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(RESEARCH ARTICLE)

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Estimation of lamivudine, tenofovirdisoproxil fumarate and efavirenz in bulk and pharmaceutical dosage form by RP HPLC method

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Abstract

The main aspiration of this research work is to assess and validate the stability indicating RP-HPLC for concurrent estimation of Lamivudine, tenofovir disoproxil fumarate and efavirenz in bulk and marketed formulation. The exploration was carried out by using Hypersil BDS column, (150×4.6mm, particle size 5µ) and PDA detector at 258nm. The mobile phase containing Methanol:0.1% TFA Buffer: acetonitrile (30:40:30v/v) in isocratic mode pumped into a column at a flow rate 0.8mL/min. The method was validated according to ICHQ2 (R1) guidelines. The linearity was perceived in the range of 10-50 µg/mL for Lamivudine, Tenofovir disoproxil fumarate and 20-100 µg/mL for Efavirenz respectively. The method was accurate with% recovery of 100.41%, 99.69% and 99.75% for Lamivudine, Tenofovir disoproxil fumarate and Efavirenz respectively. The percentage relative standard deviation was NMT 2.0, indicating that it was precise and robust. Under stress instances, execution of forced degradation studies such as acidic (0.1N HCl), basic (0.1N NaOH), oxidative (3% H_2O_2), photolytic (60%RH) and thermal (80^{0}C). The extent of degradation was accomplished within the acceptable limits i.e., 5-20%. The developed method was sophisticated, precise, accurate and can be utilized for the estimation of lamivudine, ten of ovirdisoproxil fumarate and efavirenz in bulk and tablet dosage form.

Keywords: Lamivudine; Tenofovirdisoproxil fumarate; Efavirenz; HPLC; Forced degradation studies

1. Introduction

1.1. Lamivudine [11,12]

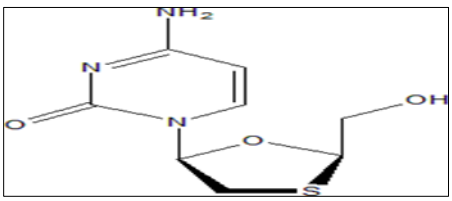

Figure 1 Structure of Lamivudine

- IUPAC name: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one
- Molecular Formula: $C_8H_{11}N_3O_3S$

* Corresponding author: Narla Sunitha

1.2. Tenofovir disoproxilfumarate [13-14]

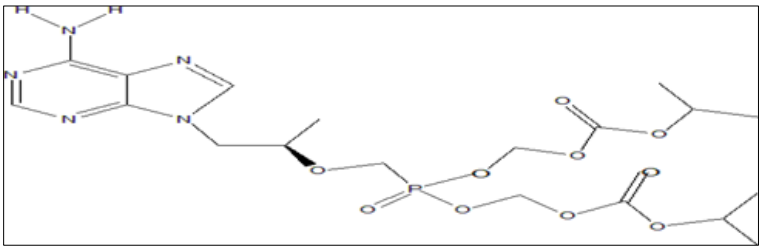

Figure 2 Structure of Tenofovir Disoproxil Fumarate

- Chemical Name :[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl]oxymethylpropan-2-carbonate;but-2-enedioicacid
- Molecular Formula :$C_{23}H_{34}N_5O_{14}P$

1.3. Efavirenz [15-17]

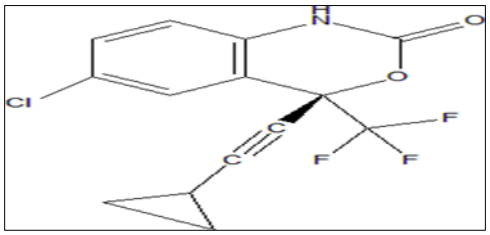

Figure 3 Structure of Efavirenz

- Chemical Name:(S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4 (trifluoromethyl)-2H-3,1-benzoxazin-2-one.
- Molecular Formula : $C_{14}H_9ClF_3NO_2$

2. Material and methods

2.1. Determination of wavelength by UV-Visible Spectrophotometric method

2.1.1. Preparation of Standard stock solution (1000µg/ml)

Weigh accurately 10mg of Lamivudine, 10mg of Tenofovir Disoproxil Fumarate and 10mgof Efavirenz accurately and transfer into a three different 10mL clean dry volumetric flask with 7mL of diluents to dissolve and volume made upto the mark with diluents (1000µg/mL).

2.1.2. Selection of wavelength

The wavelengths of Lamivudine,Tenofovir Disoproxil Fumarate and Efavirenz were determined separately by scanning the spectrum from 200 to 400 nm using the UV-Visiblespectrophotometric technique. Scanning was done with 10 µg/mL solutions. By over laying the individual spectra ,the detection wavelength was found to be 258nm.

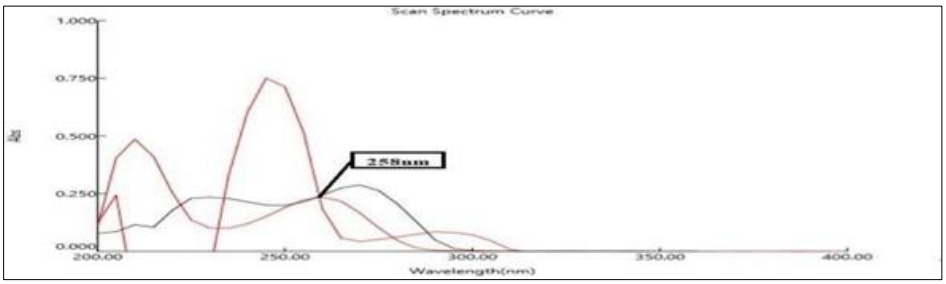

Figure 4 Combined spectrum of Lamivudine,Tenofovir Disoproxil Fumarate and Efavirenz

2.1.3. Preparation of standard stock solution

Accurately weigh 10 mg of Lamivudine, then it was transferred into a 10 ml clean, dry volumetric flask, to which 3/4 of the volume of HPLC water was added, to dilute the remaining 10ml was made up with HPLC water.

2.1.4. Preparation of Tenofovir Disoproxil fumarate standard stock solution

Accurately weigh 10 mg of Tenofovir Disoproxil fumarate, then it was transferred into a 10ml clean, dry volumetric flask, to which 3/4 of the volume of methanol was added to dilute the remaining 10ml was made up with methanol.

2.1.5. Preparation of Efavirenz standard stock solution

Accurately weigh 10 mg of Efavirenz, then it was transferred into a 10 ml clean, dry volumetric flask, to which 3/4 of the volume of methanol was added to dilute the remaining10ml was made up with methanol.

2.2. System suitability

From the stock solutions of lamivudine of 0.3ml, TDF of 0.3ml and Efavirenz 0.6mL were pipette out and transferred into a clean, dry10mL volumetric flask, and the remaining volume was filled up with diluent. Six injections of 10 µL of this solution were used to measure the parameters of retention duration, resolution, plate count,and tailingfactor.

2.3. Specificity

2.3.1. Standard solution preparation

From the stock solutions of Lamivudine of 0.3ml, TDF of 0.3ml and Efavirenz of 0.6mL,were pipette out and transferred into a clean, dry 10mL volumetric flask, and the remaining volume was filled up with diluent.

2.3.2. Sample solution preparation

Weigh accurately 53 mg of tablet powder and transferred into 10ml volumetric flask. Then,7 mL of diluent was added and mixed with a cyclone mixer. The volume was then made upto the specified level using the diluent and filtered through a 0.45 Millipore Nylon filter.Pipette 0.3 mL of solution into a volumetric flask that holds 10 mL, then add diluent to fillthe flask to the required level.

2.4. Accuracy

Preparation of 50% Solution: Weigh accurately 26.5mg of tablet powder and transferred into10ml volumetric flask. Then,7mL of diluents was added and mixed with a cyclone mixer. The volume was then made upto the specified level using the diluents and filtered through a 0.45 Millipore Nylon filter. Pipette 0.3 mL of solution into a volumetric flask that holds 10mL,then add diluents to fill the flask to the required level.

Preparation of 100% Solution: Weigh accurately 53 mg of tablet powder and transferred into 10ml volumetric flask. Then,7mLof diluents was added and mixed with a cyclone mixer. The volume was then made upto the specified level using the diluents and filtered through a 0.45 Millipore Nylon filter. Pipette 0.3mL of solution from into a volumetric flask that holds 10mL.

Preparation of 150% Solution: Weigh accurately 79.5 mg of tablet powder and transferred into10ml volumetric flask. Then, 7mL of diluents was added and mixed with a cyclone mixer. The volume was then made upto the specified level using the diluents and filtered through a 0.45 Millipore Nylonfilter. Pipette 0.3mLof solution from into a volumetric flask that holds 10mL.

3. Results and discussion

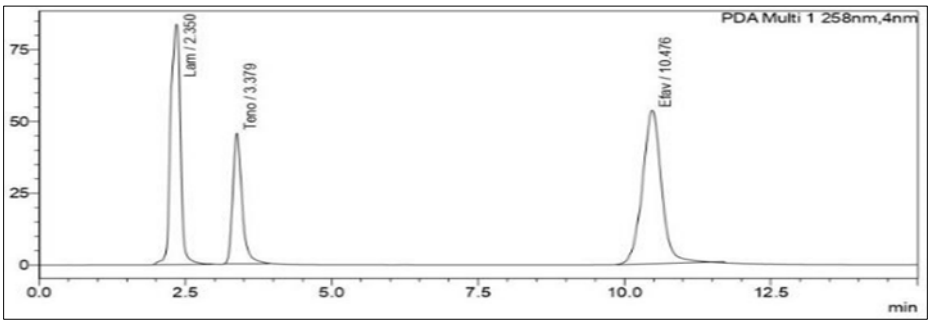

Figure 5 Combined Spectrum of LAMI, TDF,EFV

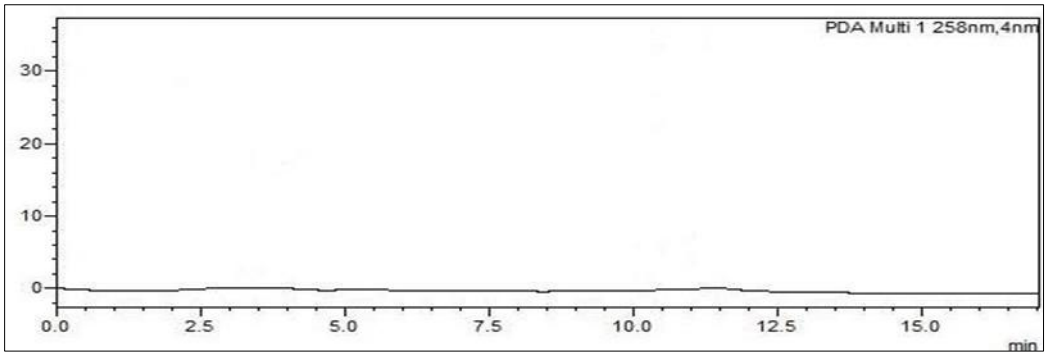

Figure 6 Chromatogram of sample

3.1. System suitability

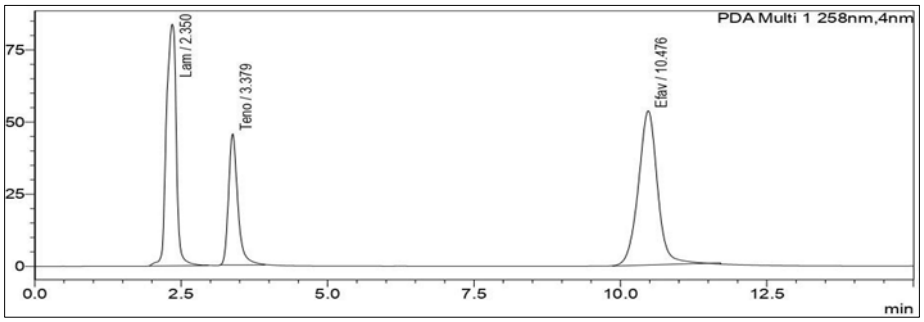

Figure 7 System Suitability Chromatogram

3.2. Specificity

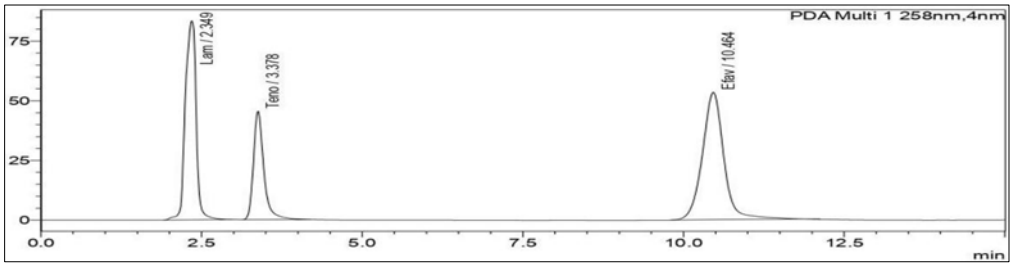

Figure 8 Standard Chromatogram

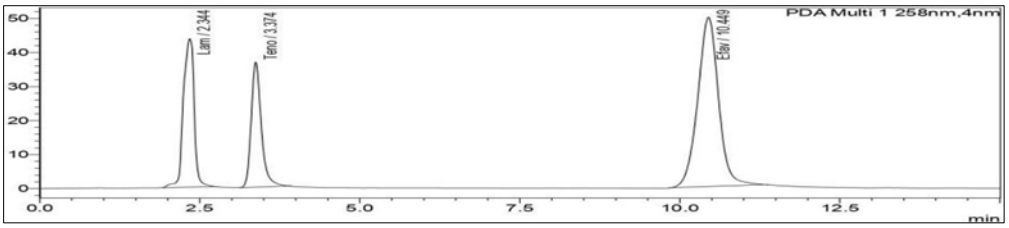

Figure 9 Sample Chromatogram

3.3. Linearity

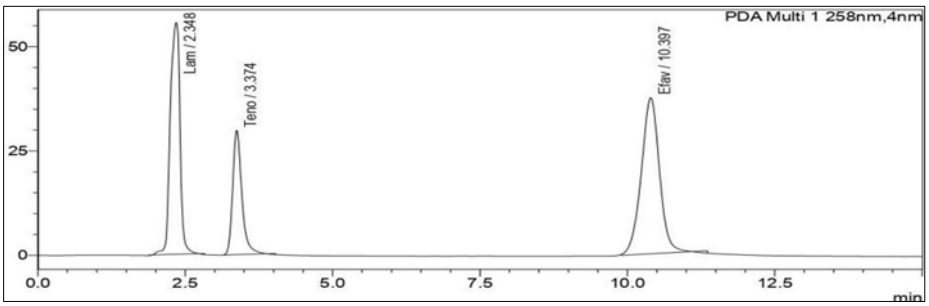

Figure 10 Lamivudine Linearity

3.4. Accuracy

Table 1 Accuracy data

%Level	LAMI				
	Std peak area	**Sample Peak area**	**% Recovery**	**Average recovery**	**Mean %recovery**
50%	977790	491232	100.29	100.43%	100.41%
	977790	492423	100.19		
	977790	493025	100.81		
100%	977790	985367	100.43	100.36%	
	977790	986367	100.39		
	977790	985654	100.26		
150%	977790	1469754	100.07	100.44%	
	977790	1479891	100.85		
	977790	1475651	100.42		
% Level	**TDF**				
	Std peak area	**Sample Peak area**	**% Recovery**	**Average recovery**	**Mean %recovery**
50%	507290	256589	100.57	100.58%	99.69%
	507290	258954	101.15		
	507290	254859	100.04		
100%	507290	507148	99.23	99.23%	
	507290	508452	99.34		
	507290	507589	99.12		

150%	507290	758956	99.20	99.25%	
	507290	759189	99.32		
	507290	759589	99.24		

3.5. Precision

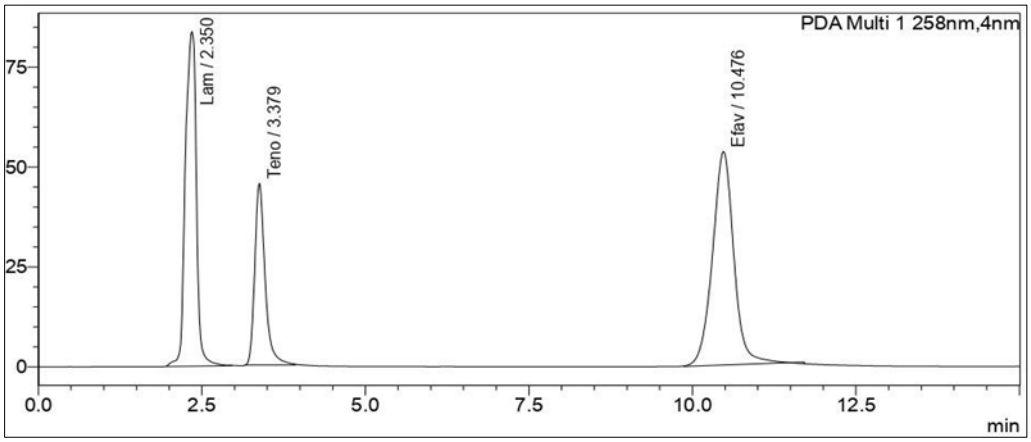

Figure 11 System precision Chromatogram

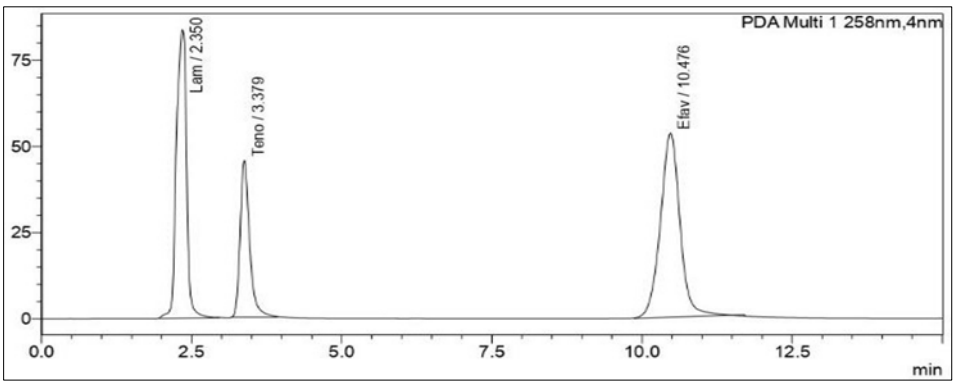

Figure 12 Method precision Chromatogram

3.6. LOD and LOQ

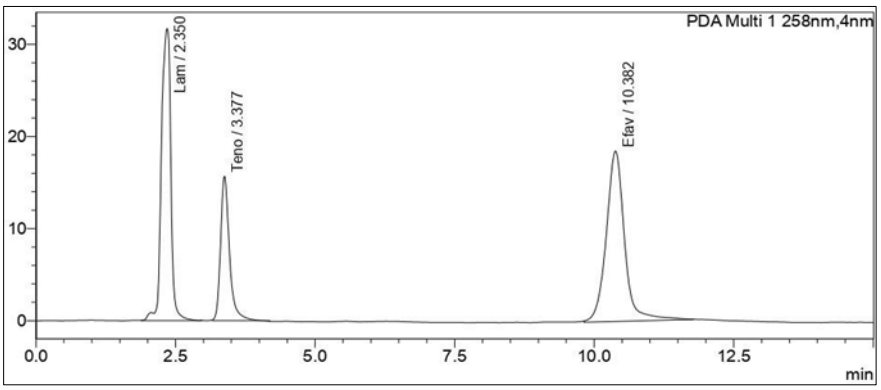

Figure 13 LOD Chromatogram

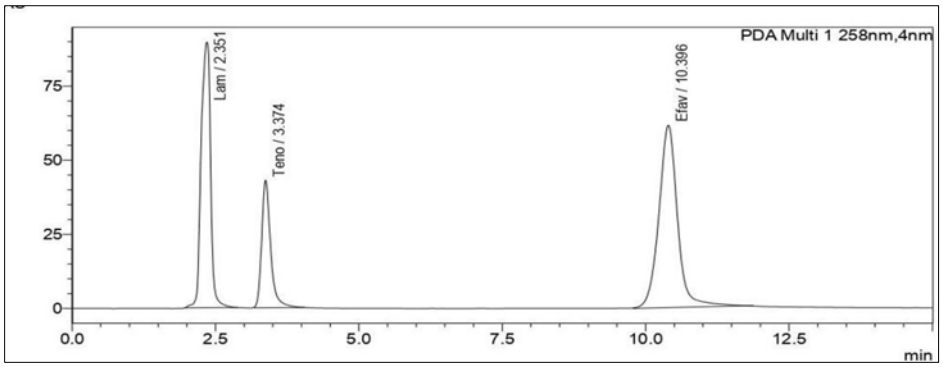

Figure 14 LOQ Chromatogram

3.7. Change in flow rate

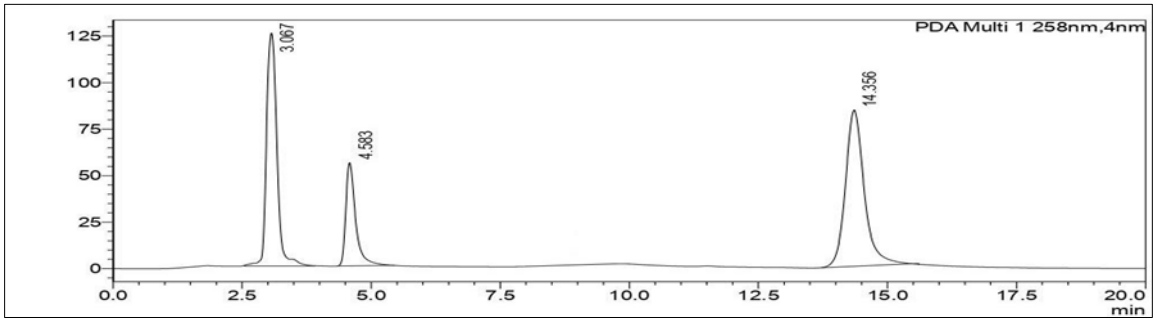

Figure 15 Chromatogram of change in flow rate-0.6ml/min

3.8. Change in wavelength

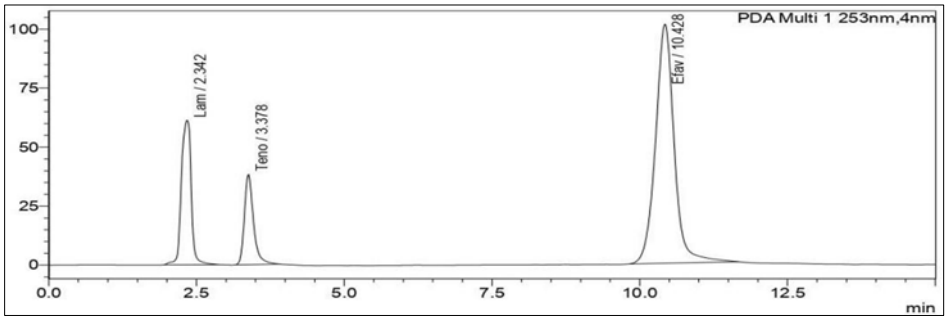

Figure 16 Chromatogram of change in wavelength-253nm

% Assay: The formulation (VANADAY®) contains 300mg of LAMI, 300 mg of TDF and 600mg of EFV. The sample solution was prepared in the same manner as the standard solution,and10mLof it was injected into chromatographic apparatus.

3.9. Forced degradation stuides:

Table 2 Data of Degradation Studies of LAMI, TDF, EFV in Tablet Formulation by RP-HPLC analysis

S. No	Degradation study	Degradation conditions	Peak area			%Assay		
			LAM	TDF	EFV	LAM	TDF	EFV
01	Tablet assay	None	975367	506788	1228852	99.45%	99.20%	99.17%
02	Acid degradation	0.1N HCL	918967	475773	1148852	93.70%	93.13%	92.72%

03	Base degradation	0.1N NaOH	938967	485773	1188852	95.74%	95.09%	95.94%
04	Oxidative degradation	3%H_2O_2	916093	465773	1158852	94.43%	93.13%	95.94%
05	Thermal degradation	80 °C	917093	462873	1157852	93.51%	90.61%	93.44%
06	Photolytic degradation	60% RH	926093	475773	1168852	94.43%	93.13%	94.33%

4. Conclusion`

A simple, rapid and specific RP-HPLC method was developed and validated for the concurrent estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz inbulk and marketed formulation. The exploration was carried out by using Hypersil BDScolumn, (150×4.6mm, particle size 5µ) with PDA detector at 258nm. The mobile phase containing Methanol:0.1% TFA buffer: acetonitrile (30:40:30v/v/v) was pumped into a column at a flow rate 0.8ml/min. The method was validated according to ICH Q2(R1) guidelines. According toa review of theliterature, no stability-indicating RP-HPLC procedures for concurrent estimation had been done for Lamivudine, tenofovir di soproxil fumarate with efavirenz.The current work exemplifies the creation and stability testing of a straight forward, precise, and accurate on current estimation method.

Compliance with ethical standards

Acknowledgments

We are thankful to the SIMS College of Pharmacy management for providing their support and guidance without which its may not be possible.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] https://www.oreilly.com/library/view/pharmaceutical-analysis / 9789332515659 / xhtml/chapter001.xhtml#:~:text=The%20pharmaceutical%20analys is % 20 is % 20 a ,the % 20 structure % 20 of % 20 the % 20 compounds.

[2] Introduction topharmaceutical analysis https : // www . lacity college . edu / Departments/Chemistry/documents/Chemistry-102-Experiments-Documents/ intro 2 qual_ 1456 au81-doc

[3] Snyder LR , Kirkland JJ , Joseph LG . Practical HPLC method development , 2nd Edition , Wiley inter science; NewYork,1997,1-56.

[4] Siddiqui M.R. , .AlOthman ZA , and Rahman N. ,Analytical techniques in Pharmaceutical Analysis: A Review , Arabian Journal of Chemistry , 2017 , vo l10 , Pp .S 1409–S1421.

[5] Analysis https://www.pharmatutor.org/articles/chromatography-introduction

[6] Skoog, AD and West MD, Principles of instrumental analysis, Saunders golden, Japan, 1985, 3rd edition, Pp.212-213.

[7] Satoskar S Bhandarkarand SD Ainapure SS .“Pharmacology andpharmacotherapeutics”17 th edition, popular prakashan, Mumbai, India2001

[8] Yadav Vidushi, Bharkatiya Meenakshi, A Review on HPLC Method Development and Validation, Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Science,2017, vol 2,Issue 6,Pp.166-178.

[9] “International Conference on Harmonisation, Draft Guideline on Validation of Analytical Procedures: Definitions and Terminology, Federal Register, Volume 60, March 1, 1995, 11260.

[10] Keyur Ahir, Sumer Singhetal "A Review Article on Development of Forced Degradation and Stability Indicating Studies for Drug Substance and Drug Product", Journal of pharmaceutical science and bio scientific research. 2019. Volume 9(2) Pp no:165-172.

[11] LAMIVUDINE https://pubchem.ncbi.nlm.nih.gov/compound/Lamivudine

[12] https://go.drugbank.com/drugs/DB00709

[13] Tenofovir Disoproxil Fumerate https : // pub chem . ncb i . nlm . nih . gov / compound /Teofovir-Disoproxil-Fumarate

[14] https://go.drugbank.com/drugs/DB00300

[15] .EFAVIRENZhttps://pubchem.ncbi.nlm.nih.gov/compound/Efavirenz

[16] https://go.drugbank.com/drugs/DB00625

[17] https://www.ncbi.nlm.nih.gov/books/NBK542316/

[18] Giri Prasad VS et al., "Development and Validation of a Rp-Hplc Method for Simultaneous Estimation of Lamivudine, Tenofovir Disoproxil Fumarate and Efavirenz in a CombinedTablet Dosage Form" International Journal of Pharmacy and Pharmaceutical Sciences, 2013,vol5suppl3,ppno:116-121.

[19] Sreekanth nadig et al., "A stability indicating RP-HPLC method for simultaneous estimation of Emtricitabine , Tenofovirdisoproxil fumarate and Efavirenzin pharmaceutical dosage forms " , International journal of Research in pharmaceutical sciences ,2013 ,4(2), Pp:391-396.

[20] Srinivasa Rao A et al., "Stability Indicating Method For The Simultaneous Estimation of Tenofovir, Emtricitabine and Efavirenz In Pure and Pharmaceutical Dosage Form By Rp-HPLC " , International journal of advance research in science and engineering, 2016, vol5 ,issue 05 ,Pp :188-200.

[21] Ramreddy Godela etal. ," Concurrent estimation of lamivudine , tenofovir disoproxilfumarate ,and efavirenz in blended mixture and triple combination tablet formulation by a new stability indicating RP-HPLC method", Future Journal of Pharmaceutical Sciences, 2021, 7:94, Pp:1-12.

[22] Vaibhav S Adhao etal ., " Reverse phase-liquid chromatography assisted protocol for simultaneous determination of lamivudine and tenofovir disoproxil fumarate in combined medication used to control HIV infection : an investigative approach" Future Journal of Pharmaceutical Sciences,2021,7:90,Pp:1-11.