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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR LAMIVUDINE IN COMPARISON TO HPLC METHODS

K. P. R. Chowdary*, K. Ravi Shankar and Prathyusha. Ravi

Vikas Institute of Pharmaceutical Sciences, Nidigatla Road, Rajahmundry- 533103.

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***Correspondence for Author**
Prof. K. P. R.Chowdary
Vikas Institute of Pharmaceutical Sciences, Nidigatla Road, Rajahmundry- 533103.

ABSTRACT

Lamivudine is a potent nucleoside analog reverse transcriptase inhibitor (nRTI) used as an antiretroviral drug. Though several HPLC methods are reported for the estimation of Lamivudine no spectrophotometric methods are available .The objective of the present study is to develop a simple, rapid, accurate, precise UV spectrophotometric method for the estimation of Lamivudine in bulk and in pharmaceutical formulations, which can be considered as a useful alternative to the expensive HPLC methods. The method developed was validated with regard to linearity, precision, accuracy, selectivity, sensitivity, LOD, LOQ robustness, ruggedness, as per ICH guidelines and compared with two HPLC methods reported in the literature. The UV spectrophotometric method developed is based on measurement of absorbance at 280 nm in either water (or) 0.01 M HCl. In both the solvents the λ_{max} was found at 280nm. The UV absorbance values were not affected by changing pH. Analysis of absorbance measurements indicated that the drug solutions were stable when stored at $+4^{oc}$ and at RT 28 ^{o}C for 2 weeks. The method obeyed beer's law in the concentration range of 1-10µg/ml in both the solvents. The UV spectrophotometeric method developed is comparable to the HPLC methods with regard to Linearity, LOD, LOQ, accuracy, precision and recovery. In the case of UV spectrophotometeric method linearity was observed in a lower concentration range when compared to HPLC methods. LOD, LOQ, accuracy, precision and recovery parameters are almost similar in both UV spectrophotometeric and HPLC methods. In addition the method developed was sufficiently rugged and robust. The UV spectrophotometeric method developed was simple, rapid and less time consuming and cost

effective when compared to HPLC methods for the routine analysis of Lamivudine in quality control testing.

KEYWORDS: Lornoxicam, UV Spectrophotometric method, Validation, HPLC methods.

INTRODUCTION

Lamivudine is a potent nucleoside analog reverse transcriptase inhibitor (nRTI) used as an antiretroviral agent that inhibits replication of retroviruses in combination with zidovudine in the management of HIV (human immunodeficiency virus). It is marketed by GlaxoSmithKline with the brand names Zeffix, Heptovir, Epivir, and Epivir-HBV. Lamivudine is also used for treatment of chronic hepatitis B.

Several analytical methods have been reported for the determination of lamivudine either individually or in combination with other anti-retroviral drugs in the dosage forms and in biological fluids. These methods include visible spectrometry[1] high performance liquid chromatographic (HPLC) [2-10], liquid chromatographic mass spectrometric (LC-MS) [11-16], capillary electophoretic [17-26] and HPTLC [27]. Literature on UV spectrophotometric methods for the estimation of Lamivudine is scanty. The objective of the present study is to develop and validate a sample, accurate and precise UV spectrophotometric method for Lamivudine, which can be considered as a useful alternative to the much expensive HPLC methods. The method developed was validated with regard to linearity, precision, accuracy, selectivity, sensitivity, LOD, LOQ, robustness, ruggedness as per ICH guidelines.

EXPERIMENTAL

Materials

Lamivudine was a gift sample M/s Eisai Pharma technology Pvt Ltd, Vishakhapatnam Methanol, hydrochloric acid, sodium hydroxide, dihydrogen phosphate(Merck / BDH grade) were procured from commercial sources. Pharmaceutical preparations of Lamivudine were procured from local pharmacies. All other materials used were of pharmacopoeial grade.

Methods

Standard drug solution

Lamivudine (25mg) was weighed accurately and dissolved in water in a 25 ml volumetric flask. The solution was made up to volume with water.

Determination of λ_{max}

The standard drug solution was suitably diluted with two solvents namely (i) water (ii) 0.01M HCl to obtain a concentration of 10 µg of drug in 1 ml solution in each case .The absorbance of these drug solutions was measured at different wavelengths in the range 200-400nm to obtain the UV absorption spectra of Lamivudine .The effect of pH and storage temperature on the λ_{max} was also evaluated.

Construction of calibration curve

Calibration plots were constructed after the analysis of 6 different concentrations with each concentration was measured 6 times. The standard drug solution was suitably diluted with water (or) 0.01M HCl to obtain a series of dilutions containing 1,2,4,6,8,10 µg of Lamivudine in one ml of solution .The absorbance of these solutions was measured at 280 nm in a LABINDIA double beam UV spectrophotometer using water/ 0.01M HCl as blank.

Validation of the Method

The method developed was validated with regard to linearity, precision, accuracy, selectivity, sensitivity, LOD, LOQ, robustness, ruggedness as per ICH guidelines.

Assay of Tablets

One market brand of Lamivudine tablets and two formulated tablets of Lamivudine were assayed by the proposed method to evaluate its application in dosage form quality control. In each case five tablets were weighed and finely powdered in a dry mortar and mixed thoroughly .Tablet powder equivalent to 10mg of drug was taken and assayed for drug content .The tablet powder was transferred to a 100 ml volumetric flask, water was added and mixed thoroughly to dissolve the drug and made up to volume (100ml) with water .The solution was filtered through Whatman filter paper No: 1.The solution was suitably diluted to obtain a concentration equivalent to 10µg/ml. The absorbance of the solution was measured at 280nm .Lamivudine content was calculated using the standard calibration curve.

RESULTS AND DISSCUSION

UV absorption spectrum of Lamivudine was recorded by measuring absorbance of standard drug solution (10µg/ml) in the wavelength range 200-400 nm .The UV spectrum of Lamivudine was run in the two solvents i.e., water and 0.01M HCl. As Lamivudine is sufficiently soluble in water and 0.01M HCl, these fluids were selected for the preparation of working solutions for taking the UV spectra with better resolution. The UV absorbance

spectra of Lamivudine are shown in Fig.1. From the UV absorbance spectrum the wavelength at which maximum absorbance was observed is recorded as $\lambda_{max.}$ The λ_{max} of Lamivudine was found to be 280 nm in both water and 0.01 M HCl. The λ_{max} was same in both the solvents tested.

The effect of pH on the absorbance at λ_{max} value was studied by measuring the absorbance of solutions containing 10µg/ml of drug in buffers of different pH's (4.5, 6.8, 7.4 and 8.0) .The UV absorbance values were not affected by changing pH. The standard drug solution was stored at two different conditions i.e., $+4^{°c}$ and at RT (28 $°^{c}$) for 2 weeks. During this period the solutions were analyzed and the absorbance of solutions of different concentrations were measured and compared with those of standard drug solution freshly prepared .No differences were found between the absorbences of the solutions of stored ones and freshly made. Based on these observations it was concluded that the drug is stable under the conditions (temperature) mentioned above for at least two weeks.

Calibration curves are constructed after analysis of six different concentration with each concentration replicated six times (n=6). Standard calibration curves were constructed by plotting absorbance value on Y-axis against concentration of lamivudine on X-axis. A model calibration curve of Lamivudine is shown in Fig.2. The slope, intercept, linearity range and correlation coefficient values in the regression analysis of data are given Table 1. Good linear relationships were observed between concentration and absorbance in each case with correlation co-efficient greater than 0.985. The method obeyed beer's law in the concentration range of 1-10µg/ml in both the solvents.

The limit of detection (LOD) (k=3.3) and limit of quantification (LOQ) (k=10) of the method were estimated according to the ICH definitions, (C_1=k.S_0/s) where C_1=LOD (or) LOQ, S_0 = standard error of blank determinations, s = The slope of standard curve, k = The constant related to the confidence interval. The standard errors of absorbance measurements for blank solutions were found to be 0.0008 and 0.0024 for the methods using water and 0.01M hydrochloric acid respectively. The LOD and LOQ values of the methods are given in Table 1. The LOD and LOQ were found to be 0.0448µg/ml and 0.13µg/ml in the case of water and 0.844µg/ml and 2.56µg/ml in the case of 0.01M hydrochloric acid respectively.

Accuracy of the method was tested by analysing three concentrations of Lamivudine in the linear range in 6 replicates on the same day and on 3 consecutive day's .The results are given

in Table 2. Accuracy was expressed as error (%).The error values were found to be in the range -0.8 to -1.6% indicating the high accuracy of the method.

Three tablet formulation of Lamivudine were assayed by the proposed method. The results are given in Table 3. The drug content estimated was in the range of 100±3% of the labeled content .As such the method was found suitable for rapid analysis of drug content in tablet formulations.

The recovery studies were carried out by spiking placebo (starch, lactose, talc and magnesium stearate mixture) and commercial tablets with lamivudine at 75% (7.5 µg/ml), 100% (10µg/ml), 125 % (12.5 µg/ml) of the standard solution concentration. The results are given in Table 3. The percentage recoveries of the three concentrations were found to be in the range 97--99.5% .The high percentage recoveries indicate no interferences from ingredients and excipients present in the formulations. The low error (%) values and high recovery values indicated that the method has high accuracy.

For precision three different concentrations of Lamivudine in the linear range were analysed in six independent series on the same day (intraday precision) and on three consecutive days (inter day precision). The data were evaluated using the calibration curve and the results are given in Table 2. The RSD values were in the range 0.92 to1.82%. The low intraday and inter day RSD values and also the low RSD values obtained from the analysis of tablets indicated that the precision of the method developed was good. The low RSD values of intraday and Interday precision indicated high repeatability of the method.

The robustness of the method was tested by making deliberate small changes in wave length and solvent concentration. For ruggedness the analysis were performed by different analyst (students) and in a different instruments (UV spectrophotometer). A tablet sample containing 10µg/ml was analysed six times. The results obtained under varying conditions were closed to those obtained under standard conditions (Table 4). Therefore the method developed is rugged or robust under small changes in experimental conditions.

Comparison with reported HPLC methods

For comparison the validation parameters of UV spectrophotometric method developed are compared with those of two reported HPLC methods in the literature for Lamivudine. The results of comparison of the methods are given in the Table 5. The results of comparison

indicated that the UV spectrophotometeric method developed is comparable to the HPLC methods with regard to Linearity, LOD, LOQ, accuracy, precision and recovery. In the case of UV spectrophotometeric method linearity was observed in a lower concentration range when compared to HPLC methods. LOD, LOQ, accuracy, precision and recovery parameters are almost similar in both UV spectrophotometeric and HPLC methods. In addition the method developed was sufficiently rugged and robust. The UV spectrophotometeric method developed was simple, rapid and less time consuming and cost effective when compared to HPLC methods for the routine analysis of Lamivudine in quality control testing.

CONCLUSION

The UV spectrophotometric method developed is based on measurement of absorbance at 280 nm in either water (or) 0.01 M HCl. The UV absorbance values were not affected by changing pH and temperature. The method obeyed beer's law in the concentration range of 1-10µg/ml in both the solvents. The UV spectrophotometeric method developed is comparable to the HPLC methods with regard to Linearity, LOD, LOQ, accuracy, precision and recovery. In the case of UV spectrophotometeric method linearity was observed in a lower concentration range when compared to HPLC methods. LOD, LOQ, accuracy, precision and recovery parameters are almost similar in both UV spectrophotometeric and HPLC methods. In addition the method developed was sufficiently rugged and robust. The UV spectrophotometeric method developed was simple, rapid and less time consuming and cost effective when compared to HPLC methods for the routine analysis of Lamivudine in quality control testing.

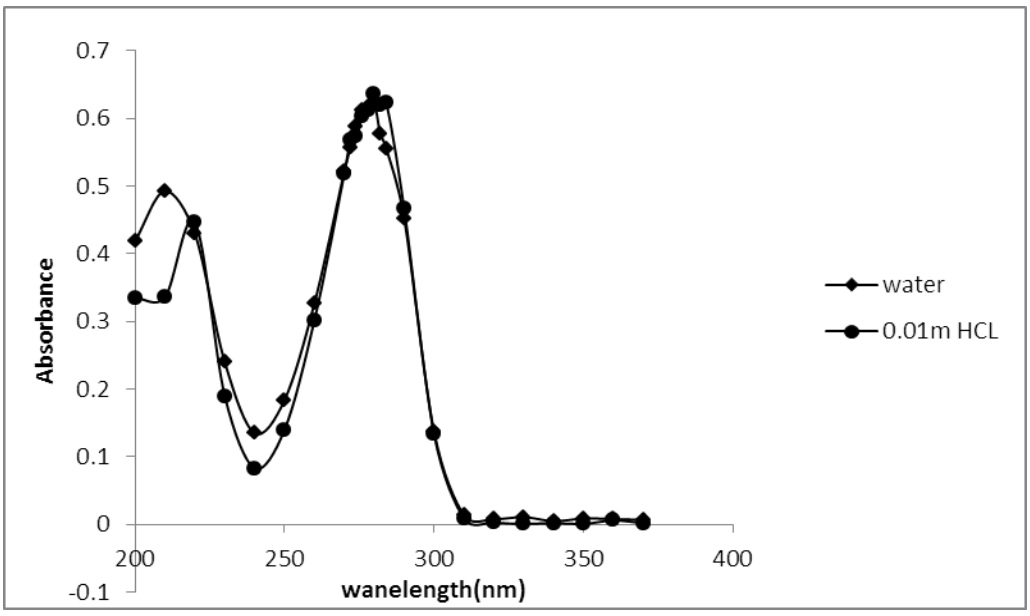

Fig.1: UV absorbance spectrum of Lamivudine in water and in 0.01 M HCl

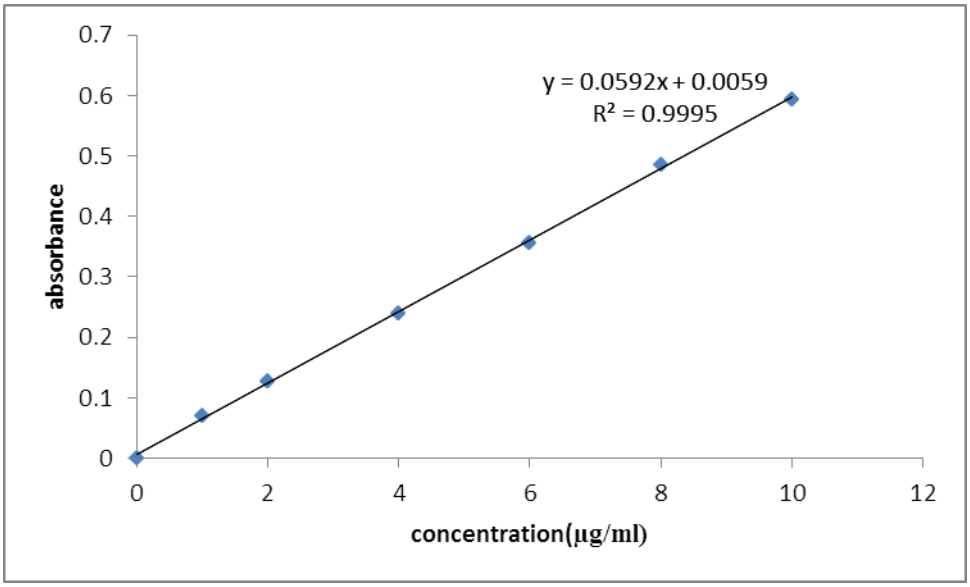

Fig .2: Calibration curve for the estimation of Lamivudine

Table 1: Validation Parameters of the U.V Spectrophotometric Method Developed (n=6)

Solvent	Slope (average)	Intercept (average)	Correlation coefficient (average)	Linearity range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
Water	0.0588 s.d:0.0003 s.e:0.0001	0.005 s.d:0.002 s.e:0.0008	0.9978	1-10	0.0448	0.13
0.01M HCl	0.0078 s.d:0.006 s.e:0.0027	0.007 s.d:0.0067 s.e:0.0024	0.983	1-10	0.844	2.56

Table 2: Precision and Accuracy of the Method (Intra and Interday)

Amount added (µg/ml)	Interday Amount found (µg/ml) Trials						Average	±SD	Accuracy (Error %)	Precision (%)
	1	2	3	4	5	6				
Day-1										
2.5	2.49	2.47	2.48	2.49	2.47	2.48	2.48	0.068	-0.8	1.02
5	5.25	4.8	4.9	4.8	4.9	4.9	4.92	0.152	-1.6	1.55
10	10	9.27	9.9	10.0	10.0	9.9	9.84	0.261	-1.6	1.82
Day-2										
2.5	2.52	2.48	2.49	2.48	2.49	2.49	2.49	0.089	-0.4	1.8
5	4.95	4.97	4.96	4.97	4.95	4.96	4.96	0.179	-0.8	1.3
10	10.1	9.8	9.9	9.8	9.9	9.7	9.86	0.124	-1.4	0.9
Day-3										
2.5	2.5	2.3	2.4	2.49	2.47	2.44	2.48	0.068	-0.8	1.02
5	5.25	4.8	4.9	4.8	4.9	4.9	4.92	0.152	-0.8	1.55
10	10	9.27	9.9	10.0	10.0	9.9	9.84	0.261	-1.6	1.82

Table 3: Estimation of Lamivudine in Dosage Forms

Lamivudine Tablets	Amount labeled (mg/tablet)	Amount estimated (mg/tablet)	Amount added (mg)	Amount found (mg)	Recovery (%)
Lamivir -1	100	99.2	10	9.9	99
Lamivir -2	100	97.8	10	9.7	97
Formulated tablets (F_1)	100	97.5	10	9.6	96
Formulated tablets (F_2)	100	98.4	10	9.8	98

Table 4: Results of Testing of Robustness and Ruggedness of the Method Developed

S.No	Condition	Mean±s.e	RSD (%)	Error (%)
1	Water	9.95±0.01	0.100	-0.5
2	0.01N HCl	9.98±0.03	0.300	-0.2
3	0.02N HCl	9.85±0.01	0.101	-1.5
4	Wavelength 280 nm	9,88±0.02	0.202	-1.2
5	Wavelength 278 nm	9.89±0.04	0.404	-1.1
6	Wavelength 282 nm	9.87±0.03	0.303	-1.3
7	Different Analyst	9.92±0.02	0.207	-0.8
8	Different Instrument	9.76±0.03	0.307	-1.4

Table 5: Comparison of the Validation Parameters of the UV Spectrophotometeric Method Developed and Reported HPLC Methods

S.No	Validation Parameter	UV Spectrophotometric Method	HPLC[28] Method-1	HPLC[29] Method-2
1	Linearity µg/ml	1-10	15-90	40-100
2	LOQ 10µg/ml	0.13 (water) 2.56 (0.01N HCl)	1.85	0.03
3	LOD 10µg/ml	0.0448 (water) 0.844 (0.01N HCl)	0.61	0.004
4	Accuracy (Error %)	-1.07	-0.386	1.4
5	Recovery (%)	99.17	99.85	99.61
6	Precision (%) (intraday)	1.02-1.82	0.006	0.301
7	Precision (%) (interday)	0.9-1.82	0.354	0.548
8	Ruggedness	Rugged	Rugged	Rugged
9	Robustness	Robust	Robust	Robust

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