Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND TENOFOVIR IN TABLET DOSAGE FORM BY RP- HPLC

V. MOHAN GOUD[1], A. SRINIVASA RAO[2], S. RANJITH KUMAR[1]

[1]Department of Pharmaceutical chemistry, Joginpally B.R Pharmacy College, [2]Bhaskar Pharmacy College, Yenkapally, Moinabad, R.R. Dist. A.P. India. Email: mohanvanga@yahoo.com

Received: 13 May 2013, Revised and Accepted: 06 Jun 2013

ABSTRACT

A simple, rapid reverse – phase high performance liquid chromatographic method has been developed and validated for the simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pure and in tablet dosage form.

Objective: To develop and validate a high performance liquid chromatographic method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pure and in tablet dosage form.

Method: The estimation was carried out on a Phenomenax Luna C18 (150 mm x 4.6 mm i.d., particle size 5µm) column with a mixture of acetonitrile: methanol: water in the ratio of 30:50:20 (v/v) as mobile phase. UV detection was performed at 258 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form.

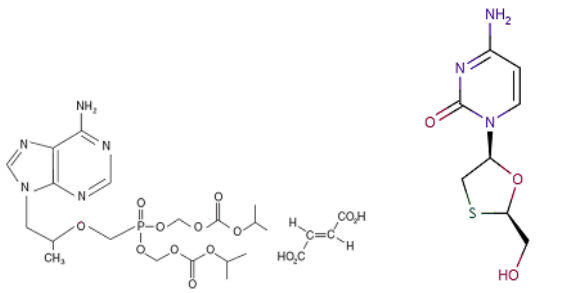
Results: The retention time was 2.166 and 5.127 min. for lamivudine and tenofovir disoproxil fumarate, respectively. The flow rate was 1.0 mL min^{-1}. The calibration curve was linear over the concentration range of 20-60ppm mL^{-1} for both lamivudine and tenofovir disoproxil fumarate. The LOD and LOQ values were found to be 2.97 and 9.98 for lamivudine, 3.04 and 9.94 for tenofovir disoproxil fumarate, respectively.

Conclusion: The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pure and in tablet dosage form.

Keywords: Lamivudine and tenofovir, RP-HPLC, Validation.

INTRODUCTION

Tenofovir chemically, it is 9-[(R)-2-[[bis [(Isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate (1:1). Is an antiretroviral agent belonging to the class of nucleotide reverse transcriptase inhibitor. Lamivudine chemically it is (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H) pyrimidinone, is a synthetic nucleoside analogue with potent activity against human immune deficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity[1].



Tenofovir disoproxil fumarate (TDF) Lamivudine (LAM)

Fig. 1

Lamivudine and Tenofovir is a new drug combination. Literature reveals different methods for their analysis in their formulations [2-4]. But our present plan is to develop a new, simple, precise & accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated as per ICH norms [5-6].

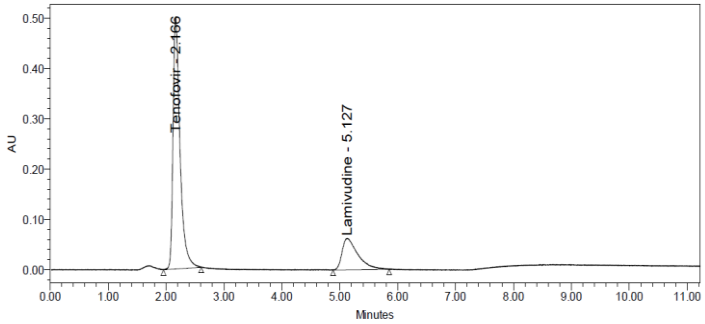
MATERIALS AND METHODS

Apparatus and chromatographic parameters

A Waters HPLC with Alliance with Auto sampler with Empower 2.0 software with Phenomenax Luna C18 (150 mm x 4.6 mm i.d., particle size 5µm) column and UV detector was employed in this study. An Edwa pH meter Afcoset digital balance and ambient column oven were the other instruments used for this study.

Reagents and solutions

HPLC grade Acetonitrile and Methanol, a GR grade/Merck Potassium di hydrogen phosphate, HPLC grade water and Lamivudine and tenofovir drug was used in the study. A mixture of acetonitrile: methanol: water in the ratio of 30:50:20 (v/v) as a mobile phase at a pH 3.0 adjusted with ortho phosphoric Acid and it is also used as a diluent for preparing the working solution of drug. The mobile phase was degassed in ultrasonic water bath for 5 minutes and filtered through 0.45µm filter under vacuum filtration.



Preparation of the Lamivudine & Tenofovir Standard & Sample Solution

Accurately weighed and transferred 10 mg of Lamivudine and Tenofovir working standard and drug sample into a different 10mL clean dry volumetric flasks, added about 7mL of Diluent and sonicate to dissolve it completely and made volume up to the mark with the same solvent. Mixed well and filtered through 0.45µm filter.

Method devlopment

Three trials were performed for the method development and the best peak with least fronting factor was found to be the third peak with RT= 2.166 for Lamivudine and 5.127 for Tenofovir.

Method validation

Precision

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria

The % RSD for the area of five standard injections results should not be more than 2% Precision result for lamivudine:

S. No.	RT	Peak area	Average peak area	Standard deviation	% RSD
1	2.282	1313235	1344089	23777.66	1.76
2	2.312	1326776			
3	2.344	1347962			
4	2.351	1368872			
5	2.358	1363598			

Precision results for tenofovir:

S. No.	RT	Peak area	Average peak area	Standard deviation	% RSD
1	3.433	458218	455995	2942.648	0.645325
2	3.557	452495			
3	3.623	453221			
4	3.639	457145			
5	3.704	458898			

Accuracy

Injected the standard solutions of Accuracy -50%, 100% and 150% and calculated the Amount found, Amount added for Lamivudine and tenofovir and the individual recovery and mean recovery values.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

For Lamivudine:

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	703289	5.0	5.0	100.0%	100.5%
100%	1398216	10.0	9.98	99.8%	
150%	2199166	15.0	15.7	101.3%	

For Tenofovir:

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	239738	5.0	4.98	99.7%	100.4%
100%	480445	10.0	9.99	99.9%	
150%	733711	15.0	15.2	101.7%	

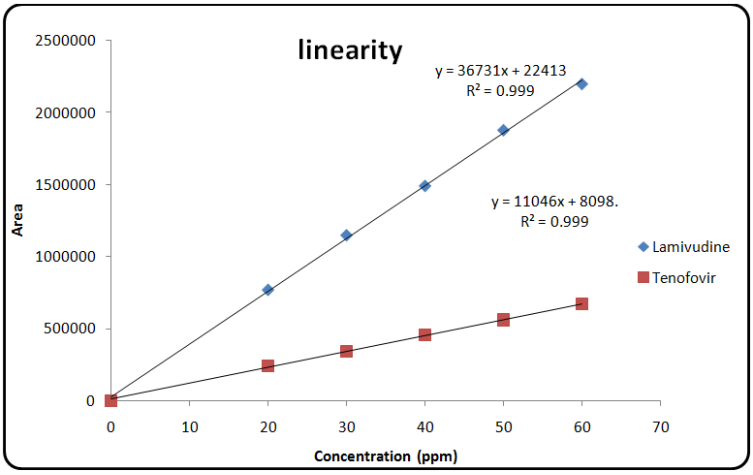
Recovery studies

To determine the accuracy and precision of the proposed method recovery studies were carried out. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were within the limits. The % recovery, Mean recovery and %Relative standard deviation value for Lamivudine and tenofovir drug was found to be 99.8-101.3% and 99.7-101.7% respectively.

Linearity and Calibration Curve

Working dilutions of Lamivudine and tenofovir in the range of 20-60ppm was prepared by taking suitable aliquots of working standard solutions of drug in different 10ml volumetric flask and diluting up to the mark with mobile phase. 20µl quantity of each dilutions was injected in to the column at a flow rate of 0.7ml/min. the drug in the elute was monitored at 258 nm and the corresponding chromatograms were recorded. From these the mean peak areas were calculated and a plot of concentration vs peak areas was constructed. The regression of the plot was computed by least square regression method. The slope and intercept value for calibration curve for lamuvidine was y=36731x+22413 (R^2=0.999) and tenofovir was y=11046x+8098 (R^2=0.999) founded respectively.

Linearity graph



X-Axis = Concentration, **Y**-Axis = Peak area

Limit of detection and limit of quantification

Limit of Detection (LOD) is the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level.

The LOD for Lamivudine and tenofovir was found to be 2.97 and 3.04 respectively.

Limit of Quantification (LOQ) is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level.

The LOQ for Lamivudine and tenofovir was found to be 9.98 and 9.94 respectively.

Robustness

Robustness is determined by making deliberate changes in the chromatographic conditions like change in flow rate, mobile phase composition and temperature and evaluated for the impact on the method. It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust.

	Sample	Rt	Area	Height	USP Plate count	USP tailing
More org	Lamivudine	2.422	1378798	171546	2358.0	1.7
Less org	Lamivudine	2.384	1404976	159808	2910.4	1.8
More org	Tenofovir	3.200	499679	50843	2616.1	1.6
Less org	Tenofovir	5.128	453297	27049	2840.1	1.7
More flow	Lamivudine	2.010	1150303	165118	2069.9	1.7
Less flow	Lamivudine	2.960	1690740	161204	2158.1	1.8
More flow	Tenofovir	3.060	402322	43574	2713.8	1.7
Less flow	Tenofovir	5.244	519208	36602	3536.2	1.7

RESULTS AND DISCUSSION

A simple, rapid and precise method has been developed and validated for the drugs Lamivudine and tenofovir. The estimation was carried out with a mixture of acetonitrile: methanol: water in the ratio of 30:50:20 (v/v) as mobile phase. Precision of the methods were studied by making repeated injections of the samples and system precision values were determined. The retention time was 2.166 and 5.127 min. for lamivudine and tenofovir disoproxil fumarate, respectively. The calibration curve was linear over the concentration range of 20-60ppm mL^{-1}. The LOD and LOQ values were found to be 2.97, 3.04 and 9.98, 9.94 for lamivudine and tenofovir disoproxil fumarate, respectively. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-HPLC method developed was very much suit for routine analysis.

S. No.	Parameter	Acceptance criteria	Observed value	
			Lamivudine	Tenofovir
1	Assay	95-105%	100.8%	99.7%
2	Accuracy	95-105%	100.5%	100.4%
3	Precision	RSD within 2%	1.76%	0.65%
4	Linearity	R^2 not less than 0.99	R^2=0.999	R^2=0.999
5	LOD	S/N=3	2.97	3.04
6	LOQ	S/N=10	9.98	9.94

CONCLUSION

The proposed study describes new and simple RP-HPLC method for the estimation of Lamivudine and tenofovir. The method validated was found to be simple, accurate and precise. Therefore the proposed study method can be used for quantification of Lamivudine and tenofovir in bulk and pharmaceutical dosage form.

REFERENCES

1. Bojja Soumya, Thimmaraju Manish Kumar and Nerella Raghunandhan. Simultaneous Determination of Tenofovir disoproxil fumarate and Lamivudine by UV Spectro photometric Method. International Journal of Pharmacy and Pharmaceutical Science Research, 2012; 2(1): 9-15.
2. Chandana shveta, Kasture A.V and Yeole P.G. Simultaneous spectrophotometric determination of Lamivudine and tenofovir in tablets. Indian journal of pharmaceutical sciences, 2005; 67 157; 627-629.
3. Yannis Dotsikas, Constantinos kousoulos, Georgia tsatsou and yannis L. loukas. Development of rapid method for the determination of lamivudine and tenofovir in human plasma using liquid; liquid extraction based on 96-well format micro-tubes and liquid chromatography/tandem mass spectrometry. Journal of Rapid communication in mass spectrometry, 2005; 19(14): 2055-2061.
4. Mikko Niemi, Kari T kivisto, Janne T Backmann and Pretti J neuvonen. Effect of lamivudine on the pharmacokinetics and pharmacodynamics of tenofovir. British journal of clinical pharmacology, 2000; 50(6): 591-595.
5. Synder K.L, Krikland J.J and Glajch. J.L:Practical HPLC Method Development 2nd Edn, Wiley-Interscience Publication, USA, 1983; 1-10.
6. International conference on harmonization “Validation of analytical procedures Methodology”, 14, Federal Register Nov.1996; 1-8.