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A validated stability indicating LC-MS compatible RP-HPLC assay and dissolution methods for marketed formulation Stribild

U. Harini *, A. K. M. Pawar

Department of Pharmaceutical Analysis and Quality Assurance, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India.

ABSTRACT

Objective: The prime objective of the current work is to develop a simple, rapid, efficient, economical and stability indicating LC-MS (liquid chromatography–mass spectroscopy) compatible RP - HPLC (reverse phase – high performance liquid chromatography) method for the analysis of emtricitabine (EMT), tenofovir disoproxil fumarate (TDF), cobicistat (COB) and elvitegravir (ELV) in bulk, marketed formulation (Stribild) and in *In-vitro* dissolution method.

Method: The chromatography was achieved on Unisol C18 column (250 × 4.0 mm, 3 µ) with a mobile phase combination of acetate buffer (adjusted with dilute glacial acetic acid to pH 4) and acetonitrile in gradient mode at a flow rate of 1mL/min and the detection was performed at 260 nm using PDA (photo diode array) detector. Forced degradation studies were performed and the % degradation under various stress conditions was calculated. The developed RP-HPLC method was applied for Stribild tablets to study the dissolution profile.

Results: The retention times for emtricitabine, tenofovir disoproxil fumarate, cobicistat and elvitegravir were 5.7, 12.1, 16.3 and 19.4 min respectively. The % degradation was below 20% which is within the limits. The percent drug release was found to meet USP specification, i.e. not less than 80% of amount of labeled drug EMT, TDF, COB and ELV dissolved in 30min.

Conclusion: The method was validated as per ICH guidelines and all the validation parameters were within the compendial requirements. The proposed method can be successfully adopted for the analysis of Stribild tablets in pharmaceutical industries.

Keywords: Stribild, emtricitabine, tenofovir disoproxil fumarate, cobicistat, elvitegravir.

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***Address for Correspondence:**

Dr. A. K. M. Pawar, Assistant Professor, Department of Pharmaceutical Analysis and Quality Assurance, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

1. INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus (a sub group of retroviruses) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening infections and cancers. STRIBILD, the first integrase inhibitor- based single tablet regimen containing emtricitabine (EMT), tenofovir disoproxil fumarate (TDF), cobicistat (COB) and elvitegravir (ELV) for HIV-1 infection, meets current WHO treatment guidelines for a complete fixed-dose, once daily highly active antiretroviral therapy (HAART) regimens. HAART is the name given to aggressive treatment regimens used to suppress HIV viral replication and the progression of HIV disease. Stribild is a pill combination of four active ingredients-150 mg of Elvitegravir, 150 mg of Cobicistat, 200 mg of Emtricitabine and 300 mg of Tenofovir disoproxil fumarate (equivalent to 245 mg of Tenofovir Disoproxil) and is taken once a day [1-3]

Emtricitabine: The chemical name of Emtricitabine (EMT) is 6-(3-Chloro-2-fluorobenzyl)-1-[(2S)-1 hydroxy-3-methyl butan-2-yl]-7-methoxy-4-oxo-1, 4-dihydro quinoline-3-carboxylic acid. Emtricitabine is the (-) enantiomer of a thio analog of cytidine, which differs from other cytidine analogs in that it has a fluorine in the 5-position. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. It has a molecular formula of $C_8H_{10}FN_3O_3S$ and a molecular weight of 247.25. It is a white to off-white crystalline powder with a solubility of approximately 112 mg/mL in water at 25°C. It has the following structural formula

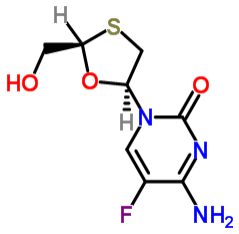

Figure 1: Structure of Emtricitabine

Tenofovir disoproxil fumarate: Tenofovir disoproxil fumarate (TDF) is a fumaric acid salt of the bis-isopropoxy carbonyl oxy methyl ester derivative of tenofovir. The chemical name of TDF is 9-[(R)-2- [[bis [[(isopropoxy carbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate. TDF belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs). It has a molecular formula of $C_{19}H_{30}N_5O_{10}P \bullet C_4H_4O_4$ and a molecular weight of 635.51. It is a white to off-white crystalline powder with a solubility of 13.4 mg/mL in water at 25 °C. It has the following structural formula

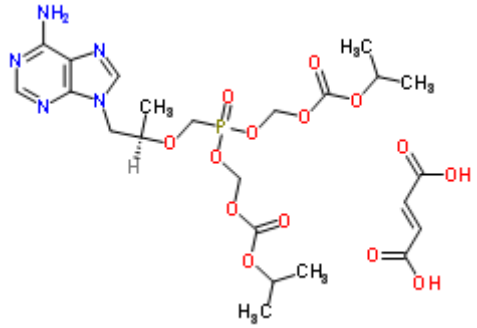

Figure 2: Structure of Tenofovir disoproxil fumarate

Cobicistat: The chemical name for Cobicistat (COB) 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-4-yl) butanol] amino}-1,6-diphenyl hexan-2-yl] carbamate. Cobicistat acts as an HIV integrase inhibitor. Cobicistat is the only other booster approved for use as a part of HAART, Cobicistat has no anti-HIV activity of its own. Cobicistat is a potent inhibitor of cytochrome P450 3A enzymes, including the important CYP3A4 subtype. It also inhibits intestinal transport proteins, increasing the overall absorption of several HIV medications. It has a molecular formula of $C_{40}H_{53}N_7O_5S_2$ and a molecular weight of 776.0. It is adsorbed onto silicon dioxide. Cobicistat on silicon dioxide is a white to pale yellow solid with a solubility of 0.1 mg/mL in water at 20 °C. It has the following structural formula

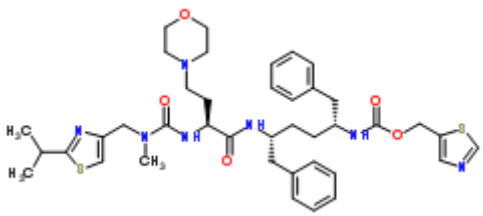

Figure 3: Structure of Cobicistat

Elvitegravir: The chemical name of Elvitegravir (ELV) is 6-(3-Chloro-2-fluorobenzyl) - 1- [(2S)-1hydroxy-3-methyl butan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. Elvitegravir is a drug used for the treatment of HIV infection. It acts as an integrase inhibitor. It has a molecular formula of $C_{23}H_{23}Cl\ F\ NO_5$ and a molecular weight of 447.9. Elvitegravir is a white to pale yellow powder with a solubility of less than 0.3 µg/mL in water at 20 °C. It has the following structural formula

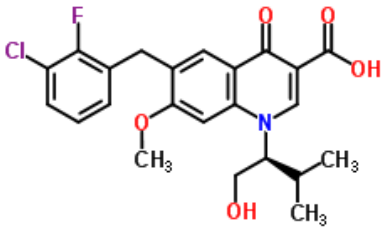

Figure 4: Structure of Elvitegravir

Objective

Literature survey reveals that there are very few RP - HPLC methods available for the estimation of EMT, TDF, COB and ELV individually, but with very poor resolution between the retention time of the four drugs and the degradation products obtained in the forced degradation studies and there is no dissolution method reported till to date for this quad pill. Hence there is a need to develop and validate a new LC-MS compatible stability indicating RP-HPLC method for developing assay and dissolution methods for the marketed formulation Stribild [4-12]

2. METHODS

2.1 Apparatus:

An Agilent Infinity 1260 HPLC system equipped with quaternary pumps G1311C, Degasser G4225A, Auto sampler G1329B, Thermostatted column compartment G1316A with PDA detector G4212B was used. The software used for data acquisition was Open LAB CDS EZ Chrom A.04.05. Lab India D_5 8000 Dissolution test apparatus, Mettler Toledo ME 204 weighing balance, Magnetic stirrer, Kemi Hot air oven, Eutech 700 pH meter, Double distillation apparatus.

2.2 Chemicals:

Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat, Elvitegravir working standards and Stribild tablets were kindly given as gift samples by Aurobindo Pharma Limited, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include ammonium acetate, sodium hydroxide, hydrochloric acid, 30% hydrogen peroxide, ortho phosphoric acid, glacial acetic acid and potassium dihydrogen phosphate, tween 80 were purchased from E. Merck Limited, Mumbai, India.

2.3 Assay Method

2.3.1 Selection of Detection Wavelength:

Drug solutions of 10 µg/mL were prepared for the four API working standards individually and scanned over the range of 200-400 nm in UV/VIS Spectrophotometer using methanol as blank. The λ max of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were found at 283 nm, 259 nm, 240 nm and 258 nm respectively. The optimum wavelength was selected based on isosbestic

point at 260 nm which represents the wavelength where the sample solution (Stribild) has an absorption maximum. Higher wavelength also helps in reducing interferences from common excipients used in the formulation.

2.3.2 Preparation of Buffer:

Accurately weighed 1.54 g of ammonium acetate and dissolved in 1000 mL of water. The pH was adjusted to 4.0 with 30% glacial acetic acid and filtered through 0.45 µ nylon filter and degassed.

2.3.3 Preparation of Diluent:

Diluent-1: HPLC grade methanol was used as 1st diluent.

Diluent-2: The second diluent was prepared by using a mixture of methanol: buffer in the ratio 60: 40.

2.3.4 Preparation of Blank:

The diluent-2 prepared by using a mixture of methanol: buffer in the ratio 60: 40, was injected as blank solution.

3.3.5 Preparation of Placebo Solution:

300 mg of placebo was weighed and transferred to 50 mL volumetric flask to which 30 mL of diluent-1 was added and sonicated for 15min at room temperature and made up to final volume to prepare stock solution. The stock solution was filtered through 0.45 µ nylon filter and 3 mL of filtered solution was transferred to 50 mL volumetric flask and made up to final volume with diluent-2.

2.3.6 Preparation of Working Standard Solution:

Accurately weighed and transferred about 20 mg of Emtricitabine, 30 mg of Tenofovir disoproxil fumarate, 15 mg of Cobicistat and 15 mg of Elvitegravir working standards into a 10 mL clean and dry volumetric flask and 5 mL of methanol was added and sonicated for 10 min to dissolve. The final volume was made up with methanol and filtered through 0.45 µ nylon filter. 1 mL from the above filtered stock solution was taken and made up to 10 mL with diluent-2. The final concentrations of EMT, TDF, COB and ELV were about 200 ppm, 300 ppm, 150 ppm and 150 ppm respectively.

2.3.7 Preparation of Sample Solution:

Ten tablets were weighed and average weight was calculated. The tablets were crushed with a mortar and pestle. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred to a 100 mL volumetric flask. Approximately 50 mL methanol was added and the mixture was sonicated for 15min with intermittent shaking, then 20 mL of water was added and sonicated for 30 min. The contents were restored to room temperature and diluted to final volume with methanol to furnish stock solution. The stock solution was filtered through 0.45 µ nylon filter and 10 mL of the filtered solution was transferred to a 50 mL volumetric flask and made up to volume with diluent-2. The final concentrations of EMT, TDF, COB and ELV were about 200 ppm, 300 ppm, 150 ppm and 150 ppm respectively. The optimized chromatographic conditions are mentioned in table 1.

Table 1: Optimized Chromatographic Conditions

<table>
<tr><th>Parameter</th><th>Optimized conditions</th></tr>
<tr><td>Stationary phase (column)</td><td>Unisol C18 column (250 mm × 4.0 mm, 3 µ)</td></tr>
<tr><td>Mobile phase</td><td>Acetate buffer and acetonitrile in gradient mode
<table>
<tr><th>Time (min)</th><th>Buffer (% v/v)</th><th>Acetonitrile (% v/v)</th></tr>
<tr><td>0.01</td><td>90</td><td>10</td></tr>
<tr><td>5</td><td>60</td><td>40</td></tr>
<tr><td>10</td><td>50</td><td>50</td></tr>
<tr><td>13</td><td>40</td><td>60</td></tr>
<tr><td>15</td><td>20</td><td>80</td></tr>
<tr><td>20</td><td>10</td><td>90</td></tr>
<tr><td>23</td><td>50</td><td>50</td></tr>
<tr><td>28</td><td>90</td><td>10</td></tr>
</table></td></tr>
<tr><td>Flow rate</td><td>1 mL/min</td></tr>
<tr><td>Injection volume</td><td>15 µL</td></tr>
<tr><td>Column temperature</td><td>30°C</td></tr>
<tr><td>Detection</td><td>Performed at 260 nm using PDA detector</td></tr>
<tr><td>Run time</td><td>30 min</td></tr>
</table>

2.4 Validation of the Developed Method

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness.

2.5 Forced Degradation Studies

2.5.1 Acid degradation:

Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir working standard solutions (1mg/mL) were individually treated with 5mL of 0.1N HCl at room temperature for 24 hours and then neutralized with 0.1N NaOH. The solutions were further diluted to required concentrations with diluent-2.

2.5.2 Alkali degradation:

Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir working standard solutions (1mg/mL) were individually treated with 5mL of 0.1N NaOH at room temperature for 24 hours and then neutralized with 0.1N HCl. The solutions were further diluted to required concentrations with diluent-2.

2.5.3 Oxidative degradation:

Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir working standard solutions (1mg/mL) were individually treated with 30% H_2O_2 at room temperature for 24 hours. The solutions were further diluted to required concentrations with diluent-2.

2.5.4 Thermal degradation:

Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir working standards of 10mg were taken individually into petri dishes and kept in a hot air oven at 70° C for 24 hours. The stock solutions were prepared with diluent-1 and further diluted to required concentrations with diluent-2.

3.5.5 Photolytic degradation:

Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir working standard solutions (1mg/mL) were prepared individually, and exposed to sunlight for 8hours on three consecutive days. Then they were further diluted to required concentrations with diluent-2.

2.6 Dissolution Method:

Application of developed RP-HPLC method for the dissolution studies of Stribild tablets.

2.6.1 Preparation of Dissolution Medium (2.0% polysorbate 80 in 0.01N HCl):

20g of polysorbate 80 was transferred to 1000mL of 0.01N HCl and mixed.

2.6.2 Dissolution parameters:

Medium: 0.1 N Hydrochloric acid

Volume: 1000 mL

Apparatus: USP Apparatus-II (paddle) with stainless steel helical sinkers

RPM: 100 rpm

Temperature: 37.0° ± 0.5°C

Time: 10, 20, 30, 40, 50 and 60 minutes

2.6.3 Preparation of Working Standard Solution:

Refer to preparation of working standard solution in assay method-3.3.6.

2.6.4 Preparation of Sample Solution:

The parameters of dissolution apparatus were set as mentioned above. One Stribild tablet was placed into each of the six dissolution vessels, one vessel with dissolution medium without sample and the dissolution test was performed. At the specified time intervals, 10 mL of the sample solution was withdrawn from each dissolution vessel. The solution was filtered through 0.45 µ membrane filter. 10 mL of fresh dissolution medium maintained at 37.0°C ± 0.5°C was replaced after every time point sampling. 5 mL of this solution was transferred to 20 mL volumetric flask and diluted to volume with dissolution medium and mixed.

2.6.5 Procedure:

The same procedure and the optimized chromatographic conditions (refer table-1) developed for assay method was also applicable for dissolution method. The system suitability was evaluated by injecting 10 µL of working standard solution five times into the chromatograph. Then blank (dissolution medium), working standard and samples from six dissolution vessels collected at each specified time interval were made up to final concentration and injected into the chromatograph, the chromatograms were recorded and peak areas were measured. The amount of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir dissolved in dissolution medium at specific time intervals were calculated and the dissolution profile was studied.

3. RESULTS AND DISCUSSION

In order to achieve good resolution between all the four drugs, different buffers with pH-conditions such as phosphate buffer at pH 3.0 and trifluoro acetic acid and different proportions of solvents like methanol and acetonitrile were tested. However, in acetate buffer (adjusted with dilute glacial acetic acid to pH 4) and acetonitrile in gradient mode achieved good satisfactory results at a flow rate of 1.0 mL/min. The retention times of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were found to be 5.7, 12.1, 16.3 and 19.4 min respectively. The chromatograms of blank, placebo, working standards of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat, Elvitegravir, four standard API's mixture and Stribild sample solution were shown in figures 1 to 8.

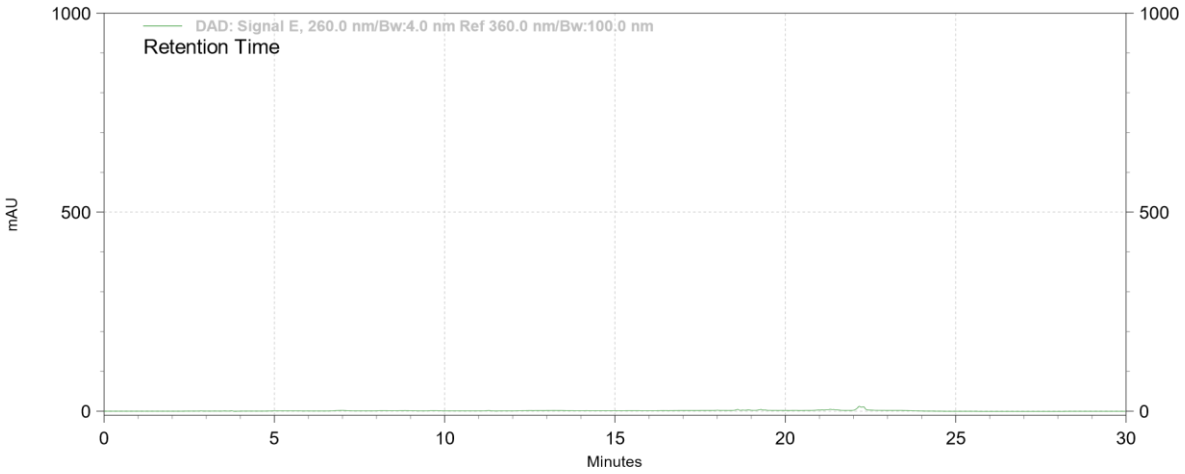

Figure 1: Chromatogram of Blank

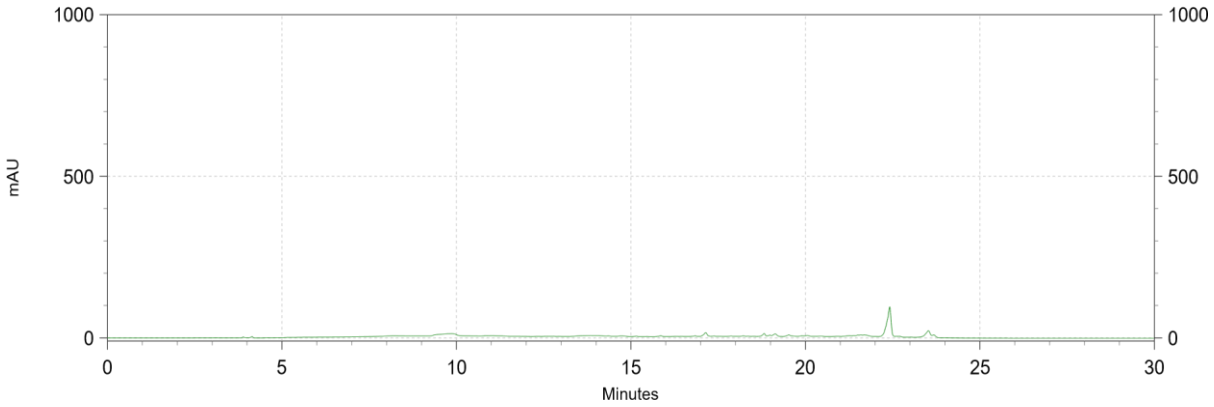

Figure 2: Chromatogram of Placebo

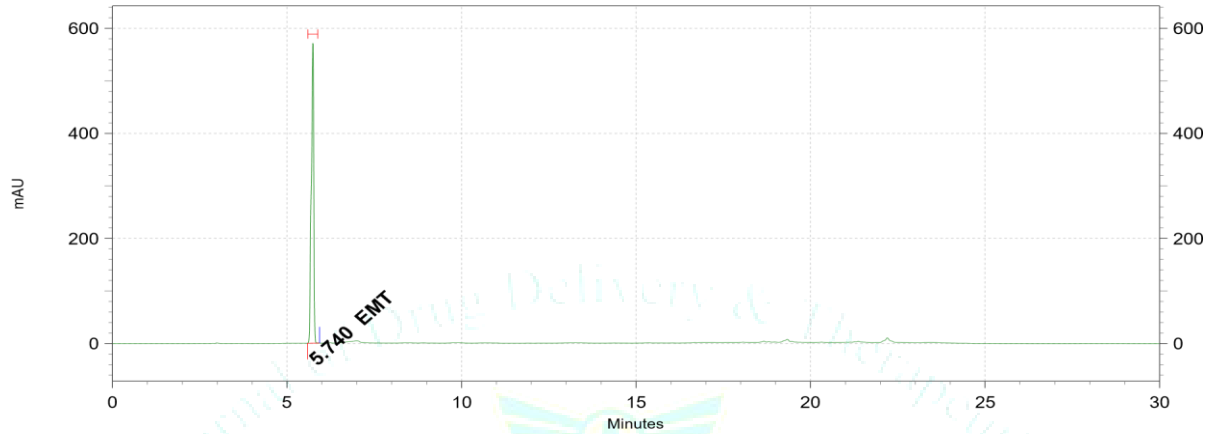

Figure 3: Chromatogram of Emtricitabine working standard

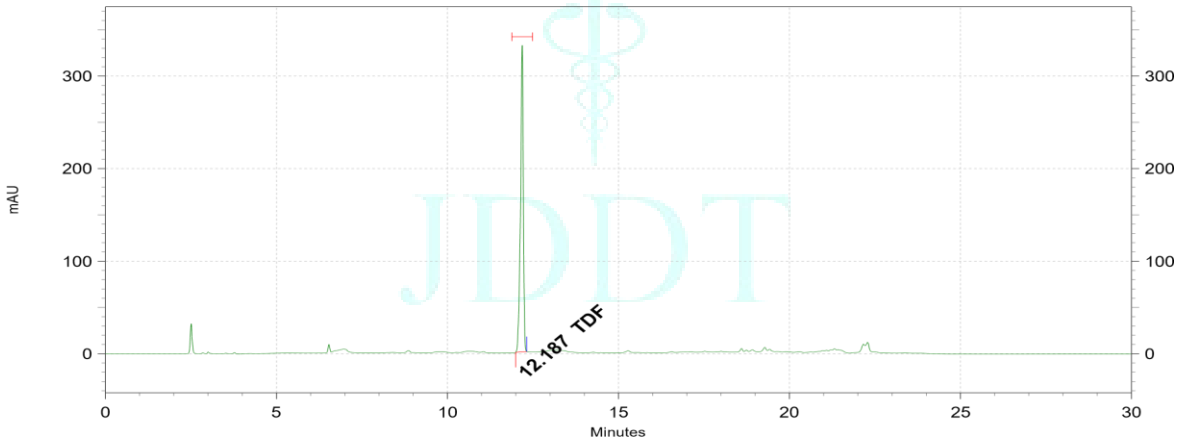

Figure 4: Chromatogram of Tenofovir disoproxil fumarate working standard

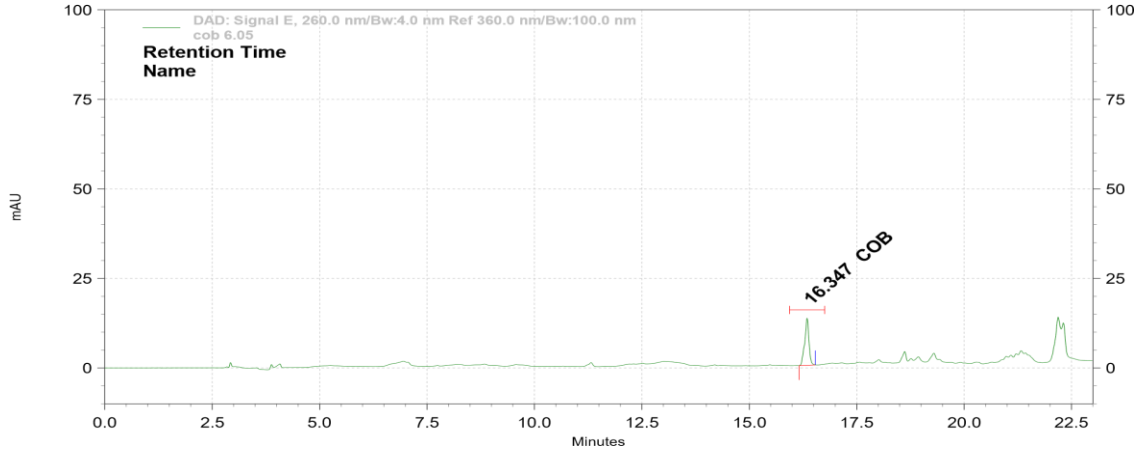

Figure 5: Chromatogram of Cobicistat working standard

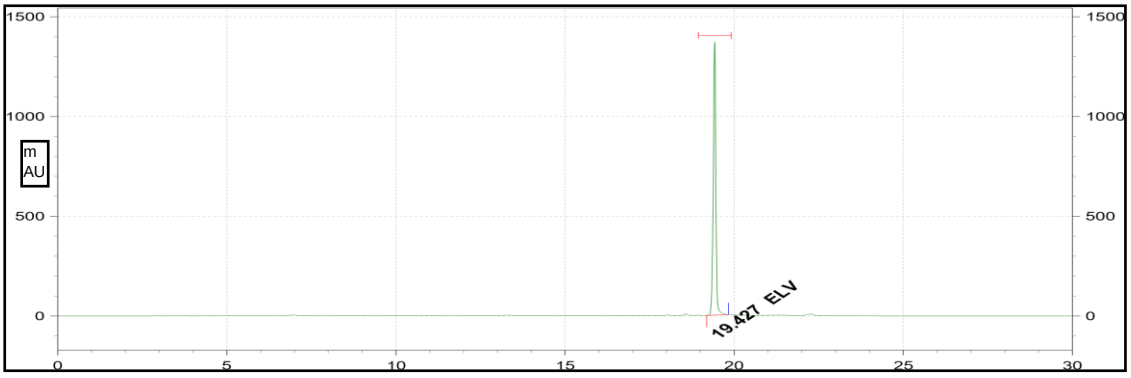

Figure 6: Chromatogram of Elvitegravir working standard

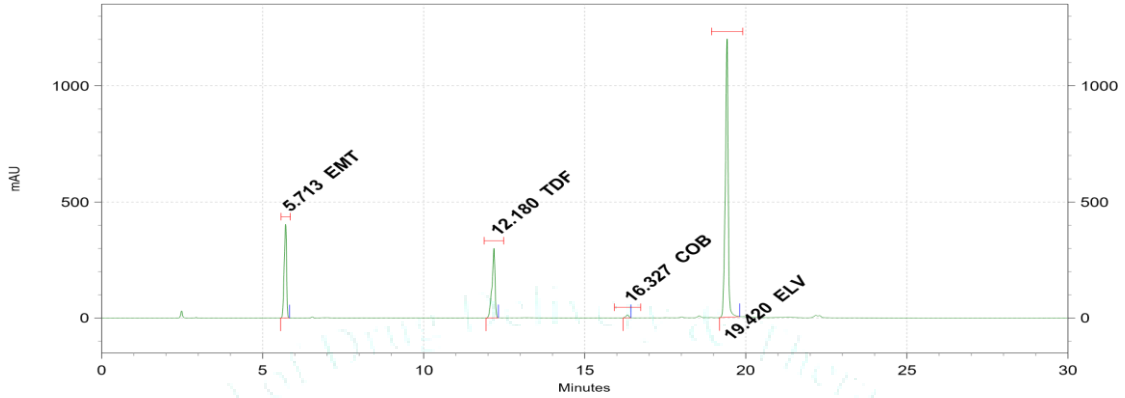

Figure 7: Chromatogram of four Standard API's mixture

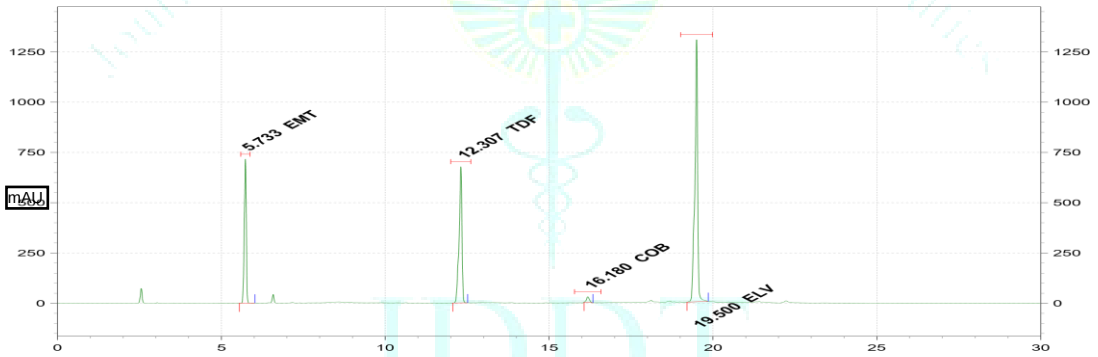

Figure 8: Chromatogram of Stribild sample

4.1 Assay:

Analysis of Stribild tablets was performed by the proposed method and the percent assay of the formulation was calculated in triplicate. The assay percentage of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were found to be within limits in the sample Stribild tablets and the results are shown in table 2.

Table 2: Assay results of Stribild tablets

S.NO.	Drug name	Label claim (mg)	Amount found(mg)	Assay (%) *
1	Emtricitabine	200	199.2	99.6
2	Tenofovir	300	297.5	99.2
3	Cobicistat	150	148.7	99.1
4	Elvitegravir	150	150.8	100.5

* = Average assay% of 3 replicate injections

4.2 Method Validation:

4.2.1 System suitability:

10µL of standard solution was injected five times into the chromatograph, the chromatograms were recorded and the peak areas were measured. The column efficiency for EMT, TDF, COB and ELV peaks should not be less than 5000 plate counts and tailing factor should be between 0.80 to 2.0. RSD for peak areas from five replicate injections should not be more than 2.0%. Parameters such as number of theoretical plates, average area and peak tailing were determined and all the parameters were within the limits. Results are shown in table 3.

Table 3: System suitability data

Parameters	EMT	TDF	COB	ELV
Theoretical plates*	47155	10206	15700	25735
Tailing factor*	0.9	1.2	0.8	1.3
Average peak area*	185376024	169302341	8113118	624388448
Standard deviation	843249.78	554654.95	51989.9	5236220
% RSD	0.45	0.33	0.64	0.84

* = Average result of 6 replicate injections

4.2.2 Specificity:

The specificity of the analytical method was established by injecting the 100 µg/mL concentration solutions of diluent (blank), placebo, working standards and sample solution individually to investigate interference from the representative peaks. From the obtained chromatograms in figures 1 to 8 it can be inferred that there were no co-eluting peaks at the retention time of EMT, TDF, COB and ELV, which shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analyte of interest.

4.2.3 Linearity:

Linearity was evaluated by analyzing different concentrations of the standard solutions of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir solutions. 6 working standard solutions ranging between 25-150, 37.5-225, 18.75-112.5 and 18.75-112.5 µg/mL were prepared and injected (n = 3). The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient, the calibration curves are shown in figures 9 to 12.

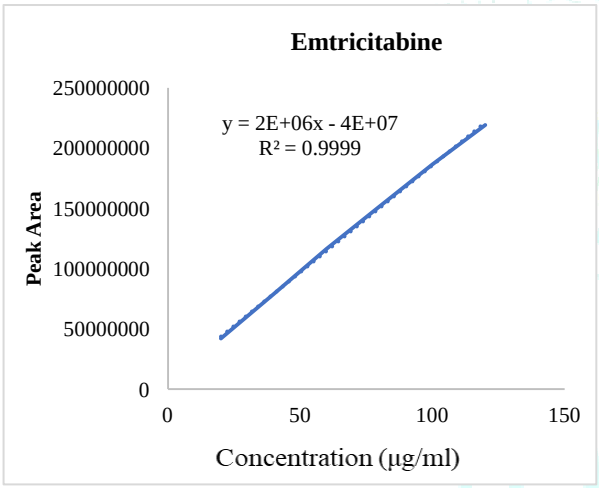

Figure 9: Calibration curve for EMT

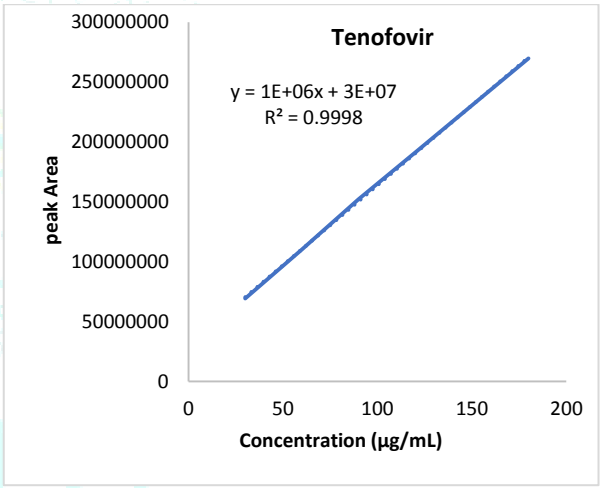

Figure 10: Calibration curve for TDF

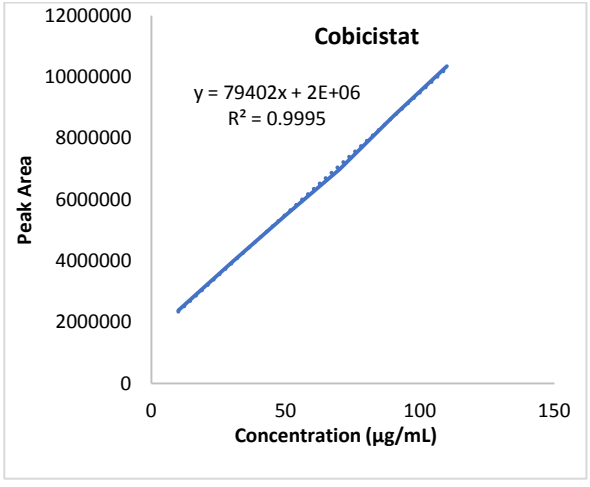

Figure 11: Calibration curve for COB

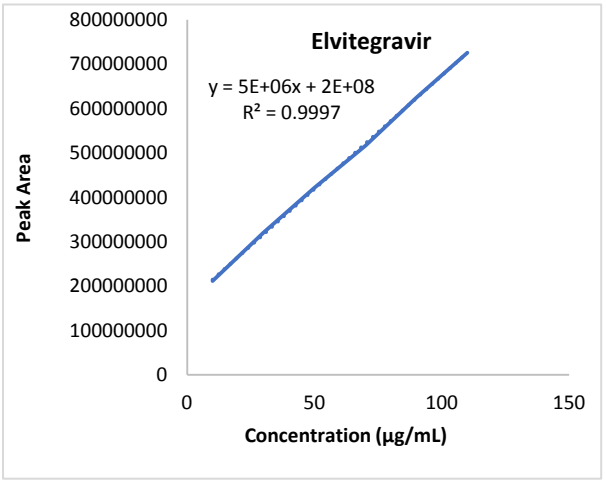

Figure 12: Calibration curve for ELV

4.2.4 Limit of detection and limit of quantification:

Limit of detection (LOD) and limit of quantification (LOD) of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were determined by calibration curve method. Solutions of EMT, TDF, COB and ELV were prepared in linearity range and injected (n = 3). Average peak areas were plotted against concentration. These were calculated by using following equations (ICH, Q2 (R1)). The LOD and LOQ values are reported in table 4.

LOD = 3.3 × σ/S and LOQ = 10 × σ/S

Where σ = the standard deviation of the response and S = slope of the calibration curve.

4.2.5 Precision:

Repeatability/method precision was determined in accordance with ICH guidelines. Six replicate injections (n=6) of same concentration (100 µg/mL) were analyzed. Determinations were performed on the same day as well as on consequent days (reproducibility/ruggedness). % RSD for Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir were found to be within acceptable limit of ≤ 2. Hence the method is precise, reproducible and rugged for 48 hours study and the results are summarized in table 5 and 6.

Table 4: LOD and LOQ data

Drug name	LOD (µg/mL)	LOQ (µg/mL)
EMT	0.92	2.78
TDF	1.04	3.14
COB	0.66	2.01
ELV	0.37	1.12

Table 5: Method Precision (repeatability) data for EMT, TDF, COB and ELV

Repeatability (Peak Areas)				
S. NO.	**EMT**	**TDF**	**COB**	**ELV**
1	185374024	169302345	8102589	624389254
2	184956826	169123548	8154265	628975425
3	184685792	168548781	8078251	629457821
4	185168745	168745985	8054654	619658724
5	184142589	169974623	8169824	618957214
6	184298745	167489524	8015786	627854625
Mean	184771120.2	168864129.3	8095894.8	624882177.2
SD	486118.4	836120.6	58901.5	4672456.8
% RSD	0.26	0.49	0.72	0.74

Table 6: Intraday and Inter-day Precision data for EMT, TDF, COB and ELV

Drug name	Time interval	% Assay *	Statistical parameters
EMT	0hrs	101.4	Mean = 101.5
	8hrs	101.7	SD = 0.15
	Day 1	101.4	%RSD = 0.15
	Day 2	101.6	
TDF	0hrs	100.7	Mean = 100.9
	8hrs	100.2	SD = 0.59
	Day 1	101.1	%RSD = 0.59
	Day 2	101.6	
COB	0hrs	101.4	Mean = 100.9
	8hrs	100.6	SD = 0.44
	Day 1	100.5	%RSD = 0.44
	Day 2	101.2	
ELV	0hrs	101.6	Mean = 101.3
	8hrs	101.3	SD = 0.34
	Day 1	100.8	%RSD = 0.34
	Day 2	101.4	

* = Average assay of 6 replicate injections

4.2.6 Accuracy:

To the placebo solution, known amounts of standard Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir corresponding to 50, 100 and 150% of target concentrations were added. Mean recovery (%) for EMT, TDF, COB and ELV are 98.97, 99.99, 100.20 and 100.17 respectively and these results are within acceptable limit of 98-102 %. The % RSD for EMT, TDF, COB and ELV are 0.5, 0.9, 0.7 and 0.8 respectively and these results were within limit of ≤ 2. Hence the proposed method is accurate and the results are summarized in table 7.

Table 7: Results of Accuracy for EMT, TDF, COB and ELV

Drug name	Conc. (%)	Amount taken* (µg/mL)	Amount spiked* (µg/mL)	Amount recovered* (µg/mL)	% recovery *	Statistical parameters
EMT	50	30	15	45.2	100.4	Mean %: 100.1
	100	30	30	59.5	99.2	SD: 0.83
	150	30	45	75.6	100.8	%RSD: 0.83
TDF	50	50	25	74.8	99.7	Mean %: 100.3
	100	50	50	100.6	100.6	SD: 0.55
	150	50	75	125.9	100.7	%RSD: 0.55
COB	50	20	10	30.2	100.6	Mean %: 100.1
	100	20	20	39.6	99	SD: 0.99
	150	20	30	50.4	100.8	%RSD: 0.98
ELV	50	20	10	30.3	101	Mean %: 101.1
	100	20	20	40.6	101.5	SD: 0.36
	150	20	30	50.4	100.8	%RSD: 0.36

* = Average result of 3 replicate injections

4.2.7 Robustness:

The method remained unaffected by deliberate small changes in parameters like flow rate and column temperature. Below tabulated % RSD values of % assays and retention times were within the tolerance limits and indicate that the method is robust. The results are reported in table 8.

Table 8: Robustness study results of EMT, TDF, COB and ELV

Drug name	Parameter	Change in ratio	%Assay *	Statistical parameters
EMT	Flow rate	0.9 mL	99.8	Mean %: 100.5
		1.0 mL	100.6	SD: 0.51
		1.1 mL	100.9	% RSD: 0.51
	Column temperature	28 °C	100.5	Mean %: 100.5
		30 °C	100.2	SD: 0.3
		32 °C	100.8	% RSD: 0.29
TDF	Flow rate	0.9 mL	100.6	Mean %: 100.8
		1.0 mL	100.4	SD: 0.58
		1.1 mL	101.5	% RSD: 0.58
	Column temperature	28 °C	100.6	Mean %: 100.6
		30 °C	100.4	SD: 0.15
		32 °C	100.7	% RSD: 0.15
COB	Flow rate	0.9 mL	99.5	Mean %: 100.1
		1.0 mL	100.2	SD: 0.56
		1.1 mL	100.6	%RSD: 0.56
	Column temperature	28 °C	100.8	Mean %: 101.2
		30 °C	101.6	SD: 0.40
		32 °C	101.3	%RSD: 0.40
ELV	Flow rate	0.9 mL	100.7	Mean %: 101.4
		1.0 mL	101.6	SD: 0.62
		1.1 mL	101.9	%RSD: 0.62
	Column temperature	28 °C	100.7	Mean %: 100.7
		30 °C	100.5	SD: 0.15
		32 °C	100.8	%RSD: 0.15

* = Average result of 3 replicate injections

4.3 Forced Degradation Studies:

For all the forced degradation samples, the purity angle formed was less than purity threshold, this indicates that there is no interference from degradants in quantitating Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir peaks in Stribild tablets. There was no purity flag observed and the % degradation was less than 20%. Thus, this method is considered to be "stability indicating". The results are reported in table 9. The chromatograms of drugs which showed specific degradation peaks which are well separated from the main drug peak with good resolution after forced degradation studies are reported in figures 13 to 18.

Table 9: Forced degradation studies at different stress conditions

Stress Condition	% Degradation	Purity Angle	Purity Threshold	Pass/ Fail
Emtricitabine				
Control	---	0.156	0.441	No
Acid	3.4	0.132	0.330	No
Alkali	16.8	0.144	0.341	No
Oxidation	18.6	0.285	0.546	No
Thermal	2.4	0.399	0.652	No
Photostability	3.6	0.124	0.332	No
Tenofovir disoproxil fumarate				
Control	---	0.186	0.541	No
Acid	17.6	0.182	0.360	No
Alkali	18.7	0.244	0.421	No
Oxidation	3.1	0.212	0.584	No
Thermal	2.3	0.412	0.674	No
Photostability	2.8	0.184	0.392	No
Cobicistat				
Control	---	0.136	0.481	No
Acid	10.8	0.122	0.230	No
Alkali	14.5	0.184	0.311	No
Oxidation	9.6	0.285	0.546	No
Thermal	9.8	0.364	0.782	No
Photostability	10.3	0.224	0.432	No
Elvitegravir				
Control	---	0.256	0.441	No
Acid	13.2	0.232	0.338	No
Alkali	1.2	0.274	0.461	No
Oxidation	2.5	0.215	0.512	No
Thermal	1.4	0.297	0.552	No
Photostability	1.7	0.184	0.375	No

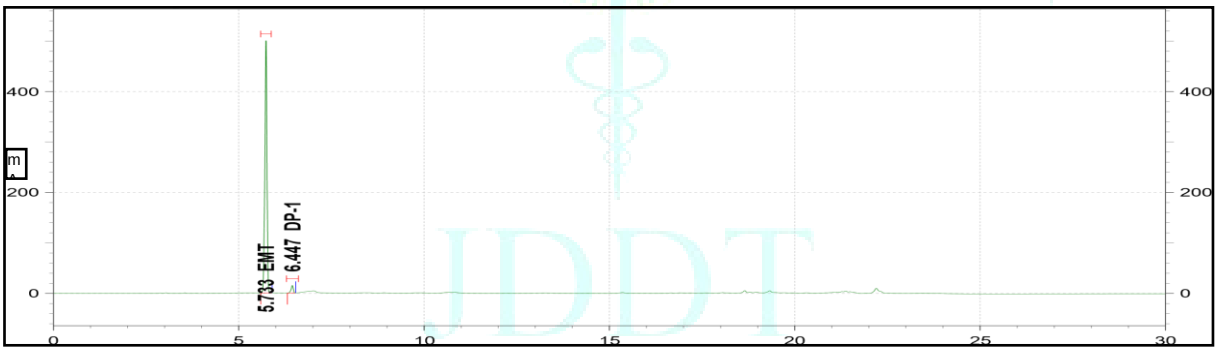

Figure 13: Chromatogram of acid hydrolysis of Emtricitabine

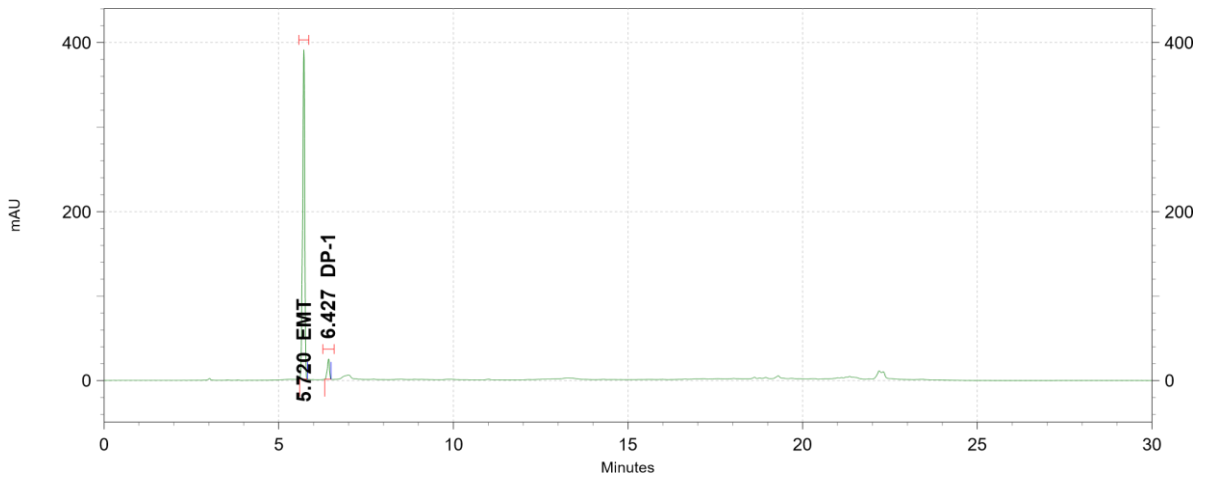

Figure 14: Chromatogram of alkali hydrolysis of Emtricitabine

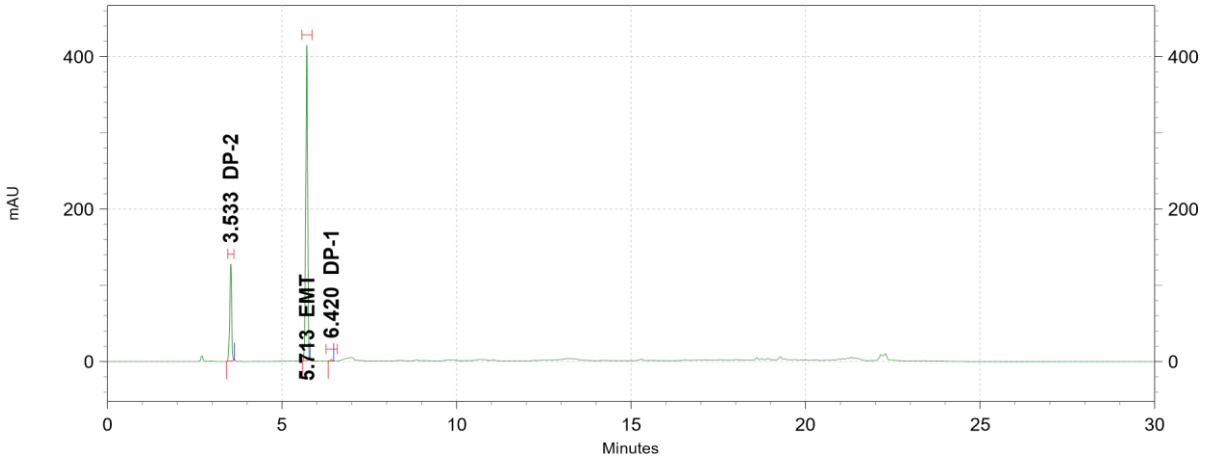

Figure 15: Chromatogram of oxidation of Emtricitabine

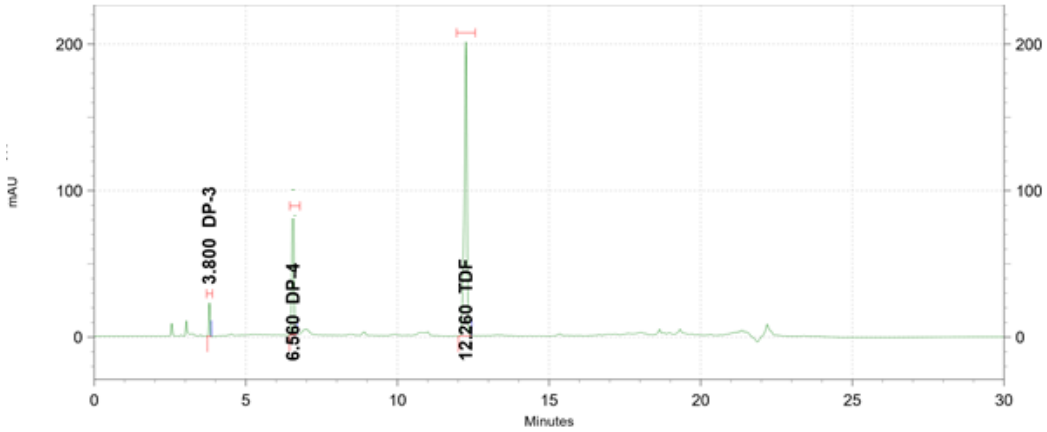

Figure 16: Chromatogram of acid hydrolysis of Tenofovir disoproxil fumarate

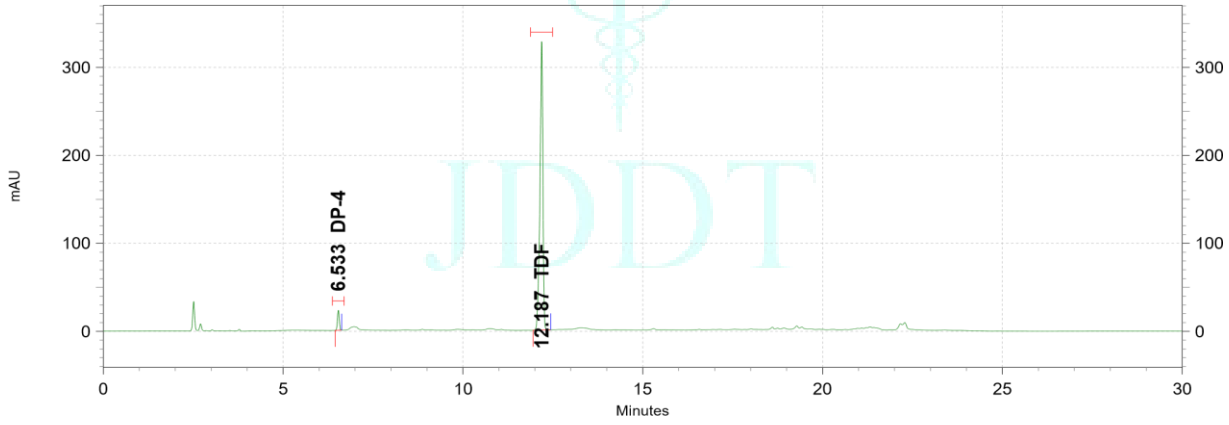

Figure 17: Chromatogram of alkali hydrolysis of Tenofovir disoproxil fumarate

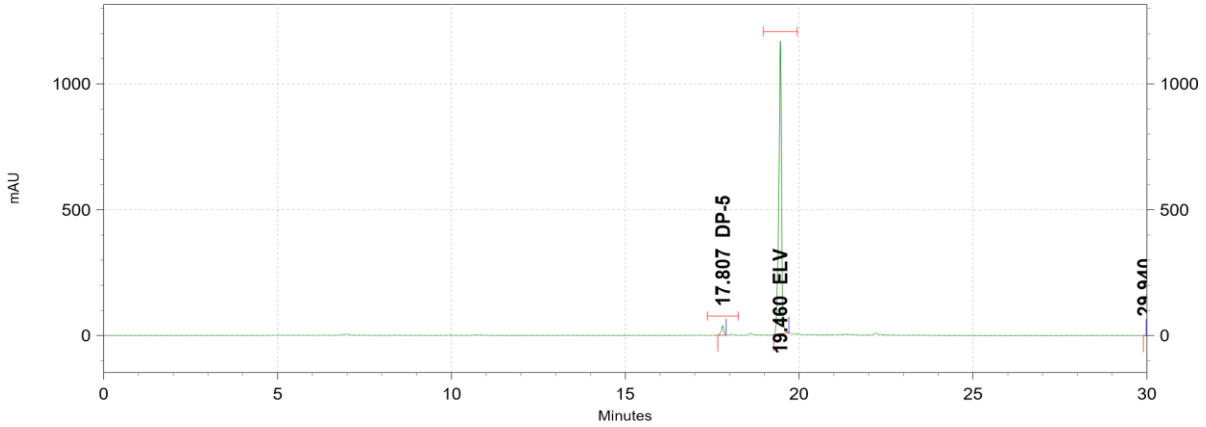

Figure 18: Chromatogram of acid hydrolysis of Elvitegravir

4.4 Dissolution Method:

The validated RP-HPLC method developed was successfully applied for the *In-vitro* dissolution method for analysis of Stribild tablets. The release rate of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir from immediate release Stribild tablets was determined using United State Pharmacopoeia dissolution testing apparatus II (paddle method). The percent drug release was found to meet USP specification, i.e. not less than 80% of amount of labeled drug EMT, TDF, COB and ELV dissolved in 30min.

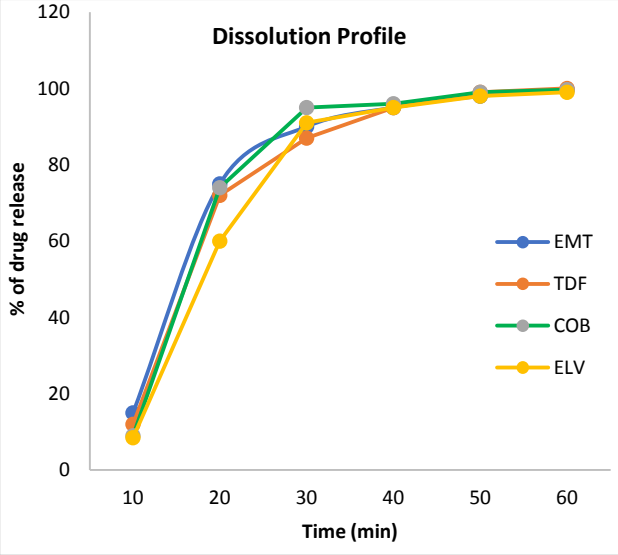

Figure 19: *In-vitro* dissolution profile of Stribild tablets

There was no interference of blank with the standard and sample chromatograms. The *In-vitro* dissolution profile of cumulative percentage of drug released verses time is presented in figure 19.

4. CONCLUSION

A simple and rugged LC-MS compatible RP-HPLC method has been described for simultaneous determination of Emtricitabine, Tenofovir disoproxil fumarate, Cobicistat and Elvitegravir in active pharmaceutical ingredients, assay for marketed formulation (Stribild) and to study the dissolution profile in Stribild tablets without any additional pretreatment. The proposed method was validated in accordance with ICH guidelines by testing its parameters which include system suitability, specificity, precision, linearity, LOD, LOQ, accuracy and robustness. The method was very specific to separate the peaks of active pharmaceutical ingredients from the degradation products which were obtained with good resolution and purity angle less than purity threshold showing peak purity of the four drugs after forced degradation studies. Thus, stress induced studies prove the effectiveness of the proposed stability indicating RP-HPLC method which can be adopted in routine analysis of Stribild tablets in pharmaceutical industries.

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Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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