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Journal of Chromatography A, 764 (1997) 265–277

JOURNAL OF
CHROMATOGRAPHY A

Supercritical fluid and enhanced-fluidity liquid extraction of phenolics from river sediment

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Received 12 June 1996; revised 9 September 1996; accepted 21 October 1996

Abstract

The extractions of eleven phenolic and nitroaromatic compounds from a spiked river sediment by methanol–CO₂ mixtures under either supercritical conditions or enhanced-fluidity conditions were studied. Supercritical CO₂ extractions and Soxhlet extractions were also performed for comparison. Enhanced-fluidity liquids, prepared by mixing large proportions of common organic solvent with low-viscosity liquid CO₂, were used in an attempt to strengthen the solvating power of supercritical CO₂. The effect of methanol proportion (0–30% mole fraction) in the extraction fluid was examined over the temperature range 50–150°C. The influence of sample treatment with water, acid and base was individually investigated. The extraction by ternary liquid mixtures of methanol–water–CO₂ was also tested. The effectiveness of various extraction conditions was evaluated in comparison to Soxhlet extraction.

Keywords: Sediment; Enhanced-fluidity liquid extraction; Extraction method; Sample preparation; Phenolic compound; Nitrophenol; Chlorophenol

1. Introduction

Phenolic compounds are widely involved in commercial applications, such as coal conversion, petroleum refining, paper manufacturing, dye synthesis and photo processing [1,2]. They are also used in the synthesis of pesticides, herbicides, fungicides and antiseptics for agricultural uses. Due to the widespread presence of phenolic compounds in our environment and their high toxicity [3], many phenolic compounds are listed as US Environmental Protection Agency (EPA) priority pollutants [4]. As an increasing concern to human health, efforts have been devoted to quantitating phenolic compounds

from environmental samples, such as waste water, ashes, sediments and soils [5–19].

The use of supercritical fluid extraction (SFE) has greatly advanced the quantitation of organic pollutants in solid environmental matrices. Compared to the conventional liquid–solid extraction methods (Soxhlet, sonication), SFE is faster and uses minimal organic solvent. The low viscosity of supercritical fluids affords faster mass transfer and faster equilibration, and, thus, usually better efficiency than conventional methods. Supercritical CO₂ is the most frequently used fluid in SFE due to its low critical temperature and pressure (31°C, 73 atm; 1 atm = 101 325 Pa), low toxicity, chemical inertness, low cost and ease of removal upon depressurization. Extraction with supercritical CO₂ has been successfully used in extractions of nonpolar or moderately

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polar compounds [20–22], but it is less efficient in extracting high-molecular-mass or polar compounds from complex matrices.

Phenols are moderately to highly polar compounds; some of them have notably low pK_a values. To achieve better efficiency in the extractions of phenolic compounds from solid matrices using supercritical CO_2 , several approaches have been explored. Ashraf-Khorassani et al. [16] examined the effects of directly adding a small amount of polar modifiers, including water, acetonitrile, dichloromethane, methanol and acetone to the matrix in the extraction of phenol, 2,6-dimethylphenol and 3,5-dimethylphenol from sand and top soil. Richards and Campbell [17] reported the positive attributes of adding 2% methanol as modifier in the supercritical fluid extractions of a few phenolic compounds from soil. Lopez-Avila and Dodhiwala [18] studied the effect of various modifiers on the extraction of 14 phenolic compounds from four solid matrices, florisil, sand, alumina and silica gel using supercritical CO_2 . Recoveries of <50% were reported. Methods other than the use of modifiers in the extraction of phenolic compounds were also reported. Lee et al. [9,10] showed that in situ acetylation, which transforms chlorinated phenols to more nonpolar extractable derivatives, is an effective alternative. Hawthorne et al. [19,20] showed increased extraction yields of chlorophenols with supercritical CO_2 at temperatures over 200°C. Yet, studies of extracting a wide selection of phenolic compounds from solid matrices are rare and investigations on extraction method development are also limited.

Enhanced-fluidity liquid extraction was developed as a complementary method to extract supercritical CO_2 -defying analytes, such as high-molecular-weight or polar compounds, from complex matrices [24–27]. An enhanced-fluidity liquid is prepared by combining a commonly associated organic solvent, such as methanol, to a large proportion of a high fluidity (low viscosity) liquid, such as CO_2 . With these mixtures, the low viscosity attribute of liquid CO_2 is retained, resembling that of supercritical CO_2 , while the solvating power of organic solvent is also implemented. For example, at 25°C, 170 atm, the viscosity of liquid CO_2 is ~0.1 cP, and that of a methanol- CO_2 (20:80 mol%) liquid mixture is ~0.13 cP, compared to 0.6 cP for pure methanol [28].

The high fraction of polar solvent is expected to increase the solubility of the analyte in the extractant, increase the solvent's ability to desorb bound analytes and provide ready penetration of analytes through the water layer at the surface of the matrix. The extractions of polynuclear aromatic hydrocarbons and phenols from sea sand [24,25], ODS [26] and house dust [27] by enhanced-fluidity liquid have shown such promise. The advantages of using enhanced-fluidity liquids as chromatographic mobile phases were also previously demonstrated [28–30].

In the present study we report our investigations of the extraction of eleven phenolic and nitroaromatic compounds from a river sediment using methanol- CO_2 mixtures under supercritical and liquid state conditions. Extractions using pure CO_2 and Soxhlet were also performed for the purpose of comparison. The eleven phenolic and nitroaromatic compounds, phenol, *o*-cresol, *m*-cresol, 2,4-dimethylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, nitrobenzene, 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol, were selected to represent three major forms of phenol derivatives of environmental concern: methyl-, chloro- and nitrophenols. They also cover a wide range of polarity and pK_a . Methanol was chosen because of its high polarity and miscibility with CO_2 over a wide range of compositions. Phenolic and nitroaromatic compounds were spiked onto the sediment and the sample was allowed to equilibrate for a period of no less than three weeks before extractions were performed. To investigate the interactions between the analytes and the sediment as well as to optimize the extraction conditions, extensive studies were conducted on common factors which affect the recovery, such as pressure, temperature, extraction fluid composition, moisture content and pH.

2. Experimental

2.1. Materials

Phenol, 2-chlorophenol, *o*-cresol, *m*-cresol, nitrobenzene, 2,4-dimethylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, 2-fluorophenol, 2,4,6-tribromophenol, biphenyl and *p*-*tert*-

butylphenol were purchased from Aldrich (Milwaukee, WI, USA) and 2,4-dinitrophenol, stabilized with 10–15% water, and 4,6-dinitro-*o*-cresol, stabilized with 11% water, were purchased from Chem-Service (West Chester, PA, USA). All chemicals have $\geq 98\%$ purity and were used as received. Methylene chloride (99%, Fisher Optima grade, Fairlawn, NJ, USA) and methanol (100%, J.T. Baker, HPLC grade, Phillipsburg, NJ, USA) were used as received. SFE/SFC-grade CO_2 was obtained from Air Products (Allentown, PA, USA). The sediment SRM-2704, a river sediment from Buffalo, New York, was purchased from the National Institute of Standard and Technology (NIST) and was used as received. This sediment contains 3.5% carbon and 0.8% water as indicated by the NIST. The pH of the sediment in aqueous solution was 6.95.

2.2. Sample preparation

A portion (20–30 g) of sediment was accurately weighed and saturated with methylene chloride in an amber glass bottle. The sediment was spiked at 15 $\mu\text{g/g}$ concentration for all the compounds except 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol, which were at 23 $\mu\text{g/g}$ concentration. The spiked sample was stored in a refrigerator and mixed daily for 2 weeks while remaining sealed. The solvent was then evaporated under gentle nitrogen stream and the sample was resealed and shaken daily for another week before being used for extractions.

The concentration of phenolic compounds spiked onto the sediment in this study is higher than that found expected on most environmental matrices, but similar to that found in industrial soil and railway bed soil [19,20]. The spiked and aged sediment was used instead of a naturally contaminated sediment to test the current extraction method on a wide selection of phenolic compounds. Naturally contaminated samples may differ from spiked samples in concentration of pollutants, sites and mechanism of adsorption and aging period, as well as the analyte-carrying solvent (methylene chloride in the present case). The adsorption of pollutant by a solid matrix is also known to be influenced by the organic content [31]. However, the spiking method used in this study was different from most spiking procedures where the analytes are spiked onto the sediment and then

extracted shortly thereafter (typically minutes to hours). Previous reports showed that sediment aged for 1–4 days with selected polynuclear aromatic compounds [32] and for 1–2 days with chlorobenzene [33] reached equilibrium adsorption conditions. Therefore, our 3 week aging process is expected to also provide equilibrium conditions between the analytes and the sediment.

2.3. Extraction apparatus and method

An ISCO SFXTM 220 automated supercritical fluid extractor with a model 260D syringe pump (Lincoln, NE, USA) was used for all extractions. Extractions with pure CO_2 were performed by directly filling the syringe pump with CO_2 from the gas cylinder. When methanol- CO_2 mixtures were used as the extraction fluid, two syringe pumps were employed for mixture preparation. First, a known amount of methanol was filled into the empty extraction pump, then a specific amount of pure CO_2 was transferred from a second pump that contained pure CO_2 and was maintained at a constant pressure of 238 atm. The CO_2 volume needed to reach a certain mole ratio of methanol- CO_2 in the extraction pump was calculated based on CO_2 density at 238 atm and the temperature of the pump. The methanol- CO_2 mixture was pressurized to 238 atm (except when otherwise specified) in the extraction pump and allowed to equilibrate for at least 12 h before use. When a methanol-water- CO_2 mixture was used as the extraction fluid, a methanol-water mixture was first prepared and then used to prepare a methanol-water- CO_2 mixture as described above for methanol- CO_2 mixture preparation.

A 2.5 ml stainless-steel vessel, equipped with a 2 μm frit at the inlet and 0.5 μm frit at the outlet, was used for the extraction. A 1.00 g sample of sediment was sandwiched between two layers of clean sand to fill the vessel completely. When extraction started, a 1 min hold time (except when otherwise stated) was used to equilibrate after the extraction vessel was pressurized. The flow-rate was maintained at $\sim 0.3 \text{ min}^{-1}$ by adjusting the length of a 30 μm I.D. fused-silica tubing that was used as a restrictor. The extracted analytes were collected at room temperature in 5 ml methylene chloride with 10 μl each of 1.5 mg/ml 2-fluorophenol and 2,4,6-tribromophenol added as surrogates. Fraction collection experiments

were performed by changing the collection vial after a selected volume of extraction fluid (0.5, 1, 1.5, 2, 3, 4, 6, 9 and 12 ml) had passed through the extraction vessel. Extracts in methylene chloride were concentrated to approximately 25 μl under gentle nitrogen flow. After evaporation, the vial was filled to 1 ml with methylene chloride and the extracts were then transferred to an autosampler vial, to which 10 μl of a 1.5 mg/ml *p*-tert-butylphenol (internal standard) and biphenyl (GC time reference compound) solution was added.

Extracts were analyzed using a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a split-splitless injection port and a flame ionization detection (FID) system. An HP 7673 autosampler was used with a splitless injection of 1 μl . A 30 m \times 0.25 mm I.D. (1 μm film thickness) SPB-5 silica capillary column (Supelco, Bellefonte, PA, USA) was used. The initial oven temperature of 40°C (1 min hold) was followed by a 30°C/min ramp to 100°C (2 min hold). Finally, a 10°C/min ramp to 280°C (1 min hold) allowed baseline separation of all compounds. The temperatures of the injector and the FID system were 290 and 300°C, respectively. Instrument control and data acquisition and analyses were performed using the HP 3365 ChemStation software.

For calibration-curve construction, a stock solution containing 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol at 150 $\mu\text{g}/\text{ml}$ and the rest of the compounds at 100 $\mu\text{g}/\text{ml}$ was serially diluted to six standard solutions, with the concentration of 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol in the range 6–30 $\mu\text{g}/\text{ml}$ and that of the rest of the compounds in the range 4–20 $\mu\text{g}/\text{ml}$. The experimental data were fitted to linear lines with correlation coefficients >0.98 for all the compounds.

2.4. Soxhlet extraction

A 1.00 g sediment was extracted in a micro-Soxhlet extractor (Ace Glass) with 15 ml methylene chloride at the rate of about 15 min solvent cycle time. The solvent was replaced with fresh 15 ml methylene chloride after 1, 2, 4, 6, 8, 12 and 24 h. Extracts were concentrated and analyzed as described above.

3. Results and discussion

3.1. Collection efficiency study

To compare the extraction recoveries from different experiment conditions, the analyte collection system must behave optimally and impartially. There are three commonly used 'off-line' collection methods: liquid, solid surface and solid-phase sorbent trapping [34]. When high proportions of modifier are used, solid-phase trapping can be problematic for the collection of analytes with a wide range of volatility [35]; therefore, a liquid-phase collection system was considered.

To test methylene chloride as a liquid trapping system under different conditions, 5.0 ml of methylene chloride was spiked with eleven phenolic analytes and the two surrogates, 3.0 ml of either pure carbon dioxide or methanol-CO₂ (20:80 mole fraction) mixture at 50°C, 100°C and 150°C was passed through the collection vial. One-way analysis of variance (ANOVA) of the data showed that the changes in recoveries with temperature or extraction fluid composition were not significant for most of the analytes. The collection efficiencies for the spiked compounds using CO₂ and methanol-CO₂ (20:80 mole fraction) at 100°C are listed in Table 1. The compounds are listed in the order of their appearances in the gas chromatogram, which is also the order of decreasing volatility. In general, the collection efficiency increased with the decreasing volatility of the compounds. Relatively high recoveries were achieved and variations with temperature or extraction fluid composition were small; thus the methylene chloride trapping system was considered an acceptable method for use in this study.

3.2. Effect of pressure

Three pressures (170 atm, 238 atm and 306 atm) were studied for extractions by pure CO₂ and methanol-CO₂ (10:90 mol%) mixture at 50°C. At this temperature for all the pressures used, pure CO₂ is supercritical and the methanol-CO₂ mixture is an enhanced-fluidity liquid. No pressures lower than 170 atm were tested to ensure single-phase extraction conditions [36] during the depressurization period. Triplicate extractions were performed at each con-

Table 1
Collection efficiency as a function of extraction composition

Extraction fluid compound	CO ₂ /100°C (<i>n</i> =4) ^a	Methanol–CO ₂ (20:80)/100°C (<i>n</i> =6)
Phenol ^c	85±6 ^b	94±2
2-Chlorophenol	83±5	91±2
<i>o</i> -Cresol ^c	86±4	92±2
<i>m</i> -Cresol	91±7	97±3
Nitrobenzene ^c	93±12	87±3
2,4-Dimethylphenol	91±4	94±1
2,4-Dichlorophenol	95±9	95±2
2,4,6-Trichlorophenol	96±5	96±2
2,4-Dinitrophenol	119±10	99±10
4,6-Dinitro- <i>o</i> -cresol	100±3	100±3
Pentachlorophenol	112±9	100±3
2-Fluorophenol ^{c,e}	87±5	95±4
2,4,6-Tribromophenol ^{c,d}	101±5	110±2

^aNumber of replications.

^bAverage recovery±standard deviation.

^cSurrogate.

^{d,e}Compound which showed significant change in recovery with temperature, ^d, or composition, ^e, by one-way ANOVA.

dition. For the supercritical CO₂ extractions, no significant variation in extraction yield was observed for all analytes as a function of pressure, as indicated by one-way ANOVA at a 95% confidence level. For the methanol–CO₂ (10:90 mol%) mixture, the extraction yields for only two compounds (2,4-dinitrophenol and pentachlorophenol) changed significantly with pressure variation. The highest extraction yields for 2,4-dinitrophenol and pentachlorophenol were found with pressures of 306 atm and 238 atm, respectively. Thus, for the pressure range studied, the recoveries are relatively independent of the pressure. Therefore, no higher pressures were tested and a moderate pressure, 238 atm, was chosen for further extraction experiments in this study.

3.3. Variation of extraction yields with temperature and methanol composition

Extractions were performed at three temperatures (50°C, 100°C and 150°C) for each methanol–CO₂ mixture composition (0, 10, 20, 30 mol% methanol). Each extraction recovery reported is an average of at least three extractions. To conserve space, Table 2 shows only the extraction yields from the use of 10:90 and 20:80 mol% methanol–CO₂ extractants and their one-way ANOVA results as examples.

The critical temperature of CO₂ is 31.1°C, while

the approximate critical temperatures for the 10, 20 and 30% methanol–CO₂ mixtures are 40–50°C, 50–60°C [36] and 100°C [37], respectively. The variation of recovery with temperature was seen to be highly dependent on the extraction fluid composition. The extraction yields using pure CO₂ did not vary significantly for most of the analytes when the temperature was increased from 50°C to 100°C with the exception of the three least volatile compounds. When the temperature was increased from 100°C to 150°C, the extraction yields for most analytes decreased significantly. However, when 10 mol% methanol was added to CO₂ extractions at 150°C gave the best extraction recoveries for most compounds. When methanol–CO₂ (20:80 mol%) was used, extractions at 100°C gave the best recoveries for most analytes. Increasing the temperature from 100°C to 150°C lowered the recoveries, especially for 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol. The highest methanol mole fraction tested was 30%, which did not show notable changes in extraction yields over the temperatures studied (50–150°C). Only two of the analytes, 2,4-dichlorophenol and pentachlorophenol, showed statistically significant differences with higher observed extraction yields at 150°C compared to 50 or 100°C.

The kinetics of extractions at different temperatures are illustrated in Fig. 1. The recovery of 2,4,6-

Table 2

Effect of temperature and extraction fluid compositions on recoveries relative to the spiking level

Compound	50°C	100°C	150°C	50°C	100°C	150°C	100°C	100°C
	10% methanol			20% methanol			CO ₂	30% methanol
Phenol	74±8	82±9	94±10	68±2 ^b	98±11	74±7	61±3 ^c	84±2
2-Chlorophenol	47±8	50±5	64±8	48±1 ^b	62±4	44±3	36±1 ^c	48±2
<i>o</i> -Cresol	58±3	59±5	65±6	53±1 ^b	66±3	51±4	47±1 ^c	54±0
<i>m</i> -Cresol	60±4	57±6	59±5	50±0 ^b	66±5	46±5	43±2 ^c	53±3
Nitrobenzene	51±9	63±6	71±3	51±1 ^b	69±3	57±2	54±2 ^c	59±4
2,4-Dimethylphenol	54±6	57±13	54±9	53±2	60±17	57±4	49±2	37±11
2,4-Dichlorophenol	61±2 ^a	64±7	75±2	55±1 ^b	73±6	63±1	43±3 ^c	61±6
2,4,6-Trichlorophenol	71±5 ^a	73±11	96±7	64±0 ^b	84±6	75±0	48±4 ^c	75±3
2,4-Dinitrophenol	57±4 ^a	57±4	86±8	58±18 ^b	84±5	40±18	56±1 ^c	89±7
4,6-Dinitro- <i>o</i> -cresol	38±6	50±9	60±13	29±2 ^b	59±9	26±3	48±14	43±4
Pentachlorophenol	69±3	73±13	77±13	51±16	101±24	86±10	67±7 ^c	67±4

^{a,b}Compound showed significant change for temperature with methanol–CO₂ (10:90 mol%), ^a, or methanol–CO₂ (20:80 mol%), ^b, extractant by one-way ANOVA.

^cCompound showed significant change for methanol composition at 100°C by one-way ANOVA.

trichlorophenol extracted using the methanol–CO₂ (20:80 mole fraction) mixture is plotted against the extraction fluid volume at three different temperatures. Increasing extraction rate was observed with increasing temperatures; however, the overall yield observed for extraction at 150°C was lower than that at 100°C.

The effect of adding methanol was evaluated at 50, 100 and 150°C, separately. The recoveries at 100°C and their one-way ANOVA results were listed in Table 2 as examples. Increasing the proportion of methanol from 0 to 10% and to 20% gradually increased the extraction recoveries for most of the analytes, while further increases to 30% caused

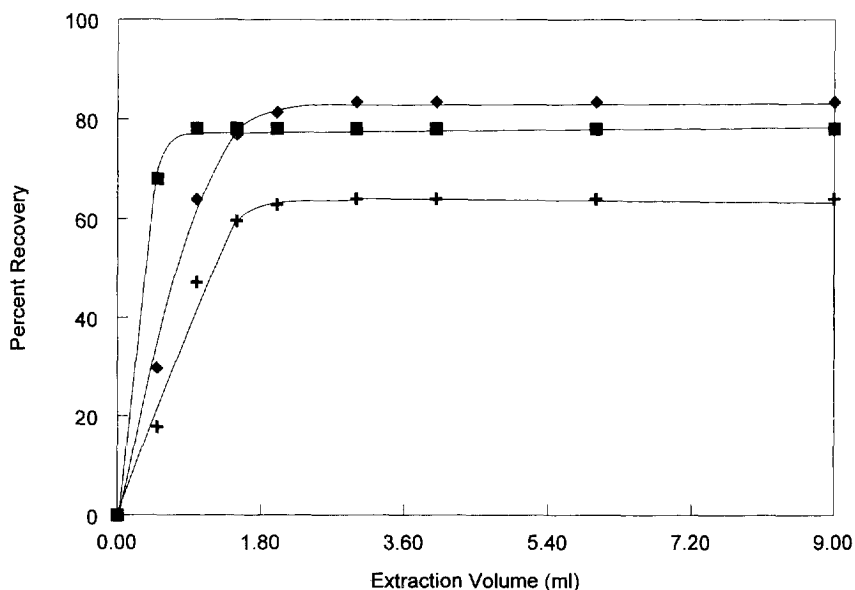


Fig. 1. Percentage recovery of 2,4,6-trichlorophenol vs. extraction volume using methanol–CO₂ (20:80 mole fraction) mixture at different temperatures: 50°C (+), 100°C (◆) and 150°C (■).

diminished extraction yields. The optimum extraction yields for most compounds was found at conditions of 100°C and 20% methanol or 150°C and 10% methanol. A *t*-test comparison (at the 95% confidence level) of the extraction yields for these two conditions showed no significant difference in the results for all the analytes. Methanol proportions >30% were not tried due to the observed recovery decreases at 100°C and 150°C using 30% methanol composition. Similar to elevating temperature, increasing the proportion of methanol also accelerated the extraction rate. Fig. 2 illustrates this effect for the extraction of 2,4,6-trichlorophenol.

A two-way ANOVA with temperature (50, 100 and 150°C) and composition (pure CO₂, 10%, 20% and 30% methanol) as factors was performed to examine the overall effect on the extraction yield. All the analytes showed significant differences in recovery with extraction-fluid composition changes, while all except three analytes (2-chlorophenol, *o*-cresol and 2,4-dimethylphenol) showed significant differences with temperature variation. The interaction between composition and temperature was also statistically significant for all except one (2,4-dinitrophenol). Therefore, extraction-fluid composi-

tion showed the largest influence on the extraction yield, followed by temperature variation and finally by the composition and temperature interaction for most of the analytes.

Several possible mechanisms have been proposed for why a polar modifier may enhance the extraction efficiency and the extraction rate. These include increasing the solubility of the analytes, competitively replacing bound analytes from active sites, interacting with the analyte-matrix complex and swelling matrix and making the extraction fluid more accessible to the internal cavities of the matrix. Therefore, whether a specific polar modifier exerts a positive impact on the extraction is very much matrix and analyte dependent. Increasing temperature is also known to be an effective way of increasing the extraction efficiency, but in a more matrix-independent way. Adding methanol and increasing temperature both have their negative attributes, such as increasing the viscosity of the extraction fluid by the former and lowering the extraction fluid density by the latter. When they are used together, predicting the proper combination of the two to achieve the maximal augmentation is not straightforward, as shown in this study. Since the extraction at 100°C gave high

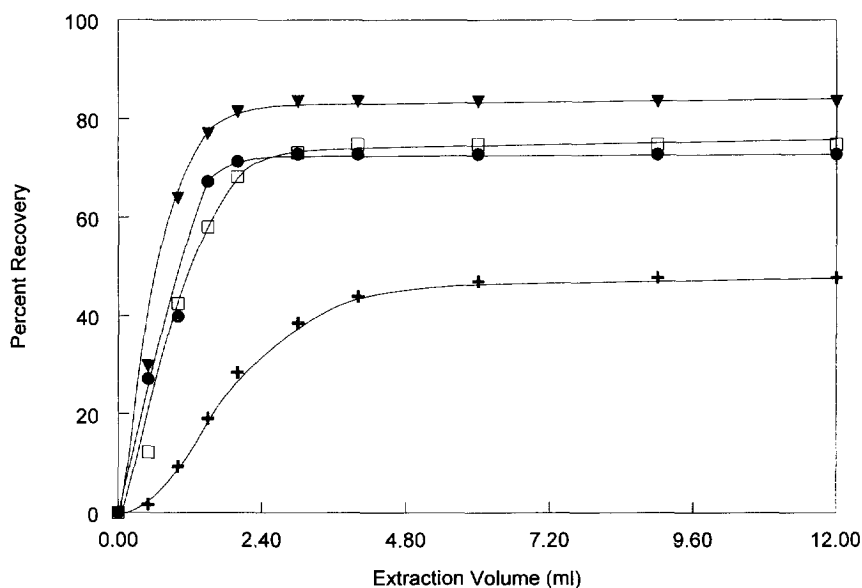


Fig. 2. Percentage recovery of 2,4,6-trichlorophenol vs. extraction volume at 100°C using extraction fluid with different methanol fractions: pure CO₂ (+), 10% (●), 20% (▼) and 30% (□) methanol mole fraction in CO₂.

recoveries (with 20% methanol), this temperature was used for the remaining studies.

3.4. Effect of moisture content

Environmental matrices, such as sediment, contain varying amounts of moisture. To study the effect of the moisture content on the extraction, a specific amount of water is usually added to the sample prior to extraction. Differing results were previously reported on the effect of moisture on the extraction of polar compounds [7,14,23,38–40].

Snyder et al. [38] reported that adding up to 5% (w/w) moisture to top soil increased the extraction yields for a series of organophosphate and organochlorine pesticides extracted by supercritical CO₂. Further increases in added water dropped the recoveries. Ashraf-Khorassani et al. [14] found that adding 1–10% (w/w) water was detrimental to the extraction of phenol from top soil using supercritical CO₂. On the other hand, Hess et al. [7] showed that 10% (w/w) moisture improved the extraction of phenol from soil using supercritical CO₂. The positive effects of moisture in the extraction of alkaloids from poppy straw [39] and zoalene and its metabolites from chicken liver [40] were also reported. The effect of moisture therefore seems difficult to predict.

In order to evaluate the effect of moisture content on the extraction of phenols from this river sediment, 100 µl and 200 µl water was directly applied onto the top of a 1.00 g sample in the extraction vessel separately. In contrast to preceding extractions, where 1 min static steps were used to equilibrate the extraction fluid with the sample before dynamic extractions, when the sediment sample was treated with a modifier, a 10 min static period was used to provide more time for the added modifier to interact with the matrix and the analytes. To examine whether the prolonged static time has affected the recovery when other conditions are held constant, extractions with 10 min static steps (without water added) were performed using 10:90 and 20:80 mol% methanol–CO₂ mixtures. A *t*-test comparison (with a 95% confidence level) of the results with the 1 min and 10 min static steps indicated that when methanol–CO₂ (10:90) was used as the extractant, no significant differences were detected for all the

analytes, while when methanol–CO₂ (20:80) was used as the extractant, the recoveries for 9 out of the 11 analytes did not show significant differences. Thus, the length of static equilibrium per se did not influence the total recovery significantly when other conditions were the same.

Extractions were done by using pure CO₂, 10:90 or 20:80 mol% methanol–CO₂ mixtures at 100°C and 238 atm. For the sake of brevity, the results of only the methanol–CO₂ (10:90 mol%) are shown in Table 3. For extractions with pure CO₂, the addition of 100 µl water (10%, w/w) did not significantly affect the extraction yields for most analytes, but when the 100 µl water treated sample was extracted using the methanol–CO₂ (10:90) mixture improvements in recovery were seen for nearly all the compounds. Extraction recoveries for nine out of the eleven analytes were increased by ca. 30%. A *t*-test between extractions using a methanol–CO₂ (10:90) mixture with and without 100 µl water treatment showed that the recoveries of all the compounds except four analytes, 2,4-dimethylphenol, 2,4,6-trichlorophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol, were statistically higher with the addition of 100 µl water. The 200 µl water treatment failed to show recoveries as high as the 100 µl water treatment. The recoveries from the extraction of 100 µl water-treated sediment with methanol–CO₂ (10:90) mixture exhibited the highest average recoveries for nearly all the analytes. One-way ANOVA of the three conditions with methanol–CO₂ (10:90) as the extractant showed that most analytes exhibited significant changes in recovery with water treatment (refer to Table 3). On the other hand, when extracted by methanol–CO₂ (20:80) mixture, adding either 100 µl or 200 µl water did not show any improvements in recovery; on the contrary, lower recoveries were seen.

The effect of moisture on extraction yield may be controlled by: increased solvent strength of the CO₂–water mixture, competition with the analyte-active site complex and efficient swelling of the solid matrix by water [41]. The positive attributes of adding water may be better manifested when a methanol–CO₂ mixture is used as the extractant instead of pure CO₂, due to the fact that water has much higher solubility in a methanol–CO₂ mixture than in CO₂ alone [42]. This may explain why better

Table 3
Effect of various conditions on the recovery relative to the spiking level

Extraction fluid	pK _a	Methanol-CO ₂ (10:90 mol%)			Methanol-water-CO ₂		Methanol-CO ₂ (10:90 mol%)	
		Water treatment			Ternary complex		HCl	TMAOH ^a
		0 μl	100 μl	200 μl			0.001 M	60 mM
Phenol	10.0 ^b	85 ± 2 ^c	110 ± 2	102 ± 4 ^d	96 ± 13	106 ± 12	100 ± 4	
2-Chlorophenol	8.6	52 ± 8	70 ± 4	64 ± 10 ^d	48 ± 4	66 ± 4	52 ± 4	
<i>o</i> -Cresol	10.2	56 ± 9	82 ± 5	66 ± 1 ^d	65 ± 6	76 ± 2	56 ± 9	
<i>m</i> -Cresol	10.1	58 ± 11	84 ± 4	64 ± 8 ^d	69 ± 9	71 ± 0	65 ± 4	
Nitrobenzene	–	65 ± 8	84 ± 3	65 ± 7 ^d	71 ± 12 ^e	67 ± 9	78 ± 7	
2,4-Dimethylphenol	10.6	57 ± 9	63 ± 7	43 ± 4	63 ± 8	65 ± 4	36 ± 8	
2,4-Dichlorophenol	7.9	68 ± 8	88 ± 0	70 ± 7 ^d	69 ± 6	77 ± 2	74 ± 3	
2,4,6-Trichlorophenol	6.2	75 ± 19	102 ± 11	79 ± 5	90 ± 14	88 ± 1	87 ± 4	
2,4-Dinitrophenol	4.1	59 ± 6	98 ± 3	78 ± 16 ^d	104 ± 13	86 ± 3	90 ± 5	
4,6-Dinitro- <i>o</i> -cresol	4.7	48 ± 6	57 ± 20	50 ± 9	91 ± 14	41 ± 3	62 ± 20	
Pentachlorophenol	4.5	78 ± 16	101 ± 9	71 ± 1 ^d	93 ± 15	83 ± 6	93 ± 6	

Temperature was 100°C for all studies.

^aAqueous solution.

^bpK_a data obtained from [44].

^cAverage recovery ± standard deviation.

^{d,e}Compound which showed significant change in recovery with water treatment using methanol-CO₂ (10:90), ^d, or with ternary methanol-water-CO₂ composition, ^e, by one-way ANOVA.

results were obtained when a 100 μl water addition is combined with methanol– CO_2 (10:90) extractant than when pure CO_2 was used. Thus, the effect of water and methanol is complementary in a sense in the extraction of polar compounds. The low miscibility of CO_2 with water may have contributed to the observed decreases in recovery when 200 μl water was used in these experiments.

When the data from the extraction using the 100 μl water-treated sediment with methanol– CO_2 (10:90 mol%) were compared by *t*-test to that from the extraction with the methanol– CO_2 (20:80 mol%) mixture which gave the best extraction results so far, the recoveries of seven compounds were statistically higher using the 100 μl water-treated sediment with methanol– CO_2 (10:90). Thus, the crucial impact of water on the extraction recoveries is documented. A certain amount of moisture may improve the overall extraction recovery. However, Fig. 3 shows that the extraction rate was slower. The extraction of pentachlorophenol is shown as an example. Clearly, when water was added, larger volumes (or longer times) were needed to reach the maximal extraction recovery.

3.5. Extraction using methanol–water– CO_2 ternary mixture

Motivated by the significant gains in recovery by increasing the moisture content in the sample, we tested further the effect of extraction by premixed methanol–water– CO_2 ternary mixtures at the same temperature (100°C). Premixing water with the extraction fluid differs from directly adding water into the sample in that the former provides continuous supply of water during the dynamic extraction and the latter emphasizes a high concentration of water during the static extraction period. Four ternary mixtures of methanol–water– CO_2 were prepared: 9:1:90, 18:2:80, 24:6:70 and 32:8:60 (mole ratios) of methanol–water– CO_2 . All of these mixtures are enhanced-fluidity liquids under the experimental condition (100°C and 238 atm) [42]. The highest average recoveries for most of the analytes were obtained with the methanol–water– CO_2 (18:2:80) mixture. However, one-way ANOVA showed that none but nitrobenzene exhibited significant change in extraction recovery among the four solvent mixtures studied. A common feature in the

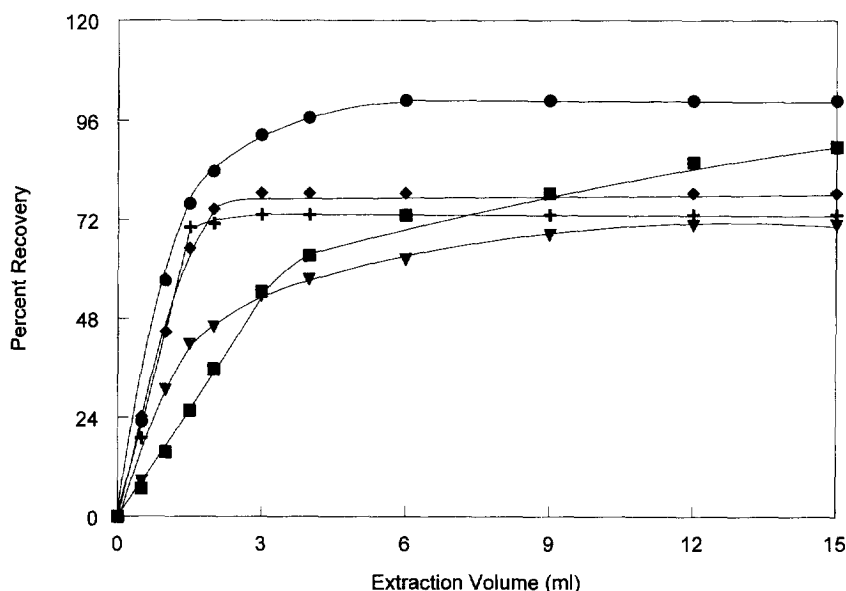


Fig. 3. Percentage recovery of pentachlorophenol vs. extraction volume at 100°C using different extraction fluids or static period: methanol– CO_2 (10:90) mixture (+), methanol– CO_2 (10:90) mixture and 100 μl water to the sediment (●), methanol– CO_2 (10:90) mixture and 200 μl water to the sediment (▼), ternary mixture of methanol–water– CO_2 (9:1:90 mole fraction) (■), methanol– CO_2 (10:90) mixture and 10 min static equilibrium period (◆).

results using these mixtures was that they extracted 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol efficiently, especially 4,6-dinitro-*o*-cresol, which was not quantitatively recovered by adding water into the extraction vessel or with any other conditions studied. For example, 4,6-dinitro-*o*-cresol was recovered to 61% by the 20% methanol extractant, but its recovery was increased to more than 90% when the methanol–water–CO₂ (18:2:80) mixture was used. For the purpose of brevity, Table 3 shows the results of the extraction using only the methanol–water–CO₂ (18:2:80) mixture.

3.6. Effect of pH and an organic base

Any factor which promotes the desorption of analytes from the matrix or increases the solubility of the analytes is expected to elevate the extraction efficiency. Keeping analytes in neutral form may facilitate their extraction. The phenols and nitroaromatics examined here have pK_a values ranging from 4 to 10 (listed in Table 3). Thus, an acidic environment is needed to retain all the analytes as undissociated compounds. Acidifying the medium has been a common practice to increase the efficiency of liquid–liquid extractions of phenols from aqueous media [5]. Also, the addition of an organic base, tetramethylammonium hydroxide (TMAOH), was shown to give higher recoveries for low pK_a phenols from graphitized carbon black [43]. It was proposed that low-pK_a phenols may be adsorbed as phenoxide ions and that displacement of these anions by OH⁻ and subsequent formation of ion pairs with tetramethylammonium ions may be responsible for the observed effect.

To test the effect of added acid and TMAOH, the following aqueous solutions were used to treat the sediment sample, as was done for the moisture simulation: 100 μl of 0.001 M HCl and 100 μl of 60 mM TMAOH. A 10 min static equilibrium was performed before the dynamic extraction. The pH for HCl and TMAOH aqueous solutions is 3 and 12.8, respectively.

Significant improvements in recovery were seen for most analytes extracted using the methanol–CO₂ (10:90) mixture when the sample was treated with 100 μl HCl, compared to that without any treatment (Table 3). However, the improvements were not as

large as when the sample was treated by 100 μl pure water. Compared with the treatment by HCl and pure water, TMAOH showed lower average recoveries, except for three phenols with pK_a values lower than 5: 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol and pentachlorophenol, whose recoveries are comparable to that treated by HCl or water.

3.7. Comparison of Soxhlet extraction with current extractions

Table 4 compares the extraction yields from some of the extraction conditions with those from the Soxhlet extraction. Except for extraction with supercritical CO₂, all other conditions gave substantially higher recoveries than found by Soxhlet extraction. A *t*-test comparison (95% confidence level) of the data for the 100 μl water-treated sediment with methanol–CO₂ (10:90 mol%) (column 3) to that from the extractions using enhanced-fluidity liquid methanol–water–CO₂ (18:2:80 mol%) (column 4) showed that the recoveries of four compounds were significantly different: three of them were better extracted by the former and the last one, 4,6-dinitro-*o*-cresol, was extracted by the latter. Results shown in this study illustrate that the phase of the extraction solvent (supercritical or enhanced-fluidity mixture) did not correlate with the extraction recovery. Both phase states can give high recovery, depending on the operating conditions.

3.8. Summary

In the extraction of phenolic compounds, pressure variation was least effective. Methanol addition significantly enhanced the percentage recovery of the analytes, but the optimal fraction was modulated by temperature. Proper combination of methanol addition and raising temperature was better than either used individually. Treating the sample with a small amount of water prior to extraction had a notable effect, while the degree of effectiveness is dependent on the extraction fluid composition. Treatment of sample by acid or TMAOH did not show improved extraction recovery relative to the adding of a small amount of pure water. The ternary mixture extractant methanol–water–CO₂ functioned as effectively as treating the sample with water and extracting with

Table 4

Comparison of Soxhlet, SFE and enhanced-fluidity liquid extraction (EFE) at 100°C

Compound	Soxhlet	% Recovery relative to the Soxhlet extraction		
		SFE	Modified SFE	EFE
		CO ₂	10/90 MeOH–CO ₂ (100 µl H ₂ O)	MeOH–water–CO ₂ (18:2:80)
Phenol	78±11 ^a	79 ^b	141	123
2-Chlorophenol	54±12	67	130	89
<i>o</i> -Cresol	61±10	79	134	107
<i>m</i> -Cresol	59±9	73	142	117
Nitrobenzene	58±12	93	144	122
2,4-Dimethylphenol	58±8	84	108	109
2,4-Dichlorophenol	63±7	68	140	110
2,4,6-Trichlorophenol	74±8	65	137	121
2,4-Dinitrophenol	61±9	92	161	170
4,6-Dinitro- <i>o</i> -cresol	46±13	104	124	198
Pentachlorophenol	81±25	83	125	115

^aAverage recovery±standard deviation of Soxhlet extractions relative to the spiked amount.^bThe percent recoveries of extractions relative to the Soxhlet extraction.

methanol–CO₂ mixture. The ternary enhanced-fluidity liquid with nearly 20% methanol added was as good as or better than the supercritical mixtures and can be considered as an effective alternative in extracting polar compounds from complex matrices.

Acknowledgments

The authors thank ISCO for loaning the SFX 220 extractor. We also thank the US Environmental Protection Agency (EPA) for financially supporting this project. Although the research described here was funded by the US EPA, it has not been subject to Agency review, and, therefore, does not necessarily reflect the views of the Agency, and no official endorsements should be inferred.

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