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SIMULTANEOUS DETERMINATION OF LAMIVUDINE, TENOFOVIR AND EFAVIRENZ IN LAMIVUDINE, TENOFOVIR DISPROPOXIL FUMARATE AND EFAVIRENZ TABLETS BY STABILITY INDICATING ISOCRATIC RP-HPLC METHOD WITH PDA DETECTOR

Manikya Rao T*, T. Prabhakar, G. Girija Sankar, PVL Naidu and N.Jyothi

Andhra University, Department of pharmaceutical sciences, Visakhapatnam, Andhra Pradesh, India

***Corresponding author e-mail:** manikvision@gmail.com

ABSTRACT

A simple fast, accurate, precise and cost effective isocratic RP-HPLC method is developed for simultaneous determination of Lamivudine, Tenofovir and Efavirenz in tablet formulation. Lamivudine, Tenofovir and Efavirenz are considered as a very potent regimen in therapy-naıve patients and therefore, it is recommended as first-line therapy. It is indicated for the treatment of human immunodeficiency virus-1 (HIV-1) infection in adults and adolescents with virologic suppression to HIV-1 RNA levels of < 50 copies/ml on their current combination antiretroviral therapy for more than three months. Patients must not have experienced virological failure on any prior antiretroviral therapy. The retention times of Lamivudine, Tenofovir and Efavirenz were found to be 2.3, 3.6 and 13.6 minutes respectively. The method was linear over the range of 25 to 45 ppm with r2 = 0.999 for Lamivudine, 25 to 45 ppm with r2 = 0.999 for Tenofovir and 50 to 90 ppm with r2 = 0.999 for Efavirenz. Mean recovery for Lamivudine Tenofovir and Efavirenz were 100.5, 99.4 and 99.9 respectively. The method found simple, accurate, precise, and linear over the given range, rugged and robust.

Keywords: Lamivudine, Tenofovir and Efavirenz, RP-HPLC, PDA detector.

INTRODUCTION

Highly active antiretroviral therapy (HAART) has brought new hope for those people who live with HIV/AIDS by decreasing the morbidity and mortality among people infected with HIV. Highly active antiretroviral therapy also has improved the quality of life among the people who live with HIV/AIDS. Combination therapy is preferred to be the gold standard for the treatment of AIDS so as to maximize potency, minimize toxicity, diminish the risk for resistance development and reduction of pill burden to once-daily dosing so as to optimize the patient's compliance and reduce the treatment costs. The Nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors as multi-drug combinations are effective in the therapy of Human Immunodeficiency Virus (HIV) infection and are used as a part of Highly Active Anti-Retroviral Therapy, for the treatment of HIV [1, 2]. The daily regimen containing Efavirenz, Lamivudine and Tenofovir disoproxil fumerate is virologically and immunologically effective, well tolerated and safe with benefits in the lipid profile in the majority of patients.[3]

Lamivudine is chemically (2R, cis)-4-amino-1-(2-(hydroxylmethyl-1,3-oxathiolan-5-yl)- (1H) pyrimidin -2 -one, is a nucleoside reverse transcriptase inhibitor[4,5]. The estimation of Lamivudine using UV-visible spectroscopy[6] and HPLC has been reported.[7] Lamivudine drug is a synthetic nucleoside analogue with activity against HIV-1 and Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70

mg/mL in water at 20°C. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3.

Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors, which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir disoproxil fumerate is chemically 9-((R)-2- ((bis(((isopropoxycarbonyl)oxy)methoxy) phosphinyl)methoxy)propyl) adenine fumerate, is a nucleotide analogue reverse transcriptase inhibitor[4,5] Tenofovir disoproxil fumerate has been determined in spiked human plasma by HPLC[8]. Tenofovir disoproxil fumerate in combination with other drugs by using RP-HPLC has been reported. [9]

Chemically Efavirenz is (4*S*)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1*H*-3,1-benzoxazin-2-one. Efavirenz is a nonnucleoside reverse transcriptase inhibitor and is used as a part of highly active antiretroviral therapy for the treatment of human immunodeficiency virus. The usual dosage of efavirenz is 600mg per day. Human immunodeficiency viruses (HIV) are lent viruses, a family of mammalian retroviruses evolved to cause chronic persistent infection[10]. Efavirenz is used widely in the developed world because of its convenience, effectiveness, and long-term tolerability.

Several analytical and bio analytical methods have been reported for Lamivudine, Tenofovir and Efavirenz individually and combination determination by simple UV spectrophotometer and as well as by RP-HPLC[8, 13, 11, 12, 14&15] with UV detector.

EXPERIMENTAL

Chemicals and Standards: Potassium dihydrogen ortho-phosphate, ortho-phosphoric acid GR grade, methanol was HPLC grade were from Merck. PVDF filters from Axiva. Hydrochloric acid from Merck and Sodium hydroxide from Rankem. Working standards from Hetero drugs, Tablets formulation marketed sample.

Instrument: Waters HPLC with software empower 2 with photo diode array detector with gradient elution and an auto sampler with HP computer.

Buffer solution: Dissolved 7.0 g of Potassium dihydrogen ortho phosphate in 1000 mL water, pH adjusted to 3.5 with ortho phosphoric acid.

Mobile phase: Mixed buffer and methanol in the ratio of 35:65 (v/v) filtered through 0.45 µ membrane filter and sonicated for 5 min.

Chromatographic condition: The chromatographic separation was achieved using Column: C-18 column, Hypersil 150 mm × 4.6 mm, 5 µm, ; Wavelength: 260 nm; Flow rate: 1.0 mL/min; Injection volume: 20 µL; Column temperature: Ambient, Run time: 16 min.

Preparation of stock solutions: Prepared different solution having the concentration 1000 ppm of Lamivudine, Tenofovir and Efavirenz in diluent.

Mixed standard solution: Transferred 0.35ml of Lamivudine, Tenofovir & 0.7ml of Efavirenz of above stock solution to a 10 mL volumetric flask and made up to volume with diluent to attain a concentration of Lamivudine, Tenofovir 35ppm and Efavirenz 70 ppm.

Sample preparation: Twenty tablets were weighed and crushed finely, powder approximately 51mg (equivalent to 100 mg of Lamivudine, Tenofovir and 200 mg) of Efavirenz was transferred to 100 mL volumetric flask and added 80 mL of diluent, sonicated for 5 min, cooled to room temperature and made up to volume with diluent filtered the above sample through 0.45 µ PVDF filter. Transferred 3.5 ml of above solution in 10 ml volumetric flask and made up to volume with diluent to attain same concentration as of standard, filtered through 0.45 µ PVDF filter. Injected equal volumes of diluent, placebo preparation, standard preparation separately in six replicates and sample once in equilibrated HPLC system and recorded the chromatograms and measured the response in terms of peak area. System suitability parameters maintained during method validation are theoretical plates not less than 2000, tailing factor not more than 2.0, relative standard deviation for six replicates of standard solution is not more than 2.0 %.

Degradation studies: Specificity is the ability of method to measure the analyte response in the presence of its potential impurities and degradation products. The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances in the drug product. The aim of this work was to decide the stability indicating capacity of the method.

RESULTS AND DISCUSSION

Specificity: The method is found specific and there is no blank or placebo interference.

Accuracy: Accuracy was studied at three concentration level at 50, 100 and 150 % level of working concentration. Each level was studied in triplicate. Each preparation was prepared independently by spiking analyte in the placebo. Per cent recovery was calculated by comparing the response obtained in spiked sample with those obtained in standard and obtained results (Tables 1, 2 and 3). The present analytical method found to be well accurate and results obtained are between 98-102 %.

Linearity: The standard stock solutions were prepared and diluted linearly and linearity was established, the concentration of the solutions and correlation coefficient was given in Tables 4 to 6.

Precision: To check the system precision (repeatability) for peak response obtained with five replicates of standard at specified concentration. The % RSD found to be within 2.0 %. To check repeatability (method precision) of the method, six individual sample preparations from same batch were prepared and injected the % RSD with six sample found to be within 2.0 %.The results obtained were presented in tables 7 to 10.

System suitability: The relative standard deviation for the peak areas of Lamivudine, Tenofovir and Efavirenz in standard solution was found to be less than 2.0 %.

Solution stability: Standard and samples solution stability was studied above 48 hours and found stable against the freshly prepared standard.

Ruggedness and robustness: The ruggedness of the method was studied by analyzing the sample on two different days on two different systems and two different columns by same manufacturer, results found to be well within acceptable limits. Robustness of an analytical method was studied by changing the pH of the buffer and found to acceptable for organic addition, the 5 % change in organic was also not influencing the resolution in between the peaks, hence method validation concludes the method was rugged and robust.

CONCLUSION

A stability indicating HPLC method was developed which is specific, precise, accurate, linear and robust for the quantification of, Efaviren, Lamivudine, Tenofovir Disoproxil Fumarate. Separation of its potential impurities produced during forced degradation studies and very well separation of Excipients from active pharmaceutical ingredients in the dosage forms, indicates that the developed method was specific and stability indicating. The shorter run times with isocratic mode revels that the method is simple and fast. The same method was subjected for analytical method validation and found to be accurate, precise and linear over the range, the method also found to be rugged.The degradation data resulted that peak purity angle is less than that the peak threshold, hence there is no co-elution, peaks are pure and the method is considered as stability indicating method and can be used for stability samples testing in quality control of the product.

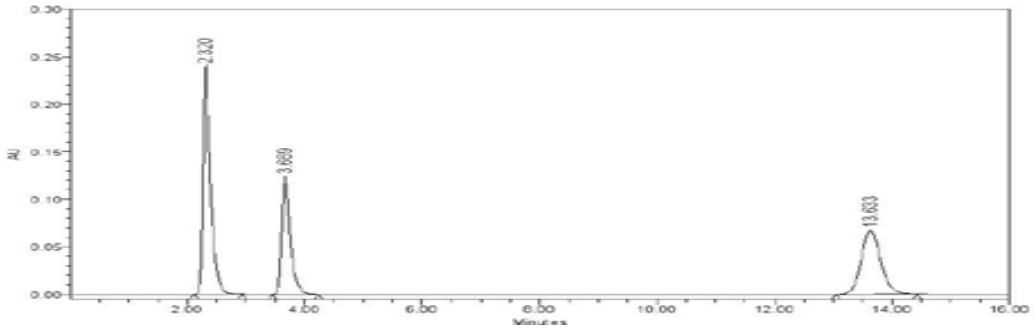

Fig 1: Specimen chromatogram for standard

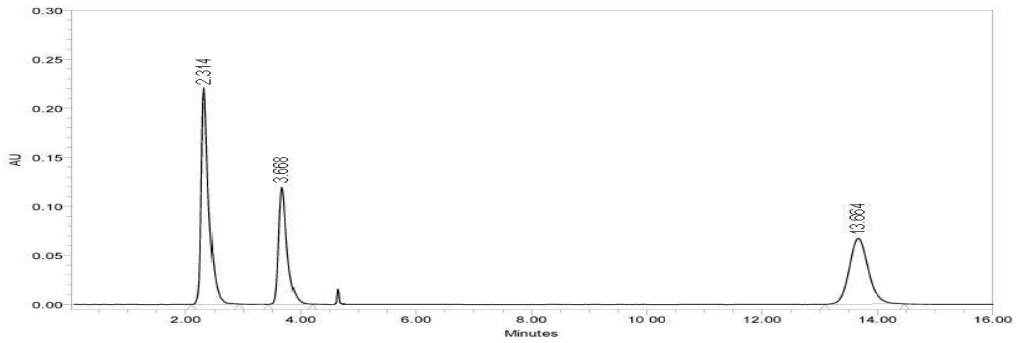

Fig 2: Acid degraded sample

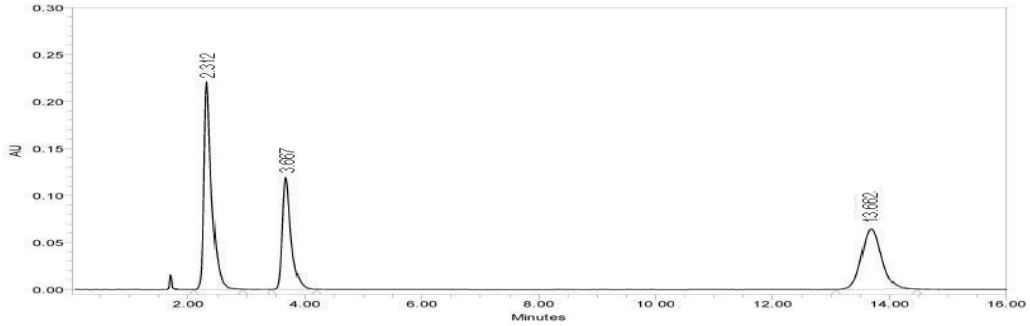

Fig 3: Base degraded sample

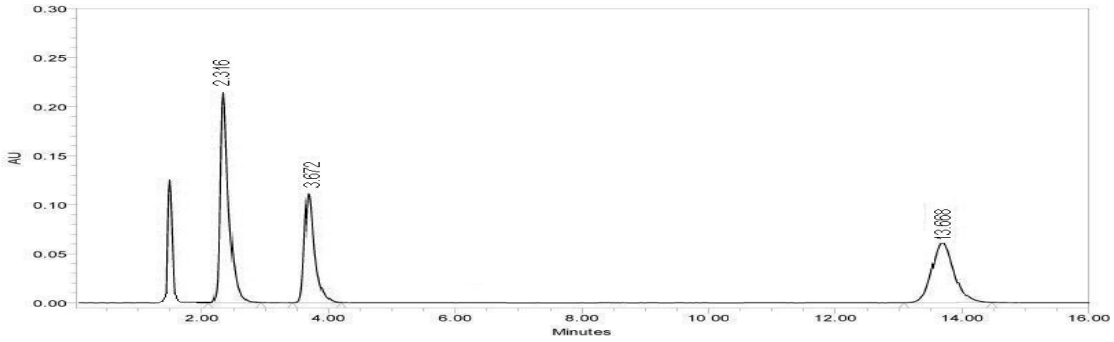

Fig 4: Peroxide degraded sample

Tables 1: ACCURACY OF LAMIVUDINE BYPLACEBO SPIKED RECOVERY METHOD

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2809306	5.4	5.5	101.8%	100.5 %
100%	3834196	10.0	10.0	100.4%	
150%	9194872	15.7	15.6	99.3%	

TABLES 2: ACCURACY OF TENOFOVIR BYPLACEBO SPIKED RECOVERY METHOD

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1614127	5.4	5.3	98.1%	99.4%
100%	2192457	10.0	9.94	99.4%	
150%	5453310	15.7	15.8	100.6%	

TABLES 3: ACCURACY OF EFAVIRENZ BYPLACEBO SPIKED RECOVERY METHOD

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2147664	5.4	5.47	101.2%	99.9%
100%	3008441	10.0	9.97	99.9%	
150%	4891270	15.7	15.5	98.7%	

Tables 4: LINEARITY OF LAMIVUDINE

Linearity level	**Conc. in ppm.**	**Experimental area (a)**	**Predicted area (y)**	**Residuals (b)**
I	25.20	1746278	1715992.6	30285.4
II	30.24	2089734	2134418.5	-44684.5
III	35.28	2561065	2552844.4	8220.6
IV	40.32	2967741	2971270.3	-3529.3
V	45.36	3399404	3389696.2	9707.8
Correlation	0.9991			
Intercept (c)	-376136.9			
Slope (m)	83021.0			

Tables 5: LINEARITY OF TENOFOVIR

Linearity level	**Conc. in ppm.**	**Experimental area (a)**	**Predicted area (y)**	**Residuals (b)**
I	25.13	971665	966601.8	5063.2
II	30.15	1251017	1263228.6	-12211.6
III	35.17	1571641	1559855.4	11785.6
IV	40.2	1849293	1856482.2	-7189.2
V	45.22	2155661	2153109	2552
Correlation	0.9998			
Intercept (c)	-516532.2			
Slope (m)	59030.2			

Tables 6: LINEARITY OF EFAVIRENZ

Linearity level	**Conc. in ppm.**	**Experimental area (a)**	**Predicted area (y)**	**Residuals (b)**
I	50.45	1325229	1311902	13327.4
II	60.54	1523856	1530484	-6628.3
III	70.63	1726162	1749067	-22905
IV	80.72	1980035	1967650	12385.3
V	90.81	2190053	2186232	3820.6
Correlation	0.9990			
Intercept (c)	218988.1			
Slope (m)	21663.3			

Tables 7: SYSTEM PRECISION

Injection	Lamivudine area	Tenofovir area	Efavirenz area
Injection-1	1927340	1116964	1411973
Injection-2	1863389	1089297	1380819
Injection-3	1868477	1088011	1386136
Injection-4	1938553	1134236	1424455
Injection-5	1912180	1113667	1406621
Average	1901988	1108435	1402001
Standard Deviation	34265.2	19680.0	18202.7
%RSD	1.80	1.78	1.30

Tables 8: METHOD PRECISION FOR LAMIVUDINE

Method precision	Test wt(g)	Avg. Weight	Test Area-1	Test Area-2	Avg Area	Mg/Unit	% Assay
Sample-1	45.19	1306	1941061	1969606	1955333	297.25	99.08
Sample-2	44.87		1922031	1919938	1920984	294.11	98.04
Sample-3	44.12		1903001	1874456	1888728	294.08	98.03
Sample-4	45.21		1937255	1912516	1924885	292.49	97.50
Sample-5	45.12		1960091	1969606	1964848	299.16	99.72
Sample-6	44.36		1903001	1899195	1901098	294.41	98.14
						AVG	98.42
						SD	0.82
						%RSD	0.83

Tables 9: METHOD PRECISION FOR TENOFOVIR

Method precision	Test wt(g)	Avg. Weight	Test Area-1	Test Area-2	Avg Area	Mg/Unit	% Assay
Sample-1	45.19	1306	1130155	1146775	1138465	300.80	100.27
Sample-2	44.87		1119075	1117856	1118465	297.62	99.21
Sample-3	44.12		1107995	1099131	1103563	298.65	99.55
Sample-4	45.21		1127939	1109103	1118521	295.40	98.47
Sample-5	45.12		1141235	1119075	1130155	299.07	99.69
Sample-6	44.36		1107995	1105779	1106887	297.93	99.31
						AVG	99.42
						SD	0.60
						%RSD	0.60

Tables 10: METHOD PRECISION FOR EFAVIRENZ

Method precision	Test wt(g)	Avg. Weight	Test Area-1	Test Area-2	Avg Area	Mg/Unit	% Assay
Sample-1	45.19	1306	1429336	1429336	1429336	597.21	99.54
Sample-2	44.87		1415323	1413781	1414552	595.25	99.21
Sample-3	44.12		1401310	1395704	1398507	598.50	99.75
Sample-4	45.21		1426533	1387296	1406915	587.58	97.93
Sample-5	45.12		1443349	1415323	1429336	598.14	99.69
Sample-6	44.36		1401310	1394303	1397806	594.97	99.16
						AVG	99.21
						SD	0.67
						%RSD	0.68

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