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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1156–1160
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Determination of oxcarbazepine by Square Wave Adsorptive Stripping Voltammetry in pharmaceutical preparations

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Received 6 June 2006; received in revised form 13 September 2006; accepted 18 September 2006
Available online 3 November 2006

Abstract

A procedure for the determination of oxcarbazepine (OXC) by Square Wave Adsorptive Stripping Voltammetry (SWAdSV) has been optimized. Selection of the experimental parameters was made using experimental design methodology. The detection limit was 1.74×10^{-7} mol dm^{-3}. This method was used to determine oxcarbazepine in pharmaceutical preparations.

Keywords: Oxcarbazepine; Epilepsy; Square Wave Adsorptive Stripping Voltammetry; Experimental design

1. Introduction

Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz[*b*,f]azepine-5-carboxamide, OXC) (Fig. 1) is the keto form of 10-hydroxycarbamazepine. As a blocker of pre- and postsynaptic voltage-dependent sodium channels in the central nervous system, it has been in therapeutic use for a few years in the treatment of partial and generalized seizures, trigeminus neuralgia, affective disorders and spasticity [1,2].

It is rapidly metabolised to its pharmacologically active dihydro metabolite (10,11-dihydro-10-hydroxy-5H-dibenz[*b*,f]azepine-5-carboxamide, dihydrooxcarbazepine).

HPLC [3–7] is the most commonly used technique for the determination of OXC in pharmaceutical products and biological fluids. Despite the presence of redox groups in this molecule, as far as the authors are aware, no electrochemical technique has yet been used in its determination.

Electrochemical techniques provide an interesting alternative to the chromatographic methods that are widely used at present in the determination of OXC. Together with the recognized advantage of the relatively low cost of electrochemical instrumentation, one should bear in mind the high sensitivity of methods, such as stripping voltammetry, based on the adsorption exhibited by numerous organic compounds on some electrodes. This paper presents a procedure for the determination of OXC using Square Wave Adsorptive Stripping Voltammetry (SWAdSV).

Numerous experimental variables can affect the response when using stripping voltammetry techniques, which calls for optimization of the variables to enable accurate measurements under the best possible conditions. In the improvement of any analytical procedure, special precautions need to be taken when choosing the experimental conditions, all the more so when it is a matter of trace level determination of species. An appropriately designed experiment [8,9] provides signals of far superior quality to those measured in an experiment that has not been optimized. Likewise, the use of experimental designs allows a reduced number of experiments to explore a wide experimental range. They are more efficient than the "one-at-a-time" experiments since they permit interactions to be detected between factors that might otherwise lead to false conclusions. Experimental design has successfully been employed in the optimization of experimental variables in electroanalytical techniques [10–15]. As a result, in our work, experimental design has been used to establish appropriate experiments that will lead to the optimization of the influencing variables, such as, potential, deposition time (E_{dep}, t_{dep}) and pH value.

2. Experimental

2.1. Reagents

Analytical grade chemicals not subjected to any further purification processes were used. All solutions were prepared with

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doi:10.1016/j.jpba.2006.09.029

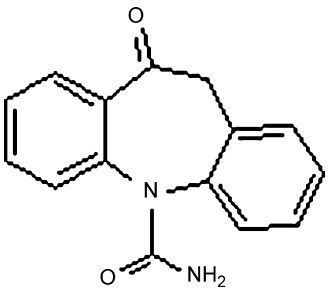

Fig. 1. Chemical structure of oxcarbazepine.

deionized water obtained using a Barnstead NANO Pure II system. Nitrogen (99.99%) was used to remove dissolved oxygen.

Britton–Robinson solutions were used as buffers. A 0.04 mol dm^{-3} Britton–Robinson buffer solution for the *o*-boric, *o*-phosphoric and acetic acids was prepared using Merck analytical grade reagents. Solutions of different pH values were prepared from this by the addition of 0.2 mol dm^{-3} sodium hydroxide (analytical-reagent grade, Merck, Darmstadt, Germany).

Oxcarbazepine was kindly provided by Novartis International Pharmaceutical Ltd. (Cork, Ireland). Solutions of oxcarbazepine were prepared by dissolving appropriate amounts of oxcarbazepine in water.

Commercial capsules of TRILEPTAL® were obtained from Novartis. TRILEPTAL® film-coated tablets contain the following inactive ingredients: colloidal silicon dioxide, crospovidone, hydroxypropyl methylcellulose, magnesium stearate, mycrocristalline cellulose, polyethylene glycol, talc and titanium dioxide and yellow iron oxide.

2.2. Apparatus

Voltammetric measurements were taken using a μAutolab (Eco Chemie) with a Metrohm Model 663 VA electrode stand and a multimode electrode (MME) operating in the hanging mercury drop electrode (HMDE) mode. An Ag/AgCl 3 mol dm^{-3} KCl reference electrode and a platinum wire auxiliary electrode were also used.

The pH of the solution was measured with a Crison Model 2002 (Barcelona, Spain) pH meter.

2.3. Software

Data analysis was processed with a STATGRAPHICS PLUS software package [16] for the experimental design process, PROGRESS [17] for the robust regression, DETARCHI [18,19] for the detection limit and PARVUS [20] was used in the multivariate calibration.

2.4. Procedure

Voltammetric measurements were taken using the following procedure: the solution was purged using nitrogen and stirred for 300 s, the deposition potential was then applied for the time and potential as determined for each experiment. The solution was left to rest for an equilibrium time of 5 s, then a cathodic scan from 0 V (initial potential) to −1.5 V (final potential) was started and the voltammogram recorded. The other main experimental parameters were as follows: mercury drop size, 0.52 mm^2; stirring rate in the deposition period, 1500 rev min^{-1}; frequency, 25 Hz.

In order to determine the concentration of OXC in TRILEPTAL® tablets the following procedure was carried out: the tablet was pulverized with a pestle and finally dissolved in water. The insoluble portion of the tablet was eliminated by filtration. The analysis of the OXC content was carried out by addition of an aliquot of the drug solution to the electrochemical cell using the voltammetric procedure described above.

3. Results and discussion

3.1. Optimization of the experimental variables

Preliminary experiments showed that in SWAdSV technique oxcarbazepine shows a well-defined reduction peak in acid media. As it is known in this technique, the response obtained, peak intensity, is notably influenced by variables, such as deposition time, t_{dep}, deposition potential, E_{dep} and pH. The experimental design was used as a tool for the optimisation of the intensity.

Previous studies in aqueous samples of oxcarbazepine in the presence of Britton–Robinson buffer at different pH levels had confirmed that a well-defined reduction peak only appeared at pH 4, for which reason it was decided to fix this factor and complete optimization of the two remaining factors. A central composite design [21–24] was chosen for this stage, its purpose being to arrange the two factors, E_{dep} and t_{dep} and their interactions according to their influence on the peak current. Subsequently, experiments with all possible combinations were carried out. The values which correspond to the high (+) and low (−) levels and to the central point (0) for each factor are as follows:

$$E_{dep}(+) = -0.3\,\text{V}, t_{dep}(+) = 120\,\text{s};$$

$$E_{dep}(-) = -0.6\,\text{V}, t_{dep}(-) = 40\,\text{s};$$

$$E_{dep}(0) = -0.45\,\text{V}, t_{dep}(0) = 80\,\text{s}.$$

The response to be optimized was the intensity ($-i_p$), at a potential of −1.04 V, of an oxcarbazepine sample at a concentration of 10^{-6} mol dm^{-3}. Table 1 shows the results obtained for this experimental design. From analysis of the variance (ANOVA) in Table 2, it can be seen that a second order function is adequate to model the data because the lack of fit is not significant at the 95% confidence level. It can also be deduced that the only significant factor is the AA interaction. However, a maximum can be observed in Fig. 2, which corresponds to a E_{dep} value of −0.44 V and an accumulation time of 83 s.

As a result of the above discussion, the optimum conditions for the determination of OXC by means of SWAdSV are:

$$\text{pH } 4, \quad t_{dep} = 83\,\text{s}, \quad E_{dep} = -0.44\,\text{V}.$$

Table 1
Results of the 2^2 central composite design for optimization of experimental variables in OXC determination by SWAdSV

E_{dep} (V)	t_{dep} (s)	$-i_p$ (nA)
−0.60	40	20.75
−0.30	100	20.28
−0.60	120	19.49
−0.30	120	23.34
−0.66	80	17.24
−0.24	80	18.72
−0.45	23	18.66
−0.45	137	23.32
−0.45	80	40.89
−0.45	80	38.44
−0.45	80	31.45

[OXC] = 10^{-6} mol dm^{-3}; pH 4.

Table 2
ANOVA with the data in Table 1

Effect	SS	DF	MS	F_{ratio}	P_{level}
A:E_{dep}	3.744	1	3.744	0.160	0.731
B:t_{dep}	8.800	1	8.800	0.370	0.606
AA	467.976	1	467.976	19.500	0.048[a]
AB	4.666	1	4.666	0.190	0.702
BB	326.031	1	326.031	13.59	0.066
Lack of fit	7.456	3	2.485	0.100	0.951
Pure error	47.992	2	23.996		
Total	690.326	10			
			$R^2 = 0.919678$		

SS, sum of squares; DF, degrees of freedom; MS, mean squares; F_{ratio}, MS_{factor}/MS_{error}; P_{level}, probability level.
[a] Significant factor at $\alpha = 0.05$.

The reduction peak of OXC under optimum conditions is shown in Fig. 3.

3.2. Calibration and detection limit

Once the optimum parameters for the analysis were chosen, a calibration graph was constructed in aqueous solution. The existence of anomalous points [17] would normally lead to incorrect adjustments altering the sensitivity and the detection limit. In order to avoid this problem, Least Median Squares (LMS) regression was used whose criterion is to minimize the median

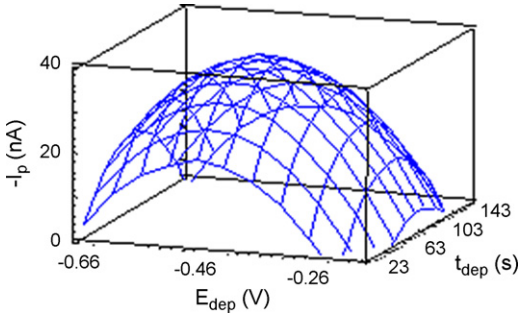

Fig. 2. Response surface for the 2^2 central composite design for optimization of experimental variables in OXC determination by SWAdSV in aqueous solution.

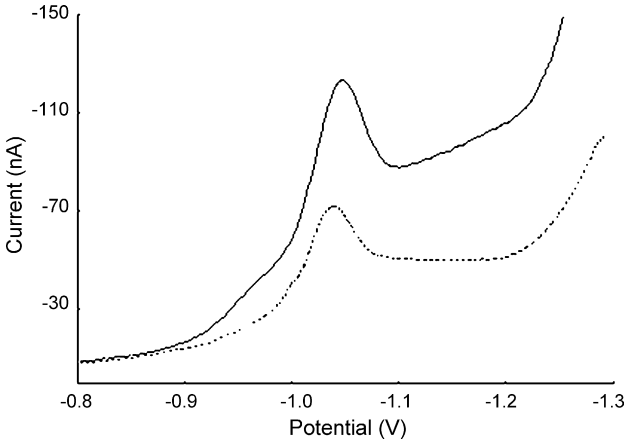

Fig. 3. Square Wave Voltammogram obtained for OXC (- - -) in Britton–Robinson buffer, pH 4, t_{dep} = 83 s and E_{dep} = −0.44 V. Square Wave Voltammogram obtained for Trileptal® (—) (theoretical concentration of OXC 1.6×10^{-6} mol dm^{-3}), in Britton–Robinson buffer, pH 4, t_{dep} = 83 s and E_{dep} = −0.44 V.

squares of the differences between the experimental and the calculated values. LMS regression has the advantage of being able to detect anomalous points, be they outlier or leverage points, in cases where a linear range is sought and when at least 50% of the data is aligned.

The strategy followed consisted of two steps, the first of which used LMS regression to detect anomalous points; taken as outlier points, if the absolute value of the standardised residual was greater than 2.5, and as leverage points, if the absolute value of their resistant diagnostic was greater than 2.5. When both of these parameters were above 2.5, the anomalous point was considered as an outlier-leverage point. In the second step, wherever anomalous points were detected they were eliminated and an Ordinary Least Squares (OLS) regression was performed to obtain optimal precision and accuracy of both slope and intercept.

In this case, to obtain the linear range, three calibrations were made between 3×10^{-7} and 1.7×10^{-6} mol dm^{-3} of OXC.

An important characteristic of an analytical method is the detection limit—that is the smallest concentration of the analyte, which can be detected with a specified degree of certainty. According to ISO 11843 [25], the capability of detection of any analytical procedure has to be performed assuming the probability of false positive and false negative. To solve this problem, the method developed by Clayton and Hines [26] was applied. They proposed the matter as a hypothesis test taking into account the relationship between signal and concentration. It is clear that the detection limit depends not only on the probability of false positive (α values), but also on the probability of false negative (β values). The representation of beta values versus the corresponding concentration is, in fact, a detailed description of an analytical process in terms of its capacity to detect. This detection plot was proposed by Liteanu [27]. The description of this method can be found in greater detail in previous works [17,28–31]. In this case, the detection plots were constructed using the Clayton method and the DETARCHI calculation program [18].

Table 3
Detection limit and signal ($\alpha = \beta = 0.05$ and one replication)

	First calibration			Second calibration			Third calibration		
	LS	LMS	LS	LS	LMS	LS	LS	LMS	LS
Number of data	8	8	7	9	9	8	9	9	7
Sensitivity (nA mol^{-1} dm^3)	2.109	2.077	2.297	2.170	2.080	2.120	2.163	1.879	2.027
Intercept (nA)	0.797	1.533	−0.269	−2.416	−1.024	−1.459	−2.454	−1.041	−1.720
Coefficient of determination (R)	0.977	0.988	0.994	0.989	0.998	0.996	0.992	0.996	0.996
Residual standard deviation	1.872		0.884	1.458		0.849	1.274		0.735
Detection current (nA)		1.838			0.498			0.007	
Detection limit (mol dm^{-3})		1.771×10^{-7}			1.796×10^{-7}			1.646×10^{-7}	

Calibration parameters, detection limits and detection signals obtained from the different calibrations are summarized in Table 3. In all cases $\alpha = 0.05$ and $\beta = 0.05$ were chosen for one replication.

3.3. Determination of oxcarbazepine in real samples

The concentration of oxcarbazepine in commercial capsules of TRILEPTAL® (Novartis Farmacéutica, Barcelona, Spain) with a known concentration of analyte was determined using the SWAdSV method, so as to evaluate the accuracy of the proposed method.

Due to the presence of excipients that interfered with the analysis of the drug, a univariate calibration gave rise to poor results in the analysis of OXC in pharmaceutical samples. Therefore, a multivariate calibration was performed.

A PLS model based on cross-validation was constructed with 14 samples of known concentration ranging between 5×10^{-7} mol dm^{-3} and 1.8×10^{-6} mol dm^{-3} of OXC. All the voltammograms were digitalized which accounts for the readings of intensities at 224 potentials between 0 and −1.5 V.

Multivariate PLS calibration is achieved by constructing latent variables, which are linear combinations of the original variables. The number of latent variables is a meta-parameter of the procedure, the value of which is estimated from the calibration data. This may be accomplished by minimizing PRESS as a function of k (internal validation), giving us

$$\text{PRESS (K)} = \sum_{i=1}^{m} (\mathbf{c}_i - \mathbf{c}_{k/i})^2$$

In this equation, $\mathbf{c}_i$ is the vector of concentrations corresponding to the ith sample and $\mathbf{c}_{k/i}$ is the vector of concentrations estimated with the PLS of k latent variables constructed without the ith sample.

The calculation of PRESS was performed with three cancellation groups; that is to say, a PLSC model was constructed three times for a number of latent variables, eliminating 5, 5 and 4, respectively, from the 14 voltammograms. The minimum PRESS is reached for the number of latent variables that give the maximum cross-validation variance. According to this criterion, more than 99.3% of the cross-validation variance is explained by taking six latent variables.

The model based on PLS cross-validation described above was applied to a set of nine solutions containing a sample of the pharmaceutical product TRILEPTAL®. Fig. 3 shows the Square Wave Voltammogram obtained in the optimised conditions for TRILEPTAL®. Good agreement was obtained between the amount found by the PLSC model constructed (311.16 ± 13.51 mg) with $n = 9$ and $\alpha = 0.05$ and the value supplied by the manufacturer (300 ± 15 mg). These results were also checked using HPLC as a reference technique obtaining (296.22 ± 15.09) mg $n = 3$, $\alpha = 0.05$.

Through the performance of a hypothesis test for the means of two normal distributions of unknown and equal variance, both the mean obtained through electrochemistry and that obtained through HPLC were shown to be equal since the test statistic fell outside the critical region which implies acceptance of the null hypothesis H_0.

Acknowledgements

The financial support made available by the Junta de Castilla y León (Burgos 08/04), the Ministerio de Ciencia y Tecnología (BQU2001-1126) and the Ministerio de Educación y Ciencia (MAT2005-01767) are gratefully acknowledged. M. Encarnación Burgoa Calvo also thanks the Consejería de Educación de la Junta de Castilla y León y el Fondo Social Europeo for the F.P.I. grant.

References

[1] P. Benetello, Pharmacol. Res. 31 (1995) 155–162.
[2] T. Tomson, S.I. Johannessen, Eu. J. Clin. Pharmacol. 55 (2000) 697–705.
[3] M.L. Qi, P. Wang, L.J. Wang, R.N. Fu, J. Pharm. Biomed. Anal. 31 (2003) 57–62.
[4] R. Mandrioli, N. Ghedini, F. Albani, E. Kenndler, M.A. Raggi, J. Chromatrogr. B 783 (2003) 253–263.
[5] H. Levert, P. Odou, H. Robert, J. Pharm. Biomed. Anal. 28 (2002) 517–525.
[6] K.M. Matar, P.J. Nicholls, M.I. Alhassan, A. Tekle, J. Clin. Pharm. Ther. 20 (1995) 229–234.
[7] F. Bugamelli, C. Sabbioni, R. Mandrioli, E. Kenndler, F. Albani, M.A. Raggi, Anal. Chim. Acta 472 (2002) 1–10.
[8] G.E.P. Box, N.G. Hunter, J.S. Hunter, Estadística para Investigadores. Introducción al diseño de experimentos, Análisis de Datos y Construcción de Modelos, Reverté, Barcelona, 1989.
[9] E. Morgan, Chemometrics: Experimental Design, ACOL, Wiley, New York, 1991.
[10] P. Gratteri, S. Furlanetto, S. Pinzanti, R. Leardi, Electroanalysis 7 (1995) 1161–1164.

[11] O. Domínguez, S. Sanllorente, M.J. Arcos, Electroanalysis 11 (1999) 1273–1279.
[12] O. Domínguez, S. Sanllorente, M.A. Alonso, M.J. Arcos, Electroanalysis 13 (2001) 1505–1512.
[13] M.J. Gómez, O. Domínguez, M.A. Alonso, M.J. Arcos, Talanta 62 (2004) 457–462.
[14] I. Paolicchi, O. Domínguez, M.A. Alonso, M.J. Arcos, Anal. Chim. Acta. 511 (2004) 223–229.
[15] M.E. Burgoa, O. Domínguez, M.J. Arcos, Anal. Chim. Acta 549 (2005) 74–80.
[16] Statgraphics PLUS for Windows (1994–1999) Version 4.0 by Statistical Graphics Corp.
[17] P.J. Rousseuw, A.M. Leroy, Robust Regression and Outlier Detection, Wiley, New York, 1989.
[18] L.A. Sarabia, M.C. Ortiz, Trends Anal. Chem. 13 (1994) 1–6.
[19] M.C. Ortiz, L.A. Sarabia, 12th International Symposium on Microchem Tech PII, vol. 12, 1992, p. 212.
[20] M. Forina, R. Leardi, C. Armanino, S. Lanteri, PARVUS: An Extendable Package of Programs for Data Exploration. Classification, Correlation, Version 1.3. Available from the authors. Instituto di Analisi e Tecnologie Farmaceutiche de Alimentari, Universitá di Genova, 1994.
[21] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, Chemometrics: A Textbook, Elsevier, Ámsterdam, 1988.
[22] M.A. Alonso, O. Domínguez, M.J. Arcos, Electroanalysis 14 (2002) 634–637.
[23] O. Domínguez, M.A. Alonso, M.J. Arcos, Electroanalysis 14 (2001) 1083–1089.
[24] O. Domínguez, M.A. Alonso, M.J. Arcos, Anal. Chim. Acta 521 (2004) 215–221.
[25] ISO 11843-1, Capability of Detection, Genéve, 1997.
[26] C.A. Clayton, J.W. Hines, P.D. Elkins, Anal. Chem. 59 (1987) 2506–2514.
[27] C. Liteanu, I. Rica, Statistical Theory Methodology of Trace Analysis, Ellis Horwood, Chichester, 1980.
[28] A. Herrero, M.C. Ortiz, M.J. Arcos, J. López Palacios, Analyst 119 (1994) 1585–1592.
[29] S. Sanllorente, M.C. Ortiz, M.J. Arcos, J. López Palacios, Electroanalysis 8 (1996) 285–292.
[30] D.L. Massart, L. Kaufman, P.J. Rousseuw, A. Leroy, Anal. Chim. Acta 187 (1986) 171–179.
[31] M.C. Ortiz, M.J. Arcos, J.V. Juarros, J. López Palacios, L.A. Sarabia, Anal. Chem. 65 (1993) 678–682.