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RP-HPLC Method Development and Validation of Lamotrigine

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Abstract

A simple, rapid and precise method was developed for the quantitative determination of lamotrigine in tablets. The method was based on RP-HPLC. Chromatographic separation was performed on a Supelco C18 (25cm X 4.6mm and i.d., 5µm) column using a mobile phase of methanol and 0.05 M potassium dihydrogen orthophosphate (65: 35v/v) adjusted the pH 4.5 with dilute orthophosphoric acid. The following system conditions were maintained throughout development and validation i.e., flow rate 1ml/min, column was maintained at room temperature and the detected by a UV-wave length at 270 nm. The lamotrigine was well resolved on the stationary phase and the retention time was 3.7 minute. The method was validated and shown to be linear for lamotrigine in 20-100 µg /ml. the correlation coefficient for lamotrigine is 0.9998 respectively. The method was validated for Precision, Accuracy, LOD and LOQ were determined to be 15ng/ml and 5 ng/ml respectively.

Keywords: Lamotrigine, RP-HPLC, Method development and validation.

Introduction

Lamotrigine [6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine] is a broad spectrum anti-epileptic drug, chemically different from other anti-convulsants. [1-3]

The mechanism of action of lamotrigine is inhibition of the release of excitatory neurotransmitters (aspartate and glutamate) and also involvement of the blocking of voltage dependent sodium channels. [4] Lamotrigine is effective for treatment of partial and generalized tonic, clonic seizures as a single drug or as an adjuvant with other anti epileptic drugs.[5] The aim of the present study was to develop and validate a simple, isocratic RP-HPLC method for the

determination of lamotrigine in tablets. The developed method was validated using ICH guidelines for validation. [6]

Results and Discussion

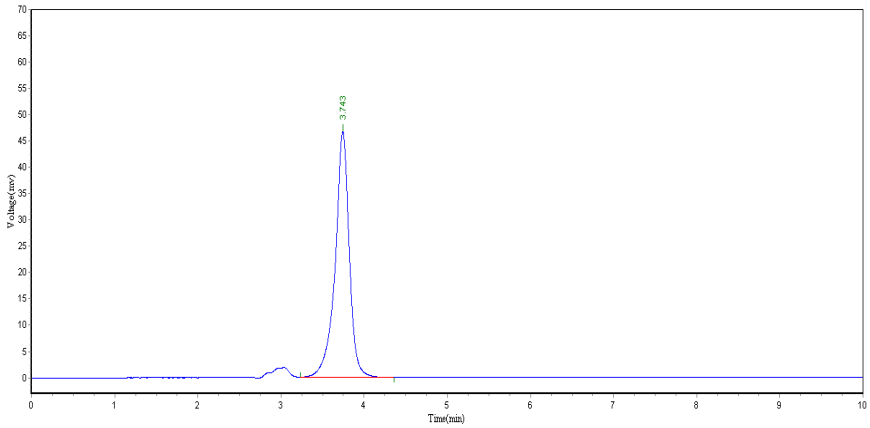
The lamotrigine is soluble in methanol. Different combinations of solvents were tried in order to separate them from mixed standards. Two different mobile phase were tried i.e Methanol:Water (70:30), Acetonitril: Ammonium acetate buffer 0.05M (80:20) having pH 5. Satisfactory separation was obtained with the mobile phase of Methanol: Potassium dihydrogen ortho phosphate (65:35v/v) adjusted the pH 4.5 with dilute orthophosphoric acid. The retention time of lamotrigine was found to be 3.74 min (Fig: 1).The detection of lamotrigine was carried out at 270 nm as its UV spectra showed appreciable absorbance at this wavelength. The regression equation obtained for lamotrigine was Y=9618.2x-13964 (r = 0.9998) in the concentration range of 20-100µg/ml (Fig: 2).The correlation coefficient value shows that the method is linear. System suitability tests were carried out as per USP XXIV [7] on freshly prepared standard stock solutions of lamotrigene and parameters obtained are summarized (Table 1).

Intra and inter-day precision studies were carried out and results show that the method is reproducible. Limit of detection and limit of quantification were found to be 15 ng/ml and 5 ng/ml (Table 1) respectively. The results obtained by the proposed method were close to the label claim of the drug (Table 2).The low value of the standard deviation indicates that the method is accurate. To study the accuracy of the proposed method, recovery experiments were also carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at three different concentrations. The values of percentage recovery showed that the proposed method is accurate (Table 3). Hence the developed method is accurate, precise, repeatable and reproducible and can be used for routine analysis of lamotrigine.

Table: 1Validation and System Suitability Parameters

Parameters	Lamotrigine
Retention time	3.74
Asymmetry	0.766
Resolution	1.832
Theoretical plates	2802
Calibration range	20-100µg/ml
Limit of detection	0.015µg/ml
Limit of quantification	0.005µg/ml

Fig. 1: Typical chromatogram of Lamotrigine



Chromatogram showing well resolved peak of Lamotrigine

Fig. 2: Linearity curve

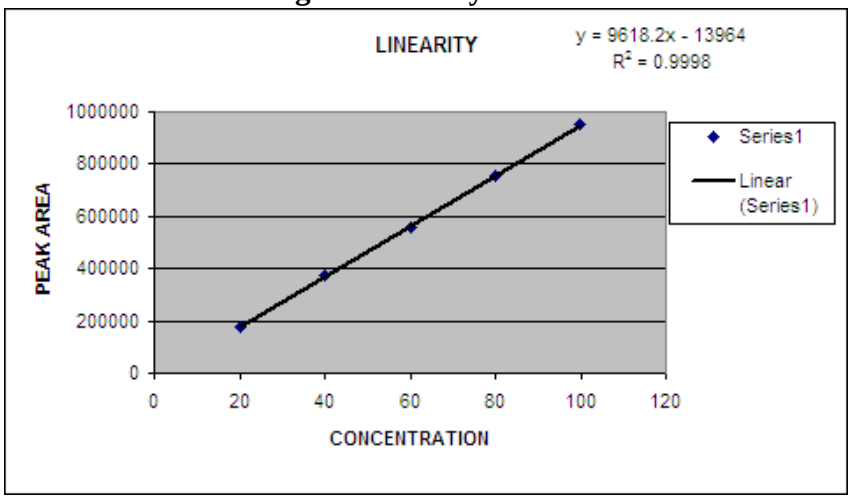


Table: 2

Analysis of Lamotrigine in Tablets			
Formulation	Label amount (mg)	Amount Found (mg)*	% of drug found ± SD*
Tablet	100	98.54	98.54 ± 0.82

*Mean value ± standard deviation of three determinations; Tablets is lamitor-DT, manufactured by Torrent Pharma Ltd., Ahmedabad. It contains 100mg of lamotrigine.

Table: 3. Recovery Studies

Amount of sample taken µg/ml	Amount of standard drug added	Amount of drug recovered	% drug recovered
60	0	59.52	99.2
60	0	59.88	99.8
60	0	60.06	100.1
60	10	69.38	99.1
60	10	70.03	100.04
60	10	69.72	99.6
60	20	79.52	99.4
60	20	79.17	98.96
60	20	79.62	99.15
60	30	89.26	99.17
60	30	89.61	99.56
60	30	89.06	98.95

Materials and Methods

Lamotrigine as the reference standard was provided by Torrent Pharma as a gift sample. The chemicals of analytical reagent grade purchased from various sources. All solvents for analysis including water for HPLC grade obtained from fisher Quallizene and Merck.

Instrument

The liquid chromatographic system used was an isocratic HPLC Shimadzu system consisting of pump (LC-10ATVP, Shimadzu, Japan), UV-Vis detector (SPD-10A Shimadzu) equipped with chromtech N2000 software and a Rheodyne(7725i) sample injector fitted with a 20µl sample loop. The chromatographic separation was carried out on Supelco C18 (250X4.6 mm i.d. 5µm) column. The mobile phase was 0.05M Potassium dihydrogen ortho phosphate –Methanol (35:65 v/v) adjusted to pH 4.5 with dilute ortho phosphoric acid and filtered with whatman filter paper. All the separations were performed isocratically at a flow rate of 1ml /min at room temperature. The peak area was determined using a UV detector at a wave length of 270nm.

Preparation of standard stock solution

Standard stock solutions of the drug were prepared by dissolving 25 mg of lamotrigine in mobile phase and the volume was made upto 25ml with the mobile phase to get the solution concentration1000µg/ml, the solution was filtered through whatman filter paper (No.1) and further diluted to obtained final concentration of 100µg/ml. Calibration curves were prepared by taking appropriate aliquots of standard lamotrigine stock solutions in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration of 20, 40, 60, 80 and 100 µg/ml. standard solutions (n=5), the sample solution was injected through 20µl loop and chromatograms were obtained using 1.0ml/min flow rate. The effluent was monitored at 270nm. Calibration curve was constructed by plotting Area under the curve versus concentration and regression equation was computed.

Preparation of working standard solution

Ten tablets were weighed accurately and finely powdered. Tablets powder equivalent to 100mg lamotrigine was taken in 50ml volumetric flask and the volume was made up to the mark with mobile phase. Further, 1ml of this solution was diluted to 10 ml with mobile phase to obtained final concentration of 60 µg/ml. The solution was filtered using whatman filter paper (No.1), and was sonicated for 15 min. Sample solutions were chromatographed (n=3), and concentration of lamotrigine in tablet samples were calculated using regression equation.

Conclusion

The proposed developed methods is most economical, simple, sensitive, précise and accurate. It can be used for routine determination of lamotrigine in bulk as well as in tablet formulation.

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