and Acetonitrile in the ratio (60:40%v/v) was used as mobile phase.

Preparation of Standard and Sample solutions

12.25mg of Bictegravir,50mg of Emtricitabine and 6.25mg of Tenofovir alafenamide working standards were dissolved in 25 ml of diluents.

1ml of above stock solution was diluted to 10ml with diluents in order to get a concentration of 50µg/ml of Bictegravir,200µg/ml of Emtricitabine and 25µg/ml of Tenofovir alafenamide respectively.

10 Tablets (BIKTAVY) were weighed accurately and the average weight was calculated.An amount equivalent to 12.25mg of Bictegravir,50mg of Emtricitabine and 6.25mg of Tenofovir alafenamide was dissolved in 25ml of diluent.Filtered the solution and diluted 1ml of the above solution to 10ml with diluent.

METHOD VALIDATION

The developed method was validated in compliance with International Conference on Harmonization (ICH) guidelines [11,12].

Specificity

The specificity of the method was determined by comparing the drug solution with the placebo solution with drug peak.

Accuracy

Accuracy of the method was determined by %recovery. The drug solution along with sample was prepared in three concentration levels 50%,100%, and 150%. Then the %recovery was calculated.

Precision

The Precision of the method was estimated by injecting the six solutions of the standard into the HPLC system and %relative standard deviation(%RSD) was calculated.

Linearity

The Linearity of the method was developed by preparing series of dilutions ranging from 12.5µg/ml to 75µg/ml for Bictegravir,50µg/ml to 300µg/ml for Emtricitabine and 6.25µg/ml to 37.5µg/ml for Tenofovir alafenamide respectively and injecting them into HPLC system.

Ruggedness

Ruggedness was determined by injecting the six solutions of the standard into HPLC for different days. The %RSD was calculated.

Robustness

Robustness of the method was determined by varying the optimized analytical conditions such as mobile phase composition by ±5%,flow rate by ±0.1ml/min and column temperature by ±5ºC.

Solution stability

Solution stability was estimated by optimizing the standard drug solution after storage for 24hrs under laboratory conditions.

Forced degradation studies

Forced degradation studies[13] were carried out for drug by exposing the drug solution to the various stress conditions such as acidic(2N Hydrochloric acid for 30min at 60ºC),basic (2N Sodium hydroxide for 30min at 60ºC),Oxidation(refluxing the drug solution with 20%H_2O_2),neutral(refluxing the drug in water for 6hrs at 60ºC),photolytic(exposing the drug

solution to UV light by keeping the solution in UV chamber for 7 days or 200 watt hrs/m^2 in photo stability chamber),thermal(drug solution was placed in oven at 105ºC for 6hrs) conditions.

RESULTS AND DISCUSSION:

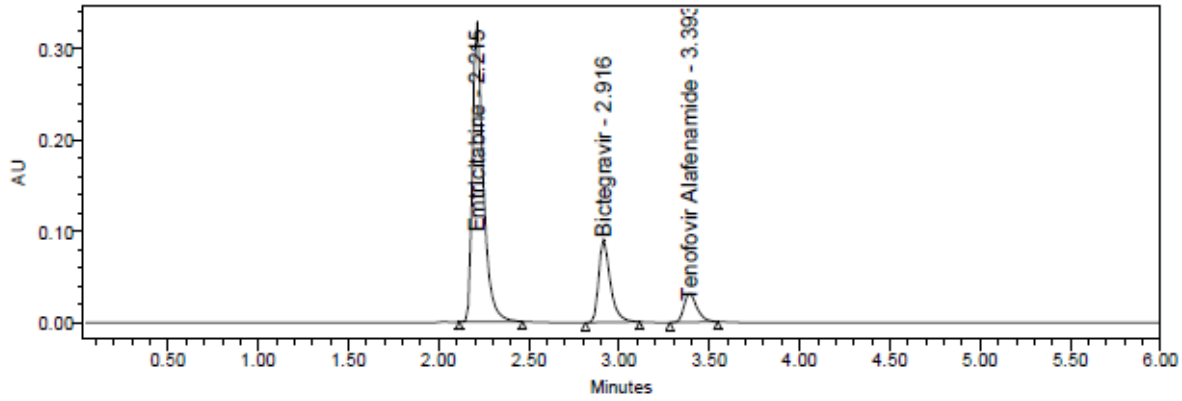

Fig. 4: Standard chromatogram

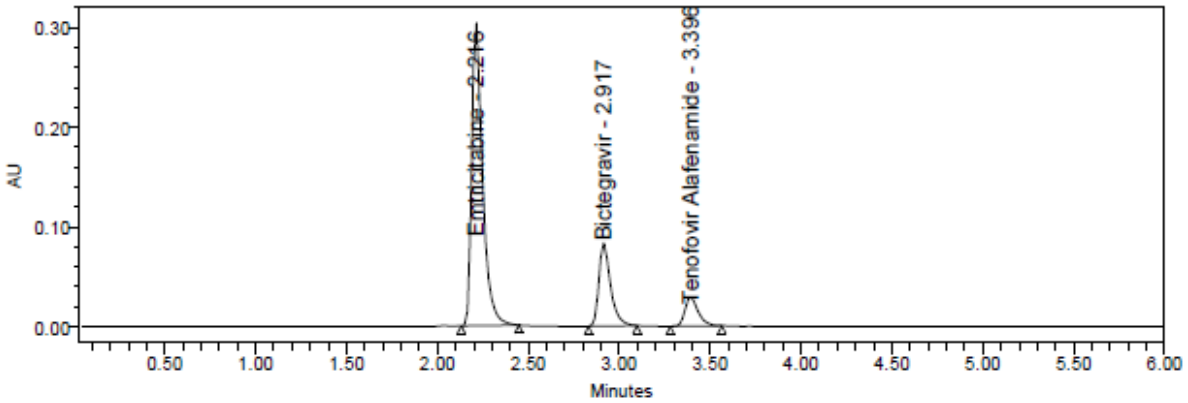

Fig. 5: Sample chromatogram

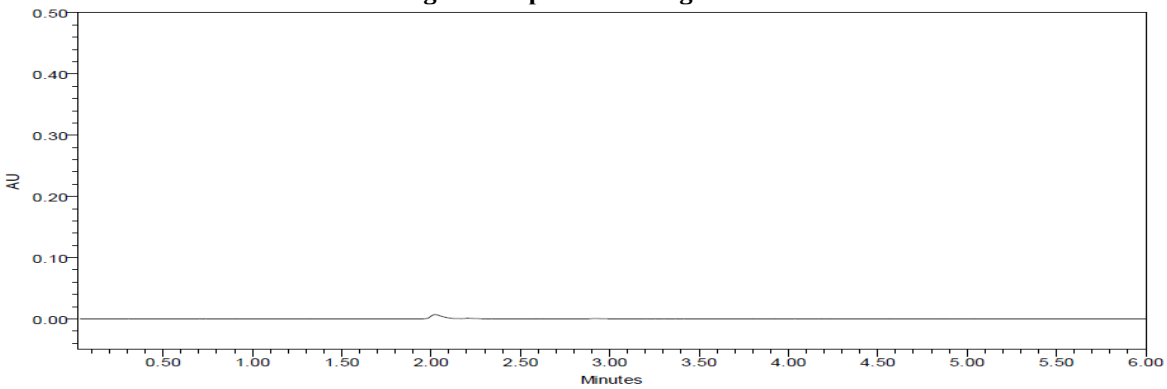

Fig. 6: Blank chromatogram

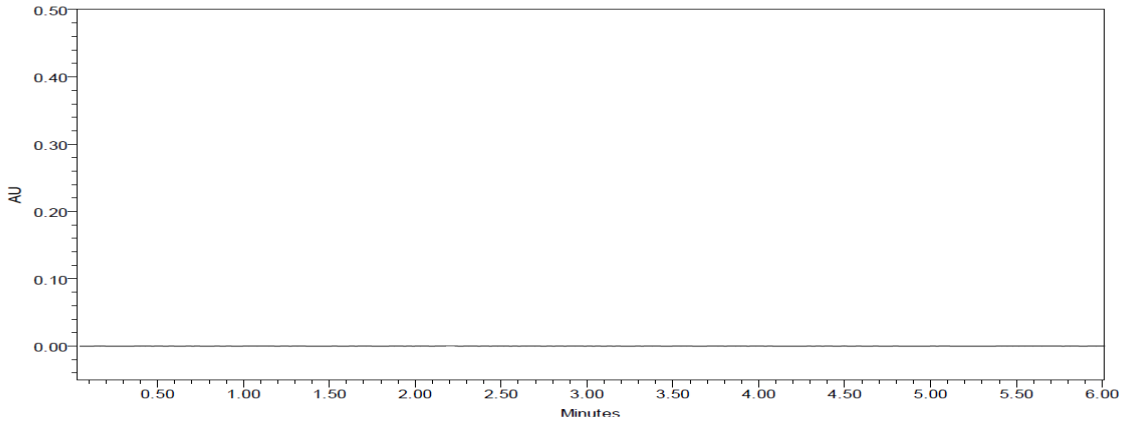

Fig. 7: Placebo chromatogram

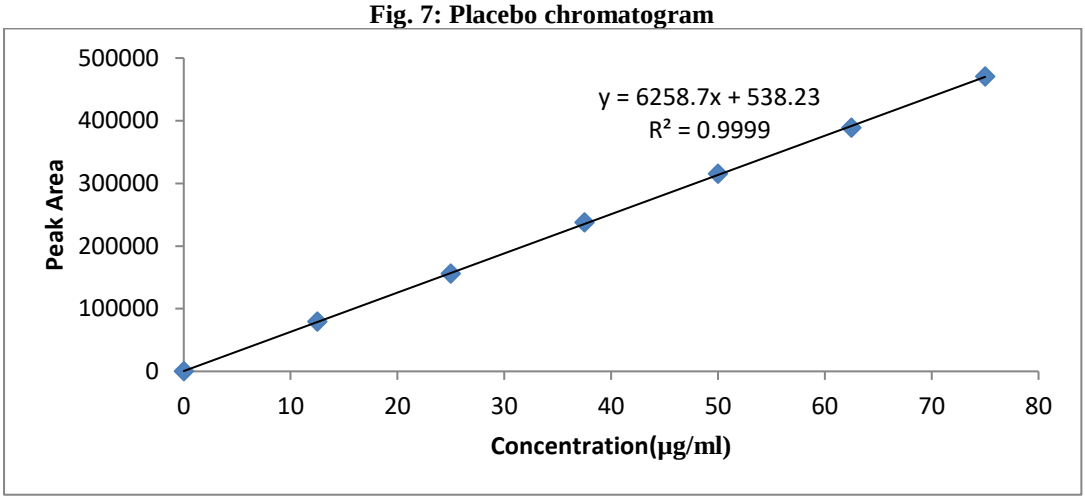

Fig.8: Linearity plot of Bictegravir

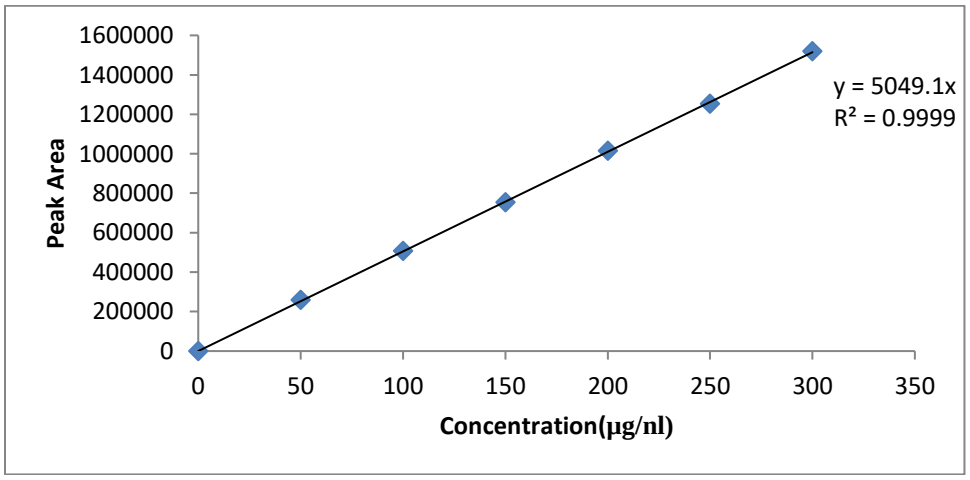

Fig.9: Linearity plot of Emtricitabine

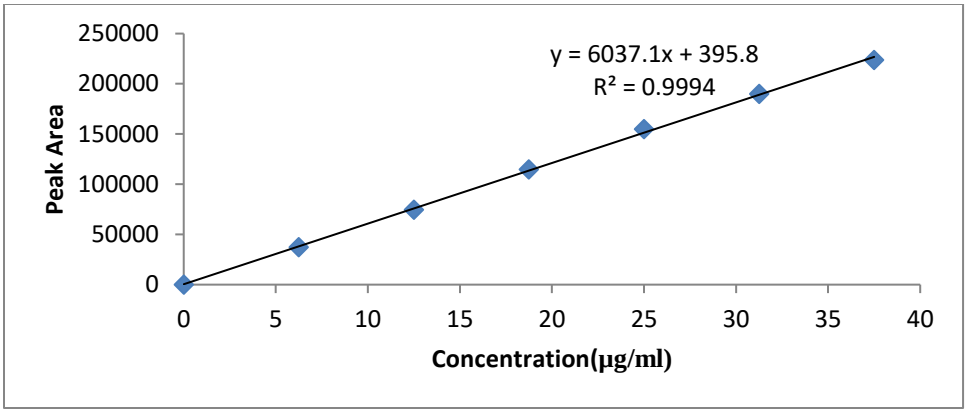

Fig.10: Linearity plot of Tenofovir alafenamide

Table 1: System suitability and validation parameters results

Parameter	Result		
	BIC	**FTC**	**TAF**
USP Plate count	10044	6315	10734
USP Tailing factor	1.4	1.5	1.3
USP Resolution	6.0	-	**3.7**
Precision(%RSD)	0.6	0.4	0.8
Accuracy %	99.32-100.73	99.24-100.49	99.0-100.32
Specificity	Specific, No interference		
Linearity range(µg/ml)	12.5-75	50-300	6.25-37.5
Correlation coefficient,r^2	0.999	0.999	0.999
LOD(µg/ml)	0.14	0.80	0.07
LOQ(µg/ml)	0.42	2.44	0.22
Ruggedness(%RSD) Day 1	0.6	0.4	0.2
Day 2	0.8	0.4	0.6
Robustness(%RSD) Flow rate –	0.4	0.2	1.0
Flow rate+	0.9	1.0	0.7
Column temp.–	0.9	0.7	0.4
Column temp.+	0.2	0.9	0.4
M.P composition–	0.3	0.6	0.4
M.P composition+	0.7	0.9	0.2
Solution stability(%RSD) Day 1(0 hr)	0.6	0.4	0.6
Day 2(24 hr)	0.8	0.6	0.8
%Assay	100.13	99.80	99

Table 2: Forced degradation studies result

Stress condition	BIC		FTC		TAF	
	%Assay	%Degradation	%Assay	%Degradation	%Assay	%Degradation
Acid	92.33	7.67	93.60	6.40	94.23	5.77
Base	94.73	5.27	94.23	5.77	94.74	5.26
Neutral	99.11	0.89	100.05	-0.05	99.17	0.83
Peroxide	95.59	4.41	92.12	7.88	94.37	5.63
Photolytic	98.33	1.67	98.19	1.80	98.49	1.51
Thermal	96.04	3.96	96.23	3.77	96.33	3.67

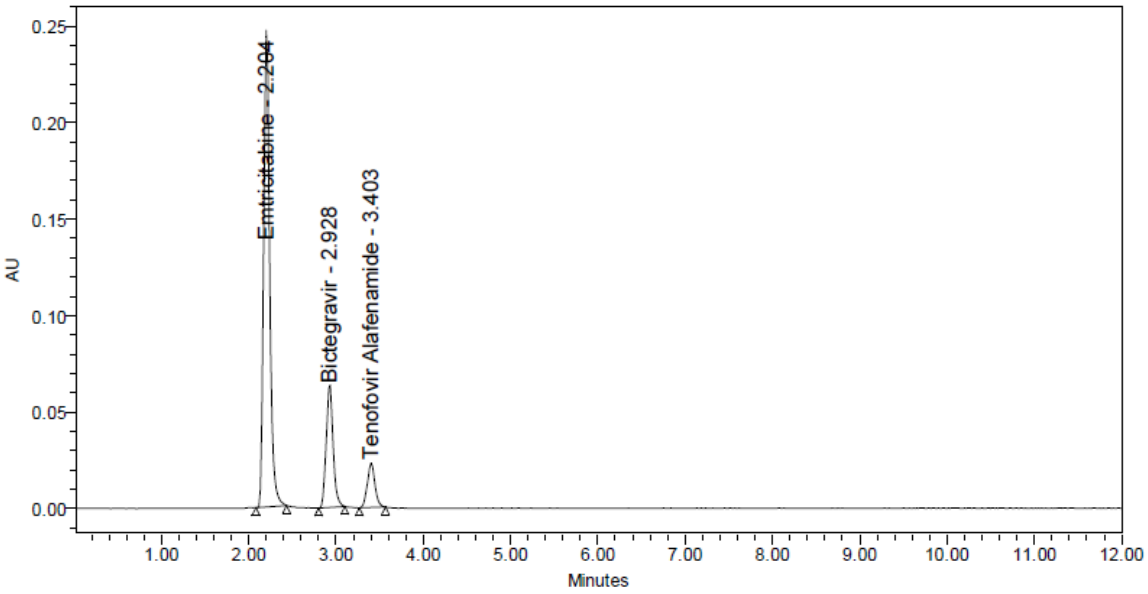

Fig. 12: Acid degradation chromatogram

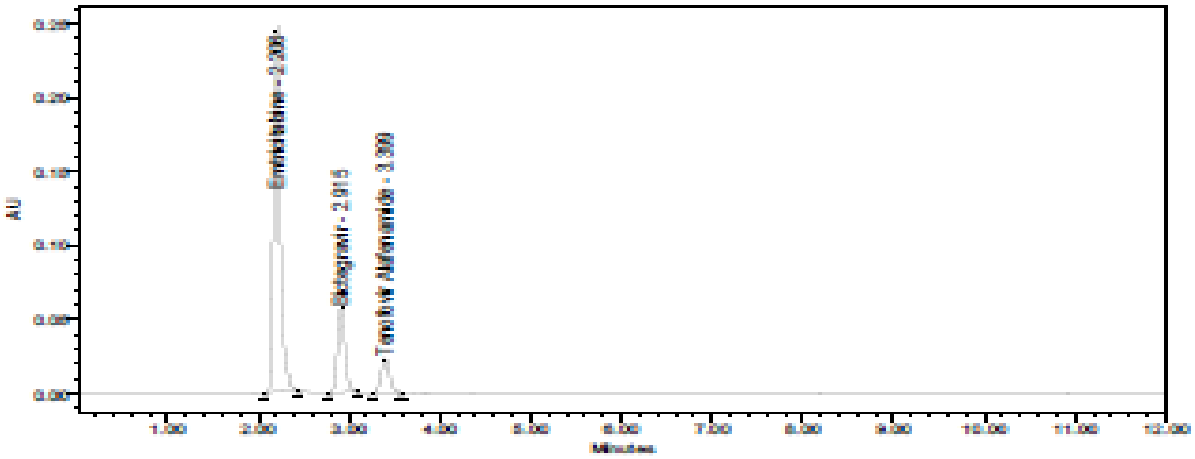

Fig. 13: Base degradation chromatogram

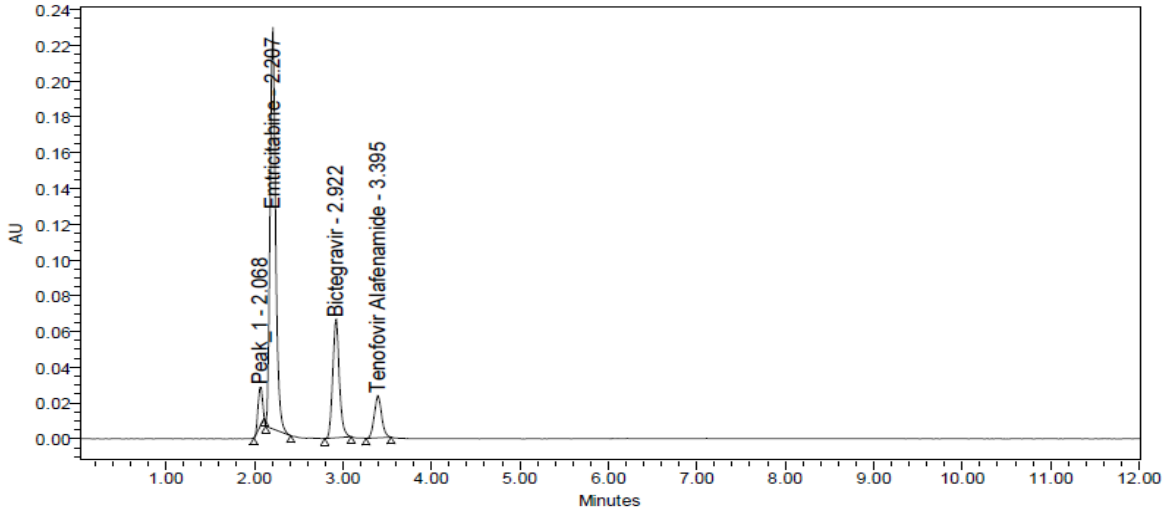

Fig. 14: Peroxide degradation chromatogram

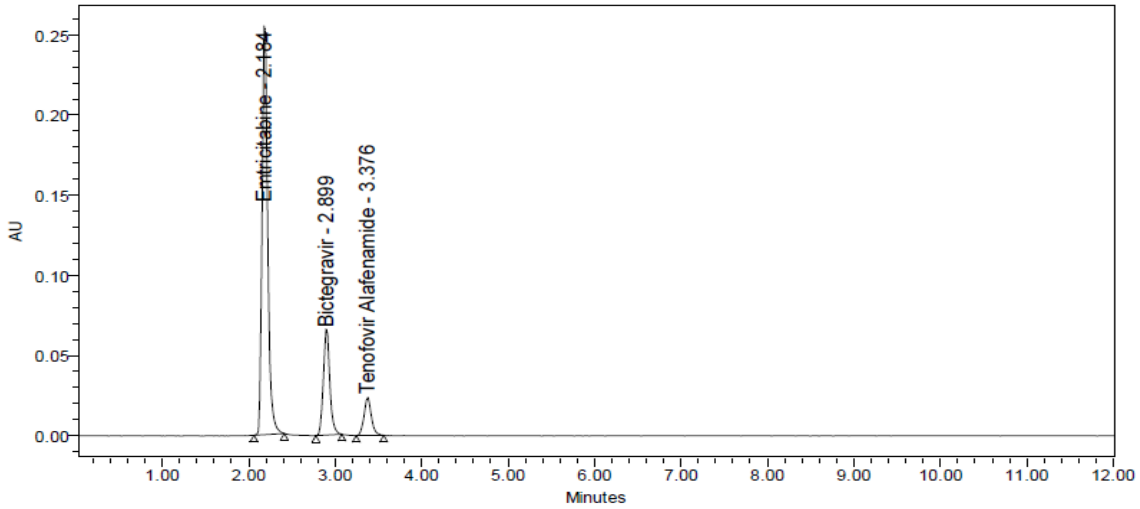

Fig. 15: Water stress study

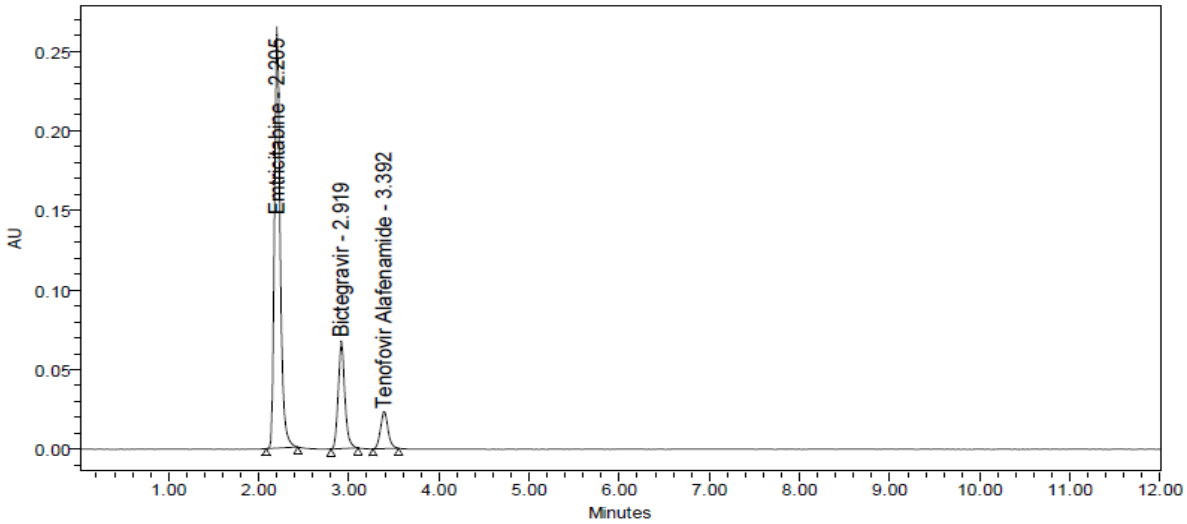

Fig. 16: Photostability Degradation

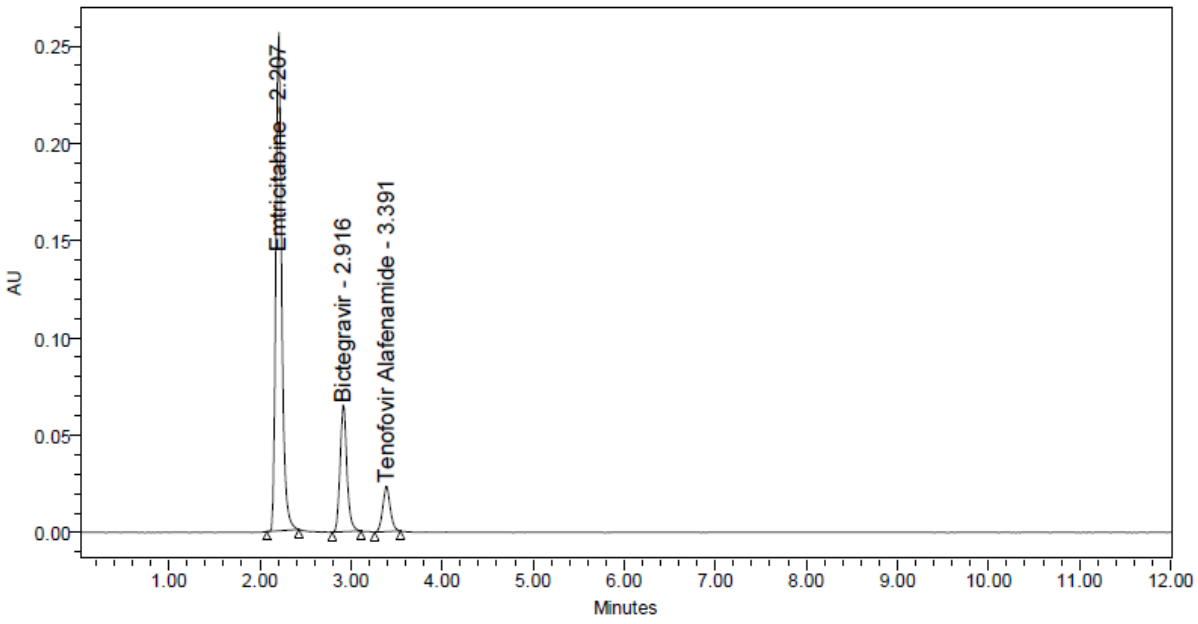

Fig. 17: Dry heat study chromatogram

For the development ofamethod for the estimation of Bictegravir, Emtricitabine and Tenofovir alafenamide initially many mobile phases and many columns were tried to elute the drug peaks with less tailing factor and more plate count.

Waters HPLC with empower 2 software, Kromasil C18 (250X 4.6mm,5µ) column and 0.01N Buffer(KH_2PO_4):Acetonitrile(60:40%v/v) as mobile phase were selected based on peak parameters. The detection wavelength was found to be 272nm.

Prepared standard solution, sample solution, and blank solution were injected into the HPLC system and system suitability parameters were noted as summarized in Table1 along with chromatograms as shown in fig.4,5 and 6 respectively.

The developed method was found to obey Beer's law in the concentration range of 12.5µg/ml-75µg/ml for Bictegravir,50µg/ml-300µg/ml for Emtricitabine and 6.25µg/ml-37.5µg/ml for Tenofovir alafenamide with a correlation coefficient of 0.999 for BIC,FTC and TAF respectively.

A linearity graph was plotted between concentration and peak area as shown in fig8,9 and 10 and results are summarized in Table 1.

The method was found to be accurate as the %recovery wasfound to be 99.32%-100.73% for Bictegravir, 99.24%-100.49% for Emtricitabine and 99.00%-100.32% for Tenofovir alafenamide respectivelyand was within the limits.The %RSD was found to be 0.6 for Bictegravir, 0.4 for Emtricitabine and 0.8 for Tenofovir alafenamide respectively, whichindicates that the method was precise.The method was found to be specific, as there is no interference of retention time of placebo peak with that of drug peak.The placebo chromatogram was shown in fig 7.

Forced degradation studies results indicate that the drug was found to be stable in various stress conditions as net degradation was found to be within the limits. The chromatograms were shown in fig12 to 17 and the results were summarized in Table 2.

CONCLUSION:

A specific, accurate, precise stability indicating method was developed for the estimation of Bictegravir, Emtricitabine and Tenofovir alafenamide in pharmaceutical dosage form using HPLC. The method was validated by using various validation parameters and the method was found to be linear, precise, accurate,specific and robust. From the degradation, studies conducted it is concluded that Bictegravir,Emtricitabine, and Tenofovir alafenamide were stable at more concentrations of acid, base, peroxide, thermal, UV and water stress study conditions. The run time was 6 min which enables

rapid quantitation of many samples in routine and Quality control analysis of tablet formulations.

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