



SENSITIVE MULTIRESIDUE QUANTIFICATION OF PROCYMIDONE AND TEBUTHIURON ON CARBON PASTE ELECTRODE BY DIFFERENTIAL-PULSE VOLTAMMETRY

M. R. FIDÉLIS¹, L. L. OKUMURA¹, A. F. de S. SILVA¹, A. GURGEL^{1,*} and A. A. SACZK²

¹ Federal University of Viçosa, Department of Chemistry, CEP 36570900, Viçosa(MG), Brazil

² Federal University of Lavras, Department of Chemistry, CEP 37200000, Lavras(MG), Brazil

*e-mail: agurgel@ufv.br

ABSTRACT: *Qualitative electrochemical characterization of procymidone (PRO) and tebuthiuron (TBH) was carried out by cyclic voltammetry using a carbon paste electrode. The studies suggest that analyte mass transfer is controlled by diffusion, and that oxidation of PRO and TBH occurs by means of irreversible electronic transfer of one electron. Oxidation of PRO and TBH occurs close to +0.820 V and +1.075 V (vs. Ag|AgCl, KCl 3.0 mol L⁻¹), respectively, in a 0.10 mol L⁻¹ KOH solution as supporting electrolyte. Quantitative analyses were carried out by differential-pulse voltammetry, a technique which is more sensitive and selective. Detection and quantification limits were determined for PRO and TBH in the absence of matrix, in a potable-water matrix, and in a nonpotable-water matrix, for which the ranges of reproducibility, intermediate precision and recovery rates were (1.01 and 4.20 %), (4.08 and 9.56 %), and (90.6 and 115 %) for PRO, and (1.59 and 3.92 %), (4.84 and 7.46 %), and (91.3 and 119 %), for TBH, respectively. The results indicate that the new method is selective, simple and cheap to simultaneously quantify PRO and TBH in water samples.*

KEYWORDS: Procymidone; Tebuthiuron; Agrochemicals; Carbon paste electrode; Differential-pulse voltammetry.

1. INTRODUCTION

Some of the most important advances that occurred in agricultural activities were the introduction of fertilizers and specific machines, and the development and use of agrochemicals, also known as pesticides or biocides (Silva *et al.*, 2005). These chemicals are destined for the protection of crops and urban areas against the harmful action of some forms of animal and/or vegetal lives (Noyrod *et al.*, 2014).

Several formulations of agrochemicals are traded in Brazil, which are more frequently consumed in crops such as soybean, cotton, sugar cane, corn, coffee, citric fruit and rice (MAPA, 2010). According to recent data reported by Anvisa, the Brazilian agency for sanitary regulation, acquired with their Program of Analyses of Agrochemical Residues in Food (PARA) in 2013, at least one third of the collected fruit and vegetables samples were considered as unsuitable, with various inadequacies, such as much higher amounts of agrochemicals than the limits allow and the presence of active ingredients that are inappropriate for the crops in which they were detected, or products that are simply forbidden by the Brazilian legislation (ANVISA, 2009).

In spite of the advantages of using agrochemicals in agricultural activities, their intensive use has negatively affected the environment as a whole, impairing human and animal health. According to Silva *et al.* (2005), human exposure to agrochemicals is regarded as a public-health problem worldwide. This fact is a continuous cause of concern for national sanitary authorities, since agrochemicals are the second most relevant source of intoxication in Brazil, only behind the improper use of medicine by the population (Bastos *et al.*, 2011).

The intensive use of agrochemicals has increased the amount of their residues in environments as diverse as water, soil and air, affecting food sources, the flora and the fauna (Mcknight *et al.*, 2015). The distribution of such compounds in the various environments depends on their physicochemical properties. Agrochemical residues in water, for example, may bond to particulate matter in suspension and deposit as sediments or be absorbed by organisms, in which they can accumulate. The residues can be transported by the aqueous system within the water currents or inside the bodies of the aquatic organisms. Some compounds may also return to the atmosphere by volatilization, which is a clear indication that there is a continuous interaction of the residues with the sediments and water that is ultimately affected by the movement of the water bodies, turbulence and temperature. This interaction may increase the amount of time of exposure of the aquatic organisms to the toxic compounds (Gerónimo *et al.*, 2014).

The standards of water quality are based on a series of parameters and their limits are established from scientific criteria that assess their risks for the environment and human and animal health. In Brazil, the Federal resolution N° 357 proposed on March 17th, 2005, by the National Council for the Environment (CONAMA), regulates the manipulation of water bodies and establishes conditions for effluent emissions (CONAMA, 2005). Maintaining the proper quality indices of water bodies is always a major challenge for environmental technology, and various separation processes can be devised for each specific purpose.

Numerous agrochemicals are available, and novel compounds are continuously produced or synthesized. Among them, the fungicide procymidone (PRO) and the herbicide tebuthiuron (TBH) are often used in agricultural activities, particularly in the cultivation of fruit and vegetable. The chemical structures of PRO [N-(3,5-dichlorophenyl)-1,2-dimethyl-cyclopropane-1,2-dicarboximide] and TBH [1-(5-*tert*-Butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea] are shown in Figure 1.

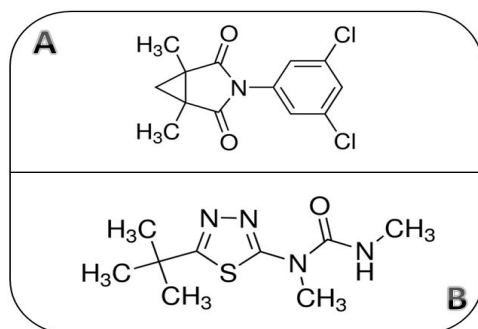


Figure 1 – Chemical structures of the agrochemicals (A) PRO and (B) TBH.

According to reported data, the analyses and determination of both procymidone and tebutiuron is mostly made by means of chromatographic techniques, coupled to several types of detectors, such as electron capture, fluorescence, visible ultraviolet, flame ionization, mass spectroscopy, among others. These techniques may be disadvantageous, for they may require a complex analytical structure to produce data, and may be costly and time-consuming. Tables 1 and 2 indicate some of the analytical techniques for the determination of PRO and TBH, respectively.

Table 1 – Analytical techniques for the determination of PRO.

| Analytical technique | Matrix | LOD* / LOQ** | Recovery | Reference |
|--|---|---|----------------------------------|--------------------------------|
| Capillary electrophoresis coupled to mass spectroscopy | Apples, grapes, oranges, pears, strawberries and tomatoes | NR*** / 0.05 mg kg ⁻¹ (all) | 71 – 80 % | Rodríguez <i>et al.</i> , 2002 |
| QuEChERS -GC-MS | Celery and soil | 1.80 and 1.65 mg kg ⁻¹ / 5.99 and 5.51 mg kg ⁻¹ | 74.9 – 100.8 % and 82.5 – 92.5 % | Chen <i>et al.</i> , 2010 |
| GC-ECD | Vegetables <i>Brassica</i> | 0.010 / 0.005 mg kg ⁻¹ | 99.56 – 108.43 % | Łozowicka <i>et al.</i> , 2012 |
| LC–ECI-MS/MS | Tomatoes | 0.001 mg kg ⁻¹ / 0.003 mg kg ⁻¹ | 94 – 108 % | Andrade <i>et al.</i> , 2015 |
| QuEChERS - GC-MS/MS | Cow milk | 3.3 µg L ⁻¹ / 10.0 µg L ⁻¹ | 81.6 – 127.6 % | Bandeira <i>et al.</i> , 2014 |
| LP-GC-MS-MS | Avocado | 0.37 µg kg ⁻¹ / 1.22 µg kg ⁻¹ | 72 – 99 % | Moreno <i>et al.</i> , 2006 |
| HPLC–DAD | Grapes | 0.05 mg kg ⁻¹ / 0.13 mg kg ⁻¹ | 75 – 95 % | Otero <i>et al.</i> , 2003 |
| GC-MS/MS | Cereals | 0.04 mg kg ⁻¹ / 0.10 mg kg ⁻¹ | 72 – 124 % | Walorczyk, 2007 |
| GC-MS | Grapes, wine and wort | NR*** / 0.0483 – 0.0509 mg kg ⁻¹ | 94.0 – 107.3 % | Navarro <i>et al.</i> , 2000 |
| GC-MS-SIM | Commercial fruit juice | 0.1 µg L ⁻¹ / 0.3 µg L ⁻¹ | 90.5 – 106.1 % | Albero <i>et al.</i> , 2005 |

*Limit of detection; **Limit of quantification; ***NR = Not reported

Although several publications can be found on the determination of both PRO and TBH in various matrices, no methodologies have been developed for the simultaneous analysis of these agrochemicals. Voltammetric techniques can be introduced with great potential to devise optimized methodologies to quantify these agrochemicals in water bodies. However, only two scientific articles discuss the selective quantification of PRO and TBH by voltammetry, and these have actually been reported by our research group (Assis *et al.*, 2014; Fulgêncio *et al.*, 2013). Based on this fact, and considering the toxicity that agrochemicals pose to human health and biodiversity, studies on the efficient monitoring of these compounds must be intensified, aiming for the reduction of contamination risks (Dores and De-Lamonica-Freire, 2001). In view of this, the main objective of this work is to present a novel electroanalytical methodology that can be successfully employed to investigate the

contamination risks to water bodies by the agrochemicals procymidone (PRO) and tebuthiuron (TBH) simultaneously.

Table 2 – Analytical techniques for the determination of TBH.

| Analytical technique | Matrix | LOD* / LOQ** | Recovery | Reference |
|------------------------------------|----------------|---|----------------|----------------------------------|
| HPLC-UV | Soil | 0.040 mg kg ⁻¹ / NR*** | 85 – 119 % | Lourencetti <i>et al.</i> , 2008 |
| HPLC-UV | Water | 8 µg L ⁻¹ / 22 µg L ⁻¹ | 90.3 – 101.3 % | Ferracini <i>et al.</i> , 2005 |
| GC with photometric flame detector | Sugar cane | 0.01 mg L ⁻¹ / NR*** | 48 – 81 % | Loh <i>et al.</i> , 1978 |
| HPLC-UV | Tomatoes | 18 mg kg ⁻¹ / 55 mg kg ⁻¹ | 80 – 117 % | Melo <i>et al.</i> , 2005 |
| HPLC-UV | Soil | 0.01 / 0.02 mg kg ⁻¹ | 90 – 103 % | Bicalho <i>et al.</i> , 2010 |
| GC-MS | Cow milk | 0.03 – 0.09 µg mL ⁻¹ | 75 – 98 % | Rutherford <i>et al.</i> , 1995 |
| HPLC-UV | Rice and maize | 0.030 and 0.032 mg kg ⁻¹ / 0.090 and 0.096 mg kg ⁻¹ | 76.9 – 94.5 % | Mou <i>et al.</i> , 2008 |
| HPLC-UV | Water | 10 µg L ⁻¹ / 35 µg L ⁻¹ | 94.9 – 110.0 % | Brondi and Lanças, 2005 |

*Limit of detection; **Limit of quantification; ***NR = Not reported

2. MATERIAL AND METHODS

2.1. Chemicals and Standard Solutions of Procymidone (PRO) and Tebuthiuron (TBH)

All chemicals (with the highest grade available) were obtained from Sigma-Aldrich and used without further purification (used as received). All solutions and subsequent dilutions were prepared daily, using Milli-Q water (Millipore, USA, 18 MΩ cm⁻¹). The standard solutions of both PRO and TBH were supplied by PESTANAL (analytical standard, USA), and were prepared in methanol at concentrations of 17.60 mmol L⁻¹ (5.00 g L⁻¹) and 43.80 mmol L⁻¹ (10.00 g L⁻¹), respectively. These solutions were stored in transparent glass flasks and kept in a desiccators at room temperature, which ranged between 293 K and 297 K throughout all the experimental procedure.

2.2. Voltammetry Measurements

The voltammetry measurements were carried out with a modular potentiostat/galvanostat, model PGSTAT 128N (Metrohm Pensalab), interfaced with a computer with the software General Purpose Electrochemical System (GPES), version 4.9. A 50.0-mL electrochemical cell stoppered with a Teflon[®] lid was also used, with holes to insert the conventional arrangement of three electrodes (work, reference and auxiliary), and a capillary for the deaeration of the solution with nitrogen gas (White Martins, Brazil). The reference and auxiliary electrodes were Ag|AgCl, KCl 3.0 mol L⁻¹ and a platinum wire, respectively.

Ten different supporting electrolyte solutions were tested for the simultaneous analysis of PRO and TBH, namely: (a) ammonium buffer (pH 9.4); (b) carbonate buffer (pH 10.0); (c) tetrabutylammonium tetrafluoroborate (BF₄TBA) in N,N-dimethylformamide (DMF); (d) lithium chloride; (e) chromium(III) chloride; (f) hydrochloric acid; (g) potassium chloride; (h) potassium nitrate; (i) sodium hydroxide; and (j) potassium hydroxide, all at the concentration of 0.10 mol L⁻¹. The criteria for choosing the best supporting electrolyte were stable and reproducible Faraday currents, voltammograms with well-defined peaks, higher-intensity currents and lowest peak potential values. It could be observed that the analyses of PRO and TBH can be carried out with the best voltammetric responses by using NaOH and KOH as supporting electrolytes. In particular, the use of KOH promotes the oxidation of both agrochemicals at a slightly lower peak potential, with lower standard deviation in the replicate results.

The study of voltammetric parameters applied for real samples is paramount when sensitive, precise and reproducible measurements are required. To comply with this, the effects of several parameters of three different techniques, namely linear sweep voltammetry (LSV), square-wave voltammetry (SWV) and differential-pulse voltammetry (DPV), were investigated. The univariate analyses of the electrochemical parameters of these techniques were carried out at least in triplicate by using the solution containing both PRO and TBH standards at the concentration of 1.00 mmol L⁻¹ in 10.0 mL of the supporting electrolyte (KOH 0.10 mol L⁻¹). In all cases, the choice of the best instrumental parameters was made by considering the following criteria: higher intensity of the peak current (I_p), lowest peak potential (E_p), lowest peak width at mid-height ($w_{1/2}$), and lowest standard deviation of the analysis.

2.3. Carbon Paste Electrode (CPE)

The carbon paste (CPE) used as work electrode was prepared by mixing graphite powder (Merck, Germany) and mineral oil (Acros Organic, Belgium). Some mass compositions (% w/w) were tested to produce a paste with chemical consistency that promoted sensitive, reproducible voltammetric responses in the analyses of PRO and TBH. To achieve this, investigations were made with the following (graphite powder)-(mineral oil) compositions: (a) (80 %)-(20 %); (b) (75 %)-(25 %); (c) (70 %)-(30 %); (d) (65 %)-(35 %); and (e) (60 %)-(40 %). In all cases, graphite powder is the major component. Each mixture was then dispersed in n-hexane (Sigma-Aldrich, USA) and homogenized with a magnetic stirrer (Corning, PC-420D) until complete evaporation of the solvent. The paste samples were then allowed to air dry, after which they were finally inserted in the proper work electrode hole (Metrohm®, area of 0.283 cm²) in the electrochemical cell. For the simultaneous determination of PRO and TBH, it was necessary to clean the surface of the work electrode between each measurement by changing the paste of the electrode. All analyses were carried out at least in triplicate.

2.4. Analytical Curves

Analytical curves were plotted in the absence and presence of matrix, by analyzing the solutions containing the PRO and TBH standards in six different concentrations which

simultaneously ranged between 2.283 and 7.991 mg L⁻¹, in a KOH 0.10 mol L⁻¹ supporting electrolyte solution, with a total volume of 10.0 mL in the electrochemical cell.

From the analytical results obtained for each agrochemical in the same concentrations, analytical curves were constructed by plotting the responses of current intensity (I_{pa}) as a function of the analyte concentration, using the DPV technique. From this relationship, data fitting was carried out with linear regression using the least-square method.

To construct the analytical curve in the absence of matrix, aqueous solutions of the supporting electrolyte (KOH 0.10 mol L⁻¹) were made with Milli-Q[®] water within the same concentration range of the standards described above. The analytical curves in the presence of matrix (potable water and nonpotable water) were constructed by preparing the supporting electrolyte (KOH 0.10 mol L⁻¹) solutions in each medium and adding the standards at concentrations within at the same range described above, to complete the same final volume of 10 mL. The limits of detection (*LOD*) and quantification (*LOQ*) were calculated with Equations (1) and (2), following the recommendation of IUPAC.

$$LOD = \frac{3 \times S_b}{b} \quad (1)$$

$$LOQ = \frac{10 \times S_b}{b} \quad (2)$$

where S_b is the standard deviation of 12 blank readings of current intensity taken precisely at the oxidation peak potential for PRO and TBH, and b is the slope of the analytical curve (IUPAC, 1978).

2.5. Water Samples

The potable-water samples used in the simultaneous determination of PRO and TBH, supplied by the Department of Chemistry of the Federal University of Viçosa (UFV), were collected within five consecutive days. The nonpotable-water samples also used in the assays were collected from the surface of a lake in the main UFV campus in four different points. This lake is situated close to where the Water Treatment Division of UFV manages the supply of water for the entire campus, at a flowrate of 50 L s⁻¹. All samples were collected in 1.0-L transparent glass flasks and stored under refrigeration for conservation until the analyses were made.

3. RESULTS AND DISCUSSION

3.1. Study of the Composition of the Carbon Paste Electrode

The first step of this work was to examine the effect of the composition of the carbon paste electrode (CPE) on the voltammetric response in the analysis of the 0.10 mol L⁻¹ KOH solution containing PRO or TBH, both at a concentration of 1.00 mmol L⁻¹. The CPEs prepared with different amounts of graphite powder and mineral oil were tested by means of

cyclic voltammetry, with potential ranging between +0.60 V and +1.25 V vs. Ag|AgCl, KCl 3.0 mol L⁻¹, and potential scan rate of 100 mV s⁻¹. The anodic peak potentials observed for PRO and TBH were +0.820 V and +1.075 V vs. Ag|AgCl, KCl 3.0 mol L⁻¹, respectively.

Random results were obtained for the value of I_{pa} in the oxidation of PRO and TBH when using different (graphite powder : mineral oil) ratios, but the maximum was detected with the (75 % graphite powder)-(25 % mineral oil) CPE. This is demonstrated by the plots shown in Figure 2. Therefore, subsequent voltammetric studies were carried out with a work electrode with such composition.

Figure 3 shows the cyclic voltammograms for both agrochemicals. It can be noted that the intensity of the anodic potential current (I_{pa}) in the oxidation of TBH is lower when using the CPE with the lowest amount of graphite powder (60 % w/w), and remains practically constant with all other CPEs.

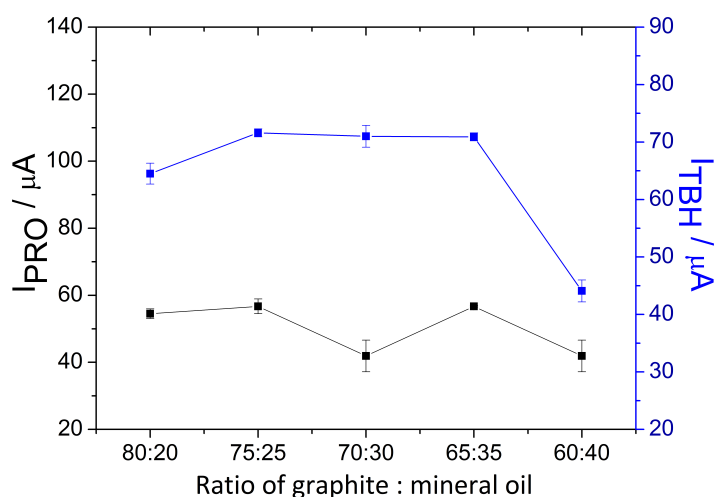


Figure 2 – Variation of the intensity of anodic current (I) for PRO and TBH as a function of the composition of the work electrode (graphite powder : mineral oil ratio). Experimental condition: $v = 100 \text{ mV s}^{-1}$.

3.2. Electrochemical Characterization of PRO and TBH by Cyclic Voltammetry (CV)

Based on the previous results, a range of potential was established from +0.60 to +1.25 V vs. Ag|AgCl, KCl 3.0 mol L⁻¹, using the CPE with 75 % in mass of graphite powder, in the KOH 0.10 mol L⁻¹ supporting electrolyte. Both oxidation peaks were monitored, one at +0.820 V for the oxidation of PRO, and another at +1.075 V for the oxidation of TBH.

At first, the absence of a peak in the reversed scanning orientation in cyclic voltammetry (CV) suggested that the oxidation of PRO and TBH occurs by irreversible

electronic transfer (Figure 3). However, such absence only does not characterize the electronic transfer as irreversible, since chemical reactions coupled with the electronic process may be associated in the system, thereby rapidly consuming any species formed on the electrode surface by the direct scanning of the electrode (Brett and Brett, 1993).

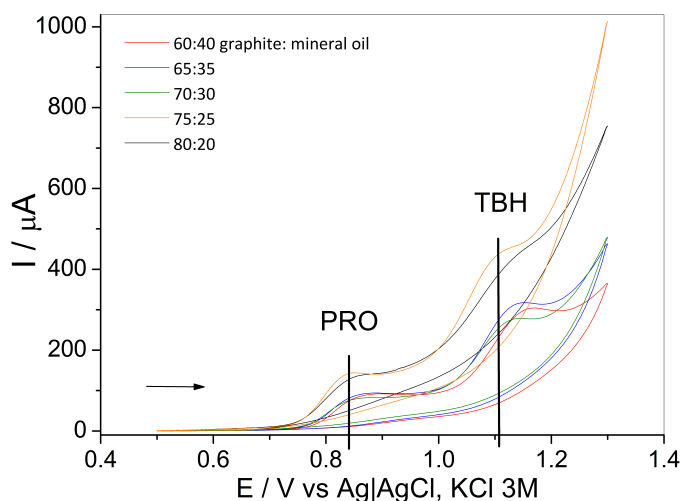


Figure 3 – Cyclic voltammograms acquired by analyzing solutions of PRO and TBH (1.00 mmol L^{-1}) in $\text{KOH } 0.10 \text{ mol L}^{-1}$ using CPEs with different compositions (% w/w).

According to Nicholson and Shain (1964), the proposition that the oxidation of PRO and TBH is irreversible is corroborated by the fact that the parameter ($I_{pa}/v^{1/2}$), known as the current function, is practically independent of the potential scan rate. This linear behavior was also observed for PRO and TBH.

The influence of the potential scan rate on the voltammetric responses of PRO and TBH was also investigated with CV. It can be seen in Figure 4 that the relationship between the anodic peak current with the square root of the potential scan rate is linear, for both PRO ($r^2 = 0.9924$) and TBH ($r^2 = 0.9978$). Such linearity for both agrochemicals indicates that the mass transfer is predominantly controlled by diffusion of the species to the electrode surface.

3.3. Optimization of Voltammetric Parameters for LSV, SWV and DPV

Table 3 presents a summary of the study of voltammetric parameters acquired by the techniques LSV, SWV and DPV in the simultaneous determination of PRO and TBH. In order to compare the voltammetric responses of such techniques, and aiming to choose the most suitable electrochemical technique to determine the agrochemicals simultaneously, a 1.00 mmol L^{-1} solution of PRO and TBH was assayed with each technique operating with the optimal selected parameters, and the results are shown in Figure 5.

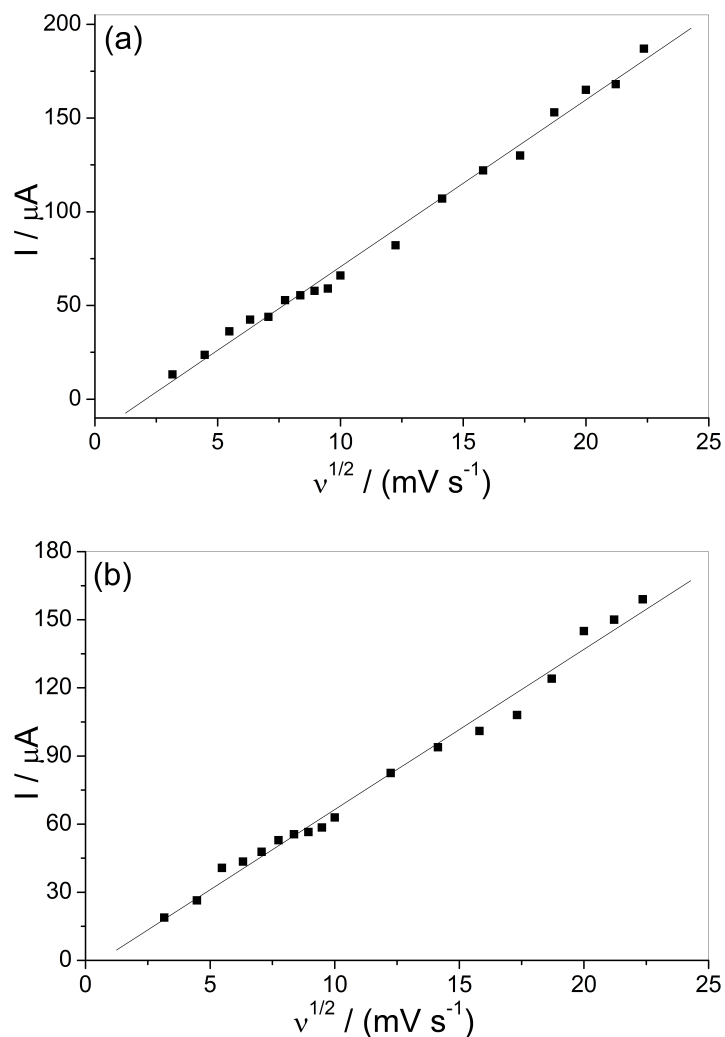


Figure 4 – Variation of the anodic peak current with the square root of the potential scan rate for (a) PRO 1.00 mmol L⁻¹ and (b) TBH 1.00 mmol L⁻¹. Experimental conditions: KOH 0.10 mol L⁻¹ as supporting electrolyte solution and CPE (75:25 % graphite:mineral oil) as work electrode.

Table 3 – Voltammetric parameters of the LSV, SWV e DPV techniques for the simultaneous analysis of PRO and TBH.

| Technique | Parameter | Range | Optimal value |
|-----------|--------------------------|-----------------------------|------------------------|
| LSV | Step increment (I_v) | 0.5 – 20 mV | 1.0 mV |
| | Scan rate (v) | 10 – 130 mV s ⁻¹ | 120 mV s ⁻¹ |
| SWV | Amplitude (A_p) | 10 – 100 mV | 80 mV |
| | Frequency (F) | 10 – 100 Hz | 100 Hz |
| | Step increment (I_v) | 0.5 – 20 mV | 7.5 mV |
| DPV | Scan rate (v) | 2 – 50 mV s ⁻¹ | 8 mV s ⁻¹ |
| | Amplitude (A_p) | 10 – 250 mV | 175 mV |
| | Pulse time (t_p) | 2 – 100 ms | 2 ms |

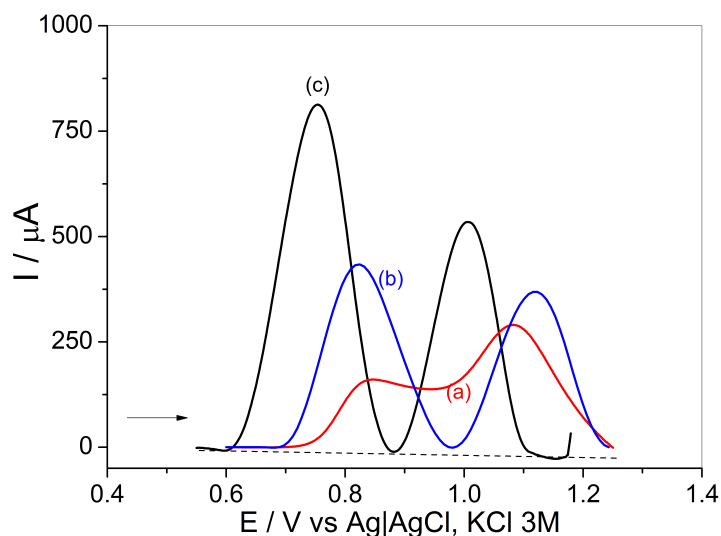


Figure 5 – Comparison of voltammograms in the analysis of a 1.00 mmol L⁻¹ solution of PRO and TBH in KOH 0.10 mol L⁻¹ with a (75:25 / graphite:mineral oil) CPE by (a) LSV, (b) SWV, and (c) DPV.

It was observed that the sensitivity of the applied methodology is higher when higher I_{pa} values are obtained, whilst the selectivity is favoured by oxidation occurring with E_{pa} close to zero (Pacheco, 2013). In view of this, DPV was selected as the best technique to simultaneously quantify PRO and TBH, based on the superposition of the voltammograms shown in Figure 5, since higher sensitivity and selectivity were obtained with DPV than with LSV and SWV.

3.4. Voltammetric Methodology and Analytical Curves

After choosing the best conditions with regards to the composition of the work electrode and the parameters involved in DPV, the analytical curve was constructed for three different conditions, namely: (a) in the absence of matrix (Milli-Q water, Millipore, USA, 18 MΩ cm⁻¹); (b) in potable water; and (c) in nonpotable water from a UFV lake. Voltammetric measurements were made in different concentrations of the PRO and TBH standards, with 10.0-mL samples, however by simultaneously varying them from 2.283 to 7.991 mg L⁻¹, in KOH 0.10 mol L⁻¹, and using the CPE.

Figure 6 shows the voltammograms acquired in the simultaneous analysis of the agrochemicals in the absence of a matrix for each concentration. It can be seen that the current intensity increases with increasing PRO concentration, but decreases with increasing TBH concentration. This is possibly due to interference of PRO on the determination of TBH, but this did not impair the development of the methodology, since the response of both compounds was linear within the concentration range investigated.

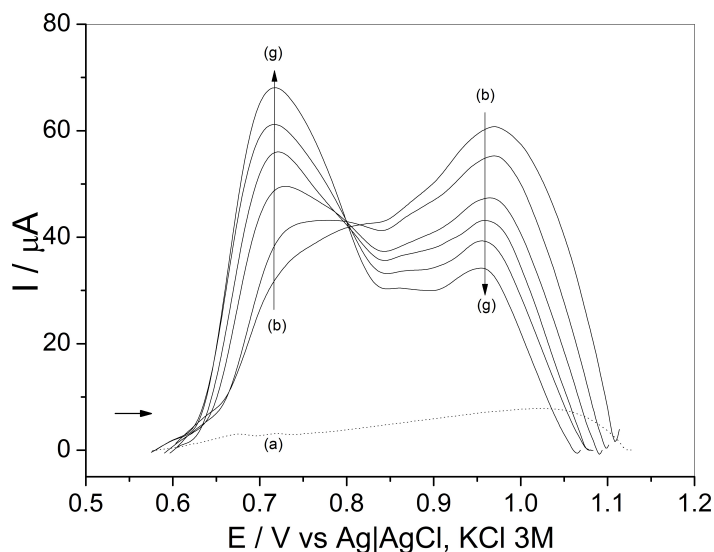


Figure 6 – Differential-pulse voltammograms for the oxidation of PRO and TBH on CPE at different concentrations: (a) blank; (b) 2.283 mg L⁻¹; (c) 3.425 mg L⁻¹; (d) 4.566 mg L⁻¹; (e) 5.708 mg L⁻¹; (f) 6.849 mg L⁻¹; (g) 7.991 mg L⁻¹. KOH 0.10 mol L⁻¹ as supporting electrolyte solution. DPV voltammetric conditions: $\nu = 8 \text{ mV s}^{-1}$; $A_p = 175 \text{ mV}$; $t_p = 2 \text{ ms}$.

From the least-squares regression fit of the current intensity with the concentration of analytes, the analytical curves for PRO and TBH were constructed (Figure 7). The same methodology was applied in the simultaneous quantification of PRO and TBH in the other water matrices, both potable and nonpotable. Particularly for TBH, the y-coordinates (I_{pa}) of the analytical curves were multiplied by (- 1) and inverted correspondingly.

One possible explanation for the decrease in I_{pa} when determining TBH by simultaneously increasing the concentration of both compounds is the occupation of more active sites on the electrode surface by PRO (or some product from its oxidation), since the kinetics of such oxidation reaction between PRO and the electrode surface is faster and occurs with lower E_p . Interestingly, it is possible to identify a point for both agrochemicals, irrespective of their concentration, with the same I_{pa} (42.3 μA) at the same oxidation potential (+0.802 V).

According to Figure 7, it is possible to identify the negative effect of the presence of matrix on the sensitivity of the methodology devised for TBH. As for PRO, the influence varied with the nature of the matrix, being positive with potable water and negative with nonpotable water. It can be concluded that the matrix effect is important on the analysis of both agrochemicals, and their determination must be made by adding a standard.

The correlation coefficients (r^2) obtained by linear regression using the least-squares method for the concentration of agrochemicals and the current intensity are shown in Table 4. The values of LOD and LOQ were also calculated for all curves, with Equations (1) and (2), respectively, and are shown in Table 5.

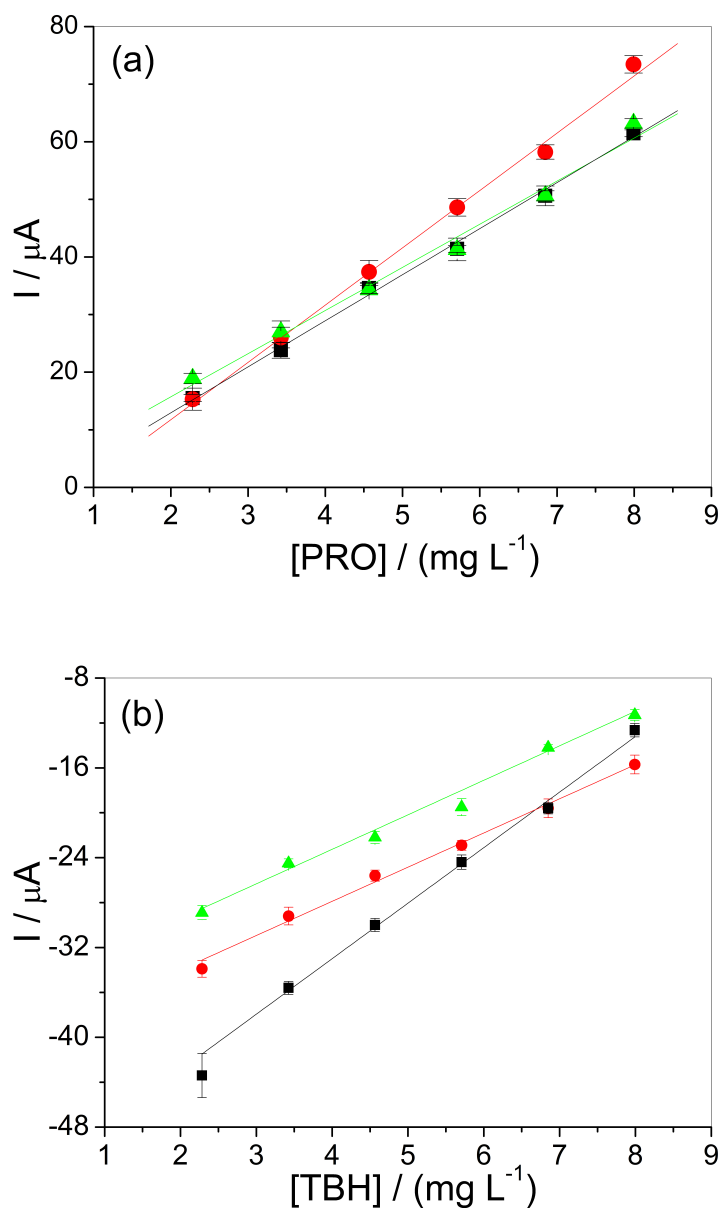


Figure 7 – Analytical curves obtained with DPV for (a) PRO and (b) TBH in (■) absence of matrix, (●) potable water, and (▲) nonpotable water.

According to ANVISA (2009), the maximal limit of TBH residues in pastureland is 20.0 mg kg^{-1} , which is much higher than the values of *LOD* and *LOQ* determined by the voltammetric methodology proposed in this work. For PRO, the maximal limit of residues ranges between 0.05 and 5.0 mg kg^{-1} for several crops, which indicates that the values of *LOD* and *LOQ* of the methodology proposed herein are higher for some crops only.

Table 4 – Linear regression least-square fit data of the analytical curves for the quantitative determination of PRO and TBH using the DPV method.

| Agrochemical | Matrix | Linear regression equation | r^2 |
|--------------|------------------|--|--------|
| PRO | Absence | $I_p(\mu\text{A}) = 3.071 + 7.998 C_{\text{PRO}}(\text{mg L}^{-1})$ | 0.9977 |
| | Potable water | $I_p(\mu\text{A}) = 8.149 + 9.948 C_{\text{PRO}}(\text{mg L}^{-1})$ | 0.9949 |
| | Nonpotable water | $I_p(\mu\text{A}) = 0.186 + 7.644 C_{\text{PRO}}(\text{mg L}^{-1})$ | 0.9904 |
| TBH | Absence | $I_p(\mu\text{A}) = 52.782 - 4.947 C_{\text{TBH}}(\text{mg L}^{-1})$ | 0.9959 |
| | Potable water | $I_p(\mu\text{A}) = 40.071 - 3.046 C_{\text{TBH}}(\text{mg L}^{-1})$ | 0.9911 |
| | Nonpotable water | $I_p(\mu\text{A}) = 35.551 - 3.074 C_{\text{TBH}}(\text{mg L}^{-1})$ | 0.9910 |

Table 5 – Limits of detection and quantification for PRO and TBH using the DPV method.

| Agrochemical | Matrix | LOD | | LOQ | |
|--------------|------------------|--------------------|------------------------|--------------------|------------------------|
| | | mg L^{-1} | $\mu\text{mol L}^{-1}$ | mg L^{-1} | $\mu\text{mol L}^{-1}$ |
| PRO | Absence | 0.525 | 1.84 | 1.75 | 6.16 |
| | Potable water | 0.486 | 1.71 | 1.62 | 5.70 |
| | Nonpotable water | 0.398 | 1.40 | 1.33 | 4.68 |
| TBH | Absence | 0.574 | 2.51 | 1.91 | 8.37 |
| | Potable water | 0.589 | 2.58 | 1.96 | 8.58 |
| | Nonpotable water | 0.534 | 2.34 | 1.78 | 7.80 |

Potable and nonpotable water samples were analyzed under the same conditions as those selected to construct the analytical curves in the absence of matrix using the CPE. To assess the applicability of the proposed voltammetric-based method in the analysis of water samples, three potable water and three nonpotable water samples were analyzed. The corresponding voltammograms were acquired for sample solutions containing the supporting electrolyte and various aliquots of potable or nonpotable water. No anodic peak could be detected for any of the six samples, which demonstrated that traceable residues of PRO and TBH did not exist in these matrices.

The procedures for PRO and TBH analyses followed the standard addition method carried out after addition of known amounts of the agrochemicals to various samples (simultaneously, in three concentration levels, namely: 2.854 mg L⁻¹; 4.566 mg L⁻¹ and 6.279 mg L⁻¹). The DPV results clearly demonstrated a linear relationship for all samples. Hence, the electrochemical response was also satisfactory for the agrochemicals detected simultaneously. The recovery percentage was also calculated and the results for each concentration are listed in Table 6.

Table 6 – Results of the apparent recovery assays of PRO and TBH in water samples.

| Concentration (mg L ⁻¹) | Apparent recovery percentage (%) | | | |
|--|----------------------------------|-------------|------------------|------------|
| | Potable water | | Nonpotable water | |
| | PRO | TBH | PRO | TBH |
| 2.854 | 90.6 – 94.7 | 96.1 – 97.6 | 100 – 113 | 99.1 – 103 |
| 4.566 | 95.5 – 104 | 115 – 119 | 105 – 115 | 117 – 119 |
| 6.279 | 106 – 108 | 91.3 – 99.2 | 94.8 – 96.5 | 92.8 – 104 |

Taking into account that the acceptable limits of recovery percentage in the analysis of residues must be within 70 and 120 %, with precision of up to ± 20 % (Ribani *et al.*, 2004), it is concluded that the analyses of all samples were satisfactory. Besides, deviations on the values of recovery percentages were due uniquely to aleatory errors, indicating that the methodology devised in this work is not tendentious. In addition, the reproducibility of the technique ranged between 1.01 and 4.20 %, with intermediate precision between 4.08 and 9.56 %, for PRO. The same data for TBH were: reproducibility between 1.59 and 3.92 % and intermediate precision between 4.84 and 7.46 %.

4. CONCLUSIONS

A novel, simple, rapid and relatively cheap electroanalytical methodology was devised for the first time to simultaneously determine two agrochemicals (procymidone, PRO; and tebuthiuron, TBH) in water samples, using the differential-pulse voltammetry (DPV) technique. A carbon paste work electrode was prepared with a mixture of graphite powder and mineral oil at a composition of 75:25 % w/w and a KOH 0.10 mol L⁻¹ solution was used as supporting electrolyte in the quantification of the agrochemicals in potable and nonpotable water samples. The analytical signals exhibited by PRO and TBH showed anodic peak potentials close to +0.820 V and +1.075 V vs. Ag|AgCl, KCl 3M, respectively. It was demonstrated that it is possible to apply the methodology proposed herein to determine residues of PRO within the detection and quantification limits of 0.486 mg L⁻¹ and 1.62 mg L⁻¹ in potable water samples, and 0.398 mg L⁻¹ and 1.33 mg L⁻¹ in nonpotable water samples, respectively. Simultaneously, it is also possible to quantify residues of TBH within the detection and quantification limits of 0.589 mg L⁻¹ and 1.96 mg L⁻¹ in potable water, and 0.534

mg L⁻¹ and 1.78 mg L⁻¹ in nonpotable water, respectively. The methodology is therefore selective, precise and simple, and can be implemented with a relatively low cost. Besides, recovery assays have also demonstrated that the method is exact. Finally, the possibility of carrying out *in loco* analyses renders the proposed methodology a very attractive tool in the field, since the direct and simultaneous determination of agrochemicals like PRO and TBH is viable, with low instrumental and experimental cost.

5. ACKNOWLEDGEMENTS

The authors are indebted to the Department of Chemistry of the Federal University of Viçosa (UFV, Brazil), for their support. The authors also gratefully acknowledge financial support granted by CNPq and FAPEMIG.

6. REFERENCES

- ALBERO, B.; SANCHÉZ-BRUNETE, C.; TADEO, J. L. Multiresidue determination of pesticides in juice by solid-phase extraction and gas chromatography–mass spectrometry. *Talanta*, v. 66, n. 4, p. 917-924, may 2005.
- ANDRADE, G. C. R. M.; MONTEIRO, S. H.; FRANCISCO, J. G.; FIGUEIREDO, L. A.; BOTELHO, R. G.; TORNISIELO, V. L. Liquid chromatography–electrospray ionization tandem mass spectrometry and dynamic multiple reaction monitoring method for determining multiple pesticide residues in tomato. *Food Chemistry*, v. 175, p. 57-65, may 2015.
- ANVISA (Agência Nacional de Vigilância Sanitária). **Programa de Análise de Resíduos de Agrotóxicos em Alimentos. Nota Técnica para divulgação dos resultados do PARA de 2008**. Brasília: ANVISA; 2009. Available at: <http://www.anvisa.gov.br/divulga/noticias/2009/pdf/150409_para.pdf>. Accessed on April 15th, 2016. (In Portuguese)
- ASSIS, A. P.; OKUMURA, L. L.; SACZK, A. A.; OLIVEIRA, M. F. New Voltammetry-Based Technique for the Determination of Tebuthiuron in Crystal and Brown Sugar Samples. *Journal of the Brazilian Chemical Society*, v. 25, n. 1, p. 27-35, 2014.
- BANDEIRA, D. D.; MUNARETTO, J. S.; RIZZETTI, T. M.; FERRONATO, G.; PRESTES, O. D.; MARTINS, M. L. Determinação de resíduos de agrotóxicos em leite bovino empregando método QuEChERS modificado e GC-MS/MS. *Química Nova*, v. 37, n. 5, p. 900-907, apr. 2014. (In Portuguese)
- BASTOS, L. H. P.; CARDOSO, M. H. W. M.; NÓBREGA, A. W.; JACOB, S. C. Possíveis fontes de contaminação do alimento leite, por agrotóxicos, e estudos de monitoramento de seus resíduos: uma revisão nacional. *Cadernos de Saúde Coletiva*, v. 19, n. 1, p. 51-60, 2011.

- BICALHO, S. T. T.; LANGENBACH, T.; RODRIGUES, R. R.; CORREIA, F. V.; HAGLER, A. N.; MATALLO, M. B.; LUCHINI, L. C. Herbicide distribution in soils of a riparian forest and neighboring sugar cane field. **Geoderma**, v. 158, n. 3-4, p. 392-397, sep. 2010.
- BRETT, C. M. A.; BRETT, A. M. O. **Electrochemistry: Principles, Methods and Applications**. New York: Oxford University Press, 1993.
- BRONDI, S. H. G.; LANÇAS, F. M. Development and validation of a multi-residue analytical methodology to determine the presence of selected pesticides in water through liquid chromatography. **Journal of the Brazilian Chemical Society**, v. 16, n. 3b, p. 650-653, may 2005.
- CHEN, L.; LI, X.-S.; WANG, Z.-Q.; PAN, C.-P.; JIN, R.-C. Residue dynamics of procymidone in leeks and soil in greenhouses by smoke generator application. **Ecotoxicology and Environmental Safety**, v. 73, n. 1, p. 73-77, jan. 2010.
- CONAMA (Conselho Nacional do Meio Ambiente). **Resolução N° 357**. Diário Oficial da União, Brasília. v. 53, p. 58-63, 2005. Available at: <<http://www.mma.gov.br/port/conama/res/res05/res35705.pdf>>. Accessed on May 5th, 2016. (In Portuguese)
- DORES, E. F. G. C.; DE-LAMONICA-FREIRE, E. M. Contaminação do Ambiente Aquático por Pesticidas. Estudo de Caso: Águas Usadas para Consumo Humano em Primavera do Leste, Mato Grosso - Análise Preliminar. **Química Nova**, v. 24, n. 1, p. 27-36, 2001. (In Portuguese)
- FERRACINI, V. L.; QUEIROZ, S. C. N.; GOMES, M. A. F.; SANTOS, G. L. Método para a Determinação de Hexazinone e Tebutiuron em Água. **Química Nova**, v. 28, n. 3, p. 380-382, feb. 2005. (In Portuguese)
- FULGÊNCIO, A. C. C.; SACZK, A. A.; OLIVEIRA, M. F.; OKUMURA, L. L. New Voltammetry-Based Analytical Method for Indirect Determination of Procymidone in Brazilian Apples. **Food Analytical Methods**, v. 7, n. 1, p. 31-38, jan. 2014.
- GERÓNIMO, E. D.; APARICIO, V. C.; BÁRBARO, S.; PORTOCARRERO, R.; JAIME, S.; COSTA, J. L. Presence of pesticides in surface water from four sub-basins in Argentina. **Chemosphere**, v. 107, p. 423-431, jul. 2014.
- IUPAC (International Union of Pure Applied Chemistry). Nomenclature, symbols, units and their usage in spectrochemical analysis – II. Data interpretation. **Spectrochimica Acta Part B: Atomic Spectroscopy**, v. 33, n. 6, p. 241-245, 1978.
- LOH, A.; WEST, S. D.; MACY, T. D. Gas chromatographic analysis of tebutiuron and its metabolites in grass, sugarcane, and sugarcane by-products. **Journal of Agricultural and Food Chemistry**, v. 26, n. 2, p. 410-413, mar. 1978.
- LOURENCETTI, C.; de MARCHI, M. R. R.; RIBEIRO, M. L. Determination of sugar cane herbicides in soil and soil treated with sugar cane vinasse by solid-phase extraction and HPLC-UV. **Talanta**, v. 77, n. 2, p. 701-709, dec. 2008.

- ŁOZOWICKA, B.; JANKOWSKA, M.; KACZYŃSKI, P. Pesticide residues in *Brassica* vegetables and exposure assessment of consumers. **Food Control**, v. 25, n. 2, p. 561-575, jun. 2012.
- MAPA (Ministério da Agricultura, Pecuária e Abastecimento. **Projeções do Agronegócio: Brasil 2009/2010 a 2019/2020**. Brasília: MAPA; 2010. Available at: <http://www.agricultura.gov.br/arq_editor/file/Ministerio/planos%20e20programas/projecoes_web1.pdf>. Accessed on May 5th, 2016. (In Portuguese)
- McKNIGHT, U. S.; RASMUSSEN, J. J.; KRONVANG, B.; BINNING, P. J.; BJERG, P. L. Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams. **Environmental Pollution**, v. 200, p. 64-76, may 2015.
- MELO, L. F. C.; COLLINS, C. H.; JARDIM, I. C. S. F. High-performance liquid chromatographic determination of pesticides in tomatoes using laboratory-made NH₂ and C₁₈ solid-phase extraction materials. **Journal of Chromatography A**, v. 1073, n. 1-2, p. 75-81, may 2005.
- MORENO, J. L. F.; LIÉBANAS, F. J. A.; FRENICH, A. G.; VIDAL, J. L. M. Evaluation of different sample treatments for determining pesticide residues in fat vegetable matrices like avocado by low-pressure gas chromatography–tandem mass spectrometry. **Journal of Chromatography A**, v. 1111, n. 1, p. 97-105, apr. 2006.
- MOU, R.-X.; CHEN, M.-X.; ZHI, J.-L. Simultaneous determination of 15 phenylurea herbicides in rice and corn using HPLC with fluorescence detection combined with UV decomposition and post-column derivatization. **Journal of Chromatography B**, v. 875, n. 2, p. 437-443, nov. 2008.
- NAVARRO, S.; BEARD, A.; NAVARRO, G.; VELA, N.; OLIVA, J. Multiresidue method for the rapid determination – in grape, must and wine – of fungicides frequently used on vineyards. **Journal of Chromatography A**, v. 882, n. 1-2, p. 221-229, jun. 2000.
- NICHOLSON, R. S.; SHAIN, I. Theory of Stationary Electrode Polarography. Single Scan and Cyclic Methods Applied to Reversible, Irreversible, and Kinetic Systems. **Analytical Chemistry**, v. 36, n. 4, p. 706-723, apr. 1964.
- NOYROD, P.; CHAILAPAKUL, O.; WONGSAWAT, W.; CHUANUWATANAKUL, S. The simultaneous determination of isoproturon and carbendazim pesticides by single drop analysis using a graphene-based electrochemical sensor. **Journal of Electroanalytical Chemistry**, v. 719, p. 54-59, apr. 2014.
- OTERO, R. R.; GRANDE, B. C.; GANDARA, J. S. Multiresidue method for fourteen fungicides in white grapes by liquid–liquid and solid-phase extraction followed by liquid chromatography–diode array detection. **Journal of Chromatography A**, v. 992, n. 1-2, p. 121-131, apr. 2003.

- PACHECO, W. F.; SEMAAN, F. S.; ALMEIDA, V. G. K.; RITTA, A. G. S. L.; AUCÉLIO, R. Q. Voltamétrias: Uma Breve Revisão Sobre os Conceitos. **Revista Virtual de Química**, v. 5, n. 4, p. 516-537, aug. 2013. (In Portuguese)
- RIBANI, M.; BOTOLLI, C. B. G.; COLLINS, C. H.; JARDIM, I. C. S. F.; MEL O, L. F. C. Validação em Métodos Cromatográficos e Eletroforéticos. **Química Nova**, v. 27, n. 5, p. 771-780, jun. 2004. (In Portuguese)
- RODRÍGUEZ, R.; PICÓ, Y.; FONT, G.; MAÑES, J. Analysis of thiabendazole and procymidone in fruits and vegetables by capillary electrophoresis–electrospray mass spectrometry. **Journal of Chromatography A**, v. 949, n. 1-2, p. 359-366, mar. 2002.
- RUTHERFORD, B. S.; SHACKELFORD, D. D.; ULMER, J. J. Determination of Tebuthiuron and Metabolites in Bovine Milk by GC-MSD. **Journal of Agricultural and Food Chemistry**, v. 43, n. 8, p. 2110-2116, aug. 1995.
- SILVA, J. M.; SILVA, E. N.; FARIA, H. P.; PINHEIRO, T. M. M. Agrotóxico e trabalho: uma combinação perigosa para a saúde do trabalhador rural. **Ciência e Saúde Coletiva**, v. 10, n. 4, p. 891-903, dec. 2005. (In Portuguese)
- WALORCZYK, S. Development of a multi-residue screening method for the determination of pesticides in cereals and dry animal feed using gas chromatography–triple quadrupole tandem mass spectrometry. **Journal of Chromatography A**, v. 1165, n. 1-2, p. 200-212, sep. 2007.



QUANTIFICAÇÃO SENSÍVEL MULTIRESIDUAL DE PROCIMIDONA E TEBUTIURON EM ELETRODO DE PASTA DE CARBONO POR VOLTAMETRIA DE PULSO DIFERENCIAL

RESUMO: *A caracterização eletroquímica de procimidona (PRO) e tebutiuron (TBH) foi realizada por voltametria cíclica usando um eletrodo de pasta de carbono. Os estudos sugerem que a transferência de massa de analitos é controlada por difusão, e que a oxidação de PRO e TBH ocorre próximo aos potenciais de +0.820 V e +1.075 V (vs. Ag|AgCl, KCl 3.0 mol L⁻¹), respectivamente, em uma solução 0.10 M de KOH como eletrólito de suporte. Análises quantitativas foram realizadas por voltametria de pulso diferencial, uma técnica que é mais sensível e seletiva. Os limites de detecção e de quantificação foram determinados para PRO e TBH na ausência de matriz, em uma matriz de água potável, e em uma matriz de água não-potável, obtendo-se faixas de reprodutibilidade, precisão intermediária e taxas de recuperação entre (1.01 e 4.20 %), (4.08 e 9.56 %), e (90.6 e 115 %) para PRO, e entre (1.59 e 3.92 %), (4.84 e 7.46 %), e (91.3 e 119 %), para TBH, respectivamente. Os resultados indicam que a nova técnica é seletiva, simples e barata para quantificar simultaneamente PRO e TBH em amostras de águas.*

PALAVRAS-CHAVE: Procimidona; Tebutiuron; Compostos agroquímicos; Eletrodo de pasta de carbono; Voltametria de pulso diferencial.