Simple removal of humic and fulvic acid interferences using polymeric sorbents for the simultaneous solid-phase extraction of polar acidic, neutral and basic pesticides

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Abstract

A styrene–divinylbenzene (St–DVB) sorbent with a specific surface area of ca. 1000 m^2 g^{-1} packed in a disposable cartridge was investigated and compared with C_{18} silicas and other St–DVB polymers such as those entrapped in Empore disks and the LC-grade PRP-1 or PLRP-S with specific surface areas around 500 m^2 g^{-1}. A much higher retention was obtained for polar pesticides and acidic pesticides were also retained in their ionic form. The recoveries obtained on percolating 500 ml of drinking water samples at pH 7 spiked with a mixture of seventeen acidic, neutral and basic pesticides through a cartridge packed with 200 mg of SDB-1 sorbent (1060 m^2 g^{-1}) were above 80% for each analyte. Another important result was that humic and fulvic acids were not co-extracted and co-eluted on percolating a sample adjusted to pH 7, so no clean-up was necessary. Application to the analysis of a 500-ml river Seine water sample spiked at 0.1 μg l^{-1} showed that detection limits in this range can be obtained without any additional clean-up step.

Keywords: Sorbents; Environmental analysis; Water analysis; Humic acid; Fulvic acid; Organic acids; Pesticides

1. Introduction

Pesticides applied in agriculture are characterized by different physico-chemical parameters and chemical properties. With the old scheme of extraction using liquid–liquid extraction (LLE), there used to be a differentiation between the analysis of acidic pesticides and that of neutral and basic pesticides. The scheme is now continued using solid-phase extraction (SPE) since the widely used C_{18} silica sorbents can only trap acidic compounds in their non-ionic form after previous acidification of the samples [1–6]. The reported multi-residue analyses including acidic, neutral and basic pesticides have mainly been performed using on-line techniques which coupled the SPE of the samples adjusted to pH 2–3 with LC analysis [6–9]. However, when natural samples are acidified co-extraction of humic and fulvic acids occurs, which generates a large, unresolved peak at the beginning of the chromatogram or a hump in the middle, depending

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on the mobile-phase gradient applied for the separation [9–11]. The same problem has been encountered using off-line SPE with cartridges [12,13]. As a consequence, the detection limits, which are in the 0.1 μg l^{-1} range for drinking water samples, are closer to 0.5 μg l^{-1} for surface water samples. A clean-up step is therefore required to obtain lower detection limits for natural waters, but with the inherent consequence of increasing the analysis time and the risk of loss and contamination of samples [13].

New apolar styrene–divinylbenzene (St–DVB) copolymer sorbents with specific surface areas around 1000 m^2 g^{-1} have recently been made available in SPE cartridges by several companies. Until now, very few apolar St–DVB copolymers were available in disposable cartridges and it was difficult to obtain them commercially. Empore disks containing an apolar copolymer with a specific area around 400 m^2 g^{-1} have been used for off-line extractions [14,15]. The LC-grade polymers used as stationary phases have more commonly been used in precolumns (mainly PRP-1 or PLRP-S) for on-line purposes. These polymers have specific surface areas around 500 m^2 g^{-1}. The advantages of using St–DVB copolymers instead of C$_{18}$ silicas are the higher retention of analytes (20–50 times higher with PRP-1) and a wider pH range (0–14) [15–18]. One can therefore expect a much higher retention with these new polymeric sorbents. This has been shown by some of the companies providing these new polymers, since they have measured recoveries of 100% for a 1-l sample spiked with deisopropylatrazine using 200 mg of sorbent whereas recoveries lower than 25% were obtained with 500-mg C$_{18}$ cartridges and with a 500-ml sample.

The objective of this work was (i) to investigate the retention of analytes using these new St–DVB polymers in comparison with C$_{18}$ silicas or PLRP-S and PRP-1 copolymers, (ii) to study the extraction of acidic pesticides at pH >3 in order to decrease the co-extraction of humic acids and (iii) to investigate the potential for multi-residue analysis without prior acidification of the samples.

2. Experimental

2.1. Apparatus

LC analyses were performed with a Varian LC System Workstation including a Varian Star 9010 solvent-delivery system and a 9065 Polychrom diode-array detector. The analytical column was connected to a Rheodyne (Cotati CA, USA) valve. The extraction procedure was performed with an Spe-12 vacuum glass manifold (J.T. Baker, Deventer, Netherlands).

2.2. Stationary phases and columns

A 25 cm × 4.6 mm I.D. analytical column prepacked with Supelcosil LC-18-DB 5-μm octadecylsilica (Supelco, Bellefonte, PA, USA) was used for the separation of mixtures containing polar compounds (see Fig. 1). A 25 cm × 4.6 mm I.D. analytical column prepacked with Bakerbond Narrow Pore 5-μm octadecylsilica (J.T. Baker) was used for the separation of mixtures containing neutral and acidic compounds (see Figs. 2 and 3). The cartridges used for preconcentration were 3-ml disposable extraction cartridges packed with 200 mg of SDB-1 apolar copolymer (J.T. Baker). Empore 3M disks containing C$_{18}$ and SDB were obtained from J.T. Baker. PLRP-S precolumns (10 × 2 mm I.D., 15–25-μm particle size) (Polymer Laboratories, Church Stretton, UK) and a PRP-1 column (100 × 4.6 mm I.D., 10-μm particle size) (Hamilton, Reno, NV, USA) were used for retention measurements.

2.3. Chemicals

HPLC-grade acetonitrile was obtained from J.T. Baker and methanol from Prolabo (Paris, France). LC-quality water was obtained from J.T. Baker or was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were purchased from Prolabo, Merck or Fluka.

The various pesticides were supplied by Riedel-de Haën (Seelze, Germany), Promochem (Wesel, Germany) or Cluzeau (Sainte Foy La Grande, France). Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol and stored at 4°C. They were used for the preparation of diluted standard solutions and for spiking water samples. No change in the chromatogram of the standard solutions was observed during the 4 months of this study. The final spiked samples did not contain more than 0.5% of methanol.

2.4. Procedure

Measurements of log k'_w on C_{18} disks were made by using a 5 cm × 4.6 mm I.D. column prepacked with a stack of small disks of alternately 4- and 6-mm diameter. This kind of stacking was chosen to limit the wall effect. For SDB disks, the column (3 cm × 4.6 mm I.D.) was prepacked in the same way. With regard to the SDB-1 sorbent, a column (5 cm × 4.6 mm I.D.) was prepacked with the phase taken from disposable cartridges after they had been emptied.

Off-line extraction steps using cartridges were performed using a Baker Spe-12 vacuum glass manifold. The procedure used for the extraction step was as follows: (1) conditioning the cartridge with 5 ml of methanol, (2) washing the cartridge with 10 ml of LC-grade water, (3) percolating the sample (10 ml min^{-1}), (4) washing the cartridge with 5 ml of LC-grade water, (5) drying the cartridge with an air flow for 30 s, (6) desorption with 4 ml of methanol, (7) evaporation under a stream of nitrogen, (8) adding 200 μl of acetonitrile–LC-grade water (2:8) of pH 3 and (9) direct injection of 50 μl of the residue solution. After the desorption with methanol, 50 μl of a mixture containing methanol and ammonia (4:1, v/v) were added before the evaporation step. Under these basic conditions, acidic compounds are ionized and therefore cannot be volatilized during the evaporation step.

3. Results and discussion

3.1. Retention of polar pesticides on new St–DVB polymer

SPE can be described to a first approximation as a simple chromatographic process and retention of compounds occurs provided that they are not eluted by the water of the aqueous sample itself. A relevant parameter in SPE is the sample volume that can be handled for preconcentration without any breakthrough. Werkhoven-Goewie et al. [19] have shown that the breakthrough volume, V_b, can be easily calculated from LC data and particularly by measuring log k'_w, the retention factor of the solute in pure water. Values of log k'_w are usually determined from chromatographic measurements using analytical columns packed with the sorbents of interest and mobile phases composed of water–methanol mixtures, from the linear relationship observed between log k' and the percentage of methanol. Such a relationship has been widely observed for alkylsilicas and has been also reported for PRP-1 apolar copolymers [17,20].

In this study, values of log k'_w were determined from LC measurements using columns packed either with C_{18} silicas or with each of the four apolar copolymers, SDB entrapped in Empore disks, PRP-1 and PLRP-S, which are LC stationary phases used in precolumns, and SDB-1, used in available off-line cartridges. The characteristics of these four sorbents are reported in Table 1. Table 2 compares the log k'_w values of a few polar pesticides, which were determined from the relationship between log k' and the percentage of methanol. Although only a few values were obtained, the retention factors in water were found to be similar using PRP-1 or SDB polymers, which have very close specific surface areas. These values are ten times higher than those obtained with C_{18} silica and are in agreement with previous studies. The effect of the increase in specific surface area to around 1000 m^2 g^{-1} is considerable, as shown by the increase in k'_w values on SDB-1 by a factor between 15

Table 1
Characteristics of solid-phase extraction sorbents

Sorbent	C_{18} disk	SDB disk	PRP-1 precolumn	PLRP-S precolumn	SDB-1 cartridges
Amount of sorbent (mg)	450	450	20–80	20–80	200
Average particle diameter (μm)	11	6.8	5		
Particle diameter (μm)		1.4–10.9		15–25	43–123
Specific surface area (m^2 g^{-1})	535	350	415	550	1060

and 200 compared with SDB or PRP-1 and by a factor between 125 and 600 compared with C_{18} silica. Therefore, the interest in using such a sorbent for extracting polar pesticides which are insufficiently retained on C_{18} silica cartridges is obvious. This is illustrated in Table 3, which shows the recoveries obtained on percolating 500 ml of water spiked with polar pesticides (values of log P_{ow} characterizing the hydrophobicity of the solute are <2) through a C_{18} disk containing 450 mg of sorbent and 1 l of the same sample through an SDB disk containing 450 mg of sorbent and through a 200-mg SDB-1 cartridge. With C_{18} disks the recoveries are very low for the more polar compounds (oxamyl, DIA) and between 50 and 70% for DEA, carbendazim and aldicarb. Using the SDB disks, oxamyl and DIA are still not totally recovered (27 and 53%, respectively), and all recoveries are above 80%

with only 200 mg of SDB-1 polymer. Fig. 1 shows the chromatograms obtained when analysing 1 l of drinking water, (A) not spiked and (B) spiked with 0.1 μg l^{-1} of a mixture containing polar

Table 3
Recoveries (%) of extraction obtained for several polar compounds in LC-grade water spiked at 0.1 μg l^{-1}

Sorbent	Log P_{ow}	C_{18} disk	SDB disk	SDB-1 cartridge
Oxamyl	−0.47	<3	27	82
DIA	1.1	21	53	92
DEA	1.5	58	93	100
Carbendazim	1.56	62	84	88
Aldicarb	1.1–1.5	69	72	90
Simazine	1.96	95	90	94

Sample volume: 0.5 l on C_{18} and 1 l on SDB and SDB-1. Water–octanol constant (log P_{ow}) from Ref. [21].

Table 2
Comparison of the logarithms of the retention factors in water obtained with C_{18} silica and various St–DVB copolymers (log k'_w values extrapolated from the log k' vs. percentage of methanol relationships)

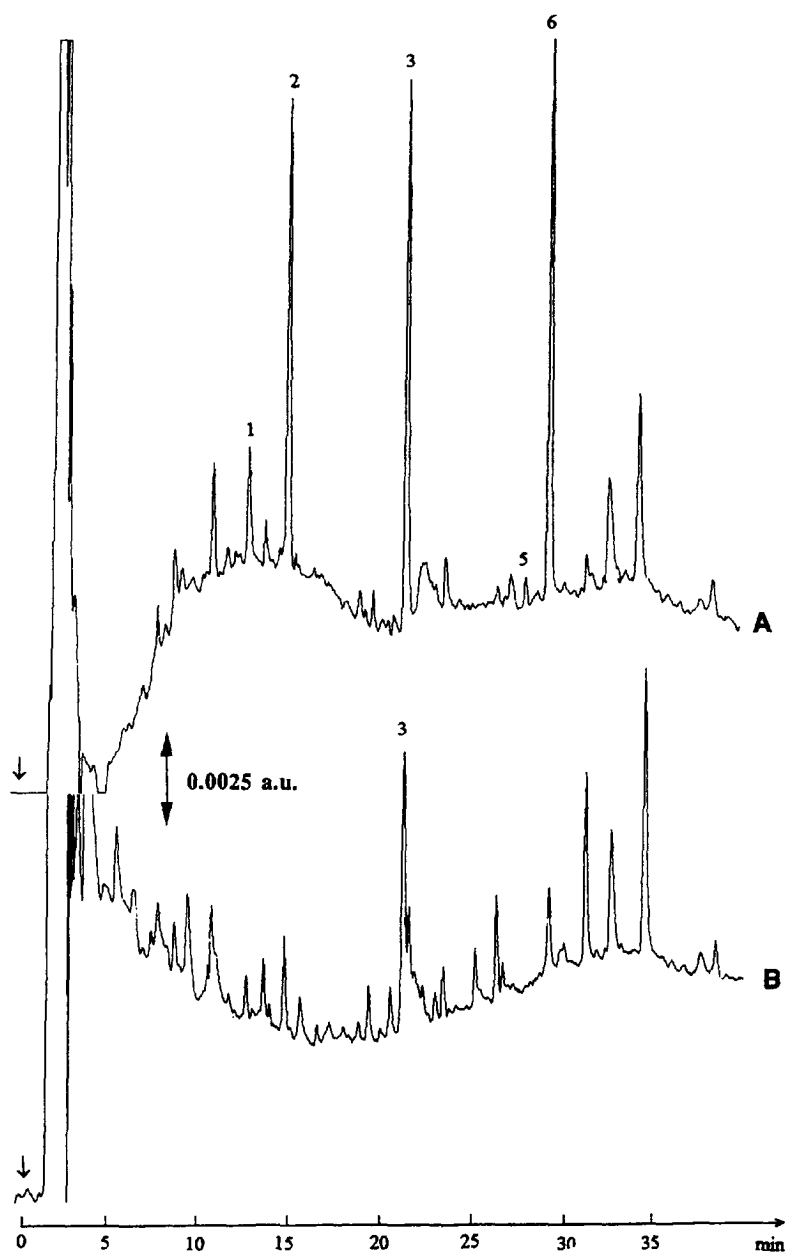| Compound	Log k'_w			
	C_{18}	PRP-1	SDB	SDB-1
DIA	2.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.2	4.4 ± 0.3
DEA	2.7 ± 0.1	3.5 ± 0.3	3.5 ± 0.2	4.8 ± 0.3
Simazine	3.4 ± 0.1	>4	4.1 ± 0.2	5.9 ± 0.3
2-Chlorophenol	2.9 ± 0.1		3.6 ± 0.2	
Oxamyl	1.7 ± 0.1		2.8 ± 0.2	4.1 ± 0.3
Aldicarb	2.5 ± 0.1		4 ± 0.2	5.3 ± 0.3
Carbendazim				5.7 ± 0.3
Chloridazon	2.3 ± 0.1		3.8 ± 0.2	

Fig. 1. Preconcentration of 1 l of drinking water (A) spiked with 0.1 μg l^{-1} of each pesticide and (B) not spiked. Analytical conditions: flow-rate, 1 ml min^{-1}; loop, 50 μl; mobile phase, acetonitrile gradient with 0.005 M phosphate buffer at pH 7, gradient from 10% to 20% acetonitrile from 0 to 12 min and from 20 to 40% from 12 to 25 min; UV detection at 220 nm. Peaks: 1 = oxamyl; 2 = DIA; 3 = DEA; 4 = carbendazim; 5 = aldicarb; 6 = simazine.

pesticides. The diode-array detector allowed the determination of deethylatrazine at a concentration of 0.040 ± 0.004 μg l^{-1} in the drinking water sample.

3.2. Recoveries of acidic pesticides at pH 7

When natural water samples are acidified, humic and fulvic acids are co-extracted and co-eluted and therefore render difficult the analysis of the more polar pesticides eluted at the beginning of the chromatogram in the broad peak or hump corresponding to the humic acids. Previous studies have shown that the interference peak due to humic and fulvic acids was obtained with drinking water and was much larger with surface waters [7–13]. However, it decreased on increasing the pH of the percolated sample from 2 to 7 [12]. Taking into account the much higher retention of the solutes on the SDB-1 copolymer, the retention of the acidic pesticides in their ionic form was investigated. Table 4 reports the recoveries obtained on percolating 500 ml of drinking water spiked with a mixture containing both acidic and neutral compounds through cartridges

Table 4
Recovery (%) of extraction obtained on C_{18} and SDB-1 cartridges for 500 ml drinking water at pH 7 and spiked at 0.1 μg l^{-1} with a mixture of neutral and acidic compounds

No.	Compound	SDB cartridge	C_{18} cartridge
1	Chloridazon	94	100
2	Dicamba	78	<3
3	Aldicarb	88	96
4	Methoxuron	100	100
5	Simazine	105	100
6	Cyanazine	86	105
7	Bentazone	102	5
8	Atrazine	100	100
9	Carbaryl	104	95
10	Isoproturon	98	94
11	Ioxynil	85	31
12	MCPP	100	27
13	Difenoxuron	102	81
14	2,4DB	99	38
15	2,4,5T	100	10
16	Metolachlor	98	102
17	Dinoterb	97	30

Average R.S.D. 3–5% ($n = 3$).

packed with 500 mg of C_{18} silica and with 200 mg of St–DVB polymer. As expected, acidic pesticides (dicamba, bentazone, ioxynil, MCPP, 2.4-DB, 2,4,5-T and dinoterb) are well retained on SDB-1 at pH 7 even in their ionized form, with recoveries higher than 80% for each pesticide. In contrast, the recoveries of acidic pesticides on C_{18} silica are lower than 40% and the more polar acidic compounds such as dicamba and bentazone are not retained at all. These results show clearly that acidification of samples is not required for the extraction of acidic pesticides using the SDB-1 polymer.

3.3. Co-extraction of humic and fulvic acids at different sample pH

The effect of the co-extraction of humic and fulvic acids is shown in Fig. 2, where the chromatograms corresponding to the extraction of drinking water samples spiked with 0.1 μg l^{-1} of each pesticide listed in Table 4 and adjusted to (A) pH 3 and (B) pH 7 using the SDB-1 polymer are presented. When the sample is acidified to pH 3, a broad peak is obtained at the beginning of the chromatogram, rendering impossible the detection of the polar compounds eluted in this interfering peak. At pH 7, humic and fulvic acids are not co-extracted, as can be seen by the flat baseline from the beginning to the end of the chromatogram. This can be explained by the fact that humic and fulvic acids contain many phenolic groups which lead to polyionized compounds that are not retained by the St–DVB polymer. Moreover, the large molecules of humic acids were shown to have a different configuration depending of the pH, so that acidic groups can interact easily at acidic but not at neutral pH. It was also shown that at pH 7 humic and fulvic acids are in the colloidal fraction and are therefore excluded when percolating the water samples.

Each of the compounds listed in Table 4 can be detected below the 0.1 μg l^{-1} level with a simple extraction of 500 ml of drinking water samples. It was therefore of interest to study surface waters under the same conditions. A sample of 500 ml of river Seine water from Paris
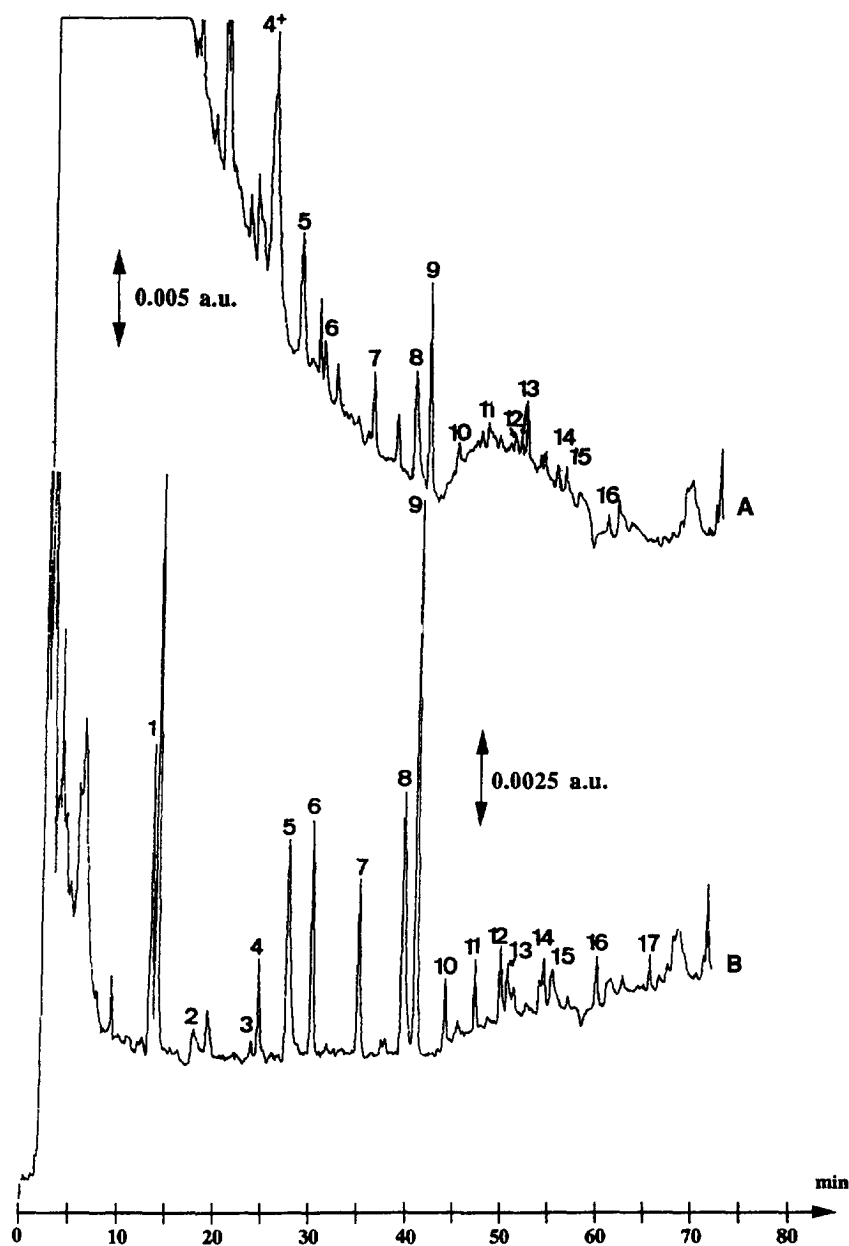
Fig. 2. Effect of the pH of the sample on the preconcentration of 500 ml of drinking water spiked at 0.1 μg l^{-1}. Sample (A) adjusted to pH 3 with perchloric acid and (B) not adjusted (pH 7). Analytical conditions: flow-rate, 1 ml min^{-1}; loop, 50 μl; mobile phase, acetonitrile gradient with 0.005 M phosphate buffer acidified to pH 3 with HClO$_4$, gradient from 10% to 30% acetonitrile from 0 to 10 min and from 30% to 77% from 10 to 80 min; UV detection at 220 nm. Peaks as in Table 4.

was extracted without any pH adjustment using the same procedure as for drinking water samples. The chromatogram obtained with detection

at 220 nm is presented in Fig. 3. Although some interferences are still present, the results are remarkable because the spiking level is 0.1 μg l^{-1}
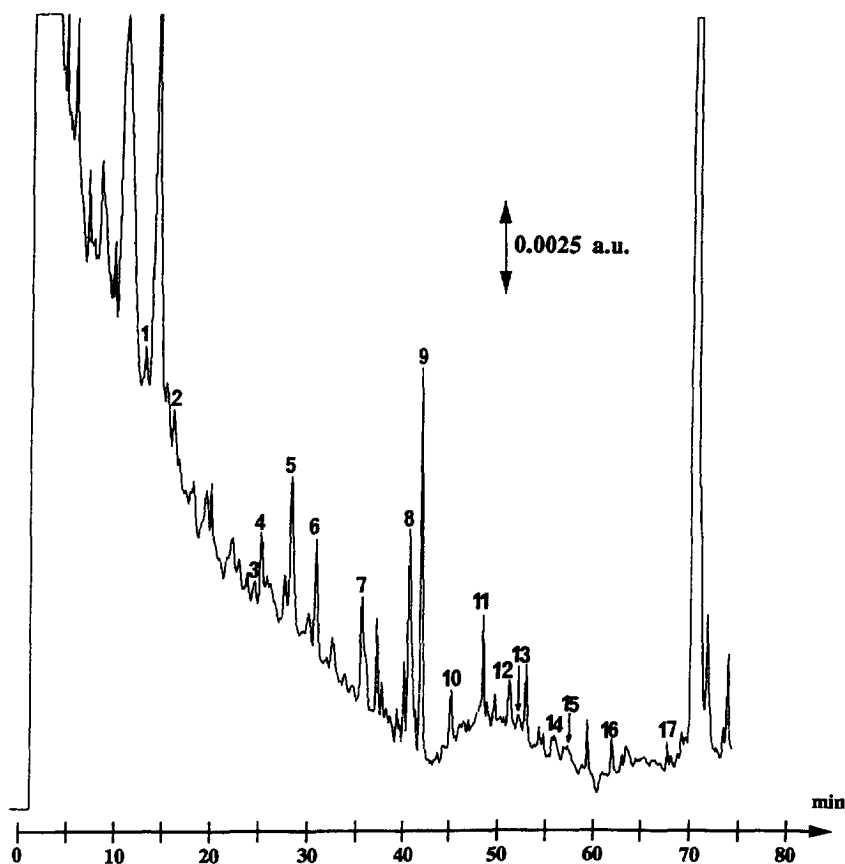
Fig. 3. Preconcentration of 500 ml of river Seine water at pH 7 and spiked with 0.1 μg l^{-1} of each pesticide. Experimental conditions as in Fig. 2.

and each pesticide can be detected at this level in a fairly contaminated surface water sample without any clean-up.

4. Conclusion

The advantage of the new polymers with high specific surface areas over C_{18} silicas for extracting polar pesticides has been demonstrated. Since acidic pesticides can be extracted from water without acidifying the samples, simple removal of the interferences due to humic and fulvic acids can thus be obtained. The sample handling step in the trace-level determination of micropollutants in water is an active area of

research with emphasis on more selective sorbents and sorbents able to trap polar analytes. One step in that direction has been achieved with these new polymeric sorbents.

Acknowledgements

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