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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE, COBICISTAT, EMTRICITABINE AND ELVITEGRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new simple, precise, selective, accurate and rapid RP-HPLC stability indicating method had been developed and validated for simultaneous quantitative determination of Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir in bulk and pharmaceutical dosage form using Kromasil C18 (250×4.6 mm, 5µm) in isocratic mode. The optimized mobile phase consists of Orthophosphoric acid buffer: Acetonitrile (55:45 %v/v). The flow rate was 1.0 mL/min and eluents were detected at 240 nm using PDA detector. The method was linear in the range of 20 -120 µg/ml for Emtricitabine, 30-180 µg/ml for Tenofovir, 15-90 µg/ml for Cobicistat and 15-90 µg/ml for Elvitegravir. Degradation studies were studied for Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir under various stress conditions, all the degradation peaks were resolved effectively using developed method with different retention times. The developed method was validated according to ICH guidelines.

Key words: Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir, Acetonitrile, Buffer, RP-HPLC.

INTRODUCTION

Tenofovir is a nucleotide analog of deoxyadenosine monophosphate, with activity against HIV-1, -2 and Hepatitis B virus (HBV). The chemical name of tenofovir disoproxil fumarate (TDF) is 9-[(R)-2-[[*bis*[[(isopropoxycarbonyl)oxy]-methoxy] phosphin yl]methoxy]propyl]adenine fumarate [1,2]. The chemical name of cobicistat (CBT) is 1,3-thiazol-5-ylmethyl[(2R,5R)-5-{[(2S)2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl) amino]-4-(morpholin-4yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate. Cobicistat is a pharmacokinetic enhancer, is a effective mechanism-based inhibitor of cytochrome P450 3A4, an enzyme that metabolizes medicinal compounds in the body. Inhibition of CYP3A-mediated metabolism by cobicistat enhances the systemic exposure of CYP3A4 substrates, mainly drugs like elvitegravir, where bioavailability is decreased and half-life is reduced by CYP3A-dependent metabolism [3,4]. Emtricitabine (ETC) is a fluorinated derivative of lamivudine, an analog of deoxycitidine. The chemical name of Emtricitabine is 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Its molecular formula is C22H18N6 •HCl and its molecular weight is 402.88. Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in

chain termination [4,5]. Elvitegravir (EVG), the second integrase inhibitor used in treatment-naïve and treatment-experienced HIV-1 infected adults. The chemical name of elvitegravir is 6-(3-chloro-2-fluorobenzyl)-1-[(2S)-1hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [6].

Elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (Stribild), manufactured by Gilead Sciences, Inc, is a combination antiretroviral agent approved by the FDA as a complete regimen for the treatment of HIV-1 infection in adults [7-10]. Various UV, HPLC and LC/MS/MS assay methods were reported in the literature for the estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir individually and in-combination with other drugs. These methods include; UV spectroscopy method [11-13], Ion pair HPLC method [14], HPLC method [15-18], HPTLC method [19-20] and LC/MS/MS [21-23]. On the contrary to the best of our knownledge, there is no official method for the stability-indicating simultaneous estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir by RP-HPLC in tablet dosage form. Hence, we planned to develop and validate a new method for stability-indicating simultaneous determination of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir in bulk drug and pharmaceutical dosage form. The new method is capable of separating all four active analytes present in the dosage form.

MATERIALS AND METHOD

Chemicals and solvents: Emtricitabine, Cobicistat, Tenofovir disoproxil fumarate and Elvitegravir were obtained as gift samples from Mylan Laboratories Limited, Hyderabad, India. The commercial Pharmaceutical tablets of STRIBILD containing 200 mg, 300 mg, 150 mg and 150 mg of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir respectively (Marketed by Gilead Sciences) were procured from local pharmacy. Orthophosphoric acid- HPLC grade (Fisher Scientific), Acetonitrile-HPLC grade (Merck), Sodium hydroxide-GR grade (Merck), Hydrochloric acid (Merck), Hydrogen peroxide (Merck) and water for HPLC- Milli-Q grade.

Instrumentation: The chromatographic separations were performed using HPLC-Waters alliance (Model-2695) consisting of an in-built auto sampler, a column oven and 2996 PDA detector. The data was acquired through Empower-2-software. The column used was Kromasil C18 (250×4.6mm i.d, 5µm particle size). Meltronics sonicator was used for enhancing dissolution of the compounds. Elico pH meter was used for adjusting the pH of buffer solution. All weighing was done on Sartrious balance (model AE-160).

Chromatographic conditions: The mobile phase consists of OPA buffer:Acetonitrile in the ratio of 55:45% v/v. The mobile phase was pumped from solvent reservoir in the ratio of 55:45 %v/v to the column in the flow rate of 1.0 ml/min whereas run time set was 11 min. The separation was performed on Kromasil C18 (250×4.6mm i.d, 5µm particle size) column and the column was maintained the temperature of 30°C and the volume of each injection was 10 µL. Prior to injection, the column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluents were monitored at 240 nm.

Preparation of buffer solution: (0.1% OPA Buffer): Diluted 1ml of concentrated Orthophosphoric acid to 1000ml with HPLC grade water and degas to sonicate.

Preparation of mobile phase: Buffer and Acetonitrile taken in the ratio 55:45%v/v. Filter through 0.45 µm filter under vacuum filtration.

Preparation of standard solution: (80 & 120 & 60 & 60 PPM): Accurately Weighed and transferred 8 mg & 12 mg & 6 mg & 6 mg of Emtricitabine and Tenofovir, Cobicistat and Elvitegravir working Standards into a individual 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1 ml was pipetted out in to a 10 ml volumetric flask and then make up to the final volume with diluent and thus we have (80µg/ml Emtricitabine & 120µg/ml Tenofovir & 60µg/ml Cobicistat & 60µg/ml Elvitegravir).

Preparation of sample solution: 20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 60 mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.4ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Label Claim: 200mg Emitricitabine + 300mg of Tenofovir + 150mg of Cobicistat + 150mg Elvitegravir

Validation of Proposed method: The developed method was validated as per the ICH (International Conference on Harmonization) guidelines with

respect to System suitability, Precision, Specificity, Forced degradation studies, Linearity, Accuracy, Limit of detection and Limit of quantification.

Linearity: Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml were taken from stock solution of concentration 0.8 mg/ml Emtricitabine, 1.2 mg/ml Tenofovir, 0.6 mg/ml Cobicistat and 0.6 mg/ml Elvitegravir and then diluted up to mark with diluent. Such that the final concentrations were in the range 20ppm-120ppm for Emtricitabine, 30ppm-180ppm for Tenofovir, 15ppm-90ppm for Cobicistat and 15ppm-90ppm for Elvitegravir. Volume of 10µl of each sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peak area vs. concentration was observed in the range of study. The observations and calibration curve were shown in Table-1 and Fig. 2,3,4,5.

Optimized Chromatographic conditions and system suitability parameters for proposed HPLC method for Emtricitabine, Tenofovir, Cobicistat and Elvitegravir

Parameter		
Chromatographic conditions		
Instrument	**:**	Waters 2695, High performance Liquid Chromatography
Flow rate	**:**	1 ml/min
Column	**:**	Kromasil C18, 250 x 4.6 mm, 5µ.
Detector wave length	**:**	240nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	11 min
Diluent	**:**	Water:Acetonitrile (50:50)
Mode of separation	**:**	Isocratic mode

System precision: Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Standard solution of Emtricitabine (80µg /ml), Tenofovir (120µg/ml), Cobicistat (60µg/ml) and Elvitegravir (60µg/ml) were prepared as per procedure and injected for 6 times. Results for responses are shown in Table-3.

PRECISION: Method precision and Intermediate precision study of Emtricitabine, Tenofovir, Cobicistat and Elvitegravir were carried out by estimating corresponding responses for 6 times on the same day and on consecutive days for the concentration of 80µg/ml for Emtricitabine, 120µg/ml for Tenofovir, 60µg/ml Cobicistat and 60µg/ml for Elvitegravir. The percent relative standard deviation (%RSD) was calculated which was within the acceptable criteria of not more than 2.0. The results were shown in Table-4.

ACCURACY (Recovery studies): To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were found to be within the limits. The accuracy was expressed as the percentage of the respective active analytes recovery. The results were shown in Table-2.

Specificity: The specificity of the method was performed by injecting blank solution(without any sample) and then a drug solution of 10µl injected into the column, under Optimized chromatographic conditions, to demonstrate the separation of four molecules Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific. The chromatogram was shown in figure-7.

Limit of Detection and Limit of Quantification: LOD and LOQ were calculated using the following formula LOD = 3.3(SD)/S and LOQ= 10 (SD)/S, where SD = standard deviation of response (peak area) and S= slope of the calibration curve. Limit of Detection and Limit of Quantification were found to be 0.097 µg/ml and 0.295 µg/ml respectively for Emtricitabine, 0.086 µg/ml and 0.260 µg/ml respectively for Tenofovir, 0.136 µg/ml and 0.412 µg/ml respectively for Cobicistat and 0.231 µg/ml and 0.699 µg/ml respectively for Elvitegravir as per ICH guidelines. The results were shown in Table-7.

ROBUSTNESS: Robustness was carried by varying three parameters from the optimized chromatographic conditions such as making small changes in flow rate (±0.1ml/min), mobile phase composition (±5%) and column temperature (±5ºC). It was observed that the small changes in these operational parameters did not lead to changes of retention time of the peak of interest and the %RSD was not more than 2.0. The degree of reproducibility of the results proven that the method is robust. The results were shown in Table-5 .

System suitability: The system suitability was determined by making six replicate injections from

freshly prepared standard solutions. The observed RSD values were well within usually accepted limits (≤2%). Theoretical plates, tailing factor of Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir were determined. The results are all within acceptable limits summarized in Table-8.

FORCED DEGRADATION STUDIES: Forced degradation studies were performed to demonstrate the optimized method is stability indicating. To prove the method which can be able to measure accurately active pharmaceutical ingredient in presence of degradants which are expected to be formed during different types of degradations applied to the drug sample.

For forced degradation analysis, aliquots of stock were separately treated with 1ml of 2N HCl (Acid stability), 1ml of 2N NaOH (Alkaline stability), 1ml of 20% H2O2 (Oxidative degradation), exposure of standard drug solution at 105ºC for 6 hrs (dry heat degradation), photo stability degradation (exposure of drug at 200 watt hours/m^2) and neutral degradation (refluxing with water at 60ºC for 6 hours. Stability of these samples was compared with fresh sample on the day of analysis. The HPLC chromatograms of degraded products show no interference at the respective analyte peaks, hence the method was specific and stability indicating. The chromatograms were shown in figures 9 and the results were shown in Table-6. The detailed degradation for each condition is as follows:

Oxidation: To 1 ml of stock solution of Emtricitabine, Tenofovir, Cobicistat and Elvitegravir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 120µg/ml, 60µg/ml and 60µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of s tock solution of Emitricitabine and Tenofovir and Cobicistat and Elvitegravir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 80µg/ml, 120µg/ml, 60µg/ml and 60µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution of Emitricitabine and Tenofovir and Cobicistat and Elvitegravir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 80µg/ml, 120µg/ml, 60µg/ml and 60µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105^0C for 6 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 120µg/ml, 60µg/ml and 60µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 120µg/ml, 60µg/ml and 60µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to obtain 80µg/ml, 120µg/ml, 60µg/ml and 60µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

The main aim for development of chromatographic method was to get reliable method for quantification of Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir from bulk and pharmaceutical dosage form and which will be applicable for the degradation products also. Different chromatographic conditions were employed for the analysis of the Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir in both bulk and pharmaceutical dosage form. Finally the analysis was performed by using OPA Buffer: Acetonitrile in the ratio of 55:45 %v/v at a flow rate 1.0 ml/min. Samples were analysed at 240 nm at an injection volume of 10 µL and separation was carried by using Kromasil C18, (250 x 4.6 mm, 5µ) column. The retention time and tailing factor were calculated. The retention time of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were found to be 2.198, 2.791, 5.228 and 5.893 respectively. The proposed column was selected

which gave a sharp and symmetrical peak with 1.29 tailing factor and theoretical plates of 6546 for Emtricitabine, 1.34 tailing factor and theoretical plates of 6217 for Tenofovir and 1.31 tailing factor and theoretical plates of 6161 for Cobicistat and 1.02 tailing factor and theoretical plates of 11132 for Elvitegravir. The resolution between the active analyte peaks found to be within the acceptable limit. The calibration curve was linear over the concentration range of 20-120 ppm for Emtricitabine, 30-180 ppm for Tenofovir, 15-90ppm for Cobicistat and 15-90 ppm for Elvitegravir. Six different concentrations of Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir in the given range were prepared and injected into HPLC. The linearity of the method was statistically confirmed. RSD values for accuracy and precision studies obtained were less than 2.0% which revealed that developed method was accurate and precise. The system suitability parameters were given in Table-8.

Forced degradation studies concluded that the all the degradant peaks obtained during degradation were well resolved from the main drugs i.e. Emtricitabine, Tenofovir, Cobicistat & Elvitegravir. And the peak purity was passed i.e. purity angle was less than purity threshold as per Empower-2 software. Hence the method is found to be stability indicating.

Therefore proposed validated stability indicating method was successfully applied to determine Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir in Bulk and Pharmaceutical dosage form.

CONCLUSION

The developed method is accurate, simple, rapid and selective & proved to be stability indicating for the simultaneous estimation of Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir in Bulk and pharmaceutical dosage form. The sample preparation is simple, the analysis time is short and the elution is by isocratic method. To our present knowledge, no attempts have yet been made to estimate this multidrug mixture by stability indicating analytical procedure. All the active ingredients were profitably resolved with good resolution and quantified. The retention time of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were found to be 2.198, 2.791, 5.228 and 5.893 respectively. The validation parameters like system suitability, linearity, accuracy, robustness, solution stability, specificity, limit of detection and limit of quanititation were found to be within the acceptance limits. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. Forced degradation studies of different conditions shows that all the degradants were well resolved from these main drug peaks and able to quantify the Tenofovir disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravir in presence of degradants & excipients which proved that the method is found to be stability indicating. Hence the proposed method can be conveniently adopted for the routine quality control analysis in the bulk and combined formulations.

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Table-1: Linearity

S.No	% level	ETC (ppm)	ETC Area	TDF (ppm)	TDF area	CBT (ppm)	CBT area	EVG (ppm)	EVG area
1	25	20	496336	30	528125	15	52011	15	466449
2	50	40	1026119	60	1066262	30	103604	30	920879
3	75	60	1472432	90	1605198	45	152359	45	1374986
4	100	80	2039792	120	2141096	60	203606	60	1814717
5	125	100	2488680	150	2628443	75	253741	75	2252619
6	150	120	3015872	180	3204307	90	309115	90	2757144

Table-2: Accuracy:

Emtricitabine			Tenofovir			Cobicistat			Elvitegravir		
Amount Added (µg/ml)	**Amount Found (µg/ml)**	**% Recovery**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**%Recovery**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**%Recovery**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**% Recovery**
40	40.36	100.9	60	60.45	100.8	30	30.13	100.4	30	29.58	98.6
40	39.19	98.0	60	60.83	101.4	30	29.93	99.8	30	30.05	100.2
40	39.77	99.4	60	59.84	99.7	30	30.05	100.2	30	29.78	99.3
80	80.99	101.2	120	119.57	99.6	60	60.05	100.1	60	59.05	98.4
80	79.49	99.4	120	120.32	100.3	60	59.91	99.9	60	60.29	100.5
80	79.63	99.5	120	121.64	101.4	60	59.20	98.7	60	59.55	99.3
120	121.95	101.6	180	179.75	99.9	90	89.33	99.3	90	89.80	99.8
120	120.66	100.6	180	178.81	99.3	90	89.63	99.6	90	90.08	100.1
120	119.61	99.7	180	182.30	101.3	90	88.54	98.4	90	88.92	98.8
Average		100.0			100.4			99.6			99.4
SD		1.141			0.809			0.691			0.739
RSD		1.141			0.807			0.694			0.744

Table-3: System precision:

INJECTIONS	**Areas**			
	Emtricitabine	**Tenofovir**	**Cobicistat**	**Elvitegravir**
1	1969852	2156625	209963	1867126
2	1965957	2122677	205476	1834549
3	1972192	2150561	208587	1862351
4	1964438	2146773	207869	1867217
5	1972918	2150510	209305	1859201
6	1937116	2158376	207973	1861507
AVG	1963746	2147587	208196	1858659
S.D	13471	12936	1553	12233
%RSD	0.69	0.60	0.75	0.66

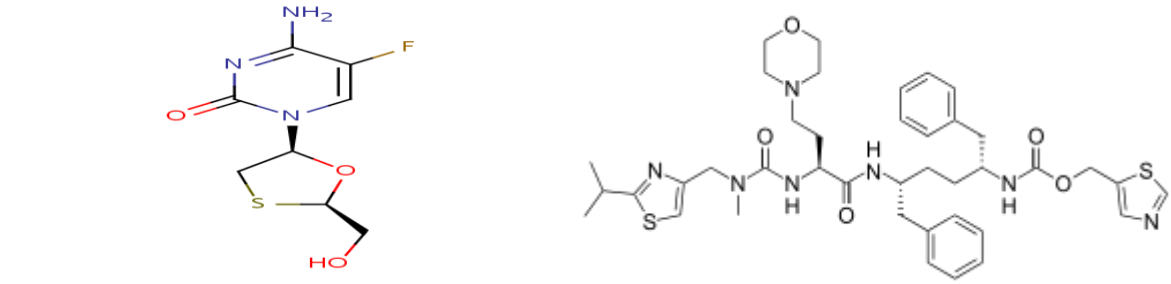
Table-4: Precision:

Table-4A: Method precision

Sample Preparations	%Assay			
	Emtricitabine	Tenofovir	Cobicistat	Elvitegravir
Sample-1	100.31	98.17	99.55	98.06
Sample-2	98.09	98.36	98.96	99.06
Sample-3	100.31	101.75	101.48	101.00
Sample-4	101.77	100.81	99.29	100.86
Sample-5	98.68	101.58	100.45	98.14
Sample-6	99.68	98.63	99.80	99.24
AVG	99.81	99.88	99.92	99.39
S.D	1.311	1.68	0.916	1.282
%RSD	1.31	1.68	0.92	1.29

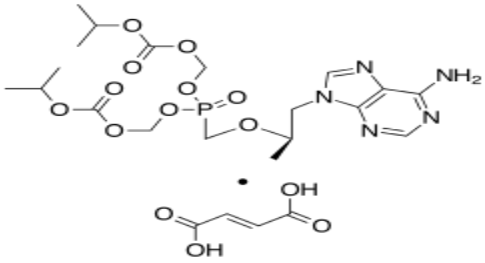
Table-4B: Intermediate precision

Sample Preparations	%Assay			
	Emtricitabine	Tenofovir	Cobicistat	Elvitegravir
Sample-1	100.1	98.34	101.25	99.32
Sample-2	97.89	98.53	102.89	101.26
Sample-3	100.1	101.92	101.6	101.12
Sample-4	101.56	100.99	99.81	98.39
Sample-5	98.48	101.76	101.86	99.49
Sample-6	99.48	98.63	100.13	98.31
AVG	99.60	100.03	101.26	99.65
S.D	1.306	1.71	1.141	1.286
%RSD	1.31	1.71	1.13	1.29



(A)

(B)



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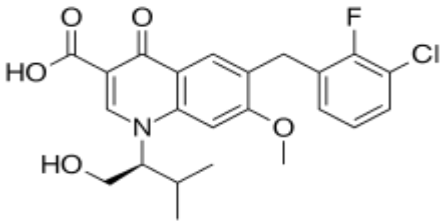

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Figure 1: The Chemical Structures of Emtricitabine (A), Cobicistat (B), Tenofovir Disoproxil fumarate (C), Elvitegravir (D)

Table-5: Compilation Robustness study results

S. No.	Parameter	System suitability	ETC	TDF	CBT	EVG
1	As such	**Retention Time**	2.198	2.791	5.228	5.893
		Plate Count	6546	6217	6161	11132
		Tailing factor	1.29	1.34	1.31	1.02
2	Flow Rate (0.9ml/min)	**Retention Time**	2.440	3.099	5.821	6.526
		Plate Count	7971	7624	6986	12787
		Tailing factor	1.21	1.38	1.28	1.02
3	Flow Rate (1.1ml/min)	**Retention Time**	2.007	2.558	5.364	4.837
		Plate Count	6532	6489	11493	6385
		Tailing factor	1.28	1.34	1.03	1.24
4	Column Temperature (25°C)	**Retention Time**	2.210	2.879	5.745	6.200
		Plate Count	7481	6938	7132	12051
		Tailing factor	1.30	1.41	1.20	1.05
5	Column Temperature (35°C)	**Retention Time**	2.196	2.727	4.845	5.592
		Plate Count	7166	7088	6413	11710
		Tailing factor	1.30	1.35	1.29	1.05
6	Mobile phase composition (60:40v/v)	**Retention Time**	2.195	2.721	4.845	5.590
		Plate Count	7544	7091	6447	11719
		Tailing factor	1.32	1.35	1.31	1.04
7	Mobile phase composition (50:50v/v)	**Retention Time**	2.210	2.879	5.745	6.200
		Plate Count	7481	6938	6968	12087
		Tailing factor	1.30	1.41	1.25	1.05

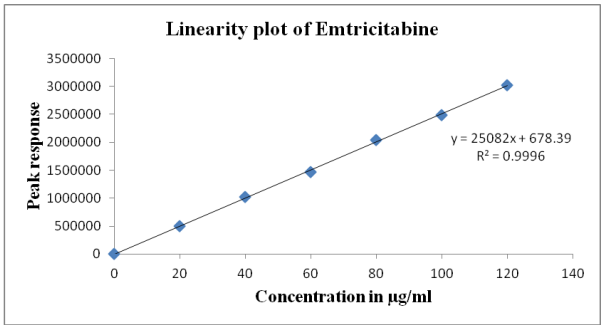

Figure-2: Linearity of Emtrictabine:

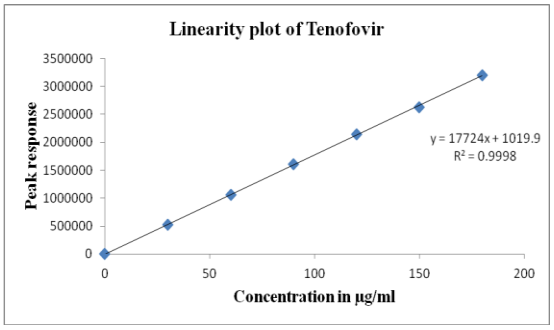

Figure-3: Linearity of Tenofovir

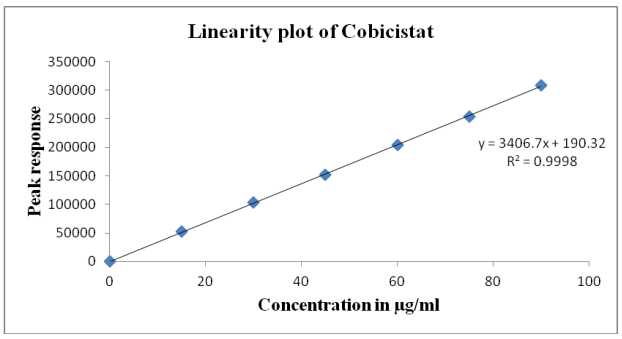

Figure-4: Linearity of Cobicistat:

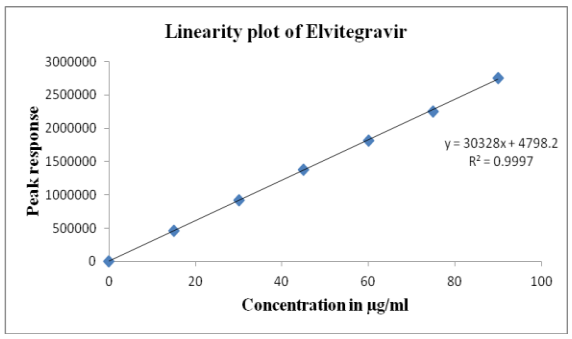

Figure-5: Linearity of Elvitegravir:

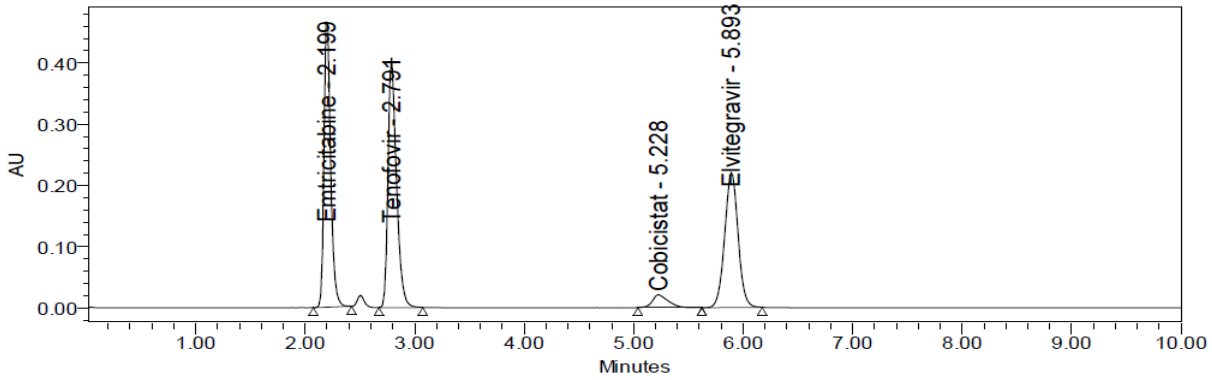

Figure 6: Typical Chromatogram of Standard

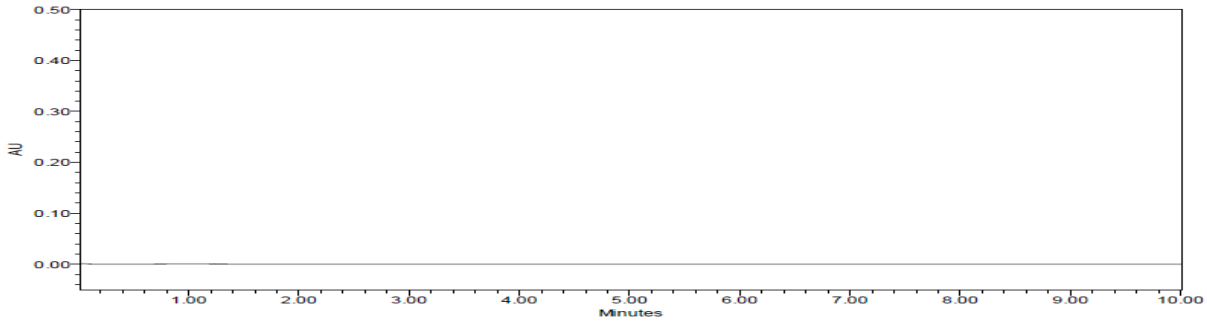

Figure 7: Typical Chromatogram of Placebo

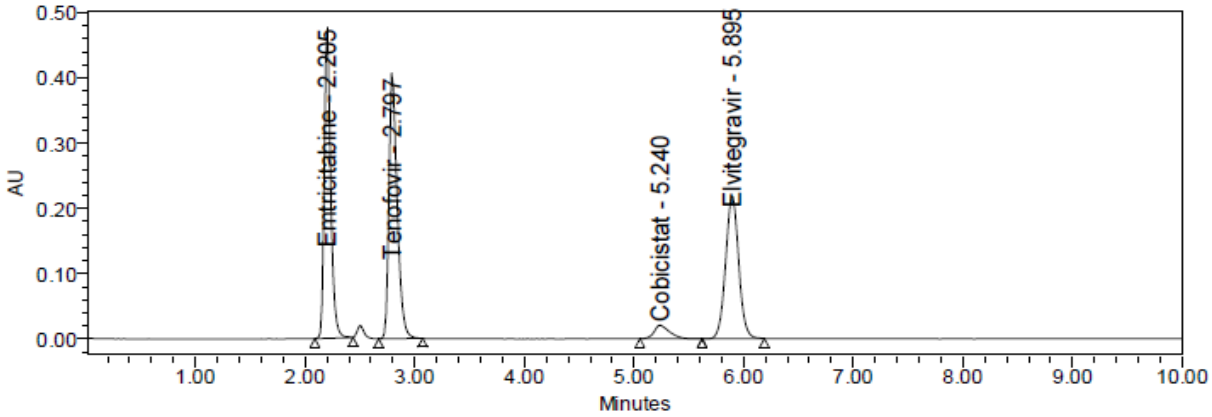

Figure 8: Typical Chromatogram of Sample

Table-6 Forced Degradation Study results:

Sr.No	Injection	%Assay	Purity Angle	Purity Threshold	Purity Flag
1	**Controlled sample**				
	Emtricitabine	99.81	0.301	1.348	No
	Tenofovir	99.88	0.110	0.292	
	Cobicistat	99.92	0.121	0.395	
	Elvitegravir	99.39	0.032	0.247	
2	**Acid Degradation**				
	Emtricitabine	93.86	0.315	1.252	No
	Tenofovir	93.25	0.107	0.245	
	Cobicistat	93.90	0.134	0.337	
	Elvitegravir	93.54	0.035	0.228	
3	**Base Degradation**				
	Emtricitabine	93.99	0.311	1.252	No
	Tenofovir	93.59	0.105	0.244	
	Cobicistat	93.46	0.141	0.360	
	Elvitegravir	94.01	0.033	0.228	
4	**Peroxide Degradation**				
	Emtricitabine	94.63	0.305	1.252	No
	Tenofovir	92.06	0.113	0.245	
	Cobicistat	92.75	0.131	0.334	
	Elvitegravir	91.72	0.033	0.228	
5	**Thermal Degradation**				
	Emtricitabine	96.78	0.408	1.254	No
	Tenofovir	97.60	0.107	0.247	
	Cobicistat	97.91	0.138	0.357	
	Elvitegravir	97.69	0.039	0.232	
6	**UV Degradation**				
	Emtricitabine	97.62	0.342	1.251	No
	Tenofovir	98.46	0.107	0.242	
	Cobicistat	98.83	0.143	0.324	
	Elvitegravir	99.06	0.032	0.226	
7	**Water Degradation**				
	Emtricitabine	98.67	0.307	1.251	No
	Tenofovir	98.85	0.112	0.243	
	Cobicistat	99.04	0.127	0.319	
	Elvitegravir	99.60	0.034	0.226	

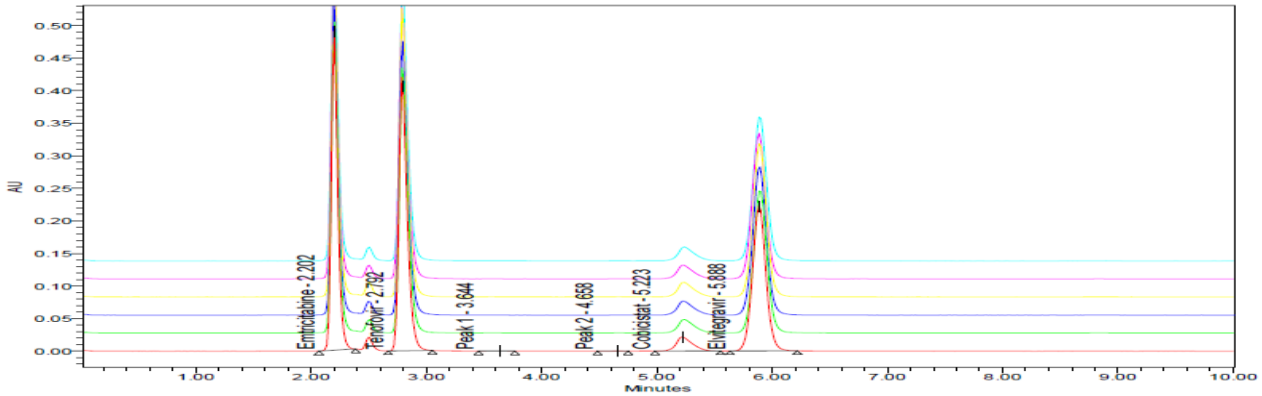

Figure 9: Typical overlay chromatograms of forced degradation samples

Table-7: Characteristics of HPLC method:

Drug	Parameters defined	Obtained value
Emtricitabine	Linearity range (ppm)	20-120 ppm
	Regression coefficient(r^2)	0.999
	Intercept	678
	Slope	25082
	LOD (ppm)	0.097
	LOQ (ppm)	0.295
Tenofovir	Linearity range (ppm)	30-180 ppm
	Regression coefficient(r^2)	0.999
	Intercept	1020
	Slope	17725
	LOD (ppm)	0.086
	LOQ (ppm)	0.260
Cobicistat	Linearity range (ppm)	15-90 ppm
	Regression coefficient(r^2)	0.999
	Intercept	190
	Slope	3406
	LOD (ppm)	0.136
	LOQ (ppm)	0.412
Elvitegravir	Linearity range (ppm)	15-90 ppm
	Regression coefficient(r^2)	0.999
	Intercept	4798
	Slope	30327
	LOD (ppm)	0.231
	LOQ (ppm)	0.699

Table-8: System suitability results:

System suitability parameters	Result				Acceptance criteria
	EMT	TDF	CBT	EVG	
Retention Time	2.198	2.791	5.228	5.893	For information
% RSD for area count of six replicate injection of standard	0.7	0.6	0.8	0.7	NMT 2.0
Tailing factor	1.29	1.34	1.31	1.02	NMT 2.0
Theoretical plates	6546	6217	6161	11132	NLT 2000
Resolution	N/A	3.8	18.5	3.5	NLT 2.0

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