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

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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 competitive inhibition of reverse transcriptase and incorporation into viral DNA [2]. Chemically, bis(isopropyloxy-carbonyloxy methyl-ester 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-5-yl]-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-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one [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 spectroscopic6 and RP-HPLC7 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].
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 Disoprorxil 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 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

<table>
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<tr>
<th>Drug</th>
<th>Label Claim (mg)</th>
<th>% Label Claim estimated* (Mean ± S.D)</th>
<th>% R.S.D</th>
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<tr>
<td>TDF</td>
<td>300</td>
<td>99.75 ± 0.3070</td>
<td>0.3077</td>
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<tr>
<td>LAM</td>
<td>300</td>
<td>99.64 ± 0.3469</td>
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<td>EFV</td>
<td>600</td>
<td>99.30 ± 0.1867</td>
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*Mean of six determinations, R.S.D is relative standard deviation

Table 2. Recovery Studies

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<th>Drug</th>
<th>Sample No.</th>
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<th>Amount added (µg/ml)</th>
<th>Amount estimated *(µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>% Recovery*</th>
<th>S.D</th>
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*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.


