



# Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine from Pharmaceutical Preparation

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**Abstract:** A new, simple, specific, accurate and precise RP-HPLC method was developed for determination of lamivudine in pure and tablet formulations. A Thermo BDS C18 column in isocratic mode, with a mobile phase consisting of 0.01 M ammonium dihydrogen orthophosphate buffer adjusted to pH 2.48 by using formic acid and methanol in the ratio of 50:50 was used. The flow rate was set at 0.6 mL/min and UV detection was carried out at 264 nm. The retention time of lamivudine and nevirapine were 2.825 min and 4.958 min respectively. The method was validated for linearity, precision, robustness and recovery. Linearity for lamivudine was found in the range of 50-175 µg/mL. Hence, it can be applied for routine quality control of lamivudine in bulk and pharmaceutical formulations.

**Keywords:** RP-HPLC, Lamivudine, Nevirapine, Tablet formulations.

## Introduction

Lamivudine (3TC) is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It needs to be phosphorylated to its triphosphate form before it is active. 3TC-triphosphate also inhibits cellular DNA polymerase. Lamivudine is often given in combination with zidovudine, with which it is highly synergistic. Chemically, lamivudine is designated as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one<sup>1</sup>. Nevirapine (NEV) was used as an internal standard. Nevirapine is a non-nucleoside reverse transcriptase

inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type 1. Nevirapine is structurally a member of the dipyrindodiazepinone chemical class of compounds. The chemical name of nevirapine is 11-cyclopropyl-4-methyl-5, 11-dihydro-6H-dipyrido [3,2-b:2',3'-e][1,4] diazepin-6-one<sup>2</sup>.

Literature survey revealed that few analytical methods have been reported for the estimation of lamivudine by using monolithic silica HPLC columns<sup>3</sup>, in human serum<sup>4</sup>, in human plasma, saliva and cerebrospinal fluid<sup>5</sup>. One method was reported for simultaneous assay with stavudine in combination tablets by derivative spectrophotometry and chromatography<sup>6</sup>. Other method was reported for simultaneous assay with zidovudine by derivative spectrophotometry and chromatography<sup>7</sup>. The objective of the present study is to develop a simple HPLC method and to validate it for the rapid and accurate determination of 3TC in bulk drugs and in tablet dosage form. The proposed method was validated as per ICH guidelines Q2A<sup>8</sup>.

## Experimental

All analytical works were performed on HPLC Shimadzu LC 2010 CHT series equipped with quaternary constant flow pump, auto injector, SPD10 AVP Shimadzu Photodiode Array Detector and LC Solution Version 1.22 SP1 Software. Thermo BDS hypersil C18 column (250 mm × 4.6 mm 5 μ) forms the stationary phase. A calibrated single pan balance *i.e.* Sartorius CP 225 D, Velp scientific vortex mixer, pH meter of Labindia, Enertech Fast Clean Ultrasonic cleaner were also used during the analysis. The reference standards of lamivudine and nevirapine were procured from Cipla LTD, Goa. The tablet (Lamivir HBV<sup>®</sup>) was purchased from the local market. All chemicals and reagents used were of AR/HPLC grade and HPLC water was prepared from Milli-Q in the lab.

### *Preparation of mobile phase and standard stock solution*

The mobile phase was prepared by mixing 500 mL of 0.01 M ammonium dihydrogen orthophosphate buffer (the pH was adjusted to 2.48 with formic acid) with 500 mL of methanol. The mobile phase was sonicated 10 minutes and then it was filtered through a 0.45 μ membrane filter paper. An accurately weighed quantity of 25 mg was transferred to 100 mL volumetric flask, which was then dissolved and made up to volume with mobile phase in order to get 250 μg/mL.

### *Preparation of internal standard solution*

An accurately weighed quantity of 25 mg of nevirapine was taken in 25 mL of volumetric flask and dissolved in mobile phase, sonicated for 10 min and made up to the mark with mobile phase. Appropriate dilutions of stock solution were made with mobile phase to get working standard solution of drug containing 25 μg/mL.

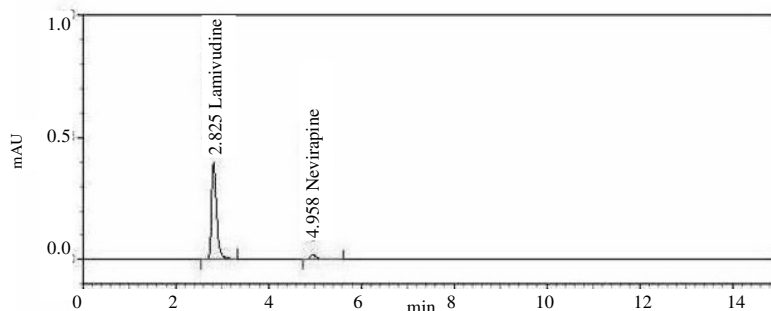
### *Optimised chromatographic conditions*

RP HPLC analysis was performed by isocratic elution with flow rate of 0.6 mL/min. The mobile phase containing 500 mL of 0.01 M ammonium dihydrogen orthophosphate buffer (pH 2.48) and methanol in the ratio of 50:50 (v/v) to obtain well-resolved peaks of lamivudine ( $R_t = 2.825$  min) and nevirapine ( $R_t = 4.958$  min), respectively is as shown in Figure 1. Wavelength of maximum absorption was selected by Photo diode array UV detector at 264 nm.

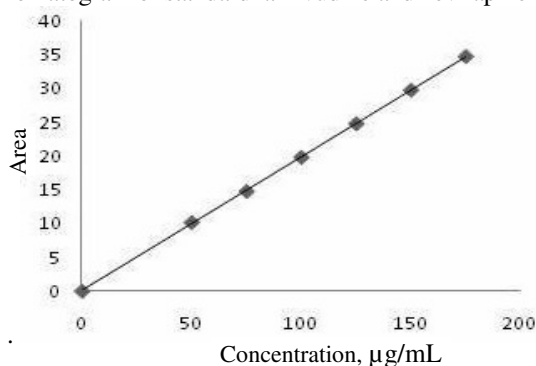
### *Calibration curve of lamivudine*

Standard stock solution 2, 3, 4, 5, 6 and 7 mL of lamivudine were transferred into 10 mL volumetric flasks and spiked with 1 mL (25 μg/mL) of internal standard stock solution and diluted with mobile phase in order to get final concentrations 50, 75, 100, 125, 150 and

175  $\mu\text{g/mL}$  of lamivudine. 20  $\mu\text{L}$  of working standard solutions were injected in to the HPLC system ( $n=6$ ) and the ratio of AUC of the drug peak to that of internal standard were calculated. A calibration curve was plotted (Figure 2) by taking concentration of drug on x-axis and the ratio of AUC of drug peak to that of internal standard on y-axis. The linearity table of lamivudine is shown in Table 1.



**Figure 1.** A typical chromatogram of standard lamivudine and nevirapine as an internal standard.



**Figure 2.** Calibration curve of lamivudine.

**Table 1.** Linearity table of lamivudine.

Analyte	Conc., $\mu\text{g/mL}$	Area
Lamivudine	50	10.1763
	75	14.7712
	100	19.8047
	125	24.7732
	150	29.7418
	175	34.7187

The developed method was validated in terms of linearity, accuracy, specificity, limit of detection and limit of quantitation, intra-day and inter-day precision and repeatability of measurement.

#### *Analysis of the marketed formulations*

Twenty tablets were weighed accurately and crushed to fine powder. Accurately weighed quantity of powder equivalent to 150 mg of lamivudine was dissolved in 100 mL of volumetric flask with the mobile phase. The flask was sonicated for 20 min and then the solution was filtered using Whatmann filter paper no.1. Appropriate volumes of the aliquot were transferred into five different 20 mL volumetric flasks and spiked with 1 mL (25  $\mu\text{g/mL}$ ) of internal

standard solution and the volume was made up to the mark with mobile phase to obtain 100 µg/mL of lamivudine. The solution was sonicated for 10 min and injected under above chromatographic conditions and peak areas were measured. The results are shown in the Table 2.

**Table 2.** Analysis of marketed formulation.

Analyte	Label claim, mg/tab	Amount found, mg/tab	C.I.	SD	% RSD	SE	t
Lamivudine	150	150.396	100.264±0.4866	0.3919	0.3909	0.1753	1.5063

## Results and Discussion

### *Method development*

The method was chosen after several trials with various proportions of buffer and methanol and at different pH values. A mobile phase consisting of buffer (pH 2.48) and methanol in the ratios of 50:50 was selected to achieve best chromatographic peak and sensitivity. The flow rate 0.6 mL/min and a Thermo BDS C18 column of 4.6 µ particle sizes, a detection wavelength of 264 nm and an injection volume of 20 µL and 25 °C temperatures for the HPLC system were found to be the best for the analysis. System suitability tests were carried out as per USP XXIV requirements. System suitability tests were carried out on freshly prepared standard stock solution of lamivudine (100 µg/mL) and the results of parameters were obtained by five replicate injections. The system suitability results are shown in Table 3.

**Table 3.** System suitability.

Parameter	Results of 3TC
Asymmetry factor	1.45
Retention Time, min	2.825
Theoretical plates	5304.693
Repeatability	0.891(%RSD)

### *Method validation*

The proposed method has been validated for the determination of lamivudine in bulk as well as tablet dosage form using following parameters:

#### *Linearity*

The linearity range was found in between 50-175 µg/mL. The linear regression equation of lamivudine is  $Y = 0.197x + 0.060$  and co-relation coefficient ( $r^2$ ) = 0.999.

#### *Specificity*

The peak purity of lamivudine and internal standard nevirapine were assessed by comparing the retention time ( $R_t$ ) of standard lamivudine and nevirapine. Good correlation was also found between the retention time of standard and sample of lamivudine and nevirapine.

#### *Precision*

Precision study was performed to find out intra-day and inter-day (within three days) variations in the estimation of lamivudine of different concentrations with the proposed method. Percentage relative standard deviation (%RSD) was found to be less than 1% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 4.

#### *Accuracy*

It was found out by recovery study using standard addition method, known amounts of standard lamivudine was added to pre-analyzed samples at a level from 50% up to 100% and then subjected to the proposed HPLC method. Results of recovery studies are shown in Table 5.

**Table 4.** Precision data for the proposed method.

Analyte	Intraday measurement			Inter-day measurement		
	Conc, $\mu\text{g/mL}$	Area	%RSD	Conc, $\mu\text{g/mL}$	Area	%RSD
3TC	75	14.8066	0.2647	75	14.7117	0.3211
	100	19.8965	0.4798	100	19.9559	0.7323
	125	24.8777	0.4489	125	24.9327	0.6375
	150	29.7168	0.1346	150	29.5501	0.3840

**Table 5.** Recovery study of lamivudine.

Analyte	Formulation, $\mu\text{g/mL}$	Amount of standard drug added, $\mu\text{g/mL}$	Amount of standard drug recovered, $\mu\text{g/mL}$	C.I.	%RSD	SE	t
Lamivudine	75	37.5	37.616	99.919 $\pm 1.095$	0.883	0.394	0.204
	75	56.25	56.604	100.142 $\pm 0.540$	0.434	0.194	0.730
	75	75	75.042	99.794 $\pm 0.752$	0.607	0.271	0.757

*SD: Standard deviation, % SE: Percent standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level =  $R \pm ts/n$ , R: Mean percent result of analysis of Recovery study ( $n = 5$ ). Theoretical 't' values at 95% confidence level for  $n - 1$  degrees of freedom  $t(0.05, 4) = 2.776$ .*

#### Robustness

It was done by making small changes in the chromatographic conditions and found to be unaffected by small changes like  $\pm 0.1$  changes in pH and 2% change in volume of the mobile phase .

#### Conclusion

The modalities adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries was obtained in the range of 99 to 100. The results of analysis of average recoveries obtained in each instance were compared with the theoretical value of 100 percent by means of Student's 't' test. As the calculated 't' values are less than theoretical' values (Table 5), it is concluded that the results of recoveries obtained in agreement with 100 percent for each analyte. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The lower limit of detection and the limit of quantitation were found to be 0.0399 and 0.1211 $\mu\text{g/mL}$  respectively. This demonstrates that the developed HPLC method is new, simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms.

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