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Journal of Chromatography A, 808 (1998) 257–263

JOURNAL OF CHROMATOGRAPHY A

Short communication

Solid-phase microextraction for quantitative analysis of organophosphorus pesticides in environmental water samples

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Received 30 October 1997; received in revised form 13 February 1998; accepted 16 February 1998

Abstract

Solid-phase microextraction (SPME) is a relatively new technique that appears as a convenient and efficient extraction method in contrast with more complex techniques used for pesticide residue analysis based on liquid–liquid and solid-phase extraction. This extraction procedure involves the absorption of analytes into a polymeric film coated onto a fine silica fiber directly dipped in the aqueous sample. An SPME procedure for the determination of 12 organophosphorus pesticides in clean environmental water samples at low ng/ml concentration level has been developed by optimising variables involved in extraction and desorption. The absorption equilibrium has been estimated by mathematical treatment of the process using an expression that describes experimental absorption time profiles. The method was evaluated according to the reproducibility, linearity range and limits of detection using two different fiber coatings: 100 μm polydimethylsiloxane and 85 μm polyacrylate. The limits of detection obtained using nitrogen–phosphorus detection ranged between 0.01 and 0.2 ng/ml with relative standard deviations lower than 15% at the 1 ng/ml level. The method showed good linearity between 0.1 and 10 ng/ml with regression coefficients ranging between 0.97 and 0.999. Determination of organophosphorus pesticides in water samples in concentration below 0.1 ng/ml can be easily carried out with this fast, economic and solvent-free SPME procedure.

Keywords: Water analysis; Extraction methods; Pesticides; Organophosphorus compounds

1. Introduction

For decades, the extensive use of organophosphorus pesticides in predominantly agricultural areas has been favoured over the more persistent organochlorine pesticides mainly because of their quicker degradation rates [1]. This fact supposes an environmental risk which has risen to an increasing social concern with respect to the presence of these compounds in drinking waters.

Determination of pesticides in environmental water samples relies on the use of complex chromatographic instrumentation but also requires the application of sample extraction procedures (usually with preconcentration steps) in order to isolate analytes, remove interferent substances and achieve the sensitivity required for drinking water pollution control (maximum residue level 0.1 μg/l). This has been carried out by using both liquid–liquid extraction (LLE) with organic solvents [2,3], and solid-phase extraction (SPE) with different adsorbents [4–6]. In both cases, but mainly in LLE, some disadvantages can be pointed to, mainly related with the time and solvent consumption, or the introduction of

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0021-9673/98/$19.00
PII: S0021-9673(98)00138-1

interferences due to the extraction procedure (from solvents or cartridges).

A new approach that has become commercially available is solid-phase microextraction (SPME), developed in Canada by Pawliszyn and co-workers [7–9]. Several advantages can be pointed out in relation to this technique, as it is solvent free, uses the whole sample for analysis, requires only small sample amounts and the fibers are highly reusable (up to more than 100 injections) [10]. Compounds are extracted into the polymeric phase according to their affinity towards the coating and then they are thermally desorbed directly in the gas chromatographic injector.

During the last few years, the SPME has been applied to the determination of a variety of analytes in several types of aqueous samples. The compounds investigated include usually several groups of low polarity volatile organics [11–13], petroleum derivatives [14], BTEX (benzene, toluene, ethylbenzene and xylenes) [15], polycyclic aromatic hydrocarbons (PAHs) [15–17] or organometallic compounds [18,19]. More recently SPME has also been applied to pesticide determination in aqueous matrices including organochlorine [20,21], triazine herbicides [10,22,23] and organophosphorus compounds [22,24–27].

This paper will focus on the optimisation and validation of an SPME procedure for the determination of several organophosphorus pesticides in water samples, with special detail in the discussion on the effect of several variables affecting the extraction efficiency. Special emphasis has been given to the study of time dependency of extraction efficiency by applying an equation published by Ai [28]. Mathematical treatment of data has provided information about absorbed amount, absorption rate and estimated equilibrium time.

2. Experimental

2.1. Reagents

99% purity pesticide standards were obtained from Dr. Erhenstorfer (Germany) and used without further purification. Stock solutions were prepared by weighting and dissolving in acetone and were stored at −18°C. Working solutions used to fortify water samples were prepared in acetone by dilution and stored at 4°C, and were renewed every week. Fortified water samples were prepared by adding the appropriate volume of the acetone standard solution to HPLC-grade water (ultrapure water system, Barnstead) in a volumetric flask, maintaining less than 1% acetone in the aqueous samples. Sodium chloride of analytical grade was used after purification by heating at 300°C overnight.

2.2. Instrumentation

SPME fibers were obtained from Supelco with two stationary phases: polydimethylsiloxane (PDMS) (7 and 100 μm film thickness) and polyacrylate (PA) (85 μm thickness). The SPME fibers were conditioned as recommended by the manufacturer by heating them in the injection port of the chromatographic system during 0.5–2 h at 250–300°C depending on the fiber coating.

Analyses were performed using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an splitless injector and nitrogen–phosphorous detection (NPD) system. The GC system was fitted with a 25 m×0.2 mm I.D., 0.33 μm Ultra 2 (5% Phe Me Silicone) column (Hewlett-Packard). Detector temperature was held constant at 270°C. Injector temperature, as well as initial oven program temperature, were investigated under the study, while the temperature programme used was: variable initial temperature; then 30°C/min to 170°C; then 4°C/min to 270°C (hold time 5 min).

2.3. Analytical recommended procedure

The fiber was immersed into the sample (3 ml containing 15% NaCl) and maintained there for 60 min under stirring at ambient temperature. After extraction, the fiber was thermally desorbed during 4 min into the glass liner of the GC injection port (250°C for PA coating and 270°C for the PDMS fiber) for subsequent analysis, maintaining initial oven temperature at 60°C. Quantitation of samples was made using a three-level calibration curve of aqueous standards (between 0.1–10 ng/ml using HPLC-grade water) extracted in the same way that samples and using peak area measurements.

3. Results and discussion

Optimisation of the SPME procedure was carried out by considering separately two main stages: absorption and desorption. Both steps were optimised separately, starting with the thermal desorption of compounds. In this way, an extraction procedure was established with initial conditions of extraction time (30 min, under magnetic stirring), sample volume (3 ml of spiked water at 5 ng/ml level), and NaCl content (10%) according to the literature [22–24]. As the effect of the stationary phase on the fiber should be remarkable, through all the experiences two different fibers were used: 100 μm PDMS and 85 μm PA. All determinations were carried out in duplicate except for those related with precision studies where a minimum of six extractions were made.

Optimum desorption conditions were obtained by testing the main variables involved: injector temperature, desorption time and depth of fiber into the injector glass-liner. Desorption temperatures tested were 250, 270 and 290°C (selected according to the recommended temperature range indicated by the manufacturer). Desorption times ranging from 1 to 5 min were also checked to obtain the optimum value referred to the maximum detector response for all compounds. Optimum values were found to be 270°C and 250°C for the PDMS and PA fibers, respectively, with a desorption time of 4 min. During the desorption the oven temperature was maintained isothermal with some additional 30 s, before starting column heating ramp. The effect of fiber depth into the liner was also checked, results obtained showing that the deeper the fiber was in the injector glass-liner (the closer to the column entrance) the higher the peak areas were obtained.

The effect of extraction time was studied by extracting replicate samples (5 ng/ml fortification level) subsequently desorbed using the optimum conditions indicated above. Extraction time values between 5 and 120 min were used. Analyte mass absorbed into the fiber (obtained by external standard calibration) as a function of extraction time were fitted to the equation given by Ai [28], who derives a time dependent equation for analyte mass absorbed into the fiber coating:

$$n = n_0\left[1 - e^{(-at)}\right] \quad (1)$$

where n and n_0 are the amounts of analyte absorbed at a time (t) and at the equilibrium, respectively, a is a parameter that measures how fast an absorption equilibrium can be reached in the SPME process.

Fig. 1 shows an example of the amount of analyte mass absorbed in the fiber as a function of the extraction time for some of the pesticides tested using the PDMS fiber. Experimental data were fitted to the Eq. (1) using MicroCal Origin software, obtaining n_0 and a for each compound using the two fibers (PDMS and PA). These values, together with the log P_{ow} (obtained from Noble [29]) are given in Table 1.

As expected, pesticides with different chemical characteristics showed different behaviours when submitted to SPME. Thus, when using the PDMS fiber, compounds with higher log P_{ow} (>3.8) were the more extensively absorbed at equilibrium, with values ranging from 4.6 ng (ethion) to 10.0 ng (phosalone) due to their higher affinity to the fiber coating. Compounds with higher polarity were absorbed in a minor extension on the equilibrium (even not absorbed at all in the case of some polar compounds also tested as dimethoate with log $P_{ow} = 0.8$). The use of the polyacrylate fiber (more polar coating) improved the extraction of the more polar compounds (specially for malathion and fenamiphos) showing an increase in the extracted amount in

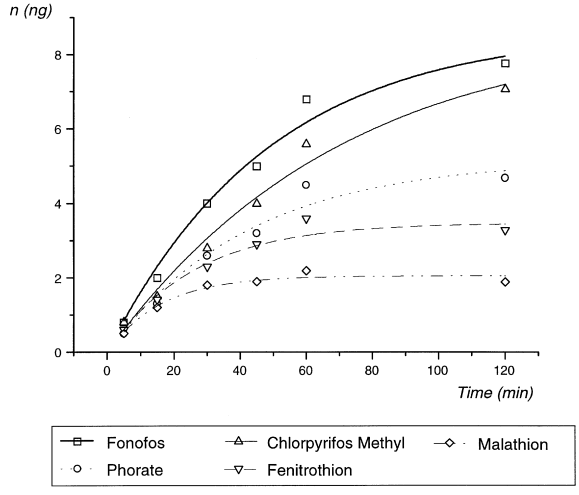

Fig. 1. SPME absorption time profiles for five of the organophosphorus pesticides studied from aqueous solution using the PDMS fiber. Plotted lines are regressions using Eq. (1).

Table 1
Parameters obtained by mathematical fitting of absorbed amount vs. extraction time (spiked HPLC-grade water samples at the 5 ng/ml level)

Pesticide	Log P_{ow}	Polyacrylate fiber			Polydimethylsiloxane fiber		
		n_0 (ng)	a	t_{eq} (h)	n_0 (ng)	a	t_{eq} (h)
Phorate	4.2	6.1	0.0124	4	5.2	0.0235	2
Fonofos	3.9	15.0	0.0049	10	8.7	0.0205	2
Chlorpyrifos-methyl	4.3	3.0	0.0114	4	8.9	0.0142	3
Fenitrothion	3.4	3.6	0.0098	5	3.6	0.0374	1
Malathion	2.9	5.0	0.0094	5	2.1	0.0614	1
Fenthion	4.1	3.6	0.0167	3	6.7	0.0163	3
Chlorfenvinphos	3.8	15.0	0.0064	8	7.6	0.0107	4.5
Methidathion	2.4	3.6	0.0037	14	2.2	0.0161	3
Fenamiphos	3.2	1.9	0.0221	2	0.2	0.0729	0.7
Ethion	5.1	0.94	0.0599	1	4.6	0.0131	4
Phosalone	4.3	2.4	0.0097	5	10.0	0.0157	3

Chemical properties of pesticides.

relation with the PDMS fiber. On the contrary, the less polar pesticides were less effectively extracted when using the PA fiber, with a decrease in equilibrium absorbed amount of 50–75% in relation to the use of PDMS fiber (chlorpyrifos-methyl, fenthion, ethion and phosalone, log $P_{ow}>4$).

Using the values obtained for the fitted equation, equilibrium time (defined as the time it takes for the fiber to absorb 95% of the equilibrium extractable mass) can be calculated (Table 1). As it can be seen, clear differences can be established between the two types of fiber (PDMS and PA) as organophosphorus pesticides tested reached equilibrium in 0.7–4.5 h for PDMS fiber while for the PA fiber it was necessary an extraction time of 1–14 h to reach equilibrium.

Finally, although the equilibrium time for pesticides studied here is clearly higher than 60 min (except for fenitrothion, malathion and fenamiphos), this time was selected for further experiments as a compromise, because it allows to extract more than 60% (PDMS fiber) and more than 40% (PA fiber, except for fonofos, chlorfenvinphos and methidathion with values between 20 and 32%) of the maximum extractable amount in a total time comparable to that of the chromatographic determination (including injection, heating and cooling). In a similar way, it has been pointed out [28] that working under non-equilibrium conditions is feasible when experimental conditions (specially extraction time) are kept constant, as there is a linear relationship between extracted amount and initial concentration.

The next step was the study of the effect of NaCl concentration in the sample. It is well known that the salting out effect is of great help when extracting polar pesticides from water using LLE or SPE [2,4]. The same effect should be expected when applying SPME, and, in fact, this has been pointed out in some papers [22,25]. A final concentration of 5 to 20% NaCl in the water sample was tested, the results showing variable behavior depending on the characteristics of each pesticide.

Thus, regarding to the PDMS fiber, it can be observed that those compounds with higher water solubility (malathion, chlorfenvinphos, methidathion and fenamiphos, with values ranging from 145 to 400 mg/l) showed an increase in extraction yield with the addition of increasing NaCl concentrations. However, no effect or even a decrease in extraction yield was observed for compounds with low water solubility. For the PA fiber the general behaviour observed indicated that the addition of NaCl improved the extraction efficiency (see Fig. 2). This variable effect of the NaCl addition is in good agreement with that presented in the literature by several authors [21,24,30,31]. Finally, 15% of NaCl was added to all samples in further experiments.

The last variable checked was the sample volume used for extraction, considering its effect over the detector response obtained. Thus for the majority of compounds (in both fibers) the maximum response was obtained when 3 ml of sample were used. In relation to this, Gorecki and Pawliszyn [32] in a

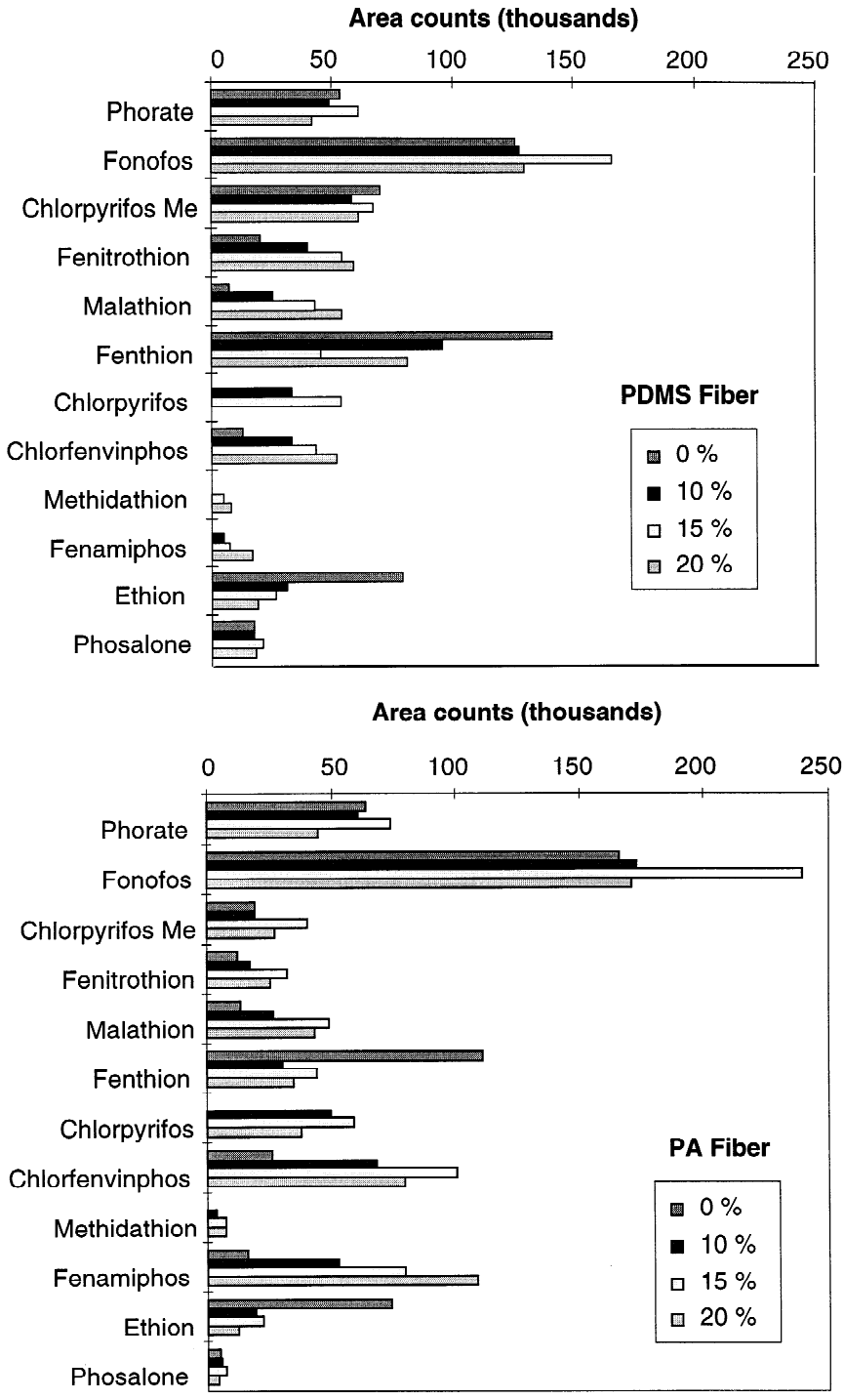

Fig. 2. Dependence of extraction efficiency on sodium chloride concentration in the water sample (0 to 20%) using both PDMS and PA fibers.

recent paper discussed the effect of sample volume on the extracted amount concluding that high sample volumes should be used in order to obtain the best results, always considering an equilibrium situation, which is not the case of the present paper for most of pesticides. However, for fenitrothion, malathion, fenamiphos (PDMS fiber) and ethion (PA fiber) which are extracted in equilibrium situation, the extracted amount increased with the sample volume, as indicated [32].

Finally, the analytical characteristics of the recommended method were obtained, including linear dynamic range, precision and detection limits for both fibers (Table 2). A series of aqueous solutions (HPLC-grade water) in concentrations ranging between 0.1 and 10 ng/ml were extracted (two replicates except for the 1 ng/ml sample which was extracted six times for precision study) and analysed using GC–NPD. The SPME procedure applied showed a linear behaviour in the range tested with r^2 values ranging between 0.98 and 0.999 (PDMS fiber) and between 0.97 and 0.997 (PA fiber). Detection limits, calculated as three-times background noise (Table 2) are comparable to those obtained when applying a LLE procedure (500 ml of water sample adjusted to a final volume of 0.5 ml with hexane), which ranged from 0.01 (fonofos) to 0.2 mg/l (phosalone) [2].

Relative standard deviations (R.S.D.s) obtained were lower when using the PA fiber (6 to 13%) than when using the PDMS fiber (7 to 19%). Precision obtained here is similar or better than other values presented in the literature for the determination of pesticides in water samples at sub-ppb levels using the SPME approach [23–25,30]. An additional consideration has to be done in relation to the fact that, according to Magdic and Pawliszyn [20], higher R.S.D.s have to be expected when, as in this case, the extraction is carried out under non-equilibrium conditions.

Finally, the SPME procedure was applied to a spiked groundwater sample (0.4 ng/ml level, $n=3$) the chromatograms being quantified using a three-level calibration curve obtained after extraction of aqueous standards (0.1–10 ng/ml in HPLC-grade water). Calculated concentrations ranged between 0.37 (chlorpyrifos) and 0.45 (fenitrothion), thus the calculated values showed a maximum deviation of 12% over the true value. Fig. 3 shows an example chromatogram obtained using the PDMS fiber.

4. Conclusions

This paper has outlined the process for method development of an SPME procedure to be applied to the determination of organophosphorus pesticides in clean water samples. This solvent-free method shows good precision, linear dynamic range over at least three orders of magnitude and detection limits in the

Table 2
Analytical performance of the recommended SPME procedure using PDMS- and PA-coated fibers for extraction of spiked groundwater samples

	Polydimethylsiloxane coated fiber		Polyacrylate coated fiber	
	R.S.D. (%)	LOD (ng/ml)	R.S.D. (%)	LOD (ng/ml)
Phorate	14	0.02	8	0.1
Fonofos	8	0.02	6	0.006
Chlorpyrifos-methyl	7	0.02	10	0.03
Fenitrothion	7	0.03	10	0.05
Malathion	17	0.04	9	0.05
Fenthion	10	0.03	9	0.05
Chlorpyrifos	15	0.03	11	0.02
Chlorfenvinphos	9	0.04	9	0.05
Methidathion	8	0.5	10	0.12
Fenamiphos	16	0.05	11	0.05
Ethion	19	0.04	8	0.12
Phosalone	7	0.04	13	0.03

Relative standard deviations ($n=6$, fortification level 1 ng/ml) and limits of detection.

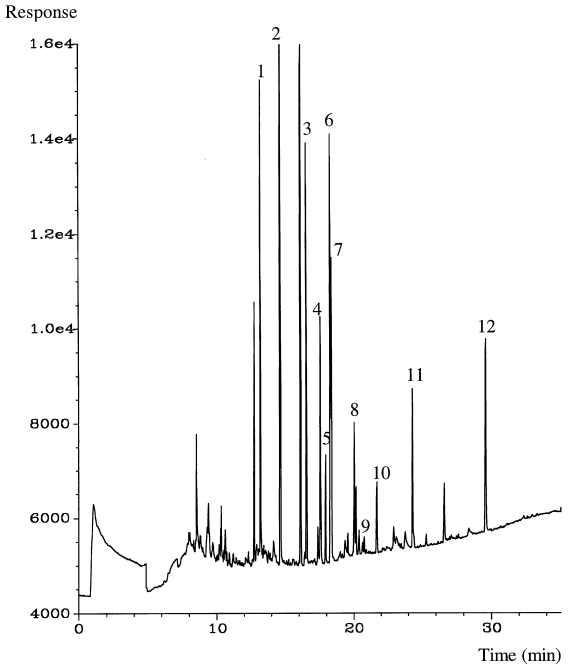

Fig. 3. GC–NPD chromatogram of a fortified groundwater sample (0.5 ng/ml) extracted by SPME using a 100 μm polydimethyl siloxane fiber. (1) Phorate, (2) fonofos, (3) chlorpyrifos methyl, (4) fenitrothion, (5) malathion, (6) fenthion, (7) chlorpyrifos, (8) chlorfenvinphos, (9) methidathion, (10) fenamiphos, (11) ethion, (12) phosalone.

parts-per-trillion level. Thus, it is highly recommendable for the routine determination of pesticides in water samples at low concentration levels with some additional advantages: low sample volume, simplicity of extraction, low cost per sample and capability of automation.

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