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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF LAMIVUDINE FROM PHARMACEUTICAL PREPARATION

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ABSTRACT

A simple, sensitive, rapid, robust and reproducible method for the determination of Lamivudine in bulk and pharmaceutical formulation (Tablets) was developed using reverse phase high performance liquid chromatographic method (RP-HPLC). The RP-HPLC analysis was performed isocratically on XTERRA C_{18} (4.6X150mm) , analytical column using a mobile phase consisting of methanol and water in the Ratio of 50:50v/v, with a flow rate of 0.6ml/min.The analyte was monitored with UV detector at 270 nm. The developed method Lamivudine elutes at a retention time of 3.05 min. The proposed method is having linearity in the concentration range from10 to50 µg/mL of Lamivudine. The present method was validated with respect to system suitability, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), ruggedness, robustness.The proposed method can be readily utilized for bulk drug and pharmaceutical formulations.

Key words: RP-HPLC, Method development and validation, Lamivudine, Xterra C_{18} column.

INTRODUCTION

Lamivudine is chemically 1[(2R,5S)-2-(Hydroxy methyl)-1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity. Lamivudine 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 alsoinhibits cellular DNA polymerase. The mechanism of action of lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.The literature survey (Mandloi DK et al., 2009; Krishnareddy NV et al., 2011; Patro SK et al., 2010) reveals that there is some HPLC methods have been reported. The aim of the present study was to develop and validate a simple, isocratic RP-HPLC (Remington, 2007; Skoog, 2004; Chatwal GR, 2004) method for the determination of lamivudine in tablets. The developed method was validated using ICH guidelines for validation (ICH, 1995).

Today, RP-HPLC is the most popular analytical technique for separating complex mixtures in the chemical, pharmaceutical and biotechnological industry. RP-HPLC is the opposite of normal-phase chromatography, with a nonpolar stationary phase and a polar, largely aqueous mobile phase. The most common stationary phases used are octadecyldimethyl (C_{18}) phases with silica as the solid support. Silica has a small pH range (3 to 8) where mixtures can be separated without degradation of the column performance. Above pH 8, silica supports dissolve and destroy the column. Below pH 3, the silicon-carbon bond is cleaved, and the column is destroyed. The separation is achieved by analytes having different interactions with the stationary phase. In RP-HPLC, solutes are separated using their hydrophobicity. A more hydrophobic solute will be retained on the column longer than a less hydrophobic one. Also, polar solutes

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will interact with the silica surface to cause peak tailing. The mobile phase is one of the two components involved in the separation process. Water is generally one of the components of a binary mixture in RP-HPLC. Water is considered to be the weak component of the mobile phase and does not interact with the hydrophobic stationary phase chains. The RP-HPLC method reported in this study was validated in accordance with the International Conference on Harmonization (ICH) guideline (ICH, 1997) and best practice (Shabir GA *et al*., 2007; Shabir GA *et al*., 2003; USFDA, 2000). Specificity, linearity, precision (repeatability and intermediate precision), accuracy, robustness, limit of detection and limit of quantitation were evaluated.

MATERIALS AND METHODS

Methanol (HPLC grade), Lamivudine as the reference standard was purchased from Matrix laboratories, Distilled water was de-ionised by using a Milli-Q system (Millipore), Lamictal tablets and the chemicals of analytical reagent grade purchased from various sources, Pump (Waters Alliance 2695) , Detector (UV – Visible Model 2487 , Injector Autosampler (20µl) , The chromatography Column C_{18} XTERRA (150mm) , Elio PH – Meter, A & D – Digital Balance.

CHROMATOGRAPHIC CONDITIONS

The mobile phase consisted of a mixture of methanol- HPLC water (50:50 v/v). The flow rate was set to 0.6 ml /min,Injection volume 20µl , The Column used is C_{18} XTERRA (150mm). The detection wavelength was set to be at 270 nm.RP-HPLC analysis was performed isocratically at room temperature.

METHOD DEVELOPMENT (Lloyd R Snyder *et al*., 2007; Synder KL *et al*., 1983)

Preparation of Standard Solution

Accurately weighed and transfered 10mg of Lamivudine Working standard into a 100 ml volumetric flask and added about 70 mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further pipetted 1 ml of the stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

Preparation of Sample Solution

Weighed 5 Lamivudine Tablets and calculated the average weight. Accurately weighed and transfered the sample equivalent to 10 mg of Lamivudine into a 100 ml volumetric flask. Added about 70 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Further pipetted 1ml of the stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

RESULTS AND DISCUSSIONS

HPLC separation of Lamivudine was carried out on a Xterra C18 column by an isocratic elution with methanol-HPLC water (50:50 v/v). The flow rate was constant at 0.6 ml/min and the column temperature was at room temperature (24±1°). The UV wavelength was set at 270 nm. No interference from diluents, impurities, or excipients present in the pharmaceutical formulation was observed at this detection wavelength. Before each run LC column was equilibrated with the mobile phase for about 15 min. A sharp, symmetrical peak was obtained for Lamivudine when analyzed under these conditions a. This retention time enable rapid determination of the drug, which is important for routine quality control analysis.

System suitability test was established from five replicate injections of a solution containing Lamivudine 10µg/ml. The percent relative standard deviation (RSD) of the peak area was calculated. The peak tailing for drug was measured. A useful and practical measurement of peak shape, the peak tailing and theoretical plate count was determined. Column plate number was determined using the formula, N = 5.54(t R /w h) 2, where w h is the bandwidth at 50% of peak height. The proposed method met these requirements within the United States Pharmacopoeia (USP) accepted limits (Tailing factor < 1.5, Theoretical plates > 2000). The stability of Lamivudine in solution was investigated in the method development phase. Five solutions containing 10 µg/ml of Lamivudine were tested. The solutions were stable during the investigated time and the RSD was < 1.0% for retention time (min), peak area and height. The solutions were shown to be stable with no significant change in Lamivudine concentration over this period.

METHOD VALIDATION (Yuri Kazakevin *et al*., 2007; Bently *et al*., 1985; David Harvey *et al*., 2000; Sethi PD, 2006)

LINEARITY

Appropriate amounts of Lamivudine stock solutions were diluted with mobile phase to give concentration of 10, 20, 30, 40 and 50 µg/ml. Each solution was injected calibration plot was prepared. Linearity was evaluated by linear least-squares regression analysis. Good linearity was observed over the concentration range evaluated (10-50 µg/ml) as shown in the linearity curve in figure 3. The correlation coefficient was found 0.999.

PRECISION

The precision of the method was investigated with respect to repeatability and intermediate precision. The repeatability (intra-day precision) of the method was evaluated by assaying five replicate injections of the Lamivudine at 100% of test concentration (30 µg/ml) on the same day. The %RSD of the retention time (min) and peak area were calculated. Intermediate precision (inter-day precision) was demonstrated by evaluating the relative peak area percent data the LC system at three different

Table 1. Separation Characteristics of Lamivudine analysed under Optimized Conditions and Method Validation of Lamivudine

PARAMETERS	LIMIT	OBSERVATION
System suitability	Theoretical Plates should not less than 2000. Tailing factor should not more than 2.0	Theoretical plates: 3681.6 Tailing factor:1.33
Precision: A)System Precision B).Method precision	 RSD NMT 2.0% RSD NMT 2.0%	 0.81 0.14
Linearity	Correlation coefficient NLT 0.99	0.999
Accuracy	%Recovery range- 98-102 %	100.34%
Robustness(Flow,Mobile phase)	System suitability parameters should comply	Complies
LOD	S:N Ratio should be about 3	2.73
LOQ	S:N ratio should be about 10	9.52

The system is suitable for tailing factor, theoretical plate, and resolution

Table 2. Recovery studies of Lamivudine from samples with known concentrations

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found ((µg/ml)	% Recovery	Mean Recovery
50%	788312	4.83	5.05	98.10%	100.34%
100%	1743148	10.29	9.97	101.84%	
150%	2540029	15.1	14.7	101.10%	

Table 3. Robustness of the method

Conditions	Value	System suitability Results	
		USP Plate count	USP Tailing
Mobile phase (±10%)	60:40	3142.33	1.32
	50:50	3681.60	1.33
	40:60	4053.82	1.31
Flow rate(ml/min)	0.5	3960.97	1.35
	0.6	3681.60	1.33
	0.7	3588.22	1.31

Figure 1. Typical LC chromatogram of the Lamivudine sample for the system suitability

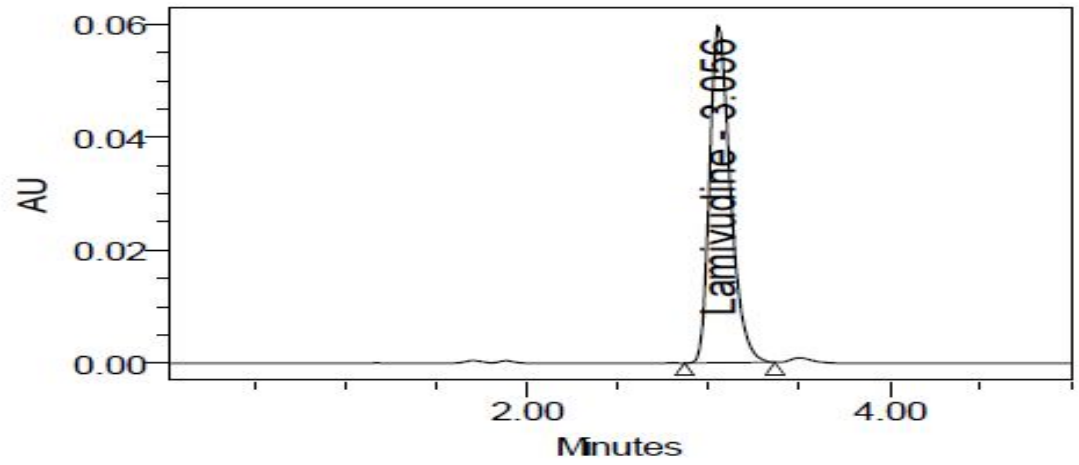


Figure 2. Typical LC chromatograms obtained for precision (a) & (b) are for precision (c),(d) are for intermediate precision

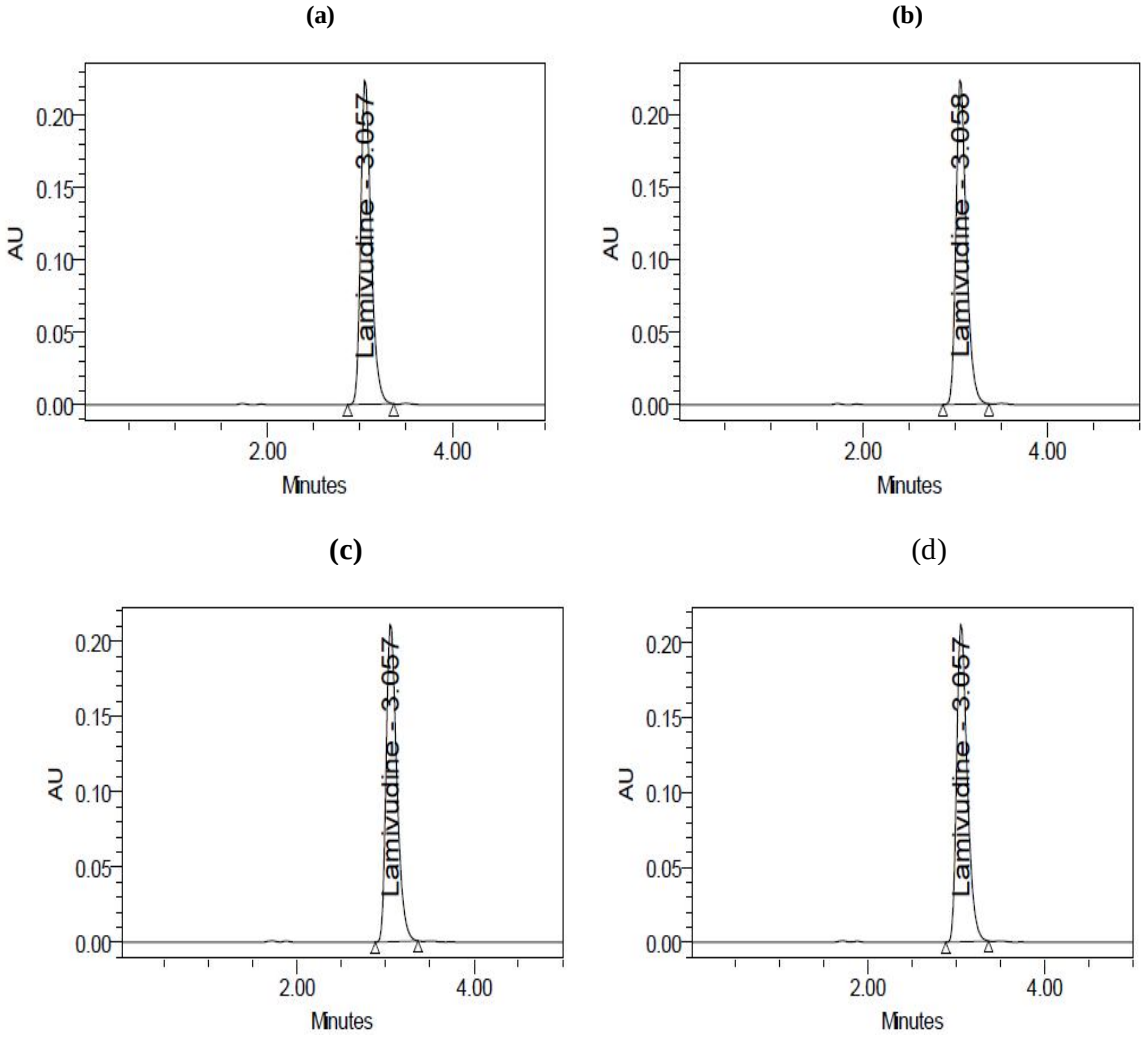


Figure 3. The linearity curve for the Lamivudine sample (10 to 50µg/ml)

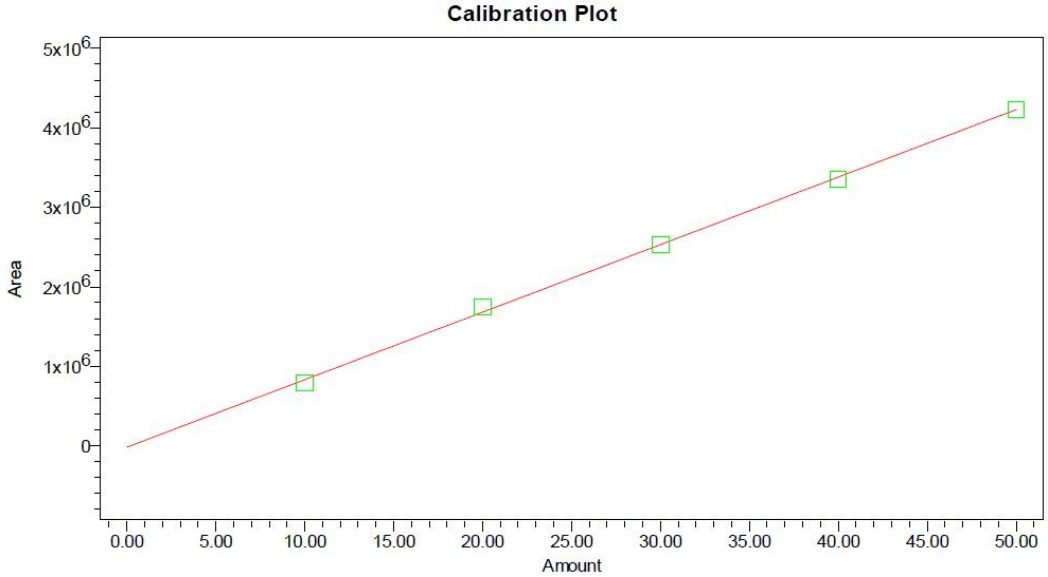
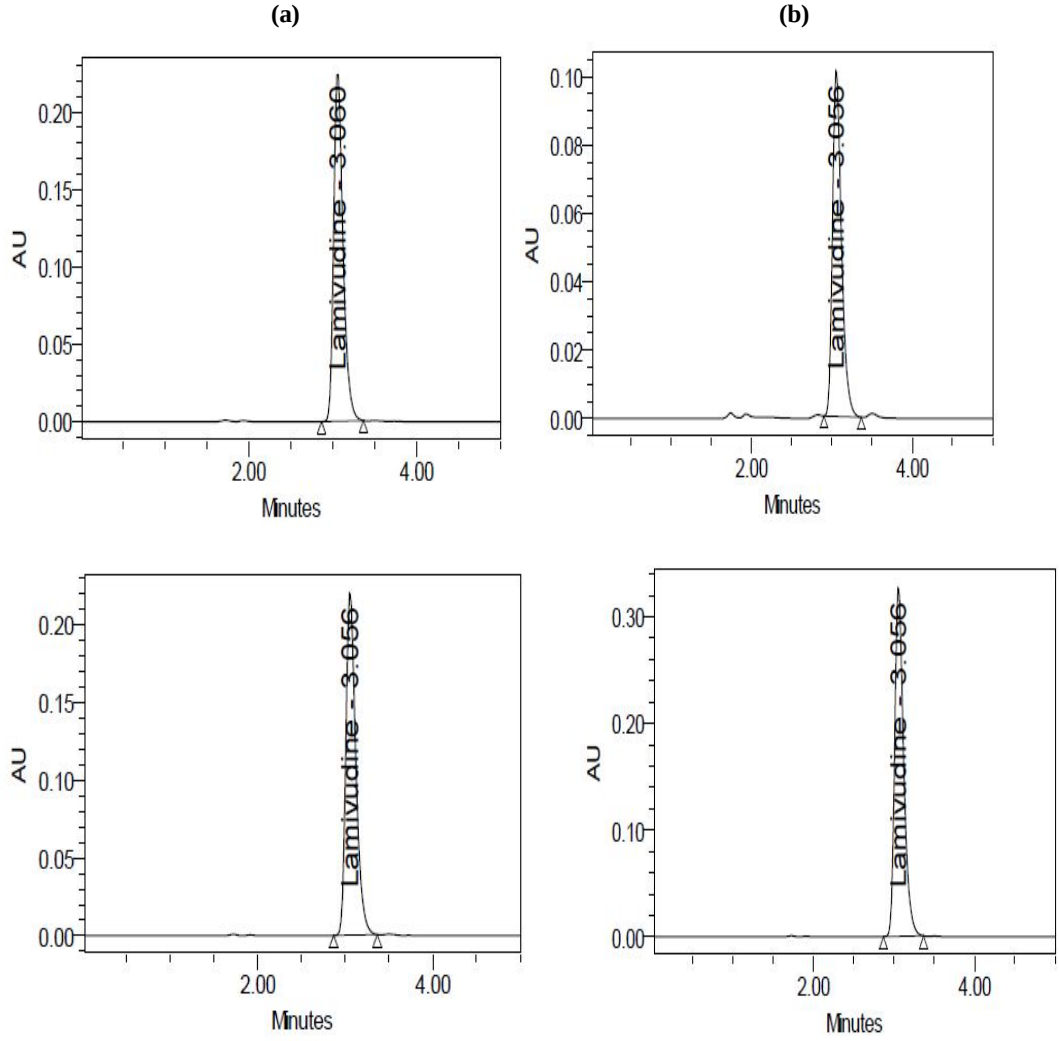


Figure 4. Typical chromatogram of accuracy for standard and sample, (a) standard; (b),(c) and (d) are for sample concentrations of 50%, 100% and 150% respectively



concentration levels (50%, 100%, and 150%) that cover the assay method range (10-50 µg/ml). The %RSD of the system was calculated from the individual relative percent peak area mean values at the 50%, 100%, and 150% of the test concentration. The intra-day (*n*= 5) and inter-day (*n*= 3) %RSD are given in table. All the data are within the acceptance criteria of 2%.

ACCURACY

Accuracy of the method was evaluated by fortifying a Lamivudine sample solution (with respect to the target assay concentration) with three known concentrations of reference standard (10, 20 and 30 µg/ml). Percent recoveries were calculated form differences between the peak areas obtained for fortified and unfortified solutions. Good recoveries were obtained within the acceptance criteria (98.0-102.0%) as shown in Table 2. No significant differences were observed between amounts of Lamivudine added and the amounts found.

LOD & LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) tests for the procedure were evaluated by serial dilutions of Lamivudine stock solutions in order to obtain signal-to-noise ratios (s/n) of ≈3:1 and ≈10:1, respectively. The LOD value for Lamivudine was found to be 0.01µg/ml (s/n = 2.73,) and LOQ (*n* =6) was 0.04 µg/ml (s/n = 9.52) as shown in Table 1.

ROBUSTNESS

Robustness of the method was evaluated by the analysis of Lamivudine under different experimental conditions such as changes in the organic composition of the mobile phase and flow rate. The percentage of methanol in the mobile phase was varied ±10%, the flow rate was varied ±0.2 ml/min. Their effects on the USP plate count, USP tailing at 10%, recovery and repeatability were studied. Deliberate variation of the method conditions had no significant effect on assay data or on chromatographic performance, indicating the robustness of method and its suitability for routine use and transfer to

other laboratories. The results from robustness testing are presented in Table 3.

DISCUSSION

A RP-HPLC method with UV detection for the assay of Lamivudine was developed and validated. The results showed that the method is very selective, no significant interfering peak was detected; accurate, with the percentage recoveries > □99; and reproducible, with the %RSD < 1%. The method was sensitive; a little as 0.01µg/ml could be detected with the LOQ of0.04µg/ml. The method involves use of a simple methanol with the HPLC water and minimum sample preparation, encouraging its application in quality control for analysis of Lamivudine in bulk samples, raw materials and final dosage forms.

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REFERENCES

Bently and Drivers. Text book of pharmaceutical chemistry,8th Edition, oxford university press, London , 1985, 1-3.

Chatwal GR and Anand SK. Instrumental Methods of Chemical Analysis, Himalaya Publishing House, New Delhi, 2004, 21-57.

David Harvey. Modern analytical chemistry, Mcgraw hill publishers, Toronto, 2000, 38-47.

Guidance for Industry: Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Draft Guidance. Rockville, MD. *US FDA*. 2000.

International Conference on Harmonization, "Q2A: Text on Validation of Analytical Procedures," Federal Register, 60(40), 1995, 11260-11262.

International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register, 62(96), 1997, 27463–27467.

Krishnareddy NV, Phani RS and Ramesh Raju R. New RP - HPLC Method Development for Analysis and Assay of Lamivudine in Formulation. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(1), 2011, 220-223.

Lloyd R Snyder, Joseph J, Kirkland Joseph, Glash, Chatwal Anand. Mass spectrometry Practical HPLC method development. Instrumental analysis. 2007, 2.624-2.639.

Mandloi DK, Tyagi PK, Rai VK, Dey S, Ashada RK, Mohanra P. Method development and validation of RP- HPLC in the application of invitro dissolution study of Lamivudine in bulk drug and tablet formulation. *Journal of Chemical and Pharmaceutical Research*, 1(1), 2009, 286-296.

Patro SK, Swain RS, Patro VJ, Choudhury SK. Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine from Pharmaceutical Preparation. *E-Journal of Chemistry*, 7(1), 2010, 117-122.

Remington. The science and practice of pharmacy, volume I, 21st edition, Lipincott Williams & Wilkins publishers, Newyork, 2007, 621-626.

Sethi PD. HPLC-Quantitative analysis of pharmaceutical formulations, CBS publishers& distributors, New Delhi, 2006, 118-120.

Shabir GA, Lough WJ, Shafique AA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. *J Liq Chromatogr Relat Technol*, 30, 2007, 311-33.

Shabir GA. Validation of HPLC methods for Pharmaceutical Analysis: Understanding the differences and similarities between Validation reqirements of the U.S Food and Drug administraton, The U.S Pharmacopoiea and International Conference on Hanmonization. *J Cromatogr. A*, 987, 2003, 57-66.

Skoog, Holler, Nieman. Principles of instrumental analysis, 5th edition, Harcourt college publishers, fortworth, 2004: 725-739.

Synder KL, Krikland JJ and Glajch JL. Practical HPLC Method Development, 2nd Edition, Wiley-Interscience Publication, USA, 1983, 1-10.

Yuri Kazakevin, Rosario Lobrutto. HPLC for pharmaceutical analysis, Wiley interscience, John wiley & sons Inc. New Jersey, 2007, 347-490.