Original Article

Development and Validation of UV/Visible Spectrophotometric Method for the Estimation of Lamotrigine in Bulk and Pharmaceutical Formulations

Arti Mohanr*[1] and S.K.Ghosh[2]

[1]Department of Pharmaceutical Sciences, Dibrugarh university, Dibrugarh, Assam-786004
[2]Maalla reddy Institute of Pharmaceutical Sciences, Secunderabad.

Address for Correspondence

Maalla reddy Institute of Pharmaceutical Sciences, Secunderabad.
E-mail: artishivliha @yahoo.co.in

ABSTRACT

Lamotrigine is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder A simple, sensitive, accurate and reproducible UV/visible spectrophotometric method was developed for the determination of Lamotrigine in bulk and pharmaceutical dosage forms. The solvent used was distilled water and wavelength corresponding to maximum absorbance for the drug was found at 304 nm. Drug obeyed beer's law in the concentration range of 20 – 100 µg/ml. with a correlation coefficient of 0.9992. The linear regression equation obtained was y=0.0073x+0.0081, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters such as Linearity, Accuracy, Precision and Robustness as per the ICH guidelines. The % recovery value which is close to 100% indicates reproducibility of the method and absence of interference of the excepients present in the formulation. The authors conclude that the proposed spectrophotometric method for the estimation of Lamotrigine can be used for routine analysis of Lamotrigine in bulk as well as in tablet dosage form.

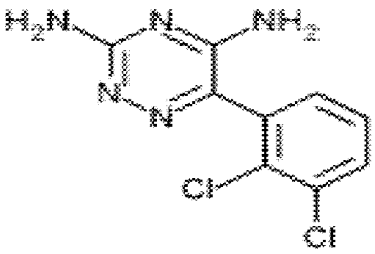
Keywords: Lamotrigine, Spectroscope, Absorbance, Wavelength.

INTRODUCTION

Lamotrigine is chemically 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine[1]. Lamotrigine is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder[2]. For epilepsy it is used to treat partial seizures, primary and secondary tonic-clonic seizures, and seizures associated with Lennox-Gastaut syndrome Chemically unrelated to other anticonvulsants (due to Lamotrigine being a Phenyltriazine). Lamotrigine has relatively few side-effects and does not require blood monitoring in monotherapy. Lamotrigine also acts as a mood stabilizer[3]. The recommended initial dosing begins at less than 1 mg for epilepsy. Generally, the

therapeutic range for epilepsy is 300mg to 500mg a day. Lamotrigine dosages are generally increased and decreased relatively gradually. A therapeutic response may require weeks or months of subsequent dose escalations, and very small differences in dosage often have noticeably different effects, much more so than with most other psychiatric medications; as little as 10% more or less may make a noticeable difference.

Previously reported spectro-photometric method as observed in literature survey gives a UV method which uses methanol as a solvent[4] which might not be suitable for oral dosage form. The present communication using distilled water as a solvent is therefore more convenient, accurate and reproducible.



Lamotrigine Structural Formula

MATERIALS AND METHODS

Instrumentation

A visible double beam spectrophotometer with a matched pair of 1 centimetre quartz cell was employed for measuring the absorbance of all the solutions.

Chemicals and Reagents

Lamotrigine was obtained as a gift sample from Organosis Ltd, Noida. (U.P.) and Analytical reagent grade Acetonitrile in distilled water was used as a cosolvent .

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10mg of Lamotrigine in 10 ml of AR grade acetonitrile and the volume was made up to 100 ml with distilled water. The final concentration of this stock solution being 100µg/ml

Determination of λ max

By appropriate dilution of standard stock solutions of Lamotrigine in distilled water containing 20µg/ml of Lamotrigine, dilutions were made and scanned on Shimadzu 160A a visible double beam spectrophotometer in the range of 200- 800 nm against distilled water as blank. Wavelength of maximum absorption was determined for drug. Lamotrigine showed maximum absorbance at 304 nm.

Preparation of standard solution

Stock solution samples were diluted with distilled water to prepare a series of concentration of 10–100µg/ml. The solutions were scanned and their absorbencies were measured at 304 nm using acetonitrile in distilled water as blank. All estimations were done in triplicate and the average values were reported.

Method validation

The method was validated for several parameters like Linearity, Accuracy, Precision, Robustness according to ICH guidelines[5,6].

RESULT AND DISCUSSION

Linearity

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analysed. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis. The drug showed

linearity in the range of 20 – 100µg/ml. with a correlation coefficient of 0.9992. The slope, intercept, correlation-coefficient and optical characteristics are summarized in Table 1and 2 and Figure 1.

Accuracy

Accuracy of the proposed method was determined using recovery studies. Accuracy was determined by spiking known amounts of the analyte into the placebo formulation (F1, F2 and F3) across the specified range of the analytical procedure to obtain 40, 50 and 60 µg/ml (80, 100 and 120%). At each level, solutions were prepared in triplicate and the accuracy was evaluated in terms of percent recovery. (Table 3)

Percent Recovery was calculated using the formula

[%Recovery = 100 x Mean Experimental Concentration/ Theoretical Concentration].

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. The precision of the assay method was determined by repeatability (intra-day) and intermediate precision (inter-day).The intraday precision was evaluated by analyzing six samples of 50µg/ml of the test concentration (n=6) at an interval of half an hour each.

Similarly interday precision was evaluated on two consecutive days (n = 12). Interday precision was evaluated by 3 samples at an interval of 1 hour on day 1 and 3 samples at an interval of 1 hour on day 2.The concentration of the drug was determined and the value of relative standard deviation (%R.S.D) of the assay method was calculated. The precision result showed a good repeatability with percent relative standard deviation less than 2. (Table 4 and 5)

Robustness

Robustness was determined by carrying out analysis by two different analyst and also by carrying out the analysis on two different instruments and the respective absorbance was noted and the results was indicated as SD. Four sample solutions each containing 50µg/ml were prepared and analyzed in two different U.V. visible spectrophotometers (Hewlett Packard 8453 and Shimadzu 160A) immediately after preparation. (Table 6)

CONCLUSION

The linear calibration curve was obtained at concentration range 20 – 100 µg/ml. with a correlation coefficient (0.9992), Slope (0.0073) and Intercept (0.0081).

The proposed method was reproducible because results obtained with in inter-day and intra-day were in acceptable limit. The results of assay and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of La,motrigine in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENT

I express my most cordial and humble thanks to my eminent guide respected guide Dr. S.K. Ghosh, HOD, Dept of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, for his constructive and meticulous guidance. And I am immensely thankful to Organosis Ltd, Noida. (U.P.) for providing me the bulk drug as gift sample for my Research work.

REFERENCES

1. Indian Pharmacopoeia (2010) 6th Edition, Ministry of Health and Family Welfare,pp. 1566 (India: Controller of Publications, Govt. of India).

2. Barbosa L, Berk m, Vorster M. A double-blind, randomized, placebo-controlled trial of augmentation with Lamotrigine or placebo in patients concomitantly treated with fluoxetine for resistant major depressive episodes *J Clin Psychiatry 2003;* 64 (4): 403–7.
3. Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs, *Nat Rev Neurosci 2004;* 5 (7): 553–564.
4. R.S. Talekar, A.S. Dhake, D.B. Sonaji AND V.K. Maurya. Spectro-photometric determination of Lamotrigine, *Indian J Pharm Sci* 2000; 62(1) :51-52.
5. ICH, Q2A validation of analytical procedure, Methodology International Conference on Harmonization, Geneva, October 1994.
6. ICH, Q2B Validation of analytical procedure, Methodology International Conference on Harmonization, Geneva, March;1996.

Table 1. Concentration and absorbance obtained for standard plot of Lamotrigine in distilled water

Sr. No.	Concentration in µg/ml	Absorbance
1	0	0
2	20	0.16
3	40	0.308
4	60	0.445
5	80	0.604
6	100	0.734

Table 2. Optimum conditions, optical characteristics and statistical data of the regression equation for Lamotrigine

PARAMETERS	VALUE
Absorption maximum (nm)	304
Beer's Law limit (mcg/ml)	20-100
Correlation coefficient	0.9992
Regression equation	Y=Ax-b
Slope(A)	0.0073
Intercept (b)	0.0081

Table 3. Percentage recovery for Lamotrigine according to the proposed method

S. No.	Initial Amount (mg)	Add of known qty of pure drug (to 100 ml of placebo formulation)	Total Theoretical drug concentration in µg/ ml	Mean Experimental drug concentration found in µg/ ml ± S.D.	% Recovery (±.S.D)
1	0 mg	4 mg	40	40 ± 0.03	100 ± 0.01
2	0 mg	5 mg	50	49 ± 0.10	98 ± 0.00
3	0 mg	6 mg	60	60 ± 0.00	100 ± 0.00

Table 4. Intraday Precision for Lamotrigine

Time in mins	Absorbance N=3	Total Theoretical drug concentration in µg/ml	Total Experimental drug concentration found in µg/ml
30	0.3731,0.3730,0.3731	50	49.99± 0.011
90	0.3732,0.3731,0.3731	50	50.00± 0.000
150	0.3731,0.3732,0.3731	50	50.00± 0.000

Table 5. Interday Precision for Lamotrigine

Time in mins	Absorbance N=3	Total Theoretical drug concentration in µg/ml	Total Experimental drug concentration found in µg/ml
30	0.3731,0.3730,0.3730	50	49.98 ± 0.02
90	0.3731,0.3731,0.3730	50	49.99± 0.01
150	0.3730,0.3731,0.3731	50	49.98± 0.01

Table 6. Robustness data for Lamotrigine

Sr.No.	Spectrophotometer 1 (Perkin Elmer Lambda 25 UV/VIS spectrometer)		Spectrophotometer 2 (Shimadzu 160A)	
	Abs	Conc	Abs	Conc
1	0.3795	50.88	0.3765	50.47
2	0.3789	50.80	0.3765	50.47

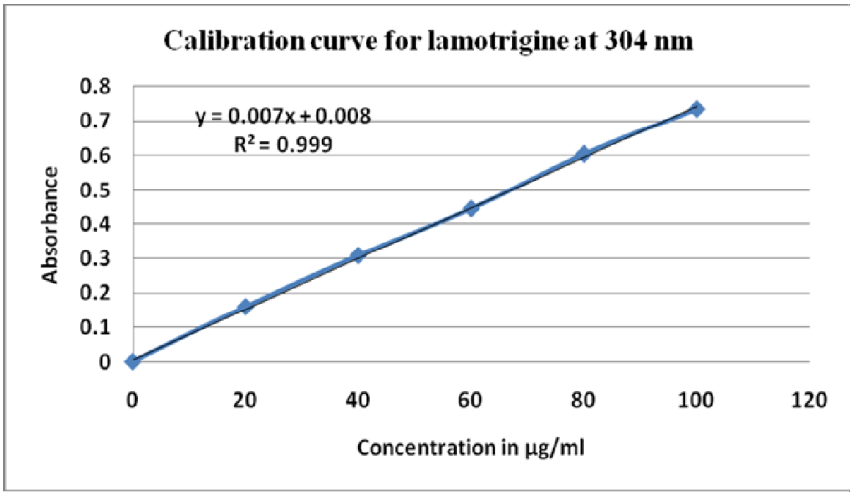


Figure 1. Calibration curve for Lamotrigine at 304nm