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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC ASSAY METHOD FOR DETERMINATION OF LAMOTRIGINE IN TABLET FORMULATION

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ABSTRACT

The objective of the current study was to developed simple, precise and accurate isocratic reversed-phase stability indicating high performance liquid chromatography (HPLC) assay method and validated for determination of lamotrigine in solid pharmaceutical dosage forms. Isocratic RP-HPLC separation was achieved on a Thermo C18 column (250 mm x 4.6 mm i.d., 5 µm particle size) using mobile phase of Acetonitrile: Buffer (1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH 6 with OPA, 40:60, v/v) at a flow rate of 1.0 ml/min and the detection was carried out at 225 nm by using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The retention time was 5.013 $\pm$ 0.3 min and the method was linear in the drug concentration range of 5-25 µg/ml with a correlation coefficient 0.999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. The accuracy (recovery) was between 99.830and 100.208 %. Degradation products produced as a result of stress studies did not interfere with detection of lamotrigine and the assay can thus be considered stability indicating.

Keywords: Lamotrigine, Method Development, Validation, RP-HPLC, Stability indicating

INTRODUCTION

Stress testing is a part of developmental strategy under the International Conference on Harmonization (ICH) requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies [1-3]. It is suggested that stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a wide range of pH values. It is also recommended that analysis of stability sample should be done through the use of a validated stability testing methods. Lamotrigine (Fig.1), 6-(2,3-dichlorophenyl)-1,2, 4-triazine-3,5-diamine is a novel anticonvulsant drug used in the treatment of epilepsy, bipolar disorder and pain syndromes.

In psychiatry, there are several studies reporting the efficacy of lamotrigine in the treatment of bipolar disorder, schizophrenia, cocaine dependence, post-traumatic stress disorder and borderline personality disorder. In these studies, lamotrigine was seen to be well tolerated, to be an effective mood stabilizer and to be significantly more effective in schizophrenia when given as adjunctive therapy to clozapine.

However it requires medical attention when combined with other antipsychotics except for clozapine due to possible iatrogenic worsening of psychiatric symptoms. For epilepsy it is used to treat partial seizures, primary and secondary tonic-clonic seizures and seizures associated with Lennox-Gastaut syndrome. Lamotrigine also acts as a mood stabilizer. It is the first medication since lithium to be granted approval by the U.S. Food and Drug Administration (FDA) for the maintenance treatment of bipolar type I. chemically unrelated to other anticonvulsants, lamotrigine has relatively few side-effects and does not require blood monitoring in monotherapy. The exact way lamotrigine works is unknown. Some think that it is a Na+ (sodium) channel blocker, though it is interesting to note that lamotrigine shares very few side effects with other, unrelated anticonvulsants known to inhibit sodium channels, (e.g. oxcarbazepine), which may suggest that lamotrigine has a different mechanism of action. Lamotrigine is inactivated by hepatic glucuronidation [4-9]. The USP describes an HPLC assay with UV detection for the assay of lamotrigine in pure and tablet form [10]. On the other hand, the BP describes a non-aqueous acid-base titration for the assay of the pure drug [11]. Moreover, techniques as HPLC [12, 13] and TLC [13] were used to quantify lamotrigine in the presence of its related impurities. The drug was also determined in tablet form using a TLC method [14]. The Literature also contains a spectrophotometric direct UV method[15] as well as a spectrofluorimetric one utilizing the reaction with o-phthalaldehyde [16] for its determination in tablet form. Pertaining to stability-indicating studies, 2 HPLC methods for the separation of the investigated drug from its forced degradation products were recently reported [15, 17]. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines [18]. The aim of our investigation was to develop a validated LC method for the determination of lamotrigine in the presence of its degradation products in pharmaceutical tablet dosage form.

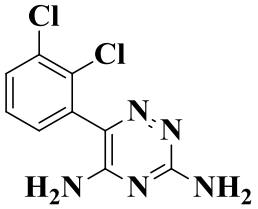


Figure 1 Chemical structure of lamotrigine

Materials and Methods

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data. A Thermo C18 (250 X 4.60 mm), 5µm column, a Lichrocart, HPLC guard cartridge system.

Reagents and chemicals

Lamotrigine were obtained as pure sample from Alembic Pharmaceuticals Ltd., Baroda (India), as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Tablet Lamepil 25mg IPCA Laboratories Pvt. Ltd, India was purchased from local market.

Chromatographic conditions

The isocratic mobile phase consisted of Acetonitrile: Buffer (1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH 6 with OPA, 40:60, v/v) flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 225 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

Accurately weighed 10 mg of lamotrigine was transferred into 10 ml volumetric flask, dissolved in 5ml of Acetonitrile and volume was made up to 10ml with Acetonitrile to get concentration of solution 1000 µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml to get concentration of 100µg/ml (Stock-B).

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 5, 10,15,20,25 µg/ml for lamotrigine.

Sample preparation

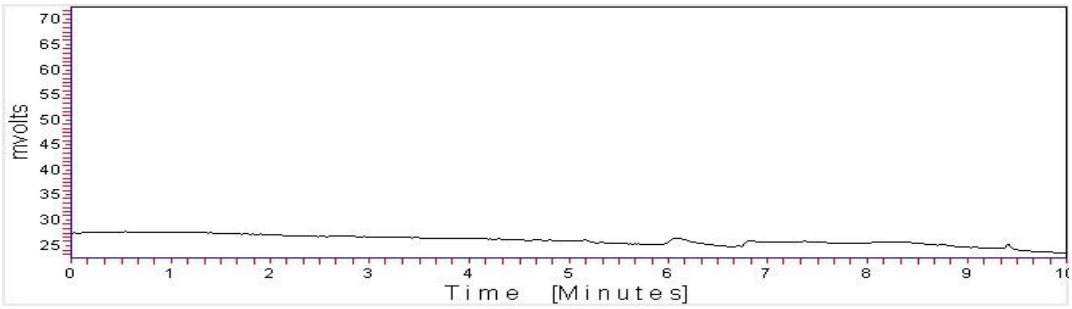
For analysis of the tablet formulation, weight equivalent to weight 10mg of lamotrigine was transferred to 10ml volumetric flask and dissolved Acetonitrile. The solution was shaking vigorously for 20mins and filtered through Whatman filter paper no. 41, then volume was made up to mark with Acetonitrile. From the above solution 1ml of solution was taken and diluted to 10 ml with Acetonitrile to get a solution

containing 100µg/ml. From the above solution 1ml of solution was taken and diluted to 10ml with Acetonitrile to get a solution containing 10µg/ml of lamotrigine. The amounts of lamotrigine in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation.

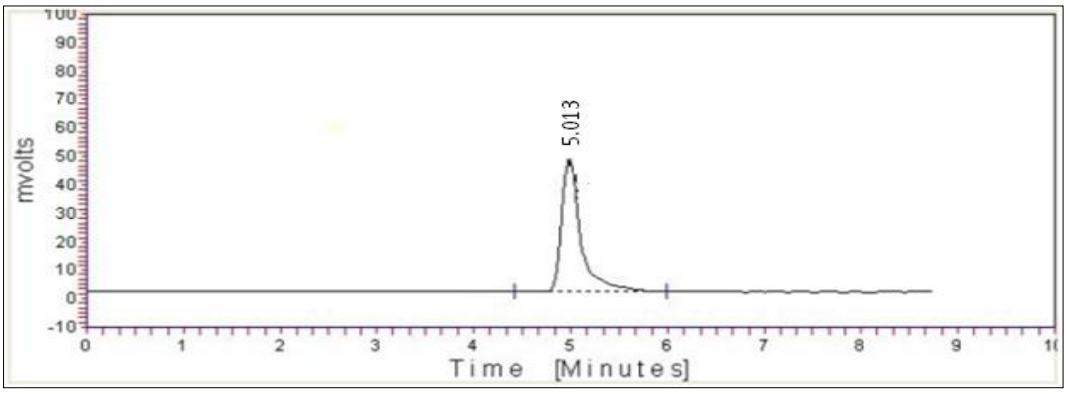
Results and discussion

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water, and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile: buffer (1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH 6 with OPA, 40:60, v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for lamotrigine was observed to be 5.013 $\pm$ 0.3 min. Total time of analysis was less than 7 min. The maximum absorption of lamotrigine was detected at 225 nm, and this wavelength was chosen for the analysis Fig. 2.



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) lamotrigine (15µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for lamotrigine was 3077.33.

Table 1 Results of system suitability parameters

Parameters	Lamotrigine
AUC*	830.464
No. of Theoretical Plates*	3077.33 ± 21.00
Tailing Factor*	1.176
Retention time*	5.013
HETP*	0.252
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25 µg/ml for lamotrigine Fig 3. The linearity was represented by a linear regression equation as follows:

Y (lamotrigine) = 81.5conc. + 9.826 (r2 = 0.999)

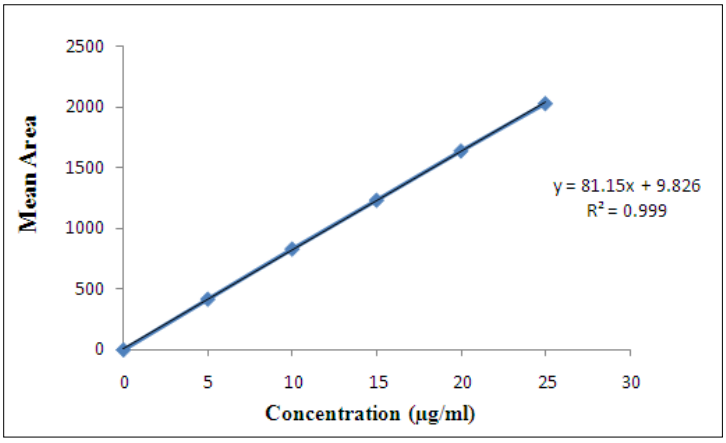


Figure 3 Calibration curve of lamotrigine

Accuracy

Method accuracy was performed by adding known amounts of lamotrigine to the preanalysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100%, and 120% of the nominal analytical concentration (10μg/ml for lamotrigine). Each level was made in triplicate table 2. The mean percentage recoveries obtained for lamotrigine was 100.20% and RSD was less than 2.

Table 2 Results of recovery study

Statistical data	Lamotrigine		
	80%	**100%**	**120%**
% Mean*	100.20	99.70	99.83
SD*	0.382	0.173	0.127
%R.S.D*	0.381	0.174	0.127

*Mean of nine determinations (three replicates at three concentration level)

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations, and results were found within acceptable limits (RSD < 2) as shown in table 3.

Table 3 Statistical data for precision

Statistical parameter	Lamotrigine		
	Mean*	**S.D***	**R.S.D***
Repeatability	99.90	0.154	0.168
Intermediate Precision (I) (A day to day)	97.297	0.499	0.513
(II) Analyst to Analyst	99.98	0.145	0.148
Robustness	98.87	0.64	0.64

*Mean of 15 determinations (three replicates at five concentration level)

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase, temperature and flow rate were made to check the method's capacity to remain unaffected. The method is found robust as RSD is again found < 2.0 table 4.

Table 4: Result of Robustness of Formulation

Compound		**% RSD in Normal**	**Changed Condition n=6**
Temperature		- 5 ^{o}C	+ 5 ^{o}C
Lamotrigine	0.89	1.1	1.25
Flow rate		(-10%)	(+10%)
Lamotrigine	0.48	0.55	0.89
Mobile phase ratio		- 2 %	+ 2 %
Lamotrigine	0.89	0.95	1.05

Analysis of Tablets

The concentration of lamotrigine in the tablet formulation was found to be 101%. The low values of % RSD indicate that the method is precise and accurate in table 5.

Table 5 Results of tablet analysis

S.NO.	Parameter	Sample Lamotrigine
1	% Found	101.23
2	S.D.	0.666
3	% R.S.D.	0.664
4	SEσ*	0.342

* Mean of nine determinations

Forced Degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on lamotrigine powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected at regular intervals Shown in Table 6.

Acid degradation

50 mg of lamotrigine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of lamotrigine.

Base degradation

50 mg of lamotrigine sample was taken into a 50 ml round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of lamotrigine.

Hydrolytic degradation

50 mg of lamotrigine sample was taken into a 50 ml round bottom flask, 50 ml of water was added and the contents were mixed well and kept for constant stirring for 48 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of lamotrigine.

Oxidative degradation

50 mg of lamotrigine sample was taken into a 50 ml round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of lamotrigine.

Thermal degradation

50 mg of lamotrigine sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks.

Photolytic degradation

The lamotrigine was exposed to sunlight during the daytime (70,000–80,000 lux) for 2 days.

Table 6 Forced degradation studies of lamotrigine

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.9	0
Acidic hydrolysis	86.45	13.55
Alkaline hydrolysis	92.12	7.88
Oxidative degradation	90.85	9.15
Thermal degradation	93.12	6.88
Photolytic degradation	90.12	9.88

Conclusion

According to the ICH guidelines, the safety, quality, and/or efficacy of a drug substance is liable to inadequate storage conditions. Therefore, the need for stability-indicating methods appears indispensable to establish the stability and purity profiles of a drug substance. In this work, lamotrigine was exposed to 7 different stress conditions among which considerable degradation occurred in acidic, basic, and oxidative media. Lamotrigine appears more sensitive to acid degradation. The proposed HPLC method was capable of quantifying low levels of lamotrigine and effectively resolving it from forced degradation impurities. The developed HPLC assay was also applied to the analysis of tablets and the ensuing results showed no statistically significance differences from those obtained by a reference method.

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