(Research Article)

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"RP-HPLC Method Development and Validation of Lamotrigine in Tablet Dosage Form"

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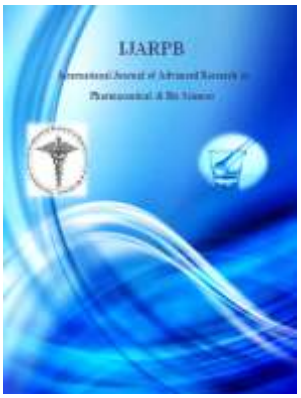

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ABSTRACT

A simple, rapid and precise method was developed for the quantitative determination of Lamotrigine in tablet dosage from. The method was based on RP-HPLC. Chromatographic separation was performed on a fortis C18 (150mm X 4.6mm and equivalent 5µm) column using a mobile phase of acetonitrile (ACN) and phosphate buffer (0.05mol) ratio 20:80 at pH 2.5 adjusted with dilute orthophosphoric acid. The following system conditions were maintained throughout development and validation i.e., flow rate of mobile phase 1ml/min, column temperature at 40˚C and the detecting of drug by a UV-wave length at 270 nm. The Lamotrigine was well resolved on the stationary phase and the retention time was 5.1 minute. The method was validated the drug was shown to be linear for drug in 10-30 µg /ml. the correlation coefficient (r^2) for the drug was 0.998. The Precision, Accuracy, LOD and LOQ were determined to validate the method.

KEY WORDS: Lamotrigine, RP-HPLC, Method development, Validation and C18 Column.

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INTRODUCTION

Lamotrigine [6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine] is a broad spectrum antiepileptic drug, chemically different from other anti-convulsants.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[1,3]. Lamotrigine is effective for treatment of partial and generalized tonic, chronic seizures as a single drug or as an adjuvant with other anti epileptic drugs[4].

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 LC solution 2010 software and a sample injector fitted with a 20µl sample loop. The chromatographic separation was carried out on Fortis C18 (150cm X 4.6mm and equivalent 5µm) column. The mobile phase was 0.05M Potassium dihydrogen ortho phosphate: ACN (80:20 v/v) adjusted to pH 2.5 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.

MATERIALS AND METHODS

Lamotrigine as the reference standard was provided from Torrent Pharma. 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.

Experiment

Preparation of solutions

Buffer Preparation

1.36g of potassium dihydrogen Orthophosphate was dissolved in l000ml water 1 ml triethaylamine(TEA) and pH 2.5 was adjusted with orthophosphoric acid and mixed well.

Diluent preparation

Water: Methanol in the ratio of (40:60) v/v was used.

Standard Preparation (20ppm)

Accurately 40.0 mg of Lamotrigine was weighed & transferred into volumetric flask of 100ml. About 60 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol and mixed. 5 ml of this solution was diluted to 100ml with diluent (water: methanol 40:60) and mixed.

Sample Preparation (20ppm)

10 tablets were weighted accurately and transfer into volumetric flask 500ml, about 250ml of Methanol was added and sonication was performed for 40minutes with vigorous shaking. After the complete dissolution of tablets, the solution was brought to room temperature and the volume was made up with methanol. 5 ml of this solution was diluted to 250 ml with diluent and mixed. Filter the above solution through 0.45µ filter.

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Selection of detection wavelength

Lamotrigine showed the absorbance at 270nm. So the wavelength selected for the determination of Lamotrigine was 270nm as shown in figure no. 1.

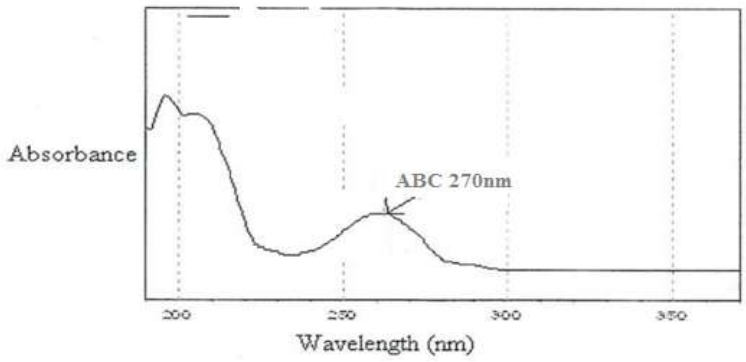

Figure No. 1: UV spectra of LamotriginePreparation of Standard Curve:

Preparation of Standard Curve

Linearity was determined at eleven levels over the range of 10 ppm to 30 ppm. A standard stock solution was prepared and further diluted to attain concentrations of 10ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm and each preaparation was injected in duplicates. The mean area at each level was calculated and a graph of mean area versus concentration in mcg/ml was plotted as shown in Table no. 1 & figure no.2. The correlation co-efficient, y-intercept, slope of regression line and residual sum of squares were calculated and recorded.

Table No. 1 Preparation of Linearity solution for ABC

S. No.	Linearity Level (W.r.t)	Actual Conc. in ppm	Mean Peak Area
1	50%	10	681480
2	75%	15	1016084
3	100%	20	1271759
4	125%	25	1626457
5	150%	30	1934250

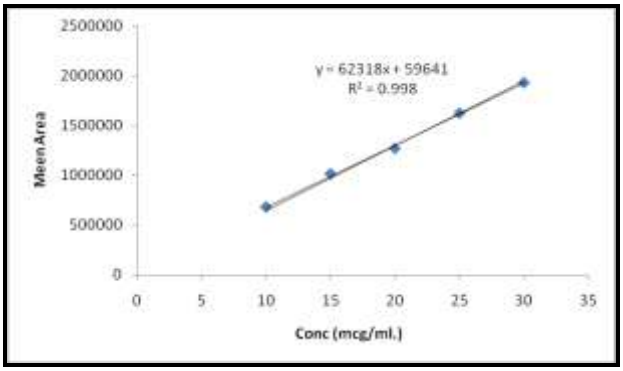

Figure No 2: Linearity curve of Lamotrigine

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PROCEDURE

Blank preparation was injected in RP-HPLC. Standard preparation was run and chromatograms were recorded. The chromatograms were checked for compliance of system suitability test and necessary changes were made to meet the system suitability requirements. Several trails were taken following this procedure and the results are shown as in table no. 2 and figure no. 3.

Table No 2: Observation for method development for lamotrigine.

Sr. No.	Trials	Observation	Remarks
1	Buffer : MeOH (85:15 % v/v) Flow rate 1.0 ml/min Column:- Fortis C18, 150mm x 4.6mm; 5µ Buffer:- pH 2.5 KH_2PO_4 Buffer	Tailing, High Retention time	Not Satisfactory
2	Buffer : MeOH (85:15 % v/v) Flow rate 1.0 ml/min Column:- Fortis C18 150mm x 4.6mm; 5µ Buffer:- pH 2.5 KH_2PO_4 Buffer	Retention time satisfactory but low theoretical plate	Not Satisfactory
3	Buffer : ACN (90:10%v/v) Flow rate 1.0 ml/min Column:- Fortis C18 150mm x 4.6mm; 5µ Buffer: - pH 2.5 KH_2PO_4 Buffer	High Retention time.	Not Satisfactory
4	Buffer : ACN(85:15%v/v) Flow rate 1.00 ml/min Column:- :-Forts C18 150mm x 4.6mm; 5µ Buffer: - pH 2.5 KH_2PO_4 Buffer	High Retention time.	Not Satisfactory
5	Buffer: CAN (80:20 %v/v) Flow rate 1.2 ml/min Column:- :-Fortis C18 150mm x 4.6mm; 5µ Buffer: - pH 2.5 NaH_2PO_4 Buffer	Peak Shape was good , Retention time 5.10 min.	Satisfactory

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FINAL TRIAL

Column : Fortis, (150mm x 4.6mm), 5µm or equivalent
Flow rate : 1.0 mL/minute
Detector : UV
Detector wavelength : 270 nm
Injection volume: 20µL
Run time : 10 minute
Diluent : Water: Methanol (40:60) v/v
Mobile phase : Buffer: Acetonitrile: (80:20) v/v

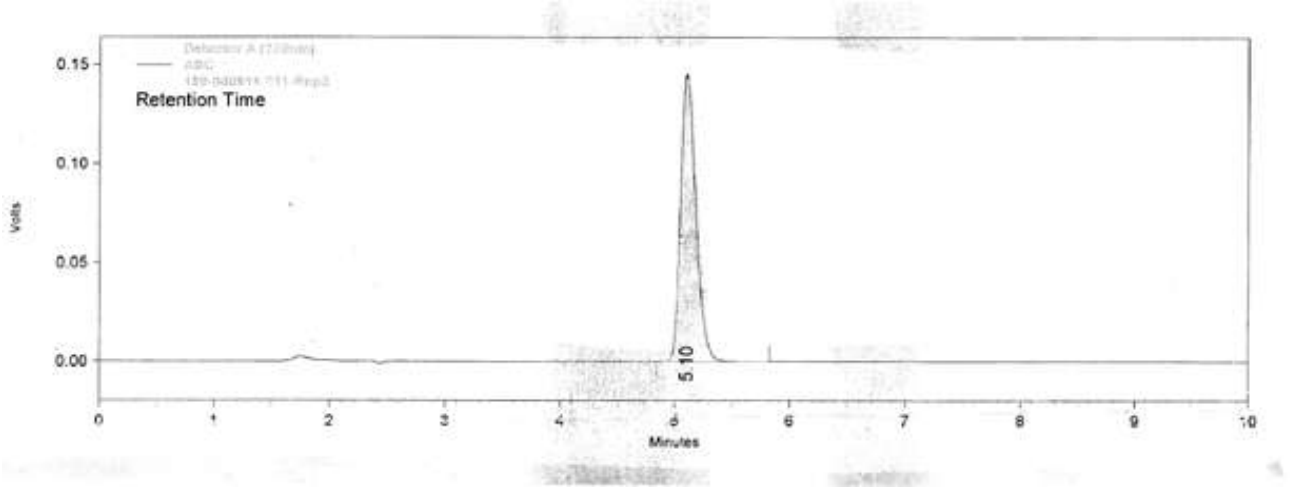

Figure No 3: Chromatogram of Lamotrigine Standard

RESULTS AND DISCUSSION

Method optimization results

Selection of proper column

Fortis, (150cm x 4.6mm), 5µm or equivalent

Selection of chromatographic conditions

Optimized chromatographic conditions for estimation of Lamotrigine finalized as shown in Table No.2.

Table No 2: Optimized chromatographic conditions

Column	Fortis,(150 x 4.6mm),5µm or equivalent
Flow rate	1.0 mL/minute
Detector	UV
Detector wavelength	270 nm

Injection volume	20µL
Run time	10 minute
Diluent	Water: Methanol (40:60) v/v
Mobile phase	Buffer : ACN: (80:20) v/v

The trails with Buffer: Methanol shows hat the peak for lamotrigine drug was resolved in a single HPLC trial run. In gradient start with gradient of Buffer: Methanol it was observed that the tailing & peak produced were not too good, thus the mobile phase was changed. In gradient use ACN at this time peak was satisfactory but retention time was around 9 min. As the ACN was increased in gradient system, good & sharp peak at 5.10 min. were obtained. The assay of lamotrigine tablet was done by this method and 99.17% result was obtained.The optimized chromatographic conditions and validation parameters are given below in table no. 3.

Table No 3: Result of different parameter

Parameter		**Drug**
Specificity		Specific
Linearity	Regression equation y=mx+c	y = 62318x+59641
	Slope	62318
	Intercept	59641
	Correlation coefficient (r^2)	0.998
Accuracy (Recovery) n=6	Level 1	99.9
	Level 2	100.3
	Level 3	98.2
Precision Method precision (Repeatability)		99.3
Intermediate Precision (Ruggedness)		99.4
Robustness (% RSD), n=3		< 2
System Suitability		1.1%

Accurately spiked known quantity of sample in the placebo and the actual quantity recovered were determined to measures the accuracy of the method. In similar manner the placebo was injected and compared with standard to determine the specificity interference with standard drug.

There was no interference occurred. This measured the ability of the method to access the analyte specifically in the presence of excipients and other degradants.

In precision the system precision and method precision were determined. The system precision there was repetition of injection with similar batch tablet and observation of the response. The method precision there was repetition of injection from the different batch tablet and observation of the response. In this manner the method precision and system precision was performed. In the study linearity and range were also observed, in which the linear relation between the concentration and the result were found satisfactory.

CONCLUSION

Based on the results, obtained from the analysis of forced degraded samples using described method, it can be concluded that there was no other co-eluting peaks of interference from excipients and degradation products due to variable stress components with the main peaks. The method is specific for the estimation of Lamotrigine in presence of degradation products. The proposed method shows good agreement with all validation parameters. The optimized method is precise, accurate and robust and so it can be applied as stability indicating for the estimation of Lamotrigine in tablet dosage form. The developed method can be used for the analysis of routine quality control test. In the present work the RP-HPLC method for the estimation of Lamotrigine in solid dosage form has been developed. The proposed method is simple, precise and accurate and do not suffer from any interferences due to common excipients. The newly developed methods can be used in pharmaceutical industry for routine quantitative estimation of Lamotrigine 20mg, 50mg, 100mg, 250mg in tablet dosage form. The developed method was validated as per ICH guidelines and can be used for the analysis of routine quality control sample.

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