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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMUVIDINE AND ZIDOVUDINE IN SOLID DOSAGE FORM BY RP-HPLC METHOD

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Abstract:

A simple rapid, accurate, precise and reproducible validated reverse phase HPLC method was developed for the determination of Lamivudine, Zidovudine in bulk and pharmaceutical dosage forms. The quantification was carried out using Hypersil C18 (150X4.6mm, 5µm) column running isocratic way using mobile phase comprising of Buffer:Methanol:Ammonium acetate buffer (40:60 v/v) pH 3.8 as mobile phase at a flow rate of 1.0 ml/min and wavelength at 260nm., and injection volume of 20µL, with a flow rate of 1.0mL/min. The retention times of Lamivudine, Zidovudine was found to be 3.465, 8.510. The method was validated intermsoflinearity, precision, accuracy, LOD, LOQ and robustness in accordance with ICH guidelines. The linearity ranges of the proposed method lies between 0.075mg/mL to 0.125mg/mL, with correlation coefficient of r2=0.9999, 0.9998 for Lamivudine, Zidovudine. The assay of the proposed method was found to be 99.98%, 99.96%. The recovery studies were also carried out and mean% Recovery was found to be 100.7%, 100.28%,. The %RSD from reproducibility was found to be <2%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Lamivudine, Zidovudine in bulk and in Pharmaceutical dosage form.

Keywords: Lamivudine, Zidovudine, RP-HPLC, Symmetry C18, Tablets, Validation.

1 Introduction

Lamivudine is chemically4-amino-1-[(2R,5S)-2(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2 dihydropyrimidin-2-onecytosine and used as an antiretroviral activity. Lamivudine is an analogue of cytidine. It can inhibitboth 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. 3TCtriphosphate also inhibits cellular DNA polymerase. Zidovudine is chemically1-(3-AZiDO-2,3-dideoxy–β–D-erythro–pentofuranosyl)-5–methyl and used as an antiretroviral activity. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi - component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances.[1,2]The literature survey [2-6]reveals that there are some HPLC methods have been reported for the

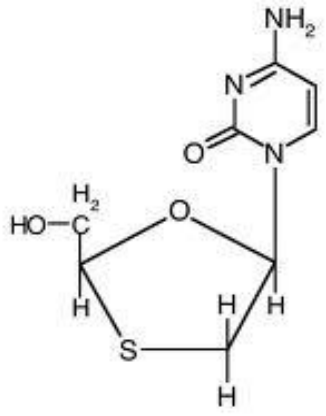

Figure.1.Structure of Lamivudine

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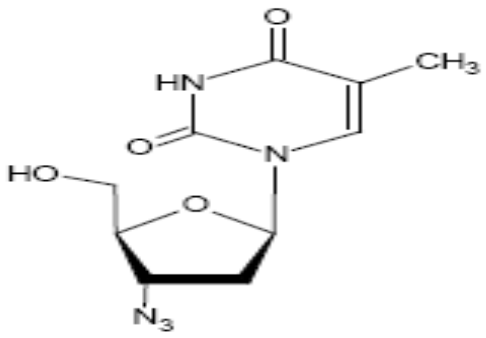

Figure.2. Structure of Zidovudine

analysis of Lamivudine and Zidovudine. But the present study is to develop an accurate, simple and low cost and reliable HPLC method for simultaneous estimation of Lamivudine and Zidovudine in solid dosage form.

Literature review reveals very few methods are reported for the assay of Lamivudine, Zidovudine in Tablet dosage forms using RP-HPLC method. The reported HPLC methods were having disadvantages like high flow rate, more organic phase and use of costly solvents. The proposed RP-HPLC method utilizes economical solvent system and having advantages like better retention time, less flow rate, very sharp and symmetrical peak shapes. The aim of the study was to develop a simple, precise, economic and accurate RP- HPLC method for the estimation of Lamivudine, Zidovudine in Tablet dosage forms.

2 Materials and Methods

UV-1800 Schimadzu double beam UV-VISIBLE spectrophotometer with 1cm matched quartz cells. WATER HPLC equipped with UV-VIS detector and the column used was hypersil C18 (150*4.6mm, 5µ). The data acquisition was performed by using borwin 1.5 solutions software.

CHEMICALS AND REAGENTS:

Lamivudine Zidovudine pure samples were were received as gift samples from micro labs Pharmaceuticals GOA (India). “Duovir- E” marketed by CIPLA was purchased from local pharmacy. Other chemicals all are of HPLC grade.

PREPARATION OF MOBILE PHASE:

A mobile phase was prepare by using 7.7 g of Ammonium Acetate was dissolved in 1000ml of HPLC water and pH was adjusted to 3.8 ± 0.05 with Glacial Acetic Acid, filtered and degassed. Then Methanol sonicated and filtered through 0.45µ Nylon filter. A suitable quantity of degassed mixture of methanol and Buffer in the ratio of 40:60 v/v was prepared and filtered through 0.45µ filter under vacuum filtration.

Preparation of standard stock solutions

10 mg of Lamivudine and Zidovudine were accurately weighed separately and transferred to separate 10 ml volumetric flasks, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Lamivudine and Zidovudine respectively. 1 ml of above stack solution transferred in 10 ml volumetric flask and the volume was made with mobile phase. The concentration of Lamivudine and Zidovudine 100µg/ml. respectively

Preparation of sample solution:

Five tablets were weighed and finely powdered and a powder quantity equivalent to 150mg Lamivudine, 300mg of Zidovudine were accurately weighed and transferred to a 100ml volumetric flask and 50ml of diluent was added to the same. The flask was sonicated for 20 min and volume was made up to the mark with diluent. Transferred 5ml of solution into a 50ml volumetric flask and dilute up to the mark with diluent so as to obtain a concentration of 150,300µg/mL mixed well and injected. The amount present in each tablet was calculated by comparing the area of standard Lamivudine, Zidovudine and tablet sample.

Selection of Mobile phase

Lamivudine and Zidovudine was injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation. After several permutation and combination, it was found thatHypersilC18(150X4.6mm,5µm)columnruninis

ocraticwayusing mobile phase comprising of Buffer : Methanol : Ammonium acetate buffer (40:60 v/v)pH 3.8 as mobile phase at a flow rate of 1.0 ml/min and wavelength at 260nm., and injection volume of 20µL, with a flow rate of 1.0mL/min. The retention times of Lamivudine, Zidovudine was found to be 3.465, 8.510, gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase contains about 60 volume of Buffer [pH 3.8] and 40 volume of Methanol as it gave high resolution of Lamivudine and Zidovudine with minimum tailing.

RESULTS AND DISCUSSION Method development: The development of HPLC method for the analysis of Lamivudine and Zidovudine various trial was conducted. The optimized chromatographic condition were given in table no 1.

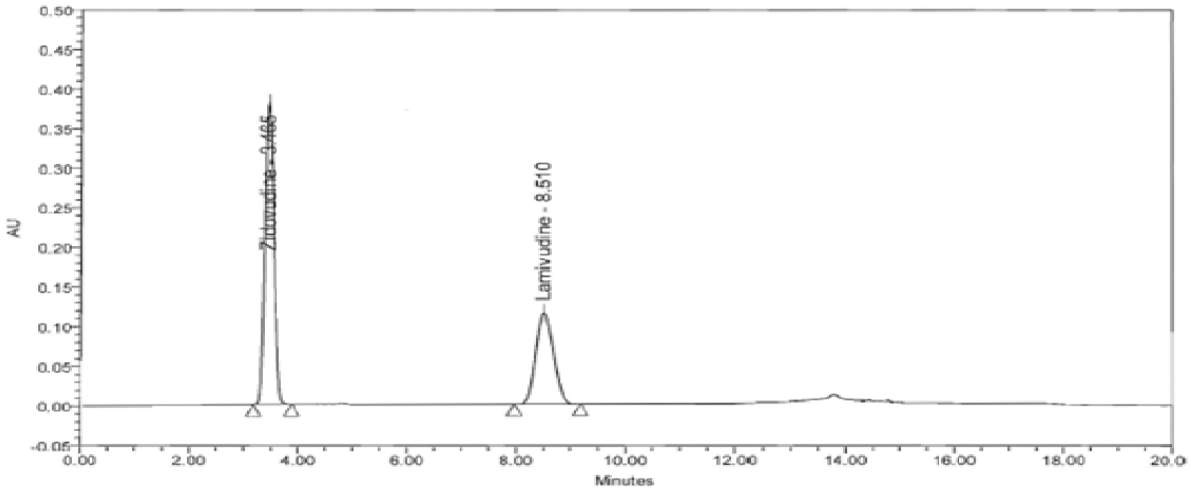

Fig no 1.Chromatogram of Optimized Chromatographic Conditions (trial 7)

Table no 1. Optimized Chromatographic condition

Parameter/ conditions	Description/Values
Column name	Inertsil ODS C_{18} (250×4.6) mm, 5µ
Detector	UV- Detector
Flow rate	1.0 ml/min
Injection volume	10 µl
Wavelength	260 nm
Column Temp.	45ºC
Sample cooler Temp.	25 ºC
Run time	20 min
Buffer	Ammonium acetate buffer pH 3.8
Mobile phase	Buffer 1 : Methanol (60:40%v/v)

Linearity-The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of samples within a given range. A series of standard concentrations were prepared from 50% to 150% of the targeted concentration of lamivudine, Zidovudine. A linearity graph of concentration (µg/ml) versus average area response was plotted for Lamivudine, Zidovudine peaks and the correlation coefficient was calculated. Lamivudine is y=1708X +16068andr2=0.999,Zidovudine y=32822X +12632 r2=0.999. A linear graph of Lamivudine, Zidovudine was obtained between 75 to 225 ppm and 150 to 450ppm respectively.

Table no. 2. Observation Optimized Chromatographic condition

Name	RT (min)	Area (µv*sec)	USP Plate Count	USP Tailing	Purity Threshold	Purity Angle
Lamivudine	8.510	2576984	3206	1.1	0.213	0.023
Zidovudine	3.465	4136663	2270	1.2	0.226	0.025

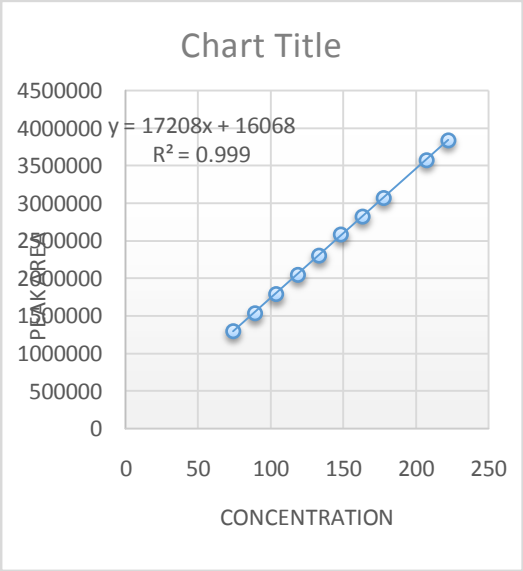

Fig no 2 Linearity Plot of Lamivudine

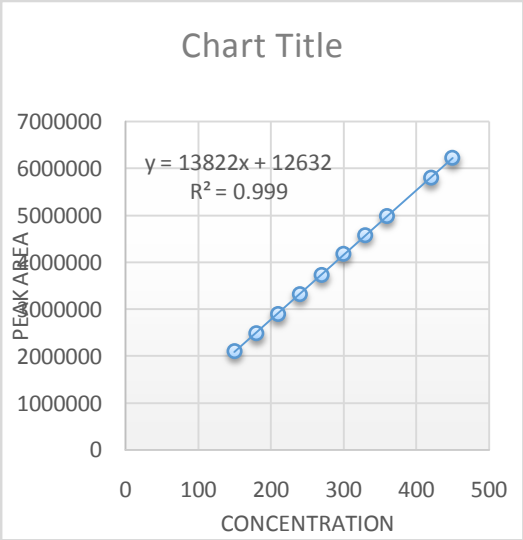

Fig no. 3. Linearity plot of Zidovudine

Accuracy (Recovery):-
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy study was conducted by spiking the known amount of active ingredients into the placebo at three different levels (50%, 100% and 150% of target concentration). The samples were analysed as per the proposed test procedure and the % recovery for each spiked level was calculated. % RSD of Lamivudine is 0.23.and Average recovery is % RSD of Zidovudine is 0.2 andAverage recovery is 100.63 the % RSD at each spike level should be NMT 2.0. The overall % RSD for % recovery for all spike level should be NMT 2.0. The % recovery at each spike level should be NLT 98.0 and NMT 102.0 of the added amount

In result of Lamivudine& Zidovudine in giventable no.2 and 3the samples were analyzed as per the proposed test procedure and the % recovery for each spiked level was calculated. % RSD of Lamivudine is 0.23.and Average recovery is % RSD of Zidovudine is 0.2 andAverage recovery is 100.63 The % RSD at each spike level should be NMT 2.0. The overall % RSD for % recovery for all spike level should be NMT 2.0. The % recovery at each spike level should be NLT 98.0 and NMT 102.0 of the added amount.

Table no 2. Results for Accuracy of Lamivudine

Target Conc. In ppm	Wt. taken	mg Spiked	Area Inj.1	Area Inj.2	Avg. Area	mg recovery	% recovery	Avg. Recovery	% RSD
50	75.10	75.73	1279417	1276337	1277877	74.00	99	99.2	0.3
50	75.10	74.73	1285016	1280660	1282838	74.28	99.4		
100	150.10	149.36	2559448	2559448	2559448	148.16	99.2	99.1	0.2
100	150.00	149.27	2559448	2550205	2554062	147.74	99.0		
150	225.00	223.90	3831334	3836364	3833849	222.00	99.2	99.4	0.2
150	225.00	223.90	3844585	3848891	3846738	222.74	99.5		
							Overall Recovery		0.23

Table no.3 Results for Accuracy of Zidovudine

Target Conc.	Wt. taken	mg Spiked	Area Inj.1	Area Inj.2	Avg. Area	mg recovery	% recovery	Avg. Recovery	% RSD
50	149.9	148.78	2056962	2054815	2055888.5	148.9	100.1	100.3	0.3
50	150	148.88	2067394	2062483	2064938.5	149.6	100.5		
100	100	297.75	4112511	4112049	4112280	297.9	100.1	100	0.1
100	300.2	297.95	4100850	4104480	4102665	297.2	99.8		
150	450	446.63	6171550	6182766	6177158	447.5	100.2	100.4	0.2
150	450.1	446.72	6194279	6202097	6198188	449.0	100.5		
							Overall Recovery	100.63	0.2

In result of Lamivudine & Zidovudine in giventable no.2 and 3the samples were analyzed as per the proposed test procedure and the % recovery for each spiked level was calculated. % RSD of Lamivudine is 0.23.and Average recovery is % RSD of Zidovudine is 0.2 andAverage recovery is 100.63 The % RSD at each spike level should be NMT 2.0. The overall % RSD for % recovery for all spike level should be NMT 2.0. The % recovery at each spike level should be NLT 98.0 and NMT 102.0 of the added amount

Precision:- System precision-Weigh accurately about 10 mg of Lamivudine and Zidovudine 10 mg of working standard into a 100 ml volumetric flask, add 75 ml of mobile phase and sonicated to dissolve, dilute to volume with mobile phase and mix it well. Filter the solution through 0.45µ nylon filter; discard first few ml of filtrate. Take 2 ml of above solution and dilute up to 10 ml in volumetric flask. (Concentration of Lamivudine is about 20µg/ml andZidovudine is about 20µg/ml).

Table no.4 Peak Results for System Precision

Lamivudine			Zidovudine		
Inj.	**RT (min)**	**Area (µV*sec)**	**Inj.**	**RT (min)**	**Area (µV*sec)**
1	8.537	2459166	1	3.478	3992161
2	8.530	2458898	2	3.474	3990845
3	8.534	2447830	3	3.471	4008455
4	8.545	2456131	4	3.478	4001858
5	8.540	2452200	5	3.475	4017468
6	8.441	2552618	6	3.480	4015544
7	8.538	2555432	7	3.478	4004339
8	8.535	2558654	8	3.480	4001858
9	8.533	2556675	9	3.485	4017468
10	8.538	2554467	10	3.478	4015544
Mean		2555496	Mean		2046321
% RSD		0.4	% RSD		0.5

Ten replicate of 20 ppm injections of were injected into the HPLC system. The % RSD for Result of systemprecision study of lamivudine and Zidovudinein given Table no.4ten replicate injections were injected into the HPLC system. For the peak responses of ten replicate injections The % RSD ofLamivudine 0.4 and Zidovudine 0.5.The % RSD for the peak responses of ten replicate injections should be NMT 1.

Method Precision-Weigh accurately about 10 mg of Lamivudine and 10 mg of Zidovudine the peak responses of ten replicate injections should be NMT 1.0.

working standard into a 100 ml volumetric flask, add 75 ml of mobile phase and sonicated to dissolve, dilute to volume with mobile phase and mix it well. Filter the solution through 0.45µ nylon filter; discard first few ml of filtrate. Take 5 ml of above solution and dilute up to 10 ml in volumetric flask. (Concentration of Lamivudine is about 50µg/ml and Zidovudine is about 50µg/ml).

Table no.5 Method Precision Results for Zidovudine

Test No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
RT	3.478	3.480	3.478	3.475	3.471	3.474
Area (Injection 1)	3992161	3990849	4008152	4001858	4017468	4015544
Mean	4004339					
% RSD	0.0065					

Table no.6 Method Precision Results for Lamivudine

Test No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
RT	8.537	8.536	8.520	8.505	8.495	8.498
Area (Injection 1)	2494823	2497668	2502040	2504593	2511236	2510257
Mean		2503436				
% RSD		0.0065				

Result of method precision lamivudine and Zidovudine in given Table no.5,6.a homogenous sample of a single batch of 50 ppm should be analyzed six times. This indicates whether a method is giving his indicates whether a method is giving consistent results for a single batch. The % RSD for the six determinations is 0.0065.The % RSD for the six determinations should be **NMT 2.0**

Table no.7 Robustness study-

Parameter	Modification	% Recovery			
		Area of peak	Lamivudine	Area of peak	Zidovudine
pH	1.8	2558461	98.84	4130267	99.27
	2	2568556	99.23	4115289	98.91
	2.2	2597547	100.35	4105303	98.67
Buffer composition (%)	34	2569332	99.26	4138589	99.47
	35	2568556	99.23	4128603	99.23
	36	2560790	98.93	4156063	99.89
Flow rate(ml/min)	0.9	2554060	98.67	4129851	99.26
	1.0	2568297	99.22	4175202	100.35
	1.1	2562084	98.98	4130267	99.27
Temperature (°C)	43	2574768	99.47	4175202	100.35
	45	2569850	99.28	4129851	99.26
	47	2585640	99.89	4128603	99.23

Table no 8. System suitability parameters

Sr. No	Parameter	Variations			
		pH		Buffer composition	
		1.8	2.2	64%	59 %
1	Theoretical plates	4363	5706	4312	6588
2	Tailing factor	1.15	1.14	1.13	1.14
3	%RSD of Area	1.68	1.05	1.37	1.02
4	%RSD of RT	0.32	0.45	0.18	0.72

Robustness study-RSD of Lamivudine and Zidovudinewere examined and found to be well within the limit of 2.0%. The plate count and asymmetry factor was well within the acceptable USP limits, ensuring that the proposed method was robust and was capable of providing data of acceptable quality.This test is performed by deliberate variations in the HPLC conditions. Results found are within limit and indicate that method is robust.

Results of the robustness study in given table no.9, 10, and 11. Showed that the elution order and resolution for Lamivudine and Zidovudine were not significantly affected. RSD of Lamivudine and Zidovudine were examined and found to be well within the limit of 2.0%. The plate count and asymmetry factor was well within the acceptable USP limits, ensuring that the proposed method was robust and was capable of providing data of acceptable quality. This test is performed by deliberate variations in the HPLC conditions. Results found are within limit and indicate that method is robust

Table no.9 Parameter and Variations

Sr. No	Parameter	Variations			
		3.Flow Rate		4.Temperature	
		0.9ml	1.1ml	43°C	47°C
1	Theoretical plates	5312	4871	5488	4954
2	Tailing factor	1.24	1.18	1.08	1.05
3	%RSD of Area	0.53	1.88	0.74	0.07
4	%RSD of RT	0.54	0.71	0.37	0.18

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Ruggedness:

% RSD between the test result obtained should not be more than 2% for assay method.It is performed by analyzing the stock standard and sample after 12, 18 and 24 hrs.at room temperature along with initially prepared standard at that interval and calculating the % assay, % RSD value and checking for system suitability parameter.

Table no.10 Ruggedness- Solution Stability

Ruggedness- Solution Stability				
Sr. No	**% Assay**			
	Area of peak	**Lamivudine**	**Area of peak**	**Zidovudine**
Initial	2583310	99.8	1462101	100.1
12 hrs Room Temperature	2598841	100.4	1467943	100.5
18 hrs Room Temperature	2585899	99.9	1462101	100.1
24 hrs Room Temperature	2593664	100.2	1457719	99.8

Result of Ruggedness: Solution Stability in given table no.12,In Ruggedness sample after 12, 18 and 24hrs at room temperature along with initially prepared standard at that interval and calculating the % assay and calculated area peak of Lamivudine andZidovudine respectively. On Initial Temperature, area peakof Lamivudine is2583310, on 12 hrs. Room TemperatureArea of peak is 2598841, on18 hrs. Room Temperature area peak is 2585899,24 hrs. Room Temperature area of peak is 2593664.On initial Temperature, area peakof Zidovudine is1462101, on 12 hrs. Room temperature,Area of peak is 1467943, on18 hrs. Room Temperature area peak is,1462101,24 hrs. Room Temperature area of peak is 1457719

METHOD VALIDATION

Accuracy (Recovery)
The accuracy was carried out by adding known amounts of each analyte corresponding to three concentration levels (50, 150, and 250) of the labeled claim to the excipients. The accuracy results were expressed as percent analyte recovered by the proposed method.

Precision
The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement.
System precision
Method Precision

The method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, ruggedness.

Linearity-
Standard solutions of Lamivudine and Zidovudine prepared at different concentrations level i.e. 50ppm, 100ppm, 150ppm, 200ppm, 250ppm was used for this purpose. The peak areas of the chromatograms were plotted against the concentrations ofLamivudine and Zidovudine obtain the calibration curves. These five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients. The dilution preparation from stock solution is shown in the table no 11.

Table no. 11. Preparation for Linearity study

Drug	**Concentration of sample (ppm)**	**Volume taken from stock solution (ml)**	**Adjusted volume of Diluent up to (ml)**
Lamivudine and Zidovudine	50	0.5	10
	100	1	10
	150	1.5	10
	200	2	10
	250	2.5	10

Limit of Detection and Limit of Quantification
The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:LOD Where, σ = the standard deviation of the response S = slope of calibration curve of analyte. The LOD for Lamivudine was found 0.0686µg/ml and Zidovudine0.03391µg/ml µg/ml.The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula:LOQ Where, σ = the standard deviation of the responseS = slope of calibration curve of analyte.The LOQ of Lamivudine 0..2080 µg/ml and Zidovudine 0.1027 µg/ml.

Robustness
It is the measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability under normal usage.

Ruggedness
% RSD between the test result obtained should not be more than 2% for assay method.It is performed by analyzing the stock standard and sample after 12, 18 and 24hrs.at room temperature along with initially prepared standard at that interval and calculating the % assay, % RSD value and checking for system suitability parameter

CONCLUSION

The method described for estimation of Lamivudine and Zidovudine found to be simple, sensitive, accurate, precise, rapid and economical. Hence method could be successfully employed for routine analysis in tablet dosage form.

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DECLERATION OF CONFLICT INTEREST

Author declares no conflict of interest.

Table no 12. Calculation of LOQ and LOD

	Limit of Detection		**Limit of Quantification**	
Zidovudine	**LOD = 3.3 × SD /S**	**0.03391µg/ml**	**LOQ = 10×SD/S**	**0.1027 µg/ml**
	3.3*13822/1344978.7		**10*13822/1344978.7**	
Lamivudine	**LOD = 3.3 × SD /S**	**0.0686µg/ml**	**LOQ = 10×SD/S**	**0.2080 µg/ml**
	3.3*17208/827893.9		**10*17208/**827893.9	

REFERENCE

1) Mendum J, Denny R.C, and Thomas M.N. Vogel's Text book of Quantitative Analysis, 6th Edition. Pearson education ltd., 2004.
2) Beckett A.H. and Stenlake J.B. Practical Pharmaceutical Chemistry, 4thEdition, Part 2, CBS Publishers and Distributors, 2002, pp. 227-237.
3) NaserL.Rezk, et al., studied on Simultaneous determination of six HIV nucleoside analogue reverse transcriptase inhibitors and nevirapine by liquid chromatography with ultraviolet absorbance detection.
4) GeethaRamachandran, A.K. Hemanth Kumar, SoumyaSwaminathan, P.Venkatesan, V. Kumaraswami and David J. Greenblatt. Simple and rapid liquidchromatography method for determination of efavirenz in plasma. Journal of chromatography B. 835(1-2): 131-135 (2006)
5) Dailly E., Thomas L., Kergueris M. F., Jolliet P.andBourin M., High-performance liquidchromatographic assay to determine the plasmalevels of HIV-protease inhibitors (amprenavir,indinavir, nelfinavir, ritonavir and saquinavir) and the non-nucleoside reverse transcriptase inhibitorNevirapine) after liquid-liquid extraction, J.Chromatogr. B. Biomed. Sci. and Appl., 2001,758, 129-135.
6) Summer R. Lewis, Catherine A. White, Michael G. Bartlett Simultaneous determination of Abacavir and Zidovudine from rattissue using HPLC with UV detection, Journal of Chromatography B. 2007; 850: 45-52.