World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION AND ESTIMATION OF LAMIVUDINE AND TENOFOVIR DRUG WITH ITS BULK FORM AND TABLET FORMULATION

[1]Manasa Kola, [2]Dr.B Raj Kamal

[1]M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District.

[2]Professor and Principal, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District.

Received: 02-09-2024 / Revised Accepted: 17-09-2024 / Published: 26-09-2024

ABSTRACT:

A straight forward and robust technique was devised to simultaneously estimate the concentrations of Lamivudine and Tenofovir in tablet dose form. The chromatogram was passed through an Inertsil C18 150 mm (4.6 x 150mm, 5µm) Mobile phase that included a Buffer (Ammonium Acetate) consisting of 90% Methanol. 10% of the buffer was pushed through the column at a flux rate of 1.2 ml/min. Thermal condition was regulated at 26°C. The selected optimised wavelength was 245.0 nm. The observed retention times for Lamivudine and Tenofovir were 2.247 minutes and 2.879 minutes, respectively. The relative standard deviation (RSD) obtained for Lamivudine and Tenofovir were 0.5 and 0.7 correspondingly. %The recoveries achieved for Lamivudine and Tenofovir were 101.07% and 99.98% respectively. The linear optical density (LOD) and limit of quantification (LOQ) values derived from the regression equations of Lamivudine and Tenofovir were 0.18, 0.19, and 0.54, 0.56 correspondingly. There are two regression equations for Lamivudine: y = 8523.9x + 11099 and y = 12735x + 2600.A reduction in retention times and a corresponding drop in run time made the devised approach easy and cost-effective for use in routine quality control tests in industries.

Key Words: Lamivudine, Tenofovir, RP-HPLC

INTRODUCTION

Human immunodeficiency virus (HIV) is a virus that attacks the body's immune system. Acquired immunodeficiency syndrome (AIDS) occurs at the most advanced stage of infection. HIV targets the body's white blood cells, weakening the immune system.[1] Antiretroviral drugs are drugs that can work against retroviruses. In general, the term "antiretroviral drug" is used for anti-HIV drugs. Since the discovery of HIV, there has been a desperate need to develop easy and convenient methods to evaluate antiretroviral drugs.[2] Lamivudine and Tenofovir disoproxil are the Antiretroviral drug in 1 tablet for the prevention of HIV.

Tenofovir disoproxil is a nucleotide analogue reverse transcriptase inhibitor used in the treatment of Hepatitis B infection and used in the management of HIV-1 infection. It is chemically known as bis({[(propan-2-yloxy)carbonyl]oxy}methyl){[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methanephosphonate.[3]
Tenofovir disoproxil is also an ingredient in several combination products, all of which are indicated either alone or in combination with other antiretrovirals for the treatment of HIV-1 infection.[4] This drug prevents viral

Address for Correspondence: Manasa Kola**,** M. Pharmacy, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Maisammaguda, Secunderabad, Hyderabad, 500100, Medchal District.; **E-Mail:** manasakola901@gmail.com.

How to Cite this Article: Manasa Kola. A Novel Rp-Hplc Method Development and Validation For Determination and Estimation of Lamivudine and Tenofovir drug with its Bulk form and Tablet Formulation. World J Pharm Sci 2024; 12(03): 45-56; https://doi.org/10.54037/WJPS.2022.100905

DNA chain elongation through inhibition of enzymes necessary for host cell infection viral replication in HIV-1 and Hepatitis B infections.[5,6]

Lamivudine is a reverse transcriptase inhibitor used to treat HIV and hepatitis B infections. It is chemically known as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.[7]

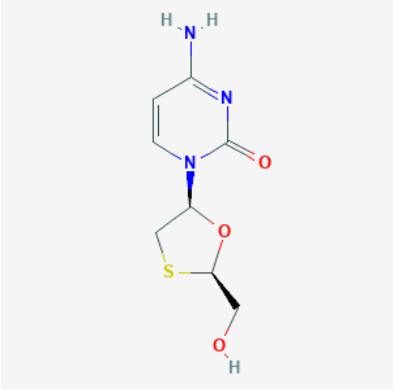

Figure 1 : structure of Lamivudine

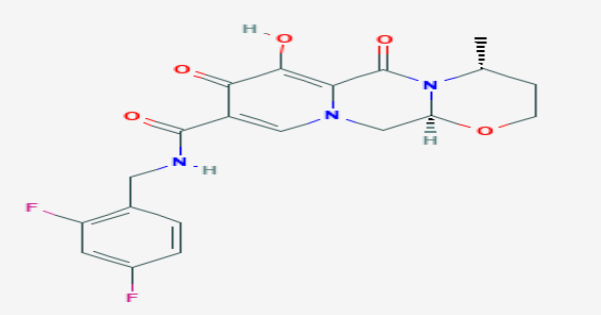

Figure 2: Structure of Tenofovir

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Lamivudine, Tenofovir, and their medicinal dose form using RP-HPLC.[8-15] must be validated and developed as per ICH guidelines.

Materials and Methods: Spectrum pharma Research Solution provide with Lamivudine and Tenofovir pure drugs (API) gift samples and Combination Lamivudine and Tenofovir tablets (Tenolam) received from local market. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: The primary objective of this study is to provide a highly exact, accurate, sensitive, specific, consistent, and efficient analytical method for the simultaneous quantification of Lamivudine and Tenofovir in their pure form and throughout tablet formation.

Table 1: Chromatographic Conditions

Mobile phase	Ammonium Acetate and Methanol (90:10)
Flow rate	1 ml/min
Column	Inertial C18(4.6 x 150mm, 5µm
Detector wave length	245 nm
Column temperature	26°C
Injection volume	10µL
Run time	5.0 min
Buffer	0.1N Ammonium acetate

Preparation of Standard stock solutions: Accurately Weighed and transferred 30 mg of Lamivudine and 30 mg of Tenofovir working Standards into a 50 ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes, and make up to the final volume with diluents. (600ppm of Lamivudine and 600 ppm of Tenofovir)

Preparation of Standard working solutions (100% solution): 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (60 ppm of Lamivudine and 60 ppm of Tenofovir)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 250ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1200µg/ml of Lamivudine and 1200 µg/ml of Tenofovir)

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml of Lamivudine and 60 µg/ml of Tenofovir).

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Lamivudine (60ppm) and Tenofovir (60ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should be not more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Table 2: System suitability results

S.no	Lamivudine			Tenofovir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.252	14261	1.39	2.871	10527	1.39	6.3
2	2.253	15639	1.45	2.872	10156	1.42	6.6
3	2.254	16316	1.50	2.873	9974	1.43	6.6
4	2.266	16795	1.33	2.884	10725	1.34	6.6
5	2.267	15612	1.34	2.885	11018	1.35	6.4
6	2.268	15669	1.35	2.886	11074	1.36	6.3

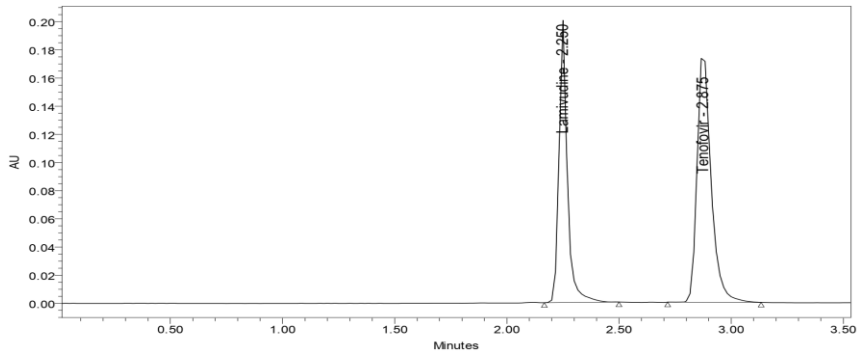

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Lamivudine	2.250	514889
Tenofovir	2.875	768301

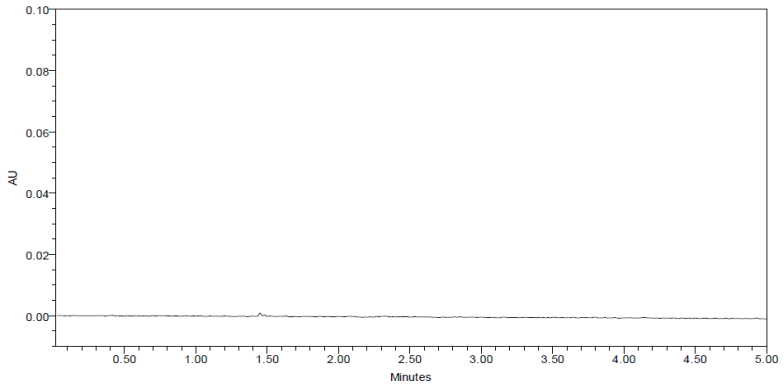

Figure.4 Blank

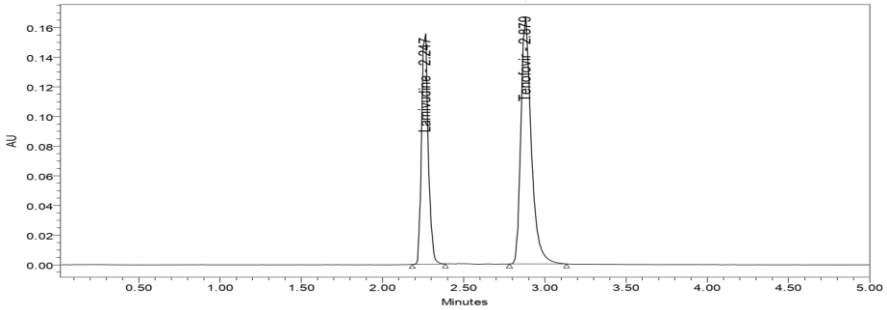

Figure 5: Specificity of Lamivudine and Tenofovir

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 6, 7

Table 4: Calibration data of Lamivudine and Tenofovir

Lamivudine		Tenofovir	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
15	138614	15	193008
30	267723	30	383857
45	392621	45	577507
60	523485	60	763656
75	652724	75	964516
90	776448	90	1144542

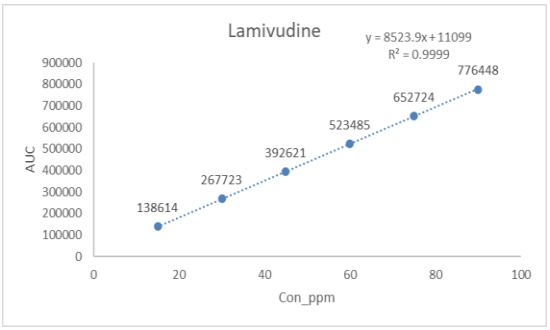

Figure 6 Calibration curve of Lamivudine

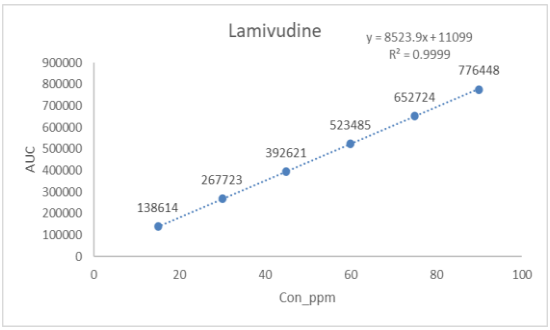

Figure 7 Calibration curve of Tenofovir

Table 5: regression data

Parameter	Lamivudine	Tenofovir
Conc range (µg/mL)	15-90µg/ml	15-90µg/ml
Regression Equation	y = 8523.9x + 11099	y = 12735x + 2600.9
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Lamivudine and Tenofovir

	Lamivudine			Tenofovir		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	30	30.19	100.63	30	29.90	99.67
	30	30.19	100.62	30	29.87	99.58
	30	30.52	101.73	30	29.72	99.07
100%	60	60.87	101.44	60	60.52	100.87
	60	60.71	101.18	60	59.68	99.46
	60	60.81	101.35	60	59.58	99.30
150%	90	91.00	101.11	90	91.07	101.18
	90	90.24	100.27	90	90.52	100.57
	90	91.16	101.29	90	90.10	100.11
% recovery	**101.07**			**99.98**		

System precision was performed and the data was shown in table 8

Table 7: System precision of Lamivudine and Tenofovir

S. No	Area of Lamivudine	Area of Tenofovir
1.	521154	762995
2.	525491	768530
3.	525491	763175
4.	521651	772775
5.	520264	773939
6.	525880	773939
Mean	**523322**	**769226**
S.D	**2561.1**	**5156.4**
%RSD	**0.5**	**0.7**

The % RSD for the peak areas of Lamivudine and Tenofovir obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Lamivudine and Tenofovir and shown in table 8.

Table 8: method Precision

S. No	Area of Lamivudine	Area of Tenofovir
1.	525988	761308
2.	521637	768734
3.	528967	774980
4.	529826	762281
5.	522833	761558
6.	521510	772249
Mean	525127	766852
S.D	3690.3	5973.1
%RSD	0.7	0.8

From the above results, the % RSD of method precision study was within the limit for Lamivudine and Tenofovir.

Robustness: Robustness conditions like Flow minus (1.1ml/min), Flow plus (1.3ml/min), mobile phase minus (85B:15A), mobile phase plus (95B:5A), temperature minus (21°C) and temperature plus(31°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Lamivudine and Tenofovir.

Condition	%RSD of Lamivudine	%RSD of Tenofovir
Flow rate (-) 0.7ml/min	0.7	0.4
Flow rate (+) 0.9ml/min	0.6	0.3
Mobile phase (-) 65B:35A	0.2	0.1
Mobile phase (+) 75B:25A	0.2	0.6
Temperature (-) 27°C	0.3	0.2
Temperature (+) 33°C	0.6	0.3

Sensitivity:

Table 10: sensitivity of Lamivudine and Tenofovir

Molecule	LOD	LOQ
Lamivudine	0.09	0.28
Tenofovir	0.10	0.30

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxdation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

Table 12: degradation data

Type of degradation	Lamivudine			Tenofovir		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	489126	93.28	6.72	725691	93.96	6.04
Base	493281	94.07	5.93	728612	94.34	5.66
Peroxide	496242	94.64	5.36	729075	94.40	5.60
Thermal	515988	98.40	1.60	758013	98.15	1.85
Uv	509012	97.07	2.93	768120	99.46	0.54
Water	516896	97.07	2.93	769661	99.66	0.34

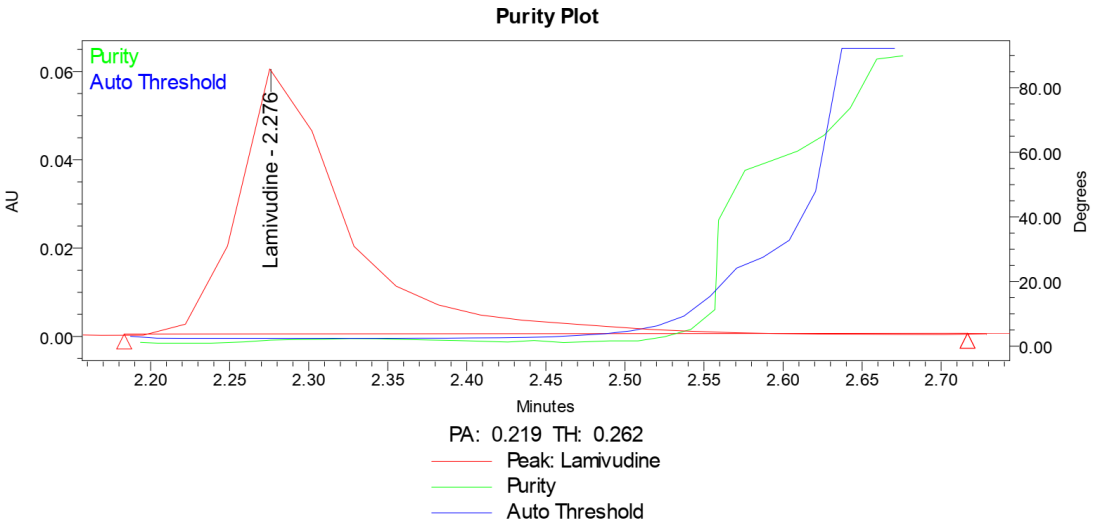

Figure 8: Purity plots for Acid Condition for Lamivudine

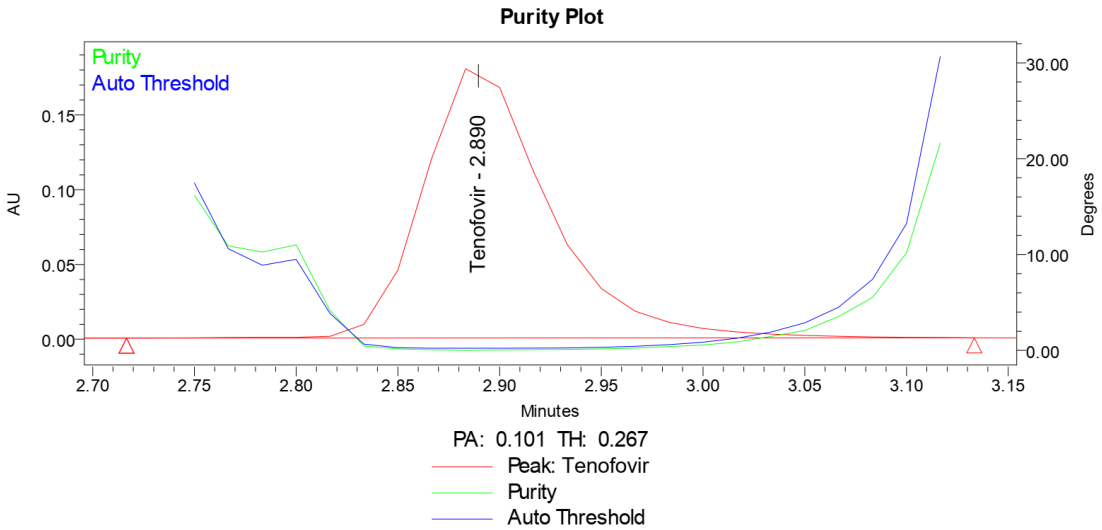

Figure 9: Purity plots for Acid Condition for Tenofovir

Assay: Tenolam Tablet, bearing the label claim Lamivudine 300mg, Tenofovir 300mg. Assay was performed with the above formulation. Average % Assay for Lamivudine and Tenofovir obtained was 100.14% and 99.29% respectively.

Table 13: assay data

	Lamivudine			Tenofovir		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	521154	525988	100.31	762995	761308	98.57
2	525491	521637	99.48	768530	768734	99.54
3	525491	528967	100.88	763175	774980	100.35
4	521651	529826	101.04	772775	762281	98.70
5	520264	522833	99.71	773939	761558	98.61
6	525880	521510	99.45	773939	772249	99.99
Avg	523322	525127	100.14	769226	766852	99.29
Stdev	2561.1	3690.3	0.70	5156.4	5973.1	0.8
%RSD	0.5	0.7	0.7	0.7	0.8	0.8

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10: formula of assay

Conclusion:

The study's results will help a lot with checking the quality of affordable medications that contain Lamivudine and Tenofovir. This might be because the study used a simple way to prepare the samples, which only needed a short analysis time and a small amount of mobile phase. After testing two medicines together in a single dose, the data showed that the newly developed analysis method was very close to being 100% effective.

ACKNOWLEDGEMENT:

The authors are thankful to, Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical science, Affiliated to JNTUH India and Spectrum Pharma Research Solutions, Hyderabad, Telangana, India.

REFERENCES :

1. https://www.who.int/news-room/fact-sheets/detail/hiv aids#:~:text=Human%20immunodeficiency%20virus%20(HIV)%20is,cells%2C%20weakening%20the%20immune%20system.
2. AshishSwarup Verma, Iqram Govind Singh, Ruby Bansal, Anchal Singh, HIV and Antiretroviral Drugs, Animal Biotechnology, 20
3. https://go.drugbank.com/drugs/DB00300

4. FDA Approved Drug Products: Atripla (efavirenz, emtricitabine, and tenofovir disoproxil fumarate) tablets for oral use
5. Duwal S, Schutte C, von Kleist M: Pharmacokinetics and pharmacodynamics of the reverse transcriptase inhibitor tenofovir and prophylactic efficacy against HIV-1 infection. PLoS One. 2012;7(7):e40382. doi: 10.1371/journal.pone.0040382. Epub 2012 Jul 11. [Article]
6. Delaney WE 4th, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, Miller MD: Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob Agents Chemother. 2006 Jul;50(7):2471-7. doi: 10.1128/AAC.00138-06
7. https://go.drugbank.com/drugs/DB00709
8. manoranjani m et al., method development and validation for simultaneous quantification of Lamivudine and Tenofovir in bulk and pharmaceutical dosage form and their forced degradation study by rp-hplc, asian j pharm clin res, vol 12, issue 2, 2019, 119-123.
9. uttam prasad panigrahy et al., a novel validated rp-hplc-dad method for thesimultaneous estimation of Lamivudine and Tenofovir inbulk and pharmaceutical dosage form with forceddegradation studies, international journal of chemtech research,2015: 8(10) 317-337.
10. dr. gampa vijay kumar et al., development and validation of rp hplc method for simultaneous estimation of Lamivudine and Tenofovir in pharmaceutical dosage form, iajps 2018, 05 (12), 16746-16755.
11. srikanth inturi et al., a validated novel rp-hplc method development for the estimation of Tenofovir hydrochloride in bulk and softule dosage forms, pelagia research library, 2011, 2 (5): 223-234.
12. kalavati et al.., development and validation of stability indicating uplc method for the estimation of Tenofovir in bulk and its pharmaceutical dosage form, international journal of pharmacy and pharmaceutical research, 2019; vol. 14 (3): 144-154.
13. mangesh harole et al., a validated stability indicating rp-hplc method for simultaneous determination of Lamivudine and palenoserton in pharmaceutical formulations,world journal of pharmacy and pharmaceutical sciences.