Method Development and Validation of Emtricitabine and Tenofovir Disoproxil Fumarate by RP-HPLC Method

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***Abstract*—A simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in bulk and in pharmaceutical dosage forms using a Waters HPLC system. Chromatographic separation was achieved on an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm particle size) maintained at ambient temperature. The mobile phase consisted of water and methanol in the ratio of 85:15 (v/v). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 261 nm using the PDA detector.**

***Index Terms*—Emtricitabine and TDF (Tenofovir Disoproxil Fumarate), RP-HPLC.**

I. INTRODUCTION

Truvada® is a combination drug containing Emtricitabine and TDF. Emtricitabine acts as a Nucleoside reverse transcriptase inhibitor. Emtricitabine is a cytidine analog which, when phosphorylated to emtricitabine 5'-triphosphate, competes with deoxycytidine 5'-triphosphate for HIV-1 reverse transcriptase. As HIV-1 reverse transcriptase incorporates emtricitabine into forming DNA strands, new nucleotides are unable to be incorporated, leading to viral DNA chain termination. Inhibition of reverse transcriptase prevents transcription of viral RNA into DNA, therefore the virus is unable to incorporate its DNA into host DNA and replicate using host cell machinery.TDF, (15-16) on the other hand, Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analog reverse transcriptase inhibitors (NtRTIs) which block reverse transcriptase, an enzyme necessary for viral production in HIV-infected individuals. This enables the management of HIV viral load through decreased viral replication.

II. MATERIALS AND METHODS

Preparation of Stock solution: Two 100 ml volumetric flasks were each filled with 25 mg of Tenofovir Disoproxil Fumarate and 20mg of precisely weighed Emtricitabine. The mixtures were sonicated for 20 minutes. Then, 10ml of each solution was taken and placed in a 50ml volumetric flask. Mobile phase was added to each flask, and after 10 minutes, the mixtures were sonicated again.

Preparation of working standard solution: Stock solutions of Emtricitabine and Tenofovir Disoproxil Fumarate, as previously mentioned were used to prepare working solutions with concentrations ranging from 20 to 80 ppm. These solutions were sonicated and filtered through a 0.45 µm membrane.

III. RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (13-16).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Emtricitabine (20µg/ml) and TDF (20µg/ ml) were injected into the HPLC system. The peak purity data of Emtricitabine and TDF were compared. There should not be any interference at the retention time of the main peaks.

Linearity: Linearity for the drugs Emtricitabine and TDF (16-19) was determined by preparing the standard solutions at six concentrations levels in six replicates in the range of 20-80µg/ml. The linearity charts of Emtricitabine and TDF was shown in the

figure no 2&3. The correlation coefficient was found to be 0.9999 and 0.9999 for Emtricitabine and TDF respectively. Linearity results were tabulated in table2.

Accuracy: Accuracy (18-19) was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analyzed for %RSD which should not be more than 2.0. The % recovery was calculated and the results was reported in table no.3&4.

Precision: The precision (19-20) of the analytical method was studied by injecting six replicates of standard containing 20µg/ml of Emtricitabine and 20µg/ml of TDF which were injected into HPLC system. The % RSD was calculated and the results were reported in the table no.5 & 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection (18-20) was defined as the concentration which yields a signal – to – noise ratio 3:1 whereas the limit of quantification (19-20) was calculated to be the lowest concentration that could be measured with signal - to – noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines (18-19). Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no.8.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

Table1: Optimized Chromatographic conditions

Parameters	Method
Stationary Phase(column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol and Water (85:15)
Flow rate (ml/min)	1.0 ml/min
Run time(minutes)	12 min
Temperature in the column (°C)	Ambient
Volume of injection loop (□l)	20 µl
Wavelength of detection (nm)	261 nm
Drug RT (min)	4.645 min for Emtricitabine and 6.209 for TDF.

Figure 1: Optimized chromatogram

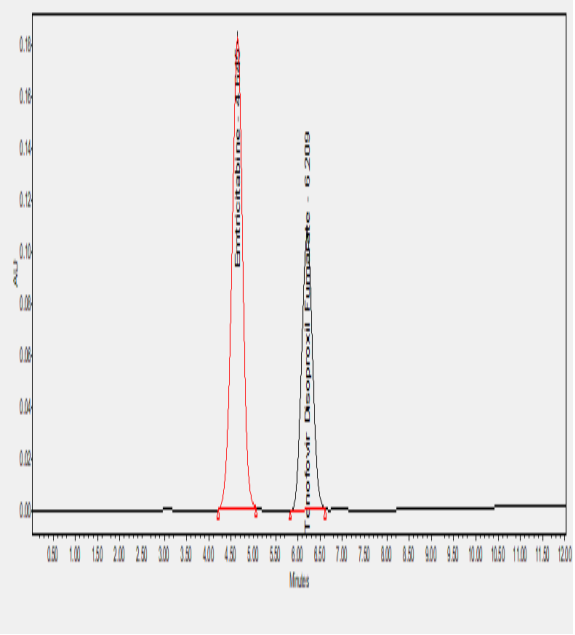

Table 2: Linearity data of Emtricitabine and TDF

Emtricitabine		TDF	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
20	1530203	20	879959
30	2189411	30	1324049
40	2964288	40	1805597
50	3633846	50	2251110
60	4336870	60	2715788
70	4971811	70	3177769

Figure 2: Calibration Curve of Emtricitabine

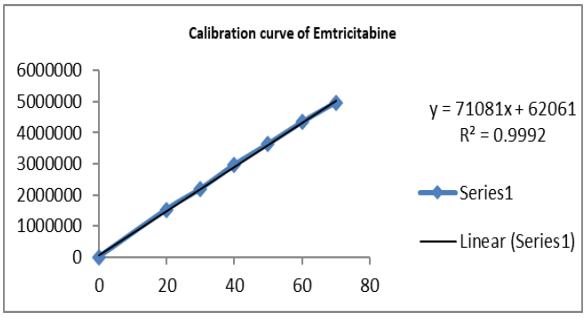

Figure 3: Calibration Curve of TDF

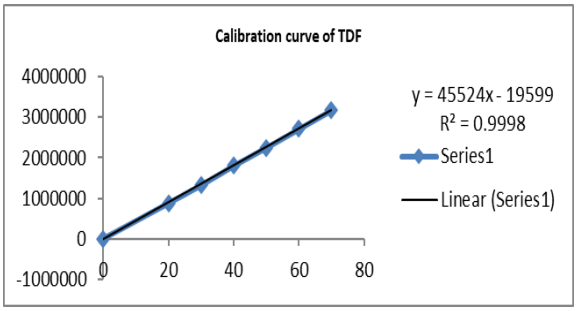

Table 3: Accuracy data of Emtricitabine

Concentration	Amount added	Amount found	% Recovery	Statistical Analysis of % Recovery	
% of spiked level	(ppm)	(ppm)			
50% - 1	20	19.83	99.73	Mean	99.87
50% - 2	20	20.12	100.11	%RSD	0.89
50% - 3	20	19.84	99.14		
100 % - 1	40	40.13	100.14	Mean	99.95
100 % - 2	40	39.88	99.90		
100% - 3	40	39.82	99.52	%RSD	0.56
150% - 1	60	60.16	100.21	Mean	100.134
150% - 2	60	60.16	100.21		
150% - 3	60	60.12	100.10	%RSD	0.211

Table 4: Accuracy data of TDF

Concentration	Amount added	Amount found	% Recovery	Statistical Analysis of % Recovery	
% of spiked level	(ppm)	(ppm)			
50% - 1	20	19.86	99.62	Mean	99.87
50% - 2	20	20.16	100.34		
50% - 3	20	19.99	99.91	%RSD	0.67
100 % - 1	40	40.13	100.10	Mean	100.06
100 % - 2	40	40.14	100.11		
100% - 3	40	40.06	100.02	%RSD	0.543
150% - 1	60	59.92	99.84	Mean	100.32
150% - 2	60	60.12	100.13		
150% - 3	60	60.11	100.10	%RSD	0.95

Table 5: System Precision data of Emtricitabineand TDF

S. No	Emtricitabine	TDF
1	2924324	1803256
2	2931021	1803452
3	2924320	1806345
4	2923742	1803421
5	2920768	1804427
Mean	2924835	1804180
SD	3759.258	1294.998
% RSD	0.128529	0.071778

Table 6: Method Precision data of Emtricitabineand TDF

S. No	Emtricitabine	TDF
1	2931412	1804137
2	2933675	1803764
3	2930791	1802461
4	2931182	1803357
5	2930452	1805320
6	2932121	1804721
Mean	2931606	1803960
SD	1162.698	1010.849
% RSD	0.039661	0.056035

Table 7: LOD and LOQ data ofEmtricitabineand TDF

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Emtricitabine	0.13	0.41
TDF	0.13	0.41

Table 8: Robustness data of Emtricitabineand TDF

S No	Drug Name	Condition	Peak area	% RSD
	Emtricitabine	Decreased Flow rate of 0.8 ml/min	2848970	0.049
		Increased Flow rate of 1.2 ml/min	2937386	0.039
	TDF	Decreased Flow rate of 0.8 ml/min	1793783	0.179
		Increased Flow rate of 1.2 ml/min	1816590	0.029

Table 9: Assay data Emtricitabine and TDF

S. No	Peak area of Emtricitabine	% Assay	Peak area of TDF	% Assay
1.	2930864	100.96	1803461	100.166
2.	2931341		1803324	
3.	2934467		1804621	
4.	2932713		1803342	
5.	2930546		1806742	
6.	2935742		1804612	

IV. CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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