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Original Article

DEVELOPMENT AND VALIDATION OF BIOANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE, LAMIVUDINE AND EFAVIRENZ IN FORMULATION BY ADVANCED DERIVATIVE UV-SPECTROSCOPY

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ABSTRACT

A *selective and sensitive derivative spectrophotometry method was developed to detect and quantify tenofovirdisoproxilfumarate, lamivudine and efavirenz in rat plasma. After liquid-liquid extraction the analyte were analysed. Mixture of methanol and phosphate buffer pH.5.0 (60:50 v/v) was used as solvent. The method was based on the measurement of absorbance at zero crossing points of three drugs 260.8nm, 271nm, and 247.6 nm respectively. The method was validated according to USFDA guidelines over the range of 100-3200 ng/mL for tenofovirdisoproxilfumarate and lamivudine and 200-6400ng/mL for efavirenz, with correlation coefficient values (R2) 0.999 for tenofovirdisoproxilfumarate and efavirenz, and 0.997 for lamivudine. The absolute recovery for analyte achieved from spiked plasma sample was consistent and reproducible. The % CV reported for precision and accuracy determinations across four validation runs (LLOQ, LQC, MQC and HQC) were less than 15. LLOQ was found to be 100ng/mL for tenofovirdisoproxilfumarate and lamivudine and 200ng/mL for efavirenz.*

Keywords: *TenofovirDisoproxilFumarate, Lamivudine, Efavirenz, Derivative Spectrophotometry, Bioanalytical Method Validation, USFDA guidelines.*

INTRODUCTION

TenofovirDisoproxilFumarate is Chemically named as [[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl]oxymethyl propan-2-yl carbonate; (E)-but-2-enedioic acid[1]. Tenofovirdisoproxil is a medication used to treat Chronic Hepatitis B and to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretrovirals. It may be used for prevention of HIV/AIDS among those at high risk before exposure, and after a needlestick injury or other potential exposure[2].

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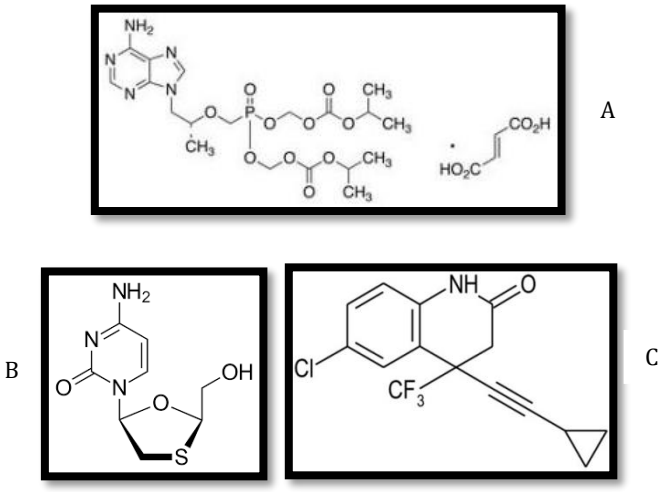

Fig. 1: Chemical Structure of TenofovirDisoproxilFumarate (A), Lamivudine (B) & Efavirenz (C)

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While some of the research publications referred from different techniques like UV-Spectrophotometry methods [7, 8], RP-HPLC techniques [9, 10]. This study establishes a validated UV-Spectrophotometry method per ICH Q2 (R1) guidelines [11, 12].

MATERIALS AND METHODS:

Table No.1: Standards and Sample

S. No.	Name	Supplier
1	Tenofovirdisoproxilfumarate	Gift sample from hetero drugs limited
2	Lamivudine	Gift sample from aurobindopharma limited
3	Efavirenz	Gift sample from hetero drugs limited
4	Tenolam-E	Hetero labs limited

Table No.2: Chemicals and Reagents

S. No.	Name	Grade	Supplier
1	Methanol	AR	RANKEM
2	Water	Double distilled	SD FINE
3	Sodium hydroxide	AR	SDFCL
4	Potassium dihydrogen phosphate	AR	RANKEM
5	Di sodium EDTA	AR	SDFCL
6	Acetonitrile	AR	RANKEM

Table No. 3: List of instruments

S. No.	Equipment	Make/model
1	Electronic Balance	Shimadzu, BL220H
2	pH meter	Elico, LI 127
3	Ultra Sonic Bath Sonicator	PCI Analytics, 6.5lit 200H
4	UV-Visible Spectrophotometer	Shimadzu, 1800
5	Refrigerated centrifuge	Eltek
6	Quick freezer	Remi, RQFV-170

Animals used for Plasma:

Male Albino Wistar rats (180-200 gm) were obtained from National Institute of Nutrition, Hyderabad.

Method Development and Optimization in Rat Plasma:

1. Selection of Solvent:

Solvent selection is the first step involved in the method development. Solvent is selected based on the solubility of the drugs. Five solvents were selected for the trial and five trials were done using acetonitrile: water, ethanol, methanol, acetate buffer and methanol: phosphate buffer pH 5.0 (60:50 v/v).

Trials were performed by preparing solutions of concentration of 10µg/mL of all three drugs separately using selected solvents and the absorption spectra were recorded and the suitable solvent was selected based on the absorbance observed for all the three drugs without interferences.

2. Preparation of Standard and Sample Solutions:

2.1. Preparation of Phosphate buffer pH-5.0: 4.2gm of potassium dihydrogen orthophosphate is taken in 1000ml volumetric flask and added with 1.08gm of sodium hydroxide and made up the volume with water. pH was checked using pH meter. If necessary pH was adjusted using orthophosphoric acid or NaOH.

2.2. Preparation of Solvent: Solvent consists of methanol and phosphate buffer pH-5.0 (50:50 v/v). A mixture of methanol and phosphate buffer pH-5.0 was prepared by taking 500mL methanol and 500mL of phosphate buffer pH-5.0 and the solution was sonicated for 10min.Samples of pooled plasma were used as both blank and for spiking throughout the validation studies.

2.3. Preparation of Standard Stock solution:

Standard stock solution-I (1000µg/mL of TDF and LAM and 2000µg/mL EFZ): Appropriately 100mg of tenofovirdisoproxilfumarate, lamivudine, and 200mg efavirenz was weighed into a 100mL volumetric flask, 30mL of solvent (methanol: phosphate buffer pH-5.0) was added, allowed to dissolve and volume was made up to the mark with solvent to give solution containing 1000µg/mL of TDF and LAM and 2000µg/mL of EFZ solution.

Standard stock solution-II (100µg/mL of TDF and LAM and 200µg/mL EFZ): 1 mL of 1000µg/mL solution was transferred into 10 mL volumetric flask and the volume was made up to the mark with solvent. This gives 100µg/mL solutions of tenofovirdisoproxilfumarate, lamivudine and 200µg/mL efavirenz.

Standard stock solution-III (10µg/mL of TDF and LAM and 20µg/ml of EFZ):1 mL of 100µg/mL was transferred into 10mL, volumetric flask and the volume was made up to the mark with solvent. This gives 10µg/mL solutions of tenofovirdisoproxilfumarate, lamivudine and 20µg/mL efavirenz.

2.4. Preparation of sample stock solution:

Sample stock solution-I (1000µg/mL of TDF and LAM and 2000µg/mL of EFZ):Twenty tablets of fixed dose combination tablets (Tenolam E manufactured by hetero labs Ltd., Hyderabad containing 300mg of TDF and LAM and 600mg of EFZ) were weighed and finely powdered. The powder equivalent to 100mg was weighed and transferred to 100mL volumetric flask containing 25ml of solvent and sonicated for 30mins. The flask was shaken and volume was made up to the mark with solvent to

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obtain a solution of 1000µg/mL of TDF and LAM and 2000µg/mL of EFZ solution. The solution was centrifuged at 300rpm for 10min.

Sample stock solution-II (100µg/mL of TDF and LAM and 200µg/mL of EFZ):From the sample stock-I solution, 1ml was transferred to a 10mL volumetric flask and the volume was made up to the mark with solvent to get a solution containing 100µg/mL of TDF and LAM and 200µg/mL of EFZ.

Sample stock solution-III (10µg/mL of TDF and LAM and 20µg/mL of EFZ):From the sample stock-I solution, 1ml was transferred to a 10mL volumetric flask and the volume was made up to the mark with solvent

3. Selection of Wavelength:

10 µg/mL individual solution of TDF, LAM and EFZ were prepared separately and were scanned in the range of 200-400nm to determine the absorption maximum for the drugs.

4. Selection of Derivative Mode:

10 µg/mL individual solution of TDF, LAM and EFZ were prepared separately and were scanned in the range of 200-400nm in derivative mode to determine the zero crossing point of the three drugs

5. Selection of Concentration Range:

From the working mixed standard stock solution-II, appropriate aliquots like 0.01ml, 0.02ml, 0.04ml, 0.08ml, 0.16m, 0.32ml solutions were transferred in to 10ml volumetric flask. The solution in each tube was made up with solvent to obtain working standard concentration ranging from 0.1-32µg/mL of TDF and LAM and 0.2-64µg/mL of EFZ.

The absorbance of these solutions was measured at 260.6nm, 271nm, and 247.4nm respectively.

6. Optimization in Plasma:

Plasma separation and treatment:The blood samples were collected from retro-orbital puncture into eppendorf tube containing disodium EDTA (20 mg disodium EDTA in 1 ml water, 1 ml of blood requires 50 µL of disodium EDTA). Plasma was separated from blood samples by centrifugation at 6000 rpm for 10 minutes. After separation plasma is collected and stored at -20oc for further use.

Extraction of plasma: 100 µL of plasma was added in 400 µL of acetonitrile, mixture was vortexed for 1 minute and centrifuged at 3000 rpm for 10 minutes at 4oc. The supernatant organic layer was separated and used in method development.

Blank Plasma preparation: The organic layer was separated from processed blank plasma, make up to 10ml with solvent (methanol: phosphate buffer pH.5.0) and directly measured in spectral range without spiking the standard.

Standard preparation: The organic layer was separated from processed blank plasma. Standard solutions of tenofovirdisoproxilfumarate, lamivudine and efavirenz10 µg/mL were spiked and analysed. Blank plasma was also analysed.

Analytical Method Validation:

All analytical procedures were validated according to USFDA guidelines 'BioanalyticalMethod Validation'.

1. Selectivity and sensitivity (LLOQ):

Selectivity was performed to determine that no absorbance of impurity or any other substance is recorded, other than the λmax of tenofovirdisoproxilfumarate, lamivudine and efavirenz respectively.

Sensitivity is the lower limit of quantification (LLOQ). The lowest concentration of the calibration curve where the analyte response was more than five times the blank response was selected as LLOQ which is 100ng/ml for tenofovirdisoproxilfumarate, lamivudine and 200ng/ml efavirenz.

2. Linearity:

Preparation of Quality control (QC) Standards in Plasma: Calibration samples were prepared by spiking with appropriate amounts of standards into 100µL of control plasma.

From 10µg/ml stock solutions of drugs, 50, 100, 200, 400, 800, 1600µL samples of tenofovirdisoproxilfumarate, lamivudine and 100, 200, 400, 800, 1600, 3200µL efavirenz were taken and spiked in 100µL control plasma. Volume was made up to 5 mL with solvent to get 100, 200, 400, 800, 1600, 3200ng/mL of tenofovirdisoproxilfumarate, lamivudine and 200, 400, 800, 1600, 3200, 6400ng/mL of efavirenz.

All the standard solutions were scanned and respective absorbances were recorded. The concentrations calculated from calibration curve for quality control standards were showing deviations less than 15% of the nominal concentrations. Six nonzero standards met the criteria for verification of linearity.

Calibration curve was constructed between absorbance and concentration (ng/mL) and linear regression analysis was performed for checking linearity between two parameters.

3. Precision:

The precision of an analytical method defines the degree of closeness of agreement between a series of measurements obtained from multiple samplings of a homogeneous sample of biological matrix.

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Standard drug concentrations were prepared by spiking with appropriate amounts of standard into 100µL of control plasma. From 10µg/mL stock solutions of individual drug 50, 150, 400, 1200µL of TDF, LAM and 100, 300, 800, 2400µL EFZ were taken and spiked into 100µL control plasma separately in each graduated tube. Volume was made up to 5 mL with solvent to get 300, 800, 2400ng/mL of TDF, LAM and 600, 1600, 4800ng/mL of EFZ. 300ng/mL- LQC, 800ng/mL– MQC and 2400ng/mL- HQC for TDF, LAM and 600ng/mL- LQC, 1600ng/mL– MQC and 4800ng/mL- HQC for EFZ.

3.1. Repeatability: The repeatability was checked by using standards of TDF, LAM and EFZ to ensure that the analytical system was precise. Repeatability results obtained for six replicates of standard solutions of TDF, LAM and EFZ, % CV is calculated.

3.2. Intraday precision and Inter day precision: Variations of results within the same day (intraday) and variation of results between days (inter day). The intra-day precision of the proposed method was determined on standard drug solutions at varying concentration levels (LLOQ, LQC, MQC and HQC) by analyzing five replicates of each sample as a batch in a single assay run on the same day. The Inter-assay precision was determined by analyzing the same samples on the next two, days and %CV was calculated.

4. Accuracy:

Samples were prepared by spiking with appropriate amount of sample in 100µL of control plasma. Sample solutions are prepared in such a way to get the concentration of 100ng/mL- LLOQ, 300ng/mL- LQC, 800ng/mL- MQC and 2400ng/mL- HQC for TDF, LAM and 200ng/mL- LLOQ, 600ng/mL- LQC, 1600ng/mL- MQC and 4800ng/mL- HQC for EFZ. To these solutions MQC (800ng/ML of TDF, LAM and 1600ng/ML of EFZ) concentration of mixed standard (1:2 proportionality same as sample) were spiked. These solution are spiked into 100µL control plasma to get levels in the range of LLOQ, LQC, MQC and HQC (100, 300, 800, 2400ng/mL of TDF and LAM and 200, 600, 1600, 4800ng/mL of EFZ).The four standard solutions absorbance was measured and %CV was calculated.

5. Recovery:

Recovery studies was determined at LQC, MQC, HQC levels (300, 800, 2400ng/mL of TDF and LAM and 600, 1600, 4800ng/mL of EFZ). Concentrations were prepared by spiking the sample solutions into 100µL of control plasma. Here the recovery studies are performed by two steps determination in treated plasma and determination in untreated plasma. In a treated plasma process the required quantities of sample solutions are directly spiked into extracted plasma (control) to get respective concentrations and measured absorbance. In a untreated plasma process the required quantities of sample solutions are directly spiked into plasma to get respective concentrations, 400µL of solvent is added and centrifuged at 3000rpm for 10mins, after centrifugation the supernatant is collected and volume was make up to 5mL and measured absorbance. Extraction of sample from plasma was done by liquid-liquid extraction technique using methanol: phosphate buffer pH.5.0 and further dilutions to desire extent were made with same solvent.

The absorbances obtained from both the methods are used to calculate % recovery. The limits for % recovery in bioanalytical method are 80-110%.

6. Stability studies:

Stability of sample solutions was checked in terms of freeze and thaw stability, short-term temperature stability, stock solution stability studies.

6.1. Freeze and Thaw Stability: Drug stability was determined after three freeze-thaw cycles. Three aliquots of each of higher (2400, 2400, 4800ng/mL) and lower concentrations (100, 100, 200ng/mL) of TDF, LAM, EFZ respectively were frozen for 24 hours and then thawed unassisted at room temperature. When thawed completely, the same samples were refrozen for 24 hours under the same conditions as before. Similarly the freeze and thaw cycles were repeated twice more and then analysed on the third cycle.

6.2. Short-term temperature stability: Three aliquots of each of the higher (2400, 2400, 4800ng/mL) and lower concentrations (100, 100, 200ng/mL) of TDF, LAM, EFZ respectively were thawed at room temperature for 24 hours and then analysed.

6.3. Stock solution stability: The stability of stock solutions of drug higher (2400, 2400, 4800ng/mL) and lower concentrations (100, 100, 200ng/mL) of TDF, LAM, EFZ respectively were evaluated by placing these concentrations at elevated temperature for 6 hours and then analysed.

RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis. 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 (Fig. 2) shows that at λmax of TDF(260.8nm), LAM (271nm), and EFZ(247.6nm)occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with 95.6 %(TDF), 94.8 % (LAM), and93.6 %(EFZ) 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.

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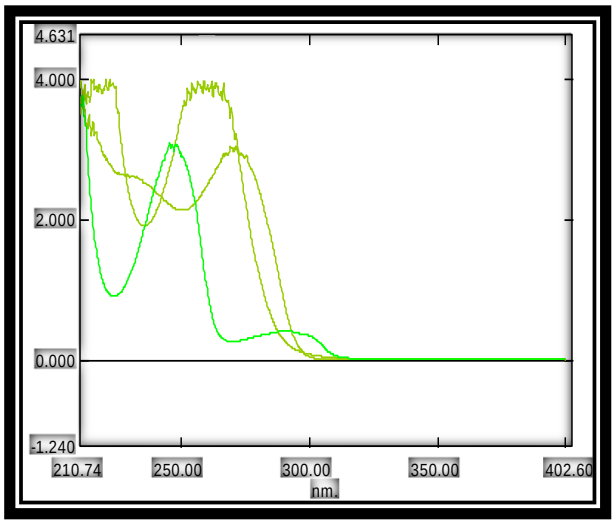


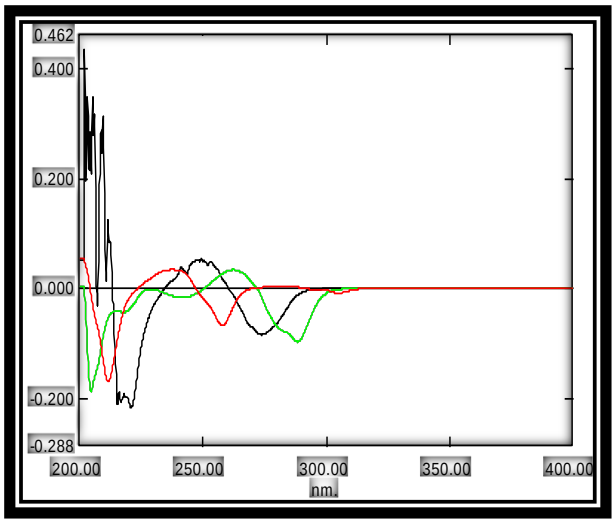


Fig. 2: Overlay spectrum of TDF, LAM and EFZ and first order derivative spectrum of TDF, LAM, EFZ

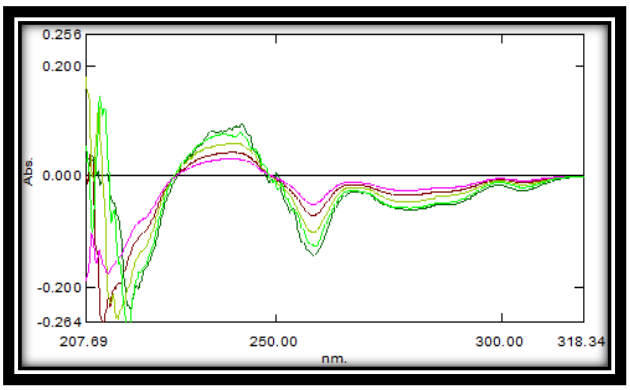
Table No. 4: Optimized Conditions of Derivative Spectroscopy

Parameters	TDF	LAM	EFZ
Beers law range	100-3200ng/ml	100-3200ng/ml	200-6400ng/ml
Coefficient of correlation	0.999	0.997	0.998
Regression equation	y = 0.0006x + 0.1970	y = 0.0005x + 0.3727	y = 0.0001x + 0.1916
Sandell's sensitivity	0.4016	0.256	0.4651
Slope	0.0006	0.0005	0.0001
λmax	260.6nm	271 nm	247.4nm

Table No. 5: Calibration curve data of TenofovirDisoproxilFumarate, Lamivudine & Efavirenz

S. No.	TenofovirDisoproxilFumarate		Lamivudine		Efavirenz	
	Conc.(ng/mL)	Abs.*	Conc.(ng/mL)	Abs. *	Conc.(ng/mL)	Abs.*
1	100	0.249	100	0.39	200	0.215
2	200	0.295	200	0.45	400	0.243
3	400	0.412	400	0.59	800	0.302
4	800	0.671	800	0.78	1600	0.411
5	1600	1.133	1600	1.15	3200	0.62
6	3200	1.995	3200	1.891	6400	1.05
Y-Intercept		0.0006x + 0.1970		0.0005 x + 0.3727		0.0001 x + 0.1916
R2		0.999		0.998		0.999

*average of five determinations



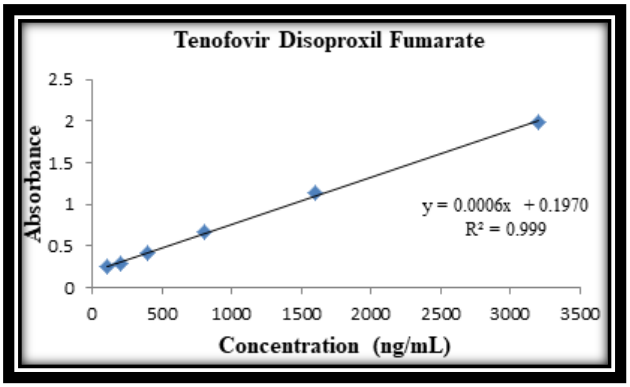


Fig. 3: Overlay first order derivative spectrum& Calibration curve of tenofovirdisoproxilfumarate

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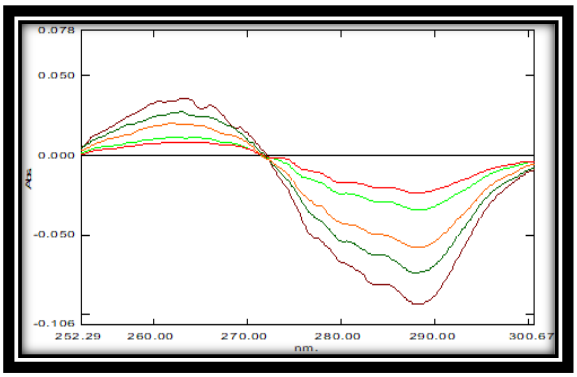


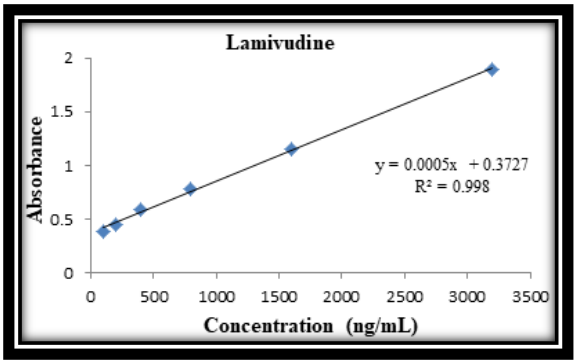
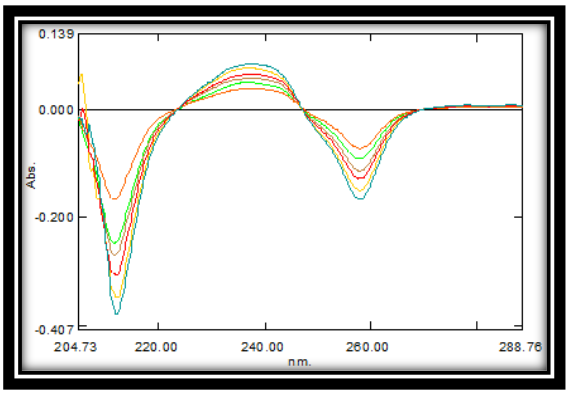


Fig. 4: Overlay first order derivative spectrum& Calibration curve of lamivudine



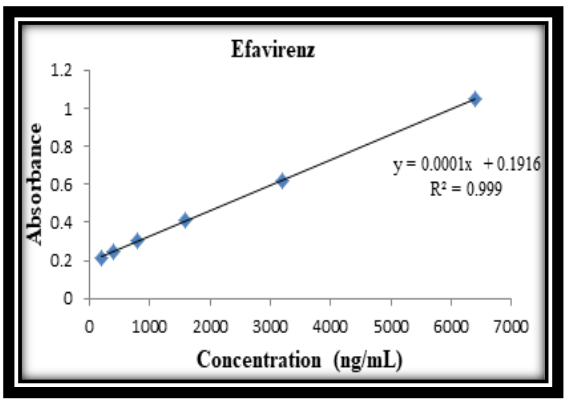


Fig. 5: Overlay first order derivative spectrum& Calibration curve of efavirenz

Table No.6: Accuracy data of TDF, LAM and EFZ

Accuracy level	Drugs	Conc. (ng/mL)	Mean Conc. from Stdcurvea(ng/mL)	SD	%CV
LLOQ	TDF	100	98	0.002645	1.03
	LAM	100	96	0.003082	0.73
	EFZ	200	194	0.004062	1.92
LQC	TDF	300	295	0.003082	0.82
	LAM	300	252	0.004582	1.09
	EFZ	600	584	0.004183	1.67
MQC	TDF	800	731	0.003082	0.48
	LAM	800	792	0.004582	0.59
	EFZ	1600	1574	0.004582	1.01
HQC	TDF	2400	2008	0.013527	0.96
	LAM	2400	2172	0.006557	0.4
	EFZ	4800	4764	0.004582	0.68

[a] Average of five determinations

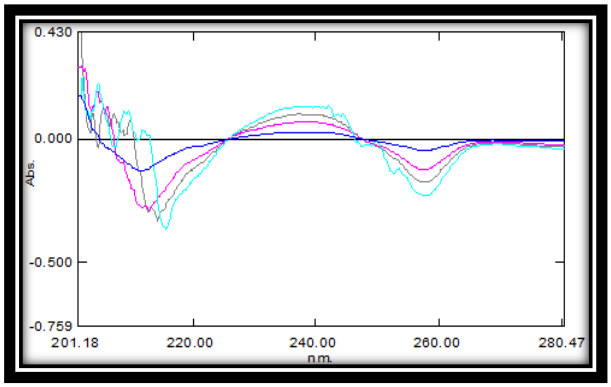


Fig. 6: First order derivative spectrum mixed standard solution

Table No. 7: Recovery data of TDF, LAM and EFZ

Conc.	Drug	Conc. (ng/ml)	A		B		Relative Recovery (%)
			Abs.[a]	Conc.[b]	Abs.[a]	Conc.[b]	
LQC	TDF	300	0.373	293	0.364	278	94.9
	LAM	300	0.495	250	0.483	220	88.2
	EFZ	500	0.246	534	0.239	474	88.7
MQC	TDF	800	0.633	776	0.628	718	92.5
	LAM	800	0.792	838	0.786	818	97.6
	EFZ	1600	0.374	1824	0.365	1734	95
HQC	TDF	2400	1.359	1936	1.353	1926.6	99.5
	LAM	2400	1.466	2186	1.453	2160	98.8
	EFZ	4800	0.711	5194	0.702	5104	98.2

[a,b] Average of five determinations

A: sample prepared by spiking externally in blank plasma; B: extracted samples; C: (B/A)×100

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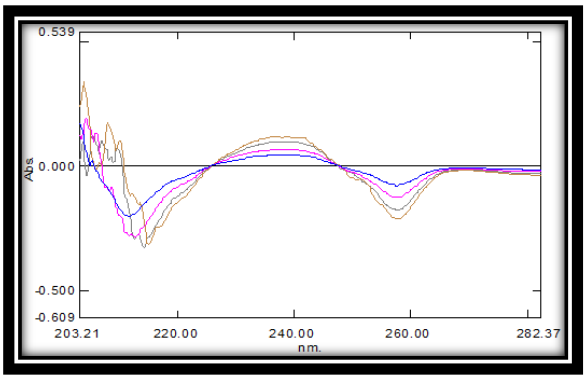
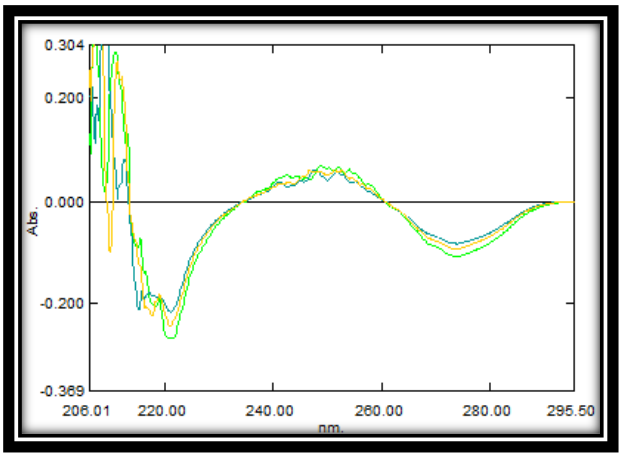


Fig. 7: First order derivative spectrum mixed standard solution

Table No. 8: Intra-day precision data of TDF, LAM and EFZ

Conc.	Drug	Mean Absorbance[a]			Meanb±Standard deviation	%CV
		Morning	Afternoon	Evening		
TDF	LLOQ(100)	0.221	0.223	0.229	0.224±0.004183	1.86
	LQC (300)	0.348	0.353	0.358	0.352±0.00591	1.68
	MQC (800)	0.526	0.530	0.532	0.0.529±0.0382	0.440
	HQC(2400)	1.386	1.391	1.396	1.0071±0.00591	0.425
LAM	LLOQ(100)	0.408	0.413	0.418	0.412±0.00514	1.24
	LQC (300)	0.472	0.476	0.478	0.475±0.00308	0.6
	MQC (800)	0.774	0.780	0.785	0.779±0.00556	0.713
	HQC(2400)	1.628	1.632	1.635	1.631±0.040036	0.220
EFZ	LLOQ(100)	0.201	0.209	0.214	0.208±0.00655	3.1
	LQC (300)	0.258	0.263	0.265	0.262±0.00360	1.37
	MQC (800)	0.380	0.383	0.391	0.384 ±0.00574	1.49
	HQC(2400)	0.221	0.223	0.229	0.224±0.004183	1.86



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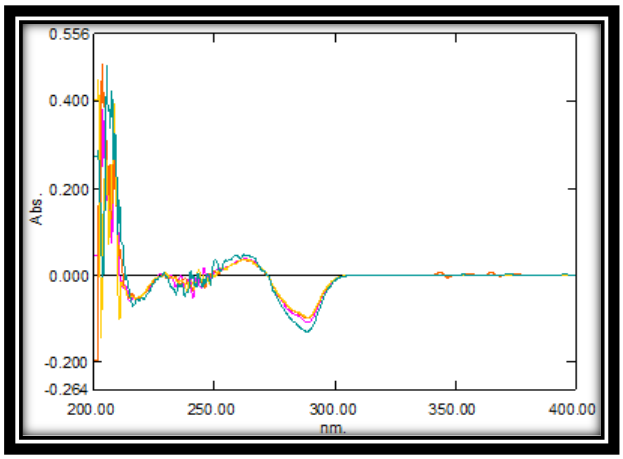
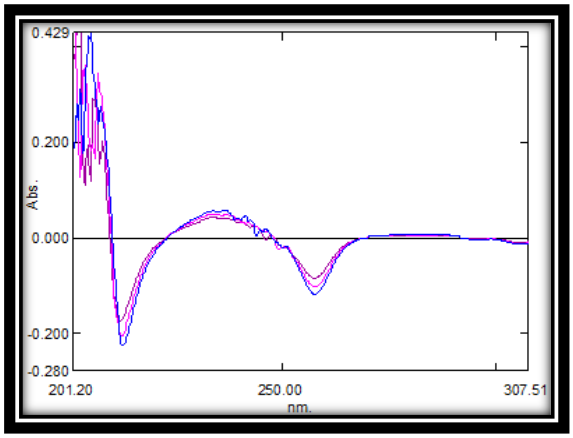


LAM



EFZ

Fig. 8: Intra-day precisionSpectrum

Table No. 9: Inter-Day Precision data of TDF, LAM and EFZ

Drug	Conc. (ng/ml)	Mean Abs[a]		Meanb±SD	%CV
		Day 1	Day 2		
TDF	LLOQ (100)	0.215	0.219	0.217±0.00282	1.29
	LQC (300)	0.324	0.329	0.326±0.00360	1.104
	MQC (800)	0.540	0.545	0.542±0.00360	0.660
	HQC (2400)	1.324	1.329	1.326±0.00360	4.778
LAM	LLOQ (100)	0.392	0.397	0.394±0.0036055	0.915
	LQC (300)	0.475	0.479	0.477±0.0028284	0.592
	MQC (800)	0.754	0.760	0.756±0.0044721	0.591
	HQC (2400)	1.451	1.456	1.453 ±0.0036055	0.240
EFZ	LLOQ (100)	0.203	0.209	0. 206±0.0042426	1.63
	LQC (300)	0.245	0.248	0.246±0.0022360	0.908
	MQC (800)	0.321	0.326	0.323±0.0033166	1.026
	HQC (2400)	0.613	0.617	0.615±0.0028284	0.45

[a,b] Average of five determinations

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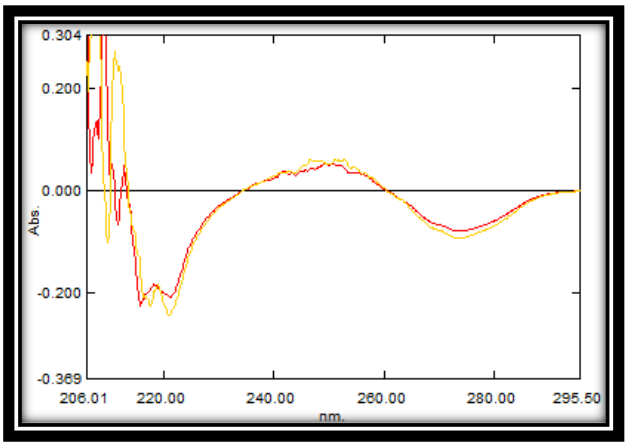
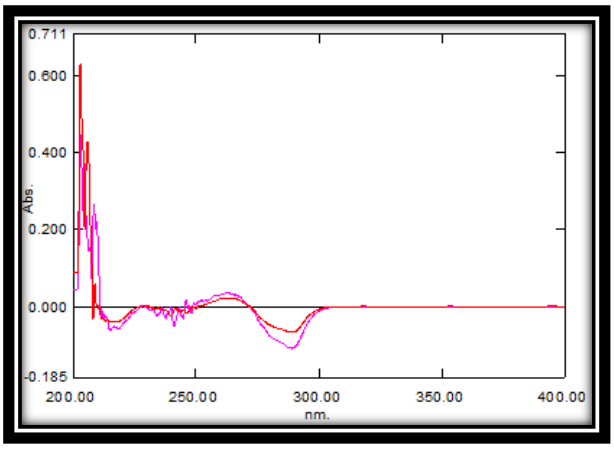
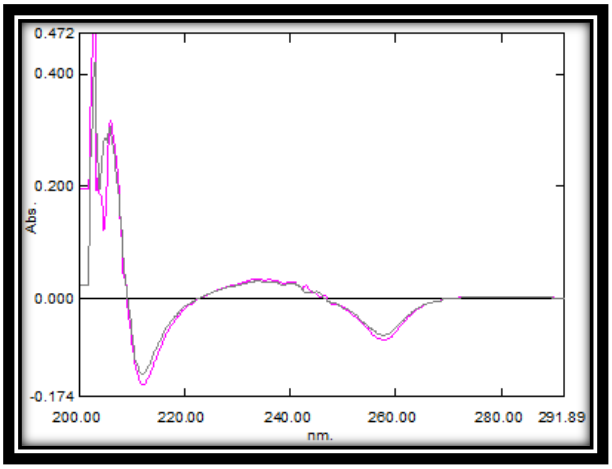


TDF



LAM



EFZ

Fig. 9: Inter-Day Precision spectrum

Table No.10: Freeze and thaw stability data of TDF, LAM and EFZ

Parameter	Drugs	Conc. (ng/mL)	Time (hr)	Mean abs.a	Mean Conc. b ±SD	%CV
LLOQ	TDF	100	0	0.336	131.45±0.005	1.50
			72	0.329		
	LAM	100	0	0.431	108±0.00640312	1.5
			72	0.422		
	EFZ	200	0	0.225	125±0.005	2.26
			72	0.218		
HQC	TDF	2400	0	1.399	2008.3±0.0042426	0.3
			72	1.402		
	LAM	2400	0	1.451	2166±0.0056568	0.38
			72	1.459		
	EFZ	4800	0	0.642	4564±0.0084852	1.3
			72	0.654		

[a,b] Average of five determinations

Table No.11: Stability data of TDF, LAM and EFZ

Parameter	Drugs	Conc. (ng/mL)	Time (hr)	Mean abs.a	Mean Conc. b ±SD	%CV
LLOQ	TDF	100	0	0.327	111±0.0036055	1.11
			24	0.322		
	LAM	100	0	0.428	104±0.0046904	1.10
			24	0.421		
	EFZ	200	0	0.229	198±0.0028284	1.24
			24	0.225		
HQC	TDF	2400	0	1.406	2010.3±0.0036055	0.25
			24	1.401		
	LAM	2400	0	1.465	2182±0.00223606	0.15
			24	1.462		
	EFZ	4800	0	0.648	4534±0.0036055	0.55
			24	0.643		

[a,b] Average of five determinations

Table No.12: Stability data of TDF, LAM and EFZ

Parameter	Drugs	Conc. (ng/mL)	Time (hr)	Mean abs.a	Mean Conc. b ±SD	%CV
LLOQ	TDF	100	0	0.331	98.5±0.00223606	0.687
			6	0.328		
	LAM	100	0	0.422	96±0.0028284	0.673
			6	0.418		
	EFZ	200	0	0.226	195±0.0036055	1.61
			6	0.221		
HQC	TDF	2400	0	1.408	2013.3±0.0036055	0.25
			6	1.403		
	LAM	2400	0	1.459	2168±0.0036055	0.24
			6	1.454		
	EFZ	4800	0	0.646	4524±0.0028284	0.43
			6	0.642		

[a,b] Average of five determinations

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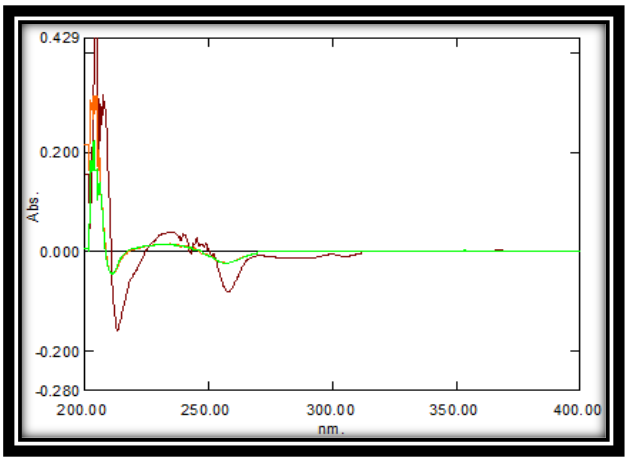

Fig. 10: Freeze and Thaw Stability Spectrum

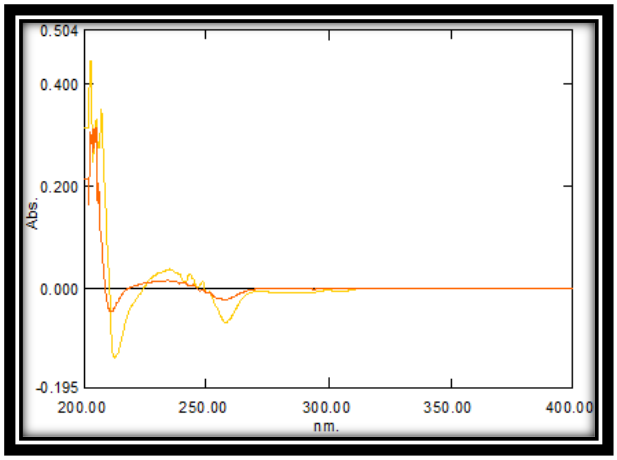

Fig.11: Short term Temperature Stability Spectrum

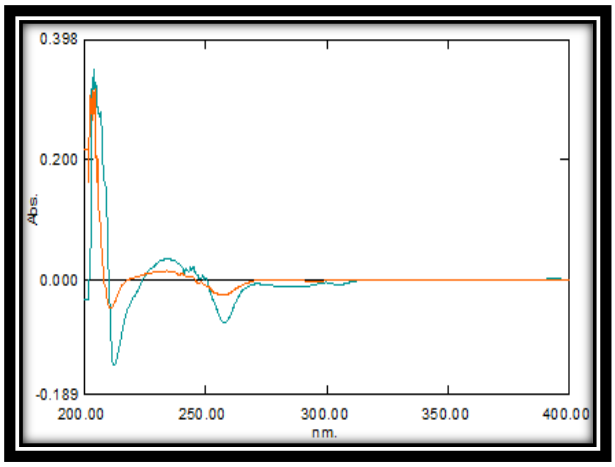

Fig.12: Stock solution Stability Spectrum

CONCLUSION

A new simple, sensitive bioanalytical method was developed and validated for estimation of tenofovirdisoproxilfumarate, lamivudine and efavirenz in spiked rat plasma. The method was found to be linear in the concentration range of 100-3200 ng/mL for tenofovirdisoproxilfumarate and lamivudine, 200-6400 ng/mL for efavirenz. Analysis was carried out at λmax 260.6nm, 271nm and 247.4nm respectively. The bioanalytical method was validated according to USFDA guidelines. All the parameters validated were within the acceptable criteria.

The developed derivative spectrophotometric method for simultaneous estimation of tenofovirdisoproxilfumarate, lamivudine and efavirenz was sensitive for determining these three drugs in nanogram level in spiked rat plasma.

As the sensitivity of derivative spectrophotometry is high when compared to conventional UV, this method can be applied for pharmacokinetic studies of newly developed formulation of tenofovirdisoproxilfumarate, lamivudine and efavirenz.

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