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QUANTIFICATION AND ANALYTICAL VALIDATION OF LAMOTRIGINE BY LIQUID CHROMATOGRAPHIC METHOD THROUGH ISOCRATIC SEPARATION

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Abstract

Introduction: A reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Lamotrigine in pharmaceutical dosage forms. **Materials and methods:** The chromatographic separation of Lamotrigine was achieved on a Luna C_{18} column (250mm×4.6 mm, 5µm particle size), Agilent LC1220 HPLC system with UV detection (VWD detector) at 270nm. The optimized mobile phase was consisted of Methanol: 0.05M Potassium dihydrogen phosphate buffer (P^H adjusted to 4.5 with diluted formic acid) (60:40 v/v). The flow rate was 1ml /min and effluents were monitored at 270nm. Chromatogram showed the main peak at a retention time of 2.977min. **Results:** The method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and ruggedness. The linearity was found in the concentration range of 10-120µg/ml. The Correlation coefficient was 0.999. The regression equation was found to be Y = 12632x+12440. The limit of detection and limit of quantitation for estimation of Lamotrigine was found to be 0.09 µg / ml and 0.28 µg / ml respectively. **Conclusion:** Recovery of Lamotrigine was found to be in the range of 99.7-100.08%. Proposed method was successfully applied for the quantitative determination of Lamotrigine in pharmaceutical dosage forms as per ICH guidelines.

Keywords: Lamotrigine, RP-HPLC, VWD Detector, ICH Guidelines.

INTRODUCTION

Lamotrigine is a member of sodium channel blocking class of antiepileptic drugs used in the treatment of patients with epilepsy and bipolar disorder (Reid et al., 2013). Lamotrigine is chemically known as 6-(2, 3-dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine was shown in Figure 1.

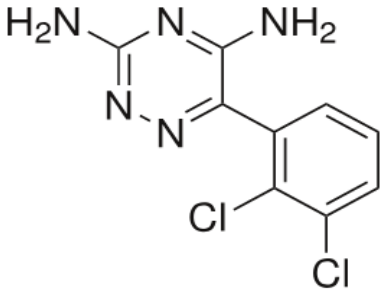

Figure 1: Chemical Structure of Lamotrigine

Literature review tells that very few analytical methods have been reported for the determination of Lamotrigine which includes Quantitative determination of lamotrigine in bulk and dosage form by UV Spectrophotometry (Navdeep et al., 2011).

Development and Validation of UV/Visible Spectrophotometric Method for the Estimation of Lamotrigine in Bulk and Pharmaceutical Formulations (Arti et. al., 2013). Spectrophotometric Estimation of Lamotrigine in Tablets (Jayanna et. al., 2016). Development and validation of a new HPLC method for determination of Lamotrigine and clinical application (Jebabli et al., 2015). A new simultaneous RP-HPLC method for development and validation of Lamotrigine tablets (Vidya Sagar et al., 2011). Development and validation of a new HPLC method for determination of lamotrigine and related compounds in tablet formulations (Emami et al., 2006). Development and validation of spectrophotometric, TLC and HPLC methods for the determination of lamotrigine in presence of its impurity (Youssef et al., 2007). Development of a validated stability indicating LC method for lamotrigine (Srinivasulu et al., 2009). Rapid HPLC analysis of the antiepileptic lamotrigine and its metabolites in human plasma (Saracino et al., 2007).

Development and validation of a stability indicating HPLC assay method for determination of Lamotrigine in tablet formulation (Vijay et al., 2011). A Simple Development and Estimation of Lamotrigine Tablets by HPLC (Selvadurai, 2012). Rapid and sensitive LC-MS/MS method for quantification of lamotrigine in human plasma: application to a human pharmacokinetic study (Hotha et al., 2012). Liquid chromatography tandem mass spectrometry method for the estimation of lamotrigine in human plasma: Application to a pharmacokinetic study (Santosh et al., 2013). Estimation of Lamotrigine in human plasma by LCMS/MS (Srinivasa et al., 2016). The present study was aimed to develop a novel, simple, economic and validated RP-HPLC method for the estimation of Lamotrigine according to ICH guidelines (Shabir, 2003).

MATERIALS AND METHODS

Chemicals and Reagents:

Lamotrigine bulk drug were kindly provided as gift sample by Smilax Labs, Hyderabad, India. Potassium dihydrogen phosphate (Merck Chemical Company, AR-Grade), Formic acid (Merck Chemical Company, AR-Grade), Water (Merck Chemical Company, HPLC-Grade) and Methanol (Merck Chemical Company, HPLC-Grade) were used in the study. Lamictal® tablet contain Lamotrigine 50mg is obtained from a local pharmacy manufactured by Glaxo smithkline Pharmaceuticals Ltd., India.

Instrumentation:

The chromatography was performed on Agilent LC1220 HPLC system, equipped with VWD detector and EZ Chrome software, Luna C_{18} column (250mm×4.6 mm, 5µm particle size) was used as stationary phase. All weights were taken on electronic balance (Model: CA123, Make: Contech), pH Meter (Model: 3 Star, Make: Global) and Sonicator (Model: UCB 70, Make: Life care) were used in the study.

Chromatographic Conditions:

In this work a reverse phase Luna C_{18} column with 250 × 4.6 mm i.d. and 5 µm particle size was chosen as stationary phase and mobile phase consisting of mixture of Methanol: 0.05M Potassium dihdrogen phosphate buffer (P^H adjusted to 4.5 with diluted formic acid) (60:40 v/v) was delivered at a flow rate of 1.0ml/min and detector wavelength at 270 nm. Injection volume was 20µl. The run time was 5min and the retention time of Lamotrigine was found to be 2.977 min.

Chromatographic Parameters:

Equipment	: Agilent LC1220 HPLC system, equipped with VWD detector
Column	: Luna C_{18} (250mm×4.6 mm, 5µm particle size)
Flow rate	: 1ml/min
Wavelength	: 270 nm
Injection volume	: 20 µl
Column oven	: Ambient
Run time	: 5 Minutes

Preparation of Mobile Phase:

Solution A: Accurately weighed about 6.8g of Potassium di-hydrogen phosphate (KH_2PO_4) was taken into 1000ml beaker and dissolved to 1000ml with HPLC grade water and degassed in ultrasonic water bath and filtered through 0.45µm filter using vacuum filtration and the pH was adjusted to 4.5 with diluted formic acid.

Solution B: Methanol HPLC-Grade

Mobile Phase: Volume of Solution (A) and solution (B) taken in ratio 40:60 (v/v) and mixed well and filtered through 0.45µm membrane filter and degas for 10 minutes.

Preparation of Diluent:

Mobile phase was used as diluent.

Preparation of Standard Stock Solution:

An accurately weighed quantity of Lamotrigine 50mg was transferred to 50ml volumetric flask, dissolved in 50ml distilled water, the final volume was made with distilled water to obtain standard solution having concentration of 1000µg/ml. These stock solutions were used to prepare further dilutions.

Preparation of Sample Solution:

Lamotrigine is available as tablets containing 50mg of Lamotrigine. Lamotrigine is available in the local market with brand names LAMICTAL® (50mg, Glaxosmithkline Pharmaceuticals Ltd., India). Twenty tablets of Lamotrigine were taken and made into a fine powder of the tablets and the powder equivalent to 50mg of Lamotrigine was weighed accurately and transferred into a 50ml standard volumetric flask. The contents were dissolved in mobile phase and sonicated for 30 Minutes. This entire solution was filtered through 0.45 micron Whatmann filter paper (No. 41) and the final solution was made with mobile phase to get the solution of 1000µg/ml. 10ml of this solution was transferred to 100ml volumetric flask, volume was made with methanol. It gives 100 µg/ml. 4ml of the solution were pipetted out separately into 10ml volumetric flask and make up to the mark to give 40µg/ml concentration. The sample solution 20µl was injected and chromatographed and the peak areas were measured for Lamotrigine was shown in Figure 2 and 3 respectively. The % Assay was calculated by comparing the peak area of standard and sample chromatogram by using the formula given below and the assay result was shown in Table 1.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Average peak area of sample preparation

AS = Average peak area of standard preparation

WS = Weight of standard taken in mg

WT= Weight of sample taken in mg

P = Percentage purity of working standard

DS= Dilution factor for standard preparation

DT=Dilution factor for sample preparation

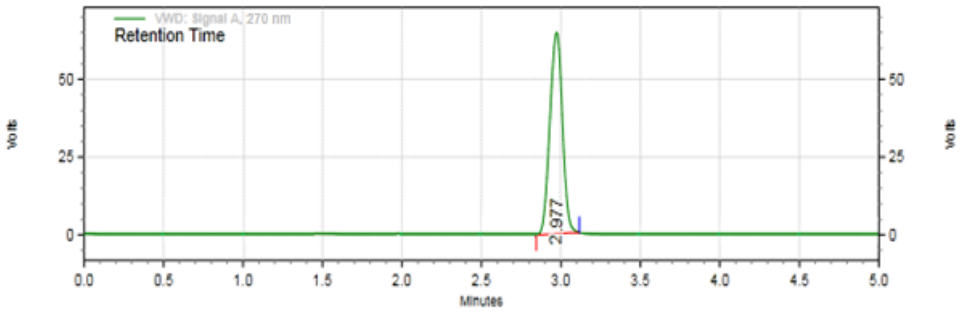

Figure 2: Standard Chromatogram of Lamotrigine

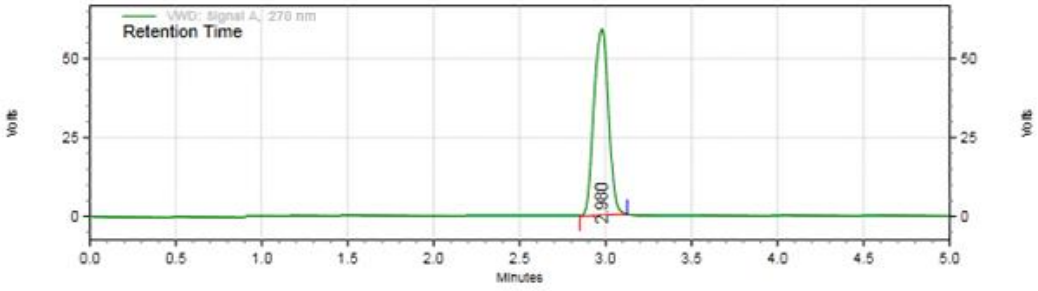

Figure 3: Sample chromatogram of Lamotrigine

Table 1: Assay of Marketed Formulation of Lamotrigine

Drug	Lamictal® Label Claim (mg)	Amount Found (mg)	% Label Claim ± % RSD (n=3)
Lamotrigine	50	49.99	99.98±0.17

RESULTS

Optimization of RP-HPLC Method:

For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Methanol :

Phosphate buffer (60:40 v/v), pH was adjusted to 4.5 with diluted formic acid using Luna C_{18} (250mm×4.6 mm, 5µm particle size).

System Suitability:

At first the HPLC system was optimized as per the chromatographic conditions. One blank followed by six replicates of a single calibration standard solution of 40µg/ml of Lamotrigine was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates and peak asymmetry were taken and results were presented in Table 2.

Table 2: System Suitability Test Parameters for Lamotrigine

Parameter (n=6)	Lamotrigine
Retention Time (Mins)	2.977
Theoretical plates	4672
Tailing factor	1.02

Specificity:

The effect of excipients and other additives usually present in the dosage form of Lamotrigine in the determination under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the blank and placebo solution into the HPLC system. The representative chromatogram of blank and placebo was shown in Figure 4 and 5 respectively.

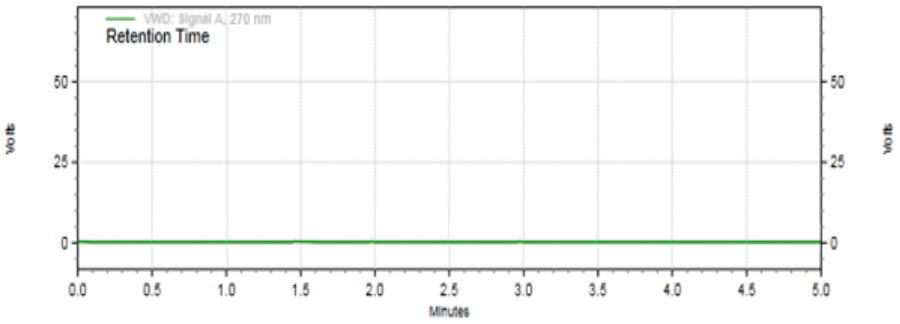

Figure 4: Chromatogram of Blank

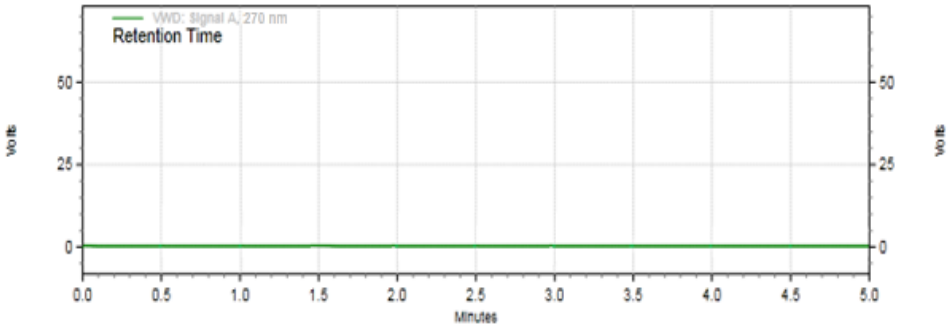

Figure 5: Chromatogram of Placebo

Linearity:

Linearity was performed by taking from stock solution (1000µg/ml) aliquots of 0.1, 0.2, 0.4, 0.8,1 and 1.2 ml were taken in 10ml volumetric flasks and diluted up to the mark

with diluent such that the final concentrations are in the range of 10-120 µg/ml. Each of these drug solutions (20µl) was injected into the chromatographic system for three times. The peak area and retention time were recorded and the mean values of peak areas were plotted against concentrations. The linearity data is presented in Figure 6 and Table 3. Acceptance Criteria: Correlation coefficient should be not less than 0.999.

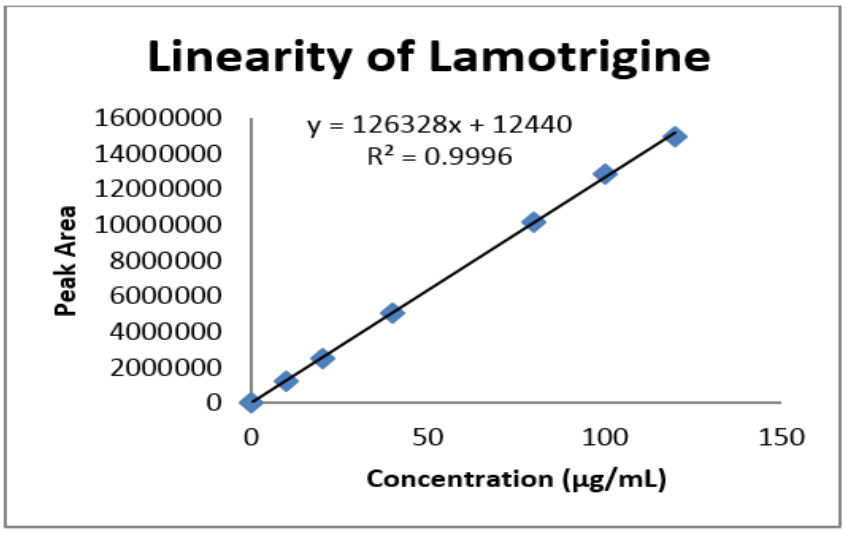

Figure 6: Linearity Graph of Lamotrigine

Table 3: Linearity Data for Lamotrigine

Linearity of Lamotrigine	
Concentration (µg/ml)	**Peak Area**
10	1262166
20	2520648
40	5043721
80	10189480
100	12831974
120	14980467

Accuracy Studies:

The accuracy of the method was determined by calculating recovery of Lamotrigine by the method of standard addition. Known amount of standard solution of Lamotrigine at 50%, 100% and 150% was added to a pre quantified sample solution and injected into the HPLC system. The mean percentage recovery of Lamotrigine at each level was calculated and the results were presented in Table 4. Acceptance Criteria**:** The % Recovery for each level should be between 98.0 to 102.0%.

Table 4: Recovery Study Data of Lamotrigine

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S_1:50%	20	19.95	99.75	Mean-99.7
S_2:50%	20	19.83	99.15	S.D-0.53
S_3:50%	20	20.04	100.20	%RSD-0.53
S_4:100%	40	40.04	100.10	Mean-100.08
S_5:100%	40	40.03	100.08	S.D-0.01
S_6:100%	40	40.03	100.08	%RSD=0.01
S_7:150%	60	59.91	99.85	Mean-99.99
S_8:150%	60	60.1	100.17	S.D-0.16
S_9 :150%	60	59.98	99.97	%RSD-0.16

Precision Studies for Lamotrigine:

Method Precision (Repeatability):

Twenty tablets were accurately weighed and tablet powder equivalent to 50mg of Lamotrigine were taken into 50ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent and filtered through 0.45 µm nylon membrane filter. Further pipette out 0.4ml from the above Lamotrigine sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 40µg/ml of Lamotrigine. A homogenous sample of a single batch analysed six times and was checked whether the method is giving consistent results. The %RSD for the area of six replicate injections was calculated as mentioned in Table 5. Acceptance Criteria: The % RSD for the peak area of six sample injections should not be more than 2%.

Table 5: Method Precision Data for Lamotrigine

Lamotrigine		
S. No.	**Concentration (µg/ml)**	**% Assay**
1	40	100.02
2	40	99.79
3	40	100.13
4	40	99.87
5	40	100.12
6	40	99.78
Average		99.95
SD		0.16
%RSD		0.16

System Precision:

The system precision was carried out to ensure that the analytical system is working properly. The standard preparation concentration of 40µg/ml of Lamotrigine was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in Table 6. Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Table 6: System Precision Data for Lamotrigine

Lamotrigine		
S. No.	**Concentration (µg/ml)**	**Peak Area**
1	40	5043891
2	40	5043623
3	40	5043842
4	40	5044839
5	40	5047322
6	40	5053110
Average		5046105
SD		3374.84
%RSD		0.07

Intermediate Precision/Ruggedness:

The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on different laboratory by different analyst and different days. The sample preparation concentration of 40µg/ml of Lamotrigine was injected six times into the HPLC and the %RSD for the area of six replicate injections was

calculated as mentioned in Table 7. Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Table 7: Ruggedness data for Lamotrigine

Ruggedness Data for Lamotrigine								
Laboratory-1 (% Assay)-HPLC-1					**Laboratory-2 (% Assay)-HPLC-2**			
	Analyst-1		**Analyst-2**		**Analyst-1**		**Analyst-2**	
Conc. (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
40	100.02	99.78	100.1	100.2	99.98	100.09	100.1	100.13
40	100.03	100.1	100.4	100.01	99.79	99.81	100.03	100.2
40	100.1	100.3	99.89	99.98	100.21	100.01	99.84	100.03
40	100.2	100.1	100.1	100.1	100.14	100.03	100.02	99.89
40	99.98	100.5	100.02	100.2	100.1	100.1	99.93	100.1
40	100.1	99.79	100.04	100.03	99.84	100.2	100.05	100.3
Average	100.07	100.10	100.09	100.09	100.01	100.04	100.00	100.11
SD	0.08	0.28	0.17	0.10	0.17	0.13	0.09	0.14
%RSD	0.08	0.28	0.17	0.10	0.17	0.13	0.09	0.14
Intermediate precision within-laboratories variations (n=24)								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Average	100.09				**Average**	100.04		
SD	0.09				**SD**	0.05		
%RSD	0.09				**%RSD**	0.05		
Reproducibility between laboratories (n=48) (% Assay)								
Average	100.06							
SD	0.03							
%RSD	0.03							

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD of Lamotrigine was calculated and shown in Table 8. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Lamotrigine was calculated and shown in Table 8.

Table 8: Summary of Validation Parameter for Lamotrigine

Parameters	**RP-HPLC method**	
	Lamotrigine	
Linearity range (µg/ml)	10-120	
Slope	12632	
Intercept	12440	
Correlation coefficient	0.999	
LOD (µg/ml)	0.09	
LOQ (µg/ml)	0.28	
Method Precision (% RSD, n=6)	0.16	
System precision (% RSD, n=6)	0.07	
Ruggedness (% RSD, n=24)	Lab-1	Lab-2
	0.09	0.05
Reproducibility (% RSD, n=48)	0.03	
% Accuracy	99.7-100.08	
Robustness (% RSD, n=6)	Less Flow rate	More Flow rate
	0.05	0.11
	Less Organic phase	More Organic phase
	0.45	0.16

Robustness:

As part of the Robustness, deliberate change in the flow rate and mobile phase proportion of ±10% was made to evaluate the impact on the method. The results reveal that the method is robust. The results are summarized in Table 9.

Table 9: Summary of Robustness (Change in Flow Rate and Mobile Phase) for Lamotrigine

Parameters	Mean peak Area(n=3)	S.D	%R.S.D	RT	Theoretical plates
Flow rate 0.9ml/min	5E+06	2409.9	0.05	3.101	4419
Actual flow rate 1ml/min	5E+06	417.69	0.01	2.977	4672
Flow rate 1.1ml/min	5E+06	5305.8	0.11	2.871	4940
10% less organic (54:46)	5E+06	22503	0.45	2.874	4381
Actual mobile phase (60:40)	5E+06	417.69	0.01	2.977	4672
10% more organic (66:34)	5E+06	8000.8	0.16	2.889	4021

DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Lamotrigine were obtained with a mobile phase containing a mixture of Phosphate buffer (pH adjusted to 4.5 with diluted formic acid) and Methanol (40:60, v/v) was delivered at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was achieved with VWD detection at 270nm based on peak area. The retention time of Lamotrigine was found to be 2.977min. Linearity was established for Lamotrigine in the range of 10-120µg/ml with correlation coefficient 0.999 and mean accuracies were found to be is 99.7% to 100.08% for Lamotrigine, which indicates accuracy of the proposed method. The % RSD values of accuracy for Lamotrigine were found to be < 2 %. The % RSD value of method precision was 0.16% for Lamotrigine and % RSD value of system precision was 0.07% for Lamotrigine. The % RSD value of reproducibility is 0.03% for Lamotrigine reveal that the proposed method is precise. LOD value for Lamotrigine was found to be 0.09µg/ml and LOQ value for Lamotrigine were found to be 0.28µg/ml. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough. These data show that the proposed method is specific and sensitive for the determination of Lamotrigine.

CONCLUSION

RP-HPLC method for the estimation of Lamotrigine in their bulk and pharmaceutical dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Lamotrigine in the range of 10-120µg/ml with correlation coefficient 0.999. The percentage recovery of drug was achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Lamotrigine in their bulk and pharmaceutical dosage form.

Acknowledgement

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