

# Analytical method development and validation for the simultaneous estimation of lamivudine and tenofovir disoproxil fumarate by RP-HPLC method

## Abstract

A simple, accurate, precise and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method has been developed, which can separate and quantitatively estimate Lamivudine and Tenofovir disoproxil fumarate in mixed dosage form. The chromatographic separation for Lamivudine and Tenofovir disoproxil fumarate was achieved with mobile phase containing acetonitrile and phosphate buffer PH 3.5 (80:20% v/v), reverse phase Thermo scientific™ Hypersil™ BDS 5 $\mu$  C18 120A (250 $\times$ 4.60mm i.d.) column in isocratic mode at room temperature and UV detection at 260nm. The compounds were eluted at a flow rate of 1.2ml/min. The retention times of Lamivudine and Tenofovir disoproxil fumarate were found to be 2.33min and 2.68min respectively. The above method was validated in terms of system suitability, linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) in accordance with ICH guidelines. The method was rapid, simple, economical and suitable for routine quality control analysis.

**Keywords:** lamivudine, tenofovir disoproxil fumarate, analytical method development, validation, RP-HPLC

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## Introduction

Lamivudine (LAM) (4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one) is nucleoside analogues with a structure that consists of a pyrimidine base, which is N-substituted at the 1-position with a 3'-thia derivative (1,3-oxazolidine) of the ribose moiety that is characteristic of nucleosides. It is 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). This compound belongs to the class of organic compounds known as 3'-thia pyrimidine nucleosides.<sup>1</sup>

Tenofovir disoproxil fumarate (TDF) (a prodrug of tenofovir) ({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methyl) phosphonic acid, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. *In vivo* tenofovir disoproxil fumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate.<sup>2</sup>

Both the drugs are marketed as combined dose tablet formulation in the ratio of 300:300 mg LAM: TNF. Literature survey revealed that there is no method reported for the simultaneous estimation of these drugs, some methods for estimation of individual drugs or with other drugs UV-spectrophotometry,<sup>3-8</sup> RP-HPLC<sup>9</sup> are available. HPLC method is more sensitive compared to UV; therefore, there is a challenge to develop RP-HPLC method for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate. The present study was involved in a research effort aimed at developing and validating a simple, accurate, precise and economical RP-HPLC method for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate mixed dosage form.

## Materials and methods

Acetonitrile (HPLC grade) and Water (HPLC grade) were obtained from Merck specialties private limited, Mumbai, India. Orthophosphoric acid (GR grade) was obtained from S.D. Fine Chemicals Ltd, Mumbai, India. Potassium Dihydrogen orthophosphate (KH<sub>2</sub>PO<sub>4</sub>) was obtained from Merck specialties private limited Mumbai, Dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>) S.D Fine Chemical Ltd, Mumbai. Pure drugs of Lamivudine and Tenofovir disoproxil fumarate were obtained as gift samples from Hetero labs (Hyderabad, India). TENOLAM tablets manufactured by Hetero Pharmaceutical Limited purchased from local pharmacy are used for the analysis. The label claim states that this formulation contains 300mg of Lamivudine and 300mg of Tenofovir disoproxil fumarate.

## Instrumentation and chromatographic condition

HPLC method development and validation were done on SHIMADZU (Japan) liquid chromatography equipped with LC-20AD pump, LC 20A UV/Vis detector and Rheodyne 7725i injection with a 20 $\mu$ L loop. For instrument control, data acquisition and processing, the chromatographic system was interfaced to LC solutions software. Other instruments included are Shimadzu electronic balance BL-220H (SHIMADZU corporation, Japan), Value 1 stage vacuum pump Model: VE115, Fast clean ultrasonic cleaner. The column used for chromatographic separations was reverse phase Thermo Scientific™ Hypersil™ BDS 5 $\mu$  C18 120A (250 $\times$ 4.60mm i.d). The analytical wave length was set as 232nm and samples of 20 $\mu$ L were injected. The chromatographic separations were accomplished using mobile phase consisting of Acetonitrile: phosphate buffer PH-3.5 (80:20%v/v), filtered through 0.45 $\mu$ m filter using Value 1 stage vacuum pump and desecrated in fast clean ultrasonic cleaner. Mobile phase was pumped in isocratic mode at a flow rate of 1.2ml/min at room temperature (Figure 1 & 2).

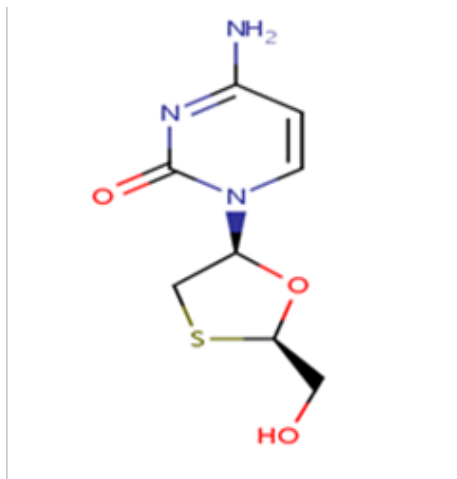


Figure 1 Structure of Lamivudine.

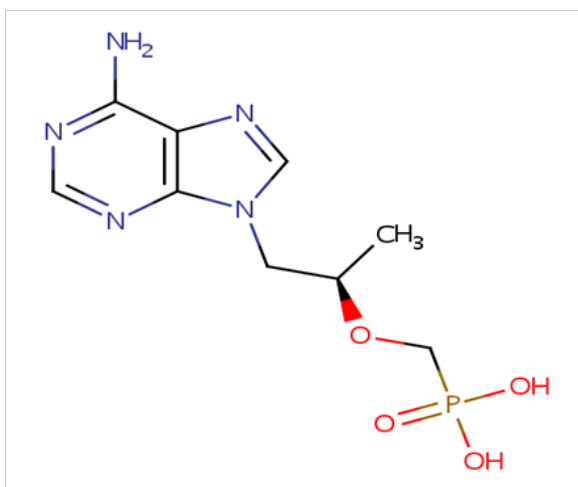


Figure 2 Structure of Tenofovir disoproxil fumarate.

### Preparation of standard solutions

Stock solutions were prepared by dissolving 10mg of Lamivudine and 10mg of Tenofovir disoproxil fumarate, in 10ml of acetonitrile separately. Aliquots of the standard stock solutions of Lamivudine and Tenofovir disoproxil fumarate were transferred into 10ml volumetric flasks and solution was made up to the volume to yield required concentrations of Lamivudine and Tenofovir disoproxil fumarate. A typical chromatogram obtained from the analysis of drugs using the developed method is shown in Figure 3.

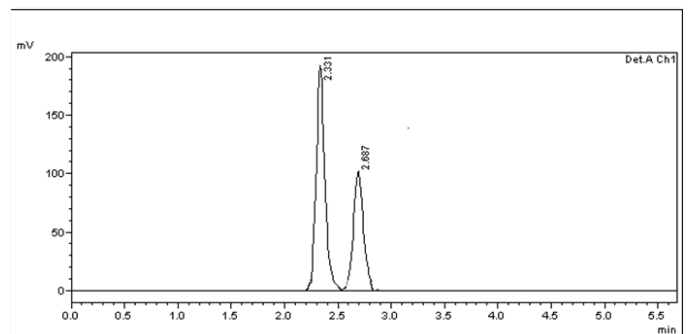


Figure 3 Chromatogram of standard solution (10µg/ml Lamivudine and 10µg/ml Tenofovir).

### Preparation of sample solution for assay

Ten TENOLAM tablets each containing 300mg of Lamivudine and 300mg of Tenofovir disoproxil fumarate were weighed, average weight was calculated and powdered. A quantity equivalent to 100mg of Lamivudine and 100mg of Tenofovir disoproxil fumarate was weighed and transferred into 10ml volumetric flask. It was extracted with acetonitrile. The volumetric flask was sonicated for 5minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with methanol and filtered. Suitable aliquots of formulation solution were prepared and injected to HPLC to obtain concentration in the linearity range (Figure 4).

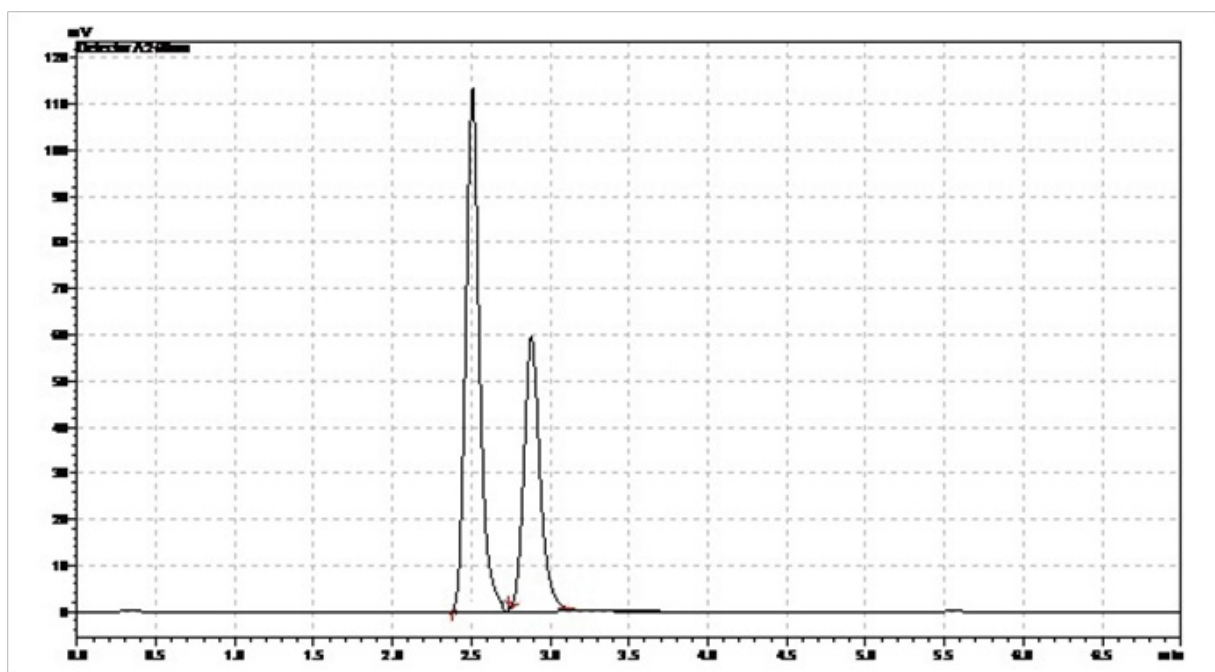


Figure 4 Chromatogram of formulation (10µg/ml Lamivudine and 10µg/ml Tenofovir)

## Results and discussion

### Method Development

For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters like symmetry, theoretical plates and capacity factor. Symmetrical peaks with good separation (retention time for Lamivudine is 2.331 and Tenofovir disoproxil fumarate is 2.687) were obtained with reverse phase Thermo Scientific™ Hypersil™ BDS 5μ C18 120A (250×4.60mm i.d) column. The mobile phase containing Acetonitrile: phosphate buffer PH-3.5(80:20% v/v) was used at a flow rate of 1.2ml/min. The optimum wavelength for detection and quantification was at 260nm, at which good detector response was obtained for both the results are given in Table 1.

**Table 1** Summary of validation parameters

Parameters	Lamivudine	Tenofovir disoproxil fumarate
Linearity range(μg/ml)	15-35	15-35
Correlation coefficient	0.999	0.999
Slope	41816	30551
Intercept	26084	4169
LOD(μg/ml)	0.4632	1.46
LOQ(μg/ml)	1.544	4.29
Recovery (%)	99.11%	99.62%
80	99%	99.33%
100	99.39%	99.79%
120	1.05	0.721
Precision (RSD %)	1.063	0.942
Intraday(n=6)	3369	3389
Interday(n=6)	1.22	1.23
Theoretical plates	2.6	
Tailing factor		
Resolution		

### Method validation

As per ICH guidelines, the method validation parameters checked were linearity, precision, accuracy, limit of detection and limit of quantification.<sup>10</sup>

### Linearity and range

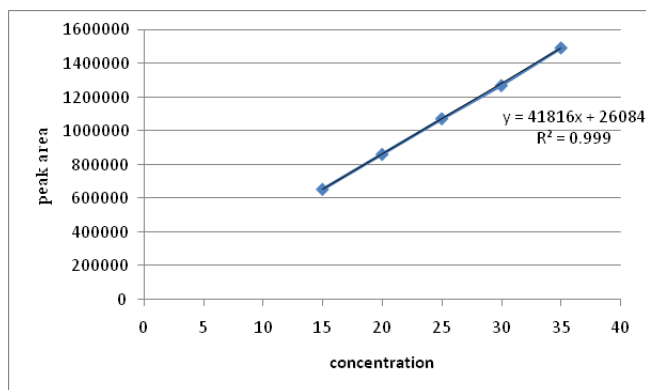
Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 15-35μg/ml for Lamivudine and 15-35μg/ml for Tenofovir disoproxil fumarate. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficients were found to be 0.999 and 0.999 for Lamivudine and Tenofovir disoproxil fumarate respectively (Figure 5 & 6). The results are given in Table 1.

### Precision

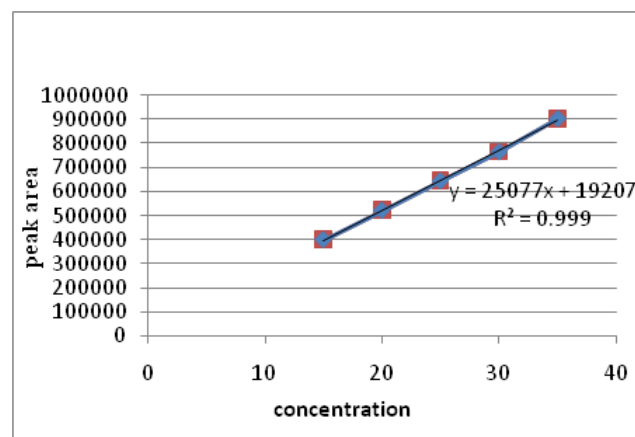
The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done at two levels (intraday and inter day). Intraday precision was done by analyzing the intermediate concentration of each drug for six times. Interday precision was measured over three consecutive days for the same drug concentrations for six times. The %RSD values were found to be calculated for each of them and the low RSD values indicate that the method is precise. The results are given in Table 1.

### Accuracy

Recovery studies were carried out by applying the method to drug sample to which known amount of standard Lamivudine and Tenofovir disoproxil fumarate corresponding to 80%, 100% and 120% of label claim had been added. At each level of the amount three determinations were performed. The results are given in Table 1.



**Figure 5** Calibration graph of Lamivudine.



**Figure 6** Calibration graph of Tenofovir by RP-HPLC.

### Sensitivity

LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by standard calibration curves. The results are given in Table 1.

### System suitability studies

System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As), tailing factor etc., were studied. The results are given in Table 1.

### Analysis of marketed formulation

The proposed procedures were successfully applied for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate in the formulation and the drug contents in each sample were calculated by comparison with the appropriate standard solution of the drug. The results obtained were in agreement with label claim. The results of analysis are given in (Table 2) (Figure 4).<sup>11-13</sup>

**Table 2** Analysis of marketed formulation

Drug	Amount labeled (mg)	Amount found(mg)	%Assay	%RSD*
Lamivudine	300	299.02	99.67	0.65
Tenofovir	300	301.13	100.3	0.715

\*Mean of three observations

## Conclusion

RP-HPLC methods enable the quantitation of Lamivudine and Tenofovir disoproxil fumarate in oral dosage form with good accuracy and precision, either in laboratory prepared samples or in pharmaceutical dosage forms. The good recoveries were obtained in all cases as well as the reliable agreement with the reported procedure proved that the proposed methods could be applied efficiently for determination of Lamivudine and Tenofovir disoproxil fumarate in oral dosage form with satisfactory precision. This method is considered simple, reliable, economical providing satisfactory accuracy, precision with lower limits of detection and quantification more sensitive. More over the shorter duration of analysis for Lamivudine and Tenofovir disoproxil fumarate makes the reported method suitable for routine analysis in mixed dosage form.

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## Conflict of interest

The author declares no conflict of interest.

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