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Short Communication

Determination of carbaryl and some organophosphorus pesticides in drinking water using on-line liquid chromatographic preconcentration techniques

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ABSTRACT

Reversed-phase high-performance liquid chromatography (HPLC) was adapted for the determination of trace concentrations of carbaryl and seven organophosphorus pesticides in drinking water. Between 100 and 300 ml of water sample is passed through a 1.5-cm precolumn, packed with C_{18}-bonded silica or styrene–divinylbenzene copolymer (PRP-1) sorbent at a flow-rate of 3 ml/min. The HPLC system is then switched to an acetonitrile–water gradient elution programme. The analytes that have been concentrated on the precolumn are eluted and separated on a 15-cm C_{18} analytical column and are determined by measuring their UV absorption at 254 nm. This wavelength was selected as the optimum for the simultaneous determination of these pesticides. The preconcentration yields of the examined solutes obtained with the two types of precolumn are almost identical. Band broadening is avoided by a suitable choice of the C_{18} precolumn and the analytical column. With 200 ml of tap water, the recoveries for most of the examined pesticides were *ca.* 90%, except for carbaryl (54%). The detection limits are in the range 0.03–0.2 μg/l.

INTRODUCTION

Organophosphorus pesticides (OPPs) are currently used in agriculture and animal husbandry for crop protection and control of ectoparasites. However, although the OPPs are generally used alone, they may also be applied in conjunction with the carbamate insecticide carbaryl for the control of pests showing resistance to OP compounds [1,2]. Because of their widespread use, they have been found in groundwaters, surface

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waters, lagoons and drinking water at concentrations varying from 20 ng/l up to 127 μg/l [3–7]. There is an increasing need for rapid, reliable methods to measure pesticide concentrations in waters.

Determinations of OPPs are generally carried out by gas chromatography (GC) with nitrogen–phosphorus detection (NPD) [3,8,9] or flame photometric detection (FPD) [10,11]. However, these methods are inapplicable to carbamates, which are too thermally labile. Azinphos-methyl, parathion and some polar OPPs are also difficult to determine by GC [12]. The use of liquid chromatographic (LC) methods is suitable for thermally labile and polar pesticides. Nevertheless, it should be taken into account that UV detection in LC is usually at least 2.5 orders of magnitude less sensitive than GC–FPD and GC–NPD [13]. This has led to the development of postcolumn reactions to enhance detection in LC. The great potential of postcolumn LC systems has recently been demonstrated [2,14–16]. Postcolumn reaction detection has been used for the trace determination of N-methylcarbamates in surface water samples [17]. Moreover, the monitoring of these compounds and OPPs at concentrations lower than the ppb (μg/l) level requires a trace enrichment step. Sample preconcentration based on solid-phase extraction (SPE) has been shown to be a good alternative to time-consuming liquid–liquid extraction and can be used in both off-line and on-line GC and LC methodologies. Several methods using off-line SPE have been developed for the LC–UV determination of selected carbamates and OPPs in aqueous samples [18–23]. However, disadvantages and problems still remain, such as sample/analyte dilution, possible contamination and lengthy sample preparation procedures. Many of these drawbacks can be avoided by using on-line enrichment on a precolumn packed with a suitable sorbent. In this case, the adsorbed analytes are then eluted directly from the precolumn into the analytical column. This technique has been used in the determination of many organic pollutants in aqueous samples [24,25], such as chlorophenoxy acid [26,27], chlorotriazine [28–30] and organochlorine [31], carbamate [32] and OP [33] pesticides. In the last instance, the precolumn used was packed with XAD-2 resin. However, the disadvantage of XAD resins is the generation of artifacts that are subsequently laborious to eliminate.

In this paper, we report the development of an HPLC method using on-line enrichment for the determination of carbaryl and seven OPPs in drinking water samples. The parameters investigated included analytical LC separation, the optimum wavelength for the simultaneous determination of these pesticides, the rate of sample loading on to the precolumn, the nature of the sorbent used in the precolumn and the dependence of the recovery on the sample volume. The eight pesticides chosen are of concern for the Tunisian environment [34–36].

EXPERIMENTAL

Solvents

HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, UK) and methanol from Prolabo (Paris, France). LC-quality water was prepared by purifying demineralized water with a Milli-Q filtration system (Millipore, Bedford, MA, USA).

Pesticides

The pesticide standards (Fig. 1) were purchased from several suppliers: parathion, parathion-methyl and azinphos-ethyl from Fluka (Buchs, Switzerland), azinphos-methyl and carbaryl from Serva (Heidelberg, Germany), diazinon from Supelco (Bellefonte, PA, USA) and phosmet and fenitrothion from Société Tunisienne des Engrais Chimiques (Mégrine, Tunisia). The purities of the individual standards ranged from 97.5 to 99.5%.

Standard preparation

Stock solutions of selected pesticides were prepared by weighting and dissolution in methanol. Milli-Q-purified water samples were spiked with these solutions at the ppb (μg/l) level. The final standard solutions did not contain more than 0.5% of methanol.

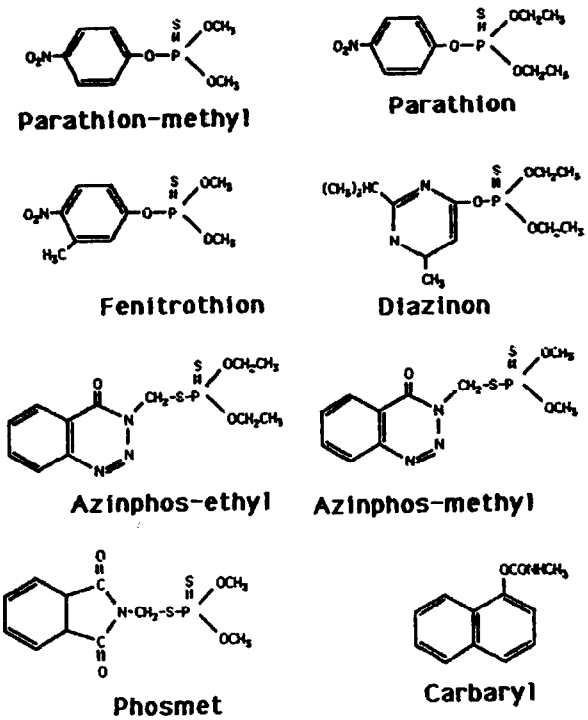

Fig. 1. Structures of the selected pesticides.

Apparatus

Precolumn elution and analysis were carried out with a Varian (Palo Alto, CA, USA) Model 5000 liquid chromatograph equipped with an Applied Biosystems (Ramsey, NJ, USA) Spectroflow 757 variable-wavelength UV detector. An auxiliary Varian Model 2010 pump was used to deliver the sample to the precolumn. Precolumn and analytical column switching was effected via a Rheodyne (Berkeley, CA, USA) Model 7010 valve. Chromatograms were recorded with a Servotrace recorder (Sefram, Paris, France).

Stationary phases and columns

The analytical column was a 150×4.6 mm I.D. stainless-steel column prepacked with 5-μm nucleosil C_{18} octadecylsilica (Macherey–Nagel, Düren, Germany). Samples were preconcentrated on a 10×2.1 mm I.D. stainless-steel precolumn prepacked with 5-μm RP-18 octadecylsilica (Merck, Darmstadt, Germany) or a 15×3.2 mm I.D. stainless-steel precolumn prepacked with 7-μm PRP polystyrene–divinylbenzene copolymer (Brownlee Columns, Applied Biosystems).

Preconcentration step

The sample loop of the injection valve (Fig. 2) was replaced with the RP-18 or PRP-1 precolumn. An auxiliary pump was used to deliver the sample solution to the precolumn via the waste vent line in the injection valve. The sample amount delivered was calculated as outlined previously. As the injector is in the "Load" position (Fig. 2), the effluent passes directly out of the valve into a waste container. Simultaneously, eluent from the reservoirs is being delivered via the other loop path to the analytical column and maintains this column in an equilibrated condition. Before each preconcentration, the precolumn was equilibrated with 10 ml of pure acetonitrile and 10 ml of LC-grade water at pH 7.

Separation step

Once the desired sample volume has been enriched, the injection valve is switched to "inject" and, simultaneously, the selected gradient is initiated, directing the eluent flow through the precolumn in a back-flush elution model. As the sample is eluted from the precolumn it enters the analytical column for completion of the separation step.

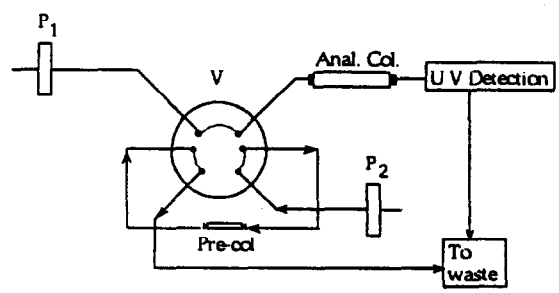

Fig. 2. Experimental set-up for the on-line preconcentration and analysis of water samples. P_1, P_2 = pumps; and V = valve; Anal. Col. = analytical column; Pre-col. = precolumn. During the preconcentration step V is in the "Load" position and P_2 delivers samples. During the analysis V is in the "Inject" position and P_1 delivers mobile phase.

RESULTS AND DISCUSSION

Analytical separation and detection

The pesticides studied (Fig. 1) included a carbamate and two OP groups, namely phosphorothionates and phosphorodithioates. The reversed-phase LC analysis of these compounds is usually performed using C_8- or C_{18}-bonded silicas [1,2,21,32,37,38]. When using a water–acetonitrile eluent, the OP pesticide separation is achieved in a reasonable time and without peak broadening if the acetonitrile concentration is greater than 50%. However, the retention of carbaryl is low.

This work started with the setting of a mobile phase gradient suitable for both the carbaryl and the OP pesticides previously sorbed on a precolumn. The gradient slope is then an important factor. In fact, when the water–acetonitrile gradient is steep, the retention time of carbaryl is short hence interference problems arise with co-extracted semi-polar compounds sorbed on the precolumn during the enrichment step; further, when the water–acetonitrile gradient is gentle, the risk of band broadening increases, especially when the precolumn sorbent is more hydrophobic than the analytical column packing.

With the gradients adopted, a fair resolution was achieved for the OP pesticides but the carbaryl retention was delayed. Table I gives the retention times of the pesticides for one of the two gradients selected.

The pesticides studied display maximum absorption bands at various wavelengths. However, these products display absorption bands between 250 and 300 nm. For our analytical conditions, 254 nm appears to be an acceptable compromise as we obtained similar responses for all the pesticides except phosmet, which exhibits a relatively small response. The instrumental detection limit (signal-to-noise ratio = 3:1) of phosmet is 3.5 ng (Table I). For the other insecticides it is in the range 0.6–1.5 ng. Hence the technique is less suitable for phosmet.

On-line preconcentration conditions

The on-line preconcentration permits the study of all the trapped compounds on the precolumn and also a reduction in the volume of the examined water samples and an improve-

TABLE I

RETENTION TIMES, LIMITS OF DETECTION (LOD) IN LC–UV AND DEPENDENCE OF RECOVERIES ON PERCOLATED SAMPLE VOLUME OF PESTICIDES STUDIED

Mobile phase, acetonitrile–water; gradient, 40% to 60% of acetonitrile in 15 min. UV detection at 254 nm. Recoveries were based on the averages of two determinations; amount of each pesticide in each percolated sample = 50 ng.

Compound		Retention time (min)	LOD[a] (ng)	Recovery (%)					
No.	Name			Percolated sample volume					
				Milli-Q water			Tap water		
				100 ml	200 ml	300 ml	100 ml	200 ml	300 ml
1	Carbaryl	8.7	1.5	98	90	80	64	54	NE[b]
2	Azinphos-methyl	12.9	1.2	100	98	90	74	78	60
3	Phosmet	13.9	3.5	97	98	95	92	91	93
4	Parathion-methyl	14.7	0.7	98	99	95	88	82	78
5	Azinphos-methyl	16.3	0.7	97	100	99	92	96	90
6	Fenitrothion	16.9	0.8	98	98	100	90	90	88
7	Parathion	18.75	0.6	100	97	98	92	88	83
8	Diazinon	19.75	0.9	100	100	98	81	87	82

[a] Signal-to-noise ratio = 3.
[b] Not evaluated.

ment in the detection limit. In order not to decrease the quality of the chromatographic separation, the on-line preconcentration technique involves precolumns with dimensions matching those of the analytical column. For a 150 × 4.3 mm I.D. column, the precolumn dimensions must be 15 × 4 mm I.D. In such cases, the most often used adsorbent size is 5 or 10 μm.

In this work, C_{18} and PRP-1 packed precolumns were tested. Preconcentration trials followed by an analytical separation for various volumes of Milli-Q-purified water spiked with various amounts of the pesticides were performed. The chromatograms obtained (Fig. 3) show the separation achieved by the two precolumn models. The peak broadening in the chromatograms resulting from enrichment on PRP-1 is significant; it is difficult to obtain quantitative results with this sorbent. For this reason, we used the C_{18} packed precolumn in subsequent work.

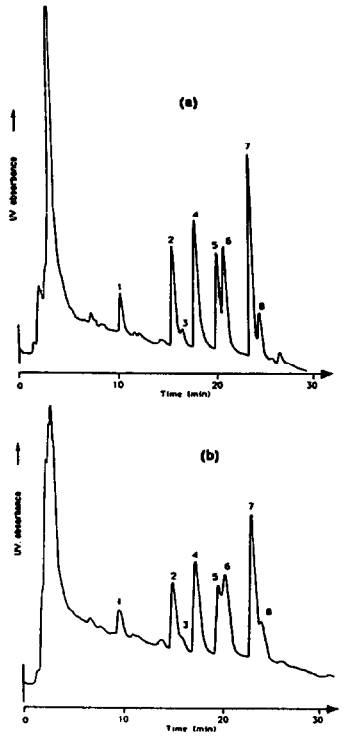

Fig. 3. RP-LC–UV traces for the on-line preconcentration of 100-ml Milli-Q-purified water samples spiked with carbaryl and seven OPPs. Preconcentration using the precolumns packed with (a) RP-18 silica and (b) PRP-1. Peak numbering corresponds to Table I. The individual concentrations of the pesticides ranged between 1 and 3 μg/l. The analytical column (15 × 0.46 cm I.D.) was packed with Nucleosil RP-18 (5 μm). Mobile phase, acetonitrile–water; gradient, 40% to 60% acetonitrile in 20 min. UV detection at 254 nm; attenuation 0.05 a.u.f.s.

The amount of solute recovered from the precolumn does not depend on the preconcentration flow-rate. These rates were changed in 1 ml/min increments from 2 to 7 ml/min. However, in order to avoid high column backpressures at flow-rates exceeding 5 ml/min, which would reduce the precolumn lifetime, a flow-rate of 3 ml/min was adopted.

Preconcentration, recovery and detection limit

Table I gives the recoveries for the preconcentrated pesticides for various volumes of Milli-Q-purified water and tap water. This recovery was calculated by means of the method described by Subra *et al.* [39]. The sample volumes were increased from 10 to 300 ml and the concentration decreased in order to have the same amount in each percolated sample. If breakthrough does not occur, the amount preconcentrated for each analyte on the precolumn is constant and the peak heights obtained after on-line elution are constant. When breakthrough occurs, the peak height decreases. Recoveries were calculated from the ratio between the peak height obtained for the sample volume studied and that obtained for a 10-ml sample. The recoveries of most of the preconcentrated pesticides from the Milli-Q-purified water reached 100% for treated volumes up to 300 ml. The decrease in the recovery of carbaryl as soon as the percolated water volume reaches 200 ml is important, however. These results show that the breakthrough on the C_{18} precolumn is greater than 300 ml for the OP pesticides on the one hand and between 100 and 200 ml for carbaryl on the other. Moreover, the observed recoveries for tap water preconcentration are, in every instance, lower than those obtained for Milli-Q-purified water. This discrepancy is not related to the amount of pesticides preconcentrated or to the breakthrough. It may arise from incomplete adsorption of trapped compounds on the precolumn owing to the formation of pesticides–humic substances complexes as suggested by

Johnson *et al.* [40]. These complexes may be poorly extracted by the octadecyl-bonded silicas.

When the proposed analytical procedure was applied to tap waters, the co-extracted compounds gave a large number of unresolved peaks at the start of the chromatogram, the intensity of which depends on the treated water volume. This part of the chromatogram affects the determination of early-eluted compounds especially when the concentrations of the latter are low. Fig. 4 illustrates the importance of this unresolved part of the chromatogram for the preconcentrates of 300 ml of tap water spiked with 0.1 ppb of each pesticide and also in the case of a blank. It is not then possible to determine carbaryl. This emphasizes the importance of the analytical gradient, as discussed earlier. Moreover, with such a drinking water sample, the solution to this problem, as has been reported [22], is to delay the carbaryl peak slightly. A very simple method without any further clean-up exists for drinking water control; it only requires optimization of the gradient.

The detection limits and the linearity of the solute peak height with concentration were assessed by adding increasing amounts to 200 ml of water, which is a compromise volume of samples of Milli-Q-purified and drinking water samples, and using the whole on-line preconcentration procedure. The detection limits were calculated for a signal-to-noise ratio of 3; in the baseline drift due to interferences, it was arbitrarily assumed that 0.5 cm was the minimum peak height that could be measured with reasonable confidence. The detection limits obtained were 0.2 μg/l for both phosmet and carbaryl. The detection limits for the other insecticides were in the range 0.03–0.06 μg/l. The calibration graphs were linear over the concentration range 0.05–1 μg/l (five data points). The regression coefficients obtained were satisfactory: azinphos-methyl, 0.9981; parathion-ethyl, 0.9994; azinphos-ethyl, 0.9976; fenitrothion, 0.9978; parathion, 0.9984; and diazinon, 0.9989.

Using the reported method, the precolumn can be re-used for analysing up to twenty 200-ml water samples without showing marked deterioration. Peak tailings are the result of possible perturbations that are often observed. Nevertheless, the precolumn can be used more times if the sample volumes are of the order of 100 ml or less. Moreover, there is no need to dry the precolumn before LC determinations, unlike the case with SPE enrichment using either on-line [9] or off-line [40] GC analysis. The described technique is amenable to automation and the whole set-up is robust.

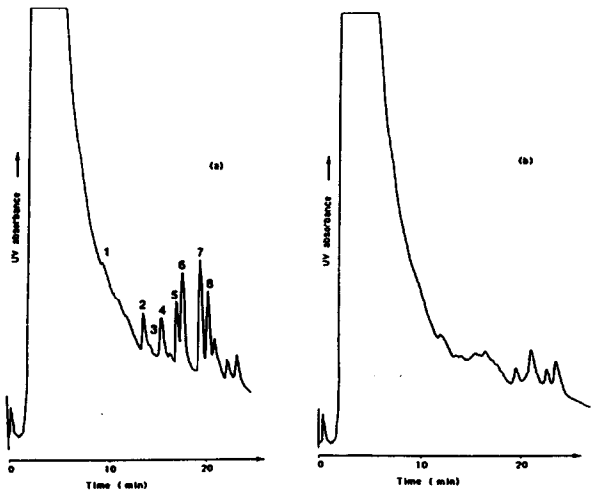

Fig. 4. RP-LC–UV traces for the on-line preconcentration of 300 ml of Paris tap water (October 1991) (a) spiked with 0.1 μg/l of each pesticide and (b) not spiked. Peak numbering corresponds to Table I. Preconcentration using the precolumn packed with RP-18 silica. Mobile phase, acetonitrile–water; gradient, 40% to 60% of acetonitrile in 15 min. UV detection at 254 nm; attenuation 0.01 a.u.f.s. Other conditions as in Fig. 3.

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