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Electrochemical quantification of chlorpyrifos in white (*Allium cepa* L.) and green (*Allium fistulosum* L.) onions

Cuantificación voltamétrica de clorpirifos en cebolla de bulbo (*Allium cepa* L.) y cebolla de rama (*Allium fistulosum* L.)

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Abstract

The Linear sweep voltammetry method (LSP) of a common pesticide such as chlorpyrifos (CP) an organophosphate pesticide (OPP) in white (*Allium cepa* L.) and green onions (*Allium fistulosum* L.) was investigated. A hanging mercury drop electrode (HMDE) Ag/AgCl was used as reference electrode and platinum as a counter electrode. The OPP was extracted from the onion through liquid-liquid partition using acetone/dichloromethane as the solvent. Validated method was compared with GC/ECD as a reference method. CP concentration was not different between the two analytical techniques; the analysis was performed with a confidence level of 90%, corresponding to the polarographic method. CP concentration was 0.8400 ± 0.062 ppm in *Allium cepa* and 0.062 ± 0.300 ppm in *Allium fistulosum* L. These values exceed the ADI and ARD values for chlorpyrifos established by current regulations.

Key words: Chlorpyrifos; Linear Sweep Polarography; onion; organophosphates

Resumen

En el trabajo se cuantificó la concentración de clorpirifos (CP), un plaguicida organofosforado (POF) en cebolla de bulbo (*Allium cepa*) y en cebolla de rama (*Allium fistulosum* L.) utilizando el método polarográfico de barrido lineal (LSP). El estudio fue realizado usando como buffer solución Britton-Robinson, electrodo de trabajo gota estática de mercurio (HMDE) Ag/AgCl como electrodo de referencia y platino como electrodo auxiliar. El POF en cebolla se extrajo por reparto liquido-liquido, utilizando acetona/diclorometano como solvente. El método polarográfico validado fue comparado con GC/ECD, como validación de referencia. La concentración de CP determinada por ambas técnicas de análisis no presentó diferencia significativa ($P > 0.01$), correspondiente al método polarográfico. La concentración de CP en cebolla junca (*Allium cepa*) fue calculada en 0.8400 ± 0.062 ppm y en la cebolla cabezona (*Allium fistulosum* L.) en 0.062 ± 0.300 ppm, estos valores sobrepasan el LMR y el IDA, establecidos por la normativa vigente (FAO/OMS, 2000, 2006).

Palabras clave: Clorpirifos; Cebolla; Organofosforados; Polarografía de Barrido Lineal

Introduction

Organophosphorous pesticides (OPP), most of which are esters and thioesters of phosphoric and thiophosphoric acids are widely used throughout the world (Chang, et al., 2009). Chlorpyrifos (O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) (CP) is an OPP that has been extensively applied in agricultural and household pest control since 1965 (Centner, 2018). They have applied in Boyacá (Colombia) for agricultural pest control in onion, tomato and potato crops. They enter freshwater and saltwater ecosystems primarily as spray drift and it can produce residues on agricultural products, contamination of soils and aquatic environments. The Environmental Protection Agency of the United States (EPA) and the World Health Organization (WHO) have determined that these pesticides are mutagenic rate risks and reproductive effects and fetotoxicity, classifying them as 1b (highly hazardous), and included in the Prior Informed Consent (PIC) Procedure (ICP, Rotterdam Convention) which applies to highly dangerous pesticides to human health (EPA, 1996). FAO (1986) sets maximum allowable concentrations of each type of OPP, for CP the acceptable daily intake is 0-0.01 mg/kg body weight. The excessive use of pesticides creates negative consequences for ecosystems, farmers and consumers, and highly residual foods such as tomatoes, potatoes, spinach and lettuce where most pollutants are stored (Del Puerto, et al., 2014). This widespread use poses a potential risk to human health because OPPs inhibit acetyl cholinesterase and lead to the modification of cholinergic signaling (Pope, et al., 2005). CP is dangerous for infants, young children and pregnant women (Silver, et al., 2017). It can cause developmental disorders, autoimmune disorders, and increase probability of chromosomal aberrations (Li, et al., 2015). Therefore, there is a growing interest in quantifying CP residues in agricultural products and foods all over the world.

Chlorpyrifos has been analyzed using a wide range of different analytical techniques, such as High-Performance Liquid Chromatography (HPLC) (Otieno, et al., 2013), Gas Chromatography-Mass Spectrometry (GC-MS) (Bauer, et al., 2018; Rai, et al., 2016), infrared micro-imaging (Li, et al., 2012.) and electrochemical detection on different electrode surfaces (Chen, et al., 2017).

White onion (*Allium cepa* L.) is an important crop in the world due to its diverse uses. Its bulb is used raw, sliced in salads or as ingredient in various traditional dishes with meat or vegetables (Moghbeli, et al., 2019). About 93 million tons of onions were produced in 4.9 million hectares in the world (FAO, 2016). Green onion (*Allium*

fistulosum L.) is an important ingredient of american cuisine. It has different nutrients such as carbohydrates, proteins, lipids, minerals (magnesium, calcium, potassium and iron), vitamins (A, C, E, K) and a lot of fiber that facilitate digestion and avoid different problems like colon diseases and constipation (Sakakibara, et al., 2003). Onion is used as a test material to detect genotoxic effects of different chemicals (Datta, et al., 2018), for that reason in this article, electrochemical quantification of chlorpyrifos in white and green onions was investigated.

Experimental

Reagents and apparatus. Lorsban® pesticide was acquired in agrochemical business house of Tunja city (Colombia) the active ingredient (AOAC, 1990) was obtained through liquid-liquid extraction proposed by Leoni et al. (1992). All solutions were prepared with water purified in a Milli-Q system from Millipore Corporation. Prior to all measurements, solutions were deaerated by passing N₂ gas (SS White Martins) for approximately 15 min.

The electroanalytical equipment was a computer controlled BAS CV 50W electrochemical analyzer from Bioanalytical Systems, Inc. (West Lafayette, IN) with three-electrode cell with Ag/AgCl reference electrode, a platinum wire as auxiliary electrode and a drop mercury electrode as working electrode.

Preparation of standard and work stock solutions. Britton Robinson electrolyte solution at pH 13 was employed. CP Stock solution of 1000 ppm was prepared in ethanol. Intermediate solution of 100 ppm was prepared from stock solution. Standard solutions were 0.0, 0.4, 4.0, 8.0, 12.0, 16.0 and 20.0 ppm. The concentrations selection was made based on maximum allowable concentrations for the active compound permitted by FAO (1986). All standard and work solutions were stored at 4°C before use.

Sample Analysis. Green onion (*Allium fistulosum* L) and white onion (*Allium cepa* L) representative samples (5 kg) were collected at random according to an experimental design that covered the entire onion market within the city of Tunja, from Aquitaine and Samacá, respectively, on peak market days: Tuesdays and Fridays, during two seasons with broad seasonal variations under optimal health and commercial maturity stage (intermediate maturity). 3 mL of standard solution, 1 mL of onion extract and 3 mL of the electrolyte solution were mixed and adjusted to pH 13. Then, this mixture was transferred to the polarographic cell, the computer was programmed according to the previous optimization parameters, and measurements were conducted.

Validation method. It took the following main steps: cleaning electrode system, extraction of active principles, selection of instrumental conditions: initial potential, ultimate potential, deoxygenation of the sample, size of the mercury drop, quiet time, sensitivity, scan speed, and potential range. The following were used for the method validation: detection limit (DL), quantitation limit (QL), precision, accuracy, range of use, sensitivity and uncertainty. Recovery (%) was calculated by equation 1 (Wani, et al. 2019).

$$\text{Recovery (\%)} = \frac{\text{Residue in ppm}}{\text{Fortification level}} \times 100 \quad \text{Eq. 1}$$

Chlorpyrifos results in white and green onions by Linear Sweep Polarography were compared with those obtained in conventionally accepted techniques: GC/ECD, performed in a gas chromatograph (GC) HP PLUS Series 6890 (Hewlett-Packard, Palo Alto, California, USA), equipped with an electron capture detector (ECD). The column used in analysis was DB-5 [5%-phenyl-poly (metilsiloxane), 30m x 0.25 mm x 0.25 μm]. The injection was performed in splitless mode (Viny = 1 μL). Data processing was performed with SPSS 2012.

Results and discussion

Verifying potential mercury resistance and the auxiliary electrode (platinum). Mercury resistance ($0.30 \pm 0.11 \, \Omega$) was measured by a multimeter Univolt DT-888 model. The optimal value should be $0.00 \, \Omega$. We proceeded by cleaning the electrolysis, obtaining a value of $0.110 \pm 0.048 \, \Omega$. The measured potential of the auxiliary electrode was $6.02 \pm 0.11 \, \Omega$. This value indicates that the platinum electrode was within the tolerable potential range: 0-20 Ω , being suitable for use in the polarographic technique.

Response Equipment for CP signal. With the aim of discovering whether the selected target (ethanol) interfered with the analysis technique for CP exploratory sweep was conducted by selecting a range of -1000 to -2200 mV, generating a signal at a potential of -1905 mV. The presence of this signal is confirmed by choosing a shorter potential range. To avoid this interference a potential range of -600 to -1500 mV was chosen which showed no signal. With the selected potential range, (-600 to -1500 mV) ran a standard of CP for the potential of electroactivity, the analyte had two signals (-720 and -940 mV). The final range of working potential was -600 to -1100 mV (Figure 1), which allowed further improvement of the economy and speed of the analysis.

Redox potential. Cyclic voltammetry (CV) method can identify accurately the redox

potential of electrochemical species that provide information about whether or not the species evaluated is reversible. The CP has a reversible reduction reaction, i.e. CP for this measurement technique is first reduced (-825 mV and 671 mV) and then oxidized (-716 and 867 mV). This is evidence that it presents intensity analyte: one negative (reduction) and the other with a positive sign (oxidation) (Figure 2).

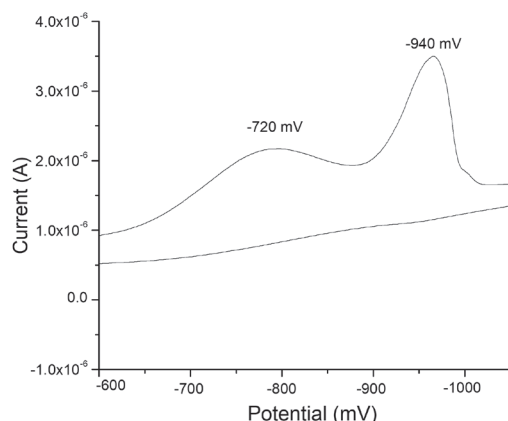


Figure 1. Sweep polarograms of chlorpyrifos. Standard of CP for the potential of electroactivity, the analyte had two signals: -720 and -940 mV. The second polarogram corresponds to blank.

Selecting the working pH. The optimum working pH selection was assessed in order to observe the relationship pH versus the current electroactive species analyzed (Liu, et al. 2001). Examined the relationship of pH vs. hydrolysis of chlorpyrifos, concluding that the greatest degree of hydrolysis was in a basic pH. Manisankar and others (2005) conducted a five OPP quantification using electrochemical technique by square wave voltammetry and determined that pH 13 show the greatest reduction of chlorpyrifos.

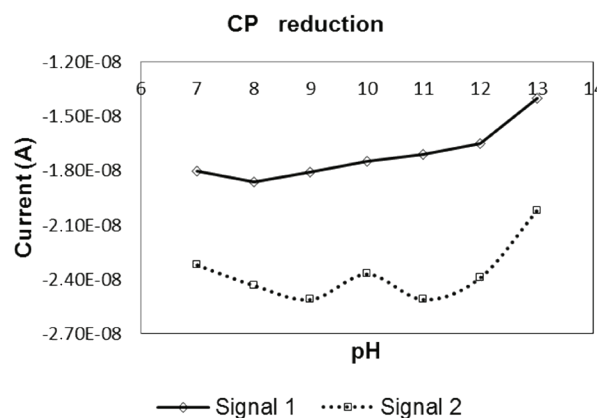


Figure 2. CP reduction behavior.

The CP in the reduction process generates two species and each generates a respective signal. The first signal occurs at -637.3 ± 32.3 mV and the second -795.6 ± 28.1 mV. The current carrying capacity of both species occurs at pH 13. The direction of the applied potential is negative-positive, indicating that the reaction is carried out cathodic i.e. electroactive species undergo a reduction process.

Parameters of the polarographic measure.

Ethanol was selected as the solvent for CP and it was used as a supporting electrolyte in a Britton-Robinson (BR) buffer to pH 13. Consecutive tests were developed which were modified and optimized using the following device parameters: deoxygenation of the sample: 5 minutes, working potential range: -600 to -1100 mV, initial potential: -600 mV, final potential 1100 mV, sensitivity: 1 uS, mercury drop size: 8, quiet time: 5 seconds and scan speed and sampling interval: 10 mV/s.

CP reduction. Hydrolysis is an important way to eliminate OPP. Generally, this process involves the breaking of one of the phosphoric acid esters or thiophosphoric acid and it is strongly dependent on pH and other constituents of the solution. The hydrolysis can be initiated via a nucleophilic or electrophilic attack (Smolen, and Stone, 1995).

CP has three ester linkages that are candidates for the start hydrolytic, two ester bonds and tertiary alkyl phosphate ester bond (pyridyl). Smith et al. (1967.) found two possible mechanisms of PO hydrolysis: neutral and alkaline hydrolysis. Neutral hydrolysis of chlorpyrifos and other organophosphate esters involve nucleophilic water attacks in less saturated carbon (weak center) with the loss of the alkyl groups. Alkaline hydrolysis and other organophosphorus esters are initiated by a nucleophilic attack of the hydroxyl ion to the phosphorus atom (strong core) causing a loss of acid group output alcoholic or phenolic group. The catalysis of the alkaline hydrolysis is assumed to be due to second order kinetics (SN2) (Macalady and Wolfe, 1983; Smith, Watson, and Fischer et al, 1967).

Calibration curve. Graphed current generated (or response signal in Amps) versus concentration (ppm) obtaining the linearity range (Figure 3). CP polarographic presents two signals, the first signal defines better the polarographic wave, which has a lower standard deviation, which is determined for purposes of quantification of chlorpyrifos in natural samples and was made based on the first signal generated by the analyte.

Validation of the method for CP quantification

Method attributes. Attributes CP quantification method by linear sweep Polarography obtained are summarized was applied in Table 1. The validated method in onion extracts.

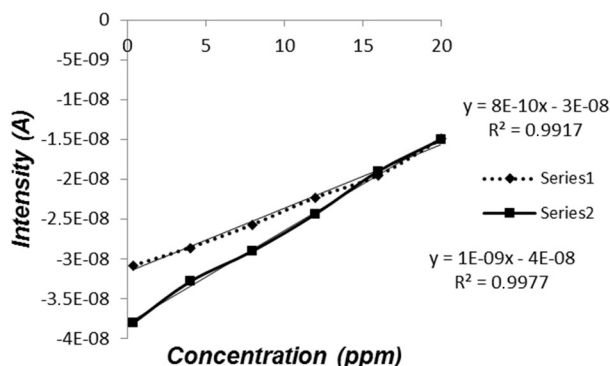


Figure 3. CP Linearity Range ($\text{mg} \times \text{L}^{-1}$).

Reproducibility of the method was evaluated in Welsh onion extracts, evaluating the current operation under standardized conditions with CP patterns. In total four trials were performed in one day, each with 6 replicates and two analysts, for a total of 48 trials, using the same equipment and the same basis for preparing extracts of the standards. The white onion does not generate any signal that might cause interference with the polarographic method and require adjustments. The readings were made with a completely randomized design.

Table 1. Attributes CPF validation method.

Attributes	Value
Confidence level	90.000%
Detection Limit	0.207 ppm
Quantification limit	1.268 ppm
Sensitivity	3.000×10^{-8} A/ppm
Accuracy in a high range	107.910%
Accuracy in a low range	91.517%
Precision	$\pm 5.321 \times 10^{-10}$ A
Recovery (%)	112.290 %
Total uncertainty	± 0.062 ppm

The variance analysis concerning reproducibility determined that the current intensities and sample standard natural + 6 standard tests performed by the two analysts in the validation process did not vary significantly, the calculated F value was less than the F acceptance criteria. It was determined that the analytical method has a 90% confidence level.

Quantitative determination of CP in green and white onion

The validated method was applied to green and white onion extracts. The polarograms showed no signal. Solutions were made from the sample and every team assessed the response, choosing the solution 1:3 V: V (sample: ethanol). Later volumes were optimized sample and standard solution CP (3 ppm). The best response was obtained by adding to the cell 3mL of the polarographic standard, 3 mL of the electrolyte solution and 1 mL of the native sample (Figure 4).

CP concentration in the sample was calculated according to Equation 1 given by the AOAC 970.53 method (AOAC, 1990). CP content was 0.300 ± 0.062 ppm in white onion and 0.840 ± 0.062 ppm in green onion. CP levels from Aquitaine and Samacá towns exceed the limits of Acceptable Daily Intake (ADI) suggested by the NTC CIPAC MT 36 and 29 and FAO which sets the maximum intake level at 0-0.01 mg/kg body weight, and the MRL of 0.2 mg/kg (FAO/WHO, 1986). Given that the onion is a very consumed food and pollution control reports are not conducted by governmental entities, the values can be inferred that there is an imminent risk of contamination with chlorpyrifos, as and possibly other agricultural products with greatest danger to the child population in whom the baby food MRL is 10 times smaller than the accepted in adult (Lu, Knutson, Fisker-Andersen,

and Fenske, 2001; Holland, 1996). The strong tendency to use POFs, ranked toxicological categories I and II (CP) in cultural practices of food products are generating residual dangers as cholinesterase inhibitors, responsible for catalyzing the hydrolysis of acetylcholine to choline and acetic acid. Monitoring pesticide residues in food is important to determine the degree of population exposure and prevent possible long-term toxicological consequences and changes in cultural practices.

Comparison of results

Linear Sweep Polarography versus reference method GC/ECD. Reliability checks polarographic measurement reading a standard 2 ppm and two extracts from the array of work, compared to the results of the reference method GC/ECD. Table 2 shows the concentrations determined by GC/ECD CP in the samples. The values reported by the laboratory chromatography of CP quantifying submitted samples was performed to correct the concentration due to dilution and the amount of biological material used. We can observe that certain concentrations of CP in the three samples of polarography scan are superior to the reference method. This difference was assessed by analysis of variance, which references the standard concentration of 2 ppm. The analysis indicated that it did not show significant difference in the concentration of CP standard determined by the two analysis techniques. The calculated 'F' value was less than the critical F acceptance. The analysis was performed with a confidence level of 90%, corresponding to the polarographic method.

Table 2. CP concentrations in samples determined by linear sweep polarography and GC / ECD

Sample	Concentration (ppm)	
	Linescan polarography (Confidence level 90%)	CG/ECD (Confidence level 95%)
Green onion: MNCI-1	0.840	0.383
White onion: MNCC-1	0.300	0.118
Standard 2 ppm: SBPC-1	1.600	1.270

Conclusion

The analytical method of Linear Sweep Polarography for the quantification of the organophosphate pesticide chlorpyrifos in green and white onions is valid and reliable. This method was compared to GC/ECD as a reference method. The CP standard concentration determined by

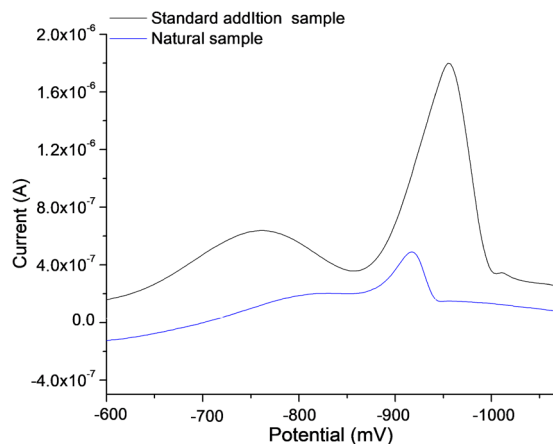


Figure 4. Polarograms natural sample and standard addition sample.

both analytical techniques showed no significant difference. The analysis was performed with a confidence level of 90%, corresponding to the polarographic method. The residual concentration of CP in green onion and white onion sold in the city of Tunja exceeds by 420% and 150% respectively the maximum permitted levels of ADI (0.01 mg/kg body weight) and RML (0.2 mg/kg) established by current regulations CIPAC MT 36, NTC 29, and *Codex Alimentarius* (FAO/WHO). The methodology developed may be used in other agricultural products for quantification of POP. The Analytical Laboratory of the Environmental Chemistry research group (GIQUA) has the necessary conditions to assess CP content matrices of agricultural origin, with an uncertainty of ± 0.062 ppm.

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