Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (10):85-91
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

High performance liquid chromatography with PDA detector for combined determination of emtricitabine, tenofovir and efavirenz

Som Shankar Dubey* **and Mahesh Duggirala**

Department of Chemistry, Gitam Institute of Technology, Gitam University, Visakapatnam

---

ABSTRACT

A simple, rapid, precise and accurate reverse-phase HPLC method was developed and validated for the simultaneous determination of emtricitabine, tenofovir and efavirenz in commercial tablets. The method has shown adequate separation for emtricitabine, tenofovir and efavirenz. Separation was achieved on Inertsil C18 (250 mm × 4.6mm; 5 µm) column using isocratic method with 0.1%OPA: Methanol (55:45) system at room temperature and the detection was carried out at 260 nm using photodiode array (PDA) detector. The linearity of the proposed method was investigated in the range of 20-60µg/ml (r^2=0.9999), 30-90µg/ml (r^2=0.9999), 60-180µg/ml (r^2=0.9988) for emtricitabine, tenofovir and efavirenz respectively. The limit of detection (LOD) was 0.066, 0.1540 and 0.353 for emtricitabine, tenofovir and efavirenz respectively. The limit of quantification (LOQ) was 0.290, 0.0773 and 1.176 for emtricitabine, tenofovir and efavirenz respectively. The relative standard deviation (RSD) of six replicates is less than 2%. This HPLC method is applied successfully to the simultaneous quantitative analysis of emtricitabine, tenofovir and efavirenz in commercial tablets.

Key words: Emtricitabine, Tenofovir, Efavirenz, HPLC, Simultaneous Determination.

INTRODUCTION

Emtricitabine [1,2], 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (Figure 1), is a nucleoside reverse transcriptase inhibitor belonging to the class of compounds known as 3'-thia pyrimidine nucleosides.. 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.

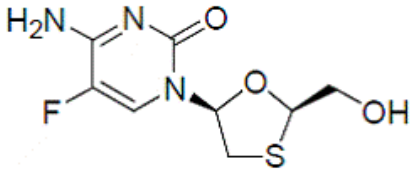

Figure 1: Structure of emtricitabine

Tenofovir [3,4] chemically known as ({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid (Figure 2). Tenofovir belongs to the class of compounds known as 6-aminopurines. Tenofovir inhibits the activity of

HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Specifically, the drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain.

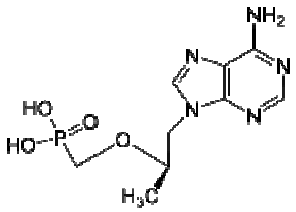

Figure 2: Structure of tenofovir

Efavirenz [5,6] is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus (HIV) type 1. Efavirenz belongs to the class of compounds known as benzoxazines.Chemically, it is described as (4S)-6-chloro-4-(2-cyclopropyl ethynyl)-4-(trifluoromethyl)-2, 4-dihydro-1H-3, 1-benzoxazin-2-one (Figure 3). Efavirenz is also used in combination with other antiretroviral agents as part of an expanded postexposure prophylaxis regimen to prevent HIV transmission for those exposed to materials associated with a high risk for HIV transmission.

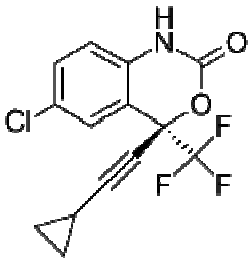

Figure 3: Structure of efavirenz

The literature reports, many methods for simultaneous quantitative determination of Emtricitabine, Tenofovir and Efavirenz in bulk, tablet dosage form, capsule dosage form and human plasma. These methods include simultaneous estimation of Emtricitabine, Tenofovir and Efavirenz by UV spectrophotometry [7, 8, 9] HPTLC [10] and HPLC [11-15] method.

The aim of the present investigation is to develop and validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of Emtricitabine, Tenofovir and Efavirenz in bulk and in its combined pharmaceutical formulation.

MATERIALS AND METHODS

Apparatus

A Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software is used in the present investigation.

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 is 0.1%OPA and methanol in the ratio of 55:45 *v/v*. The mobile phase is filtered before using, through millipore membrane filter and degassed for 15 min by sonication.

Chromatographic conditions

Inertsil C18 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of the Emtricitabine, Tenofovir and Efavirenz. The column temperature was maintained at 30 ± 1°C. The separation

was carried out under isocratic elution. The flow rate was maintained 1.0 ml/min. The injection volume was 10µl. The selected drugs were analyzed using a PDA detector covering the range of 200–400 nm.

Standard solutions

The standard stock solution was prepared by dissolving 200 mg of emtricitabine, 300 mg of tenofovir and 600 mg of efavirenz in 100 ml mobile phase. Working standard solutions equivalent to 20-60 µg/ml emtricitabine, 30-90 µg/ml tenofovir and 60-180 µg/ml efavirenz was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

Sample Solution

Ten tablets were weighed and crushed to a fine powder. The powder equivalent to dissolving 200 mg of emtricitabine, 300 mg of tenofovir and 600 mg of efavirenz was taken in a 100 ml volumetric flask containing 20 ml of mobile phase, sonicate for 20 min and made up to mark with the same solvent. The resultant mixture was filtered through 0.45 µm filter paper. The resultant solution was diluted appropriately with the mobile phase to get a final concentration of 200 µg/ml emtricitabine, 300 µg/ml tenofovir and 600µg/ml efavirenz.

RESULTS AND DISCUSSION

HPLC parameters optimization

Chromatographic parameters such as mobile phase composition, wavelength of detection, column and column temperature were optimized to achieve better efficiency of the chromatographic system. Two HPLC analytical columns, Zorbax C18 (150 mm x 4.6 mm x 5 µm) and Inertsil C18 (250 mm x 4.6 mm x 5 µm) were tested during method development. The system suitability parameters like tailing factor, resolution, and plate count were taken into consideration. Based on the above said parameters Inertsil C18 (250 mm x 4.6 mm x 5 µm) column was finalized for simultaneous analysis of emtricitabine, tenofovir and efavirenz. Different composition of mobile phases containing a mixture (*v/v*) of 0.1M CH_3COONH_4, methanol and 0.1%OPA were assessed in order to get apt composition of mobile phase. Finally the mixture of 0.1% OPA and methanol in the ratio of 55:45(*v/v*) was selected as optimal as it produced well defined and well resolved peaks of emtricitabine, tenofovir and efavirenz at a flow rate of 1.0 ml/min and with column temperature of 30^0C. For the detection and quantification of emtricitabine, tenofovir and efavirenz, 260 nm was selected as the optimum wavelength. The retention time for emtricitabine, tenofovir and efavirenz was found to be 2.993, 7.129 min and 9.096min, respectively. A typical chromatogram is given in Figure 4.

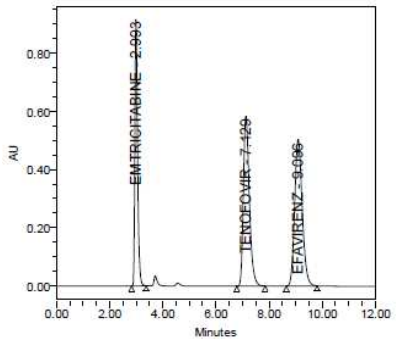

Figure 4: Chromatogram of emtricitabine, tenofovir and efavirenz

Method validation

The optimized RP-HPLC method for simultaneous assay of emtricitabine, tenofovir and efavirenz was validated according to ICH guidelines [16, 17] with respect to system suitability, linearity, sensitivity, accuracy, precision and robustness.

System suitability

Prior to analysis, the chromatographic system must satisfy system suitability test requirements. System suitability test was assessed from five replicate injections of the standard solution containing 200, 300 and 600 µg/ml emtricitabine, tenofovir and efavirenz, respectively. All the three peaks were well resolved and the precision of injections for all the peaks were acceptable. The percent relative standard deviation of the emtricitabine, tenofovir

and efavirenz peaks area responses were determined to be less than 1. The USP tailing factor and USP plate count were also calculated. The results of system suitability in comparison with the required limits are shown in Table 1 and are found to be within the accepted limits.

Table 1: System suitability of the method

Parameters	Results			Recommended limits
	Emtricitabine	Tenofovir	Efavirenz	
Retention time	2.993	7.129	9.096	-
Peak area	6516518 (%RSD – 0.5)	9209798 (%RSD – 0.6)	10064097 (%RSD – 0.6)	RSD ≤1
USP resolution	-	13.67	4.14	> 1.5
USP plate count	4290	4894	4896	> 2000
USP tailing factor	1.37	1.35	1.17	≤ 2

Linearity and range

The linearity of the HPLC method was determined, for the simultaneous assay of emtricitabine, tenofovir and efavirenz, by analyzing five different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the three drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 200 µg/ml emtricitabine, 300 µg/ml tenofovir and 600µg/ml efavirenz. The linearity parameters such as regression equations and regression coefficients are given in Figures 5,6 and 7. The results show a good correlation between the peak areas of the three drugs and their corresponding concentrations.

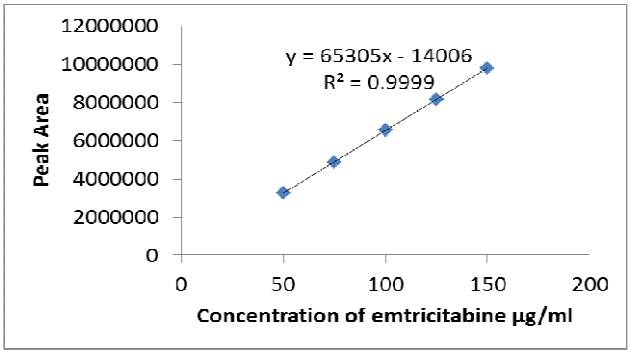

Figure 5: Linearity curve of emtricitabine

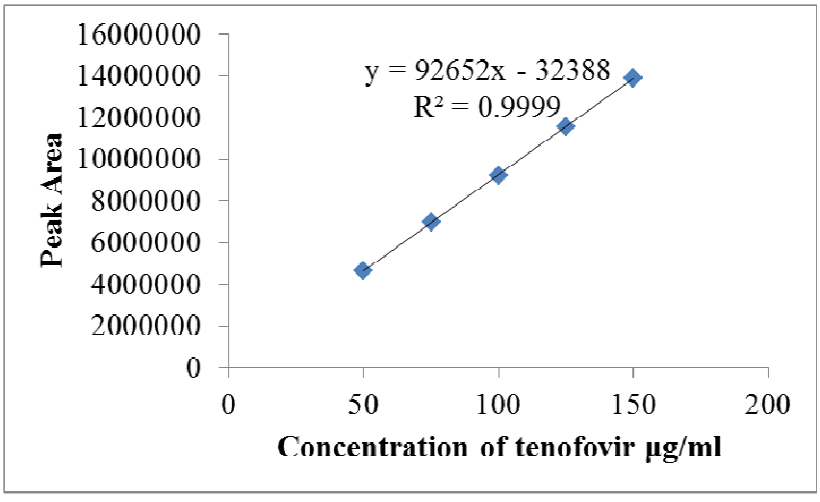

Figure 6: Linearity curve of tenofovir

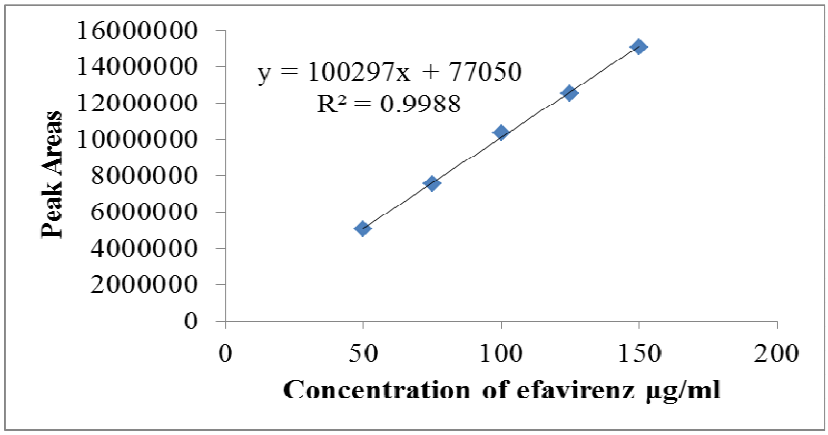

Figure 7: Linearity curve of efavirenz

Sensitivity

The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ). LOD was found to be 0.066 µg/ml, 0.1540 µg/ml and 0.353 µg/ml for emtricitabine, tenofovir and efavirenz, respectively (signal to noise ratio of 3:1). LOQ was found to be 0.220 µg/ml, 0.5133 µg/ml and 1.176 µg/ml for emtricitabine, tenofovir and efavirenz, respectively (signal to noise ratio of 10:1). The low values of LOD and LOQ demonstrate sufficient sensitivity of the HPLC method.

Precision

Precision was determined by injecting six standard solutions of emtricitabine, tenofovir and efavirenz with 200,300 and 600 µg/ml concentrations, respectively. The peak areas were determined. Relative standard deviation of emtricitabine, tenofovir and efavirenz peaks was then calculated to represent precision. The results are summarized in Table 2. The low % RSD values indicated that the method is precise.

Table 2: Precision of the method

Emtricitabine (200 µg/ml)		Tenofovir (300 µg/ml)		Efavirenz (600 µg/ml)	
Peak area	**%RSD**	**Peak area**	**%RSD**	**Peak area**	**%RSD**
6512630	0.05	9204211	0.03	10047271	0.28
6517531		9208534		10078508	
6515340		9205694		10047477	
6519176		9201320		10061658	
6511380		9206424		10005694	
6513552		9207430		10011359	

Accuracy

Accuracy of the method was evaluated by recovery studies at three concentration (50%, 100%, and 150%) levels by standard addition method. The mean percentage recoveries obtained were in the range of 99-100% for all the three drugs (Table 3). The good % recovery values showed the method to be highly accurate.

Table 3: Accuracy of the method

Drug	Spiked Level	µg/ml added	µg/ml found	% Recovery	% Mean
Emtricitabine	50%	19.800	19.82	100	100
	50%	19.800	19.82	100	
	50%	19.800	19.79	100	
	100%	39.600	39.63	100	100
	100%	39.600	39.63	100	
	100%	39.600	39.66	100	
	150%	59.400	59.47	100	100
	150%	59.400	59.44	100	
	150%	59.400	59.47	100	
Tenofovir	50%	30.000	29.88	100	100
	50%	30.000	29.89	100	
	50%	30.000	29.92	100	
	100%	60.000	59.79	100	100
	100%	60.000	59.78	100	
	100%	60.000	59.79	100	
	150%	90.000	89.93	100	100
	150%	90.000	90.15	100	
	150%	90.000	89.67	100	
Efavirenz	50%	59.400	59.45	100	100
	50%	59.400	59.50	100	
	50%	59.400	59.55	100	
	100%	118.800	118.93	100	100
	100%	118.800	119.26	100	
	100%	118.800	118.45	100	
	150%	178.200	178.41	100	100
	150%	178.200	178.35	100	
	150%	178.200	178.36	100	

Robustness

In order to show the robustness of the method, system suitability parameters were evaluated by slightly varying flow rate and column temperature. The parameters used to define robustness were retention time, USP tailing factor and USP plate count. The results showed (Table 4) that slight variations in method parameters had a negligible effect on the analysis.

Table 4: Robustness of the method

Drug	Parameter	Retention time	Peak area	USP Plate Count	USP Tailing
Emtricitabine	Flow 1	3.678	8154266	4585	1.42
	Flow 2	2.537	5602490	3917	1.44
	Temperature 1	3.671	8062743	4445	1.43
	Temperature 2	2.530	5514877	3879	1.40
Tenofovir	Flow 1	8.846	11425345	5482	1.38
	Flow 2	6.190	7898337	4584	1.38
	Temperature 1	8.857	11350965	5586	1.35
	Temperature 2	6.176	7795871	4521	1.37
Efavirenz	Flow 1	11.250	12522932	5370	1.16
	Flow 2	7.909	8701290	4197	1.21
	Temperature 1	11.256	12429156	5670	1.17
	Temperature 2	7.896	8555025	4371	1.20

CONCLUSION

A RP-HPLC method has been reported for simultaneous estimation emtricitabine, tenofovir and efavirenz. The proposed method gives good resolution of the above said drugs. The validation of developed method was done as per ICH guidelines and proved that method to be simple, sensitive, precise, accurate and robust. The validated method was successfully applied to the determination of commercially available pharmaceutical dosage form. Hence, the method can be used for the routine quality control analysis of pharmaceutical dosage forms containing emtricitabine, tenofovir and efavirenz.

Acknowledgement
The authors thank Department of Chemistry, Gitam Institute of Technology, Gitam University, Visakhapatnam for providing necessary facilities.

REFERENCES

[1] Lim SG, Ng TM, Kung N .F. *Arch. Intern. Med.* **2006**, 166, 49–56.
[2] Liu KZ, Hou W, Zumbika E, Ni Q. *J Zhejiang Univ Sci B* .**2005**, 6, 1182–7.
[3] Deeks SG, Barditch-Crovo P, Lietman PS. *Antimicrob. Agents Chemother.* **1998**,42,2380–4
[4] Delahunty T, Bushman L, Robbins B, Fletcher CV. *J. Chrom. B.***2009**, 877, 1907–1914.
[5] Ford N, Mofenson L, Kranzer, K, Medu L, Frigati L, Mills E. J, Calmy A. *AIDS* **2010**,24, 1461–1470.
[6] Gatch MB, Kozlenkov A, Huang RQ, Yang W, Nguyen JD, González-Maeso J, Rice KC, France CP, Dillon GH, Forster MJ, Schetz JA. *Neuropsychopharmacology* **2013**,38, 2373–84
[7] Murugan S, Pranabesh Sikdar, Subhashis Debnath, Niranjan Babu M. *International Journal of Pharmaceutical Research & Analysis*, **2013**, 3, 29-32.
[8] S. Venkatesan and N. Kannappan. *International Scholarly Research Notices*, **2014**, pages 8.
[9] Anindita Behera1, Aurobinda Parida, Amit Kumar Meher, Dannana Gowri Sankar, Swapan Kumar Moitra, Sudam Chandra Si. *Int.J. PharmTech Res*.**2011**, 3, pp 1874-1882.
[10] HPTLC Maithilee Joshi, AP Nikalje, M Shahed, M Dehghan. *Indian Journal of Pharmaceutical Sciences*, **2009**, 71, pp 95-97.
[11] HPLC C. Palavan, LA Ramaprasad, P Srinivasu, JVLN Seshagiri Rao. *Am. J. PharmTech Res.* **2013**, 3,548-554.
[12] N Appala Raju and Shabana Begum. *J. Pharm. and Tech*. **2008**, 1, pp 522-525.
[13] Prashant S. Devrukhakar,Roshan Borkar,Nalini Shastri,and K. V. Surendranath. *Chromatography*,**2013**,pages 8
[14] Rajesh Sharma1 and Pooja Gupta**.** *Eurasian J. Anal. Chem.***2009**, 4, 276-284.
[15] Arun Ramaswamy, Anton Smith Arul Gnana Dhas. *Arabian Journal of Chemistry*, **2014**, 1-7.
[16] International Conference on Harmonization, ICH Guidelines, Validation of Analytical Procedures Technical Requirements for Registration of Pharmaceuticals for Human Use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November **2005**.
[17] International Conference on Harmonization, Validation of Analytical Procedure, Text and Methododlogy Q2 (R1), IFMA, Geneva, Switzerland, **2005**.