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A New RP-HPLC method for the Simultaneous Estimation of Emtricitabine, Efavirenz and Tenofovir in Tablet Dosage forms.

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ABSTRACT

An accurate, precise and reproducible high performance liquid chromatographic method was developed for quantitative estimation of emtricitabine, efavirenz and tenofovir disoproxil fumarate simultaneously in tablet dosage forms. Separation of the drugs was achieved within 15.0 min on a Xterra RP-18 column (150 x 4.6 mm; 5µ) by gradient elution using mixtures of ammonium acetate buffer and acetonitrile as the mobile phase. The analytes in the eluate were monitored at 260 nm. The retention times obtained for emtricitabine, efavirenz and tenofovir disoproxil fumarate were 4.611, 9.098 and 7.512 min respectively. The calibration curves were linear over the range of 20.11-60.33 µg/mL for emtricitabine, 60.28-180.45 µg/mL for efavirenz and 30.13-90.18 µg/mL for tenofovir disoproxil fumarate. The performance of the method was validated according to ICH guidelines. The method was found to be suitable for accurate determination of these drugs in tablet dosage forms without any interference from excipients or endogenous substances.

Keywords: Emtricitabine, Efavirenz, Tenofovir disoproxil fumarate, Determination, HPLC, Gradient elution.

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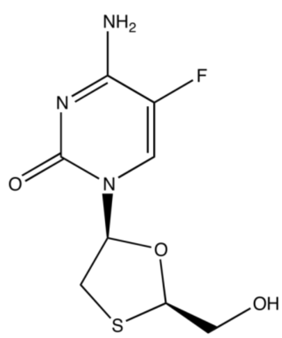
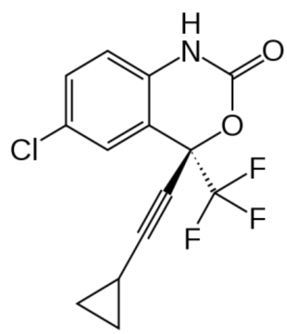
INTRODUCTION

Emtricitabine (4-amino-5-fluoro-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one), an analogue of cytidine, and Tenofovir disoproxil fumarate (({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid), are nucleoside reverse transcriptase inhibitor (NRTI) that are used for the treatment of HIV infection[1]. Efavirenz ((4*S*)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1*H*-3,1-benzoxazin-2-one**)** is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active antiretroviral therapy for the treatment of a human immunodeficiency virus type 1 [2]. The chemical structures of these drugs are shown in Figure 1. Both nucleoside and non-nucleoside reverse transcriptase inhibitors inhibit the same target, the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. Unlike NRTIs, which bind at the enzyme's active site, NNRTIs act allosterically by binding to a distinct site away from the active site known as the NNRTI pocket.


(A)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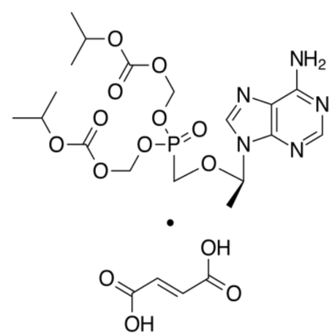
(C)

Figure 1: Chemical structures of emtricitabine (A), efavirenz (B) and tenofovir disoproxil fumarate (C)

A literature survey revealed that very few liquid chromatography[3-8] techniques have been reported for the simultaneous determination of emtricitabine, efavirenz and tenofovir disoproxil fumarate in pure drug, pharmaceutical dosage forms and biological samples. Hence, the authors have attempted to develop a simple, rapid, precise and accurate method for the simultaneous estimation of these drugs in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH)[9] for the simultaneous determination of emtricitabine, efavirenz and tenofovir disoproxil fumarate in bulk and in tablet dosage forms.

MATERIALS AND METHODS

Drugs, chemicals and solvents

Emtricitabine, efavirenz and tenofovir disoproxil fumarate working standards were obtained from Hetero Labs Ltd. (Hyderabad, India) as gift samples. Ammonium acetate (extrapure) and glacial acetic acid were purchased from Qualigens Chemicals Limited. HPLC grade methanol and acetonitrile were purchased from Merck Limited. HPLC grade water was prepared using Millipore Milli-Q system.

Equipment and chromatographic conditions

The chromatographic system consisted of Waters Alliance liquid chromatography (model 2695) fitted with Diode array detector (model 2996) using Empower2 software as data handling system. Xterra RP-18 column (150 x 4.6 mm; 5µ) was used for this method. All chromatographic runs were carried out in a gradient mode with a flow rate of 1.0 mL/min. Ammonium acetate buffer was prepared by dissolving 9.2 g of ammonium acetate in 1000mL of water and adjusting the pH to 4.6 with glacial acetic acid, followed by filtration through 0.45µ filter and sonication. The mobile phase consisted of mobile phase-A (Ammonium acetate buffer) and mobile phase-B (Acetonitrile). The detector wavelength was set at 260 nm. The injection volume was 10 µL and the column was kept at 22°C.

Preparation of diluent

Ammonium acetate buffer and methanol was mixed in the ratio of 50:50 v/v and used as diluent.

Preparation of working standard solution of emtricitabine, efavirenz and tenofovir disoproxil fumarate

Individual standard stock solutions of emtricitabine, efavirenz and tenofovir disoproxil fumarate were prepared by weighing 40.22 mg of emtricitabine, 60.125 mg of efavirenz and 60.28 mg of tenofovir disoproxil fumarate into three separate 100 mL volumetric flasks and dissolving in 60 mL of methanol. The volumes were made up with further quantity of methanol and mixed well.

5 mL of emtricitabine, 10 mL of efavirenz and 5mL of tenofovir disoproxil fumarate standard stock solutions were transferred into a 50 mL volumetric flask, diluted to volume with diluent and mixed to get concentrations of 40 µg/mL, 120 µg/mL and 60 µg/mL of emtricitabine, efavirenz and tenofovir disoproxil fumarate respectively. This was used as the working standard solution.

Calibration curve

Calibration curve was performed by preparing mixed standard solutions of emtricitabine, efavirenz and tenofovir disoproxil fumarate at different concentration levels including working concentration mentioned in experimental condition. Ten microlitres of each concentration was injected into the HPLC system. The response was read at 260 nm and the corresponding

chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually.

Estimation of the drugs from tablet dosage forms

Five Atripla tablets (Cipla Limited) were weighed and transferred into a 500 mL volumetric flask. About 100 mL of buffer was transferred into the volumetric flask and shaken for 20 min on a rotary shaker. 200 mL of methanol was added and sonicated for 20 min with intermittent shaking. It was diluted to volume with methanol and mixed well.

2 mL of the above solution was transferred into a 100 mL volumetric flask and the volume was made up to mark with diluent and mixed. This solution was filtered through 0.45µ nylon membrane filter and used as sample solution. The above solution was then chromatographed six times. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by the regression equation of the method.

RESULTS AND DISCUSSION

Using mobile phase-A and B in gradient as shown in the Table 1, base line separation for the peaks of emtricitabine, efavirenz and tenofovir disoproxil fumarate was achieved. Under these conditions, the retention times for emtricitabine, efavirenz and tenofovir disoproxil fumarate were found to be 4.611, 9.098 and 7.512 min respectively. The proposed method was also applicable to tablet formulations.

Table 1: Gradient Program

S.No.	Time (min)	% Mobile Phase-A	% Mobile Phase-B
1	0.00	100	0
2	4.00	70	30
3	6.00	20	80
4	10.00	50	50
5	11.00	100	0
6	15.00	100	0

Specificity

A good analytical method should be able to measure the analytes accurately in the presence of suspected interferences such as blank, excipients, and degradation products. Figure 2 shows chromatographic base-line separation of emtricitabine, efavirenz and tenofovir disoproxil fumarate. Figure 3 demonstrates that no interferences were found at the retention times of emtricitabine, efavirenz and tenofovir disoproxil fumarate in their dosage forms due to excipients.

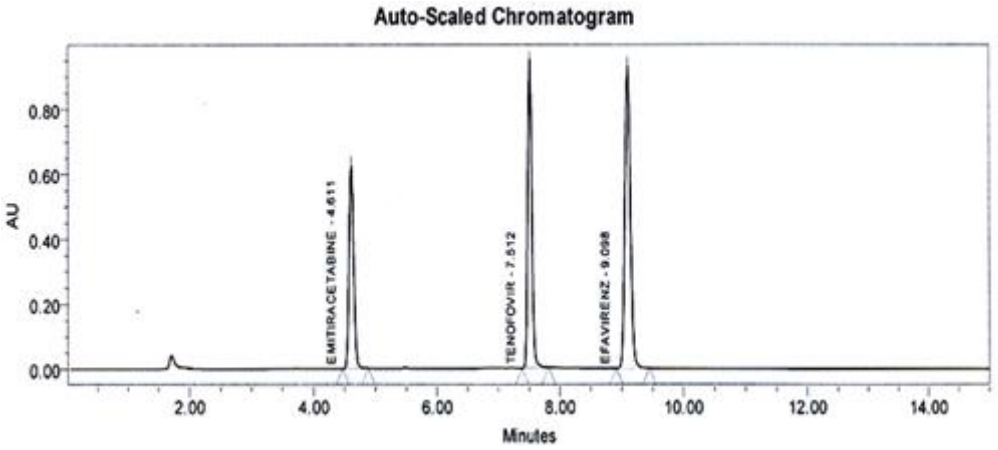

Figure 2: Representative chromatogram obtained from analysis of emtricitabine, efavirenz and tenofovir disoproxil fumarate from working standard solution.

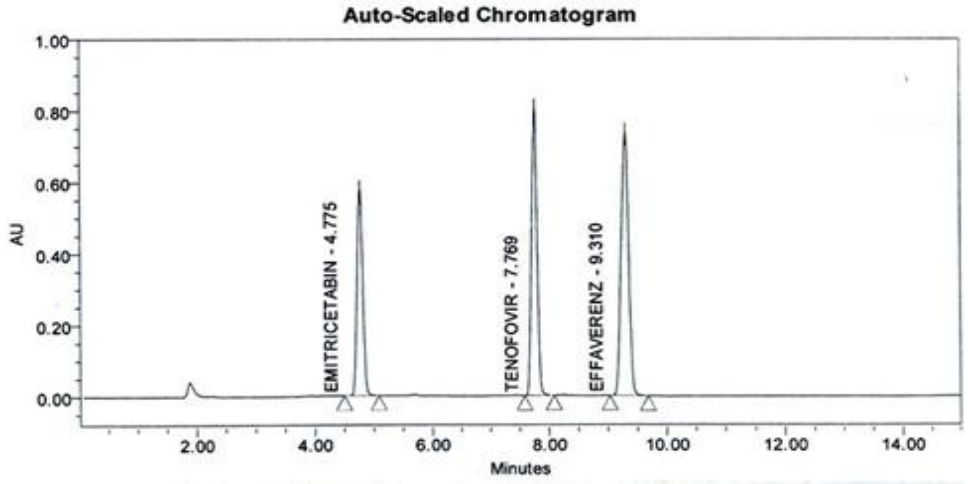

Figure 3: Representative chromatogram obtained from analysis of emtricitabine, efavirenz and tenofovir disoproxil fumarate from formulation sample solution.

Linearity

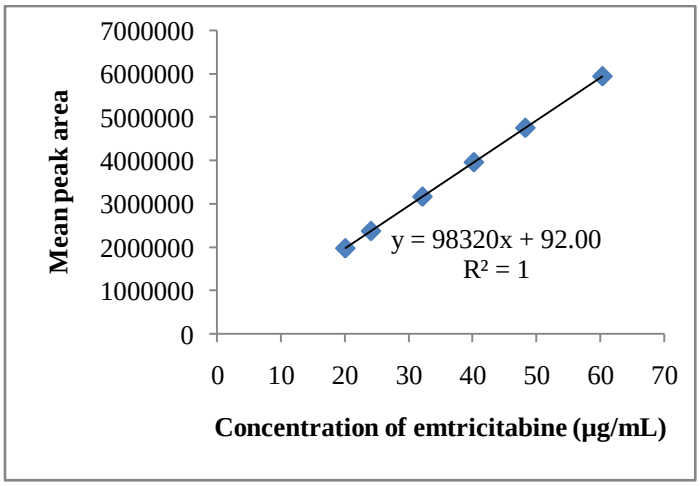

Figure 4: Linearity plot for emtricitabine

The regressions of the plots were computed by least square regression method and were shown in the Figures 4, 5 and 6. The calibration curves (n=3) constructed for each drug were linear over the concentration range of 20.11-60.33 µg/mL for emtricitabine, 60.28-180.45 µg/mL for efavirenz and 30.13-90.18 µg/mL for tenofovir disoproxil fumarate. The correlation coefficients were greater than 0.999 and the %RSD for each concentration studied was less than 2%.

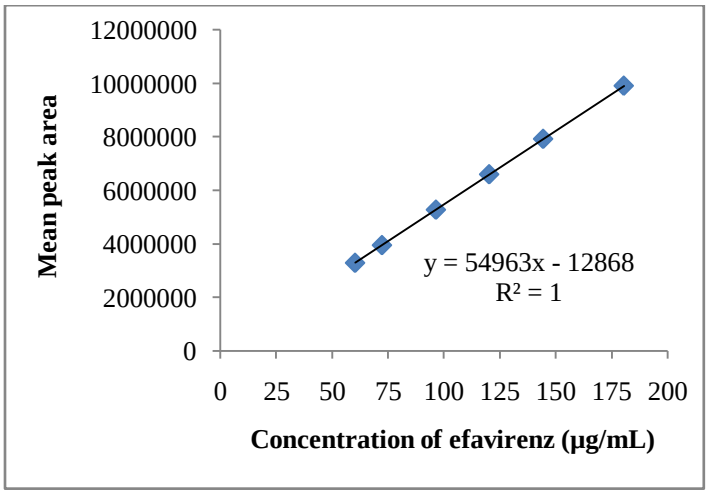

Figure 5: Lineaity plot for efavirenz

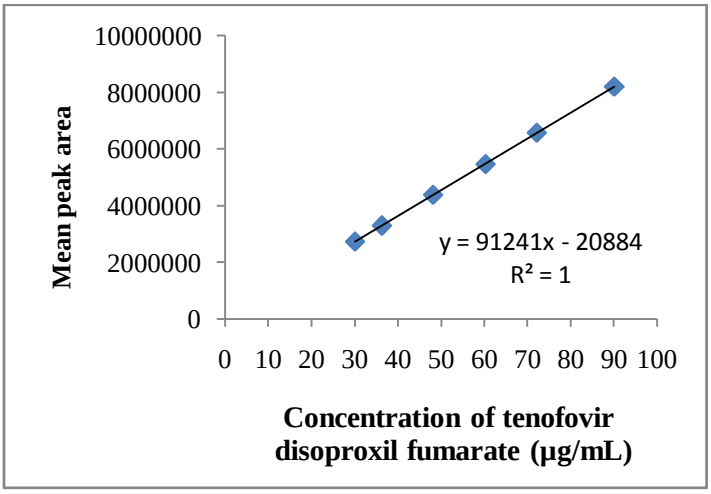

Figure 6: Linearity plot for tenofovir disoproxil fumarate

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in Table 2. The high percentage of recovery indicates that the proposed method is accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Five replicate injections of working standard solution were chromatographed and the percentage RSD of the peak areas obtained was calculated. The

precision study data is represented in Table 3. From the data obtained the developed RP-HPLC method was found to be precise.

Table 2: Accuracy data of the proposed method

Analyte	Amount of the analyte taken(µg/mL)	Mean recovery (µg/mL) ± SD(n=3)	% Mean recovery ± SD
Emtricitabine	20.11	19.93 ± 0.09	99.10 ± 0.43
	40.22	39.89 ± 0.14	99.18 ± 0.36
	60.33	59.85 ± 0.08	99.20 ± 0.13
Efavirenz	60.28	59.96 ± 0.16	99.46 ± 0.26
	120.25	119.533 ± 0.29	99.40 ± 0.24
	180.45	180.45 ± 0.32	100.00 ± 0.18
Tenofovir disoproxil fumarate	30.13	30.06 ± 0.18	99.77 ± 0.60
	60.28	60.11 ± 0.24	99.72 ± 0.40
	90.18	90.25 ± 0.25	100.08 ± 0.27

Table 3: Precision data for the proposed method

Analyte	Intra-day precision		Inter-day precision	
	Mean peak area ± SD (n=5)	%RSD	Mean peak area ± SD (n=5)	%RSD
Emtricitabine	3887583 ± 21272.84	0.54	3883485 ± 22219.63	0.57
Efavirenz	6554656 ± 52897.06	0.81	6540663 ± 35294.11	0.39
Tenofovir disoproxil fumarate	5331552.8 ± 2950.22	0.55	5331894 ± 20619.17	0.54

System Suitability Parameters

System suitability parameters were studied with six replicates of working standard solution and the parameters are presented in Table 4.

Table 4: System suitability parameters of the proposed method

Parameter	Emtricitabine	Efavirenz	Tenofovir disoproxil fumarate
Retention time (min)	4.611	9.098	7.512
Tailing factor	1.1	1.0	1.0
Theoretical plates	11496	24553	31109
HETP	0.013048	0.006109	0.004822

Method Suitability

The commercial tablet formulation, Atripla (Cipla Limited) was analyzed by the proposed method and the results are shown in Table 5. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of drugs in pharmaceutical dosage forms.

Table 5: Recovery of emtricitabine, efavirenz and tenofovir disoproxil fumarate

Drug	Labeled amount (mg)	Amount recovered (n=6)	% Recovery
Emtricitabine	200	200.26	100.13
Efavirenz	600	599.84	99.97
Tenofovir disoproxil fumarate	300	299.46	99.82

CONCLUSION

The proposed RP -HPLC method is sensitive, precise and accurate and can be used for the routine quality control analysis for the simultaneous determination of the emtricitabine, efavirenz and tenofovir disoproxil fumarate in their tablet dosage forms.

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