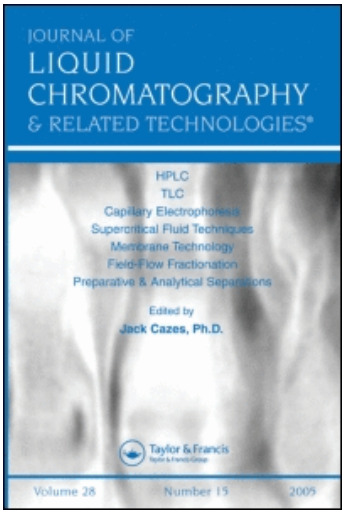

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Direct Supercritical Fluid Extraction of Alkylphenols from Spiked and Wastewater Samples Using Extraction Cells Equipped with Hydrophobic Membrane-Assemblies

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ABSTRACT

Akylphenols (4-nonylphenol, 4-(1,1,3,3-tetramethylbutyl)-phenol and 2,6-*bis*(1,1-dimethylethyl)-4-ethylphenol) have been directly extracted from water samples using neat supercritical carbon dioxide and collected

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on an octadecyl silica (ODS) solid phase trap. The extraction cells were equipped with home-built assemblies containing hydrophobic membranes. The assemblies were inserted at each end of the extraction cell to maintain the water sample inside the extraction cell. In this case, no sample pretreatment step, such as freeze-drying or solid phase extraction (SPE) of the water samples, was needed prior to the supercritical fluid extraction (SFE). Distilled water, spiked with an alkylphenol standard solution at a level of 25 mg/L, was used as a model sample to investigate extraction efficiency as well as collection capability. To obtain quantitative recoveries, a fractionated extraction/elution procedure was adopted. The alkylphenols were finally determined in a leachate water sample and in an industrial effluent sample, where the levels were in the range of 0.2–10 mg/L.

Key Words: Direct supercritical fluid extraction; Aqueous samples; Alkylphenols.

INTRODUCTION

In comparison with the huge number of scientific papers dealing with SFE of solid samples, the numbers of investigations of aqueous matrices are very limited. The vast majority of these investigations deal with the determination of distribution coefficients of a variety of organics to support process scale SFE.[1–4] However, some applications of analytical SFE have been reported for fluid samples, such as the determination of oil in water,[5] persistent organic pollutants in water,[6,7] phenolic compounds from an aqueous hydrolysate of spruce,[8] drugs from biological fluids,[9] and vitamins from milk.[10]

SFE of aqueous samples can be performed in either an indirect or a direct manner. Indirect SFE involves a pretreatment step of the sample by solid phase extraction (SPE)[6,11–15] or freeze-drying of the water sample[16] before placing it in the extraction cell. In the former case, the analytes are adsorbed on a disk or cartridge before elution with SFE, and selectivity can be extended by the choice of SPE phase. An advantage for both SPE and freeze-drying is that large sample volumes can be handled relatively easy. The major disadvantage is that indirect SFE is time-consuming with many manual steps.

Direct SFE of aqueous samples is usually performed by mixing the aqueous sample with a support material in the extraction cell prior to extraction;[9,10,17–19] alternatively the whole aqueous sample is mechanically retained inside the extraction cell.[20–22] In a few cases, direct SFE has also been connected on-line to SFC-MS or LC-MS.[23,24] The advantage of extract-

ing directly from water is that no sample preparation is required, as the aqueous sample can be placed directly into the extraction chamber.[25] A disadvantage of using a support material is that interaction between analyte and adsorbent may be so strong that neat carbon dioxide will not be sufficiently polar for breaking the adsorption forces.

Specially designed cells for direct SFE were first reported by Hedrick and Taylor.[20–22] They successfully determined a variety of compounds, such as phenol and caffeine, by letting the supercritical fluid re-circulate through the water sample before transferring the extract into the final chromatographic system. A home-built cell has also been used recently in applications concerning extractions of PCBs and PAHs from spiked water samples.[26–28] An early paper by Thiebaut et al.[29] showed a set-up where the aqueous sample was mixed with the supercritical fluid. After completed extraction the mixture was separated by means of a hydrophobic membrane. Kane et al. have utilized another special designed vessel for the extraction of surfactants in water, where the supercritical fluid bubbled through the bulk water sample.[15] To enable the supercritical fluid to have a larger contact area towards the water sample, a standard solvent filter was placed at the inlet of the extraction cell. This approach improved analyte recoveries. A similar cell was used by Barnabas and co-workers for extractions of organochlorine pesticides.[30]

One difficulty when using SFE for aqueous samples is to maintain the liquid in the extraction cell with a certain risk of breakthrough to the collection device. Since the water solubility in neat carbon dioxide varies from 0.4 mol% at 80 bar to about 0.8 mol% at 400 bar and 50°C, some co-extraction of water is unfortunately inevitable.[25] The change of pH when water is exposed to supercritical carbon dioxide also needs to be considered. Basic compounds will then be protonated in the carbon dioxide/water mixture, and therefore, more difficult to extract.[25] A few studies have been performed on the effects of pH during extraction of organics from water samples.[31,32] In these experiments, pH only played a minor role on analyte recovery.

In this study, with direct SFE of aqueous samples, hydrophobic membranes were used to keep the aqueous sample inside the extraction cell. Three alkylphenols, 4-nonylphenol (NP), 4-(1,1,3,3-tetra-methylbutyl)-phenol (MBP), and 2,6-*bis*(1,1-dimethylethyl)-4-ethylphenol (MEEP) were used as model substances. The recent large interest in the analysis of this type of organic pollutants, which originate from the degradation of alkylphenolethoxylates, depends on the fact that such compounds have been identified as endocrine-disrupting chemicals (i.e., mimic the action of natural hormones).[33,34] Since these pollutants can be present at relatively high levels in industrial effluent waters, they were considered to be well suited for evaluating the new technical solution for direct SFE.

EXPERIMENTAL

Samples

Spiked water samples were prepared directly in the extraction cell by adding 50 μL of an alkylphenol standard solution (1.0 mg/mL of each alkylphenol in methanol) to a volume of 2 mL distilled water.

Leachate samples were collected from a landfill outside Emmaboda, Sweden. Wastewater samples, effluent from a textile industry, were collected in Porto, Portugal. All samples were stored in sealed glass beakers at 4°C.

Chemicals

NP, MBP, and MEEP were all of 98% purity and purchased from Promochem AB, Ulricehamn, Sweden. The chemical structures of NP, TMBP, and MEEP are shown in Fig. 1.

Standard stock solutions (1.0 mg/mL) were prepared for each alkylphenol in methanol. Working standards of mixtures of the compounds (0.1–40 μg/mL) were prepared by diluting aliquots of the stock solutions in methanol. Methanol and acetonitrile were of HPLC grade and delivered by Merck (Darmstadt, Germany). All water used was of p.a. quality or better. Orthophosphoric acid (85%, analytical-reagent quality) was from Merck (Darmstadt, Germany). The extraction medium used for SFE was carbon dioxide (4.8 grade; AGA, Sweden). Support material used in the SFE experiments was stainless steel beads (300–385 μm, Anval, Torshälla, Sweden).

Instrumentation

SFE

The SFE system used was a Hewlett Packard 7680T extraction unit (Wilmington,USA) equipped with a Hewlett Packard 1090 LC pump for addition of modifier. During the extraction, the analytes were collected on a Hewlett Packard standard trap filled with octadecyl silica (ODS). After completed extraction, the analytes were eluted from the trap at 40°C with 1.5 mL of methanol. The cells used for the extraction in this report were modified Hewlett Packard 7 mL extraction cells made of stainless steel with standard pressure-tight seals. At each end of the stainless steel cell, an assembly containing a hydrophobic membrane (0.2 μm Fluoropore, Millipore, Bedford, MA) was placed to maintain the water sample in the extraction cell. In this

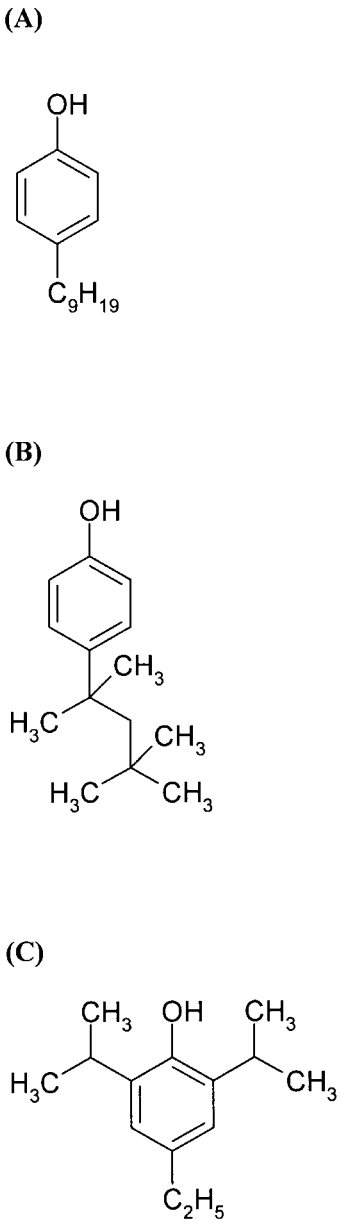

Figure 1. Chemical structures of the alkylphenols investigated. (A) 4-Nonylphenol (NP) $pK_a = 10.4$, (B) 4-(1,1,3,3-tetramethylbutyl)-phenol (TMBP), $pK_a = 10.2$, (C) 2,6-*bis*(1,1-dimethylethyl)-4-ethyl-phenol (MEEP), $pK_a = 12.8$.

new assembly, the hydrophobic membrane was placed between a porous stainless steel frit (0.5 μm) placed closest to the sample, and a washer placed closest to the inlet/outlet of the carbon dioxide. The cell had a total internal volume of 4 mL. In Fig. 2 the cell arrangement is shown in detail.

This configuration of the extraction cell allows the supercritical fluid to flow through the water sample, and since a membrane assembly also covers the outlet, only dissolved analytes can be eluted from the extraction cell to the solid phase trap. Another advantage of using hydrophobic membrane assemblies is the possibility to extract markedly larger sample volumes compared with a cell filled with support material.

Extraction of Analytes Spiked on Stainless Steel Beads

Initial experiments to investigate the extractability of the target alkylphenols from an inert material was performed by adding 50 μL of a standard solution (1.0 mg/mL of each alkylphenol in methanol) onto stainless steel beads with no membrane assemblies present. The stainless steel material volume

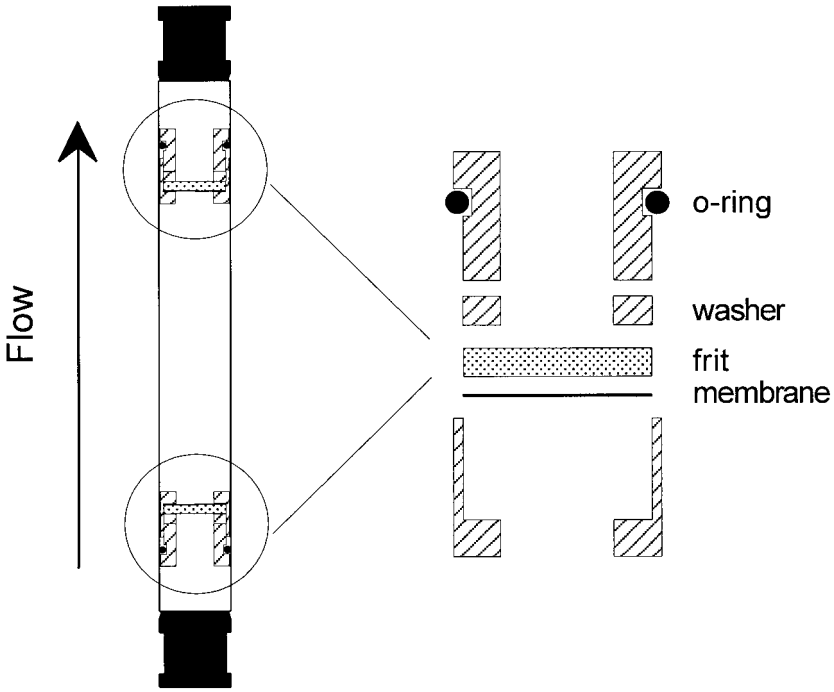

Figure 2. Extraction cell with membrane-containing assemblies.

was 5 mL, filling up the extraction vessel to ca. 2/3. These experiments were carried out using neat carbon dioxide at the following conditions; 40°C, 138 bar (0.75 g/mL) for 5–30 min dynamic extraction with flow rate set at 4 mL/min. The trap temperature was varied between 40°C and 100°C, and the analytes were eluted from the solid phase trap with 1.5 mL of methanol.

Extraction of Analytes from Aqueous Samples

Further investigations were focused on extracting the alkylphenols from 2 mL spiked distilled water. After closing the extraction cell, it was shaken thoroughly for 1 min using a vortex mixer. Extractions were performed by varying different parameters such as flow rate, temperature, pressure, extraction time, and trap temperature. Neat carbon dioxide was used as extracting solvent. Finally, the genuine environmental aqueous samples were extracted using the optimized extraction procedure.

Liquid Chromatography

All extracts were analyzed by a LC system consisting of a LKB 2150 high-pressure pump (LKB, Bromma, Sweden) run at 1 mL/min, a manual LC injector (Valco Instruments, Houston, USA), and a UV-detector LDC/Milton Roy Spectromonitor (Milton Roy Co., Riviera Beach, USA) with the wavelength set to 280 nm. The injection volume was 20 μL and the alkylphenols were separated on a LiChrospher® 60 RP-select B column, 250 × 4 mm (5 μm) from Merck (Darmstadt, Germany) equipped with a guard column LiChrospher® 60, 4 × 4 mm (Merck). The mobile phase was composed of acetonitrile and 0.8% phosphoric acid (80 : 20, v/v). This is a slightly modified LC method developed by others.[35] Chromatographic data were collected with Borwin software (JMBS Developments, Le Fontanil, France).

RESULTS AND DISCUSSION

Solubility Study

The extractability of the alkylphenols from stainless steel beads using neat supercritical carbon dioxide was found to be satisfactory at 138 bar, 40°C (0.75 g/mL), 4 mL/min using a trap temperature of 80°C. Recoveries of 99% (NP), 98% (MBP), and 103% (MEEP) were obtained after a 10 min dynamic extraction with RSD values of 1.0%, 4.6%, and 3.9% ($n = 5$) for the three analytes, respectively. The influence of trap temperature was

tested at 40°C, 60°C, 80°C, and 100°C. The recoveries for all three analytes were between 90% and 110% ($n = 2$) at all temperatures, demonstrating no significant effect of trap temperature when extracting analytes spiked on stainless steel beads.

Extraction of Spiked Water

Initial Experiments

In order to find an appropriate working temperature for the trap, when working with spiked water samples, a few experiments were performed at the same conditions (138 bar, 40°C, 4 mL/min for 10 min) varying the trap temperature between 40°C and 110°C. It was then observed that increasing the trap temperature from 40°C to 80°C caused no large differences in recoveries, which were always in the range of 30–40% ($n = 2$) for all temperatures independent of analyte. However, when increasing the trap temperature to 100°C, a decrease in recovery to 5% for MEEP was observed. Increasing the trap temperature to 110°C showed that no MEEP could be recovered. The reason for this is not known, but might be due to analyte breakdown. It was, therefore, decided to work at a medium temperature of 80°C, which gave low condensation of water in the trap, and no significant losses of analytes due to breakdown. All further experiments were performed at this trap temperature.

The initially chosen extraction conditions (138 bar, 40°C, 4 mL/min for 10 min) combined with the chosen trap temperature of 80°C were repeated with spiked water in order to get information about the repeatability. This experiment gave recoveries of 37% (NP), 40% (MBP), and 27% (MEEP) with RSDs below 5% ($n = 3$) for each alkylphenol. The recovery values are much lower than with no water present, but very repeatable. These low recoveries were considered to be dependent on decreased extraction efficiency for water samples and/or analyte breakthrough in the trap, as compared to analytes extracted from spiked stainless steel beads where no water was co-extracted into the trap. Both low extraction efficiency and breakthrough in the trap were investigated in detail below.

Extraction Efficiency Experiments

First, experiments were performed to study the influence of different parameters on the extraction efficiencies of the analytes. In all these experiments, the trap temperature was kept at 80°C. Extractions were first studied at lower flow rates (1 and 2 mL/min) using 40 mL of carbon

dioxide, corresponding to an extraction time of 40 and 20 min, respectively. The extraction pressure and temperature were set to 138 bar and 40°C. The obtained recoveries were 32% and 37% (NP), 36% and 40% (MBP), and 27% and 27% (MEEP) at 1 and 2 mL/min, respectively ($n = 2$). These results indicate that the transport of the alkylphenols into the bulk flow of carbon dioxide is fast enough even at the highest flow rate (4 mL/min). This is an example of an extraction controlled by a solubility/elution step.[36] Extraction rates from samples like this will benefit from faster flow rates, and by increased solubility of the analyte in the supercritical fluid. In an attempt to verify that the solubility/elution step is dominating the extraction process, the extraction temperature was increased to 60 and 80°C, keeping the pressure constant (138 bar) at 4 mL/min for 10 min. This caused a decrease in density from 0.75 g/mL at 40°C to 0.55 and 0.37 g/mL at 60°C and 80°C, respectively. The obtained recoveries were 43% and 28% (NP), 45% and 28% (MBP), and 38% and 29% (MEEP) at 60°C and 80°C, respectively ($n = 2$). Eventhough the decrease in recovery was not very pronounced at the lowered densities it still partly verified the above predication. The extraction process was further investigated by increasing the pressure to 207 bar at 40°C, corresponding to a density of 0.84 g/mL. This resulted in improved recoveries of 58%, 66%, and 54% for NP, MBP, and MEEP, respectively ($n = 2$). However, when increasing the pressure to 276 bar the recoveries decreased to 40%, 46%, and 43% for NP, MBP, and MEEP, respectively ($n = 2$). These results apparently contradicted that higher density, leading to improved solubility, would increase the recovery. However increasing the density also leads to higher water content in the effluent from the extraction cell and, thus, increased risk for condensation and analyte breakthrough. This will lead to bad trapping performance and, thus, lowered recoveries. Low recoveries in SFE are often interpreted as low extraction efficiency, while the problem might be bad trapping performance.[37] Consequently, it was assumed that the collection of the analytes also had an influence on the recoveries, and was therefore, investigated in more detail to find possible losses of analytes from the trap during the extraction.

Fractionated Extraction/Elution

To handle problems with limited trap capacity, a fractionated extraction/elution procedure has previously been developed for samples containing large amounts of fat.[10,38] This methodology is based on a periodical elution of the trap during the extraction. This methodology was now applied to alkylphenols in water. The alkylphenols were extracted for 40 min with elution of the trap every 10 min, using extraction conditions of 207 bar, 40°C, 4 mL/min, trap

temperature 80°C, which had been found to be the best of above. The extraction profiles for the three analytes with a periodical trap elution every 10 min (dotted lines, Fig. 3) are compared with recoveries obtained with elution after extractions with varying lengths from 10 to 40 min (columns, Fig. 3).

Fractionated extraction/elution gives recoveries of 98%, 94%, and 80% for NP, MBP, and MEEP, respectively. These recoveries are much higher than those obtained using a single elution step. In the latter case, the recoveries of the analytes are very similar after 10 and 40 min, demonstrating that breakthrough of analytes occurs after 10 min of extraction. This implies that water is co-extracted during the process, leading to decreased trapping capacity by rinsing out of the analytes and/or by changing the properties of the adsorbing surface. When the extraction time in Fig. 3 was extended to a total of 120 min, with elution of the trap every 10 min, the recoveries for NP and MBP leveled off at a total recovery of 105%, while for MEEP it reached a final value of 83%. From these findings it was concluded that 40 min extraction time was enough for satisfactory recoveries.

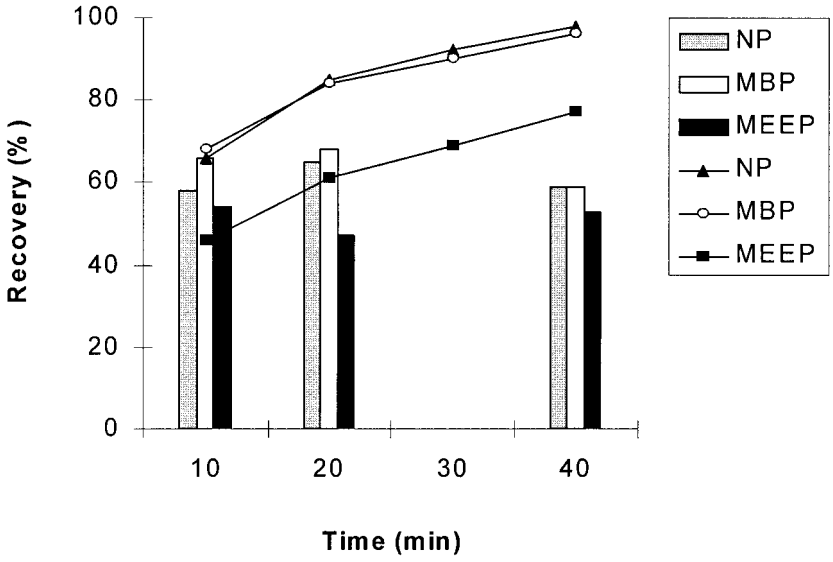

Figure 3. Recovery of analytes from spiked water, comparing fractionated extraction/elution every 10 min (lines), and a single elution step after various extraction times (columns). SFE conditions: pressure 207 bar, temperature 40°C (density 0.84 g/mL), flow rate 4 mL/min. Sample volume: 2 mL. Each data point is the mean of two measurements.

Once it had been verified that a fractionated extraction/elution procedure gave quantitative or close to quantitative recoveries, a number of experiments were performed at other temperatures and pressures in order to select appropriate conditions for the final method. In these experiments, the trap was eluted after 2, 5, 10, 20, 30, and 40 min of extraction. The reason for increasing the number of elutions during the first 10 min was to test if this gave increased recoveries of MEEP. Extraction profiles for the two best conditions are shown in Fig. 4, while the resulting recovery values for all investigated conditions together with RSDs are collected in Table 1.

The mildest extraction conditions (138 bar and 40°C) showed best recovery and RSD values (Table 1). When increasing the density, decreased recoveries were obtained with higher RSD values. Even lower recoveries were found when the temperature was increased keeping the density constant at 0.75 g/mL. Comparing the recovery of MEEP after 10 min extraction in Fig. 4B with the profile previously obtained in Fig. 3, it is clear that no losses of MEEP occurs during a single elution step of 10 min. The recoveries

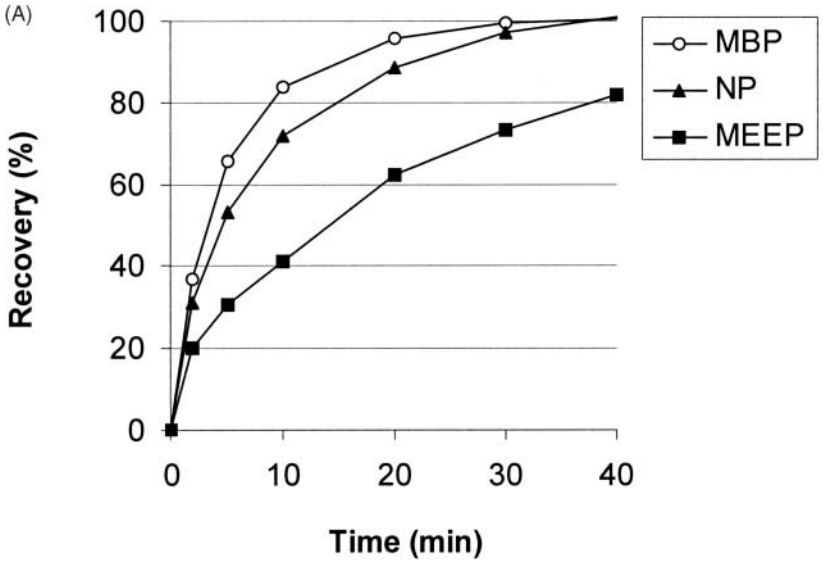

Figure 4. Recovery of analytes from spiked water with fractionated extraction/elution at 2, 5, 10, 20, 30, and 40 min. (A) SFE conditions: pressure 138 bar, temperature 40°C (density 0.75 g/mL), flow rate 4 mL/min. (B) SFE conditions: pressure 207 bar, temperature 40°C (density 0.84 g/mL), flow rate 4 mL/min. Sample volume: 2 mL. Each data point is the mean of two measurements.

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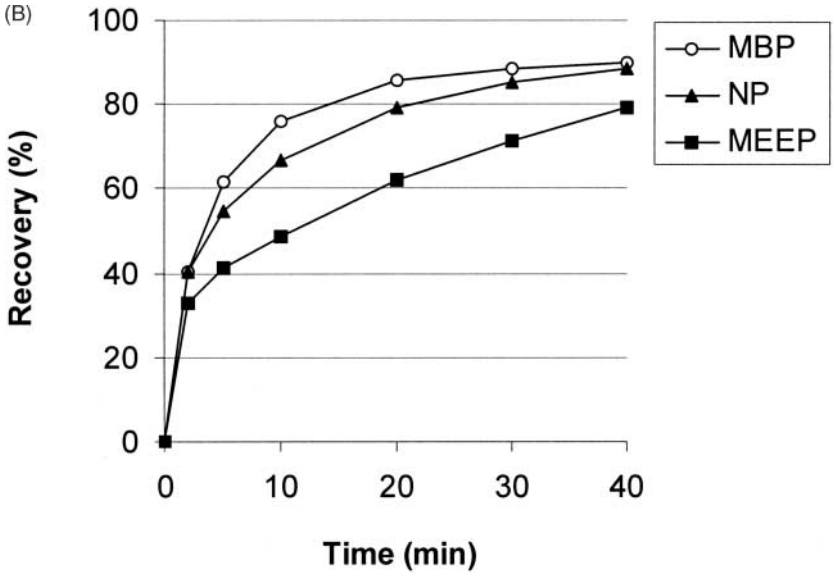

Figure 4. Continued.

of MEEP are after 10 min 46% and 49%, in Figs. 3 and 4, respectively. Decreases in recoveries at higher densities and temperatures are most probably dependent on analyte trapping problems (Table 1), but the reason is not completely known. Even so, extraction conditions of 138 bar, 40°C at 4 mL/min for 40 min, with a fractionated extraction/elution procedure were chosen as the final optimized conditions. These mild extraction conditions are also favorable due to decreased co-extraction of unwanted matrix components.

Until now, a sample volume of 2 mL was used while the maximum sample volume possible is 4 mL with the assemblies mounted. Since larger volumes give lower detection limits, 4 mL spiked water samples were investigated using the best conditions. The average recoveries obtained were 98%, 98%, and 86% for NP, MBP, and MEEP, respectively. Obviously, the recoveries do not differ significantly when the sample volume was increased from 2 to 4 mL, which means that with the chosen conditions, sample volumes up to 4 mL can be used. The final method includes an extraction of 4 mL sample, which with the fractionated extraction procedure results in six fractions at 1.5 mL. With an injection volume of 20 μL on each fraction, the detection limits, measured as three times the noise level of the baseline of the chromatogram, are 0.05, 0.04, and 0.06 mg/L for NP, MBP, and MEEP, respectively.

Table 1. Extractions of spiked water performed at different conditions using a fractionated extraction/elution procedure with elution of the trap after 2, 5, 10, 20, 30, and 40 min extraction ($n = 3$).

			NP		MBP		MEEP	
Temperature (°C)	Pressure (bar)	Density (g/mL)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
40	138	0.75	99	2.4	102	2.4	83	4.0
40	207	0.84	88	3.3	90	5.4	80	3.6
40	276	0.90	80	9.8	86	8.2	69	12
60	221	0.75	70	15	72	9.2	63	26
80	311	0.75	64	27	65	23	49	26

Note: Flow rate 4 mL/min. Sample volume: 2 mL. The obtained recoveries from each fraction are added together.

Table 2. Determination of alkylphenols from genuine water samples performed at 138 bar and 40°C using a fractionated extraction/elution procedure with elution of the trap after 2, 5, 10, 20, 30 and 40 min extraction ($n = 2$).

Sample	NP (mg/L)	MBP (mg/L)	MEEP (mg/L)
Leachate water from a landfill	0.084	0.15	<0.060
in Emmaboda, Sweden	<0.050	0.060	<0.060
Industrial effluent from Porto,	11	<0.040	<0.060
Portugal	10	<0.040	<0.060

Note: Flow rate 4 mL/min. Sample volume: 4 mL.

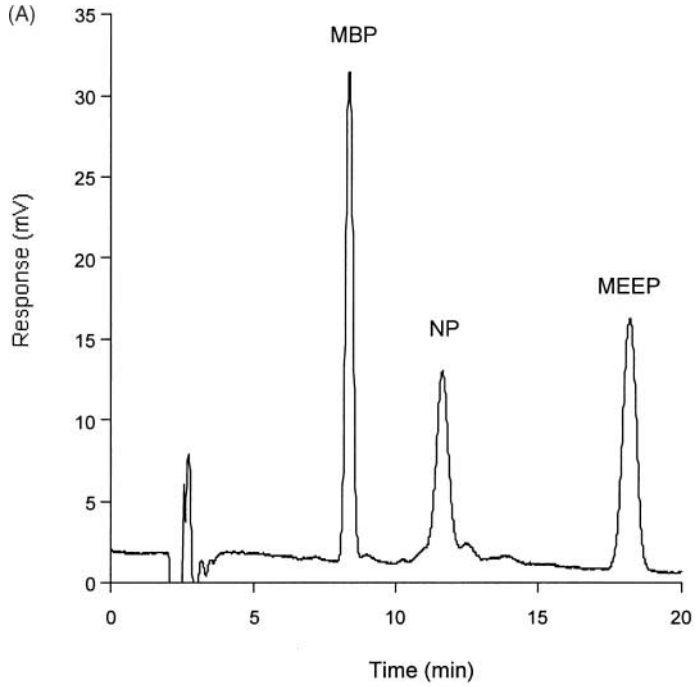

Figure 5. Chromatograms obtained from a standard solution, 5 ppm of each alkylphenol (A) and after direct SFE of Portuguese industrial wastewater (B) and Swedish leachate sample (C). SFE conditions: pressure 138 bar, temperature 40°C (density 0.75 g/mL), flow rate 4 mL/min. Fractionated extraction/elution at 2, 5, 10, 20, 30, and 40 min. Sample volume: 4 mL. LC conditions were as described in Experimental section. Note that the *y*-axes in the chromatograms have different scales.

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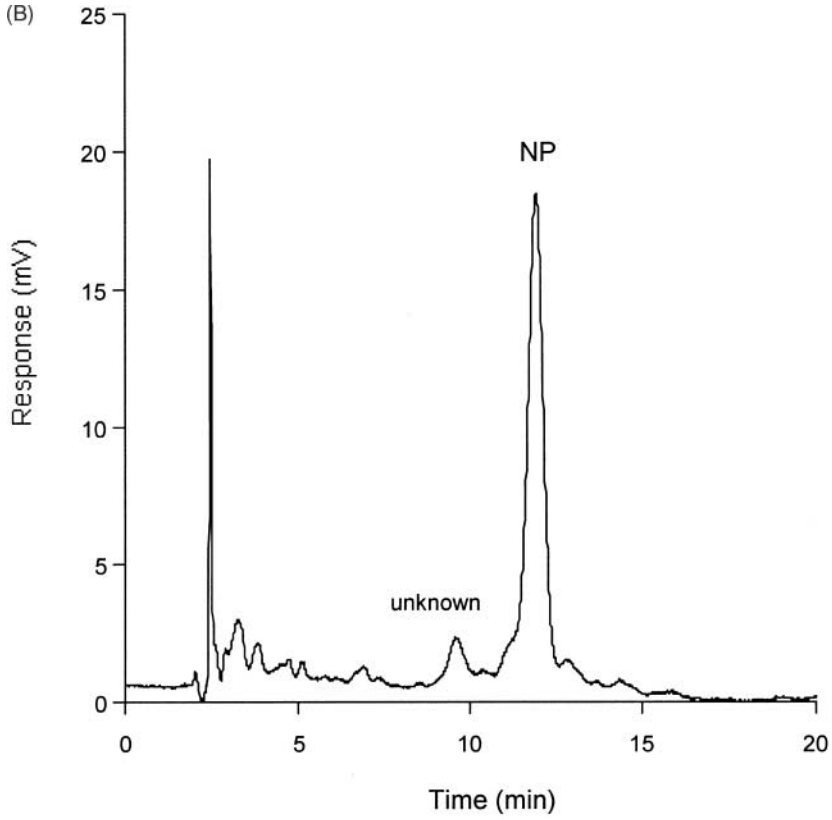

Figure 5. Continued.

Extraction of Genuine Environmental Aqueous Samples

Swedish leachate water and Portuguese industrial effluent wastewater were finally extracted using the optimized extraction conditions; 138 bar, 40°C at 4 mL/min for 40 min extraction with elution after 2, 5, 10, 20, 30, and 40 min. Results are shown in Table 2.

NP and MBP were found at levels near their detection limits in the Swedish leachate water, which has an impact on the precision. A high concentration of NP was found in the Portuguese industrial waste effluent, while MBP was not present in detectable levels. MEEP could not be found in any of the genuine water samples.

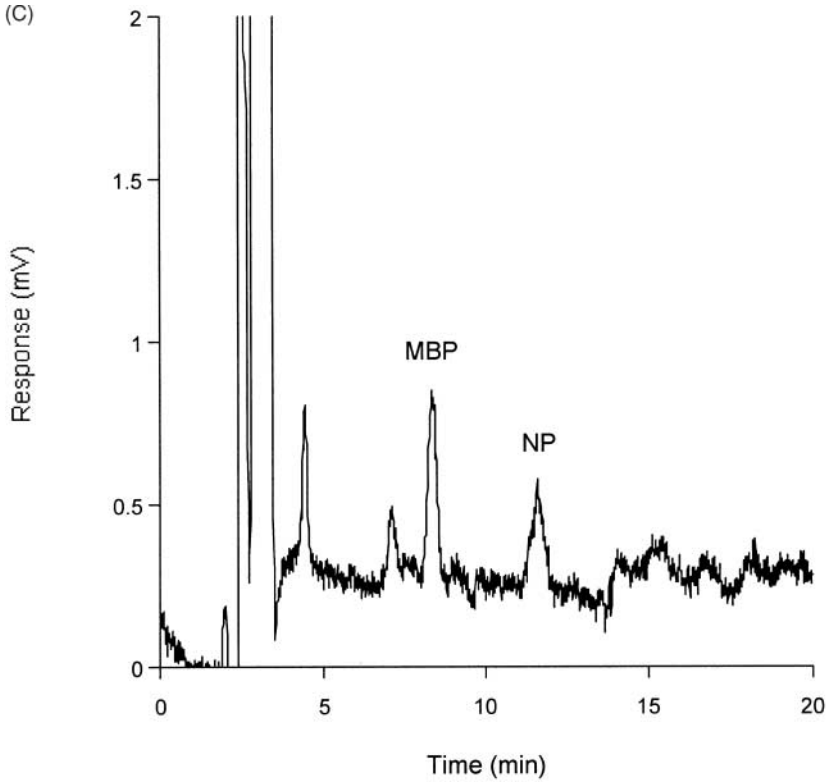

Figure 5. Continued.

Selectivity of Direct SFE

The direct extraction of industrial wastewater did not cause any considerable co-extraction of non-target matrix components. Chromatograms obtained from a standard solution, as well as from genuine water samples, are shown in Fig. 5.

Despite that the Portuguese industrial wastewater gave a very dirty visual impression, the extracts were completely uncolored and no external clean up was needed (Fig. 5B).

CONCLUSIONS

The results in this work show that it is possible to use assemblies with hydrophobic membranes to maintain aqueous samples in the extraction cell.

This makes it possible to perform direct SFE with a minimum of sample handling. It is clear that co-extraction of water influences the recoveries using an adsorbent trap as collection device, leading to analyte breakthrough. However, with a fractionated extraction/elution procedure quantitative recoveries can be obtained as demonstrated for some alkylphenolic substances. It is believed that with the developed methodology other types of neutral or weakly acidic substances in aqueous matrices can be handled as well.

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