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Six Spectroscopic Methods for Simultaneous Estimation of Lamivudine and Tenofovir in Tablets

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ABSTRACT

Six new, simple, rapid, sensitive, accurate, precise and economical spectrophotometric methods were developed and validated for simultaneous determination of Lamivudine (LAM) and Tenofovir (TEN) in bulk and pharmaceutical dosage forms. Method A is Simultaneous equation method, where LAM and TEN were determined at 270.8 nm and 260.6 nm. Method B is first derivative zero crossing point method (FDZ) where LAM and TEN were determined at 260 nm and 249 nm, respectively. Method C is second derivative dual wavelength (DWL) where ΔA at 255 nm and 269 nm for LAM ≠ 0 and ΔA at 281 nm and 267 nm for TEN ≠ 0. Method D is first derivative of ratio spectra derivative method, where peak amplitudes at 223.80 nm for LAM and 245.60 nm for TEN were used. Method E is ratio difference spectroscopic method where ΔA at 255.2 nm and 210.6 nm (TEN 3 µg/ml is used as the divisor) of the zero order divisor spectra were measured for the determination of LAM. Whereas ΔA at 255.4 nm and 283.8 nm (LAM 9 µg/ml is used as the divisor) of the zero order divisor spectra were measured for the determination of TEN. Method F is amplitude factor method where LAM is determined at 266 nm and TEN was determined at 393 nm. The two compounds were simultaneously determined in the concentration ranges of 3-15 µg/ml for both LAM and TEN. The methods were validated according to the ICH guidelines.

Keywords: Lamivudine; Tenofovir; Simultaneous estimation; First derivative zero crossing point; Second derivative dual wavelength method; Ratio difference; Ratio spectra derivative; Amplitude factor method and Validation.

INTRODUCTION

Lamivudine (LAM), chemically it is (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H) pyrimidinone, is a synthetic nucleoside analogue with potent activity against human immune deficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.2956 m.mol (Figure 1). TEN and LAM are extensively used for antiretroviral therapy for HIV infection to delay disease progression by prolonged suppression of HIV replication. The combination may be given to patients, those are not responding to mono-therapy of HIV.

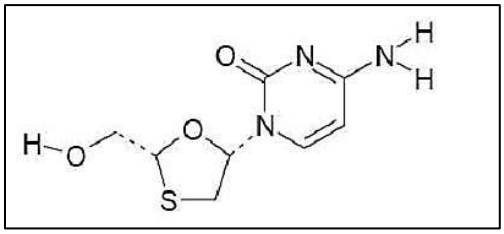

Figure 1: Chemical structure of lamivudine

Tenofovir (TEN) Chemically, it is ({[2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl]N oxy}methyl)phosphonic acid. It has a molecular formula of $C_9H_{14}N_5O_4P$ and a molecular weight of 287.2123 m.mol (Figure 2).

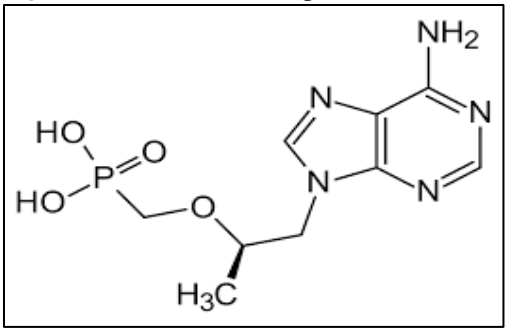

Figure 2: Chemical structure of tenofovir

TEN is the first nucleotide analog approved for HIV-1 treatment and remains in cells for longer periods of time than many other antiretroviral drugs. Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination [1-4]. Literature survey has revealed a number of analytical methods for determination of both drugs are official in compendial LAM and TEN. UV methods [5-21], plasma hyphenated techniques [22] HPTLC [23] and RP- HPLC methods [24-35]. Hence it has much attracted us to carry out the development of simultaneous determination of Tenofovir (TEN) and Lamivudine (LAM) by UV spectrophotometric method with distilled water as solvent system. The present study illustrates the method development and validation for simultaneous determination of both drugs in their combinations.

EXPERIMENTAL SECTION

Apparatus

Spectrophotometer system was of Shimadzu UV-1800 Spectrophotometer with two matched 1cm quartz cells using the following spectral parameters; a single fast scan mode and a slit width (1 cm), connected to a computer loaded with Shimadzu UV-Probe 2.34 software and used for all the absorbance measurements and data manipulation. The first derivative of the working standard solutions were tresses with smoothing at smoothing factor ($\Delta\lambda$) = 16 and multiplying the entire spectra with a scaling factor = 10.

Materials and Method

LAM and TEN working standards were kindly gifted by micro Laboratories Pvt. Ltd., Bangalore, India. The tablet TENOLAM (HETERO DRUGS) each one tablet containing of LAM 300 mg and TEN 300 mg was procured from local market and used for analysis of marketed formulation. On basis of solubility studies distilled water is used as common solvent (eco-friendly).

Preparation of Standard Stock Solution

Standard stock solutions of LAM stock solution -I (1000 µg/ml) and TEN stock solution-I (1000 µg/ml) were prepared by dissolving 25 mg of LAM and 25 mg of TEN in 25 ml distilled water. LAM working solution stock solution -II (100 µg/ml) was prepared by transferring 2.5 ml from LAM stock solution to a 25 ml volumetric flask and diluted up to the mark with distilled water. TEN working solution stock solution- II (100 µg/ml) was prepared by transferring 2.5 ml from TEN stock solution to a 25 ml volumetric flask and diluted up to the mark with distilled water. Appropriate and accurate volume aliquots of the stock solutions were transferred to 10 ml calibrated flasks and made up to volume with distilled water.

Aliquots Preparation

Aliquots of LAM (0.3,0.6,0.9,1.2 and 1.5) stock solution-II (100 µg/mL) were accurately transferred separately into 10 mL volumetric flasks then completed to volume with distilled water to prepare concentrations ranging from 3-15 µg/mL of LAM.

Aliquots of TEN (0.3, 0.6, 0.9,1.2, and 1.5 mL) stock solution-II (100 µg/mL) were accurately transferred separately into 10 mL volumetric flasks then completed to volume with distilled water to prepare concentrations ranging from 3-15 µg/mL of TEN.

Sample Preparation

Ten tablets were weighed to get the average weight and grounded. An amount of powder equivalent to 100 mg of tablet powder was transferred to a 100 mL volumetric flask and added 70 mL of distilled water and sonicated for 30 min. The volume was made up with solvent to obtain a solution containing 1000 µg mL^{-1}. The solution was filtered using 0.45 µm membrane filter paper and then transfer 1ml of above stock solution diluted with distilled water to produce 100 µg mL^{-1}. From 100 µg mL^{-1} transfer 0.9 ml to produce 9 µg mL^{-1}.Scan in the range of 200-400 nm
Applicability of methods to the formulation (Table 1).

Table 1: Applicability of proposed methods to marketed formulations

S.No	Methods	Label Claim	Tenofovir (*% ± SD Found)	Lamivudine (*% ± SD Found)
1	SEM	300:300 mg	99.66 ± 0.02	100.1 ± 0.05
2	FDZ		100.45 ± 0.02	100.19 ± 0.01
3	DD^2-DWL		99.55 ± 0.02	98.36 ± 0.06
4	RSD		101.5 ± 0.01	99.91 ± 0.02
5	RDS		100.7 ± 0.02	100.6 ± 0.03
6	AFM		99.91± 0.02	99.47± 0.06

RESULTS AND DISCUSSION

Method A: Simultaneous Estimation

Linearity of the both drugs was is in the range of 3-15 µg/ml was constructed by measuring the absorbance at its own wavelength in

In this spectrophotometric method the spectra of the binary mixture containing LAM and TEN were measured at 270.8 nm and 260.6 nm which are λmax of LAM and TEN. Pharmaceutical formulation was successfully analyzed using the developed method.

Simultaneous equation using λmax of one drug and λmax of another drug was derived as:

For Lamivudine:

$C_x = (A_2\ a_{y1} - A_1\ a_{y2}) / (ax_2\ a_{y1} - a_{x1}\ a_{y2})$

For Tenofovir:

$C_{y\ =} (A_1\ a_{x2} - A_2\ a_{x1}) / (ax_2\ a_{y1} - a_{x1}\ a_{y2})$

a_{x1} = Absorptivity of LAM at 270.8 nm
a_{x2} = Absorptivity of TEN at 260.6 nm
a_{y1} = Absorptivity of LAM at 270.8 nm
a_{y2} = Absorptivity of TEN at 260.6 nm
A_1 = Absorbance of unknown at 270.8 nm
A_2 = Absorbance of unknown at 260.6 nm

Method B: First Derivative Zero Crossing Method (FDZM)

In this 1st derivative zero-crossing spectroscopic method the amplitudes of the 1st order derivative of the spectra of the binary mixture containing LAM and TEN were measured at 260 nm (zero crossing of TEN) for determination of LAM and at 249 nm (zero crossing of LAM) for the determination of TEN.

Method C: Second Derivative Dual Wavelength Method

In this 2nd derivative dual wavelength spectrophotometric method the difference between absorbance at 255 nm and 269 nm (Difference is zero for TEN) of the 2nd derivative spectra of the binary mixture containing LAM and TEN were measured for the determination of LAM. Similarly the difference between absorbance at 267 nm and 281 nm (Difference is zero for LAM) of the 2nd derivative spectra of the binary mixture containing LAM and TEN were measured for the determination of TEN.

Method D: Ratio Spectra Method

In this ratio spectra derivative spectroscopic method the amplitudes of the 1st order derivative of the spectra of the binary mixture containing LAM and TEN were measured at 223.80 nm (where TEN was used as divisor) for determination of LAM and at 245.60 nm (where LAM was used as divisor) for the determination of TEN.

Method E: Ratio Difference Method

In this ratio difference spectrophotometric method the difference between absorbance at 210.6 nm and 255.2 nm (TEN 3 µg/ml is used as the divisor) of the zero order divisor spectra of the binary mixture containing LAM and TEN were measured for the determination of LAM. Similarly the difference between absorbance at 255.4 nm and 283.8 nm (LAM 9 µg/ml is used as the divisor) of the zero order divisor spectra of the binary mixture containing LAM and TEN were measured for the determination of TEN.

Method F: Amplitude Factor Method

In this amplitude factor spectrophotometric method (P- factor) the amplitudes of the 1st derivative of the spectra of the binary mixture containing LAM were measured at 275.6.0 nm and for determination of TEN at 360.0 nm (zero crossing of LAM). Pharmaceutical formulations were successfully analyzed using the developed methods.

Validation of Proposed Methods

The methods is validated as per ICH guide lines. The method show good linearity, precision, accuracy. The linearity range of lamivudine and tenofovir was found to be 3-15 µg/ml and 3-15 µg/ml respectively. The accuracy was determined by three different levels 50%, 100%, 150% of the target concentration of the active ingredient, by adding know amount of concentration for previously analyzed of sample (Tables 2 and 3). Precision was studied to determine the intraday variation by performing nine replicates, %RSD was calculated and also inter-day variation by performing six replicates, %RSD was calculated. The LOD and LOQ were calculated from the standard calibration curves.

Table 2: Recovery studies of lamivudine for proposed methods

S.no	Formulation (µg/ml)	Std added (µg/ml)	Total amount of sample (µg/ml)	Amount of sample recovered(µg/ml)						% Recovery ±%RSD					
				SEM	FDZ	DD^2-DWL	RSD	RDS	AFM	SEM	FDZ	DD^2-DWL	RSD	RDS	AFM
1	6	3	9	8.54	8.87	8.62	8.72	8.69	9.142	98.6 ± 0.05	98.61 ± 0.05	95.71 ± 0.04	96.89 ± 0.06	96.55 ± 0.02	101.58 ± 0.02
2		6	12	11.64	11.75	12.05	11.85	12.09	12.28	97.86 ± 0.02	97.91 ± 0.01	100.41 ±0.02	98.75 ± 0.03	100.75 ± 0.01	102.38 ± 0.03
3		9	15	14.65	14.62	14.96	14.92	14.56	16.41	97.06 ± 0.04	97.46 ± 0.02	99.73 ± 0.01	99.46 ± 0.04	97.06 ± 0.04	107.61 ± 0.04

Table 3: Recovery studies of tenofovir for proposed methods

S.no	Formulation (µg/ml)	Std added (µg/ml)	Total amount of sample (µg/ml)	Amount of sample recovered(µg/ml)						% Recovery ±%RSD					
				SEM	FDZ	DD^2-DWL	RSD	RDS	AFM	SEM	FDZ	DD^2-DWL	RSD	RDS	AFM
1	6	3	9	8.98	8.93	8.75	8.93	8.95	9.157	99.26 ± 0.02	99.23 ± 0.02	97.22 ± 0.02	98.81 ± 0.04	99.44 ± 0.03	101. ± 0.037
2		6	12	11.97	11.53	11.83	12.05	11.89	12.615	99.40 ± 0.01	96.11 ± 0.05	98.58 ± 0.03	100.4 ± 0.02	99.08 ± 0.05	105.1± 0.01
3		9	15	14.98	14.83	14.39	14.94	14.99	15.624	99.84 ± 0.04	98.86 ± 0.02	95.93 ± 0.01	99.62 ± 0.03	99.93 ± 0.04	104.1± 0.04

Std- Standard; % RSD- relative standard deviation

The residual standard deviation of the regression line or the standard deviation of the y-intercepts of regression lines was used as the standard deviation. LOD and LOQ were calculated with equations LOD=3.3 σ/S and LOQ=10 σ/S; where, σ is the Standard deviation of the response and S is the slope of the calibration curve (Table 4). The proposed method was successful applied for analysis of samples in marketed formulation by performing three replicate for ensuring the reproducibility. Summary of validation parameters are mentioned as follows.

Table 4: Validation sheet for proposed methods

S.no	Linearity (µg/ml)		Precision		Repeatability		LOD(µg/ml)		LOQ(µg/ml)		Wavelength selected(nm)	
	LAM	TEN	LAM	TEN	LAM	TEN	LAM	TEN	LAM	TEN	LAM	TEN
SEM	3-15µg/ml		0.739	1.057	0.735	1.097	0.164	0.135	0.499	0.411	270.8	260.4
FDZ			1.64	1.823	1.632	1.839	0.503	0.2	0.742	0.609	260	249
DD^2-DWL			1.561	1.576	1.931	1.576	0.203	0.128	0.614	0.388	255&269	267 & 281
RSD			0.152	1.485	0.169	0.733	0.503	0.2	0.742	0.6	223.8	245.6
RDS			1.702	0.952	0.147	0.343	0.258	0.218	0.781	0.72	210.6 & 255.2	255.4 & 283.8
AFM			0.9475	1.98	1.45	1.25	1.715	0.2712	18.5	3.9	266	393

Optimization of Parameters

Though formulation is official in compendia but the method of analysis is varied by comparing statistically with official compendial method of analysis. It found that there is no any significant difference between them. Based on the solubility studies it confirms that both drugs are soluble in water. The zero order absorption spectra of pure drugs were severely overlapped show in Figure 3, method A(SEM), in this spectrophotometric method the spectra of the binary mixture containing LAM and TEN were measured at 270.8 nm and 260.6 nm which are λmax of LAM and TEN. Method B - 1st derivative zero-crossing spectroscopic method the amplitudes of the 1st order derivative of the spectra of the binary mixture containing LAM and TEN were measured at 260 nm (zero crossing of TEN) for determination of LAM and at 249 nm (zero crossing of LAM) for the determination of TEN (Figure 4).

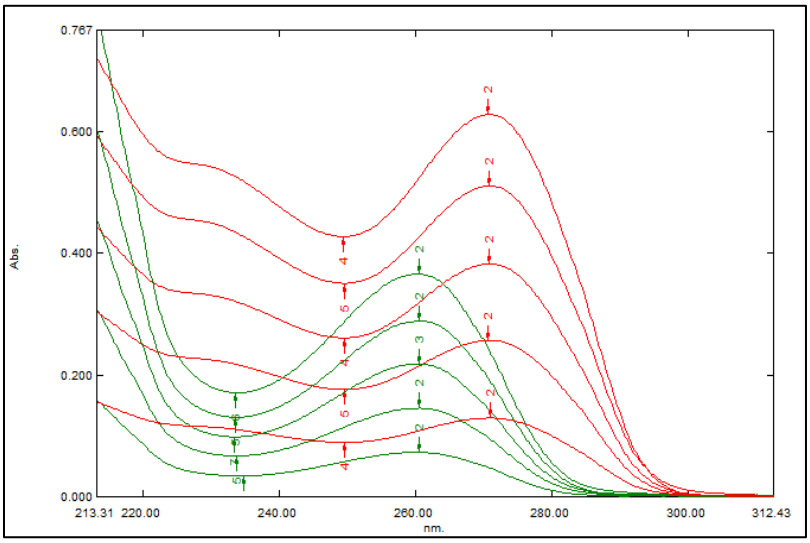

Figure 3: Overlay spectra of LAM and TEN

Method C (DD^2 dual wavelength spectrophotometric method ΔA ≠0 at 255 nm and 269 nm for LAM (ΔA=0 for TEN) of the 2nd derivative spectra of the binary mixture containing LAM and TEN were measured for the determination of LAM. Whereas ΔA≠0 at 267 nm and 281 nm for TEN (ΔA=0 for LAM) of the 2nd derivative spectra (Figure 5). Method D is first derivative of ratio spectra derivative method, where peak amplitudes at 223.80 nm for LAM and 245.60 nm for TEN were used for their determination (Figure 6). Method E is ratio difference spectroscopic method where ΔA at 255.2 nm and 210.6 nm (TEN 3 µg/ml is used as the divisor) of the zero order divisor spectra of the binary mixture containing LAM and TEN were measured for the determination of LAM. Whereas ΔA at 255.4 nm and 283.8 nm (LAM 9 µg/ml is used as the divisor) of the zero order divisor spectra of the binary mixture containing LAM and TEN were measured for the determination of TEN (Figure 7). Method F is amplitude factor method where LAM is determined at 266nm and TEN was determined at 393 nm, whereas LAM zero amplitudes, (Figure 8).

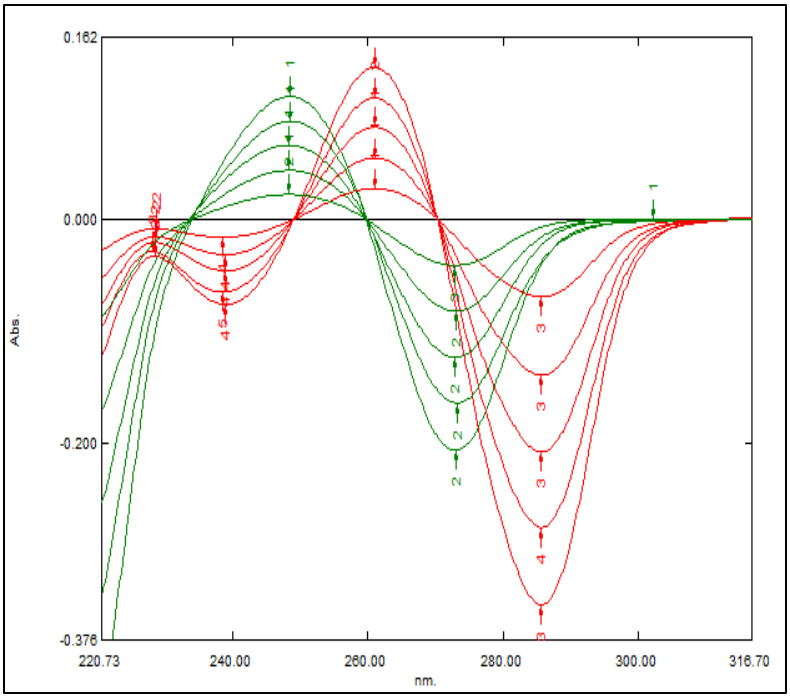

Figure 4: Zero crossing point of LAM and TEN

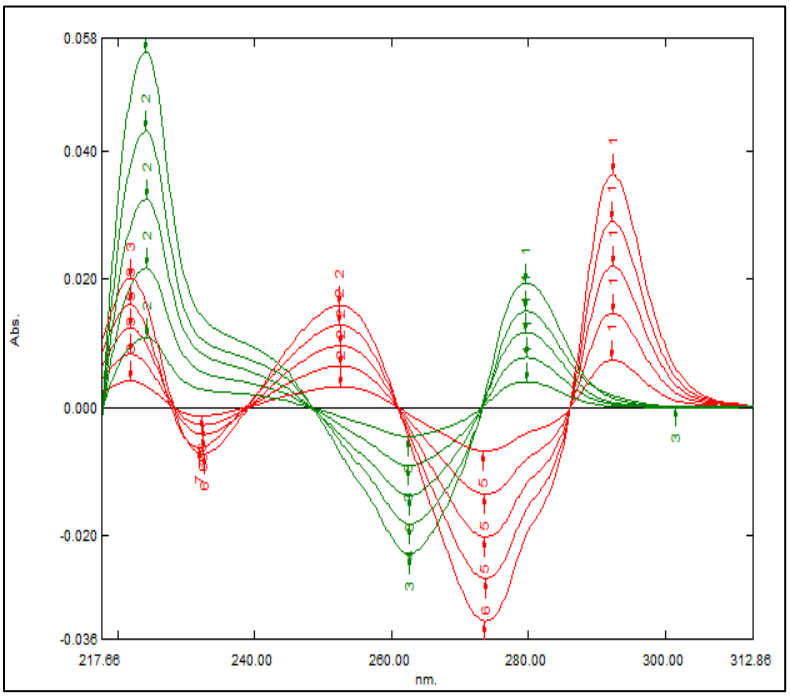

Figure 5: Second derivative dual wavelength of LAM and TEN

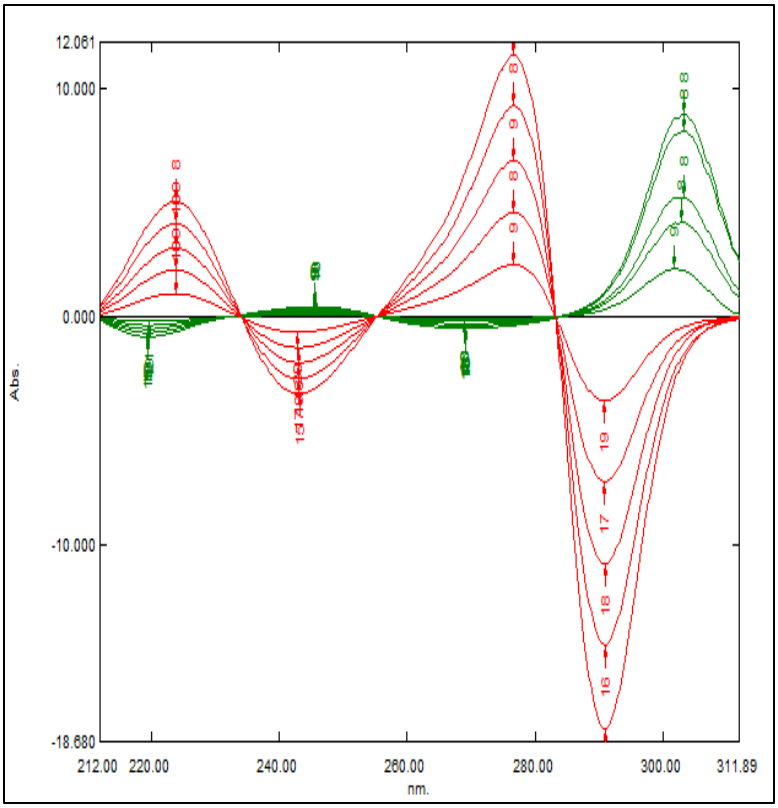

Figure 6: Ratio spectra derivative of LAM and TEN

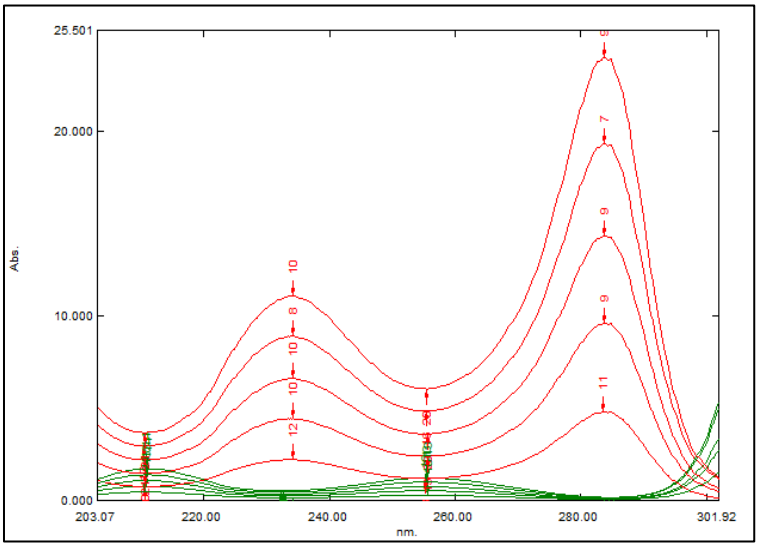

Figure 7: Ratio difference of LAM and TEN

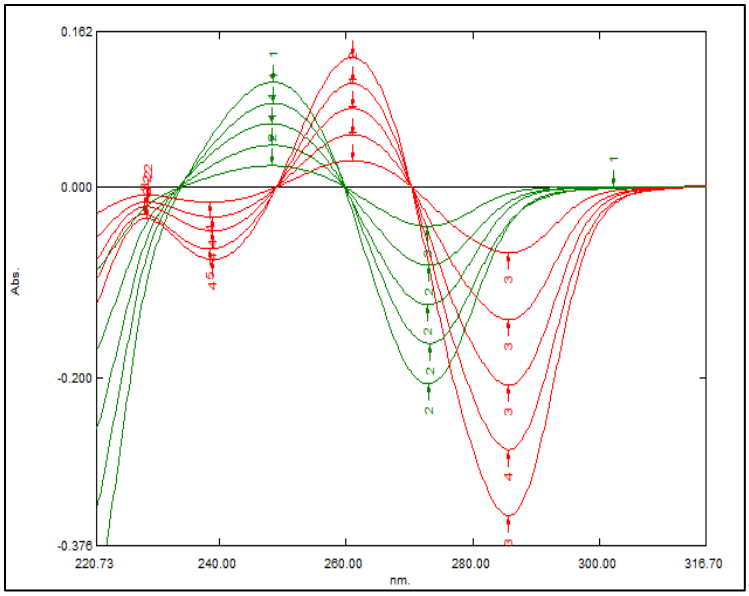

Figure 8: Amplitude factor method of LAM and TEN

RESULTS AND DISCUSSION

Statistical comparison of two different reported methods with new developed method by calculating F-value and t-test to the developed method.

To further confirm the analytical method validation, a single factor analysis of variance (ANOVA) of the linear regression data measurements was performed to evaluate the linearity of the proposed method. Statistical significance was established by a P-value <0.05, which indicates that the model is explained by the proposed regression at a 95% confidence interval. There is no any significant difference among the developed six spectrophotometric methods of analysis.

CONCLUSION

The mentioned spectrophotometric methods was validated and successfully applied for simultaneous determination of lamivudine and tenofovir in binary mixtures and in their available dosage form. The proposed procedure is simple and do not require sophisticated techniques or instruments. This method is simple, rapid, sensitive, accurate, precise, economical and eco-friendly for the routine analysis of lamivudine and tenofovir.

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REFERENCES

[1] The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 15th edition, RSC publishing, Royal Society of Chemistry, Cambridge, UK, **2013.**
[2] Goodman, Gilman. The Pharmacological basis of Therapeutics, 10th edition, Mc Grawhill, New York, **2001**.
[3] SC Sweetman. Martindale the complete drug information, 36th edition, Pharmaceutical Press, UK, **2009**.
[4] KD Tripathi. Essential of medical pharmacology, 7th edition, Jayapee Brother's Medical Publication, India, **2013**.
[5] Indian Pharmacopoeia. The Indian Pharmacopoeia Commission. Ghaziabad: Govt. of India Ministry of Health and Family Welfare, **2010**, 3.

[6] British Pharmacopoeia. The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA). Great Britain: The Department of Health, **2013**, 3407-3409, 6433-6435.
[7] United States Pharmacopoeia. Rockville: United States Pharmacopoeial Convention, Inc., USA, **2013**, 3, 1.
[8] CJ Gnana Babu; GV Kumar. *Int J Chem Technol Res*. **2009**, 1, 4.
[9] AV Singh; KN Lila; RP Nihar. *J Pharm Anal*. **2011**, 1(4), 251-257
[10] K Chaithanya; A Padma; A Swapna; K Swaroopa Rani; P Ramalingam. *Int J Chem Technol Res*. **2011**, 3, 2.
[11] KC Madhu; PO Ukoha; AA Attama. *Am J Anal Chem*. **2011**, 2, 849-856.
[12] PV Rajesh; CP Karunasree; G Dharmamoorthy; K Padmini; CH Sudeer. *Int J Pharm Technol*. **2012**, 2(1), 15-19.
[13] HB Charushila; NH Shivanand. *Int J Med Pharm Sci*. **2013**, 3(1), 39-44.
[14] R Kumar; Y Rupa; M Chaitanya. *Int J Pharm Res Anal*. **2014**.
[15] S Malipatil; M Nandedher. *J Indian Counc Chem*. **2009**, 26, 67-69.
[16] PA Nevase; HM Nimje; RJ Oswal; RV Antre; SS Kshirsagar. *Int J Sci Res Dev*. **2011**, 3(3), 11.
[17] TS Rani; K Sujatha; K Chitra; DM Jacob; R Yandapalli; D Manasa; B Sushma. *RRJPA*. **2012**, 1(1), 9-12.
[18] M Balaji; DG Moorthy; RV Perumal; KS Sumanth; KP Channabasavaraj. *Int J Pharm Res Anal*. **2012**, 2(1), 14-17.
[19] MS Varsha; NR Babu; Y Padmavathi; PR Kumar. *Int Curr Pharm J*. **2015**, 4(4), 378-381
[20] MI Anees; MS Baig. *Int J Curr Microbiol Appl Sci*. **2015**, 4(2), 756-763
[21] B Soumya; TM Kumar; N Raghunandhan. *Int J Pharm Pharm Sci Res*. **2012**, 2(1), 9-15.
[22] G Baheti; JJ Kiser; PL Havens; CV Fletcher. *Antimicrob Agents Chemother*. **2011**, 5294-5299.
[23] CD Trivedi; RB Mardia; BN Suhagia; SP Chauhan. J Pharm Sci Biosci Res. **2012**, 2(2), 73-76.
[24] T Sharma; N Mishra; S Moitra; DG Sankarg. *Asian J Pharm Clin Res*. **2012**, 5(3), 108-110.
[25] S Havele; SR Dhaneshwar. J Sci Technol. **2012**, 34(6), 615-622.
[26] S Manavarthi; GS Chhabra. *Der Pharma Chemica*. **2014**, 6(2), 401-409.
[27] P Chandra; AS Rathore; L Sathiyanarayanan; KR Mahadik. *J Chilean Chem Soc*. **2011**, 56, 2.
[28] AK Karunakaran; K Kamarajan; V Thangarasu. *Eurasian J Anal Chem*. **2012**, 7(2), 56-66.
[29] K Hymavathi; DM Babu; P Afroz. *Int J Adv Res Pharm Bio Sci*. **2012**, 1(3), 277-284.
[30] AK Kumar; KK Chaitanya; NS Babu. J Global Trends Pharm Sci. **2013**, 3(3), 849-852.
[31] A Gorja; J Bandla. *Int J Adv Res Pharm Sci*. **2013**, 2(1), 22-32.
[32] PH Sonawane; PS Panzade; MA Kale. *Asian J Biomed Pharm Sci*. **2013**, 3(16), 27-30.
[33] M Goud; S Avanapu; SR Kumar. *Int J Adv Pharm Sci*. **2012,** 5(3), 215-218.
[34] AS Mali; PA Salunke; SD Barhate; MM Bari. *World J Pharm Res*. **2015**, 4, 3.
[35] BH Diana; BK Shaik; KS Kumari. *Int J Asian Philos Assoc*. **2015**, 5(1), 10-13.