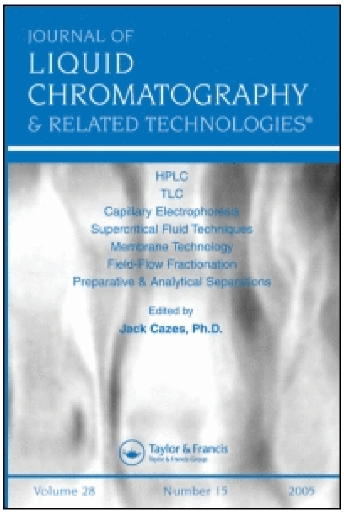

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Determination of Diazinon and Fenitrothion in Environmental Water and Soil Samples by HPLC

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ABSTRACT

High performance liquid chromatography (HPLC) methods have been developed for the determination of the organophosphorus pesticides, diazinon, and fenitrothion in environmental water and soil samples; a simple and rapid sample preparation procedure using solid-phase extraction being developed in the case of water samples. In soil samples, the analytical procedure proposed consisted of a 10-min ultrasonic extraction of the target compounds from a 20 g soil sample with 20 mL of acetonitrile and, after centrifugation, the filtering and concentration of the supernatant in an SC110H Speed Vac Plus concentrator to evaporate

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solvents. The quantification of diazinon and fenitrothion was performed using UV photodiode detection at 245 and 267 nm, respectively. The average recoveries from spiked water and soil samples at various concentration levels exceeded 86% with relative standard deviations of 1.8–5.1%. It was possible to determine concentrations of these pesticides in the range 0.1–2 ng/g in water and soil samples with a good level of reproducibility and accuracy.

Key Words: Organophosphorus insecticides; Diazinon®; Fenitrothion®; Water analysis; Soil analysis; Solid-phase extraction.

INTRODUCTION

Environmental pollutant compounds have been increasingly studied in recent decades because of their toxicity, persistence, and bioaccumulation, the main steps in environmental analysis being described in the literature. They are (i) sampling and sample preparation; (ii) clean-up and/or extraction; and (iii) final separation and qualitative and quantitative determination. Since analytes can be contained in a wide variety of matrices (e.g., aqueous samples can include all kinds of salt water, surface and treated fresh water, and industrial water; solid samples can include soils, sediments, and other types of solid waste and air samples), a considerable amount of effort is being made to deal with the handling of environmental samples before chromatographic techniques are applied. In this study, SPE has been used as a clean-up and preconcentration technique in order to improve the selectivity and detection limits of the analytes. For a complete extraction, the sorptive capacity of the solid-phase extractant must, of course, be suitable to handle the sum of the analytes. While sorptive capacities may vary widely, the weight of sorbent must usually be at least 1000 times the total weight of analytes, so, for example, a 10-mL sample containing a total analyte concentration of 10 μg/mL would require 100 mg or more of solid sorbent. Assuming the total adsorptive capacity is adequate, distribution of the analytes between the liquid sample and the solid SPE phase must be weighted in favor of the latter.

Available methods for diazinon and fenitrothion analysis include enzyme-linked immunoassay,[1,2] gas chromatography,[3–9] high performance liquid chromatography (HPLC),[10–13] HPLC with mass spectrometric detection (LC–MS),[8,14,15] and capillary electrophoresis (CE).[16] The purpose of this work was to develop rapid, simple, and sensitive methods for the determination of diazinon and fenitrothion (Fig. 1) by HPLC with ultraviolet (UV) detection using SPE in environmental water and soil samples.

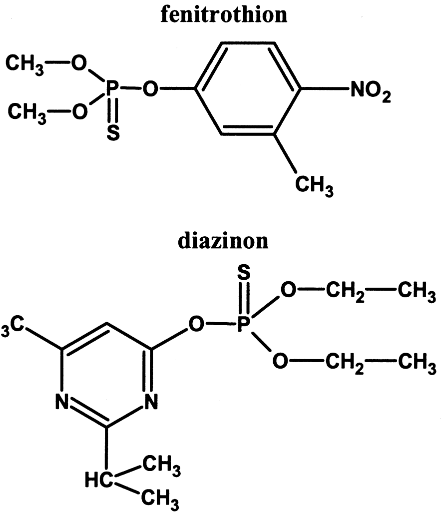

Figure 1. Structures of diazinon and fenitrothion.

EXPERIMENTAL

Chemicals and Reagents

The pesticides diazinon (98.5%) and fenitrothion (96.3%) were purchased from Riedel de Haën of Hannover, Germany. All of the solvents (methanol, acetonitrile, and diethyl ether) were HPLC grade and purchased from Merck of Barcelona, Spain. 4-Nitrobenzyl bromide (99%) was used as an internal standard and purchased from Aldrich of Steinheim, Germany. All water used in this study was purified using a Milli-Q water purification system (18 MΩ resistance) (Millipore, Bedford, MA, USA).

Equipment

An Alliance® HPLC System liquid chromatograph, (Waters, Mildford, MA), equipped with a 2695 Separations Module and a 996 Diode Array Detector (DAD) was used, and separation was carried out using a Phenomenex C18[2] Luna Column (5 µm; 150 × 4.60 mm) (Torrence, CA).

An ultrasonic bath Ultrasons Selecta, (Selecta, Barcelona, Spain) and a Beckman GS-GR centrifuge (Fullerton, CA, USA) were used for the extraction of the pesticides from the soils, and to separate the soil particles from the supernatant, respectively.

Two types of SPE cartridges were evaluated in the study: the HLB cartridges containing 200 mg of sorbent [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] and Sep-Pak Plus C-18 cartridges containing 500 mg of sorbent, both from Waters Corporation (Milford, MA). For solid-phase extraction a Manifold (Waters, Milford, MA) and an SC110A Speed Vac Plus concentrator (Savant Instruments, Farmingdale, NY) were used for solvent evaporation.

Chromatographic Conditions

High performance liquid chromatography analysis of diazinon and fenitrothion was isocratically performed in a reversed-phase C-18 column with a mobile phase consisting of acetonitrile/water (65/35, v/v) at ambient temperature. The injection volume was 100 μL and the flow-rate was 1 mL/min. The most appropriate detection wavelengths of the UV detector, suggested from the UV spectra of each pesticide, were 245 for diazinon and 267 for fenitrothion.

Stock and Standard Solutions

A stock solution of each pesticide was prepared at 500 mg/L with acetonitrile. This stock solution was further diluted with Milli-Q water to prepare additional working solutions to obtain final concentrations of 10, 1, 0.1, and 0.01 μg/mL. 4-Nitrobenzylbromide served as the internal standard (I.S.), while a working solution of 20 μg/mL was prepared in Milli-Q water. From working solutions containing pesticide and I.S., calibration standards were prepared in Milli-Q water to construct the calibration curve for water and soil samples. Concentrations of the calibration standards ranged from 0.05 to 30 μg/mL. The stock and working solutions were stored in the dark at −20°C and the calibration standards were stored at 5°C for up to two days.

Preparation of Water Samples

Water samples were prepared by adding μL amounts of the pesticide working solutions to the appropriate volume of pesticide-free water sample, to produce a concentration in the range of 0.1–50 ng/mL, the process being applied to ground, drinking, and river water samples collected from local sources and River Bernesga in the city of León (Spain), which were filtered through a 0.45-μm filter (Millipore, Bedford, MA) before the SPE process. Preliminary experiments were carried out to optimize the main parameters affecting the SPE of diazinon and fenitrothion from aqueous solutions to achieve high reproducible recoveries using Sep-Pak C-18 Plus and Oasis HLB. The cartridges were activated with 6 mL of methanol/diethyl ether (10/90, v/v) and subsequently rinsed with 6 mL of methanol, and then equilibrated with 6 mL of water. A 200-mL aliquot water sample spiked with the appropriate amount of working solutions was passed through the extraction cartridge at a flow rate of 10 mL/min in a vacuum, and then the pesticide was eluted with 2 mL of methanol and 6 mL of methanol/diethyl ether (10/90, v/v). The solvent was left for a few minutes in the cartridge before being slowly eluted at 2 mL/min. The eluate was collected in a glass vial and evaporated to dryness in SC110A Speed Vac Plus concentrator. The residue was reconstituted in 0.4 mL mobile phase by sonicating for 1 min and then analyzed. The efficiency of the SPE method tested was evaluated by comparison of the peak areas obtained in each case with those of the calibration lines of spiked water standard solutions.

Preparation of Soil Samples

Soil samples were spiked in the range 1–500 ng/g with working solutions of pesticide and then mixed well by hand and shaken for 5 min. A 20-g spiked soil sample was extracted for 10 min by ultrasonic agitation with 20 mL of acetonitrile. The suspensions were then centrifuged at 2000 *g* for 10 min, and the supernatant removed and filtered through a glass-fiber filter. The acetonitrile fraction was evaporated to dryness using an SC110A Speed Vac Plus concentrator. The residue was reconstituted like the water samples and analyzed.

Quantification

The calibration standard solutions of the pesticide were prepared at six different concentrations between 0.05 and 30 μg/mL, using the procedure described above, and calibration graphs were constructed of the ratio of the pesticide/I.S. peak areas vs. pesticide concentration. Unknown concentrations of pesticide in the water and soil samples were calculated by interpolation from the calibration graphs by a least-squares regression treatment.

RESULTS AND DISCUSSION

Selectivity

Suitable HPLC conditions concerning separation and retention of the diazinon and fenitrothion for water and soil samples were obtained by using a mobile phase of acetonitrile–water (65/35, v/v). Figures 2–5 show the chromatograms from analysis of these pesticides in water and soil samples. Irrelevant interfering peaks are visible in samples analyzed at the retention time of the I.S. and pesticides of interest. Minor changes to the organic modifier content of the mobile phase, were occasionally required to accommodate column efficiency, loss, or interference from atypical water and soil samples.

Recovery

Water Samples

Considerable trace enrichment is necessary to obtain detection limits of ng/mL levels. A pre-concentration of about three orders in sensitivity should be obtainable by the SPE procedure.[17,18] To evaluate the potential of trace enrichment of diazinon and fenitrothion, samples of spiked Milli-Q water were analyzed, detector responses to them being compared with responses to calibration standards with identical pesticide concentrations.

Table 1 shows the recoveries of diazinon and fenitrothion from spiked river water at different concentrations, with excellent results for the two pesticides in river water samples filtered through Oasis HLB and Sep-Pak cartridges. The recovery values obtained were in the 92–102% and 86–97% ranges for Oasis HLB and Sep-Pak cartridges, respectively. A comparison of the retention capabilities of these cartridges revealed no significant differences in the retention of these pesticides. The sample volume/sorbent ratio, eluent solvent, and volume are important parameters to obtain consistent recovery.[19]

Soil Samples

Mean % recoveries and standard deviations for pesticides extracted from soil samples with the ultrasonic method were 94 ± 4. Acetonitrile was chosen for its greater eluting power in reversed-phase chromatography.

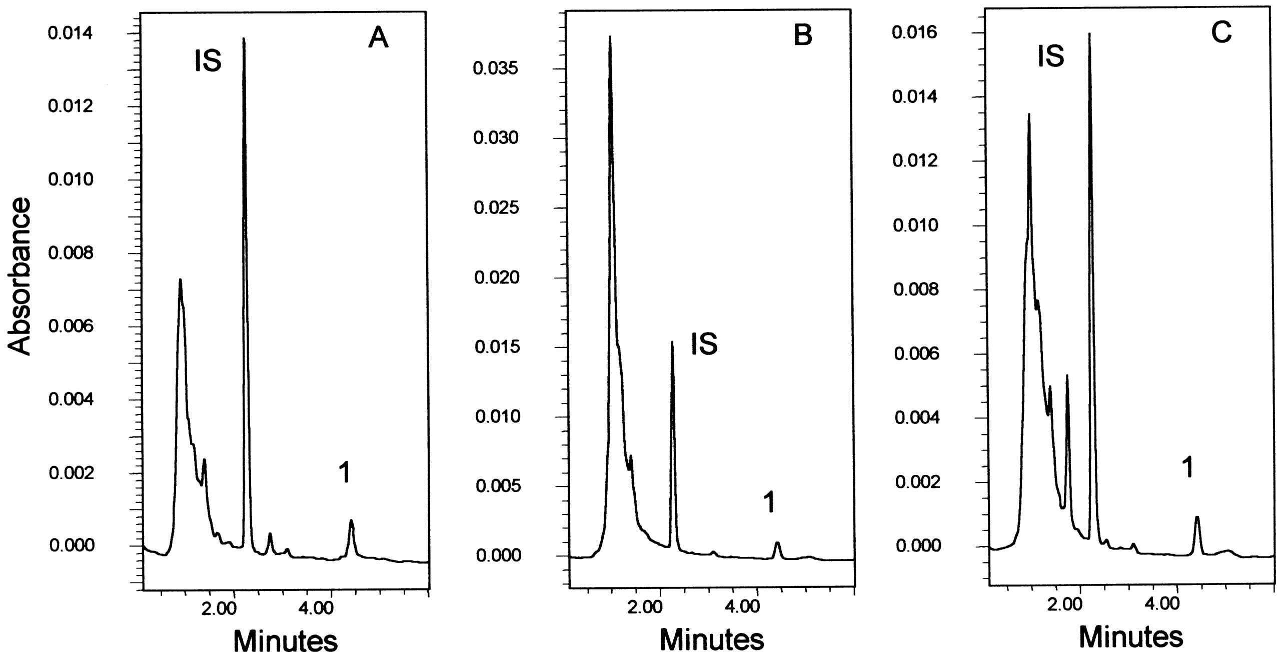

Figure 2. HPLC–DAD chromatograms for the environmental water samples spiked with diazinon (1) and preconcentrated by SPE with Oasis HLB as sorbent. (A) Drinking water (1 ng/mL). (B) Ground water (1 ng/mL). (C) River water (1 ng/mL). Analysis conditions: C-18 column (15 cm × 4.6 cm I.D.); injection volume 100 µL; mobile phase: acetonitrile/water (65/35, v/v); 245 nm UV absorbance. IS = Internal Standard.

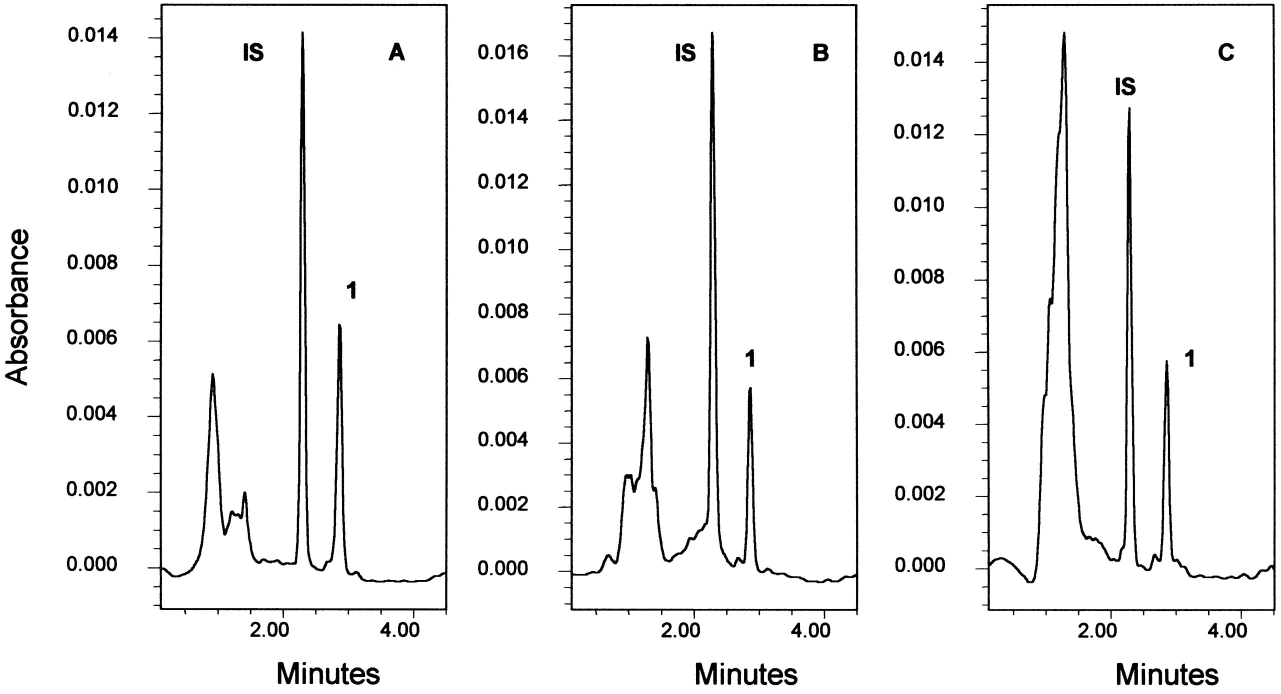

Figure 3. HPLC–DAD chromatograms for the environmental water samples spiked with fenitrothion (1) and preconcentrated by SPE with Oasis HLB as sorbent. (A) Drinking water (1.5 ng/mL). (B) Ground water (1.3 ng/mL) and (C) River water (1.3 ng/mL). Analysis conditions: C-18 column (15 cm × 4.6 cm I.D.); injection volume 100 µL; mobile phase: acetonitrile/water (65/35, v/v); 267 nm UV absorbance. IS = Internal Standard.

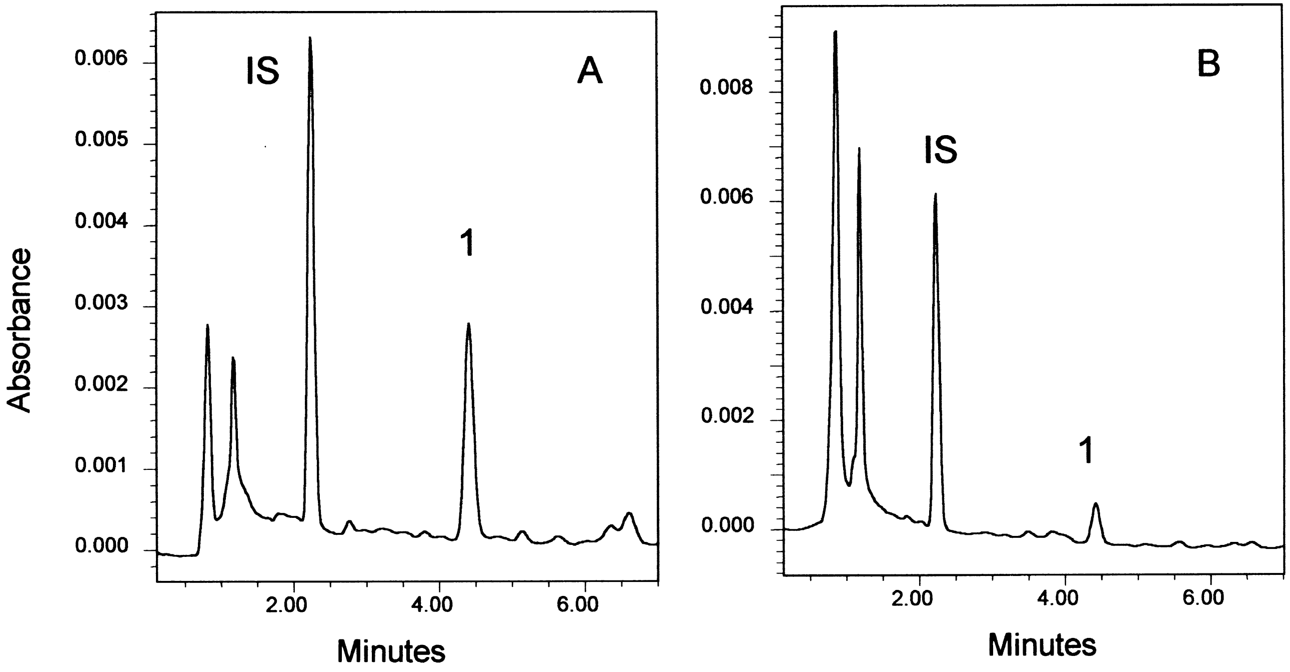

Figure 4. HPLC–DAD chromatograms obtained after ultrasonic extraction with acetonitrile of a soil spiked with diazinon (1). Spiking level: (A) (200 ng/mL) and (B) (35 ng/mL). For analysis conditions see Fig. 2. IS = Internal Standard.

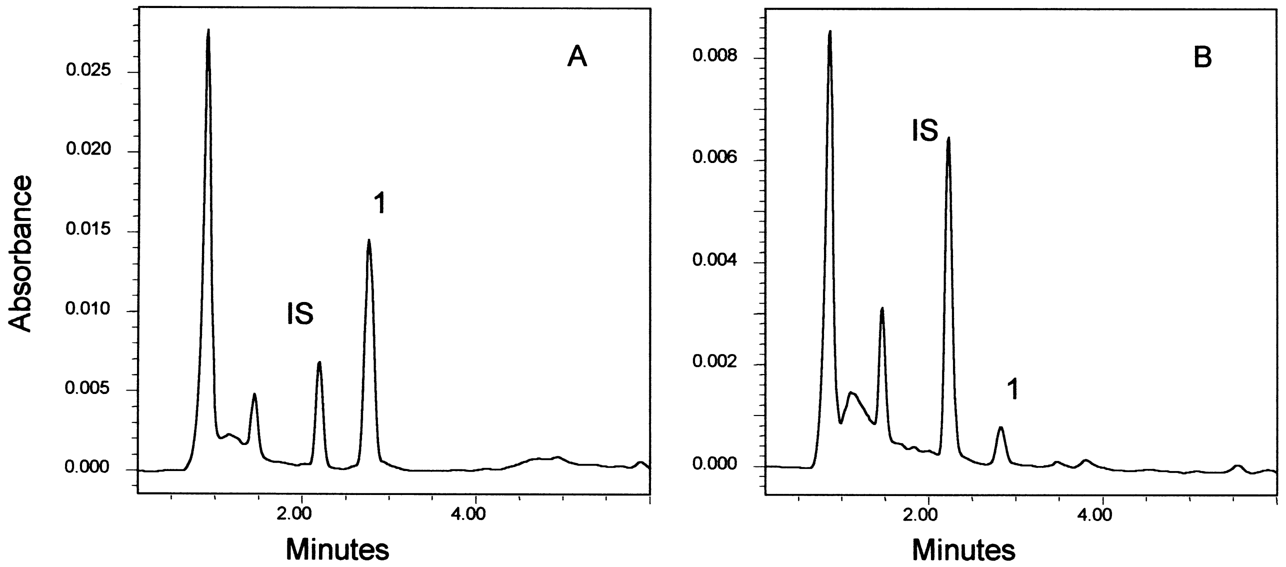

Figure 5. HPLC–DAD chromatograms obtained after ultrasonic extraction with acetonitrile of a soil spiked with fenitrothion (1). Spiking level: (A) (250 ng/mL) and (B) (15 ng/mL). For analysis conditions see Fig. 3. IS = Internal Standard.

Table 1. Recoveries of diazinon and fenitrothion extracted from 200 mL of spiked river water after SPE on 200 mg of Oasis HLB and 500 mg of Sep-Pak C-18 plus.

	200 mg Oasis HLB				500 mg Sep-Pak C-18			
	Diazinon		Fenitrothion		Diazinon		Fenitrothion	
Concentration added (ng/mL)	Recovery rate (%)	RSD (%)	Recovery rate (%)	RSD (%)	Recovery rate (%)	RSD (%)	Recovery rate (%)	RSD (%)
1	92	4.8	96	5.1	91	4.7	86	4.3
15	98	2.3	102	3.0	95	1.8	93	4.1
50	96	3.5	99	1.9	97	3.2	94	2.5

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Linearity and Sensitivity

The water standard solutions of the pesticides were prepared at six different concentrations between 0.1 and 50 ng/mL, and treated in the manner described above. With the I.S. method, and a linear relationship between the peak-area ratios and the pesticide concentrations in the range studied, regression analysis of the data revealed a correlation coefficient of ≥ 0.99 for both pesticides. The limits of quantification (LOQs, 0.1 and 0.2 ng/mL for fenitrothion and diazinon, respectively) were determined by evaluating the level of pesticide necessary to produce a peak with a signal-to-noise ratio of 5, assuming a 200 mL sample aliquot and a 0.4 mL final volume of extract. Higher or lower volumes of sample extracted will result in an increased or decreased LOQ.

The soil standard samples of the pesticides were prepared at five different concentrations between 1 and 500 ng/g and treated in the manner described above. For soil samples, the LOQs were 1 and 2 ng/mL for fenitrothion and diazinon, respectively.

Application of the Methods

The final optimized method was applied for the analysis of spiked, real samples of water and soil in order to observe the effect of the matrix in the recoveries, separation, and interfering peaks. Recoveries of diazinon and fenitrothion from drinking, ground, and river water are over 93%, and over 85% for soil samples. The HPLC chromatograms of the pesticide standards, after their addition to 200 mL of environmental water and soil samples and SPE in Oasis HLB and Sep-Pak C-18 cartridges, are shown in Figs. 2–5.

The optimized methods were used to ascertain the degradation kinetics of pesticides in soil samples in different physico-chemical conditions.

Reproducibility

The reproducibility of the methods developed for diazinon and fenitrothion in ground water were determined by adding known amounts of these pesticides to blank ground water. The within-run and between-day of the methods were evaluated using three concentrations of pesticide in ground water samples. The results obtained are shown in Table 2, from which one can see that for within-run the relative standard deviations (RSD) ranged from 1.9 to 5.1%. The between-day RSD values varied from 2.7 to 7.3%, which are higher than the within-run assays. The greatest increases in the RSD values are within the 1 and 25 ng/mL concentration levels.

Table 2. Reproducibility of diazinon and fenitrothion in spiked ground water.

		RSD (%)	
Pesticide	Concentration added (ng/mL)	Within-run	Between-day
Diazinon	1	4.3	6.8
	15	4.5	3.7
	50	2.6	3.8
Fenitrothion	1	5.1	7.3
	15	3.6	5.1
	50	1.9	2.7

CONCLUSIONS

In this study, we have developed methods for the determination of diazinon and fenitrothion in differents water and soil samples. These pesticides in the low ppb range can be efficiently concentrated from drinking, ground, and river water, and aqueous soil extracts by SPE with Oasis-HLB and Sep-Pak. Recoveries from water and soil samples were in the range of 86 to 102%. The optimized methods, which gave baseline separation for diazinon and fenitrothion, are fast, sensitive, accurate, and applicable to the determination of these pesticides in environmental samples.

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