

# Electroanalytical Determination of Fenthion in *Passiflora alata* Tincture Samples

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## Abstract:

In this work a direct, simple, fast and sensitive method to determine Fenthion was developed using a Boron-Doped Diamond Electrode and Square Wave Voltammetry. The method was applied into *Passiflora alata* herbal medicinal tinctures derivate from passion fruits. None previous treatment such as derivation or pre-concentration of the analyte was done. Cyclic Voltammetry results showed well defined irreversible oxidation peaks at around 1.27 V into the pH range of 2.0 to 8.0. Square Wave Voltammetry was used to analytical determination and the analysis parameters, such as frequency, amplitude and pH from support electrolyte were optimized. A calibration curve was obtained, and it was observed a sensitivity of 0.38 A/mol L<sup>-1</sup> and linearity of 0.995. The limits of detection and quantification were 8.0x10<sup>-8</sup> mol L<sup>-1</sup> (21 µg L<sup>-1</sup>) and 2.5x10<sup>-7</sup> mol L<sup>-1</sup> (70 µg L<sup>-1</sup>), respectively. Recovery studies were applied using *Passiflora alata* samples fortified with Fenthion in concentrations of 2.5 µmol L<sup>-1</sup> and 4.0 µmol L<sup>-1</sup>, followed by the standard addition of the pesticide aliquots. It was observed that the complex matrix does not interferes in the method. The recovery results were about 98.4 and 112.0%, reinforcing that the method is suitable to determine the analyte in target complexes samples, such as Herbal Medicinal Plants.

**Keywords:** fenthion; boron-doped diamond electrode; square wave voltammetry; medicinal plants

## 1. Introduction

The international commerce of products derived from medicinal plants has demonstrated high growth and also the interest in the research and manufacture of herbal medicines [1-4]. As for example, in recent years there has been an increase in the marketing of herbal medicines with indication for mental disorders, for example the *Passiflora edulis* and the *P. incarnata*. The *Passiflora L* species, especially *Passiflora edulis* Sims. *F. flavicarpa* Deg. (Passionfruit-tart) and *Passiflora alata* Dryander (Passionfruit-sweet) are widely used for a variety of purposes. Among them, a *Passiflora alata* Dryander (leaves) is described due to their anxiolytic and tranquilizers [5-8].

Based on the expansion of the medicinal plants market also raises the concern about rational use of medicinal plants, both in relation to their natural constituents regarding the presence of possible contaminants [3, 9-11]. Like in other cultures, the medicinal plants are susceptible to attack from insects and disease, which makes usual the application of these pesticides in their cultivation. In this way, the safe use of pesticides requires analytical procedures to determine endogenous compounds and also exogenous such as pesticides [1, 10-12]. In this sense, the World Health Organization (WHO) [13] warns about the need for regulation of pesticide limits for these substances, similar that already occurs with foods samples. Also, there are several works in literature that reinforce the importance of this kind

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of control [3, 9, 14].

Generally, analytical procedures to determine pesticides in complex matrices such as medicinal plants are based on chromatographic methods [10, 11, 15]. Furthermore, WHO [13] also recommends these methods but warns that some pesticides may be lost in the clean-up and extraction processes. However, these methods are in general time and solvent consuming. In this sense, electroanalytical techniques have been developed as alternative for the chromatographic ones being simple, fast, and allow the *in-situ* detection with low consumption of organic solvents. Among these techniques, voltammetric methods offer the possibility to determine the pesticides directly in the samples without pre-treatment or prior separations with good selectivity and sensitivity [16-22].

Among traditional carbonaceous materials such as glassy or paste carbon electrodes, the boron-doped diamond electrode (BDD) has increasingly attracted interest due some features such as wide potential window, high chemical resistance and low residual currents [23]. These properties resulted in several works that applies the BDD electrode to determine pesticides in complex matrices samples [21, 24-26]. As for example, Maynard *et al.* [21], have demonstrated that the BDD electrode combined with square wave voltammetry technique can be used in the determination of contaminants such as fat-soluble pesticides. In this sense, voltammetric techniques allied to the BDD electrode can be used as methodologies for evaluation of pesticides in herbal medicines samples.

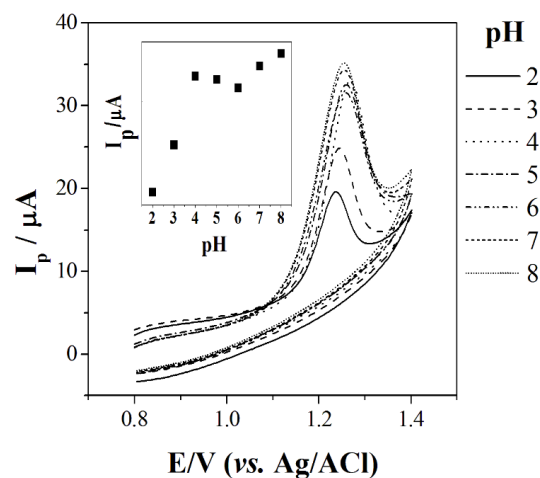
Fenthion (*O,O*-dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate) is an organophosphate insecticide (Class II, according to EPA) used against several cultures such as *Passionfruit* cultures [27, 28]. However, direct electroanalysis of Fenthion, without any preconcentration, derivation or modified electrode as sensor or biosensor still unexplored [29-32]. Galeano Diaz *et al.* [30], developed an electroanalytical method to determine Fenthion in samples of Olive Oil and river water using Square-wave adsorptive-stripping voltammetry (AdSV) and a mercury electrode. However, the method was based on a previous oxidation of Fenthion to its metabolite, fenthion-sulfoxide, by using  $\text{KMnO}_4$ . The metabolite gives rise to a peak due to an

adsorptive-reductive process at  $-0.786$  V. Also, Fenthion was isolated from olive oil by carrying out a solid-liquid extraction procedure using silica cartridge, followed by a liquid-liquid partitioning with acetonitrile.

Thus, the objective of this work was to develop an electroanalytical methodology using the BDD electrode, without any preconcentration or pretreatment to determine Fenthion pesticide into *Passiflora alata* samples.

## 2. Results and Discussion

Initially, the electrochemical behavior of FEN was evaluated using Cyclic Voltammetry and BDD electrode in BR buffer for pH varying from 2 to 10. The voltammograms are shown in Figure 1.

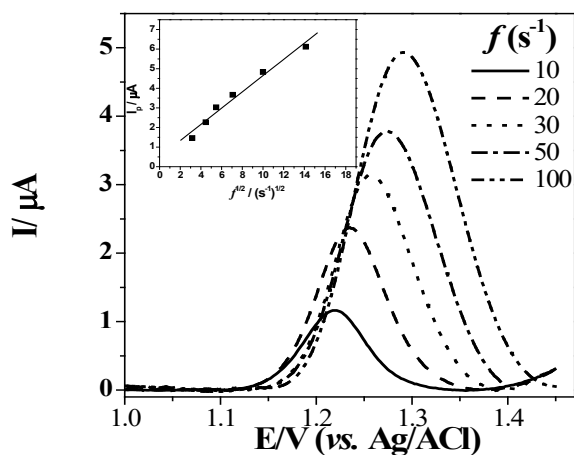


**Figure 1.** Cyclic Voltammetry of FEN ( $5.0 \times 10^{-5}$  mol  $\text{L}^{-1}$ ) using a BDD electrode for different pHs. Support electrolyte of BR buffer,  $\nu = 100$  mV  $\text{s}^{-1}$ . Inset: peak current ( $E_p = 1.27$  V) versus pH.

By the analysis of the voltammograms from Figure 1 it was observed an oxidation peak in approximately 1.27 V (vs. Ag/AgCl) with irreversible features for all measured pHs. It was also observed that peak current intensities ( $I_p$ ) were influenced by pH variation (inset of Figure 1). In pH varying from 2.0 to 4.0 it was observed a linear increase of current signals which remains almost constant above this value up to pH 8.0. The measurements were not evaluated in pH above 8.0 due to the silicon substrate could be affected in highly basic media damaging the electrode. pH 4.0 was chosen for the following SWV study.

Aiming to reach faster analyses with good reproducibility, repeatability and lowest limit of detection (LOD), Square Wave Voltammetry (SWV) was applied to determine FEN pesticide. SWV parameters such as frequency and amplitude were optimized using a BR Buffer (pH 4.0). Figure 2 shows the obtained voltammograms varying the frequency from 10 to 100 s<sup>-1</sup>.

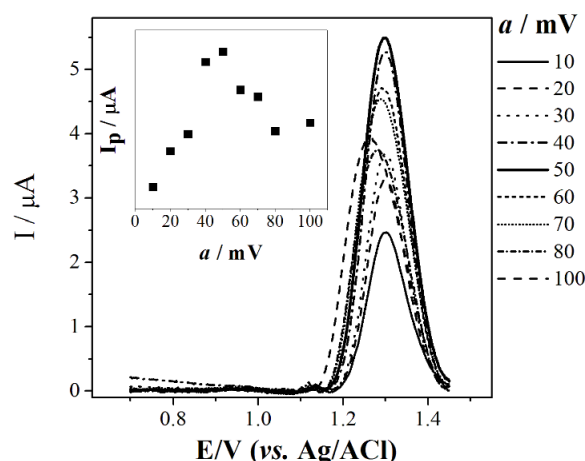
By the analysis from voltammograms (Figure 2) it was observed an increase of peak currents, with a slight displacement of peak potentials (E<sub>p</sub>) to more positive values as function of the applied frequency parameter. Analyzing the inset curve (Figure 2) it was also observed a linear relationship between the peak currents (*I*<sub>p</sub>) and the frequency square root (*f*<sup>1/2</sup>) which is generally associated with electrode processes controlled by mass transport that is (in this case) by semi-infinite linear diffusion [33]. Aiming to reach better sensitivity, the frequency of 100 s<sup>-1</sup> was chosen for the study of amplitude parameter (*a*). Figure 3 presents the SWV results of FEN varying the amplitude (*a*) parameter.



**Figure 2.** SWV of FEN ( $5.0 \times 10^{-5}$  mol L<sup>-1</sup>) versus frequency (s<sup>-1</sup>). Support electrolyte of Na<sub>2</sub>HPO<sub>4</sub> 0.1 mol L<sup>-1</sup> (pH 4.0), *a* = 50 mV,  $\Delta E_s$  = 2 mV. Inset: Current peak (*I*<sub>p</sub>) versus frequency square root (*f*<sup>1/2</sup>).

In totally reversible redox systems, the analytical sensitivity in SWV is highly affected by varying the amplitude parameter (*a*). In general, selectivity is not affected for amplitudes values higher than 50 mV. By the analysis of voltammograms from Figure 3 it was observed that peak currents (*I*<sub>p</sub>) increase up to 50 mV. For amplitude values above than 50 mV it was

observed a decrease of peak currents (Figure 3, Inset). This result is in agreement with literature for totally irreversible process [34]. Thus the 50 mV amplitude was chosen.



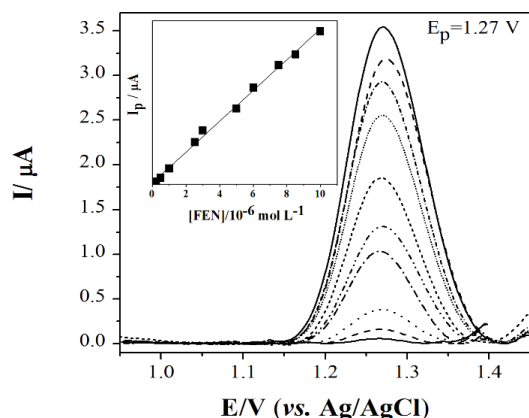
**Figure 3.** SWV of FEN ( $5.0 \times 10^{-5}$  mol L<sup>-1</sup>) in Na<sub>2</sub>HPO<sub>4</sub> 0.1 mol L<sup>-1</sup>, pH 4.0, *f* = 100 s<sup>-1</sup> and  $\Delta E_s$  = 2 mV). Inset: peak currents (*I*<sub>p</sub>) vs. amplitude (*a*).

In SWV, the scan rate is the product of frequency and step. In this sense, higher step values can affect the method sensitivity. However, the selectivity of the method can be affected by higher step values due to lower resolution occasioned by the peak enlargement. Thus, the chosen SWV parameters for the following studies were: step ( $\Delta E$ ) of 2.0 mV, frequency (*f*) of 100 s<sup>-1</sup> and an amplitude (*a*) of 50 mV. Figure 4 shows the SWV results for the standard addition of FEN within the concentration range from  $2.50 \times 10^{-7}$  to  $10.0 \times 10^{-6}$  mol L<sup>-1</sup>.

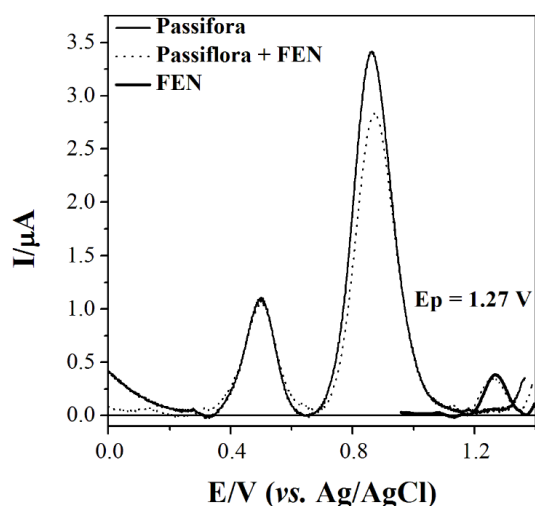
Analyzing the results from Figure 4, it was observed a linear growth of peak currents (*I*<sub>p</sub>) as function of FEN concentration (inset of Figure 4). The sensitivity and linearity were 0.38 A/mol L<sup>-1</sup> and 0.995, respectively. The relative standard deviation (RSD) for a FEN concentration of  $5.0 \times 10^{-6}$  mol L<sup>-1</sup> was 1.8 % (*n* = 10). The calculated limits of detection (LOD) and quantification (LOQ) were  $8.0 \times 10^{-8}$  mol L<sup>-1</sup> (21 μg L<sup>-1</sup>) and  $2.5 \times 10^{-7}$  mol L<sup>-1</sup> (70 μg L<sup>-1</sup>), respectively.

According to Brazilian National Sanitary Surveillance Agency [28], the maximum limit of residues (MLR) for FEN is about 0.1 mg/kg, depending of culture type. Aiming to evaluate the applicability of the method, samples of *Passiflora*

*alata* tinctures were fortified with FEN. Figure 5 shows a comparison of the SWV results for the *Passiflora alata* sample with and without the FEN fortification as well as the voltammogram of FEN in the support electrolyte of NaHPO<sub>4</sub> 0.1 mol L<sup>-1</sup> (without *Passiflora alata*).



**Figure 4.** SWV results of FEN for different concentrations. Support Electrolyte of Na<sub>2</sub>HPO<sub>4</sub> 0.1 mol L<sup>-1</sup> (pH 4.0),  $a = 50$  mV,  $f = 100$  s<sup>-1</sup> and  $\Delta E_s = 2$  mV. **Inset:** peak currents ( $I_p$ ) versus FEN concentration (mol L<sup>-1</sup>).

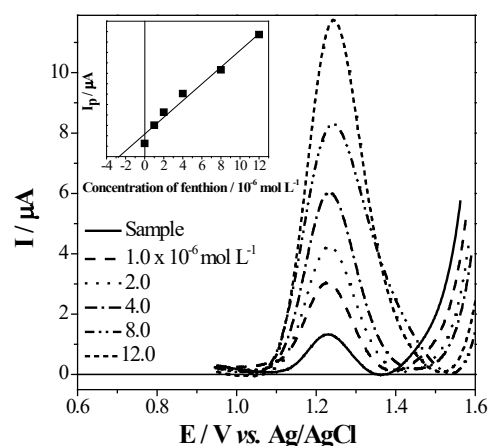


**Figure 5.** SWV results with *Passiflora alata* sample (line, —), *Passiflora alata* fortified with FEN,  $1.0 \times 10^{-6}$  mol L<sup>-1</sup>, (dot, .....), and FEN in Na<sub>2</sub>HPO<sub>4</sub>, 0.1 mol L<sup>-1</sup>, pH 4.0 (Black line, —). SWV parameters:  $a = 50$  mV,  $f = 100$  s<sup>-1</sup> e  $\Delta E_s = 2$  mV.

The SWV result from *Passiflora alata* sample showed two well defined peaks in 0.50 (1) and 0.87 V (2), vs. Ag/AgCl. Compounds into *Passiflora alata* are rich on *Flavonoids*. In this sense these peaks were attributed to *Flavonoids*

oxidation over the BDD electrode [35, 36]. After fortification with FEN pesticide ( $1.0 \times 10^{-6}$  mol L<sup>-1</sup>) it was obtained an additional peak at 1.27 V which was also observed in FEN (without *Passiflora alata*) showing the same electrochemical behavior with similar shape, peak potential ( $E_p$ ) and current intensity ( $I_p$ ). This result was attributed to complete absence of any interaction between the matrix (*Passiflora alata*) and FEN pesticide. Thus, based on absence of interaction between the matrix sample and FEN pesticide as well as the complete separation of all observed peaks, the applicability of the method was evaluated in following.

In the recovery studies (Figure 6), samples with 0.5 mL of *Passiflora alata* (without any previous treatment or separation) were fortified with FEN aliquots and added to the electrochemical cell with 9.5 mL of the support electrolyte. The FEN resulting concentrations were 2.5  $\mu$ mol L<sup>-1</sup> and 4.0  $\mu$ mol L<sup>-1</sup>, respectively. The SWV analyses were performed by subsequent standard additions of FEN pesticide. The obtained voltammograms from recovery study of FEN (2.5  $\mu$ mol L<sup>-1</sup>) into *Passiflora alata* sample, as well as the resulting analytical curve, are presented in inset of Figure 6. All the experiments were done in triplicate.



**Figure 6.** SWV results of *Passiflora alata* fortified with FEN ( $2.5 \times 10^{-6}$  mol L<sup>-1</sup>) followed by standard addition of the pesticide. Support electrolyte of Na<sub>2</sub>HPO<sub>4</sub> 0.1 mol L<sup>-1</sup> (pH 4.0),  $f = 100$  s<sup>-1</sup>,  $a = 50$  mV,  $\Delta E_s = 2$  mV. **Inset:** Analytical curve extracted from voltammograms.

Table 1 shows the summarized results of recovery studies of FEN in both samples with

concentrations of  $2.5 \mu\text{mol L}^{-1}$  and  $4.0 \mu\text{mol L}^{-1}$ , respectively. The recovery results were about 98.4 and 112.0%. These results reinforce that this electroanalytical methodology, using the BDD electrode and SWV, is suitable to determine the FEN pesticide into complex matrices such as *Passiflora alata* samples with excellent reproducibility, repeatability and sensitivity.

**Table 1.** Recovery results of FEN, in *Passiflora alata* samples, using BDD electrode and SWV.

Sample	*Spiked ( $10^{-6} \text{ mol L}^{-1}$ )	*Recovered ( $10^{-6} \text{ mol L}^{-1}$ )	Percentual (%)
1	2.5	$2.4 \pm 0.3$	$98.4 \pm 3.1$
2	2.5	$2.5 \pm 0.3$	$100.0 \pm 3.2$
3	2.5	$2.8 \pm 0.2$	$112.0 \pm 2.5$
4	4.0	$4.3 \pm 0.3$	$107.5 \pm 3.2$
5	4.0	$4.0 \pm 0.2$	$100.0 \pm 2.3$

\*concentrations into the electrochemical cell.

### 3. Material and Methods

**Reagents:** All chemicals were of analytical grade. Aqueous solutions were prepared using ultra-purified water from Milli-Q system (Millipore Corporation). A phosphate buffer solution ( $\text{Na}_2\text{HPO}_4$   $0.1 \text{ mol L}^{-1}$ ) was used as support electrolyte.

**Instruments:** An electrochemical analyzer AUTOLAB® PGSTAT128N (ECO CHEMIE, Netherland) was used for all voltammetric measurements with GPES software.

**Apparatus:** A conventional three-electrode cell was used with Ag/AgCl ( $\text{KCl } 3.0 \text{ mol L}^{-1}$ ) as reference and Pt wire as auxiliary electrodes. The working electrode was a BDD film (8000 ppm boron). Prior to the experiments, the BDD electrode was submitted to an anodic treatment ( $+3.0\text{V}$  vs Ag/AgCl for 10 min) to remove the hydrophobicity of the film. After that, a cathodic treatment was realized ( $-3.0\text{V}$  vs Ag/AgCl for 10 min) to conditioning the surface. The cathodic treatment was conducted for 30 sec to recover the electrode surface, when necessary.

**Samples:** The *Passiflora alata* tincture was purchased from a local commercial trade. Aliquots from the sample were fortified with FEN with the concentrations of  $5.0 \times 10^{-5}$  and  $10.0 \times 10^{-5} \text{ mol L}^{-1}$  and were used for the recovery studies. Analytical curves were obtained by the standard addition

method using an aliquot of 0.5 mL from samples fortified with FEN. The aliquots were added into the electrochemical cell with 9.5 mL of the support electrolyte of  $\text{Na}_2\text{HPO}_4$  ( $0.1 \text{ mol L}^{-1}$ ), pH 4.0. The final FEN concentrations into the electrochemical cell were  $2.5 \times 10^{-6} \text{ mol L}^{-1}$  and  $4.0 \times 10^{-6} \text{ mol L}^{-1}$ . All the experiments were performed in triplicate.

### 4. Conclusions

Cyclic voltammetry results showed a well-defined oxidation peak of FEN pesticide at about 1.27 V, with irreversible features (once no reverse redox processes was observed) using the BDD electrode. The SWV results showed also that the FEN oxidation process was pH independent once no displacement of peak potential was observed. An analytical curve using SWV and the analysis parameters were optimized. The results showed good linearity, reproducibility and lower limits of detection and quantification.

The applicability of the method was evaluated with recovery studies of FEN pesticide into *Passiflora alata* samples (Medicinal Plant). It was observed that the *Passiflora alata* sample do not interferes in the electrochemical behavior of FEN. The results showed that this method using the BDD electrode and SWV is simple, fast and efficient to determine FEN pesticide direct into small volume of herbal medicinal plants samples without any pretreatment or pre-concentration. Thus, aiming the increasingly consuming and uses of herbal medicinal plants as well as the importance in the quality control of the pharmaceuticals from vegetal origin, the voltammetric method combined with BDD electrode is an alternative to determine pesticides.

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