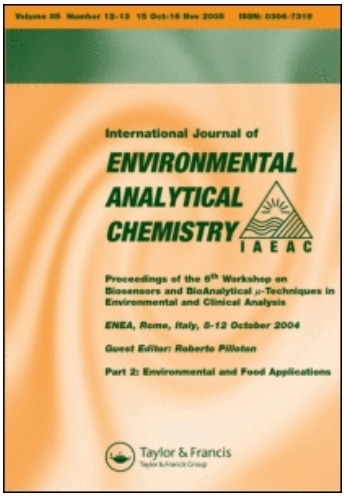

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ASSESSMENT OF SUPERCRITICAL FLUID EXTRACTION OF PENTACHLOROPHENOL FROM AQUEOUS SAMPLES

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Three methods of extraction of analytes from water were compared: liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid CO_2 (SFE). The method for SFE was altered to reduce time and amount of solvent used. Only 2 mL of methanol were required for SFE. The recovery of the analyte (pentachlorophenol, PCP) from 200 ppb aqueous solution was 86 % ± 9 % with LLE, 69 % ± 10% with SPE and 60 ± 13% with SFE. The SFE was however done without acidification to pH 2 before extraction. The recovery was not affected by the presence of fulvic acid (20 mg/L) with LLE and SPE, but was reduced somewhat with SFE (from 60 to 46 %). There was no significant decrease in recovery of PCP from river water with 37 ppm suspended solids and 6.4 ppm dissolved organic carbon.

Keywords: supercritical fluid extraction; liquid-liquid extraction; solid phase extraction; fulvic acids; pentachlorophenol; aqueous solutions

INTRODUCTION

Sample preparation is one of the most important steps in chemical analysis. It counts for two-thirds of the total analysis time and has a significant share in the cost of analysis due to the use of high purity solvents and laboratory waste production. Furthermore, poor recoveries will lead to underquantitation of the analyte and misinterpretation of the result.

Liquid-liquid extraction is the most commonly used technique because it is easy to use and does not require expensive equipment; however, it requires considerable amounts of organic solvents. Solid phase extraction is also easy to use and to automate; however, in preconditioning the disk, about 30 mL of solvent is required and the disposition of the solid presents a problem.

Although supercritical fluid extraction (SFE) for organic compounds from diverse matrices have been reported[1], most of the reports have focused on solid samples (soils, adsorbents, activated carbon). Few experiments have been done in the direct extraction of organic compounds from aqueous samples[2,3,4,5]; moreover, the application of SFE to real aqueous samples has not been reported. Experiments comparing SFE with LLE and SPE are reported here.

Natural waters contain a diversity of dissolved and particulate inorganic and organic constituents. Dissolved organic matter (DOM) in natural water is composed mainly of humic substances (HS) in concentrations as high as 100 mg/L[6]. Because of the polyelectrolytic nature of the HS, they have the ability to bind to various inorganic and organic compounds.

It was hypothesized that the current extraction techniques may be able to extract free analytes, but not those bound to dissolved organic matter (DOM) if the interactions between the analytes and DOM are strong. The amount of such analytes present in the sample could, therefore, be underestimated.

Recognizing the importance of DOM in the behavior of organic compounds, fulvic acids were selected to represent DOM in natural water. Pentachlorophenol (PCP) was chosen as a representative compound of the chlorophenol class. It has been used in sawmills, preservation of leather, and as herbicide in pineapple, sugar cane and rice fields[7].

The purposes of this work were thus:

a. To assess the effectiveness of the direct extraction of pentachlorophenol (PCP) with $SFCO_2$ as a sample preparation method for analytical purposes, and compare it to other methods of extraction, specifically liquid-liquid extraction (LLE) and solid phase extraction (SPE).
b. To evaluate the sensitivity of the aforementioned techniques to the presence of fulvic acids
c. To evaluate the effectiveness of SFE when using raw river water spiked with PCP.

EXPERIMENTAL

Reagents

All the reagents were HPLC grade or better, and were used as received. The purity of the PCP was higher than 98%. Fulvic acids were provided by the Department of Water and Environmental Studies in Lipköping, Sweden; they

were obtained from the Oulopki River, Finland. The water sample, from the Ottawa River, was supplied by the Britannia Water Purification Plant.

Sample preparation

Working solutions were prepared from the stock standard solution containing 400 mg/L of pentachlorophenol in methanol. A stock solution containing 200 mg/L of fulvic acids was prepared by dissolving 200 mg in deionized water. The stock solutions were stored at 4 °C in the dark prior to their use. The following solutions were prepared and used for the extractions:

a. PCP, 0.200 mg/L (200 ppb), in deionized water (PCP/W).
b. PCP, 0.200 mg/L in 20 mg/L of fulvic acid solution (PCP/FA).
c. Ottawa River water (real sample) was spiked with PCP to have a final concentration of 0.200 mg/L.

Standards were prepared for every set of extractions by taking aliquots from the stock PCP solution to get concentrations of 0, 0.50, 0.75, 2.25 and 4.25 mg of PCP/L in methanol.

Supercritical fluid extraction

The apparatus used has been described elsewhere[8], but some modification were made. The CO_2 was supplied as liquid, 99.5% purity (CO_2 high pressure liquid, BOC Gas). The cylinder was connected to the pump (mini Pump®, Milton Roy); the pump head was chilled with glycol solution at 0°C. Stainless steel tubes 1/8" o.d. and 1/16" connected the pump to a pressure gauge (Span Instruments 0–6000psi) and the pressure gauge to the extraction vessel, respectively. A valve was placed between the extraction vessel and the restrictor. The restrictor used was a fused silica capillary (41μm i.d. × 30 cm length) connected to the system with a 1/16" Swagelock and graphite ferrule[9]. Part of the tubing and capillary were heated with an electric heater and ceramic insulators. The end part of the capillary (2 cm) was submerged in methanol contained in a vial which was immersed in a water bath at room temperature. Earlier experiments had showed aerosol formation, and part of the methanol was trapped in the bubble flow meter. To ensure that there was no analyte loss, a Teflon tube was connected from the first vial to a second vial, which was placed in an ice bath. The bubble flow meter was connected to the last one.

The extractions were performed at 3000 psi (204 atm), T = 21 °C, $SFCO_2$ density 0.94 g/mL, and $SFCO_2$ flow rate of 1.2 mL/min. The density was obtained from the SF-Solver (TM) program provided by ISCO, Inc. 1992.

Liquid-liquid extraction

The sample (20 mL ± 0.03 mL) was placed in a 125 mL separatory funnel. The sample was acidified with 9 M H_2SO_4 to pH 2 and shaken thoroughly. Three successive extractions were performed by shaking the sample with 2 mL of dichloromethane (HPLC grade, Fisher) for 5 min periods and allowing the phases to separate for 15 min. The extracts were collected in a preweighed vial.

Some problems were encountered in the analysis by HPLC when dichloromethane (DCM) was used; therefore, the sample was allowed to dry in the dark at room temperature and restored with a small amount of methanol. In order to assess the suitability of the drying procedure, for each set of extractions two standards were dried and restored at the same conditions.

Solid phase extraction

A standard 47 mm filtering apparatus was used (Millipore). The disk Poly(styrendivinyl-benzene)SDB (Empore ^{TM}) was placed in the apparatus. The disk was conditioned as follows: 10 mL of acetone was poured into the beaker; the disk was let to soak for 3 min; then vacuum was applied for 1 min. The same procedure was repeated with 10 mL of DCM. Finally, 10 mL of methanol was poured in, the vacuum was applied, but the disk was not allowed to dry. This is the critical step since the extraction relies on how well the disk is wetted. The 20 mL sample (acidified to pH 2 with H_2SO_4) was passed through the disk, with the vacuum applied. After drying, the PCP was eluted with 6 mL of DCM which was collected in a preweighed vial. This was allowed to dry in the dark and restored with methanol prior to analysis.

HPLC analysis

The analysis of the extracts was done by high pressure liquid chromatography (HPLC) (Varian 9010/9050) on a reverse phase column: 46 mm by 25 cm RP-C_{18} with particle size 5 μm (Zorbax) equipped with a guard column of the same packing. The mobile phase was 95% methanol 5% (0.5% acetic acid), with a flow rate 1.5 mL/min and a pressure of 130 atm. The detector used was Varian 9050 UV-VIS detector. The maximum absorbance was obtained at a wavelength of 224 nm.

The volume of both standards and samples injected into the column was 30 μL. At these conditions, the retention time for PCP was 3.0 (± 0.1) min. The standards and samples were injected three times. The criteria for the existence of a peak is predetermined by setting the signal to noise ratio to a value of 5.

In order to quantify the extracts, standards of PCP were prepared and injected, followed by the extracts. The calibration curve was constructed by plotting the area (average of the three injections) versus the amount of PCP injected. Neither a surrogate nor an internal standard was added to the extract.

RESULTS AND DISCUSSION

Supercritical fluid extraction (SFE) extraction profiles

In order to get the extraction profiles for PCP, a number of vials were used. For example, in the course of a single extraction, the vial was replaced after each 20 mL of $SFCO_2$, giving three extracts. Figure 1 is a representation of the profiles for the two kinds of samples (PCP in deionized water and PCP in 20 mg/L of Fulvic Acids). The results suggest that the presence of FA makes the extraction of PCP less effective.

Effect of fulvic acids in the extraction method

Although the samples were stored in the dark at 4 °C to avoid analyte losses, it was considered necessary to perform a control test as follows. The sample con-

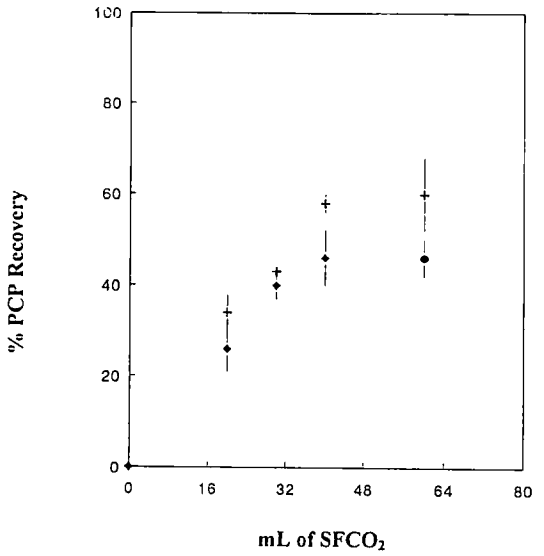

FIGURE 1 Extractions profiles of pentachlorophenol at 21 °C. Without fulvic acids (PCP/W, †) and with fulvic acids (PCP/FA, ♦). Minimum of three experiments per point. The error bars indicate the standard deviation

taining PCP in deionized water was extracted by each method (LLE, SPE and SFE) at one day and at 10 days after its preparation. In doing this the differences in the recoveries between methods will be due to the presence of FA and not due to any analyte decay. The samples (PCP/W and PCP/FA) were prepared at the same time and under the same conditions. Results indicated no differences in recoveries.

The results shown in table 1 indicate that the percent of recovery obtained by LLE and SPE was not affected by the presence of fulvic acids at 20 mg/L (Figure 2). This suggests that the interactions between PCP and fulvic acids at pH 2 are relatively weak. These interactions can be overcome by the DCM used in LLE and the styrenedivinylbenzene groups in the Empore^{TM} disk; however, the interactions might not be fully overcome by the carbon dioxide (CO_2) in SFE. With $SFCO_2$ the recovery was reduced from 60 % (without FA) to 46% (with FA). There was no overlap of one standard deviation (Figure 1 at 60 mL of $SFCO_2$ and Figure 2).

TABLE I PCP recovery with different methods. The samples were extracted on the 10 th day after their preparation ([PCP]= 0.200 mg/L). **Mean:** Percent of recovery for each extraction. **PCP in water:** PCP spiked in deionized water (final concentration of 0.200 ppm). **PCP in FA:** PCP spiked in 20 mg FA/L solution (0.200 mg/L PCP). **LLE:** liquid-liquid extraction with dichloromethane. **SPE:** solid phase extraction Poly(styrenedivinylbenzene)SDB (Empore^{TM}), dichloromethane as eluting solvent. **SFE:** Supercritical fluid extraction, trapping in methanol

Sample	*Extraction method*		
	LLE	***SPE***	***SFE***
PCP in water			
Mean	86 %	69 %	60 %
% RSD	9	10	13
# cases	5	5	4
PCP in FA			
Mean	83 %	71 %	46 %
% RSD	12	6	9
# cases	6	3	5
Real sample			
Mean	87 %	63 %	55 %
% RSD	5	10	11
# cases	5	6	5

The weak interactions between FA and PCP can be explained as suggested in the literature[10]. For acid/basic compounds, two kinds of interactions are possible. Ion exchange is unlikely to be an important mechanism for humic substances when the compound is an acid, but hydrophobic bonding could occur, as it increases as the solute becomes more nonpolar. Active surfaces for hydrophobic bonding include aliphatic side chains on the fulvic acids.

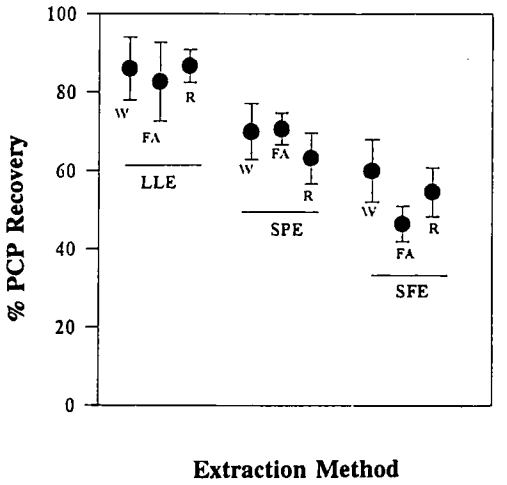

FIGURE 2 Pentachlorophenol recovery obtained by different methods. The average(•) and error bars (± 1 std) are plotted for each extraction method: LLE, SPE and SFE. Pentachlorophenol was spiked into deionized water (**W**), into 20 mg/L fulvic acid solution (**FA**) and into Ottawa river water

Pentachlorophenol has a pK_a of 4.35[11] and is thus relatively acidic. The pK_{ow} values reported are 3.69[12] and 5.01[13], suggesting intermediate lipophilicity. It was expected that, on one hand, interaction between PCP and FA should be present. On the other hand, extractions of PCP by SF CO_2 should be effective since water in contact with $SFCO_2$ has a pH about 2.9[14]. The solubility of CO_2 in water is substantial and increases when increasing the pressure (at constant temperature)[15]. Carbonic acid is formed and its deprotonation lowers the pH.

It is believed that the way by which the sample is prepared might have some effect on the interactions. In this study the sample was unbuffered, and made from deionized water which has a pH about 5.6 (± 0.2). It is known that fulvic acids act as a buffer in natural water. The pH of the solution after adding the 20 mg/L FA was in the order of 5.2 (±0.2). No significant change was observed after adding the PCP (final concentration of PCP of 0.200 mg/L). At pH 5.2, 12.5 % of the initial PCP is in the protonated form.

Comparison between extraction methods

In order to compare the performance of each extraction, one kind of sample will be selected (PCP/FA). The highest recovery was obtained by LLE followed by SPE and SFE. Based on one standard deviation, the difference in recovery between LLE and SPE is not statistically significant, but that between LLE and SFE is significant (Figure 2).

Reports indicate that at the SFE experimental condition (3000 psi) the pH of the solution is about 2.85–2.9[16]. The partitioning of the PCP to the $SFCO_2$ is favored by this pH lowering, as at that pH 96.5 % of the PCP are in the protonated, more hydrophobic form. On the other hand, the lower the pH the more protonation of the FA sites. The pH is lowered to 2 with the addition of H_2SO_4 prior the LLE and SPE. The SFE experiments were done without this prior acidification step; this may be partially responsible for the lower recoveries.

Pentachlorophenol extractions from Ottawa river water

Most of the results reported in the literature described extractions from spiked reagent water, which differs considerably from natural river water. The results, therefore, do not reflect what actually happens in real samples since there is no consideration of interactions between organic compounds and suspended solids, and organic compounds and DOM.

Pentachlorophenol was spiked into the Ottawa river water, and it was extracted by LLE, SPE and SFE.

Results of the water quality of the Ottawa river were provided by the Britannia Purification Plant. Of all the organic compounds listed in the drinking water surveillance program, 2,4,6-trichlorophenol was reported in concentrations of the order of 0.05 ppb. The average amount of dissolved organic carbon and dissolved solids are 6.4 and 37.2 ppm respectively.

The results are in accordance with those obtained with the fulvic acid solution: the extraction efficiencies ranked as LLE > SPE > SFE. In spite of the emulsion formed in LLE, the recoveries were higher, but the time needed for the phases to separate was longer than for the previously described LLEs.

CONCLUSIONS

The presence of fulvic acids (FA), at concentration of 20 mg/L, does not interfere in the extraction efficiency by LLE and SPE. Graphical results showing one standard deviation (Figure 4) suggest that the presence of FA does lower the recovery of PCP with SFE (46 % vs 60 % without FA).

The results obtained in this work show some disadvantages and some advantages in the use of SFE for analytical purposes. In terms of solvent usage, SFE is most favorable; only 2 mL of methanol are required. The SFEs were also done without use of H_2SO_4 for acidification to pH 2.

It may be possible to overcome some of the disadvantages of SFE, as relatively little research has been done in optimizing direct SFE from water. Some further optimization of SFE was reported here, as the PCP was trapped directly into the solvent (methanol) used for the quantitation. Further optimization is likely to be possible in the time taken by using a higher flow rate. Although flow rate does not affect the extraction efficiency[17], it would reduce the time of extraction. However, a redesign of the SFE equipment is required to increase the $SFCO_2$ flow through a higher pumping speed. The advantages of the reduction of solvent and of time may eventually make SFE as suitable as LLE or SPE for standard use.

Acknowledgements

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