Rapid and Sensitive Determination of 4-Nitrophenol, 3-Methyl-4-nitrophenol, 4,6-Dinitro-*o*-cresol, Parathion-methyl, Fenitrothion, and Parathion-ethyl by Liquid Chromatography with Electrochemical Detection

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Liquid chromatography with electrochemical detection has been used to determine various nitropesticides, DNOC, fenitrothion, and parathion (methyl and ethyl), and some of their main metabolites, 4-nitrophenol for parathion (methyl and ethyl) and 3-methyl-4-nitrophenol for fenitrothion, by using indirect detection. Analysis of them in river water samples has been performed without a preconcentration step. The recovery efficiencies of the tested compounds yielded values between 96 and 112% at the fortification level of 0.5 ppb in a river water sample, and their relative standard deviations were between 1 and 15%. The detection limits of these compounds ranged between 0.05 and 0.14 ppb.

Keywords: *Pesticides; river water; HPLC; electrochemical detection*

INTRODUCTION

Due to the indiscriminate use of pesticides, serious problems in the environment are emerging and they are an important risk to human health. Therefore, the determination of pesticides in environmental samples is one example of the general and more frequently occurring problem of determining traces of a single component or a group of special components in different matrices. Hence, an increasing number of analytical methods are being developed to detect the presence of those compounds and their degradation products, some of which are more toxic that the original pesticide.

The EU has set a maximum admissible concentration of 0.1 μg/L for individual pesticides and their related compounds in drinking water (EEC, 1980).

4-Nitrophenol, 3-methyl-4-nitrophenol, 4,6-dinitro-*o*-cresol (DNOC), parathion-methyl, fenitrothion, and parathion-ethyl are related aromatic compounds with one or more nitro groups.

DNOC is used as a herbicide to combat diseases that attack a great variety of crops such as wheat, corn, and potatoes, but its residues can reach surface water and sediments, normally due to rain.

Parathion (ethyl and methyl) and fenitrothion are widely used as organophosphorus insecticides. 4-Nitrophenol is a degradation product of parathion (ethyl and methyl) and 3-methyl-4-nitrophenol a degradation product of fenitrothion. Both compounds can also enter the environment. These degradation products are very soluble in water. Figure 1 shows the chemical structures of the studied compounds.

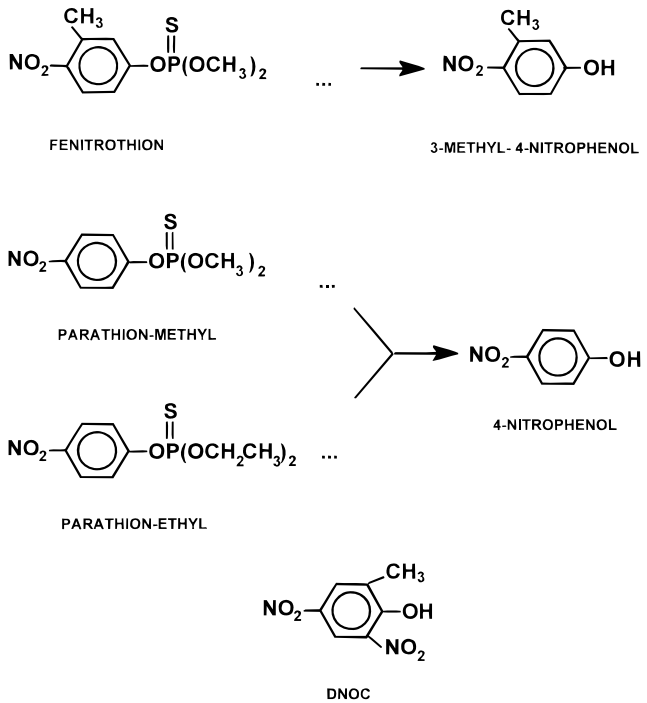

Figure 1. Chemical structures of the studied compounds.

Multiresidue analytical approaches have been applied to the gas−liquid chromatographic (GLC) separation and selective detection of volatile organohalogen, organophosphorus, and organonitrogen pesticides in food (Luke et al., 1981). Volatile organonitrogen pesticides can be detected with a electroconductivity detector in the nitrogen mode. However, because of the large number of natural and manmade organonitrogen compounds, the practical selectivity is less than that desired. In addition, many toxic organonitrogen con-

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taminants are not amenable to GLC because they are nonvolatile and/or heat labile.

The detection techniques (electron capture, flame photometric, and thermionic detectors) do not selectively detect the nitro functionality, or the detector responds to another functionality in the compound. In addition, nitrophenols require derivatization (Johansson, 1978; Shanfik et al., 1973) before determination by GLC.

In comparison to GC-based techniques (Ballesteros et al., 1993; Ogierman, 1982; Coutts et al., 1980), reversed phase liquid chromatography (RPLC) is a more suitable technique for the direct determination of polar analytes in water because derivatization is usually unnecessary, and in the analysis of aqueous samples the mobile phase system is fully compatible, so that there is a high potential for automation.

The nitrophenols have been chromatographed directly by HPLC-UV (Roseboom et al., 1981; Buckman et al., 1984; Menezes et al., 1998; Sojo et al., 1997; Chang et al., 1995; Ameno et al., 1995). However, because of the multitude of UV-absorbing compounds, the UV detector does not have the selectivity and sensitivity needed for a residue determinative technique.

The analysis by RPLC-UV of environmental water is usually complicated by a large excess of polar interferences, for example, anions and humic and fulvic acids, making the determination of more polar analytes eluting in the first part of the chromatogram difficult or impossible.

UV detection is still the most widely applied principle in LC analysis, for both research and application purposes. Proper trace enrichment is included in the procedure. In practice, however, these procedures are time-consuming and can result in incomplete extraction; analyte losses are further drawbacks of these tedious operations.

Organonitro compounds are electrochemically active and therefore can be detected electrochemically. Electrochemical detection (ED) can provide a fair degree of selectivity for the nitro functionality. The purpose of the work reported here was to determine the feasibility of using HPLC with ED to separate and detect a variety of organonitro pesticides and their toxic degradation products in Guadiana river water samples (Badajoz, Spain).

The chromatographic responses of the compounds were monitored indirectly by oxidative coulometric detection of the coulometrically reduced organonitro functionality by means of a porous graphite detector. This technique overcomes the problems of high background currents and oxygen interference observed in the direct reductive detection of organonitro compounds.

The electrochemical behavior of fenitrothion and its metabolites has been examined (Katagi et al., 1989). LC procedures with ED for the determination of one or more of these types of compounds in different matrices can be found in the literature (Carabias-Martínez et al., 1993) including fenitrothion, parathion, and parathion-methyl in river water by HPLC with dual electrochemical (reductive−oxidative) detection after preconcentration of 100 mL of river water with C18 cartridges. Pulsed amperometric detection at 1.25 V of 4-nitrophenol has also been reported (Carrieri et al., 1998). DNOC has been determined using a coulometric detector after LC separation (Kuribayashi et al., 1994).

Studies comparing LC followed by UV spectrophotometric detection of DNOC and with amperometric detection have also been carried out (Yao et al., 1991).

EXPERIMENTAL PROCEDURES

Apparatus. The studies were carried out on a chromatographic system consisting of a solvent reservoir, a degassing unit (79700 in-line vacuum degasser from Waters), to remove the oxygen, a double-piston HPLC pump 420 from Kontron equipped with a damper, a six-way injection valve (7125 Rheodyne) containing a 40 μL loop, and a Nova-Pak C18 analytical column (150 × 3.9 mm, 4 μm) with a Symmetry C18 guard column. The coulometric detection system consisted of a conditioning cell (model 5021 from ESA) and a high-performance analytical cell (70% efficiency; model 5011 from ESA) composed of two porous carbon working electrodes, in series together with the associated reference (hydrogen/palladium) and counter electrodes. The coulometric detector system is controlled by a Coulochem II (from ESA) potentiostat; the signal was acquired by a personal computer equipped with the Integration Pak software supplied by Kontron Instruments for chromatographic data processing.

Optimization of the mobile phase composition was carried out on a Hewlett-Packard HPLC instrument model 1100 equipped with a degasser, quaternary pump, manual six-way injection valve, diode array detector, and Chemstation Software package for instrument control, data acquisition, and data analysis.

The voltammograms for the organonitro compounds were obtained with an Autolab computer-controlled potentiostat (Eco Chemie) PSTAT 10 equipped with a Metrohm (Herisau, Switzerland) 663 VA stand. This system was connected to a PC 80386/25 MHZ equipped with the General Purpose Electrochemical System (GPES3) version 3.2 software package. The stand included an Ag/AgCl−3 M KCl reference electrode, a platinum-wire counter electrode, and a working glassy carbon electrode.

Reagents. 4-Nitrophenol, 3-methyl-4-nitrophenol, DNOC, parathion-methyl, fenitrothion, and parathion-ethyl (content > 99%) were obtained from Riedel-de Haën (Seelze, Germany) and used without further purification. Stock solutions of each compound in methanol were prepared by weighting. Diluted standards were prepared by suitable dilution of the stock solution with mobile phase. All solutions were kept in a refrigerator at 4 °C.

HPLC grade water was obtained from a Water Pro PS (Labconco) system. Methanol for chromatography from Merck (Darmstadt, Germany) was used.

Sodium perchlorate from Merck and glacial acetic acid from Romil were also used. All chemicals were of analytical reagent grade.

HPLC Operating Parameters. The mobile phase was methanol/water (60:40) containing 0.01 M sodium perchlorate and 0.5% in glacial acetic acid. It was filtered and degassed before use. The flow rate was 1.0 mL/min.

The conditioning cell was set at −600 mV, and electrodes 1 and 2 of the analytical cell were set at −1300 and +700 mV, respectively. A cleaning step for electrode 1 was made prior to injection of the sample or standard by applying a potential of +1300 mV for 7 min.

General Procedure for the Determination of the Studied Compounds. Standards containing known concentrations of the pesticides and their degradation products in mobile phase were filtered through 0.45 μm nylon filter membranes and degassed by passing oxygen-free nitrogen for a minimum time of 1 min before their injection in the chromatographic system. Standards and samples were degassed to guarantee an effective reduction of our compounds in the first electrode and to preserve the more possible unaltered surface of this electrode (because it is not necessary to reduce at the same time the oxygen which is present in greater concentration than our compounds). On the other hand, it is convenient to be able to monitor the upstream

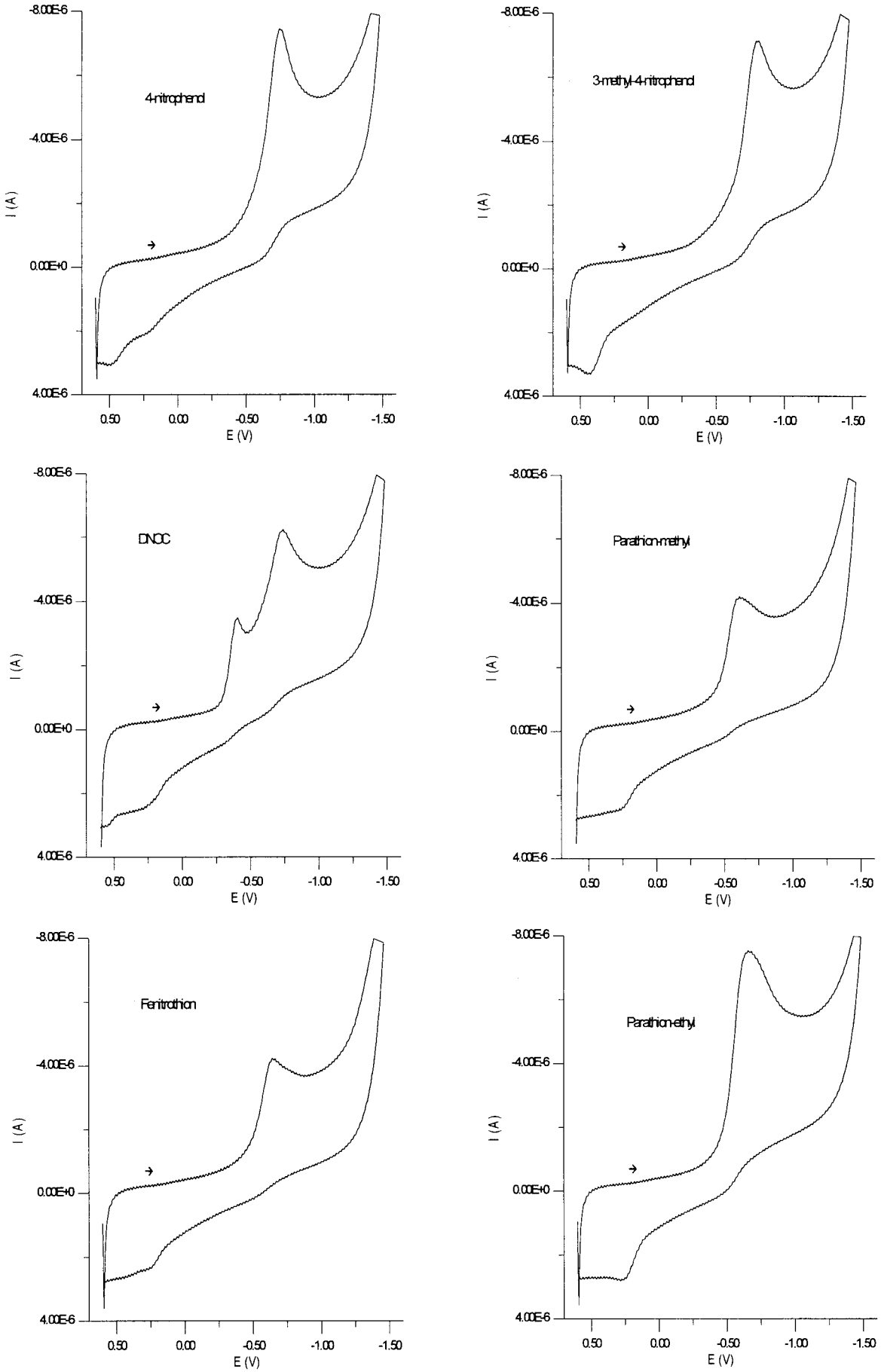

Figure 2. Cyclic voltammograms of the six organonitro compounds. Analyte concentrations: 8×10^{-5} M for 4-nitrophenol, 3-methyl-4-nitrophenol, and parathion-ethyl; 3×10^{-5} for DNOC, parathion-methyl, and fenitrothion. Scan range: + 0.6 to −1.5 V.

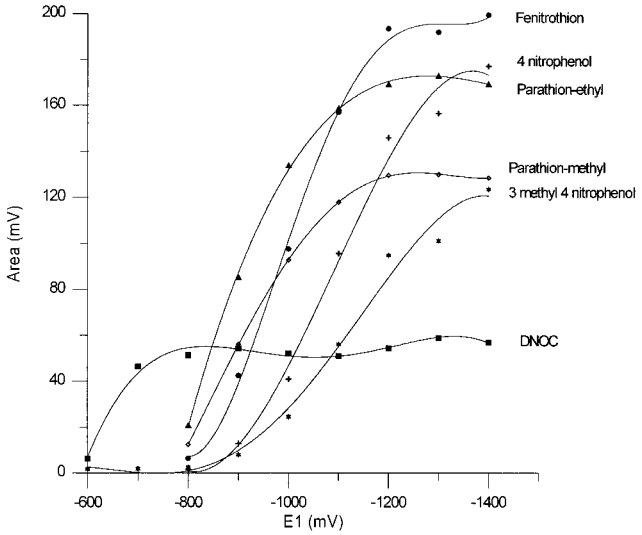

Figure 3. Hydrodynamic curves of reduction of the six compounds in the porous carbon electrode of the analytical cell. Chromatographic conditions: methanol/water (60:40) made 0.01 M in sodium perchlorate and 0.5% in glacial acetic acid; flow rate, 1.0 mL/min (1 V = 1 μA).

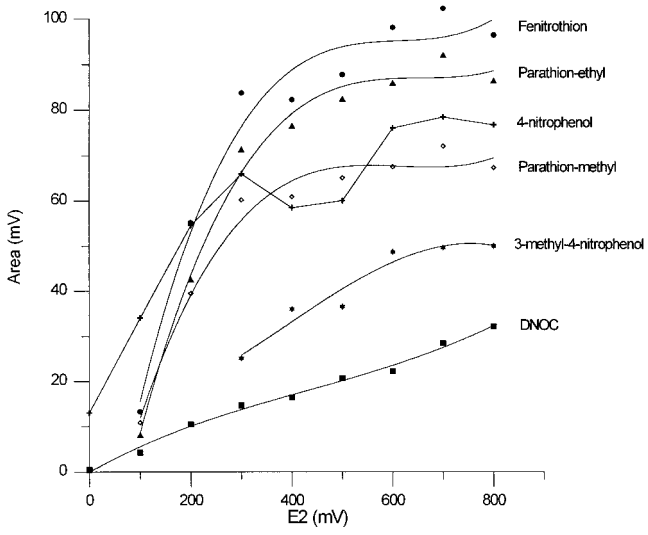

Figure 4. Hydrodynamic curves of oxidation of the six compounds in the porous carbon electrode of the analytical cell. Chromatographic conditions: methanol/water (60:40) made 0.01 M in sodium perchlorate and 0.5% in glacial acetic acid; flow rate, 1.0 mL/min (1 V = 2 μA).

electrode current to check that the system continues to be stable. Two chromatograms per sample were obtained, and the mean of their peak areas was used as analytical signal.

Aliquots of river water (spiked with different amounts of the pesticides and their degradation products studied) were filtered and analyzed directly following the general procedure without a preconcentration step.

RESULTS AND DISCUSSION

Optimization of the mobile phase composition was done by using photometric detection due to the faster stabilization of the baseline taking into account the necessary presence of an electrolyte (in order to maintain a constant ionic strength and to ensure the conductivity of the mobile phase) and the effects of pH on electrochemical detection. Different mobile phases were tested with a methanol content ranging from 50 to 80%. It can be seen that problems occur with 4-nitrophenol and parathion-ethyl, which showed poor chromatography in these mobile phases. Finally, a mobile phase of methanol/water in a 60:40 proportion made 0.01 M in sodium perchlorate and 0.5% in glacial acetic acid was selected. The six compounds were eluted in 15 min.

The cyclic voltammograms of 4-nitrophenol (8×10^{-5} M), 3-methyl-4-nitrophenol (8×10^{-5} M), DNOC (3×10^{-5} M), parathion-methyl (3×10^{-5} M), fenitrothion (3×10^{-5} M), and parathion-ethyl (8×10^{-5} M) for the scan range of +0.6 to −1.5 V on a glassy carbon working electrode and using the selected mobile phase are presented in Figure 2.

The voltammograms show reductive and oxidative peaks. This permits direct and indirect electrochemical detection by HPLC. The reference and working electrodes used to obtain the voltammograms are different from those used in the high-performance analytical cell, and the results are also different under hydrodynamic conditions. For these reasons our HPLC signals are shifted toward more negative potential values than the peak potentials observed in the voltammograms (~0.70 V). At first we tried to develop a procedure for the direct reductive detection, obtaining the hydrodynamic curves of reduction by changing the reduction potentials (from −0.80 to −1.60 V) and monitoring the response of this electrode. However, the background current grew more than the peaks, being observed relatively small peaks, which means that the necessary potential values to get the reduction of these compounds were too high and, consequently, a high and noisy baseline was obtained that produced a poor sensitivity.

To avoid the high background currents, we decided (as an alternative detection approach) to detect the nitro functionality indirectly, in other words, by the oxidative detection of the coulometrically reduced organonitro compounds. This kind of detection provides a greater signal/noise ratio and, also, this detection type could eliminate some possible interferences.

After each injection into the HPLC system, we found that the electrochemical detector became "poisoned", specifically the first electrode (electrode 1), where the pesticides were reduced. This results in a lower response for the subsequent sample. For this reason an electrochemical cleaning step of the first electrode was made prior to each injection. The influence of the potential and cleaning step duration was examined. It was found that a period of 7 min at +1300 mV was sufficient to restore the electrode surface. After the cleaning step and before the start of the run, the measuring potentials were restored gradually (from lower values to the desired values); the time of the stabilization was ~10 min. At the end of this work we did not find any change in the characteristics of the electrode due to the application of the required potential values.

Hydrodynamic curves were obtained with the HPLC system to select the detector operating reduction and oxidation potentials. Hydrodynamic curves for the reduction of each compounds were obtained (Figure 3) by changing the applied reduction potential on electrode 1 while monitoring the response of electrode 2. This second electrode was maintained at a constant oxidation potential (+800 mV). The conditioning cell was set at −600 mV to contribute to the elimination of oxygen in the mobile phase.

According to the shape of the hydrodynamic curves and to obtain the highest signal-to-noise ratio, a potential value of −1300 mV for electrode 1 was selected.

Table 1. Analytical Figures of Merit Using Peak Area and Peak Height[a]

compound	correl coeff (r)	analytical sensitivity[b] (ng^{-1})	RSD (%), $n = 7$ (0.1 ng)	detection limit (ppb)
		Peak Area		
4-nitrophenol	0.9931	54	8	0.11
3-methyl-4-nitrophenol	0.9928	53	11	0.08
DNOC	0.9995	203	2	0.05
parathion-methyl	0.9977	95	4	0.06
fenitrothion	0.9938	46	8	0.12
parathion-ethyl	0.9956	68	5	0.14
		Peak Height		
4-nitrophenol	0.9894	44	13	0.14
3-methyl-4-nitrophenol	0.9782	30	13	0.11
DNOC	0.9973	88	5	0.05
parathion-methyl	0.9940	59	10	0.10
fenitrothion	0.9785	31	9	0.10
parathion-ethyl	0.9868	39	7	0.20

[a] Concentration range: 0.01−0.4 ng (1 V = 10 nA). [b] Analytical sensitivity: slope of calibration curve/residual mean (Skoog et al., 1994).

Table 2. ANOVA Test: Linearity Test for Peak Area

	source of variation					
	set means about the line (lack of fit)		within line (pure error)			
compound	sum of squares of deviations	DF[a]	sum of squares of deviations	DF	F_{exptl}	F_{theor}
4-nitrophenol	2.04×10^2		2.51×10^2		0.81	3.71
3-methyl-4-nitrophenol	7.49×10^1		3.46×10^1		2.16	
DNOC	1.28×10^2	3	2.84×10^2	10	0.45	
parathion-methyl	1.52×10^2		8.95×10^1		1.69	
fenitrotion	1.61×10^1		3.19×10^2		0.05	
parathion-ethyl	1.28×10^2		1.27×10^2		1.00	

[a] Degree of freedom.

Table 3. Results Obtained in the Analysis of the Nitro Compounds Studied in Spiked River Water Samples

compound	added (ppb)	recovery ± RSD (%)
4-nitrophenol	1	104 ± 7
	0.5	97 ± 15
3-methyl-4-nitrophenol	1	90 ± 23
	0.5	112 ± 13
DNOC	1	99 ± 2
	0.5	96 ± 6
parathion-methyl	1	104 ± 3
	0.5	104 ± 1
fenitrothion	1	107 ± 3
	0.5	99 ± 5
parathion-ethyl	1	88 ± 3
	0.5	105 ± 12

Hydrodynamic curves of oxidation were obtained (Figure 4) by scanning the applied oxidation potential on electrode 2 to select the best oxidation potential. Electrode 1 is maintained at the constant reduction potential (−1300 mV). The conditioning cell was set at the same previous potential (−600 mV). An oxidation potential of +700 mV was selected.

The potential of the conditioning cell was changed to −1300 mV, but because a significative increase of the peak heights was not obtained, its potential was maintained at −600 mV so as not to force the electrode with a very high potential.

Mobile phase flow rates (between 0.5 and 1.5 mL/min) were tested to optimize detection. We found in general higher signals at lower flow rate values as expected. A value of 1 mL/min was selected as a compromise between detector response and chromatographic separation.

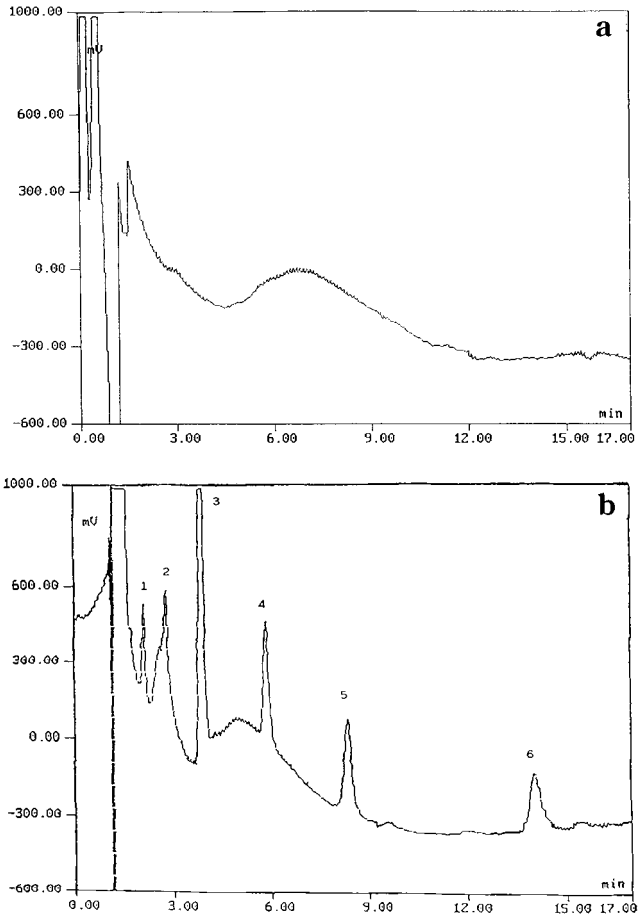

Figure 5. RPLC-ED chromatogram of (a) unspiked river water and (b) river water sample spiked at 0.5 μg/L level for each compound. The sample was analyzed according to the proposed method. Peaks: 1, 4-nitrophenol; 2, 3-methyl-4-nitrophenol; 3, DNOC; 4, parathion-methyl; 5, fenitrothion; 6, parathion-ethyl (1 V = 10 nA).

Determination of the Six Compounds. By using the experimental chromatographic conditions previously selected, calibration graphs were obtained in the range 0.5−20 μg/L. Samples were prepared in triplicate, and each sample was injected two times into the chromatograph. The average peak areas or peak heights were plotted against the concentration as a calibration curve. The results obtained using peak heights were less reproducible in general. The correlation coefficient and the detection limits, calculated using the signal-to-noise ratio (S/N) of >3 (n = 3) for river water samples, are presented in Table 1.

Due to the previous results, an analysis of variance (ANOVA) allowed us to evaluate coupling of the experimental points obtained by measuring peak area with a linear model. Data about the validation of the lineal model are summarized in Table 2. There is linearity over all of the concentration range study.

The developed method has been applied to the direct analysis (without a preconcentration step) of the studied compounds in river water samples spiked at two concentration levels. The results were acceptable with regard to both recovery and repeatability (see Table 3). A chromatogram of river water sample spiked at the 0.5 μg/L level for each compound is presented in Figure 5, together with the chromatogram of an unspiked river water sample.

Guadiana River water samples from a selected location in Badajoz (Spain) were analyzed by means of the developed procedure. Residues of these compounds and their degradation products were not detected.

Conclusions. A sensitive and selective RPLC-ED method was developed for the subtrace level determination of DNOC, fenitrothion, parathion (methyl and ethyl), and some of their degradation products, including 4-nitrophenol for parathion (methyl and ethyl) and 3-methyl-4-nitrophenol for fenitrothion, in river waters. The results demonstrate the possibility of their easy determination in river water samples in the low parts per billion level.

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