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Effect of the various parameters governing solid-phase microextraction for the trace-determination of pesticides in water

J. Dugay*, C. Miège, M.-C. Hennion

Laboratoire Environnement et Chimie Analytique (URA CNRS 437), Ecole Supérieure de Physique et de Chimie Industrielles de Paris, 10 Rue Vauquelin, 75231 Paris Cedex 05, France

Abstract

The parameters governing solid-phase microextraction (SPME) are investigated, with emphasis on the determination of the partition coefficients, *K*, and their use to predict the selection of a fibre, depending on the respective characteristics of the fibres and the analytes. Film thickness and stability of the compounds can interfere with the determination of *K* values. The time profile curves were determined for twelve pesticides having a wide range of water solubilities and polarities and using four fibres (polydimethylsiloxane, polydimethylsiloxane–divinylbenzene, Carbowax–divinylbenzene and polyacrylate). Although the affinity order was different for the four coatings, no correlation was found between the respective characteristics of the fibres and solutes. The two fibres containing divinylbenzene were shown to have the highest affinities and the polydimethylsiloxane had the lowest affinity. The polyacrylate fibre which is the more polar commercial fibre did not provide the highest affinities for the more polar and water-soluble analytes. The important parameters for quantitative analysis have been evaluated. The calibration curves were similar when one analyte of interest was present on its own in a drinking water sample, or when eleven other pesticides were present at the same concentration or when much higher concentrations of other analytes were present in the sample. Linearity was obtained over a wide range of concentrations in drinking water samples. Detection limits are in agreement with European regulatory levels in drinking water for most of the analytes using solid-phase microextraction–gas chromatography–nitrogen–phosphorus detection (SPME–GC–NPD). In contaminated surface water samples, the chromatograms are relatively clean and most of the compounds can be detected at levels lower than 0.5 μg/l. © 1998 Elsevier Science B.V.

Keywords: Extraction methods; Solid-phase microextraction; Water analysis; Partition coefficients; Environmental analysis; Pesticides

1. Introduction

Solid-phase microextraction (SPME) is a new, fast and simple analytical technique which uses coated fused-silica fibres to extract analytes from aqueous or gaseous samples. In addition, SPME requires a small volume of sample. Subsequent analyses are currently performed by gas chromatographic analysis and the analytes are desorbed in the injector of the gas chromatograph. This method was introduced by Pawliszyn and his group, some seven years ago, and represents a further advance as a solvent-free alternative to the extraction of organic compounds from water samples [1–4]. The extraction and desorption processes in the injector gas chromatograph can be fully automated using a conventional autosampler [5]. Nowadays, six different coatings are commercially available for SPME–gas chromatography (GC). Polydimethylsiloxane (PDMS) and polydimethylsiloxane containing divinylbenzene (PDMS–

*Corresponding author.

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DVB) are considered as the less polar coatings, whereas polyacrylate (PA) is the more polar one. A recent new coating is made of Carbowax containing divinylbenzene (CW–DVB). A Carboxen–PDMS coating was also recently introduced on the market and is more appropriate for small and rather volatile molecules. These coatings are available in different thicknesses.

The SPME method has been applied to the trace determination of organic micropollutants, including volatile organic compounds (VOCs) [6–15], phenol and its derivatives [16–18], polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) [19–21]. More recently, the method was applied to fatty acids [22] and other environmental pollutants, such as organophosphorus pesticides, nitrogen-containing pesticides, triazine- and 2,6-dinitroaniline pesticides [23–32].

From the studies published to date, it appeared that selection of a fibre according to the physicochemical parameters of the compounds was difficult. In classical solid-phase extraction with a conventional stationary phase, it is easy to predict the compounds that would be extracted with good recoveries from the chromatographic data or from a good knowledge of the analyte's retention time using the extraction phase of interest as an analytical stationary phase. The process involved in SPME is different and is based on a partition process that cannot be related to chromatographic data, thus explaining the difficulty involved in predicting the extraction properties of the fibres according to the analyte's properties. Moreover, quantitative aspects when several compounds are in competition in an unknown sample with matrix effects has received very little attention. In this paper, we will study in detail the determination of the partition coefficients based on the characteristics of the fibres and of the solutes, especially their water-solubility and their hydrophobicity, according to the water-partition coefficient. Attention will be given to the effect of the number of compounds to be determined and their relative concentrations on the quantitative results. Nitrogen- and phosphorous-containing pesticides will be taken as models, because of the selectivity of nitrogen–phosphorus detection (NPD) and the performance of the method will be presented in real samples using GC–NPD.

2. Experimental

2.1. Reagents

The various pesticides were supplied by Riedel-de-Haën (Seelze, Germany) or Cluzeau (Sainte Foi la Grande, France). Stock solution of selected solutes were prepared by weighing and dissolving them in methanol (Baker–Mallinckrodt, Noisy le Sec, France) for spiking samples processed by SPME or in *tert*.-butyl methyl ether (Prolabo, Paris, France) for direct GC injection. These solutions were stored at 4°C and used for the preparation of diluted standard solutions and for spiking water samples. Deionized water was obtained from a Milli-Q water system (Millipore, St. Quentin-en-Yvelines, France). Sodium dihydrogenphosphate, disodium hydrogenphosphate and sodium chloride (of quality >99.5%) was purchased from Prolabo. Triphenylphosphate was obtained from Fluka (St. Quentin Fallavier, France).

2.2. SPME fibres

The fibre assemblies purchased were coated with either 100, 30 or 7 μm PDMS, 65 μm PDMS–DVB, 65 μm CW–DVB and 85 μm PA (Supelco, St. Quentin Fallavier, France). All fibres were conditioned in the hot injector part of the gas chromatograph according to instructions provided by the supplier.

2.3. Instrumentation

Analyses were carried out in a Varian 3400 CX GC system associated with a Varian 8200 CX AutoSampler from SPME (Varian, Les Ulis, France). The chromatograph was equipped with a 1078 split/splitless injector and a NPD system. The injector and detector temperatures were set at 280 and 300°C isothermal, respectively. Helium was used as the carrier gas at 1 ml/min and NPD was at 3.2 A intensity and maximum range (range 12) with air at 175 ml/min, nitrogen at 30 ml/min (make-up gas) and hydrogen at 4.25 ml/min.

Pesticides were separated using a DB-5.625 column (J&W Scientific, Les Ulis, France), 30 m× 0.25mm I.D., with a phase thickness of 0.25 μm,

using the following temperature programme: 70°C, held for 2 min, then increase by 20°C/min to 170°C, finally increase by 4°C/min to 250°C and hold for 10 min.

2.4. Calibration of the nitrogen–phosphorus detector

The response of the NPD system was calibrated for each analyte by direct injection in the splitless mode of standard pesticide mixtures dissolved in *tert*.-butyl methyl ether at six different concentrations: 1, 5, 10, 20, 30 and 40 mg/l. Each standard solution contained an internal standard (I.S.), triphenylphosphate, at 40 mg/l. The presence of I.S. allowed us to estimate, by two repeated injections of 4 μl, the real volumes of standard pesticide mixtures that had been injected. Area counts were also plotted against the amount injected (ng), to produce a calibration curve.

2.5. SPME analytical procedure

Standard solutions or water samples were adjusted to pH 7 with concentrated phosphate buffer and the ionic strength was fixed at 4 *M* using solid sodium chloride. An 11-ml volume of sample was placed in each autosampler vial with a magnetic stirring bar, then the vials were sealed with hole-caps and PTFE-lined septa. A laboratory-made stirring system was added to the autosampler. The samples were stirred before and during extraction. The fibre was then exposed to the aqueous phase for an appropriate time period, with stirring at room temperature (26±2°C). After extraction, the fibre was directly exposed to the hot injector of the GC system for analysis. The linear purge was closed during the desorption of the analytes from the SPME fibre in the split/splitless injector (2 min delay time). Thermal desorption of the pesticides was carried out at 280°C.

3. Results and discussion

Twelve nitrogen- or phosphorus-containing pesticides with various chemical functionalities were selected and these are listed in Table 1. They are representative of various groups of pesticides that are widely used in Europe and their physicochemical properties cover a wide range of water solubilities (20–8000 mg/l) and hydrophobicities with log K_{ow} (water–octanol partition coefficient) values in the range 1.5–4. These pesticides are not volatile and, therefore, only aqueous extraction will be considered.

Organic pollutants are adsorbed from aqueous samples by the solid-phase coating of a silica fibre support. The analytes are then directly transferred to the injector of a gas chromatograph using a modified syringe assembly where they are thermally desorbed

Table 1
Selected nitrogen and phosphorus pesticides, class, water-solubility and water–octanol partition coefficients

Class	Name	Molecular formula	Solubility in water (mg/l)	Log K_{ow}
Triazines	Atrazine	$C_8H_{14}ClN_5$	33	2.50[a]
	Prometon	$C_{10}H_{19}N_5O$	750	2.99[a]
	Terbutryn	$C_{10}H_{19}N_5S$	22	3.65[b]
Triazole	Triadimefon	$C_{14}H_{16}ClN_3O_2$	64	3.11[a]
Thiocarbamates	Cycloate	$C_{11}H_{15}NOS$	75	3.88[a]
	EPTC	$C_9H_{19}NOS$	375	3.20[a]
	Vernolate	$C_{10}H_{21}NOS$	90	3.84[a]
Amide	Napropamide	$C_{17}H_{21}NO_2$	73	3.30[a]
Chloracetamide	Metolachlor	$C_{15}H_{22}ClNO_2$	488	2.90[a]
Organophosphorus	Dichlorvos	$C_4H_7Cl_2O_4P$	8000	1.42[a]
	Fenamiphos	$C_{13}H_{22}NO_3PS$	700	3.30[a]
Organochlorine	Norflurazon	$C_{12}H_9ClF_3N_3O$	28	2.45[a]

[a]From [33].
[b]From [34].

and analyzed. In contrast to conventional off-line extraction methods, the total amount of extracted sample is used for the determination by GC. The miniature cylindrical geometry of this apparatus permits rapid mass transfer during extraction and then desorption of the concentrated extract into the GC system. In the first step, the coated fibre is exposed to the sample and the target analytes partition from the sample matrix into the coating. The fibre with the concentrated analytes is then transferred to a GC system, where compounds are thermally desorbed, separated and quantified. Therefore, optimization of the SPME procedure consists of (i) selecting the phase and the thickness of the phase, (ii) optimizing desorption times and temperatures, (iii) optimizing the amount extracted by generating time profiles for each analyte in order to select the exposure time for the fibre under stirring conditions and (iv) testing to determine if the method is quantitative.

Some factors have been extensively studied and will no longer be considered, such as modification of the ionic strength or adjustment of the pH value. This is particularly important for the extraction of water-soluble compounds, because the more soluble the analyte is in water, the lower is the affinity of that analyte for the fibre coating. Therefore, the amount of analyte extracted by the fibre can be increased by reducing the solubility of the analyte, which can be achieved by the addition of salt or by pH adjustment. Because of the analytes we were interested in, experimental conditions were selected in order to have both a constant pH (7) and ionic strength in the samples [23,25,29]. Samples were adjusted to pH 7 when necessary by the addition of a concentrated phosphate buffer and the ionic strength was modified using solid sodium chloride. In the same manner, the desorption conditions were optimized according to previous studies. Having a higher temperature and a longer desorption time than is strictly necessary is advantageous in order to achieve complete desorption in real unknown samples and to clean the fibre between experiments.

3.1. Experimental determination of the partition coefficient

The theoretical partition process has also been extensively studied [1,2]. In a finite volume of sample, V_{aq}, the number of moles, n_s, of analyte extracted by the fibre at equilibrium is dependent on the initial concentration of analyte in solution, C°_{aq}, according to:

$$n_S = \frac{KV_S V_{aq} C^{\circ}_{aq}}{KV_S + V_{aq}} \quad (1)$$

where K is the partition coefficient of the analyte between the stationary phase and the aqueous phase at equilibrium and V_s is the volume of polymeric stationary phase. The dynamics of such a model have been mathematically modelled for both stirred and unstirred solutions, by equations for the diffusive and connective transport of analytes in the sample matrix and the extractive phase [2]. The model predicts that rapid extractions in less than 1 min are possible if the solution is completely mixed [35]. Without mixing, the time required to reach equilibrium is limited by diffusion in water. With inefficient mixing, an unstirred layer of water remains next to the fibre, which limits the rate of extraction because the analytes must first diffuse across this static layer. In practice, stirring with a magnetic bar fails to provide perfect mixing, so the static water layer increases equilibration times to a few minutes [3]. Therefore, all extractions described in this study have been performed under optimized and reproducible stirring conditions. It can also be seen from the above equation that the mass extracted and the linear range depend on the partition coefficient and the volume of the stationary phase. The choice of stationary phase is therefore important. The equilibration time depends on the partition coefficient. The higher the K value, the larger the amount extracted at equilibrium.

In order to experimentally determine K, one needs accurate values of V_s, V_{aq} and n_s, since Eq. (1) can be transformed into:

$$K = \frac{n_s V_{aq}}{V_s(V_{aq} C^{\circ}_{aq} - n_s)} \quad (2)$$

The volume of the aqueous phase was set at a constant volume of 11 ml, using 15 ml flasks in the autosamplers. The volume of the solid phase, V_s, is defined as:

$$V_s = \pi L(e^2 + ea) \quad (3)$$

where e is the film thickness, a is the diameter of the fibre of the silica rod, which was 110 ± 10 μm according to the manufacturer.

The accuracy of the n_s values are not often discussed. Due to the partition process, it is impossible to use any I.S. or surrogate and n_s can only be determined using external standardisation. According to previous work [5,23,27], the amounts extracted in a SPME process range between a few ng to 200 ng of analyte. When performing such a standardisation from direct injection of sample volumes from 2 to 4 μl using the splitless mode, as used for the desorption of the SPME syringe and NPD, R.S.D. values in the range 6–13% are obtained, as shown in Table 2.

Taking into account the precision on both V_s and n_s, and the fact that equilibrium should be reached before n_s values can be determined, the partition coefficient values can only be approximated.

3.2. Effect of film thickness on the K values

The K values should be a criteria for selecting the best conditions. As fibres of different thickness exist, it is important to investigate this effect on the determination of K values. At first glance, K values should not depend on the thickness of the fibre, provided that the concentration of the solute in the fibre is proportional to the volume of the solid phase. However, the partition process may depend on diffusion within the fibre and not reach all of the fibre's volume. PDMS fibres were selected as they are available in various thicknesses, 7, 30 and 100 μm. Fig. 1 shows the time profiles for eight analytes, with the extracted amounts obtained when the extraction time was varied from a few to 150 min for the three thicknesses of the PDMS fibres also being shown. First, n_s increases with film thickness, as expected. According to Eq. (1) and to the four orders of magnitude difference between V_s and V_{aq}, the effect of increasing the film thickness is first to increase n_s. It was also established that the film thickness has an effect on the sorption kinetics, and it was shown that the time required to reach 90% of the equilibrium n_s values (T_{90}) varied with film thickness, provided that the solution was perfectly stirred, according to:

$$T_{90} = e^2/2Ds \quad (4)$$

Ds being the molecular diffusion coefficient of the analyte within the fibre [2]. Looking at Table 3, where the n_s values are reported, as are the equilibrium time and the corresponding K values for each analyte and each thickness, it can be seen that the time required for equilibrium to be reached is longer for the 100 μm fibre than for the 30 μm fibre. This equilibration time increases with the amount extracted and is in the range of 100–150 min when n_s is above 25–30 ng, which corresponds roughly to 5% of the amount present in the samples (550 ng). However, depending on the analytes, variation of the equilibration time with e^2 can be verified for metolachlor, prometon, atrazine, dichlorvos or triadimefon, since there is an average of a factor of ten difference between their respective equilibration times with the 100 and 30 μm coatings, which roughly corresponds to the e^2 ratio. For compounds such as terbutryn, fenamiphos and napromide, the variation with e^2 is not verified and this cannot be explained by inadequate stirring since the stirring conditions were similar for the eight analytes.

The partition coefficients were calculated for each analyte and each film and the results are given in Table 3. For the 7 μm coating, we do not think that reliable values were obtained since the amounts extracted are very low and there is also some imprecision regarding the film thickness. Moreover, this thickness is not appropriate for the trace analysis

Table 2
Calibration coefficients and corresponding R.S.D. values of the NPD using direct injection of standards solutions

Compound	Response NPD (area counts/ng injected)	R.S.D. (%) ($n=26$)
Dichlorvos	7500	11
EPTC	1200	7
Vernolate	1100	6
Cycloate	1000	8
Prometon	2500	7
Atrazine	3800	10
Terbutryn	3000	8
Metolachlor	680	9
Triadimefon	2000	11
Fenamiphos	7800	10
Napropamide	1700	11
Norflurazon	2100	13

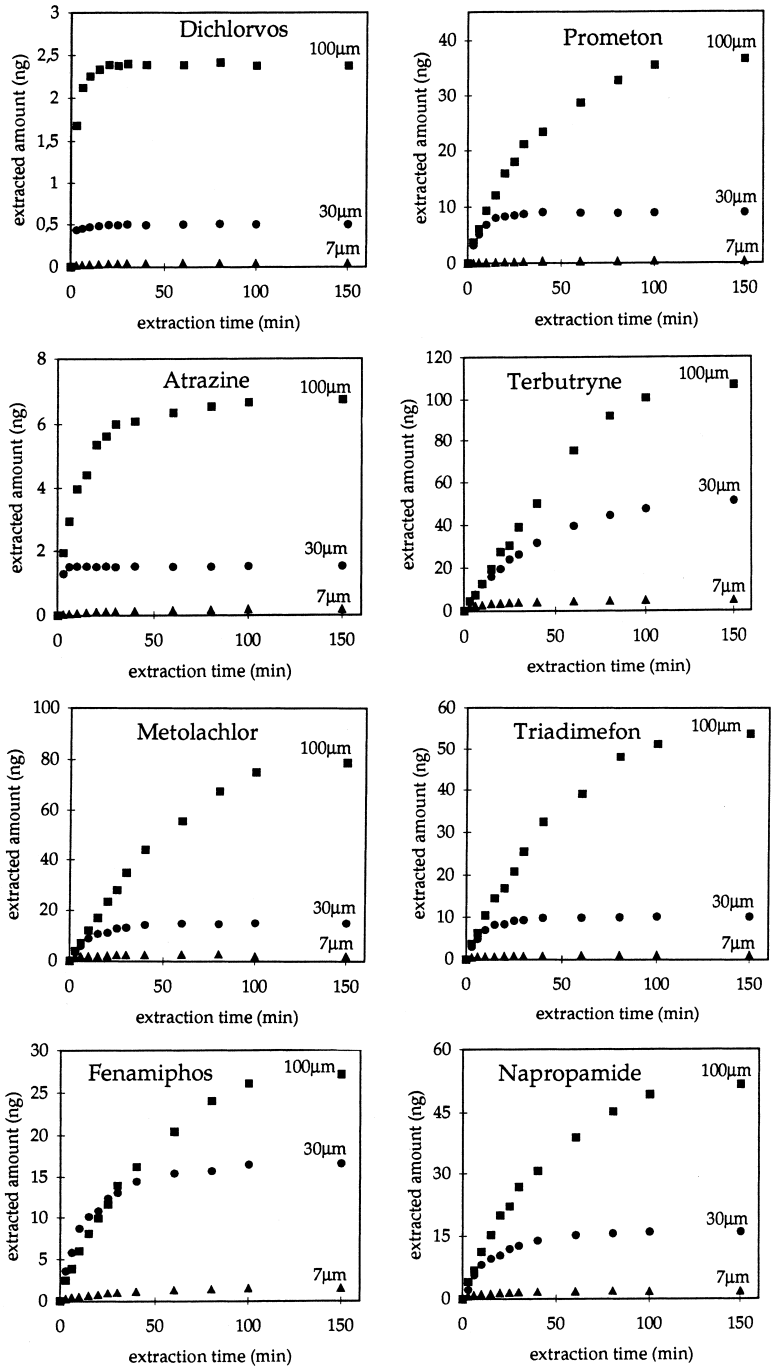

Fig. 1. Time profile curves for eight pesticides, represented by the extracted amount as a function of the exposure time, obtained with three different thicknesses of the polydimethylsiloxane fibre. Experimental conditions: Sample volume, 11 ml, stirring, Milli-Q water with 4 *M* NaCl at pH 7 and spiked with 50 µg/l of each analyte; desorption temperature, 280°C; desorption time, 5 min.

Table 3
Variation of the equilibration time, T_{eq}, extracted amounts, n_s, at equilibrium and of the partition coefficients, K, with the film thickness of the PDMS fibre

PDMS film thickness (μm)	100			30			7	
Coating volume ($\times 10^4$ cm^3)	6.6			1.3			0.26	
Compounds	T_{eq} (min)	$n_s^{a,b}$ (ng)	$K^{a,c}$	T_{eq} (min)	$n_s^{a,b}$ (ng)	$K^{a,c}$	$n_s^{a,b}$ (ng)	$K^{a,c}$
Terbutryn	150–200	110	4100	100–150	52	8800	5	4000
Metalochlor	150–200	79	2800	20–30	15	2400	2	1500
Triadimefon	150–200	54	1800	20–30	10	1600	1	800
Napropamide	150–200	52	1700	40–60	16	2500	2	1500
Prometon	150–200	37	1200	15–25	9.1	1400	0.5	400
Fenamiphos	150–200	28	900	50–70	17	2700	1.5	1000
Atrazine	50–70	6.8	210	3–5	1.6	250	0.2–0.3	200
Dichlorvos	20–30	2.4	70	<3	0.5	80	nd[d]	nd

[a] Milli-Q water spiked with 50 μg/l, with stirring, sample volume, 11 ml; pH=7, 4 *M* NaCl.
[b] Mean of three replicate experiments, average R.S.D. values of 7–10%.
[c] R.S.D. in the range 10–20%.
[d] not determined.

of pesticides. Therefore, only results from the 100 and 30 μm fibres will be compared. For the compounds metolachlor, prometon, atrazine, dichlorvos and triadimefon, the values of K are similar, whereas for the three other compounds, terbutryn, napromide and fenamiphos, the K values are lower (1.5–3-fold) with the 100 μm coating than with the 30 μm one. These results, together with the relative variation of the equilibration times, indicates that diffusion is either lower or incomplete in the 100 μm coating for these three compounds.

The results obtained in this section show that K values can vary with film thickness, depending on the analyte. This fact should be taken into account and can contribute to the difficulty of comparing results obtained with fibres of various types but also with different thicknesses. As an example, the K values in Table 2 can provide opposite results when comparing affinities of the PDMS coating for triadimefon and fenamiphos, depending on whether the values were measured with the 100 or the 30 μm film thickness. When fibres are available with different thicknesses, comparison of the times required for equilibrium to be reached for different thickness ratios allows one to rapidly estimate whether or not K will depend on the film thickness.

3.3. Effect of analyte stability

The times profiles drawn for three compounds, cycloate, S-ethyl dipropylthiocarbamate (EPTC) and vernolate, using the different film thicknesses were shown to be different from those determined in Fig. 1 and are represented in Fig. 2a. The amount extracted first increases with exposure time and then decreases after 80 min for cycloate, but after less than 50 min for vernolate, so that no equilibrium is reached. Since these pesticides belong to the group of thiocarbamates, a lack of stability was suspected to be responsible of these time profiles. Moreover, when comparing them to the time profiles given in Fig. 1, it is clear that, although an apparent plateau is observed for the cycloate, the equilibration times do not correspond to those observed for other compounds with similar extracted amounts, and the ratio between the amount extracted with the 100 and 30 μm fibres is higher. These thiocarbamates are included in the EPA National Priority Survey (NPS) list and the lack of stability was shown for EPTC, when compounds were not stored correctly at 4°C for fourteen days [36]. In order to investigate their stability, these three compounds were studied separately by measuring the peak area after a stirring

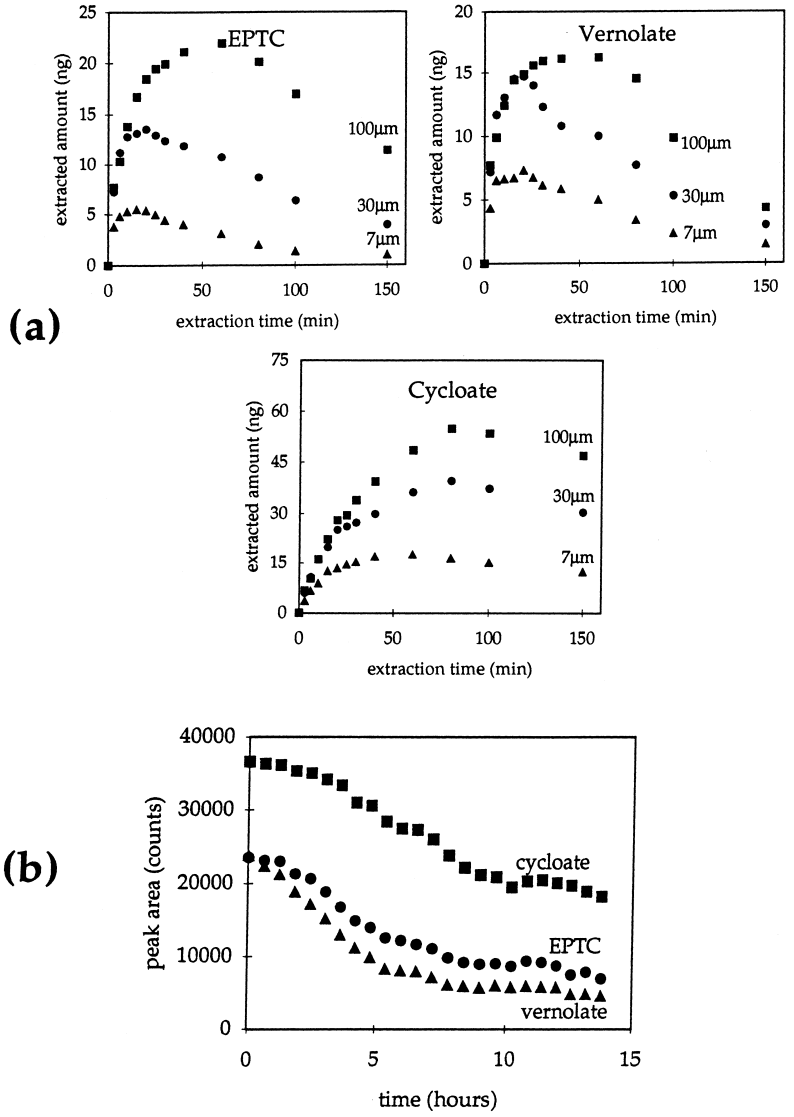

Fig. 2. Effect of the non-stability of the analytes demonstrated by (a) time profiles constructed as in Fig. 1 with different thicknesses of the PDMS fibre and (b) variation of the peak areas of solutes with the amount of time that the samples were stirred for, at 26°C in the autosampler, before the SPME process. Experimental conditions: (a) as in Fig. 1; (b) Fibre, 65 μm CW–DVB; 30 min extraction; sample volume, 11 ml; stirring, Milli-Q water with 4 *M* NaCl at pH 7 and spiked with 50 μg/l of each analyte.

period with an extraction time set at 30 min. In the autosampler, the temperature was determined to be 26±2°C. Fig. 2b represents the variation of the peak areas with stirring time before the SPME process. The lack of stability under the experimental conditions is very rapid for EPTC and vernolate. These results show that the stability of the analytes can be influenced by the SPME conditions, i.e., stirring for at least 30 min, and samples exposed to a temperature of around 25°C, because of the position of the autosampler on top of the chromatograph, sometimes for several hours. On another hand, this provides a rapid and appropriate method for checking the stability of organic compounds at trace levels in

water with a lot of potential for identification of degradation products when SPME–GC is coupled to mass spectrometry.

3.4. Effect of the nature of the fibre and the properties of the analyte on K values

Two factors are to be taken into account for the selection of the fibre; the equilibration time and the amount extracted. Selection of the fibre is generally guided by the polarity of the analytes and, when available, published *K* values are taken as a guide for fibre selection. Comparisons of the performances of PDMS and PA coatings have been presented. PDMS fibres were preferred for the extraction of non-polar pesticides with very low solubility in water, such as organochlorine pesticides and some of the non-polar organophosphorus compounds, whereas the more polar PA fibre was shown to be more appropriate for the more polar nitrogen-containing herbicides and for phenols [16,23,25,37]. The 95 μm PA fibre has shown much higher affinity than the 100 μm PDMS fibre for the extraction of fatty acids [22]. However, the PA coating was also shown to have some affinity for non-polar analytes because the structure of the PA coating consists of a hydrocarbon chain backbone with polar ester side chains [16,27]. PDMS, PDMS–DVB, CW–DVB and PA have recently been compared for the extraction of two organophosphorus pesticides, malathion and parathion [30]. The decreasing order of affinity is as follows: 65 μm PDMS–DVB, then the 85 μm PA and, finally, the 65 μm CW–DVB fibre.

3.4.1. Effect of the nature of the fibre on n_s and K values

Four fibres (100 μm PDMS, 65 μm PDMS–DVB, 65 μm CW–DVB and 85 μm PA) were compared by determining the time profile curves using the same conditions as those reported in Fig. 1. Table 4 gives the extracted amounts and the respective *K* values. When comparing the n_s and *K* values obtained with the PDMS fibre to those obtained using the other fibres, there is not a great difference except for atrazine, norflurazon and, to a lesser extent, dichlorvos. The PDMS–DVB fibre had the highest values for almost all of the compounds. However, the weakly polar CW–DVB fibre had good affinity for many analytes and the polar PA coating was not much worse in terms of extraction efficiency. Few published values can be used for comparison. Boyd-Boland and Pawliszyn [23] found similar *K* values for atrazine (2000) and metolachlor (4000) using a 95-μm PA fibre. Magdic et al. [27] found *K* values

Table 4
Extracted amount, n_s, at equilibrium and partition coefficient, *K*, obtained from time profile curves performed with various fibres

Fibre	PDMS (apolar)		PDMS-DVB (apolar)		CW-DVB (weakly polar)		PA (polar)	
Film thickness (μm)	100		65		65		85	
Coating volume ($\times 10^4$ cm^3)	6.6		3.6		3.6		5.2	
Compound	$n_s^{a,b}$ (ng)	$K^{a,c}$	$n_s^{a,b}$ (ng)	$K^{a,c}$	$n_s^{a,b}$ (ng)	$K^{a,c}$	$n_s^{a,b}$ (ng)	$K^{a,c}$
Terbutryn	110	4100	93	6200	83	5400	87	4000
Metalochlor	79	2800	120	8500	55	3400	72	3200
Triadimefon	54	1800	100	7100	71	4500	81	3700
Napropamide	52	1700	50	3100	47	2900	42	1700
Prometon	37	1200	75	4800	41	2500	36	1500
Atrazine	6.8	210	49	3000	45	2700	48	2000
Fenamiphos	28	900	26	1500	32	1900	27	1100
Dichlorvos	2.4	70	26	1500	6.5	370	2.7	100
Norflurazon	0.4	10	10	620	11	680	14	550

[a]Milli-Q water spiked with 50 μg/l, stirring, sample volume, 11 ml; pH=7, 4 *M* NaCl.
[b]Mean of three replicate experiments, average R.S.D. values of 7–10%.
[c]*K* determined with R.S.D. values in the range 10–20%.

of 130 and 300 using 100 μm PDMS and 85 μm PA fibres, respectively, for dichlorvos. Our values are a little lower, being 70 and 100, respectively.

These results show that the two coatings, PDMS–DVB and CW–DVB, extract the moderately polar compounds under study (log K_{ow} values in the range 1.4–3.7) with the highest affinity. Since they have the same film thickness, the comparison is more accurate and the introduction of DVB helped to increase the affinity. In classical SPE, DVB polymer phases have been shown to provide the highest recoveries for the extraction of more polar compounds [38].

The difference between the partition process involved in SPME and the chromatographic process involved in classical SPE can clearly be seen by examining the n_s values. In a SPE process, the recoveries are closed to 100%, provided that the extraction phase has been correctly selected in order to retain the analyte in water [39]. Here, all the samples contained 550 ng of analyte and the n_s values obtained for the 100 μm PDMS–DVB fibre ranged from 10 ng for norflurazon to 120 ng for metolachlor.

3.4.2. Effect of the nature of the fibre on the equilibration time

For thirteen out of a set of twenty organophosphorus pesticides, a shorter equilibration time was observed using the 85 μm PA fibre rather than the 100 μm fibre, and an equal or lower equilibration time was obtained for the other compounds [27]. In our experiments, small differences between the four fibres were observed, the equilibrium time being more dependant on the amount extracted for all the fibres and being in the range of 100–150 min when n_s value was highest.

3.4.3. Relationship between water-solubility and the hydrophobicity of the analytes

Correlations have been shown between K values and the solubility or octanol–water constant, K_{ow}. However, these correlations have been observed mainly for relatively homogeneous groups of compounds [16,22,25,40,41].

Our results show that the affinity order obtained with the three fibres are different, but, as described above, the film thickness can change some results. However, for two of the fibres, PDMS–DVB and CW–DVB, with similar thicknesses, the order is also different. Obviously, there is no relationship between the water solubility of the analyte when compounds with different functionalities are considered. As an example, let us consider the three compounds with the lowest water solubility, terbutryn, atrazine and norflurazon (22, 28 and 33 mg/l, respectively). With the PDMS fibre, atrazine and norflurazon has had lowest K values, but terbutryn has the highest K value. With the other coatings, the K values decreasing in the order of terbutryn, atrazine and norflurazon. Similarly, no relationship was found between the K values and the log K_{ow} values for the four coatings of interest. The greatest differences in K values between the coatings were obtained for the more soluble and the less hydrophobic compounds.

Combinations between solubility and log K_{ow} values can be considered. As an example, metolachlor and triadimefon have similar log K_{ow} values (2.9 and 3.1) but different water solubilities (488 and 64 mg/l, respectively). On the non-polar PDMS and PDMS–DVB coatings, we could expect the less soluble compound to have more affinity, but the opposite is observed, since metolachlor has a higher K value. Atrazine and terbutryn have similar solubilities but different log K_{ow} values. The less hydrophobic compound, atrazine, has a lower K value than terbutryn for all of the fibres. This is an expected result for the non-polar fibre, where hydrophobic interactions can be predominant, but it is less expected with the polar fibre. However, this points out the occurrence of hydrophobic interactions with the PA fibre due to its non-polar backbone structure.

In conclusion, solubilities and/or hydrophobicities are not sufficient to explain the observed affinities. This is a difference with SPE based on a chromatographic process, where the extraction parameters (selection of the phase and amount, recoveries, sample volume) can be predicted from log K_{ow} values using alkyl-bonded silicas or apolar copolymers, for instance [39]. Since some trends are observed in homogeneous series, the structure of the compounds should certainly be taken into account for affinity as well as for some more specific interactions. The partition process is certainly more difficult to predict than the chromatographic process and only guidelines can be given.

3.5. Quantitative analysis: Effect of competition on the partition process

Usually, when several compounds are analysed simultaneously, the extraction time is often shorter than the equilibration times of all of the compounds. Equilibration times are often set in the range of 20 to 40 min, depending on the analysis time. With an autosampler, the chromatographic analysis of a sample occurs at the same time as the extraction of the subsequent sample, so that analysis and extraction have the same duration. A shorter equilibrium time may affect the sensitivity and precision of the method. Under such conditions, it is important that the extraction time should be monitored carefully, because when equilibrium is not established, slight deviations in the extraction times may result in deviations in the amounts extracted. Compounds with low K values are not affected, since equilibrium is usually reached within 30 min.

An extraction time of 30 min was selected using the 65 μm CW–DVB fibre, for testing to see if the method was quantitate and for application to real samples. In order to increase the precision of the measurements, it is necessary to repeat the experiments at least twice. However, it is important to use separate vials for each experiment. Fig. 3 shows that consecutive experiments lead to non-reproducible results because the amount extracted can represent as much as 20–30% of the initial amount in the sample. When using autosamplers, it is also recommended to check that the fibre is cleaned at each run and to run some blanks in between experiments.

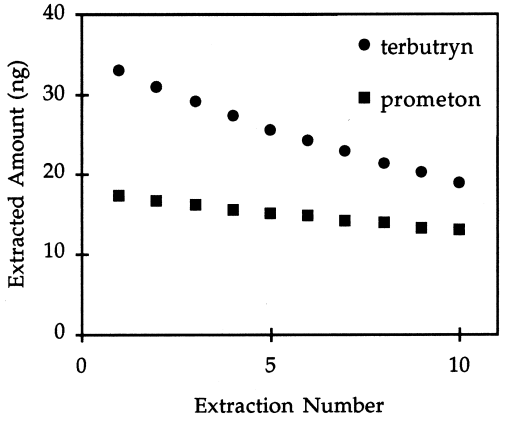

Fig. 3. Reproducibility of the amount extracted using in the same flask and the 65 μm CW–DVB fibre. The experimental conditions are as in Fig. 2b.

Quantitative measurements are not made by standardisation of the detector in order to have the real extracted amounts. Usually, spiked solutions are made with a known amount of a mixture of analytes and calibration curves are drawn by relating the peak area obtained when desorption occurs to the concentrations used for spiking the samples. Such experiments have been performed with drinking water samples. However, in real samples, the number of analytes and their concentrations are unknown, and some matrix effects exist that can modify the calibration curves. In order to be sure that no competition occurs between the analytes in the partition process, calibration curves were determined by spiking drinking water samples with each analyte separately. It is important to verify that the occurrence of a compound at a high concentration does not modify the partition of a targeted analyte at trace levels. Calibration curves of drinking water samples spiked with atrazine, at concentrations in the range of 0.1 to 10 μg/l, were determined under various conditions, i.e. (i) alone in the sample, (ii) in the presence of the eleven other pesticides (given in Table 1), each at the same concentration and, finally, (iii) in the presence of a 500 μg/l solution of prometon and terbutryn. Our results show that the three calibration curves are similar: (i) $y=1310x-70$, (ii) $y=1370x-130$ and (iii) $y=1350x-90$ with $r>0.997$ for most calibration curves, according to the corresponding R.S.D. These results are important and, therefore, the partition process is reproducible using these conditions, indicating that the method is quantitative for these pesticides.

The linearity of the method was verified over the range 0.5–50 μg/l using samples spiked with mixtures of the twelve analytes. Regression coefficients above 0.997 were obtained. It is worthwhile to mention that, for the three compounds that were shown to be unstable (Fig. 2), a constant 30 min exposure time lead to correct calibration curves.

Most of the pesticides were extracted in the range 0.5–10 μg/l with R.S.D. values in the range 2–15%. This precision is in the range of reported values in the literature using GC–NPD. The reproducibility

between fibres was found to be good. With more than 30 extractions from aqueous standards using three different fibres, the percentage difference for each comparison of the fibres was less than 5%, which corresponds to published data [42].

3.6. Application and detection limits in real samples

SPME was applied first to drinking water samples using the same conditions as those applied for the

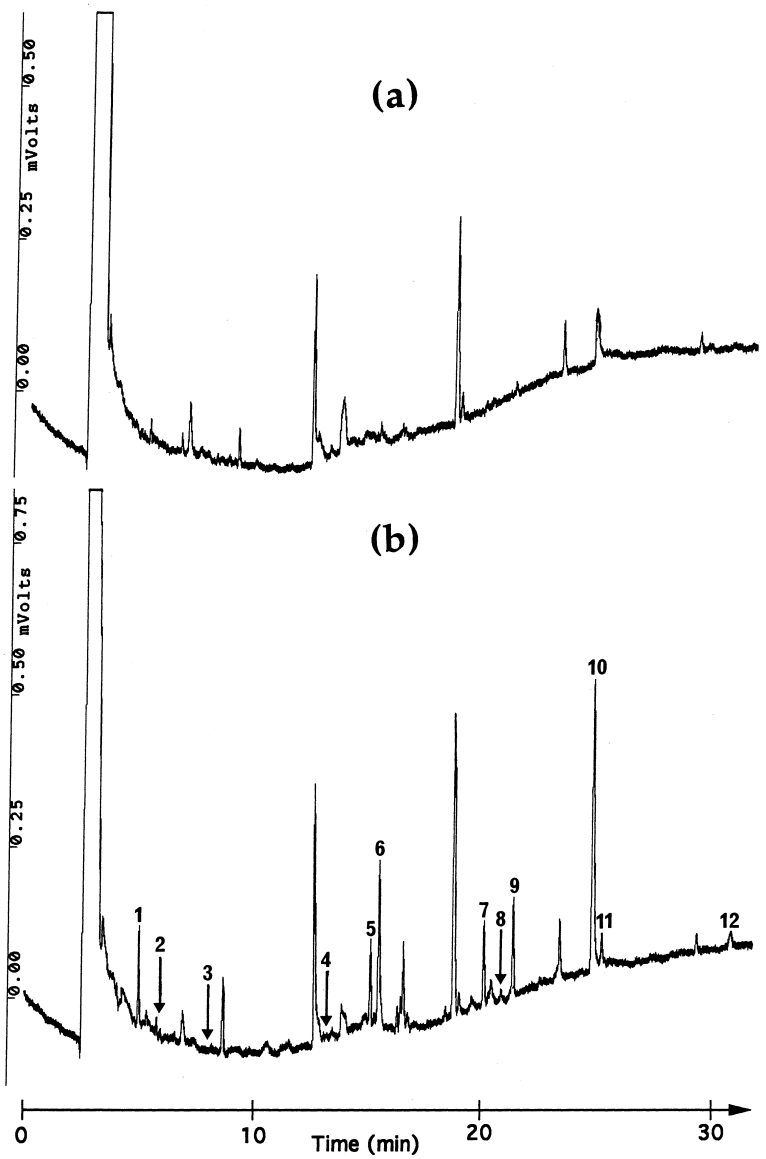

Fig. 4. SPME–GC chromatogram corresponding to the analysis of drinking water (a) non-spiked and (b) spiked with 0.1 μg/l of each pesticide. Experimental conditions as in Fig. 2b. Peak identity: 1=Dichlorvos; 2=EPTC; 3=vernolate; 4=cycloate; 5=prometon; 6=atrazine; 7=terbutryn; 8=metolachlor; 9=triadimefon; 10=fenamiphos; 11=napropamide; 12=norflurazon. Other experimental conditions as in Fig. 2b.

calibration of the method. Fig. 4 shows the chromatogram corresponding to a non-spiked sample and to the same sample spiked with 0.1 μg/l of each pesticide. The detection limits depend on the n_s values and on the detection mode. They are reported in Table 5 and are in the range 0.02–0.3, depending on the analytes. For more than half of the twelve compounds, these detection limits can allow quantification at the 0.1 μg/l level. For the others, the detection limits are too low to meet the EU regulatory levels, but this limitation can be solved using GC–MS for which detection limits were reported to be much lower than for GC–NPD [25,26]. Moreover, GC–MS allows multiresidue analysis. Since MS is required for confirmation and GC–MS is now available in many environmental laboratories, SPME–GC–MS can be a good routine method for the rapid monitoring of drinking water at the 0.1 μg/l level required by EU regulations. If one wants to keep the SPME–GC–NPD system with a confirmation column, then an enrichment factor of ten to twenty may be required for some pesticides, which can be easily achieved by a simple preconcentration step using a C_{18} cartridge. Recently, an interesting application was presented for analysis of PAHs in soils, by coupling extraction with subcritical water and SPME–GC [43].

Monitoring surface water samples is also necessary for quality control purposes, with detection limits in the range of 0.5–1 μg/l. Fig. 5 shows the chromatogram corresponding to surface water samples, non-spiked and spiked with 1 μg/l of each pesticide. First, the non-spiked sample shows many peaks, but detection at the 1 μg/l level can be easily distinguished from the non-spiked sample. The surface water sample comes from the River Seine in Paris, which is quite contaminated, and the relatively clean corresponding chromatogram is due, in part, to the selectivity of NPD. The detection limits in surface water are given in Table 5 and are in the range 0.04–0.7 μg/l using GC–NPD. Peaks 2, 3 and 4 cannot be detected at the 1 μg/l level on this chromatogram because their instability was not taken into account and the samples were last to be analysed because of their place in the autosampler. In the non-spiked sample, there is some uncertainty regarding the occurrence of peaks 7 and 10 and since a product cannot be identified by its retention time, a confirmation column or GC–MS should be used. Our aim was to estimate the performance of the method with real samples, therefore, further attempts at identification were not made. Humic and fulvic substances do not seem to be extracted by the CW–DVB fibre, or, if they are, it is only to a small extent.

Table 5
Detection limits (LOD) and precision (R.S.D.) for SPME coupled with GC–NPD of selected pesticides. Comparison with standard US EPA method

Compound	SPME–GC–NPD			EPA method 507
	Drinking water	River Seine water		LLE–GC–NPD
	LOD[a,b] (μg/l)	LOD[a,b] (μg/l)	R.S.D.[a,c] (%)	LOD[b,d] (μg/l)
Dichlorvos	0.03	0.08	9	2.5
EPTC	0.2	0.5	15	0.2
Vernolate	0.3	0.7	18	0.1
Cycloate	0.2	0.5	13	0.2
Prometon	0.04	0.1	3	0.3
Atrazine	0.03	0.06	3	0.1
Terbutryn	0.03	0.06	6	0.2
Metolachlor	0.2	0.4	4	0.7
Triadimefon	0.04	0.1	8	0.6
Fenamiphos	0.02	0.04	6	1.0
Napropamide	0.1	0.2	8	0.2
Norflurazon	0.1	0.2	6	0.5

[a]65 μm CW-DVB coated fibre, 30 min extraction with stirring, sample volume of 11 ml, pH = 7, 4 *M* NaCl.
[b]With $S/N = 3$.
[c]At the 10 μg/l level ($n = 6$).
[d]Liquid–liquid extraction coupled with GC–NPD, drinking water [44].

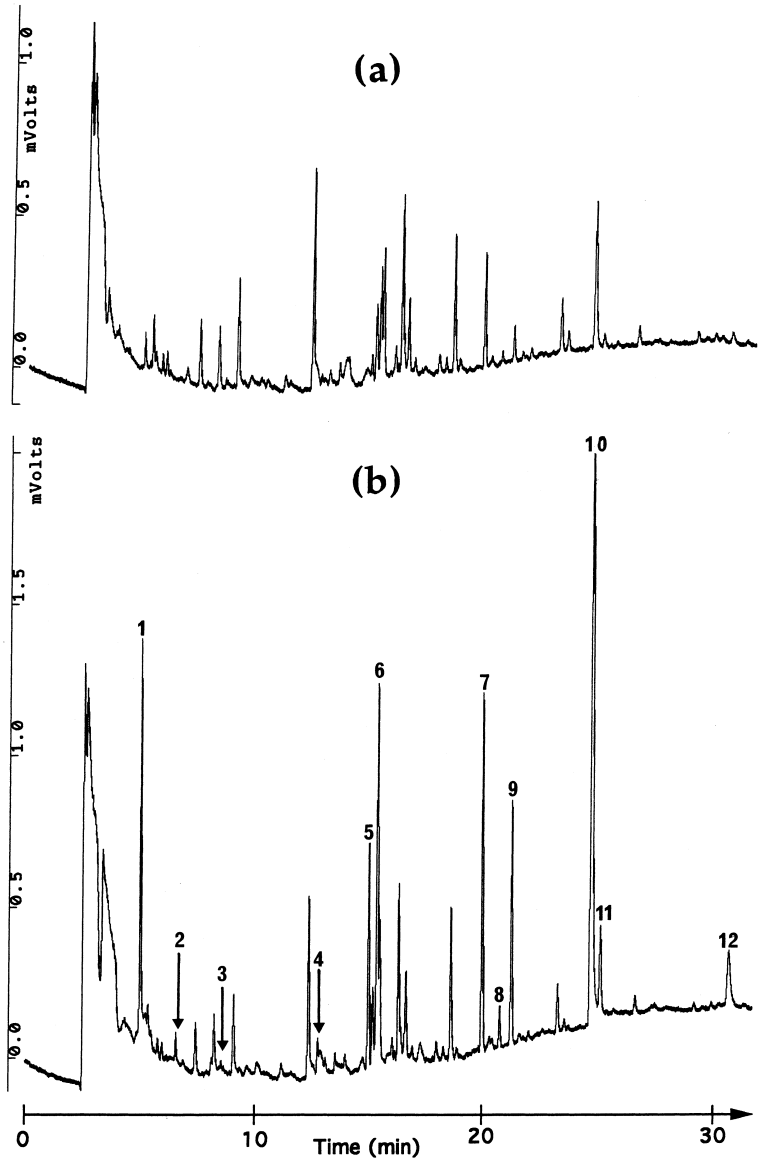

Fig. 5. SPME–GC chromatogram corresponding to the analysis of River Seine water (a) non-spiked and (b) spiked with 1 μg/l of each pesticide. Conditions were the same as in Fig. 4.

This is because the SPME method is not an exhaustive method but is an equilibrium method. In exhaustive methods, such as LLE or most SPE methods, the first aim is to obtain quantitative transfer of target analytes into the extracting phase in order to have 100% recoveries and a consequence of this is that selectivity is often lost because many matrix components are co-extracted. Equilibrium methods are more selective because they take full advantage of differences in the extracting-phase-matrix distribution constants to separate target analytes from interferences [35]. However, one must think that the

effect of the sample matrix on the only SPME step is difficult to estimate because it is usually combined with the selectivity of the GC detector.

Each fibre can be re-used many times with real samples, e.g. more than 50 times for drinking water samples and less, around 30 times, for surface water samples. Fibres have been used more than 100 times with distilled water and one report indicated that the fibre had to be replaced after 27 analyses in run-off water [42].

4. Conclusion

SPME is certainly a promising new and solvent-free method for the handling of aqueous samples prior to GC analysis. Its important features are its simplicity, low cost, rapidity and the sensitivity of the combination of SPME–GC with appropriate detection modes.

Linearity is obtained over a wide range of concentrations, and the detection limits are in agreement with regulation levels. As it is based on an equilibration step, recoveries are not 100%, but the examples presented above show that precision and reproducibility are similar to those obtained with other conventional extraction methods. Moreover, one advantage of this method over exhaustive extraction methods is a reduction in the co-extraction of humic substances. However, one drawback is that predictions of the amounts of compounds extracted are very difficult and no straightforward relationship has been shown between the partition coefficient, the nature of the fibres and the characteristics of the analytes. Therefore, quantitative analysis can be performed provided careful calibration with spiked solutions has been performed previously using exactly the same conditions as those used for running unknown samples. The fact that co-extracted unknown analytes do not modify the calibration curve is a strong indication of the reliability of the quantitative data.

The current limitations are the fact that there are limited types of fibres, especially for the extraction of the more polar pesticides. More specificity and selectivity are expected with the constant progress in polymer technology and further developments are certainly to be expected in the near future with this new approach.

Acknowledgements

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