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

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND TENOFOVIR IN BULK AND TABLET DOSAGE FORM

Dr. K. Atchuta Kumar[1], T. Uma Maheswara Rao[2]

[1]Professor, Srinivasa Rao College of Pharmacy (Approved by PCI, New Delhi, & Affiliated to Andhra university, VSP, NAAC Accredited – 'B' Grade) An ISO 9001:2015, ISO 14001:2015 Certified Institution Visakhapatnam – 530041 India.

[2]M. Pharmacy, Department of Pharmaceutical Analysis, SRINIVASA RAO COLLEGE OF PHARMACY (Approved by PCI, New Delhi, & Affiliated to Andhra university, VSP, NAAC Accredited – 'B' Grade) An ISO 9001:2015, ISO 14001:2015 certified institution VISAKHAPATNAM – 530041 India.

Received: 06-09-2023 / Revised Accepted: 14-09-2023 / Published: 20-09-2023

ABSTRACT

A simple, accurate, exact approach was devised to estimate Lamivudine and Tenofovir in tablet form. Chromatogram was analyzed using Kromosil C18 150 mm (4.6 x 150mm, 5µm). Mobile phase: Buffer (Disodium phosphate) 75% Acetonitrile: 25% injected through column at 1 ml/min. The temperature was 30°C. Optimal wavelength was 250.0 nm. Lamivudine and Tenofovir had 2.250 and 2.875min retention times. %RSD of Lamivudine and Tenofovir were 0.4 and 0.2.% Lamivudine recovered 99.92% and Tenofovir 99.62%. Lamivudine and Tenofovir regression models yielded LOD, LOQ values of 0.11, 0.32, and 0.27, 0.83. Lamivudine regression equation is y = 8638.7x + 10272, Tenofovir 12913x + 5402. The technique devised was straightforward and affordable for routine quality control tests in industries since retention and run times were reduced.

Keywords: Lamivudine, Tenofovir, RP-HPLC

INTRODUCTION

HIV is a sexually transmitted infection (STI). It can also be spread by contact with infected blood and from illicit injection drug use or sharing needles. It can also be spread from mother to child during pregnancy, childbirth or breastfeeding. Without medication, it may take years before HIV weakens your immune system to the point that you have AIDS. There's no cure for HIV/AIDS, but medications can control the infection and prevent progression of the disease. Antiviral treatments for HIV have reduced AIDS deaths around the world, and

Address for Correspondence: Dr. K. Atchuta Kumar, Professor, Srinivasa Rao College of Pharmacy (Approved by PCI, New Delhi, & Affiliated to Andhra university, VSP, NAAC Accredited – 'B' Grade) An ISO 9001:2015, ISO 14001:2015 Certified Institution Visakhapatnam – 530041 India.; **E-Mail:** tirlangirobinhood@gmail.com

How to Cite this Article: Dr. K. Atchuta Kumar, T. Uma Maheswara Rao. Development And Validation of Rp-Hplc Method For The Simultaneous Estimation Of Lamivudine And Tenofovir In Bulk And Tablet Dosage Form. World J Pharm Sci 2023; 11(01): 62-76; https://doi.org/10.54037/WJPS.2022.100905.

international organizations are working to increase the availability of prevention measures and treatment in resource-poor countries.[1]

Lamivudine and Tenofovir are the drugs, which are used for the treatment of HIV.

Lamivudine:A reverse transcriptase inhibitor and zalcitabine analog in which a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV).[2]

Tenofovir: It is an acyclic nucleotide diester analog of adenosine monophosphate.[4] In the most strict sense and due to the fact that it presents a phosphate group bound to the nitrogenous base, it is determined as an actual nucleotide analog.[4] The antiviral activities of tenofovir were first reported in 1993 and this agent was commercially available since 2008 in the form of tenofovir disoproxil and tenofovir alafenamide in order to obtain oral bioavailability.[3,5]

This combination product is used with other HIV medications to help control HIV infection. It helps to decrease the amount of HIV in your body so your immune system can work better. This lowers your chance of getting HIV complications (such as new infections, cancer) and improves your quality of life. This product is a combination of 2 different drugs: lamivudine and tenofovir. Lamivudine is called a nucleoside reverse transcriptase inhibitor and tenofovir is called a nucleotide reverse transcriptase inhibitor. Lamivudine and tenofovir are often called NRTIs. Lamivudine/tenofovir is not a cure for HIV infection. To decrease your risk of spreading HIV disease to others, continue to take all HIV medications exactly as prescribed by your doctor. Use an effective barrier method (latex or polyurethane condoms/dental dams) during sexual activity as directed by your doctor. Do not share personal items (such as needles/syringes, toothbrushes, and razors) that may have contacted blood or other body fluids. Consult your doctor or pharmacist for more details.[6]

They are available in market with the brand name Cimduo and Temixys and this drugs are also available with many other combinations for example, The fixed-dose combination of doravirine, lamivudine and tenofovir disoproxil fumarate (trade name: Delstrigo) has been approved in Germany since November 2018 for the treatment of HIV-1 in adults.

The human immunodeficiency virus (HIV) multiplies in cells of the immune system and destroys them. Without proper treatment, the immune system of most HIV patients is weakened so much over time that they become seriously ill. This stage is referred to as AIDS (acquired immune deficiency syndrome).

Although the currently available medications can't cure HIV, they can prevent immunodeficiency for many years. But the medication may become ineffective over time, allowing the viruses to multiply more again.

The fixed-dose combination of doravirine, lamivudine and tenofovir disoproxil fumarate inhibits certain proteins that the virus needs in order to multiply.[7]

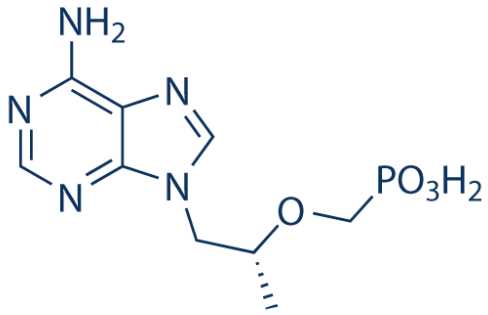

Figure 1: Structure of Tenofovir

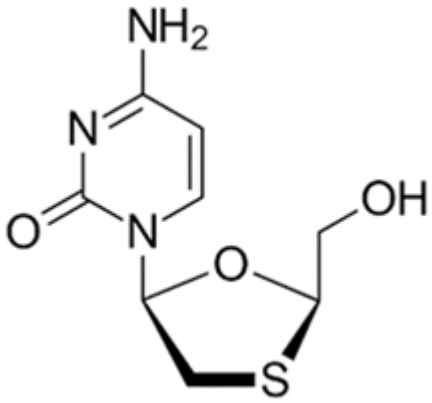

Figure 2: Structure of Lamuvidine

According to a literature review, there are some techniques for the simultaneous estimate of these medicines as well as others for assessment of the drugs alone or in combination with other drugs. Utilizing UV-Spectrophotometry RP-HPLC . There is no established technique for the stability-indicating simultaneous measurement of Lamivudine and Tenofovir by RP-HPLC in pharmaceutical dosage form, according to a survey of the literature. The primary goal of this work is to provide an efficient, quick, and accurate RP-HPLC approach for estimating of Lamivudine and Tenofovir in medicinal dose and tablet form. According to ICH recommendations, a proven approach was also used to estimate the amounts of Lamivudine and Tenofovir. [8-14]

MATERIALS AND REAGENTS

Lamivudine and Tenofovir pure drugs were received from Spectrum Pharma research solutions, Hyderabad. The combination tablet Lamivudine and Tenofovir (Telekast Plus) was purchased from a local pharmacy store. Rankem in India provided all of the chemicals and buffers utilised in this method like Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen Ortho phosphate buffer, Ortho-phosphoric acid, Distilled water.

Instrumentation and Chromatographic Conditions

For the development and validation method, an automated sample injector was employed with a WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of buffer:

Buffer:

0.1N Potassium dihydrogen Ortho phosphate: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q

water added and degas to sonicate and finally make up the volume with water then added 1ml of Trimethylamine then PH adjusted to 3.3 with dil. Orthophosphoric acid solution.

Buffer: (0.1% Orthophosphoric acid): 1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

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 each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (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).

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation of Lamivudine and Tenofovir drug material in accordance with the ICH criteria.

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.

Linearity: stock solutions of Lamivudine and Tenofovir is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy:

Preparation of Standard 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 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°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.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above

solutions 0.1ml each of Lamivudine and Tenofovir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Lamivudine and Tenofovir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

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.

Degradation studies:

Oxidation: To 1 ml of stock solution of Lamivudine and Tenofovir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 60µg/ml& 60µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Lamivudine and Tenofovir, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 60µg/ml& 60µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Lamivudine and Tenofovir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 60µg/ml& 60µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 1hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 60µg/ml & 60µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 600µg/ml & 600µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 60µg/ml & 60µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr at a temperature of 60º. For HPLC study, the resultant solution was diluted to 60µg/ml & 60µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

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).

RESULTS AND DISCUSSIONS:

Table 1. System suitability table

S.No	Lamivudine			Tenofovir			
Injection	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	RS
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	1.5	2.873	9974	1.43	6.6
4	2.266	16795	1.33	2.884	10925	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	11094	1.36	6.3

Table 2. Specificity data

Sample name	Retention time (Mins)	Area
Lamivudine	2.250	514889
Tenofovir	2.857	768301

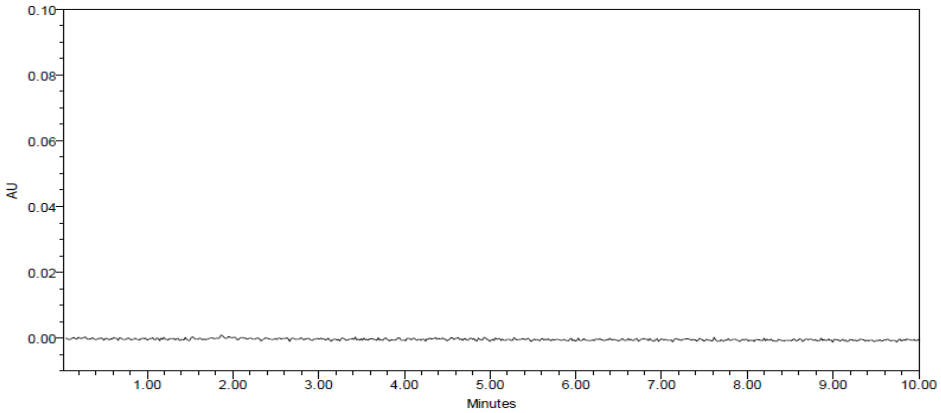

Figure 3. Blank Chromatogram

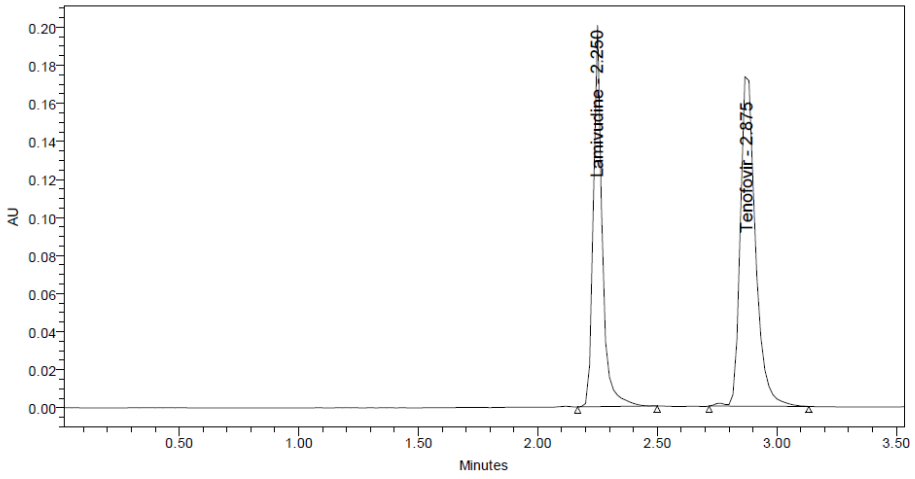

Figure 4. Specificity Chromatograms of Lamivudine and Tenofovir

Linearity

Table 3. Linearity table for Lamivudine and Tenofovir:

Lamivudine		Tenofovir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
15	142026	15	204515
30	281172	30	392948
45	404673	45	586007
60	523158	60	781670
75	655878	75	977173
90	786185	90	1163176

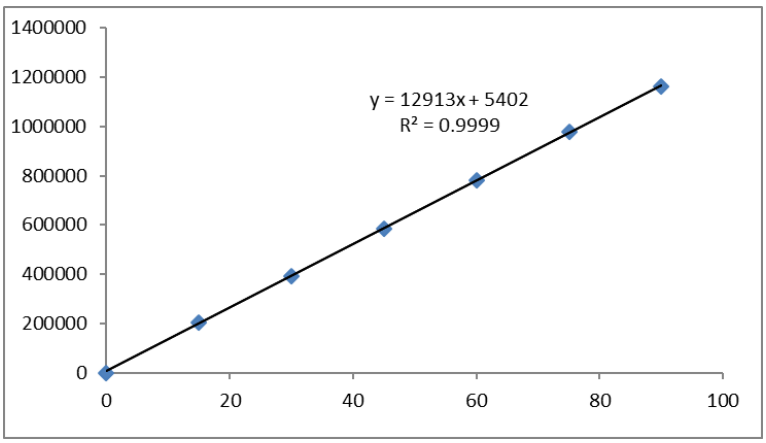

Figure 4. Calibration curve of Tenofovir

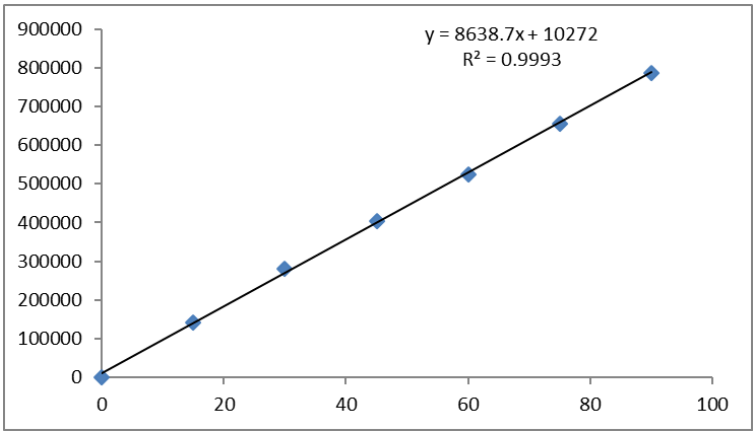

Figure 5. Calibration curve of Lamivudine

Accuracy: Table 4. Accuracy table of Lamivudine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	60	101.00	99.92%
	30	60	99.52	
	30	60	99.33	
100%	60	60	100.72	
	60	60	101.15	
	60	60	100.63	
150%	90	60	99.02	
	90	60	98.80	
	90	60	99.14	

Table 5. Accuracy table of Tenofovir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	30	60	99.92	99.62%
	30	60	98.03	
	30	60	99.33	
100%	60	60	99.47	
	60	60	100.42	
	60	60	99.47	
150%	90	60	99.12	
	90	60	100.50	
	90	60	100.35	

System Precision: With regard to the working strength of Lamivudine and Tenofovir, six duplicate injections of the standard solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 6. System precision

S. No	Area of Lamivudine	Area of Tenofovir
1.	521059	773059
2.	518867	774890
3.	516786	774248
4.	522296	773099
5.	522970	774274
6.	521037	776361
Mean	**520503**	**774322**
S.D	**2297.3**	**1231.2**
%RSD	**0.4**	**0.2**

The % RSD for the peak areas of Lamivudine and Tenofovir obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Lamivudine and Tenofovir allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Table 7. Method precision

S. No	Area of Lamivudine	Area of Tenofovir
1.	527361	772730
2.	526312	773313
3.	518623	777163
4.	523660	779519
5.	522394	774714
6.	523536	774803
Mean	**523648**	**775374**
S.D	**3089.3**	**2544.9**
%RSD	**0.6**	**0.3**

Results shows, the % RSD of Repeatability study was within the range for Lamivudine and Tenofovir is (<2%)

Table 8. Robustness

S.No.	Condition	%RSD of Lamivudine	%RSD of Tenofovir
1	**Flow rate (-) 0.9ml/min**	0.2	0.2
2	**Flow rate (+) 1.1ml/min**	0.7	0.1
3	**Mobile phase (-) 60B:40A**	0.2	0.1
4	**Mobile phase (+) 70B:30A**	0.7	0.3
5	**Temperature (-) 25°C**	0.2	1.0
6	**Temperature (+) 35°C**	0.4	0.9

Table 9. Forced degradation for Lamivudine and Tenofovir

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

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table 10. Degradation results of Lamivudine and Tenofovir

Type of degradation	Lamivudine		Tenofovir	
	% RECOVERED	% DEGRADED	% RECOVERED	% DEGRADED
Acid	93.46	6.54	93.44	6.56
Base	94.10	5.90	93.69	6.31
Peroxide	95.10	4.90	93.77	6.23
Thermal	97.00	3.00	97.43	2.57
Uv	97.29	2.71	98.69	1.31
Water	97.29	2.71	99.40	0.60

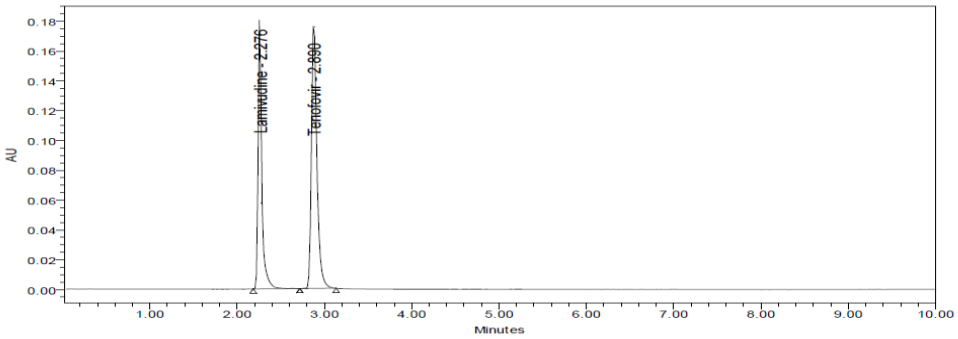

Figure 6: Acid chromatogram of Lamivudine and Tenofovir

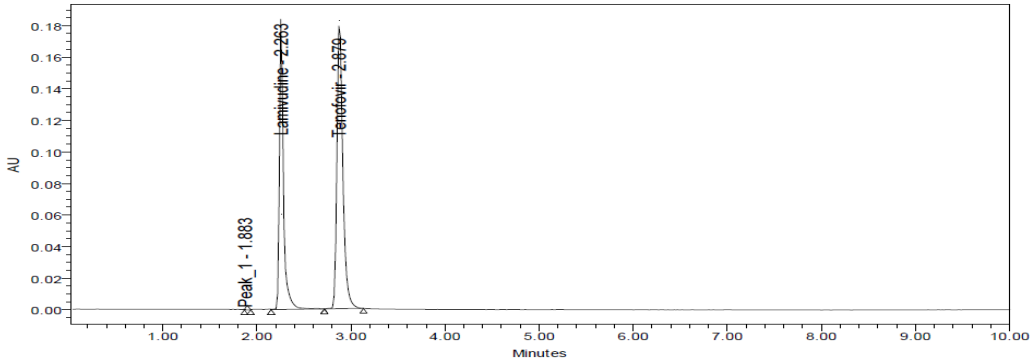

Figure 7: Base chromatogram of Lamivudine and Tenofovir

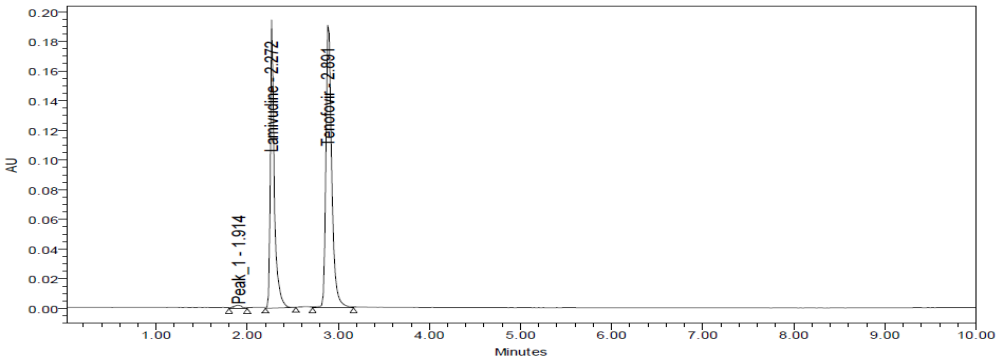

Figure 8: Peroxide chromatogram of Lamivudine and Tenofovir

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: (TEMIXYS) bearing label claim, Lamivudine 300mg and Tenofovir 300mg, assay was carried out by injecting sample into HPLC System.

Table 11. Assay Data of Lamivudine

S.no	Standard Area	Sample area	% Assay
1	521059	527361	100.91
2	518867	526312	100.71
3	516786	518623	99.24
4	522296	523660	100.20
5	522970	522394	99.96
6	521037	523536	100.18
Avg	**520503**	**523648**	**100.20**
Stdev	**2297.3**	**3089.3**	**0.59**
%RSD	**0.4**	**0.6**	**0.6**

Table 12. Assay data of Tenfovir

S.no	Standard Area	Sample area	% Assay
1	773059	772730	99.40
2	774890	773313	99.47
3	774248	777163	99.97
4	773099	779519	100.27
5	774274	774714	99.65
6	776361	774803	99.66
Avg	**774322**	**775374**	**99.74**
Stdev	**1231.2**	**2544.9**	**0.3**
%RSD	**0.2**	**0.3**	**0.3**

Table 13. Assay outcome for Lamivudine and Tenofovir

Drug Name	Label claim dose	%Assay	Brand Name
Lamivudine	300mg	100.20	**TEMIXYS**
Tenofovir	300mg	99.62	

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Lamivudine and Tenofovir in tablet dosage form. The method was found to be accurate, precise, robust and specific. Retention time of Lamivudine and Tenofovir were found to be 2.250 min and 2.875 min. %RSD of the Lamivudine and Tenofovir were and found to be 0.6 and 0.3 respectively. %Recovery was obtained as 99.92% and 99.62% for Lamivudine and Tenofovir respectively. LOD, LOQ values obtained from regression equations of Lamivudine and Tenofovir were 0.11, 0.32 and 0.27, 0.83 respectively. Regression equation of Lamivudine is y = 8638.7x + 10272, and y = 12913x + 5402 of Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmaceutical Analysis, Srinivasarao College of Pharmacy, visakapatnam, Andhra Pradesh, India, and Spectrum Pharma Research Solution, Hyderabad, Telangana, India, for providing Lamivudine and Tenofovir gift samples

REFERENCES

1. Mayo clinic, https://www.mayoclinic.org/diseases-conditions/hiv-aids/symptoms-causes/syc-20373524
2. Lamivudine from https://go.drugbank.com/drugs/DB00709
3. Ray AS, Fordyce MW, Hitchcock MJ: Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus. Antiviral Res. 2016 Jan;125:63-70. doi: 10.1016/j.antiviral.2015.11.009. Epub 2015 Nov 27.
4. Margolis AM, Heverling H, Pham PA, Stolbach A: A review of the toxicity of HIV medications. J Med Toxicol. 2014 Mar;10(1):26-39. doi: 10.1007/s13181-013-0325-8.
5. Ridruejo E: Treatment of chronic hepatitis B in clinical practice with entecavir or tenofovir. World J Gastroenterol. 2014 Jun 21;20(23):7169-80. doi: 10.3748/wjg.v20.i23.7169.
6. https://www.webmd.com/drugs/2/drug-174913/lamivudine-tenofovir-disoproxil-fumarate-oral/details
7. Institute for Quality and Efficiency in Health Care (IQWiG); 2006
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,
11. Iajps 2018, 05 (12), 16746-16755.
12. 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.
13. 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.

14. 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.