ISSN: 0975-766X
CODEN: IJPTFI
Available Online through ***Research Article***
www.ijptonline.com

SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF LAMOTRIGINE IN TABLET FORMULATION

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Received on 05-08-2011 *Accepted on 18-08-2011*

Abstract:

The objective of the current study was to develop a simple, precise and accurate Spectrophometric assay method and validated for determination of Lamotrigine in solid pharmaceutical dosage forms. Standard stock solution was prepared in methanol and distilled water in the ratio of 50:50. The λmax of Lamotrigine was found to be 308 nm. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 16-640 µg mL^{-1} with a correlation coefficient 0.9990. The precision (relative standard deviation- RSD) amongst six-sample preparation was 0.98 % for repeatability and the intermediate precision [RSD] amongst six-sample preparation was 0.79 %. The accuracy (recovery) was between 98.82 and 100.61 %.

Keywords: Lamotrigine, Assay method, Spectrophotometry, Development and Validation.

Introduction

Lamotrigine is chemically 6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine and its molecular formula is C9H7N5Cl2, and molecular weight is 256.09 gm/mole. Lamotrigine, an antiepileptic drug (AED) of the phenyltriazine class, is chemically unrelated to existing antiepileptic drugs, has been used successfully to treat essential trigeminal neuralgia (TN) [1-4].

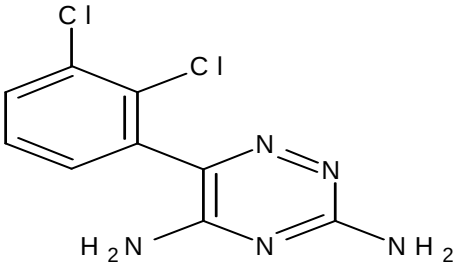

Figure-1: 6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine

Quantitative methods for the determination of Lamotrigine in tablets using Gas Chromatographic, High Perfomance Thin Layer Chromatography and HPLC methods for the determination of Lamotrigine in pharmaceutical dosage forms or in biological fluids are reported[5-10]. Gas chromatographic, HPTLC methods simply used for estimation of Lamotrigine from pharmaceutical formulation and HPLC methods also applied for the determination of Lamotrigine. So far to our present knowledge, no such validated stability indicating spectrophometric assay method for the determination of Lamotrigine in pharmaceutical formulation was available in literature. Moreover spectrophometric method can be the first choice of chromatographers among the High performance liquid chromatography; Reflectance Near-Infrared and Gas Chromatography methods. So, development is based on spectrophometric method. This paper deals with the validation of the developed method for the assay of Lamotrigine from its dosage form (tablets).

Experimental

Materials

Lamotrigine standard of was provided by Aarti Drugs Ltd., Boisar (India). Lamotrigine tablets containing 308mg lamotrigine and the inactive ingredient used in drug matrix were obtained from market. Analytical grade methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India).

Instrumentation

The spectrophotometer system used to perform development and validation of this assay method was UV-Visible double beam spectrophotometer PharmaSpec 1700 (Shimadzu, Kyoto, Japan) with matched quartz cells (1cm).

Diluent Preparation

Methanol and Water (50:50, v/v) used as a diluents.

Standard preparation

A lamotrigine standard stock solution containing 500µg/ml was prepared in a 50 ml volumetric flask by dissolving 25.00 mg of lamotrigine and then diluted to volume with methanol as diluents.

Further 2 ml of this stock solution in 25 ml volumetric flask and make up to mark with diluents. (Final concentration of standard solution is 40µg/ml)

Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 250 ml volumetric flask. About 50 ml diluents was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with methanol. The sample was filtered through 0.45µm nylon syringe filter. The concentration obtained was 500 µg/ml of lamotrigine.

Further take 2 ml this filtrate solution in 25 ml volumetric flask and make up to mark with diluents. The concentration obtained was 40 µg/ml of lamotrigine.

Method validation

Specificity study

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution.

Linearity

Seven points calibration curve were obtained in a concentration range from 16-64 µg/ml for lamotrigine. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y =29.75x-0.018 with correlation coefficient 0.9990.

Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of Lamotrigine test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition.

Accuracy

An accuracy study was performed by adding known amounts of Lamotrigine to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in duplicate.

Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the spectrophotometric conditions. The factors chosen for this study were the change in diluent composition [methanol-water (55: 45 and 45: 55, v/v)], and by different analyst study.

Solution stability

The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2 - 5° C and tested at interval of 12, 24, 36 and 48 hours. The responses for the aged solution were evaluated using a freshly prepared standard solution.

Result and discussion

The λ maxima of Lamotrigine in standard and test preparation was found to be 308 nm from its spectrum (Fig. a, b).

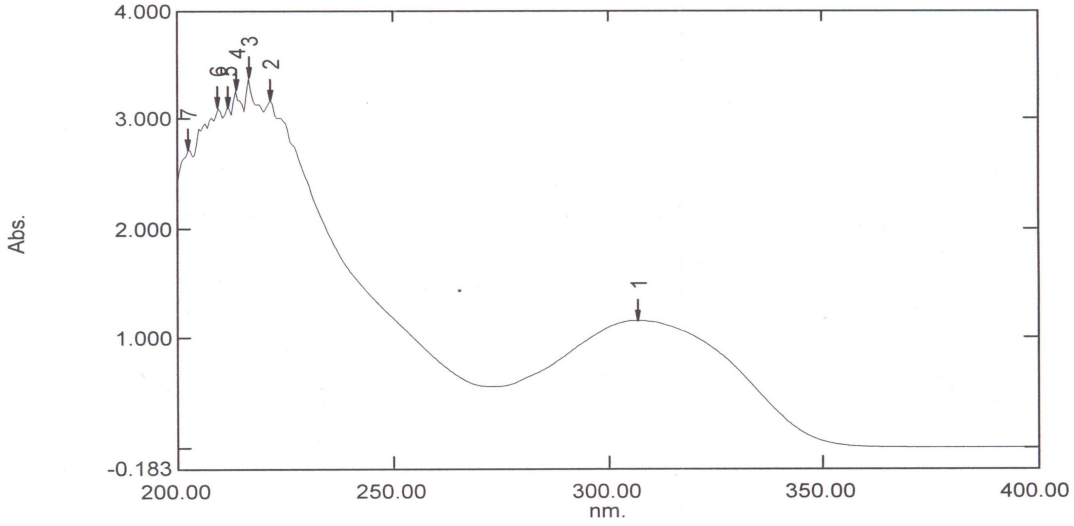

Fig. a: UV spectrum of Lamotrigine in Standard solution

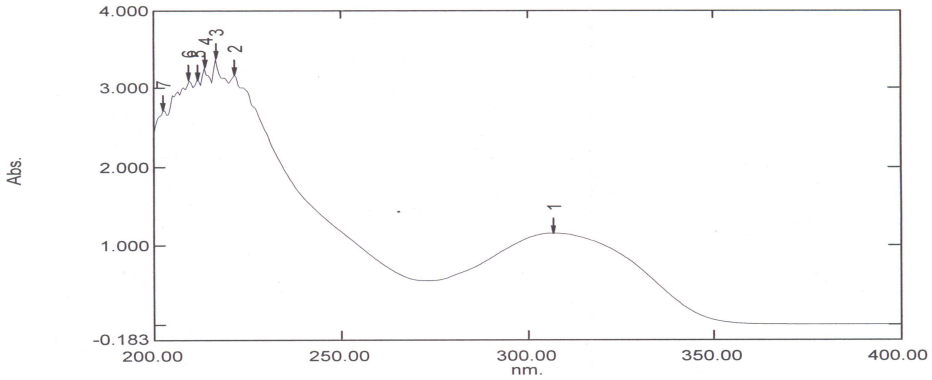

Fig. b: UV spectrum of Lamotrigine in Sample solution

Lamotrigine showed linear absorption from 16-64 µg/mL. The correlation coefficient (r) was found to be 0.9990.(Fig. c) The stability of solutions of formulation was determined by measuring the absorbance at 308 nm at periodic intervals. There was no considerable change in the absorbance at this wavelength up to 3 hours indicating that the solution was stable for at least 3 hours. Commercial formulations containing Lamotrigine were analyzed by proposed method. The precision (relative standard deviation- RSD) amongst six-sample preparation was 0.98 % for repeatability and the intermediate precision [RSD] amongst six-sample preparation was 0.79 % indicating that the method has required precision. The accuracy (recovery) was between 98.82 and 100.61 %. The validation results are presented in Table 1-4. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

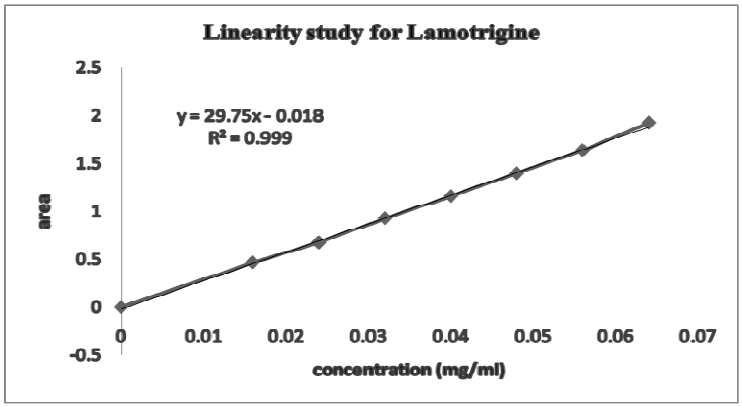

Fig. c: Linearity curve for Lamotrigine

Table-1: Evaluation data of precision study.

Set	Intraday (n = 6)	Interday (n = 6)
1	101.8	100.5
2	101.2	99.0
3	99.1	98.2
4	101.3	98.6
5	100.6	99.0
6	101.5	99.3
Mean	101.1	99.1
Standard deviation	0.99	0.78
% RSD	0.98	0.79

Table-2: Evaluation data of accuracy study.

Level (%)	Amount added concentration [a] (mg/ml)	Amount found concentration [a] (mg/ml)	% Recovery	% RSD
50	0.01960	0.01953	99.65	1.82
100	0.04040	0.03992	98.82	1.13
150	0.06067	0.06104	100.61	0.20

[a] Each value corresponds to the mean of three determinations

Table-3: Evaluation data of solution stability study.

Level (%)	Amount added concentration [a] (mg/ml)	Amount found concentration [a] (mg/ml)	% Recovery	% RSD
50	0.01960	0.01953	99.65	1.82
100	0.04040	0.03992	98.82	1.13
150	0.06067	0.06104	100.61	0.20

Table-4: Evaluation data of robustness study.

Level (%)	Amount added concentration [a] (mg/ml)	Amount found concentration [a] (mg/ml)	% Recovery	% RSD
50	0.01960	0.01953	99.65	1.82
100	0.04040	0.03992	98.82	1.13
150	0.06067	0.06104	100.61	0.20

Conclusion

A new analytical method has been developed to be routinely applied to determine Lamotrigine in pharmaceutical dosage form. In this study, the developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

Acknowledgements

The authors are thankful for facilities & grants given under UGC- Special Assistance Programme (DRS) Department Research Support (Sanction letter No. 540/6/DRS/2004 (SAP-I) Dt. 26/03/2004, and Department of Science & Technology New Delhi Fund for Improvement of Science & Technology (FIST) (Sanction letter No. SR/FST/CSI-072/2003 Dt. 24/12/2003 and Department of Chemistry, Saurashtra University, Rajkot – 360 005 (INDIA) and Department of Chemistry, KSKV Kachchh University, Bhuj – 370 001 (INDIA) for providing facilities.

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