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RAPID SEPARATION AND ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE IN MARKETED FORMULATIONS USING RP-HPLC METHOD AND ITS VALIDATION

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ABSTRACT

The goal of the present investigation was carried out to develop a validated analytical RP-HPLC method for simultaneous separation and estimation of Lamivudine and Zidovudine for pharmaceutical formulations. The chromatographic separation was accomplished on Welchrom RP-C_{18} Column (250 mm X 4.6 mm; 5µm), Shimadzu LC-20AT Prominence Liquid Chromatograph and with a mixture of 10 mM Phosphate buffer (pH 3.6): acetonitrile (50:50, v/v). The flow rate was fixed at 1.2 mL/minute and the analysis was performed using Shimadzu SPD-20A Prominence UV-Visible detector at 241 nm. The Lamivudine and Zidovudine were separated within three minutes. The retention time for Lamivudine and Zidovudine was found to be 2.393 minutes and 2.7 minutes respectively. The calibration plots were linear over the concentration range of 2-10 µg/mL for Lamivudine (r^2=0.9995) and 4-20 µg/mL for Zidovudine (r^2=0.9991). There was no interference due to commonly used excipients. The % RSD for both the drugs were calculated and showed less than 2% which obviously indicates that the present method was said to be highly precise. Regarding accuracy of the developed method the %RSD were also found less than 2% which shows the method is completely accurate. The method was very sensitive with regard to limit of detection and limit of quantitation for Lamivudine and Zidovudine were found to be 0.181 µg/mL, 0.576 µg/mL and 0.551 µg/mL, 1.747µg/mL respectively. The mean assay values for Lamivudine and Zidovudine were arrived at 98.88% and 99.70 % respectively. The developed RP HPLC method was found to be simple, rapid, sensitive, highly precise and accurate and can be successfully employed for simultaneous estimation of Lamivudine and Zidovudine in pharmaceutical formulations.

Keywords: RP HPLC Method, Simultaneous estimation, Lamivudine, Zidovudine.

INTRODUCTION

Lamivudine (LAM) and Zidovudine (ZID) are currently among the most widely prescribed anti retroviral drugs in hospitals. They are official in IP and USP. LAM and ZID combination has significant therapeutic importance. The combination of at least three drugs treatment is known as highly active antiretroviral therapy (HAART). Using a HAART protocol, HIV replication is stopped, the presence of HIV-RNA in the plasma is decreased to undetectable levels and patient survival is significantly increased.

LAM is utilized in the treatment of Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). Lamivudine is chemically (2R,cis)-4- amino-l-(2-hydroxymethyl-l, 3-oxathiolan-5-yl)-(l H)-pyrimidin-2-one with the molecular formula $C_8H_{11}N_3O_3S$ and a molecular weight of 229.256 g/mol. LAM is soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol. ZID is a potent inhibitor of HIV replication, acting as a chain-terminator of viral DNA during reverse transcription. It ameliorates immunologic function, partially reverses the HIV-produced neurological dysfunction, and enhances certain other clinical abnormalities related with AIDS. Zidovudine is chemically1-(3-azide-2, 3-di de oxy-β-D-ribofuranosyl)-5-

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methyl Pyrimidin-2,4(1H, 3H) –dione with the molecular formula $C_{10}H_{13}N_5O_4S$ and with a molecular weight of 267.25g/mol. ZID is soluble in water, alcohol, acetone, ethanol and sparingly soluble in denatured alcohol.

Zidolam Tablet consists of LAM (150 mg) and ZID (300 mg). Zidolam Tablets are used in antiretroviral combination therapy for the treatment of HIV infection. Zidolam Tablet reduces the amount of HIV in the body and keeps it at a low level. It also increases CD4 cell counts. CD4 cells are a type of white blood cells that plays prominent role in maintaining a healthy immune system to fight against infection.

Literature survey revealed that there were few analytical methods have been reported for the simultaneous estimation of above titled drugs individually, tertiary or in combination with some other drugs in biological samples as well as pharmaceutical dosage forms by spectrophotometry [1-7], HPLC [8-18], LC-MS [19]. However most of the available methods have limitations such as long run time, poor resolution, uneconomical and low sensitivity. So based on the above mentioned reasons, infact an attempt has been made to develop a simple, precise, accurate, reproducible and robust RP-HPLC method for the simultaneous determination of LAM and ZID in pharmaceutical dosage form.

EXPERIMENTAL

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutically pure samples of LAM and ZID were obtained as a gift sample from Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid purchased from Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) purchased from Merck Pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid used was of HPLC grade and obtained from Merck Specialties Private Ltd., Mumbai, India. Commercial formulation (ZIDOLAM Tablets) were procured from local drug market. ZIDOLAM Tablets contain 150 mg of Lamivudine and 300 mg of Zidovudine manufactured by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India.

Instrument Specifications

Quantitative HPLC was performed on a isocratic high performance liquid chromatograph (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250mm, 5µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were used in this present study.

Chromatographic Conditions

LAM and ZID were analyzed by various reversed phase columns like C_8 and C_{18} columns. Among C_8 and C_{18} columns, C_{18} (250mmX4.6mm, 5µm) column was selected. Various combinations of acetonitrile, phosphate buffer and methanol with triethylamine as column modifier were tested. The mixture of 10 mM Phosphate buffer (pH adjusted to 3.6 using triethylamine) and Acetonitrile in ratio of 50:50 v/v was selected as mobile phase and UV detection wavelength of 241 nm with a flow rate of 1.2 mL/min. Injection volume was 20 µL, with ambient temperature, run time was 4 minutes and retention time was 2.393 minutes for LAM and 2.7 minutes for ZID.

Preparation of Reagents and Standards

a. Mobile phase

A 10 mM Phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water. To this 55 mL of 0.1M phosphoric acid was added and pH was adjusted to 3.6 with triethylamine. The above prepared buffer and acetonitrile were mixed in the proportion of 50:50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

b. Preparation of Standard Stock Solution

LAM (10 mg) and ZID (20 mg) drug were accurately weighed and transferred into a 10 ml clean, dry volumetric flask and about 5ml of diluent was added and sonicated for about 10 minutes to dissolve the drugs completely and made up to the mark with the mobile phase to obtain final concentration of 1000 µg/mL of each LAM and ZID (standard stock solutions A_1 and A_2 respectively). From the above stock solution A_1 and A_2, 1 mL aliquots were pipette in to two separate volumetric flasks and dissolved in 5mL of solvent and made up to the mark with CH_3CN and phosphate buffer (50:50) to obtain a final concentration of 100 µg/mL.(Standard stock solutions B_1 and B_2 respectively). The standard solution was filtered through 0.45 µm nylon membrane filter and degassed by sonicator.

c. Working standard solution

Lamivudine

From the above standard stock solutionB_1 appropriate aliquots ranging from 0.2 mL to 1 mL (1mL=100 µg/mL) was pipette out in to a series of 10 mL volumetric flasks. The volume was made up to the mark with diluent to obtain a concentration range, ranging from 2-10 µg/mL (2, 4, 6, 8, 10 µg/mL).

Zidovudine

From the above standard stock solution B_2,appropriate aliquots ranging from 0.4 mL to 2 mL (1mL=100 µg/mL) was pipette out in to a series of 10 mL volumetric flasks. The volume was made up to the mark with diluent to obtain a concentration range, ranging from 4-20 µg/mL (4, 8, 12, 16, 20 µg/mL).

d. Preparation of Sample solution

Sample was prepared by selecting twenty Tablets randomly, weighed and finely powdered. The average weight of the Tablets was determined from the weight of twenty Tablets. From the prepared sample, a portion of powder equivalent to the weight of one Tablet was accurately transferred into 200 ml volumetric flask and 20 ml diluent was added to it. The volumetric flask was sonicated for 15 minutes with intermittent shaking for complete dissolution. The solution was then made up to volume with diluent. The resulting solution was then filtered through 0.45 µm membrane filter. First 2 mL of the filtrate was discarded and then 5 ml of the filtrate was diluted to 25 ml with diluent. Further 5 ml of the resulting solution was diluted to 50 ml with diluent and mixed. Finally, 20 microlitres of the prepared test solution was injected and chromatogram was recorded for the same, and the amounts of the drugs were calculated.

e. Selection of Analytical wavelength

The superimposed UV spectra of various diluted solutions of LAM and ZID in mobile phase were taken into account by using UV spectrophotometer. The isobestic point of maximum absorbance was observed at 241nm and this wavelength was observed for detection of Lamivudine and Zidovudinewhich is detailed in Figure 3.

Optimization of mobile phase and Method development

The mobile phase and stationary phase play a prominent role on resolution, peak shape, theoretical plates, and asymmetry. To attain symmetrical peaks with decorous resolution, various chromatographic conditions was investigated and optimized for the estimation of LAM and ZID, such as mobile phase with different composition, pH, and various stationary phases. For obtaining appropriate mobile phase for the analysis, various mixtures of acetonitrile, methanol HPLC grade water, phosphate buffer were tested with different compositions at various pH (3.6,6.8) and 10 mM, 20 mM, 30 mM concentrations of phosphate buffer was added to enhance polarity of the mobile phase. Eventually it was demonstrated that highly symmetrical, sharp peaks, good resolution of LAM and ZID peaks were obtained in a short time when utilized Welchrom C_{18} column with a mixture of 10 mM Phosphate buffer (pH 3.6): acetonitrile (50:50, v/v) with flow rate of 1.2 mL/minute at the analytical wavelength 241nm.

METHOD VALIDATION

The developed method of analysis was validated as per the ICH Q2 (R1) guidelines [20] for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection and limit of quantitation.

System suitability

System suitability tests are an integral part of chromatographic system. To determine its effectiveness, certain system suitability test parameters (resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count (NLT 3000)) were checked by repetitively injecting the drug sample solution of LAM and ZID to check the reproducibility of the system. Initially the HPLC system was stabilized for forty minutes. One blank followed by six replicates of a single sample solution of LAM and ZID was injected to check the system suitability. The % RSD of the peak areas and retention time for the LAM and ZID are within the limits of less than 2% which shows the system suitability. The results for system suitability results are tabulated in Table 1.

Specificity

Specificity of the method is performed by separate injections of LAM and ZID standard and sample. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc., Specificity of the method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the Tablet formulation and also that there is good correlation between the retention times of standard and sample. The specificity results are tabulated in Table 2.

Linearity

Aliquots of primary working standard solution consisting LAM and ZID were diluted in such a way to get the eventual concentrations of LAM and ZID in the range of 2-10µg/mL and 4-20 µg/mL respectively. The linearity graphs for the proposed assay methods were plotted over the concentration range. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values. A calibration curve was plotted between concentration and peak area response and statistical analysis of the calibration curve was performed. Results are represented in Table 3 and Table 4.

Accuracy (Recovery studies)

Accuracy of an analytical procedure is the closeness of agreement between the conventional true value or an accepted reference value and the value found. The accuracy was determined by adding a known amount of standard drug to the fixed amount of pre-analyzed

Tablet solution. Accuracy studies were performed for LAM and ZID at three different levels (80%, 100% and 120%) and the mixtures were analyzed in triplicate by the proposed method. Known amount of standard LAM and ZID 80%, 100% and 120% of predetermined sample was added to a pre quantified Tablet sample. The % recovery was calculated and the results are presented in Table 5.

Precision

Precision of an analytical procedure is the closeness of agreement (Degree of scatter) between a series of measurements obtained from multiple sampling of the sample homogeneous sample under the prescribed conditions. Precision was estimated by intra-day and inter-day study and carefully evaluated by carrying out the assay and analyze corresponding responses 6 times on the same day and on different days for the sample solution. The percent relative standard deviation (% RSD) was calculated. This is within the acceptable criteria of not more than 2.0. The values of percentage of RSD obtained in intra and inter day precision results are presented in Table 6.

Robustness

The robustness of the developed method was determined by analyzing the samples under a variety of conditions of the method parameters such as variation of the pH of the buffer, flow rate, detection wavelength and mobile phase composition. It was observed that there were no significant effect on chromatographic parameters which demonstrated that the developed method was robust in nature. The complete results are shown in Table 7.

Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. Results are represented in Table 8.

Limit of detection and Limit of quantitation

The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.LOD and LOQ were calculated by formula LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= slope of the calibration curve. The LOD and LOQ of LAM and ZID by proposed method are abridged in Table 9.

Analysis of marketed formulation

The proposed validated method was successfully applied to determine the LAM and ZID in their Tablet dosage form. 20 µL of sample solution was injected into Liquid Chromatograph. The assay was repeated for six times and the amount of the drug present per Tablet was estimated from calibration equation. The mean % recovery was determined.

Table 1. Optimized chromatographic conditions and system suitability parameters

Parameter	**Chromatographic conditions**	
Instrument	SHIMADZU LC-20AT Prominence liquid chromatograph	
Column	WELCHROM C_{18} Column (4.6 X 250mm, 5µm)	
Detector	SHIMADZU SPD-20A Prominence UV-Vis detector	
Diluents	10mM Phosphate Buffer(pH 3.3) : Acetonitrile (50:50, v/v)	
Mobile phase	10mM Phosphate Buffer(pH 3.3) : Acetonitrile (50:50, v/v)	
Flow rate	1.2mL/min.	
Detection wave length	UV at 241nm.	
Run time	4 minutes	
Column back pressure	103-109 kgf	
Temperature	Ambient temperature (25 ^{o}C)	
Injection Volume	20µL	
	Lamivudine	**Zidovudine**
Retention time (t_R)	2.393minutes.	2.7 minutes.
Theoretical plates[th.pl] (Efficiency)	7933	9087
Resolution	-	2.753
Tailing factor (asymmetry)	1.119	1.137

Table 2. Specificity study

Name of the solution	**Retention time, (t_R)min.**
Mobile phase	No peaks

Placebo	No peaks
Solution containing a concentration of LAM, 10µg/mL and ZID, 20µg/mL.	Peaks at 2.397 min and 2.7 min for LAM and ZID respectively.

Table 3. Calibration data

	Lamivudine		Zidovudine	
S.No.	**Concentration, µg/mL**	**Peak area, mV.s.**	**Concentration, µg/ML**	**Peak area, mV.s.**
1.	0	0	0	0
2.	2	148.656	4	59.741
3.	4	281.764	8	123.309
4.	6	425.67	12	186.512
5.	8	577.929	16	251.228
6.	10	709.392	20	302.94

Table 4. Linear regression data

Parameter	Lamivudine	Zidovudine
Detection wavelength(λ_{max})	277nm	266 nm
Linearity range (µg/mL)	2-10 µg/mL	4-20 µg/mL
Regression equation (Y = aX + b)	Y = 71.12x + 1.614	Y = 15.37x + 0.214
Slope(a)	71.12	15.37
Intercept(b)	1.614	0.214
Standard error of slope (S_a)	71.066	15.374
Standard error of intercept (S_b)	1.900	0.214
Standard error of estimation (S_y)	4.485	3.711
Regression coefficient (R^2)	0.9995	0.9991
% Relative standard deviation* i.e., Coefficient of variation(CV)	0.608	0.219
Percentage range of errors (Confidence limits) 0.05 significance level 0.01 significance level	0.123063 0.161733	0.485722 0.638413

*Average of 6 determinations; acceptance criteria < 2.0.

Table 5. Accuracy Study

S. NO	Recovery Level	Lamivudine					Zidovudine				
		Amount added (µg/mL)		Amount found (µg/mL)	% Recovery	% RSD	Amount added (µg/mL)		Amount found	% Recovery	% RSD
		Std.	Test				Std.	Test			
1	80%	8	5	12.97	99.76	0.5994	8	5	12.95	99.61	0.6007
2		8	5	13.02	100.15		8	5	13.09	100.69	
3		8	5	12.98	99.84		8	5	12.96	99.69	
4	100%	10	5	15.05	100.33	0.7087	10	5	15.13	100.8	0.8704
5		10	5	14.98	99.86		10	5	14.98	99.86	
6		10	5	14.97	99.8		10	5	14.87	99.13	
7	120%	12	5	16.96	99.76	0.4507	12	5	17.05	100.29	0.4764
8		12	5	16.95	99.70		12	5	16.95	99.70	
9		12	5	17.01	100.05		12	5	16.89	99.35	

#average of triplicate injections

Table 6. Precision study

Drug name	Precision	Intra day	Inter day		
			Day 1	Day 2	Day 3
Lamivudine	Mean % recovery	425.83	424.38	422.67	420.92
	SD	0.1538	0.8935	0.6759	0.6262
	%RSD	0.0361	0.2105	0.1599	0.1487
Zidovudine	Mean % recovery	185.67	183.22	180.28	177.85
	SD	0.6071	1.0208	0.6683	0.9057
	%RSD	0.3269	0.5571	0.3707	0.5092

Table 7. Robustness data

S.NO	Parameter	Used	Retention time (t_R), min.		Plate count[$]		Peak asymmetry[#]		Remarks
			LAM	ZID	LAM	ZID	LAM	ZID	
1	Flow rate (±0.2 mL/min)	1.0 mL/min	2.405	2.729	7939	9091	1.119	1.136	Robust
		1.2 mL/min	2.393	2.700	7933	9087	1.119	1.137	Robust
		1.4 mL/min	2.380	2.692	7927	9079	1.118	1.137	Robust
2	Detection wavelength (±5 nm)	236 nm	2.393	2.700	7933	9087	1.118	1.137	Robust
		241 nm	2.393	2.700	7933	9087	1.119	1.137	Robust
		246 nm	2.393	2.700	7934	9086	1.119	1.136	Robust
3	Mobile phase composition (±2 % v/v)	52:48, %v/v	2.398	2.714	7947	9095	1.117	1.136	Robust
		50:50, %v/v	2.393	2.700	7933	9087	1.119	1.137	Robust
		48:52, %v/v	2.390	2.690	7921	9079	1.118	1.138	Robust

Acceptance criteria (Limits):
[#]Peak Asymmetry < 1.5, [$] Plate count > 3000, *significant change in Retention time.

Table 8.Ruggedness

S.NO	Conditions	Lamivudine		Zidovudine	
		Retention time	Peak area	Retention time	Peak area
1	Instrument - 1	2.397	709.392	2.700	302.94
2	Instrument - 2	2.381	711.426	2.717	305.641
3	Analyst - 1	2.397	709.32	2.700	302.946
4	Analyst - 2	2.399	706.274	2.711	304.251

Table 9. LOD & LOQ

S.No	Parameter	Result	
		Lamivudine	Zidovudine
1	Limit of Detection(µg/ml)	0.181	0.576
2	Limit of Quantitation (µg/ml)	0.551	1.747

Table 10. Assay results

S.no	Formulation	Labeled amount		Mean ± SD		%Assay		% RSD	
		LAM	ZID	LAM	ZID	LAM	ZID	LAM	ZID
1	**Zidolam Tablets**	150mg	300mg	148.32 **± 0.9**	299.12 **±0.65**	98.88%	99.70%	0.608	0.219

Figure 1. Chemical structure of Lamivudine

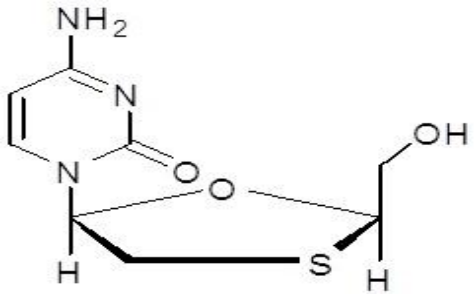


Figure 2. Chemical structure of Zidovudine

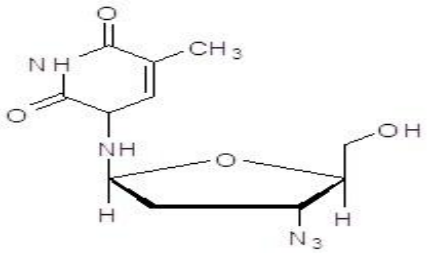


Figure 3. Overlain UV Spectra of Lamivudine and Zidovudine

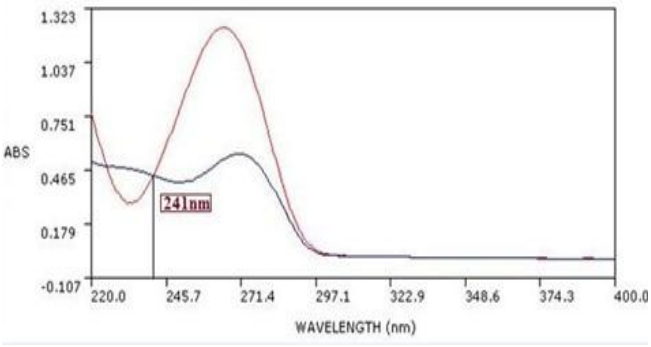


Figure 4. Calibration plot of Lamivudine

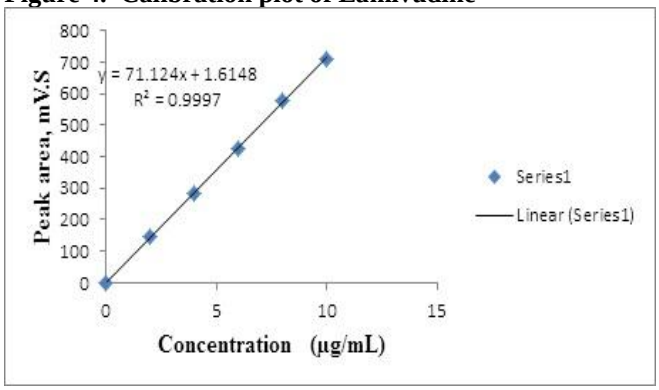


Figure 5. Calibration plot of Zidovudine

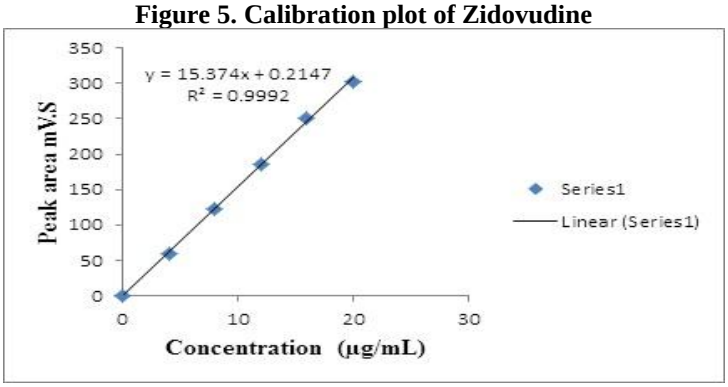


Figure 6. Chromatogram of blank solution

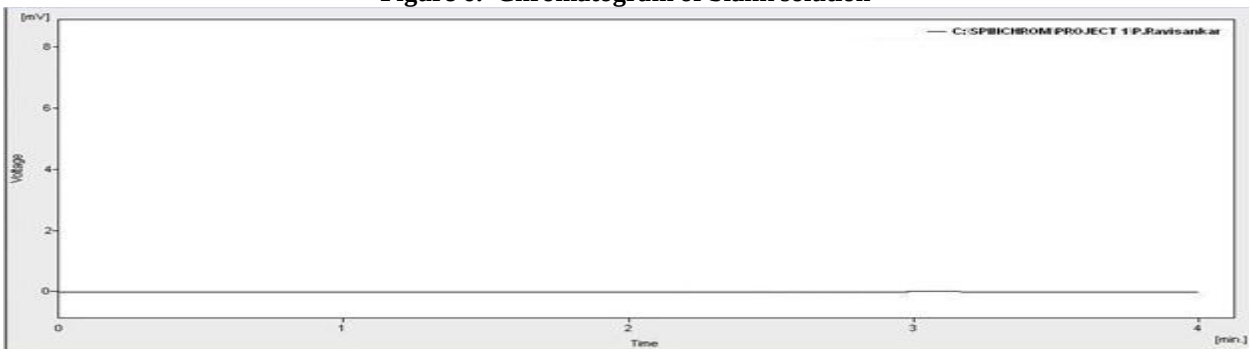


Figure 7. Standard chromatogram of Lamivudine

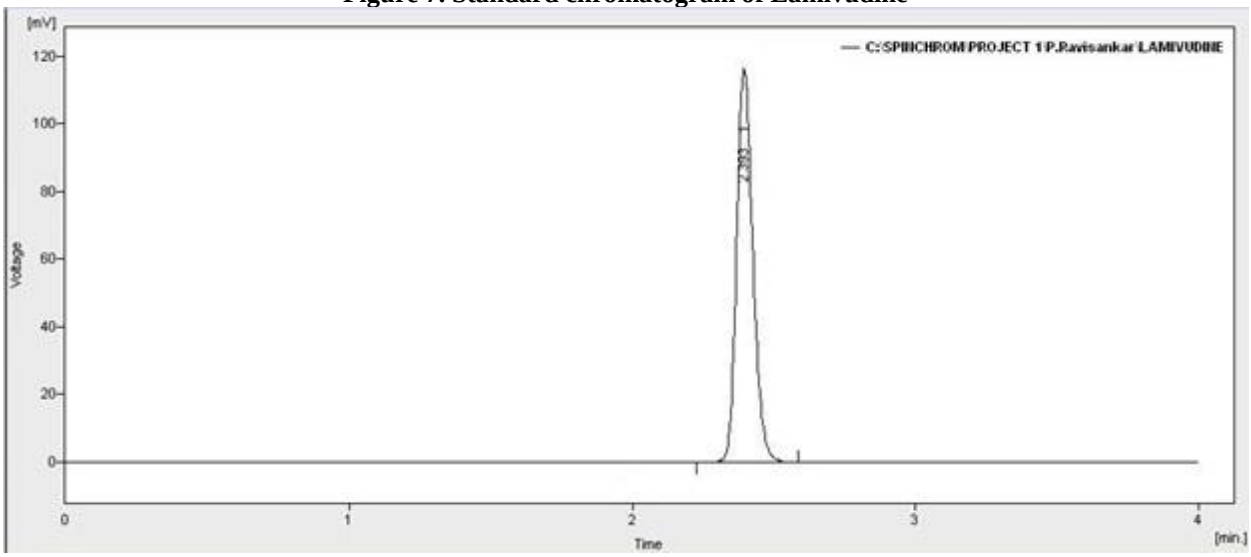


Figure 8. Standard chromatogram of Zidovudine

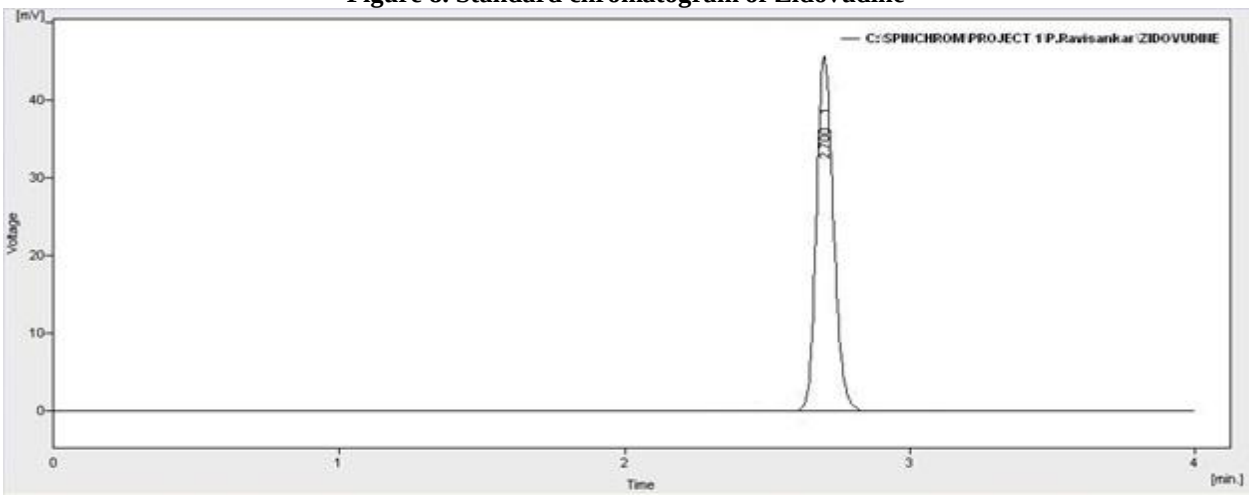


Figure 9.Chromatogram of LAM and ZID sample drug

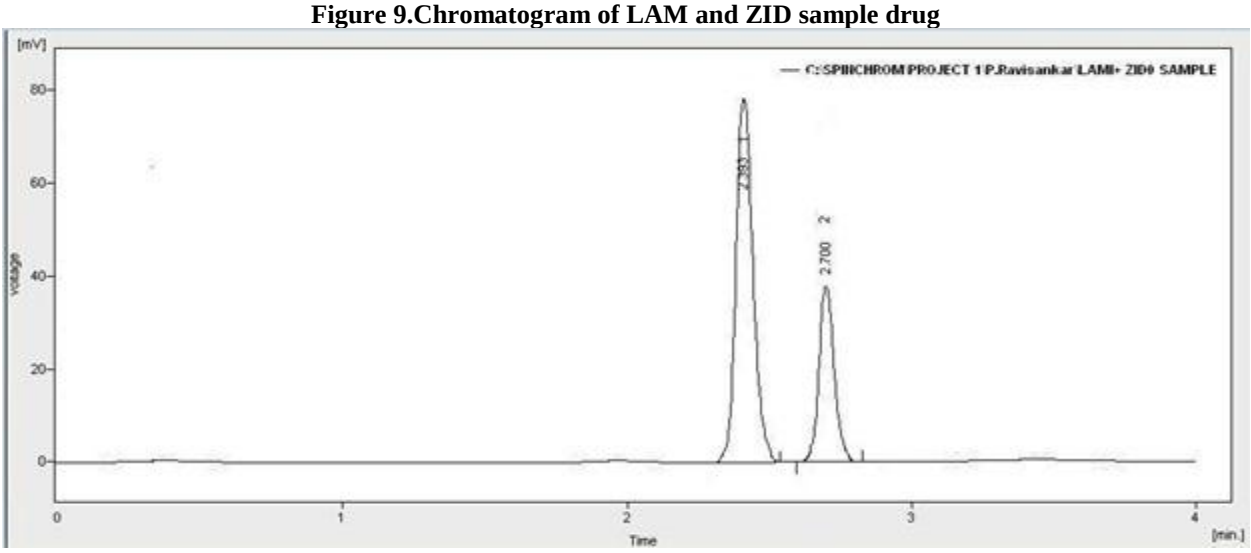


Figure 10. Standard chromatogram of LAM (2 µg/mL) and ZID (4 µg/mL)

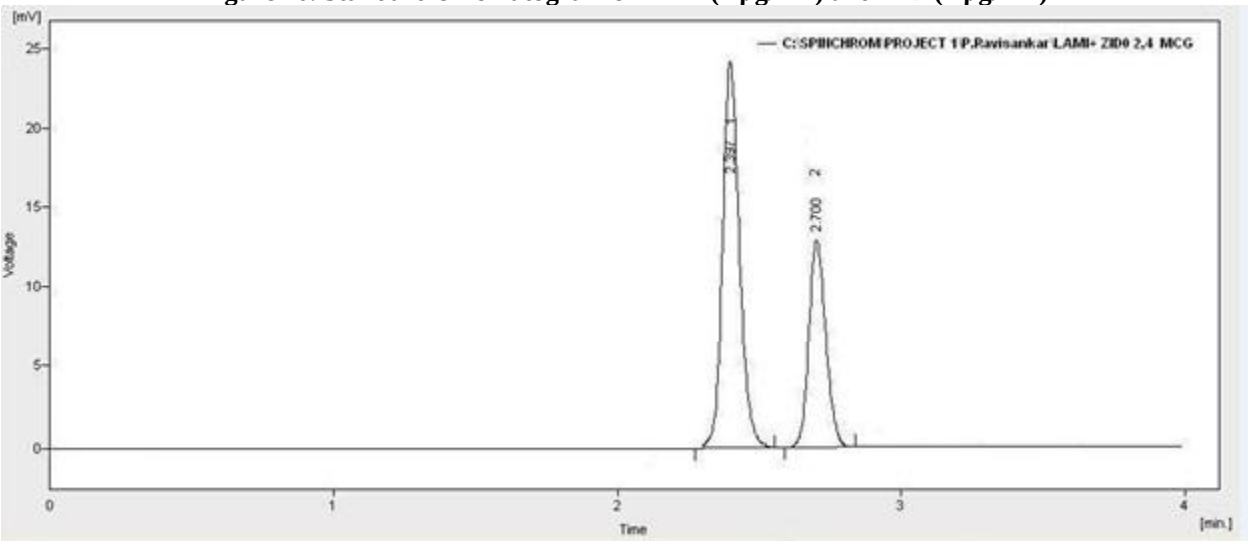


Figure 11. Standard chromatogram of LAM (4 µg/mL) and ZID (8 µg/mL)

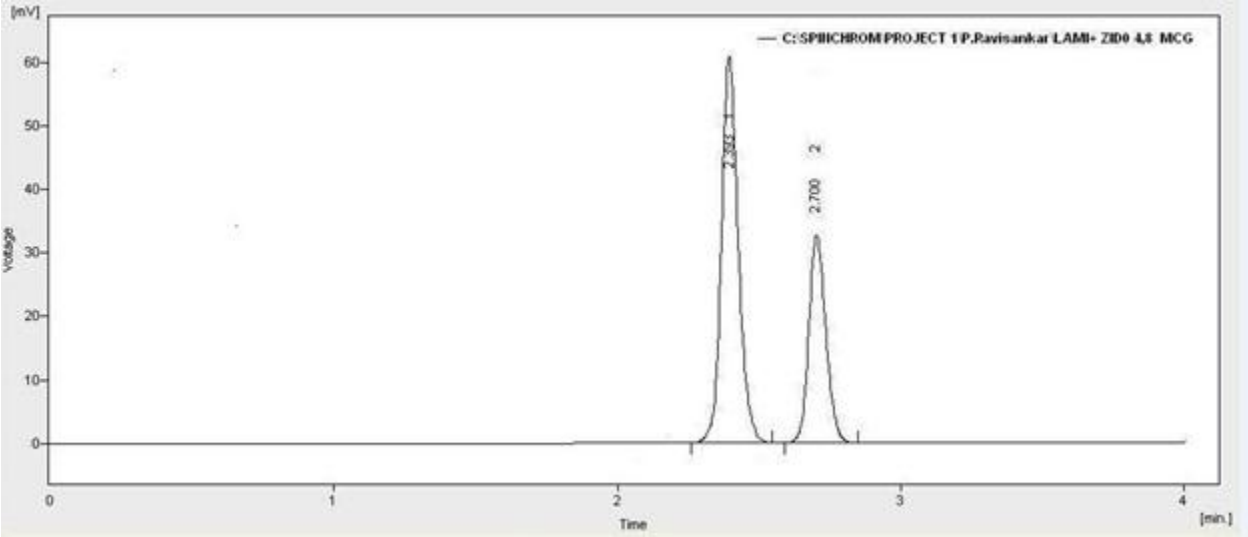


Figure 12. Standard chromatogram of LAM (6µg/mL) and ZID (12 µg/mL)

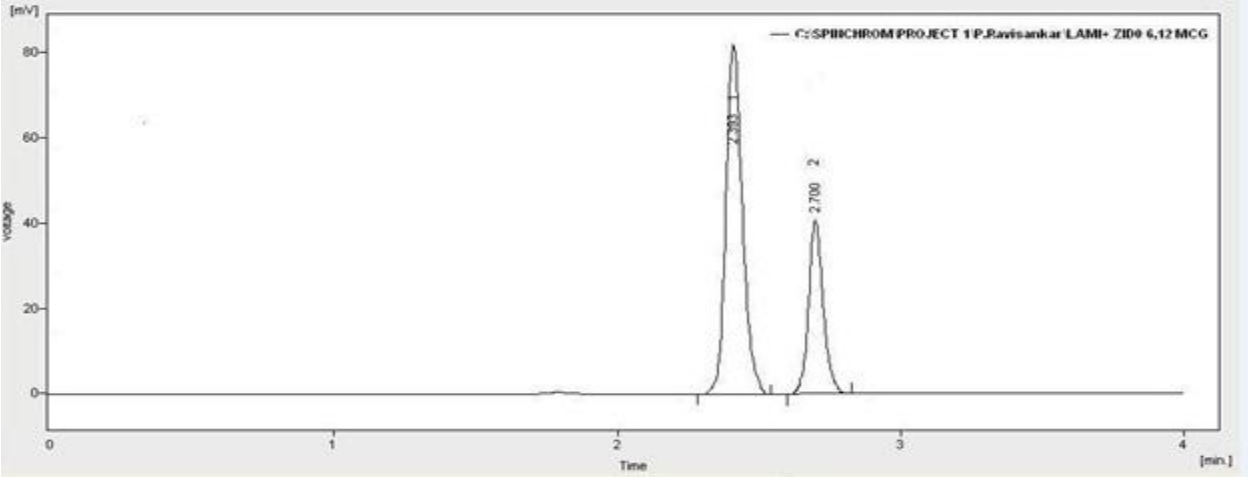


Figure 13. Standard chromatogram of LAM (8 µg/mL) and ZID (16 µg/mL)

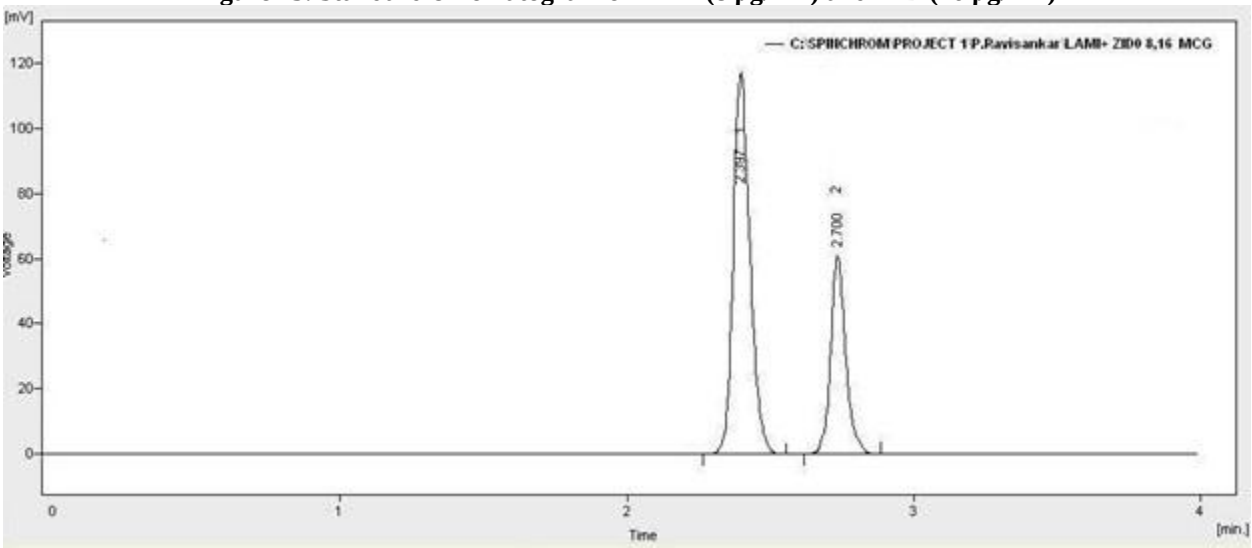
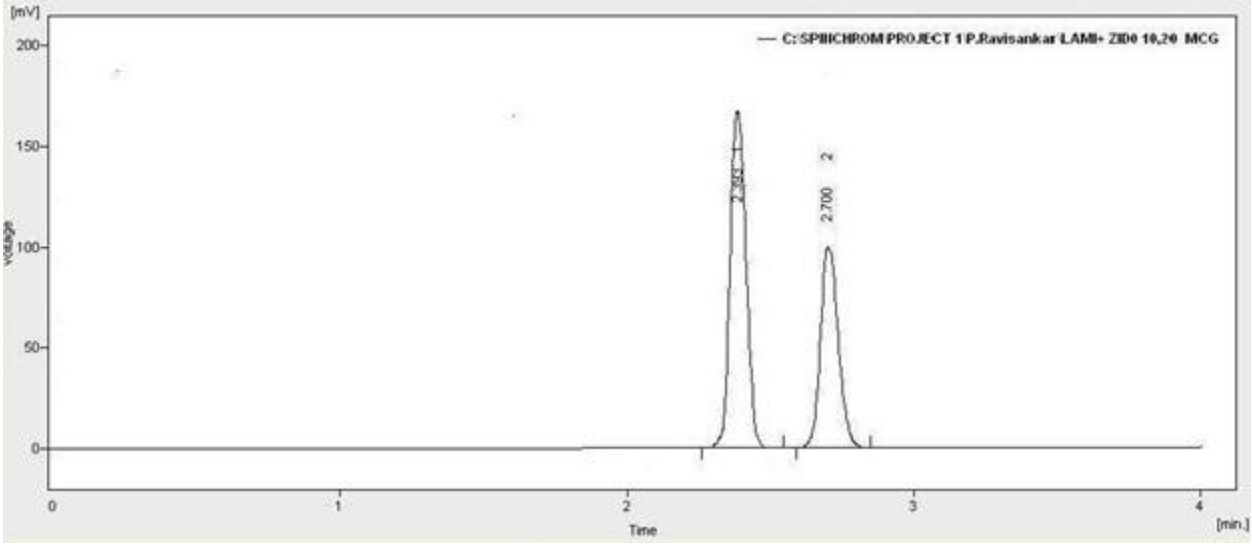


Fig 14. Standard chromatogram of LAM (10 µg/mL) and ZID (20 µg/mL)



RESULTS AND DISCUSSION

The method was developed with a RP-HPLC Welchrom C_{18} column with a 4.6 mm inner diameter, 250 mm length and 5 micron particle size was preferred and the best chromatographic separation was attained by adjusting chromatographic conditions like mobile phase and detection wavelengths. In order to attain the desired system suitability criteria and chromatographic conditions various parameters such as flow rate, detector wavelength, different modifications of mobile phase composition, column dimension, particle size, injection volume were adjusted before to the development of this method to attain the best resolution and peak shapes and to minimize run time analysis. In the present work, after some trials, it was found that the mixture of phosphate buffer (pH-3.6) and acetonitrile in a composition of 50:50, %v/v as mobile phase resulted in symmetric peak at 241nm in short runtime. The pH of buffer was corrected to 3.6 using triethylamine. Different column types and lengths were tried regarding other chromatographic parameters. UV overlain spectra of these drugs showed that these drugs absorbed appreciably at 241 nm (Figure 3), so that this wavelength was chosen as the detection wave length. Flow rate used was set to 1.2 mL/minute. Chromatograms showed a peak of LAM at retention time of 2.393 min and peak of ZID at retention time of 2.7 min respectively. The specificity was determined to test the interference of commonly used excipients. The comparison of standard and blank chromatograms indicates no co-eluting peak between the two main peaks in the chromatograms as well as it was decorously resolved, well shaped peaks also indicates the specificity of the method. Therefore, it was concluded that the method is specific. The specificity

results are summarized in Table 2. All the system suitability parameters were evaluated. Since there are no marked changes in the system suitability parameters, the method was capable to remain unaffected by small variation of tested variables for robustness study. Bench top solution stability was conducted to the working solutions of the drugs and the results suggest that the solutions are Table up to 48 hours in the routine and operational and environmental conditions. The calibration curve was obtained for a series of concentration in the range of 2-10 µg/mL for LAM and 4 - 20 µg/mL for ZID respectively and it was found to be linear. The calibration data is shown in Table 3. The regression data analysis of LAM and ZID is presented in Table 4. The regression equation obtained from linearity plot for LAM was Y = 71.12x + 1.614 with R^2=0.9995and for ZID was Y = 15.37x + 0.214 with R^2=0.9991 which shows that this method had good linearity. The calibration graphs of LAM and ZID are shown in Figure 4 and Figure 5 respectively. Figure 6 represents the chromatograph of the blank solution and the standard individual chromatograms of LAM and ZID are shown in Figure 7and Figure 8. The sample chromatogram of LAM and ZID is shown in Figure 9. The standard chromatograms of LAM and ZID are shown in Figures 10 to Figures 14. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e. multiple level recovery studies. A known amount of standard drug was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in Table 5. Generally the mean percentage recovery of LAM at each level was not less than 99.7% and not more than 100.33%, for ZID the mean percentage recovery was not less than 99.13% and not more than 100.8%. Precision was studied to find out intra and inter day variations in the test methods of LAM and ZID for the six times on the same day and on different days. The values of % RSD (< 2.0) indicate that the proposed method is quite precise and reproducible and results are shown in Tables 6. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition etc.; It was observed that there were no marked changes in the chromatograms. Infact the parameters are within the limit which indicates that the method has robustness and suitable for routine use. The Robustness results are presented in Table 7.Ruggedness was performed by two different analysts and two instruments under same experimental condition. The % RSD was calculated. The results were reported to be within the limits. The results are tabulated in Table 8.The calculated LOD and LOQ are found to be 0.181 µg/mL and 0.551 µg/mL for LAM and 0.576 µg/mL and 1.746 µg/mL for ZID showed that the method is specific and sensitive to estimate these drugs at low concentration level. The results of LOD and LOQ are shown in Table 9.Eventually the proposed validated method was eventually applied for quantitative determination of the marketed formulation ZIDOLAMTablets. 20 µL of sample solution was injected into liquid chromatograph and chromatogram was recorded and the mean assay value was found to be 99.88 ± 0.9 % for LAM and 99.70 ± 0.65 % for ZID. Satisfactory results were achieved. The mean % found for all two drugs were in good agreement with the label claim and results are presented in Table 10.

CONCLUSION

The present proposed research study by the author describes the estimation of LAM and ZID available as combination Tablet dosage forms and was carried out by utilizing RP-HPLC. The linearity of the proposed method was in the range of 2-10 µg/mL for LAM and 4-20 µg/mL for ZID respectively. The LOD and LOQ of LAM were 0.181µg/mL and 0.551µg/mL and for the estimation of ZID were 0.576 µg/mL and 1.747 µg/mL respectively. The total run time of the above titled anti retroviral agents were four minutes with an elution window of one minute.

The developed method has several advantages like decorous linearity, decrease the time needed for analysis and low flow rate used in this method consumes less solvent consumption, improved resolution as compared with the previous methods which makes the method more economical than the existing methods in practice. The developed RP-HPLC method for the quantification of LAM and ZID was found to be simple, specific, highly sensitive, fast, economical, precise and extremely accurate with robustness. Therefore this method can be recommended for the routine analysis of LAM and ZID in quality control and clinical laboratories.

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