Ecophysiology, Crop Metabolism, Technology, Production and Seed Physiology/ Ecofisiología, Metabolismo de Cultivos, Tecnología, Producción y Fisiología de Semillas

doi: http:// dx.doi.org/10.15446/acag.v64n4.44521 e-ISSN 2323-0118

Validation of the voltammetric method for the determination of residues of paraquat applied to potato crops

Validación del método voltamétrico para la determinación de residuos de paraquat aplicado en cultivos de papa

Adriana Elizabeth Lara Sandoval*, José Mauricio García Colmenares and Sandra Patricia Chaparro Acuña

Research group in Environmental Chemistry Faculty of Sciences. Universidad Pedagógica y Tecnológica de Colombia. Tunja, Boyacá, Colombia. *Corresponding author: adriana.lara@uptc.edu.co

Rec.: 21.07.2014 Acep.:04.11.2014

Abstract

Pesticides quantification is an expensive work and it is inaccessible for farmers and health authorities and supervisory entities. This paper describes a simple, economical and accessible methodology for quantification of carbofuran, methomyl and mancozeb in potato (*Solanum tuberosum*). The square wave electrochemical voltammetric method to quantify Paraquat was validated. It used Britton-Robinson solution buffer as supporting electrolyte, glassy carbon as working electrode, Ag/AgCl as reference electrode and platinum as counter electrode. Herbicide was extracted from potato through solid-liquid partition methodology, the results obtained by voltammetry were compared with UV-Vis spectroscopy, suggesting that that the voltammetric method produces accurate and reliable results. The proposed method was successfully applied for the determination of paraquat in potato samples with satisfactory results.

Key words: Agricultural products, detection, electrochemistry, herbicides, paraquat.

Resumen

La cuantificación de plaguicidas es un proceso costoso y de difícil acceso para los cultivadores y entidades de salud y vigilancia. En este trabajo se presenta la metodología de voltametría de onda cuadrada para la cuantificación de paraquat (gramoxone) en papa (*Solanum tuberosum*). El método de voltametría de onda cuadrada fue validado para la cuantificación de este herbicida en papa. El estudio fue realizado usando como electrolito de soporte solución buffer Britton-Robinson, electrodo de trabajo de carbón vítreo, Ag/AgCl como electrodo de referencia y platino como electrodo auxiliar. El herbicida en papa fue extraído por reparto sólido-líquido y los resultados obtenidos fueron contrastados por espectroscopía UV-Vis,lo que permitió deducir que el método voltamétrico genera resultados precisos y confiables. El método propuesto fue exitosamente aplicado para la cuantificación de paraquat en muestras de papa con resultados satisfactorios.

Palabras clave: Detección, electroquímica, herbicidas, paraquat, productos agrícolas.

Introduction

Pesticides are more often used in the Department of Boyaca, Colombia to control pests on potato crops (ICA, 2011). The use of these compounds have shown to be one of the practices that have contributed most to the increase in agricultural production in recent decades in about 50%, together with the use of synthetic fertilizers, irrigation systems and organic-synthetic components. Currently, those practices are essential to meet the growing global demand for food (Ministry of Social Protection, 2003); but their indiscriminate use has negative consequences for ecosystems, farmers and consumers. It has resulted to leave high doses of residues in foods like tomatoes, along with potatoes, spinach and lettuce, which are the products with higher accumulation of contaminants (Larsbo *et al.*, 2009).

Paraquat is a non-selective, post-emerging, contact herbicide which is applied to the foliage of weeds and has a broad spectrum of control. Regular applications might generate residuals whose toxicological significance should be evaluated regularly. This herbicide is extremely toxic to humans (LD50 = 35 mg/kg) and animals (LD50 = 110-150 mg/kg) by all sources of exposure (The Harmoudi*et al.*, 2013). Maximum Residue Limits (LMR) for paraquat in root crops is at 0.05 mg/kg (50 mg/g) (Codex Alimentarius, 2013). The Environmental Protection Agency of the United States (EPA) and the World Health Organization (OMS) have identified several compounds of this type to have risk for mutagenic, reproductive and fetus-toxicity effects. Also, they are included in the PIC procedure (ICP, Rotterdam Convention, 1998) applied to extremely dan-gerous human health pesticides. In acute toxicity studies using laboratory animals, paraquat has been shown to be highly toxic by inha-lation, having a toxicological category I (the highest of four levels) for acute inhalation effects. Through oral ingestion, this compound is moderately toxic (Category II), and slightly toxic (Category III) through dermal contact (Environmental Protection Agency of the United States, 1997). This herbicide enters the body through the skin, respiratory or digestive; Its life-time in the body is relatively short with about 12 to 120 hours (Rios, 2012). Although its use has been shown to be safe, poisoning cases might occur. If so, accumulation of reactive oxygen species (ROS) and toxic free radicals are accumulated in various organs, especially the lungs leading to oxidative damage (Paixao *et al.*, 2002).

The used methods for the determination of paraquat, like coupled gas chromatography to mass spectrometry (GC/MS) or UV-Vis spectrophotometry, have been considered as reference methods reported by the Codex Alimentarius and AOAC, respectively. Literature shows different techniques to analysis paraquat, including: spectrophotometry (Rai *et al.*, 1997), liquid chromatography (Taguchi *et al.*, 1998) and flow analysis (Saad *et al.*, 1998). However, innovative and accessible methodologies are needed such as voltammetry, providing an alternative broad spectrum, economical, versatile and environmentally friendly. The current methods are often expensive and only few universities and research institutes are able to quantify this herbicide. As a consequence, few studies related to this compound have been done.

Electrochemistry is an option for the analysis of traces of heavy metals, anions, sulfides, nitrites, nitrates and other organic compounds in water, food, chemicals products and electroplating baths to ppt, ppb and ppm levels. Some pesticides such as paraquat has processes of oxidation and/or reduction when receives small electrical pulses. Thus, a current electricity response can be quantified electrochemically. Rühling *et al.* (1998), Souza and Machado (2005), Monk *et al.* (1999), Luciana *et al.* (2010) evaluated the electrochemical behavior of paraquat by vol-tammetry differential pulse with mercury electrodes, by voltammetry square wave with microelectrodes of gold, by cyclic voltammetry with mercury electrodes, and by voltammetry of modified wave.

The aim of this study was to validate the electrochemical method using glassy carbon electrode as a working element for the quantification of paraquat in potatoes. Since this electrode produces no waste, reducing environmental pollution, also, the analyst has a wider range of work.

Materials and methods

Reactives and equipment

The reagents used were analytical grade: 98% sodium hydroxide, 99.8% boric acid, 85% phosphoric acid (of Merck); 99.9% paraquat (1,1'-dimethyl-4,4'-dichloro bipyridine) from Sigma; 99.5% acetic acid, 85% sodium dithionite of Panreac, and nitrogen gas. All solutions were prepared with deionized water (Millipore System) with conductivity 0.05 us.

The optimal operating and calibration conditions were verified on the devices in order to validate the method, like the following: polarography BAS CV 50W equipped with a voltammetric analyzer, working electrode of glassy carbon BASI MF-2070, reference electrode Ag/AgCl BASI MF-2052, auxiliary platinum electrode BASI MW-1032, Aventure Ohaus analytical balance and Sartorius Schott potentiometric CG 842. The comparison of results from the square wave voltammetry against the spectra-photometric method UV-Visse performed in a UV- Vis Genesys 10 UV.

Working standard solutions. A stock solution of 1000 mg/l pesticide in deionized water was prepared. It was 'pipetted' 10 ml of stock solution to a ball of 100 ml. Then, deionized water was gauged and adjusted to a 100 mg/l concentration, of which 10 ml was taken to the ball of 100 ml. Afterwards, the ball was gauged with deionized water. The final concentration of the working solution was 10 mg/l.

Supporting electrolyte (Britton Robinson buffer). A 100 ml ball was filled with 0618 g boric acid, 0.56 ml acetic acid and 0.48 ml ortho-phosphoric acid. Then, pH 5 was adjusted with NaOH 0.1N and deionized water.

Voltammetry parameters. The voltammetry conditions were optimized, adjusting the deoxygenation time, rest time, working range, sensitivity, wave amplitude and wavelength through analysis in triplicate.

Paraquat electrochemical behavior. The electrochemical process of paraquat is quasi-reversible, as described by Souza *et al.* (2005). Paraquat has two oxidation processes. The first is present at 663 ± 50 mV (Equation 1), followed by the second oxidation (Equation 2) at -947 ± 50 mV; finally a dimer formation occurs with two quasi reversible positive charges (Equation 3) (Souza and Machado, 2005).

$$\mathrm{Par}^{+2}+\mathrm{e}^{-} \leftrightharpoons \mathrm{Par}^{+} \qquad \text{(Equation 1)}$$

$$\mathrm{Par}^{+} + \mathrm{e} \leftrightharpoons \mathrm{Par}^{0} \qquad \text{(Equation 2)}$$

And for the subsequent formation of a dimer:

$$\mathrm{Par}^{0}+\mathrm{Par}^{2} \leftrightharpoons {}^{+}\mathrm{Par}_2^{2+} \qquad \text{(Equation 3)}$$

Validation of the voltammetric technique

In the pre-validation method, besides checking the calibration of equipment and volumetric material, the instrumental conditions were selected as: the initial potential, the final potential, the deoxygenation of the sample, the quiet time, the sensitivity, the wave amplitude and the range of potential. The linearity of the method (0.5 to 3 mg/l) was carried out for validation, so the main attributes of the method/validation were determined as follows: detection limit (DL) (Eurachem, 2005), the quantification limit (QL) (Eurachem, 2005), the accuracy of the standard deviation and coefficient of variation, the accuracy of rate of recovery, the useful range, sensitivity and uncertainty. Six batches were assessed, including a white, a high standard, a low standard, two natural samples (potato) and a fortified sample with a high and low standard (2.7 mg/l and 500 g/l) with added herbicide to the mentioned concentration solutions. Analysis were performed by two laboratory technicians.

To determine the accuracy of the volumetric method fortified samples were used (Eurachem, 2005). Thus, a known volume of standard solution was mixed with 3 ml of potato extract and 4 ml of the electrolytic solution, adjusted to pH 5. The obtained mixture was transferred to a electrochemical cell, and the equipment was scheduled under the previous parameter of optimization to be measured afterwards. The results were compared with those obtained in contrast samples by spectrophotometric method 969.09 (AOAC, 1990).

Treatment of natural samples

The method of solid-liquid extraction, described by Qiu and Nian (2008) and Leoni *et al.* (1992), was modified. They proposed an extraction liquid partition solid with acetone and dichloromethane as solvent. Instead, a sohxlet extraction was additionally performed with dichloromethane to obtain herbicide extracts from the treated sample. The sample was dissolved in deionized water for the voltammetric measurement. The representative 5 kg of potatoes as samples were obtained from the central supply Abastos in the south of the city Tunja- Boyacá.

Experimental design

Evaluation was made in a 2^6 factorial design with random triplicate samples. 64 trials were performed in total, allowing the evaluation of the safety and reliability of the method. Significant differences were determined by boxplot using SPSS 18 statistical software for Windows.

Results and discussion

Signal response of the equipment

An exploratory sweep by the technique of cyclic voltammetry VC (Figure 1a) and voltammetric square wave OSWV (Figure 1b) was performed. The last technic was selected for the validation process because of its higher sensitivity, which is an advantage for the quantitation of very small amounts (Figure 1b). In trial did not showed signals that could interfere with the chemical analysis.

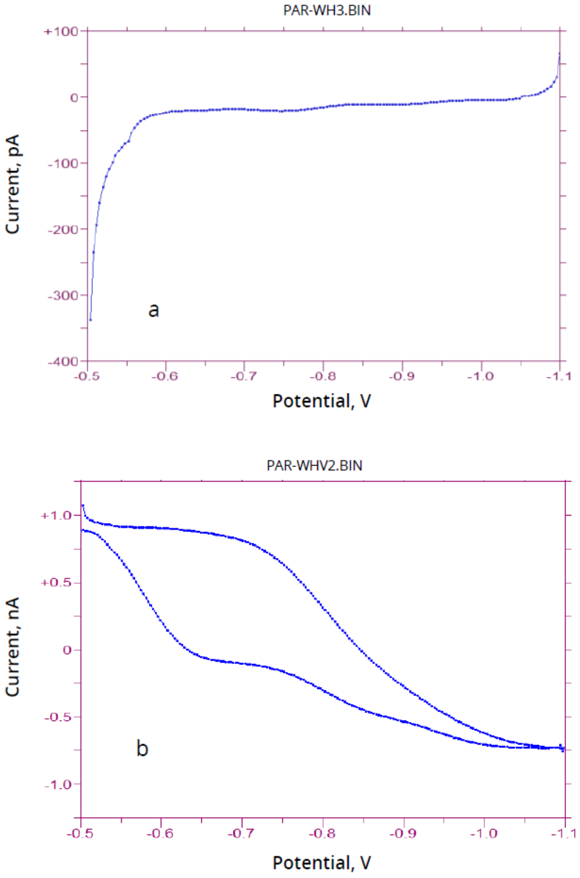

Figure 1. Scanning screening with: (a) cyclic voltammetry, (b) square wave voltammetry.

A standard was analyzed to know its electro-activity potential by VC, which can accurately identified the potential of an electrochemical redox species, provides information about the evaluated species, and determine if the reaction is reversible or not. Moreover, the technical parameters of square wave voltammetry (OSWV) were optimized in a range of -300 mV to -1600 mV, obtaining an optimum range of -500mV to -1100mV, optimized sensitivity between 100 uA/V to 100 nA/V in 1 uA/V, quiet time of 2-20 seconds with an optimal value of 5 seconds, evaluated wave amplitude between 15 mV to 50 mV with an optimum value of 25mV, wave frequency of 15 Hz to 16 Hz, based in 15 Hz, and deoxygenation time between 1-10 min, optimized by 5 min.

The potential in paraquat was determined by the anodic method, showing two oxidation potentials: -663 and -947mV (Figure 2a). The second signal presented better statistical parameters, therefore validation and quantification of the herbicide took place in the parent study. Rühling *et al.* (1998) studied the presence of three pesticides including paraquat, using the technique of differential pulse voltammetry (DPV) and mercury drop electrode, and found an oxidation potential of -600 and -1400 mV. Souza and Machado (2005) used gold microelectrodes by the OSWV analysis technique to quantify the herbicide and found oxidation potentials of -640 and -940 mV. Monk *et al.* (1999) studied the electrochemical behavior of paraquat in aqueous medium using cyclic voltammetry and detected two peaks. The first with -700 mV and the second with -1200 mV, compared to the mercury drop electrode (SCE). The first peak is generated by the formation of the radical cation and the second by the formation of the neutral species; according to these authors, this process is followed by a chemical dimerization.

Selection of the calibration curve

Standard solutions of paraquat were 0.5, 0.8, 1.0, 1.5, 2.0, 2.5 and 3 mg/l, which were used to plot the response by the equipment, intensity (amps) vs. concentration (mg/l). So, the linear range of the method and the construction of the calibration curve showed a correlation coefficient of 0.993 (Figure 2b).

Solid/liquid extraction of paraquat

The method of solid-liquid extraction described by Qiu and Nian (2008) and Leoni *et al.* (1992) is based on a wet basis and requires a lot of dichloromethane and acetone. The proposed and modified method of this study was performed on a dry basis and Soxhlet system. Thus, up to 60% of solvents were saved with an extraction efficiency of 93%, which is 16% more than the reference method. Other methodolo-

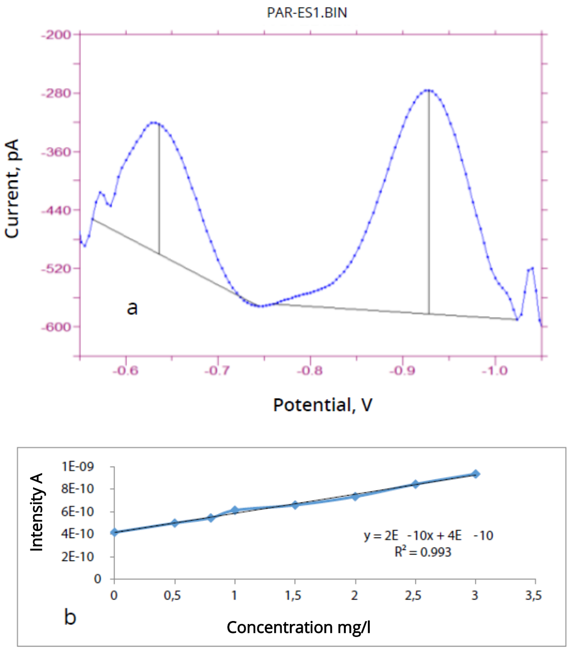

Figure 2. Voltammogram of Paraquat by OSWV (a) and calibration curve (b).

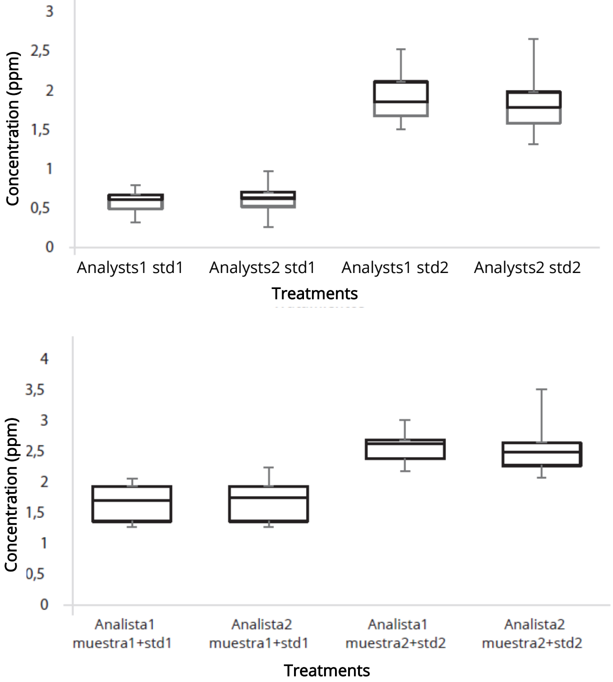

Figure 3. Analysis of dispersion for the performed treatment by two analysts.

gies of paraquat extraction in potato obtained an extraction efficiency lower than 90% according to García *et al.* (2014).

Validation of the OSWV method

The attributes of the volumetric method were: detection limit 50 ug/g, quantification limit 150µg/g, sensitivity 1,69E-09I /ug per gram, high added recovery of 81.28% , low added recovery of 90.16%, accuracy of 86.63%, exactitude of 96.90%, uncertainty ± 0.06 ug/g and confidence level of 95%.The responses of standards and natural samples plus standard, for the six conducted trials by two analysts during the validation process, did not vary significantly, which demonstrates the robustness of the used method (Figure 3).

The measurements of standard-2 and sample-1 showed higher dispersion because these concentrations are higher than the standard-1 and sample-1 plus added, however, no significant differences were presented ($P > 0.05$) (Table 1).

To assess whether the validation method was, or was not in a state of statistical control, in other words, if they were just acting as common or random causes inherent in the method and was a stable and predictable process, control charts were developed. When there are special reasons, the process is out of statistical control. Control charts detect the existence of these causes at the time they occur, allowing immediate action (Feingenbaum, 1994). When the values of the mean concentrations by the two analysts were obtained in 10 measurements of a high standard paraquat, the obtained concentrations were within the control limits. So, the process and validation random variables were controlled.

Table 1. Analysis of variance for the content for Paraquat in potato in the two factors with one sample per group: sample-1 + add-1 (M1A1).

Variations	S.S.	D. of L.	Average of the square	Calculated F'	Probability	Critical value for 'F'
Repetitions	0.270589548	5	0.05411791	0.490402584	0.773566688	5.050329058
Analysts	0.01766566	1	0.01766566	0.160081672	0.705602606	6.607890974
Error	0.551770232	5	0.110354046	—	—	—
Total	0.84002544	11	—	0.650484256	1.479169294	11.65822003

Results by square wave voltammetry vs. the UV-Vis spectrophotometry

Once the process for quantification of paraquat was validated, the voltammetric method was applied to potato extracts, confirming the reliability of the voltammetric measurements on five fortified samples of potato (500 g/l) and standards of paraquat (0.5, 1, 2 was verified and 3 mg/l). Results were compared with those obtained by the # 969.09 method using UV-Vis spectrophotometry and were supported and validated by the AOAC (AOAC, 1990).

The specific concentrations of paraquat in samples and standards by OSWV were higher than those found with the reference method. Differences were evaluated by analysis of variance of one factor, which was taken as reference concentration standards. No differences (P> 0.05) were presented in the concentration of paraquat standards for both of these techniques. The calculated 'F' value (Standard 1: 2.829, Standard 2: 0.193; sample 1 +standard 1: 0.167, sample 2 + standard 2: 0.639) was less than the critical 'F' value acceptance (standard 1: 4.14; Standard 2: 4.15; sample 1 + standard 1: 4.18, sample 2 + standard 2: 4.14). The analysis was performed with a confidence level of 95%, corresponding to the voltammetric method (Table 2).

Table 2. Analysis of variance for the content of Paraquat in potato in the two factors with one sample per group: sample-2 + add-2 (M2A2).

Variations	S.S.	D. of L.	Average of the square	Calculated F'	Probability	Critical value for 'F'
Repetitions	0.49327693	5	0.098655386	1.974969411	0.23651398	5.050329058
Analysts	0.13672884	1	0.136728842	2.737157013	0.158944354	6.607890974
Error	0.24976434	5	0.049952868	—	—	—
Total	0.87977011	11	—	4.712126423	0.395458334	11.65822003

Conclusions

The instrumental conditions and main validation parameters by the square wave voltammetric method for quantitative determination of paraquat in potato allowed to obtain valid and reliable results with a 95% level confidence.

The developed methodology is an economical and friendly alternative in a natural environment for the determination of paraquat in potatoes, with accurate and precise results. This electrochemical method can be applied for the analysis of this herbicide or in other products of agricultural origin but, performing pretreatment to the samples.

Acknowledgements

The authors thank Colciencias and the Pedagogical and Technological University of Colombia for financial support under the Convention 0212-2013 'Detection system in situ of pesticides in the potato chain for the knowledge to farmers'.

References

Agencia de Protección Ambiental de los Estados Unidos. 1997. Available at http://www.epa.gov/oppsrrd1/REDs/factsheets/0262fact.pdf

Codex Alimentarius. 2013. Residuos de plaguicidas en alimentos y piensos. Available at http://www.codexalimentarius.net/pestres/data/pesticides/details.html?id=57

El Harmoudi, H.; Achak, M.; Farahi, A.; ahrich, S. *et al.* 2013. Sensitivedetermination of paraquat by square wave anodic stripping voltammetrywith chitin modified carbon paste electrode.Talanta 115:172-177.

García, R.; Salvador, P.; and Sánchez, F.; 2014. Rapid method based on immunoassay for determination of paraquat residues in wheat, barley and potato. Food Control 41:193 - 201.

ICA (Instituto Colombiano Agropecuario). 2011. Manejo fitosanitario del cultivo de la papa. Medidas para la temporada invernal. Produmedios. p. 5.

Leoni, V.; Caricchia, A. M.; and Chiavarini, S. 1992. Multiresidue method for quantitation of organophosphorous pesticides in vegetable and animal foods. J. AOAC. Int. 75:511-518.

Luciana, B. O.; Carlos, M. C.; and Jorge, C. 2010. Development of a sequential injection–square wave voltammetry method for determination of paraquat in water samples employing the hanging mercury drop electrode. Anal Bioanal Chem. 396:1897 - 1903.

Larsbo, M.; Stenström, J.; Etana, A.; Börjesson, E.; and Jarvis, N. J. 2009. Herbicide sorption, degradation, and leaching in three Swedish soils under long-term conventional and reduced tillage. Soil Till Res. 105:200-208.

Monk, P.M.;Turner, C.; and Akhtar, S.P. 1999. Electrochemical behaviour of methyl viologen in a matrix of paper. Electrochim.Acta.44:4817 - 26.

AOAC (Official Methods of Analysis). Edited by Kenneth Helrich, 15th Edition, Validación 969.09, 1990. p. 227.

Paixao, P.; Costa, P.; Bugalho T.; Fidalgo C.; and Pereira L.M. 2002. Simple method for determination of paraquat in plasma and serum of human patients by high-performance liquid chromatography. J. Chromatogr.B. 775:109-113.

Qiu, P. y Nian. Y. 2008. Determination of ziram in vegetable samples by square wave voltammetry. Chinese Chem. Lett. 19:1337 - 1340.

Rai, M.K.;Das, J.V.; y Gupta, V.K. 1997. A sensitive determination of paraquat by spectrophotometry.Talanta 45(2):343- 348.

Ríos, J. 2012. Paraquat. Available at: http://escuela.med.puc.cl/publ/guiaintoxicaciones/Paraquat.html 23/10/2014.

Rühling, I.; Schäfer, H.; and Ternes, W. 1999.HPLC online reductive scanning voltammetric detection of diquat, paraquat and difenzoquat with mercury electrodes. Fresenius J. Anal. Chem.364:565 - 569.

Saad, B.; Ariffin, M.; and Saleh, M.I. 1998. Flow injection potentiometric determination of paraquat in formulations and biological samples. Talanta 47(5):1231-1236.

Souza, D.; y Machado, S. A. 2005.Electrochemical detection of the herbicide paraquat in natural water and citric fruit juices using microelectrodes. Anal Chim. Acta. 546:85 - 91.

Taguchi, V. Y.; Jenkins, S. W.; Crozier, P.W.; and Wang, D.T. 1998. Determination of diquat and paraquat in water by liquid chromatography (electrospray ionization) mass spectrometry. J. Am. Soc. Mass Spectrom 8:830- 839.