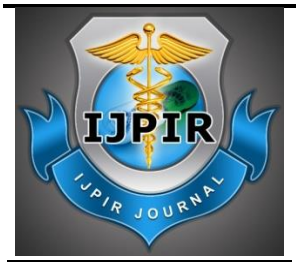
Research Article



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Method development and validation for the simultaneous estimation of lamivudine and zidovudine in pure form and marketed pharmaceutical dosage form by using RP-HPLC

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Lamivudine and Zidovudine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Phosphate buffer pH4.6, Methanol and ACN (65:25:10 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 265nm. The retention time of the Lamivudine and Zidovudine was 2.088, 6.068 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Lamivudine and 20-100mg/ml of Zidovudine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Lamivudine, Zidovudine, RP-HPLC, Validation.

INTRODUCTION

Lamivudine [1] is a synthetic nucleoside analogue with activity against hepatitis B virus (HBV) and HIV. Intracellularly, lamivudine is phosphorylated to its active metabolites, lamiduvine triphosphate (L-TP) and lamiduvine monophosphate (L-MP). In HIV, L-TP inhibits HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleoside analogue into viral DNA. In HBV, incorporation of L-MP into viral DNA by HBV polymerase results in DNA chain termination. L-TP is a weak inhibitor of mammalian DNA polymerases alpha and beta, and mitochondrial DNA polymerase. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV) to disrupt viral DNA synthesis. When phosphorylated, lamivudine can form active metabolites that compete for incorporation into viral DNA. Via DNA incorporation, lamivudine metabolites competitively inhibit the activity of the HIV reverse transcriptase enzyme and act as a chain terminator of DNA synthesis. Due to the lack of a 3'-OH group, incorporated nucleoside analogues prevent the formation of a 5' to 3' phosphodiester linkage that is essential for DNA chain elongation.

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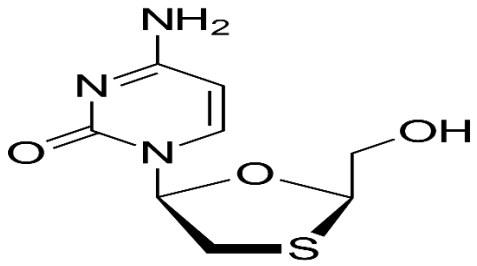

Fig-1: Chemical Structure of Lamivudine

A dideoxynucleoside compound [2] in which the 3'-hydroxy group on the sugar moiety has been replaced by an azido group. This modification prevents the formation of phosphodiester linkages which are needed for the completion of nucleic acid chains. The compound is a potent inhibitor of HIV replication, acting as a chain-terminator of viral DNA during reverse transcription. It improves immunologic function, partially reverses the HIV-induced neurological dysfunction, and improves certain other clinical abnormalities associated with AIDS. Its principal toxic effect is dose-dependent suppression of bone marrow, resulting in anemia and leukopenia. Zidovudine, a structural analog of thymidine, is a prodrug that must be phosphorylated to its active 5′-triphosphate metabolite, zidovudine triphosphate (ZDV-TP). It inhibits the activity of HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue. It competes with the natural substrate dGTP and incorporates itself into viral DNA. It is also a weak inhibitor of cellular DNA polymerase α and γ.

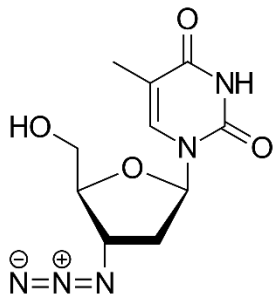

Fig-2: Chemical Structure of Zidovudine

A detailed survey of literature also revealed that scientist has previously used this simultaneous estimation method for Lamivuidne and Zidovudine in its pure form as well as in tablet dosage form.

The objective of the present study was to develop a simple, precise, accurate and rapid method for the estimation of in its pure form as well as in tablet dosage form.

MATERILAS AND METHODS

Table-1: List of Instruments Used

S.No.	Instruments and Glasswares	Model	Software	Manufactures Name
1	HPLC	SPD20A	LC Solutions	Shimadzu
2	UV spectrophotometer	T60	UV Win 5	PG Instruments
3	Digital Weighing Balance (sensitivity 5mg)	ER200A	-	Ascoset
4	pH meter	AD102U	-	ADWA
5	Sonicator	SE60US	-	Enertech
6	Volumetric flasks	Borosil	-	-
7	Pipettes and Burettes	Borosil	-	-

Table-2: List of Chemicals Used

S.No.	Chemical	Brand Names	Grade
1	Water	LICHROSOLV (MERCK)	HPLC
2	Methanol	LICHROSOLV (MERCK)	HPLC
3	Acetonitrile for HPLC	Merck	HPLC
4	Triethylamine	Merck	HPLC

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of Lamivudine and 0.3ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Lamivudine and Zidovudine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3 ml of Lamivudine and Zidovudine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD DEVELOPMENT

Selection of Wavelength

10 mg of Zidovudine and Lamivudine was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Zidovudine and Lamivudine. The isobestic point was taken as detection wavelength.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase was optimized to Buffer: Methanol: ACN in proportion 65:25:10v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, ODS and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized Chromatographic Conditions

Instrument used: SPD20A with LC Solutions software of Shimadzu model.

Temperature : 38ºC
Column : Altima C18 (4.6×150mm, 5µ)
Buffer : Phosphate buffer (pH-4.6)- Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298 g of Citric acid monohydrate in sufficient water to produce 1000mL. Adjust the pH 4.6 by using ortho phosphoric acid.
pH : 4.6
Mobile phase : Buffer: Methanol: ACN (65:25:10v/v)
Flow rate : 1ml/min
Wavelength : 265 nm
Injection volume : 10 µl
Run time : 14 min

Method Validation

System Suitability

Accurately weigh and transfer 10 mg of Lamivudine [3] and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of Lamivudine and 0.3ml of Zidovudine from the above stock solutions into a 10ml volumetric flask [4] and dilute up to the mark with diluents.

Procedure

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 found to be within the specified limits.

Specificity Study of Drug

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of Lamivudine and 0.3ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents [5].

Preparation of Sample Solution

Take average weight [6] of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Lamivudine and Zidovudine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3 ml of Lamivudine and Zidovudine above stock solution [7] into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions [8] and calculate the assay by using formula:

$$\%\ \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of drug solutions for Linearity

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely [9] and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5 µg/mL of Lamivudine & 10 µg/mL of Zidovudine)

Pipette out 0.05ml of lamivudine and 0.1ml of zidovudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 µg/ml of Lamivudine&20 µg/ml of Zidovudine)

Pipette out 0.1ml of lamivudine and 0.2ml of zidovudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 µg/ml of Lamivudine&30 µg/ml of Zidovudine)

Pipette out 0.15ml of lamivudine and 0.3ml of zidovudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 µg/ml of Lamivudine&40 µg/ml of Zidovudine)

Pipette out 0.2ml of lamivudine and 0.4ml of zidovudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 µg/ml of Lamivudine&50 µg/ml of Zidovudine)

Pipette out 0.25ml of lamivudine and 0.5ml of zidovudine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [10].

Precision

Repeatability

Preparation of Lamivudine and Zidovudine Product Solution for Precision

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume [11] up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of Lamivudine and 0.3ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD [12] for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision [13] (also known as Ruggedness) of the method, Precision [14] was performed on different days by maintaining same conditions.

Procedure

Day 1

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 found to be within the specified limits.

Day 2

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 found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate [15] to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.075ml of Lamivudine and 0.15ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve

it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.15ml of Lamivudine and 0.3ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.225ml of Lamivudine and 0.45ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added [16] for Lamivudine and Zidovudine and calculate the individual recovery and mean recovery values.

Robustness

The analysis [17] was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Lamivudine and 10mg of Zidovudine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.15ml of Lamivudine and 0.3ml of Zidovudine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Buffer: Methanol: ACN was taken in the ratio and 75: 15: 10, 55:35:10 instead 65:25:10, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

RESULTS AND DISCUSSION

Method Development

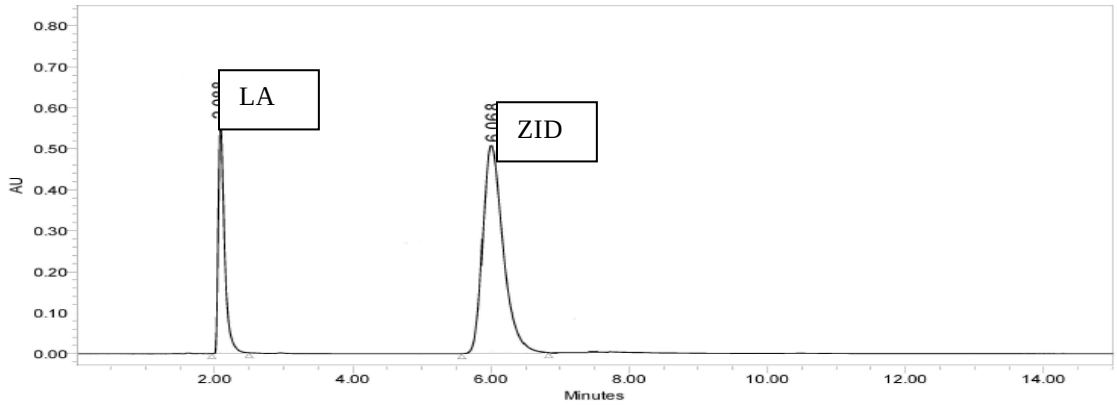

Fig-3: Optimized Chromatogram

Table-3: Peak Results for Optimized Chromatogram

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lamivudine	2.088	3425413	567933		1.0	5565.5
2	Zidovudine	6.068	1629854	517733	6.5	1.1	5355.2

Method Validation

System Suitability

Table-4: Results of system suitability for Lamivudine

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lamivudine	2.080	3569412	567917	5568.0	1.0
2	Lamivudine	2.080	3465125	517719	6359.2	1.1
3	Lamivudine	2.080	3598154	567933	5565.5	1.0
4	Lamivudine	2.081	3586491	517733	5355.2	1.1
5	Lamivudine	2.081	3582694	567917	6348.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Table-5: Results of method precession for Zidovudine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Zidovudine	6.056	3582264	567917	5568.0	1.0	6.5
2	Zidovudine	6.056	3586491	517719	5359.2	1.1	6.5
3	Zidovudine	6.056	3598154	567933	5565.5	1.0	6.5
4	Zidovudine	6.057	3564125	517733	5355.2	1.1	6.5
5	Zidovudine	6.057	3569412	562173	5568.0	1.0	6.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

Specificity

Table-6: Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.087	3425681	567917		1.0	5568.0
2	Zidovudine	6.067	16235984	517719	6.5	1.1	5359.2
3	Lamivudine	2.088	3425413	567933		1.0	5565.5
4	Zidovudine	6.068	16298543	517733	6.5	1.1	5355.2
5	Lamivudine	2.088	3465423	567933		1.0	5545.5
6	Zidovudine	6.068	16265213	517733	6.5	1.1	5352.1

Table-7: Peak Results for Assay Sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.089	3469821	567917		1.0	6568.0
2	Zidovudine	6.069	16259845	517719	6.5	1.1	5359.2
3	Lamivudine	2.090	3468547	567933		1.0	5565.5
4	Zidovudine	6.070	16287531	517733	6.5	1.1	5355.2
5	Lamivudine	2.090	3468143	567813		1.0	5391.1
6	Zidovudine	6.070	16282431	517623	6.5	1.1	5564.0

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The percentage purity of the given marketed pharmaceutical dosage form was found to be 100.915%.

Linearity

Table-8: Linearity Study of Lamivudine

Concentration µg/ml	Average Peak Area
10	1010252
20	2049374
30	3072706
40	3921068
50	4952813

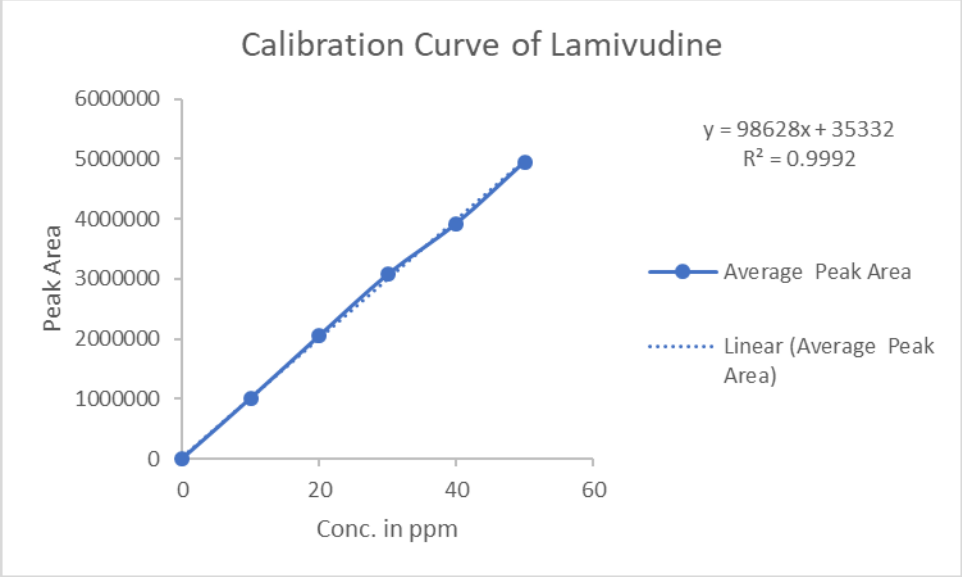

Fig-4: Calibration Graph for Lamivudine

Zidovudine

Table-9: Linearity Study of Zidovudine

Concentration µg/ml	Average Peak Area
20	8040807
40	14318417
60	21087985
80	27913928
100	34584741

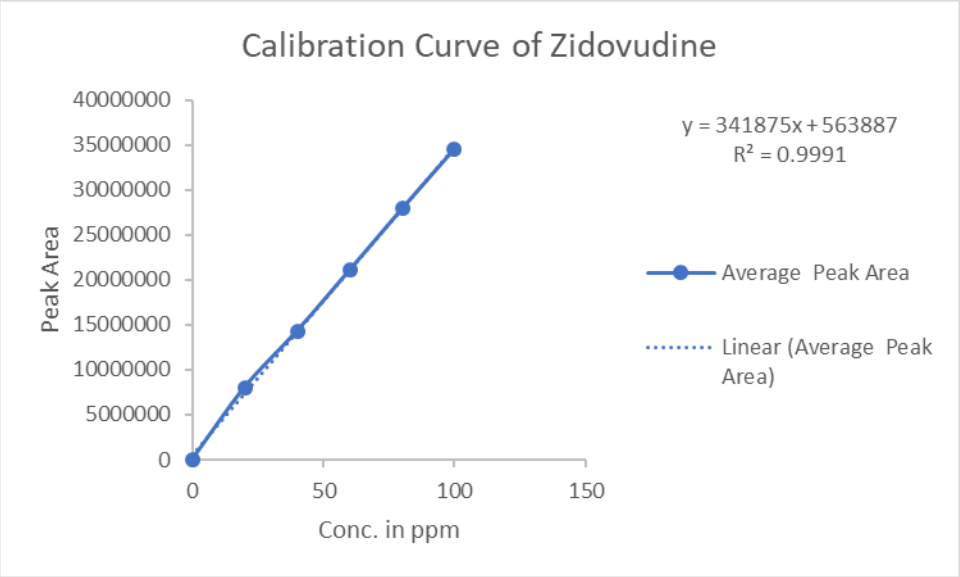

Fig-5: Calibration Graph for Zidovudine

Precision

Repeatability

Table-10: Results of Repeatability for Lamivudine

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lamivudine	2.084	3569412	567917	5568.0	1.0
2	Lamivudine	2.083	3465125	517719	5359.2	1.1
3	Lamivudine	2.082	3598154	567933	5565.5	1.0
4	Lamivudine	2.081	3586491	517733	5355.2	1.1
5	Lamivudine	2.080	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Table-11: Results of Method Precision for Zidovudine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Zidovudine	2.080	3582264	567917	5568.0	1.0	2.5
2	Zidovudine	2.081	3586491	517719	5359.2	1.1	2.5
3	Zidovudine	2.082	3598154	567933	5565.5	1.0	2.5
4	Zidovudine	2.083	3564125	517733	5355.2	1.1	2.5
5	Zidovudine	2.084	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

Intermediate Precision

Day-1

Table-12: Results of Intermediate Precision for Lamivudine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Lamivudine	2.081	3481579	567917	5568.0	1.0
2	Lamivudine	2.082	3458121	517719	5359.2	1.1
3	Lamivudine	2.083	3426581	567933	5565.5	1.0
4	Lamivudine	2.084	3465712	517733	5355.2	1.1
5	Lamivudine	2.085	3451476	567917	5568.0	1.0
6	Lamivudine	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Table-13: Results of Intermediate Precision for Zidovudine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Zidovudine	6.061	15481579	567917	5568.0	1.0	2.5
2	Zidovudine	6.062	15369852	517719	5359.2	1.1	2.5
3	Zidovudine	6.063	15248454	567933	5565.5	1.0	2.5
4	Zidovudine	6.064	15874692	517733	5355.2	1.1	2.5
5	Zidovudine	6.064	15236547	567933	5568.0	1.0	2.5
6	Zidovudine	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

Day-2

Table-14: Results of Intermediate Precision Day 2 for Lamivudine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Lamivudine	2.081	3481579	567917	5568.0	1.0
2	Lamivudine	2.082	3458121	517719	5359.2	1.1
3	Lamivudine	2.083	3426581	567933	5565.5	1.0
4	Lamivudine	2.084	3465712	517733	5355.2	1.1
5	Lamivudine	2.085	3451476	567917	5568.0	1.0
6	Lamivudine	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Table-15: Results of Intermediate Precision for Zidovudine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Zidovudine	6.061	15481579	567917	5568.0	1.0	2.5
2	Zidovudine	6.062	15369852	517719	5359.2	1.1	2.5
3	Zidovudine	6.063	15248454	567933	5565.5	1.0	2.5
4	Zidovudine	6.064	15874692	517733	5355.2	1.1	2.5
5	Zidovudine	6.064	15236547	567933	5568.0	1.0	2.5
6	Zidovudine	6.064	15217547	567133	5359.2	1.1	2.5

Mean	15404779
Std. Dev	251289.4
% RSD	1.6

Accuracy

Table-16: Results of Accuracy for Concentration-50%

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.080	1536547	567917		1.0	5568.0
2	Zidovudine	6.100	1180807	517719	2.5	1.1	5359.2
3	Lamivudine	2.081	1526986	567933		1.0	5565.5
4	Zidovudine	6.101	1046986	517733	2.5	1.1	5355.2
5	Lamivudine	2.082	1567845	566924		1.0	5568.0
6	Zidovudine	6.102	1025466	519124	2.5	1.1	5359.2

Table-17: Results of Accuracy for Concentration-100%

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.082	3065874	567917		1.0	5568.0
2	Zidovudine	6.102	2108433	517719	6.5	1.1	5359.2
3	Lamivudine	2.083	3029381	567933		1.0	5565.5
4	Zidovudine	6.101	2084929	517733	6.5	1.1	5355.2
5	Lamivudine	2.084	3012393	567917		1.0	5568.0
6	Zidovudine	6.102	2094845	517719	6.5	1.1	5359.2

Table-18: Results of Accuracy for Concentration-150%

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.080	4477263	567917		1.0	5568.0
2	Zidovudine	6.044	3094837	517719	6.5	1.1	5359.2
3	Lamivudine	2.082	4482921	567933		1.0	5565.5
4	Zidovudine	6.042	3094843	517733	6.5	1.1	5355.2
5	Lamivudine	2.082	4392832	568117		1.0	5568.0
6	Zidovudine	6.041	3148372	517689	6.5	1.1	5359.2

Table-19: Accuracy Results for Lamivudine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	15	15.2	101.9	100.9%
100%	3035883	30	30.4	101.4	
150%	4451005	45	44.7	99.4	

Table-20: Accuracy Results for Zidovudine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	30	30.07	100.2	99.6%
100%	2096069	60	59.6	99.4	
150%	3112684	90	89.3	99.3	

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Lamivudine: 1.9µg/ml

Zidovudine: 17.0µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Lamivudine: 5.9µg/ml

Zidovudine: 51.5µg/ml

Robustness

Table-21: Robustness Results of Lamivudine

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

Table-22: Robustness Results of Zidovudine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lamivudine and Zidovudine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Phosphate buffer pH4.6, Methanol, ACN (65:25:10 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.This method can be used for the routine determination of Lamivudine and Zidovudine in bulk drug and in Pharmaceutical dosage forms.

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Abbreviations

API-Active Pharmaceutical Ingredient, λmax-Maximum wavelength, RP-HPLC-Reversed Phase High Performance Liquid Chromatography, ICH-International Conference on Harmonization, LOD-Limit of Detection, LOQ-Limit of Quantitation, r2-

Correlation Coefficient, %RSD-Percentage Relative Standard Deviation.

Conflict of interests

All authors have none to declare.

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