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Development and validation of the analytical method by high performance liquid chromatography (HPLC) for Lamotrigine raw material

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ABSTRACT. Currently when all roads lead to the pursuit of total quality in production of drugs, it is essential to fully understand each phase of a production process. In this case, validation is the appropriate tool to ensure reliability of a production process involving new equipment and the analytical methodology, either in the pharmaceutical, food, computer, microelectronics area or any other area where the quality of the manufactured product is one of the main reasons for the existence of a given company. This study aimed to analyze the main aspects of the validation of analytical methods for Lamotrigine, new drug for bipolar disorder with wide use today. In conclusion, the proposed analytical method for determination of the lamotrigine content in raw materials is adequate, effective and capable of reproducing reliable results during analysis. This method is fast (running time of 10 minutes), selective, accurate, precise and robust for the determination of the drug, with no observed interfering substances in the optimum wavelength. It could be concluded that this may be a routine method for quality control laboratories to certify the quality of Lamotrigine.

Keywords: validation, Lamotrigine, HPLC, phenylthiazinic.

RESUMO. Desenvolvimento e validação do método analítico por cromatografia líquida de alta eficiência (CLAE) para a matéria prima Lamotrigina. Atualmente quando todos os caminhos levam à busca da qualidade total na produção de medicamentos, torna-se indispensável conhecer perfeitamente cada fase de um processo produtivo. Neste caso, a validação é a ferramenta adequada para garantir a confiabilidade de instalação de um processo produtivo, de equipamento novo e, inclusive, da metodologia analítica, seja do setor farmacêutico, alimentício, informática microeletrônico ou qualquer outra área onde a qualidade do produto fabricado é uma das principais razões da existência da empresa. O presente trabalho teve por objetivo analisar os principais aspectos da validação de métodos analíticos para Lamotrigina, fármaco novo para transtorno bipolar com amplo uso atualmente. Em conclusão, o método analítico proposto para determinação do teor de Lamotrigina em matéria-prima é adequado, eficaz e capaz de reproduzir resultados confiáveis durante as análises. É um método rápido (tempo de corrida de apenas 10 minutos), seletivo, exato, preciso e robusto para a determinação do fármaco, não sendo observada interferência de outras substâncias no melhor comprimento de onda. Concluímos então que este pode ser um método de rotina para laboratórios de controle de qualidade para atestarem a qualidade da Lamotrigina utilizada.

Palavras chave: validação, Lamotrigina, CLAE, feniltiazínico.

Introduction

Lamotrigine is chemically known as 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine (Figure 1) with molecular formula $C_9H_7Cl_2N_5$ and molecular weight of 256.09 daltons (MERCK INDEX, 2006). It is presented as a white to pale cream powder and has pKa 5.7. It is insoluble in water and slightly soluble in 0.1 M HCl (USP, 2000).

Lamotrigine (ANVISA, 2003b) is an antiepileptic drug in C1 list of substances subject to special control (BRASIL, 1998). It is a drug derived from phenylthiazinic indicated for cases of partial epilepsy and tonic-chronic crisis (FEELY, 1999; LEE, 1995). Other studies have shown its effectiveness and safety for treating cases of depression, including type-I bipolar depression and prophylaxis of new episodes

(CALABRESE et al., 1999, 2003; LAFER; SOARES, 2005; SACHS et al., 2000). Cheniaux et al. (2005) reported a case where the patient showed improvement of depressive symptoms without causing cognition damage.

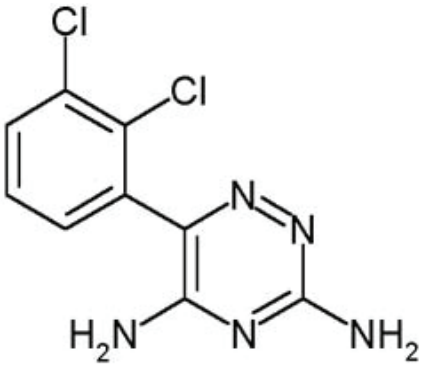

Figure 1. Structural formula of Lamotrigine.

In Brazil, Lamotrigine is marketed as Lamictal (GlaxoSmithKline), Lamitor (Torrent), Neural (Cristalia), Lamotrix (Union Chemicals) and generic drugs, with presentations in tablets of 25 to 100 mg. It has CAS registry under number: 84057-84-1.

The concern about the quality of medicines increases every year (LA ROCA et al., 2007), and not only about the commercial aspect, but legal and ethical aspects, because the health of patients depends on the quality and effectiveness of these drugs (LINSBINSKI et al., 2008; RIBANI et al., 2004). In this regard, various regulatory agencies worldwide are demanding validation methods for registration of new drugs and to ensure the quality of the marketed product (ANVISA, 2003a; GRILLO et al., 2009; ICH, 1997; SANTANA et al., 2007; VALENTINI et al., 2004), because errors from the analysis may result in irreparable financial losses (RIBANI et al., 2004).

There is great interest in developing rapid analytical methods that provide efficient, precise and accurate parameters for quantitative analysis of drugs, important data for routine analysis for quality control and development of new drug forms (RUELA et al., 2009), which could be obtained through spectrophotometric analysis.

Methods for the analysis of the drug in raw material have not been described in official literature, fact which justifies carrying out validation of this substance. Therefore, this study aimed to develop and validate a chromatographic method for routine analysis of quality control for determination of Lamotrigine in raw material.

Material and methods

Reagents and samples

The reagents used were potassium phosphate monobasic (J.T.Baker) of analytical purity, potassium hydroxide (J.T.Baker) and acetonitrile (J.T.Baker), both HPLC grade. The working standard used was Lamotrigine Work Standard Lot: LM 06007, and reference was Lamotrigine Lot: F0H256 obtained from United States Pharmacopeia (USP, 2000), with power of 99.9%.

Equipments

This study used a device to conduct HPLC-RP (High Performance Liquid Chromatography – Reverse Phase) from Merck with diode array detector model L-7455, Pump Model: L-7100; auto sampler model L-7200 and Column Quen and model: L-7300, used for the evaluation of chromatographic purity associate with the spectral scan from 200 nm to 400 nm for the purity peak test. Waters Alliance 2695 Separations Module equipment, equipped with degas, quaternary pump, automatic injector with a 100 mL loop detector and Waters 2487 Dual λ Absorbance Detector for evaluation of other parameters were used. C18 GL analytical column Science Inertsil ODS-3, diameter of 4.6 mm and length of 250 mm was also used. In order to evaluate the intermediate precision and robustness of the method employed, another C18-type analytical column, Agilent Zorbax Eclipse XDB was alternatively used, with the same dimensions as the first.

Preparation of standard solutions and samples

The mobile phase used was a mixture of potassium phosphate monobasic buffer 0.05 mol L^{-1}, pH 6.8 and acetonitrile at a ratio of 75:25 (v:v) with a flow rate of 1.0 mL min^{-1}. The best wavelength for the detection of two active ingredients was selected at 210 nm. The injection volume was 5 µL at furnace temperature of 25°C.

The preparation of the standard assay was performed by transferring 50.0 mg to a 100.0 mL in the flask, adding 30 mL of mobile phase and leading to the ultrasound until total solubilization, and then, the volume was completed with the mobile phase up to 100.0 mL. Soon after, 2 mL of this solution was volumetrically diluted to a 50 mL flask and the solution was filtered through a 0.45 µm Millex® type membrane, and a solution concentration of 0.02 mg mL^{-1} was obtained. Then, the same dilutions were performed to the test solutions to obtain the theoretical concentrations similar to that of the standard solution.

Validation method

The validation of the analytical methodology was conducted under Resolution RE no. 899 of ANVISA. The evaluations have considered the following criteria: selectivity, linearity and range of application, precision, accuracy and robustness. Furthermore, the suitability of the chromatographic system was verified from a study in which 9 injections of the same standard sample were made and parameters such as retention factor (k) and standard deviation (RSD) of chromatographic peaks were evaluated.

The selectivity was determined by analysis of 3 samples of Lamotrigine raw material. The linearity was studied in 5 concentration levels equally spaced, ranging from 32.0 to 48.0 mg mL^{-1}, all injections were repeated three times and measurements were made in triplicate. The accuracy was assessed by the analysis of 9 samples of concentrations 80, 100 and 120%. The precision of the method was evaluated on two levels: repeatability and intermediate precision. In the repeatability assessment, 6 successive determinations of the 100% solution were analyzed. In the intermediate precision evaluation, 6 successive determinations of the 100% solution were analyzed and repeated on two different days using two different analysts. The robustness and stability factors of the analytical solution were monitored for 12h.

Results and discussion

The system suitability was evaluated as if the system being used was adequate for the time of analysis. The parameters were standard deviation, spray factor (tailing), theoretical number of plates and capacity factor (K prime) as Table 1.

Table 1. Table for assessing the system adequacy.

Readings	Areas	Tailing	Plates	K Prime
1	1545235	1.057	7843	1.583
2	1575086	1.060	7513	1.589
3	1563645	1.060	7644	1.566
4	1566282	1.056	7248	1.547
5	1568976	1.061	7422	1.531
6	1574375	1.058	7434	1.527
Aceptance criteria	RSD < 2.0%	< 2.000	> 2000	1 a 10
Retrieved result	0.698	1.059	7517	1.557

The method was selective, since the chromatograms of the solvent showed no peak, while the peaks had the same retention time as the standard, as shown in Figure 2.

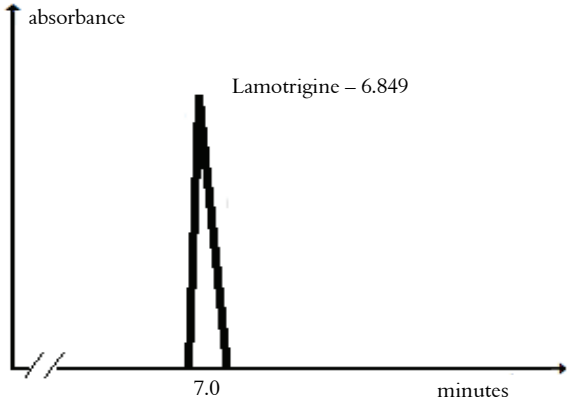

Figure 2. Chromatogram obtained for the selectivity analysis.

The method showed linearity in a range from 32.0 to 48.0 mg mL^{-1}, which corresponds to a variation from 80 to 120% in the drug grade analysis. The least squares method was used in order to verify the proximity of data points to the line pattern, and to provide results with minimum variance (PIMENTEL; BARROS NETO, 1996). The calibration curves obtained by the least squares method presented the following equation: $\hat{y} = 16212.0533x - 82182.8667$, and both showed a correlation coefficient (r^2) of 0.9999, which is consistent with the minimum criterion acceptable (ICH, 1997), which is $r^2 = 0.99$, as shown by the curve on Figure 3, therefore, it is correct to say that the linear model is perfectly suitable to the data (BRAGA; POPPI, 2004).

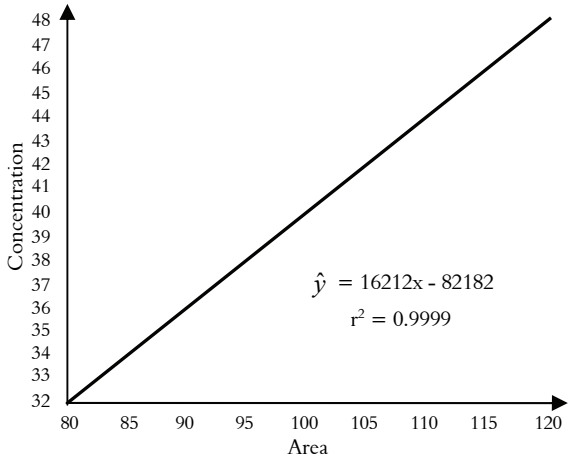

Figure 3. Linearity curve [concentration x3.75x10^4].

The accuracy was assessed on the levels of 80, 100 and 120% and the results are shown in Table 2. The recovery degree was between 99.40 and 100.89%, which is within the specifications expected from 98 to 102% (ANVISA, 2003a e b).

Table 2. Accuracy evaluation for dosage.

Concentration (mg mL^{-1})	Results found (mg mL^{-1})	Results found (%)	RSD of readings	Concentration Recovered (%)
0.0320	0.0318	99.49		
0.0320	0.0320	100.01	0.337	99.88
0.0320	0.0320	100.13		
0.0400	0.0403	100.72		
0.0400	0.0402	100.49	0.198	100.70
0.0400	0.0404	100.89		
0.0480	0.0482	100.51		
0.0480	0.0480	99.91	0.403	100.37
0.0480	0.0483	100.69		
Average recovered				100.32

To repeatability was determined by assessing 6 successive determinations of a 100% solution, and the results are shown in Table 3. The maximum RSD observed was 0.9482%. In the evaluation of the intermediate precision, the determination of 100% of solutions type to each drug were repeated in two days with different analysts, using the same chromatographic column. The RSD values were also within acceptable criteria (RSD ≤ 5%) with precision of day 1 equal to 0.8496% and to day 2 equal to 0.9548%. This fact is important because, according to Honda and Magalhães (2001) the result of an analytical method cannot fail, due to subsequent decisions on these results.

The method was also considered robust for presenting stability of analytical solutions for up to 12h after preparation (maximum variation around the theoretical concentration of 1.98%) as shown in Figure 4.

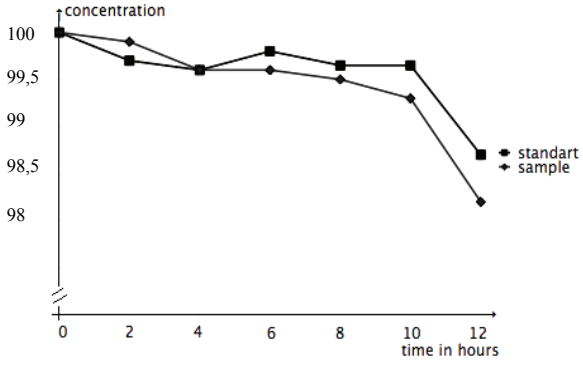

Figure 4. Solution stability.

For robustness, accurate and reliable results were obtained for samples taken at flow rate, temperature, pH, proportion and column brand of mobile phase, whereas the method of analysis did not change in the retention time, keeping up with variation of ± 6.0 minutes, as shown in Figure 5.

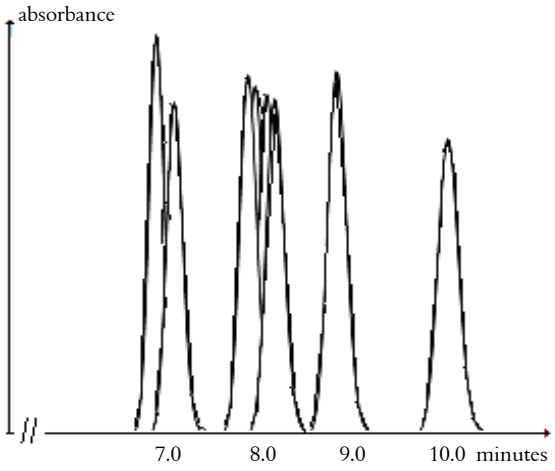

Figure 5. Chromatogram obtained for the robustness analysis using the method of Lamotrigine validation.

Conclusion

In conclusion, the analytical method for determination of Lamotrigine in raw materials is adequate, effective and able to reproduce reliable results during analysis. It is fast (running time of 10 minutes), selective, accurate, precise and robust for the drug determination, and no interference from other substances was observed in the best wavelength. It is concluded that this method could be a routine method for laboratory quality control to certify the quality of the Lamotrigine used.

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