AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

HPLC Isocratic Elution Method for Quantification of Tenofovir Disoproxil and Emtricitabine Simultaneously In Bulk and Tablet Formulation

Eegala Bheema Shankar[1*], Challa Gangu Naidu[2], Vangala Saidi reddy[1]
1.Clearsynth Research centre, Hyderabad, Telangana, India - 500076.
2.Analytical chemistry division, Indian institute of Chemical Technology, Hyderabad, Telangana, India- 500607.

ABSTRACT

An HPLC method has been developed for simultaneous estimation of tenofovir disoproxil and emtricitabine in bulk and in their tablet dosage form. Separation and analysis of both drugs was achieved on Supelco C18 (250 x 4.6 mm; 5 µm particle size) analytical column with temperature set at 25˚C. The best chromatographic condition was found as an isocratic mobile phase consisting of 0.2 M ammonium acetate (pH 4.5) and methanol in a ratio of 65: 35 (% *v/v*) at a flow rate of 1.2 ml/min for 6 minutes. The retention of emtricitabine and tenofovir disoproxil was found to be 3.020 min and 4.264 min, respectively. The method was validated according to the International Conference on Harmonization guidelines and various validation parameters (system suitability, selectivity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness) were determined. The results of validation parameters are satisfactory. Applicability of the developed and validated HPLC method was checked in tablet dosage form.

Keywords: Tenofovir disoproxil, Emtricitabine, HPLC method, Validation, Estimation.

*Corresponding Author Email: bheemashankareegala@gmail.com
Received 14 July 2017, Accepted 27 July 2017

Please cite this article as: Shankar EB *et al.*, HPLC Isocratic Elution Method for Quantification of Tenofovir Disoproxil and Emtricitabine Simultaneously In Bulk and Tablet Formulation. American Journal of PharmTech Research 2017.

INTRODUCTION

Tenofovir disoproxil is an antiretroviral drug belonging to the drug class known as nucleoside reverse transcriptase inhibitors[1,2]. Chemically, it is described as [[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl] oxy methyl propan-2-yl carbonate;(E)-but-2-enedioic acid (Figure 1). Tenofovir disoproxil acts by blocking a crucial enzyme, reverse transcriptase, which is responsible for the viral multiplication in human immunodeficiency virus infected people. Tenofovir disoproxil is prescribed for the treatment of human immunodeficiency virus and hepatitis B virus infections in combination with additional antiretroviral agents[3].

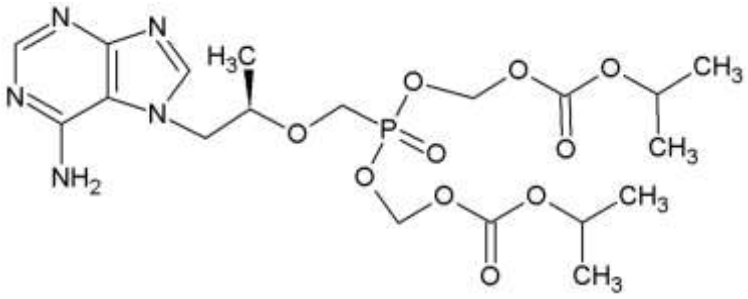

Figure 1: Structure of tenofovir disoproxil

Emtricitabine is a synthetic nucleoside analog of cytidine with activity against human immunodeficiency virus type 1 reverse transcriptase[4]. Chemically, emtricitabine is describes as 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one (Figure 2). Emtricitabine was approved for use in combination with other antiretroviral agents in adults with human immunodeficiency virus infection[5]. Emtricitabine inhibits viral replication by inhibiting the activity of human immunodeficiency virus type 1 reverse transcriptase reverse transcriptase[6].

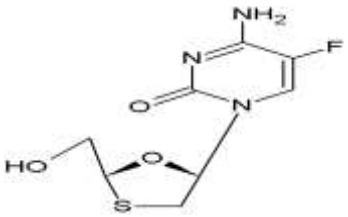

Figure 2: Structure of emtricitabine

Tenofovir disoproxil is formulated in binary mixture with emtricitabine to prevent human immunodeficiency virus from altering the genetic material of healthy T cells[7,8]. Combining the two antiviral drugs, tenofovir disoproxil and emtricitabine, in one tablet also helps in lessening of the pill burden and increases the compliance with antiretroviral therapy.

Several analytical methods have been reported for simultaneous estimation of tenofovir disoproxil and emtricitabine including first derivative spectrophotometry[9], first derivative of ratio spectra

spectrophotometry[9], simultaneous equation spectrophotometry[10], absorbance ratio spectrophotometry[10], area under curve spectrophotometry[11], dual wavelength spectrophotometry[11], ratio derivative spectrophotometry[12], absorbance corrected spectrophotometry[12], HPLC[13-19], HPTLC[20,21] and LC-MS[22-24]. In the present study an attempt was made to develop an HPLC method for the assay of tenofovir disoproxil and emtricitabine, simultaneously, in bulk and combined tablet dosage. The developed method was validated as per guidelines given by International Conference on Harmonization[25].

MATERIALS AND METHOD

Mobile phase:

The solvents and chemicals used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used was 0.2M ammonium acetate (Sd. Fine Chemicals Ltd., Mumbai) and methanol (Merck India Ltd., Mumbai) in the ratio of 65:35 *v/v*. The pH was adjusted to 4.5 with dilute orthophosphoric acid (Sd. Fine Chemicals Ltd., Mumbai). Before use, the mobile phase was filtered through millipore membrane filter and degassed for 15 min by sonication.

Instrumentation and chromatographic conditions:

In the present study Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used. Supelco C18 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous quantification of tenofovir disoproxil and emtricitabine. The column temperature was maintained at 25±1 ^{o}C. The separation was carried out under isocratic elution. The flow rate was maintained as 1.2 ml/min. The injection volume was 10 µL. The eluents were detected at 260 nm.

Standard solutions:

Tenofovir disoproxil and emtricitabine bulk samples were obtained from Lara drugs pvt Ltd., Hyderabad. The standard stock solution was prepared by dissolving 200 mg of emtricitabine and 300 mg of tenofovir disoproxil in 100 ml mobile phase. Working standard solutions equivalent to 100, 150, 200, 250 & 300 µg/ml emtricitabine and 150, 225, 300, 375 & 450 µg/ml tenofovir disoproxil was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

Sample Solution:

The tablet dosage form, Tavin-EM, manufactured by Hetero Drugs Limited, Hyderabad, India (Label claim: emtricitabine 200 mg and tenofovir disoproxil 300 mg), were purchased and used in

the present study. Ten tablets were weighed, average weight was determined and finely powdered to obtain a homogenous mixture. The powder equivalent to 200 mg of emtricitabine and 300 mg of tenofovir disoproxil was transferred to a 100 ml volumetric flask containing 30 ml of mobile phase, sonicated for 20 min and made up to mark with the mobile phase. The resultant mixture was filtered through 0.45 µm filter. The filtrate was diluted appropriately with the mobile phase to get a final concentration 200 µg/ml and 300 µg/ml of emtricitabine and tenofovir disoproxil, respectively.

Assay of emtricitabine and tenofovir disoproxil in bulk:

The working standard solutions, in the concentration of 100, 150, 200, 250 & 300 µg/ml emtricitabine and 150, 225, 300, 375 & 450 µg/ml tenofovir disoproxil, prepared from stock solution were injected into the column (10 µl) and chromatographed under optimized chromatographic conditions. Peak areas were recorded for each concentration of emtricitabine and tenofovir disoproxil. The calibration curve was plotted as concentration *vs* peak area. The regression equation was derived. The concentration of unknown was determined either from the calibration curve or regression equation derived.

Assay of emtricitabine and tenofovir disoproxil in combined tablet dosage forms:

10 µL of the sample solution prepared in the section "sample solution" was injected into the HPLC system three times. The peak areas were recorded and the content of emtricitabine and tenofovir disoproxil in combined tablet dosage form was determined from the calibration curve or from the regression equation.

RESULTS AND DISCUSSION

Method development:

The main objective of the present study was to develop a HPLC method for the estimation of emtricitabine and tenofovir disoproxil simultaneously in bulk and tablet dosage form and to obtain well resolved peaks of emtricitabine and tenofovir disoproxil. During method development, chromatographic parameters such as mobile phase composition, flow rate of mobile phase, detection wavelength, analytical column and column temperature were optimized to get improved efficiency of the chromatographic system. Two HPLC analytical columns, Phenomenex C18 (150 mm x 4.6 mm; 5 µm particle size) and Supelco C18 (250 mm x 4.6 mm; 5 µm particle size) were tested during method development. The system suitability parameters like tailing factor, resolution, and plate count were considered. Based on the above said parameters Supelco C18 column (250 mm x 4.6 mm; 5 µm particle size) was finalized. Mobile phase containing a mixture (*v/v*) of 0.2 M

ammonium acetate and methanol in different ratios were evaluated so as to obtain appropriate composition of mobile phase. Finally the mixture of 0.2 M ammonium acetate and methanol in the ratio of 65:35 (*v/v*) was selected as optimal. At a flow rate of 1.2 ml/min and with column temperature of 25±1 ^{o}C well defined and well resolved peaks of emtricitabine and tenofovir disoproxil are obtained. At the wavelength 260 nm, best detector response for emtricitabine and tenofovir disoproxil was obtained. Therefore, 272 nm was selected as the analytical wavelength for the detection and simultaneous estimation of emtricitabine and tenofovir disoproxil. Under the optimized chromatographic conditions, the retention time for emtricitabine and tenofovir disoproxil was found to be 3.020 min and 4.264 min, respectively.

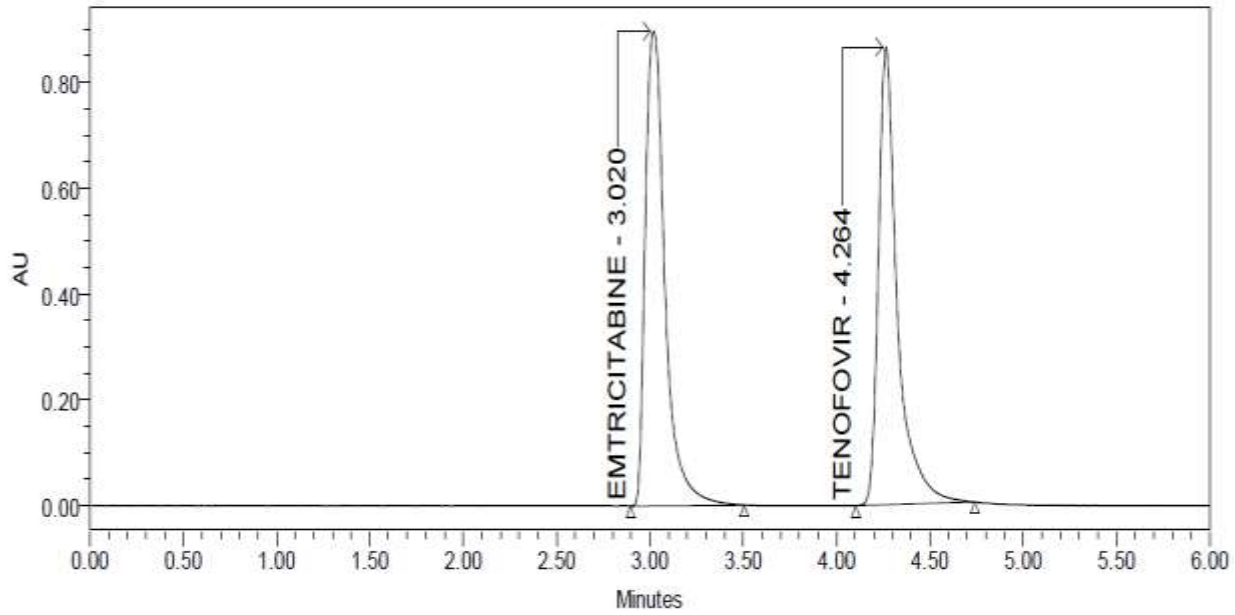

Figure 3: Chromatogram of emtricitabine and tenofovir disoproxil under optimized conditions

Method validation:

The developed method was validated for system suitability, selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per International Conference on Harmonization (ICH) guidelines[25].

System suitability studies:

Mixed standard solution of emtricitabine (200 µg/ml) and tenofovir disoproxil (300 µg/ml) solution was injected in five replicates in the HPLC system to determine system suitability. System suitability parameters established for the developed method include number of relative standard deviation of peak area, theoretical plates, resolution and tailing factor. The values obtained (Table 1) demonstrated the suitability of the system for the analysis of this drug combination.

Table 1: System suitability parameters

Parameters	Emtricitabine	Tenofovir disoproxil	Recommended limits
Retention time	2.999	4.205	-
Peak area	6462861 (%RSD – 1.044)	6050896 (%RSD - 0.150)	RSD ≤1
USP resolution	-	6.826	> 1.5
USP plate count	4548	10096	> 2000
USP tailing factor	1.77	1.73	≤ 2

Selectivity:

The selectivity of the developed HPLC method was investigated by non-interference of excipients in tablet dosage form and components of mobile phase. Selectivity of the present method was demonstrated by comparing the chromatograms of standard solution of emtricitabine (200 µg/ml) and tenofovir disoproxil (300 µg/ml)with the chromatogram of sample solution (containing emtricitabine 200 µg/ml and tenofovir disoproxil 300 µg/ml), blank mobile phase and placebo blank. The chromatograms are shown in Figures 4-7. There were no difference in the chromatograms of standard solution and sample solution (Figures 4 & 5). There are no peaks in the chromatogram of blank mobile phase and placebo blank (Figures 6 & 7). The results indicated the selectivity of the present method.

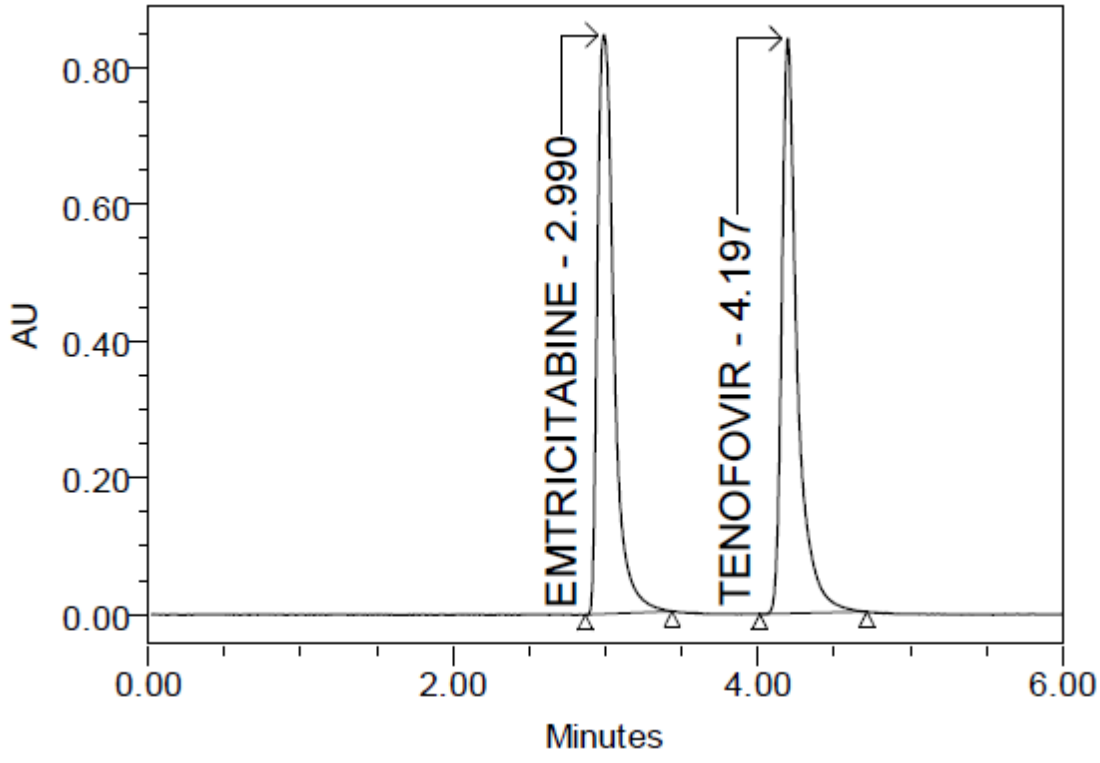

Figure 4: Chromatogram of standard drug solution

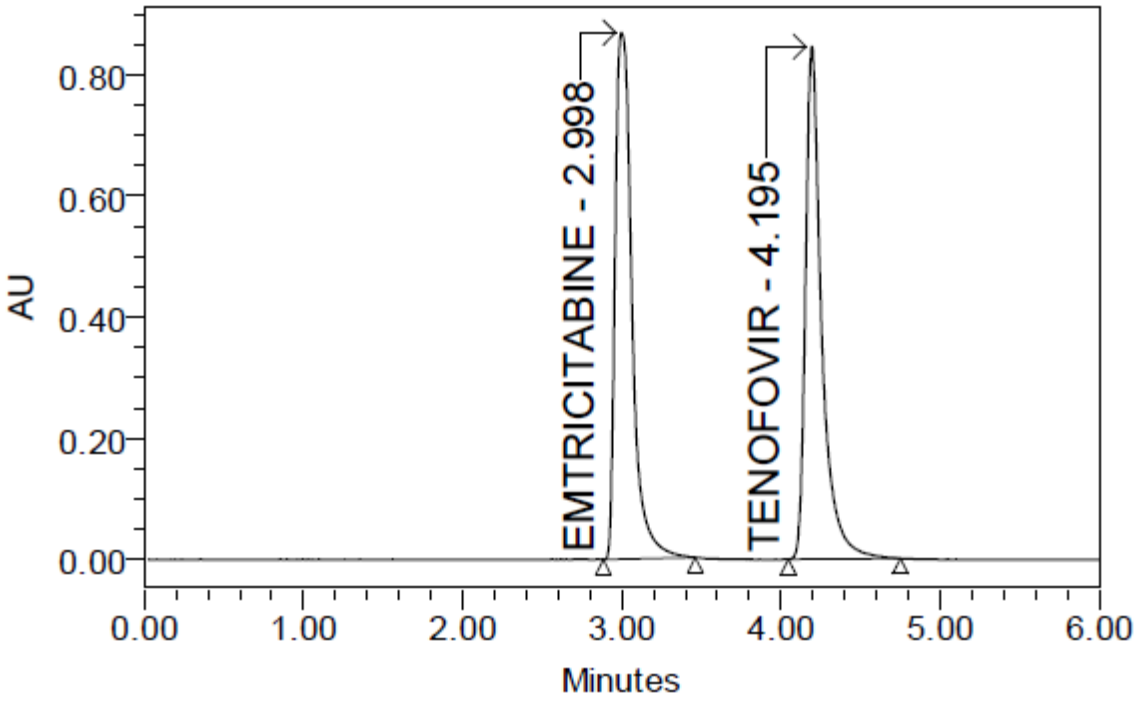

Figure 5: Chromatogram of tablet sample solution

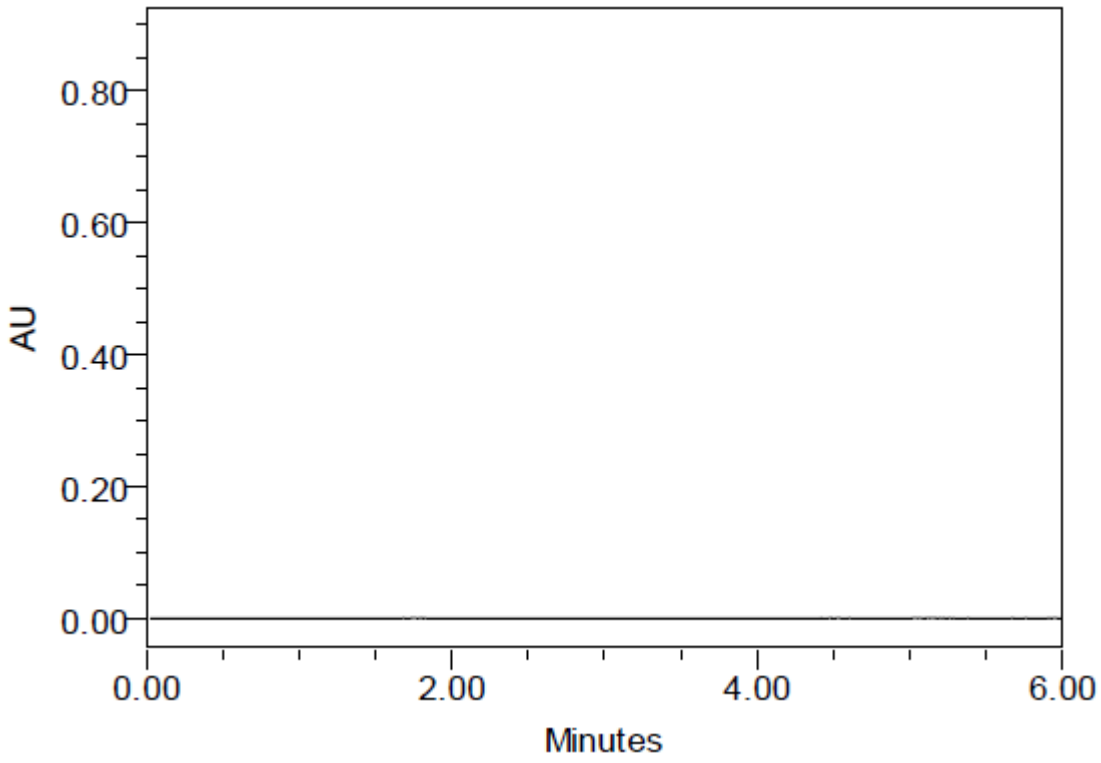

Figure 6: Chromatogram of mobile phase blank solution

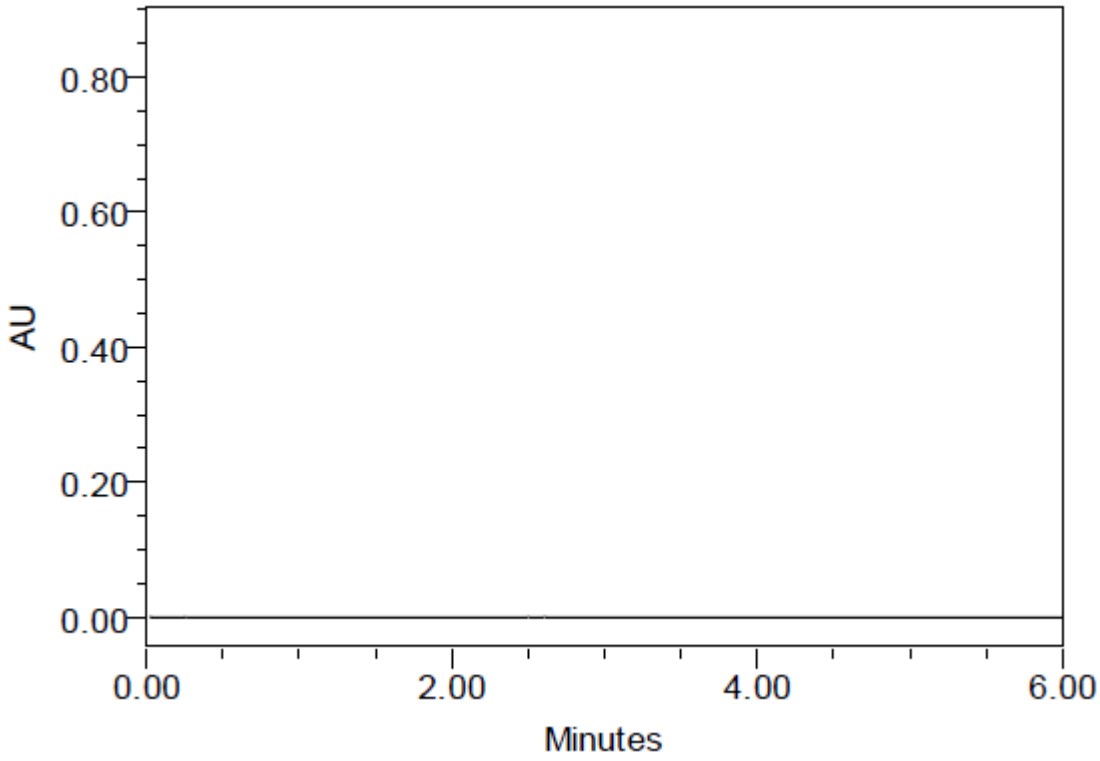

Figure 7: Chromatogram of placebo blank solution

Linearity and range:

The linearity for the present method was established by least squares regression analysis of the calibration curve. Calibration curves were linear over the concentration range of 100-300 µg/ml for emtricitabine and 150-450 µg/ml for tenofovir disoproxil with a regression coefficient (R^2) of 0.9999 for both the drugs. The results shows a good correlation exists between peak area and concentration of drugs within concentration range indicated above. The results for calibration data are shown in Table 2.

Table 2: Linearity for emtricitabine and tenofovir disoproxil by present method

Emtricitabine		**Tenofovir disoproxil**	
Concentration (µg/ml)	**Peak area**	**Concentration (µg/ml)**	**Peak area**
100	3239167	150	3020788
150	4848789	225	4536348
200	6463443	300	6052003
250	8075765	375	7564988
300	9693348	450	9079296
Regression equation:		**Regression equation:**	
y = 32271 *x* + 9967		y = 20180 *x* - 2706	
R^2 = 0.9999		R^2 = 0.9999	

y = peak area, x = concentration of drug in µg/ml, R^2 = Regression coefficient

Limit of detection (LOD), limit of quantification (LOQ):

The Limit of quantification and detection determines the sensitivity of the method. The LOD and LOQ were calculated using the following formulas (a) and (b).

(a) LOQ = 10 sd / S

(b) LOD = 3.3 sd / S

Where

sd = standard deviation of response

S = slope of the calibration curve.

The LOD of emtricitabine and tenofovir disoproxil was found to be 0.566 µg/ml and 0.879 µg/ml and the LOQ of emtricitabine and tenofovir disoproxil was 1.885 µg/ml and 2.932 µg/ml, respectively. The chromatograms of emtricitabine and tenofovir disoproxil at LOD and LOQ levels are shown in Figure 8. The results indicate that the developed method possess adequate sensitivity for the simultaneous determination of emtricitabine and tenofovir disoproxil.

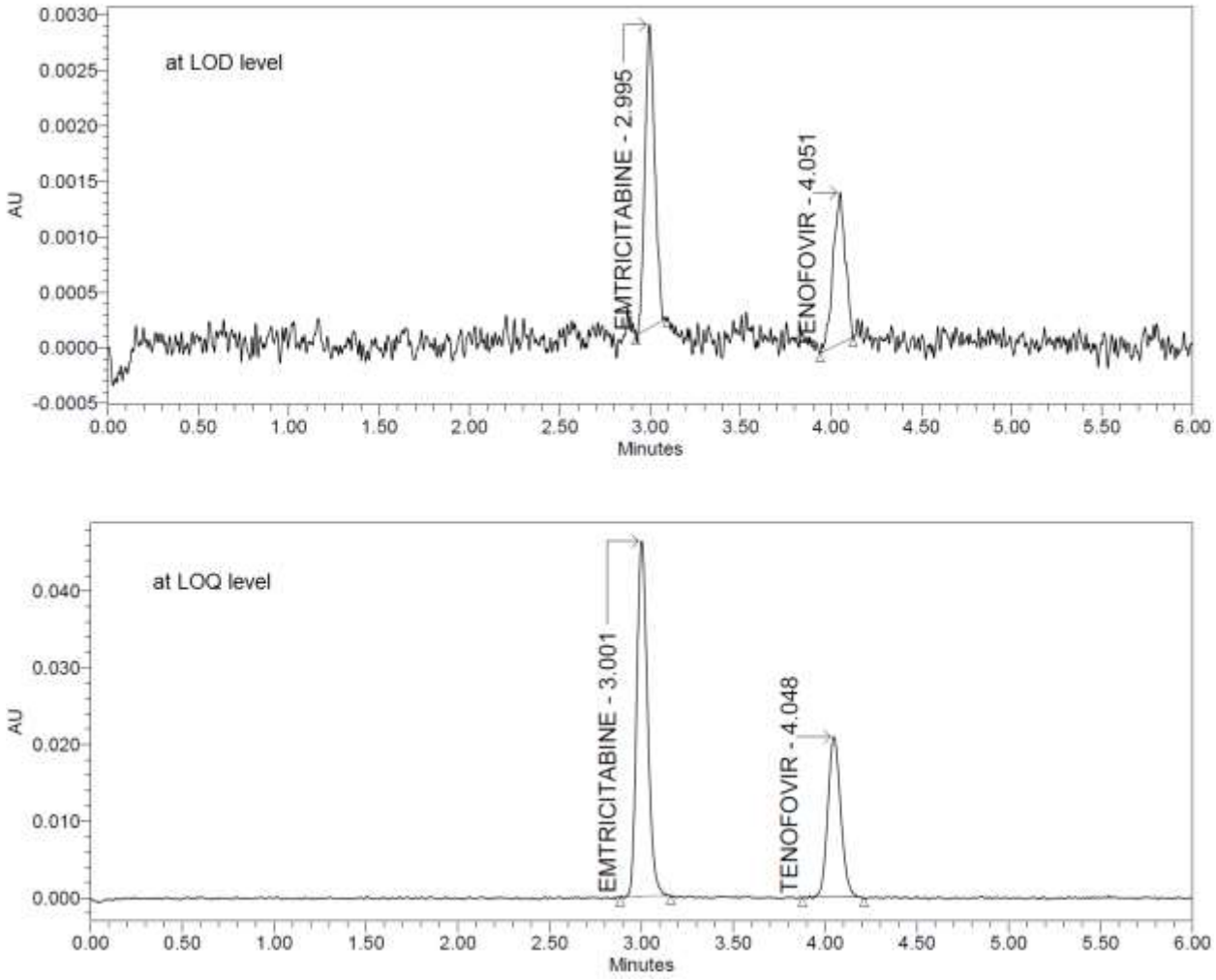

Figure 8: Chromatograms of emtricitabine and tenofovir disoproxil at LOD and LOQ levels

Precision:

The precision of the proposed method was established by analyzing five standard solutions (emtricitabine-200 µg/ml; tenofovir disoproxil-300 µg/ml). The peak area of emtricitabine and tenofovir disoproxil and their percentage relative standard deviation were calculated. The results of are presented in Table 3. The results were within the acceptable limit and indicated that the method is precise.

Table 3: Precision of the method

S.No	Emtricitabine			Tenofovir disoproxil		
	Concentration (µg/ml)	Peak Area	%RSD	Concentration (µg/ml)	Peak Area	%RSD
1	200	6464178		300	6056286	
2	200	6460798		300	6050608	
3	200	6460733	0.175	300	6055169	0.043
4	200	6435886		300	6057505	
5	200	6462315		300	6056448	
	200	6460808		300	6055628	

Accuracy:

Recovery experiments were performed to determine the accuracy of the method. The accuracy of the proposed method was established by preparing samples spiked with 50%, 100%, and 150% of the test concentration of emtricitabine and tenofovir disoproxil. Each concentration level was analyzed thrice. Mean percent recovery was calculated for each concentration.

Table 4: Accuracy of the method

Accuracy level	(µg/ml) added	(µg/ml) found	% Recovery	% Mean
		Emtricitabine		
50%	99.000	99.60	100.61	
	99.000	99.57	100.57	100.63
	99.000	99.70	100.71	
100%	198.000	199.06	100.54	
	198.000	198.96	100.49	100.52
	198.000	199.03	100.52	
150%	297.000	298.63	100.55	
	297.000	298.53	100.52	100.54
	297.000	298.66	100.56	
		Tenofovir disoproxil		
50%	148.500	148.84	100.23	
	148.500	149.01	100.34	100.34
	148.500	149.16	100.44	
100%	297.000	297.89	100.30	100.34
	297.000	298.23	100.41	

	297.000	297.96	100.32	
150%	445.500	446.79	100.29	
	445.500	446.89	100.31	100.29
	445.500	446.67	100.26	

Percent recovery was well within the acceptable limit. Results are presented in Table 4. From the data, added recoveries of standard drugs were found to be accurate.

Robustness:

The robustness test was carried out by making deliberate changes in optimized chromatographic conditions. Retention time, tailing factor, resolution and plate count were measured to demonstrate the robustness of the method. The results are shown in Table 5. In all the deliberate varied chromatographic conditions, the parameters like tailing factor, peak area and theoretical plates were within the acceptance limits, which show that the method is robust.

Table 5: Robustness of the method

Parameter varied	Retention time	Peak area	USP plate count	USP Tailing	USP resolution
Emtricitabine					
Flow rate – 1.1 ml/min	3.747	8439799	4323	1.84	-
Flow rate – 1.3 ml/min	2.463	5839251	3692	1.22	-
Column temperature - 24 ^{o}C	3.687	8829885	3633	1.36	-
Column temperature - 26 ^{o}C	2.457	5922671	4606	1.70	-
Tenofovir disoproxil					
Flow rate – 1.1 ml/min	4.921	10357991	14138	1.80	5.86
Flow rate – 1.3 ml/min	3.042	6397673	6990	1.25	3.50
Column temperature - 24 ^{o}C	4.531	9862925	7782	1.43	3.55
Column temperature - 26 ^{o}C	3.026	6486799	8658	1.77	4.00

Comparison with the reported methods:

Simultaneous determination of tenofovir disoproxil and emtricitabine by spectrophotometry[9-12], HPLC[13-19], HPTLC[20,21] and LC-MS[22-24] are found in the literature. The spectrophotometric methods[9-12], are less selective as it involves measurement in UV region where most of the tablet excipients shows absorbance. The reported HPTLC[20, 21] and LC-MS[22-24] methods are often time consuming, require expensive/sophisticated instrumentation, cumbersome procedure and required expertise operational personnel. The LC-MS[22-24] methods are not applied to combined tablet dosage forms. HPLC, because of its simplicity, sensitivity, selectivity, fair accuracy, precision and easy access in most quality control laboratories, is widely used for the analysis of therapeutic compounds used as medications. The details of reported and proposed HPLC methods are summarized in Table 6.

The decreased total run time (6 min) makes the proposed method more rapid than the reported HPLC methods[14,15,17-19]. The decreased runtime also decreases the utilization of solvents and increases the throughput of the proposed method. The proposed method has wide range of linearity than the methods of Sharma & Gupta[13], Lavanya et al.[15], Viswanath et al.[16] and Karunakaran et al.[18]. From the values of LOD and LOQ, it was observed that the proposed method is sensitive than the methods reported by Reddy et al.[14] Viswanath et al.[16], Gandhi et al. [17] and Karunakaran et al.[18]. The percent relative standard deviation and percent recovery values indicated the good precision and accuracy of the proposed method, respectively over all the reported HPLC methods[13-19]. The volume of sample (10 µl) used in the proposed method for single analysis is less than all the reported HPLC methods[13-19]. Unlike the methods of Sharma & Gupta[13] and Karunakaran et al.[18], the proposed method does not utilize triple solvent system as mobile phase. The use of triple solvent system as mobile phase increases the utilization of solvents and cost of single analysis. Most of the reported HPLC methods are not fully validated. The system suitability[14,15] and robustness[14,15,16,18] tests are not reported in some of the reported methods. Though the HPLC method reported by Rezk et al.[19] is sensitive with broad range of linearity, it suffers from drawbacks like long run time (18 min) and gradient mode of elution. These two factors increase the solvent utilization, time of analysis and cost per single analysis. Furthermore Rezk et al.[19] method is applied only to human plasma samples.

Table 6: Performance of the proposed and reported HPLC methods for the simultaneous estimation of emtricitabine and tenofovir disoproxil

Drug	Run Time (min)	Detection wavelength (nm)	Flow rate (ml/min)	Linearity (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	RSD (%)	Recovery (%)	Reference
TDF	4	260	1.5	5-50	0.039	0.117	0.124-0.216	100.02-100.08	Sharma & Gupta [13]
EMT				5-50	0.015	0.045	0.116-0.451	100.06-100.11	
TDF	10	260	1	60-360	4.6	13.65	0.226-0.374	99.54-100.43	Reddy et al., [14]
EMT				40-240	1.54	4.54	0.226-0.375	99.12-100.33	
TDF	8	NR	1	8-50	0.00851	0.0315	0.1	99.00-99.83	Lavanya et al., [15]
EMT				8-50	0.00752	0.00218	0.26	99.50-100.83	
TDF	6	270	1	3-15	0.191	0.581	0.59	99.60-100.40	Viswanath et al., [16]
EMT				2-10	0.639	1.907	0.96	99.40-101.7	
TDF	8	260	1	60-360	4.5924	13.931	0.374-0.377	99.73-99.81	Gandhi et al., [17]
EMT				40-240	1.5456	4.6839	0.227-0.251	99.74-99.84	
TDF	8	258	0.6	1-6	1.427	4.3243	0.288-0.629	100.77-101.59	Karunakaran et al., [18]
EMT				2-12	0.03003	0.091015	0.675-0.977	100.51-100.82	
TDF	18	259	NA	10- 10000*	NA	NA	3.7 to 5.2	97-103	Rezk et al.,[19]
EMT		280		10- 10000*	NA	NA	1.7 to 3.7%	98-105	
TDF	6	260	1.2	150-450	0.879	2.932	0.043	100.29-100.34	Proposed method
EMT				100-300	0.566	1.885	0.175	100.52-100.63	

EMT-emtricitabine; TDF-tenofovir disoproxil; NA-not available; NR-not reported; *- ng/ml

CONCLUSION

The present HPLC method was developed and validated for the simultaneous quantification of emtricitabine and tenofovir disoproxil in bulk and tablet dosage form. The proposed HPLC method is rapid[14,15,17-19], sensitive[14,16-18], precise[13-19] and accurate[13-19] with broad range of linearity[13,15,16,18] than the reported HPLC methods (Table 6). The proposed HPLC method was validated as stated by the guidelines of International Conference on Harmonization. The results of the validation tests were found to be satisfactory. Hence, the proposed HPLC method can be applied successfully for routine quality control analysis of emtricitabine and tenofovir disoproxil simultaneously in bulk and pharmaceutical formulation (tablet dosage form).

ACKNOWLEDGEMENTS

Authors are very grateful to the Analytical chemistry division, Indian Institute of Chemical Technology, Hyderabad and Clearsynth Research Centre, Hyderabad for their kind cooperation and generous support in running this research work.

REFERENCES

1. Chapman T, McGavin J, Noble S. Tenofovir disoproxil fumarate. Drugs 2003; 63(15): 1597-1608.
2. Antoniou T, Park-Wyllie LY, Tseng AL. Tenofovir: a nucleotide analog for the management of human immunodeficiency virus infection. Pharmacotherapy 2003; 23(1): 29-43.
3. Thompson CA. : Prodrug of tenofovir diphosphate approved for combination HIV therapy. Am J Health Syst Pharm 2002; 59(1): 18.
4. Molina JM, Cox SL. Emtricitabine: A novel nucleoside reverse transcriptase inhibitor. Drugs Today (Barc) 2005; 41 (4): 241-252.
5. Miguel G, Brookie B. Efavirenz/emtricitabine/tenofovir disoproxil fumarate fixed-dose combination: first-line therapy for all?. Expert Opin Pharmacother 2007; 8 (3): 371-382.
6. Bang LM. Scott LJ. Emtricitabine: an antiretroviral agent for HIV infection. Drugs 2003; 63 (22): 2413-2426.
7. Perry CM. Emtricitabine/tenofovir disoproxil fumarate: in combination with a protease inhibitor in HIV-1 infection. Drugs 2009; 69(7): 843-857.
8. Gazzard BG. Use of tenofovir disoproxil fumarate and emtricitabine combination in HIV-infected patients. Expert Opin Pharmacother 2006; 7(6): 793-802.

9. Mohammad HA, Azza AG, Rasha AS, Heba KA. Simple spectrophotometric methods for determination of tenofovir fumarate and emtricitabine in bulk powder and in tablets. J Spectrosc 2013; 2013: Article ID 937409, 7 pages
10. Ingale KD, Barhate AL, Kale AN, Bobade CD, Choudhari VP, Kuchekar BS. Spectrophotometric estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form by simultaneous equation and absorbance ratio methods. J Pharma Res 2010; 9 (1): 11–13.
11. Ghorpade SA, Sali MS, Kategaonkar AH, Patel DM, Choudhari VP, Kuchekar BS. Simultaneous determination of emtricitabine and tenofovir by area under curve and dual wavelength spectrophotometric method. J Chilean Chem Soc 2010; 55 (1): 115–117.
12. Choudhari VP, Ingale S, Gite SR, Tajane DD, Modak VG, Ambekar A. Spectrophotometric simultaneous determination of tenofovir disoproxil fumarate and emtricitabine in combined tablet dosage form by ratio derivative, first order derivative and absorbance corrected methods and its application to dissolution study. Pharm Methods 2011; 1 (2): 47–52.
13. Sharma R, Gupta P. A validated RP-HPLC method for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in a tablet dosage form. *Eurasian* J Anal Chem 2009; 4 (3): 276–284.
14. Reddy YBR, Reddy SMI, Reddy CBS. Simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form by reverse phase high-performance liquid chromatography. SOJ Chromatograph Sci 2015; 1(1): 6.
15. Lavanya B, Hariprasad P, Venkatapraveen A, Lakshmi DP, Ramireddy M. Simultaneous estimation of emtricitabine and tenofovir disproxil fumerate by HPLC method. Der Pharmacia Lettre 2012; 4 (6): 1855-1862.
16. Viswanath V, Shanmugasundaram P, Ravichandiran V. RP-HPLC method for the simultaneous estimation of tenofovir disoproxil fumerate and emtricitabine in combined tablet dosage form. Int J PharmTech Res 2013; 5 (3): 1186-1195.
17. Gandhi BM, Rao AL, Rao JV. . Method development and validation by RP-HPLC for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate. World J Pharm Pharma Sci 2015; 4 (7): 859-873.

18. Karunakaran A, Kamarajan K, Thangarasu V. A validated RP-HPLC method for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in pure and in tablet dosage form. Der Pharmacia Sinica 2010; 1 (2): 52–60.
19. Rezk NL, Crutchley RD, Kashuba ADM. Simultaneous quantification of emtricitabine and tenofovir in human plasma using high-performance liquid chromatography after solid phase extraction. J Chromatogr B 2005; 822 (1-2): 201–208.
20. Joshi M, Nikalje AP, Shahed M, Dehghan M. HPTLC method for the simultaneous estimation of emtricitabine and tenofovir in tablet dosage form. Indian J Pharma Sci 2009; 71 (1): 95–97.
21. Charushila HB, Shivanand NH. Development of validated stability-indicating simultaneous estimation of tenofovir disoproxil fumarate and emtricitabine in tablets by HPTLC. J Pharma Res 2013; 7 (2): 157–161.
22. Gomes NA, Vaidya VV, Pudage A, Joshi SS, Parekh SA. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study. J Pharma Biomed Anal 2008; 48 (3): 918–926.
23. Delahunty T, Bushman L, Robbins B, Fletcher CV. The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. J Chromatogr B 2009; 877 (20-21): 1907–1914.
24. D'Avolio A, Mauro S, Marco S, Lorena B, de Requena DG, Stefano B, Giovanni DP. A new assay based on solid-phase extraction procedure with LC-MS to measure plasmatic concentrations of tenofovir and emtricitabine in HIV infected patients. J *Chromatogr* Sci 2008; 46 (6): 524–528
25. International Conference on Harmonization, Validation of Analytical Procedure, Text and Methodology Q2 (R1), IFMA, Geneva, Switzerland, 2005.