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Development and validation of a capillary electrophoresis method for the determination of lamotrigine in pharmaceutical dosage form

Opracowanie i walidacja metody elektroforezy kapilarnej do oznaczania lamotryginy w preparacie farmaceutycznym

INTRODUCTION

Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a broad-spectrum antiepileptic agent that is indicated for the adjunctive and monotherapy treatment of partial seizures both with and without secondary generalization. Lamotrigine inhibits voltage-sensitive sodium channels, stabilizing neuronal membranes and modulating presynaptic transmitter release of excitatory amino acids such as glutamate and aspartate. Lamotrigine inhibits also high voltage-activated calcium channels [2]. Lamotrigine is a lipophilic weak base, and it is well absorbed after oral administration. The chemical structure of lamotrigine is shown in Fig. 1.

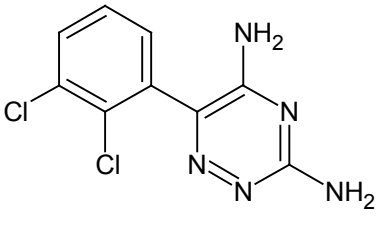

Fig. 1. Chemical structure of lamotrigine

Literature data on the determination of lamotrigine in pharmaceuticals describe the application of HPLC [5,8,9], spectrophotometric [1,9], TLC [4,9], and ion-selective electrode [6] methods. Two different voltammetric techniques were also presented for the determination of lamotrigine in pharmaceutical preparations: differential pulse adsorptive stripping voltammetry [3,7], and square

wave adsorptive stripping voltammetry [3]. There is no data in the literature on the application of capillary electrophoretic (CE) method for the analysis of lamotrigine in pharmaceutical dosage forms. Since lamotrigine is frequently used in the therapy of epilepsy, its measurement in formulations is widely practiced. Therefore, simple and validated methods are continually required for the determination its concentration in different preparations. This paper describes the application of capillary electrophoresis method for quantitative analysis of lamotrigine in tablets.

\MATERIALS AND METHODS

C h e m i c a l s . Lamotrigine pure substance was purchased from Sigma (St. Louis, MO, USA). Lamotrix tablets containing 25 mg lamotrigine per tablet were obtained commercially. Methanol (Merck, Darmstadt, Germany) was of analytical reagent grade.

Water used in all experiments was deionized in SolPure-7 water system (POLL Lab, Poland).

S t a n d a r d s o l u t i o n s . Stock standard solution of lamotrigine (1.0 mg/mL) was prepared by dissolving 10.0 mg pure substance in a minimal amount of methanol and then diluted to 10.0 mL with water. Standard solution at a concentration of 0.1 mg/mL was obtained by diluting stock solution 1:10 with deionized water. The procedure was repeated five times individually weighting the pure substance – lamotrigine each time. The solutions were stored in a refrigerator at the temperature of 4°C and were stable for at least 6 weeks.

C a l i b r a t i o n s o l u t i o n s . The volumes of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard solution (0.1 mg/mL) of lamotrigine were transferred to vials and filled with water to 1.0 mL volume to give solutions 5.0, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 µg/mL, respectively. Peak areas were recorded to form calibration curve. The procedure was repeated five times. Calibration curves were constructed by plotting the peak area versus the respective drug concentration.

Tablet samples. The average mass of 20 Lamotrix tablets was determined. The tablets were pulverized and amount of 0.01 g was transferred to 25-mL volumetric flask containing approximately 7 mL methanol. The mixtures were shaken mechanically for 20 min, diluted to volume with deionized water and filtered. 2.0 mL from Lamotrix solution was transferred to 5-mL volumetric flask and diluted with water. These solutions were used for electrophoretic analysis. The peak areas were recorded. The procedure was repeated 5 times, individually weighing the tablet powder each time. The amount of the substance analyzed in each tablet was calculated using the appropriate regression equation.

Running buffers. Lamotrigine samples were electrophoresed in a phosphate buffer (final pH 4.80). The pH of buffer was checked and adjusted with Hanna pH-meter HI 98150 (Hanna Instruments, Portugal).

E l e c t r o p h o r e t i c p r o c e d u r e . A Prince Technologies CE instrument with Bischoff detector UV 1010 was set at 30 kV, 30°C with detection at 210 nm. Samples were introduced by pressure injection (100 mbar) for 10 s on an untreated capillary (75 µm I.D. x 80 cm) and electrophoresed for 15 min in a running buffer. The capillary was washed for 1 min with 0.1 M of NaOH followed by 1 min with the electrophoresis buffer between injections. In the beginning and in the end of each working day capillary was rinsed with 0.1 M NaOH for15 min.

Precision. Precision of the CE assay was evaluated by injecting the series of standard solutions at 3 concentrations. The solutions containing 5.0, 40.0 and 100.0 µg/mL of lamotrigine were analyzed

5 times on the same day. Inter-day precision was assessed by analyzing the identical solutions in three consecutive days. Five determinations for each concentration were performed. Precision was expressed as the percentage relative standard deviation (%RSD) for peak area of lamotrigine (Table 1).

Accuracy. Accuracy of the method was proved by determination of lamotrigine in the laboratory-prepared mixtures at 3 levels of addition (50, 100, and 150 % of the drug concentration in tablets). Determination was repeated 5 times for each level of addition, and recoveries of lamotrigine were calculated from the amounts found. The results from the recovery studies are shown in Table 2.

RESULTS AND DISCUSSION

Capillary electrophoresis is a powerful analytical technique which is increasing in utility in the pharmaceutical industry. It is used as an alternative or complementary technique to HPLC due to its high efficiency, speed of analysis, reduction in solvent and sample consumption, and low operating cost compared to HPLC methodology.

In a CE process pH of the buffer, the voltage and temperature play important roles. The phosphate buffer pH 4.80 appeared to be optimal for determination of lamotrigine.Under these conditions substance migrated rapidly, emerging at about 9.5 min. Achieved peaks were well-shaped and symmetrical. The temperature 30°C and voltage 30 kV were chosen for analysis.

Lamotrigine has two maxima of light absorption: a weak one at 308 nm and a strong one at 210 nm. The wavelength of 210 nm (the first of the absorbance maxima for lamotrigine), was selected for the determination of lamotrigine. This wavelength permits us to visualize a clean electrophoregram without any interference from excipients.

Calibration was carried out using 7 points. For each point 5 measurements were made. The data were averaged and calibration curve calculated. Evaluated method is linear between 5.0 and 100.0 µg/mL. The calibration curve was represented by the following linear regression equation:

$$y = 2.56 \times 10^{-5} x - 9.82 \times 10^{-5} (r = 0.9996)$$

Electrophoregrams recorded for the standard solutions are presented in Fig. 2 A,B,C.

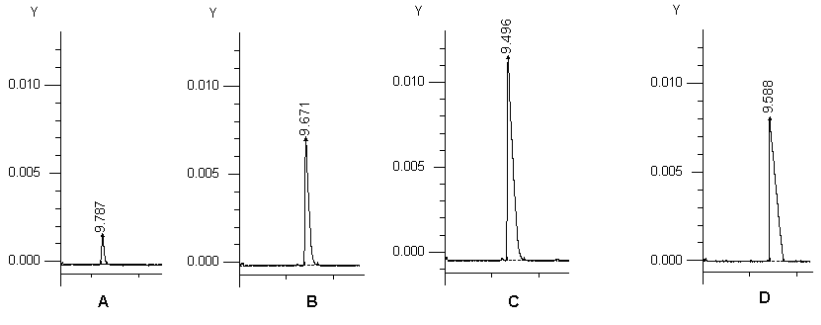

Fig. 2. Electrophoregrams of standard solutions of lamotrigine: **A** – 5 µg/mL, **B** – 40 µg/mL, **C** – 100 µg/mL; **D** - electrophoregram of lamotrigine obtained after isolation from tablets

The intraday precision for lamotrigine expressed as RSD were 1.20 and 1.45% for the lowest and the highest concentrations. The respective values for the interday precision were 1.27 and 1.58%. The results obtained are listed in Table 1.

Table 1. Precision data for lamotrigine in the standard solutions

Lamotrigine amount (μg/mL)	n	Intra-day precision RSD (%)	Inter-day precision RSD (%)
5.0	5	1.20	1.27
40.0	5	2.43	2.34
100.0	5	1.45	1.58

Accuracy of the method was assessed on the basis of determination of lamotrigine in the laboratory-prepared mixtures at 3 levels of addition (50, 100, and 150% of the drug concentration in tablets). For lamotrigine, the recovery results ranged from 100.64 to 101.16% for the lowest and the highest concentrations of the drug, with RSD values ranging from 0.71 to 0.43% (Table 2).

Table 2. Accuracy data for lamotrigine in the laboratory-prepared mixtures

Level of addition (%)		Lamotrigine	
	n	Recovery (%)	RSD(%)
50	5	100.64	0.71
100	5	101.07	0.64
150	5	101.16	0.43

Therefore, the recovery study of the active ingredient from the matrix was successful, and the proposed method is sufficiently accurate. Excipients in the tablets did not interfere in the assay. Therefore, this method is selective in relation to the declared excipients and can be considered as sufficiently selective for routine work.

Table 3. Statistical evaluation of results obtained from the determination of lamotrigine in pharmaceutical preparation

	Lamotrix tablets
Amount claimed [mg]	25
Mean amount found [mg]	25.15
Recovery [%]	100.60
Variance	0.2315
Standard deviation	0.4811
Relative standard deviation [%]	1.91
95% Confidence interval	24.785 – 25.524

The proposed CE method was successfully applied to the determination of lamotrigine in Lamotrix tablets. Results of the analysis of active substance in the pharmaceutical product were evaluated statistically; the results are shown in Table 3. For lamotrigine, total recovery from tablets was found to be 100.60% or 25.15 ± 0.4811 mg/tablet (mean ± SD, n=5). Electrophoregram obtained during analysis of pharmaceutical formulation is shown in Figure 2 D.

In conclusion, electrophoretic method described in this paper is accurate, precise and reliable for the rapid determination of lamotrigine. In addition, it don't consume reagents and samples and don't need complicated steps of extraction or derivatization.

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SUMMARY

A simple, rapid capillary electrophoretic (CE) method has been established and validated for determination of antiepileptic drug lamotrigine, pure substance and in tablets. The method was

developed by employing fused silica capillary with an effective length of 80 cm and internal diameter of 75 µm. 0.067 M phosphate buffer with pH 4.80 was used for lamotrigine analysis. The recommended applied voltage, capillary temperature and injection time were 30 kV, 30°C and 10 s, respectively. Detection was followed by direct absorptiometric measurements at 210 nm. Developed method was linear in the range of 5–100 µg/mL and had good correlation coefficient 0.9996. Intra- and inter-day precision, calculated as relative standard deviation (RSD), were better than 2.5 %. The proposed CE method was successfully applied to the assay of lamotrigine in pharmaceutical formulation. Excipients present in the tablets did not interfere in the assay.

Keywords: lamotrigine, determination, capillary electrophoresis

STRESZCZENIE

Opracowano prostą i szybką metodę elektroforezy kapilarnej do oznaczania lamotryginy w substancji i w tabletkach. Metodę poddano walidacji. Do analizy lamotryginy zastosowano niemodyfikowaną powlekaną kapilarę kwarcową o długości efektywnej 80 cm i średnicy wewnętrznej 75 µm, bufor fosforanowy o pH 4,80, temperaturę 30°C, napięcie separacyjne 30 kV i długość fali detektora UV 210 nm. Oznaczenie przeprowadzono w zakresie stężeń 5–100 µg/mL uzyskując dobrą korelację, r=0,9996. Precyzja oznaczeń w ciągu dnia oraz precyzja międzydniowa charakteryzują się wartością względnego odchylenia standardowego (RSD)< 2,5 %. Opracowaną metodę elektroforezy kapilarnej zastosowano do oznaczania lamotryginy w preparacie farmaceutycznym. Substancje pomocnicze wchodzące w skład tabletek nie wykazywały pików na elektroforegramie w opisanych warunkach analizy.

Słowa kluczowe: lamotrygina, oznaczanie, elektroforeza kapilarna