ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Stability-Indicating Reverse-Phase High-Performance Liquid Chromatographic Method Development and Validation of Lamotrigine in Bulk and Pharmaceutical Dosage Form

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Abstract

In the current study, analytical method has been validated by system suitability parameters, linearity, accuracy and percent recovery, precision, ruggedness, robustness, limit of detection, and limit of quantification. The present study of lamotrigine was achieved using Cosmosil C18 (250 nm×4.6 ID, particle size: 5 Micron) Column with mobile phase methanol: water (60:40) pH: 3 at a flow rate 0.8 ml/min with UV detection at 308 nm. The retention time for lamotrigine was found to be 4.979 min. In linearity, the correlation coefficient (R^2) for lamotrigine was found to be 0.999, slope is 39,801, and intercept was found to be 51,862 which are well within the acceptance criteria. The mean percent recovery for lamotrigine at three different levels for 50%, 100%, and 150% was found to be 99.28%, 99.30%, and 100.40%. The % RSD should not more than 2%. In precision study, interday (RSD is 0.41%) and intraday (RSD is 0.26%) are found. Forced degradation experiments were carried out by exposing standard form of lamotrigine for acid-base, oxidative, photolytic, and thermal stress conditions. Hence, the developed method is accurate, precise, repeatable, and reproducible and can be used for routine analysis of lamotrigine.

Keywords: Lamotrigine, Reverse-phase high-performance liquid chromatographic, Validation

Asian Pac. J. Health Sci., (2021); DOI: 10.21276/apjhs.2021.8.4.31

Introduction

Lamotrigine, it is one of the antiepileptic drugs, belongs from phenyltriazine class. It is used in the treatment of both epilepsy and as a mood stabilizer in bipolar disorder. Food and Drug Administration (FDA) approval for the maintenance treatment of bipolar type I, lamotrigine is the first medication. It is approved for use in more than 30 countries.[1] Basically, lamotrigine is a sodium channel blocking group of antiepileptic drugs. This may suppress the release of glutamate and aspartate, two of the dominant excitatory neurotransmitters in the CNS.[1-3] It is a sodium channel blocking antiepileptic drugs, but it could have additional actions since it has a broader spectrum of action than other sodium channel antiepileptic drugs such as phenytoin and is effective in the treatment of the depressed phase of bipolar disorder. Lamotrigine drug showing few side effects with other, unrelated anticonvulsants known to inhibit sodium channels, which further emphasizes its unique properties.[4]

It is very useful in the treatment of partial seizures, tonic–clonic seizures, and also used in treatment of Lennox-Gastaut syndrome. On literature survey, it was found that ever-increasing number of drugs and their combinations in the market leads to the need for the development of analytical methods for their quality control. The methods have to be such that it takes less time in their development as well as the best accurate and robust results should be obtained. Based on this concept, the aim of the research work is to develop reverse-phase high-performance liquid chromatographic (RP-HPLC) method and validate for simultaneous estimation of lamotrigine. Analytical chemistry is focused on the creation of new measurement tools to provide better chemical information.

In production and evaluation of new product, analytical technique, it is used to determine the concentration of drug. An appropriate analytical method is required to study the pharmacokinetic, therapeutic, and toxicological effects of new drug entities.[5]

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How to cite this article: Ghawate VB, Sikchi SD, Kedari CC, Jadhav VS, Deshmukh VK, Mote P. Stability-Indicating Reverse-Phase High-Performance Liquid Chromatographic Method Development and Validation of Lamotrigine in Bulk and Pharmaceutical Dosage Form. Asian Pac. J. Health Sci., 2021;8(4):155-159.

Source of support: Nil

Conflicts of interest: None.

Received: 12/6/21 **Revised:** 28/7/21 **Accepted:** 15/8/21

The present work is focused on developing new analytical techniques for multicomponent dosage forms.

Chemical Formula and Structure of Lamotrigine: [6-8]

Molecular formula: C9H7Cl2N5

Materials and Methods

Working Standards

Working standard of lamotrigine as gift sample was obtained from Ajanta Pharma Pvt. Ltd., Paithan, Aurangabad, Maharashtra.

Formulation

Lamotrigine tablet, USP 200 mg, and manufactured by: Cipla Ltd., Verna, Goa, India, was purchased from local market [Table 1].

Chemical and Reagents

A HPLC grade methanol, ethanol, water, sodium hydroxide, hydrogen peroxide, hydrochloric acid, potassium bromide chloroform, ethyl acetate, acetone, acetonitrile, benzene, toluene, and triethylamine were used.

Chromatographic Conditions

A RP-HPLC (Binary Gradient System) was used for the analysis. The Pump P-3000-M Reciprocating (40 MPa), Column Cosmosil C18 (250 mm×4.6 ID, particle size-5 micron), and Detector UV-3000-M were used system manufactured by Analytical Technologies Ltd. Mobile phase is methanol: water (60:40) pH: 3 at a flow rate 0.8 ml/min, sample volume used 20 µl and pressure is 10–11 MPa with UV wavelength found to be 308 nm.

Preparation of Standard Stock Solution

Standard stock solution of lamotrigine was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml from which 1 ml was further diluted with methanol to get the final concentration 100 ng/µl. Solution was filtered through Whatman filter paper (No.1) and further diluted to obtained final concentration of 100 µg/ml.

Preparation of Working Standard Solution

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 10, 20, 30, 40, and 50 µg/ml.

Preparation of Sample Stock Solution

Ten tablets (lamotrigine) were weighed accurately and finely powdered. A quantity of powder equivalent to 100 mg of lamotrigine was weighed and transferred to a 10 ml volumetric flask containing approximately 5 ml methanol. The mixture was sonicated for 15 min. The solution was filtered and diluted to volume with methanol.

Preparation of Working Sample Solution

To prepare working sample solution in different 10 ml volumetric flasks using correct aliquots and diluted up to the mark with mobile phase to obtain final concentration of 10, 20, 30, 40, and 50 µg/ml.

Selection of Mobile Phase and Chromatographic Conditions

Selection of mobile phase was done on trial and error basis using different solvent systems such as chloroform: methanol, methanol: ethyl acetate, acetone: methanol, acetonitrile: water, benzene: methanol, and toluene: methanol:triethylamine in various proportions, to obtain the satisfactory resolution between the two drugs along with desired system suitability parameters. After several trials, methanol: water (60:40 v/v) selected as optimum mobile phase with chamber saturation time 25 min, which gave good resolution and sharp peaks for the drug. Other chromatographic conditions, including run length, sample application volume, sample application positions, distance between tracks, and detection wavelength, were optimized to give reproducible R_f values and symmetrical peak shape for the drug peak.

Selection of Detection Wavelength

Standard stock solutions of lamotrigine were prepared by separately using methanol and scanned over the range of 200–400 nm and the spectra was obtained. It was observed that both the drugs showed maximum absorbance at 308 nm. Hence, it was selected as detection wavelength.

Assay

A 20 µL volume of these lamotrigine standard and sample solutions was used. After finished the setting of chromatographic conditions and stabilizing the instrument to obtain a steady baseline. The solution was injected and a chromatogram was recorded.
The percent assay for bulk drug was calculated using regression equation and peak areas of drug presented in Table 2.

Method Validation

System suitability test

System suitability tests are integral part of method development it is use to make confirm adequate performance of system. It is shown in Figure 2 and result in Table 3.

Linearity

The ability of analytical method, within a given range, it is a linearity to provide results. Different volumes of standard solution of drug were injected to obtain different concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm of lamotrigine. The linearity in terms of measured peak areas versus corresponding concentration of drugs was estimated by ordinary linear regression analysis it is shown in Figure 3 and result in Table 4.

Table 1: Marketed formulation supplier

Marketed formulation	*Content*	*Name of Manufacturer*
Lamotrigine Tab, USP 200 mg	Each tablet contain lamotrigine 200 mg	Cipla Ltd., Verna, Goa Ltd., India

Table 2: Result of high-performance liquid chromatographic assay for lamotrigine

Name of drug	*Composition (ppm)*	*% assay*	
		Bulk	*Formulation*
Lamotrigine	30	102.33	99.47

Table 3: System suitability studies of lamotrigine

	Lamotrigine
Properties	*Observed values*
Retention time	4.979
Resolution (Rs)	0.00
Theoretical plates (N)	5988
Tailing factor/asymmetry factor (T)	1.24

Accuracy (recovery study)

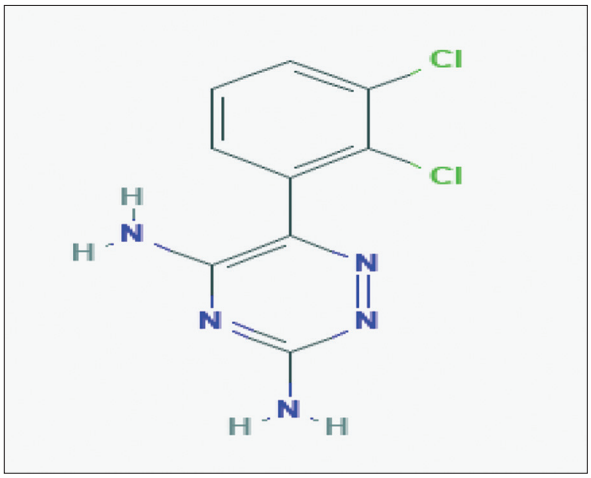
The accuracy selected method was determined by calculating the recovery studies of the test drug at three different concentration levels (50%, 100%, and 150%) by standard addition method. Three determinations were performed at each level of the amount. The % recovery found within the acceptable criteria 30 ppm – 50%, 40 ppm – 100%, and 50 ppm – 150% Recovery was obtained which is shown in Tables 5 and 6.

Table 4: Linearity results for lamotrigine

Drug	*Lamotrigine*				
Conc. (ppm)	10	20	30	40	50
Area	430,989	857,871	1,273,917	1,633,510	2,033,244
Regression equation	y = 39,801x + 51,862				
Correlation (R^2)	0.999				
Slope (m)	39,801				
Intercept (c)	51,862				

Table 5: Accuracy and recovery data for lamotrigine

S. No.	*% composition*	*Area of standard*	*Area of sample*	*% recovery*
1	50% recovery	1,273,917	1,264,800	99.28433328
2	100% recovery	1,633,510	1,622,115	99.30242239
3	150% recovery	2,033,244	2,041,358	100.3990667



AQ8 **Figure 1:** Chemical formula and structure of lamotrigine:[6-8] Molecular formula: $C_9H_7Cl_2N_5$

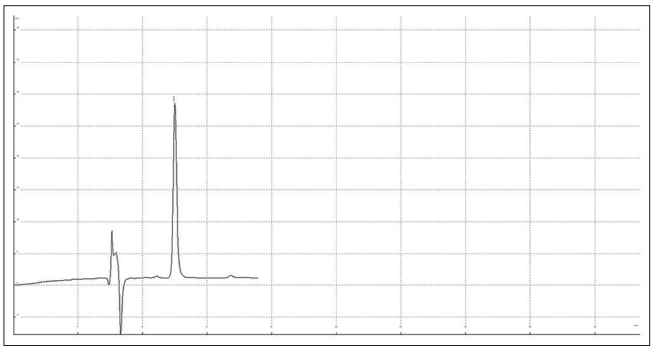


Figure 2: Optimized chromatogram for lamotrigine at 308 nm

Precision

The intraday and interday studies evaluating precision of developed method. Intraday precision was carried out by performing concentrations (30 ppm**)** on same day and peak area measured was expressed in terms of percent relative standard deviation (% RSD). The interday precision study was performed on 2 different days using mentioned concentrations of drug and % RSD was calculated. The result presented in Table 7.

Ruggedness

Ruggedness was done by making small changes in flow rate and wavelength by using solutions of Lamotrigine in different five concentration level ranging from 10 to 50 ppm. The calibration curve was constructed by area against concentration of drug and it is shown in Figure 4. The correlation coefficient for area versus concentration of analyte was calculated and is presented in Table 8.

Robustness

By making slight changes in the chromatographic conditions, robustness of the method was determined. Minimum and maximum values for flow rate were selected as 0.7 and 1.0 ml/min. Minimum and maximum values for wavelength were selected as 306 and 310 nm. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are robust. The result is shown in Tables 9 and 10.

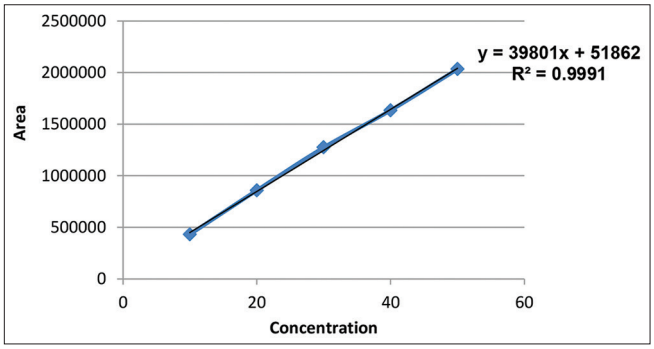


Figure 3: Linearity graph of lamotrigine

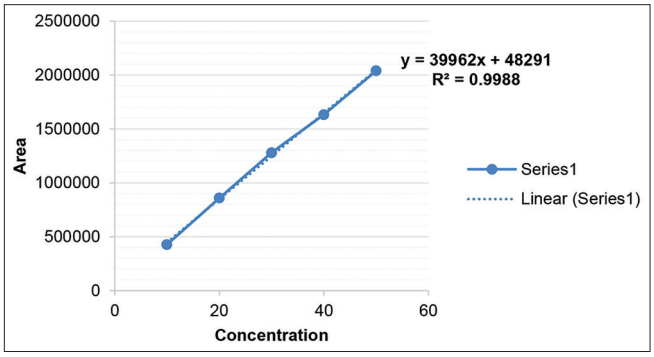


Figure 4: Ruggedness graph of lamotrigine

Table 6: Statistical data of accuracy and recovery for lamotrigine

Conc.	*Conc.*	*Area*	*Mean*	*SD*	*%SD*	*%RSD*
1	10	430,989	430,387.6667	1662.66122	0.3863171	0.386317116
	10	428,508				
	10	431,666				
2	30	1,273,917	1,270,315.667	4253.335789	0.3348251	0.334825107
	30	1,265,623				
	30	1,271,407				
3	50	2,033,244	2,030,265.667	4906.215887	0.2416539	0.241653886
	50	2,032,950				
	50	2,024,603				

Table 7: Precision results for lamotrigine

	Lamotrigine	
Sample No.	*Interday precision (area)*	*Intraday precision (area)*
1	1,273,917	1,273,917
2	1,265,623	1,265,623
3	1,271,407	1,271,407
4	1,266,892	1,271,525
5	1,276,655	1,270,879
6	1,263,018	1,266,219
Mean	1,269,585	1,269,928
SD	5256.348	3281.587
%RSD	0.41	0.26

Table 8: Ruggedness results for lamotrigine

Drug	*Lamotrigine*				
Conc. (ppm)	10	20	30	40	50
Area	427,405	858,119	1,279,434	1,632,395	2,038,349
Regression equation	y = 39,962x + 48,291				
Correlation (R^2)	0.998				
Slope (m)	39,962				
Intercept (c)	48,291				

Table 9: Robustness data for lamotrigine at different flow rate and wavelength

	Lamotrigine	
Level	*Retention time*	*Tailing factor*
Change in flow rate (ml/min)		
−1 (0.7 ml)	5.643	1.25
0 (0.8 ml)	4.975	1.24
+1 (0.9 ml)	4.456	1.24
Change in wavelength (nm)	*Retention time*	*Tailing factor*
−2 (306 nm)	4.972	1.25
0 (308 nm)	4.975	1.24
+2 (310 nm)	4.975	1.25

Table 10: Statistical data of robustness for lamotrigine

	Lamotrigine				
Change in flow rate (ml/min)	*Conc. (ppm)*	*Area*	*Mean*	*SD*	*%RSD*
−1 (0.7 ml)	20	850,051	854,282	3949.413	0.46
0 (0.8 ml)	20	857,871			
+1 (0.9 ml)	20	854,925			
Change in wavelength (nm)	*Conc. (ppm)*	*Area*	*Mean*	*SD*	*%RSD*
−2 (306 nm)	20	858,021	858,383	760.613	0.089
0 (308 nm)	20	857,871			
+2 (310 nm)	20	859,257			

Limit of Detection and Limit of Quantification (LOQ)

The limit of detection and LOQ were separately determined based on standard deviation from accuracy and slope from linearity. The results represent in Table 11.

Table 11: limit of detection and limit of quantification data for lamotrigine

Drug	*limit of detection (µg/ml)*	*Limit of quantification (µg/ml)*
Lamotrigine	0.138	0.418

Table 12: Stability data for lamotrigine

Stress condition	*Retention time*	*Area of peak*	*Degraded up to %*	*Actual % degradation*
Standard drug	4.991	2,033,244	--	--
Acidic (0.1 N HCl)	4.998	1,735,716	85.36683251	14.63316749
Alkaline (0.1 N NaOH)	4.987	1,635,021	80.41440181	19.58559819
Oxidation (3% H_2O_2)	4.987	1,879,268	92.42707712	7.572922876
Photolytic	4.999	2,007,585	98.73802652	1.261973477
Thermal	5.000	2,004,921	98.60700437	1.392995627

Forced degradation studies

Forced degradation studies are completed by different ways such as acidic, base/alkaline, oxidative, photolytic, and thermal degradation. The concentration of standard solution was prepared and used for degradation at different conditions the sample injected in HPLC and chromatogram was recorded. The result of degradation is shown in Table 12.

Results

Mobile phase: Methanol:water (60:40) pH:3, run time: 7.59 min					
Flow rate	*Time*	*Area*	*Resolution*	*T. Plate num.*	*Asymmetry*
0.8 ml/min	4.979	373,200	0.00	5988	1.24

Discussion

The HPLC conditions were optimized and adequate elution of compound observed. The main objective of this study was to develop sensitive and rapid RP-HPLC method for determination and analysis of lamotrigine in bulk and pharmaceutical dosage form using mobile phase methanol: water (60:40) pH: 3. The flow rate found to be 0.8 ml/min and wavelength on UV detection is 308 nm. The retention time for lamotrigine was found to be 4.979 min.

A good linear relationship ($r^2 = 0.999$) was observed between the concentration of lamotrigine and respective peak areas in the range of 10–50 ppm. To analyze tablet formulations, RP-HPLC method has been developed. The mean recoveries were found in the range of 98%–102%. The low %RSD values (≤2) indicated

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Table 13: Characteristic parameters of lamotrigine for proposed reverse-phase high-performance liquid chromatographic method

S. No.	*Parameters*	*Reverse-phase high-performance liquid chromatographic*
1	Calibration range (µg/ml)	10–50 of lamotrigine
2	Detection wavelength	308 nm
3	Mobile phase	Methanol:water (60:40) pH: 3
4	Flow rate	0.8 ml/min
5	Retention time	4.979 min
6	Temperature	Ambient
7	Pressure	10–11 MPa
8	Regression equation (Y)	Y=mx+c
9	Slope (m)	39,801
10	Intercept (c)	51,862
11	Correlation coefficient (r^2)	0.999
12	Interday precision (%RSD)	0.41%
13	Intraday precision (%RSD)	0.26%
14	Limit of detection (mcg/ml)	0.138
15	Limit of quantification (mcg/ml)	0.418

that method was accurate and precise. In stability study, lamotrigine undergoes different parameters, comparatively more degradation was found with alkaline and acidic shows that the degradation product does not interfere with analytical determination of lamotrigine in pharmaceutical dosage form. The degradation of drug was found to be within acceptance criteria.

Conclusion

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

Acknowledgments

The authors are thankful to Ajanta Pharma Pvt. Ltd., Paithan, Aurangabad, Maharashtra, for procurement of API drug sample and Cipla Ltd., Verna, Goa Ltd., India, for procurement of lamotrigine formulation, kind cooperation rendered in fulfilling a research work.

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AQ1: Kindly cite Table 13 in the text part
AQ8: Kindly cite Figure 1 in the text part

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