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UPLC Method for the Quantification of Elvitegravir, Cobicistat, Emtricitabine and Tenofovir Disoproxil Fumarate in Tablets Using OGD Reference Dissolution Medium

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ABSTRACT

The current paper discuss about strategic development and validation activity performed for quantification of Elvitegravir (EL), Cobicistat (CO), Emtricitabine (EM) and Tenofovir Disoproxil fumarate (TDF) in a combination drug product using Office of Generic drugs (OGD) recommended Dissolution medium. Ultra Performance Liquid Chromatographic (UPLC) technique method was chosen and developed a method with a run time of four minutes. Mobile phase A consists of 0.1% perchloric acid and Mobile phase B consists of Acetonitrile. Gradient elution technique was opted with an optimized flow rate of 0.3 ml per minutes. Acquity UPLC BEHC$_{18}$ (100 mm × 2.1 mm ID), 1.7µ particle size column is finalized for testing purpose with a detection wavelength of 260 nm. Typical retention times observed for EM, TDF, CO and EL are 0.89, 1.42, 2.01 and 2.77 min respectively. Method is found to be linear over the specified concentration of 3.71- 44.56 µg/ml of EL, 1.77-21.23 µg/ml for CO, 4.90-58.74 µg/ml for EM and 7.37-88.40 µg/ml for TDF with correlation coefficient more than 0.99. Accuracy of the drugs is found to be more than 90% in proposed OGD medium. Developed method could be useful to quantify the drugs in pharmaceutical quality control and contract research laboratories for dissolution profiles at very fast rate.

Keywords: Elvitegravir, Cobicistat, Emtricitabine, Tenofovir Disoproxilfumarate, Dissolution, RP-UPLC.

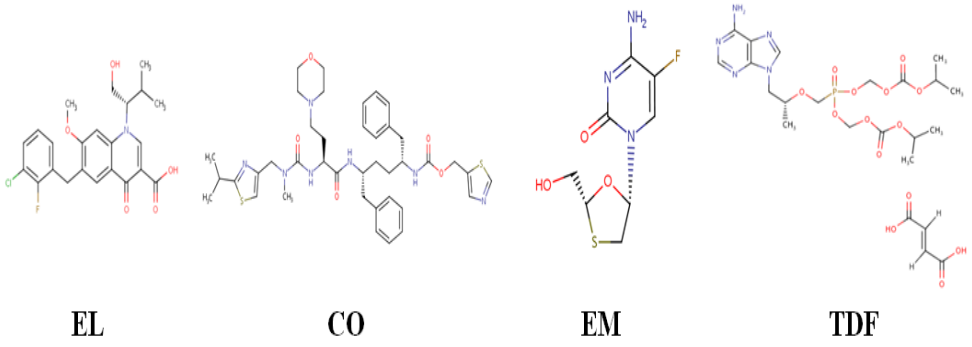
INTRODUCTION

Elvitegravir/Cobicistat/Emtricitabine/ Tenofovir Disoproxilfumarate tablet is available in Fixed Drug Combination (FDC) under the brand name Stribild. In other names, it is also called as QUAD. Stribild is prescription medicine approved by United States Food and Drug administration (USFDA) to treat in adults who have never taken medicines for HIV infection earlier [1-3]. Each tablet contains 150 mg of EL, 150 mg of CO, 200 mg of EM and 300 mg of TDF (equivalent to 245 mg of Tenofovir Disoproxil). EL is a HIV medicine known as integrase inhibitor. Chemical nameis "6-(3-Chloro-2-fluorobenzyl)-1-[(2S)-1-hydroxy-3methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid"with a molecular weight of 447.9 and an empirical formula of $C_{23}H_{23}ClFNO_5$. CO is a pharmacokinetic enhancer, which would be useful toincrease the effectiveness of EL. CO has a chemical name of "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)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate" with a molecular weight of 776.0 with an empirical formula of $C_{40}H_{53}N_7O_5S_2$.

EM is a Human Immunodeficiency Virus (HIV) Nucleoside Analog Reverse Transcriptase Inhibitor. The chemical classification of EM is Nucleoside Analog. The mechanism of action of EM is defined as a Nucleoside Reverse Transcriptase Inhibitor. EM forms emtricitabine 5'-triphosphate within the cell by phosphorylation. The action of the metabolite is to inhibit the activity of HIV reverse transcriptase both by competing with the natural substrate deoxycytidine 5'-triphosphate and by incorporation into viral DNA causing a termination of DNA chain elongation. EM has a chemical name of"5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]cytosine". It is a thio analog of cytidine with (-) enantiomer with a molecular weight of 247.24 and molecular formula of $C_8H_{10}FN_3O_3S$. TDF is a prodrug and exists as fumaric acid salt form of tenofovir. The chemical category of TDF is a nucleoside reverse transcriptase inhibitor analog of adenosine. It is mainly prescribed to treat not only for HIV and also for hepatitis B virus under chronic conditions in adults in combination with other antiviral therapeutic agents. TDF has a chemical name of "9-[(R)-2[[bis[[(isopropoxycarbonyl)oxy]methoxy] phosphinyl] methoxy] propyl]adenine fumarate (1:1)" with a molecular weight of 635.52 and a molecular formula of $C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$.

Upon survey of different research articles, journals and publications indicate that few HPLC Assay test procedures being present for quantification of EL, CO, EM and TDF in FDC products [4-10]. UPLC method is available to determine the degradants present in this formulation [11-19]. However UPLC test methods are not reported to quantify these drugs in OGD recommended dissolution medium. Since all drugs are having different polarity, it is difficult to fix a common chromatographic method with shorter run time. While performing dissolution profiles during drug product development, it is very difficult to conclude the results if it runs for longer run time. Hence to avoid such practical problems, method was targeted to develop using simple volatile buffer which is compatible with low micron ID columns with RP-UPLC. The advantage of using volatile buffers is to increase longevity of column inspite of repeated number of injections when compared against organic buffers.

Validated test procedure is specific with respective to dissolution medium and placebo. Method validation was performed as per ICH Q3 (d) guidance and found to be suitable for quantification of dissolution profiles required for EL, CO, EM and TDF in FDC product by following dissolution conditions as proposed in Office of Generic Drugs (OGD) for QUAD. 0.01 N HCl with 2% w/w Polysorbate 80 is used as Dissolution medium, USP Apparatus type II (Paddle) with a stirring speed of 100 rpm, proposed dissolution volume is 1000 ml. The specified time points are 5, 10, 15, 20 and 30 minutes respectively. Chemical structures of EL, CO, EM and TDF have been illustrated in Figure 1.



MATERIALS AND METHODS

Figure 1: Chemical structures of EL, CO, EM and TDF

Instrumentation

Waters-Acquity UPLC connected to Binary gradient-pump system which has temperature controller to column compartment with an integrated Auto sampler and Photo diode array detector (PDA). Windows based computer is loaded with Empower 2 software which acts as an interface to monitor output signals. Dissolution profiling was performed using Distek dissolution Apparatus type II system. Acquity Ethylene Bridged Hybrid technology (BEH) C_{18}, (100 × 2.1 mm ID) with 1.7 µm column was used.

Chemicals

EL, CO, EM and TDF standards, Stribild tablets from Gilead Sciences, Inc. were taken fromAurobindo Pharma limited. Ultra-pure Acetonitrile, Hydrochloric acid of GR grade and Polysorbate 80 of GR grade are taken from Merck. Ultrapure water is taken from Evoqua water purifier.

Development and optimization of UPLC technique

Aim of the current paper is to reproduce, precise and accurate results when performed for Dissolution profiling at short run time in EL, CO, EM and TDF tablets. QUAD is not official or cited in any compendial monographs. There is no RP-UPLC method being published so far for dissolution profiling test. EL, CO, EM and TDF are having different polarities. The pKa values observed for EL is about 6.6, for CO is about 6.4, for EM is about 2.65 and for TDF is about 3.75 respectively. Structural moieties of EM and TDF are showing amine functional groups which may tend to pose peak tailing due to silanol effects. To avoid this it is preferred to choose acidic mobile phase for development activity.

The OGD recommended dissolution medium contains 2% w/w Polysorbate 80. Hence care must be taken during optimizing chromatographic conditions. Due to viscous nature of the medium, there could be probable chance to accumulate back pressure after repeated number of injections which may reduce the life of the column. Especially in UPLC applications, this practical problem can be addressed in two ways i.e., by keeping column oven temperature on higher side and prefer to use volatile buffers which do not give much column back pressure. By considering these issues, method parameters were optimized accordingly. Perchloric acid is strongly acidic and volatile in nature and also a small ion pair reagent. It completely dissociates in water and provides true ion exchange selectivity when interacted with different drugs especially present in FDC products. Hence for mobile phase preparation purpose 0.1% perchloric acid was selected and considered as Mobile phase A and Acetonitrile was chosen as Mobile phase B. Since drugs are having different polarity, to get shorter run time method it was recommended to prefer gradient elution mode by keeping moderate flow rate at 0.3 ml per min. Column oven temperature was kept beyond 40°C to maintain low back pressure from column.

Ethylene bridged hybrid (BEH) technology bonded phase present in Waters Acquity column works on hydrophilic interaction and hence produces a versatile robust separation between the compounds and also can operate at wider usable pH range. Since the buffer used in mobile phase preparation contains acidic pH, it was preferred to keep reverse phase column with C18 chemistry using BEH technology. For trial purpose, Waters Acquity UPLC BEH C18 (100 mm × 2.1 mm id) with 1.7 µm particle size was used and found to be suitable for optimum separation between all drugs present in QUAD. The wavelength maxima observed for EL is about 259 nm, for CO is about 240 nm, for EM is about 288 nm and for TDF is about 260 nm (Spectral data has been mentioned) (Figure 2). To quantity all drug components; a common wavelength of 260 nm has been finalized. Using 2 µl injection volumes for all drugs has shown reproducible area counts which are found to be suitable for drug profiling using 260 nm.

Upon taking several logical gradient trials using 0.1% perchloric acid, Acetonitrile as Mobile phase A and Mobile Phase B, resolution between the drugs is found to be more than 3.0 in the optimized chromatographic conditions. Flow rate was finalized at 0.3 ml per min. Column oven temperature is finalized at 45°C, where column backpressure is found to be under control. In all robustness conditions resolution between each drug is found to be more than 2.5 and USP tailing factor is found to be less than 1.2 for all drugs. This indicates, in optimized chromatographic conditions for quantification of drugs shall not be altered with minor changes that are likely to occur during continuous runs.

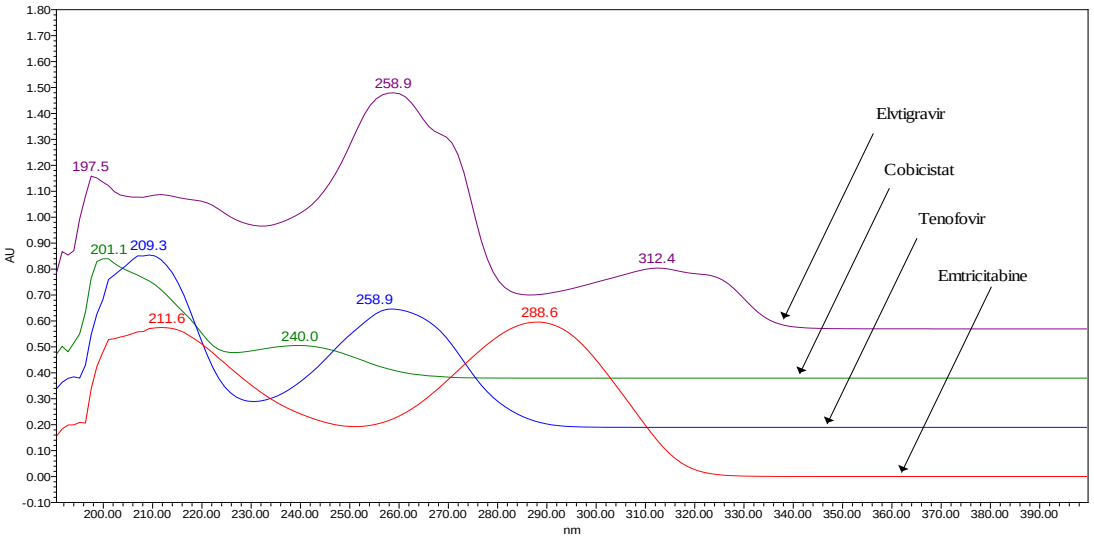


Figure 2: Spectral characteristics of EL, CO, EM and TDF

Method optimized chromatographic conditions

The finalized chromatographic conditions are given in Table 1. The Typical retention times observed for EM, TDF, CO and EL in the optimized chromatographic conditions are about 0.89, 1.42, 2.01 and 2.77 minutes respectively.

Table 1: Chromatographic conditions

Column	Waters Acquity UPLC (BEH) C18, 1.7 µ. 100 × 2.1 mm			
Detection	260 nm (PDA Detector)			
Column temperature	45°C			
Inj. volume	2 µL			
Mobile phase A	1 ml of perchloric acid in 1000 ml of water			
Mobile phase A	Degassed acetonitrile			
Diluent	10 ml of acetonitrile followed by dissolution medium for preparation of Standard and sample preparation is performed in dissolution medium only.			
Step gradient program	**Time (min)**	**Flow (ml)**	**% Elution Phase-A**	**% Elution Phase-B**
	0	0.3	60	40
	1.5	0.3	30	70
	3.2	0.3	30	70
	3.3	0.3	60	40
	4	0.3	60	40

Preparation of solutions

Preparation of standard solution

Individual Standard stock solution of EL, CO, EM and TDF were prepared at 0.9 mg/ml, 0.9 mg/ml, 1.2 mg/ml and 1.2 mg/ml, initially by dissolving in 10 ml of acetonitrile, further diluted with dissolution medium. This stock solution was diluted to prepare standard solutions at a concentration of 36.0 µg/ml, 48.0 µg/ml, 36.0 µg/ml and 72.0 µg/ml respectively using dissolution medium.

Dissolution test conditions

The dissolution profiling test was conducted for QUAD tablets as per OGD recommended dissolution medium of 2.0% polysorbate 80 in 0.01 N HCl, using USP type II apparatus (Paddle) with 100 rpm stirring speed. Dissolution medium volume is 1000 ml which was maintained at 37°C (± 0.5°C) in dissolution bowls. Samples of about 10 ml were withdrawn from the dissolution bowl at specified time points of 5, 10, 15, 20 and 30 min respectively. After each sampling, about 10 ml of dissolution medium which is maintained at 37°C is placed into each dissolution vessel.

Sample solutions are filtered using suitable filters.

Analytical method validation

Stribild tablets are available in FDC with 150 mg of EL, 150 mg of CO, 200 mg of EM and 300 mg of TDF. The same label claim tablets were considered for method validation purpose. Validations parameters covered for System suitability evaluation, Specificity, Precision (method precision and intermediate precision), Linearity, Accuracy/Recovery, stability of solutions, suitability of Filter papers and Robustness parameters as per ICH recommendation for "Validation of Analytical Procedures: Text and Methodology - Q2 (R1)".

System suitability evaluation

Standard solution was prepared and injected for five replicate injections and observed for peak area of EL, CO, EM and TDF. Theoretical plate count, tailing factor, Resolution and %RSD is evaluated.

Table 2: General system suitability data

Name of the drug	USP plate count	Tailing factor	Resolution	%RSD for replicate injections
EM	362	1.14	-	0.5
TDF	2081	1.06	3.43	0.58
CO	4201	0.94	4.73	1.33
EL	7290	0.98	5.87	0.62

Specificity

Equal proportions of excipients are mixed as per QUAD formula. This placebo powder which is equivalent to individual tablet weight is transferred to dissolution vessel which contains dissolution medium. Rotation of the paddle is maintained at 100 rpm for 60 min. Placebo solution is withdrawn from dissolution vessel and filtered through 0.22 µ PVDF filter paper and analyzed in UPLC system.

Precision

Stribild tablets were tested for precision of the method for intra and inter day precision for six individual preparations. All test samples were analyzed after subjecting it in dissolution vessels as per proposed time intervals. Measured % dissolution at every time interval and % RSD is determined for same values for every time point.

Linearity

Linearity study was assessed by preparing the test solutions ranging from 10%-120% level using concentrated standard stock solutions for each drug. Linearity curves were constructed, by plotting the concentration (µg/ml) against peak area for each drug. The calculation of regression line was employed by the method of least squares.

Accuracy

Known amounts of EL, CO, EM and TDF reference substances were transferred to dissolution bowls at 10%, 80%, 100% and 120% levels along with tablets placebo. Dissolution was run for the samples asper OGD recommended dissolution test conditions. Triplicate preparations are made at each level.

Solution stability

Standard and sample solutions were periodically injected and verified the response of the peaks from the solutions stored at bench top condition (25°C) or cooler temperature (2-8°C). The chromatograms obtained by the RP-UPLC method were evaluated for area. Tests results of area counts are compared against freshly prepared solutions of standard and sample solutions.

Filter evaluation

To demonstrate the filter paper interference, standard and sample solutions were filtered using 0.22 µ PVDF and Nylon membrane filters by initially discarding 2-3 ml of aliquots from the filters. The filters were pre saturated with dissolution medium prior to filtration. Results are compared against centrifuged sample areas.

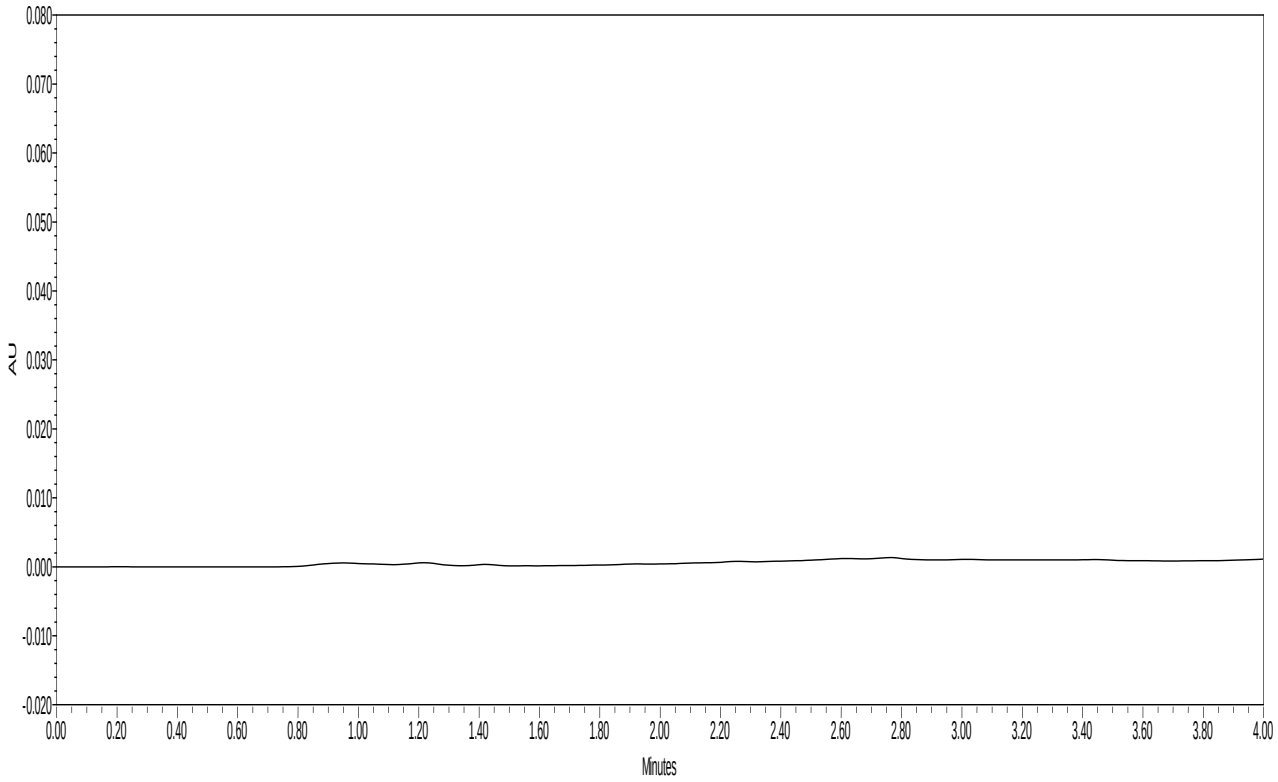
Robustness

Robustness study was assessed by making deliberate changes in the optimized chromatographic conditions and impact was noted for USP plate count, Tailing factor and resolution between each drug. Accordingly conditions were modified for flow rate of 0.3 ml (± 10%), wavelength of 260 nm (± 5 nm), temperature of 45°C (± 5°C) and Organic ratio in gradient elution (± 2% absolute). For each robustness experiment, one parameter was modified and remaining chromatographic conditions were kept as such.

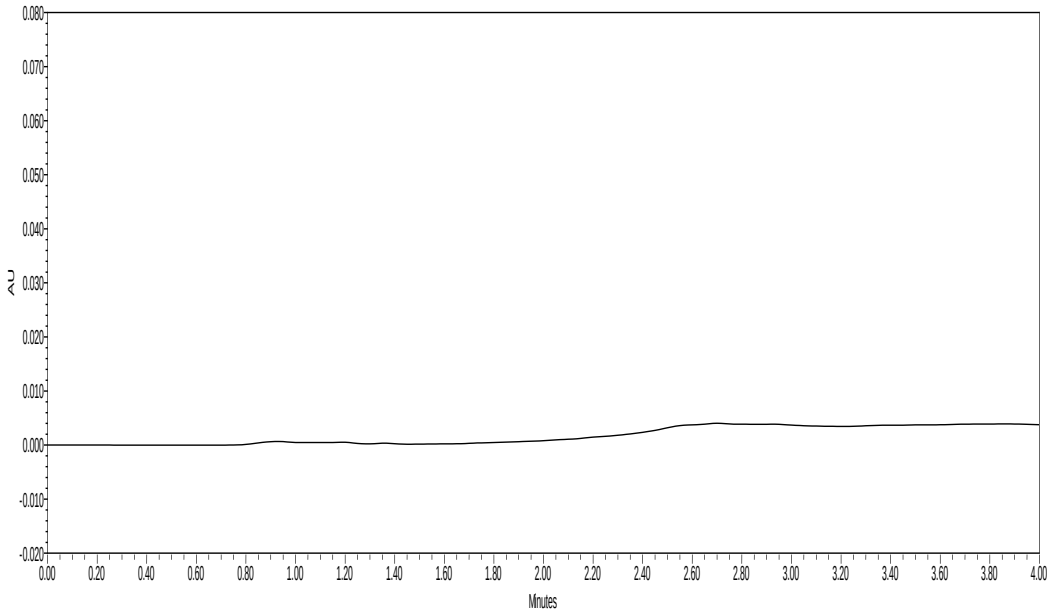
RESULTS AND DISCUSSION

Specificity

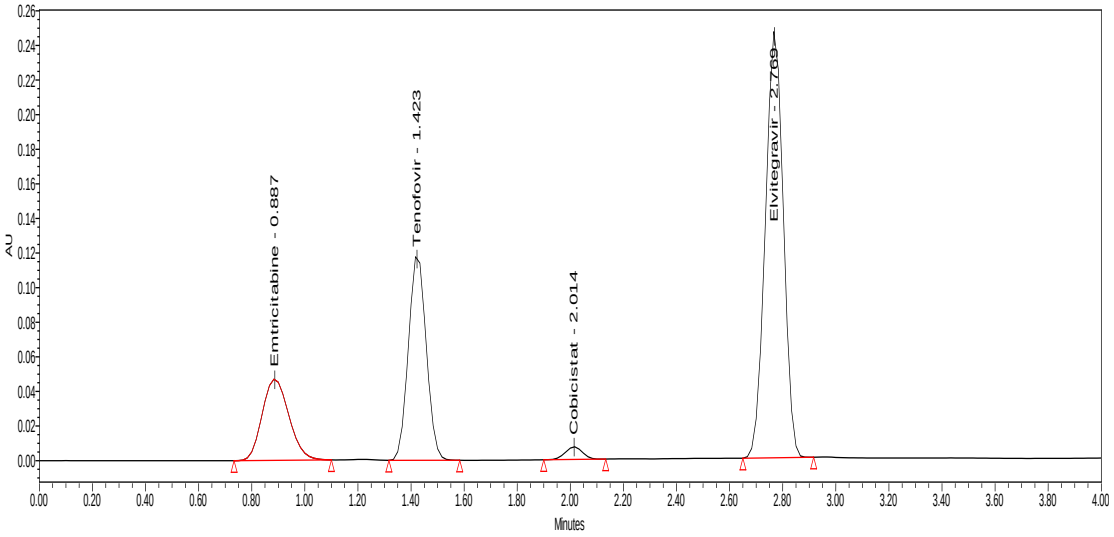
Placebo chromatogram was assessed in RP UPLC method to check interference in chromatographic data. From the placebo chromatogram it is evident to see no interference was observed from placebo mixture being used for tablet fabrication at the retention times of EL, CO, EM and TDF. Hence the developed UPLC method is found specific to quantify the drugs of EL, CO, EM and TDF in pharmaceutical formulation using standard reference solution. For chromatograms refer (Figure 3).



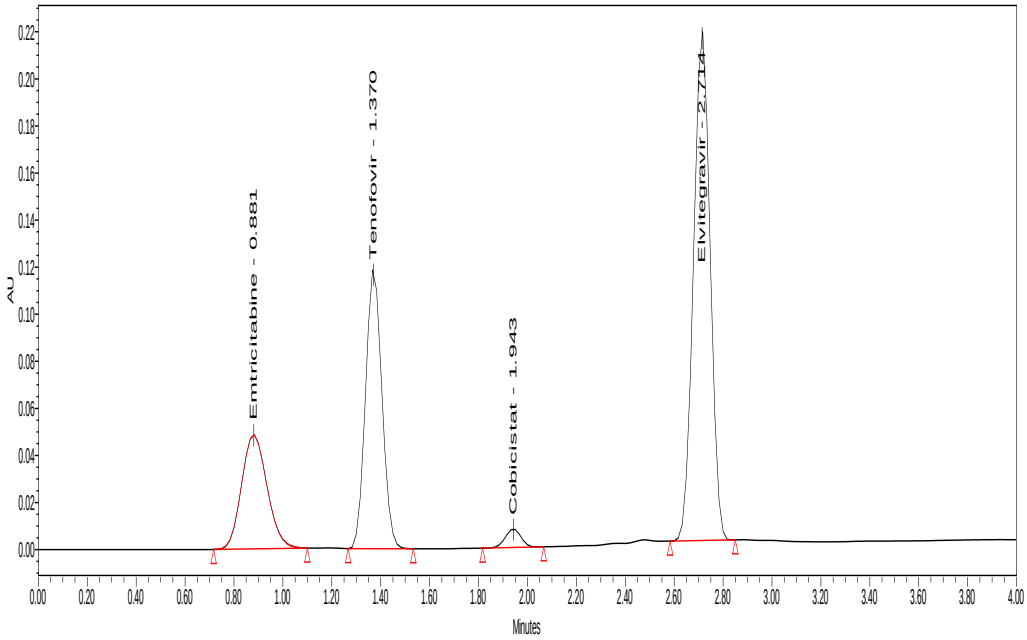
(a) Diluent chromatogram



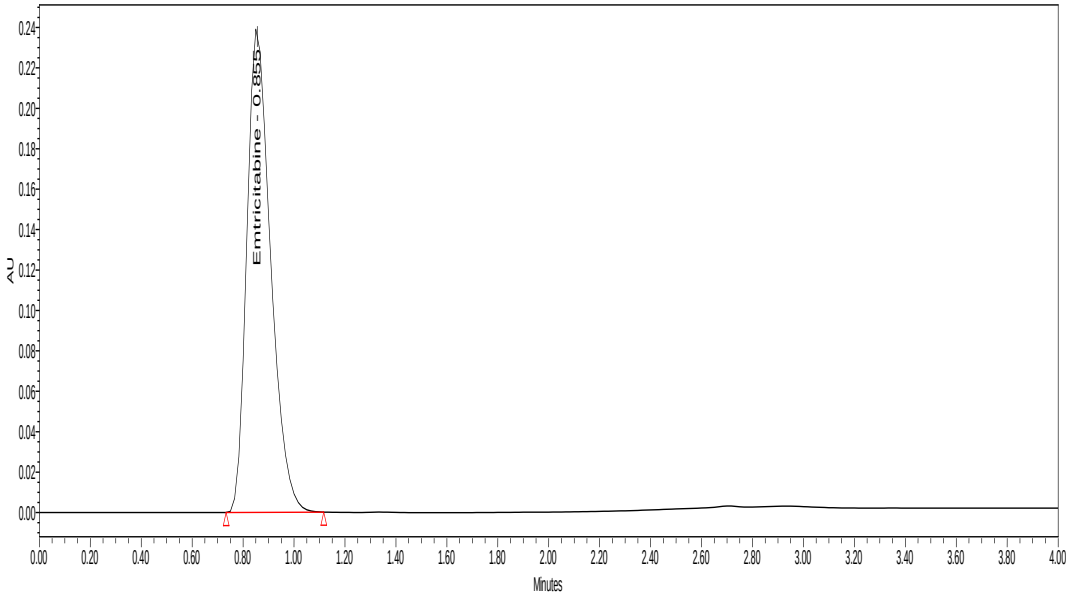
(b) Placebo chromatogram



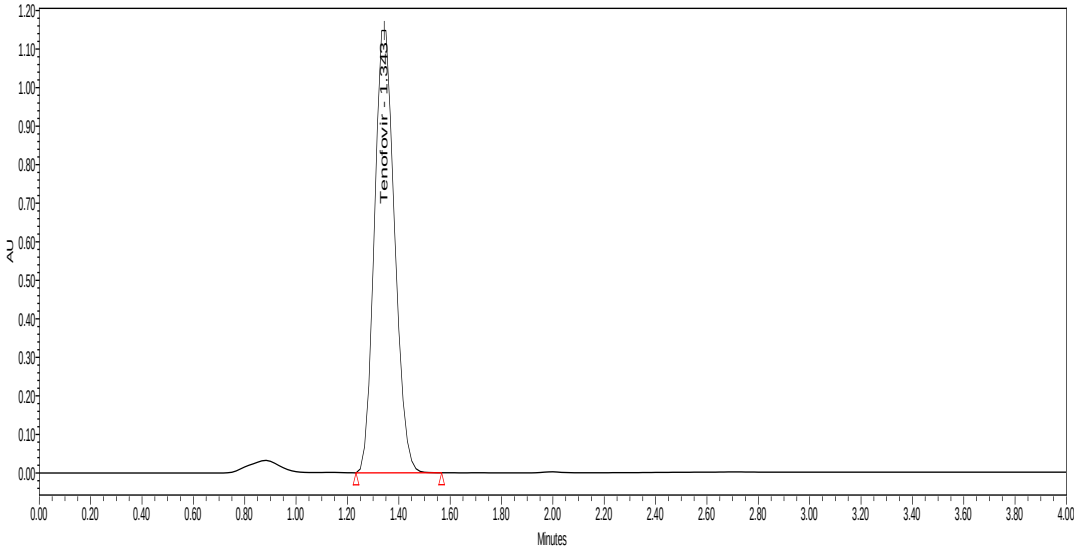
(c) Standard Chromatogram



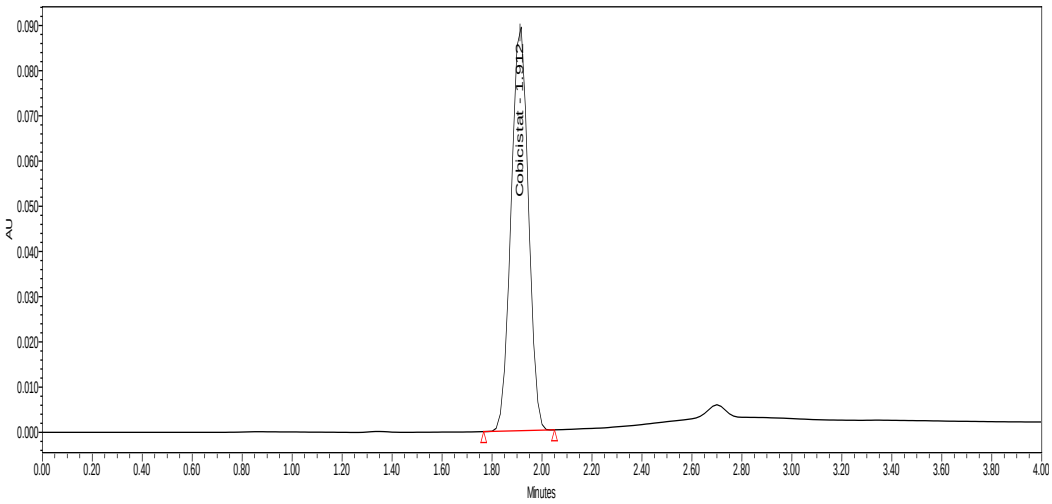
(d) Sample Chromatogram (Stribild tablets – 150 mg of EL/ 150 mg of CO/ 200 mg of EM/ 300 mg of TDF)



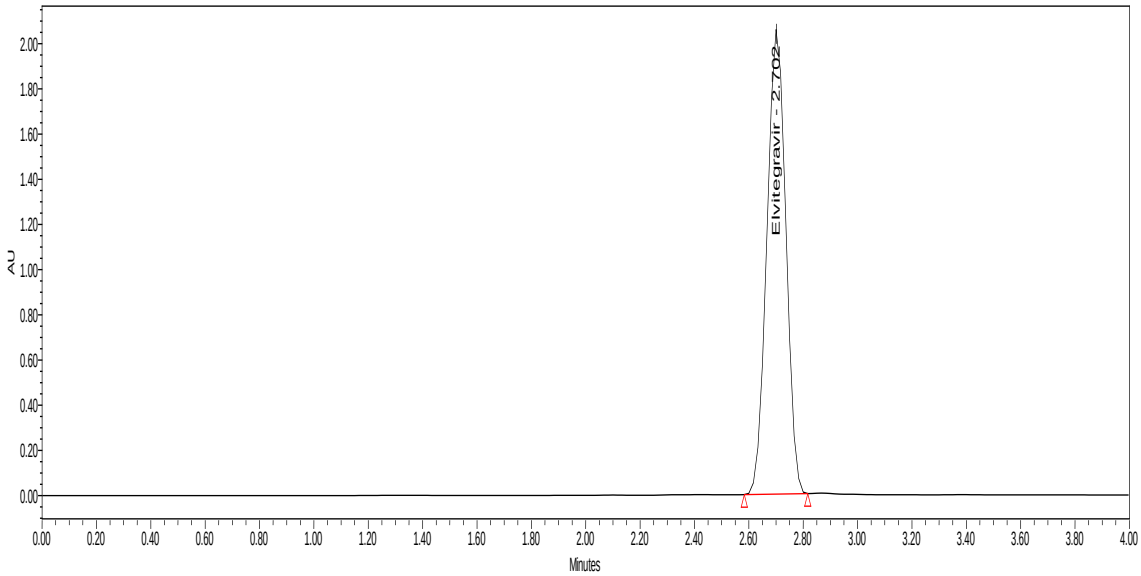
Emtricitabine (EM) Chromatogram



Tenofovir (TDF) Chromatogram



Cobicistat (CO) Chromatogram



Elvitegravir (EL) Chromatogram

(e) Individual chromatograms of EM, TDF, CO and EL

Figure 3: Chromatogram of Diluent, Placebo, Standard chromatogram, Sample chromatogram and Individual chromatograms of EM, TDF, CO and EL

Precision

The results obtained from both method precision (Intra-Day) and intermediate precision (Inter-Day) shows that the percentage RSD did not exceed 5% especially after initial release at 5 min. This demonstrates the precision of the method (Tables 3 and 4).

Table 3: Method precision results

For EL									
Sr. No	**Time (min)**	**% Release**						**Average % Release**	**% RSD**
		1	**2**	**3**	**4**	**5**	**6**		
1	5	77	72	74	77	75	74	75	2.59
2	10	85	91	84	85	91	84	87	3.89
3	15	93	90	91	93	91	90	91	1.5
4	20	96	95	93	96	95	93	95	1.44
5	30	100	100	98	100	101	98	100	1.22
For CO									
Sr. No	**Time (min)**	**% Release**						**Average % Release**	**% RSD**
		1	**2**	**3**	**4**	**5**	**6**		
1	5	95	80	86	94	86	82	87	7.06
2	10	96	94	94	96	98	95	96	1.58
3	15	96	99	99	99	97	95	98	1.8
4	20	100	99	96	102	99	99	99	1.96
5	30	101	98	98	102	98	101	100	1.86
For EM									
Sr. No	**Time (min)**	**% Release**						**Average % Release**	**% RSD**
		1	**2**	**3**	**4**	**5**	**6**		
1	5	97	91	94	97	94	94	95	2.38
2	10	98	98	101	99	98	100	99	1.28
3	15	99	98	101	100	98	100	99	1.22
4	20	100	99	101	101	99	102	100	1.21
5	30	101	99	101	101	99	101	100	1.03
For TDF									
Sr. No	**Time (min)**	**% Release**						**Average % Release**	**% RSD**
		1	**2**	**3**	**4**	**5**	**6**		
1	5	95	90	89	95	94	89	92	3.22
2	10	98	99	96	99	99	95	98	1.79
3	15	100	98	96	100	98	96	98	1.83
4	20	101	99	96	101	100	97	99	2.12
5	30	101	100	96	101	100	97	99	2.16

Table 4: Intermediate precision results

For EL									
Sr. No	**Time (min)**	**% Release**						**Average % Release**	**% RSD**
		1	**2**	**3**	**4**	**5**	**6**		
1	5	76	71	75	77	75	74	75	2.75
2	10	86	92	84	86	91	84	87	4.01
3	15	94	90	90	94	91	90	92	2.15

4	20	96	96	94	96	96	94	95	1.09
5	30	100	100	98	101	101	100	100	1.1

For CO

Sr. No	Time (min)	% Release						Average % Release	% RSD
		1	2	3	4	5	6		
1	5	79	78	83	84	84	82	82	3.15
2	10	98	98	92	96	95	91	95	3.12
3	15	96	94	96	101	94	96	96	2.67
4	20	103	97	98	100	101	100	100	2.14
5	30	99	99	97	98	100	100	99	1.18

For EM

Sr. No	Time (min)	% Release						Average % Release	% RSD
		1	2	3	4	5	6		
1	5	95	89	93	95	92	92	93	2.42
2	10	97	97	99	97	96	99	98	1.25
3	15	98	96	99	98	97	100	98	1.44
4	20	99	98	101	100	98	101	100	1.38
5	30	100	98	101	100	98	101	100	1.37

For TDF

Sr. No	Time (min)	% Release						Average % Release	% RSD
		1	2	3	4	5	6		
1	5	95	90	89	95	93	88	92	3.34
2	10	99	99	96	99	98	96	98	1.5
3	15	100	99	96	100	99	97	99	1.66
4	20	101	101	97	101	100	97	100	1.97
5	30	101	100	98	102	100	98	100	1.6

Linearity

Linearity curves were assessed for EL, CO, EM and TDF by checking the concentration versus area observed that ranges from 10%-120%. From the calibration curves extrapolated the values for correlation coefficient, slope and Y-intercept for each drug. From the results it is observed a linear relationship to all drug components with a satisfactory correlation coefficient of more than 0.995 on tested concentration range. Linearity graphs for EL, CO, EM and TDF have been depicted in Figure 4. Statistical summary of Linearity results are given in Table 5.

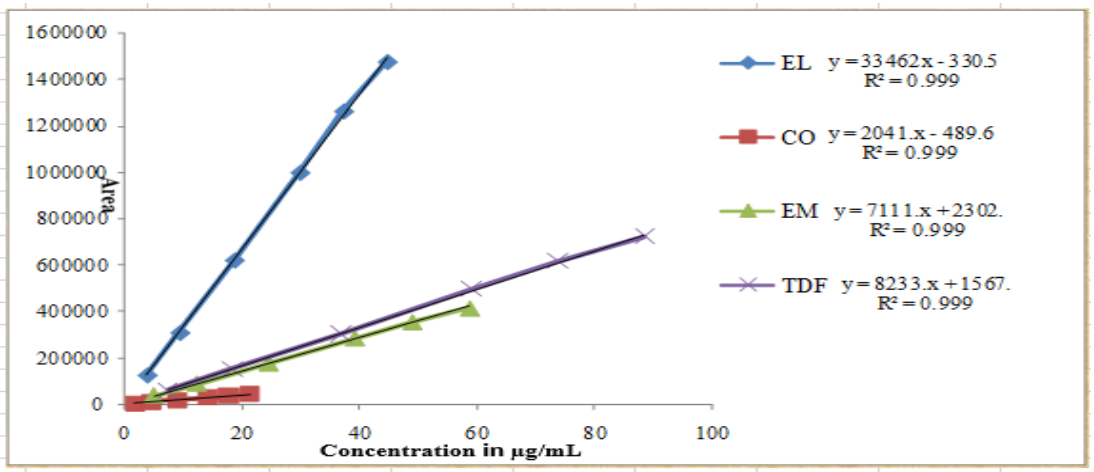


Figure 4: Typical Linearity chart for EL, CO, EM and TDF

Table 5: Statistical summary of linearity data

Name of the drug	Trend line equation	Range (µg/ml)	Intercept	Residual sum of squares	Correlation coefficient
EL	y = 33462 - 330.5	3.713-44.559	-331	12826.216	0.99977
CO	y = 2041.x - 489.6	1.769-21.233	-490	363.496	0.99978
EM	y = 7111.x + 2302.9	4.895-58.742	2303	4222.154	0.99968
TDF	y = 8233.x + 1567	7.367-88.399	1567	7411.618	0.99968

Accuracy

The accuracy expresses the agreement between the accepted value and the observed value. According to ICH guidelines or USP general chapter for validation of compendial procedures <1225>, the recovery of dissolution results shall be in the range between 95-105%. The % recovery was found within acceptable range in all specified ranges and found acceptable (Tables 6a and 6b).

Table 6a: Accuracy results for EL and CO

Level spiked at	EL					CO				
	Amount added (mg/ml)	Amount recovered (mg/ml)	% Recovery	Avg.	% RSD	Amount added (mg/ml)	Amount recovered (mg/ml)	% Recovery	Avg.	% RSD
10	3.74	3.82	102.1	101.8	0.9	3.17	3.31	104.4	100.2	3.7
	3.74	3.77	100.8			3.17	3.14	99.1		
	3.74	3.83	102.4			3.17	3.08	97.2		
50	30.04	30.46	101.4	101.8	0.4	30.14	28.73	95.3	97.1	1.6
	30.04	30.71	102.2			30.14	29.64	98.3		
	30.04	30.55	101.7			30.14	29.46	97.7		
100	37.55	38.8	103.3	103.9	0.5	37.67	37.33	99.1	99.2	1.5
	37.55	39.12	104.2			37.67	37.94	100.7		
	37.55	39.09	104.1			37.67	36.83	97.8		
120	45.05	45.8	101.7	101.5	0.4	45.21	43.74	96.7	96.7	1
	45.05	45.82	101.7			45.21	44.15	97.7		
	45.05	45.49	101			45.21	43.31	95.8		
mg/ml=milli gram/milli liter, % RSD=Percentage Related Standard Deviation										

Table 6b: Accuracy results for EM and TDF

Level spiked at	EM					TDF				
	Amount added (mg/ml)	Amount recovered (mg/ml)	% Recovery	Avg.	% RSD	Amount added (mg/ml)	Amount recovered (mg/ml)	% Recovery	Avg.	% RSD
10	5.43	5.39	99.3	98.5	1.5	7.48	7.75	103.6	102.7	1.1
	5.43	5.25	96.7			7.48	7.59	101.5		
	5.43	5.4	99.4			7.48	7.7	102.9		
50	40.71	41.46	101.8	100.8	0.8	60.25	62	102.9	102.3	0.5
	40.71	40.89	100.4			60.25	61.49	102.1		
	40.71	40.84	100.3			60.25	61.41	101.9		
100	50.88	51.64	101.5	102.1	0.7	75.31	77.78	103.3	103.7	0.6
	50.88	51.86	101.9			75.31	77.88	103.4		
	50.88	52.37	102.9			75.31	78.63	104.4		
120	61.06	61	99.9	99.5	0.6	90.38	91.66	101.4	101.1	0.5
	61.06	60.94	99.8			90.38	91.63	101.4		
	61.06	60.41	98.9			90.38	90.8	100.5		
mg/ml=milli gram/milli liter, % RSD=Percentage Related Standard Deviation										

Solution stability

No significant changes were observed in the area of EL, CO, EM and TDF when both standard and samples were analysed at room temperature of 25°C. Both standard and samples solutions of each drug is found to be stable upto 24 h. % degradation is found to be less than 2 for all drugs. Since there is no issue observed at room temperature, solution stability at cooler temperature of 2-8°C was not established.

Filter evaluation

The results obtained for filter paper evaluation is clearly indicating that there is no drug absorption is seen for any compound when analysed for both standard and sample solutions. The absolute difference between the area of unfiltered standard versus filtered standard solutions and centrifuged sample versus filtered samples were within 98-102%. This indicates that the absence of EL, CO, EM and TDF absorption by the filters used for study for PVDF and Nylon membrane filter in the dissolution test.

Robustness

Results from robustness study shows retention times for EL, CO, EM and TDF are not altering much. Also there is no considerable change being observed for system suitability parameters such as USP plate count, tailing factor and resolution between each drug. The critical attribute of USP resolution in all parameters is found to be more than 2.6 between each drug, which shows the optimized chromatographic parameters are robust in nature over tested conditions (For robustness study chromatogram refer Figure 5).

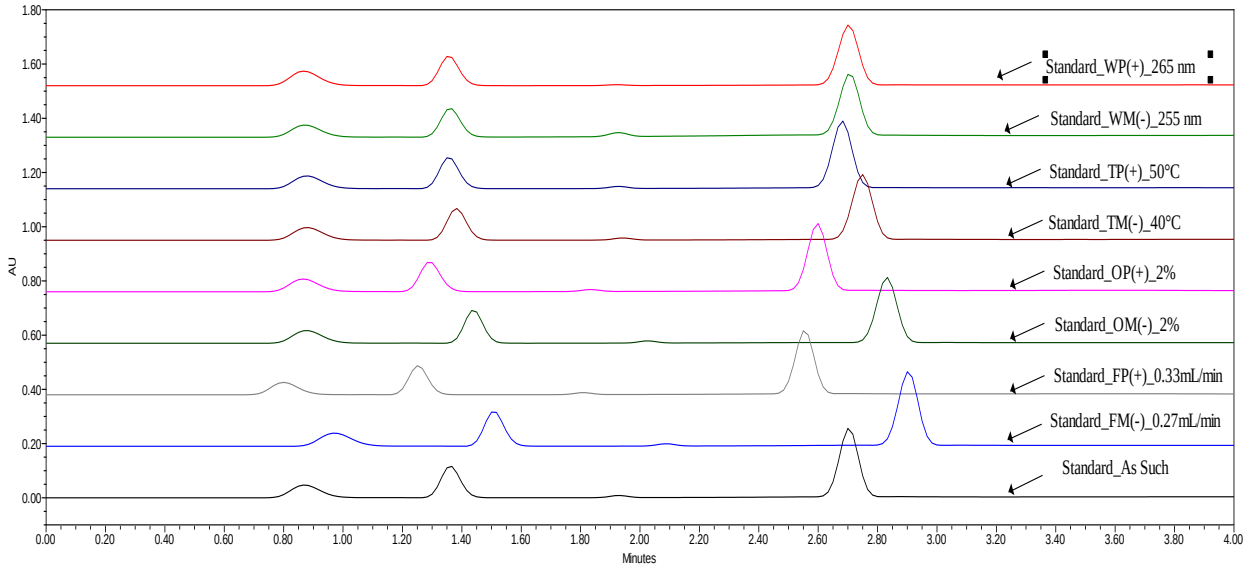


Figure 5: Robustness study chromatogram

CONCLUSION

Method was validated as per ICH general requirement for Dissolution test procedure. The result obtained from specificity experiment is showing that there is no placebo interference observed at the retention times of EL, CO, EM and TDF. Detection wavelength of 260 nm is found to specific and could able to provide precise area counts for each drug. Calibration curves depicting a proper linearity response from concentration versus area observed for each drug with correlation coefficients greater than 0.99. Recovery/Accuracy results confirming that satisfactory drug recovery is seen on proposed test concentrations for each drug. The developed and validated method could able to quantify the drugs of EL, CO, EM and TDF in tablet formulation for dissolution profiling at precise and accurate levels at very short run time. Hence developed method could be useful to pharmaceutical analytical laboratories to release the profiling results at faster rate.

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