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ISSN: 0975-7538
Research Article

A stability indicating RP-HPLC method for simultaneous estimation of Emtricitabine, Tenofovir disoproxil fumarate and Efavirenz in pharmaceutical dosage forms

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ABSTRACT

A stability indicating RP-HPLC method has been developed for quantitative determination of Emtricitabine, Tenofovir Disoproxil Fumarate and Efavirenz in Pharmaceutical dosage forms. Chromatographic separation was achieved through gradient elution. Detection wavelength was monitored at 262 nm. The retention times of the Emtricitibine, Tenofovir disoproxil fumarate and Efavirenz was about 2.6, 5.4, 7.9min respectively. The developed method was validated as per ICH guidelines. This method is found to be simple, fast and economical Hence this validated method can be used in routine quality testing of individual dosage forms and combination dosage forms of Emtricitabine, Tenofovir disoproxil fumarate and Efavirenz.

Keywords: Emtricitabine; Tenofovir disoproxil fumarate; Efavirenz; HPLC; Validation

INTRODUCTION

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV which still kills 5000 people a day. Different class of antiretroviral drugs is used in treating HIV infection by making a single dosage unit containing two or more antiretroviral drugs (Highly Active Antiretroviral therapy, or HAART) (Bartlett JG., 2006). These combination drugs, when given as a fixed dose combination product rather than individual entities has shown to improve therapy in terms of sustained virological suppression and significant reduction in the mortality rates of the HIV/AIDS infected people (Katlama C *et al.,* 1996; Palella Jr FT., 1998). Globally, eight million people living with HIV in low- and middle-income countries were accessing ART at the end of 2011(Joint United Nations Program., 2012). At the same time, an estimated 15 million people needed ART for their own health in low- and middle income countries, based on WHO's 2010 treatment guidelines (World AIDS day report., 2012). In recent years, drug companies have worked to combine complex regimens into simpler formulas, necessitating development of new HPLC methods for estimation of multiple analytes in a single method. Since the use of these drug products tremendously increasing, there is a need for controlling the quality of these drug products which are sold in the underdeveloped and developing countries. Literature survey reveals few Chromatographic methods (Montgomery ER *et al.*, 2001; Schuman M *et al.*, 2005; Matthews CZ *et al.*, 2002; Bhargavi YG *et al.*, 2012; Adeyeye MC *et al.*, 2012, Soumya B *et al.*, 2012; Kappelhoff BS *et al.*, 2003; Sharma R *et al.*, 2009) reported for the estimation of Emtricitabine, Tenofovir disoproxil fumarate and Efavirenz from pharmaceutical dosage form. With this interest a simple, economical and faster, Stability indicating Assay method was developed and validated for the simultaneous estimation of Emtricitabine, Tenofovir disoproxil fumarate and Efavirenz in formulated tablets in different combination and individually.

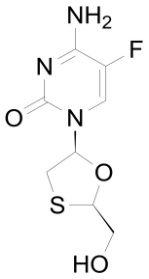

Figure 1: Chemical structure of Emtricitabine

Emtricitabine (4-amino-5-fluoro-1-[(2*S*, 5*R*)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one) having molecular structure as given in Fig. 1, Tenofovir disoproxil fumarate (9-[(R)-2 [[bis[[(isopropoxycarbonyl)oxy]- methoxy] phosphinyl] methoxy] propyl]adenine fumarate (1:1)) having molecular structure as given in Fig. 2 and Efavirenz ((4*S*)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoro methyl)-2*H*-3, 1- benzoxazin-2-one) having molecular structure

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Received on: 09-04-2013
Revised on: 22-04-2013
Accepted on: 26-04-2013

as given in Fig. 3 belongs to category of reverse transcriptase inhibitors. This combination of three medications approved by the U.S. Food and Drug Administration (FDA) in July 2006 under the brand name Atripla, provides HAART in a single tablet taken once a day (FDA, HIV/AIDS Historical timeline, 2000-2010). It results in a simplified drug regimen for many patients.

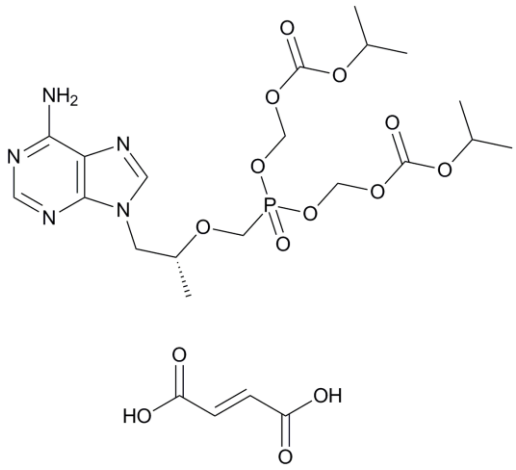

Figure 2: Chemical structure of Tenofovir Disoproxil Fumarate

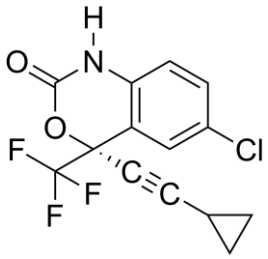

Figure 3: Chemical structure of Efavirenz

MATERIALS & METHODS

Chemicals and Reagents

All the reagents and chemicals used are of general laboratory chemicals, HPLC grade Methanol was taken from Rankem chemicals .Trifluroacetic acid was taken from ACROS chemicals. HPLC grade water was obtained from Milli Q water purification system.

Equipment

High performance Liquid chromatography system (Waters 2695 and Agilent 100 series) with quaternary gradient, auto sampler and with PDA detector was used for the study. Data was collected and processed by using Waters Empower software.

Chromatographic Conditions

The chromatographic column used was Inerstil ODS C18 250x4.6 mm, 5µm (Make GL Sciences).Separation was achieved through gradient elution .Buffer (0.05% Trifluro acetic acid in water) as mobile phase A and Methanol as mobile phase B was used as binary gradient with the flow rate of 2.0 ml/min. Detection wavelength was monitored at 262 nm. Column oven temperature was maintained at 30°C with the injection volume of 20µL. Finalized gradient program is as fallows, Time (min)/%Solution B, 0/20, 6/90, 7/100, 10/100, 11/20 and 15/20.

Sample Preparation

Diluent Preparation

Methanolic HCl (0.1N) was used as extraction diluent and further samples were diluted with the mixture of Water: Methanol in the ratio of 65:35 respectively.

Standard Preparation

A Standard solution was prepared to get a concentration of 120µg/ml for Efavirenz, 40µg/ml for Emtricitabine and 60µg/ml for Tenofovir disoproxil fumarate.

Test Preparation

Test solution was prepared by taking homogenous mixture of tablet powder equivalent to 300 mg of Efavirenz/100 mg Emtricitabine/150 mg of Tenofovir in to a 250 ml volumetric flask, added about 180 ml of Methanolic HCl, sonicated for about 30 minutes. Volume was made up to 250 ml with Methanolic HCl and mixed well. Further 5 ml of this solution was diluted to 50 ml with mixture of Water and Methanol (65:35) and filtered through 0.45 µm filters.

Experimental Design

Method development and Optimization

The main objective of the method development was to estimate simultaneously Emtricitabine, Tenofovir disoproxil fumarate and Efavirenz in formulated tablets in different combination and individually by a stability indicating Simple, Economical and faster HPLC method.

Column used (Inerstil ODS C18 250x4.6 mm, 5µm) is a generally available column in any Laboratory. All chemicals are also easily available. A gradient elution method was developed based on the polarity of individual molecule. Diluent for extraction was selected based on the solubility of drug substances.

Under above optimized conditions all three components were well separated from each other and also from main degradation impurities like Monoester impurity of Tenofovir, S-Oxide impurity of Emtricitabine and Amino alcohol impurity, Quinoline impurity of Efavirenz.

Method Validation

Above optimized analytical method was subjected for validation to check its Specificity, Precision, Accuracy, Linearity and Robustness. The principal purpose of analytical validation was to ensure that a selected analytical procedure will give reproducible and reliable results that are adequate for the intended purpose. Validation activity was planned as per the International Conference on Harmonization (ICH) guidelines (Guideline I. H. T., 2010) Procedures, experimental design and ac-

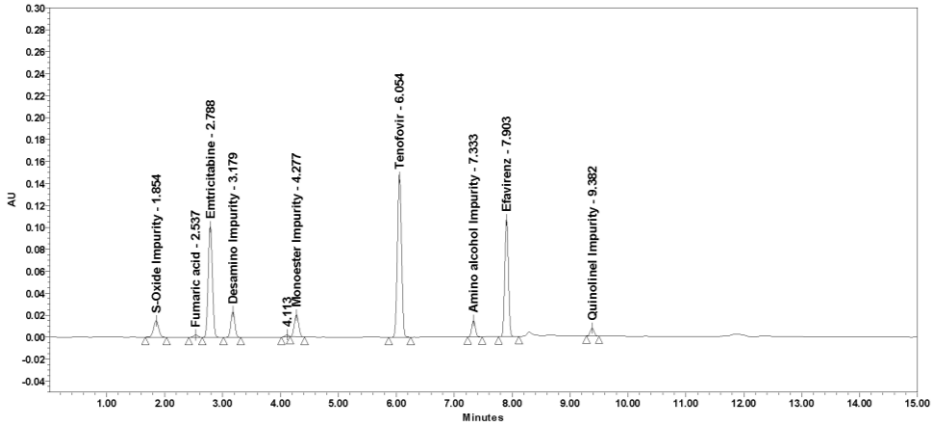

Figure 4: Typical Chromatogram of Standard solution Spiked with Known Degradent impurities

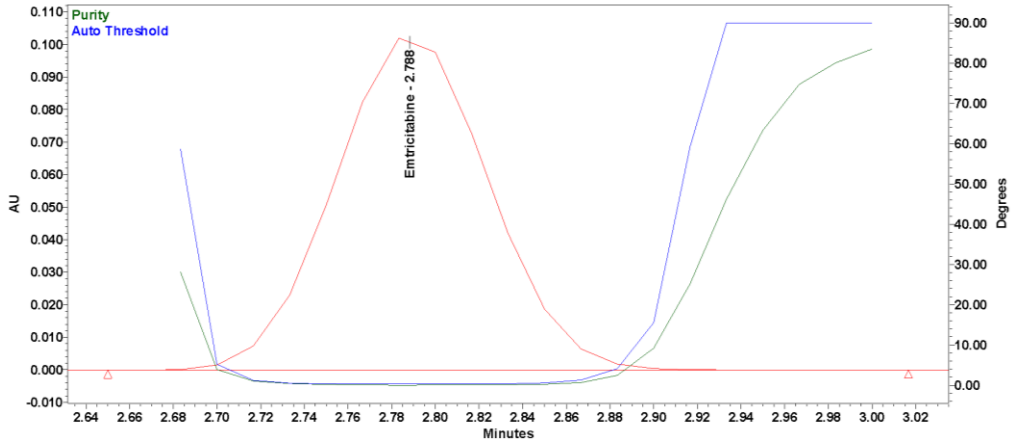

Figure 5: Peak Purity Plot of Emtricitabine from Chromatogram of Standard solution Spiked with Known Degradent impurities

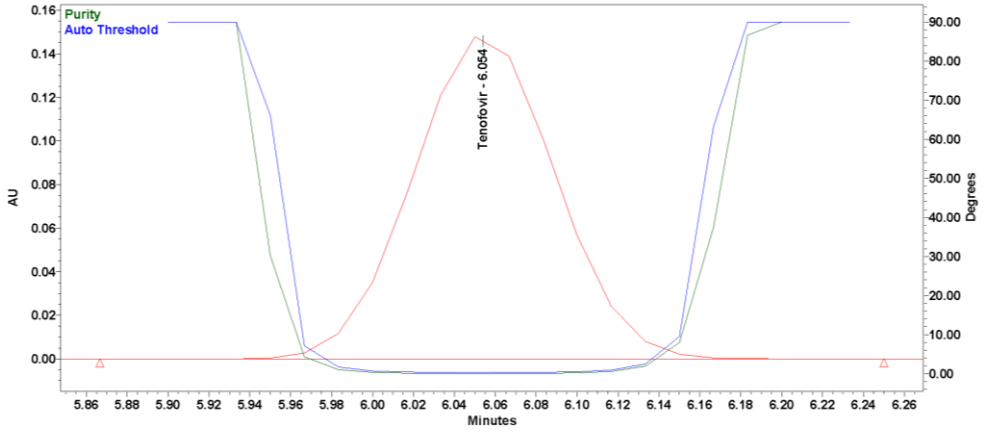

Figure 6: Peak Purity Plot of Tenofovir from Chromatogram of Standard solution Spiked with Known Degradent impurities

ceptance criteria were followed as per the general industrial practices.

RESULTS AND DISCUSSION

System suitability

As integral part of chromatographic method system suitability parameters like USP Tailing, Theoretical plates and Relative standard deviation (RSD) for replicate injections were evaluated and found to be satisfactory as per general chromatographic practices. Results are shown in Table 1.

Specificity

To demonstrate the Stability indicating power of analytical method used, Specificity was proved by injecting the all three components individually, Known impurities (degradents), placebo and Standard solution Spiked with Known degradent impurities (Fig. 4). Peak purity plot of Standard solution Spiked with Known

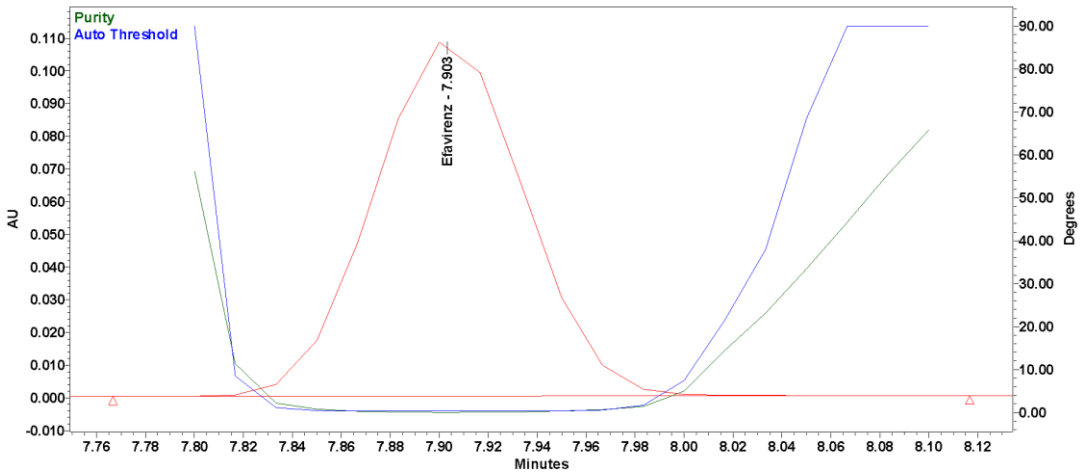

Figure 7: Peak Purity Plot of Efavirenz from Chromatogram of Standard solution Spiked with Known Degradent impurities

Table 1: Results of System Suitability Test

Name of Drug substances	Retention time (minutes)	USP Tailing factor	Theoretical plates	%RSD for replicate injections
Emtricitabine	2.6	1.0	6382	0.2
Tenofovir	5.4	1.0	29742	0.1
Efavirenz	7.9	1.0	71381	0.2

Table 2: Results of Forced degradation Studies with Peak purity details

Stress Conditions	% Degradation	Emtricitabine		Tenofovir		Efavirenz	
		PA [a]	PT [b]	PA [a]	PT [b]	PA [a]	PT [b]
Treated with 0.1N HCl for about 10 minutes	3.51	0.129	1.129	0.196	1.301	0.136	1.069
Treated with 0.1N NaOH for about 10 minutes	28.65	0.113	1.109	1.283	1.749	0.129	1.058
Treated with 3%H202 for about 10 minutes	2.88	0.127	1.129	0.194	1.238	0.134	1.053
Exposed to heat at about 90°C for about 24 hours	3.46	0.103	1.117	0.257	1.28	0.135	1.058
Exposed to heat at about 90%RH for about 24 hours at room temperature	0.57	0.105	1.096	0.145	1.192	0.126	1.046

PA = Purity Angle; PT= Purity Threshold

Note: Purity Angle should be less than Purity Threshold to meet Peak purity criteria acceptance criteria

degradent impurities has been shown in Fig. 5, 6 and 7. Specificity of analytical method has also been demonstrated by performing forced degradation studies under different stress conditions like Acid hydrolysis, Alkali hydrolysis, Oxidation by peroxide, thermal treatment and exposed to humidity. From this it was proved that possible degradents are well separated from the analytes peak, which was supported by peak purity results of each analyte peak. Results are shown in Table 2.

Linearity

Linearity of the analytical procedure was demonstrated by preparing and analyzing the standard preparations at six different levels. Linearity solutions were prepared at 25, 50, 75, 100, 125 and 150% of standard concentration for all the three analytes. From this experiment co efficient of correlation, Intercept and slope were calculated by plotting a graph between peak responses and concentrations. Calibration plots were linear over the rages tested. Correlation coefficient >0.999 for all the three components shows the good correlation between concentration and peak responses. Results are shown in Table 3.

Precision

Method precision was performed by preparing the six test preparation from same lot of tablets and they were injected in to chromatography. From this experiment Relative standard deviation (RSD) was determined for %Assay results for each analyte.The results indicates that the method is precise. Results are shown in Table 4.

Accuracy

Accuracy of the method was tested by preparing the test preparation at three different levels 50, 100 and 150% and analyzing by chromatography. Test preparations were prepared by mixing placebo and drug substances at above said levels. Test preparations were

Table 3: Results of Linearity Studies (Response Vs Concentration)

Name of Drug substances	Correlation coefficient	Intercept	Slope
Emtricitabine	0.999	1837.943	12129.559
Tenofovir	0.999	-564.333	12096.033
Efavirenz	0.999	3379.174	3933.3016

Table 4: Results of Method precision (Repeatability studies)

Sample No	% Assay of Emtricitabine	% Assay of Tenofovir	% Assay of Efavirenz
Sample 1	99.3	101.2	100.7
Sample 2	99.3	101.7	100.1
Sample 3	99.6	101.6	100.8
Sample 4	99.5	101.6	100.9
Sample 5	99.7	101.9	101.3
Sample 6	99.6	102.0	101.2
Mean	99.5	101.7	100.8
SD	0.1673	0.2805	0.4274
%RSD	0.2	0.3	0.4

Table 5: Results of Recovery Study at Different Levels

Name of Drug substances	50% level		100% level		150% level	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Emtricitabine	100.1	0.5	99.6	0.1	99	0.3
Tenofovir	101.6	0.3	101.5	0.2	101.5	0.3
Efavirenz	100.3	0.8	101.1	0.2	100.6	0.1

Note: Number of samples analyzed at each level is in triplicate

done in triplicate at each level. Amount of drug added, amount drug found, % recovery and Relative standard deviation was determined for %recovery results for each analyte. % Recovery results were ranged from 99.0 to 100.1 with %RSD 0.1 to 0.5 for Emtricitabine, 101.5 to 101.6 with %RSD 0.2 to 0.3 for Tenofovir and 100.3 to 101.1 with %RSD of 0.1 to 0.8 for Efavirenz. This indicates the capabability of method to recover the analytes from placebo matrix. Results are shown in Table 5.

CONCLUSION

A simple, faster and economical chromatographic method could be developed for the estimation of Emtricitibine, Tenofovir Disoproxil fumarate and Efavirenz in formulated products in combination or individually. Method requires all commonly available materials for analysis. Analytical method was validated as per ICH guideline and general industrial practices and proved that it is suitable for its intended purpose.

The above validated chromatographic method can be used by government agencies, government laboratories, research institutions and manufacturing companies to analyze the drug product to check the quality of it.

ACKNOWLEDGEMENTS

The authors wish to thank the management of NGSM Institute of Pharmaceutical Sciences, Mangalore, India for their support.

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