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Short communication

New design for the on-line solid-phase extraction of pesticides using membrane extraction disk material and liquid chromatography in environmental waters

A. Martín-Esteban, P. Fernández, C. Cámara*

Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain

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Abstract

A new design consisting of a microcolumn (10 cm×4.6 mm I.D.) filled with a spiral of C_{18} Empore disk material for the on-line trace enrichment of polar pesticides in environmental waters is described. The breakthrough volumes obtained were three times greater than those obtained using ten small Empore disks (4.6 mm I.D.) and were comparable to the ones obtained using the same dimensions of PRP-1 or PLRP-S precolumns. Samples were spiked with 0.1–1.0 $\mu g\ l^{-1}$ of pesticides (in drinking, ground and sea water) and were preconcentrated using solid-phase extraction onto spiral C_{18} Empore disk material precolumns followed by liquid chromatography–diode array detection. This approach allowed the determination of all pesticides studied in water at the concentration level required in Europe.

Keywords: Preconcentration; Pesticides

1. Introduction

Several pesticides are widely used throughout the world, so that these compounds, depending of their physicochemical properties [1], can migrate into surface, ground and finally into drinking water and could be degraded by different mechanisms [2]. In this respect, the EEC Directive on the Quality of Water Intended for Human Consumption allows a maximum concentration for total pesticides in drinking water of 0.5 $\mu g\ l^{-1}$ and a concentration of 0.1 $\mu g\ l^{-1}$ for individual pesticides and related compounds [3].

Consequently, it is neccesary to develop analytical methodologies which usually require an enrichment step. In the last few years, solid phase extraction (SPE) has been widely employed for the preconcentration of a variety of pesticides, using either off-line or on-line methodologies. Off-line SPE methodologies involve the use of packing materials that may contain functional groups of different polarity, such as C_8- and C_{18}-bonded silica phases [4–6], graphited carbon black [7–10] and Empore extraction disks [11–14]. In on-line SPE studies followed by LC, the most important parameter is the selection of an adequate precolumn in order to avoid band broadening of the first eluted peaks and to allow the percolation of large sample volumes. C_8- or C_{18}-bonded silica [15,16], PRP-1 or PLRP-S [17–20] and Empore extraction disks [21–24] have been employed, but in some cases the breakthrough volumes

*Corresponding author.

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of the most polar pesticides are very low, making detection, at the level required in Europe, difficult.

Recently, Van der Wal et al. [24] developed a special holder that can contain up to 25 small disks. This device would allow one to improve the breakthrough volumes, since the amount of C_{18} material is increased up to about 120 mg, but it would be necessary to use a low flow-rate (the above-mentioned authors use a flow-rate of 2 ml min^{-1} employing only seventeen disks) in order to avoid a pressure that is too high and which increases the preconcentration time.

Increasing the amount of sorbent material placed in the precolumn [25] or adding salt to the sample [26] could improve the breakthrough volumes. Therefore, the aim of this work is to develop an on-line SPE method using a new design precolumn employing C_{18} Empore extraction disk material followed by liquid chromatography–diode array detection (LC–DAD) for the determination of desisopropylatrazine, desethylatrazine, hydroxyatrazine, simazine, cyanazine, atrazine, propanil, linuron and fenamiphos.

2. Experimental

2.1. Chemicals

High purity Milli-Q water (Millipore) and RS-grade acetonitrile (Carlo Erba) were passed through a 0.45-μm nylon filter (Whatman) before use. Desisopropylatrazine, desethylatrazine, hydroxyatrazine, simazine, cyanacine, atrazine, propanil, linuron and fenamiphos were obtained from Riedel-de-Häen. Stock standard solutions were prepared in acetonitrile and stored at −20°C in the dark.

Highly pure NaCl and KH_2PO_4 were purchased from Merck.

2.2. Chromatographic conditions

Eluent delivery was provided by a ConstaMetric 4100 Series high pressure pump, from ThermoSeparations Products, coupled with a SpectroMonitor 5000 photo diode-array detector from LDC Analytical. The analytical column was a 10 cm×4.6 mm I.D. packed Hypersil 5 μm ODS from Phenomenex.

Gradient elution was as follows; from 100% A (0.002 mol l^{-1} KH_2PO_4 buffer, pH=7) and 0% B (acetonitrile) to 90% A–10% B in 5 min; from 90% A–10% B to 50% A–50% B in 40 min and returning to the initial conditons in 10 min at a flow-rate of 1 ml min^{-1}.

Quantitative measurements of peak areas by LC–UV at 215 nm (desisopropylatrazine and desethylatrazine), 220 nm (hydroxyatrazine, simazine, cyanazine and atrazine) and 250 nm (propanil, linuron and fenamiphos) were carried out. These wavelenghts were choosen in order to get the best selectivity and sensitivity.

2.3. Preconcentration conditions

C_{18} Empore extraction disks (47 mm diameter) were obtained from J.T. Baker. A stainless-steel precolumn (10×4.6 mm I.D.) was filled with a spiral of Empore disk (Fig. 1) or with ten small Empore disks (4.6 mm diameter) and was stoppered with steel frits. The precolumn was placed in a cartridge

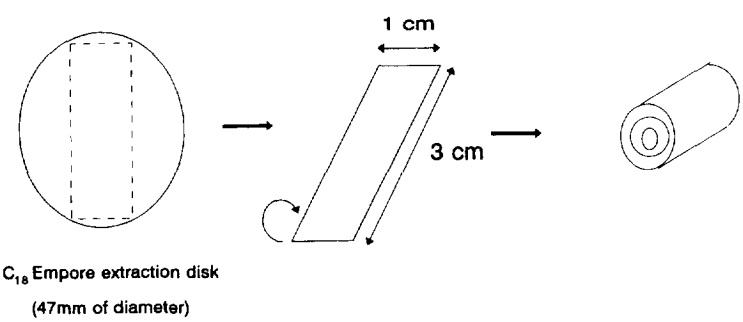

Fig. 1. Design of the preconcentration precolumn using C_{18} Empore extraction disk material.

holder (Jones Chromatography) and coupled to the loop of a Rheodyne 7725i injection valve in order to carry out the extraction and enrichment steps.

The preconcentration pump was a Waters 590 from Millipore.

2.4. Procedure

The SPE precolumn was conditioned by flushing it with 10 ml of acetonitrile and then with 10 ml of Milli-Q water at 1 ml min^{-1}. Water sample (60 ml; pH=6–7), containing 10% (w/v) NaCl, spiked with pesticides at concentrations of between 0.1–1.0 $\mu g\ l^{-1}$ were filtered on a 0.45-μm nylon filter (Whatman) and then preconcentrated on the precolumn followed by a washing step with 8 ml of Milli-Q water at a flow-rate of 4 ml min^{-1}. Then, the injection valve was switched and the analytes were eluted in back-flush mode and separated in the analytical column.

After each analysis the SPE precolumn was cleaned by flushing it with 10 ml of acetonitrile, followed by 10 ml of Milli-Q water at 1 ml min^{-1}.

The precolumn was changed after fifteen preconcentration steps, each using 60 ml of sample.

3. Results and discussion

3.1. Breakthrough volumes

The effect of the sample volume was studied using different volumes of Milli-Q water fortified with pesticides at the 0.2–10 $\mu g\ l^{-1}$ level, each one containing 80 ng of the analytes on two different precolumns of C_{18} Empore extraction disk material. The recoveries were calculated by the ratio of peak areas obtained when volumes that were lower and higher than the breakthrough volume were used.

Table 1 shows the breakthrough volumes obtained for all pesticides studied. Using the new design (spiral of C_{18} Empore disk material), the breakthrough volumes obtained for all pesticides were improved with respect to those obtained using a precolumn filled with ten small Empore disks. This behaviour can be explained because the amount of C_{18} material in the spiral precolumn is about 87 mg while that in ten small Empore disks is about 48 mg.

3.2. Salting-out effect

It has been demonstrated that the addition of NaCl to the sample can improve the breakthrough volumes of some analytes [26]. Therefore, (10% w/v) NaCl was added to the samples and the breakthrough volumes of all pesticides studied are shown in the Table 1.

Under these conditions, using a spiral C_{18} Empore disk material precolumn and adding 10% (w/v) NaCl to the sample, the breakthrough volumes of the most polar analytes are improved, allowing up to 60 ml of sample to be percolated. These breakthrough volumes are comparable with those obtained using a precolumn packed with PRP-1 [19] and PLRP-S [20].

Table 1
Breakthrough volumes (ml) of pesticides obtained by on-line SPE with different C_{18} Empore extraction disk material precolumns in Milli-Q water

Compound	Ten Empore disks	Spiral of C_{18} Empore material[a]	Spiral of C_{18} Empore material[b]
Desisopropylatrazine	17	30	60
Desethylatrazine	40	80	150
Hydroxyatrazine	40	80	160
Simazine	125	>300	>300
Cyanazine	125	>300	>300
Atrazine	170	>300	>300
Propanil	>200	>300	>300
Linuron	>200	>300	>300
Fenamiphos	>200	>300	>300

[a] Without salt addition to the sample.
[b] With the addition of 10% (w/v) NaCl to the sample.

3.3. Analytical performance and applications

The method has been applied to the determination of the above-mentioned pesticides in different matrices; drinking water, ground water (irrigated land water, Madrid, Spain) and sea water (Mediterranean Sea, Alicante, Spain).

The recoveries for all pesticides tested in drinking, ground and sea water by on-line SPE using a precolumn of spiral C_{18} Empore extraction disk material and a 60-ml water sample spiked with 0.2 $\mu g\ l^{-1}$ of each pesticide and containig 10% (w/v) NaCl were within 78–109%, with a relative standard deviation (R.S.D.) <10% (n=3). These values are acceptable, confirming a good repeatability of the method except for desisopropylatrazine, which was not recovered in ground and sea water, and desethyl- and hydroxyatrazine in sea water, where the recoveries were 38 and 32%, respectively, due to a broad interfering matrix peak. The recovery for cyanazine in ground water was 123%, due to a non-specific interference in the chromatograms.

Equations of analytical calibration graphs for all analytes in spiked drinking, ground and sea water were within the range 0.1–1 $\mu g\ l^{-1}$ [using the same experimental conditions with a sample volume of 60 ml (without salt addition for sea water)] and are shown in Table 2.

Fig. 2 shows the chromatogram obtained in the on-line preconcentration of 60 ml of drinking water at 220 and 250 nm at the 0.2 $\mu g\ l^{-1}$ level of each pesticide.

Fig. 3 shows the chromatogram obtained in the on-line preconcentration of 60 ml of unspiked ground water. Peaks corresponding to the retention times of hydroxyatrazine and cyanazine were detected. DAD identified hydroxyatrazine, but not cyanazine, by comparing the UV spectra for hydroxyatrazine (Fig. 3A) and cyanazine (Fig. 3B) of the sample and of a reference standard. The concentration of hydroxyatrazine in ground water was calculated using the standard addition method, giving a concentration of 0.64±0.04 $\mu g\ l^{-1}$.

Fig. 4 shows the chromatogram obtained in the on-line preconcentration of 60 ml of sea water spiked at the 0.5 $\mu g\ l^{-1}$ level of each pesticide at 220 nm. It is observed that the background level is too high due to elution of dissolved natural organic matter, mak-

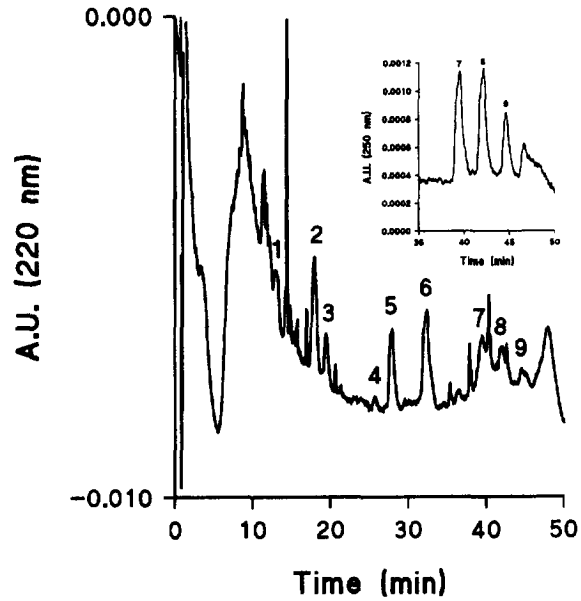

Fig. 2. Chromatograms obtained at 220 and 250 nm after on-line preconcentration of 60 ml of drinking water containing 10% (w/v) NaCl and spiked with 0.2 $\mu g\ l^{-1}$ of pesticides. Peak numbers: (1) Desisopropylatrazine; (2) desethylatrazine; (3) hydroxyatrazine; (4) simazine; (5) cyanazine; (6) atrazine; (7) propanil; (8) linuron; (9) fenamiphos. For LC conditions see Section 2.

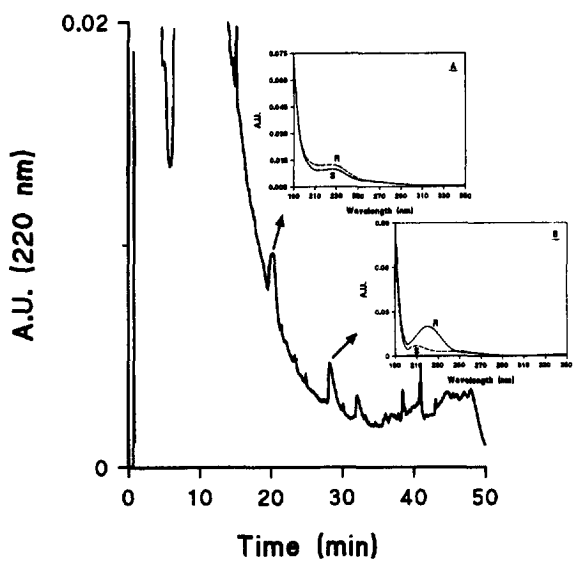

Fig. 3. Chromatogram obtained at 220 nm after on-line preconcentration of 60 ml of unspiked ground water with 10% (w/v) NaCl added. For LC conditions see Section 2. Comparasion of reference standard and unknown peak spectra for (A) hydroxyatrazine and (B) cyanazine. S refers to the sample and R to the reference.

Table 2
Calibration data for pesticide analysis (0.1–1 $\mu g\ l^{-1}$) after preconcentration of 60 ml of environmental water (10% (w/v) NaCl) in on-line SPE on a spiral C_{18} Empore extraction disk material precolumn

Compound	Wavelength (nm)	Drinking water		Ground water		Sea water[a]	
		Calibration equation[b]	R^2	Calibration equation[b]	R^2	Calibration equation[b]	R^2
Desisopropylatrazine	215	$y=-693+210\ 110x$	0.9996	–	–	–	–
Desethylatrazine	215	$y=3987+486\ 960x$	0.9994	$y=-3676+198\ 080x$	0.9967	$y=-10\ 916+277\ 439x$	0.9897
Hydroxyatrazine	220	$y=-924+182\ 123x$	0.9990	$y=96\ 612+145\ 162x$	0.9900	$y=-319+254\ 053x$	0.9999
Simazine	220	$y=-607+41\ 837x$	0.9935	$y=-2273+68\ 724x$	0.9905	$y=-3260+388\ 033x$	0.9998
Cyanazine	220	$y=-352+357\ 776x$	0.9998	–	–	$y=-6983+315\ 814x$	0.9989
Atrazine	220	$y=9270+445\ 137x$	0.9960	$y=-3634+208\ 702x$	0.9971	$y=9880+504\ 533x$	0.9971
Propanil	250	$y=-1564+208\ 735x$	0.9985	$y=1565+92\ 925x$	0.9949	$y=-2982+201\ 243x$	0.9992
Linuron	250	$y=-1096+199\ 798x$	0.9976	$y=-649+78\ 216x$	0.9994	$y=-9010+209\ 789x$	0.9958
Fenamiphos	250	$y=1044+88\ 140x$	0.9937	$y=-927+77\ 288x$	0.9974	$y=1396+216\ 626x$	0.9980

[a]Without salt addition.
[b]y=peak area; x=concentration ($\mu g\ l^{-1}$).

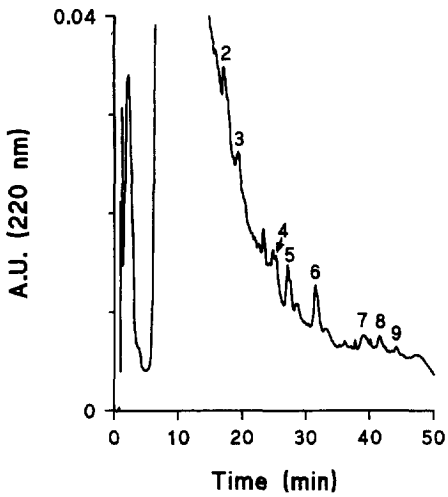

Fig. 4. Chromatogram obtained at 220 nm after on-line preconcentration of 60 ml of sea water spiked at the 0.5 $\mu g\ l^{-1}$ level with each pesticide. For peak numbers, see Fig. 2 and for LC conditions, see Section 2.

ing it difficult to obtain very low detection limits, but it is sufficient to suggest possible environmental contamination.

Table 3 shows the detection limits, calculated as three times the standard deviation of low concentrations, which are within 0.01–0.2 $\mu g\ l^{-1}$, depending on the pesticide and the type of water used. These detection limits are in agreement with the required level for the control of water in Europe.

4. Conclusions

It has been demostrated that the use of a precolumn filled with a spiral of C_{18} Empore extraction disk material and that the NaCl "salting-out" effect allow the on-line preconcentration of water samples of up to 60 ml, without exceeding the breakthrough volumes of the most polar analytes, desisopropylatrazine, desethylatrazine and hydroxyatrazine and also allow the simultaneous determination of pesticides with different chemical functionalities using LC–DAD. This proposed design allows increasing breakthrough volumes for all the pesticides tested. Furthermore, problems were not encountered using a flow-rate of 4 ml min^{-1} and this method could be an alternative to using PRP-1 and PLRP-S precolumns.

Table 3
Detection limits ($\mu g\ l^{-1}$) obtained for pesticides in different water samples using the on-line preconcentration method

Compound	Detection limit[a] ($\mu g\ l^{-1}$)		
	Drinking water	Ground water	Sea water
Desisopropylatrazine	0.04	n.d.	n.d.
Desethylatrazine	0.01	0.07	0.2
Hydroxyatrazine	0.05	0.06	0.14
Simazine	0.05	0.1	0.1
Cyanazine	0.06	n.d.	0.1
Atrazine	0.05	0.1	0.1
Propanil	0.04	0.05	0.1
Linuron	0.04	0.1	0.15
Fenamiphos	0.04	0.1	0.1

[a]Calculated as three times the standard deviation for the low concentrations.
n.d., not detectable.

The proposed method was successfully applied to the determination of desisopropylatrazine, desethylatrazine, hydroxyatrazine, simazine, cyanazine, atrazine, propanil, linuron and fenamiphos in drinking (at the maximum level allowed in Europe), ground and sea water.

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