Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF LAMIVUDINE IN TABLET DOSAGE FORM BY UPLC METHOD

RAGHUPATHI G[1]*, C PARTHIBAN[1]

[1]Department of Pharmaceutical Analysis and Quality Assurance, Malla Reddy College of Pharmacy, Maisammaguda, Dullapally, Secunderabad -14, Andra Pradesh, India. Email: parthi1617@gmail.com

Received: 21 Aug 2013, Revised and Accepted: 24 Sep 2013

ABSTRACT

The objective of this work is to develop a novel high performance liquid chromatographic method for the determination of Lamivudine in tablet dosage form. The separation was achieved by using C_{18} column with methanol: water 80:20 v/v as a mobile phase, at a flow rate of 0.2ml/min. Detection was carried out at 270 nm. The retention time of Lamivudine was found to be 0.620 min. The proposed method was ascertained by evaluating validation parameters like linearity (5-30 µg/ml) with r^2=0.999. The proposed method is successfully applied for the determination of drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies. The method can be used to analyze commercial solid dosage containing Lamivudine with good recoveries for routine analysis.

Keywords: Lamivudine, RP-UPLC, Method development and Validation.

INTRODUCTION

Lamivudine,is chemically 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidinone-2-one [1-4]. E K Kano et al, evaluated the Lamivudine in human plasma by HPLC, finally they concluded that the two Lamivudine formulations are bioequivalent in their rate and extent of absorption, and thus, may be used interchangeably [5]. Bengi Uslu etal, determined the binary mixture of Lamivudine and Zidovudine by first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography–UV methods [6]. Namita Kapoor etal, performed Simultaneous determination of Lamivudine and Stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography [7]. But there is no simple and easy method for the analysis of Lamivudine. Hence, it is necessary to develop a rapid, accurate and validated RP-UPLC method for the determination of Lamivudine in tablet dosage form. The method proved to be simple model since it does not contain a buffer system. The present study illustrates development and validation of a simple, accurate and precise procedure for determination of Lamivudine by RP-UPLC.

MATERIALS AND METHODS

Instrument

Chromatographic separation was performed on Waters UPLC system having Waters 2996 PDA detector and Rheodyne injector with 5µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and Chemicals

Methanol and water of HPLC grade were procured from Merck Ltd. API of Lamivudine received as gift sample from Dr.Reddy's. The commercial sample LAMIVI HBV (150mg) tablets are purchased from the local market.

UPLC Conditions

A Thermo scientific C_{18} (10cm×2.1mm, 1.7µm) column was used as the stationary phase. A mixture of methanol and water in the ratio of (80:20v/v) was used as a mobile phase. It was filtered through 0.2µ nylon membrane filter and degassed. The mobile phase was pumped at 0.2 ml/min. The eluents were monitored at 270nm.The injection volumes of sample and standard were 5.0µl.

Standard Solution Preparation

About 10 mg of Lamivudine standard was weighed accurately and transferred to 100 ml volumetric flask. The volume was made up to mark with the mobile phase to obtain a concentration of 100µg/ml. Further dilutions were made to obtain the concentration in the range of 5-30 µg/ml of Lamivudine and filtered through 0.2µ nylon membrane filter.

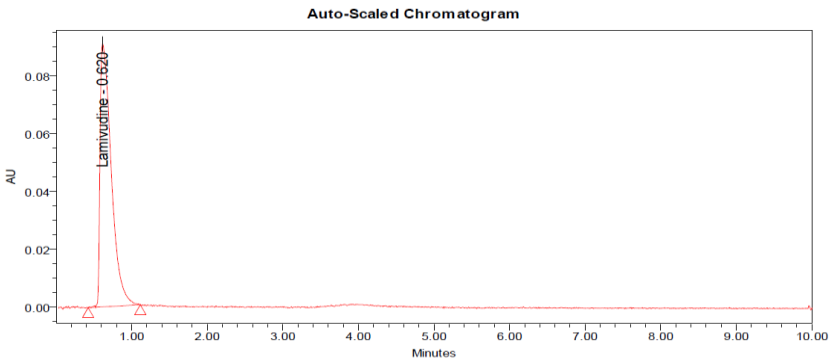

Fig. 1: Chromatogram of Lamivudine

Sample Solution Preparation

For analysis in tablet dosage form, twenty tablets were weighed. The tablets were finely powdered and powder equivalent to 150 mg of Lamivudine was accurately weighed and transferred into a 100 ml volumetric flask. The volume was made up to mark with the mobile phase to obtain a concentration of 1000µg/ml. Further dilutions were made to obtain a concentration of 5-30µg/ml and filtered through 0.2µ nylon membrane filter.

Assay of the Formulation

Five µl of standard and sample solutions were separately injected on UPLC system. The analysis was repeated in triplicates, from the peak area of Lamivudine the amount of drugs in the sample were computed. The retention time was 0.620 min as shown in fig.1. Concentrations of lamivudine in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of the drug was calculated and presented in Table 1.

Validation of the Method

Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard Lamivudine corresponding to 50%, 100%, and 150% of the label claim was added to pre analysed sample of tablet dosage form separately. The recovery studies were carried out three times, at each level of recovery. The data's of accuracy were shown in (Table. 2).

Precision

In the system precision studies, six replicate injections of the working standard solution prepared as per the proposed method and chromatograms were recorded. Standard deviation and relative standard deviation for the area was calculated and presented in table.3.

Linearity

Linearity was studied by preparing standard solutions of Lamivudine at different concentration levels. The linearity ranges were found in the range of 5-30 µg/ml. The standard calibration curve was generated (5-30 µg/ml) using regression analysis with Microsoft excel. The assay was judged to be linear as the correlation coefficient was greater than 0.999 by the least-square method as shown in fig 2.

Table 1: Assay report

Sample name	Lable claim(mg/tablet)	*Amount present(mg/tablet)	*Percentage lable claim(%w/w)
Lamivudine	150	150.26	100.17

*-average of three samples

Table 2: Accuracy of the method

Concentration	Amount Added	Amount found	Area	Mean	SD	%RSD	% Recovery
50%	7.5	7.48	935990 942693 946004	941562	4165	0.54	99.73
100%	15	15.12	1404043 1417538 1414907	1412163	7153	0.50	100.80
150%	22.5	22.38	2868084 2862588 2875349	2868674	6400	0.22	99.46

Table 3: Precision of the method

Intra-Day Precision				Inter-Day Precision			
*Mean	SD	%RSD	% Recovery	*Mean	SD	%RSD	% Recovery
1415244	5657	0.39	99.82	140690	7005	0.49	100.51

*-Mean of six samples

Table 4: Linearity report

Concentration(µg/ml)	Area
0	0
5	532565
10	936029
15	1404043
20	1872058
25	2340072
30	2808087

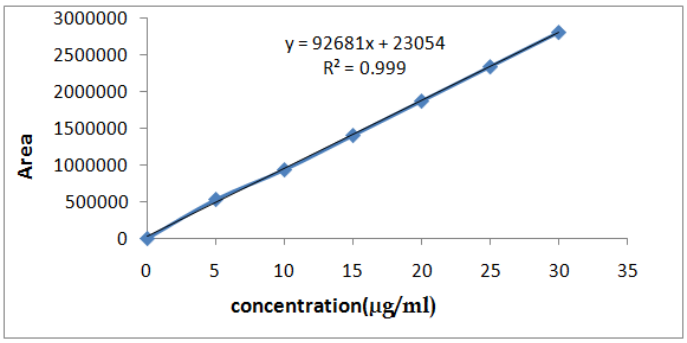

Fig. 2: Linearity of lamivudine

Table 5: Robustness of the method (change in mobile phase ratio)

Mobile Phase Ratio	R_t (Min)	Area(n=6)	SD	%RSD
81:19	0.634	141379	5265	0.37
80:20	0.621	1418944	2326	0.16
79:21	0.613	1435832	3576	0.24

Table 6: Robustness of the method (change in flow rate)

Flow Rate(ml/min)	R_t (Min)	Area(n=6)	SD	%RSD
0.1ml/min	0.835	1435313	1513	0.102
0.2ml/min	0.620	1461765	2217	0.151
o.3ml/min	0.592	1441862	4848	0.334

Table 7: System suitability report

Parameters	Lamivudine
Retention time, min	0.620
Tailing factor	0.56
Number of theoretical plates	2664
LOD	0.25µg/ml
LOQ	0.76 µg/ml

Robustness

Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase and flow rate of the mobile phase chromatographic characteristics were evaluated and presented in table.5 and 6.

RESULTS AND DISCUSSION

System suitability parameter indicates high column efficiency from the large number of theoretical plates (>2000). The degree of asymmetry was also evaluated using the tailing factor result 0.56 which did not exceed the critical value (1.5) indicating acceptable degree of peak asymmetry. The method was found to be accurate and precise as indicated by results of recovery studies and precision studies %RSD not more than 2%. The standard deviation of % assay for sample was calculated for each parameter in robustness studies and relative standard deviation was found less than 2%. The low %RSD value confirms the robustness of the method. The proposed method was ascertained by evaluating validation parameters like linearity (5-30 µg/ml) with r^2=0.999.

CONCLUSION

The reported RP-UPLC method was proved to be simple, accurate, fast and precise isocratic method has been developed for the determination of Lamivudine.

REFERENCES

1. Indian Pharmacopoeia - Addendum, The Indian Pharmacopoeia Commission, 6th edition Ghaziabad, 2010:1557-1560.
2. The United state Pharmacopoeia, Convention Inc., Twin brook Parkway, Rockville, 2003, 26.
3. Martindale, Pharmaceutical Press, 34th edition, 2005: 648.
4. The Merck Index, Merck & Co., Inc., 14th edition, Whitehouse Station. NJ, 2006:927-928.
5. Eunice Kazue Kano, Cristina Helena dos Reis Serra, Eunice Emiko Mori Koono, Simone Schramm Andrade and Valentina. Estimation of Lamivudine in human plasma by HPLC and its use in bioequivalence studies. International Journal of Pharmaceutics. 2005; 297 (1-2): 73-79.
6. Bengi Uslu and Sibel A. Ozkan. Analytica Chimica Acta. Determination of Lamivudine and Zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography–UV methods .2002; 466 (1):175-185.
7. Namita Kapoor, Sateesh Khandavilli, Ramesh Panchagnula. Simultaneous determination of Lamivudine and Stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 2006; 41: 761-765.