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Estimation of Lamotrigine by RP-HPLC Method

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Abstract: A rapid and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of lamotrigine in its pure form as well as in pharmaceutical dosage forms. Chromatography was carried out on a Luna C_{18} column using a mixture of potassium dihydrogen phosphate buffer (pH 7.3) and methanol in a ratio of 60:40 v/v as the mobile phase at a flow rate of 1.0 mL/min. The detection was done at 305 nm. The retention time of the drug was 6.1 min. The method produced linear responses in the concentration range of 10 to 70 µg/mL of lamotrigine. The method was found to be reproducible for analysis of the drug in tablet dosage forms.

Keywords: Lamotrigine, Estimation, Tablets, RP-HPLC.

Introduction

Lamotrigine[1-6], (6-(2, 3-dichlorophenyl)-1, 2, 4-triazine-3,5-diamine) is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. 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 medicament since lithium approved by food and drug administration (FDA) for the maintenance treatment of bipolar type I disorder. Chemically unrelated to other anticonvulsants, lamotrigine has relatively few side-effects and does not require blood monitoring. The exact way how lamotrigine works is unknown.

Lamotrigine is thought to exert its anticonvulsant effect by stabilizing pre synaptic neuronal membranes. The *in vitro* pharmacological studies suggest that lamotrigine inhibits voltage-sensitive sodium channels, thereby stabilizing neuronal membranes and consequently modulating presynaptic transmitter release of excitatory amino acids (*e.g.* glutamate and aspartate). Some methods based on HPLC[7-19], LC-MS[20-22], HPTLC[23] and spectrophotometry[24-26] were reported earlier for the determination of lamotrigine individually and in combination with other drugs and related substances in dosage forms and biological fluids. The present investigation by the author describes a rapid, accurate and precise RP- HPLC method for the determination of lamotrigine in tablet dosage forms.

Experimental

A Shimadzu Prominence high pressure liquid chromatographic instrument provided with a Luna C_{18} column (150 mm x 4.6 mm, 5 µ), a LC 20 AT quaternary pump, a CTO 20A column oven and an SPD 20A UV-Visible detector was employed in the study. A 20 µL Hamilton injection syringe was used for sample injection. Data acquisition was done by using LC solution software.

Methanol (HPLC grade), potassium dihydrogen phosphate (ExcelaR grade) and sodium hydroxide (ExcelaR grade) of Qualigens, India were used in the study. HPLC grade water prepared using Millipore Milli Q system was used for preparing the mobile phase mixture.

A freshly prepared binary mixture of potassium dihydrogen phosphate buffer (pH 7.3) and methanol in a ratio of 60:40 v/v was used as mobile phase and as diluent for making various working solutions. The mobile phase was filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1 mL/min. The column temperature was maintained at 25±1 ^{0}C. The detection was carried out at 305 nm.

Preparation of stock and working standard solutions

About 100 mg of lamotrigine was weighed accurately and transferred into a 100 mL volumetric flask containing 25 mL of the mobile phase. The solution was sonicated for 20 min and then the volume made up with a further quantity of the mobile phase to get 1 mg/mL solution. An aliquot of this solution was suitably diluted with the mobile phase to get a working standard of 100 µg/mL of lamotrigine.

Linearity and construction of calibration curve

The quantitative determination of the drug was accomplished by an external standard method. The mobile phase was filtered through a 0.45 µ membrane filter before use. The flow rate of the mobile phase was adjusted to 1 mL/min. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution.

Linearity of the peak area response was determined by taking measurements at seven concentration points (six replicates at each point). Working dilutions of lamotrigine in the range of 10- 70 µg/mL were prepared by taking suitable aliquots of the working standard solutions in different 10 mL volumetric flasks and diluting up to the mark with the mobile phase. Twenty microlitres of the dilutions was injected each time into the column at a flow rate of 1 mL/min. Each dilution was injected six times into the column. The drug in the eluate was monitored at 305 nm and the corresponding chromatograms were obtained. From these chromatograms, the mean peak areas were noted and a plot of concentrations over the peak areas was constructed. The regression of the plot was computed by least square regression method. The linear relationship was found to be of 10-70 µg/mL between the concentration of lamotrigine and peak area. This regression equation was later used to estimate the amount of lamotrigine in pharmaceutical dosage forms. The calibration data and regression parameters are reported in Table 1.

Table 1. Calibration of the proposed method

Concentration of lamotrigine, µg/mL	Mean Peak Area (n=6)
10	126693
20	245143
30	367715
40	488754
50	615236
60	734806
70	865947

Regression equation: y = 12302x - 45 (r=0.9999)

Estimation of lamotrigine in tablet dosage forms

Twenty tablets of Lamitor each containing 25 mg of lamotrigine were weighed and finely powdered in a mortar. From this, the average weight of a tablet is calculated (404.1 mg). An accurately weighed portion from this powder equivalent to 100 mg of lamotrigine was transferred to a 100 mL volumetric flask containing 25 mL of the mobile phase. The contents of the flask were sonicated for about 15 min for complete solubility of the drug. Then the mixture was filtered through a 0.45 µ membrane filter and made up to the volume with the mobile phase. From the above solution a dilution equivalent to 50 µg/mL (a dilution within the linearity range) was prepared using the mobile phase. Twenty microlitres of the above solution was then injected eight times into the column. The mean of the peak areas obtained was calculated and the drug content in the formulation was calculated by using the regression equation obtained for the reference sample. The same procedure is adopted for the estimation of the drug from the tablets of Lamepil containing 25 mg of lamotrigine.

Results and Discussion

The present study was aimed to develop a rapid, precise and accurate HPLC method for the analysis of lamotrigine in pharmaceutical dosage forms. In order to effect analysis of the component peaks under isocratic conditions, mixtures of methanol and potassium dihydorgen phosphate buffer in different combinations were tested as mobile phase on a C_{18} stationary phase. A binary mixture of potassium dihydrogen phosphate buffer (pH 7.3) and methanol in a ratio of 60:40v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was sharp. The retention time of the drug was found at 6.1 min. A model chromatogram is shown in Figure 1.

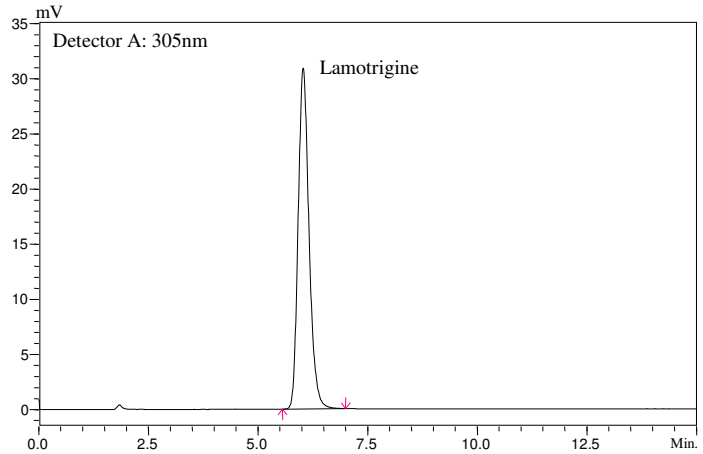

Figure 1. A model chromatogram showing the separation of lamotrigine

Each of the samples was injected six times and the same retention times were observed in all cases. The mean peak area of lamotrigine for different concentration setup was calculated and the average value at each concentration is shown in Table 1. A good linear relationship (r=0.9999) was observed between the concentrations of lamotrigine and the corresponding peak areas. The linearity range was found to be 10-70 µg/mL. The regression equation of the calibration curve between concentration of lamotrigine over its peak area was found to be $y = 12302\,x - 45$ (where y is the peak area and x is the concentration of lamotrigine). The method was validated in terms of precision, accuracy, robustness, LOD

and LOQ. The intra-day and inter-day variations studied at 30, 40 and 50 µg/mL of working drug concentrations showed a low coefficient of variation (Table 2). This reveals that the method is quite precise.

Table 1. Calibration of the proposed method

Concentration of lamotrigine, µg/mL	Mean peak area (n=6)
10	126693
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50	615236
60	734806
70	865947

Regression equation: y = 12302x - 45 (r=0.9999)

Table 2. Precision of the proposed method

Concentration of lamotrigine, µg/mL	Intra-day precision			Inter-day precision		
	Mean amount found (n=3)	Percent amount found	Percent RSD	Mean amount found (n=9)	Percent amount found	Percent RSD
30	29.95	99.83	0.49	29.85	99.50	0.35
40	39.98	99.95	0.44	39.97	99.93	0.45
50	49.68	99.36	0.24	49.78	99.56	0.38

The percent recoveries of the drug solutions were studied at three different concentrations levels. The percent individual recovery and the percent RSD values at each level were (100.02-100.05) within the acceptance limits. The results are given in Table 3. The drug content in the tablets was quantified by using the proposed method of analysis.

Table 3. Accuracy data (Triplicate values at 80, 100 and 120 percent levels)

Amount taken, µg	Amount found, µg	Percent recovery	Mean % recovery	Percent RSD
20+25 =45	45.01	100.02		
20+25 =45	44.98	99.96	100.02	0.07
20+25 =45	45.04	100.09		
25+25=50	49.98	99.96		
25+25=50	50.08	100.16	100.01	0.13
25+25=50	49.89	99.92		
25+30=55	55.09	100.16	100.05	0.20
25+30=55	55.1	100.18		
25+30=55	54.9	99.82		

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The lowest values of LOD and LOQ as obtained by the proposed method indicate the method is sensitive. The working solution of the drug was stable up to 24 hours. The tailing factor, the number theoretical plates and HETP are in the acceptable limits. Therefore, the proposed method can be used for routine quality control and analysis of the drug in bulk samples and in tablet dosage forms. The results of robustness study and system suitability parameters are given in Table 4 & 5.

Table 4. Results of the robustness study

Variations	Chromatographic parameters		
	Tailing factor	Theoretical plates	% Assay
38% of methanol in the mobile phase	1.14	13186	99.4
42% of methanol in the mobile phase	1.13	13258	99.6
Flow rate at 0.9 mL/min	1.14	12985	101.1
Flow rate at 1.1 mL/min	1.13	13150	99.8
Column oven temperature at 23 ^{0}C	1.13	13190	99.6
Column oven temperature at 27 ^{0}C	1.13	13205	99.8
pH of mobile phase at 7.25	1.14	13185	101.2
pH of mobile phase at 7.35	1.13	13900	99.8

The mean amount of lamotrigine obtained from two different tablet dosage forms is 99.96 and 100.16% as shown in Table 6 indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non interference of the commonly used excipients in the tablets and hence the method is specific.

Table 5. System suitability parameters

Parameters	Value
Theoretical plates (n)	3298
Plates per meter (N)	21988
HETP	4.5478×10^{-5}
Tailing factor (T)	1.1
LOD, µg/mL	0.102
LOQ, µg/mL	0.342

Table 6. Recovery of lamotrigine from tablets formulations

Sample	Labeled amount, mg	Amount found* ± S.D.	%Recovery ± R.S.D.
Lamitor	25 mg	25.04 ± 0.56	100.16 ± 0.02
Lamepil	25 mg	24.99 ± 0.64	99.96 ± 0.03

**Average ± standard deviation of eight determinations*

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References

1. Indian Pharmacopoiea 2007, Govt. of India, Ministry of Health and Family Welfare, Delhi, Indian Pharmacopoeial Commission, Ghaziabad, 2007.
2. USP 32 NF 27, United States Phamacopoeia, The United States Pharmacopoeial Convention, Rockville, MD, 2009.
3. Sweetman S C Martindale, The Complete Drug Reference. 34th Ed. London, Pharmaceutical Press, 2005.
4. http://www.drugbank.ca/drugs/DB00555
5. http://www.rxlist.com/cgi/generic/lamotrigine.htm
6. http://www.usp.org/pdf/EN/pendingStandards/lamotrigine.pdf

7. Papadoyannis I N, Zotou A C and Samanidou V F, *J Liq Chromatogr Relat Tech.*, 1995**, 18(13)**, 2593-2609.
8. Emami J, Ghassami N and Ahmadi F, *J Pharm Biomed Anal.,* 2006, **40**, 999-1005.
9. Stoforidis P A, Morgan D J, O'Brien T J and Vajda F J E, *J Chromatogr B Biomed Sci Appl.*, 1999, **727(1-2)**, 113-118.
10. Vidal E, Pascual C and Pou L, *J Chromatogr B Biomed Sci Appl.*, 1999, **736(1-2)**, 295-298.
11. Yamashita S, Furuno K, Kawasaki H, Gomita Y, Yoshinaga H, Yamatogi Y and Ohtahara S, *J Chromatogr B Biomed Sci Appl.*, 1995, **670**, 354-357.
12. Danilo C, Andrea S, Ugo D G and Gaetano B, *Ther Drug Monit.,* 2001, **23**, 665.
13. Hart A P, Mazarr-Proo S, Blackwell W and Dasgupta A, *Ther Drug Monit.,* 1997, **19**, 431-435.
14. Manuela C, Monica B, Erica C, Carmina C, Fiorenzo A, Roberto R and Agostino B, *J Chromatogr B*., 2005, **828**, 113-117.
15. Merce T, Miquel R, Santiago A and Jacint C, *Ther Drug Monit.,* 2000, **22**, 621.
16. Khoschsorur G A, Fruhwirth F and Baumann G H, *Chromatogr.*, 2001, **54**, 345-349.
17. Dumortier G, Pons D, Zerrouk A, Januel D, Saba G and Degrassat K, 2001, **24**, 3171. *J Liq Chromatogr Rel Tech.,* 2001, **24**, 3171.
18. Youssef N F and Taha E A, *Chem Pharm Bull,* 2007, **55(4)**, 541-545.
19. Patil K M and Bodhankar S L*, J Pharm Biomed Anal.,* 2005, **39**, 181-186.
20. Jimenez G M, Moreno A C P A, Morales L M, Espinosa L R and Martínez M M, *Bioanalysis,* 2009, **1(1)**, 47-55.
21. Olof B, Inger O and Helena N, *Ther Drug Monit.,* 2006, **28(5)**, 603-607.
22. Murali S, Angela B and Rory R, *Ther Drug Monit.*, 2008, **30(3)**, 347-356.
23. Patil K M, Agarwal A K and Bodhankar S L, *Indian J Pharm Sci*., 2004, **66(3)**, 283-286.
24. Talekar R S, Dhake A S, Sonaje D B and Mourya V K, *Indian J Pharm Sci*., 2000, **62**, 51-52.
25. Rajput S J and Patel A K, *Indian J Pharm Sci*., 2004, **66(3)**, 342.
26. Alizadeh N, Khakinahad R and Jabbari A, *Pharmazie,* 2008, **63(11)**, 791-795.
27. Validation of analytical procedures: Text and Methodology, ICH Hormonised Tripartite Guideline Q2 (R1), Commission of the European Communities, 2005.