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Optimization of a derivatization–solid-phase microextraction method for the analysis of thirty phenolic pollutants in water samples

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Abstract

Solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry has been applied to the extraction of 30 phenol derivatives from water samples. Analytes were in situ acetylated and headspace solid-phase microextraction was performed. Different parameters affecting extraction efficiency were studied. Optimization of temperature, type of microextraction fiber and volume of sample has been done by means of a mixed-level categorical experimental design, which allows to study main effects and second order interactions. Five different fiber coatings were employed in this study; also, extraction temperature was studied at three levels. Both factors, fiber coating and extraction temperature, were important to achieve high sensitivity. Moreover, these parameters showed a significant interaction, which indicates the different kinetic behavior of the SPME process when different coatings are used. It was found that 75 μm carboxen–polydimethylsiloxane and 100 μm polydimethylsiloxane, yield the highest responses. The first one is specially appropriated for phenol, methylphenols and low chlorinated chlorophenols and the second one for highly chlorinated phenols. The two methods proposed in this study shown good linearity and precision. Practical applicability was demonstrated through the analysis of a real sewage water sample, contaminated with phenols.

Keywords: Factorial design; Water analysis; Solid-phase microextraction; Headspace analysis; Phenols; Chlorophenols; Alkylphenols; Cresols

1. Introduction

Phenols are present in the aquatic environment as a result of their industrial applications. Because of their toxicity, phenols have been included in the US Environmental Protection Agency (EPA) list of priority pollutants [1–3]. Also, the European Union (EU) has classified several phenols as priority contaminants and the 80/778/EC directive states a maximum concentration of 0.5 μg/l for total phenols in drinking water. Individual concentration should be under 0.1 μg/l.

Gas chromatography (GC) is a popular technique for the analysis of phenol compounds. However, because of their high polarity, these analytes tend to give broad, tailed peaks, and these effects increase as the chromatographic column ages [4]. To avoid these drawback, several derivatization reactions have been proposed to transform phenols in less polar com-

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pounds, with better chromatographic characteristics. Phenol acetylation with acetic anhydride in presence of carbonate or hydrogencarbonate, is one of the most common derivatization procedures [5]. This reaction can be performed in aqueous samples in a few minutes with high efficiency [6].

Solid-phase microextraction (SPME), is a fast, simple, inexpensive and solvent free extraction technique [7]. It has been applied to the extraction of organic pollutants from different matrixes at trace levels [8,9]. To achieve more selective determination of different classes of compounds, the number of available coating materials has increased in recent times. The nonpolar polydimethylsiloxane (PDMS) fiber was the first polymer being used for SPME [5] and, to date, this coating is the most used one. However, according to the principle of Alike dissolves like, the polar compounds are more likely to be extracted by polar coating such as polyacrylate (PA) and Carbowax–divinylbenzene (CW–DVB).

SPME has been applied to the analysis of phenols in environmental matrices, mainly in water samples [10–18]. In most of these publications SPME is performed at ambient temperature, after adjusting pH at 2 and saturating the sample with NaCl, using PA fibers. Moeder et al. [15] compared extraction efficiency of different SPME fibers (PDMS–DVB, PDMS and PA), for some compounds including nine phenols. The responses achieved with PA and PDMS–DVB were very similar (with the exception of pentachlorophenol, which response was much more higher with PA) and considerably superior to the ones obtained by a 100 μm PDMS fiber. Phenols have also been extracted as acetylderivatives using SPME [10,12,16]. Bulchholz and Pawliszyn [10] investigated the in situ acetylation of phenols followed by SPME–GC–MS analysis. They shown the advantages of acetylation regarding the peak shape and the chromatographic separation of phenols.

In this paper, the derivatization–headspace (HS) SPME of phenols in water samples is studied. Different parameters affecting the analytical process efficiency have been optimized using a mixed level categorical factorial design. Extensive comparison between the performance of five commercial fiber coatings is given. Two of the fibers, 85 μm CAR–PDMS and 100 μm PDMS were the most suitable ones for the extraction of acetylphenols. Linearity and precision studies have been made with both fibers. The proposed methods have been applied to the analysis of the influent of a urban wastewater treatment plant.

2. Experimental

2.1. Reagents and materials

Phenol, *o*-cresol, *m*-cresol, *p*-cresol, 2,4-dimethylphenol (2,4-DMP), 2,3-dimethylphenol (2,3-DMP), 2,6-dimethylphenol (2,6-DMP), 3,4-dimethylphenol (3,4-DMP), 2,5-dimethylphenol (2,5-DMP), 2-chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2-chloro-5-methylphenol (2-Cl-5-MP), 4-chloro-2-methylphenol (4-Cl-2-MP), 4-chloro-3-methylphenol (4-Cl-3-MP), 2,6-dichlorophenol (2,6-DCP), 2,4-dichlorophenol (2,4-DCP), 3,5-dichlorophenol (3,5-DCP), 2,3-dichlorophenol (2,3-DCP), 3,4-dichlorophenol (3,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,6-trichlorophenol (2,3,6-TCP), 2,3,5-trichlorophenol (2,3,5-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,3,4-trichlorophenol (2,3,4-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP), 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP), 2,3,5,6-tetrachlorophenol (2,3,5,6-TeCP) and pentachlorophenol (PeCP) were supplied by Aldrich Chemie (Steinheim, Germany). Acetic anhydride, acetone, sodium chloride potassium carbonate and potassium hydrogencarbonate were purchased from Merck (Darmstadt, Germany). A 1000–5000 mg/ml acetone stock solution of each phenol was prepared. Different acetone standard solutions containing the target phenols were made by dilution of the stock solutions. The spiked water samples used in optimization studies were prepared by adding an appropriate amount of a phenol standard solution. A real water sample from an urban wastewater treatment plant was collected and its phenol content was analyzed.

2.2. GC–MS analysis

Analyses were carried out on a Varian 3400 GC system, equipped with a split/splitless injector, with a Varian Saturn 3000 ion trap mass spectrometer

(Varian Chromatography Systems, Walnut Creek, CA, USA). Experimental parameters were as follows: column, VA-5MS 30 m×0.25 mm I.D., 0.25 μm film; temperature program, 60 °C for 2 min, heated to 115 °C at 15 °C/min and hold for 5 min, heated to 175 °C at 3 °C/min and finally heated to 250 °C at 30 °C/min. Helium was employed as carrier gas, with a flow of 1.0 ml/min. Injector was programmed to return to the split mode after 2 min from the beginning of a run. Split flow was set at 50 ml/min. Injector temperature was held constant at 270 °C. Trap, manifold and transferline temperatures were 250, 50 and 280 °C, respectively. The mass spectrometer was used in the positive electron impact mode at 70 eV. A mass range of 40–300 u was scanned, and the detector was turned off for the first 300 s of the run. The automatic gain control was selected, and the electron multiplier was set at a nominal value of 1600 V.

2.3. HS-SPME extraction procedure

Commercially available 100-μm PDMS, 65 μm PDMS–DVB, 85 μm PA, 74 μm carboxen (CAR)–PDMS and 65 μm CW–DVB fibers housed in manual SPME holders were used (Supelco, Bellefonte, PA, USA). An aliquot of water containing the target phenols was placed in a 22 ml headspace vial. After the addition of sodium chloride, potassium hydrogencarbonate or potassium carbonate, and acetic anhydride (derivatization reagents), the vial was sealed with a headspace aluminium cap with a PTFE-faced septum. In the experiments run at 60 and 100 °C the vial was immersed in a water bath and let to equilibrate for 5 min before HS-SPME. The fiber was exposed to the headspace over the water (HS-SPME) for 5–120 min depending on the experiment. In some of the experiments the sample was magnetically stirred. The fiber was then immediately inserted into the GC injector and analysis was carried out.

3. Results and discussion

3.1. Preliminary experiments

Initial experiments were conducted to optimize the chromatographic temperature program and, thus, to achieve an adequate resolution of the 30 compounds. As can be seen in Fig. 1, the 30 compounds could be separated with the exception of 2,4- and 2,5-dichlorophenol. These compounds coelutes and have been quantified together.

Preliminary SPME experiments were performed in order to study the influence of different parameters on the derivatization–HS-SPME process. In these studies the spiked concentration was at the low ng/ml level. Five milliliters of sample were poured into a 22 ml glass vial and 100 μl of acetic anhydride and 0.2 g of $KHCO_3$ were added. The vial was sealed with a PTFE septum and an aluminum cap and then introduced in a water bath at 25 °C; a 100 μm PDMS-coated fiber was exposed to the headspace over the sample for 15 min.

Firstly, we studied the influence of the addition of NaCl by adding different amounts of this salt. The addition of salt increases the ionic strength of the solution. This makes organic compounds (mainly the more polar ones) less soluble, increasing the partition coefficients and, consequently, the SPME response. Table 1 shows the results obtained for some selected compounds. As can be seen, and in spite of the fact that the derivatization of phenols leads to less polar compounds, an important increase in sensitivity was achieved for all the target compounds. Responses were between four and 17 times higher than the ones obtained without the addition of salt, depending on the compound. NaCl sobresaturation of the samples gave the highest responses (see Table 1) and, in the rest of the experiments performed in this paper, 0.6 g of salt were added per milliliter of sample.

We also study the influence of alkali utilized and the amount of acetic anhydride (derivatization reagents) in the process. Two different bases $KHCO_3$ and K_2CO_3, in varying amounts (between 0.02 and 0.12 g/ml) were added to the sampling media in different experiments. The response obtained was similar in all cases. In all subsequent experiments, 0.02 g of $KHCO_3$ were added to each sample.

Acetic anhydride in the amount of 20–200 μl was added to 5 ml of sample. The responses obtained were similar in these experiments and, so, 4 μl/ml of derivatization reagent were added in latter experiments.

Another parameter studied was the agitation of the

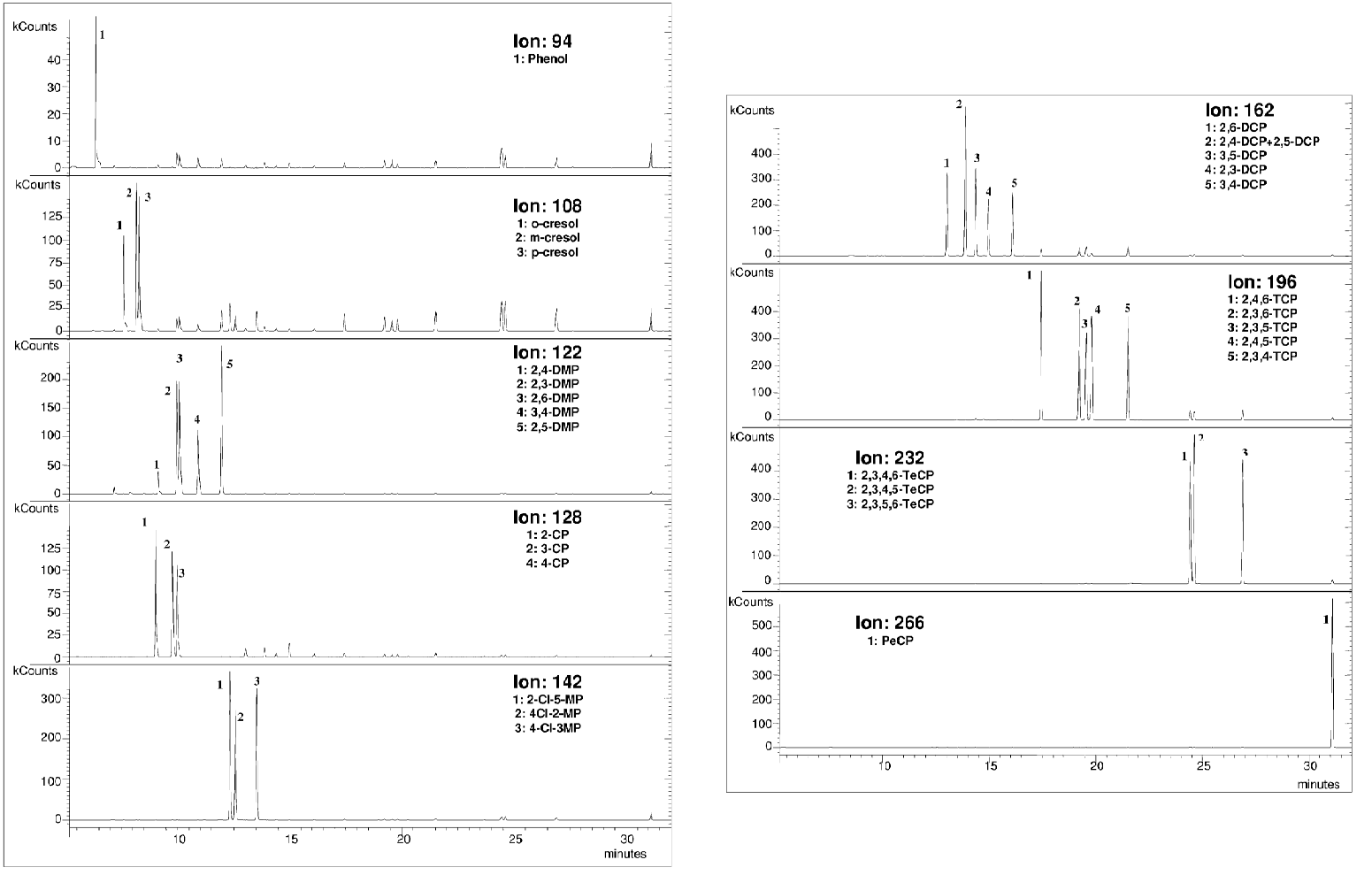

Fig. 1. Ion chromatogram obtained by HS-SPME–GC–MS with PDMS fiber for a water sample containing 10 ng/ml of each phenol.

Table 1
Salting out effect

Compounds	NaCl (% w/v)			
	0	10	20	Sobresaturation (>40%) (n=3)
Phenol	6662	12877	21688	53716±1157
m-Cresol	11672	23440	43180	138559±3886
2-CP	12892	25026	44460	137585±5931
4-Cl-3-MP	13725	27444	52891	214682±19630
2,4-DCP	16613	32260	61196	241942±23473
2,4,6-TCP	28795	65422	120368	342760±39604
2,3,5,6-TeCP	2052	4560	7531	13438±1312
PeCP	4078	10314	12842	16502±1406

Table 2
F ratios and P values obtained in the analysis of variance

Compounds	Main effects						Interactions					
	A: Fiber		B: Temperature		C: Volume		AB		AC		BC	
	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value
Phenol	389	+	61	+	6	+	57	+	398	+	1	
o-Cresol	3429	+	227	+	77	+	145	+	53	+	0.9	
m-Cresol	601	+	81	+	7	+	72	+	4	+	3	
p-Cresol	8	+	0.1		2		0.2		1		1	
2,4-DMP	634	+	78	+	19	+	23	+	15	+	2	
2,3-DMP	600	+	41	+	19	+	19	+	14	+	2	
2,6-DMP	937	+	78	+	12	+	46	+	8	+	0.9	
3,4-DMP	182	+	23	+	4		8	+	2		0.3	
2,5-DMP	110	+	53	+	1		23	+	1		2	
2-CP	1260	+	181	+	9	+	104	+	7	+	2	
3-CP	51	+	21	+	0.4		14	+	0.1		2	
4-CP	39	+	12	+	0.3		10	+	0.1		2	
2-C-5-MP	64	+	40	+	0.9		9	+	0.4		0.7	
4-C-2-MP	125	+	62	+	0.2		25	+	0.8		1	
4-C-3-MP	19	+	37	+	0.1		11	+	0.1		1	
2,6-DCP	400	+	89	+	3		7	+	0.2		0.1	
2,4-DCP	29	+	86	+	2		19	+	0.3		2	
3,5-DCP	94	+	111	+	3		19	+	0.3		2	
2,3-DCP	42	+	84	+	0.1		18	+	0.5		3	
3,4-DCP	15	+	35	+	0.3		11	+	0.2		3	
2,4,6-TCP	14	+	41	+	29	+	6	+	0.8		0.2	
2,3,6-TCP	13	+	51	+	20	+	8	+	0.6		0.5	
2,3,5-TCP	10	+	38	+	14	+	7	+	0.5		0.5	
2,4,5-TCP	16	+	32	+	13	+	11	+	0.2		0.9	
2,3,4-TCP	12	+	54	+	11	+	13	+	0.6		0.9	
2,3,4,6-TeCP	22	+	40	+	21	+	5	+	1		3	
2,3,4,5-TeCP	10	+	18	+	10	+	3		1		1	
2,3,5,6-TeCP	18	+	32	+	15	+	6	+	1		3	
PeCP	17	+	22	+	10	+	3		1		4	

+ cell, P-value<0.05; empty cell, P-value>0.05.

samples. Also in this case we could not appreciate significant differences between the results obtained with and without stirring.

3.2. Factorial design: study of the influence of the type of fiber, extraction temperature and volume of sample

A factorial design, mainly focused to study the behavior of the different available SPME coatings, was performed with the purpose of selecting the best extraction conditions affecting the derivatization–HS-SPME process. For this study, a spiked water sample with individual phenols concentration of 10 ng/ml was employed. The extraction time was 30 min. The experimental parameters studied were: kind of fiber, volume of sample and extraction temperature. The fibers included in the design were: 100 μm PDMS, 65 μm PDMS–DVB fiber, 75 μm CAR–PDMS, 65 μm CW–DVB and 85 μm PA. The temperature was set at three levels: 25, 60 and 100 °C and the volume of sample was 5 and 12 ml, depending on the experiment. In principle, all these fibers could be adequate for the extraction of acetylphenols. We chose a multi-factor categorical 5*3*2 type V resolution design, which involved 30 runs [19]. The advantage of this design is that it allows the study of the main effects and two-factor interactions.

The analysis of the results obtained, after running the 30 experiments, produces the analysis of variance (ANOVA) results summarized in Table 2. For simplicity, only *F*-ratios and *P*-values are given. The *P*-values test the statistical significance of each of the factors. When *P*-values are less than 0.05, these factors have a statistically significant effect at the

Table 3
Normalized mean values for the factor fiber

Compounds	PDMS	PA	PDMS–DVB	CAR–PDMS	CW–DVB
Phenol	1	1.5	1.9	54.4	4.5
o-cresol	1.2	1	1.5	31.0	1.3
m-cresol	2.5	1	4.0	25.8	1.3
p-cresol	1.8	1.2	3.1	29.2	1
2,4-DMP	1.3	1	1.2	12.8	1.7
2,3-DMP	9.1	1	9.3	49.0	2.5
2,6-DMP	8.5	1	9.3	61.0	2.4
3,4-DMP	1.3	1	1.3	10.0	1.8
2,5-DMP	1.3	1	1.5	5.2	1.7
2-CP	1.0	1	1.3	12.1	1.8
3-CP	1.1	1	1.5	5.3	1.4
4-CP	1.1	1	1.6	6.9	1.6
2-C-5-MP	1.1	1	1.2	3.6	1.2
4-C-2-MP	1.2	1	1.3	4.3	1.3
4-C-3-MP	1.2	1	1.3	2.7	2.0
2,6-DCP	1.4	1.1	1.1	5.7	1
2,4-DCP	1.5	1.2	1.4	2.3	1
3,5-DCP	1.4	1.1	1.3	3.0	1
2,3-DCP	1.6	1.2	1.5	2.8	1
3,4-DCP	1.3	1.1	1.4	2.5	1
2,4,6-TCP	2.0	1.5	1.4	2.0	1
2,3,6-TCP	2.2	1.6	1.5	1.7	1
2,3,5-TCP	2.1	1.2	1.4	1.3	1
2,4,5-TCP	2.5	1.3	1.9	1.0	1.1
2,3,4-TCP	2.1	1.3	1.7	1.0	1
2,3,4,6-TeCP	3.6	2.1	1.8	1.5	1
2,3,4,5-TeCP	3.1	2.0	1.4	1	1.0
2,3,5,6-TeCP	3.8	1.4	2.1	1	1.1
PeCP	6.4	2.6	2.6	1.1	1

Each value has been divided by the lowest value of each row.

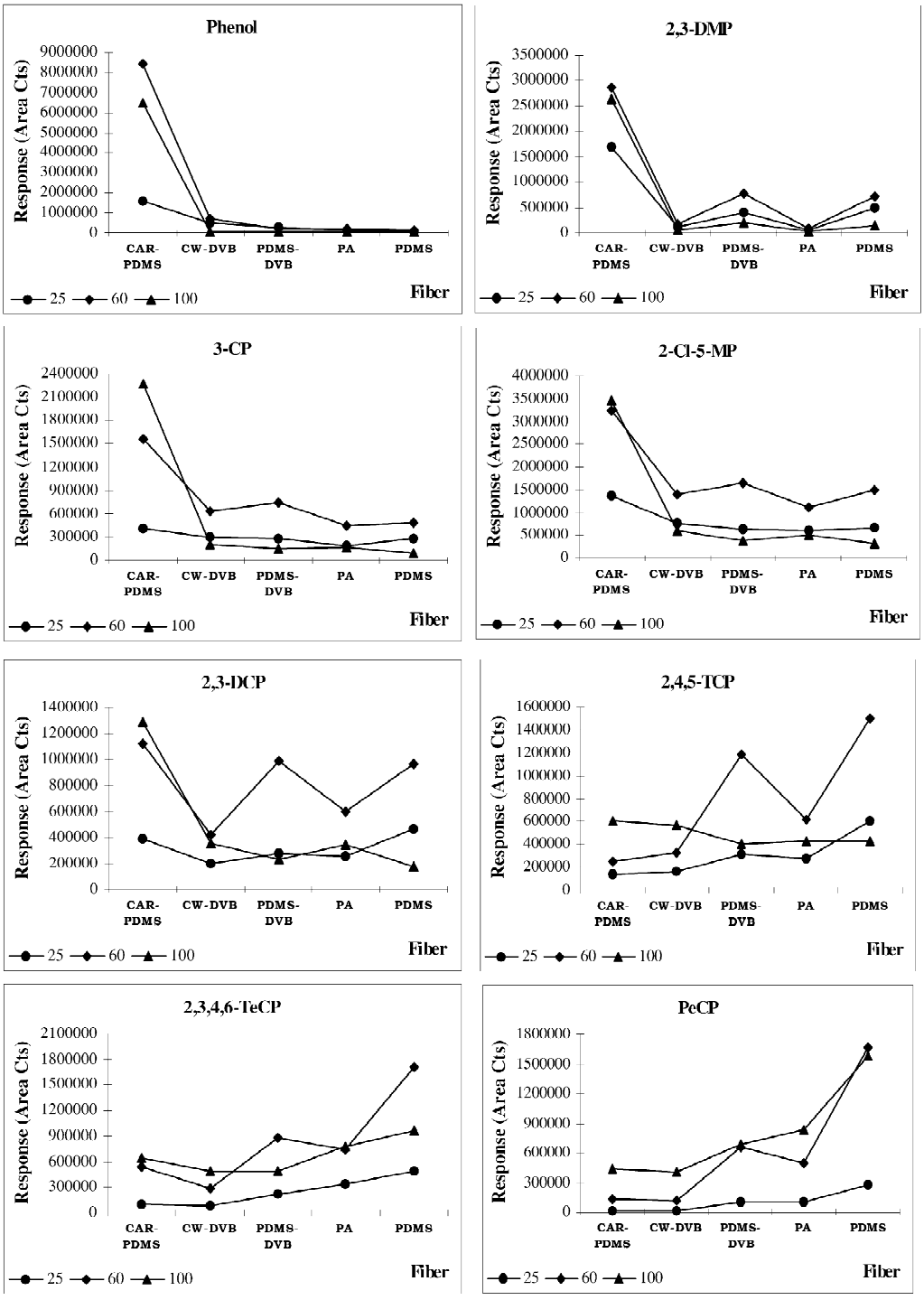

Fig. 2. Graphics showing the influence of main effects type of fiber and extraction temperature (°C).

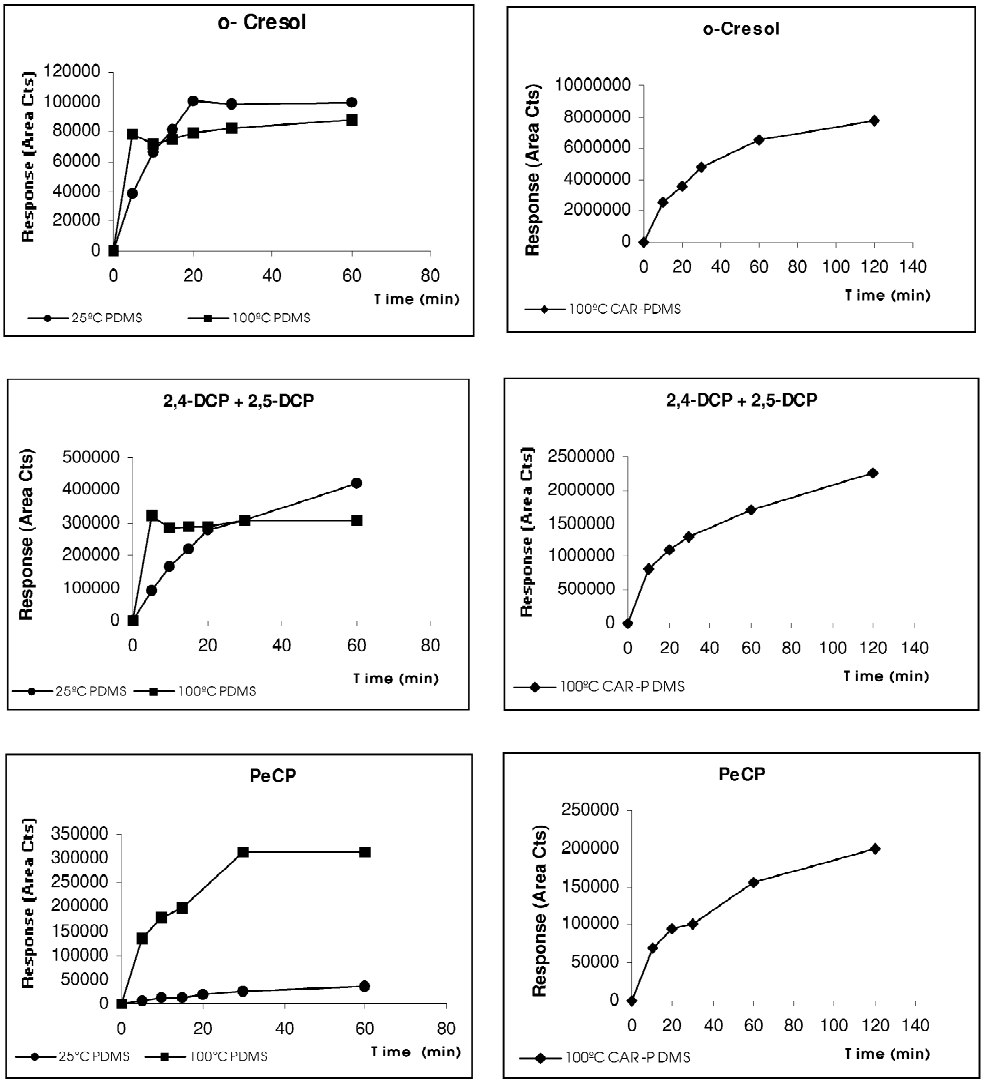

Fig. 3. Extraction time profiles obtained with PDMS and CAR–PDMS fibers.

95% confidence level. The kind of fiber and the temperature were significant for almost all the compounds. The F-ratios measures the contribution of each factor on the variance of the response. As can be seen, the most important factor affecting the SPME of the lighter phenols was the kind of fiber. On the other hand, the temperature was the most significant factor for di-, tri-, tetra- and pentachlorophenols. The volume of sample was less important than the other main factors and it was only significant for some of the target compounds, specially for the highly chlorinated phenols.

Table 3 shows the mean values obtained for the fiber factor. In this table, the responses have been divided by the lowest response in each row. Thus, the lowest response in each row is now 1. As can be seen, the lowest extraction efficiency was obtained by the PA coating for the lighter phenols (see cells with a value of 1 in Table 3) and by the CW–DVB coating for the highly chlorinated phenols. On the

other hand, CAR–PDMS fiber is the one that produces highest mean responses for the non and less chlorinated compounds and PDMS fiber provides the most efficient extraction for tri-, tetra- and pentachlorophenols. These results have been obtained averaging all the levels of the other factors. But considering that the interaction between fiber and temperature, is also significant for almost all the compounds (see Table 2) a closer look at the data must be taken. Fig. 2 shows the interaction plots for these two main factors for some representative compounds. As can be seen, 60 °C is the best extraction temperature for most of the compounds regardless of the fiber used, with the exception of CAR–PDMS fiber. For this fiber, 60 and 100 °C, yield very close results for most of the compounds. For the most highly chlorinated congeners, CAR–PDMS and CW–DVB at 100 °C gave higher responses than at 60 °C. For CAR–PDMS fiber, the least efficient extraction temperature was 25 °C for all the compounds. Nevertheless, for the other fibers, 100 °C was the least efficient temperature for most of the analytes excluding the more chlorinated ones. These results suggest that CAR–PDMS is the fiber presenting slowest kinetics. The extraction time profile for CAR–PDMS at 100 °C was compared with the one for PDMS fiber at two temperatures: 25 and 100 °C. Fig. 3 shows the extraction time curves for some representative congeners. As can be seen, the HS-SPME process is faster for PDMS. For this last fiber, the kinetic of the process at 100 °C is very fast and most of the congeners reached equilibrium conditions in only 5 min. For pentachlorophenol, equilibrium was reached after 30 min. At 25 °C, the process is much slower and equilibrium was only reached for the lighter phenols in the interval of time studied (60 min). For CAR–PDMS at 100 °C equilibrium was not reached within 120 min for any of the compounds.

To compare the efficiency of the five fibers for phenol extraction more easily, Fig. 4 shows the results obtained for the different fibers at 60 °C. As we have already mentioned this is the best extraction temperature for most of the compounds using any of the fibers studied. Again CAR–PDMS stands out as the best fiber for the less chlorinated compounds. In fact, for some compounds, the responses obtained are up to 15 times higher than the ones obtained with the

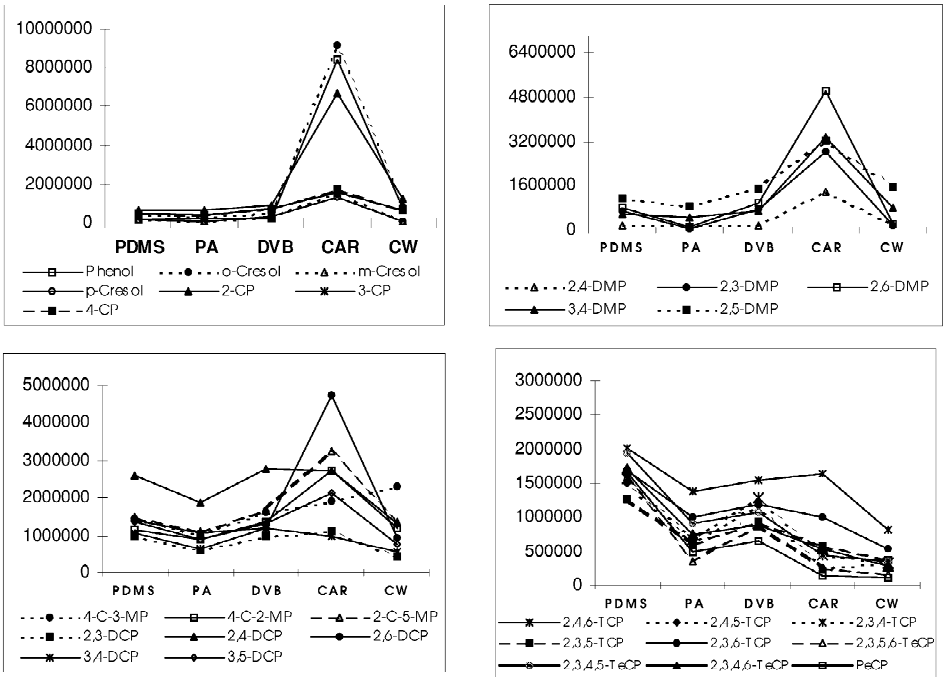

Fig. 4. Influence of the factor fiber at 60 °C.

next best extraction fiber. For the most highly chlorinated congeners, the PDMS fiber is the one that achieves the highest responses. For some of the compounds, it is important to point out that the SPME efficiency is quite similar for all the fibers studied (the difference in response is never greater than a factor of 2).

The volume was not a very important factor for most of the compounds. This factor was only significant for some methylphenols and also for the high chlorinated phenols (tri-, tetra- and pentachlorophenols). For the methylphenols, 5 ml gave slightly higher response for CAR–PDMS fiber. For the other fibers, the two volumes produced almost the same response for both volumes. For the high chlorinated phenols, the responses for 12 ml were significantly higher than the ones obtained using 5 ml for any of the fibers.

After analyzing all this data we can establish two general methods for the SPME of acetylphenols: one method using CAR–PDMS fiber, 100 °C extraction temperature, 12 ml of sample and another method using PDMS fiber, 60 °C extraction temperature, 12 ml of sample. The first method will be more appropriate for the extraction of alkyl and low chlorinated phenols and the second for the highly chlorinated phenols.

3.3. Linearity, precision and application of the HS-SPME methods

To evaluate the linearity of the HS-SPME meth-

Table 4
Linearity, precision and quantification limits of the HS-SPME procedures

Compounds	Concentration range (ng/ml)	Correlation coefficients (R^2)		Repeatability (% RSD)		Quantification limits ($S/N = 10$, ng/ml)	
		PDMS	CAR–PDMS	PDMS	CAR–PDMS	PDMS	CAR–PDMS
Phenol	0.10–10.18	0.9994	0.9999	2.8	4.6	0.061	0.001
o-Cresol	0.13–13.27	0.9988	1.0000	11.1	1.0	0.051	0.003
m-Cresol	0.11–11.29	0.9981	1.0000	0.8	8.2	0.054	0.005
p-Cresol	0.11–11.43	0.9986	0.9998	5.9	11.6	0.054	0.004
2,4-DMP	0.11–11.22	0.9989	0.9998	16.2	2.8	0.052	0.005
2,3-DMP	0.10–9.50	0.9984	0.9998	6.3	7.2	0.015	0.003
2,6-DMP	0.09–9.44	0.9984	0.9998	3.5	8.6	0.017	0.002
3,4-DMP	0.10–9.57	0.9980	0.9997	10.7	7.4	0.019	0.008
2,5-DMP	0.09–9.30	0.9979	0.9992	6.3	5.5	0.012	0.003
2-CP	0.10–10.14	0.9989	0.9998	5.3	0.7	0.030	0.012
3-CP	0.09–8.75	0.9982	0.9992	4.3	2.5	0.033	0.020
4-CP	0.09–8.52	0.9987	0.9992	8.5	4.1	0.054	0.036
2-Cl-5-MP	0.11–10.98	0.9987	0.9989	8.3	5.1	0.007	0.003
4-Cl-2-MP	0.10–9.69	0.9984	0.9994	5.0	3.7	0.030	0.013
4-Cl-3-MP	0.09–9.27	0.9985	0.9990	6.4	2.2	0.008	0.004
2,6-DCP	0.10–10.02	0.9993	0.9997	6.6	4.5	0.005	0.001
2,4-DCP + 2,5-DCP	0.11–11.10	0.9990	0.9989	5.7	7.7	0.013	0.003
3,5-DCP	0.11–11.35	0.9988	0.9990	6.2	2.4	0.029	0.005
2,3-DCP	0.10–9.73	0.9986	0.9986	5.0	7.2	0.030	0.005
3,4-DCP	0.11–10.63	0.9987	0.9991	3.3	8.5	0.025	0.006
2,4,6-TCP	0.10–9.79	0.9996	0.9988	4.9	6.3	0.002	0.003
2,3,6-TCP	0.10–9.62	0.9994	0.9986	5.5	6.5	0.007	0.014
2,3,5-TCP	0.10–10.00	0.9989	0.9976	2.3	3.7	0.007	0.015
2,4,5-TCP	0.10–9.46	0.9990	0.9975	7.9	6.2	0.021	0.040
2,3,4-TCP	0.11–10.59	0.9983	0.9966	0.3	6.3	0.010	0.014
2,3,4,6-TeCP	0.09–9.26	0.9993	0.9982	1.1	5.5	0.002	0.008
2,3,4,5-TeCP	0.08–8.31	0.9994	0.9981	6.8	6.6	0.014	0.040
2,3,5,6-TeCP	0.10–9.66	0.9977	0.9969	6.3	3.8	0.004	0.010
PeCP	0.10–9.46	0.9989	0.9980	5.8	4.9	0.003	0.010

ods, calibration studies were performed with PDMS and CAR–DVB fibers. The concentration range was from 0.1 to 10 ng/ml. The two fibers exhibited a directly proportional relationship between the extracted amount of phenols and its initial concentration in the sample. The correlation coefficients (R^2), shown in Table 4, demonstrated a directly proportional relationship between the extracted amount of phenols and its initial concentration in the sample.

The precision of the experimental procedure was also evaluated. A series of 5 HS-SPME consecutive of a water sample with 10 ng/ml of each phenol gave a relative standard deviation (RSD) ranging from 0.3 to 12% for PDMS and from 0.7 to 12% for CAR–PDMS (Table 4).

The quantification limits (signal-to-noise ratio of 10) are presented in Table 4. They were lower than 0.1 ng/ml for all phenols with both fibers.

Finally, a real contaminated water sample, the influent of an urban wastewater treatment plant, was analyzed. Some of the compounds included in this study were found in the sample and its concentration was evaluated using both HS-SPME methods. For the quantification of this sample standard addition protocol was performed. Table 5 shows the results obtained. As can be seen the concentrations given by both fibers were very close with the exception of *p*-cresol. Matrix effect was also evaluated for this sample by spiking the wastewater with all the target compounds at a concentration level of 10 ng/ml. For most of the analytes, with the exception of tetrachlorophenols and pentachlorophenol, no matrix effect was observed.

Table 5
Phenol concentration (ng/ml) found in the influent of an urban wastewater treatment plant

Compound	PDMS	CAR–PDMS
Phenol	0.74±0.08	0.69±0.04
p-Cresol	1.1±0.1	2.1±0.4
2,3-DMP	0.055±0.003	0.073±0.002
2,6-DMP	0.17±0.01	0.19±0.02
3,4-DMP	3.3±0.5	2.1±0.1
2,4-DCP+2,5-DCP	0.20±0.01	0.17±0.01
PeCP	0.41±0.06	0.37±0.02

4. Conclusions

In this paper the different parameters affecting the HS-SPME of acetylphenols have been studied. Phenols were derivatized in situ by adding $KHCO_3$ and acetic anhydride to the sampling vial. The addition of NaCl produced a very significant increment of extraction efficiency. A factorial design, mainly focused to study the behavior of the different available SPME coatings, was carried out. Two of the five coating, 85 μm CAR–PDMS and 100 μm PDMS were the most suitable fibers: the first one for the extraction of methyl and low chlorinated phenols, and the second one for the high chlorinated phenols. On the other hand, PA and CW–PDMS were the least efficient extraction fibers. The best extraction temperature for 30 min of extraction was 100 °C for CAR–PDMS and 60 °C for PDMS. The kinetics for both fibers were also studied and compared. CAR–PDMS kinetic is considerably slower than PDMS. At 100 °C equilibrium with this fiber has not been achieved for any of the compounds. On the other hand, PDMS equilibrium at 100 °C is achieved in 5 min for most of the target analytes. These two fibers (CAR–PDMS and PDMS) shown good linearity and precision for the 30 compounds included in this study.

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