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Original Research

A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMERATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:

A novel, simple, precise and accurate method developed for the estimation of Lamivudine and tenofovir disoproxil fumarate (TDF) in bulk drug form has been established. Lamivudine and tenofovir are well known drugs and used in treatment of HIV- I. The method was performed by using $C1_8$ column, ODS Hypersil column with UV detection at 262nm by using Acetonitrile and water in ratio 55:45. The retention time was found to be 2.8 and 6.8 min for Lamivudine and tenofovir disoproxil fumarate (TDF). The linearity was found in range of 6- 14µg/ml for Lamivudine and 10- 50µg/ml for Tenofovir disoproxil fumarate with flow rate 1ml/min. the method was validated for linearity, accuracy, precision and robustness as per ICH guidelines. This method is suitable for simultaneous analysis for both the nucleoside analog reverse- transcriptase inhibitors Similarly, the %RSD value for precision was also found to be within the acceptable limit. The method was validated according to international conference of harmonization guidelines in terms of accuracy, precision, specificity, robustness, linearity and other aspects of analytical validation. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method. Developed method was rapid and convenient which could be successfully applied for the routine control of both the component.

Keywords: RP-HPLC, Lamivudine and Tenofovir and Validation; Robustness and ICH Guidelines.

Introduction:

Tenofovir disoproxil fumarate (Fig-1) and Lamivudine (Fig-2) are widely used anti-retroviral drugs in the categories of NRTIs i.e. nucleotide analogues reverse transcriptase inhibitors [1-4]. These drugs are used for the prevention and clinical management of acquired immune deficiency syndrome (AIDS) with multiple complications [5-8]

Lamivudine is commonly called 3TC used in the treatment of HIV/AIDS and also treat chronic hepatitis B where the virus that causes complicated liver inflammation. It slow down and prevent damage to immune system and reduce the risk of developing AIDS related illnesses. Lamivudine help fight the virus and slow the ability to damage liver [9-12]

Tenofovir is type of anti-HIV medicine called a nucleoside reverse transcriptase inhibitor (NRTI). It is always used in combination with other antiviral agents to treat patients with HIV. It is used in the form of pro- drug as Tenofovir Disoproxil Fumarate [13-15]. Tenofovir is not cure for HIV infection but decrease risk of spreading HIV disease to others. It also used to treat the certain type of liver

infection called chronic hepatitis B infection [16-18]. The literature review revealed that there are several methods available for single component analysis for lamivudine and Tenofovir (TDF) [19-20].

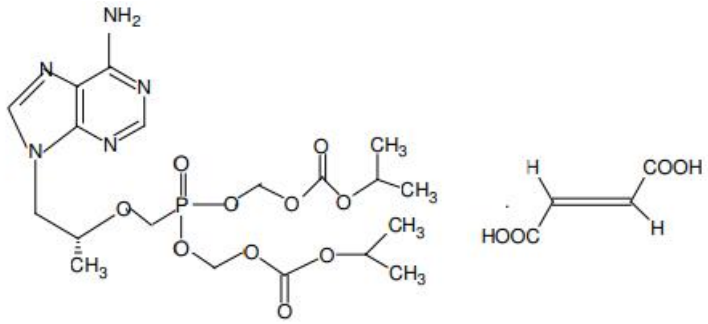

Figure 1: Tenofovir (TDF)

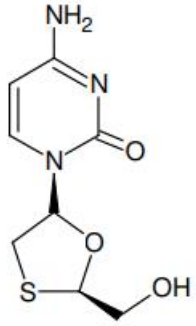

Figure 2: Lamivudine

Fig.01 &2. Chemical structure of Tenofovir and Lamivudine

MATERIAL AND METHOD

Instrument The lambda max and iso-absorptive point were determined by UV –spectrophotometer using Lab India with UV win software. HPLC (Shimadzu) prominence LC 20 AD, manual sampler, software LC solution and detector (UV-visible), Column C-18, Thermo scientific octadecylsilane Hypersil (ODS), ultra sonicater, vacuum filter, analytical balance. Selection of wavelength the selection of wavelength 10 µg/ml concentration of lamivudine and 10µg/ml concentration of tenofovir was prepared in 55:45 with ACN: water respectively. The result show iso-absorptive point that was observed at 262 nm (figure 3)

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Selection of chromatographic condition

The isocratic mode with mobile phase Acetonitrile and water in ratio 55:45 with flow rate 1ml/min. The resulting chromatograms were recorded and the chromatographic responses were measured.

Analytical Method Validation

A calibration curve was plotted with the concentration range 6- 14µg/ml for lamivudine and 10-50µg/ml for tenofovir (TDF). The method was developed and validated as per ICH guidelines. The parameters were studied linearity, accuracy, precision (intraday and interday precision and repeatability) and robustness and the amount recovery, percentage recovery and mean recovery for the same was calculated.

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Preparation of standard stock solution

Lamivudine standard stock solution Standard lamivudine 100 mg was weighed and transferred to a 100 ml clean and dry volumetric flask and dissolved into the HPLC grade sample solution (ACN: Water in the ratio 55:45) then volume was made up to the mark with solution containing 1000µg/ml conc. Then 10 ml of solution was pipette out and transferred to 100 ml clean and dry volumetric flask, made up its volume with solvent to get 100 µg/ml conc. solutions.

TDF standard stock solution Standard tenofovir (TDF)

100 mg was weighed and transferred to a 100 ml clean and dry volumetric flask and dissolved into the HPLC grade sample solution (ACN: Water in the ratio 55:45) then volume was made up to the mark with solution containing 1000 µg/ml conc. Then 10 ml of solution was pipette out and transferred to 100 ml clean and dry volumetric flask, made up its volume with solvent to get 100 µg/ml conc. Solutions

Chromatographic conditions The mobile phase consisting of Acetonitrile: water (55:45) was used and absorbance was measured at 262 with the run time 15 min and the flow rate was set at 1.0 ml/min respectively. Preparation of mobile phase Mobile phase was prepared by mixing HPLC grade acetonitrile and water in ratio of 55: 45 respectively, and the chromatographic conditions were made for separation of the drugs at the wavelength of 262nm. Degassing is done before the use of mobile phase.

Forced degradation studies

The drug substance was subjected to forced degradation under acidic, basic and neutral conditions. The acidic (0.1 M HCl) and basic (0.1 M NaOH) hydrolyses were carried out by refluxing in a water bath for 60 min. Before injection into the chromatograph, the solutions were neutralized using either NaOH or HCl. Oxidative stress studies were carried out at room temperature for up to 1 h in 3% H2O2. For all degradation studies in solution, 1 mg/mL drug concentration was used.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

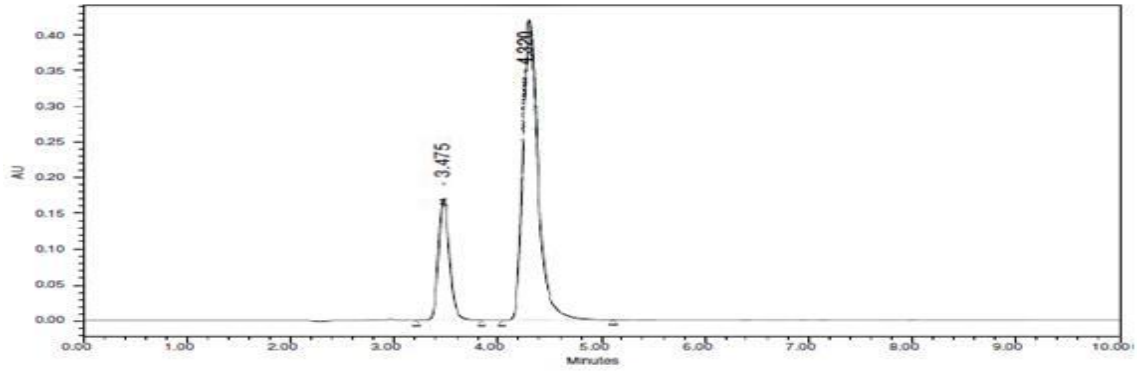
An LC method described by Reddiah et al. was used as a starting point [15]. Since this method was able to separate lamivudine in lamivudine-zidovudine-abacavir combination, the separation conditions of the method were improved to make it suitable for separation of both tenofovir disoproxil fumarate and lamivudine. The method uses an Intensil® ODS-3V (250 mm × 4.6 mm, 5µm) column at 50°C and sample compartment at 5°C. Two mobile phases were used: methanol as mobile phase A and a mixture of buffers (2.3 g/L ammonium dihydrogen phosphate and 1.32 µg/L of diammonium hydrogen phosphate, pH 3.9) mobile phase B. The gradient used was: 0 min 97/3; 15 min 97/3; 70 min 60/40; 80 min 40/60; 82 min 97/3; and 90 min 97/3. Several trials were conducted using the adopted method. It was observed that at 70 min (60/40), there was poor separation of tenofovir disoproxil fumarate and hence the gradient system was adjusted to enhance the separation (Figure 2). Chromatographic conditions were optimized by changing the gradient composition at 70 min to 50/50 and reducing the column temperature to 30°C due to the instability of tenofovir. Different experiments were performed to optimize the elution gradient and adequate separation of the two drugs. The optimized mobile phase was composed of methanol as mobile phase A and mixture of buffers (2.3 µg/L ammonium dihydrogen phosphate and 1.32 µg/L of diammonium hydrogen phosphate, pH 3.9) as mobile phase B.

METHOD VALIDATION

Method robustness the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small changes. When the method is applied place in a different laboratory by different analysts and equipment. In this study, the influence of three chromatographic parameters on the

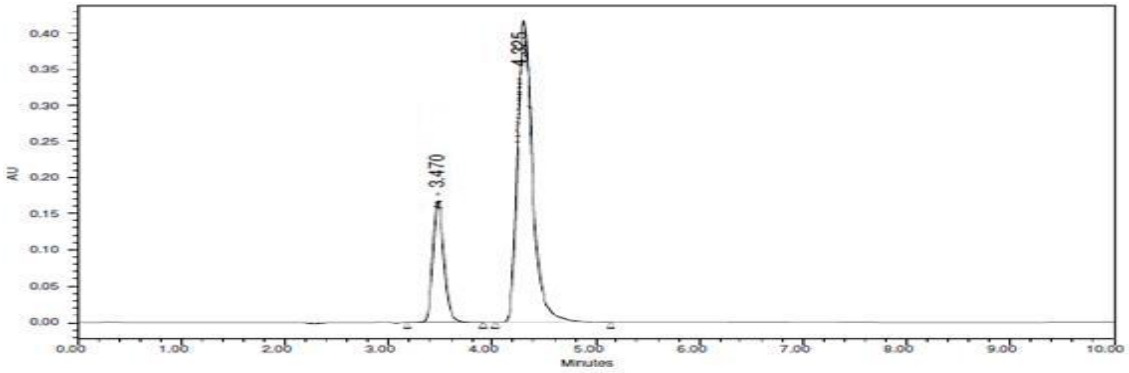
separation was investigated. The parameters examined were the flow rate of the mobile phase, the column temperature and the pH of the mobile phase. Each of these parameters was investigated at three levels, low (-1), central (0) and high (+1) (Table 2). Their effects on the separation, between lamivudine and tenofovir were evaluated by means of a central composite face centered design using Modde 4.0 statistical graphic software (Umetrics, Umea, Sweden). A central composite face centred design which prescribes 17 experiments was applied.

The central composite design permitted the response surface to be modelled by fitting a second-order polynomial model (Figure 3). A positive effect means that an increase of the factor value increases the response while a negative gives the opposite response. The interactive effect of temperature and pH on the theoretical plates of lamivudine showed maximum response at intermediate pH and lowest extreme of temperature. For TDF, temperature had negligible effect on k' while a decrease in pH caused a significant decrease with least effect observed at intermediate pH. However, the tailing factor for TDF decreased with increased temperature and was highest at intermediate pH (Figure 3). Specificity and selectivity Excipients mixture that were used for preparation of LT tablets without the APIs was used as a placebo in order to check possible interference with the analyte peaks during analysis. No interference was observed between analytes, placebo and solvent chromatograms (Figure 4).



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lamivudine	3.475	1246124	170215	22.67		1.07	5754
2	Tenofovir	4.320	4239565	421542	77.33	3.89	1.06	4898

Fig.3: Chromatogram for Standard



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lamivudine	3.470	1239565	165986	22.76		1.05	5547
2	Tenofovir	4.325	4229956	418954	77.24	3.89	1.06	4986

Fig.4: Chromatogram for Sample

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Assay Calculations:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100s$$

Assay Calculations for Tenofovir

$$\text{assay \%} = \frac{4230110}{4242603} \times \frac{400.5}{100} \times \frac{100}{328} \times \frac{99.80}{100} X328X\ 100$$

$$= 99.63\%$$

Assay Calculations for Lamivudine:

$$\text{Assay \%} = \frac{1237414}{11240388} \times \frac{30}{100} \times \frac{100}{328} \times \frac{99.80}{100} x\ 328X\ 100$$

Table.1: System Suitability data for Lamivudine

S No	Lamivudine	
	RT	Area
1	3.486	1239517
2	3.486	1241754
3	3.486	1246030
4	3.486	1244401
5	3.485	1247129
6	3.486	1251757
Average	3.486	1245098
Standard Deviation	0.0004	4293.1
%RSD	0.0117	0.345

Table.2: System Suitability data for Tenofovir

S No	Tenofovir	
	RT	AreaS
1	4.313	4209541
2	4.312	4212874
3	4.312	4232293
4	4.312	4228294
5	4.311	4250605
6	4.311	4248839
Average	4.312	4230408
Standard Deviation	0.001	17311.94
%RSD	0.02	0.41

s

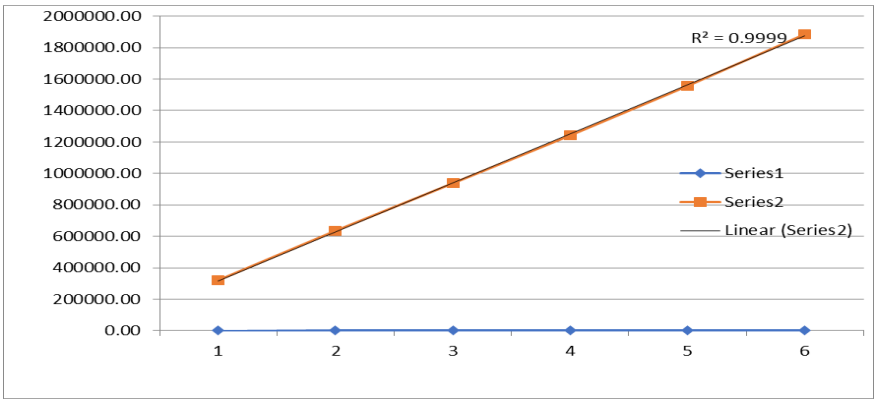

Fig.5: Linearity graph for Lamivudine

Table.3: Linearity results for Lamivudine

Conc. (mcg)	Area
3.75	318579
7.50	632307
11.25	940714
15.00	1242080
18.75	1555290
22.50	1882846
Correlation coefficient	0.999

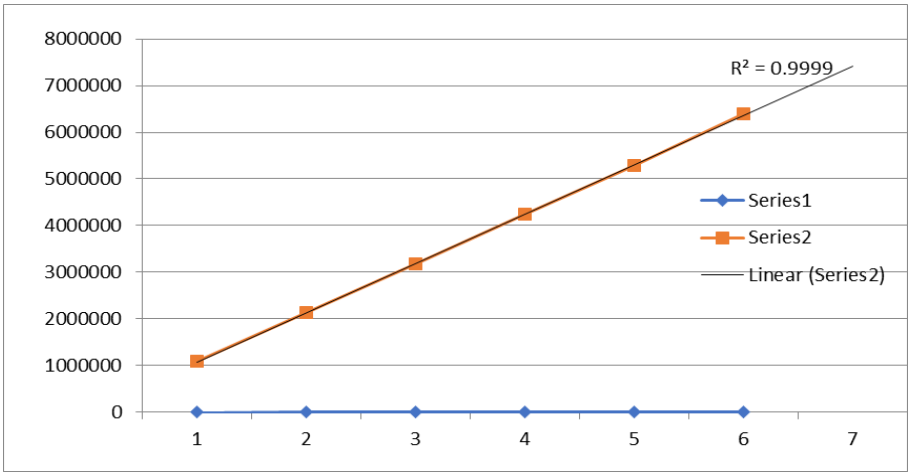

Fig.6.: Linearity graph for Tenofovir

Table.4. Linearity results for Tenofovir

Conc.(mcg)	Area
50	1083182
100	2140868
150	3178742
200	4234699
250	5283960
300	6383864
Correlation coefficient	0.999

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Accuracy Results:

Table.5: Accuracy Results of Lamivudine and Tenofovir

	Accuracy-- 80%		**Accuracy-- 100%**		**Accuracy-- 120%**	
	Lam	Tenofovir	Lam	Tenofovir	Lam	Tenofovir
S No	**Area**	**Area**	**Area**	**Area**	**Area**	**Area**
Injection-1	989546	3393262	1238232	4222121	1495695	5072521
Injection-2	993025	3353232	1247565	4220120	1488513	5035654
Injection-3	994545	3366565	1239665	4219232	1477756	5091236
Avg	**992372**	**3371020**	**1241821**	**4220491**	**1487321**	**5066470**
amt Recovered	**79.41**	**79.35**	**99.20**	**99.32**	**119.02**	**119.27**
%Recovery	**99.26**	**99.19**	**99.20**	**99.32**	**99.18**	**99.39**

PRECISION:

Precision of the method was determined as described under experimental work and the corresponding chromatograms and results are shown below

Method Precision Results:

Table 6. Method Precision results for Lamivudine and Tenofovir

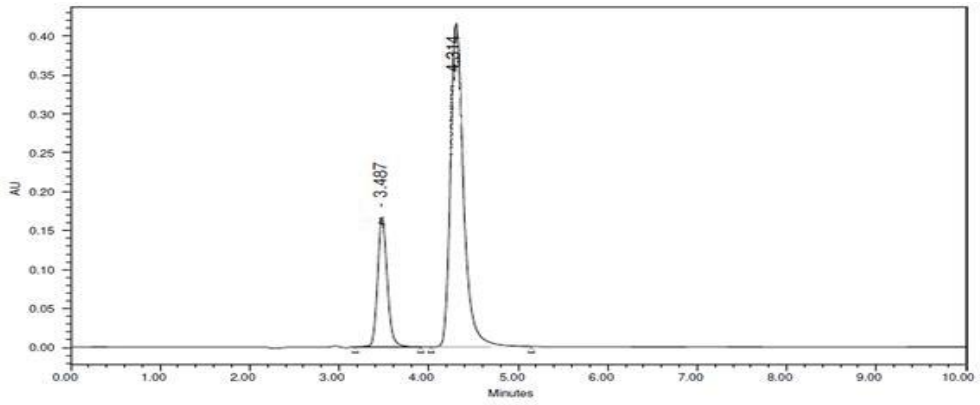
S No	**Name**	**Lamivudine**		**Tenofovir**	
		RT	Area	RT	Area
1	M-Precision-1	3.481	1220596	4.323	4201252
2	M-Precision-2	3.477	1226595	4.319	4199998
3	M-Precision-3	3.489	1230155	4.315	4222215
4	M-Precision-4	3.485	1229899	4.316	4201213
5	M-Precision-5	3.486	1221999	4.315	4215222
6	M-Precision-6	3.488	1245656	4.316	4212121
Average		3.484	1229150	4.317	4208670
Standard Deviation		0.0045	8998.4	0.003	9210.24
%RSD		0.1305	0.732	0.07	0.22

SYSTEM PRECISION:

Table.7: System Precision Results for Lamivudine and Tenofovir

S No	**Name**	**Lamivudine**		**Tenofovir**	
		RT	Area	RT	Area
1	S-Precision-1	3.486	1239517	4.313	4209541
2	S-Precision-2	3.486	1241754	4.312	4212874
3	S-Precision-3	3.486	1246030	4.312	4232293
4	S-Precision-4	3.486	1244401	4.312	4228294
5	S-Precision-5	3.485	1247129	4.311	4250605
6	S-Precision-6	3.486	1251757	4.311	4248839
Average		3.486	1245098	4.312	423408
Standard Deviation		0.0004	4293.1	0.001	17311.94
%RSD		0.0117	0.345	0.02	0.41

SPECIFICITY:



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lamivudine	3.487	1232691	167875	22.65		1.05	5856
2	Tenofovir	4.314	4220012	416589	77.35	3.89	1.03	4986

Fig.7: Chromatogram for Specificity of Standard

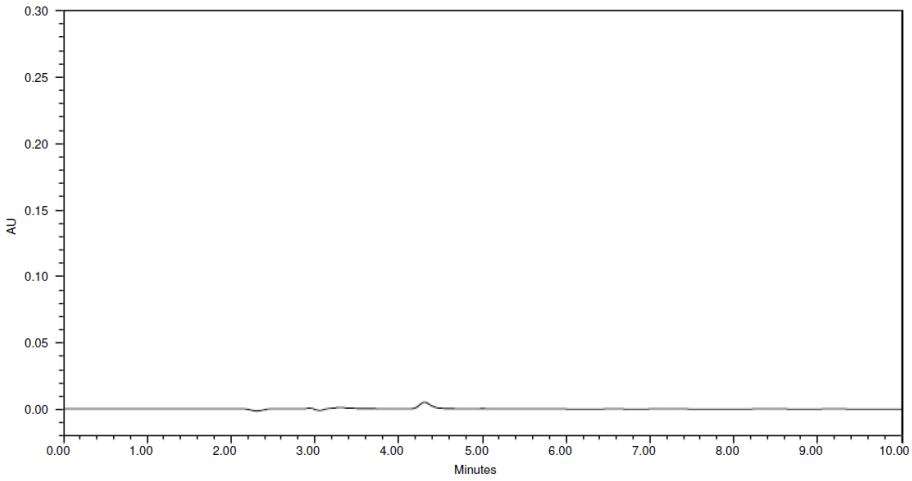
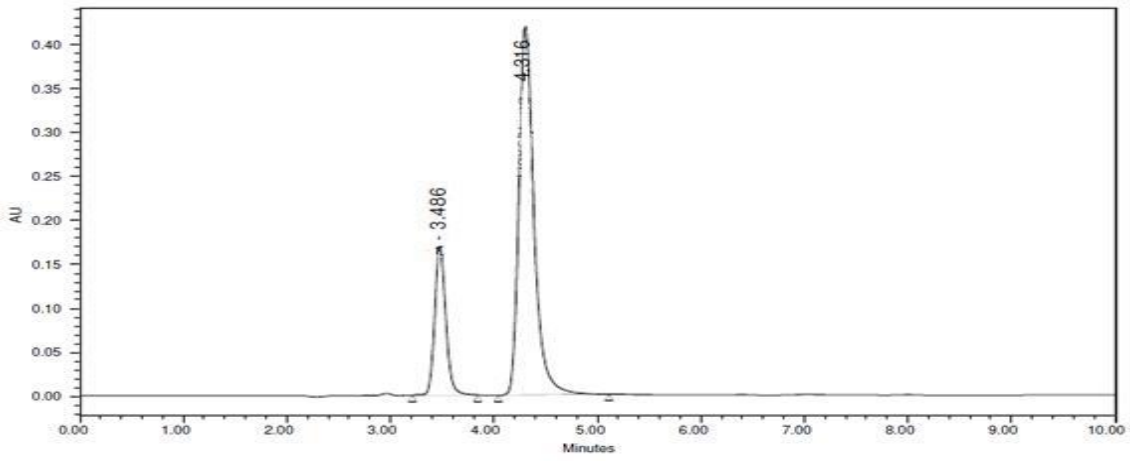


Fig.8.: Chromatogram for Specificity of Placebo



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lamivudine	3.486	1219652	168784	22.67		1.05	5351
2	Tenofovir	4.316	4224152	419452	77.33	3.89	1.07	4745

Fig.9. Chromatogram for Drug sample after Placebo

LOD and LOQ Results:

LOD and LOQ for Lamivudine:

Limit of detection = 3.3xs.d/slope
= 3.3 x10702 / 82241.8 =0.429
Limit of quantization =10.1xs.d/slope
=10.1x 10702 /82241.8 =1.31

LOD and LOQ for TENOFOVIR:

Limit of detection= 3.3 x s.d/slope
=3.3 x17425/20955 =2.74
Limit of quantization =10.1 x s.d/slope
=10.1 x17425/20955 =8.31

Table.8: Statistical data for Lamivudine and Tenofovir by HPLC method

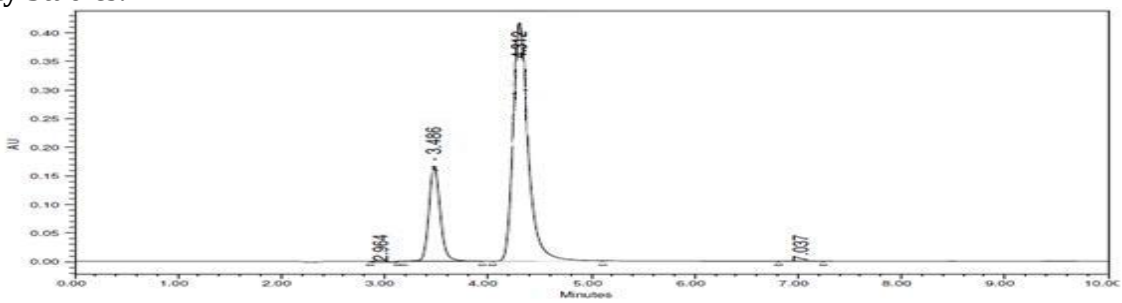
Parameter	Lamivudine	Tenofovir
Linearity range(µg/ml)	3.75-22.50	50-300
Slope	82241	20955
Limit of detection (µg/ml)	0.429	2.74
Limit of Quantization(µg/ml)	1.31	8.31

Robustness

Table.9: Robustness results for Lamivudine and Tenofovir

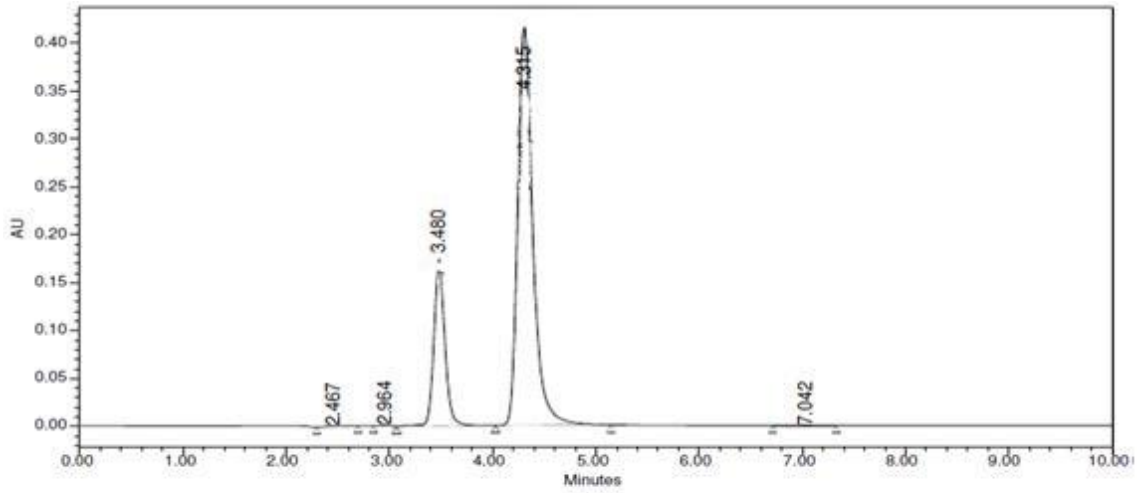
S.No.	Lamivudine		Tenofovir	
	RT	Area	RT	Area
Standard				
1	3.487	1232691	4.314	4220012
Robust-1 Flow -1				
2	3.107	1122733	3.841	3791183
Robust-2 Flow-2				
3	3.971	1434649	4.915	4879613
Robust-3 Column Oven Temperature-1				
4	3.506	1253860	4.338	4252684
Robust-4 Column Oven Temperaure-2				
5	3.467	1250755	4.304	4273151

Stability Studies:



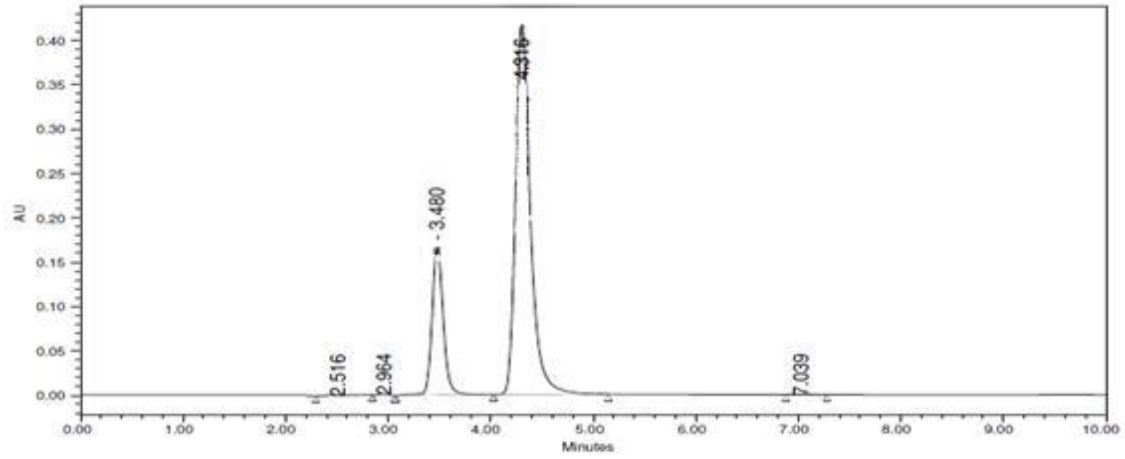
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		2.964	10158	1512	0.19		0.89	7093
2	Lamivudine	3.486	1204401	167541	22.67	3.15	1.16	5211
3	Tenofovir	4.312	4218294	417466	77.04	3.75	1.16	4589
4		7.037	5416	441	0.10	9.35	0.99	7091

Fig.10. Chromatogram for Stability Indicting at 5º C



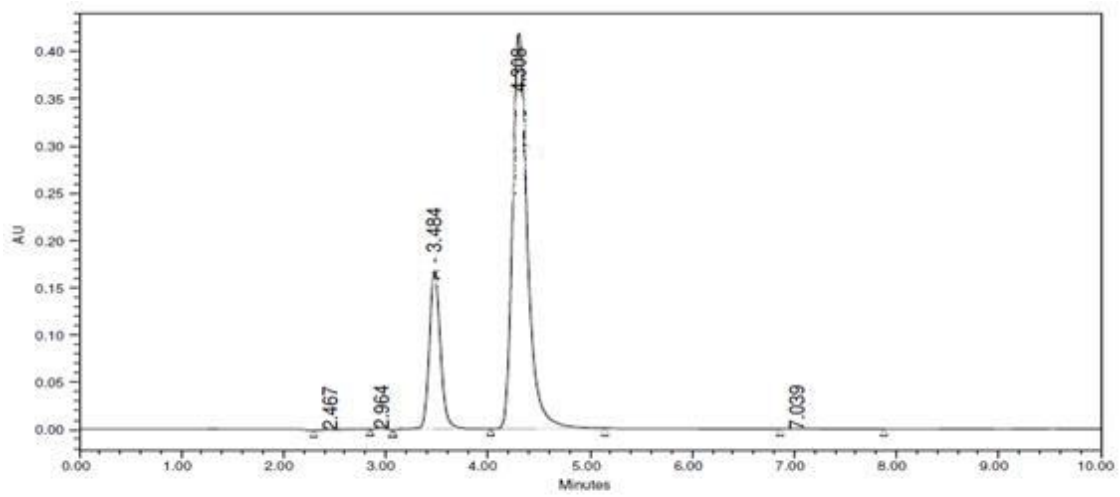
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		2.467	10088	757	0.18		0.59	2112
2		2.964	10158	1699	0.18	2.96	1.04	5367
3	Lamivudine	3.480	1186962	167709	23.02	2.99	1.14	5199
4	Tenofovir	4.315	4125875	415931	76.50	3.75	1.36	4604
5		7.042	6127	459	0.11	9.15	1.02	6504

Fig.11. Chromatogram for Stability Indicating At 25º c/60% RH



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		2.516	16545	865	0.30		1.27	2338
2		2.964	10843	1827	0.20	1.12	1.04	5450
3	Lamivudine	3.480	1214391	168278	23.01	3.00	1.14	5176
4	Tenofovir	4.316	4222293	417437	76.42	3.74	1.36	4589
5		7.039	4195	375	0.08	9.74	1.22	8442

Fig.12 Chromatogram for Stability Indicating At 40º C/75%RH



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		2.467	16911	924	0.30		0.54	2211
2		2.964	11345	1921	0.20	3.04	1.04	5495
3	Lamivudine	3.484	1227345	168970	22.97	3.00	1.14	5185
4	Tenofovir	4.308	4220325	419359	76.43	3.74	1.16	4585
5		7.039	5539	424	0.10	8.95	1.19	7234

Fig.13. Chromatogram for Stability Indicating at Room TEMP (25ºC)

CONCLUSION

In the present study a new RP-HPLC method was developed for Stability indicating and simultaneous estimation of Lamivudine and Tenofovir Disoproxil Fumarate in Pharmaceutical dosage forms and Bulk drugs. The analysis is resolved by using a on C_{18} Inertsil 5µ, 250mm×4.6mm column using phosphate buffer: acetonitrile: methanol (40:20:40) as mobile phase the flow was quite satisfactory. The flow rate was 0.8ml/min and the analyte were monitored at 257nm at which better detector response for drugs were obtained. The retention time for Lamivudine and Tenofovir Disoproxil Fumarate were 3.4min and 4.5min respectively. The method was validated for system suitability, accuracy, precision, linearity and ruggedness. The system suitability parameters were within limit; hence it was concluded that the method was suitable to perform the assay. It was also used for determining lower concentration of drug in its solid dosage forms. Therefore, it was concluded that the proposed method can be used for analysis of Lamivudine and Tenofovir Disoproxil Fumarate in Pharmaceutical dosage forms.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

References:

1. Sharma BK(2001). Instrumental methods of chemical analysis.23th edition. Goel publishing House Meerut; Introduction to Analytical chemistry.
2. (1996) The International Conference on Harmonization (ICH)- Validation of Analytical procedures; Methodology(Q2B), Food and Drug Administration, USA**.**
3. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: World Health Organization, 2016.
4. Backett AH, Stenlake JB, Practical of pharmaceutical chemistry. 4th ed. New Delhi. CBS publishers & distributers, p. 284 (2005) Venkatesan S, Kannappan N. Simultaneous

spectrophotometric method for determination of emtricitabine and tenofovir disoproxil fumarate in three component tablet formulation containing rilpivirine hydrochloride. International Scholarly Research Notices, p 9(2014)

5. Lloyed R, Joseph J et al. Practical HPLC method development. 2nd ed. Jhon Willy and Sons, New York: p. 653.
6. S. Raffanti, D. Haas, Antimicrobial Agents: Antiretroviral Agents, McGraw-Hill, New York, 1990.
7. Kapoor Namita, Khandavilli Sateesh, Panchagnula Ramesh. Simultaneous determination of lamivudine, stavudine and nevirapine in antiretroviral fixed dose combinations by high performance liquid chromatography, Analytica Chimica Acta, 570, 41–45 (2006)
8. Markwalder JA, Seitz SP, Crist DD and Multlib AE. Synthesis of putative metabolites of the non neucleoside reverse trancriptase inhibitor. National medicinal chemistry symposium, 14 – 18 (1998)
9. Sonara KV, Sapkalea P, Jadhav A. Development and validation of uv spectroscopic method for estimation of lamivudine in tablet dosage form, International Journal of Current Pharmaceutical Research, 9, 86- 89 (2017)
10. Anjaneyulu. N, Nagakishore. R, Nagaganesh. M, Muralikrishna. K. Development and validation of RP HPLC method for the simultaneous estimation of lamivudine and tenofovir disproxilfumerate in combined dosage form. Asian Journal of Biomedical and Pharmaceutical Sciences, 23, 7-11 (2013)
11. Krishna S, Subramanian S, Muthuraman MS. RP-HPLC Analytical Method Development And Validation For Lamivudine And Zidovudine In Pharmaceutical Dosage Forms, International Journal of PharmTech Research, 5, 1321-1331 (2013)
12. Patro SK, Swain SR, Patro VJ, Choudhary NSK. Development and validation of high performance liquid chromatographic method for determination of lamivudine from pharmaceutical preparation. E-Journal of Chemistry, 7, 117- 122 (2010)
13. Bhavsar DS, Patel BN, Patel CN. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form, Pharm Method, 3, 72-78 (2012)
14. Kumar D, Rao GS. Simultaneous Determination of Lamivudine, Zidovudine and Abacavir in Tablet Dosage Forms by RP HPLC Method, E-Journal of Chemistry, 7,180-184 (2009)
15. Kapoor N., Khandavilli S, Panchagnula R. Simultaneous determination of lamivudine, stavudine and nevirapine in antiretroviral fixed dose combinations by high performance liquid chromatography, Analytica Chimica Acta, 570, 41- 45(2006)
16. Deepali G, Elvis M. UV Spectrophotometric Method for Assay of the Anti-Retroviral Agent Lamivudine in Active Pharmaceutical Ingredient and in its Tablet Formulation, Journal of Young Pharmacists, 2, 417-149 (2010)
17. Uppalapati Y, Ghosh B, Deshpande K, Tadimarri VS. Method Development and Validation for the Simultaneous Estimation of Emtricitabine and Tenofovir in Pharmaceutical Dosage Forms by RP-HPLC, Journal of Drug Development and Delivery, 1:1, 07-12 (2018)
18. Akram N, Umamahesh MD. A New Validated RP-HPLC Method for the Determination of Emtricitabine and Tenofovir AF in its Bulk and Pharmaceutical Dosage Forms, Journal of Chemical and Pharmaceutical Sciences, 10, 54- 59 (2017)
19. Gorja Ashok, Sumanta Mondal. Development and Validation of Stability Indicating Method for the Simultaneous Quantification of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine Hydrochloride in Pharmaceutical Dosage Forms by RP-HPLC, Saudi Journal of Medical and Pharmaceutical Sciences, 15,175-183 (2018
20. Anandakumar Karunakaran (2012). Analytical method development and validation for simultaneous estimation of Lamivudine and Tenofovir. Eurasian J Anal Chemistry. Volume; 56-66.

21. Anjaneyulu. N(2013). Validation for simultaneous estimation of Lamivudine and Tenofovir. Asian Journal of Biomedical and Pharmaceutical Sciences. Vol 3, Issue 23, 7-11
22. Sindhura D (2013). Analytical Method Development and Validation for Simultaneous Estimation of Lamivudine, Zidovudine and Efavirenz by RP-HPLC. International Journal of Research in pharmacy and Biotechnology.pp;583-588.
23. Naga sandhya and Manikanta (2013). Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz. pp;147-155.