SIMULTANEOUS DETERMINATION OF PARATHION AND P-NITROPHENOL IN VEGETABLE TISSUES BY DERIVATIVE SPECTROPHOTOMETRY

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Abstract. A first derivative spectrophotometric method has been developed for the determination of parathion and p-nitrophenol in vegetable tissues. Ethanol was used as solvent for extracting the compounds from the tissues and subsequently the samples were evaluated against a vegetable tissue blank, directly by derivative spectrophotometry. The simultaneous determination of these compounds can be carried out using the zero-crossing approach for parathion at 253.0 nm and for p-nitrophenol at 273.1 nm. In the samples each analyte was determined in the presence of one another in the ranges between 4.9 to 3883.5 μg g^{-1} for parathion and 4.9 to 3285.3 μg g^{-1} for p-nitrophenol. The detection limits (3σ) were found to be 1.5 and 1.4 μg g^{-1} for parathion and p-nitrophenol, respectively. The relative standard deviations were in all instances less than 1.8%. The proposed method was applied to the determination of the analytes in spiked leafs of corn. The results show a good recovery and they are in agreement with those obtained by polarography.

Keywords: derivative spectrophotometry, parathion, p-nitrophenol, simultaneous determination, tissues vegetables

1. Introduction

Parathion (I) was introduced to the market at the end of the forties by the Cyanamid Company and the Bayer Lever-Kusen. Despite of its toxicity, it is still applied in vegetable tissues as pesticide. Among the organophosphorus pesticides, parathion shows a smaller toxicity, but its resulting hydrolysis product p-nitrophenol (II) which is the main contaminant of the commercial product, presents a higher toxicity.

Traditionally, spectrophometric (Raju *et al*., 1989; Ramachandran *et al*., 1996 and Shivhare *et al*., 1990), chromatographic (Van-Nieuwkerk,1987 and Abe *et al*., 1979) and electrochemical (Toral *et al*., 1990) methods have been used for the parathion determination. In the work of Toral *et al*., it is described also the simultaneous determination of I and II, similarly as it was reported by using supercritical fluid-extraction with immunoassay (Wong *et al*., 1991). On the other hand, when the sample contains parathion, p-nitrophenol and other pesticides or metabolic products, a separation step is needed previous to the instrumental determination.

In this context, diverse methods by high performance liquid chromatography have been proposed (Thapar *et al*., 1995; Carabias-Martínez *et al*., 1993 and Lee *et al*., 1997). Electrochemical techniques have been also described for the detection and determination of parathion, p-nitrophenol together with other pesticides or nitrophenols (Budnikov *et al*., 1996 and Hernandez *et al*., 1993).

In this work, it is proposed a new method for the simultaneous determination of parathion and p-nitrophenol, which is based on the use of digital derivative spectrophotometry. This method is simple, easy to implement in any routine laboratory, because it requires instrumental of low cost and provides reliable results. The method was applied to the determination of the analytes in vegetable tissues and the results were compared with those obtained by polarography (Toral *et al*., 1990). This method presents the advantage that the measures can be carried out directly on the ethanolic extract without a clean-up step as those proposed by other authors (Patil *et al*., 1993 and Dorea *et al*., 1996 and Oh-Shin *et al*., 1997).

2. Experimental

2.1. Apparatus and instruments

A Shimadzu UV-1603/PC spectrophotometer, with a software kit 3.7 (P/N 206-60570-04) and cells of 10.0 mm was used for measurement of the absorbance and the derivative spectra. For all solutions, the first derivative spectra were recorded, over range from 400 to 200 nm against ethanol, at a scan speed of 480 nm min^{-1}, with a $\Delta\lambda = 200$ nm.

The polarographic experiments were carried out using a CV-27 (Bioanalytical Systems, Lafayette, IN, U.S.A.). A laboratory- made damping assembly was constructed for polarographic measurements. A dropping-mercury electrode as working electrode, a saturate calomel as the reference electrode and a platinum coil as the counter electrode were used.

2.2. Reagent solutions

All reagents used were of analytical reagent grade. The solutions were prepared with ethanol Merck p.a. Parathion (I) (EPA Research, Triangle Park, NC, USA) and p-nitrophenol (II) (Merck) were used in all experiments. 1×10^{-2} M standard solutions of I and II were prepared dissolving the appropriate quantity of each substance in ethanol. Other ranges of concentrations were prepared by appropriate dilution using the same solvent.

The supporting electrolyte for polarography was prepared by diluting 20 mL of pyridine (12.3M)and 10 mL of formic acid (98–100%) with 120 mL of 0.1M tetramethiammonim chloride (TMAC) solution. The resulting solution had a pH = 4.5. Gelatin solution (0.5%) was used as a maximum suppressor.

2.3. RECOMMENDED PROCEDURE

2.3.1. *Calibration Graph for Determination of II and I in Mixtures*

Aliquots of the stock solution of II and I were simultaneously diluted in ethanol over the concentration range between 2.5×10^{-7} to 2.5×10^{-4} mol L^{-1}. The calibration graph was carried out for each compound in presence of 6×10^{-5} mol L^{-1} of the other compound. In all cases the corresponding absolute values of the first derivative spectra at 253.0 nm and 273.0 nm for I and II, respectively, were evaluated and were plotted against the corresponding concentrations, using: 250 nm as $\Delta\lambda$ differentiation, 4 as smoothing factor, 10000 as scale factor.

2.3.2. *Procedure for Determination of Parathion and P-Nitrophenol in Vegetal Tissues Enriched with II and I*

Samples of corn leaf were weighed and powdered. Powder samples equivalent to 500–600 mg were accurately weighed with an approach of $\pm$ 0.01 mg. The samples were enriched with a well-known quantity of parathion and p-nitrophenol and leached in 7 mL of ethanol during 30 min. The extracts were filtered and transferred into separate 10 mL calibrated flasks and diluted to the mark. The extracts were then diluted four times. The contents of the flasks were evaluated by first derivative spectra versus a corn leaf blank.

2.3.3. *Procedure for Determination of Parathion and P-Nitrophenol in Vegetal Tissues*

Leafs of corn were fumigated after 20 days of initiate the cultivation. The fumigation was carried out according to the procedure recommended by Bayer (C) Germany. It is based of an aqueous emulsion of 1.2 mL L^{-1} of parathion (Folidol-M 54%). Then the same procedure was followed as the described above for the spiked samples.

2.3.4. *General Procedure for Simultaneous Determination of II and I in Corn Leaf by Polarography*

Aliquots of the corn leaf extract were diluted with 15 mL of supporting electrolyte and 1 mL of gelatin solution. The final solution was de-oxygenated with oxygen-free nitrogen and analyzed using d.c. polarography. The standard addition technique was used for determination. The height of the mercury column, h, was previously determined, in order to obtain a constant mercury flow-rate, m, and drop time, t.

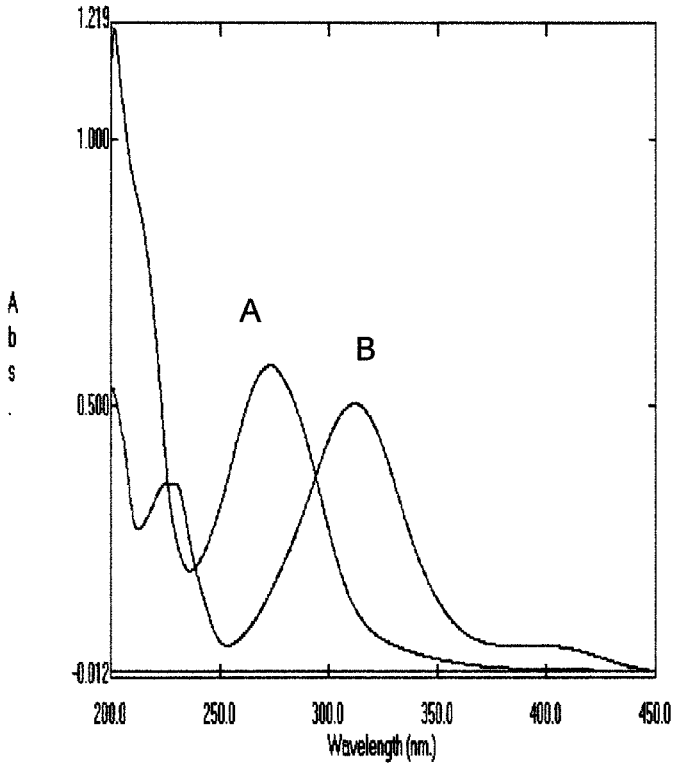

Figure 1. Absorption spectra of parathion and p-nitrofenol dissolved in ethanol measured against ethanol. (A) parathion 1×10^{-5} mol L^{-1}; (B) p-nitrophenol 1×10^{-5} mol L^{-1}.

3. Results and Discussion

3.1. Selection of the extracting solvent

The spectral behavior of parathion and p-nitrophenol in different solvents such as dimethylsulfoxide, acetonitrile, dimethylformamide methanol, and ethanol was studied. In all cases the relation between the molar absorptivities are similar. In this context, all solvents could be used for the determination. However, ethanol was selected because it is the best extractant for parathion and p-nitrophenol from vegetal tissues.

3.2. Spectral features

The zero-order spectra of parathion and p-nitrophenol using ethanol as solvent are shown in Figure 1. The spectrum of both compounds appear overlapped almost totally and only p-nitrophenol could be determined directly considering the wavelength region between 400 to 450 nm. In order to carry out simultaneous

determinations of multicomponents when the spectra are overlapped, there are described some chemometric approaches which have been successfully applied in for this purpose. Derivative spectrophotometry and multiwavelength evaluation methods are well-know examples of this type of approaches. In this work, we adopted derivative spectrophotometry for resolution of spectral bands, because this approach is simpler and it does not require too much mathematical data treatment.

In this work the digital derivative spectrophotometry technique was used where the numeric spectral data are processed by a software incorporated on-line with the spectrophotometer. Traditionally, the software dedicated to the differentiation of the classic spectra allows varying the value of $\Delta\lambda$ (14–15). When low values are assigned to this variable, it is possible to reach a higher spectral resolution, but in many cases the noise is also increased. Savitzky *et al*. (Savitzky *et al*., 1964) proposed an automated method for removal of the noise from the derivative spectra. In this method, the smoothing and differentiation of data is carried out by simplified least squares procedures, which does not require a complex computational treatment to obtain information of quality. Under this differentiation procedure, $\Delta\lambda$ cannot be modulated. In this differentiation road, the resolution is sacrificed in benefit of to increases strongly the signal/noise ratio, favoring the accuracy of the results and the precision of the method. In this work this approach was used.

3.3. Selection of spectral variables

3.3.1. *Derivative Order*

Derivative spectra of solutions containing separately the respective analytes were recorded, in order to optimize the derivative order. As can be seen in Figures 2a–2d, the first derivative spectrum presents the higher sensitivities and also presents a good resolution for the simultaneous determination. In this context the first derivative spectrum was selected. Higher derivative orders were discarded because when the noise is attenuated, the signal decreases and the distortion effect increases.

3.3.2. *Selection of the Smoothing Factor*

By using the first derivative of the spectrum, the smoothing factor was varied taking into account values that are defined by defect depending on the $\Delta\lambda$ selected. For a $\Delta\lambda$ value of 200 nm, the smoothing factor can be 2, 4, 8 or 16. These numbers are in relation to the range of wavelengths used for scanning the spectrum. As can be seen in the Figure 3, when the smoothing factor increases, the heights of derivative signal decreased, but the noise decreased more significantly, given rise to higher signal/noise ratios. A value of 4 was selected, because in these conditions good sensitivities are obtained, without sacrificing the signal/noise ratio.

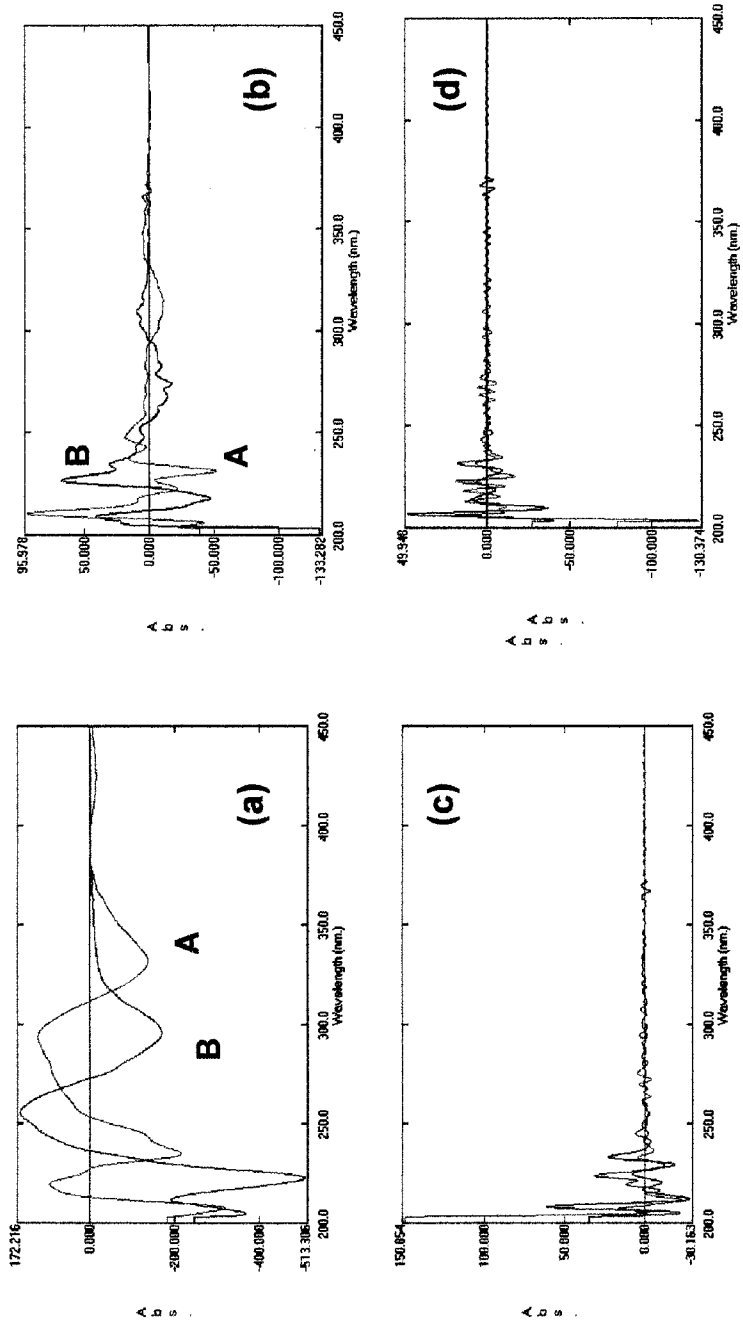

Figure 2. First, second, third and fourth derivative spectra of parathion and p-nitrophenol dissolved in ethanol measured against ethanol. (a) First derivative spectra (b) Second derivative spectra (c) Third derivative spectra and (d) Fourth derivative spectra (A) parathion 1×10^{-5} mol L^{-1}; (B) p-nitrophenol 1×10^{-5} mol L^{-1}.

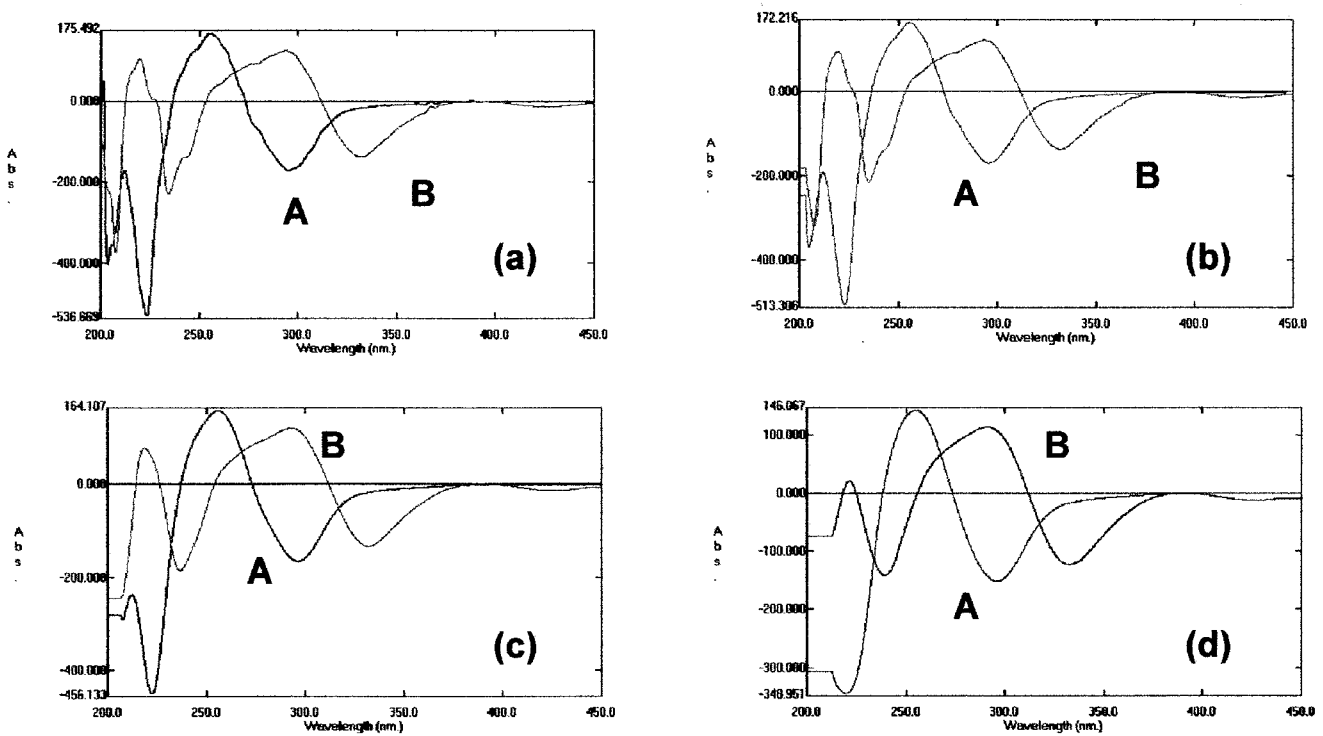

Figure 3. Effect of smoothing factor on first derivative spectra of parathion and p-nitrophenol. (A) Parathion, 1×10^{-5} mol L^{-1} (B) p-nitrophenol 1×10^{-5} mol L^{-1} (a) smoothing factor, 2; (b) smoothing factor, 4; (c) smoothing factor, 8; (d) smoothing factor, 16.

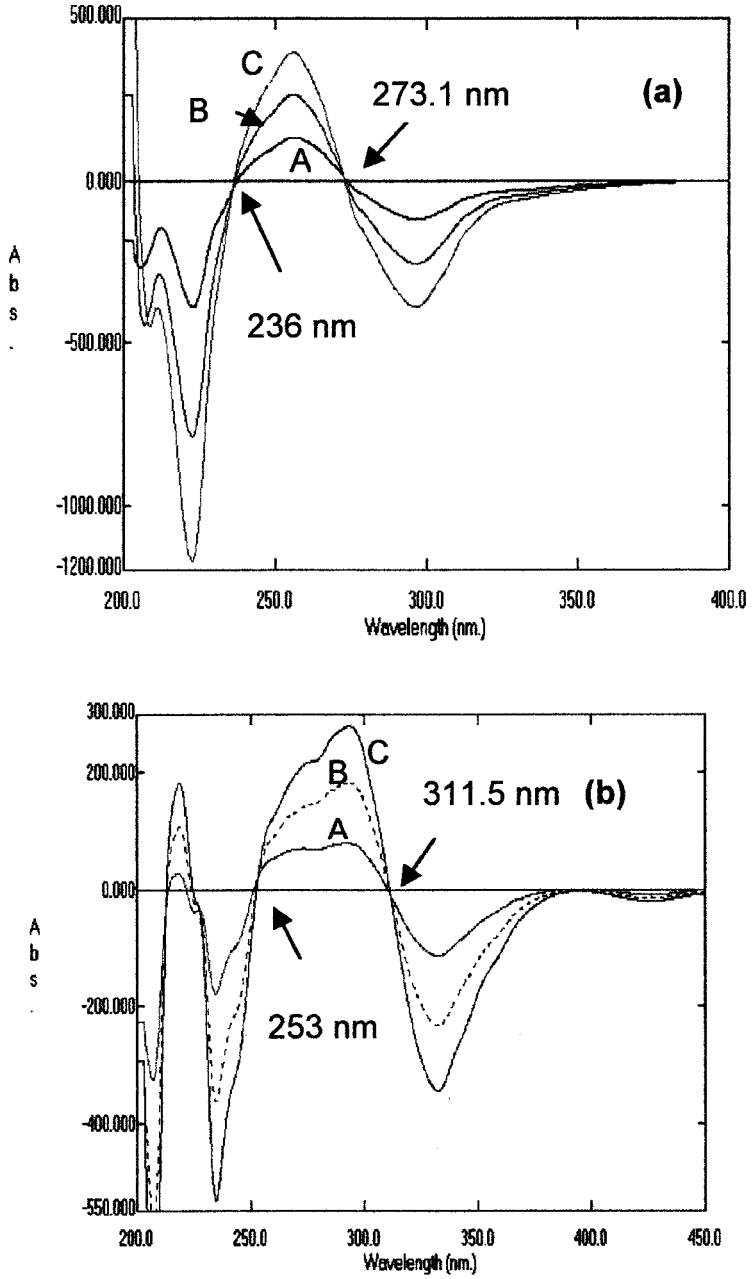

Figure 4. (a) Effect of parathion concentration on the first derivative spectra: (A) 4.0×10^{-5} mol L^{-1}; (B) 8.0×10^{-5} mol L^{-1}; (B) 8.0×10^{-5} mol L^{-1}; (C) 12.0×10^{-5} mol L^{-1}; (b) Effect of p-nitrofenol on the first derivative spectra: (A) 4.0×10^{-5} mol L^{-1}; (B) 8.0×10^{-5} mol L^{-1}; (C) 12.0×10^{-5} mol L^{-1}.

3.3.3. *Selection of Wavelength*
The first derivative spectrum of p-nitrophenol present a zero crossing at 253.0 nm consequently when this compound is present together with parathion, all the absorption at this wavelength can be attributed the later. Similarly parathion present a zero crossing at 273.0 nm, in which p-nitrophenol can be determinate in mixtures. The effect of the concentration of the analytes over both zero crossing points was studies. To a solution containing the individual compound in a concentration range between 4.0×10^{-5} to 12.0×10^{-5} M, the first derivative spectra was recorded by using a smoothing factor of 4. As can be seen in Figure 4, the zero crossing values selected are independent of the concentration. In this context 253.0 and 273.0 nm were selected for the determination of parathion and p-nitrophenol, respectively.

3.3.4. *Selection of Scale Factor*
By using a solution of 1×10^{-5} M of each individual compound, and a smoothing factor of 4, the scale factor was studied in the range between 10^0 and 10^{11}. The signals were evaluated at 253.0 nm (H_1) and at 273.1 nm (H_2) for parathion and p-nitrophenol, respectively. Ethanol was also evaluated at the same conditions against air. In all cases the shape of the spectra did not present alterations, which would be indicating that the amplification does not produce distortion of the spectra. Further, the analytical signal/signal blank ratio was found constant in the range between 200 and 400 nm. A factor scale of 10000, was selected because it allows that the reading of the values be appropriated for the analytic measurements. When smaller scale factors are used, the approached values are obtained and when the factors are higher, the values should be approximate according to the approach of the calculates significant.

3.3.5. *Analytical Parameters*
By using the selected conditions, linear graphs of derivative units (H) versus analyte concentration were obtained in the concentration ranges between and 2.5×10^{-7} to 2.0×10^{-4}M for parathion and 3.7×10^{-7} to 2.5×10^{-4}M for p-nitrophenol. The equations of the regression lines obtained where:

Parathion; $H_1 = 3.31 \times 10^6 \times C\ (\text{mol L}^{-1}) - 0.156$
$r = 0.999 \quad (\lambda = 253.0\ \text{nm})$
p − Nitrophenol; $H_2 = 1.71 \times 10^6 \times C\ (\text{mol L}^{-1}) + 0.565$
$r = 0.999 \quad (\lambda = 273.1\ \text{nm})$

Where, H is in derivative units.

Taking into account the standard deviation (σ) of 11 independent first derivative measurement of ethanol used as blank at the analytical wavelengths 253.0 nm and 273.0 nm and the sensitivities (S) of the method, the following detection limits (3σ/S) were found: 7.7×10^{-8} and 1.1×10^{-7} for parathion and p-nitrophenol, respectively. The determination ranges were found to be $2.5 \times 10^{-7} - 2.0 \times 10^{-4}$ mol L^{-1} for parathion and $3.7 \times 10^{-7} - 2.5 \times 10^{-4}$ mol L^{-1} for p-nitrophenol.

TABLE I
Recovery of I and II on standard mixtures

Relation	Added concentration mg L^{-1}		Found concentration[a] / mg L^{-1}	
I:II	I	II	I	II
3:1	6.00	2.00	6.10 ± 0.2	2.06 ± 0.3
1:2	2.00	4.00	2.00 ± 0.2	3.97 ± 0.2
1:2	4.00	8.00	3.96 ± 0.3	7.95 ± 0.4
1:4	2.00	8.00	1.97 ± 0.4	7.93 ± 0.3
1:5	2.00	10.00	1.95 ± 0.3	9.86 ± 0.4

[a] Average of five determinations.

According to these analytical parameters both analytes can be simultaneously determined in 0.6 g of vegetable tissues in the ranges between 4.9 to 3883.5 μg g^{-1} for parathion and 4.9 to 3285.3 μg g^{-1} for p-nitrophenol. The detection limits (3σ) were found to be 1.5 and 1.4 μg g^{-1} for parathion and p-nitrophenol, respectively.

The repeatability of the method was studies with 11 independent standard solutions that contain 1.0×10^{-6} mol L^{-1} of the both compound, obtaining a relative standard deviation of 1.5 and 1.8%, for parathion and p-nitrophenol, respectively.

In order to assess the accuracy of the proposed method, recovery experiments were performed on standard mixtures containing different known amounts of both analytes. The concentration of each compound was determined by using the linear equations given above. The results obtained are shown in Table I. The results shown that the content of each compound can be reliably determined in mixtures at different molar ratio.

3.4. PRACTICAL APPLICATION

3.4.1. *Recovery in Vegetal Tissues Spiked with II and I*

In order to validate the proposed method, corn leaf were enriched with different molar ratio of parathion and p-nitrophenol and the measurements were carried out using a blank of vegetal tissue, according to the procedure given above. In all cases a recovery between 98.2 to 102.2% with a relative standard deviation minor that 3% was obtained.

The method developed was applied for simultaneous determination of I and II in corn leaf, according to the procedure given in experimental. Table II shows the results for the determination of I and II in subsequent days after fumigation. When the concentrations are high, the results are in accord with those obtained by polarography. It is necessary to point out that this method allows to determine sufficiently

TABLE II
Determination of I and II in corn leaf after fumigation

Day	Found concentration/μg g^{-1}			
	Proposed method		Polarographic method	
	I	II	I	II
2	165 ± 1.2	*	164 ± 1.0	*
4	120 ± 1.1	*	119 ± 0.9	*
6	100 ± 1.0	*	101 ± 0.9	*
8	60 ± 1.1	*	< 98[a]	*
10	35 ± 1.0	40 ± 0.7	< 98[a]	39 ± 0.5
12	10 ± 0.5	50 ± 0.6	< 98[a]	50 ± 0.5
14	*	50 ± 0.7	< 98[a]	49 ± 0.6

* Detection limit of the polarographic method.

low concentrations to detect the parathion degradation, which is considered that occurs in a total period of 15 days.

4. Conclusion

The proposed method is a good alternative for the simultaneous determination of parathion and p-nitrophenol in vegetal tissues. The method presents good analytical properties and performance, the results are accurate and the method is precise. Also, this method require simple instrumental and he procedure is simple and rapid.

Acknowledgments

The authors are grateful to the National Fund for Development of Sciences and Technology (FONDECYT), project 1990033 for financial support.

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