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Development and validation of a capillary electrophoresis method for determination of oxcarbazepine and zonisamide in pharmaceuticals

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Abstract: A simple, rapid capillary electrophoretic (CE) method has been evaluated and validated for determination of two newer antiepileptic drugs: oxcarbazepine and zonisamide, as pure substances and in tablet form. 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 7.10 was used for oxcarbazepine analysis and 0.1 M acetate buffer with pH 4.58 was used for zonisamide determination. 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 (oxcarbazepine) or 240 nm (zonisamide). Developed methods were linear in the range of 5–100 µg/mL, and had good correlation coefficients (r=0.9999 for oxcarbazepine and r=0.9995 for zonisamide). Intra- and inter-day precision, calculated as relative standard deviation (RSD), were better than 2.0 %. The proposed CE methods were successfully applied to the assay of oxcarbazepine and zonisamide in pharmaceutical formulations.

Key words: capillary electrophoresis, oxcarbazepine, zonisamide

INTRODUCTION

Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz[b,f]azepine-5-carboxamide) is a new antiepileptic drug, licensed for clinical use as monotherapy and adjunctive treatment for partial seizures, and generalised tonic-clonic seizures in children and adults. After oral administration, it is rapidly metabolised to a pharmalogically active monohydroxy metabolite (10-hydroxy-carbazepine). It acts by blocking voltage-sensitive sodium channels, and also reduces voltage-activated calcium currents in striatal and cortical neurons, which contributes to the inhibition of repetitive neuronal firing and the reduction of synaptic impulsive activity [1, 2].

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) is licensed for the adjunctive therapy for partial onset seizures. Its mode of action is based on blocking voltage-dependent sodium and calcium channels. It also increases dopaminergic and serotoninergic neurotransmission, weakly inhibits carbonic anhydrase, and protects neurons from free-radical damage [1, 2]. The chemical structures of oxcarbazepine and zonisamide are shown in Figure 1.

Literature data on the determination of oxcarbazepine in pharmaceuticals describe the application of HPLC [3], spectrophotometric [4], Square Wave Adsorptive Stripping Voltammetry [5] and TLC [6, 7] methods. One high-performance liquid (HPLC) was reported in the literature for the determination of zonisamide in pharmaceutical preparations (capsules) [8]. Two HPLC methods were also presented for the determination of zonisamide in the bulk drug [8, 9]. No studies using capillary electrophoresis (CE) have been conducted determining oxcarbazepine and zonisamide in pure and pharmaceutical preparations. Because oxcarbazepine and zonisamide are used in the therapy of epilepsy, simple and validated methods are continually

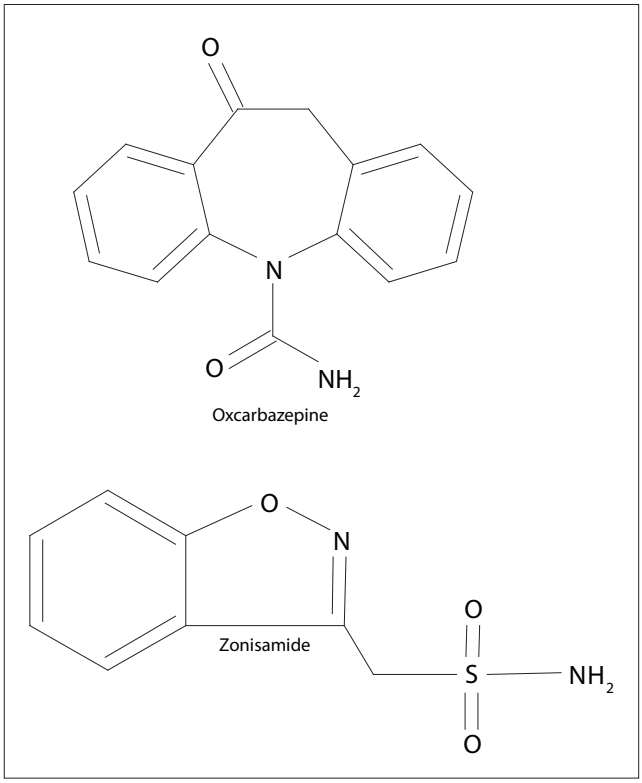

Figure 1 Chemical structures of oxcarbazepine and zonisamide.

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required for their determination in different samples. This study presents a simple and rapid capillary electrophoresis method for the quantification of oxcarbazepine and zonisamide in pharmaceutical preparations.

MATERIALS AND METHODS

Chemicals. Oxcarbazepine pure substance was purchased from Sigma (St. Louis, MO, USA). Zonisamide pure substance and Excegran tablets (100 mg of zonisamide per tablet) were obtained from Dainippon Pharmaceutical Co., Ltd. (Japan). Apydan tablets containing 600 mg oxcarbazpine per tablet were obtained commercially. Methanol (Merck, Darmstadt, Germany) was of analytical reagent grade.

Ammonium hydroxide (25%) and acetic acid were purchased from POCH, Gliwice, Poland. Water used in all experiments was deionized in SolPure-7 water system (POLL Lab, Poland).

Standard solutions. Stock standard solution of oxcarbazepine (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. Stock standard solution of zonisamide (1.0 mg/mL) was obtained in the same way. Standard solutions at a concentration of 0.1 mg/mL were obtained by diluting stock solutions 1:10 with deionized water. The solutions were stored in a refrigerator at the temperature of 4°C, and were stable for at least 6 weeks.

Calibration solutions. The volumes of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard solutions (0.1 mg/mL) of oxcarbazepine or zonisamide 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. Each of them was injected to capillary 5 times. Peak areas were recorded to form calibration curves. Calibration curves were constructed by plotting the peak area versus the respective drug concentration.

Tablet samples. The average masses of 20 Apydan and 20 Excegran tablets were determined. The tablets were pulverized, and amounts of 0.01 g (both Apydan and Excegran tablets) were transferred to two 25-mL volumetric flasks containing approx. 7 mL methanol. The mixtures were shaken mechanically for 20 min, diluted to volume with deionized water and filtered. 1.5 mL from Apydan solution and 2.0 mL from Excegran solution were transferred to 10-mL volumetric flasks 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. Zonisamide samples were electrophoresed in a 0.1 M acetate buffer adjusted to pH 4.58. Oxcarbazepine was determined using phosphate buffer (final pH 7.10). The pH of buffers was checked and adjusted with Hanna pH-meter HI 98150 (Hanna Instruments, Portugal).

Electrophoretic procedure. A Prince Technologies CE instrument with Bischoff detector UV 1010 was set at 30 kV, 30°C with detection at 210 nm (oxcarbazepine) or 240 nm (zonisamide). 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. At the beginning and at the end of each working day, capillary was rinsed with 0.1 M NaOH for 15 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 oxcarbazepine or zonisamide were analyzed 5 times on the same day. Inter-day precision was assessed by analyzing the identical solutions on 3 consecutive days. Five determinations for each concentration were performed. Precision was expressed as the percentage relative standard deviation (%RSD) for peak area of oxcarbazepine and zonisamide (Table 1).

Table 1 Precision data for oxcarbazepine and zonisamide in the standard solutions[a].

Amount (μg/mL)	Oxcarbazepine RSD (%)		Zonisamide RSD (%)	
	Intraday	Interday	Intraday	Interday
5.0	1.90	1.97	1.02	1.19
40.0	1.04	0.94	0.50	0.73
100.0	0.40	0.53	0.11	0.27

[a] n=5.

Accuracy. Accuracy of the method was proved by determination of oxcarbazepine and zonisamide 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 both drugs, and each level of addition and recoveries of the drugs were calculated from the amounts found. The results from the recovery studies are shown in Table 2.

Table 2 Accuracy data for oxcarbazepine and zonisamide in the laboratory-prepared mixtures[a].

Level of addition (%)	Oxcarbazepine		Zonisamide	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
50	100.76	0.65	99.37	0.54
100	100.18	0.19	99.87	0.68
150	100.23	0.24	99.56	0.41

[a] Results are the average of 5 determinations.

RESULTS AND DISCUSSION

In a CE process pH of the buffer, the voltage and temperature play important roles. The phosphate buffer pH 7.10 appeared to be optimal for determination of oxcarbazepine.Under these conditions, the substance migrated rapidly, emerging at about 6 min. Determination of zonisamide was optimal in acetate buffer pH 4.58, with time of migration of about 8 min. Achieved peaks were well-shaped and symmetrical. The temperature 30°C and voltage 30 kV were chosen for analysis.

Calibration was carried out using 7 points. For each point, 5 measurements were made. The data were averaged and

calibration curves calculated. Evaluated methods are linear between 5.0 - 100.0 μg/mL. The calibration curves were represented by the following linear regression equations:

$y = 1.11 \times 10^{-5} x - 5.06 \times 10^{-7}$ ($r = 0.9999$) oxcarbazepine

and

$y = 6.39 \times 10^{-6} x - 1.73 \times 10^{-5}$ ($r = 0.9995$) zonisamide.

The intraday precision for oxcarbazepine expressed as RSD were 1.90 and 0.40 % for the lowest and the highest concentrations. The respective values for the interday precision were 1.97 and 0.53 %. The intraday RSD values for zonisamide ranged from 1.02-0.11 % for the lowest and the highest concentrations. The interday RSD values were 1.19 and 0.27 %, respectively. The results obtained are listed in Table 1.

Accuracy of the method was assessed on the basis of determination of oxcarbazepine and zonisamide in the laboratory-prepared mixtures at 3 levels of addition (50, 100, and 150% of the drug concentration in tablets). For oxcarbazepine, the recovery results ranged from 100.76-100.23% for the lowest and the highest concentrations of the drug, with RSD values ranging from 0.65-0.24%. For zonisamide, the recovery results ranged from 99.37-99.56% for the lowest and the highest concentrations of the drug, with RSD values ranging from 0.54-0.41% (Table 2).

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

The proposed CE methods were successfully applied to the determination of oxcarbazepine in Apydan tablets and zonisamide in Excegran tablets. Results of the analysis of both substances in the pharmaceutical products were evaluated statistically; the results are shown in Table 3. For oxcarbazepine, the recovery was found to be 99.99 % or 599.93 ± 6.76 mg/tablet. For zonisamide, total recovery from tablets was found to be 98.69 % or 98.69 ± 0.61 mg/tablet (mean ± SD, n=5).

Table 3 Statistical evaluation of results obtained from determination of oxcarbazepine in Apydan tablets and zonisamide in Excegran tablets.

	Apydan tablets	Excegran tablets
Amount claimed [mg]	600	100
Mean amount found [mg]	599.93	98.69
Recovery [%]	99.99	98.69
Variance	45.7779	0.3779
Standard deviation	6.7659	0.6147
Relative standard deviation [%]	1.13	0.62
95% Confidence interval	594.73-605.14	98.34-99.03

CONCLUSION

The electrophoretic methods described in this paper are accurate, precise and reliable for the rapid determination of oxcarbazepine and zonisamide. They show adequate linearity, and matrix effect occurred. In addition, they do not consume reagents, and samples and do not need complicated steps of extraction or derivatisation.

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