Journal of Chromatography, 594 (1992) 297–307
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 812

Effects of collection solvent parameters and extraction cell geometry on supercritical fluid extraction efficiencies

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(First received August 6th, 1991; revised manuscript received October 18th, 1991)

ABSTRACT

Supercritical fluid extraction (SFE) collection efficiencies of 66 compounds with a wide range of volatility and polarity were examined. Good collection efficiencies required efficient partitioning of the analyte into the collection solvent after depressurization, and factors including collection solvent polarity and temperature were found to be more important than collection solvent volume and height. Heating the collection solvent with a heat gun to avoid plugging of the outlet restrictor resulted in 20–50% losses of the more volatile analytes, while >90% trapping of all test analytes could be attained by controlling the solvent temperature at 5°C. Extraction cell geometry ("long, narrow" *versus* "short, broad" vessel) at constant internal volume and the orientation of the extraction cell were found to have negligible effects on the extraction rates of polycyclic aromatic hydrocarbons (PAHs) from railroad bed soil and flavor and fragrance compounds from lemon peels. The supercritical fluid flow-rate also had little effect on the extraction rate of native PAHs provided that it was sufficient to sweep the cell dead volume every *ca.* 3 min.

INTRODUCTION

The use of supercritical fluids for the extraction of organic compounds from a wide variety of matrices is rapidly increasing because of the attractive properties that supercritical fluids exhibit and their potentially wide applicability for sample extractions. Supercritical fluids have lower viscosities and higher solute diffusivities than liquid solvents, which improves mass transfer and reduces the extraction time needed, and the solvent strength of a supercritical fluid is a function of density and can be controlled by simply changing the pressure or temperature. Supercritical fluids such as carbon dioxide can be inexpensive, available in high purity and chemically inert, and as they are gases under ambient conditions the need for liquid solvents and concentration steps is nearly eliminated.

There have been several reports of different methods for trapping analytes after the SFE depressurization step, including collection in an open vessel [1–3], collection on sorbent resins such as Tenax, C_{18}, silica gel and XAD traps [4–6], collection on cryogenically cooled surfaces [7] and on-line methods coupled to various chromatographic instrumentation [8–11]. In this investigation, a simple off-line analyte trapping technique was investigated which consisted of depressurizing the supercritical fluid into an organic collection solvent [12–14]. This method was chosen because it has been the most commonly used method in supercritical fluid extraction (SFE) studies, is relatively simple and inexpensive to perform and because the extracts are immediately ready for chromatographic analysis using conventional injection techniques.

There have been numerous reports of quantitative extractions using supercritical fluids [15], but most have dealt with fairly non-volatile species that are more easily trapped using the collection methods mentioned above. However, many analytes of interest for SFE exhibit a high vapor pressure, which may make collection after SFE difficult. For such analytes, low recoveries may be wrongly attributed to poor extraction efficiency when the real problem is poor collection of extracted analytes on depressurization of the supercritical fluid. In addition,

0021-9673/92/$05.00

many real environmental and natural matrices contain high concentrations of water that can freeze and cause restrictor plugging owing to the rapid cooling from the carbon dioxide depressurization. Heating the collection solvent is an effective method to prevent the restrictor from becoming blocked, but may further reduce the collection efficiencies of volatile analytes.

This paper describes the collection efficiencies of 60 environmentally hazardous pollutants from the US Environmental Protection Agency (EPA)'s semivolatile target compound list and six flavor and fragrance compounds. It must be emphasized that the investigations reported here were focused on determining the collection efficiencies under SFE conditions, not SFE extraction efficiencies, and thus are based on the extraction of test analytes that were added to relatively inert matrices. Even though the use of such spikes is not always a valid approach to determining extraction efficiencies (as spikes do not necessarily interact with the same matrix active sites as native analytes), spiking is an effective and reliable method for determining trapping efficiencies as the spiked analytes are introduced into the collection system under the identical SFE conditions experienced by native analytes. The effect of different solvent trapping conditions on SFE collection efficiencies, including collection solvent polarity, solvent volume and height, solvent temperature and supercritical fluid flow-rate, were investigated. In addition, the effects of SFE flow-rate, cell geometry and cell orientation on the extraction rates of native (not spiked) polycyclic aromatic hydrocarbons (PAHs) from railroad bed soil and native flavor and fragrance compounds from lemon peel are discussed.

EXPERIMENTAL

Samples and standards

Standards (0.6 mg/ml each) of 60 compounds from the semivolatile target compound list were prepared in methylene chloride and stored at 0°C until used. An additional standard of six flavor and fragrance compounds in methylene chloride (100 mg/ml each) was prepared and stored in the same manner. Spiking levels for the semivolatile pollutants and the flavor and fragrance compounds were 18 μg and 600 μg of each compound, respectively. Internal standards added to each extract for gas chromatographic (GC) analysis after SFE were 10 μg of 1,4-dichlorobenzene-d_4 for the semivolatile pollutants and 500 μg of *n*-heptadecane for the flavor and fragrance compounds.

Two real samples with different physical and chemical characteristics were chosen to investigate the effect of cell geometry and orientation on SFE extraction rates of native analytes. Railroad bed soil was chosen because it contained native PAHs of environmental interest. Lemon peel was selected to represent a non-homogeneous, odd-shaped (not conforming to the extraction vessel shape and therefore increasing the cell dead volume) matrix that contained a wide range of volatile components such as monoterpenes and sesquiterpenes, and because it also had a high water content (85% as determined by oven drying overnight at 100°C) which consistently led to restrictor plugging from frozen water unless the collection solvent was heated during SFE. Prior to extraction, the railroad bed soil was sieved to <2 mm to remove any debris and fresh lemon peel was cut into strips of *ca.* 10 mm × 2 mm × 1 mm.

Supercritical fluid extractions

All supercritical fluid extractions were performed using SFC-grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, USA) and an ISCO (Lincoln, NE, USA) Model 260D syringe pump operated at 400 atm. The extraction cell temperature was maintained at 50°C using a thermostatically controlled tube heater. Extractions were performed using 2.5-ml "long, narrow" (132 mm × 5 mm I.D.) or 2.5-ml "short, broad" (33 mm × 10 mm I.D.) extraction cells from Keystone Scientific (Bellefonte, PA, USA). The flow-rate of supercritical fluid through the extraction cells was controlled by a 10-cm outlet restrictor (26 μm I.D. unless noted otherwise, resulting in a flow-rate of *ca.* 0.6 ml/min measured as a liquid at the pump and a gaseous carbon dioxide flow-rate of *ca.* 300 ml/min) made from fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA). All organic solvents used were of pesticide grade.

Collection efficiencies were determined by filling the 2.5-ml "long, narrow" extraction cell with 3.5 g of 70–80-mesh glass beads for the flavor and fragrance compounds or 3.5 g of clean sea sand for the

semivolatile pollutants, and spiking the standard solutions into the center of the glass beads or sea sand. The cell was immediately sealed to prevent any loss of the volatile spike components, placed inside the tube heater and the samples were extracted for 5–30 min. Extracted analytes were collected into 3.7-ml (33 mm × 12 mm I.D.), 7.4-ml (48 mm × 14 mm I.D.) or 15.0-ml (59 mm × 18 mm I.D.) vials containing 2.5–10.0 ml of collection solvent. Solvent volume was maintained by small additions of solvent during SFE. Heat was applied to the collection solvent using a heat gun or a temperature-controlled block made from aluminum (75 mm × 50 mm × 38 mm) with four holes (26 mm × 23 mm I.D.) bored into it for the sample vials. Water was placed between the vial and the heating block to ensure proper thermal transfer was achieved.

The effect of cell geometry and orientation was investigated using 3 g of railroad bed soil or 1 g of lemon peel placed inside one of the 2.5-ml extraction cells ("long, narrow" or "short, broad"). The extraction vessel was sealed and placed inside the thermostated tube heater, and the native analytes were extracted from the sample into a 7.4-ml vial containing 5 ml of methylene chloride. The collection solvent was maintained at 5°C using the temperature-controlled block (no provision was made to cool the block to 5°C; however, as discussed later, the solvent temperature rapidly drops to 5°C on beginning SFE). The flow-rate of the supercritical fluid was varied from 0.15 to 1.2 ml/min using 10-cm lengths of 15, 24, 26, 29 or 32 μm I.D. restrictors. Fractions of the extracts were collected at timed intervals over a period of 100 min to compare extraction rates for different extraction cell geometries, cell orientations and flow-rates. Each fraction was immediately spiked with the appropriate internal standard and analyzed.

Gas chromatographic analysis

All GC analyses were done with a Hewlett-Packard Model 5890 gas chromatograph with flame ionization detection and hydrogen as the carrier gas. The injections were performed in the split mode with a 20:1 splitting ratio into a wide-bore (25 m × 0.32 mm I.D., 0.17 μm film thickness) HP-1 (Hewlett-Packard, Avondale, PA, USA) or a wide-bore (25 m × 0.32 mm I.D., 0.17 μm film thickness) HP-5 fused-silica capillary column. The injector and detector temperatures were maintained at 300°C. Compound identifications were confirmed using a Hewlett-Packard Model 5985 gas chromatograph–mass spectrometer and by the injection of standards.

RESULTS AND DISCUSSION

Collection efficiencies during supercritical fluid extraction

As previously discussed, SFE of wet (*e.g.*, containing more than *ca.* 1% water) real samples often requires heating to prevent restrictor plugging from ice formation. To mimic an extraction scheme when the sample contains significant amounts of water, mild heating (heated every 30 s for 5 s during the first 5 min and thereafter heated every 2 min for 5 s) with a heat gun was applied to the collection solvent, and the collection efficiencies were determined for several different collection solvents of differing polarity and boiling point (GC calibration standards were prepared in each solvent tested to ensure that any differences in SFE collection efficiencies that were observed between solvents were not a result of differences in the GC analysis that might be caused by the solvent). The effect of mild heating on collection solvent temperature is shown in Fig. 1. As shown in Table I for 40-min extractions, analyte losses of up to 60% occurred, with a general trend in

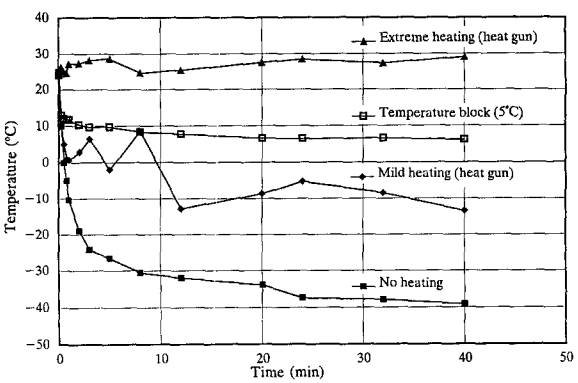

Fig. 1. SFE collection solvent temperature with and without heating during SFE. Supercritical CO_2 flow-rate was *ca.* 0.6 ml/min (*ca.* 300 ml/min gaseous CO_2) into 5 ml of methylene chloride. Mild heating (heated every 30 s for 5 s during the first 5 min and thereafter heated every 2 min for 5 s) and extreme heating (heated every 30 s for 15 s during the first 5 min and thereafter heated every 2 min for 15 s) were performed for 40 min using a heat gun. The temperature-controlled block was set at 5°C as described in the text.

TABLE I

COLLECTION EFFICIENCIES OF SEMIVOLATILE POLLUTANTS INTO VARIOUS TRAPPING SOLVENTS WHILE USING MILD HEATING

Compound	Recovery (%)[a]				
	Methylene chloride	Chloroform	Acetone	Methanol	Hexane
Phenol	77.4 (2.4)	72.3 (3.1)	67.6 (1.6)	54.9 (11.6)	42.5 (1.6)
Di(2-chloroethyl) ether	73.1 (5.6)	68.7 (2.6)	72.5 (1.1)	56.4 (13.8)	60.5 (5.6)
1,3-Dichlorobenzene	70.4 (5.5)	66.5 (3.1)	57.4 (0.9)	53.4 (19.2)	54.5 (2.7)
1,4-Dichlorobenzene	76.8 (3.0)	72.5 (3.4)	69.9 (1.4)	53.4 (13.0)	44.2 (4.7)
1,2-Dichlorobenzene	77.6 (4.9)	74.3 (3.5)	74.9 (0.8)	57.8 (13.3)	45.6 (4.6)
N-Nitrosodipropylamine	77.6 (7.3)	77.3 (2.8)	75.9 (1.4)	55.8 (10.7)	48.8 (6.2)
4-Methylphenol	78.5 (3.6)	74.8 (3.7)	70.4 (1.1)	54.7 (16.0)	46.4 (5.8)
Nitrobenzene	82.2 (3.6)	82.3 (4.6)	73.7 (1.3)	57.7 (10.2)	60.4 (1.6)
Isophorone	79.7 (3.9)	74.9 (3.0)	73.2 (1.4)	55.9 (15.6)	50.5 (2.7)
2-Nitrophenol	80.0 (5.0)	79.6 (5.3)	82.0 (0.2)	60.8 (8.3)	56.8 (5.1)
2,4-Dichlorophenol	77.7 (4.5)	75.4 (5.2)	67.6 (1.3)	63.8 (18.6)	55.5 (5.9)
1,2,4-Trichlorobenzene	78.7 (5.5)	76.2 (3.4)	70.4 (1.3)	59.4 (17.9)	55.9 (4.9)
4-Chloro-3-methylphenol	83.6 (6.1)	86.8 (5.9)	81.4 (3.9)	49.7 (15.4)	72.0 (0.9)
Hexachlorocyclopentadiene	78.2 (1.4)	77.1 (4.4)	70.1 (1.6)	58.6 (16.3)	60.9 (1.9)
2,4,5-Trichlorophenol	83.9 (2.9)	83.6 (3.8)	85.5 (1.8)	59.4 (3.4)	71.1 (1.8)
2,4,6-Trichlorophenol	85.3 (3.9)	85.4 (4.8)	87.4 (2.4)	57.9 (4.9)	73.4 (1.9)
2-Chloronaphthalene	82.5 (4.0)	81.9 (2.6)	79.8 (1.7)	60.2 (11.2)	67.1 (2.9)
2-Nitroaniline	86.4 (1.4)	86.4 (2.5)	88.4 (1.3)	57.3 (4.7)	72.4 (3.1)
Acenaphthylene	83.7 (3.8)	85.4 (1.7)	82.7 (1.4)	60.0 (8.3)	66.3 (9.9)
Dimethyl phthalate	85.5 (3.1)	91.6 (2.0)	87.1 (0.9)	59.9 (2.8)	76.7 (2.8)
2,6-Dinitrotoluene	85.9 (3.3)	90.1 (1.1)	88.1 (0.8)	60.9 (3.0)	76.7 (3.3)
Acenaphthene	84.1 (3.5)	85.1 (3.6)	82.9 (1.4)	60.6 (7.3)	72.2 (2.0)
3-Nitroaniline	85.8 (3.9)	87.1 (2.8)	88.3 (2.2)	54.5 (5.6)	74.1 (6.5)
Dibenzofuran	84.2 (4.3)	85.8 (3.1)	86.1 (0.9)	63.1 (5.8)	73.9 (5.1)
2,4-Dinitrotoluene	92.3 (4.4)	86.9 (4.5)	96.4 (3.0)	60.1 (11.0)	67.8 (1.8)
Fluorene	85.5 (3.9)	88.1 (3.7)	87.4 (1.2)	61.8 (3.4)	76.1 (2.6)
4-Chlorophenyl phenyl ether	85.5 (2.8)	88.4 (4.3)	86.6 (1.2)	62.1 (3.9)	75.5 (2.3)
Diethyl phthalate	87.3 (1.7)	90.7 (4.4)	88.9 (0.8)	59.9 (2.7)	79.3 (3.3)
4-Nitroaniline	87.0 (8.3)	89.1 (3.5)	89.6 (3.6)	48.2 (13.3)	53.2 (20.6)
N-Nitrosodiphenylamine	86.5 (8.3)	90.2 (6.2)	91.8 (2.4)	64.6 (2.9)	74.0 (11.3)
4-Bromophenyl phenyl ether	86.4 (2.3)	94.3 (9.8)	89.3 (1.2)	60.2 (3.6)	81.7 (6.4)
Hexachlorobenzene	86.0 (2.1)	90.6 (8.7)	88.7 (0.8)	61.7 (3.2)	79.4 (1.2)
Phenanthrene	86.9 (2.9)	89.0 (3.9)	90.9 (1.7)	59.7 (3.5)	79.1 (4.0)
Anthracene	87.5 (2.6)	88.0 (3.7)	89.6 (1.6)	58.9 (3.8)	78.7 (3.5)
Dibutyl phthalate	90.4 (1.8)	89.4 (1.7)	96.6 (3.5)	57.5 (10.2)	81.8 (2.5)
Fluoranthene	89.1 (2.9)	89.9 (4.9)	91.5 (3.0)	56.2 (7.1)	78.3 (2.3)
Pyrene	89.5 (3.1)	90.8 (1.7)	92.8 (1.8)	57.7 (6.7)	79.6 (4.5)
Butylbenzyl phthalate	89.4 (2.4)	89.4 (3.6)	92.1 (2.7)	54.7 (9.8)	77.3 (3.8)
Chrysene	90.4 (3.5)	91.9 (4.5)	92.1 (2.0)	56.7 (9.2)	80.3 (3.5)
3,3'-Dichlorobenzidine	88.8 (2.4)	88.9 (4.4)	91.6 (4.4)	49.6 (17.8)	76.8 (3.2)
2-Ethylhexyl phthalate	92.2 (2.4)	90.5 (2.5)	98.6 (7.0)	56.1 (10.0)	90.7 (1.3)
Dioctyl phthalate	91.1 (1.5)	88.9 (3.5)	90.9 (1.6)	53.9 (13.6)	77.8 (4.5)
Benzo[*b*]fluoranthene	91.6 (2.3)	88.1 (3.0)	91.5 (2.9)	54.9 (15.8)	77.3 (2.5)
Benzo[*k*]fluoranthene	91.5 (2.3)	88.5 (3.5)	91.5 (2.8)	55.7 (14.6)	77.9 (2.9)
Benzo[*a*]pyrene	92.2 (2.1)	86.9 (4.5)	91.4 (3.7)	56.9 (17.9)	76.3 (1.8)
Dibenzo[*a,h*]anthracene	93.7 (4.7)	82.3 (2.4)	90.0 (7.6)	63.2 (12.2)	72.6 (2.8)
Benzo[*ghi*]perylene	93.2 (2.2)	81.1 (2.3)	87.6 (6.5)	66.5 (11.2)	71.4 (1.7)

[a] Values in parentheses are relative standard deviations (%) for triplicate 40-min extractions.

recoveries based on the volatility of the analyte (the compounds in Table I are listed in order of GC retention indices) and analyte solubility in the collection solvent. Based on a comparison of chloroform (b.p. = 60.9°C) and methylene chloride (b.p. = 40°C), the boiling point of the collection solvent did not affect the collection efficiencies as both of these solvents showed similar losses of 10–25% of the tested compounds. Acetone yielded trapping efficiencies similar to methylene chloride and chloroform, but methanol failed to collect *ca.* 35–50% of each of the species. Hexane was the poorest collection solvent for several of the most volatile test species, but was better than methanol for the less volatile components. As methylene chloride was the best overall collection solvent (and a good solvent for GC), it was used to test the collection efficiencies for the test species in subsequent studies.

The cooling effect on the collection solvent (methylene chloride) that results from carbon dioxide depressurization with and without solvent heating for 40 min is shown in Fig. 1. Without any heating the temperature of the collection solvent rapidly drops to −25°C, then slowly approaches −40°C. All of the semivolatile pollutants were quantitatively collected (>95%) into methylene chloride with no heating, but this is an unrealistic experimental approach because the collection solvent becomes so cold that real samples containing significant amounts of water can cause restrictor plugging from freezing water (such plugging has been observed for nearly all samples that we have encountered which contain more than *ca.* 1% water). As previously discussed, this problem is easily solved by using a heat gun to warm the restrictor and collection solvent but, as shown in Fig. 1, this results in temperature fluctuations that depend on the degree of heating and that can approach the boiling point of methylene chloride. As shown in Table I, even mild heating with the heat gun resulted in significant losses of the more volatile analytes. To avoid the solvent temperature fluctuations resulting from the heat gun, an alternative method of heating the collection solvent, the temperature-controlled block, was designed and tested. Fig. 1 shows that when the temperature-controlled block was set at 5°C, the collection solvent rapidly cooled to *ca.* 6°C and the block was able to maintain a relatively constant solvent temperature. (Note that the block contains no cooling device and was at room temperature at the beginning of the SFE step. The temperature profile results from the cooling effect of the expanding carbon dioxide combined with the block heater set to turn on at 5°C.) The temperature-controlled block set at 5°C also eliminated restrictor plugging from freezing water during SFE of wet samples.

Collection efficiencies of the semivolatile pollutants from the target compound list were again determined (40-min extractions and methylene chloride as the collection solvent) using the temperature-controlled block set at 5°C. As shown in Table II, the collection efficiencies of all of the compounds on the list were good, with a range of 92–104%. The collection efficiencies also showed excellent reproducibility, with the relative standard deviations for all of the test species being <6% for triplicate spike extractions.

As the temperature-controlled block was effective in eliminating restrictor plugging from ice formation during SFE of wet samples, such as lemon peel, and yielded quantitative collection of the semivolatile pollutants, this method of collection was used to test the collection efficiencies of several additional flavor and fragrance compounds including α-pinene, carvone, eugenol, cedrene, cedrol and santonin. The collection efficiencies achieved during the 10-min SFE using different trapping solvents (5 ml, resulting in a 33-mm solvent height) were again found to depend on the solubility and volatility of the test analytes. Acetone and toluene behaved similarly and recovered *ca.* 90% of all the compounds except α-pinene, which was only 76% and 78% recovered, respectively. Methanol was able to trap 100% of the santonin and *ca.* 90% of the remaining compounds, but only trapped 79% of the α-pinene. Hexane was an excellent trapping solvent for all of the compounds (>95%) except santonin, which was only 69% recovered. Methylene chloride was able to trap >90% of all of the components that were tested. Since methylene chloride was also the best collection solvent for the semivolatile pollutants, it was again chosen as the collection solvent in the remainder of the collection experiments.

It should be noted that some losses of the flavor and fragrance compounds can occur when concentrating the extracts under a gentle stream of nitrogen. For example, when extracts collected in

TABLE II

COLLECTION EFFICIENCIES OF SEMIVOLATILE POLLUTANTS IN METHYLENE CHLORIDE HELD AT 5°C

Compound	Recovery (%)[a]	Compound	Recovery (%)[a]
2-Chlorophenol	96.8 (1.1)	Dimethyl phthalate	100.1 (1.9)
Phenol	97.8 (0.3)	2,6-Dinitrotoluene	99.0 (2.0)
Di(2-chloroethyl) ether	100.3 (1.9)	Acenaphthene	98.3 (1.4)
1,3-Dichlorobenzene	99.8 (2.5)	3-Nitroaniline	93.2 (6.4)
1,4-Dichlorobenzene	101.4 (2.7)	Pentachlorophenol	97.8 (1.4)
1,2-Dichlorobenzene	99.8 (2.2)	Dibenzofuran	98.1 (5.0)
Benzyl alcohol	95.4 (1.2)	2,4-Dinitrotoluene	99.6 (1.8)
Di(2-chloroisopropyl) ether	95.5 (0.6)	Fluorene	99.7 (2.1)
2-Methylphenol	95.1 (1.5)	4-Chlorophenyl phenyl ether	100.4 (1.0)
Hexachloroethane	95.6 (1.8)	Diethyl phthalate	97.1 (4.0)
N-Nitrosodipropylamine	97.9 (1.6)	4-Nitroaniline	102.9 (5.2)
4-Methylphenol	98.9 (2.0)	N-Nitrosodiphenylamine	101.1 (4.6)
Nitrobenzene	100.0 (2.2)	4-Bromophenyl phenyl ether	99.7 (1.7)
Isophorone	98.6 (2.5)	Hexachlorobenzene	99.9 (3.5)
2-Nitrophenol	99.0 (0.6)	Phenanthrene	100.3 (2.8)
2,4-Dimethylphenol	95.4 (0.9)	Anthracene	97.0 (5.1)
2-Chloroethoxymethane	103.9 (1.9)	Dibutyl phthalate	99.1 (2.6)
2,4-Dichlorophenol	98.5 (1.4)	Fluoranthene	96.1 (4.5)
1,2,4-Trichlorobenzene	99.7 (1.0)	Pyrene	99.1 (4.6)
Naphthalene	98.6 (1.3)	Butylbenzyl phthalate	98.4 (2.1)
4-Chloroaniline	97.6 (2.5)	Benz[*a*]anthracene	98.6 (1.2)
Hexachlorobutadiene	97.8 (1.6)	Chrysene	100.9 (1.4)
2-Methylnaphthalene	97.5 (2.6)	3,3′-Dichlorobenzidine	92.4 (4.1)
4-Chloro-3-methylphenol	100.5 (1.3)	2-Ethylhexyl phthalate	99.5 (2.9)
Hexachlorocyclopentadiene	103.5 (1.7)	Dioctyl phthalate	96.7 (2.3)
2,4,5-Trichlorophenol	97.7 (1.7)	Benzo[*b*]fluoranthene	97.0 (3.6)
2,4,6-Trichlorophenol	99.0 (2.3)	Benzo[*k*]fluoranthene	98.6 (3.2)
2-Chloronaphthalene	99.5 (1.4)	Benzo[*a*]pyrene	96.8 (2.2)
2-Nitroaniline	98.3 (2.4)	Dibenzo[*a,h*]anthracene	92.7 (5.3)
Acenaphthylene	99.4 (1.7)	Benzo[*ghi*]perylene	98.8 (3.8)

[a] Values in parentheses are relative standard deviations (%) for triplicate 40-min extractions.

methylene chloride were evaporated from 5 to 1 ml under a gentle stream of nitrogen, 7% of the α-pinene was lost and *ca.* 4% of the other compounds were lost. To avoid these losses, no concentration of the extracts was performed for the flavor and fragrance recovery studies.

To investigate whether losses of analytes occur during SFE because they are purged out of the collection solvent by the high flow-rate of gaseous carbon dioxide or because they fail to partition into the collection solvent, the flavor and fragrance standard was spiked into a 7.4-ml vial containing 5.0 ml (33-mm height) of methylene chloride, and carbon dioxide was allowed to bubble through the spiked solvent for 5–30 min under normal SFE conditions. Table III shows the effect of purging under different supercritical fluid flow-rates and purging times. At 0.3 and 0.6 ml/min, there are no significant losses ($< 5\%$) except for α-pinene, which showed a 6% loss at 0.3 ml/min and a 13% loss at 0.6 ml/min after 30 min. At 1.2 ml/min, α-pinene showed a 24% loss and the losses of the other flavor and fragrance compounds ranged from 4 to 7% after 30 min. As none of the test species showed significant losses after 5 min, these results demonstrate that excessively high flow-rates coupled with long extraction times may result in lower overall recoveries of volatile analytes because of purging losses.

To determine the effect of collection solvent volume on the trapping efficiencies of the flavor and

TABLE III

CO_2 PURGING EFFECT ON FLAVOR AND FRAGRANCE COMPOUND LOSSES

Compound	Flow-rate (ml/min)[a]	Amount remaining (%)[b]	
		5 min	30 min
α-Pinene	0.3	98.8 (0.8)	94.5 (3.3)
Carvone		100.1 (0.6)	101.7 (1.9)
Eugenol		100.2 (0.8)	101.7 (2.4)
Cedrene		100.5 (1.1)	101.1 (2.1)
Cedrol		100.4 (1.9)	101.2 (3.0)
Santonin		100.3 (1.3)	100.8 (3.1)
α-Pinene	0.6	96.9 (3.4)	86.8 (0.9)
Carvone		99.4 (2.9)	100.4 (1.2)
Eugenol		99.4 (2.3)	100.5 (1.5)
Cedrene		99.1 (2.6)	99.7 (1.8)
Cedrol		99.9 (2.3)	100.6 (1.1)
Santonin		98.7 (1.2)	98.1 (1.5)
α-Pinene	1.2	98.3 (0.6)	76.6 (3.1)
Carvone		100.6 (1.8)	93.4 (3.1)
Eugenol		100.9 (0.5)	94.9 (3.7)
Cedrene		100.6 (0.6)	92.7 (3.2)
Cedrol		99.8 (0.6)	95.7 (3.0)
Santonin		99.3 (0.3)	95.8 (3.3)

[a] Flow-rate of CO_2 measured as a liquid at the pump.
[b] Values in parentheses are relative standard deviations (%) for triplicate extractions.

fragrance compounds, collection for 10 min into different solvent volumes (2.5, 5.0 and 10.0 ml) with a supercritical fluid flow-rate of 0.6 ml/min was tested. The results in Table IV show that the collection solvent volume was less important for efficient trapping than was originally expected. When only 2.5 ml of collection solvent (22-mm solvent height) were used, the losses of analytes ranged from 6 to 13% and when 5.0 or 10.0 ml of collection solvent were used the losses were similar, ranging from 7 to 10%. It should also be noted that the solvent height can possibly affect the collection efficiencies because the analytes need a certain amount of time after the depressurization step to diffuse into the collection solvent. At a constant bubble rise rate, a greater solvent height should permit longer solvent–analyte contact and thus increase the chances that the analyte will be trapped in the collection solvent. This was investigated by using two collection vials of differing dimensions (48 mm × 24 mm I.D. and 33 mm × 12 mm I.D.), each containing 2.5 ml of collection solvent (resulting in solvent heights of 8 and 22 mm, respectively). The results in Table IV show that, with the exception of α-pinene, the 8-mm collection solvent height trapped up to 11% less of the flavor and fragrance compounds than the 22-mm solvent height. Even though the collection solvent height did affect the recoveries of the flavor and fragrance compounds, the differences in the recoveries were still small, which indicates that the mass transfer of the analyte from the gaseous carbon dioxide into the collection solvent is very fast, and the collection solvent height is not as important as might be expected. As the results for collection into 2.5, 5.0 and 10.0 ml were nearly identical, a 5.0-ml collection solvent volume (33-mm solvent height) was arbitrarily selected for convenience to be used throughout the remainder of the collection experiments.

TABLE IV

COLLECTION EFFICIENCIES OF FLAVOR AND FRAGRANCE COMPOUNDS INTO VARIOUS TRAPPING SOLVENT VOLUMES AND HEIGHTS

Compound	Trapping efficiency (%)[a]			
	2.5 ml		5.0 ml	
	8 mm[b]	22 mm[b]	33 mm[b]	41 mm[b]
α-Pinene	87.8 (1.5)	86.6 (1.4)	90.0 (4.1)	90.4 (1.6)
Carvone	82.6 (1.3)	93.3 (2.0)	91.1 (1.1)	92.3 (1.7)
Eugenol	84.3 (0.6)	92.6 (1.6)	89.6 (1.0)	91.1 (1.9)
Cedrene	86.7 (0.5)	92.6 (1.7)	92.3 (2.7)	92.2 (1.6)
Cedrol	90.9 (1.2)	92.4 (1.4)	90.4 (2.6)	92.3 (2.1)
Santonin	90.2 (1.5)	93.8 (0.3)	92.5 (2.5)	91.9 (0.7)

[a] Values in parentheses are the relative standard deviations (%) for triplicate 10-min extractions.
[b] Solvent height in the collection vial using 48 mm × 24 mm I.D., 33 mm × 12 mm I.D., 48 mm × 14 mm I.D., and 59 mm × 18 mm I.D. vials.

The effect of supercritical fluid flow-rate on the trapping efficiencies of the flavor and fragrance compounds was also determined using 5.0 ml of collection solvent over a range of flow-rates from 0.3 to 1.2 ml/min for 10 min. There was little effect on the recoveries of these analytes based on supercritical fluid flow-rate. For example, at 0.3 ml/min

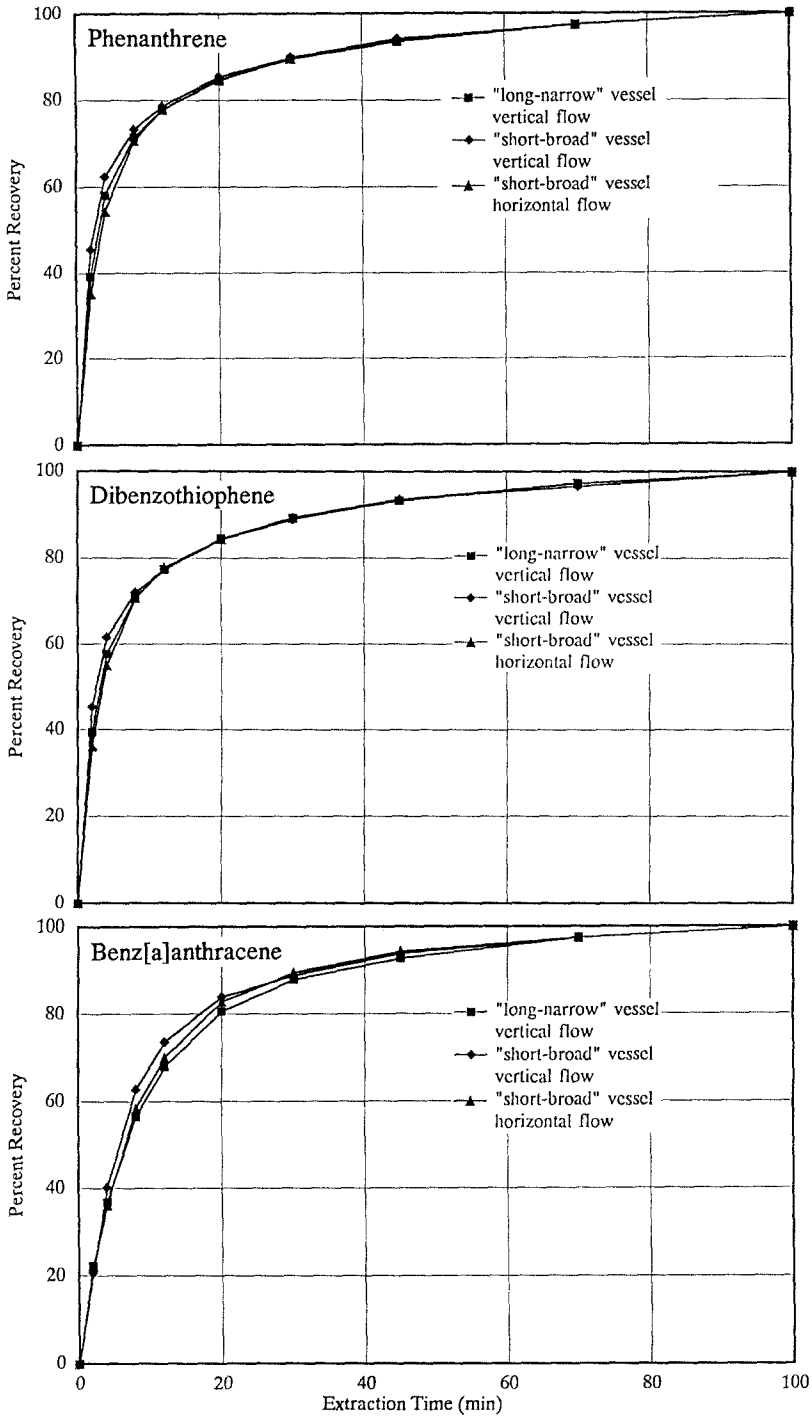

Fig. 2. SFE rates of native (not spiked) phenanthrene (top), dibenzothiophene (middle) and benz[*a*]anthracene (bottom) from 3 g of railroad bed soil using a "long, narrow" extraction vessel (132 mm × 5 mm I.D.) with vertical flow, a "short, broad" extraction vessel (33 mm × 10 mm I.D.) with vertical flow and a "short, broad" extraction vessel with horizontal flow. A 100% recovery was based on 100 min of SFE with carbon dioxide.

α-pinene showed losses of only 10% whereas at 1.2 ml/min the losses were 15%. Similarly, the least volatile compound, santonin, had losses of 5% at 0.3 ml/min and 7% at 1.2 ml/min. Whereas, as discussed earlier, high flow-rates coupled with long extraction times were responsible for volatile analyte losses due to purging (*i.e.*, the losses occurred after the analytes were dissolved in the collection solvent as shown in Table III), these results demonstrate that trapping efficiencies were relatively independent of flow-rate.

Effect of extraction cell geometry, flow-rate and cell orientation on supercritical fluid extraction rates from real samples

The effect of extraction cell geometry and orientation on SFE rates (extraction efficiency *versus* extraction time) was investigated using two extraction cells with different dimensions (132 mm × 5 mm I.D. and 33 mm × 10 mm I.D.) but the same internal volume (2.5 ml). The extraction rate curves (400 atm, 50°C) for native PAHs ranging from 2-methylnaphthalene to benzo[*a*]pyrene and heteroatom-containing PAHs such as dibenzofuran and dibenzothiophene from 3 g of railroad bed soil, and for monoterpenes, oxygenated monoterpenes and sesquiterpenes (α-pinene, nerol, limonene and $C_{15}H_{24}$) from 1-g samples of lemon peel were determined for a 100-min extraction period. Although a previous study reported that chromatographic retention of PAHs spiked on a sorbent was increased when using a "long, narrow" vessel instead of a "short, broad" vessel [16], we observed no significant differences in extraction rates from real samples. As shown in Fig. 2 for phenanthrene, dibenzothiophene and benz[*a*]anthracene and in Fig. 3 for limonene and nerol, extraction cell geometry and orientation (horizontal *versus* vertical) had virtually no effect on SFE rates of native PAHs and flavor and fragrance compounds from the railroad bed soil and the lemon peel.

The effect of supercritical fluid flow-rate on the extraction rates of native (not spiked) PAHs and heteroatom-containing PAHs from 3-g samples of railroad bed soil was also investigated at supercritical fluid flow-rates ranging from 0.15 to 1.2 ml/min using the 2.5-ml "long, narrow" extraction cell. As shown in Fig. 4 for phenanthrene and dibenzothiophene, flow-rates from 0.3 to 1.2 ml/min did not have an appreciable effect on the extraction rates of

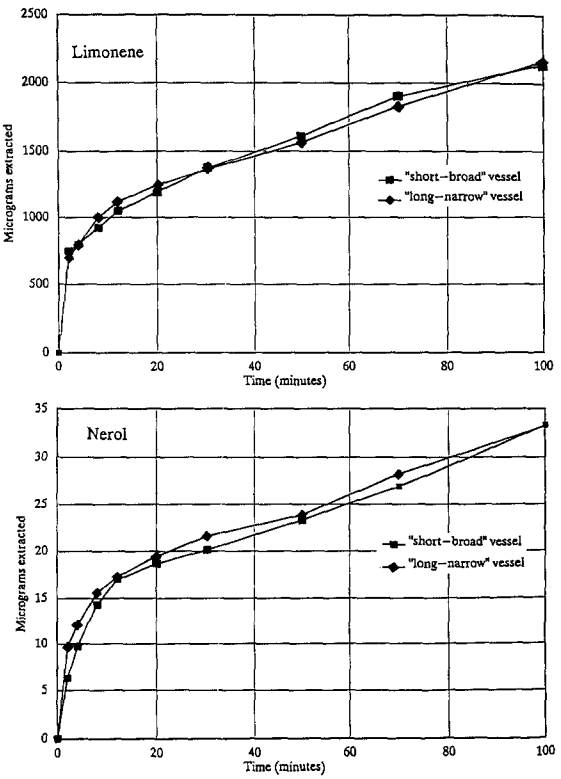

Fig. 3. SFE rates of limonene (top) and nerol (bottom) from 1 g of lemon peel using a "short, broad" extraction vessel (33 mm × 10 mm I.D.) and a "long, narrow" extraction vessel (132 mm × 5 mm I.D.).

these PAHs from the 3-g samples. When the flow-rate was reduced to 0.15 ml/min, the recovery rates were significantly slower, as would be expected by dead volume considerations since *ca.* 6–7 min would be required to sweep 1 void volume (estimated to be *ca.* 50% of the total internal volume of the extraction vessel) of the extraction cell. As reported by Bartle *et al.* [17], extraction rates of PAHs from soil show kinetic limitations that can mimic a diffusion-controlled process of the analytes in the sample matrix. As the SFE rate for this sample is probably limited by another rate-controlling mechanism (*e.g.*, analyte–matrix–supercritical fluid interactions), increasing the supercritical fluid flow-rate might not be expected to have a large effect on the extraction kinetics unless the supercritical fluid becomes saturated with analytes (*e.g.*, SFE of fats from meats [3]). For the railroad bed soil sample (and many environ-

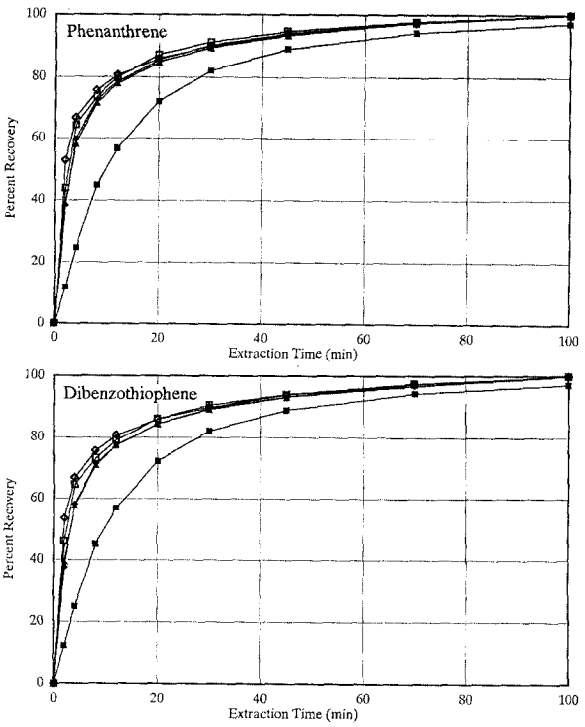

Fig. 4. Effect of supercritical fluid flow-rate on the extraction rates of phenanthrene (top) and dibenzothiophene (bottom) from 3 g of railroad bed soil. A 100% recovery was based on 100 min of SFE with carbon dioxide. Flow-rates (ml/min): ■ = 0.15; ◆ = 0.3; ▲ = 0.6; □ = 0.9; ◇ = 1.2.

mental samples) the concentration of the analytes present on the sample matrix is well below their saturation solubility limit in supercritical carbon dioxide. For example, the saturation concentration of phenanthrene in carbon dioxide is *ca.* 13 mg/ml (400 atm, 50°C [18], but the total amount of phenanthrene present on the railroad bed soil (3 g) was only *ca.* 100 μg (based on 100 min of SFE with carbon dioxide at 400 atm and 50°C).

CONCLUSIONS

Improper solvent trapping conditions for analytes that have been extracted using SFE can result in losses which may be wrongly attributed to poor SFE extraction efficiencies. Proper choice of collection solvent and temperature were most important for obtaining good collection efficiencies of semivolatile pollutants and flavor and fragrance compounds, whereas solvent volume and height had minimal effect. Quantitative (90–104%) collection efficiencies of all 66 test compounds were achieved when methylene chloride collection solvent was placed in a temperature-controlled block set at 5°C, a procedure that also prevented restrictor plugging while extracting wet samples. Excessively long extraction times and high supercritical fluid flow-rates can cause losses of highly volatile analytes from the collection solvent because of purging. Extraction cell geometry and orientation had negligible effects on the extraction rates of native analytes, as demonstrated by the extraction of PAHs from railroad bed soil and flavor and fragrance compounds from lemon peel. The flow-rate of the supercritical fluid was also found to have negligible effects on the extraction rates of PAHs from railroad bed soil as long as it was sufficient to sweep the void volume of the extraction vessel in a reasonable time period.

ACKNOWLEDGEMENTS

The partial financial support of the New Jersey Department of Environmental Protection (Division of Science and Research) is gratefully acknowledged. The authors also thank ISCO (Lincoln, NE, USA) for instrument loans.

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