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Simultaneous Determination of Lamivudine and Tenofovir Disproxil Fumarate by UV Spectrophotometric Method

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Abstract
A simple and rapid UV spectrophotometric method has been developed for simultaneous estimation of Lamivudine (LAM) and Tenofovir disoproxil fumarate (TDF). The absorption maxima of both drugs were found at 268nm and 258nm and obeyed Beer's law in the range of 2-10µg/ml (y = 0.059x-0.02; r^2 = 0.998) and 2-10µg/ml (y = 0.0565x-0.017; r^2 = 0.997) respectively for LAM and TDF in water: water (50:50) solvent system. Accuracy and reproducibility of the proposed method was statistically validated by recovery studies. This method is found to be precise and accurate and can easily be employed in the laboratory for the routine estimation of drugs.
Key words: *Tenofovir disoproxil fumarate, Lamivudine, Simultaneous equation, UV spectrophotometry*

INTRODUCTION

Tenofovir [Figure 1] is chemically known as({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid. It inhibit the activity of HIV reverse transcriptase by competing with natural substrate deoxyadenosine 5'-triphosphate and ,after incorporation into DNA ,by DNA chain termination. Lamivudine [Figure 2] is chemically known as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. It is nucleoside reverse transcriptase inhibitor with activity against HIV-1 and hepatitis- B.

Literature survey has revealed a number of analytical methods for determination of both TDF and LAM. TDF was estimated individually by UV8, 13, 14, RP-HPLC15, 16 and HPTLC17. TDF with other drug combinations were determined by UV18, 19, 20, RP-HPLC21, 22 and HPTLC23. LAM was estimated individually by UV12, 24, 25, 26 and its combination with other drugs by UV27, 28 and RP-HPLC29, 30 techniques. Simultaneous estimation of TDF and

LAM with distilled water31 and methanol32 and with acetonitrile and 0.1N HCL (20:80) was reported.

Hence it has much attracted us to carry out the development of simultaneous determination of Lamivudine (LAM) and Tenofovir Disoproxil Fumarate (TDF) by UV spectrophotometric method with water : water (50:50) solvent system.

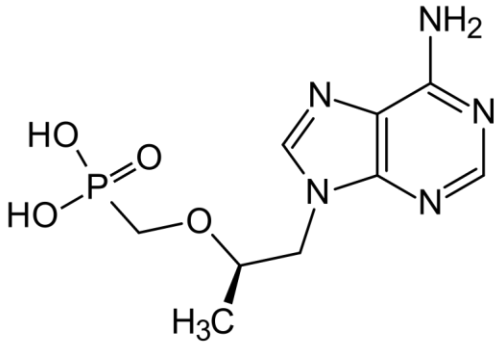

Figure 1: ({[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl]oxy}methyl)phosphonic acid

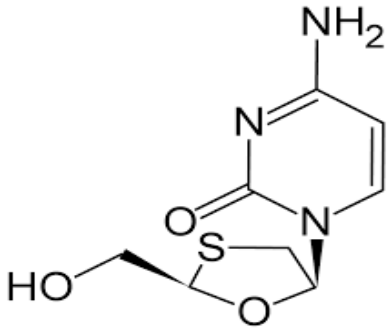

Figure 2: 4-amino-1-[(2R,5S)-2-(hydroxymethyl) -1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one

MATERIAL AND METHODS

Instrument

Schimazu 1800 UV-Vis. Spectrophotometer is a double beam, high speed scanning spectrophotometer. The instrument needs about 1 minute for initialization. The light source used is Deuterium lamp in the UV region and Tungsten halogen lamp in the visible region. To the compact body of spectrophotometer, a computer is attached which helps in data processing and manipulation. Quartz cuvette with path length 1 cm was used.

Reagents and Materials

Working standards of pharmaceutical grade Lamivudine (IP) and Tenofovir Disoproxil Fumarate (IP) were procured locally and other chemicals used were of AR grade and purchased Mylan Laboratories Ltd, Sinner, Nashik, India.

Selection of solvent system

LAM and TDF were dissolved separately in water solvent and final volume was made up with water. The absorbances of LAM and TDF at respective wavelengths were determined.

Preparation of TDF and LAM Standard Stock Solutions

Standard stock solution of LAM and TDF (10mg of each) were prepared separately in 50ml water and made up to 100ml with water to get the final concentration of 200µg/ml.

Selection of wavelength (λmax)

Standard solutions were scanned in the range of 200-400nm, against(50:50) water:water solvent system as reference. LAM(Figure-3) and TDF (Figure-4) were showed absorbance maxima (λmax) at 268nm and 258nm respectively.

Calibration standards

From the standard stock solution of LAM and TDF, different concentrations were prepared respectively in the range of 2-10µg/ml and 2-10µg/ml and measured absorbance at 268nm and

258nm (Figure-5). The calibration curves were plotted (Figure-6and Figure-7) and data presented in Table -1 and Table-2.

Validation parameters

Linearity- Linear correlation was obtained between absorbance and concentration of LAM and TDF in the range of 2 - 10µg/ml and 2 - 10µg/ml respectively. Data of regression analysis was summarized in Table- 9.

Accuracy – It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of LAM and TDF standard was added to preanalysed sample.

Precision – Precision of the method was studied as intra-day and inter- day variation and also repeatability of sample injections. Intra- day precision was determined by analyzing, the three different concentration 4 µg/mL, 6 µg/mL and 8µg/mL of LAM and 4µg/mL, 6 µg/mL and 8µg/mL of TDF respectively, for three times in the same day. Inter day variability was assessed using above mentioned three concentration analysed on two different days, over a period of one week

Repitability– It was performed by injecting sample 10mg/mL of LAM and 10 mg/mL of TDF into the system and measuring the peak area. It was repeated for six times.

Simultaneous equation method

The simultaneous Spectrophotometric determination of TDF and LAM in a acetonitrile : 0.1N HCl (20:80) solvent system is provided without reaction between these two drugs. The amount of TDF and LAM were calculated using the simultaneous equation given below

At λ_1 $A_1 = ax_1bcx + ay_1bcy$

At λ_2 $A_2 = ax_2bcx + ay_2bcy$

$$\mathbf{C(LAM)} = \frac{\mathbf{A2ay1 - A1ay2}}{\mathbf{ax2ay1 - ax1ay2}}$$

$$\mathbf{C(TDF)} = \frac{\mathbf{A1ax2 - A2ax1}}{\mathbf{ax2ay1 - ax1ay2}}$$

Where

A1 = Absorbance of Mixture at 268nm

A2 = Absorbance of Mixture at 258nm

ax1 = Absorptivity of LAM at 268nm

ax2 = Absorptivity of TDF at 268nm

ay1 = Absorptivity of LAM at 258nm

ay2 = Absorptivity of TDF at 258nm

CLAM = Concentration of Lamivudine

CTDF = Concentration of Tenofovir disoproxil fumarate

The wavelengths were selected to coincide with the absorption maxima of two drugs: the absorption spectra of two drugs should not overlap appreciably; so that LAM absorbs strongly at λ1 (268nm) and weakly at λ2 (258nm), and TDF absorbs strongly at λ2 (258nm) and weakly at λ1 (268nm). The absorbances of pure LAM and TDF in the concentration of each 6µg/ml were measured at two wavelengths 268nm and 258nm. Absorptivity values were calculated from the absorbance values of both drugs at two wavelengths. The drug content in the combination was quantified by using above simultaneous equations.

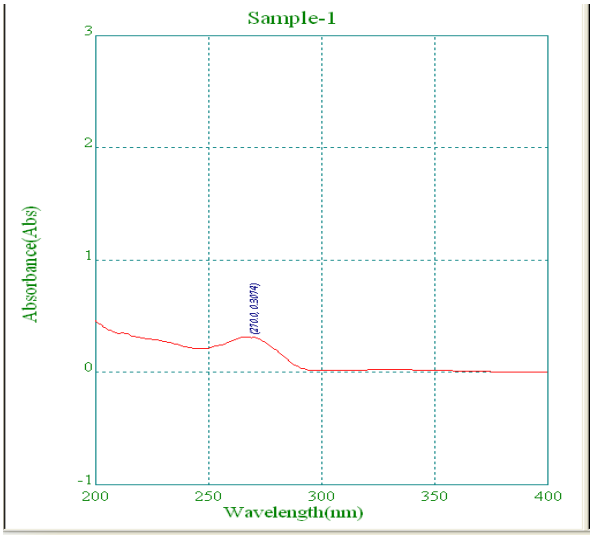

Figure-3: Scanning of LAM

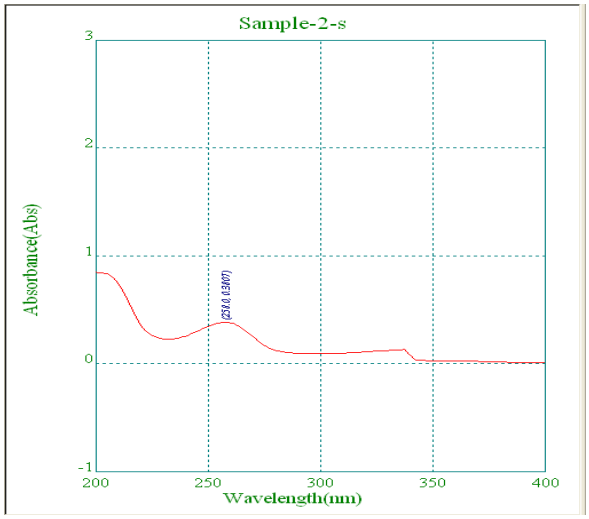

Figure-4: Scanning of TDF

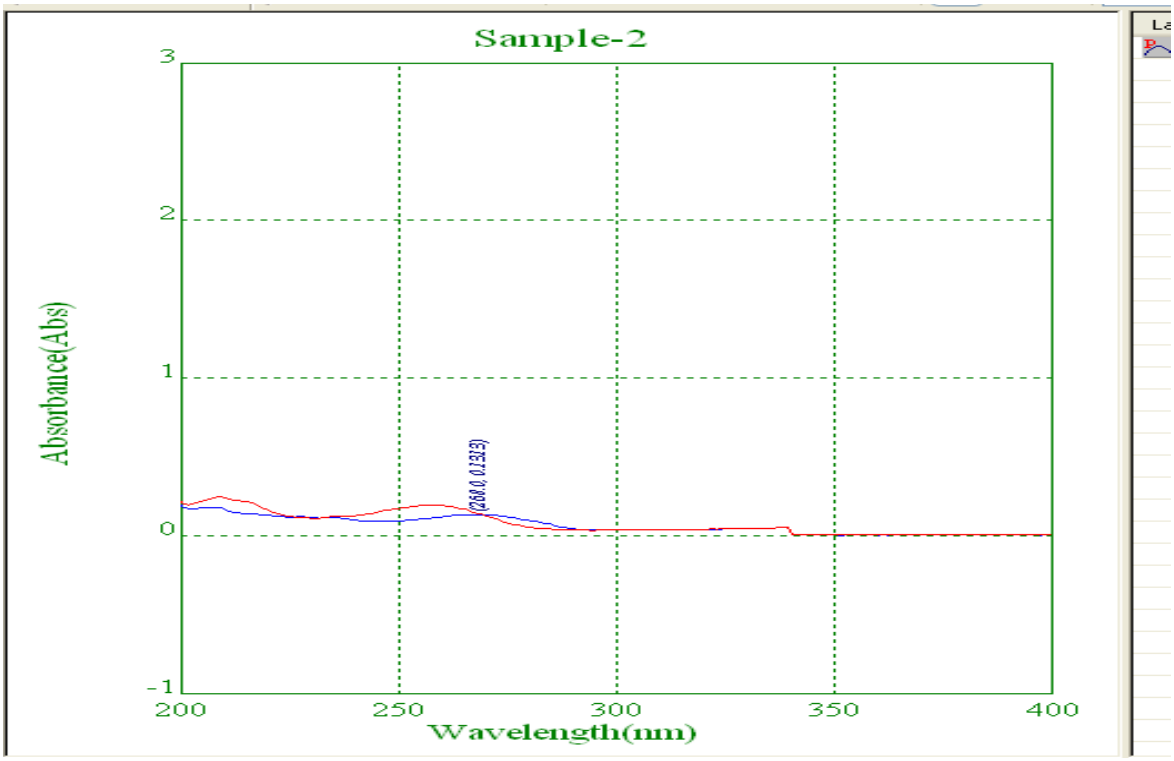

Figure-5: Overlay spectrum of LAM at 268nm and TDF at 25

Table 1: Linearity studies of Lamivudine (LAM)

Concentration of LAM[µg/mL]	Peak Area	± SD	%RSD
2	0.1	0.00	0.62
4	0.22	0.00	0.32
6	0.33	0.01	0.28
8	0.44	0.01	0.13
10	0.58	0.02	0.07

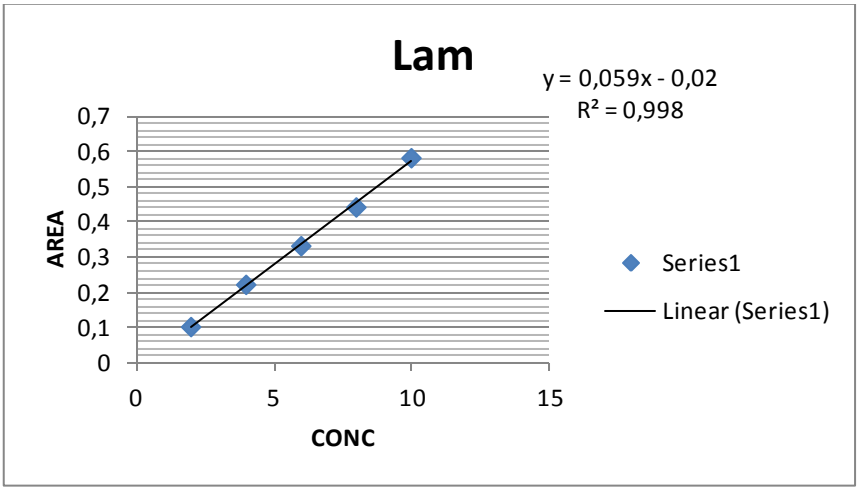

Figure – 6 : Calibration curve of Lamivudine (LAM)

Table 2: Linearity studies of Tenofovir Disproxil Fumarate (TDF)

Concentration of TDF[µg/mL]	Peak Area	± SD	%RSD
2	0.10	0.01	0.14
4	0.21	0.02	0.57
6	0.32	0.00	0.07
8	0.42	0.02	0.05
10	0.56	0.00	0.04

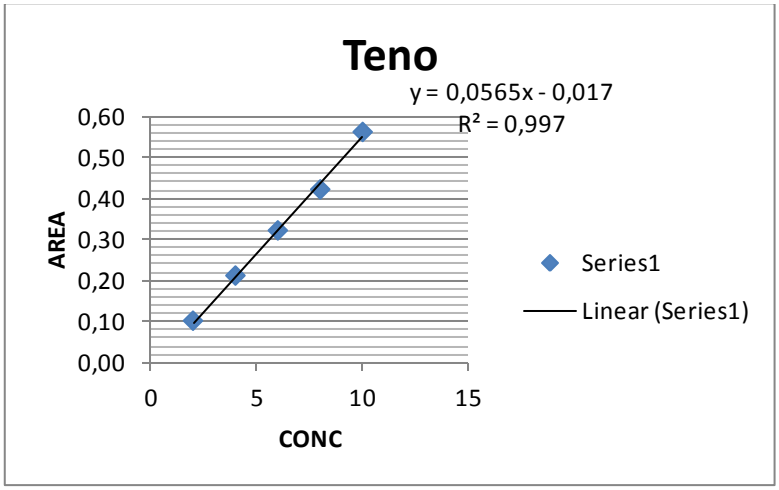

Figure – 7 : Calibration curve of Tenofovir disproxil fumarate (TDF)

Analysis of marketed formulation

Brand Name: Lamivudine and Tenofovir Disproxil Fumarate Tablets IP 300mg/300mg

Total weight of 20 tab wt. . = 21.74gms

Avgr Weight = 1.087gms./Tab

Eq.wt for 10mg= 10 X 1087 /300 = 36.23mg

Take 36.23 mg powder in 50 ml water = 200 µgm/ml each solution

Method Validation

Accuracy– The recovery experiments were carried out by the standard addition method. The recoveries obtained were 97.33±0.3% and

99.46±0.1% for LAM and TDF respectively. The high percentage recovery and low %RSD values indicate that method is accurate.(Table–3&4)

Table 3: Recovery studies of LAM

Label claim (mg/Tab)	Amount Added (mg)	Total Amount found	Amount Recovered(mg)	% RSD	% Recovery
2	1.6(80%)	3.55	1.95	0.31	97.33
2	2(100%)	3.93	1.93	0.74	96.95
2	2.4(120%)	4.39	1.99	0.36	99.75

Table 4: Recovery studies of TDF

Label claim (mg/Tab)	Amount Added (mg)	Total Amount found	Amount Recovered(mg)	% RSD	% Recovery
2	1.6(80%)	3.59	1.99	1.27	99.46
2	2(100%)	4.05	2.05	0.18	102.63
2	2.4(120%)	4.43	2.03	0.31	101.74

Precision - The RSD values for TDF and LAM were found to be 0.3003 and 0.2081% respectively. The RSD values were found to be below 2% which indicate that the proposed method is repeatable (Table-5&6).

Table 5: Recovery studies of LAM

Conc (mg/ml)	Intra-day Amount found(mg/ml)			Inter-day Amount Found(mg/ml)		
	Mean	SD	%RSD	Mean	SD	%RSD
4	0.28	0.00	0.60	0.23	0.00	0.28
6	0.43	0.00	0.80	0.34	0.00	0.10
8	0.55	0.00	0.61	0.45	0.00	0.27

Table 6: Recovery studies of TDF

Conc (mg/ml)	Intra-day Amount found(mg/m)			Inter-day Amount Found(mg/ml)		
	Mean	SD	%RSD	Mean	SD	%RSD
4	0.31	0.00	0.60	0.22	0.00	0.19
6	0.47	0.00	1.03	0.32	0.00	0.09
8	0.52	0.00	0.37	0.43	0.00	0.20

Repeatability-

Table 7: Repeatability studies on LAM

Concentration of LAM (mg/ml)	Peak Area	Amt Found	%Amt Found
8	0.4453	7.88	98.66
8	0.4587	8.11	101.41
8	0.4495	7.95	99.47
8	0.4456	7.89	98.64
8	0.4479	7.93	99.13
	Mean	7.95	99.46
	SD	0.09	1.14
	%RSD	1.17	1.15

Table 8: Repeatability studies on TDF

Concentration of TDF (mg/ml)	Peak Area	Amt Found	%Amt Found
8	0.4209	7.81	97.74
8	0.4266	7.92	99.01
8	0.4281	7.94	99.35
8	0.4213	7.83	97.83
8	0.4255	7.90	98.77
	Mean	7.88	98.54
	SD	0.06	0.72
	%RSD	0.74	0.73

Table 9 : Statistical data of regression equations and validation parameters for Lamivudine (LAM) and Tenofovir disproxil fumarate (TDF) (each value is result of nine separate determinations)

Parameters	LAM	TDF
Wavelength (nm)	268	258
Beer's law limit (µg/ml)	2-10	2-10
Regression equation*		
Intercept (c)	0.02	0.017
Slope (m)	0.059x	0.0565x
Regression coefficient (r2)	0.998	0.997

*y = mx+c; where y = absorbance at respective λ_{max}, x = concentration of the analyte

RESULTS AND DISCUSSION

Literature review indicated that various methods have been reported for the analysis of TDF and LAM by UV, RP-HPLC and HPTLC etc. But no analytical methods were reported for the estimation of these drugs using this solvent system [water: water (50:50)] in UV Spectroscopic by simultaneous equation method.

The absorption maxima of LAM and TDF were found at 268nm and 258nm respectively. In these wave lengths absorbances of LAM and TDF mixture was noted. The results showed an excellent correlation between absorbances and concentration of the drugs. Validation parameters like accuracy, precision and linearity found low %RSD values which indicates that the method is sensitive. The percentage recovery of LAM and TDF were found to be 97.33 ±0.1 and 99.46±0.3 respectively. The main advantage of the proposed method is suitability for routine determination of LAM and TDF without their prior separation.

CONCLUSION

Simple UV spectrophotometric methods were developed for the simultaneous determination of LAM and TDF in bulk. To the best of our knowledge, the present study is the first report for the purpose. The present method succeeded in adopting a simple sample preparation and achieved satisfactory percentage recovery and therefore it can be concluded that use of this method can be save analysis time and money. The proposed method is accurate and precise for the determination of LAM and TDF in combined form. Hence, it can be employed for routine analysis in Quality Control Laboratories.

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