Oriental Journal of Chemistry Vol. **24**(2), 645-650 (2008)

Simultaneous estimation of tenofovir disoproxil, emtricitabine and efavirenz in tablet dosage form by RP-HPLC

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(Received: May 07, 2008; Accepted: July 10, 2008)

ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method in isocratic mode has been developed for the estimation of Tenofovir disoproxil, Emtricitabine and Efavirenz in tablet dosage form. A Hypersil BDS C18, 250x4.6 mm, 5 µm partical size, with mobile phase consisting of acetonitrile and 0.03 M KH_2PO_4 water (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 60:40 v/v was used. The flow rate was 0.8 ml/min and the effluents were monitored at 260 nm. The retention times were 3.105min for Emtricitabine, 3.860 for Tenofovir disoproxil and 10.549 min for Efavirenz. The detector response was linear for Tenofovir disoproxil, Emtricitabine and Efavirenz are in the range of 6-72 mcg/ml, 4-48 mcg/ml and 12-144 mcg/ml respectively. The respective linear regression equation being Y=47439x-9882.3343 for Tenofovir disoproxil, Y=35167.413x+22780.1317 for Emtricitabine and Y=19958.961x+23536.8626 for Efavirenz. The limit of detection (LOD) for Tenofovir disoproxil, Emtricitabine and Efavirenz were found to be 0.03µg/ml, 0.04µg/ml and 0.12µg/ml respectively. The limit of quantification (LOQ) for Tenofovir disoproxil, Emtricitabine and Efavirenz were found to be 0.09µg/ml, 0.12µg/ml and 0.36µg/ml respectively. The percentage assay of Tenofovir disoproxil, Emtricitabine and Efavirenz was 98.78%, 98/57% and 98.28% respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Tenofovir disoproxil, Emtricitabine and Efavirenz in bulk drug and in its pharmaceutical dosage form.

Key words: Tenofovir disoproxil, Emtricitabine and Efavirenz, RP-HPLC, Estimation, and Tablets.

INTRODUCTION

Tenofovir disoproxil, Emtricitabine and Efavirenz [1,2,3.] are a novel formulation combining fixed doses of the nucleoside reverse transcriptase inhibitors emtricitabine (200mg) and tenofovir disoproxil fumarate (300mg) with the non-nucleoside reverse transcriptase inhibitor efavirenz (600mg) represents the first once-daily, one-tablet antiretroviral regimen.[10] Tenofovir disoproxil fumerate is chemically know as 9-[(R)-2-[[bis[[isopropoxycarbonyl]oxy]methoxy] phosphonyl] methoxy]popyl]adenine fumarate. Emtricitabine is chemically 5-fluoro-1-(2R, 5S)-[2-hydroxymethyl)-1,3-oxathiolan-5-ylcytosine[5]. Efavirenz[4] is (4S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)2-H-3,1-benzoxazin2-one. Emtricitabine[4] is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one. Literature survey reveals few Chromatographic

methods[11-13] for the determination of Tenofovir disoproxil, Emtricitabine and Efavirenz, in biological fluids along with other antiretroviral dugs. So far, only one HPLC procedure has been reported in gradient mode for the estimation of Tenofovir disoproxil, Emtricitabine and Efavirenz from pharmaceutical dosage form[14]. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Tenofovir disoproxil, Emtricitabine and Efavirenz in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method in isocratic mode for the estimation of Tenofovir disoproxil, Emtricitabine and Efavirenz in bulk drug samples and in pharmaceutical dosage form.

EXPERIMENTAL

Material and Methods

Tenofovir disoproxil, Emtricitabine and Efavirenz were obtained as a gift samples from Aurobindo Pharma Ltd, Hyderabad. Potassium dihydrogen orthophosphate was of analytical grade, and supplied by M/s S.D.Fine Chem Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available Tenofovir disoproxil, Emtricitabine and Efavirenz tablets (Vireday, Cipla) were procured from local market.

Instrument

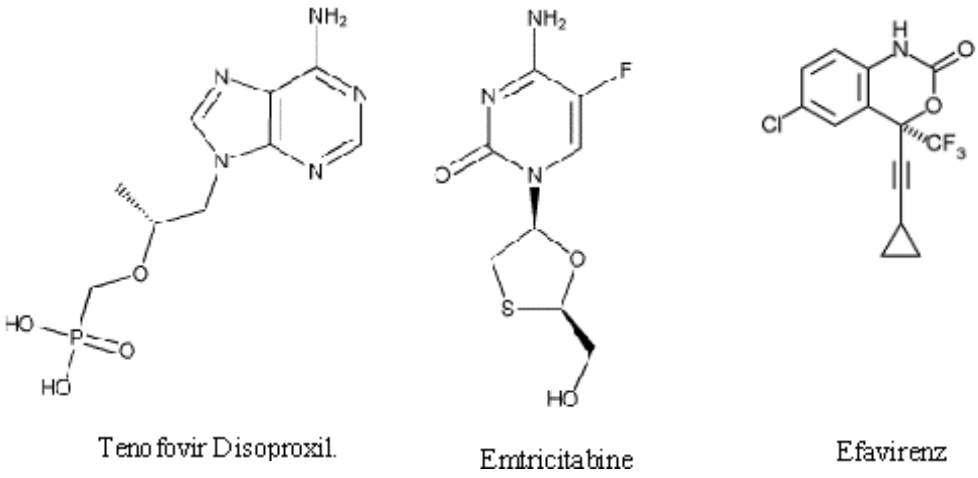
Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µl, and 2693 pump. A RP C-18 Hypersil BDS column (250x4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions

The contents of the mobile phase were acetonitrile and 0.03M KH_2PO_4 in water (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 60:40 v/v. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 ml/min. The run time was set at 15.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 260 nm.

Preparation of Standard Stock solution

A standard stock solution of the drug was prepared by dissolving 30 mg of Tenofovir disoproxil, 20 mg of Emtricitabine and 60 mg of Efavirenz in 100 ml volumetric flask containing 30 ml of diluent (60:40 acetonitrile: water), sonicated for about 15



Structures of Tenofovir disoproxil, Emtricitabine and Efavirenz

min and then made up to 100 ml with diluent to get the primary standard stock solution containing 300 mcg/ml of Tenofovir Disoproxil, 200 mcg/ml of Emtricitabine and 600 mcg/ml of Efavirenz.

Working standard solution

10 ml of the above stock solution was taken in 50 ml volumetric flask and thereafter made up to 50 ml with diluent to get the working standard solution containing 60 mcg/ml of Tenofovir Disoproxil, 40 mcg/ml of Emtricitabine and 120 mcg/ml of Efavirenz.

Preparation of sample solution

Twenty tablets (Vireday, Cipla) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 30 mg of the Tenofovir disoproxil, 20 mg of Emtricitabine and 60 mg of Efavirenz active ingredients, was mixed with 50 ml of diluent(60:40 acetonitrile: water) in 100 ml

Table 1: Linear regression data for calibration curves

Parameter	Tenofovir disoproxil	Emtricitbine	Efavirenz
Conc.range (µg/ml)	6-72	4-48	12-144
Slope (m)	47439.005	35167.413	19958.961
Intercept (b)	-9882.3343	22780.1317	23536.8626
Correlation coeff.	1.0	0.999	1.0
% RSD	0.12	0.19	0.17
Standard error of estimate	9587.8629	19107.2859	9898.6037

Table 2: Results of hplc assay and recovery studies

Amount claim (mg/tablet)			Amount Obtained(mg)* by proposed method			** % Recovery by the Proposed method		
Emtricitabine	Tenofovir disoproxil	Efavirenz	Emtrici-tabine	Tenofovir disoproxil	Efavirenz	Emtric-itabine	Tenofovir disoproxil	Efavienz
200 mg	300 mg	600 mg	199.4	298.3	592.6	98.87	98.98	98.92
200 mg	300 mg	600 mg	199.8	298.4	594.3	98.28	98.78	98.79
200mg	300 mg	600 mg	199.4	298.9	598.4	98.57	98.88	98.29

*Average of three different concentration levels. ** After spiking the sample.

Table 3: Validation summary

Parameter	Tenofovir disoproxil	Emtricitbine	Efavirenz
System Suitability			
TheoreticalPlates(N)	7281.01	7594.57	14889.29
Tailing factor	1.51	1.37	1.09
Retention time(min)	3.865	3.107	11.85
Resolution	4.33	1.78	10.13
K'	0.00	0.423	3.714
LOD (µg/ml)	0.03	0.04	0.12
LOQ (µg/ml)	0.09	0.12	0.36

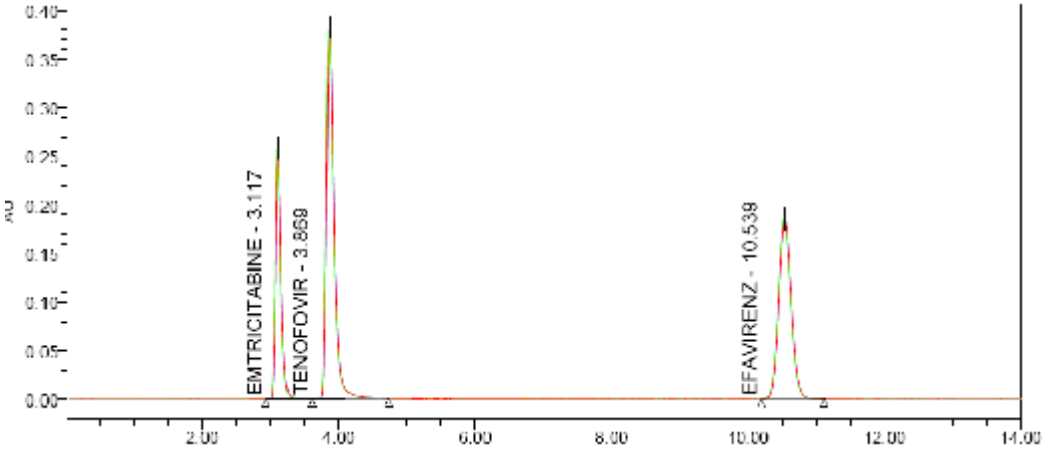


Fig 1: Typical chromatogram of tenofovir disoproxil, emtricitabine and efavirenz by RP-HPLC

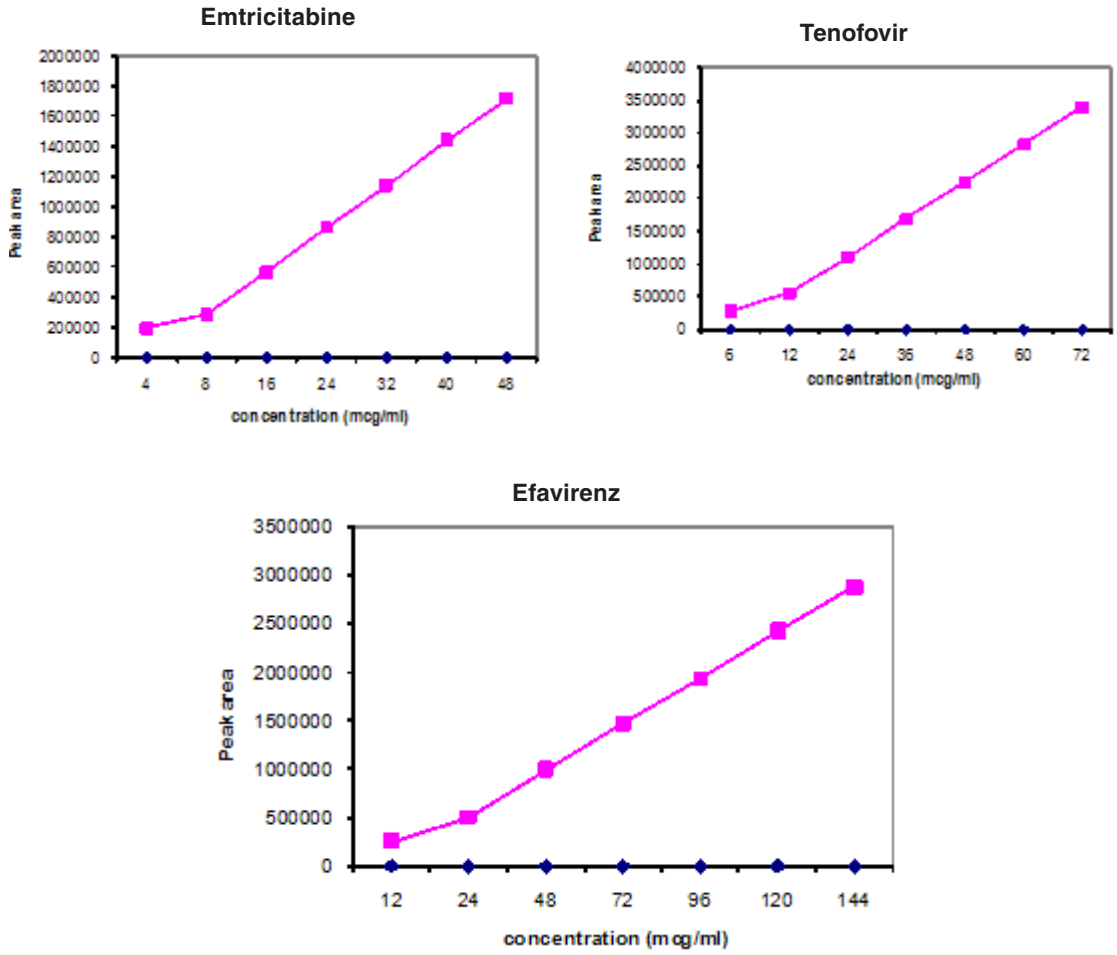


Fig. 2: Calibration curves of tenofovir disoproxil, emtricitabine and efavirenz by RP-HPLC

of volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drugs, and then filtered through a 0.45 µm membrane filter, followed by adding diluent to obtain a stock solution containing 300 mcg/ml of Tenofovir Disoproxil, 200 mcg/ml of Emtricitabine and 600 mcg/ml of Efavirenz. 10 ml of the above stock solution was taken in 50 ml volumetric flask and thereafter made up to 50 ml with diluent to get the working standard solution containing 60 mcg/ml of Tenofovir Disoproxil, 40 mcg/ml of Emtricitabine and 120 mcg/ml of Efavirenz.

Linearity

Aliquots of primary standard Tenofovir disoproxil, Emtricitabine and Efavirenz stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Tenofovir disoproxil, Emtricitabine and Efavirenz are in the range of 6-72 mcg/ml, 4-48 mcg/ml and 12-144 mcg/ml respectively. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 260 nm and a Calibration graph was obtained by plotting peak area versus concentration of Tenofovir disoproxil, Emtricitabine and Efavirenz (Fig 2). The plot of peak area of each sample against respective concentration of Tenofovir disoproxil, Emtricitabine and Efavirenz was found to be linear in the range of 6-72 mcg/ml, 4-48 mcg/ml and 12-144 mcg/ml respectively with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective linear regression equation being Y=47439x+9882.3343 for Tenofovir disoproxil, Y=35167.413x+22780.1317 Emtricitabine and Y=19958.961x+23536.8626 for Efavirenz. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

Assay

20 µl of sample solution was injected into the injector of liquid chromatograph. The retention times were 3.105min for Emtricitabine, 3.860 for Tenofovir disoproxil and 10.549 min for Efavirenz. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Recovery Studies

Accuracy was determined by recovery studies of Tenofovir disoproxil, Emtricitabine and Efavirenz, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of Tenofovir disoproxil, Emtricitabine and Efavirenz. Parameters that were studied to evaluate the suitability of the system are given in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) for Tenofovir disoproxil, Emtricitabine and Efavirenz were found to be 0.03µg/ml, 0.04µg/ml and 0.12µg/ml respectively. The limit of quantification (LOQ) for Tenofovir disoproxil, Emtricitabine and Efavirenz were found to be 0.09µg/ml, 0.12µg/ml and 0.36µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

From the typical chromatogram of Tenofovir disoproxil, Emtricitabine and Efavirenz as shown in fig 1, it was found that the retention times were 3.105min for Emtricitabine, 3.860 for Tenofovir disoproxil and 10.549 min for Efavirenz. A mixture of acetonitrile and 0.03 M KH_2PO_4 in water (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 60:40 v/v was found to be most suitable to obtain a peaks well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.9999) was observed between the concentration range of linear in the range of 6-72 mcg/ml, 4-48 mcg/ml and 12-144 mcg/ml for Tenofovir disoproxil, Emtricitabine and Efavirenz respectively. Low values of standard deviation are indicative of the high precision of the method. The assay of Tenofovir disoproxil, Emtricitabine and

Efavirenz tablets was found to be 98.78%, 98/57% and 98.28% respectively. Based on the recovery studies it was found that about 98.88% of Tenofovir disoproxil, 98.57% of Emtricitabine and 98.28% of Efavirenz was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of Tenofovir disoproxil, Emtricitabine and Efavirenz within a short analysis time.

ACKNOWLEDGEMENTS

The authors are grateful to M/s Hetero Drugs, Hyderabad for the supply of Tenofovir disoproxil, Emtricitabine and Efavirenz as a gift samples and to the Management, Sultan-Ul-Uloom college of Pharmacy, Hyderabad, for providing the necessary facilities to carry out the research work.

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