Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Issue 2, 2013 ISSN - 0974-2441

Research Article

SIMULTANEOUS DETERMINATION OF STAVUDINE AND LAMIVUDINE IN PHARMACEUTICAL DOSAGE FORMS BY RP –HPLC

C. JOTHIMANIVANNAN, S.ANANDA THANGADURAI, M.RAMYA KRISHNA*, CH.ARJUN, V.M.K.GOWTHAM

Department of Pharmaceutical Analysis, Swamy Vivekananda College of pharmacy, Elayampalayam- 637205, Tamil Nadu, India.
Email: ramya2426@gmail.com

Received: 11 October 2012, Revised and Accepted: 7 December 2012

ABSTRACT

A simple, rapid and precise method is developed for the quantitative simultaneous determination of Stavudine and Lamivudine in bulk and pharmaceutical dosage forms by RP-HPLC method. Separation of the drug was achieved on a reverse phase $C_{18,45\mu}$ (250 X 4.6 mm) using a mobile phase consisting of HPLC grade water and methanol in the ratio of 90:10 v/v. Isocratic elution at a flow rate of 1 ml/min was employed, the linearity is obtained in the range of 20-100 µg/spot for both Stavudine and Lamivudine. The Wavelength at which assay method studied was 257nm. The retention time of Stavudine and Lamivudine were found to be 5.621 min. and 4.176 min respectively with good resolution. The accuracy and reliability of the method was assessed by evaluation of precision (intra-day and inter-day % RSD >2 for Stavudine and Lamivudine) in accuracy 99.2-101.5% recovery was obtained for Stavudine and Lamivudine respectively, Robustness and Ruggedness in accordance with ICH guidelines. Based on its simplicity, rapidness and high precision, the developed HPLC method may be used for the simultaneous estimation of Stavudine and Lamivudine in quality control studies and routine analysis.

Keywords: RP-HPLC, Stavudine, Lamivudine.

INTRODUCTION

Stavudine is a nucleoside analog reverse transcriptase inhibitor (NARTI) is indicated for the treatment of HIV infection[1].Lamivudine (2', 3'-dideoxy-3'-thiacytidine) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI). It has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV[2]. Stavudine in combination with Lamivudine has been shown to have synergistic antiretroviral activity. The mechanism of action involved is both drugs act by inhibiting the reverse transcriptase of HIV and by terminating the growth of the DNA chain.

Literature survey reveals that various methods were reported for the individual determination of Stavudine and Lamivudine in pharmaceutical dosage forms[3-8]. The present work describes a simple, precise and accurate HPLC method for the simultaneous estimation of Stavudine and Lamivudine in Pharmaceutical dosage forms.

The present method was validated according to the International Conference on Harmonization (ICH) for the simultaneous estimation of Stavudine and Lamivudine in bulk and pharmaceutical dosage forms[9].

MATERIALS AND METHODS

All chemicals used are of analytical grade, and HPLC grade methanol and water (E.Merck Ltd., Mumbai) were used. The pure drugs Stavudine and Lamivudine were procured from Hetero Chemicals and Drugs Ltd. Hyderabad, which was certified to be 99.5% and 99.7% respectively.

Instrument

The chromatographic separation was carried out on Perkin Elmer HPLC model (with rheodyne injector having 20µl fixed loop. Chromatographic analysis was performed using C_{18} column and particle size of 4.5µ with internal diameter (250 X 4.6 mm). Shimadzu electronic balance (AX-200) was used for weighing purpose.

ASSAY PREPARATIONS

Preparation of standard solution

An accurately weighed quantity 100 mg of each Stavudine and Lamivudine were weighed into separate 100ml volumetric flask, dissolved in the diluent and volume was made up to the mark with the diluent to obtain the concentration of 1000µg/ml. From the 1000µg/ml stock solution, 10ml is pippeted out in a 100 ml volumetric flask and made up the volume using diluent to obtain the concentration of 100µg/ml.

Chromatographic conditions

Column	Perkin Elmer, C18,(250x4.6mm),4.5µ
Wavelength	257nm
Injection volume	20 µl
Run time	10min
Flow rate	1ml/min
Diluent	Methanol : Water(90:10)

Sample preparation

Twenty tablets were weighed accurately and powdered. Powder equivalent to 150mg was weighed and transferred to 100 ml volumetric flask and dissolved in small quantity of methanol by sonicating the flask for 15mins and was made up to volume with diluent. The solution was filtered through 0.45µ membrane filter. 10ml of above solution was pippeted out in a 100 ml volumetric flask and made up to the volume with diluent.

From the above sample solution, 20µl solutions were injected into the chromatographic system and chromatogram was recorded. The peak area values of Stavudine and Lamivudine were calculated. By calculating the AUC, the amount of Stavudine and Lamivudine solution were estimated. The results of analysis were shown in Fig 3.

Validation of the developed method

The developed method was validated as per ICH guidelines. The parameters studied for validation were linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification and ruggedness.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of sample solutions were made and the response factor of drug peak and % RSD were calculated. In the inter-day variation studies, six repeated injections of sample solutions were made for three consecutive days and response factor of drugs peak and % RSD were calculated and presented in Table no 1

Linearity

Under the experimental conditions described above, linear calibration curves for both Stavudine and Lamivudine were obtained with five concentration level each. Peak area (A) and concentration (C) of each drug substance was subjected to regression analysis to calculate the regression equation and the correlation coefficients. The linearity range of Stavudine and Lamivudine was 20-100µg/ml. The regression equation for Stavudine is y=3184x+878.6 and y=3230x+1731 for Lamivudine and the correlation coefficients were (R^2=0.998) for Stavudine and Lamivudine respectively. Results were tabulated in table no.3. Calibration Curve of Stavudine and Lamivudine was shown in Fig 3& 4.

Accuracy

The accuracy of the method was confirmed by recovery studies. To the preanalysed formulation known amount of standard is added. Accuracy of the method was carried out by standard addition method at three levels of concentrations (80%, 100%, and 120%). The results of recovery (%) and RSD were shown in table no.3. The amount of drug recovered was calculated and the percentage recovery was found to be in the range of 99.2-101.5%.

Limit of detection and limit of Quantitation

LOD is the lowest amount of analyte in a sample that can be detected. LOQ is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances.LOD and LOQ were calculated from the calibration curves of Stavudine and Lamivudine. The results were tabulated in Table no.7.

Robustness

The robustness study was performed by slight modification in flow rate, pH of the buffer and composition of the mobile phase. It was observed that when flow rate was changed Rt had changed significantly. The results of robustness study were shown in Table no. 8 & 9.

Ruggedness

The ruggedness of the method was determined by performing the same assay by different analysts to check the reproducibility. The solutions were analyzed in triplicate and the %RSD was calculated and presented in the Table no 10.

Table 1 Precision result for Stavudine and Lamivudine.

	AUC	
S.No	Stavudine	Lamivudine
1	341971.2	323556.8
2	342854.1	321457.11
3	344964.0	323346.1
4	341923.5	323452
5	345497	323546
6	343687	323658.2
Mean	343482.3	323169.7
SD	1510.4	845.5
RSD %	0.43	0.26

Table 2: Tabulation of Slope and correlation Coefficient.

S.NO	Parameter	STAV	LAM
1.	Slope	3456	3229
2.	Correlation-coefficient	0.999	0.999

Table 3: Linearity studies on Stavudine and Lamivudine

Stavudine		Lamivudine	
Conc.(mcg)	**Area**	**Conc.(mcg)**	**Area**
20	64789	20	68311
40	129422	40	124781
60	193215	60	199189
80	248842	80	263570
100	323556	100	321973

Accuracy Studies on Stavudine and Lamivudine

Table 4: 80%Spiking.

S. No	STAV	LAM
1	305674.1	295924.4
2	305675.6	295928.22
3	305671.1	295929.10
Avg	305672.7	295926.6
amt. recovered%	89.39	91.36
%Recovery	99.32	101.52

Table 5: 100%Spiking

S. No	STAV	LAM
1	381253.1	359658.2
2	381251	359652.0
3	381249.0	359655.6
Avg	381251.9	359654.8
amt. recovered%	111.49	111.04
%Recovery	101.36	100.94

Table 6: 120%Spiking

S. No	STAV	LAM
1	450271.1	424274.1
2	450270.2	424271.3
3	450277	424278.1
Avg	450274.1	424278.5
amt. recovered%	131.68	130.99
%Recovery	101.29	100.2

Table 7: LOD and LOQ

Parameters	Stavudine	Lamivudine
LOD	3.52µg/ml	0.825µg/ml
OQ	.61µg/ml	.501µg/ml
LOQ	10.61µg/ml	2.501µg/ml

Robustness Studies on Stavudine and Lamivudine

Table 8: Flow Rate 0.9ml/min

		AUC
S.NO	**Stavudine**	**ivudine**
1	341977	323659
2	343108.0	323859
3	343689	324999
Mean	342924.9	324172.3
SD	870.6	722.8647
RSD %	0.252839	0.223

Table 9: Flow Rate 1.1ml/min

		AUC
S.NO	**Stavudine**	**Lamivudine**
1	341962.0	323459
2	343911.8	323359
3	343890	323299
Mean	343254.3	323372.3
SD	1119.243	80.82904
RSD %	0.3245	0.025

Table10: Ruggedness studies of Stavudine and Lamivudine

	AUC	
S.NO	**Stavudine**	**Lamivudine**
1	341972	323556.8
2	343778.0	323596.5
3	344689	323452.2
Mean	343007.9	323177.2
SD	1278.867	841.4948
RSD %	0.72839	0.460382

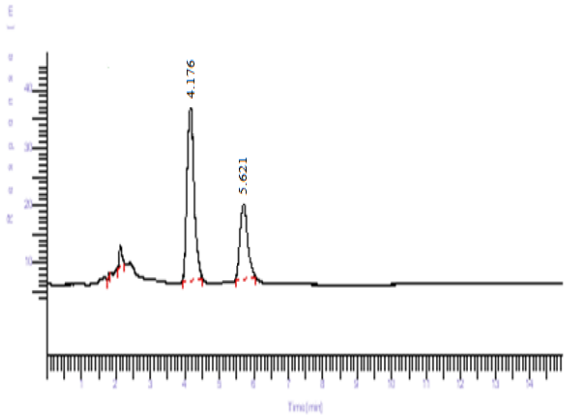

Fig 1: Chromatogram of assay for Combined Drug.

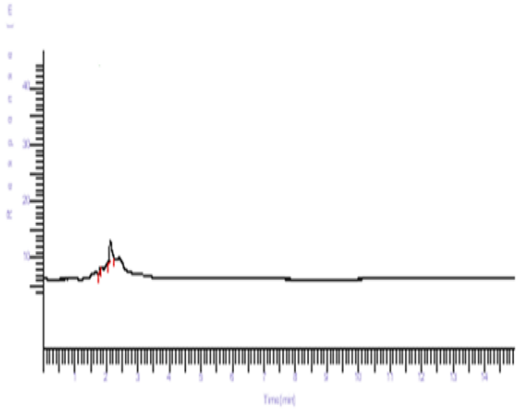

Fig 2: Chromatogram of Blank Solution.

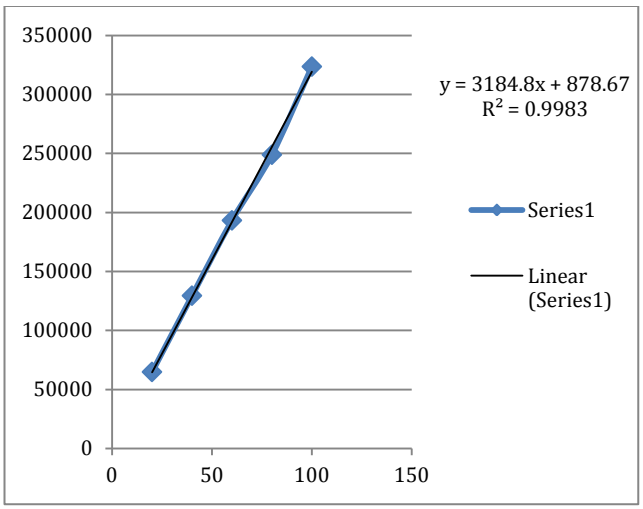

Fig 3: Linearity of Stavudine.

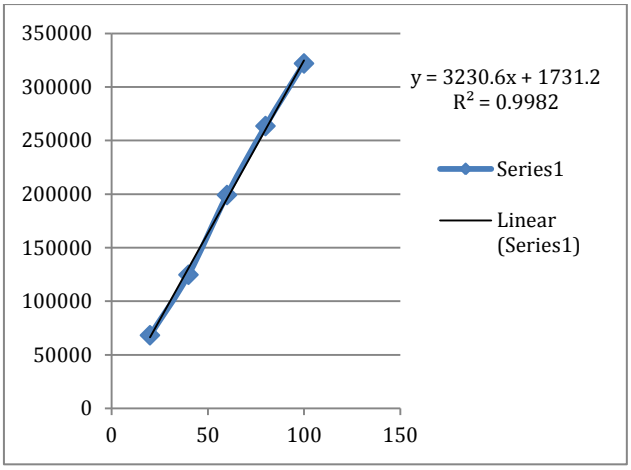

Fig 4: Linearity of Lamivudine.

RESULTS AND DISCUSSION

To optimize the RP-HPLC method parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of water and methanol in the ratio of 90:10 v/v and 1.0 ml/min flow rate was maintained. The chromatogram obtained for sample and blank was shown in Fig 1 and 2 .The retention time of Lamivudine was 4.176 min and for Stavudine was 5.621 min

Validation Parameters

Precision

From the results shown in precision table, it was found that % RSD for Stavudine is 0.43 and for Lamivudine is 0.26 .Hence the %RSD is less than2% for both drugs, which indicates that the proposed method has good precise. Results are shown in Table no. 1.

Linearity

The calibration was linear in concentration range of 20-100ppm for Stavudine and Lamivudine respectively. The regression equation for Stavudine is y=3184x+878.6 and y=3230x+1731 for Lamivudine and the correlation coefficients were (r^2=0.998) for both drugs. The calibration curves were shown in Fig 3& 4 and the results were tabulated in table no.2&3.

Accuracy

The percentage recovery of Stavudine and Lamivudine, in drug sample was ranged from 99.32-101.36 which indicates that the method was accurate .The results were shown in table no. 4, 5& 6.

LOD and LOQ

The LOD was calculated to be 3.52 µg/ml for Stavudine and 0.825µg/ml for Lamivudine. The LOQ of Stavudine and Lamivudine were found to be 9.61µg/ml and 2.501µg/ml, respectively. As results are within the acceptance criteria the given method was said to be accurate. The results were shown in table no 7.

Robustness

When flow rate was changed Retention time had changed significantly. When the flow rate was increased the retention time was decreased and vice versa. While changing the above parameter the %assay was observed to be within limit. % RSD for Stavudine is 0.32 and for Lamivudine is 0.025. The results were shown in table no. 8&9.

Ruggedness

From the results shown in ruggedness table, it was found that % RSD is less than2% for both drugs, which indicates that the proposed method has good precise. % RSD for Stavudine is 0.72 and for Lamivudine is 0.46. The results were given in table no. 10.

CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Stavudine and Lamivudine offers simplicity, selectivity, precision and accuracy. It produces symmetric peak Shape, good resolution with rapid retention time for both drugs while comparing with the existing method. So this method can be applicable for the simultaneous estimation of Stavudine and Lamivudine in quality control studies for bulk and pharmaceutical dosage form.

ACKNOWLEDGEMENTS

Authors are thankful to the management of Swamy Vivekananda College of Pharmacy for providing necessary facilities.

REFERENCE

1. http://Www.Wikipedia.Org/Wiki/Stavudine.
2. http://www.wikipedia.org/wiki/Lamivudine.
3. ABiksham Babu, ARamu, SVenkatarao, SNeeharika, Spectrophotometric Determination of anti retro viral drug Stavudine in bulk and pharmaceutical dosage form, Rasayan Journal of Chemistry, 2011;4(2) : 336-342.
4. A.K.Hemanth Kumar, V.Sudha, Geetha ramachandran, Comparison of HPLC & spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products Indian J Med Res, October 2010;132: 390-394.
5. Parthiban, C.Geetha, Simultaneous determination of Lamivudine, Stavudine and Nevirapine in antiretroviral fixed dose combinations by High performance liquid chromatography. International Journal of Biological & Pharmaceutical Research. 2012; 3(1): 158-163.
6. S. k. patro, S.R.Swain, V.J. Patro and N.S.K.Choudhury Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine from Pharmaceutical Preparation, E-Journal of Chemistry, 2010; 7(1):117-122.
7. V Reddy Panditi, Anjaneyulu Vinukonda, Validated First Order Derivative Spectroscopic Method for the determination of Stavudine in Bulk and Pharmaceutical Dosage Forms, International Journal of Chem Tech Research, 2011; 3(1): 18-22.
8. The United State Pharmacopoeia 32 (2009), National Formulary 27, U.S. Pharmacopeia convention Inc., Rockville, MD; 1956-57.
9. ICH Guidelines, Q2A, Validation of Analytical Procedures: Definition & Terminology, Geneva, Switzerland.