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RESEARCH ARTICLE

Simultaneous Estimation of Lamivudine and Tenofovir disoproxil fumarate in Bulk and Combined Pharmaceutical Dosage Form by HPLC method

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ABSTRACT

A simple, precise, selective and rapid reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Tenofovir and Lamivudine in bulk and combined dosage form. The mobile phase used was mixture of phosphate buffer (6.5 mM) adjusted to pH 2.5 with orthophosphoric acid and acetonitrile (50:50 v/v). The Inertsil C18 column (15 cm x 4.6 mm, 5 µm) was used and drugs were detected by UV detector at 260 nm. The retention time of Lamivudine and Tenofovir were found to be 2.04 and 3.54 min respectively. The method was linear in the concentration range of 60-140 µg/ml and 180-420 µg/ml with correlation coefficient (r^2) of 0.998 and 0.999 for Lamivudine and Tenofovir respectively. The method was validated according to ICH guidelines with respect to accuracy, precision, specificity and can be used to determine drug content of marketed formulation.

Keywords: RP-HPLC, UV detection, Lamivudine, Tenofovir, validation, ICH guidelines

1. INTRODUCTION

Lamivudine, chemically 4-amino-1-[(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Lamivudine is reverse transcriptase reported to be active against HIV-1, HIV-2 and hepatitis B virus. Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver[1-3]. Tenofovir disoproxilfumarate (TDF) belongs to the class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which blocks reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Chemically TDF is 9[(R)-2-[[bis [[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate. Tenofovir is the first nucleotide analog approved for HIV-1 treatment[4]. The literature survey suggests UV method[5] and HPLC method[6,7] for lamivudine and RP-HPLC method[8,9] and HPTLC[10] for Tenofovir. Tenofovir is also determined in plasma[11]. The aim of the present study was to develop simple, rapid, accurate, specific and precise method for the estimation of lamivudine and tenofovir in the bulk and pharmaceutical formulation.

2. MATERIALS AND METHOD

Tenofovir and Lamivudine as pure drugs were obtained as gift samples from Torrent Pharmaceuticals Gujrat, India. Acetonitrile, Triethylamine, Sodium dihydrogen orthophosphate dihydrate and Orthophosphoric acid, (all HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai, India. High purity water was prepared using Milli Q grade purification system.

The HPLC system of Shimadzu (LC-2010 CHT) with ultraviolet detector was used for analysis. The data acquisition was performed by LC Solution Software. The analysis was performed by using HPLC column Inertsil (150 × 4.6 mm; 5 µm) with flow rate of 1ml/min and at ambient temperature. The mobile phase composition was phosphate buffer (6.5 mM) adjusted pH to 2.5 with Orthophosphoric acid and Acetonitrile (50:50 v/v). The injection volume was 20 µl and UV detector was used at 260 nm. Mobile phase was filtered through 0.45 µm nylon filter (Millipore) using filtration assembly with vaccum pump (Rocker pump 400, Today's) and ultrasonicated

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using ultrasonic waterbath (Model UCB 100, Spectralab) for degassing. The retention time obtained for Lamivudine and Tenofovir were at 2.04 and 3.54 min respectively.

Stock standard solutions were prepared by dissolving separately Lamivudine (100 mg) and Tenofovir (300 mg) in separate volumetric flask and sonicated for 15 min. (1000 µg/mL and 3000 µg/mL). The stock solutions were sufficiently diluted with mobile phase to give 100 µg/mL and 300 µg/mL solutions.

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 100 mg of Lamivudine and 300 mg of Tenofovir was transferred to volumetric flask containing mobile phase. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter paper. The stock solution was further diluted sufficiently with mobile phase to get sample solution of drug concentration of 100 µg/mL and 300 µg/mL for Lamivudine and Tenofovir respectively.

The proposed method was validated according to the ICH guidelines[12,13]. The method was validated for its linearity range, accuracy, precision (repeatability and intermediate), sensitivity and specificity.

The standard calibration curves were prepared with five concentrations ranging from 60-140 µg/mL and 180-420 µg/mL for Lamivudine and Tenofovir respectively in triplicate into the HPLC system keeping injection volume constant. The 10µL Aliquots of each solution were injected under the operating chromatographic condition described above and chromatogram was recorded. The typical chromatograms are shown in Fig. 1 The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

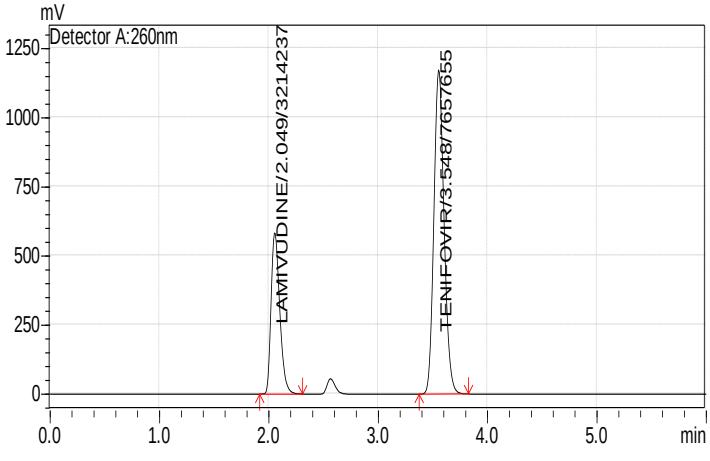

Fig. 1: Typical chromatogram of Lamivudine and Tenofovir at 260 nm

The accuracy of the method was at three different levels, 80%, 100% and 120% was determined by calculating recovery of Lamivudine and Tenofovir by the standard addition method. The solutions were prepared in triplicates and the accuracy is expressed as % recovery.

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of Lamivudine and Tenofovir (100 µg/ml and 300 µg/ml respectively)

The intermediate precision of the method was determined on different day using column of different make of same dimensions. The standard solutions were injected for six times and area was measured. The % RSD for all injections was within the specified limits.

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentration of standard solution using the HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equation as per ICH guidelines.

LOD = 3.3 X σ /S

LOQ = 10 X σ /S

3. RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation, good peak symmetry and best resolution was obtained with a mobile phase consisting of phosphate buffer (6.5 mM) adjusted to pH 2.5 with orthophosphoric acid and acetonitrile (50:50 v/v). Quantification was achieved with UV detection at 260 nm based on peak area. The retention time for Lamivudine and Tenofovir were 2.04 and 3.54 min respectively. Suitability of chromatographic system was monitored by calculating tailing/asymmetry factor and theoretical plates.

The calibration graphs were linear for both drugs and the system adhered to Beer's law over the concentration range 60-140 µg/mL and 180-420 µg/mL for Lamivudine and Tenofovir respectively. Linearity was evaluated by triplicate analysis of five standard working solutions equivalent to 60-140 µg/ml, 180-420 µg/ml of Lamivudine and Tenofovir. The overlain Chromatogram of Lamivudine and Tenofovir revealed at 260 nm (Fig. 2).

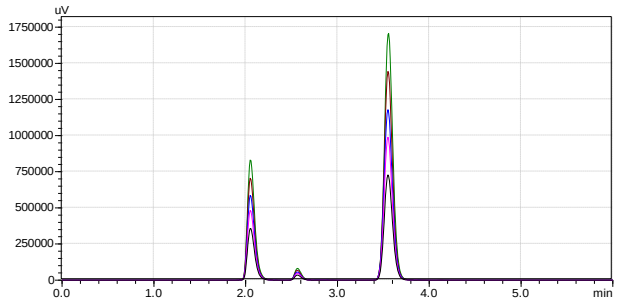

Fig. 2: Overlain view of linear chromatograms of Lamivudine and Tenofovir at 260 nm

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope as per ICH guidelines. The results showed that, the method is sensitive for the determination of Lamivudine and Tenofovir. System suitability and validation parameters are given in Table 1.

Parameter	Lamivudine	Tenofovir
Linearity range (µg/ml)	60-140	180-420
Slope (m)	9.21525	1.29517
Intercept (c)	-1.28862	-0.44198
Correlation coefficient(r^2)	0.9988904	0.9990452
Retention time (min)	2.049	3.451
Area	3214788	7653426
Height	585727	1176525
Tailing factor	1.438	1.196
Theoretical plates	2717.01	5981.02
LOD (µg/ml)	3.35	3.55
LOQ (µg/ml)	10.14	10.75

Table 1: Validation and system suitability parameters

Accuracy was determined by calculating the % recovery. The method was found to be accurate with % recovery 104.91% and 105.05% for Lamivudine and Tenofovir respectively. The high values indicate that method is accurate and are shown in Table 2.

% Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	*% Recovery	Mean Recovery
Lamivudine					
80	2787554.66	80	83.50	104.49	
100	3483552.66	100	104.92	104.92	104.91
120	4145554.02	120	126.38	105.32	
Tenofovir					
80	6741539.01	180	188.31	104.62	
100	8358875.6	300	315.36	105.12	105.05
120	10022213.66	420	442.72	105.41	

Table 2. Recovery study of tadalafil (tablet) n=3

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Lamivudine and Tenofovir combined test solution in the same equipment at a concentration value of 100 µg/ml and 300 µg/ml of lamivudine and tenofovir respectivly on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak areas of the drugs were determined and precision was reported as % RSD which clearly indicates the method is precise. (Table 3)

Precision	Lamivudine	Tenofovir
Method Precision (Average Area and % RSD)	3476785 and 0.242	842000 and 0.25
Mean Assay	105.12%	105.39%
Intermediate Precision (Average Area and % RSD)	3475185 and 0.232	842501 and 0.27
Mean Assay	104.97%	105.02%

Table 3. Method and intermediate precision studies of lamivudine and tenofovir

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Lamivudine and Tenofovir. The specificity and selectivity of the method was tested under optimum conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Lamivudine and Tenofovir. The validated HPLC method was adopted for the quantification of Lamivudine and Tenofovir in their combined pharmaceutical dosage form and the results of analysis are given in Table 4. The contents of the pharmaceutical dosage form were found to be in the range of 100±10% with RSD less than 2% which indicate suitability of the method for routine analysis of Lamivudine and Tenofovir in pharmaceutical dosage form.

Drug	Dosage	Sample Conc.	Amount of drug estimated	% of drug estimated
Lamivudine	100 mg	100 µg	102.22 µg	102.22
Tenofovir	300 mg	300 µg	307.12 µg	102.37

Table 4: Analysis of tablet by RP-HPLC

4. CONCLUSION

The proposed study describes a new RP-HPLC method using simple mobile phase for the estimation of Tenofovir and Lamivudine in combined pharmaceutical dosage formulations. The method was validated and found to be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Tenofovir and Lamivudine in the pharmaceutical dosage form. The percentage recovery shows that, the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

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Conflict of Interest: None Declared

