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RP-HPLC Analytical Method Development And Validation For Lamivudine And Zidovudine In Pharmaceutical Dosage Forms

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Abstract: A validated RP-HPLC method for concurrent estimation of lamivudine and zidovudine in pharmaceutical dosage form has been established. The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. XTerra column (150mm x 4.6 mm) was found to be ideal as it gave a good peak shape and resolution at 0.5ml/min flow. The proposed method was found to be fast, accurate, precise, reproducible and rugged and can be used for simultaneous analysis for both the nucleoside analog reverse- transcriptase inhibitors.
Keywords: RP-HPLC, lamivudine, zidovudine.

Introduction

Reverse transcriptase inhibitors like lamivudine and zidovudine [1-5] have been used in combination to treat patients with HIV. The treatment is used to prevent or prolong the onset of acquired immune deficiency syndrome (AIDS), which can lead to a variety of fatal complications. Lamivudine is also used in lower doses to treat patients with chronic hepatitis B in whom the virus has replicated and caused liver inflammation [6-10]. Zidovudine is used along with other antiviral medications to treat patients with HIV. Zidovudine is also used in pregnant women to reduce the risk of HIV transmission from a mother to an unborn child. The literature review reveals that few RP-HPLC methods for the estimation of lamivudine and zidovudine alone and in combination with other drugs[11-17]. Few methods are also reported for estimation of both drugs from formulation. Hence the present work was intended to develop a RP-HPLC method for the simultaneous determination with simple, rapid, greater sensitivity and faster elution.

Materials and methods

Apparatus

A Waters RP-HPLC instrument equipped with software (Empower, 2695 separation module) and an UV-Visible or DAD detector, manual injector with 20 µl loop, and XTerra C_{18} (150 mm x 4.6 mm i.d., 5 µm particle size). The UV/VIS spectrophotometer used was LABINDIA UV 3000^{+}. The pH meter was of Adwa-AD1020 make and pipettes and burettes were of Borosil make.

Reagents and Materials

The drug samples of lamivudine and zidovudine was procured from Dr.Reddy's Pharma, India. Methanol,acetonitrile ,water (HPLC grade LICHROSOLV, Merck.), 0.22µm filter (Millipore,Bangalore.) Pharmaceutical formulation of lamivudine and zidovudine were purchased from a local pharmacy.

Chromatographic Conditions

XTerra C_{18} (150 mm x 4.6 mm i.d., 5 µm particle size). Initially the mobile phase tried was methanol: Ammonium acetate buffer and acetonitrile: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3), acetonitrile in proportion 55: 45 respectively. Flow rate was set as 0.5 ml/min. The mobile phase was degassed before use. Detection wavelength was 271 nm and the injection volume as 20 µl, and Temperature: 25 $\pm 3^0$ C and the total run time was 8 min.

Standard Solution Preparation

Accurately weighed amount of 10mg lamivudine and 10 mg zidovudine were taken to a 10 ml cleaned and dried volumetric flask. This was then diluted with 7 ml of diluent and was sonicated. The volume was made to100 ml with the same solvent. This was marked and labeled as Stock solution. Further, an amount of 0.3 ml lamivudine and zidovudine each were pipetted from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent and the final concentration was 75 ppm.

Sample Solution Preparation

Accurately weighed 10 mg of lamivudine and 20mg zidovudine hydrochloride sample were taken into a 100 ml clean dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and volume made up to the mark with the same solvent. This was taken as Stock solution. Further, 0.75 ml of lamivudine and zidovudine of the above stock solution is pipetted into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

A calibration curves were plotted over a concentration range 10-50 ppm for lamivudine and zidovudine. These solutions were injected into the HPLC under the optimized conditions. Recorded the chromatograms and measured the peak responses. The spectrum and area of lamivudine and zidovudine standards were analysed and drawn a plot between the concentration (mcg/ml) Vs area and reported the slope, intercept.

Accuracy (% Recovery)

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. These solutions were filtered through 0.45µ membrane and then each concentration three replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. The chromatograms of these solutions are analysed and the amount recovery and percent recovery and mean recovery for the same was calculated.

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 calculated as mean and percentage RSD.

Limit Of Detection (for Lamivudine)

Preparation of 30µg/ml solution

Accurately weighed and transferred 10mg of lamivudine working standard into a 10ml clean dry volumetric flask added about 7 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).

Further pipetted 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.06% solution At Specification level (0.018µg/ml solution)

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipetted 0.06ml of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Recorded the chromatograms and measured the peak responses.

Limit Of Quantification (for Lamivudine)

Preparation of 30µg/ml solution

Accurately weighed and transferred 10mg of lamivudine working standard into a 10ml clean dry volumetric flask added about 7 mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stocksolution).Further pipetted 0.3ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.06% solution At Specification level (0.2µg/ml solution)

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.Pipetted 0.06ml of 1µg/ml solution into a 10 ml of volumetric flask and diluted up to the mark with diluents Recorded the chromatograms and measured the peak responses.

Limit Of Detection: (for Zidovudine)

Preparation of 30µg/ml solution

Accurately weighed and transferred 10mg of zidovudine working standard into a 10ml clean dry volumetric flask added about 7 ml of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).

Further pipetted 0.03 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.095% solution At Specification level (0.028µg/ml solution)

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent Pipetted 0.095ml of 1µg/ml solution into a 10 ml of volumetric flask and diluted up to the mark with diluent. Recorded the chromatograms and measured the peak responses.

Limit Of Quantification: (for Zidovudine)

Preparation of 30µg/ml solution

Accurately weighed and transferred 10mg of zidovudine working standard into a 10ml clean dry volumetric flask added about 7 ml of Diluent and sonicated to dissolve it completely and made volume up to the

mark with the same solvent. (Stock solution). Further pipetted 0.3ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.3% solution At Specification level (0.09µg/ml solution)

Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Pipetted 0.3ml of 1µg/ml solution into a 10 ml of volumetric flask and diluted up to the mark with diluent.Recorded the chromatograms and measured the peak responses.

Robustness

The robustness is measure of its capacity to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability during normal usage hence the following are performed by slight variations in parameters.

Variation of flow

The assay content of the sample was measured by change in the flow rate 0.4ml/min to 0.6ml/min.

Assay Procedure

A solution of 20 µL standard, sample seperately were injected into the chromatographic system and areas for the lamivudine and zidovudine peaks were measured and the percentage assay calculated by using the formulae.Recorded the chromatogram and measured the peak responses. Calculated the mean and persentage RSD for the same.

$$\text{Assay percentage} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = label claim of drug mg/ml.

Results and discussions

Method development

Three trials were conducted to optimize the simultaneous detection of both the drugs and these data are tabulated in table 1. Thus it can be safely be inferred that trial 3 (figure 1), is the best method for detection of both the drugs as the chromatogram observed had lamivudine and zidovudine peaks are well separated.

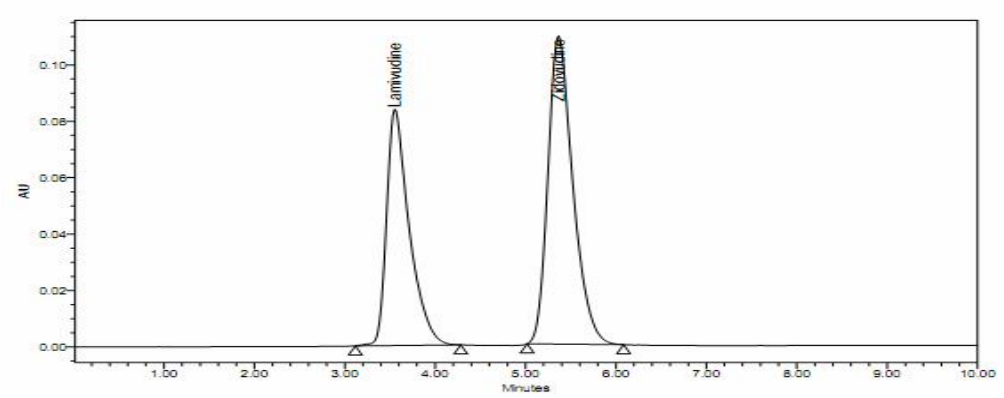

Figure 1: Chromatogram for lamivudine and zidovudine

Table 1: Table showing list of optimized chromatographic parameters

	Trial1	Trial 2	Trial 3
Column	Symmetry C18;XTerra	Symmetry C18;XTerra	Symmetry C18;XTerra
Flow rate	0.7ml/min	1.0ml/min	0.5ml/min
Mobile phase	Buffer:Acetonitrile	Buffer:Acetonitrile	Buffer:Acetonitrile
pH	3.5	4.5	3.0
Rt(Lamivudine)	3.556	3.556	3.556
Rt(Zidovudine)	5.364	5.364	5.364

System Suitability

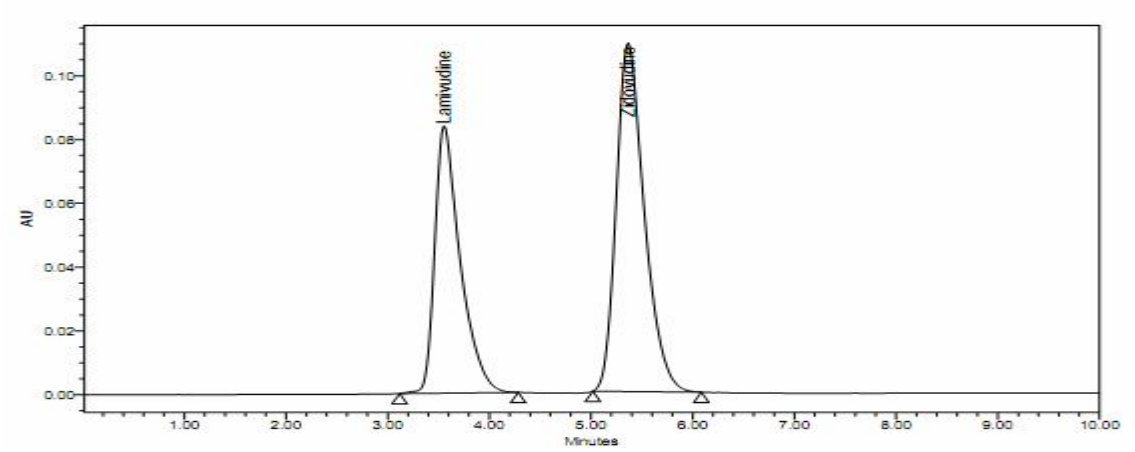

Figure 2: Chromatogram for system suitability

Table 2: System suitability parameters for lamivudine and zidovudine

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Symmetry Factor
1	Lamivudine	3.556	1454895	83907	970.35	1.59	1.6
2	Zidovudine	5.364	2091554	109282	1791.70	1.32	1.3

The Resolution between lamivudine and zidovudine (figure 2 and table 2) was not less than 2 and the theoretical plates for lamivudine and zidovudine was not less than 2000.the tailing factor for lamivudine and zidovudine was not less than 0.9 and not more than 2, thus indicating the suitability.

Precision

The results of repeatability experiments are tabulated in the table 3. The developed method was found to be precise and the RSD values were <2%, hence under the limits of the ICH guidelines.

Table 3: Table for precision of lamivudine and zidovudine

Standard injections	Lamivudine AREA	Zidovudine AREA
Injection-1	2403786	1892706
Injection-2	2492050	1971723
Injection-3	2498315	1958626
Injection-4	2491704	1956498
Injection-5	2495157	1984128
Average	**2476202**	**1952736**
Standard deviation	**40570.39**	**35349.72**
% RSD	**1.63**	**1.81**

Accuracy And Linearity

The peak recovery values are the indicators for accuracy, and they have been found to be well below the permissible limits. The linearity range was found to lie from 10ppm to50ppm of lamivudine, 10ppm to 50ppm 0f zidovudine and chromatograms are shown below.

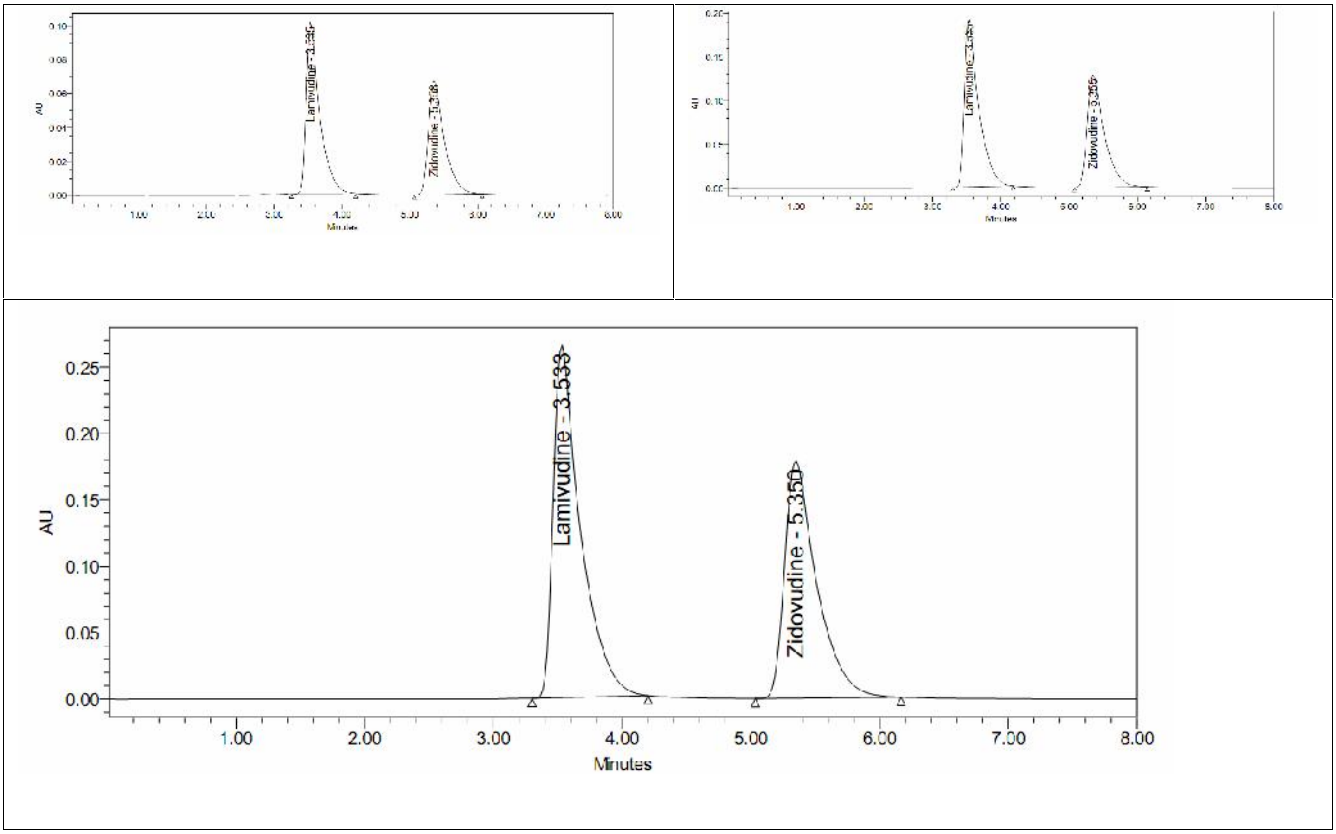

Figure 3: Chromatograms for accuracy

Table 3: Accuracy (recovery) data for lamivudine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1384929	5	5.04	100.8%	100.8%
100%	2804629	10	10.2	102.1%	
150%	3877682	14.2	14.1	99.4%	

Table 4: Accuracy (recovery) data for zidovudine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1098849	5.1	5.14	100.9%	100.1%
100%	2215169	10.3	10.38	100.7%	
150%	3073816	14.6	14.4	98.6%	

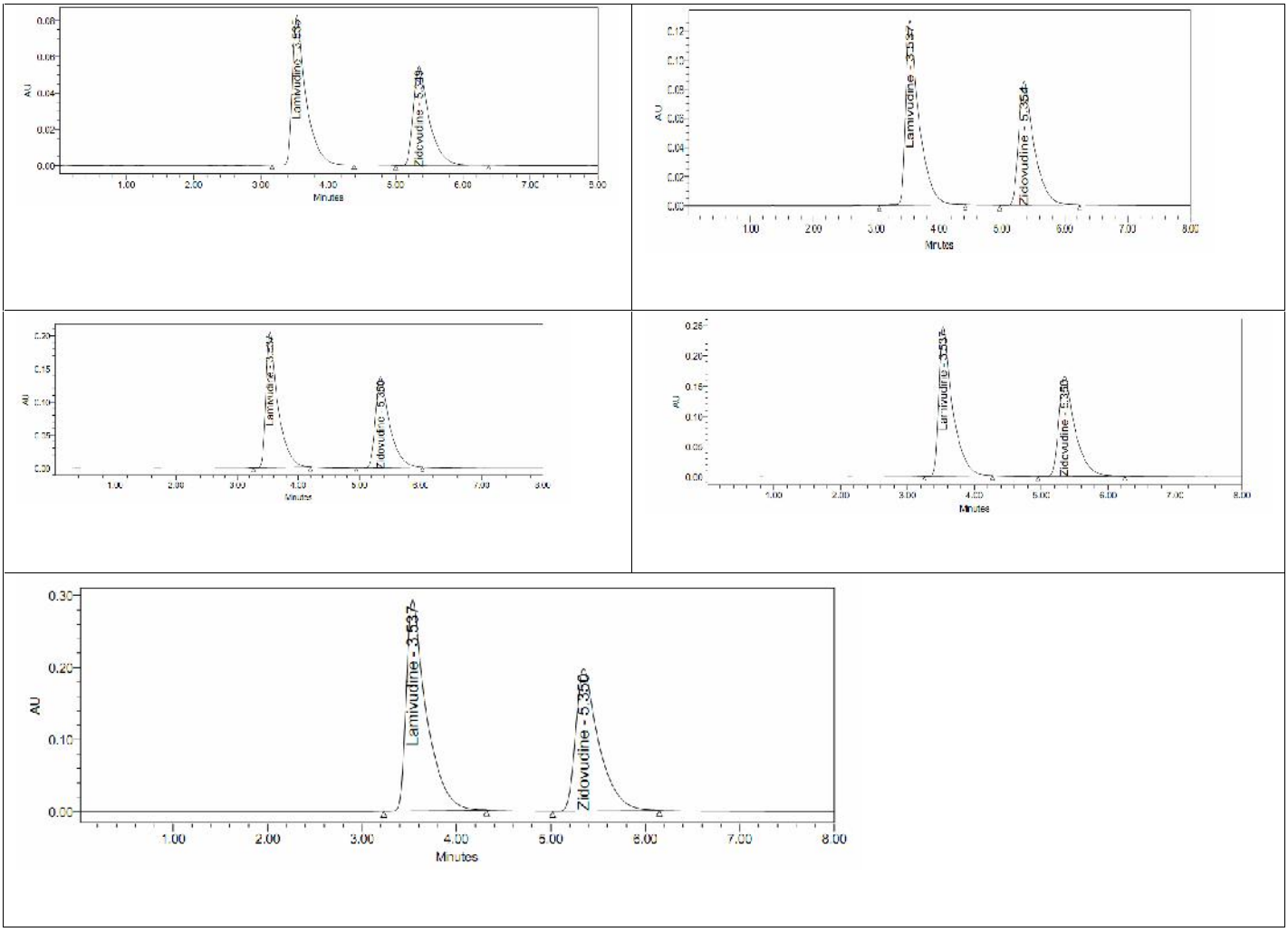

Figure 4: Chromatogram for linearity concentration

Table 5: Area of different concentration of lamivudine and zidovudine

Concentrations (mcg/ml)	Lamivudine area	Zidovudine area
10	1142992	901788
20	1862897	1456684
30	2794602	2200558
40	3497638	2786015
50	4279842	3382081

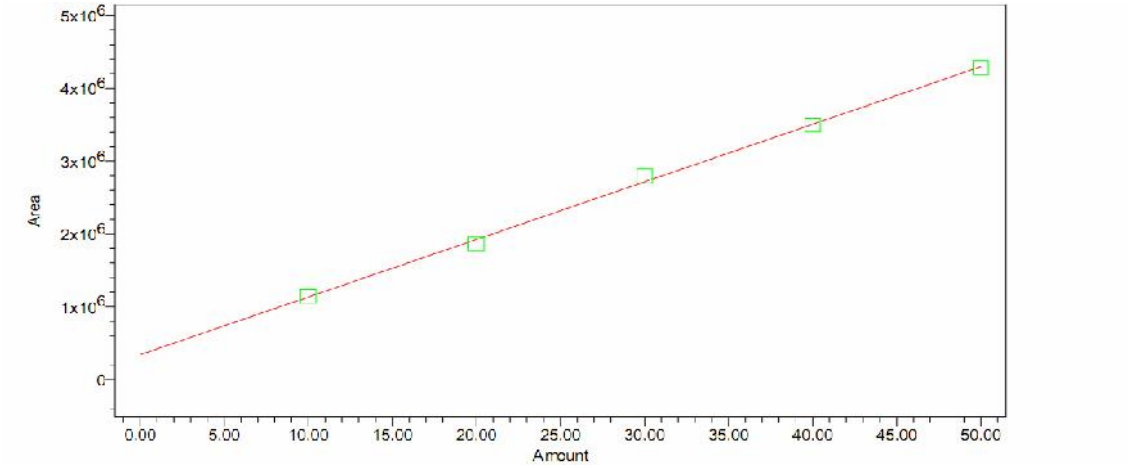

Figure 5: Calibration graph for lamivudine at 271 nm

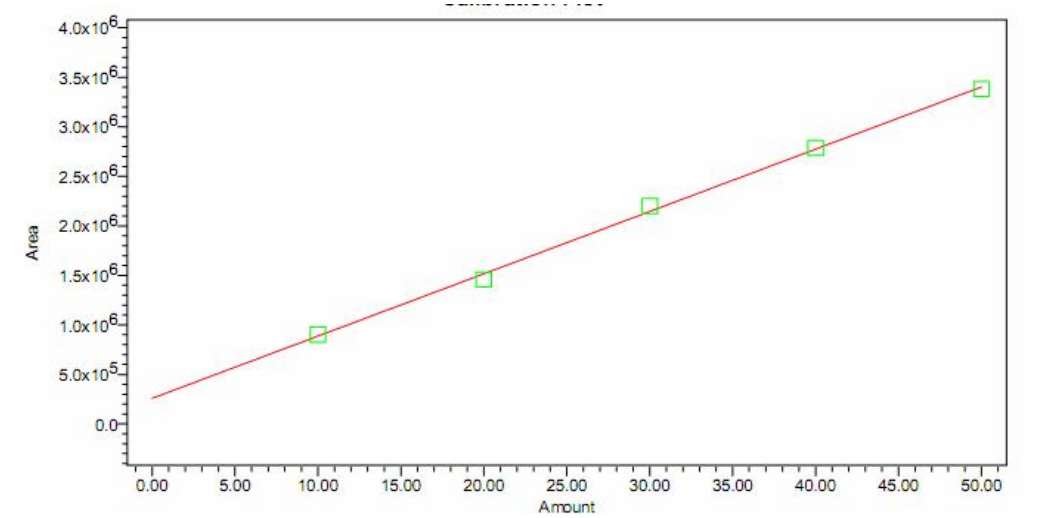

Figure 6: Calibration graph for zidovudine at 271 nm

Table 6: Analytical performance parameters of lamivudine and zidovudine

Parameters	**Lamivudine**	**Zidovudine**
Slope (m)	62866	55641
Intercept (c)	20154	33951
Correlation coefficient (R^2)	0.999	0.999

- Correlation coefficient (R^2) is not less than 0.999

Limit Of Detection For Lamivudine And Zidovudine

The limits of detection and quantification was also done, based on the calibration curves for lamivudine and zidovudine. Based on the signal to noise ratios, The LOD was found to be less than 3:1 for lamivudine and zidovudine the LOQ was also found to be 10:1 respectively.

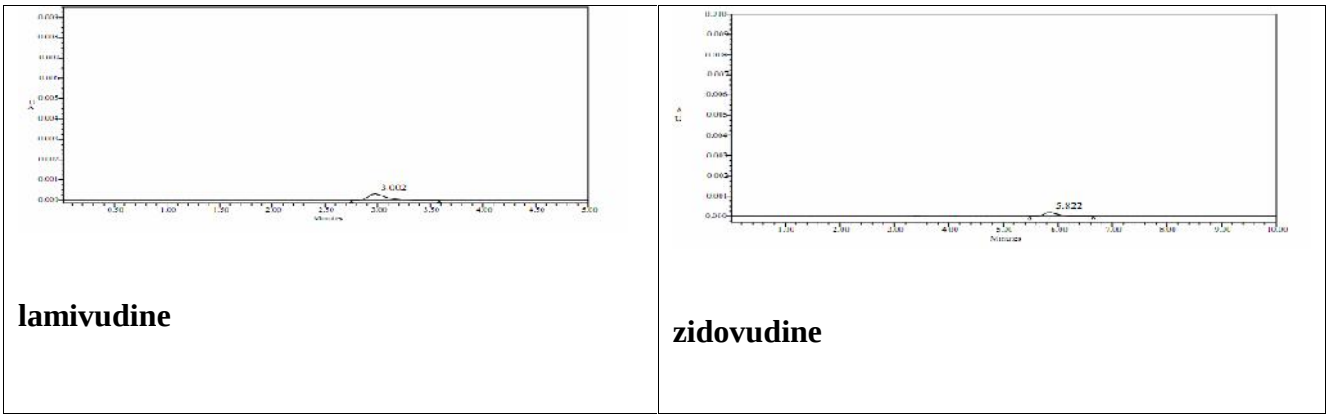

Figure 7: Chromatogram of lamivudine and zidovudine showing LOD

Table 7: LOD for lamivudine and zidovudine

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Lamivudine	52	152	2.92
Zidovudine	52	158	3.03

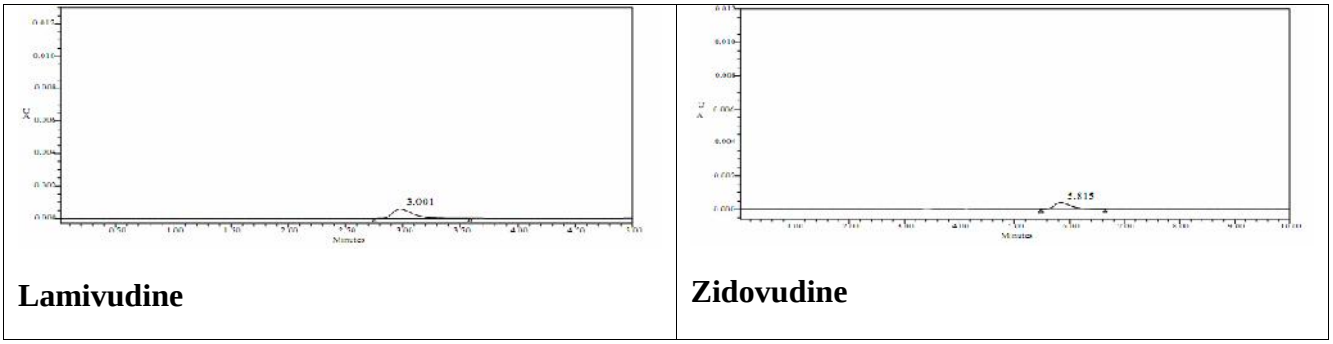

Figure 7: Chromatogram of lamivudine and zidovudine showing LOQ

Table 8: LOQ for lamivudine and zidovudine

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Lamivudine	52	516	9.92
Zidovudine	52	530	10.19

Conclusion

The estimation of lamivudine and zidovudine was done by RP-HPLC. The Phosphate buffer was p^H 3 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 55:45 % v/ v. A C18 column contains octadecylsilane chemically linked to porous silica particles was used as stationary phase. UV detector was used at 271 nm. The solutions were chromatographed at a constant flow rate of 0.5 ml/min. The linearity range of lamivudine and zidovudine were found to be rom 10-50 µg/ml.of lamivudine and zidovudine Linear regression coefficient was not more than 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of. Lamivudine and zidovudine LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements .it can be also inferred that the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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