Research Article

ISSN: 2230-7346

Journal of Global Trends in Pharmaceutical Sciences
Vol.2, Issue 2, pp -177-186, April-June 2011

RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF EMITRACITABINE, TENOFOVIR AND EFAVIRENZ IN PHARMACEUTICAL DOSAGE FORM

V.P.V.S.Koteswara Rao[1], Kotta Kranthi Kumar*[2], VenkataRaveendranath.T[2,] LeelaMadhuri.P[2], K.Sasikanth[1,]

1. Nova College of Pharmacy, Vegavaram, Jangareddygudem, W.G.Dist-534447, A.P, India

2. Narasaraopeta Institute of Pharmaceutical Sciences, Kotappakonda Road NARASARAOPETA-522601, Guntur, Andhra Pradesh, India.

***Corresponding Author E-mail:** kranthikumarkotta@gmail.com

ABSTRACT

The main objective of the present work is to develop a new simple RP-HPLC method the for simultaneous estimation of Emtricitabine,Tenofovir and Efavirenz.Chromatography was carried on an column Xterra RP-18 using gradient composition of Ammonium acetate buffer as mobile phase A and Acetonitril as mobile phase B at a flow rate of 1.0 ml/min with detection at 260nm. The retention times of the Emtricitabine, Tenofovirdisoproxilfumerate and Efavirenz was about 4.61, 7.52, 9.10 min respectively. The linearity and range was found to be in the range of 50-150µg/ml for Emtricitabine, Tenofovir and Efavirenz. The correlation coefficient of Emtricitabine, Tenofovir and Efavirenz was found to be 0.99992, 0.99998 and 0.99999respectively, which indicates a perfect correlation. The developed method was validated for accuracy, precision, and system suitability. The percentage recovery of Emtricitabine, Tenofovir, and Efavirenz was found to be 99.77% and 100.06% respectively. The good percentage recovery of the sample clearly indicates the reproducibility and accuracy of the developed method. Similarly the % RSD value for precision was also found to be within the acceptable limit. The proposed method is simple, fast, sensitive, Linear, accurate, rugged and

precise and hence can be applied for routine quality control of Emtricitabine, Tenofovirdisoproxilfumerate, and Efavirenz in bulk and in tablet dosage form.

KEY WORDS: Emtricitabine, Tenofovir, and Efavirenz, Acetonitrile

INTRODUCTION:

Emtricitabine is chemically known as 4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2 one.

Tenofovirdisoproxilfumeratefumerate is chemically know as 9-[(R)-2-[[bis [[isopropoxycarbonyl] oxy] methoxy] phosphonyl] methoxy] popyl] adenine fumarate.

Efavirenz is chemically known as (4S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)2-H-3,1-benzoxazin 2-one.

Tenofovirdisoproxilfumerate, Emtricitabine, and Efavirenz a novel formulation combining fixed doses of the nucleoside reverse transcriptase inhibitors Emtricitabine (200mg) and Tenofovirdisoproxilfumerate fumarate (300mg) with the non-nucleoside reverse transcriptase inhibitor Efavirenz (600mg) represents the first once-daily, one-tablet antiretroviral regimen. It is official in Martindale-The Extra pharmacopoeia.

EXPERIMENTAL WORK:

Instrumentation used for the Study:

Alliance-Waters 2695 separation module with Waters 2996 photo diode array detector equipped with EMPOWER software, Single pan balance, Vacuum pump with filtration kit. Stationary phase Xterra RP-18(150 x 4.6 mm)5µ particle size, sonicator, pH meter.

Reagents and Chemicals used for the Study:

1. Ammonium acetate : AR grade
2. Acetonitril : HPLC grade
3. Water : Milli-Q grade
4. Methanol : HPLC grade,
5. Working reference : Tenofovir, Efavirenz and Emtricitabine, Tablet

Chromatographic conditions:

The following optimized parameters were used as a final method for the simultaneous estimation of Emtricitabine, Tenofovir, and Efavirenz

Buffer preparation: weigh accurately 9.2g of Ammonium Acetate and dissolve it in 1000ml of Milli-Q water. Adjust the pH to 4.6 with Glacial acetic acid, filter through 0.45µm nylon membrane filter and degas.

Mobile phase-A: Ammonium acetate buffer-pH 4.6

Mobile phase-B: Acetonitrile

Chromatographic conditions:

Flow rate	**:**	1.0 ml/min
Column	**:**	Xterra RP-18, 150 x 4.6 mm,5µ
Detector wave length	**:**	260nm
Column temperature	**:**	Ambient
Injection volume	**:**	10µl
Run time	**:**	15 mins

Gradient programme of mobile phases and Retention time (min) of drugs are given in table No: 1.The three peaks were well resolved with good peak shape and symmetry.Fig:1, 2&3.

Emtricitabine Standard stock Preparation:

Weigh and transfer accurately about 40.0 mg of Emtricitabine Working Standard into a 100 ml clean dry volumetric flask, add about 60 ml of methanol, sonicate for 5 minutes, and dilute to volume with methanol

Tenofovir Standard stock Preparation:

Weigh and transfer accurately about 60.0 mg of Tenofovirdisoproxilfumerate Working Standard into a 100 ml clean dry volumetric flask, add about 60 ml of methanol, sonicate for 5 minutes, and dilute to volume with methanol.

Efavirenz Standard stock Preparation:

Weigh and transfer accurately about 60.0 mg of Efavirenz Working Standard into a 100 ml clean dry volumetric flask, add about 60 ml of methanol, sonicate for 5 minutes, and dilute to volume with methanol.

Diluted Standard:

Pipette out 10 ml of the Efavirenz standard stock solution, 5ml of Emtricitabine Standard stock solution and 5ml Tenofovir Standard stock solution and dilute to 50 ml with diluent.

Sample preparation:

Weigh five tablets and transfer into a 500ml volumetric flask. Add about 100ml of buffer and shake the volumetric flask on a rotary shaker for 20min, add 300ml of methanol and sonicate for 20min with intermittent shaking and dilute to volume with methanol. From this pipette out 2ml of sample solution into a 100ml volumetric flask, make up the volume with diluent and filter the solution through 0.45µ nylon membrane filter to obtain clear solution.

Selection of wavelength for detection of components:

Solutions of Emtricitabine, Tenofovir and Efavirenz were scanned in the UV region and spectrum was recorded. The solvent used was Ammonium acetate buffer (pH 4.6), Methanol and Acetonitrile in the ratio of 50:50. It was seen that at 260nm these compounds have very good absorbance which can be used for the estimation of compounds by RP- HPLC.

Selection of chromatographic method:

Proper selection of the method depends on the nature of the sample (ionic or ionisable or neutral molecules), its molecular weight, pka value and stability. The drugs selected in the present study are polar and so reversed phase or ion exchange chromatography can be used. The reversed phase HPLC was selected for the initial separation because of its simplicity and suitability. From the literature survey and with the knowledge of properties of the selected drugs, Xterra RP-18 column was chosen as stationary phase and mobile phase with different compositions such as solvent and buffer was used. From all the data observed, obtained, available the initial separation conditions were set to work around.

Initial separation conditions:

The following chromatographic conditions were fixed initially to improve the separation.

Effect of pH of mobile phase:

Several trials were made using different Buffer solutions of different pH range. The best separation was achieved with Ammonium Acetate and pH to 4.6.

METHOD VALIDATION:

The developed method was validated for simultaneous assay determination of Emtricitabine, Tenofovir, andEfavirenz were using following parameters.

Linearity:

Linearity was demonstrated by analyzing six different concentrations of active Compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Emtricitabine, Tenofovir, and Efavirenz which were found to be linear

in the range of 50-150 µg/ml, 50-150 µg/ml and 50-150 µg/ml respectively. Coefficient of correlation was 0.999992, 0.9999 and 0.999998 in Table No: 2, Figure-4, 5 and 6.

Precision:

To demonstrate agreement among results, a series of measurements are done with Emtricitabine, Tenofovir andEfavirenz injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.79, 0.74 and 0.74 for Emtricitabine, Tenofovir and Efavirenz- Table 3.

Calculation: Calculate the amount of each drug by using the following formula

$$\text{Tenofovir (Mg/tablet)} = \frac{A_T}{A_{ST}} \times \frac{D_S}{D_T} \times \frac{P}{100}$$

$$\text{Efavirenz (Mg/tablet)} = \frac{A_{EF}}{A_{SEF}} \times \frac{D_S}{D_T} \times \frac{P}{100}$$

$$\text{Efavirenz (Mg/tablet)} = \frac{A_{EM}}{A_{SEM}} \times \frac{D_S}{D_T} \times \frac{P}{100}$$

Where,

A_T = Average area counts of injections for Tenofovir peak in the chromatogram of sample solution.

A_{ST} = Average area count of five replicate injections for Tenofovir peak in the chromatogram of standard solution.

A_{EF} = Average area counts of injections for Efavirenz peak in the chromatogram of sample solution.

A_{SEF} = Average area count of five replicate injections for Efavirenz peak in the chromatogram of standard solution.

A_{EM} = Average area counts of injections for Emtricitabine peak in the chromatogram of sample solution.

$\mathbf{A_{SEM}}$ = Average area count of five replicate injections for Emtricitabine . peak in the chromatogram of standard solution.

$\mathbf{D_S}$ = Dilution factor of standard solution (weight÷dilution).

$\mathbf{D_T}$ = Dilution factor of sample solution.

P = Percentage purity of working standard used.

$$\textbf{\% Labeled Amount} = \frac{\text{Content of each drug (mg/tablet)}}{\text{Label claim, in mg}} \times 100$$

RESULT AND DISCUSSION:

A simple reverse phase HPLC method was developed for the simultaneous determination of Emtricitabine, Tenofovir, and Efavirenz in pharmaceutical dosage form. An Xterra RP-18 (150 × 4.6 mm), 5µ column from Waters in gradient mode, with mobile phases pH 4.6 Ammonium Acetate buffer and acetonitrile was used. The flow rate was 1.0-ml/ min and effluent was monitored at 260 nm. The retention times were 4.61 min, 7.52 and 9.10 min for Emtricitabine, Tenofovir and Efavirenz respectively. The linearity and range was found to be in the range of 50-150µg/ml for Tenofovir, Emtricitabine and Efavirenz. The correlation coefficient of Emtricitabine, Tenofovir andEfavirenz was found to be 0.99992, 0.99998 and 0.99999 respectively, which indicates a perfect correlation. As per ICH guide lines the method was validated over the range of 10–1000 µg/mL for the three analytes, and is accurate accuracies of three concentrations ranged from 99.4 for Emtricitabine, 99.8% for Tenofovir and 100% for Efavirenz.

TABLES AND FIGURES:

Table No: 1- Gradient programme

Time(min)	Flow(ml/min)	%A	%B
0.00	1.0	100	0
4.00	1.0	70	30
6.00	1.0	20	80
10.00	1.0	50	50
11.00	1.0	100	0
15.00	1.0	100	0

Observation

S.NO	Name of the peak	Retention time(min)
1.	Emtricitabine	4.61
2.	Tenofovir	7.52
3.	Efavirenz	9.10

Table: 2-Validation and system suitability parameters

Parameters	Emtricitabine	Tenofovir	Efavirenz
Linearity range µg/ml	50-150	50-150	50-150
Correlation Coefficient (r2) S.D	0.9999	0.9996	0.999998
Retention time (min) ± S.D	4.61	7.52	9.10
Tailing factor	1.08	0.99	1.01
Theoretical Plate	12051	31182	24412
Limit of detection (µg/ml)	0.4	0.3	0.3
Limit of Quantification (µg/ml)	0.12	0.9	0.36
Precision (RSD %) intraday (n=6)	0.55	0.54	0.81

Table.3: Assay of Emtricitabine, Tenofovir and Efavirenz

DRUG	Method Precision		Assay Amount found
	Mean %	RSD (%)	
Tenofovir	99.26	0.79	99.8
Emtricitabine	99.38	0.74	99.4
Efavirenz	99.11	0.74	100.1

Fig: 1- Typical chromatogram of and Emtricitabine, Tenofovir and Efavirenz (blank)

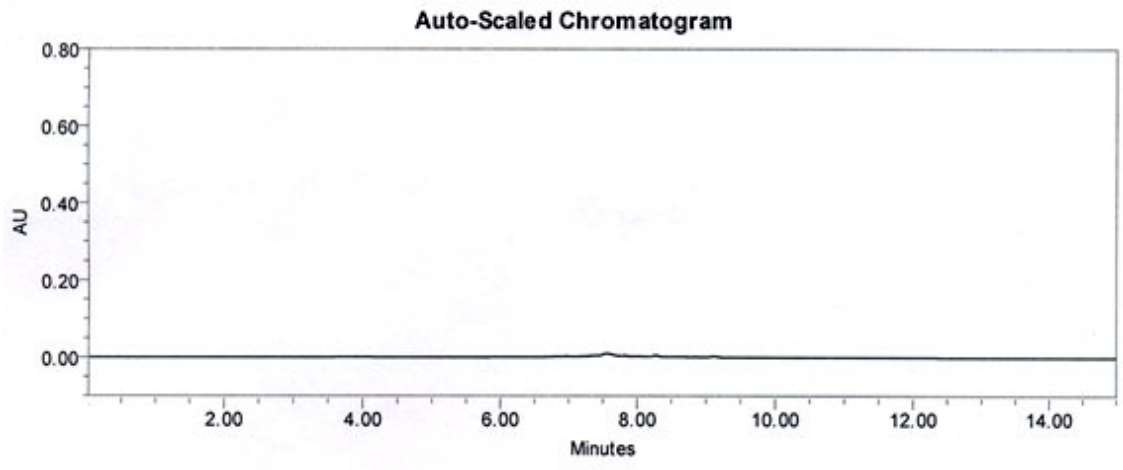


Fig: 2-Typical chromatogram of and Emtricitabine, Tenofovir and Efavirenz (Standard)

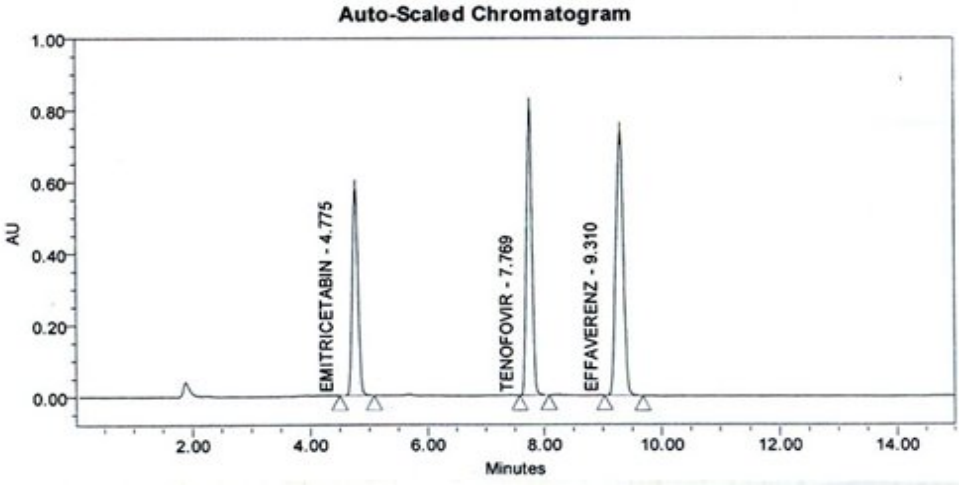


Fig: 3-Typical chromatogram of and Emtricitabine, Tenofovir, and Efavirenz (sample)

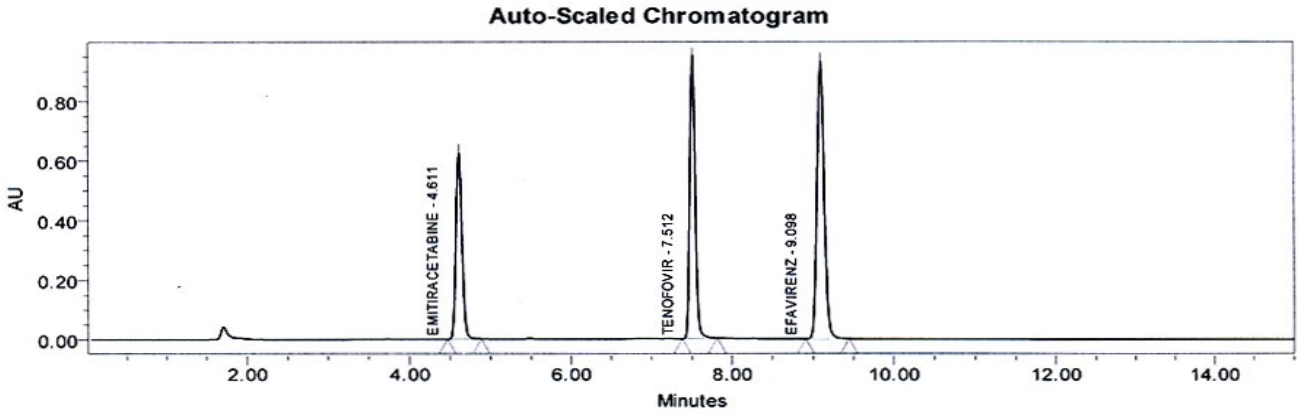


Fig: 4- Emtricitabine:

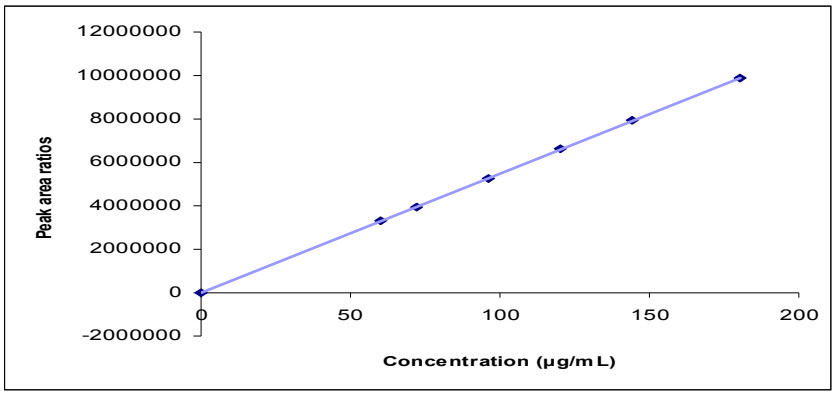


Fig: 5- TenofovirGraph:

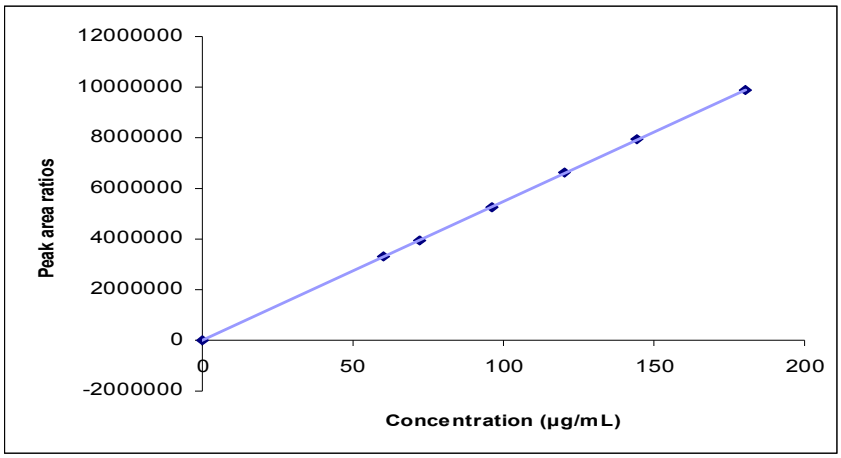
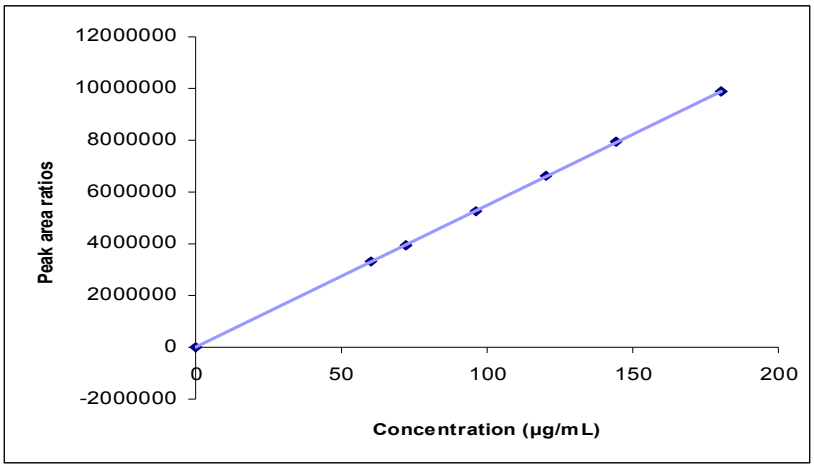


Fig: 6- Efavirenz Graph:



REFERENCES:

1. S.Budhavari, the Merck Index (monograph#3521), 14, 598, (2006).

2. S.Budhavari, the Merck Index (monograph#3565), 14, 606, (2006).

3. S.Budhavari, the Merck Index (monograph#9146), 14, 1573(2006)
4. C.SeanSweetman, Martindale-The Complete Drug Reference, 34,632, (2005).
5. C.SeanSweetman, Martindale-The Complete Drug Reference, 34,654, (2005).
6. Indian pharmacopoeia vol.2, 1071, (2007).
7. Indian pharmacopoeia vol.2, 1075, (2007).
8. Indian pharmacopoeia vol.2, 1782, (2007).
9. Ashutoshkar, Pharmaceutical drug analysis 2 nd edition. New Age Publisher 2005 P.No:16-17
10. Parimoo.p. Pharmaceutical Analysis CBS Publishers & Distributors; 2006 P. No: 24.
11. Frank a Settle. Hand Book of Instrumental Techniques for Analytical Chemistry Pearson Education 2004. P.No: 147-159.
12. P.D. Sethi, Quantitative analysis of drugs in pharmaceutical formulation, 3rd edition. CBS Publishers & Distributors. New Delhi 1997, P. No: 17-19.
13. Rebiere, Herve; Mazel, Bernard; Civade, Corinne; Bonnet, Pierre-Antoine, Journal of Chromatography B: 850(1-2), 376-383 (2007).
14. Choi, Sun Ok; Rezk, Naser L.; Kashuba, Angela D. M., Journal of Pharmaceutical and Biomedical Analysis, 43(4), 1562-1567, (2007).
15. Weller, Dennis R.; Brundage, Richard C.; Balfour, Henry H.;Vezina, Heather E. Journal of Chromatography, B: 848(2),369-373 (2007).