IJPAR |Volume 2 | Issue 3 | July-Sep - 2013 **ISSN: 2320-2831**

INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

Available Online at: www.ijpar.com

[Research article]

Analytical Method Development and Validation for Simultaneous Estimation of Lamivudine and Zidovudine in Tablet Dosage form by RP-HPLC

***M.Sravani, K.Haritha Pavani.**

Department of Pharmaceutical Analysis, Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawada, A.P, India.

ABSTRACT: A new, precise, rapid, accurate RP-HPLC method was developed for the Simultaneous Estimation of Lamivudine and Zidovudine in pharmaceutical dosage forms. Chromatographic separation was achieved with mobile phase consisting of Ammonium acetate buffer pH 4.0, Acetonitrile and THF in the ratio of 60:30:10 v/v as the mobile phase with Inert sil ODS C18 (250 × 4.6 mm I.D) 5 µm, column as stationary phase at flow rate of 1 mL/min and detection wavelength of 240 nm. The retention times of Lamivudine and Zidovudine was found to be 3.793 min and 2.547 min respectively. The method was validated in terms of Linearity, Range, Accuracy, Precision, Specificity, LOD, LOQ, Robustness and system suitability according to ICH guidelines. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis for determination of Lamivudine and Zidovudine in tablet dosage form.

KEY WORDS: Lamivudine, Zidovudine, RP-HPLC, development, validation, simultaneous estimation.

INTRODUCTION

Lamivudine (LAM) is chemically (2*R*,5*S*)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1*H*)-pyrimidinone). Lamivudine is the (-) enantiomer of 2- deoxy-3-thiacytidine, which is a nucleoside analog. The (-) enantiomer of the racemic mixture shows much less cytotoxicity than the positive enantiomer. Lamivudine has very low cellular cytotoxicity and generally less potent than zidovudine in inhibiting HIV-1 and HIV-2 replication in vitro. It is rapidly absorbed with bioavailability of approximately 80%.[1]

Zidovudine (ZID) is chemically 1-(3-azido-2,3-dideoxy-β-D-ribofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione. Zidovudine is also a nucleoside analogue which is structurally similar to thymidine and used in the management of AIDS and AIDS-related complex. It may be given to patients with early HIV infection. In patients with HIV-1 infected MT-4 cells, Lamivudine in combination with zidovudine had synergistic antiretroviral activity. It is rapidly absorbed from the gastro-intestinal tract with a bioavailability of about 60–70%. It crosses the blood–brain barrier.[2]

*** Corresponding author: M.Sravani.**
E-mail address: marupudisravani1988@gmail.com

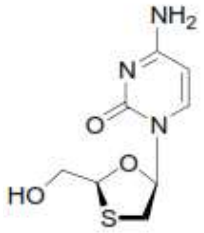

***Fig 1*: Chemical structure of LAM**

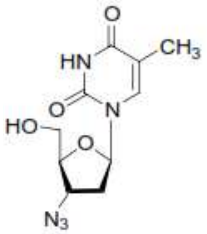

***Fig 2*: Chemical structure of ZID**

A literature survey shows that a number of HPTLC[4,5],Liquid chromatography tandem mass spectrometry[5,6],Uv spectroscopy[8] methods have been reported for the simultaneous estimation of LAM and ZID in pharmaceutical dosage forms in combination or combinations with other drugs and a few HPLC methods for simultaneous estimation of LAM and ZID in combined dosage forms were reported[9,10,11,12] . But the methods are cost effective. So my aim to develop new method with optimum pH and less run time for simultaneous estimation of LAM and ZID in tablet dosage form Accurately weigh 1.925 gm of Ammonium Acetate in 500 mL of HPLC grade water and pH is adjusted to 4.0±0.2 with O-Phosphoric acid.

.MATERIALS AND METHODS

Chemicals and reagents

Drug samples were obtained from Chandra labs Pvt. Ltd., India. Tablets (Combivir, Cipla Pharmaceuticals Pvt Ltd), Potassium Dihydrogen orthophosphate (Rankem/AR Grade), Dipotassium hydrogen orthophosphate (Rankem/AR Grade), THF (Rankem/AR Grade), ammonium acetate (Rankem/AR Grade), Acetonitrile (Merck/HPLC Grade), Water (Merck/HPLC Grade), Methanol (Merck/HPLC Grade), O-phosphoric acid (Rankem/ Reagent Grade).

INSTRUMENTATION

Shimadzu HPLC system equipped with pump (LC-10ATVP), UV detector (SPD-10ADVP), column Inertsil ODS C18 (250×4.6 mm I.D) 5 µm particle size, Rheodyne injector fitted with 20µL loop and data read out by using spinchrom software.

Chromatographic conditions

Analysis was carried out at ambient temperature. Compounds were separated isocratically with a consisting of ammonium acetate buffer pH 4.0, acetonitrile and thf in the ratio of 60:30:10 v/v as the mobile phase with Inert Sil ODS C18 (250 × 4.6 mm i.d) 5 µm, column as stationary phase at flow rate of 1 mL/min and detection wavelength of 240 nm. The mobile phase was filtered by using 0.45 µm membrane filter (millipore, bradford, ma), and sonicated in ultrasonicator for 15 minutes.

Preparation of Ammonium Acetate buffer pH 4.0±0.2

Preparation of standard stock solution

Stock solution was prepared by dissolving 50 mg of LAM and 100 mg of ZID in few mL of mobile phase in a 100 mLvolumetric flask. It sonicated and the volume was made to the mark with the mobile phase and filtered.

Working standard solution

From above stock solution 50 µg/mL of LAM and 100 µg/mL of ZID working solution was prepared, pipette out 1 mL in 10 mL volumetric flask and the volume up to the mark was made with the mobile phase. The above solution is used for precision, specificity, robustness

Sample stock preparation

Twenty tablets were weighed accurately and powdered. A quantity of powder equivalent to 50 mg of LAM and 100 mg of ZID in 100 mL volumetric flask and make up mark with mobile phase. The above solution was sonicated and filtered.

Working sample solution

From above stock solution 50 µg/mL of LAM and 100 µg/mL of ZID working solution was prepared by pipette out 1 mL in 10mL volumetric flask and the volume up to the mark was made with the mobile phase. The above solution is used for assay.

Calibration and linearity

The calibration curves were constructed in the range of 30-70 µg/mL for LAM and 60-140 µg/mL for ZID. the solution were prepared by diluting 0.6,

0.8, 1.0, 1.2, 1.4 mL of standard stock solution to 10 mL with mobile phase.

RESULTS AND DISCUSSIONS

Optimization of chromatographic conditions by using different mobile phase compositions. The optimum chromatographic conditions found with Ammonium acetate buffer pH 4.0, Acetonitrile and THF in the ratio of 60:30:10 v/v on Inert sil ODS C18 (250 × 4.6 mm I.D) 5 µm particle size with a flow rate of 1 mL/min and detection wavelength was selected as 240 nm. LAM was eluted at 3.793 min with theoretical plates 3250, tailing factor 1.725 and resolution 4.192. ZID was eluted at 2.547 min with theoretical plates 2320, tailing factor 1.742. So this condition was used for assay and the assay results were shown in Table 1.

Method validation

Method validation done according to the ICH guidelines for validation of analytical procedures

System suitability

System suitability of method was carried out to verify that the resolution and reproducibility of the system are satisfactory for the analysis to be performed. Theoretical plates, tailing factor, Resolution parameters were determined and compared against the specifications and are presented in Table 2.

Precision

System Precision

System precision was carried out by using standard preparation for six times and the result was calculated. The % RSD was found to be less than 2%. This proves the method was precise. The result was shown in Table 3.

Method Precision

Method precision was carried out by using sample preparation for six times and the result was calculated. The % RSD was found to be less than 2%, which proves the method was precise. The result was shown in Table 4.

Linearity

The linearity of method was studied by preparing different concentration levels of standard solutions. The linearity range for LAM and ZID were found to be 30-70 µg/mL and 60-140 µg/mL, respectively. The regression equation for LAM and ZID were found to be $y = 26.41x - 57.71$ and $y = 30.80x - 1319$ with coefficient of correlation, (R^2) 0.998 and 0.993, respectively.The linearity graph of LAM and ZID was shown in Fig 1 and Fig 2.

LOD and LOQ

LOD and LOQ is calculated from standard deviation of response from precision and slope from linearity

$$LOQ = 10\ \sigma / S$$
$$LOD = 3.3\ \sigma / S$$

Where

σ is standard deviation from response
S is slope from calibration curve

The LOD and LOQ for Lamivudine were found to be 1.98 µg/mL and 5.99 µg/mL respectively. The LOD and LOQ for zidovudine were found to be 3.39 µg/mL and 10.27 µg/mL respectively.

Specificity

Specificity of the method was determined by comparing the retention times of LAM and ZID of standard solution with the retention times of LAM and ZID of sample solutions. Good correlation was obtained between the retention times of standard with sample shows that there is no interference of excipients from tablet dosage form. Specificity chromatograms given in Fig 3 and Fig 4.

Accuracy

The degree of accuracy of the method was determined by recovery studies in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard LAM and ZID were added to pre-analyzed samples and were analysed in proposed HPLC method. Results of recovery studies were shown in Table 5 and Table 6.

Robustness

Robustness of the method was performed by small deliberate variation in operating conditions like wave length (±2 nm) and flow rate (±0.1mL/min). The results were shown in Table 7.

From linearity the correlation coefficient R^2 values were found to be near to 0.999 for both drugs which shows that linear relationship between concentrations versus response. The proposed HPLC method was also validated for system suitability, system precision and method precision. The % RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The LOD and LOQ for Lamivudine were found to be 1.98 µg/mL and 5.99 µg/mL respectively. The LOD and LOQ

for zidovudine were found to be 3.39 µg/mL and 10.27 µg/mL respectively, indicates the sensitivity of the method. The percentage of recovery of LAM and ZID were found to be 100.77 and 101.32 respectively shows that the proposed method is highly accurate.

Table: 1 Results for assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
LAM	150	148.09	98.72
ZID	300	298.43	99.48

Table 2 Results for system suitability parameters

Parameters	LAM	ZID
Retention times(min)	3.793	2.547
Theoretical plates	3250	2320
Tailing factor	1.725	1.742
Resolution	4.192	-

Table 3 Results for system precision

Injection	LAM		ZID	
	Retention times	Area	Retention times	Area
1	3.833	1165.912	2.583	1467.059
2	3.797	1142.959	2.550	1445.257
3	3.793	1139.959	2.547	1453.436
4	3.780	1149.783	2.537	1448.468
5	3.780	1139.774	2.537	1431.868
6	3.773	1160.209	2.530	1437.808
Average	3.793	1149.766	2.5473	1447.316
SD	0.022	11.064	0.0189	12.344
%RSD	**0.57**	**0.96**	**0.74**	**0.85**

Table 4 Results for method precision

Injection	LAM		ZID	
	Retention times	Area	Retention times	Area
1	3.727	1136.462	2.5	1419.877
2	3.767	1134.434	2.537	1421.089
3	3.743	1135.119	2.52	1420.774
4	3.797	1136.717	2.55	1445.257
5	3.78	1143.22	2.537	1437.213
6	3.781	1140.223	2.532	1436.22
Average	3.765833	1137.696	2.529333	1430.072
SD	0.023919	3.073084	0.015808	9.920191
%RSD	0.63517	0.270115	0.624982	0.693685

Fig 1 Linearity graph of LAM

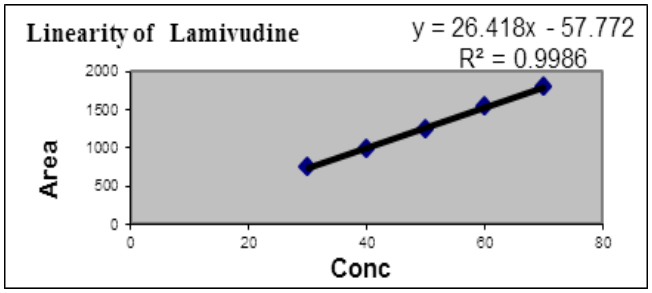

Fig 2 Linearity graph of ZID

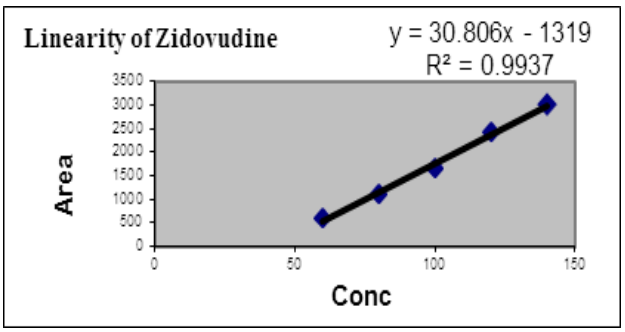

Fig 3 Chromatogram of sample

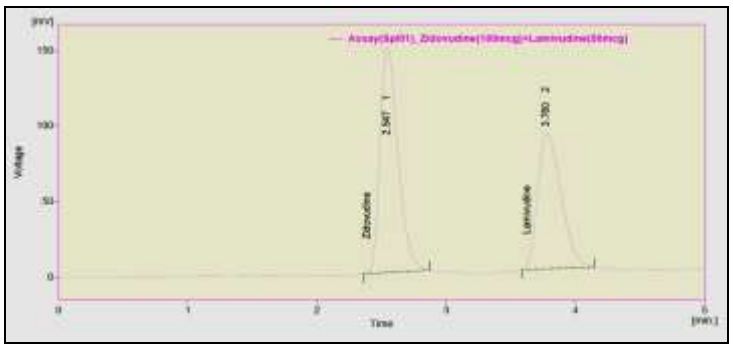

Fig 4 Chromatogram of standard

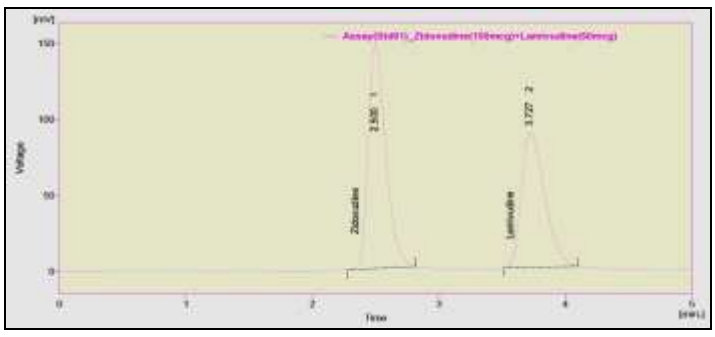

Table 5 Results for Recovery of LAM.

Conc	Amount present (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)*	Percentage Recovery *	% Mean Recovery
80%	40	10	50.59	101.18	100.77
100%	50	10	60.75	101.24	
120%	60	10	69.92	99.89	

***** Mean of three observations

Table 6 Results for Recovery of ZID.

Conc	Amount present (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)*	Percentage Recovery *	% Mean Recovery
80%	80	20	101.09	101.09	101.32
100%	100	20	121.74	101.45	
120%	120	20	141.98	101.42	

***** Mean of three observations

Table 7 Results for Robustness

Chromatographic changes		Retention time(min)		Tailing factor	
		LAM	ZID	LAM	ZID
Flow rate (mL/min)	0.8	4.727	3.157	1.692	1.650
	1	3.797	2.550	1.725	1.710
	1.2	3.167	2.113	1.533	1.605
Wavelength (nm)	238	3.803	2.540	1.628	1.636
	240	3.797	2.550	1.725	1.710
	242	3.857	2.523	1.600	1.636

***** Mean of three observations

CONCLUSION

The develop RP-HPLC method was proves to be precise, rapid, accurate, linear, specific. It can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories in near future.

ACKNOWLEDGEMENT

The author wish to thank the management of Nimra College of Pharmacy for providing facilities during the work.

REFERENCES

[1] Lamivudine Drug profile: www.drugbank.ca/drugs/DB00709
[2] Zidovudine drug profile: www.drugbank.ca/drugs/DB00495
[3] D.H. Shewiyo, E. Kaale, C. Ugullum, M.N. Sigond, P.G. Risha, B. Dejaegher, J. Smeyers–Verbeke, Y. Vander Heyden. Development and validation of a normal-phase HPTLC method for the simultaneous analysis of lamivudine, stavudine and nevirapine in fixed dosecombination tablets. Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 445–450.
[4] Palani Venkatesha, Mahesh Daggumati. Development and validation of a normal-phase HPTLC method for the simultaneous analysis of Lamivudine and Zidovudine in fixed-dose combination tablets. Journal of Pharmaceutical Analysis. 2(2), 2012,152–155.
[5] Kathryn B. Kenney, Stephen A. Wring, Richard M. Carr , Guy N. Wells ,John A. Dunn. Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass Spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 22, 2000, 967–983.
[6] Muralli Krishna Matta, Nageswararao Pilla, Jaswanth Kumar, Laxminarayana.B, Seshagirirao. Simultaneous quantitation of Lamivudine, Zidovudine and Nevirapine in human plasma by Liquid chromatography–tandem mass spectrometry and application to a pharmacokinetic study. Acta Pharmaceutica Sinica B. 2(5), 2012, 472–480.
[7] P. Nagulwar vaishali and P. bhusari kishore . Simultaneous estimation of abacavir, Lamivudine And Zidovudine in combined tablet dosage form by uv method. Ijrap, 2(2), 2011, 610-614.
[8] Yadavalli Rekha, Yellina Haribabu, Sheeja Velayudhankutty, Sosamma Cicy Eapen and Jane Mary. Method Development and Validation for the Simultaneous Estimation of Efavirenz, Lamivudine and Zidovudine through Stability indicating RP-HPLC Method *Res. J. Pharmaceutical Sci.*2(4), 2013, 10-18.
[9] J. Nijamdeen, B. Jayalakshmi, N. Senthilkumar, Vijayamirtharaj and C. Saravanan. Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Zidovudine. J. Chem. Pharm. Res., 2010, 2(3), 92-96.
[10] P.Venkatesh, Lydia Smiley and Mahesh D. Simultaneous estimation of Zidovudine and Lamivudine tablets by RP-HPLC method. International Journal of ChemTech Research 2011, 3(1), 376-380.
[11] Ravi Prakash Mahor, Versha Parcha, Yogendra Singh, Rajiv Sharma and Anil Bhandari. Development and validation of a HPLC method for simultaneous estimation of Lamivudine and Zidovudine in Tablet Dosage Forms. Der Chemica Sinica, 2(6), 2011, 12-19.
[12] CH .Venkata Reddiah , P. Rama Devi , K. Mukkanti , K.Srinivasarao. Development and validation of stability indicating HPLC method for Lamivudine, Zidovudine and Abacavir in tablet dosage forms *Int.J.Pharm.Phytopharmacol.Res* 1(5), 2005, 247-256.

****