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Research Article

Development and Validation of HLPC Method for the Estimation of Lamotrigine in Bulk and Pharmaceutical Formulations

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A simple, precise, accurate, and rapid HPLC method was developed to estimate the amount of lamotrigine in bulk and its pharmaceutical formulations. Waters Alliance HPLC system equipped with autosampler, ultraviolet detector, and Symmetry C8 (4.6 mm ID × 150 mm, 3.5 μm, Make: XTerra) column were used for the quantification of the drug. Separation was carried out at a flow rate of 0.7 mL/min. of mobile phase (acetonitrile and potassium dihydrogen phosphate buffer of pH = 7.0 in the ratio 60 : 40 v/v), and the detection was carried out at a wavelength of 215 nm. The retention time of lamotrigine was found to be 2.797 min. The linearity was obeyed in the range of concentration 5–25 μg/mL. The developed method was found to be repeatable and reproducible; hence, it can be used as an alternative method in assay of the lamotrigine in any pharmaceutical industries.

1. Introduction

Lamotrigine (LTG) (Lamictal by Glaxo SmithKline) is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. It is generally accepted to be a member of the sodium channel blocking class of antiepileptic drugs [1], but it could have additional actions in as much as it has a broader spectrum of action than other sodium channel antiepileptic drugs such as phenytoin and carbamazepine and is effective in the treatment of the depressed phase of bipolar disorder, whereas other sodium channel blocking antiepileptic drugs are not. In addition, lamotrigine shares few side-effects with other, unrelated anticonvulsants known to inhibit sodium channels, which further emphasizes its unique properties [2]. Lamotrigine is chemically known as 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, $C_9H_7Cl_2N_5$, molecular weight 256 g. Emami et al. [3] developed a HPLC method for determination of lamotrigine and related compounds in tablet formulations. Youssef and Taha [4] have developed a spectrophotometric, TLC, and HPLC methods for the determination of lamotrigine in presence of its impurity. A stability indicating LC method was developed for the determination of lamotrigine by Srinivasulu et al. [5]. Sallustio and Morris [6] reported a high-performance liquid chromatography method for quantitation of plasma lamotrigine concentrations in patients with epilepsy. Simultaneous determination of lamotrigine, zonisamide, and carbamazepine in human plasma by high-performance liquid chromatography was reported by Griner-Sosanko et al. [7]. Several HPLC methods were reported in the literature for the determination of lamotrigine in different biological fluids [8–19]. A spectroscopic method [20] in UV region (307 nm) was developed for the quantitative determination of lamotrigine in bulk and in dosage form in which linearity obeyed in the concentration range 5–50 mcg/mL. A few visible spectrophotometric methods [21–23] were developed for the determination of lamotrigine (LTG) in pharmaceutical dosage forms and urine samples using some chromogenic reagents. The aim of the present study was to develop and validate rapid, simple, and selective liquid chromatography method for LTG quality control in tablets.

2. Experimental

Waters Alliance HPLC system equipped with autosampler, binary gradient pump, and dual wavelength UV-visible detector was applied to perform the analyses. An analytical column; Symmetry C8 (4.6 mm ID × 150 mm, 3.5 μm, Make: XTerra) was used in the analysis. Chromatographic Software Empower was used for data collection and processing.

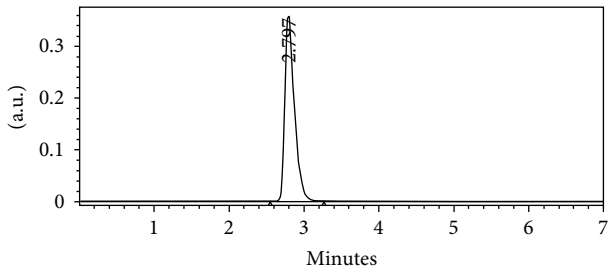

Figure 1: A typical RP-HPLC chromatogram of lamotrigine standard 15 μg/mL.

Table 1: Precision of the method.

Intraday precision		Interday precision	
Injection	Area	Injection	Area
Injection-1	3108761	Injection-1	3139181
Injection-2	3113250	Injection-2	3126470
Injection-3	3123530	Injection-3	3135139
Injection-4	3129896	Injection-4	3143320
Injection-5	3136552	Injection-5	3143734
Average	3122398	Average	3137569
Standard deviation	11483.6	Standard deviation	7120.1
% RSD	0.37	% RSD	0.23

Acceptance criteria: the % RSD should be between less than 2.0.

3. Materials and Chemicals

Lamotrigine LMG (purity 99.7%) was gifted by Dr. Reddy's Laboratories Ltd., Hyderabad. The commercially available formulations of lamotrigine were purchased from the local market. The water of HPLC was prepared by double glass distillation and filtration through 0.45 mm filters. Acetonitrile of HPLC grade was obtained from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and sodium hydroxide of analytical grade are purchased from Qualigens Fine Chemicals Ltd., Mumbai.

3.1. Preparation of Buffer Solution

(1) About 7.0 grams of potassium dihydrogen phosphate were weighed accurately, transferred into a 1000 mL beaker, and dissolved in 500 mL of HPLC grade water. The solution was sonicated for 30 min., degassed, and then made to total volume. The pH of the resulting solution was adjusted to 7.0 by adding dilute sodium hydroxide solution and filtered through 0.45 μm membrane filter.

(2) The mobile phase was prepared by adding of 600 mL acetonitrile to 400 mL of 0.7% potassium dihydrogen phosphate buffer of pH 7.0. The two solutions were mixed well, degassed for 30 min., and filtered through 0.45 μm membrane filter.

3.2. Preparation of Standard Drug Solution. Stock solution of the lamotrigine (LMG) was prepared by dissolving accurately weighed 10 mg of lamotrigine standard in 70 mL of mobile phase in a 100 mL volumetric flask, sonicated, and made up to the mark. Working standard solution of 15 μg/mL was prepared by transferring about 1.5 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with mobile phase, sonicated, and filtered through 0.45 μm filter. A series dilute solutions ranging from 5 to 25 μg/mL were prepared in similar manner and transferred into an auto sampler vial for analysis.

Table 2: Accuracy of the proposed method.

% concentration	Area	Amount added	Amount found	Percent of recovery	Mean recovery
50%	1559242	5.0	4.99	99.8%	99.96%
100%	3111570	10.0	9.96	99.6%	
150%	4656674	15.0	14.9	99.4%	

Acceptance criteria: the percent of recovery for each level should be between 98.0 and 102.0%.

Table 3: Linearity of the drug concentration with peak area.

S. no.	Concentration μg/mL	Area of the peak
1	5	1025925
2	10	2027807
3	15	3095827
4	20	4058008
5	25	5068723
Slope		16351
Intercept		78025
Correlation coefficient		0.9998

Acceptance criteria: correlation coefficient should be not less than 0.999.

3.3. Analysis of Pharmaceutical Formulations. Five tablets were accurately weighed and finely powdered in a mortar. An amount of tablet mass equivalent to 10 mg was transferred to a 100 mL volumetric flask and dissolved in 70 mL of mobile phase. Then, the flask was placed in ultrasonic bath for 15 min. The resulting suspension was diluted to volume with mobile phase and then filtered through 0.45 μm membrane. Further three different concentration solutions (i.e., 50%, 100%, and 150%) of the target concentration were prepared, and the percent of recovery was studied.

3.4. Chromatographic Conditions. The mobile phase was pumped from the solvent reservoir into the column at a flow rate of 0.7 mL/min. The column was allowed to equilibrate for 30 min. prior to the injection of 20 μL the standard. The detection of the components eluted from the column was monitored at 215 nm. The chromatogram was recorded for the five replicate injections of the working standard solution, and precision was calculated. Calibration graph was constructed by plotting peak area against concentration of five sample solutions. The assay of the drug in different pharmaceutical formulations was calculated at three different concentrations.

Table 4: Study of robustness of the proposed method.

S. no.	Flow rate mL/min.	Plate count	Tailing factor	% mobile phase	Plate count	Tailing factor
1	0.6	2573.0	1.6	10% less	2250.9	1.6
2	0.7	2365.7	1.5	Actual	2365.7	1.5
3	0.8	2461.1	1.5	10% high	2166.3	1.5

*Results for actual flow (0.7 mL/min) have been considered from assay standard.
*Results for actual mobile phase composition (60 : 40 acetonitrile : buffer) have been considered.

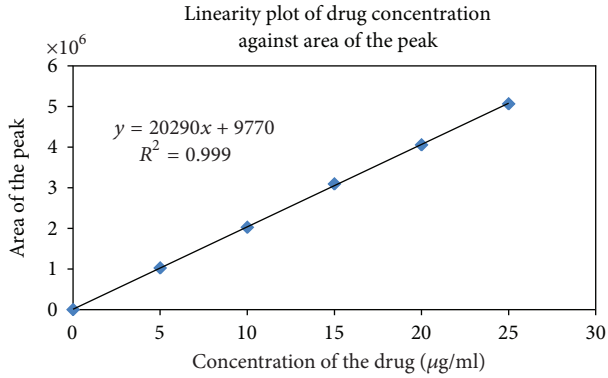

Figure 2: Linearity of the drug concentration against the area of the peak.

4. Results and Discussion

The working standard solution of concentration 15 μg/mL was injected into 20 μL loop, and the chromatogram was recorded. A typical chromatogram was presented in Figure 1. The system suitable parameters such as tailing factor (1.5) and number of theoretical plates (2365) are found to be within the limits. The retention time of the component was found to be 2.647 min. The intraday precision or interday precision of a method was expressed in terms of statistical parameters such as standard deviation and % RSD. The % RSD was calculated for five replicate measurements and found to be less than 2.0. Interday precision of the method was determined by carrying out the experiment on different days using same instrument and same column under similar chromatographic conditions. The results are presented in Table 1. The linearity of the method was studied by injecting 20 μL of working standard solutions of concentration ranging from 5 to 25 μg/mL into the column, and linearity report was obtained. A calibration curve was constructed by plotting concentration against peak area (Figure 2). The correlation coefficient, slope, and intercept were presented in Table 3. The accuracy of the method was determined from recovery experiments. The recovery studies were carried out at three different concentration levels (50%, 100%, and 150% of target concentration). The percentage recovery of the drug at three different concentration levels is presented in Table 2. Robustness of the proposed method is checked by making slight deliberate change in the experimental procedures. In the present method, a deliberate change in the flow rate and mobile phase composition is made to evaluate the impact on the method. The results are summarised in Table 4. The drug obeys linearity in the range of 5–25 μg/mL, and the correlation coefficient is found to be 0.9998. The developed method is found to be accurate and precise as indicated by recovery studies and % RSD not more than 2.0. Recovery studies are performed at 50%, 100%, and 150% concentration levels, and the results are found to be within the limits mentioned as per ICH Guidelines.

5. Conclusions

The proposed HPLC method for the determination of lamotrigine in pharmaceutical formulation was found to be sensitive, accurate, precise, simple, and rapid. Hence, the present HPLC method may be used for routine analysis of the raw materials and formulations.

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