Murugan S. et al. / Vol 3 / Issue 1 / 2013 / 29-32.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE, LAMIVUDINE AND EFAVIRENZ IN BULK AND TABLET DOSAGE FORM

Murugan S*, Pranabesh Sikdar, Subhashis Debnath, Niranjan Babu M

* Seven Hills College of Pharmacy, Tirupati-517 561, Andhra Pradesh, India.

ABSTRACT

A simple, sensitive, rapid, economic and accurate first order derivative spectrophotometric method has been developed for estimation of Tenofovir disoproxil fumarate (TDF), Lamivudine (LAM) and Efavirenz (EFV) and in bulk and in tablet dosage form. The wavelengths selected for quantitation were 249 nm for TDF (zero cross for Lamivudine but Efavirenz shows absorbance), 293 nm for Lamivudine (zero cross for Tenofovir disoproxil fumarate and Efavirenz) and 321 nm for Efavirenz (zero cross for Tenofovir disoproxil fumarate and Lamivudine). Beer's law was obeyed in the concentration range of 5-30 µg/ ml, 5-30 µg/ ml and 10-60 µg/ ml for TDF, LAM and EFV. The methods were validated as per ICH guidelines. Statistical analysis proved that the methods were accurate, precise, and reproducible for analysis of TDF, LAM and EFV in tablet dosage form. The wide linearity range, sensitivity, accuracy and simple procedure imply that the proposed technique demonstrated to be appropriate for routine analysis and quality control assay of tablet.

Keywords: Tenofovir disoproxil fumarate, Efavirenz, Lamivudine, First Order Derivative Spectroscopy.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is converted intercellularly to the diphosphate. This diphosphate halts the DNA synthesis of HIV through competative inhibition of reverse transcriptase and incorporation into viral DNA [2]. Chemically, bis(isopropyloxy-carbonyloxymethylester of (R)-9-(2-phosphonomethoxy-propyl) adenine with fumaric acid [1]. TDF has been determined in spiked human plasma by HPLC [4,5]. The estimation of TDF by RP-HPLC has been reported [6]. Lamivudine (LAM), chemically (2R,5S)-4-amino-1-[2-(hydroxyl methyl)-1,3-oxathiolan-5yl]-2(1H)-pyrimidinone [1]. It is converted intercellularly in stages to the triphosphate. This triphosphate halts the DNA synthesis of retroviruses, including HIV, through competitive inhibition of reverse transcriptase and incorporation into viral DNA [2]. The estimation of lamivudine using UV [7,8,9] spectroscopy and HPLC has been reported [3]. Efavirenz (EFV) is chemically designated as (4S)-6-chloro-4-(cyclopropylethylnyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2one [1]. It is an HIV-Protease inhibitors are antiretrovirals that act by binding reversibly to HIV-Protease there by preventing cleavage of the viral precursor polyproteins.

This results in the formation of immature viral particles incapable of infection other cells [2]. EFV has been determined by UV spectroscopic[6] and RP-HPLC[7] methods in single and in combined dosage form.

The method described is rapid, economical, precise, and accurate and can be effectively used for routine quality control analysis of tablets. The developed method was validated as per ICH norms [10-11].

Corresponding Author:- Murugan S Email:- msm_apcp07@yahoo.com

MATERIALS AND METHODS

Instrumentation

The instrument used in the present study was Shimadzu double beam UV/Visible spectrophotometer (Model UV-1700) with spectral band width of 1 nm. All weighing was done on electronic balance (Model Shimadzu AUX -220).

Reagents and chemicals

Analytically pure sample of TDF, LAM and EFV was kindly supplied by Strides Arco Labs. (Bangalore, India). The pharmaceutical dosage form used in this study was a Trioday tablets manufactured by Cipla Ltd (Goa, India) labeled to contain 300 mg of Tenofovir disoproxil fumarate, 300 mg of Lamivudine and 600 mg of Efavirenz I.P. 0.1 M NaOH was used as solvent.

Preparation of standard stock solution

Standard stock solution of Lamivudine and Tenofovir Disoproxil Fumarate were prepared by dissolving, 50 mg of Lamivudine and Tenofovir Disoproxil Fumarate in 100 ml of 0.1 M NaOH, separately to get a concentration of 500 µg/ ml. For Standard stock solution of Efavirenz 100 mg was weighed and transferred into 100 ml volumetric flask, dissolved in 0.1 M NaOH and made up to the volume with more 0.1 M NaOH to get a concentration of 1000 µg/ ml.

Study of spectra and selection of wavelength

In this method, solutions of TDF, LAM and EFV (10 µg/ ml, each), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized for first order. From the overlain spectra of these drugs, wavelength selected for quantitation were 249 nm for TDF (zero cross for LAM but EFV shows absorbance), to estimate the amount of Tenofovir Disoproxil Fumarate at 249 nm the absorbance corrected for interference method was applied. The absorbance of Tenofovir Disoproxil Fumarate was corrected for interference at 249 nm. Thus, the amount of Tenofovir Disoproxil Fumarate was found out, 293 nm for LAM (zero cross for Tenofovir disoproxil fumarate and Efavirenz) and 321 nm for EFV (zero cross for Tenofovir DF and LAM). The overlain first order derivative spectra of TDF, LAM and EFV is shown in Fig.No.1.

Analysis of Tablet Formulation

For the estimation of drugs in the commercial formulation, twenty tablets were weighed accurately. The average weight was calculated and then crushed to obtain fine powder.A quantity of tablet powder equivalent to about 100 mg of EFV was transferred to 50 ml volumetric flask; 25 ml 0.1 M NaOH was added and sonicated for 15 min, volume was then made up to the mark with 0.1 M NaOH. The resulting solution was mixed and filtered through Whatmann filter paper no 41 and filtrate was appropriately diluted to get approximate concentration of 10 µg/ ml of TDF, 10 µg/ ml of LAM and 20 µg/ ml of EFV, the concentration of TDF, LAM and EFV were determined by measuring absorbance of sample solution in first order derivative mode at 249 nm, 293 nm and 321 nm. Concentration of TDF, LAM and EFV in the diluted solution was obtained from calibration curves. Amount of TDF, LAM and EFV in mg/tab was then calculated, by multiplying the concentration obtained with dilution factor. Results of tablet analysis are shown in Table No.1.

Validation

The proposed method was validated as per ICH guidelines.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table 2.

Precision

The reproducibility of this method was determined by analyzing tablets at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision). Coefficient of variance for intra-day assay precision was found to be 0.2136 (for Tenofovir Disoproxil Fumarate), 0.3455 (for Efavirenz) and 0.2670 (for Lamivudine). Inter-day assay precision coefficient of variance was found to be 0.2144 (for Tenofovir Disoproxil Fumarate), 0.3982 (for Efavirenz) and 0.2820 (for Lamivudine).

RESULTS AND DISCUSSION

The method discussed in the present work provide a convenient and reliable way for quantitative determination of TDF, LAM and EFV in combined dose tablet formulation. The quantitative determination was carried out at wavelength range and 249 nm (for TDF), 293 nm (for LAM) and 321 nm (for EFV). Linearity for TDF and LAM was observed in the concentration range of 5-30 µg/ml and EFV was found to be linear in the concentration range of 10-60 µg/ml. Percent label claim for TDF, LAM and EFV in tablet analysis was found in the range of 99.30 to 99.75 %. Percent recovery for TDF, LAM and EFV and LAM was found in the range of 99.28% to 100.78 % with standard deviation well below 2 indicating accuracy of the method. Intra-day and Inter-day precision studies were carried out by analyzing tablet formulation, by three times on the same day and on three different days, respectively. Standard deviation and coefficient of variance for intra-day and inter-day precision studies was satisfactorily low indicating high degree of precision and reproducibility of proposed methods.

Table 1. Results of Analysis of Tablets

Drug	Lable Claim (mg)	% Label Claim estimated* (Mean ± S.D)	% R.S.D
TDF	300	99.75 ± 0.3070	0.3077
LAM	300	99.64 ± 0.3469	0.3481
EFV	600	99.30 ± 0.1867	0.1880

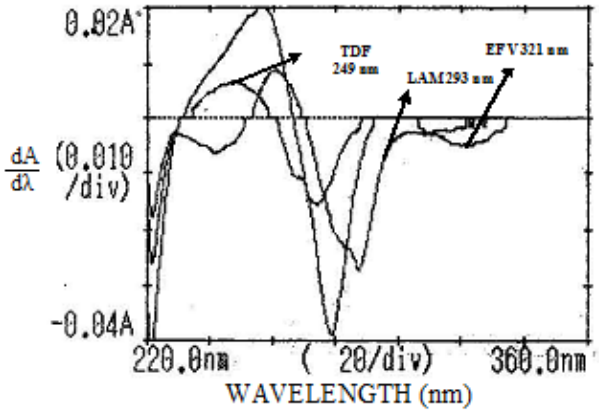
*Mean of six determinations, R.S.D is relative standard deviation

Table 2. Recovery Studies

Drug	Sample No.	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated * (µg/ml)	Amount recovered (µg/ml)	% Recovery*	S.D	% R.S.D	S.E
TDF	1 2 3	9.9754 9.9754 9.9754	8.04 9.87 11.97	18.1010 19.8889 22.0464	8.1256 9.9135 12.0710	101.06 100.44 100.84	0.3163	0.3139	0.0351
					Mean	100.78			
LAM	1 2 3	9.9642 9.9642 9.9642	7.98 9.99 12.00	17.9623 19.8367 21.9252	7.9981 9.8725 11.9610	100.22 98.82 99.65	0.7057	0.7087	0.0784
					Mean	99.56			
EFV	1 2 3	19.8613 19.8613 19.8613	17.02 20.07 23.89	36.3889 40.0591 43.7751	16.5276 20.1978 23.9138	97.10 100.63 100.09	1.9019	1.9157	0.2113
					Mean	99.28			

*Mean of Three Observation

Fig.1. First order derivative overlain spectra of 10µg/ml of TDF, LAM and EFV respectively



CONCLUSION

The validated First order derivative method employed here proved to be simple, economical, rapid, precise and accurate. Thus these can be used for routine simultaneous estimation of TDF, LAM and EFV in tablet dosage form instead of processing and analyzing each drug separately.

REFERENCES

1. Indian pharmacopoeia, New Delhi: The Indian Pharmacopoeial Commission, Ghaziabad, 2007, 1071, 1782 and 1276.
2. 2. Sean C Sweetman, editors, Martindale: The complete drug reference, 34 th ed. London: The Pharmaceutical Press; 2005, 639,648 and 655.
3. Mangaonkar K, Desai A. Simultaneous estimation of emtricitabine, tenofovir disoproxilfumarate and efavirenz from tablets by reverse phase high performance chromatography method. *Indian Drugs*, 45, 2008, 188-92.
4. Sentenac S, Fernandez C, Thuillier A, Lechat P, Aymard G. Sensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci*, 24, 2001, 317-24.

5. Kandagal PB, Manjunatha DH, Seetharamappa J, Kalanur SS. RP-HPLC method for the determination of tenofovir in pharmaceutical formulations and spiked human plasma. *Anal Lett*., 41, 2008, 561-70.
6. Mangaonkar K, Desai A. Simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in tablets by isocratic reverse phase high performance liquid chromatography method. *Indian Drugs*, 45, 2008, 119-22.
7. Uslu B, Ozkan SA. Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography-UV methods. *Anal Chim Acta*., 466, 2002, 175-85.
8. Kapoor N, Khandavilli S, Panchagnula R. Simultaneous determination of lamivudine and stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography. *J Pharm Biomed Anal*., 41, 2006, 761-5.
9. Sankar G, Reddy MV, Rajendra Kumar JM, Murthy TK. Spectrophotometric determination of lamivudine and stavudine. *Indian J Pharm Sci*., 64, 2002, 504-6.
10. Code Q2A-Text on Validation of Analytical Procedure Methodology Step-3 Consensus Guideline, ICH Harmonised Tripartite Guideline, 2005.
11. Code Q2A-Text on Validation of Analytical Procedure Methodology Step-3 Consensus Guideline, ICH Harmonised Tripartite Guideline, 1994.