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Stability indicating HPLC method for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage form

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ABSTRACT

A new HPLC method was developed and validated for the determination of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir in tablet dosage form. The chromatographic separation was achieved on a Inertsil ODS 3V(4.0 x 250mm, 5µm,) with a mobile phase combination of 0.1%TFA and Acetonitrile in gragient mode employing at a flow rate of 1.2 ml/min, and the detection was carried out by using UV detector at 242 nm. The total run time was 12 minutes. The retention time of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir were found to be 3.43 min., 4.75 min., 5.27, and 7.56 min. respectively. The performance of the method was validated according to the present ICH guidelines.

Key Words: Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir

INTRODUCTION

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Chemical name of Emtricitabine is (4-amino-5-fluoro-1-[(2*R*, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.
Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness. [1-2]. Tenofovir disoproxyl fumarate disoproxil fumarate belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs). Tenofovir disoproxyl fumarate disoproxil fumarate is a prodrug form of Tenofovir disoproxyl fumarate. Chemical name of Tenofovir disoproxyl fumarate disoproxil fumarate is ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl) propan-2-yl]oxy}methyl) phosphonic acid. Tenofovir disoproxyl fumarate blocks reverse transcriptase, a crucial virus enzyme in human immunodeficiency virus 1 (HIV-1) and hepatitis B virus infections.Tenofovir disoproxyl fumarate exhibits anti-HIV effects in humans when dosed by subcutaneous injection. Tenofovir disoproxyl fumarate is indicated for the treatment of chronic hepatitis B in adults and pediatric patients 12 years of age and older. [3-5] Cobicistat is a licensed drug for use in the treatment of infection with the human immunodeficiency virus (HIV).Cobicistat acts as an HIV integrase inhibitor. Chemical name of Cobicistat is Thiazol-5-ylmethyl *N*-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl) methyl-methyl-carbamoyl] amino]-4-morpholino-butanoyl] amino]-5-phenyl-pentyl]carbamate. 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, including atazanavir, darunavir and Tenofovir disoproxyl fumarate alafenamide fumarate. [6-7]
Elvitegravir is a drug used for the treatment of HIV infection. It acts as an integrase inhibitor. Chemical name of Elvitegravir is 6-[(3-Chloro-2-fluorophenyl) methyl]-1-[(2*S*)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxoquinoline-3-

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carboxylic acid. Elvitegravir acts as an integrase inhibitor.. According to the results of the phase II clinical trial, patients taking once-daily elvitegravir boosted by ritonavir had greater reductions in viral load after 24 weeks compared to individuals randomized to receive a ritonavir-boosted protease inhibitor.[8] The literature survey revealed that there are a very few HPLC and spectroscopic methods available for the determination of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir in pure and combined dosage forms. The present study was aimed to develop a new HPLC method for simultaneous estimation of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir in combined pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and reagents: Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir bulk drugs were made available from Pharmatrain, Kukatpally, Hyderabad. Tri Fluoroacetic acid, methanol, Acetonitrile were obtained from Merck. Commercially available STRIBLED was used for the dosage form analysis. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment.

Equipments: The Waters HPLC system with a UV or photo diode array detector was used for method development and validation. The output signal was monitored and processed by using Empower software.

Chromatographic condition: The mobile phase used 0.1%Trifluoroacetic acid buffer and Acetonitrile in the gradient mode employing at a flow rate of 1.2 ml/min. The analytical column used Inertsil ODS 3V (4.0 x 250mm, 5µm,).The detection was carried out at a wavelength of 242nm for a run time of 12 min. Diluent used as water and methanol in the ratio of 60:40 v/v.

Gradient programme:

Time	%A	%B
0.00	90.0	10.0
3.00	40.0	60.0
5.00	10.0	90.0
8.00	10.0	90.0
8.10	90.0	10.0
12.00	90.0	10.0

Preparation of standard solution: Accurately weigh and transfer about 200 mg of Emtricitabine, 300 mg of Tenofovir disoproxyl fumarate , 150mg of Cobicistat and 150mg of Elvitegravir working standard into a 100 ml clean dry volumetric flasks add Methanol and sonicate to dissolve it completely and make volume up to the mark with the methanol (Stock solution).Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Assay of Pharmaceutical Dosage form: (Sample Preparation): Twenty tablets of STRIBLED were weighed to get the average weight and then ground. An amount of powder equivalent to about 200 mg of Emtricitabine,300 mg of Tenofovir disoproxyl fumarate,150mg of Cobicistat and 150mg of Elvitegravir into a 100 ml clean dry volumetric flasks add Methanol and sonicate to dissolve it completely and make volume up to the mark with the methanol (Stock solution).Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Method development: Chromatographic parameters were preliminary optimized to develop HPLC method for simultaneous estimation of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir with short analyses time (12min), and acceptable resolution (> 2). The isoabsoprtive point of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir selected was 242 nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested along with different buffers. Different columns like X-terra, Inertsil, Inspire columns were tried. Resolution was the major problem while we are developing method. Resolution was less very less when we are using one organic phase, to increase resolution acetonitrile were used in isocratic mode. Finally separation for simultaneous determination of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir was carried out by gradient elution with a flow rate of 1.2 mL/min inertsil ODS (4.0 x 250mm, 5µm,). The standard chromatogram was shown in Fig-1.The system suitability parameters were shown in Table-1.

Fig.1: Standard chromatogram

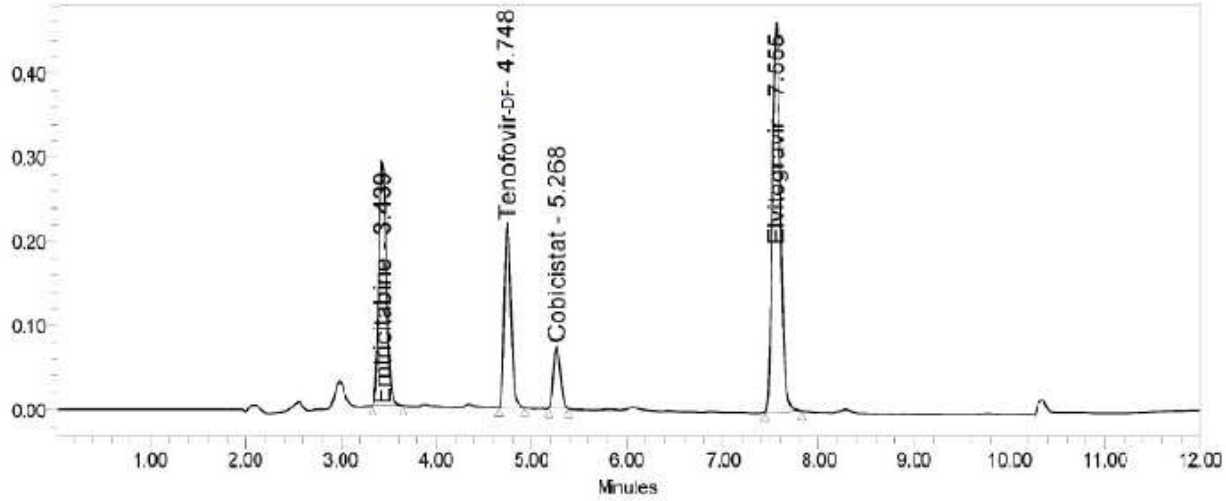


Table 1:system suitability parameters

Parameter	Emtricitabine	Tenofovir disoproxyl fumarate	Cobicistat	Elvitegravir
Retention time	3.43	4.75	5.27	7.56
USP Plate count	3122	33634	29854	36897
USP Tailing	1.53	1.24	1.10	1.35
USP Resolution		4.1	3.2	6.4

Method Validation: The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method[4].

Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in six replicate for intermediate precision and six replicate for precision. The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-3 and ID Precision in Table-4.

Table-3: Precision results for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir

S.No.	Emtricitabine	Tenofovir disoproxyl fumarate	Cobicistat	Elvitegravir
1	1518036	1016343	360286	2963810
2	1525361	1020659	360042	2963597
3	1520862	1016895	359508	2953289
4	1521059	1013990	360128	2942866
5	1527678	1021905	366055	2959222
6	1524755	1022604	362698	2966363
Average	1522959	1018733	361453	2958191
Standard deviation	3563.92	3473.96	2514.75	8803.72
% RSD	0.23	0.34	0.70	0.30

Table-4: ID Precision results for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir

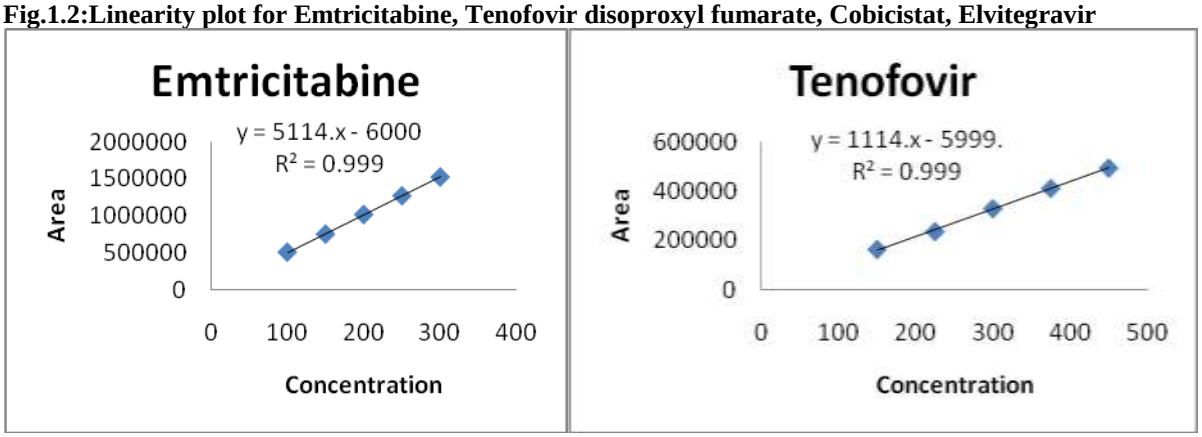
S.No.	Emtricitabine	Tenofovir disoproxyl fumarate	Cobicistat	Elvitegravir
1	1534633	1055928	361825	3001156
2	1550115	1075595	363902	3003871
3	1547737	1033812	365463	3001424
4	1559426	1067179	368294	3014152
5	1550990	1071722	368019	3002129
6	1567351	1062605	369238	3025260
Average	1551709	1061140	366124	3007999
Standard deviation	11087.37	15056.73	2893.18	9770.94
% RSD	0.71	1.42	0.79	0.32

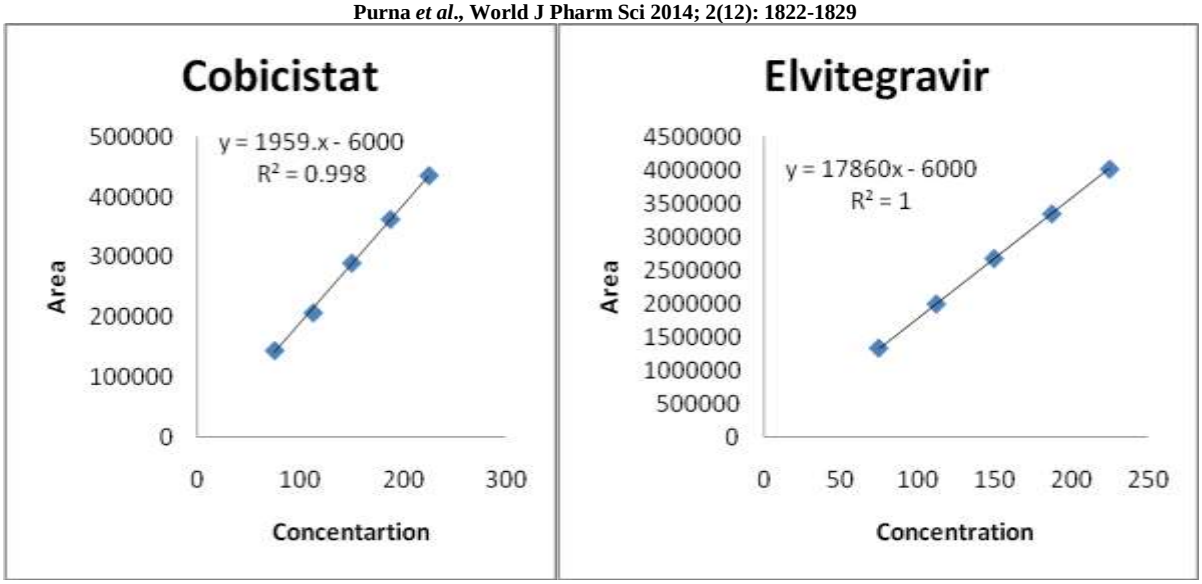
Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detector response for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations ranging from 100-300 µg/ml for Emtrcitabine, 150-450 µg/ml for Tenofovir disoproxyl fumarate, 75-225 µg/ml for both Cobicistat & Elvitegravir were prepared and analyzed. The linearity graph was plotted using concentration Vs peak area and they were shown in Fig-1.2. Slope, correlation coefficient (R) and intercept were calculated and the results were shown in Table-2.

Table 2: Regression and statistical parameters

Parameter	Emtricitabine	Tenofovir disoproxyl fumarate	Cobicistat	Elvitegravir
Concentration Range (µg/ml)	100-300	150-450	75-225	75-225
Correlation coefficient	0.999	0.999	0.999	0.999
Intercept	6000	5999	6000	6000
Slope	5114	1114	1959	17860

Fig.1.2:Linearity plot for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir





Method precision: Twenty tablets of STRIBLED were weighed to get the average weight and then ground. An amount of powder equivalent to about 200 mg of Emtricitabine, 300 mg of Tenofovir disoproxyl fumarate ,150mg of Cobicistat and 150mg of Elvitegravir into a 100 ml clean dry volumetric flasks add Methanol and sonicate to dissolve it completely and make volume up to the mark with the methanol (Stock solution).Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. The above procedure used for 6 different preparations and injected, % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-5.

Table-5: Method Precision results for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir

S.No.	Emtricitabine	Tenofovir disoproxyl fumarate	Cobicistat	Elvitegravir
1	99.36	99.08	101.32	101.85
2	100.39	98.81	101.12	101.46
3	97.89	98.24	100.02	100.41
4	99.45	98.73	99.69	101.49
5	101.91	97.94	99.98	101.22
6	97.79	97.53	99.88	98.91
Average	99.47	98.39	100.34	100.89
Standard deviation	1.56	0.59	0.70	1.08
% RSD	1.57	0.60	0.70	1.07

Accuracy: The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with preanalyed sample. For all the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from preanalyed sample area. The results were shown in Table-6.

Table 6: Accuracy Results For Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir

Analyte	% Level	Nominal Value (mg)	Found (mg)	% Recovery	Mean % Recovery
Emtricitabine	50%	5	5.04	100.86	100.34
	100%	10	9.92	99.21	
	150%	15	15.14	100.96	
Tenofovir disoproxyl fumarate	50%	5	5.06	101.21	100.58
	100%	10	9.85	98.54	
	150%	15	15.30	101.99	
Cobicistat	50%	5	5.05	101.0	100.27
	100%	10	9.92	99.17	
	150%	15	15.10	100.64	
Elvitegravir	50%	5	5.05	100.93	100.44
	100%	10	10.00	100.05	
	150%	15	15.05	100.34	

Robustness: Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio(±10%), flow rate (0.2ml/min). It was observed that there were no marked changes in system suitability parameters, which demonstrated that the developed RP-HPLC method is robust.

Table 7: Robustness Results for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir

	Emtricitabine			**Tenofovir disoproxyl fumarate**			**Cobicistat**			**Elvitegravir**		
	RT	USP Plate count	USP Tailing	RT	USP Plate count	USP Tailing	RT	USP Plate count	USP Tailing	RT	USP Plate count	USP Tailing
Flow 1	4.65	10766	1.15	5.91	31311	1.18	6.32	33691	1.12	8.75	34631	1.12
Flow 2	2.85	10534	1.14	4.23	31962	1.16	4.96	32852	1.11	7.01	34296	1.11

Flow 1- 0.9ml/min, Flow 2- 1.3ml/min

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir in their tablet dosage form. The result obtained for Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir was comparable with the corresponding labeled amounts and they were shown in Table-8.

Table 8: Assay Results

	Label claim	% Assay
Emtricitabine	200 mg	98.85
Tenofovir disoproxyl fumarate	300 mg	98.21
Cobicistat	150mg	99.84
Elvitegravir	150mg	99.98

Degradation studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Exforge using the proposed method.

Preparation of Stock Solution:

Accurately weigh and transfer about 200 mg of Emtricitabine, 300 mg of Tenofovir disoproxyl fumarate, 150mg of Cobicistat and 150mg of Elvitegravir working standard into a 100 ml clean dry volumetric flasks add Methanol and sonicate to dissolve it completely and make volume up to the mark with the methanol (Stock solution).Further pipette 5 ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

ACID degradation condition: Pipette 5ml of the above stock solution into a 50ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 80ºC for 1 hour and then neutralized with 0.1 N NaOH and make up to 50ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

ALKALI degradation condition: Pipette 5 ml of the above stock solution into a 50ml volumetric flask and add 3 ml of 0.1N NaOH was added in 20 ml of volumetric flask. Then, the volumetric flask was kept at 80ºC for 1 hour and then neutralized with 0.1N HCl and make up to 20ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal induced degradation: The sample was taken in petridish and kept in Hot air oven at 105^0 C for 48 hours. Then the sample was taken and diluted with diluents to prepare 200 ug/ml, 300 ug/ml,150 ug/ml,150µg/ml of Emtricitabine, Tenofovir DF, Cobicistat, Elvitegravir and injected into HPLC and analysed.

Oxidative degradation: Pipette 5 ml of the above stock solution into a 50ml volumetric flask 3 ml of 3% w/v of hydrogen peroxide added in 50 ml of volumetric flask and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 30 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Table: Degradation Data

	% Total Degradation
Acid	12%
Base	11%
Peroxide	16%
Thermal	2%

CONCLUSION:

In the present work a new, accurate, precise and robust HPLC method was developed and validated for estimation of Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir in pharmaceutical dosage form in accordance with the ICH parameters. The method gives good resolution between both the compounds with a short analysis time (12 min). Linearity is observed in the concentration range of 100-300 µg/ml for Emtrcitabine, 150-450 µg/ml for Tenofovir disoproxyl fumarate, 75-225 µg/ml for both Cobicistat & Elvitegravir drugs at 242 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the Emtricitabine, Tenofovir disoproxyl fumarate, Cobicistat, Elvitegravir in combined dosage form without any interference of excipients.

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