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Diazinon Determination Using High Performance Liquid Chromatography: A Comparison of the ENVI-Carb Column with the Immunoaffinity Column for the Pretreatment of Water and Soil Samples

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Abstract An immunoaffinity chromatography column was developed to remove diazinon from water and soil samples. In this paper, two types of absorbent columns, the immunoaffinity chromatography column and the ENVI-Carb column, were compared. To accomplish this, each of these columns was used to treat water and soil samples that had been spiked with diazinon at concentrations of 2.5 or 5 ng/mL (or ng/g). High performance liquid chromatography was then used to analyze the treated samples. The ENVI-Carb column recovered 87.99%–95.95% of the diazinon from water and soil with CVs of 5.08%–8.06%. The recoveries observed when the immunoaffinity chromatography column was used were slightly lower (52.61%–81.58%); however, it effectively clean up the soil samples.

Keywords Immunoaffinity · Solid phase extraction · Diazinon · Comparison

Organophosphorus compounds (OPPs) are primarily used in agriculture as pesticides. OPPs have replaced organochlorine compounds due to the persistence and accumulation of the latter in the environment (Bavcon et al. 2003). OPPs inhibit acetylcholinesterase activity in insects; however, they can also affect the nervous system of humans and animals (Muggleton et al. 2005). In addition, as a result of their widespread application, OPPs are one of the most common types of organic pollutants found in environmental matrices and food products. Therefore, OPPs pose a human and animal health hazard.

Diazinon [*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate] (Fig. 1) is a nonselective organophosphorus insecticide that is used extensively on lettuce, almonds, citrus, cotton, turf, alfalfa and other crops, as well as on fruit and nut orchard crops, foundations and landscapes and as an urban pest control (Sullivan and Goh 2000). Diazinon is available as a dust, granules, seed dressings, wettable powder and emulsifiable solution. After diazinon is applied it is often found in the surrounding soil and surface waters, as well as on the surface of plants.

Because the general population is exposed to pesticides, it is important to investigate the concentration of pesticides and their metabolites in environmental samples. Therefore, reliable methods that enable analytical chemists to evaluate a variety of compounds that include the parent pesticides as well as their metabolites are required (Hernández et al. 2005). These methods often include optimized chromatographic techniques (Gas Chromatography and High Performance Liquid Chromatography) coupled with highly sensitive detectors, such as electron-capture detectors (ECD), nitrogen phosphorous detectors (NPD), ultraviolet detectors (UV) and mass spectrometry.

The conventional extraction method used during the analysis of pesticides is liquid–liquid extraction (LLE). Although the cost of materials required for LLE is generally lower than the cost of materials required for solid phase extraction (SPE), LLE often suffers from the formation of emulsions, impurities and a low recovery of the analytes. In addition, use of the organic solvents required for LLE is not desirable from a viewpoint of environmental pollution. Therefore, SPE has gained popularity as a tool for the isolation, concentration and purification of analytes from complicated matrices. In addition, SPE involves a much

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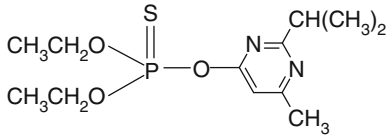

Fig. 1 The chemical structure of diazinon

simpler analytical procedure that produces cleaner extracts and higher recoveries than LLE. The non-polar octadecyl (C_{18}) bonded silica is the sorbent packing material widely used (Kumazawa and Suzuki 2000) Affinity chromatography is then used to extract the analyte based on a reversible interaction between a ligand (analyte) and a specific antibody coupled onto a chromatographic matrix. The technique is ideal for the capture of analytes or as an intermediate step in a purification protocol and can be used whenever a suitable antibody is available for the ligand of interest. In addition, single step affinity purification can save time when compared to less selective multistep procedures (Amersham Pharmacia 1995). This technique is primarily used to evaluate samples for the presence of various pesticides (Degelmann et al. 2006), veterinary drugs (Godfrey 1998) and others environmental pollutants (Thomas and Li 2000; Cichna et al. 2001).

This paper describes the development of an immunoaffinity chromatography column designed to identify and quantify the organophosphate insecticide, diazinon. A comparison of the results obtained using the immunoaffinity chromatography column and the ENVI-Carb column to extract or clean water and soil samples is also described herein.

Materials and Methods

Diazinon pesticide was purchased from the Institute of Environmental Protection, Ministry of Agriculture (Tianjin, China). Dichloromethane, hydrochloric acid, sodium chloride, acetic ether and acetic acid were obtained from Sonipharm Chemical Reagent Co., Ltd (Shanghai, China). Acetonitrile (ACN) and methanol, both gradient grade for HPLC, were delivered from Merck (Shanghai, China). Tetramethoxysilane (TMOS) were obtained from Fluka (Shanghai, China). Polyclonal antibody PNB against diazinon was prepared in coworkers' laboratory.

Analysis was performed using a Shimadzu LC system equipped with a SCL-6B controller, two LC-6A pumps and a PDA-UV detector. The analytical column was ODS C_{-18} column operated with a mobile phase consisting of pure water – MeCN (10: 90, v/v). All separations were carried out at 20°C with a flow rate of 1 mL/min.

Immunoaffinity column (IAC) was prepared according to the protocol described in a previously published paper (Kaware et al. 2006). In brief, the mixture of 2 mL TMOS, 0.1 mL MeOH, 0.1 mL of 0.04 M aqueous hydrochloric acid and 0.25 mL water was sonicated under ice-cooling for 30 min. The pre-hydrolysed TMOS was added to 2 mL of phosphate-buffered saline (PBS) containing the isolated IgG fraction (approximate 2 mg antibody). After gelation, the beaker was weighed and stored at room temperature during aging of the gel. The aging process was stopped when the gel had lost 40 $\sim$ 50% of its initial weight. The resulting silica glass was ground in a mortar and packed into a 5 mL syringe equipped with a polytetrafluoroethylene frit. The immunoaffinity column had been flushed in sequence with 20 mL of PBS, 15 mL of ACN/water (30:70, v/v), and 20 mL of PBS. The columns were stored in PBS at 4°C.

The pesticide solution containing 750 or 1,500 ng of diazinon was spiked into 300 mL tap water. Aliquots of 50 mL spiked water sample were streamed though the immunoaffinity column at a flow rate of 1 mL/min by negative pressure. Blank soil samples were spiked with diazinon, and the final concentrations were 2.5 and 5 ng/g. Aliquots of 50 g spiked soil sample were adjusted for natural humidity by 10 mL double-distill water, and the samples were extracted with three portions of ethyl acetate (15 mL and twice 10 mL). The organic phases were collected and evaporated. The residue was redissolved in 1 mL of MeCN. The analyte was eluted with 5 mL 40% MeCN: water (v/v) solution.

A SPE of ENVI-Carb method used in this study was the same as a previously reported method for the pretreatment of samples (Herrera et al. 2005). Prior to the extraction, the ENVI-Carb column (SPE Tube, 6 mL, Supelco, containing 500 mg of the bonded phase) was washed with 10 mL of methanol under vacuum followed by 10 mL of double-distill water. The column was not allowed to dry. The sample was mixed well and allowed to percolate through the column at a flow-rate of 1 mL/min under vacuum. After sample extraction, the pesticide trapped in the column was collected by using 2 $\times$ 5 mL of dichloromethane as eluting solvent. The fractions were evaporated to dryness and the residue was redissolved in 1 mL of acetonitrile.

Results and Discussion

A calibration curve was created by analyzing diazinon standard solutions ranging in concentration from 2 to 1,000 ng/mL. The peak areas of diazinon versus the concentration of the calibration standard solutions were then plotted. The correlation coefficient of the calibration curve was found to be greater than 0.9980, which indicated that the curve could be used to quantify samples that contain diazinon samples. The limit of detection (LOD) was evaluated on the basis of the mass giving a signal equivalent to 3

times that of the noise (S/N = 3). The limit of quantification (LOQ) was evaluated on the basis of the mass giving a signal equivalent to 10 times that of the noise (S/N = 10). A LOD of 2 ng/mL and a LOQ of 7 ng/mL was achieved.

The first set of experiments was designed to evaluate the ability of the ENVI-Carb column and the immunoaffinity column to bind diazinon. To accomplish this, 100 µL aliquots of diazinon standard solution were directly loaded onto the columns. The eluant was then evaluated for the presence of diazinon to determine the recovery from the columns. As shown in Table 1, the recoveries approached 100% (98.01% for the ENVI-Carb column and 85.02% for the immunoaffinity column), which indicates that the two columns could potentially be used to remediate samples that have been contaminated with diazinon.

To compare the efficacy of the ENVI-Carb column and the immunoaffinity column while treating actual samples of water and soil, samples were spiked with diazinon standard solution and the recoveries from these samples were then evaluated. For water samples, the final concentrations of diazinon were 2.5 and 5 ng/mL. The recoveries of diazinon from samples treated using the immunoaffinity column and the ENVI-Carb column are shown in Table 2. When samples that were spiked with 2.5 ng/mL were evaluated, the ENVI-Carb column recovered 91.76% of the diazinon with a CV of 5.18%. However, when the samples were spiked with 5 ng/mL, the ENVI-Carb column only recovered 87.99% of the diazinon. When soil samples that were spiked with 2.5 and 5 ng/g of diazinon were treated using the ENVI-Carb column, the observed recovery was 95.95% and 90.04% with a CV of 6.02% and 7.11%, respectively. These results indicate that the capacity of the ENVI-Carb column to treat soil samples was satisfactory.

The recovery of diazinon from water and soil samples using an immunoaffinity column was also investigated (Table 2). When water samples that were spiked with 2.5 and 5 ng/mL diazinon were treated using the immunoaffinity column, the recovery was 81.58% and 65.3% with a CV of 4.53% and 6.71%, respectively. When soil samples that were spiked with 2.5 and 5 ng/g diazinon were treated using the immunoaffinity column, the recovery was 60.78% and 52.61% with a CV of 13.37% and 16.79%, respectively. These findings indicate that the recovery of diazinon from water and soil samples by the immunoaffinity column was significantly lower than the recovery by the ENVI-Carb column (Table 2).

Taken together, these results indicate that combining a highly selective IAC sample cleanup method with a chromatography system coupled to a high sensitive detector provides no additional advantages over the use of an ENVI-Carb column alone. Therefore, even though the ENVI-Carb method is less selective, it may be more applicable.

In this study, soil samples were cleaned using an ENVI-Carb column and an immunoaffinity column. Figure 2 shows the chromatograms obtained from soil samples that were cleaned using both methods. The chromatogram of the sample prepared without being passed through either column (Fig. 2b) had a large interfering peak at the beginning and a much noisier baseline than the chromatograms of the samples prepared using the ENVI-Carb column and the immunoaffinity column (Fig. 2c, d). In addition, the chromatogram of the sample prepared using the immunoaffinity column was cleaner and smoother than the chromatogram obtained when the sample was treated using the ENVI-Carb column, which demonstrates the high selectivity of the diazinon immunoaffinity column (Fig. 2d).

Table 1 Comparison between ENVI-Carb column and immunoaffinity column in absorbing the diazinon pesticide from standard solution

	Concentration (mg/L)	Loading volume (µL)	Obtained value (average, ng)	Recovery (%)	SD (ng)	C.V. (%)	Limit of detection (µg/mL)	Dimit of quantification (µg/mL)	Linear range (µg/mL)
ENVI-Carb	1	100	98.01	98.01	4.41	4.50	2	7	2–800
IAC	1	100	85.02	85.02	5.54	6.54	2	7	2–800

Table 2 Recoveries and CVs of diazinon from water and soil samples with two kinds of SPE columns

Sample	Spiked	ENVI-Carb		IAC	
		Found (mean, ng)	Recovery & C.V. (mean, %)	Found (mean, ng)	Recovery & C.V. (mean, %)
Water	2.5 ng/mL	114.70	91.76 ± 5.18	101.97	81.58 ± 4.53
	5 ng/mL	219.98	87.99 ± 8.06	163.26	65.3 ± 6.17
Soil	2.5 ng/g	119.93	95.95 ± 6.02	75.98	60.78 ± 13.37
	5 ng/g	225.1	90.04 ± 7.11	131.53	52.61 ± 16.79

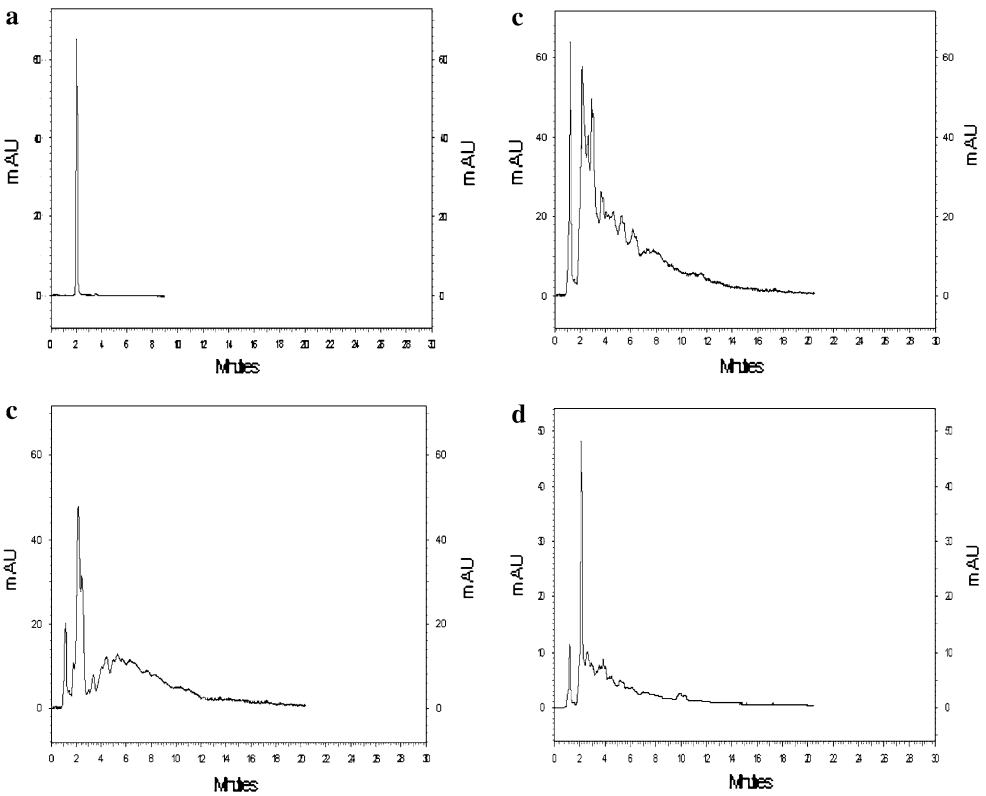

Fig. 2 Chromatograms obtained from **a** diazinon standard solution; **b** spiked soil sample unpurified by columns; **c** spiked soil sample purified by the ENVI-Carb column and **d** spiked soil sample purified by the immunoaffinity column

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