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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE BY UV-SPECTROPHOTOMETER

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ABSTRACT

A simple, rapid and precise method is developed for the quantitative simultaneous determination of Lamivudine and Tenofovir disoproxil fumarate in combined pharmaceutical-dosage forms. The method was based on UV-Spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 290 nm (λ_{max} of Lamivudine) and 287 nm (λ_{max} of Tenofovir) in P^{H}buffer: ACN(60:40v/v). For UV Spectrophotometric method, linearity was obtained in concentration range of 1-50 µg/ml for Lamivudine and 1-50 µg/ml for Tenofovir disoproxil fumarate respectively, with regression 0.995 and 0.997 for Lamivudine and Tenofovir disoproxil fumarate respectively. Recovery was in the range of 99 -103%; the value of standard deviation and % R.S. D was found to be < 2 %; shows 0the high precision of the method., in accordance with ICH guidelines. The method has been successively applied to pharmaceutical formulation and was validated according to ICH guidelines.

KEYWORDS: UV-spectrophotometer, Lamivudine, Tenofovir disoproxil fumarate.

INTRODUCTION

Lamivudine chemically it is (2R, 5S)-4-Aminol [2-(Hydroxy methyl)-1,3-oxathiolan-5yl]-2(1H)-pyrimidinedione . Lamivudine and Tenofovir disoproxil fumarate (TDF), both drugs are

active against hepatitis B virus (HBV). Due to its potency, high genetic barrier to resistance and safety during pregnancy. TDF is used to prevent HBV transmission from mother to child.[4] The multi component dosage form proves to be effective due to the combined mode of action on the body. **The specific aim/objective of the research was** to develop UV Spectrophotometric method for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate in bulk and dosage form and validate the proposed method in accordance with ICH guidelines for the intended analytical application. Tenofovir is the first nucleotide analogue approved for HIV-1 treatment. Tenofovir is also determined in plasma .Both Tenofovir disoproxil fumarate and lamivudine are official in IP and marketed as combined tablet dosage formulation in the ratio is a 300:300 mg Lamivudine and tenofovir disoproxil fumarate and were named as TENVIR-L as a brand name which was produced by Cipla Ltd.

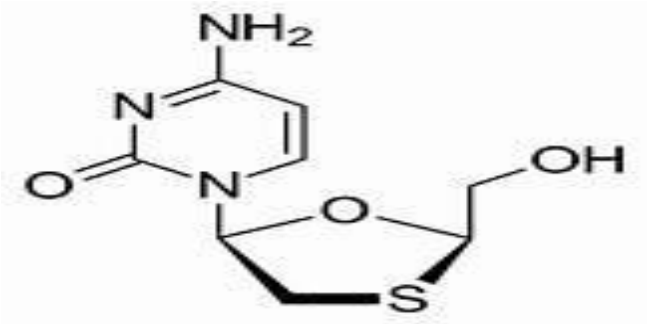

Fig 1: Structure for lamivudine.

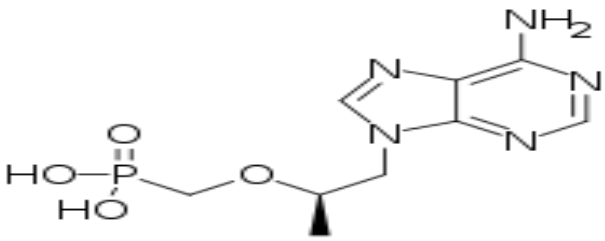

Fig. 2: Structure for tenofovir.

MATERIALS AND METHODS

MATERIALS AND REAGENTS

Working standards of Lamivudine (99.9%), Tenofovir disoproxil fumarate (99.8%) were obtained from Aurobindo pharmaceuticals Limited. Tablet containing 300 mg Lamivudine, 300 mg Tenofovir disoproxil fumarate as per label claim in combinations were procured from Aurobindo pharmaceuticals, HPLC grade methanol, acetonitrile, potassium dihydrogen phosphate and orthophosphoric acid were obtained from Merck, India.

PREPARATION OF STANDARD SOLUTION

Pure drug samples of Lamivudine and tenofovir disoproxil fumarate were dissolved separately in acetonitrile: p^H buffer (40:60 v/v) so as give several dilutions of standard in the concentration rage of 50-250 mcg/ml for Lamivudine and 50-250 mcg/ml for Tenofovir disoproxil fumarate respectively. All dilutions were scanned in wave length rage of 200-350nm.

Simultaneous equation method based on the principle that, the total absorbance of the components in a mixture is the sum of individual absorbance two wavelength selected to frame the simultaneous equation method were at 289 and 291 nm for calibration curves stock solutions of Lamivudine and Tenofovir disoproxil fumarate in the concentration range of 1-60mcg/ml and 1-60mcg/ml. The absorbance of Lamivudine and Tenofovir disoproxil fumarate were measured at 289 nm and 291 nm, calibration curves were plotted. The absorptivity's of both the drugs at both the wavelengths were determined.

PREPARTION OF SAMPLE SOULTIONS

Twenty tablets were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 300 mg of Lamivudine and 300 mg of tenofovir were transferred into a 100ml volumetric flask. The contents were ultrasonicated for 10min with methanol, made to volume and filtered through Whatman filter paper NO-41. The solution was further diluted with diluent to give concentrations of 10mcg/ml and 10mcg/ml of Lamivudine and Tenofovir respectively. Absorbances of those solutions were measured at 289 and 291nm respectively.

METHOD VALIDATION

Linearity & Range

The linearity of calibration curves (absorbance Vs concentration) in pure drug solution was checked over the concentration ranges of about 1-50 and 1-50 for Lamivudine and tenofovir disoproxil fumarate. The results were show in the table 2.

Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at level of 80%, 100%,120%. The recovery studies were carried out three times and percentage recovery and percentage relative standard deviation of the recovery were calculated and shown in table 3.

Assay

Twenty tablets were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 300mg of Lamivudine and 300 mg of tenofovir were transferred into a 100 ml volumetric flask. The contents were transferred into a 100 ml volumetric flask. The contents were ultrasonicated for 10 min with methanol, made to volume and filtered through Whatman filter paper No-41. The solution was further diluted with diluent to give concentration of 10mcg /ml and 10mcg/ml of lamivudine and tenofovir respectively. The results are shown below in table 4.

Precision

The data for Intraday and Intraday precision studies at three different concentrations in the linearity range. The %RSD values for Intraday and Intraday precision were <2% indicating that the method was sufficiently precise. The results were shown in table 6.

LOD&LOQ

The LOD &LOQ were separately determined based on standard deviation of Y- intercept of the calibration curve. The standard deviation of the Y-intercept and the slope of the calibration curves were used to calculate the LOD& LOQ by using the equations 3.3* std. dev/slope for LOD, 10*std dev/slope for LOQ. The result was shown in the table 7.

RESULTS AND DISCUSSION

Results for UV-Spectrophotometer

The absorbance and absorptivity's values at the particular wavelength of Lamivudine and Tenofovir disoproxil fumarate can be obtained from the following table λ max of Lamivudine=291 nm, λ max of Tenofovir=289 nm. Both drugs are soluble in Methanol and Acetonitrile.

Table 1: Absorbance and values at the particular wavelength of Lamivudine and Tenofovir and Tablet.

S.NO	Name of Drug	λ_1 at 289 nm	λ_2 at 291 nm
1.	Lamivudine	ax_1=0.03680233	ax_2=0.0371652
2.	Tenofovir	ay_1=0.02731832	ay_2=0.0257032
3.	Tablet	A_1=1.092	A_2=1.084

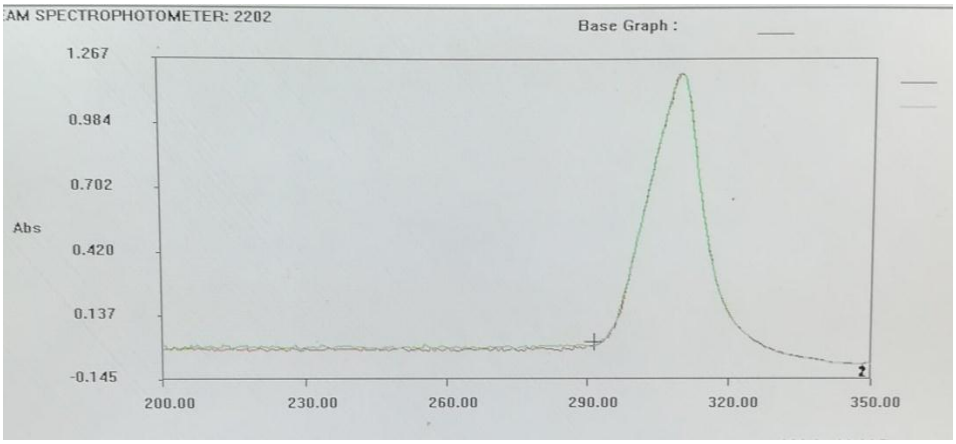

Fig. 3: Overlain Spectra of Lamivudine & Tenofovir.

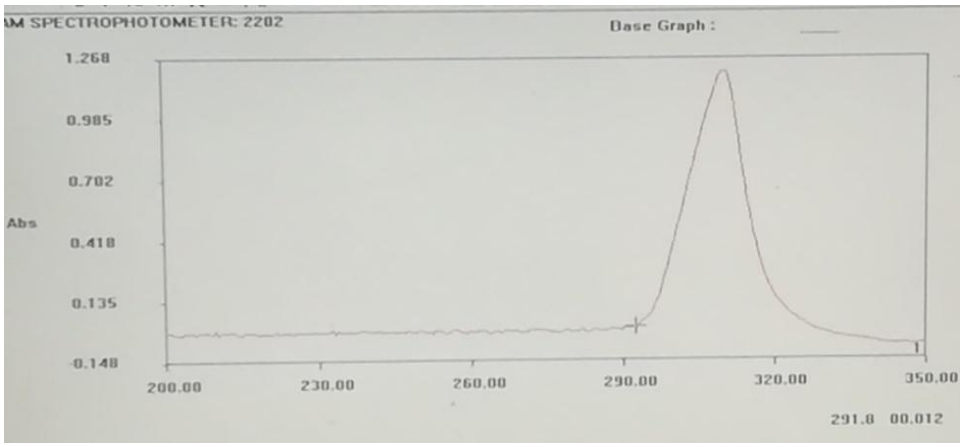

Fig. 4: Spectra of Lamivudine.

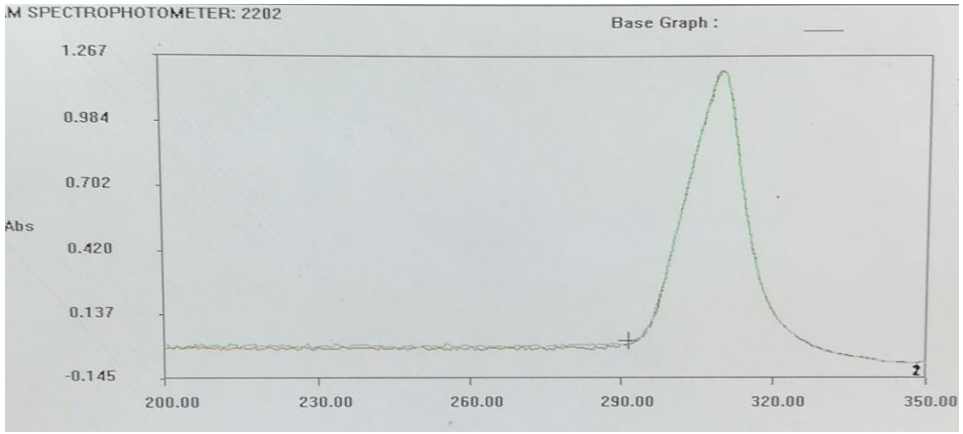

Fig. 5: Spectra of Tenofovir

$C_{x=}$ $A_1*ay_2-A_2*ay_1/ (ax_2*ay_1) -(ax_1*ay_2)$

C_y $= A_2*ax_1-A_1*ax_2/ (ax_2*ay_1) -(ax_1*ay_2)$

Therefore C_x= 20.28 µg/ml, C_y=9.952 µg/ml.

Linearity

Table 2: Linearity Values of Lamivudine and Tenofovir.

Linearity of Lamivudine

S. NO.	Concentration	Absorbance
1.	10	0.325
2.	20	0.592
3.	30	0.876
4.	40	1.312
5.	50	1.542

Linearity of Tenofovir disoproxil fumarate

S.NO	Concentration	Absorbance
1.	10	0.360
2.	20	0.755
3.	30	1.032
4.	40	1.402
5.	50	1.786

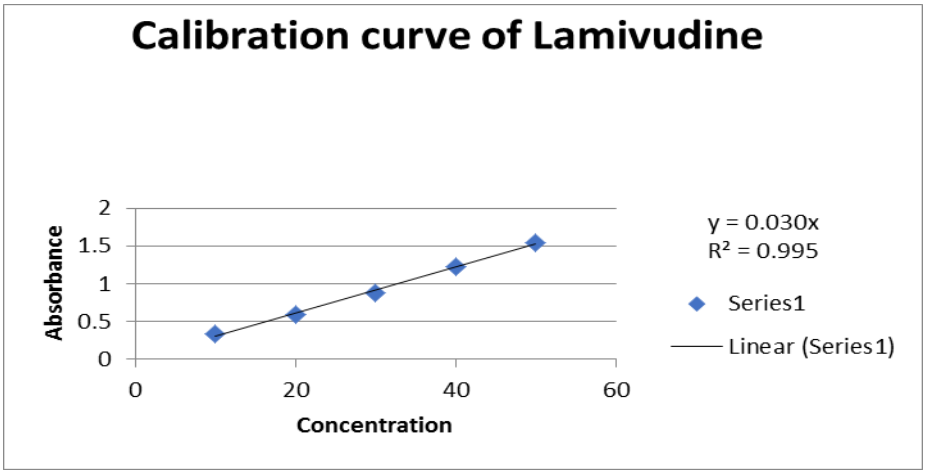

Fig. 6: Calibration curve of Lamivudine.

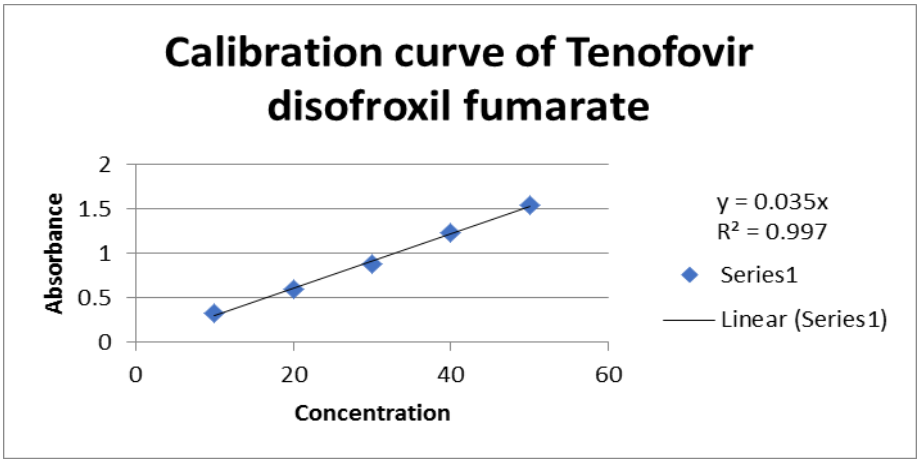

Fig. 7: Calibration curve of Tenofovir disoproxil fumarate.

Accuracy

Table 3: Recovery Reports of Lamivudine and Tenofovir

Drug	QC Conc. (µg/ml)	Amount of Drug added	Amount of Drug found(µg/ml) Mean± S.D	% RSD	% Recovery
Lamivudine	5	80% 100% 120%	8.83±0.049 10.04±0.028 11.1±0.028	0.554 0.028 0.254	98.56 100.6 101.7
Tenofovir	5	80% 100% 120%	9.04±0.014 9.93±0.021 10.88±0.014	0.155 0.213 0.129	100.3 99.23 99.2

Table 4: Accuracy values.

Drug	Label claim mg/tablet	Amount added	Amount found	% Recovery	%RSD
Lamivudine	300	300 mg	30.28 mg	100.4	0.282
Tenofovir	300	300 mg	30.11mg	99.5	0.132

Precision

Table 5: Intraday studies of Lamivudine and Tenofovir disoproxil fumarate.

DAY	Lamivudine				Tenofovir			
	289 nm		291 nm		289 nm		291 nm	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
1.	0.521	0.221	0.625	0.186	0.393	0.39	0.358	0.967
2.	0.464	0.777	0.561	0.617	0.355	0.281	0.290	0.689

Table 6: Intraday studies of Lamivudine and Tenofovir disoproxil fumarate.

	Lamivudine				Tenofovir			
	287 nm		290 nm		287 nm		290 nm	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
1.	0.521	0.221	0.62	0.186	0.397	0.39	0.358	0.967
2.	0.512	0.406	0.61	0.433	0.375	0.38	0.336	0.595
3.	0.484	0.206	0.584	0.342	0.361	0.319	0.312	0.977

LOD & LOQ

Table 7: LOD & LOQ

	Lamivudine	Tenofovir
LOD	0.264 µg/ml	0.331 µg/ml
LOQ	0.816 µg/ml	1 µg/ml

6. Range

Beers law limit for Lamivudine = 1-50µg/ml

Beers law limit for Tenofovir = 1-50µg/ml

Table 8: Parameters of UV-Spectrophotometer.

Validation Parameters	Lamivudine	Tenofovir
Mobile phase	ACN:PH buffer (60:40 v/v)	ACN:PHbuffer(60:40 v/v)
Detection wavelength	291 nm	289 nm
Beer's limit	1-50µg/ml	1-50µg/ml
Linearity	10-50 µg/ml	10-50 µg/ml
R^2	0.995	0.997
LOD	0.264 µg/ml	0.331 µg/ml
LOQ	0.816 µg/ml	1 µg/ml
Precision	% RSD < 2	% RSD < 2
Recovery	98-101%	99-101%

CONCLUSION

The Proposed UV-Spectrophotometric method is suitable technique for simultaneous determination of Lamivudine and Tenofovir in Fixed Dose Combinations (FDCs) without any interferences form each other. All the parameters for both the drugs met the criteria of ICH guidelines for method validation. The UV Spectrophotometric method is rapid, simple and cost effective. The developed method may be recommended for routine and QC analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination of Lamivudine and Tenofovir.

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