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Quantitative analysis of boldine alkaloid in natural extracts by cyclic voltammetry at a liquid–liquid interface and validation of the method by comparison with high performance liquid chromatography

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ABSTRACT

The quantitative determination of boldine alkaloid in boldo leaf extracts by employing cyclic voltammetry, at a liquid/liquid interface as well as the validation of this methodology against the reference method, high performance liquid chromatography (HPLC), are reported in the present paper. The voltammetric analysis was performed successfully and economically using two kinds of liquid/liquid interfaces: water/1,2-dicholoroethane and water/PVC (polyvinyl chloride)–gelled 1,2-dichloroethane. Linear calibration curves in the concentration range of 1.04×10^{-5} mol L^{-1} to 5.19×10^{-4} mol L^{-1} were obtained with a detection limit equal to $(6.1 \pm 0.7) \times 10^{-5}$ mol L^{-1} and the quantitative determination of this alkaloid, in complex matrixes such as boldo leaf extracts, by the electrochemical technique proposed was found to be equal to the values obtained using the standard HPLC method. The validation analysis of this methodology against HPLC demonstrated that accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), specificity and precision are acceptable. The electroanalytical technique proposed is economical and selective, involves simple equipment and can be applied for the quantitative determination of boldine alkaloid in complex matrixes such as leaf extracts without special drug separation. Moreover, cyclic voltammetry (CV) experiments applied at the liquid/liquid interface under different experimental conditions allowed us to study the transfer mechanism of boldine, and determine a value of $pK_a^w = 6.90$ for protonated boldine, from the variation of voltammetric peak current with pH.

1. Introduction

Boldine is the main alkaloid found in the tree leaf and bark of *Peumus boldus* (boldo). This compound exhibits strong free-radical scavenger and antioxidant properties [1–4] as well as other important biological effects which have been studied and revised [5]. According to pharmacopoeias and treatises on medicinal plants, boldo is employed in the form of infusion, tincture and extract. In the practice of traditional medicine, boldo preparations are generally indicated for the treatment of different conditions amongst which digestive and hepatobiliary disorders have been the most commonly mentioned [1].

Different methodologies, like colorimetric, paper electrophoresis, thin layer and gas chromatographic methods, were employed for the analytical detection and quantification of boldine [1]. The high performance liquid chromatography (HPLC) method coupled to a UV detector was developed by Pietta et al. [6] based on the two absorption maxima (282 and 303 nm) of boldine, and this procedure is currently used in different pharmacopoeias for pharmaceutical boldo preparations. Considering the use of boldo and its alkaloids in pharmaceutical manufacturing, the implementation of analytical methodologies is required to enable the highly sensitive detection of boldine in these preparations.

Several drugs have been analyzed in pharmaceutical matrixes using different electrochemical techniques [7–12]. In particular, voltammetric methods applied to the interface between two immiscible electrolyte solutions (ITIES) [13] have been adopted to (a) examine kinetic and analytical aspects of the transport of various drugs in aqueous [14–27] or gelled solutions [28,29]; (b) determine partition coefficients from transfer potentials [30]; and (c) clarify the electrochemical behavior of structurally specific and nonspecific drugs [31–34]. However, despite the large number of papers describing electroanalytical procedures at liquid/liquid

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interfaces, exceptionally few of them concern to complex real matrices [14].

In the present paper we report the quantitative determination of boldine in boldo leaf extracts, a complex matrix, by employing cyclic voltammetry (CV) at water/1,2-dichloroethane (1,2-DCE) and water/polyvinyl chloride (PVC)–gelled 1,2-dichloroethane interfaces without sample pre-treatment or pre-separation. The validation of this methodology with respect to HPLC quantification is also assessed.

2. Experimental

2.1. Materials and electrochemical cell

CV experiments were performed in a four electrode system using a conventional glass cell of 0.18 cm^2 interfacial area. Two platinum wires were used as counter electrodes and the reference electrodes were Ag/AgCl. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 1.00×10^{-2} mol L^{-1} tetraphenylarsonium chloride (TPAsCl, Sigma). Potential values (E) reported in this work include $\Delta^{o}_{tr,TPAs^+} = 0.364$ V for the transfer of the reference ion $TPAs^+$.

The base electrolyte solutions were 1.00×10^{-2} mol L^{-1} LiCl (Fluka, p.a. grade) in ultrapure water and 1.00×10^{-2} mol L^{-1} tetraphenyl arsonium dicarbollyl cobaltate (TPAsDCC) in 1,2-DCE (Dorwil p.a.). TPAsDCC was prepared by metathesis of TPAsCl (Merck, p.a. grade) and cesium dicarbollyl cobaltate (CsDCC, Lachema p.a.). The pH of the aqueous phase was varied in the range 2.00–8.23 by adding HCl or LiOH. In one set of experiments, the organic phase was gelled to achieve higher stability. For this purpose, 15% LMW-PVC (low molecular weight polyvinyl chloride) was added to the organic solution.

The electrochemical cell used was as follows:

Ag	AgCl	TPAsCl 1×10^{-2} mol L^{-1} (w′)	TPAsDCC 1×10^{-2} mol L^{-1} (o)	LiCl 1×10^{-2} mol L^{-1} (w)	AgCl	Ag

Pure boldine (Sigma Aldrich, p.a) was added to the aqueous phase (w) at a concentration range 1.00×10^{-5} to 5.20×10^{-4} mol L^{-1} and used as standard solutions.

Boldo extracts were obtained from a sample of commercial boldo leaves. These leaves were identified by Dr. Gloria Barboza, from the Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba. A voucher specimen is deposited at the herbarium of the Museo Botánico de Córdoba (CORD) as CORDf 108.

Boldo extracts containing all boldo alkaloids (boldine and other minority alkaloids) were prepared according to standard procedures by Real Farmacopea Española [35]. Briefly, 3.0000 g of dry boldo leaves was extracted three times with 150.0 mL of 10% HCL in bath water at 80 °C for 30 min. These three extracts were combined and partitioned with 300.0 mL of EtOAc/*n*-hexane (1:1) twice. Then, the aqueous phase was adjusted to pH 9.50 with ammonia and partitioned with methylene chloride, thrice. The combined organic extracts were divided into two parts and evaporated separately to dryness using a rotary evaporator. One part was dissolved in methanol and transferred to a volumetric flask of 5.00 mL. This solution (sample extract) was filtered (Durapore membrane filter HV) and directly injected into HPLC. The other fraction was diluted to 10.00 mL with a 1.00×10^{-2} mol L^{-1} LiCl solution of pH = 2.00 and used in cyclic voltammetry experiments.

2.2. Cyclic voltammetry experiments

CV experiments were performed using a four electrode potentiostat with periodic current interruption for automatic elimination of solution resistance [36]. The voltage ranged from 0.200 to 0.750 V with a potential sweep generator (L y P Electrónica Argentina). Voltammograms were recorded employing a 10 bit Computer Board acquisition card connected to a personal computer. The scan rate was varied from 0.010 to 0.125 V s^{-1}. The current peak values obtained in each experiment were used as a quantification parameter.

2.3. HPLC experiments

2.3.1. HPLC conditions

HPLC analyses were performed using a VARIAN Pro Star 325 with UV-Vis detector; Star Varian Chromatography Workstation System 5.50 Software was used for data acquisition. A C18 reversed-phase packing column (Hypersil RP18, 4.6 mm × 250 mm, 5 μm; Phenomenex, USA) was used for the separation. The UV acquisition wavelength was set at 304 nm. The injection volume was 20.0 μL. The mobile phase comprised a mixture of solution A/B (16:84), solution A: acetonitrile/diethylamine (99.8:0.2, v/v), and solution B: water/diethylamine (99.8:0.2 v/v) adjusted to pH 3.00 with formic acid. Separation was achieved under isocratic elution conditions at 1.50 mL/min flow rate.

2.3.2. Reference solution

A reference solution was prepared weighing exactly 10.0 mg of boldine reference compound and transferring it to a volumetric flask of 10.00 mL. The flask was filled with methanol and stored at 4 °C under dark conditions. The reference solution of boldine was used as external standard by preparing a range of different concentrations between 0.010 and 0.06 mg/mL. The data obtained from the analysis of each solution allowed plotting a calibration curve showing a good linearity (correlation coefficient: 0.9994 and randomly distributed residual values). The reference solutions were filtered (Durapore membrane filter HV) and injected into HPLC.

2.4. Validation of voltammetric analysis

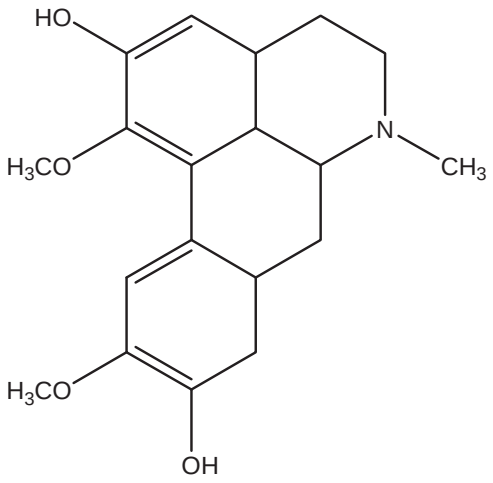
The following steps were performed for the validation of the quantitative analysis of boldine by CV at water/1,2-DCE interface [37–39].

2.4.1. Accuracy

To evaluate accuracy, the closeness of agreement between the average value obtained from a large set of voltammetric results and the accepted reference value obtained by HPLC (reference method) were compared. Accordingly, we measured the same solutions following the two methods, CV and HPLC, and then we compared the average values, standard deviation and coefficient of variation. The representative test sample consisted of a solution $(5.1 \pm 0.1) \times 10^{-4}$ mol L^{-1} in LiCl 1.00×10^{-2} mol L^{-1} and pH = 2.00. The comparison between both methods was carried out employing six portions of the same sample and each one in duplicate.

2.4.2. Linearity, limit of detection (LOD) and limit of quantification (LOQ)

To assess linearity, LOD and LOQ, calibration curves (peak current values as a function of boldine concentration) at different scan rates were plotted, from which linear regression was calculated. The boldine concentration was varied in the range



Scheme 1. Chemical structure of boldine.

1.04×10^{-5} mol L^{-1} to 5.19×10^{-4} mol L^{-1}. Each concentration was measured in duplicate. The scan rates used were 0.010, 0.025, 0.05, 0.075 and 0.1 V s^{-1}.

2.4.3. Specificity

Specificity, understood as the ability of the method to determine accurately and specifically the boldine concentration in the presence of other components in a sample matrix, was evaluated in a natural extract of boldo leaves. For this purpose, the extract obtained from 3.0000 g of boldo leaves was dissolved in 15.0 mL of a solution 1.00×10^{-2} mol L^{-1} of LiCl at pH = 2.00 (solution A). Secondly, a 1:2 dilution was performed with solution A (final volume: 10.00 mL, solution B). Solution B was electrochemically analyzed and successive aliquots of 70.0 μL of a standard solution of boldine 1.02×10^{-2} mol L^{-1} was added to solution B. Cyclic voltammograms were recorded in triplicate at different scan rates after each aggregate.

2.4.4. Precision

Precision, defined as the closeness of agreement between independent test results obtained under stipulated conditions, was assessed comparing the standard deviation and the variation coefficient shown by both methods, here CV at the water/1,2-DCE interface and HPLC. Therefore, the same standard solution of boldine was measured 12 times by both methods; in the case of CV five scan rates were employed. From concentration values determined from each method, the relative residue ($rl = (X_i - \bar{X})/\bar{X}$, where X_i is the measured concentration value and $\bar{X}$ is the average value of concentration obtained by each method) was calculated and plotted against the measure number. Standard deviation and variation coefficient were calculated from this set of experiments.

On the other hand, this set of experiments was repeated at a water/gelled 1,2-DCE interface. This modification in the system produces a more stable interface.

3. Results and discussion

3.1. Electrochemical behavior of boldine

Scheme 1 shows the molecular structure of boldine. As can be noted, this compound has a protonation–deprotonation site on the nitrogen atom present in one of the rings. Thus, at pH values below p*K*a, this molecule becomes a protonated monocation (HB^+). It is important to note that the p*K*a value for boldine is not available in the literature.

Fig. 1 shows cyclic voltammograms obtained at several scan rates for a 5.01×10^{-4} mol L^{-1} aqueous solution of boldine at pH = 2.00 in contact with an organic base solution. All the profiles exhibit the typical behavior of a reversible univalent ion transfer controlled by diffusion. The positive peak potential, $E_p{}^+ = 0.557$ V, and the peak potential difference, $\Delta E_p = E_p{}^+ - E_p{}^- = 59$ mV, were independent of the potential sweep rate, v, and of the boldine concentration (not shown). The positive peak current, $I_p{}^+$, was proportional to $v^{1/2}$ and to the equilibrium concentration of HB^+ species.

The dependence of $E_p{}^+$ and $I_p{}^+$ on pH is shown in Fig. 2a. As can be noted, a constant value of $E_p{}^+$ is observed in the full range of pH analyzed (3.00–8.23). This evidence indicates that the transfer of HB^+ from the aqueous to the organic phase occurs according to a direct transfer mechanism. Moreover, the peak current decreases with pH due to the depletion of HB^+ equilibrium concentration in aqueous phase as pH increases. From these results it was possible to determine $pK_a{}^w$ values for HB^+. Fig. 2b shows the variation of peak current value with pH. As seen, at low pH values the current reaches a maximum value since the mole fraction of HB^+ species is equal to 1 under these conditions. As pH is increased, a fraction of protonated species in aqueous phase is transformed into neutral species and, consequently, the current value decreases. The pH point at which $I_p{}^+$ value is equal to the half of the initial value corresponds to $pK_a{}^w$, since at this point protonated and neutral species have the same concentration value. In this way a value of $pK_a{}^w = 6.90$ was obtained for protonated boldine. This value was corroborated by measurements of acid base titrations.

Finally, the variation of $I_p{}^+$ with protonated boldine concentration was analyzed. Fig. 3 shows the calibration curves obtained at pH = 2.00 and several scan rates. From these results the linear range, the limit of detection and the limit of quantification were evaluated as discussed later.

3.2. HPLC results

The sample extract was analyzed by HPLC from calibration curves, according to the Real Farmacopea Española [35]. The alkaloid total content was 0.06% P/P expressed as boldine.

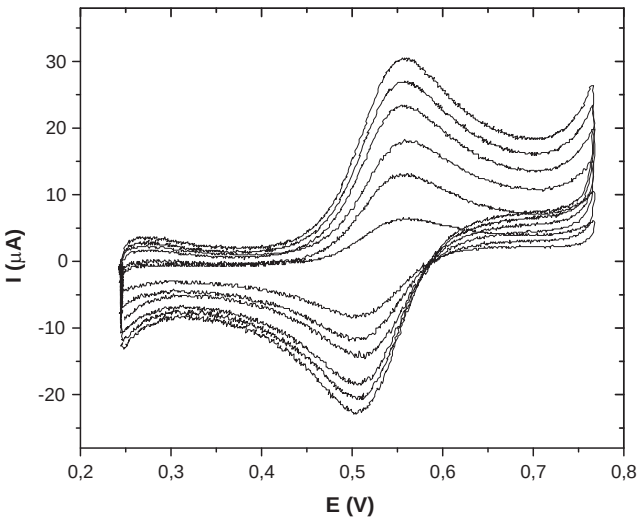


Fig. 1. Cyclic voltammograms for HB^+ transfer at different scan rates: 0.010, 0.025, 0.050, 0.075, 0.100 and 0.125 V s^{-1}. Aqueous phase composition: 5.01×10^{-4} mol L^{-1} HB^+, 1×10^{-2} mol L^{-1} LiCl, pH = 2.00. Organic phase composition: 1×10^{-2} mol L^{-1} TPAsDCC.

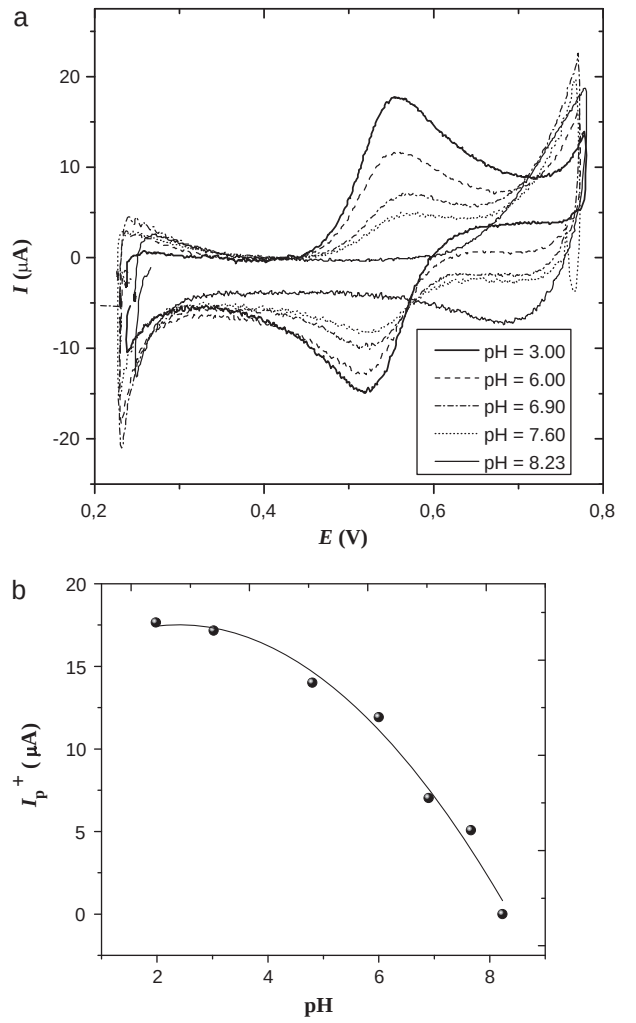


Fig. 2. (a) Cyclic voltammograms for HB^+ transfer at different pH values. Aqueous phase composition: 5.01×10^{-4} mol L^{-1} boldine, 1.00×10^{-2} mol L^{-1} LiCl. Organic phase composition: 1.00×10^{-2} mol L^{-1} TPAsDCC. Scan rate 0.050 V s^{-1}. (b) Dependence of I_p^+ with pH. Scan rate 0.050 V s^{-1}.

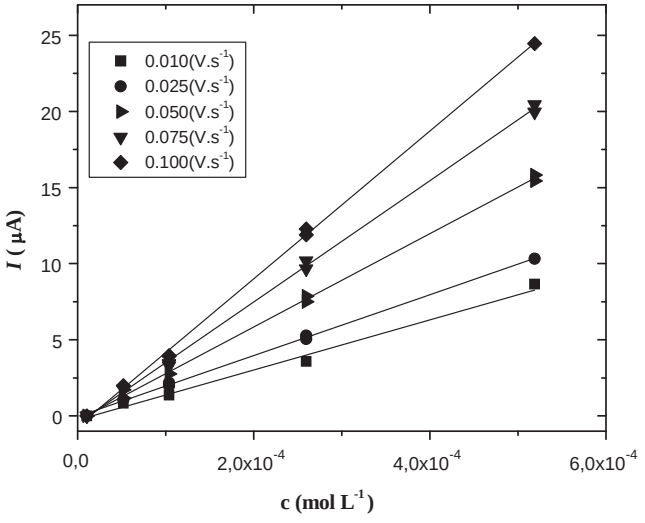


Fig. 3. Calibration curves for boldine at different scan rates. Aqueous phase composition: ×M HB^+, 1.00×10^{-2} mol L^{-1} LiCl, pH = 2.00. Organic phase composition: 1.00×10^{-2} mol L^{-1} TPAsDCC.

Table 1
Values of different parameters obtained from HPLC and CV at 0.025 (V s^{-1}) and 0.050 (V s^{-1}).

Parameters	CV 0.025 (V s^{-1})	CV 0.050 (V s^{-1})	HPLC
n (number of measure)	11	11	11
$\bar{X}$ (average (mol L^{-1}))	5.6×10^{-4}	5.4×10^{-4}	5.30×10^{-4}
s (standard deviation (mol L^{-1}))	6×10^{-5}	5×10^{-5}	8×10^{-6}
s^2 (variance (mol^2 L^{-2}))	3.6×10^{-9}	2.5×10^{-9}	6.4×10^{-11}
Coefficient of variation ($s/\bar{X}$)	10.71	9.25	1.57

3.3. Validation of voltammetric analysis

3.3.1. Accuracy

Table 1 shows different parameters (average value, standard deviation, variance and coefficient of variation) determined by both methods: HPLC and CV at two scan rates (0.025 V s^{-1} and 0.050 V s^{-1}) following the procedure described in the experimental section.

The coefficient of variation for CV is much greater than for HPLC, which may be due to mechanical instability inherent in liquid/liquid interfaces. This result could indicate that the proposed voltammetric method is not sufficiently accurate, however this parameter can be improved, as will be shown in Section 3.3.4.

With the aim of comparing the variance of both methods, a Fisher test (F-test) [37,38] was applied. In this way, the experimental F values were calculated using the following expression:

$$F_{experimental} = \frac{s_{CV}^2}{s_{HPLC}^2}$$

In these experiments the null (H_0) and the alternative (H_1) hypotheses, respectively, were as follows:

$$H_0 : s_{CV}^2 = s_{HPLC}^2$$
$$H_1 : s_{CV}^2 \neq s_{HPLC}^2$$

and the level of significance (α) was set as 0.05 (two-sided test) to get a value of "$F_{tabulated}$".

The experimental and tabulated F values found were compared each (Table 2). As can be noted, $F_{experimental}$ values obtained for both scan rates were higher than $F_{tabulated}$ values. In such a case the null hypothesis has to be rejected, the difference between both variances being statistically significant.

Therefore, in this case we applied the following "t"-test [39]:

$$t_{experimental} = \frac{\left|\bar{X}_{CV} - \bar{X}_{HPLC}\right|}{\sqrt{\frac{s_{HPLC}^2}{n_{HPLC}} + \frac{s_{CV}^2}{n_{CV}}}}$$

And the degrees of freedom (df) were obtained from the following equation:

$$df = \frac{\left(s_{HPLC}^2/n_{HPLC} + s_{CV}^2/n_{CV}\right)^2}{\frac{\left(s_{HPLC}^2/n_{HPLC}\right)^2}{n_{HPLC}-1} + \frac{\left(s_{CV}^2/n_{CV}\right)^2}{n_{CV}-1}}$$

Table 2 summarizes the results found.

Table 2
Values of $F_{experimental}$, $t_{experimental}$ (for two different scan rates), df, $F_{tabulated}$, and $t_{tabulated}$ obtained from Ref. [37].

Parameter	Scan rate			
	0.025 (V s^{-1})		0.050 (V s^{-1})	
$F_{experimental}$	56.25		39.06	
$F_{tabulated}$ ($\alpha = 0.05$)	2.98		2.98	
$t_{experimental}$	1.64		0.65	
df	10	11	10	11
$t_{tabulated}$ ($\alpha = 0.05$, two side test)	2.228	2.201	2.228	2.201

Table 3
Linear regression parameters obtained from calibration curve in Fig. 3 at a scan rate of 0.025 (V s^{-1}).

Interception value (b)	-0.04 ± 0.06
Probability of the interception value	0.5367
Slope (a)	$(200 \pm 2) \times 10^2$
Probability of the slope	<0.0001
α	0.05
R	0.999
R^2	0.999

As reported before, the null and alternative hypotheses for the equivalence between both average values were defined as:

$$H_0 : \bar{X}_{CV} = \bar{X}_{HPLC}$$
$$H_1 : \bar{X}_{CV} \neq \bar{X}_{HPLC}$$

As can be noted in Table 2, $t_{experimental}$ values are smaller than $t_{tabulated}$ ones, for both degrees of freedom and both scan rates. This comparison leads us to conclude that there is no a statistical difference between the average values for both methods and that accuracy is accepted.

3.3.2. Linearity, limit of detection (LOD) and limit of quantification (LOQ)

3.3.2.1. Linearity. Linearity, defined as the ability of the method to get test results proportional to the concentration of analyte in a given range, was evaluated from the calibration curves in Fig. 3 [40]. Table 3 summarizes the values obtained from linear regression at a scan rate 0.025 V s^{-1} (data from others scan rates are not shown for simplicity).

An important aspect in the analysis of linearity is the assessment of intercept (b) and slope (a) of the regression line, which must be statistically equal and different to zero respectively.

For the intercept we stated the null and alternative hypotheses, respectively, as:

$$H_0 : b = 0$$
$$H_1 : b \neq 0$$

To accept one of the hypotheses, a comparison between the probability of the interception value and the level of significance (α) is required. In this case (see Table 3) $\alpha <$ probability of the interception value, thus, no evidence has been found to reject the null hypothesis. It can be concluded then that "b" is statistically similar to zero. This conclusion holds for all scan rates measured.

Similarly we stated both hypotheses for the slope of the regression line:

$$H_0 : a = 0$$
$$H_1 : a \neq 0$$

The probability for the slope value is smaller than α, therefore we can reject the null hypotheses and accept the alternative one. The slope of the calibration line is thus statistically different to zero.

Finally, to assess linearity the calculation of residual values and the analysis of the residual plot need to be considered. In the evaluation of linearity, the residual values were calculated as $e_i = y - (a \times x + b)$ (where e_i is the residual value, y is the current value measured, a and b are the slope and the intercept of the calibration line respectively). The resulting e_i values were plotted against the boldine concentration (Fig. 4). As seen, the residual values are randomly distributed around zero showing no evident tendency and confirming, in this way, the linearity. The behavior previously found is valid for all scan rates analyzed.

3.3.2.2. Limit of detection and limit of quantification. LOD is often defined as the minimum amount or concentration of substance that can be reliably detected by a given analytical method; while LOQ is defined as the lowest value that can be determined with an acceptable level of precision and accuracy. For the calculation of both, LOD

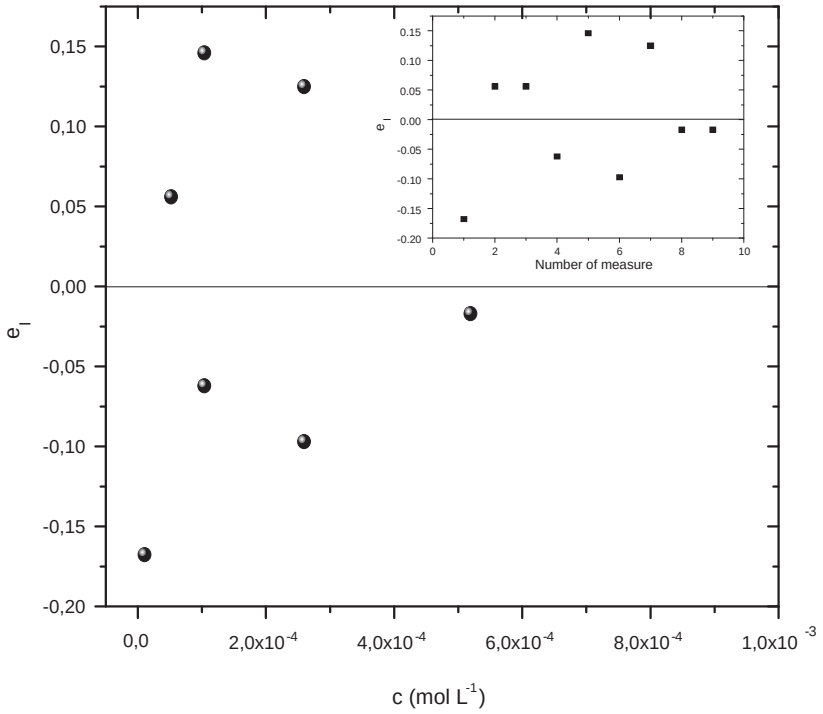


Fig. 4. Plot of residual values vs. boldine concentration for the calibration curve. Scan rate 0.025 V s^{-1}. Experimental conditions are the same than those in Fig. 3. Inset: plot of residual values vs. number of measure.

and LOQ, we used the following expressions:

$$\mathrm{LOD} = y_b + 3 \times s_{x/y}$$

$$\mathrm{LOQ} = y_b + 10 \times s_{x/y}$$

$$s_{x/y} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n-2}}$$

where y_b is the blank signal and $s_{x/y}$ is the standard deviation of the interception of the calibration regression.

The average values obtained, considering all the scan rates, were: LOD: $(6.1 \pm 0.7) \times 10^{-5}$ mol L^{-1} and LOQ: $(2.0 \pm 0.4) \times 10^{-4}$ mol L^{-1}.

3.3.3. Specificity

To evaluate specificity, we have carried out CV experiments employing a boldine solution prepared from a natural extract of boldo leaves as described in the experimental section. Hence, the effects of the sample matrix could be assessed. Fig. 5a (solid line) shows the voltammogram obtained for a solution prepared from the extract of 3.0000 g of boldo leaves diluted in 30.0 mL of an aqueous LiCl solution pH = 2.00. Two processes are evident, the first one at $E = 0.450$ V can be assigned to other components of the matrix, while the second processes at $E = 0.557$ V corresponds to the protonated boldine transfer from the aqueous to the organic phase. To corroborate this statement, the addition of standards was carried out on this solution. Fig. 5a shows the voltammograms obtained after the successive additions of 70.0 μL of a standard solution of boldine 1.02×10^{-2} mol L^{-1} to the initial solution. As expected, the current for the second process is enhanced with the successive aggregates of boldine, while the current for the first process remains constant. Fig. 5b represents the variation of the peak current at $E = 0.557$ V obtained after each addition at different scan rates, as a function of the added boldine concentration. From this calibration curve, the concentration of boldine in the extract was determined from the *x*-axis intercept. The standard addition experiments were performed on three different extracts, and after each addition voltammograms were measured in duplicate at five scan rates. The values of boldine concentration informed in Table 4 represent an average of the values obtained at the different scan rates.

For comparison purposes, a deconvolution procedure was carried out on voltammograms obtained for the extract solution (Fig. 5c). Two current peaks were defined. A boldine concentration similar to that informed in Table 4 was calculated from the current corresponding to the second process, and the calibration curve shown in Fig. 3.

As can be noted in Table 4, the values obtained for boldine concentration employing CV are similar to those obtained with the validated method (HPLC). This result leads to the conclusion that the selectivity of the methodology proposed is valid.

3.3.4. Precision

Fig. 6 shows the variation of relative residues, calculated as described in Section 2, as a function of the number of measures, for HPLC (square symbols) and CV at the water/1,2-DCE interface (cir-

Table 4
Boldine concentration obtained from CV for extracts 1, 2 and 3, and from HPLC for extract 3.

Extract	Leaves weight (g)	% P/P of boldine in leaves	*s*
1	1.0000	0.057	0.009
2	1.0000	0.061	0.005
3	3.0000	0.061	0.009
HPLC		0.060	0.001

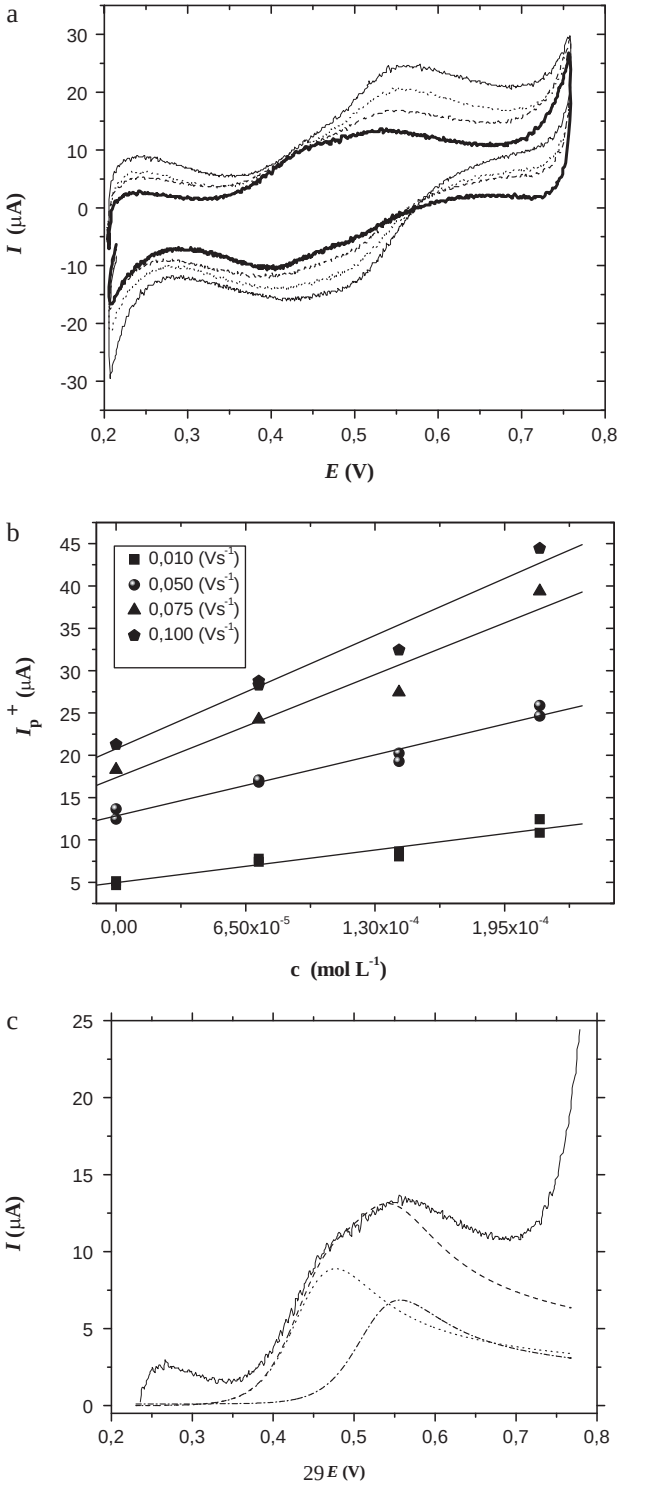


Fig. 5. (a) Cyclic voltammograms obtained after standard additions of different volumes of 1.02×10^{-2} mol L^{-1} boldine solution to an extract obtained from 3.0000 g of boldo leaves: (—) 0.0 μL, (- - -) 70.0 μL, (···) 140.0 μL, (–) 210.0 μL. Scan rate: 0.0050 V s^{-1}, pH = 2.00. (b) Lineal dependence of I_p^+ vs. concentration for the successive additions of boldine, at different scan rate. (c) Comparison between experimental voltammogram for Boldo extract (–), and a simulated voltammogram (- - -) obtained from the sum of simulated voltammograms for boldine (- · - · -) and an interferent (···). $v = 0.050$ V s^{-1}.

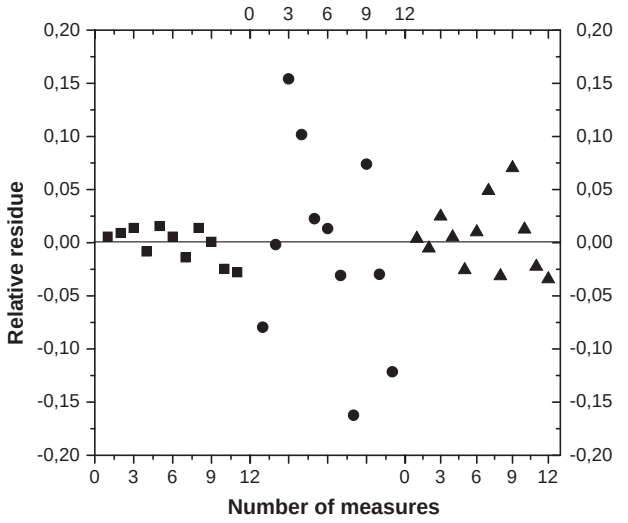


Fig. 6. Plot of relative residue ($rl = (X_i - \bar{X})/\bar{X}$) vs. number of measure for (■) HPLC, (●) cyclic voltammetry at liquid/liquid interface and (▲) cyclic voltammetry at gel/liquid interface. Scan rate 0.050 V s^{-1}. Experimental conditions are the same than those in Fig. 3.

Table 5
Comparison of coefficient of variation for CV applied to a water/1,2-DCE interface and a water/1,2-gelled DCE.

Scan rate	Interface	Coefficient of variation
0.025 (V s^{-1})	Water/1,2-DCE	10.71
	Water/1,2-gelled DCE	4.3
0.050 (V s^{-1})	Water/1,2-DCE	9.21
	Water/1,2-gelled DCE	3.28

cle symbols). The results indicate that relative residues, as well as the coefficient of variation and standard deviation values (Table 1), obtained from CV procedure are higher than those found when HPLC is employed. The high dispersion found in CV experiments might be accounted for as a result of the important mechanical instability of the liquid/liquid interface. To overcome this disadvantage, the organic phase was gelled employing PVC, and the same CV experiments were carried out. The results, shown in Fig. 6 (triangle symbols) and Table 5, evidence that an improvement in the dispersion of the values is achieved when the water/1,2-DCE interface is replaced by the water/1,2-gelled DCE interface.

The results indicate that both, the precision and accuracy, as well as the LOD and LOQ are acceptable for the quantification of boldine in extracts obtained from boldo leaves.

4. Conclusions

Boldine at a boldo leaves extract can be quantified by employing CV applied to a liquid/liquid interface. The detection and quantification limits were $(6.1 \pm 0.7) \times 10^{-5}$ mol L^{-1} and $(2.0 \pm 0.4) \times 10^{-4}$ mol L^{-1}, respectively. Linear calibration curves in the concentration range between 1.04×10^{-5}M and 5.19×10^{-4} mol L^{-1}, with correlation coefficient of 0.997, were obtained. The standard addition method is highly efficient in this kind of systems where matrix effects are present; it also requires less reactive and involves easy sample manipulation. The validation of this methodology was tested against the reference method, HPLC. Accuracy, linearity, LOD, LOQ, specificity and precision have been assessed and the results indicate that all of these parameters are acceptable compared with those corresponding to HPLC. The electroanalytical technique proposed is selective, requires simple equipment and implies low cost, and can be applied to rapid and accurate quantitative determination of boldine alkaloid in complex matrixes as leaf extracts without special drug separation.

At this point, it is worthwhile a brief discussion comparing the results presented in this paper with those obtained employing other techniques. The analytical detection and quantification of boldine have been carried out by other authors employing paper electrophoresis, calorimetric methods, thin layer and gas liquid chromatography. The assay of boldine and related alkaloids contained in crude boldo samples, in extracts, or in pharmaceutical preparations is based on the initial separation of the alkaloids by high performance liquid chromatography [1]. The methodology presented in this paper allows direct determining boldine concentration in extracts of boldo leaves without previous separation and with precision and accurate similar to that obtained employing the methods mentioned above.

Moreover, the CV experiments applied at the liquid/liquid interface under different experimental conditions, have also allowed us to study the transfer mechanism of boldine and to determine the acidic dissociation constant.

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