

A Simple Development and Estimation of Lamotrigine Tablets by HPLC

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Abstract: A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the estimation of Lamotrigine from pharmaceutical formulation. The method was carried out on a Princeton SPHER C₁₈ (250 mm x 4.6 mm i.d., 5 μ) column with a mobile phase consisting of acetonitrile: 0.3 % Triethylamine (adjusted to pH 6.5 using orthophosphoric acid) (25:75 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 305 nm. Ondansetron hydrochloride was used as an internal standard. The retention time of Lamotrigine and Ondansetron hydrochloride was 5.28 and 7.40 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method could used for the estimation of Lamotrigine in pharmaceutical dosage form and applicable for Quality control, bulk drugs and clinical aspects.

Keywords: Lamotrigine, HPLC, Validation, Tablet.

1.Introduction

Lamotrigine, is a new antiepileptic drug that is currently used as an add-on therapy or monotherapy in patients with partial and secondary generalized seizures [1]. 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, C₉H₇Cl₂N₅, mol. Wt. 256 g, is chemically unrelated with other antiepileptic drugs in current use, differing in structure and pharmacology. Lamotrigine (LTG) has a broad spectrum in antiepileptic activity [2,3]. Its efficacy against partial and generalized seizures has been demonstrated in several studies. Several methods for determination of lamotrigine and its metabolites in biological matrices have been developed including reversed-phase HPLC [4–17]. The present work describes the development of a validated RP-HPLC method with internal standard. The present RP-HPLC method was validated according to ICH guidelines [18]. A literature review revealed that at this time the HPLC method has been considered as the technique of choice for the separation and determination of lamotrigine. An official monograph of lamotrigine does not

exist in any pharmacopoeia and determination of lamotrigine and related substances in pharmaceutical formulations has not been yet described. Therefore, it is very imperative to develop a simple and suitable analytical method for the measurement of lamotrigine and related compounds in bulk and in formulations. Such methods could be easily adapted for routine and in-process quality control analysis, dissolution or similar studies. Due to the importance of bioequivalence of the dosage forms and limitations of UV spectrophotometric methods in detection of impurities, our purpose was to develop a simple, sensitive, and reliable method for simultaneous determination of the drug and respective impurities which can be applied in quality control laboratories. To achieve this aim, a liquid chromatographic assay was developed in this study.

2.Experimental

2.1.Reagents and chemicals

Acetonitrile HPLC grade was procured from JT scientific, Malaysia. Triethylamine AR grade were procured from AR Alatan Sdn Bhd, Malaysia. Water HPLC grade was obtained from a Milli-QRO water purification system. A reference standard of Lamotrigine and Ondansetron hydrochloride were procured from Cadila pharmaceuticals ltd, India.

2.2.Apparatus and chromatographic conditions

Chromatographic separation (Prominence) was performed on a HPLC C₁₈ (250 mm x 4.6mm i.d., 5 μ) Princeton SPHER column with a mobile phase consisting of Acetonitrile: Triethylamine (adjusted to pH 6.5 using orthophosphoric acid) (25:75 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 305 nm. Ondansetron hydrochloride was used as an internal standard. The mobile phase was filtered through a Millipore 0.2 μ membrane filter and degassed. The injection volume was 50 μ L and the analysis was performed at ambient temperature.

2.3.Preparation of standard solutions

Standard stock solutions of 1.0 mg/ml Lamotrigine were prepared by using a mixture of water and acetonitrile (1:1 v/v). From the standard stock solution, mixed standard solution was prepared to contain 10.0 μ g/ml of Lamotrigine and 25.0 μ g/ml of Ondansetron hydrochloride as internal standard. The solution is stored until the analysis using refrigerator.

2.4.Preparation of sample solutions

Twenty tablets, each containing 100.0 mg of Lamotrigine were weighed and finely powdered; a quantity of powder equivalent to 100.0 mg of Lamotrigine was weighed and transferred to a calibrated flask. To this 5.0 ml of 1.0 mg/ml solution of Ondansetron hydrochloride was added and the drugs were extracted with three quantities, each of 20 ml of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 10.0 μ g/ml of Lamotrigine, 25.0 μ g/ml of Ondansetron hydrochloride as internal standard and this solution was used for the estimation.

2.5.Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Lamotrigine and Ondansetron hydrochloride was found to be 5.28

and 7.40 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated.

3.Results And Discussion

3.1.Estimation of Lamotrigine in dosage forms

The HPLC procedure was optimized with a view to develop precise and stable assay method [19-24]. Both the pure drugs Lamotrigine were run in different mobile phase compositions with different C₁₈ columns (250 mm x 4.6mm i.d., 5 μ). The flow rate was also varied from 0.5 ml to 1.2 ml/min. Finally Thermo C₁₈ (250 mm x 4.6mm i.d., 5 μ) with a mobile phase of a mixture of acetonitrile and 0.3 % Triethylamine (adjusted to pH 6.5 using orthophosphoric acid) at a flow rate of 1.0 ml/min with a detection at 305 nm gave sharp and symmetrical peaks with retention time 5.28 and 7.40 for Lamotrigine and Ondansetron hydrochloride respectively. The resolution factor at the above said condition was 2.2. The typical chromatogram of sample solution is shown in Figure 1. Detection was done at 305 nm. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. Results of Analysis of formulation and recovery studies presented in Table 1. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulation (Containing 100 mg of Lamotrigine).

3.2.Method validation

3.2.1.Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table I. From the data obtained, added recoveries of standard drugs were found to be accurate. The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table II. From the data obtained, the developed RP-HPLC method was found to be precise.

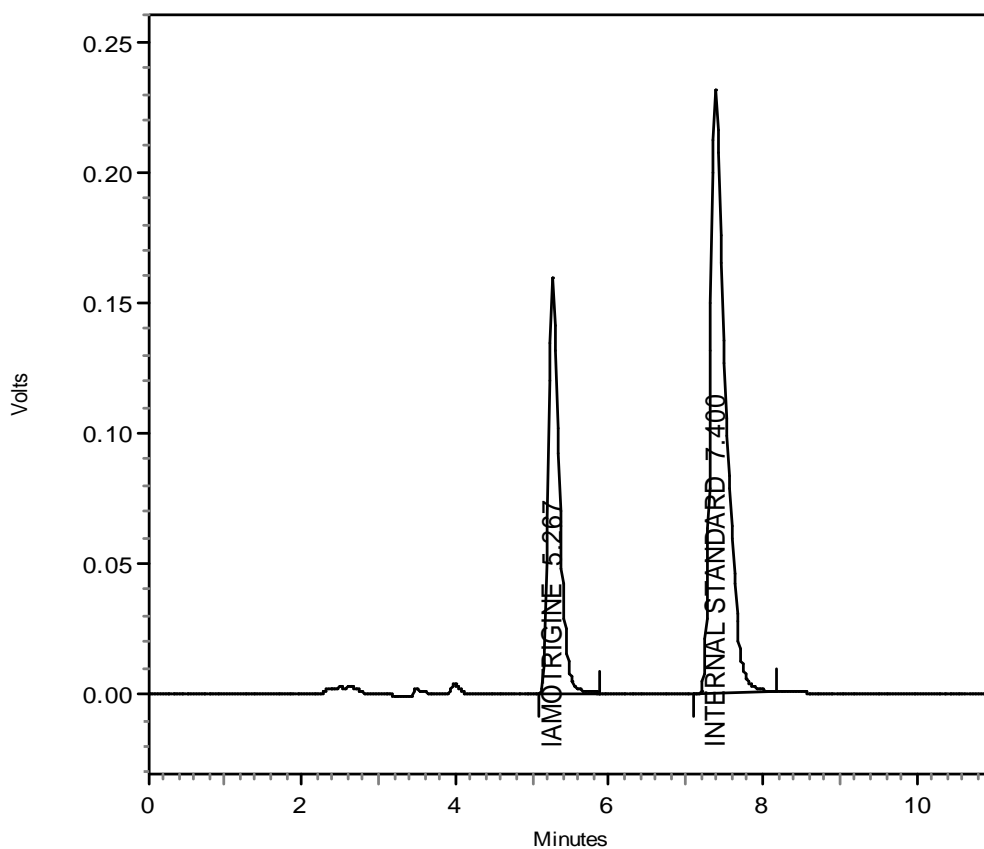
Table I. Results of analysis of formulation and recovery studies

Drug	Amount mg/ tab		% Label claim*	% Recovery*
	Labelled	Found *		
Lamotrigine	100.0	99.19 ± 1.02	99.81 ± 1.11	99.28 ± 0.92

* Average of six determinations, mean ± standard deviation

Table II. System suitability studies

S. No.	Parameters	Lamotrigine
1	Linearity range	10-125 µg/ml
2	Regression equation $Y = mx + c^*$	$0.2619x - 0.0557$
3	Correlation coefficient	0.9992
4	Theoretical plate/meter	2895.37.64
5	Resolution factor	2.22
6	LOD (ng/ml)	7
7	LOQ (ng/ml)	15

**Fig. 1 The typical chromatogram of sample solution**

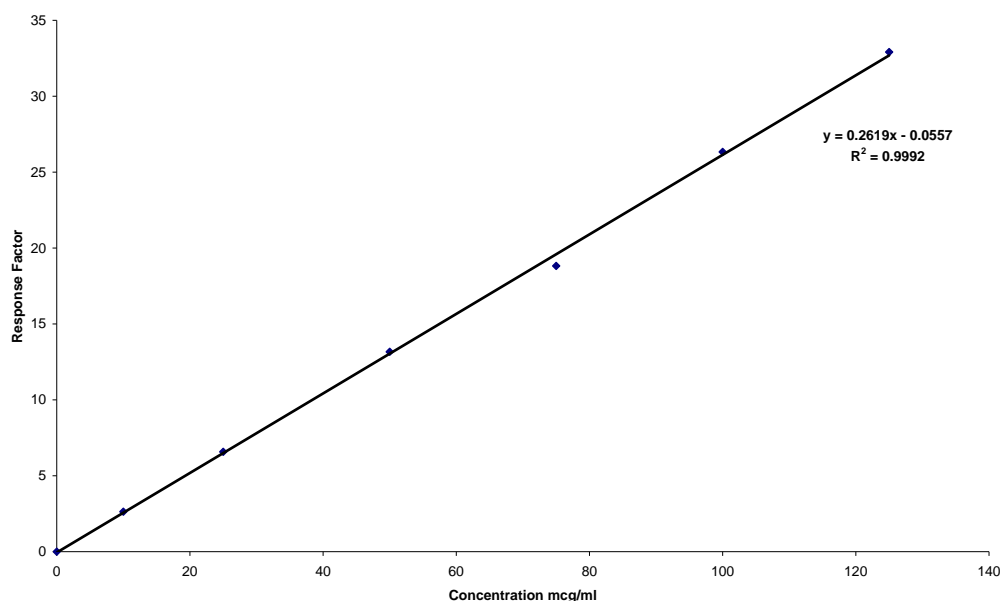


Fig. 2 Calibration Curve of Lamotrigine

3.2.2. Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 10.0 $\mu\text{g/ml}$ to $\mu\text{g/ml}$ for Lamotrigine. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was $y = 0.2619x - 0.0557$ ($R^2=0.9992$) for Lamotrigine. The calibration Curve presented in **Figure 2**.

3.2.3. Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Lamotrigine was found to be 7.0 ng/ml. 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 15.0 ng/ml respectively.

3.2.4. Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h

at room temperature. The results show that for both solutions, the retention time and peak area of Lamotrigine remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

3.2.5. System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug and the system suitability parameters fall within ± 3 % standard deviation range during routine performance of the method. The results are presented in Table II.

4. Conclusion

The use of the described HPLC method allows a selective and quantitatively accurate analysis of lamotrigine drugs in pharmaceutical dosage forms. The proposed RP-HPLC method for the estimation of Lamotrigine in pharmaceutical dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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