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Development and Validation of First-Derivative Spectrophotometric Method for the Simultaneous Estimation of Lamivudine and Tenofovir disoproxil fumerate in Pure and in Tablet Formulation

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ABSTRACT

This paper describes validated First Derivative Spectrophotometric method for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumerate in pure and in formulation. The solutions of standard and sample were prepared in distilled water. Quantitative determination of the drugs was performed at 287 nm and at 249 nm (N = 1; Δλ = 1) for Lamivudine and Tenofovir disoproxil fumerate, respectively. Proposed method was evaluated for the different validation parameters. The specificity test showed that there was no interference from excipients commonly found in the commercial pharmaceutical formulations at the analytical wavelengths of LAM and TDF. Quantification was achieved over the concentration range of 5 – 30 µg/ ml for Lamivudine and 10 – 60 µg/ ml for Tenofovir disoproxil fumerate. The mean recovery was 100.27 ± 1.2511 and 100.70 ± 1.0604 % for LAM and TDF, respectively. This method is simple, precise, and sensitive and applicable for the simultaneous determination of LAM and TDF in pure powder and formulation.

Keywords: Lamivudine, Tenofovir disoproxil fumerate, First Derivative Specrophotometry, Method Validation.

INTRODUCTION

Lamivudine (LAM) is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 4 – amino – 1 – [(2R, 5S) – 2 – (hydroxyl methyl) – 1, 3 – oxathiolan – 5 – yl] – 1, 2 – dihydro pyrimidin – 2 – one. It can inhibit both types (I and II) of HIV reverse transcriptase and also the reverse transcriptase of Hepatitis B. Tenofovir disoproxil Fumarate (TDF) is fumaric acid salt of the bis isopropoxy carbonyl oxy methyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-

[[(isopropoxcarbonyl)- oxy] methoxy] phosphinyl] methoxy] propyl] adeninefumarate [1-3]. Fig.1 show the nucleotide reverse transcriptase inhibitors (NtRTIs) used in combination for the treatment of HIV infection. Lamivudine is official in IP [4], BP [5] and USP [6]. TDF is official in IP [7]. Literature survey reveals that TDF is estimated individually by UV [8], derivative-HPLC [9], Plasma RP-HPLC [10-11] and Plasma LC/MS/MS [12-14] methods. Similarly for LAM, HPLC [15], Titrimetry [16-17] and HPLC in plasma [18-20] were reported. Few RP-HPLC [21-23] methods were reported for estimation of Emtricitabine, Tenofovir and efavirenz in pharmaceutical formulation. RP-HPLC [24] and LC-MS/MS [25] and HPTLC [26] methods were reported for the simultaneous estimation of Emtricitabine and TDF in human plasma and in formulations. Also UV [27-32], HPLC [33-39], LC – MS [40], HPTLC [41-42] and enzymatic assay [43] methods were reported for the simultaneous estimation of LAM with other antiretero viral drugs. To the best of our knowledge, there is no reported spectrophotometric or pharmacopoeial method for simultaneous determination of LAM and TDF in pharmaceutical formulations, previous to our work. Thus, efforts were made to develop fast, selective and sensitive analytical method for the estimation of LAM and TDF in their combined dosage form using first order derivative spectrophotometry method.

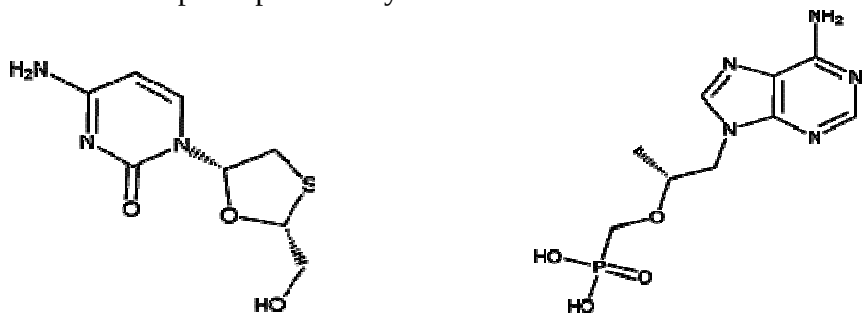

Fig 1: Chemical structures of LAM and TDF

MATERIALS AND METHODS

Experimental

Apparatus

Absorbance was measured, and derivative spectra were recorded over the wavelength range of 200-400 nm in two matched quartz cells with a 1 cm path length using a Shimadzu – 1700 Double beam UV – Visible Spectrophotometer.(Shimadzu, Japan).

Reagents and Materials

Working Standards of pharmaceutical grade LAM and TDF were obtained as gift samples from Strides Arcolabs Bangalore, India. 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. All the chemicals and reagents used were of AR grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Preparation of LAM and TDF Standard Stock Solutions

Standard stock solution of LAM and TDF (25 mg of each) were prepared separately in 50 ml in distilled water to get the final concentration of 1000 µg/ ml. From the standard stock solution of drugs, different dilutions were prepared to construct the calibration graph.

Selection of wavelength for estimation of LAM and TDF

Standard stock solutions of LAM and TDF was diluted appropriately with distilled water to obtain solutions containing 5 – 25 µg/ ml for LAM and 10 – 60 µg/ ml for TDF, respectively. Spectra of these diluted solutions were scanned in the spectrum mode between 200 - 400 nm, with the band width of 2 nm against distilled water as blank. These zero order spectra of LAM and TDF were treated to obtain corresponding first order and first order derivative spectra with an interpoint distance of 1 nm in the range of 200 – 400 nm. The derivative spectra were recorded by using digital differentiation (convolution method) with a derivative wavelength difference (Δλ) of 1nm in the range of 200 – 400 nm. No smoothing of the spectra was found to be necessary. Using memory channels, the first derivative spectra were overlapped. The wavelength 287 nm was selected for the quantification of LAM (where the derivative response for TDF was zero). Similarly, 249 nm was selected for the quantification of TDF (where the derivative response for LAM was zero). A characteristic wavelength (ZCPs) for LAM and TDF was confirmed by varying the concentration of the one component and while the concentration of the other component was constant, and vice versa.

Standard mixture solution

Mixed standard analysis was performed to validate the procedure. From the standard stock solutions of the drugs, different mixed standard solutions of 10:30, 12:28, 14:26, 16:24, 20:20, 22:18, 24:16, 26:14 and 30:10 of LAM and TDF respectively were prepared and analyzed, statistical results were within the range of acceptance i.e. %COV<2.0.

Preparation and analysis of Sample solutions

For the analysis of tablet dosage form, twenty tablets (TENVIR - L) were weighed and their average weight was determined. The tablets were then crushed to a fine powder and the tablet powder equivalent to 50 mg of TDF was transferred to a 100 mL volumetric flask and dissolved in about 80 mL of methanol. The solution was shaken for 5 min. Sonicated for 15-20 min at 100 rpm and made up to the volume with methanol. The solution was filtered through Whatman filter paper # 41. This filtrate was further diluted with mobile phase to get the final concentration of 15 µg mL^{-1} for both the drugs theoretically. The absobance of the solution at their selective wavelengths and the amount of LAM and TDF per tablet was calculated by extrapolating the peak area from the calibration curve.

Validation

The method was validated for linearity, accuracy, precision, repeatability, selectivity, and specificity in accordance with ICH guidelines [44]. Accuracy was studied by measurement of recovery at three different levels of the amount expected in the formulation. Precision was measured both intra-day and inter-day. In the intra-day study the concentrations of all three drugs were calculated three times on the same day at intervals of an hour. In the inter-day study the concentrations of all three drugs were measured on three different days. Specificity of the method was confirmed by loading the the excipients used in tablet formulation with preweighed standard drugs and the absorbance was measured. The limits of detection and quantitation of the method were studied to detect the lowest amount of analyte and quantitative determination of analyte in a sample, respectively

RESULTS AND DISCUSSION

The zero order spectra of standard solutions of LAM and TDF were found to be similar in nature when overlaying (Fig.2). It was observed that LAM and TDF contribute significantly at their corresponding λmax values of absorption. Therefore, it was thought that a derivative graphical method could be used to estimate LAM and TDF in the presence of each other.

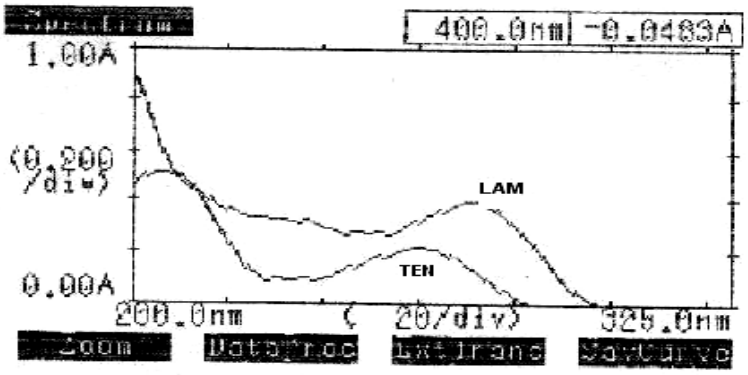

Fig.2. Overlain zero order spectrums of LAM and TEN in distilled water

The derivative spectra of different orders were obtained from the zero order spectra using digital differentiation. The principle advantages of derivative spectroscopy are the improvement of resolution of overlapping absorption bands and the accuracy and precision compared to UV absorption methods; therefore, derivative spectroscopy has been used in quantitative analysis when the analyte to be determined present in admixture with other components [45, 46]. Fig. 3 shows that the overlayed first derivative spectra could be used for determination of LAM and TDF. The spectra present well defined bands for determination of the analytes, and the sensitivities are greater. Thus first derivative was selected and the other derivatives were discarded because they showed insufficient resolution and do not present analytical advantages.

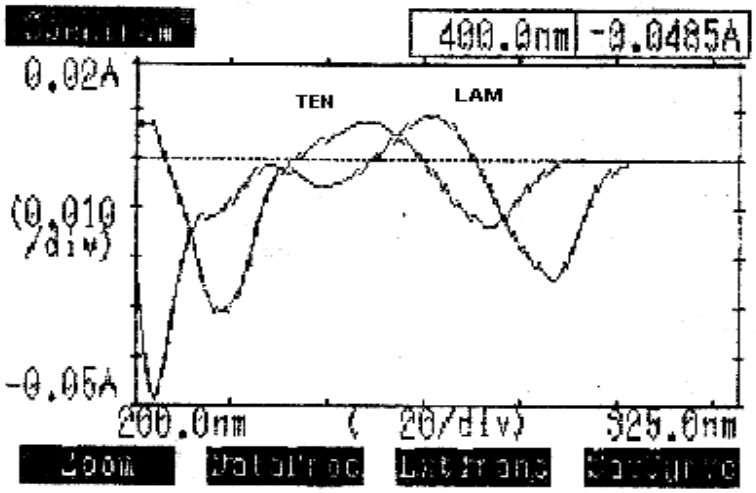

Fig.3. Overlain first derivative spectrums of LAM and TEN in distilled water

The type of solvent, degree of deviation, range of wavelength, and N value were chosen in order to optimize the conditions. The solvent selected was distilled water because it allowed sufficient spectral resolution to be obtained for the application of the peak zero method. The derivative wavelength difference ($\Delta\lambda$) depends on the measuring wavelength range and N values. Generally, the noise decreases by increasing $\Delta\lambda$. The optimal wavelength range should be chosen because broad peaks become sharper, the ratio of signal-to-noise(S/N) increases, and the sensitivity of the method increases by the degree of low pass filtering or smoothing. Therefore, a series of N value (N = 1 – 9) was tested in the first order UV spectrum of LAM and TDF in distilled water. Optimum results were obtained in the measuring wavelength range 200 – 325 nm, N = 1 ($\Delta\lambda$ = 1) for first derivative method. The first derivative spectra of LAM and TDF were found to be appropriate for the determination of LAM and TDF by having separated zero crossing points in distilled water. The first derivative spectrum of LAM has zero absorption at 249 nm, where TDF gives significant derivative response, while spectrum of TDF has zero absorption at 287 nm, where LAM gives the significant derivative response. Therefore, 287 nm was selected for estimation of LAM and 249 nm was selected for the estimation of TDF.

Validation of Proposed Method

Linearity

Linear correlation was obtained between absorbance and concentration of LAM and TDF in the range of 4 – 30 µg/ ml for LAM and 10 – 60 µg/ ml for TDF. Data of regression analysis are summarized in Table 1.

Table 1: Regression analysis of calibration curves for LAM and TEN for the proposed First Derivative Spectrophotometric method

S.No	Parameters	LAM	TDF
1	Concentration Range (µg/ ml)	5 – 30	10 – 60
2	Slope	0.00296	0.00078
3	Standard deviation of Slope	0.00002	0.00001
4	Intercept	0.0000005	0.00003
5	Standard deviation of Intercept	0.00016	0.00013
6	Correlation Coefficient	0.99989	0.99991

Accuracy

The recovery experiments were carried out by the standard addition method. The recoveries obtained were 100.27 ± 1.2511 and 100.70 ± 1.0604 % for LAM and TDF, respectively. The high percentage recovery and low % RSD values indicate that methods are accurate.

Method Precision

The RSD values for LAM and TDF were found to be 0.9906 and 1.2675 %, respectively. The RSD values were found to be below 2% which indicate that the proposed method is repeatable.

Intermediate Precision

The RSD values were found to be below 2% which indicate that the proposed methods are reproducible (Table 2).

Table 2: Summary of validation parameters for the proposed method

S.No	Parameters	LAM	TDF
1	LOD[a] (µg/ ml)	0.02411	0.0557
2	LOQ[b] (µg/ ml)	0.0730	0.1687
3	Accuracy (%)	100.27 ± 1.2511	100.70 ± 1.0604
4	Repeatability (%RSD[c] n[d] = 6)	0.9906	1.2675
	Precision (% RSD)		
6	Interday (n = 3)	0.4808 – 0.6585	0.8657 – 1.3678
7	Intra day (n = 3)	0.8008 – 1.0229	1.1667 – 1.2424

a LOD = Limit of detection. b LOQ = Limit of quantification. c RSD = Relative standard deviation. d n = Number of determination

LOD and LOQ

LOD for LAM and TDF were found to be 0.02411and 0.0557 µg/ ml, respectively. LOQ for LAM and TDF was found to be 0.0730 and 0.1687 µg/ ml, respectively. These data show that microgram quantity of both drugs can be accurately determined.

Specificity

Excipients used in the specificity studies did not interfere with the estimation of either of drugs by the proposed methods. Hence, method was found to be specific for estimation of LAM and TDF.

Robustness

Absorbance variation was found to be less than 1%. Also, no significant change in absorbance was observed during 24 h. No decomposition was observed after 24h. Hence, methods were found to be robust for estimation of LAM and TDF.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine LAM and TDF in their tablet dosage forms. The results obtained for LAM and TDF were comparable with the corresponding labeled amounts (Table 3).

Table 3: Assay results for the combined dosage form using the proposed method

Drug	Label Claim (mg/ tab)	Amount Found * (mg/ tab)	% Label Claim* ± SD	% RSD
LAM	300	302.96	100.99 ± 1.0003	0.9906
TEN	300	300.66	100.22 ± 1.1574	1.1549

CONCLUSION

Proposed method was found to be precise and accurate. The methods can be used for the routine simultaneous analysis of LAM and TDF in pharmaceutical preparations. In spite of the low concentration of LAM, method was successfully used to estimate the amount of LAM and TDF present in the tablet without the need for addition of internal standard or prior separation.

Moreover, the proposed method has the advantages of simplicity, convenience and quantification of LAM and TDF in combination and can be used for the assay of their dosage form.

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