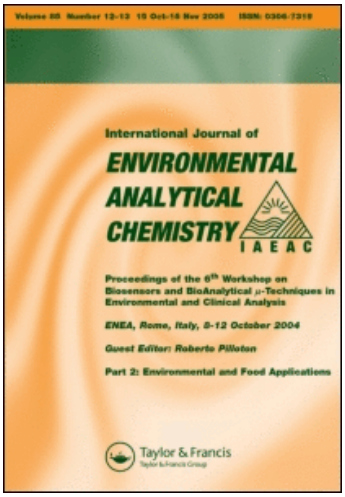

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Verónica Pino[a]; Juan H. Ayala[a]; Venerando González[a]; Ana M. Afonso[a]
[a] Department of Analytical Chemistry, Nutrition and Food Science, University of La Laguna, La Laguna, Spain

To cite this Article Pino, Verónica , Ayala, Juan H. , González, Venerando and Afonso, Ana M.(2007) 'Monitoring chlorophenols in industrial effluents by solid-phase microextraction-gas chromatography-mass spectrometry', International Journal of Environmental Analytical Chemistry, 87: 3, 159 — 175
To link to this Article: DOI: 10.1080/03067310600847252
URL: http://dx.doi.org/10.1080/03067310600847252

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 3, 15 March 2007, 159–175

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Monitoring chlorophenols in industrial effluents by solid-phase microextraction–gas chromatography–mass spectrometry

VERÓNICA PINO, JUAN H. AYALA, VENERANDO GONZÁLEZ and ANA M. AFONSO*

Department of Analytical Chemistry, Nutrition and Food Science, University of La Laguna, Campus de Anchieta, Astrofísico Francisco Sánchez s/n, E-38205, La Laguna, Spain

(*Received 3 April 2006; in final form 23 May 2006*)

A method for the determination of 19 chlorophenols in industrial effluents samples using solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry has been developed. Four kinds of different SPME fibres have been studied. Among them, the polyacrylate and carbowax®-divinylbenzene fibres were the most adequate. The extraction process was optimized by means of the experimental design, which allows the study of a large number of factors with a reasonable number of experiments. The optimized method allows the determination of the studied chlorophenols in complex matrices with a high organic content with detection limits down to 0.07 ng mL^{-1} and RSD ranging from 4.4% to 13.8%. The recovery studies with spiked real effluent samples at low levels of chlorophenols ranged from 59.8% to 142.1% for the lowest level (0.5 ng mL^{-1}) and from 79.6% to 115.8% for the highest spiked level (2 ng mL^{-1}). These results show the suitability of the proposed method to monitor chlorophenols in complex samples. 2,4,5-TCP was detected at concentrations close to its limits of detection in effluents coming from an oil refinery.

Keywords: Factorial design; Industrial effluents analysis; Solid-phase microextraction; Chlorophenols; Gas chromatography–mass spectrometry

1. Introduction

Phenolic compounds enter the environment in different ways: directly, as industrial effluents, and indirectly, as conversion products from natural and synthetic chemicals. Chlorophenols have been used as wood preservatives, fungicides, and intermediates in the production of chlorinated pesticides, and in the preparation of adhesives for more than 50 years [1]. Other sources of chlorinated phenols in the environment are the hydrolysis of phenoxyacidic herbicides [2] and the chlorination of phenol resulting from the degradation of lignin in pulp and paper mills when chlorine is used during the bleaching process [1, 3–5]. Chlorine treatment of drinking water can also produce chlorophenols [6].

*Corresponding author. Fax: +34-922-318090. Email: aafonso@ull.es

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310600847252

It is well known that chlorophenols are toxic at very low levels [7, 8] and quite persistent in the environment [9]. The influence of endocrine-disrupting chemicals for animals, humans, and environments has attracted a great deal of public attention [10, 11]. Some chlorophenols affect porphyry metabolism and have been confirmed as possessing carcinogenic (hepatocellular tumours, leukaemia) and immunosuppressive properties [12, 13].

Owing to their toxicity, both the US Environmental Protection Agency (EPA) [14] and the European Community (EC) [15] have included several phenols in their list of priority pollutants. Achieving nanogram levels of detection, for a large range of chlorophenols in a complex matrix, where organic matter exists at $mg\,L^{-1}$ concentrations, is not an easy task.

Gas chromatography (GC) is a common tool for the analysis of phenols. At a low chlorophenol concentration, peak tailing and interferences with the integration may occur [16, 17], especially when environmental samples are analysed. To overcome this problem, phenols have to be derivatizated with a suitable reagent. In general, such derivatization is an acetylating step that allows the chromatographic separation of the acetylated chlorophenols with symmetric peak shapes [18–20].

Furthermore, to achieve the necessary levels of sensitivity, an enrichment step is needed before the chromatographic analysis. Liquid–liquid extraction (LLE) [21] and solid-phase extraction (SPE) are the most commonly used techniques for the isolation and/or the enrichment of phenols [22–24]. These methods have several disadvantages: they are tedious, labour-intensive and time-consuming. The conventional extraction methods are also hazardous to human health, as they use organic solvents, and extremely expensive with respect to the disposal of solvents.

Great concern over the disposal of such toxic organic solvents and their effect on the environment has led to moves towards cleaner extraction methods such as solid-phase microextraction (SPME). The use of SPME in the analysis of chlorophenols produces acceptable chromatograms, and so it can avoid the acetylating step in some cases, especially when using polyacrylate fibre coatings [16]. If the intention is to automate the process, for screening purposes, the reduction of as many experimental steps as possible is highly desirable. The use of SPME-GC-MS in landfill leaches and soils for the determination of five and 13 chlorophenols has been described [25, 26]. In these works, the optimization of the variables was carried out using traditional univariate methods (one at a time). This procedure is only valid when the variables do not interact. In addition, it is time-consuming and costly, since it requires a large number of experiments.

Having these precedents, the need for determining chlorophenols is now recognized, being essential to achieve good seawater-quality aims in areas subjected to the influence of industrial effluents. Industrial effluent waters are characterized, among other attributes, by their high total organic carbon (TOC) (from 20 up to $1000\,mg\,C\,L^{-1}$ or more, compared to levels of 1–10 in surface waters) and by containing a high amount of particles. This means that the extraction procedures developed for surface and ground waters may not necessarily work for industrial effluent waters, since extraction will be influenced by the TOC, humic and fulvic material, and the particle content of the water matrix [27]. The aim of this work is to develop an SPME-GC-MS screening method to determine the incidence of nineteen chlorophenols at $\mu g\,L^{-1}$ levels in industrial effluents with high organic content flowing into the Canary Islands seawaters. Four kinds of SPME fibres commercially available were evaluated to determine the extraction

efficiency of these compounds. In addition, the parameters affecting the extraction process were optimized using the experimental design, allowing the selection of the optimal values with a relatively short number of experiments.

2. Experimental

2.1 *Chemicals*

The standard mixture solution of 19 chlorophenols (Phenol-Mix 10) with a concentration of $50\,ng\,\mu L^{-1}$ in acetonitrile was supplied by Dr. Ehrenstorfer (Reference Materials, Augsburg, Germany). This standard was stored at 4°C and used for the preparation of a stock standard solution of $1\,mg\,L^{-1}$ in acetonitrile. Afterwards, this stock standard solution was employed in the preparation of the working standard solutions. Acetonitrile of HPLC grade (Merck, Darmstadt, Germany) was used for such dilutions.

NaCl of analytical grade (Merck) was used for adjusting the ionic strength of the solutions. H_2SO_4 of analytical grade (Merck) was employed when adjusting the pH of the solutions.

The SPME fibres were cleaned with Milli-Q water (Millipore, Bedford, MA) after each analysis by immersion for 4–5 s to avoid damage due to crystallization of NaCl.

2.2 *Equipment*

Four different SPME fibres were used: a 100 μm poly(dimethyl)siloxane (PDMS) (Supelco 57301, Bellefonte, PA), a 85 μm polyacrylate (PA) (Supelco 57305), a 65 μm carbowax®-divinylbenzene (CW-DVB) (Supelco 57313) and a 75 μm carboxen™-poly(dimethyl)siloxane (CAR-PDMS) (Supelco 57319). The fibres were conditioned in the hot injector port of the GC according to the instructions given by the manufacturer.

The identification and quantification of chlorophenols were achieved using SPME and gas chromatography/mass spectrometry (GC/MS). GC/MS was performed on a Varian (Varian Inc., Palo Alto, CA) model 3800 Varian Saturn 2000 GC/MS system, equipped with a $30\,m \times 0.25\,mm$ i.d. WCOT CP-SIL-8 CB column (Chrompack, Middelburg, The Netherlands) and equipped with a Varian autosampler (model 8200 CX). The Saturn GC/MS workstation 5.3 software was used for data acquisition.

The temperature of the injector was maintained at 300°C for the PA fibre, at 250°C for the CW-DVB fibre, at 300°C for the CAR-PDMS fibre and at 280°C for the PDMS fibre. The desorption time of the fibres in the GC injector was 5 min.

The GC column was employed under the following temperature programme: 60°C, 4 min isothermal, $8°C\,min^{-1}$ to 120°C, then $2°C\,min^{-1}$ to 135°C, and then $8°C\,min^{-1}$ to 280°C. The carrier gas was helium, with a flow of $0.9\,mL\,min^{-1}$, linear velocity of $34.8\,cm\,s^{-1}$.

The temperature of the transfer line was maintained at 290°C. The ionization was performed with a kinetic energy of the impacting electrons of 70 eV. The temperature of the ion trap was 200°C. The MS analysis was carried out in scan mode with a mass range between 65 and 300 m/z (amu). The quantitative determination was carried out

using the mass values corresponding to the molecular ions of the different chlorophenols (SIM mode).

For the SPME analysis, an autosampler fibre holder (model 57331) from Supelco was used. In this system, it was necessary to use a 12-vial carousel, prepared for 10 mL vials (2-7389 from Supelco). This SPME system incorporates an agitation mechanism consisting of a small motor and a cam to vibrate the needle. The fibre in this design works as a stirrer. The amber vials were capped with PTFE-coated septa.

The problem with the crystallization of NaCl in the fibre has been solved by some authors when using manual injection, by washing the fibre with water prior to the injection [28]. However, this solution has the disadvantage of using manual instead of automatic injection. We preferred the automatic injection in our screening method, so we immersed the fibre in Milli-Q water after each analysis (between samples and not before the injection) to avoid damage by NaCl. Damaged fibres have lost some adsorbent, and therefore they present a decrease in their extraction efficiency. This problem was monitored by injecting a standard solution of chlorophenols after every three samples. At least 40 samples were analysed by the same fibre before it was damaged.

The pH-meter was a Crison GPL21 (Crison, Barcelona). The Statgraphic (Statistical Graphics, Rockville, MD) software package version 4.2 was used for the statistical treatment.

The glassware was first washed with detergent and deionized water, and then rinsed with deionized water, methanol (Merck), and a mixture of acetone/ethanol (1 : 1), both from Merck. Finally, the non-graduated glassware and, especially, the sample vials were dried in an oven at 550°C (to completely eliminate the presence of organic matter) and wrapped with aluminium foil before using.

2.3 *Optimization procedure*

The working standard solutions were prepared using an aliquot of the stock standard solution of chlorophenols, an adequate amount of NaCl, an adequate percentage of organic solvent (acetonitrile), and an adequate pH. These quantities were dependent on the particular experiment dictated by the experimental design.

The SPME procedure consisted in the immersion of the fibre for a fixed time (dependent on the particular experiment), while 10 mL of the working standard solution was being stirred. Afterwards, the fibre was subjected to desorption for 5 min in the GC injector.

The working standard solutions were prepared at pH 2, 35% (w/v) in NaCl and 2% (v/v) of acetonitrile during the validation of the method. The extraction time for the PA fibre in the optimized conditions was 50 min. Blanks were running periodically during the analysis to confirm the absence of contaminants.

2.4 *SPME procedure with industrial effluents*

The industrial effluents were collected in two different points in Tenerife (Canary Islands). One effluent came from an oil refinery, specifically from a water-treatment plant (a combination of chemical waters, waters from processes, and deballasting waters). This effluent had an average content of $0.25\,\mu g\,L^{-1}$ in total aliphatic hydrocarbons. The second effluent came from an industrial area located south of the

Island with different small industries (paper mill, brewery, power station, etc.) and was a combination of several waste streams. This is a well-characterized effluent for environmental purposes, with values of 320 mg C L^{-1} in total organic carbon (TOC), 10.5 mg L^{-1} in total N, 50 mg L^{-1} of oils and greases, 690 mg L^{-1} of biochemical oxygen demand (BOD), and 920 mg L^{-1} of chemical oxygen demand (COD). Therefore, the compositions of both effluents were quite different. Several samples were obtained from each sampling point.

The effluents were collected in amber glass containers using an automatic sampler refrigerated at 4°C. This system took 50 mL aliquots of effluent every hour. The total sample was the result of 24 h sampling, by combining all the 50 mL aliquots. The combined aliquots were taken and as soon as the effluents reached the laboratory, they were kept at 4°C in the dark for at least 24 h. The maximum storage time was 1 week. Afterwards, they were centrifuged at 3000 rpm for 5 min, and the aqueous supernatant was adequately transferred. This aqueous supernatant was saturated in NaCl, adjusted to pH 2 and 2% (v/v) of acetonitrile. Aliquots of this solution (10 mL) were subjected to the optimized SPME procedure.

3. Results and discussion

3.1 *Chromatographic separation*

The chromatographic conditions were optimized to achieve an adequate resolution of the target chlorophenols. Figure 1 shows a representative SPME-GC-MS chromatogram of chlorophenols in an aqueous solution with no control of pH or ionic strength. The chromatogram was obtained with the PA fibre and using a nominal concentration of 20 μg L^{-1} for each chlorophenol. The chromatographic resolutions were higher than

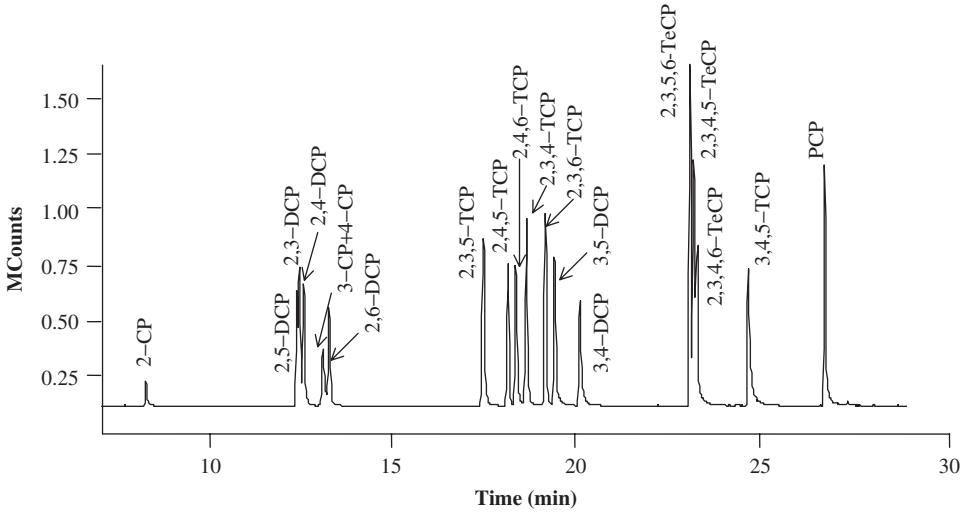

Figure 1. SPME-GC-MS chromatogram (RIC) of chlorophenols obtained with the PA fibre (20 μg L^{-1} of each chlorophenol in an aqueous solution with no control of the pH or the ionic strength).

1.5 for all compounds except for 3-chlorophenol (3-CP) and the 4-chlorophenol (4-CP). It was not possible to achieve a chromatographic resolution between them. In addition, these compounds have the same ions. Therefore, the peak was expressed as 3-CP + 4-CP. The resolution for the pair 2,3,4,5-tetrachlorophenol (2,3,4,5-TeCP)/2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) was 1.36, which could be considered acceptable. Furthermore, the chromatographic resolution between this mixture (3-CP + 4-CP) and the 2,6-dichlorophenol is not good. However, these compounds have different ions, and so an acceptable identification was achieved. Table 1 summarizes the retention time window (RTW) determined for each compound. The RTW is defined for each chlorophenol as the average of the retention times, obtained for 20 different replicas over 2 months and using three different fibres, plus or minus three times the standard deviation. This table also includes the ions used for the quantification of the chlorophenols, and their pK_a values.

3.2 *Selection of the SPME fibre*

Four kinds of commercially available SPME fibres (PA, PDMS, CW-DVB, and CAR-PDMS) were evaluated to determine their extraction efficiencies for these compounds. These experiments were carried out, maintaining the content of NaCl in the chlorophenols solution at 4% (w/v), with a content of acetonitrile of 8% (v/v), and without controlling the pH. The extraction time for all the fibres was 40 min, and the concentration of each chlorophenol was 80 ng mL^{-1}. The experiments were carried out in triplicate and using two different fibres of each kind. Figure 2 shows the extraction efficiencies for several chlorophenols expressed as peak areas. It can be observed that both the CW-DVB and the PA gave the best efficiencies. The studied fibre coatings show the following polarity order: CW-DVB > PA > CAR-PDMS > PDMS.

Table 1. Retention time window, quantifying ions and pK_a values for the studied chlorophenols (chromatographic conditions as described in section 2).

Compound name (abbreviation in parentheses)	RTW (min)	Ion (m/z)	pK_a [24]
2-Chlorophenol (2-CP)	8.29–8.62	128	8.1
2,5-Dichlorophenol (2,5-DCP)	12.26–12.61	162	7.5
2,3-Dichlorophenol (2,3-DCP)	12.31–12.67	162	7.7
2,4-Dichlorophenol (2,4-DCP)	12.41–12.76	162	7.7
3-Chlorophenol + 4-chlorophenol (3-CP + 4-CP)	12.87–13.31	128	8.9 and 9.4
2,6-Dichlorophenol (2,6-DCP)	13.04–13.40	162	6.8
2,3,5-Trichlorophenol (2,3,5-TCP)	16.75–17.29	196	6.4
2,4,5-Trichlorophenol (2,4,5-TCP)	17.41–17.92	196	6.7
2,4,6-Trichlorophenol (2,4,6-TCP)	17.61–18.18	196	6.7
2,3,4-Trichlorophenol (2,3,4-TCP)	17.91–18.46	196	6.9
2,3,6-Trichlorophenol (2,3,6-TCP)	18.50–19.05	196	5.8
3,5-Dichlorophenol (3,5-DCP)	18.92–19.50	162	8.2
3,4-Dichlorophenol (3,4-DCP)	19.72–20.27	162	8.6
2,3,5,6-Tetrachlorophenol (2,3,5,6-TeCP)	23.01–23.37	232	5.0
2,3,4,5-Tetrachlorophenol (2,3,4,5-TeCP)	23.08–23.52	232	5.1
2,3,4,6-Tetrachlorophenol (2,3,4,6-TeCP)	23.19–23.53	232	5.2
3,4,5-Trichlorophenol (3,4,5-TCP)	24.77–25.14	196	7.5
Pentachlorophenol (PCP)	26.89–27.20	266	4.9

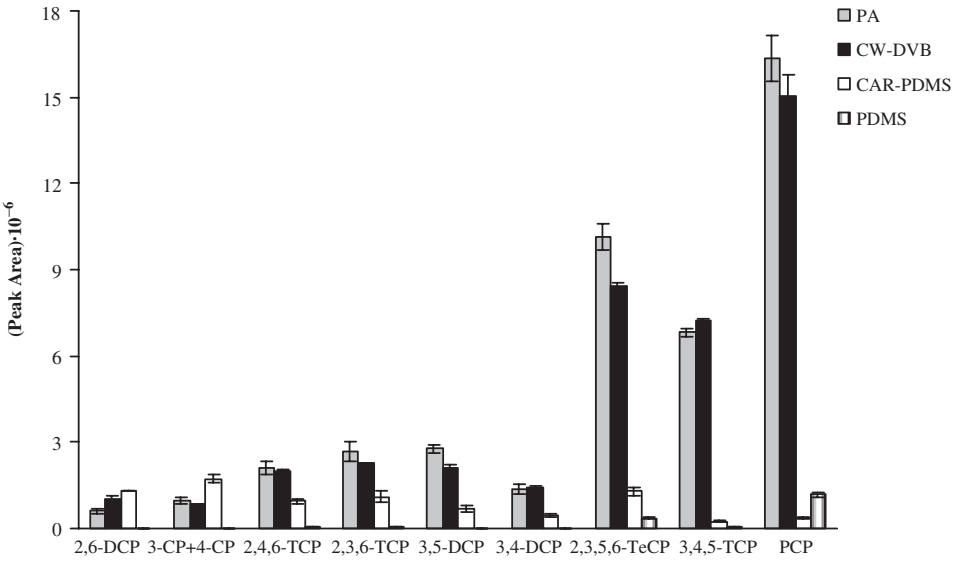

Figure 2. Extraction efficiencies for several chlorophenols expressed as peak areas as a function of the kind of fibre used. The results are the average of 2 fibres of the same kind, and three replicates for each fibre.

We found that the most polar fibres exhibited better extraction efficiencies for the studied compounds. The intermediate precisions, expressed as average relative standard deviation for all the chlorophenols studied, were 8.5, 3.9, 11.8, and 28.5% for the PA, CW-DVB, CAR-PDMS, and PDMS fibres, respectively. The PDMS had the worst intermediate precision, and this effect was particularly remarkable with mono- and di-chlorophenols. When comparing fibres of different kinds, the precision was higher for the CW-DVB followed by the PA fibre. Therefore, the PA and the CW-DVB fibres were selected as the most appropriate when determining chlorophenols. These experiments were developed with the unique purpose of selecting the appropriate SPME fibre coating. In this sense, we carried out the experiments for the four fibres under the same conditions, not yet optimized. Comparing the four fibres under the optimum conditions would lead us to the same conclusion: PA and CW-DVB are the most adequate ones.

Nonetheless, we developed the experimental design and the method validation with the PA fibre, which in general had better extraction efficiencies. It can be assumed that the obtained results can be extrapolated to the CW-DVB fibre.

3.3 *Experimental design for the optimization of the extraction process*

The experimental design allows the study of a large number of parameters and possible interaction between them with a reasonable number of experiments, and to find the optimal practical conditions [29–31]. It is more efficient, more accurate, and faster than the intuitive method, which consists in varying one factor at a time. The studies related to the determination of chlorophenols by SPME have been carried out mainly by optimizing one factor at a time [25, 26, 28]. Llompart *et al.* [32] uses the experimental design to optimize a derivatization-HSPME method in the determination of phenolic

pollutants in water samples. Chlorophenols are much better detected by direct immersion-SPME rather than HSPME, unless an acetylation step is carried out [32]. We have decided not to use acetylation in order to automate and simplify the process, so immersion was the preferred sampling mode.

This optimization study starts by selecting several variables that could potentially affect the extraction efficiency: pH, extraction time of the fibre, ionic strength (NaCl concentration), and amount of acetonitrile in solution.

The pH and ionic strength of the solution have been described as relevant variables in the extraction of chlorophenols [26, 28, 33]. The affinity of a fibre coating for the analytes is related to the octanol–water partition coefficient and to the solubility of the analytes in water. It is obvious that compounds with a high solubility (low octanol–water partition coefficient) will have a higher preference for the PA fibre. In this sense, a higher preference for tetra- and penta- chlorophenols, and lower preference for mono- and dichlorophenols, are expected. At low pH values, the percentage of ionized form of chlorophenols is negligible, and the efficiency of the extraction of chlorophenols can be increased. Therefore, it is essential to develop a design that works with acidic pH values. On the other hand, the 'salting-out' effect by adding salt into the matrix should increase the amount extracted, depending on the solubility of the chlorophenols.

With these precedents, a two-level half-fractional design (screening design) with four variables and one central point, involving 9 runs, was used as a first approach to the response surface of the extraction process [31]. The upper and lower values given to each factor were 2 and 5 for the pH, 0 and 35% (w/v) for the NaCl concentration, 15 and 100 min for the extraction time of the fibre, and 2 and 20% (v/v) for the percentage of acetonitrile in the solutions. Other variables implicated in the extraction were kept constant: volume of solution (10 mL), desorption time of the fibre in the GC (5 min) and the concentration of chlorophenols spiked (20 ng mL^{-1} in each chlorophenol).

The experimental design matrix is shown in table 2. The chronological listing of the experimental design parameters represents the statistically randomized order in which the experimental treatments were undertaken. The elemental response value used in the design was the peak area of each compound. Main effects and interactions can be evaluated by means of the Pareto chart [31]. For all chlorophenols, both pH and percentage of acetonitrile have a negative effect. On the other hand, percentages of NaCl and extraction time have a positive effect in the responses. Figure 3(a) shows the Pareto chart of the 2-chlorophenol as an example. It can be observed that the pH is the

Table 2. Experimental design parameters in the screening design.

Run	pH	% NaCl (w/v)	Extraction time (min)	Percentage of acetonitrile (v/v)
1	5	0	15	20
2	2	35	100	2
3	3.5	17.5	57.5	11
4	2	35	15	20
5	5	35	100	20
6	2	0	100	20
7	5	0	100	2
8	2	0	15	2
9	5	35	15	2

less important factor for this compound. This was valid for all the studied chlorophenols, but when comparing among chlorophenols, the effect of the pH was higher for penta- > tetra- > tri- > di- ~ mono-chlorophenols. The effect of the pH on the chlorophenols extraction efficiency is as expected, based on the pK_a values. Compounds with high pK_a values, such as mono- and most di-chlorophenols, showed no significant change in the amount adsorbed when the pH was varied from 2 to 5. However, for compounds with pK_a values between 4.7 and 7, the decrease in pH produced a higher increase in responses. Nevertheless, other factors appeared to be more important than the pH for the latter compounds. The extraction time was the main important factor for PCP and the tetra-chlorophenols, whereas the amount of acetonitrile was the main factor for the mono-, di-, and tri-chlorophenols.

To develop a total design with the four variables studied would give a large number of experiments. Therefore, it is necessary to fix one factor. Since the pH was the less significant factor and taking its negative effect into account, it was kept to value of 2. In addition, this value was selected because it was the optimum screened by the design and because of the easy adjustment with sulphuric acid. It seems that the utilization of pH < 2 would produce better results, but the recommended working range for the PA fibre is 2–11.

Once the pH was fixed, a new factorial design was established. The selected design was a central composite design, 2^3 + star with two central points and face centred, involving 16 randomized runs [31]. The levels used were: 2 and 20% (v/v) for the acetonitrile content, 15 and 100 min for the extraction time, and 0 and 35% (w/v) for the NaCl concentration. The corresponding design matrix is shown in table 3. The obtained results are much better understood by means of the Pareto charts. Figure 3(b) shows a representative Pareto chart using this central design. It can be observed that the order of significance of the factors is extraction time > acetonitrile > NaCl concentration for the tetra-chlorophenols and PCP. The order of the factor is amount of acetonitrile > NaCl concentration > extraction time for mono-, di- and tri-chlorophenols. As expected, the effect of the salt concentration on the extraction efficiency of chlorophenols is based on their water solubility. The salt concentration was a statistically significant factor for compounds such as mono-, di-,

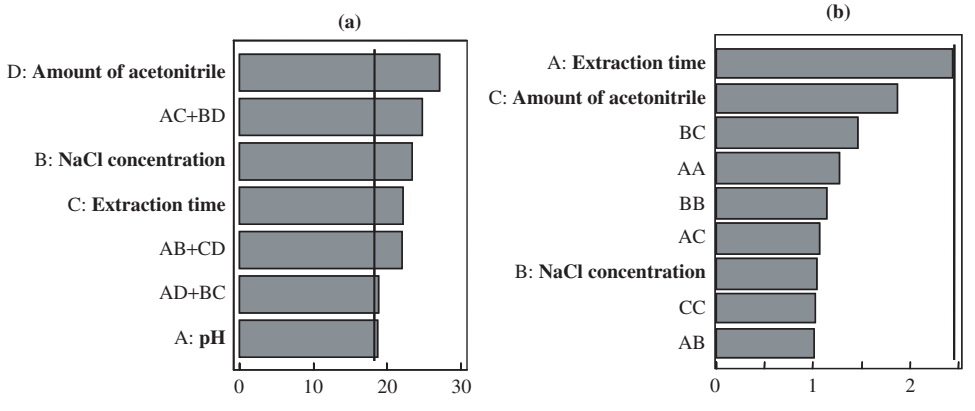

Figure 3. (a) Pareto chart for the 2-CP obtained during the screening design and (b) Pareto chart for the 2,3,4,6-TeCP in the central design.

and some trichlorophenols, which have water solubility values ranging from 1.13×10^4 mg L^{-1} (2-CP) to 450 mg L^{-1} (2,3,6-TCP). In addition, the acetonitrile content also presented a great effect for these compounds. However, the salt concentration was not statistically significant for the rest of the chlorophenols studied, which have water-solubility values ranging from 90.09 mg L^{-1} (2,3,5-TCP) to 14 mg L^{-1} (PCP). Besides, the interaction NaCl concentration × amount of acetonitrile was statistically significant for mono- and di-chlorophenols. There were no other significant interactions for the rest of the chlorophenols. Nevertheless, the interaction NaCl concentration × amount of acetonitrile always had a great effect. Figure 4(a) shows the response surface for the 2,3,4-TCP at the higher extraction time (100 min) with this model. The optimum can be seen in the region of low percentage of acetonitrile and high content of NaCl. Higher responses were obtained with higher extraction times. These results are applicable to mono-, di-, and tri-chlorophenols. Figure 4(b) shows the response surface for the 2,3,5,6-TeCP as an example of the response surface for the tetra-chlorophenols and PCP. In this last case, the optimum is achieved in the low percentage region of acetonitrile, relatively high extraction times, and intermediate NaCl content.

In order to have optimal conditions for most compounds, the optimal conditions for mono-, di-, and tri-chlorophenols were selected for all the chlorophenols. As dictated by the design, these optimal conditions were 35% (w/v) of NaCl concentration (saturation), 2% (v/v) of acetonitrile in the solution, a pH value of 2, and 100 min for the extraction time. Nevertheless, such an extraction time is too high for practical conditions. The profile times for the studied chlorophenols were obtained to decrease the extraction time.

The sorption time profiles were studied by monitoring the peak area as a function of the extraction time of the fibre. Therefore, the PA fibre was immersed in working standard solutions of 10 ng mL^{-1} of chlorophenols, 35% (w/v) in NaCl and 2% (v/v) of acetonitrile content (optimal conditions already achieved). The fibre was stirred at room temperature for increasing periods of time (from 5 to 100 min). Some of these

Table 3. Design matrix of the central design.

Run	Extraction time (min)	% NaCl (w/v)	Percentage of acetonitrile (v/v)
1	15	0	20
2	15	35	20
3	15	0	2
4	57.5	35	11
5	100	35	2
6	100	35	20
7	57.5	17.5	20
8	100	17.5	11
9	57.5	17.5	2
10	100	0	20
11	15	35	2
12	57.5	17.5	11
13	57.5	17.5	11
14	57.5	0	11
15	15	17.5	11
16	100	0	2

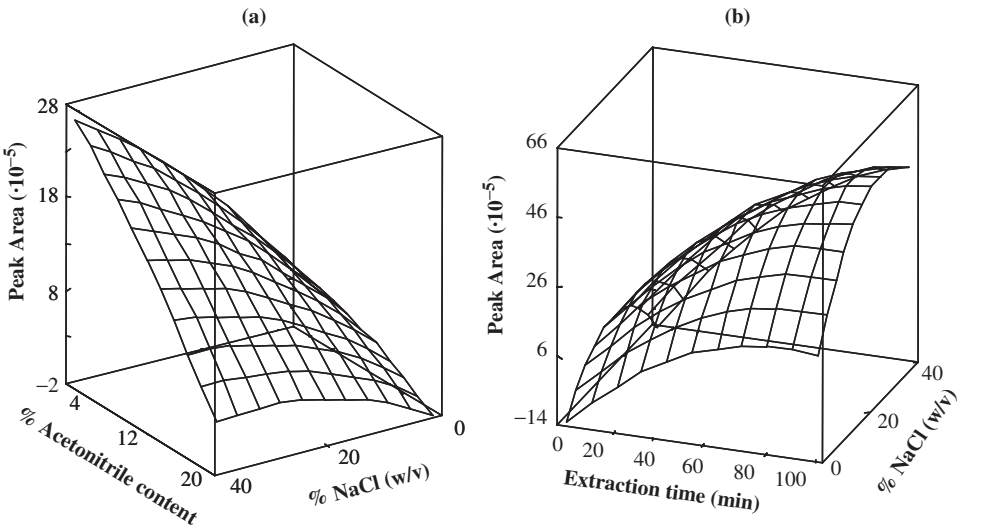

Figure 4. (a) Response surface for the 2,3,4-TCP (extraction time: 100 min) and (b) Response surface for the 2,3,5,6-TeCP (2% v/v of acetonitrile content).

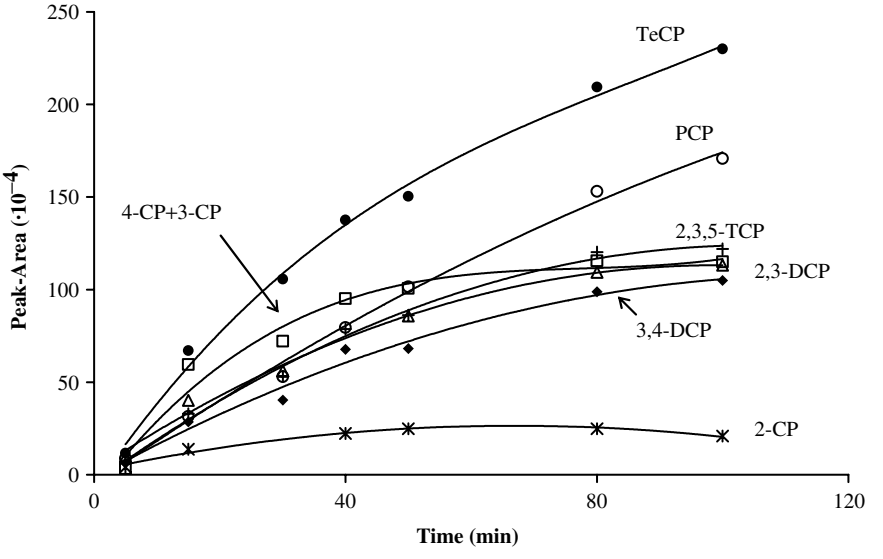

Figure 5. Extraction time profiles for representative chlorophenols. Experimental conditions are as described in the text.

profile times are shown in figure 5. It was observed that mono- and di-chlorophenols seemed to reach equilibration at extraction times around 80 min, with the exception of 3,4-DCP, 3,5-DCP, and 2,3-DCP. In these last cases, it seemed that the equilibration was close, but extraction times higher than 100 min are necessary. This behaviour is also observed with the tri-chlorophenols. For the tetra-chlorophenols and PCP, there were no signs of closeness to equilibration. For quantitative analysis, the analytes do not

need to reach equilibration. Shorter times can be used, as long as the extractions are carefully timed and the mixing conditions remain constant. Therefore, an extraction time of 50 min for the PA fibre was adopted. This extraction time is not too high, especially considering the automation of the system, and it allows the determination of all chlorophenols with sufficient sensitivity.

The type of agitation used in this study (as explained in section 2) is very convenient when automation of the process is preferred. Stir bars have been demonstrated to accumulate analytes and interference substances, with subsequent contamination problems [34]. In addition, agitation by ultrasound has been demonstrated to be inadequate for chlorophenols [25].

In order to increase the lifetime of the fibre, it was immersed in Milli-Q water after each analysis to avoid crystallization of NaCl (as explained in section 2). It would be interesting to have a modification of the SPME software (Saturn workstation 5.3) that would allow the fibre to be immersed in water for several seconds directly after each extraction (agitation of the fibre into the solution) and immediately before the injection in GC (desorption of the fibre into the injector). This washing would minimize the damage of the fibre caused by the crystallization of the NaCl, and the dust would be effectively reduced in the liner of the injector. In addition, this extra step before injection would facilitate some derivatization reactions when using SPME in other applications.

3.4 *Quality parameters of the analytical method*

To evaluate the performance of the SPME procedure under the optimized conditions, the figures of merit were studied. Table 4 illustrates the linearity, intermediate precision, detection limits, and extraction efficiencies for the studied chlorophenols.

Table 4. Linearity, intermediate precision (RSD), limits of detection and extraction efficiencies for the optimized SPME procedure.

Compound	Intercept ± SD[a]	Slope ± SD[a]	r	LOD (ng mL^{-1})	RSD (%)	Recovery (%)
2-CP	–4155.3 ± 3645.4	25 687.1 ± 720.3	0.998	0.31	12.5	100.9
2,5-DCP	–19 459.3 ± 18 196.5	84 719.7 ± 3490.0	0.996	0.34	12.8	98.2
2,3-DCP	–2467.2 ± 12 979.7	87 356.6 ± 2489.5	0.998	0.15	4.8	102.7
2,4-DCP	706.0 ± 12 356.4	76 143.2 ± 2381.9	0.997	0.11	4.4	98.1
4-CP + 3-CP	–32 005.6 ± 18 171.6	102 753.2 ± 3590.6	0.997	0.34	11.6	96.1
2,6-DCP	–3926.3 ± 9142.3	66 932.9 ± 1806.5	0.998	0.28	11.9	98.3
2,3,5-TCP	–4763.6 ± 17 361.2	89 154.5 ± 3346.7	0.996	0.13	11.3	100.4
2,4,5-TCP	983.0 ± 15 866.2	75 183.8 ± 3058.5	0.995	0.22	11.4	103.9
2,4,6-TCP	1551.3 ± 17 682.8	80 678.2 ± 3391.5	0.995	0.09	11.6	98.7
2,3,4-TCP	1276.9 ± 11816.3	79 035.2 ± 2277.8	0.997	0.21	13.6	99.7
2,3,6-TCP	–5186.7 ± 14 755.0	81 257.5 ± 2844.3	0.996	0.17	10.6	101.8
3,5-DCP	–4368.2 ± 16 063.5	89 528.1 ± 3080.9	0.997	0.13	6.5	97.2
3,4-DCP	–6987.3 ± 12 297.8	71 781.5 ± 2358.7	0.997	0.17	7.2	97.5
2,3,5,6-TeCP	–3861.4 ± 22 296.5	105 740.5 ± 4396.9	0.996	0.18	12.2	100.4
2,3,4,5-TeCP	–11 131.8 ± 27 854.1	116 271.7 ± 5492.8	0.995	0.43	12.2	99.6
2,3,4,6-TeCP	18 149.4 ± 17 960.8	153 746.2 ± 4940.7	0.998	0.07	10.5	102.9
3,4,5-TCP	–19 305.5 ± 22 647.1	110 613.5 ± 4954.0	0.995	0.58	13.8	97.6
PCP	3887.7 ± 27134.7	106 038.7 ± 5351.0	0.995	0.66	13.2	101.5

[a] $n = 7$ (seven levels by duplicate).

All chlorophenols showed good linearity with correlation coefficients (r) greater than 0.995 within the calibration range: 0.3–10 ng mL^{-1}. Intermediate precision was evaluated by doing two consecutive extractions of an aqueous standard of chlorophenols (5 ng mL^{-1}) over three different days. The obtained RSD ranged between 4.4 and 13.8%. In addition, the evaluation of the extraction efficiency was carried out under the optimized SPME conditions using aqueous standards at concentrations corresponding to an intermediate point of the calibration curve. The recoveries were good, and they oscillated between 96.1% for the 4-CP + 3-CP and 103.0% for the 2,4,5-TCP. Detection limits (LODs) were calculated as three times the standard deviation of the signal, corresponding to a solution with chlorophenol concentration close to the lowest value of the calibration working range of each chlorophenol, and analysed under the optimized procedure. This way of calculating LODs usually generates higher values, but the high LOD values are more realistic. This is because these LODs concern all of the analytical procedure, and not just the chromatographic separation. They ranged between 0.07 ng mL^{-1} for 2,3,4,6-TeCP and 0.66 ng mL^{-1} for PCP. There are practically no studies related to the determination of chlorophenols in industrial samples. A study from Lacorte *et al.* [32] has determined phenols and chlorophenols in industrial effluents by SPE-HPLC-ED achieving detection limits ranging from 2 to 60 ng mL^{-1}. The limit imposed by the European Community for the chlorophenols content in drinking water is 0.1 ng mL^{-1}. There are no regulations for the chlorophenols content in industrial effluents, but obviously these limits would be higher.

In order to prove the efficiency of the extraction process with real industrial effluents, recoveries of industrial effluent samples spiked before and after centrifugation have been obtained. Figure 6 shows some of these extraction efficiencies when analysing two different industrial effluents. The spiked level was 15 ng mL^{-1}, and the rest of the experimental conditions were maintained. It can be observed that there are slight decreases in the extraction efficiencies for the analytes during the centrifugation step. In any case, extraction efficiencies higher than 82% were obtained, and so real samples can be analysed satisfactorily under the optimized conditions.

3.5 *Analysis of industrial effluents*

The chlorophenols content in real industrial effluent samples was analysed as described in section 2. The compound 2,4,5-TCP was identified in the industrial effluent coming from an oil refinery, with a good MS identification. The Fit search is a useful tool when there are coeluting peaks, as the algorithm only looks for the Saturn library peaks of the analyte in the sample mass spectrum. The value obtained in the Fit search was 805. According to the Saturn library specifications, a Fit value around 800 altogether with a low value for the purity search shows that the spectrum of the library exists in the sample, but there is a high probability of presence of co-eluted mixed compounds. The Reverse Similarity Search (Rsim) shows the similarities between the spectrum of the sample and the spectrum of the NIST library, and assuming that the unknown spectrum has impurities. The Rsim obtained was 756. An Rsim value of 700–800 can be considered a normal identification, according to the specifications. The concentration of 2,4,5-TCP in this effluent was close to the calculated LOD of the method for this compound (0.22 ng mL^{-1}).

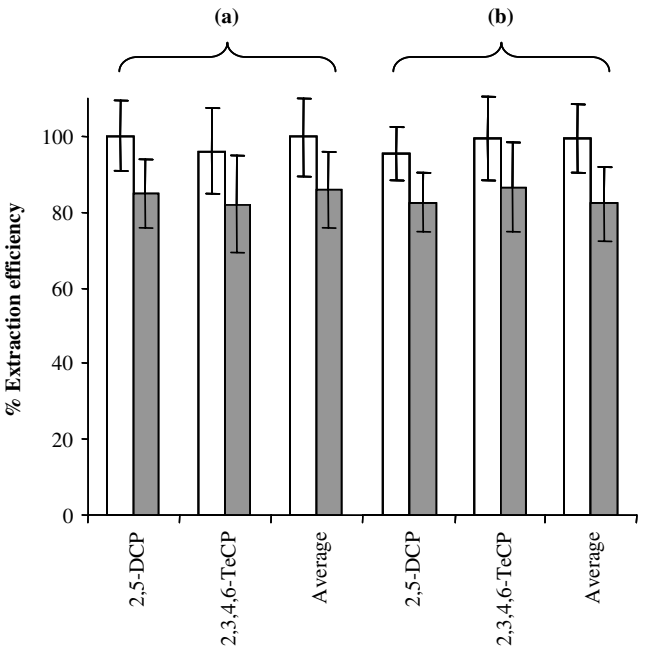

Figure 6. Extraction efficiencies with two different spiked industrial effluents (a) effluent from an oil refinery and (b) combined effluent from different waste streams. The first (white) column represents efficiencies when effluents are spiked after centrifugation. The second (grey) column represents efficiencies when effluents are spiked before centrifugation.

None of the industrial effluent samples coming from the south industrial area of Tenerife Island contained any traces of phenols. Consequently, this effluent was used as a placebo matrix to carry out the accuracy studies to validate the method. The placebo matrix was spiked at two different levels of concentration: 0.5 and $2\,ng\,mL^{-1}$ for all chlorophenols. The selected spiked levels are low in order to evaluate the efficiency of the method with low contaminated samples. Table 5 lists the relative recoveries obtained. Good recoveries, even when spiking at low levels of chlorophenols, can be observed. The average values were 105% and 121% for the effluents spiked at $2\,ng\,mL^{-1}$ and $0.5\,ng\,mL^{-1}$, respectively. At a low level ($0.5\,ng\,mL^{-1}$), as expected, the method is not as accurate, and the extraction efficiencies are generally above 100%, probably due to interferences from the matrix (matrix effect). It should be noticed that this industrial effluent has a high content in TOC ($320\,mg\,C\,L^{-1}$). Other authors have reported acceptable recovery studies (spiking at 1–3 $ng\,mL^{-1}$ levels) when the industrial effluents have low levels of TOC (between 20 and $75\,mg\,C\,L^{-1}$) using SPE-HPLC-ED. The recoveries were not that good when TOC levels were around $500\,mg\,C\,L^{-1}$ [32]. Nevertheless, these recoveries obtained by SPE are from an exhaustive extraction, whereas SPME is not an exhaustive extraction method.

In addition, the spiked samples were subjected to the overall procedure, and so the error in the calculated concentration can be used to estimate the overall error. These average relative errors varied from 11.2% to 26.4% for the $2\,ng\,mL^{-1}$ and $0.5\,ng\,mL^{-1}$ spiked concentrations, respectively.

Table 5. Recoveries of chlorophenols obtained from spiked effluent samples.

Compound	$C_{added} = 2\,ng\,mL^{-1}$		$C_{added} = 0.5\,ng\,mL^{-1}$	
	C_{found} ($ng\,mL^{-1}$) $\pm$ SD[a]	R^b (%)	C_{found} ($ng\,mL^{-1}$) $\pm$ SD[a]	R^b (%)
2-CP	2.01 ± 0.21	100.4	0.60 ± 0.11	120.1
2,5-DCP	1.73 ± 0.16	86.7	0.61 ± 0.10	122.0
2,3-DCP	2.18 ± 0.16	108.9	0.59 ± 0.14	118.2
2,4-DCP	2.24 ± 0.23	112.2	0.63 ± 0.09	126.4
4-CP + 3-CP	1.59 ± 0.05	79.6	0.63 ± 0.14	126.0
2,6-DCP	1.96 ± 0.24	98.0	0.68 ± 0.11	136.2
2,3,5-TCP	2.29 ± 0.28	114.5	0.67 ± 0.08	134.8
2,4,5-TCP	2.30 ± 0.27	115.0	0.64 ± 0.12	128.2
2,4,6-TCP	2.20 ± 0.15	110.0	0.71 ± 0.07	142.1
2,3,4-TCP	2.32 ± 0.28	116.0	0.56 ± 0.15	112.1
2,3,6-TCP	2.10 ± 0.14	105.0	0.70 ± 0.22	140.2
3,5-DCP	2.15 ± 0.15	107.5	0.64 ± 0.22	128.6
3,4-DCP	1.81 ± 0.12	90.3	0.64 ± 0.19	129.2
2,3,5,6-TeCP	1.79 ± 0.19	89.5	0.66 ± 0.21	132.8
2,3,4,5-TeCP	2.30 ± 0.07	115.0	0.52 ± 0.23	104.3
2,3,4,6-TeCP	2.32 ± 0.24	115.8	0.30 ± 0.12	59.8
3,4,5-TCP	2.21 ± 0.29	110.5	0.62 ± 0.24	124.4
PCP	2.30 ± 0.30	115.0	0.44 ± 0.22	87.2
Average recovery for all CPs	105.0		120.7	

[a] Average of three independent extractions.
[b] Mean recovery of three independent extractions.

Figure 7 shows the chromatogram of the industrial effluent spiked at $0.5\,ng\,mL^{-1}$ level. In this chromatogram, we can observe that it is possible to identify all the chlorophenols suitably, even in the presence of a high number of interferences: quite different intensity scales for the Resulting Ion Chromatogram (RIC) and for the Single Ion Monitoring (SIM) mode. It can be observed in the chromatogram that five chlorophenols (2-CP, 2,6-DCP, 3,5-DCP, 3,4-DCP, 3,4,5-TCP, and PCP) showed very low peak areas. It would be difficult to decrease their peak areas (considering the interferences from the matrix) without losing reliability in their identification. To achieve the necessary levels of reliability, $0.5\,ng\,mL^{-1}$ was considered as the lowest reliable spiked level for complex samples like the industrial effluents.

4. Conclusions

The proposed SPME-GC-MS method allows the determination of 19 chlorophenols in industrial effluent samples with high recoveries and good sensitivities. In addition, this determination is possible even in samples with a high amount of interference. A factorial design was used to optimize the variables that affect the microextraction process. The extraction time, pH, acetonitrile content, and ionic strength were the factors studied in order to obtain optimal extraction efficiencies when using the PA fibre. The maximum recoveries were obtained at low pH values, low acetonitrile content in the solution, high content of NaCl, and high extraction times. These optimized conditions are basically related to the water solubility of chlorophenols and also to the affinity of the PA coating for the molecular form of these compounds. In addition, it would be interesting to have a modification of the SPME software that would allow

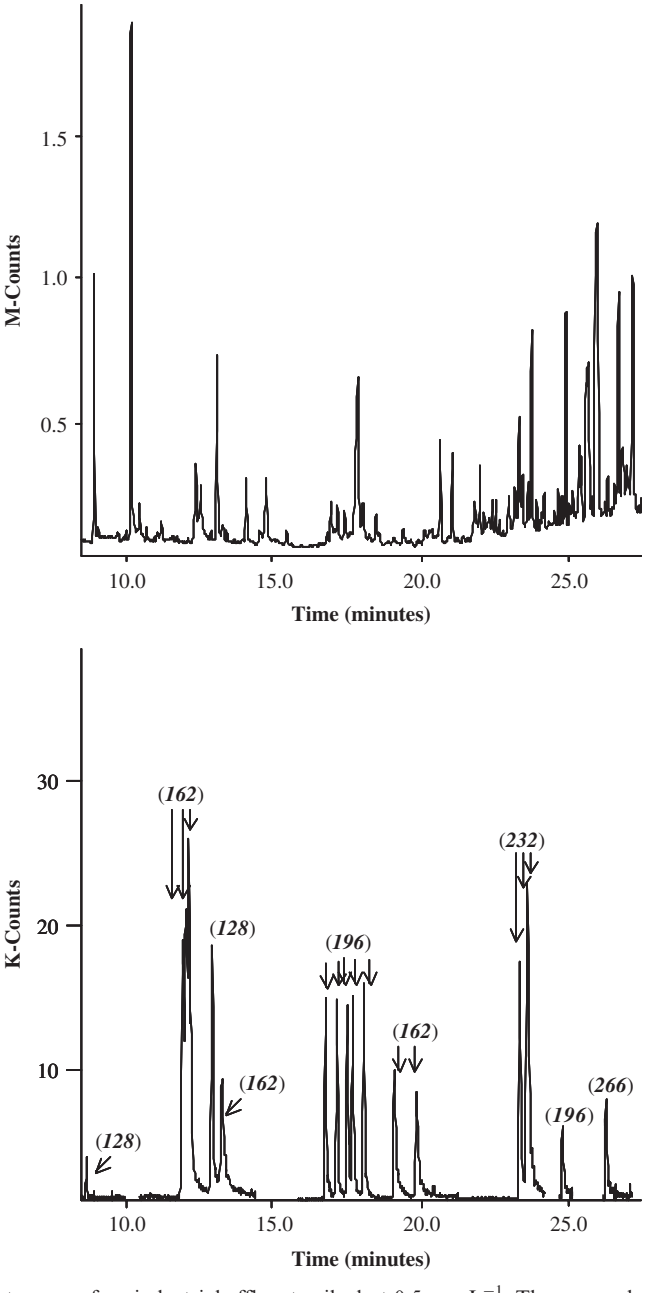

Figure 7. Chromatogram of an industrial effluent spiked at 0.5 ng mL^{-1}. The upper chromatogram shows the resulting ion chromatogram (RIC), and the lower chromatogram shows the single ion monitoring (SIM) for each chlorophenol.

the fibre to be immersed in water for several seconds directly after each extraction and immediately before the injection in the GC. This washing would minimize the damage of the fibre caused by the crystallization of the NaCl. This modification in the software would also be welcome when using automated-SPME with derivatization.

Acknowledgements

Verónica Pino would like to acknowledge the Ministerio de Educación y Ciencia the Juan de la Cierva contract with University of La Laguna. This work was supported by the project AGL-2002-02149 financed by Dirección General de Investigación del Ministerio de Ciencia y Tecnología (Spain).

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