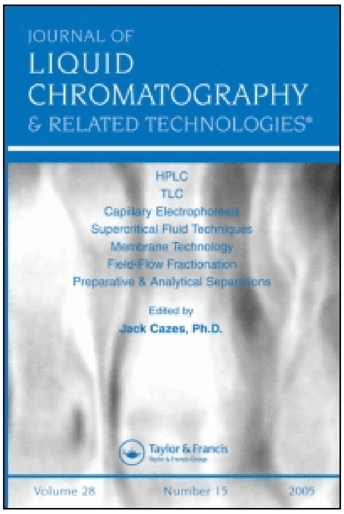

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OPTIMIZATION OF THE SEPARATION, ISOLATION AND RECOVERY OF SELECTED PESTICIDES IN WATER SAMPLES BY SOLID-PHASE EXTRACTION AND HPLC PHOTODIODE ARRAY DETECTION

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ABSTRACT

An optimized HPLC method for the analysis of selected pesticides (Atrazine, Diuron, Dichloran, Methiocarb, Folpet, Triazophos, Vinclozolin, Tetradifon and Carbophenothion) is developed by applying a solvent elution programme with two steps, isocratic and gradient mode, both in reverse phase (RP). In the isocratic step, the mobile phase is optimized by a grid search procedure over an water-acetonitrile-methanol solvent triangle. Off-line solid-phase extraction (SPE) has been combined with HPLC using a photodiode array detector (DAD) to isolate, recover and quantify the selected pesticides from water samples at ppb levels. The recoveries are higher than 75% in all cases except for atrazine in sea water (50%) and ground water (64%).

Table 1. Same propierties and characteristics of the pesticides studied

pesticide	aqueous solubility(mgL^{-1})	Potential to contaminate ground water*	Use in Europe[17]
Atrazine	30	PL	major
Diuron	42	TL	major
Dicloran	7	--	local
Methiocarb	30	TL	major
Folpet	1	IL	major
Triazofos	35	--	local
Vinclozolin	1000	TL	major
Tetradifon	0.34	--	major
Carbophenotion	1	--	local

*GUS classification(17); (PL) probable leacher, (IL) improbable leacher, (TL) transition.

INTRODUCTION

The analysis of pesticides in environmental samples by HPLC technique requires the elution of a wide variety of analytes under approximately optimum conditions. One way to get this objective, is by applying solvent programming or gradient elution techniques (1). On the other hand, HPLC using DAD provides an opportunity for the chromatographer to explore all wavelengths in the UV in order to confirm the presence of tentatively identified peaks and choose the monitoring wavelengths which maximize instrumental sensitivity (2-5) but, unfortunately it does not reach the detection limits (DLs) required (ppb levels). So, combining SPE with HPLC using selective wavelengths programming presents in many cases a good choice to get these requeriments (6-12).

In the present study a rapid, sensitive, and accurate chromatographic procedure for the analysis of nine selected pesticides with very different water solubility and currently used in Europe (Table 1) is developped. The work described proceeded in the following stages: (a) Optimization of the HPLC mobile phase by a grid search procedure (13-16) which gave the best chromatographic resolution and the least time for analysis; (b) determining the linearity, reproducibility and recoveries of the analytical method; (c) evaluating perfomance of the analytical method on different spiked samples with selected target compounds and real -drinking, sea and ground-water samples of Almería (Spain), an area concerned by the use of agrochemicals.

EXPERIMENTAL

Instrumentation

The Waters (Mildford, Massachussets) HPLC system includes a 600 E pump; a Rheodine six-part injection valve with 20 μL loop; a 990 photodiode array detector; a printer/plotter and an Olivetti microcomputer using a 991 software. HPLC separations were conducted using a Nova-Pack 3x150 mm (4 μm particle size) C_{18} column (Waters)

Prepacked cartridges Sep-Pack plus 360 mg C_{18} chemically bonded silica were used (Waters)

Chemicals and solvents

HPLC-grade solvents were used in this work. Mobile phases were degassed with helium prior to use. Distilled water was obtained from a Millipore water purification Milli-Q system. The pesticide standards -pestanal quality- summarized in Table 1 were obtained from Riedel-de Haën (Seelze, German). Solid standars were dissolved in AcN and diluted in this solvent. All solvents and samples were filtered through Millipore membrane filters before injection in the column

HPLC operating conditions

Flow rate 1 mL min^{-1}; chart speed 0.5 cm min^{-1}; Detector sensitivity 0.02 AUFS; Column at ambient temperature; Wavelengths 212, 220, 250 and 350 nm.

Solvent programming

The solvent rogramming was as follows (elapsed time, composition of mobile phase); initial, 56 % Water, 27 % AcN and 17 % MeOH; 2 minutes, 56 % Water, 27 % AcN and 17 % MeOH ; 15 minutes, 5 % Water, 90% AcN and 5% MeOH ; 20 minutes, 5 % Water, 90% AcN and 5% MeOH. Changes in the percentage of organic solvent in the mobile phase throught the gradient program ocurred linearly . An additional time of 10 min. of gradient program was enough to return the system to the initial conditions and enable for another analysis run.

Sample Pre-concentration

The 360 mg C_{18} Sep-Pack plus cartridges were conditioned with 5 mL of AcN followed by 5 mL of ultrapure water without allowing the cartridges to dry out. 400 mL of water sample was filtered out through a 0.45 μm filter, connected with PTFE tubes to the conditioned cartridges and passed through of them, at a rate of 8-10 mL min^{-1} and then sucked dry for 5 min The sample thus, concentrated were eluted with 2 mL of AcN. The extract was evaporated to 0.5±0.1 mL and diluted to 1 mL with AcN:Water (50:50) and then 20 μL were injected, so 5% of total.

For adsorption, elution and recoveries studies spiked ultrapure and real water samples with 1 μg L^{-1} of each selected pesticide were carried out.

RESULTS AND DISCUSSION

Optimization of the mobile phase

Preliminary gradient runs using AcN as organic modifier show chromatograms with an adequate resolution for vinclozolin, tetradifon and carbophenothion. However, it is not possible to separate the other six pesticides because of the closeness of their retention times.

Isocratic mode for atrazine, diuron, dichloran, methiocarb, folpet and triazophos separation was chosen. The optimum solvent composition in this isocratic form was carried out by a grid of experimental conditions over an water-acetonitrile-methanol

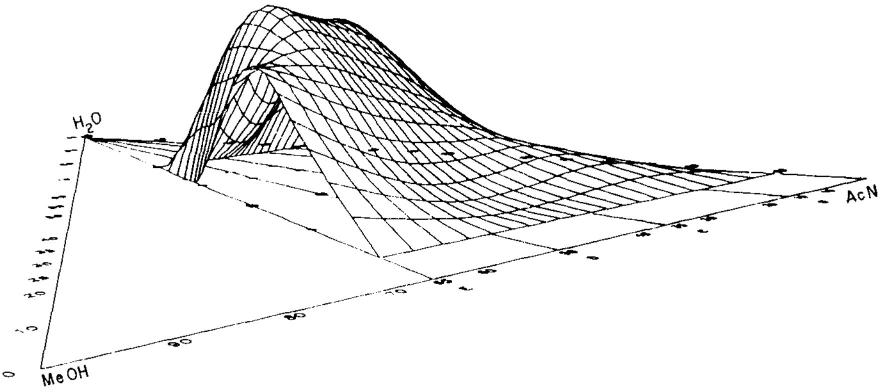

Figure 1. Response surface obtained by the present study.

solvent triangle (100% AcN, 100% MeOH and 100% water as vertexes). In order to draw the response surface and to avoid local optima were carried out chromatograms corresponding to 30 mobile phases of different composition on the triangular variable space.

The selection of an objective function (OF) as response function was based on two responses, resolution (Rs) and analysis time by applying criteria given by Deming (16). This function is defined as:

$OF = \Sigma R_S + p_1 + p_2$

where

$p_1 = 0$ for $y_i > y_{it}$; $p_1 = -\infty$ for $y_i < y_{it}$

$p_2 = 0$ for $y_i < y_{it}$; $p_2 = -\infty$ for $y_i > y_{it}$

R_S is the resolution between adjacent peaks .(R_S, is limited to a maximum value of 1.5 to avoid that ΣR_S is determined largely by the largest values of R_S) and p is the value of the penalty. y_{it}, and y_{jt} are the threshold retention time values associated to 4 and 15 minutes; yi (initial peak: atrazine) and yj (last peak: triazophos) as acceptable retention times of the initial and last peak.

The different values of OF obtained corresponding to the grid search chosen let to the graphic representation of the response surface (Figure 1). Two different zones with

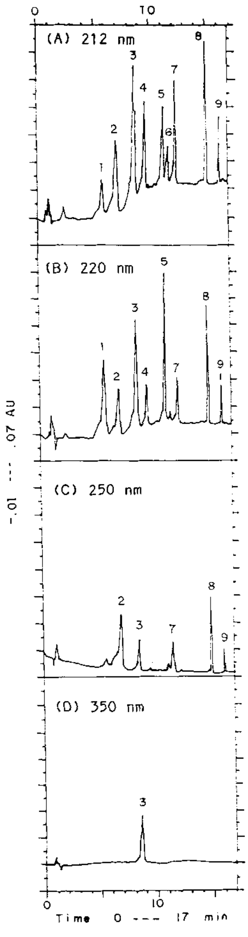

Figure 2. LC chromatograms obtained by injection of 20 μL of standard solution sample in concentration of 5 $\mu g L^{-1}$ of each pesticide. (A) 212 nm,(B) 220 nm,(C) 250 nm (D) 350 nm.Numbers above the peaks correspond with those given in Table 2 (see experimental for further details)

Table 2. Retention times (RT), average recoveries (AV) and coefficients of variation(C.V.,n=5) both in % of test compounds using off-line C_{18} extraction cartridges in environmental waters. Spiking level: 1 μgL^{-1} .Water volume 400 mL .

Pesticide	Peak	RT	Milli-Q		Drinking		Sea	
	n°	(min)	AV.	C.V.	AV.	C.V.	AV.	C.V.
Atrazine	1	5.0	85	5.0	64	5.8	50	8.3
Diuron	2	6.5	100	4.5	101	3.9	111	6.5
Dicloran	3	8.3	108	2.1	110	2.2	105	2.2
Methiocarb	4	9.3	99	2.1	95	2.2	90	3.1
Folpet	5	11.1	99.	2.5	98	2.4	75	2.8
Triazofos	6	11.6	88	2.4	80	1.4	85	6.0
Vinclozolin	7	12.4	90	2.8	78	6.0	82	2.4
Tetradifon	8	15.3	95	2.2	81	2.5	80	4.2
Carbophenothion	9	16.5	80.	5.1	75	4.5	76	7.7

high response can be seen. The first partial optimum is placed at low values of %MeOH whereas the global optimum correspond to the central zone of the solvent triangle. A solvent mixture of 56% water, 27% AcN and 17% MeOH was chosen as the best mobile phase for the HPLC separation of these six pesticides

The use of DAD detection allowed the selection of the wavelength used for the determination of each pesticide in the analytical procedure in order to get improvement of the sensitivity and selectivity. These wavelengths are 212, 220, 250 and 350 nm. In Figure 2 an example of an chromatogram of pestice mixture is shown, the retention behaviour is summarized in Table 2.

The quantitation of peaks are carried out by the external standar method, using measurements of peak areas and calibration curve for each pesticide. The linear dynamic range of the detector response has been checked for the nine selected pesticides at the wavelength of 212 nm are ranged between 3 - 60 ng for Dichloran, Methiocarb, Vinclozolin, Tetradifon and Carbophenothion: 5-60 ng for Atrazine, Diuron, Folpet and Triazophos. The correlation coefficients are higher than 0.995 in all cases.

Trace enrichment

As mentioned before, considerable trace enrichment is necessary to obtain detection limits of ppb levels. A gain of about three orders in sensitivity should be obtainable by trace enrichment. To evaluate the potential of trace enrichment of all test compounds, samples of spiked (1 μg L^{-1}) ultrapure water were analyzed. The analyses were done in duplicate.

A flow rate of 8-10 mL min^{-1} through the cartridge was found to be optimum. At a flow rate 20 mL min^{-1} breaktrhough of the earling eluting compounds (Atrazine, Diuron) was evident. The breaktrough volume was stablished in 400 mL on the average. AcN (2mL) was chosen as solvent for analytes elution due to its suitability for the RP-HPLC system. The calibration curves between 0.5 and 15.6 μg L^{-1} were linear (the correlation coefficients are higher than 0.991 in all cases). The detection limits are 0.3, 1.0, 0.2, 0.3, 0.2, 0.1, 0.1 and 0.2 μg L^{-1} for Atrazine, Diuron, Dichloran, Methiocarb, Folpet, Triazophos, Vinclozolin, Tetradifon and Carbophenothion respectively, at a ratio signal to noise equal 3. The recoveries of the pesticides were higher than 85% in all cases. The repeatability in terms of areas at various concentrations was studied using the conditions above described. The data obtained for 1 μg L^{-1} were fully satisfactory in all cases with a coefficient of variation (CV) ranged between 2.2-5.0%.

Method Perfomance

The proposed method was assesed for the analysis of spiked real water samples in order to to observe the effect of the matrix in the recoveries, separation and interfering peaks. Operating on drinking, ground and sea water at a spiking level of 1 μg L^{-1}, the recoveries of three pesticides (Diuron, Dichloran and Methiocarb) are higher than 90% with a CV from 2.1-6.6%. Five of them (Folpet Triazophos, Vinclozolin, Tetradifon and Carbophenothion) have a recovery ranged between 75-90% with a CV range of

Table 3. Analysis of environmental water samples

Sample	Pesticide (μg L^{-1})
drinking - water	--
sea- water	--
ground - water 1	methiocarb (0.2) atrazine (1.4) carbophenotion (1.2)
ground - water 2	atrazine (2.7)
ground - water 3	methiocarb (0.2)
ground - water 4	--
ground - water 5	atrazine (2.7)

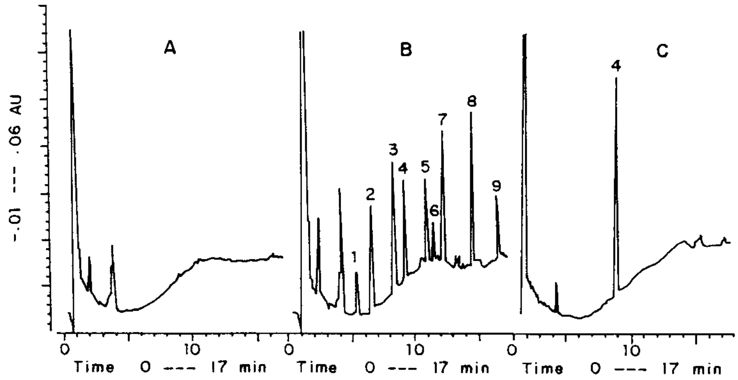

Figure 3. Typical chromatograms at 212 nm obtained from ground water SPE extract. A; No contaminated ground water. B; spiked ground water with 2 mL of pesticide standar solution in concentration of 1 μL-1 of each pesticide. C; Contaminated ground water (sample number 3, Table 3).

1,5-7.7% and most polar pesticide, Atrazine was only recovered between 50% (sea water) to 85% (drinking water) with CV of 8.3 and 5.0% respectively (Table 2).

Analysis of pesticides in environmental water samples

The proposed method was applied to determine pesticide levels in drinking, sea and ground waters of Almería (southeastern of Spain) located in an area of great development of greenhouses and the results are shown in Table 3. An example of the chromatogram corresponding to one of the analyzed samples can be seen in Figure 3. The pesticides detected in a higher number of samples were Methiocarb and Atrazine. The chromatograms corresponding to the samples of drinking and sea water did not show any identificable peak

CONCLUSIONS

An off-line SPE system based on the use of C18 bonded silica cartridges has been developped for the preconcentration of nine pesticides from environmental waters followed by LC-UV diode array analysis. The resolution and sample throughput has been increased considerabily due to the optimization of the chromatographic procedure. The repeatability and senstivity both in terms of qualitative and quantitative analysis is quite acceptable. The perfomance of the total analytical procedure has been evaluated on three types of waters, ground, drinking and sea water. The method is applied to the determiation of pesticides on real water samples.

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