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Development and validation of UV Spectrophotometric method for simultaneous estimation of Lamivudine and Tenofovir Disproxil Fumarate in the combined dosage form

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ABSTRACT

A Simple, precise, accurate and economical spectrophotometric method was developed and validated for simultaneous estimation of Lamivudine (LAM) and Tenofovir disoproxil fumarate (TDF) in combined dosage form. In simultaneous equation method, LAM and TDF were quantified using their absorptivity values at selected wavelengths, viz., 276nm and 260nm respectively in water solvent system. LAM and TDF obeyed Beer's law in the concentration range of 5-25µg/ml. Different analytical parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined as per ICH guidelines (Q2A & Q2B). % Recovery for both the drugs were in the range of 98.31-98.94% indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The simultaneous equation method permits simple, rapid and direct determination of LAM and TDF in commercially available combined dosage form without previous separations and can therefore be used for routine analysis.

Keywords: Lamivudine, Tenofovir disoproxil fumarate, Spectrophotometric analysis, Simultaneous equation method.

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INTRODUCTION

Tenofovir disoproxil fumarate(TDF) (a prodrug of tenofovir) ({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy} methyl)phosphonic acid), belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. *In vivo* TDF is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate [1]. Lamivudine (LAM) (4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one) is nucleoside analogues with a structure that consists of a pyrimidine base which is N-substituted at the 1-position with a 3'-thia derivative (1,3-oxazolidine) of the ribose moiety that is characteristic of nucleosides. It is reverse trascriptase inhibitor and zalcitabine analog in which a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat HIV-1 and hepatitis B (HBV). This compound belongs to the class of organic compounds known as 3'-thia pyrimidine nucleosides [2]. Both the drugs are marketed as combined dose tablet formulation in the ratio of 300:300mg LAM: TDF. Literature survey reveals that TDF is estimated individually by UV [3], derivative-HPLC [4], Plasma RP-HPLC [5, 6] and Plasma LC/MS/MS [7-9] methods. Similarly for LAM, HPLC [10], Titrimetry [11, 12] and HPLC in plasma [13-15] were reported. The purpose of this study was to develop simple, rapid, precise and accurate UV method for the simultaneous estimation of TDF and LAM in pure and in combined tablet dosage form by Simultaneous equation method and validate as per International Conference on Harmonization (ICH) guidelines.

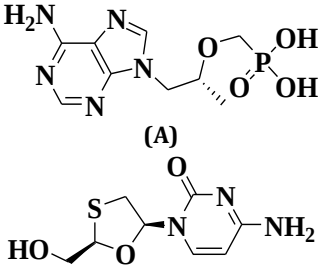

Figure 1 Chemical structure of (A) Tenofovir disoproxil fumarate (B) Lamivudine

MATERIALS AND METHODS

Reagents and chemicals

Working standards of pharmaceutical grade LAM and TDF were obtained as gift samples from Scan Research Laboratories, Bhopal. The tablet dosage form, TENVIR - L, manufactured by Cipla Limited, Mumbai, India (Label Claim: LAM 300 mg and TDF 300 mg), was procured from the local pharmacy. Milli Q water was used throughout the study. All the chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Method development

Standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml Milli Q water in 100 ml volumetric flask and the flask was sonicated for about 10 min to solubilizing the drug and the volume was made up to the mark with Milli Q water to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of LAM and TDF and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with Milli Q water that gave concentration of 100µg/ml (Stock-B).

Determination of λ_{max}

Standard solutions of 50µg/ml of LAM and 2µg/ml TDF were prepared separately from respective sub-stock solutions. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of LAM and TDF were observed at 276.0 nm and 260.0 nm, respectively. They were scanned in the wavelength range of 200-400 nm and the overlain spectrum was obtained (Figure 2-4).

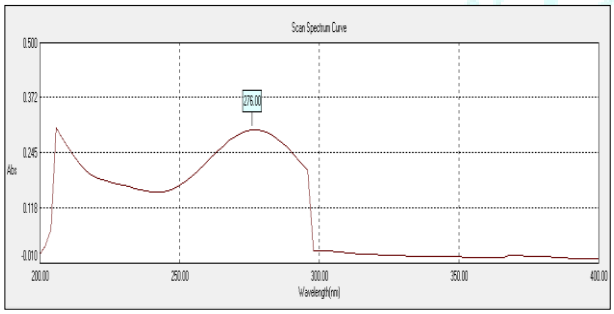

Figure 2 Determination of λ_{max} of LAM

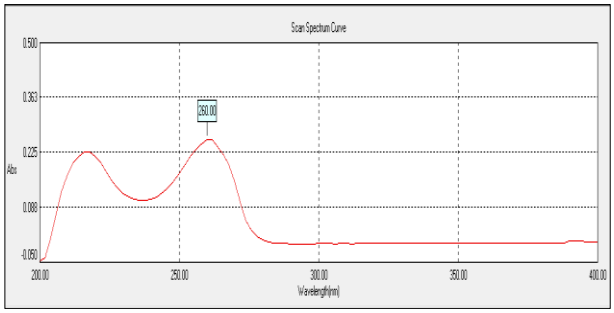

Figure 3 Determination of λ_{max} of TDF

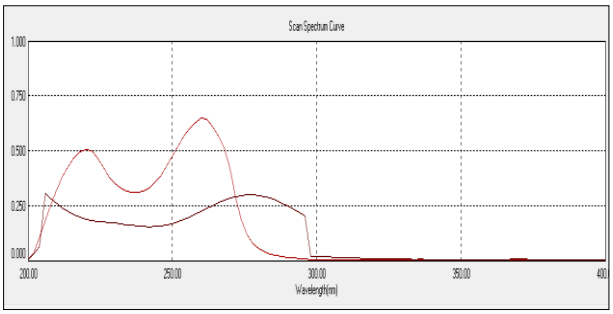

Figure 4 overlay spectra of LAM and TDF

Preparation of calibration curve

From the standard stock solution of each drug, appropriate aliquots were pipette out into a series of 10 ml volumetric flasks. The volume was made up to the mark with Milli Q water to get a set of solutions having a concentration range of 5-25µg/ml for both drugs. Triplicate dilutions of each drug solutions were prepared separately. The prepared working solutions of LAM and TDF were scanned 276 nm and 260 nm, respectively. The absorbance's were recorded and were plotted against the concentrations to obtain their respective calibration curves.

Simultaneous equation method (Vierordt's)

The overlain spectra also showed isoabsorptive points at 269 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method.

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 276.0 nm and 260.0 nm that are λ_{max} of LAM and TDF respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_{LAM} = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \text{..... Eq. (1)}$$

$$C_{TDF} = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \text{..... Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 276.0 nm and 260.0 nm respectively, ax_1 and ax_2 are absorptivities of LAM at λ_1 (276.0 i.e. λ_{max} of LAM) and λ_2 (260.0 i.e. λ_{max} of TDF) respectively and ay_1 and ay_2 are absorptivities of TDF at λ_1 and λ_2 respectively. C_{TDF} and C_{LAM} are concentrations of LAM and TDF respectively. Figure 4 represent the overlain spectra of both the drugs in 1:1 ratio and the criteria for obtaining maximum precision[i.e. absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the LAM and TDF [16].

Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2A & Q2B guidelines 2005 [17].

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in Table 1 and Figure 5 & 6.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies by using standard addition method. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in Table 2.

Precision

Precision was determined by repeatability and Intermediate precision (Day to day, Analyst-to-Analyst) of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in Table 3.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve Table 4.

Assay of Tablet Formulation

Mixed Blends of LAM and TDF were weighed and ground to a fine powder; amount equal to 100mg of TDF was taken in 100ml volumetric flask. The present in this amount of marketed tablets (TENVIR-L tablet, Cipla) was 300mg LAM. Then 50ml of Milli Q water was added and the flask was sonicated for about 10 min to solubilizing the drug present in tablet formulation and the volume was made up to the mark with Milli Q water. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with buffer to get the final concentrations of both drugs in the working range. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times Table 5.

RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis [18]. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials. To develop suitable methods of analysis, various solvents were studied. Based on sensitivity of the method , Milli Q water was selected as a solvent for the methods. Overlain spectra (Figure 4) shows that at λ_{max} of LAM (276 nm) interference of TDF and at λ_{max} of TDF (260nm) interference of LAM occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with 99.666± 0.039 (LAM), 98.311±0.145 (TDF) and 98.37±0.921 (LAM), 98.70±0.513 (TDF) respectively. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine LAM and TDF simultaneously.

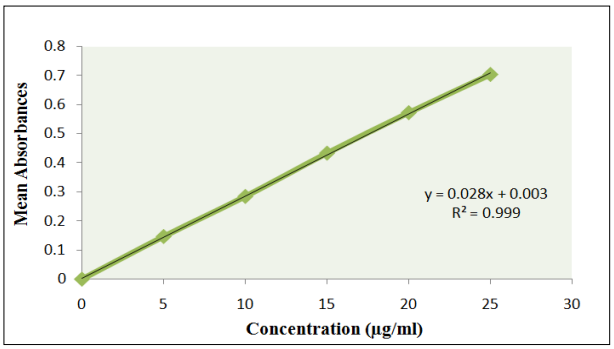

Figure 5: Calibration Curve of LAM

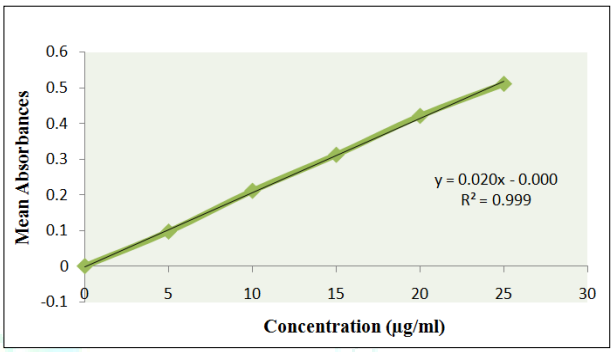

Figure 6: Calibration Curve of TDF

Table 1 Results of Linearity of LAM and TDF

PARAMETER	LAM	TDF
Concentration (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.028	0.020
Intercept (c)*	0.003	-0.000

*value of five replicate

Table 2 Results of Recovery Study

% LEVEL	% MEAN±SD*	
	LAM	TDF
80%	98.30±1.125	98.94±0.798
100%	98.37±0.921	98.70±0.513
120%	98.31±1.338	98.33±1.075

* Value of three replicate and five concentrations.

Table 3 Results of Precision

PARAMETER	% MEAN±SD*	
	LAM	TDF
Repeatability	98.719±0.166	98.887±0.143
	Intermediate precision	
Day to day	99.075±0.146	99.183±0.098
Analyst-to-Analyst	98.847±0.143	99.556±0.093
Reproducibility	99.666± 0.039	98.311±0.145

* Value of five replicate and five concentrations

Table 4 LOD and LOQ OF LAM and TDF

Name	LOD (µg/ml)	LOQ (µg/ml)
LAM	0.85	2.35
TDF	1.12	3.31

Table 5 Assay of Tablet Formulation

	% Conc. Found	
	LAM	TDF
Mean	97.75	98.47
S.D.	1.480	1.304
% RSD	1.514	1.324

*Average of three replicate

CONCLUSION

A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of LAM and TDF in their tablet formulation. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied for determination and dissolution testing of LAM and TDF in commercial tablet formulations.

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