International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-3, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Analytical Method Development and Validation of Lamotrigine by High Performance Liquid Chromatography

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Manuscript No: IJPRS/V3/I3/00341, Received On: 16/07/2014, Accepted On: 31/07/2014

ABSTRACT

In the present work, a rapid and sensitive RP-HPLC Method with UV detection (270 nm) for routine analysis of Lamotrigine in Bulk and in Pharmaceutical formulation was developed. Chromatography was performed with mobile phase containing a mixture of methanol and Phosphate buffer (70:30 v/v) with flow rate 1.1 ml min^{-1}. In the range of 20-100 µg/ml, the linearity of Lamotrigine shows a correlation co-efficient of 0.9998. Recovery of drug was found to be good (99-102%). Method was found to be reproducible with relative standard deviation (RSD) values less than 2% for intra and inter-day precision. The proposed method was validated as per the standard guidelines.

KEYWORDS

Lamotrigine, bulk, formulation, HPLC

INTRODUCTION

Lamotrigine, 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, is a novel antiepileptic drug chemically unrelated to other anticonvulsants used as an add-on therapy of seizure in children and adults[1]. It has been shown that lamotrigine is effective against partial and secondarily generalized tonic–clonic seizures as monotherapy or adjunctive treatment[2]. It's mechanism of action seems to be the inhibition of the release of excitatory neurotransmitters like aspartate and glutamate and also involvement in the blocking of voltage-dependent sodium channels[1–3]. Several methods for determination of lamotrigine and its metabolites in biological matrices have been developed including reversed-phase HPLC[2–10], gas chromatography with nitrogen phosphorus detector[11], capillary electrophoresis[12,13], chromatography–thermo spray mass spectrometry[14], immune fluorometric assay[15] and radioimmunoassay[16]. An analytical method for the detection of trace amount of the principal synthetic route indicative impurity in lamotrigine including pre concentration sample extract by normal-phase HPLC which then was analysed with a reversed-phase HPLC TSP-MS has also been reported[1]. 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[17]. An official monograph of lamotrigine does not exist in any pharmacopoeia. Therefore, it is very imperative to develop a simple and suitable analytical method for the measurement of lamotrigine in bulk and in formulations. Such methods could be easily adapted for routine and in-process quality control analysis, dissolution or similar studies.

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EXPERIMENTAL

Instrument

The specifications of HPLC instrument used are as follows. A gradient high pressure liquid chromatograph (Shimadzu, class LC-Series) with one LC-10 AT VP pumps, UV/VIS detector SPD-10A VP, CTO-10 AS VP column oven (Shimadzu), SCL-10AVP system controller (Shimadzu), a disposable guard column LC-18 (PELLIGUARD)TM, LC-18, 2 cm, supelco, Inc., Bellefonte, and a Reverse Phase C-18 Column (150mm x 4.6 mm i.d., particle size 5 µm) was used The HPLC system was equipped with the software class, N-2000 CHROMTECK (Shimadzu).

Chemicals and Reagents

All the chemicals used were of A.R. grade procured from qualigens Mumbai, S. D. Fine Chem Ltd, Merck and Spectrochem, Mumbai, India. Distilled water was used for making the solutions.

Lamotrigine was obtained as a gift sample from Alembic Ltd., Vadodara, India. Lamittor DT (100 mg tablets) from Torrent pharmaceutical Ltd India was collected from local market.

Preparation of Standard Stock Solution of Lamotrigine

100 mg of standard Lamotrigine was accurately weighed and transferred to a 100 ml volumetric flask, dissolved in 10 ml of methanol and diluted to 100ml with buffer (pH-4.5), then sonicated for 10 min. From this a working standard solution of 100 µg/ml was prepared. By using the working standard, aliquots of 20, 40, 60, 80 & 100 µg/ml were prepared with buffer of pH (4.5). 20 µL of each dilution was injected into the column with a flow rate of 1.1 ml/min.

Assay of Lamotrigine in Tablets

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of Lamotrigine was transferred to 100 ml volumetric flask containing 10 ml of methanol and the contents of the flask were sonicated for 15 min, to ensure the complete solubility of the drug, the mixture was then made upto 100 ml with buffer of pH (4.5). The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter. From this a working standard solution of 100 µg/ml of strength was prepared. Aliquots of 20,40,60,80 & 100µg/ml were made in 100 ml volumetric flasks & diluted with buffer of pH (4.5). This solution (20 µL) was injected five times into the column. The mean values of peak areas were calculated and the drug content in the tablets was quantified using the regression equation.

Chromatographic Conditions

The contents of the mobile phase were phosphate buffer solution (pH: 4.5) and methanol in the ratio of 70:30 percent v/v. The mobile phase was filtered through 0.45-µm-membrane filter and sonicate for 15 min. The flow rate of the mobile phase was maintained at 1.1 ML/min. The column temperature was set at 20±1° C and the detection was carried out by UV-Detector wavelength at 270 nm. The run time was set at 10 min and the volume of the injection loop was 20 µL. Prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The datas were acquired, stored and analyzed with the software class N-2000 Chromteck (Shimadzu).

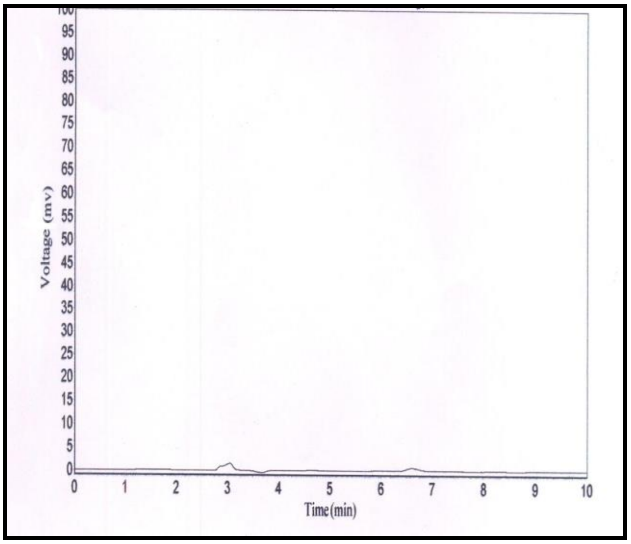

Figure 1: Chromatogram of placebo solution of Lamotrigine

The chromatogram obtained through the injection of the placebo solution did not contain any other peak at the retention time of Lamotrigine. The chromatogram peak purity tools show that the peak was 100%. Thus, it was shown that the peak at 3.707min was not due to any interference from the excipients in the formulation.

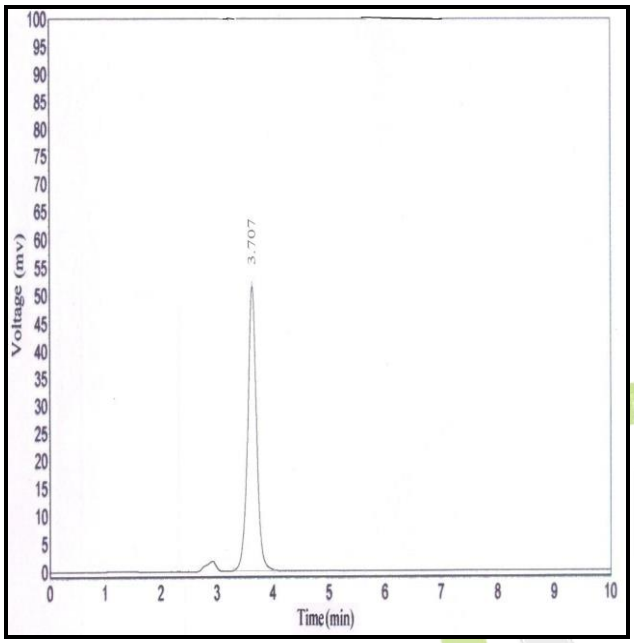

Figure 2: Chromatogram of Lamotrigine

The small peak observed at RT 2.9 is observed both in placebo and drug sample chromatogram.

The Liquid Chromatographic method was validated for the following parameters:

Calibration Procedure

The calibration curve was plotted with five concentrations of the standard drug solution 20-100 µg/ml and chromatography was repeated five times for each dilution. The linearity was evaluated by linear regression analysis. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Five determinations were carried out for each solution, peak areas were recorded for all the solutions. The correlation graph was constructed by plotting the peak areas obtained at the optimum wavelength of detection versus the injected amounts of the respective concentrations.

Linearity and Range

By using the working standard, aliquots of 20, 40, 60, 80 & 100 µg/ml were prepared with buffer of pH (4.5). Five dilutions of each of the above mentioned concentrations were prepared separately and from these five dilutions, 20 µl of each concentration of the drug were injected into the HPLC system and their chromatograms were recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

Precision

The precision of the assay was determined in terms of intra and inter-day variation in the peak area for a set of drug solution (40, 60 and 80 µg/ml) assayed five times on the same day and on three different days.

The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of standard deviation to the mean with 100(CV=SD/MEAN X 100).

Robustness

As defined by the ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ±2% variation in volume of mobile phase composition ±0.1 ml/min in flow rate of mobile phase, ±1°C for column temperature and ±0.1 variation in pH**.**

Specificity and Selectivity

The specificity of the RP-HPLC method was determined by using the parameters like retention time (t_R), resolution (R_S) and tailing factor (T_f). Here tailing factor for peaks of Lamotrigine was less than 2%. The peaks obtained for Lamotrigine was sharp and have clear baseline separation.

RESULTS AND DISCUSSION

Table 1: Calibration of RP- HPLC Method for Determination of Lamotrigine

Concentration of Lamotrigine (µg/ml)	Peak Area*
20	178655.5
40	375618.5
60	558631.3
80	753127.4
100	951069.6

*Mean of five determinations

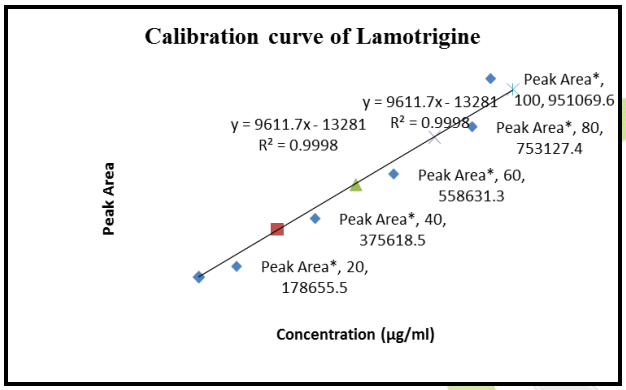

Figure 3: Calibration curve of Lamotrigine

Table 2: Results of the data analysis for the quantitative determination of Lamotrigine by the RP-HPLC

Statistical parameters	RP-HPLC
Concentration range, µg/ml	20-100
Regression equation	Y= 9611.7 X - 13281
Correlation co-efficient (r2)	0.9998
Limit of detection (LOD), µg/ml	0.005
Limit of quantification (LOQ), µg/ml	0.015

Table 3: Intra and Inter-Day Precision for Lamotrigine Assay in Tablet Formulations

Concentration of Lamotrigine (µg/ml)	Observed concentration (µg/ml) of Lamotrigine Found on			
	Intra-Day		Inter-Day	
	Mean (n=5)	C.V. (%)	Mean (n=5)	C.V. (%)
40	40.3	0.79	40.1	1.11
60	60.4	1.11	60.4	1.07
80	80.1	0.72	80.2	1.06

Table 4: Accuracy of Lamotrigine

Sample	Percentage recovery	Mean
S1-80%	99.7	99.6
S2-80%	98.9	
S3-80%	100.3	
S1-100%	100.2	100.5
S2-100%	99.7	
S3-100%	101.6	
S1-120%	100.7	100.5
S2-120%	99.6	
S3-120%	101.3	

Table 5: Assay of Lamotrigine

Formulation	Label claim (mg)	Concentration found (%)
LAMITTOR DT	100	99.7
	100	100.3
	100	101.2

*Average of three determinations

Table 6: Robustness Results for variations in Flow Rate (ml/min)

Method parameter → / Flow rate ↓	Retention time	Tailing factor
0.9	4.061	1.110
1.1	3.707	0.987
1.3	3.681	0.938

Table 7: System Suitability Parameters

Sl .No.	Parameters	Obtained Values
1	Theoretical plates (N)	3011.266
2	Tailing factor (T)	0.987

Table 8: System Evaluation of Lamotrigine

	Retention time [min]	Height [m.V.]	Area [m.V.s]	Area [%]	Tailing factor
Sample	3.707	51334.248	568719.625	100.000	0.987
Standard	3.699	49867.854	523648.286	100.000	0.963

The run time was set at 10 min and the retention time for Lamotrigine was found 3.707 min as shown in Figure 2. The sample solution was injected 5 times and the retention times were found to be same. The averages of 5 such determinations of peak areas are shown in Table 1.

When the concentrations of Lamotrigine and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (r^2 = 0.9998) was observed between the concentration of Lamotrigine and the respective peak areas in the range 20-100 µg/ml. The regression of Lamotrigine was found to be Y= 9611.7 X - 13281, where 'Y' is the peak area and 'X' is the concentration of Lamotrigine (Table 2).

The regression equation was used to estimate the amount of Lamotrigine, either in tablet formulations or in validation study (precision and accuracy). The proposed RP-HPLC method was validated for intra and inter-day variation. When the solution containing 40, 60 and 80 µg/ml of Lamotrigine were repeatedly injected on the same day, the coefficient of variance (CV) in the peak area for five replicate injections was found to be less than 2.11%. Also the inter day variation (3 days and five injections) was found to be less than 1.11% (Table 3).

A known amount of the drug solution (80, 100 and 120 µg) was added to the powder sample of the tablet formulation and subjected to the estimation of the drug for the recovery studies. There was a high recovery of Lamotrigine (99.7%, 100.3%, and 101.2%) indicating that the proposed procedure for the determination of Lamotrigine in the tablet formulation is highly accurate (Table 5).

Accuracy of the method was confirmed by analyzing 80%, 100% and 120% sample. The mean of the analysis was found to be 99.6%, 100.5% and 100.5% respectively (Table 5). This was again confirmed by analyzing a marketed sample (Lamittor DT) and getting the result as per the labeled quantity of the drug (Table 4).

Robustness of the method was tested by altering the flow rate (increasing 0.2 mg/ml and decreasing 0.2 mg/ml and it was found that there was no significance influence in the result (Table 6).

The numbers of theoretical plates are found to be more than 2000 and tailing factor less than 2. Hence the method complies with the ICH

guideline for system suitability parameter (Table 7).

In system evaluation test, both the standard and the sample have identical retention time, peak and tailing factor. So it is considered to be complies the ICH guideline for system evaluation (Table 8).

The results showed that the proposed RP-HPLC method is highly reproducible. The RP-HPLC method developed in the present study has been used to quantify Lamotrigine in tablet formulations.

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Lamotrigine in bulk drug and pharmaceutical formulations. This method can be used for the routine determination of Lamotrigine in bulk drug and in pharmaceutical formulations.

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