Supercritical fluid extraction of polycyclic aromatic hydrocarbons from a marine sediment and analyte collection via liquid–solid trapping

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ABSTRACT

A standard reference marine sediment (SRM HS-3) certified for polycyclic aromatic hydrocarbons (PAHs) was extracted with pure and toluene-modified supercritical carbon dioxide at various temperatures while the pressure was maintained at 400 atm (1 atm = 101 325 Pa). The best results were achieved with toluene-modified carbon dioxide at 140°C. Similar results were obtained when carbon dioxide was replaced with nitrous oxide. Analyte collection after off-line supercritical fluid extraction (SFE) was carried out with liquid–solid traps whereby analyte losses due to aerosol formation during the depressurization of the supercritical fluid were reduced. As the liquid–solid traps were developed from conventional clean-up systems, further clean-up steps became unnecessary in this study. Such traps yielded better PAH recoveries than SFE with analyte trapping in pure organic solvents. The results of the optimized procedure were compared with the certified PAH values, with the results obtained by Soxhlet extraction with toluene and *n*-hexane–acetone and with published SFE results. With SFE instead of conventional Soxhlet extraction, the complete time required for the extraction, evaporation and clean-up steps can be reduced from *ca.* 27 h to *ca.* 3 h per sample.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants emitted from numerous natural and anthropogenic sources (*e.g.*, traffic, industrial processes) [1]. As PAHs comprise the largest class of known chemiʼal carcinogens, the rapid and precise determination of these compounds in different matrices is very important. PAHs are usually recovered from soil by conventional liquid solvent extraction techniques (*e.g.*, Soxhlet extraction), although these methods are time consuming and require large amounts of organic solvents. Compared with these methods, extraction times can be reduced from hours to minutes using supercritical fluid extraction (SFE) with physiologically harmless carbon dioxide [2]. In this study, PAH extractions from a marine sediment were performed with supercritical fluids in order to determine the applicability of this technique and to improve critical steps such as analyte collection and clean-up.

The optimum extraction temperature was determined by extracting the marine sediment at different temperatures with carbon dioxide while the pressure was maintained at 400 atm. Organic modifiers can increase the extraction efficiency by reducing the affinity of the analytes for

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sorptive sites of the matrix and by increasing their solubility in the supercritical fluid [3]. Toluene was chosen as a modifier because it yielded better results than methanol, acetone and n-hexane in a previous study [4].

Further increases in extraction efficiency may be obtained by using the slightly more polar nitrous oxide (N_2O) instead of CO_2 [5]. As nitrous oxide is a strong oxidant, the extraction of large amounts of organic and easily oxidizable material, particularly at elevated temperatures, should be avoided [6], so that most of the analytical SFE applications are still performed with CO_2. Nevertheless, the optimized procedure was also carried out with toluene-modified nitrous oxide at different temperatures.

Poor analyte recoveries are usually attributed to poor extraction efficiencies, although they may also be caused by incomplete analyte collection after an off-line SFE step [6–8]. The conventional collection method is depressurization of the supercritical fluid into an organic solvent. In this case, analyte losses due to aerosol formation with the expanding gas may occur [6]. Analyte collection on sorbent resins such as Tenax, C_{18}, silica gel and XAD traps has been reported [8], but the use of modifiers may influence the adsorption of the analytes or even wash them out [9]. The recently developed liquid–solid trapping method [4] allows the use of modified fluids, because washed out analytes are collected in the solvent.

PAHs are normally present in low concentrations in sample matrices spanning a wide polarity range [10]. These materials may interfere with the high-performance liquid chromatographic (HPLC) determination by causing a loss of separation reproducibility and contamination of the columns. Owing to these problems, a clean-up of the extracts is necessary but also very critical for the accurate quantification of the analytes because losses may occur if the rinse solvent is not able to overcome the solute–stationary phase interactions [7].

The aim of this work was to reduce analyte losses during analyte collection and to develop a method that combines analyte collection and clean-up in one step. Several clean-up systems were used as liquid–solid traps and the results were compared with those we had obtained by conventional analyte trapping in pure organic solvents.

Finally, the results of the optimized procedure were compared with the certified values and with the published results [11].

EXPERIMENTAL

Samples and standards

All solvents were purchased from Merck (Darmstadt, Germany) in the highest purity available. Standards of individual PAHs were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The certified reference materials NIST SRM 1647b (sixteen PAHs in acetonitrile) (NIST, Gaithersburg, MD, USA) and the marine sediment HS-3 (National Research Council of Canada) were purchased from Promochem (Wesel, Germany).

The marine sediment (10 g) was treated with 1 M hydrochloric acid (20 ml) for 1 h, washed with distilled water (50 ml) and dried in air prior to SFE [4,12]. The aim of this pretreatment step was to reduce interactions between the analytes and the sorptive sites of the sediment. It increased the extraction efficiency by up to 20% for the PAHs investigated. For Soxhlet extractions the marine sediment was used as received because a conventional method should be employed. However, a wet sample would have to be dried only once, either prior to Soxhlet extraction or for SFE after the hydrochloric acid treatment step.

Silica gel (63–200 μm) (Merck) was heated at 500°C for 12 h and cooled in a desiccator to room temperature before addition of 3% (w/w) of water. Florisil (for residue analysis, 150–250 μm) (Merck) and alumina (neutral, for chromatography) (Baker, Gross-Gerau, Germany) were used as received.

Extraction of PAHs from marine sediment HS-3

Soxhlet extractions. A 70-ml Soxhlet extractor with 100 × 25 mm I.D. extraction thimbles and a 100-ml round-bottomed flask was used for all extractions. For each extraction 1.5 g of the sediment was mixed with 0.2 g of sodium sulphate and extracted with 80 ml of either toluene

or n-hexane–acetone (1:1, v/v) for 24 h in the dark. The extracts were concentrated at elevated temperatures (35–50°C) in a rotary evaporator to a volume of ca. 1 ml. Light petroleum (b.p. 40–60°C) (10 ml) was added and the solution was cleaned up as described below (clean-up procedure 1). Diethyl phthalate (50 μl) was added to the eluate prior to evaporation of the solvent under a gentle stream of nitrogen in order to prevent evaporation to dryness [13]. After dilution with 15 ml of acetonitrile, the extracts were analysed by HPLC.

Supercritical fluid extractions. All supercritical fluid extractions were performed with an SFE-703 supercritical fluid extraction system (Dionex, Idstein, Germany). The extraction cells (3.5 ml; 5 cm × 9.4 mm I.D.) were filled with silanized glass-fiber wadding (Macherey–Nagel, Düren, Germany) and 0.35 g of the marine sediment. Copper granules (2 g) were placed at the outlet end of the extraction cells in order to avoid restrictor plugging, which may be caused by sulphurous species that are present in marine sediments [4,7]. To modify CO_2, toluene (1 ml) was added to the sample and the loaded cell was heated at 120°C in the extraction oven for 15 min prior to dynamic extraction with pure carbon dioxide or nitrous oxide [4]. The cell pressure was set to 400 atm (1 atm = 101 325 Pa) and extractions were carried out at 60, 100 and 140°C for 75 min. A flow-rate of ca. 400 ml/min of gaseous CO_2 was measured after depressurization.

To prevent restrictor plugging due to frozen CO_2, the restrictor was heated to 150°C and a "dual-chamber trapping vial" [7] was used for analyte collection (Fig. 1a). This vial design prevents the heated restrictor from dipping into the analyte collection solvent (15 ml of either light petroleum or n-hexane placed in 5.5 × 2.5 cm I.D. vials), whereby evaporation losses were reduced. A transfer tube (Fig. 1a) was necessary to guide the expanded gas through the collection solvent, which was kept at ca. 3°C. For the new liquid–solid traps, the diameter of the transfer tube was reduced on the side that dips into the solvent before it was filled with silanized glass-fiber wadding and the dry solid sorbent as shown in Fig. 1b.
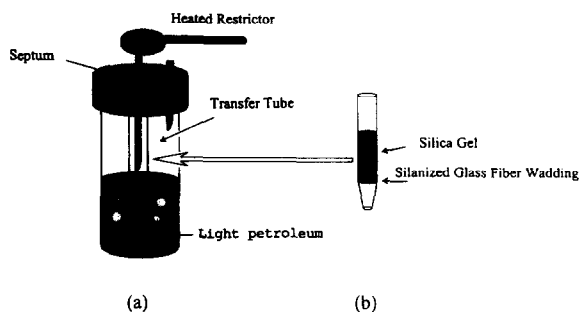

Fig. 1. Diagram of (a) the dual-chamber trapping vial and (b) the recently developed collection column, which is a modified transfer tube that is used with the dual-chamber trapping vial.

Clean-up

All clean-ups were carried out with "collection columns" that were prepared as described in Fig. 1b. The PAH recovery after the individual clean-up procedures was investigated by diluting 100 μl of the certified PAH solution SRM 1647b with 10 ml of light petroleum (clean-up procedure 1) or n-hexane (clean-up procedures 2–4) and transferring this solution directly on to the collection column. The sample flasks were rinsed with small amounts of the solvent (either light petroleum or n-hexane). For the SFE studies, the extracts were used as received from the analyte collection and handled like the standard solutions for the clean-up recovery study. The following clean-up procedures were tested.

Clean-up 1: silica gel–light petroleum–toluene. The columns were filled with 0.3 g of silica gel and washed with light petroleum (2 ml). Analyte collection during SFE and dilution of SRM 1647b for the clean-up recovery study were performed with light petroleum. PAHs were eluted with 3 ml of light petroleum–toluene (3:1, v/v) [4,14].

Clean-up 2: silica gel–n-hexane–dichloromethane. The columns were filled with 0.3 g of silica gel and washed with dichloromethane (1 ml) followed by n-hexane (1 ml). n-Hexane was used for analyte collection and dilution of SRM 1647b. PAHs were eluted with 3 ml of n-hexane–dichloromethane (9:1, v/v) [15].

Clean-up 3: Florisil–n-hexane–dichloromethane. The column was filled with 0.3 g of Florisil and washed with dichloromethane (1 ml) fol-

lowed by n-hexane (1 ml). n-Hexane was used for analyte collection and dilution of SRM 1647b. PAHs were eluted with 3 ml of n-hexane–dichloromethane (1:1, v/v) [15].

Clean-up 4: Silica gel–alumina–n-hexane–di-chloromethane. The column was filled with 0.2 g of silica gel (bottom) and 0.15 g of alumina (top). It was washed with dichloromethane (1 ml) followed by n-hexane (1 ml). n-Hexane was used for analyte collection and dilution of SRM 1647b. PAHs were eluted with 3 ml of n-hexane–dichloromethane (3:2, v/v) [16].

To minimize evaporation losses, 50 μl of diethyl phthalate were added to each extract prior to evaporation of the solvent under a gentle stream of nitrogen. Acetonitrile (1 ml for recovery studies, 5 ml of SFE studies) was added and the extracts were analysed by HPLC [4].

HPLC analysis

For HPLC analysis, an HP Series 1050 liquid chromatograph (Hewlett-Packard) with a programmable variable-wavelength UV detector and HP ChemStation software for data analysis was used. The following wavelength programme was used: 220 nm, naphthalene, acenaphthylene, acenaphthene, fluorene; 248 nm, phenanthrene, anthracene; 234 nm, fluoranthene, pyrene; 270 nm, benz[a]anthracene, chrysene; 250 nm, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene; 296 nm, benzo[ghi]perylene, dibenz-[a,h]anthracene, indeno[1,2,3-cd]pyrene [17]. Continuous degassing of the mobile phase was achieved with an HP Series 1050 on-line degassing system. The following acetonitrile (ACN)–water gradient was used as mobile phase: 0–6 min, 50% ACN; 35 min, 100% ACN (held until 45 min); 46 min, 50% ACN. The C_{18} HPLC column (Bakerbond Wide Pore octadecyl C_{18}, 250 × 4.6 μm I.D., 5 μm, 300 Å) (Baker) was heated to 30°C and initially equilibrated with acetonitrile–water (1:1, v/v) for 15 min prior to each run. The total flow-rate was set to 1 ml/min.

For identification of chromatographic peaks, retention times and UV spectra were compared with those of reference PAH compounds.

RESULTS AND DISCUSSION

In a first step, the PAH recoveries after different clean-up procedures were investigated using known amounts of the PAH standard solution SRM 1647b (certified for sixteen PAHs) as described above. The results (Table I) indicate that none of the clean-up procedures achieved a quantitative recovery for the most volatile PAHs (naphthalene, acenaphthylene and acenaphthene). As these components are to some extent soluble in water [18], losses may also occur during the pretreatment with hydrochloric acid and it was therefore not possible to determine these PAHs in this study. In contrast, the clean-up procedures 1, 2 and 4 achieved almost quantitative recoveries for PAHs with higher molecular masses. Only the Florisil–n-hexane–dichloromethane procedure (No. 3) obtained neither quantitative recoveries nor reproducible results.

Fig. 2 shows an HPLC chromatogram of an extract from SRM HS-3. Under the HPLC conditions described above, the dibenz[a,h]anth-racene–benzo[ghi]perylene pair as well as chrysene and acenaphthene were not separated from co-eluted substances so that their accurate determination was impossible with a UV detector. Better results may be obtained by using the more sensitive fluorescence detection which, however, was not available for this study. Because of this, only the following ten PAHs were determined in the marine sediment HS-3 in this study: fluorene (Fluo), phenanthrene (Phen), anthracene (Anth), fluoranthene (F), pyrene (Py), benz[a]anthracene (B[a]A), benzo-[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P) and indeno[1,2,3-cd]pyrene (I[cd]P).

To determine the optimum extraction temperature, the sediment was extracted at 400 atm and temperatures of 60, 100 and 140°C (fluid density $d = 0.89$, 0.76 and 0.64 g/cm^3) for 75 min with pure carbon dioxide. Analyte collection was carried out with light petroleum–silica gel traps. The results are presented in Fig. 3. SFE was less efficient than Soxhlet extraction with n-hexane–acetone for PAHs with higher molecu-

TABLE I

PAH RECOVERIES AFTER DIFFERENT CLEAN-UP PROCEDURES

A detailed description of each procedure is given in the text.

PAH	$\bar{x} \pm \sigma$ (%)			
	Clean-up 1: silica gel– light petroleum	Clean-up 2: silica gel– n-hexane– dichloromethane	Clean-up 3: Florisil– n-hexane– dichloromethane	Clean-up 4: silica gel– alumina– n-hexane– dichloromethane
Naphthalene	–	2.4 ± 0.1	2.3 ± 0.2	3.0 ± 0.4
Acenaphthylene	–	58.9 ± 2.6	45 ± 15	44 ± 31
Acenaphthene	40 ± 36	72.5 ± 1.8	59 ± 13	65 ± 27
Fluorene	63 ± 30	94.7 ± 0.2	74 ± 13	67 ± 9
Phenanthrene	103 ± 4	106.0 ± 1.7	101 ± 1	102 ± 3
Anthracene	97 ± 1	97.5 ± 2.7	94 ± 1	95 ± 5
Fluoranthene	103 ± 2	101.7 ± 0.3	97 ± 3	101.7 ± 0.2
Pyrene	103 ± 2	102.4 ± 0.4	98 ± 2	102.1 ± 0.5
Benz[a]anthracene	104 ± 2	103.0 ± 0.4	94 ± 9	102.5 ± 0.4
Chrysene	103 ± 2	102.9 ± 0.3	91 ± 13	100.0 ± 2.1
Benzo[b]fluoranthene	103 ± 2	103.0 ± 0.6	85 ± 19	103.0 ± 0.4
Benzo[k]fluoranthene	98 ± 5	100.4 ± 0.6	83 ± 26	97.9 ± 1.6
Benzo[a]pyrene	106 ± 5	105.0 ± 2.0	99 ± 20	90.1 ± 0.5
Dibenz[a,h]anthracene	100 ± 1	97.0 ± 0.9	68 ± 46	97.8 ± 0.6
Benzo[ghi]perylene	103 ± 2	100.8 ± 1.1	77 ± 37	102.1 ± 0.8
Indeno[1,2,3-cd]pyrene	102 ± 2	100.3 ± 0.7	74 ± 40	101.7 ± 0.9

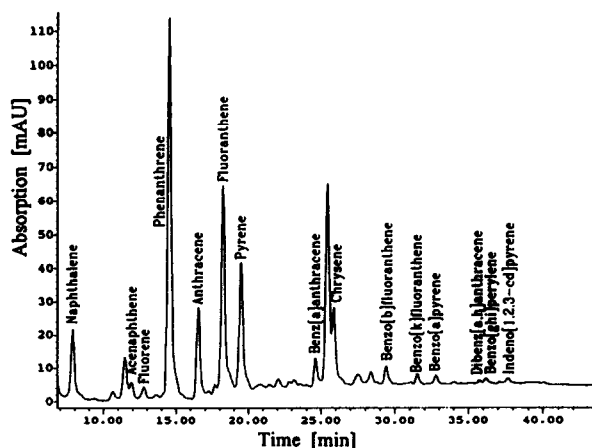

Fig. 2. Chromatogram of an extract of HS-3. Chromatographic conditions are described in the text.

lar masses (B[b]F, B[k]F, B[a]P, I[cd]P), but this discrepancy decreased with increasing temperature. The best results for these PAHs were obtained at 140°C, although the density and solvent strength of supercritical fluids decrease with increasing temperature if the pressure is held constant. This observation may be a consequence of thermal desorption effects and of increasing solute diffusivities and vapour pressures [5,6]. For PAHs with lower molecular masses, the extraction efficiency was less affected by temperature. The best results were obtained at 100°C. Both thermal desorption effects and increasing solvent strength (compared with extractions at 140°C) seem to be important in this case.

As a modifier can improve the solubility of PAHs in supercritical CO_2 and also the competition for the active sites of the sample, 1 ml of toluene was added directly to each sample. Static
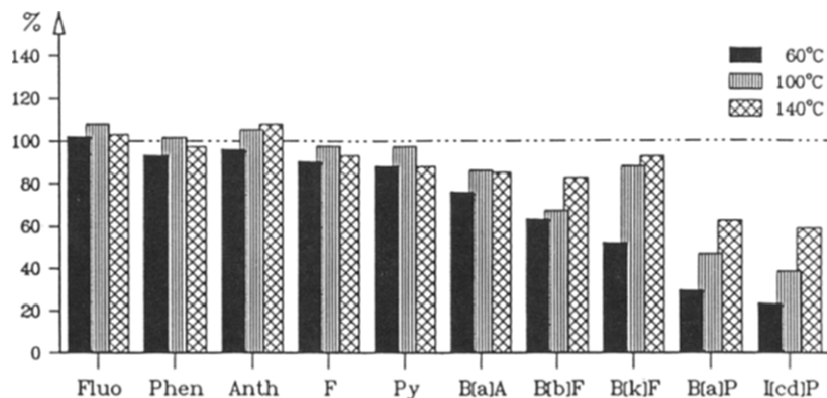
Fig. 3. Results of SFEs performed at different temperatures with pure CO_2. Fluo = fluorene; Phen = phenanthrene; Anth = anthracene; F = fluoranthene, Py = pyrene; B[a]A = benz[a]anthracene; B[b]F = benzo[b]fluoranthene; B[k]F = benzo[k]fluoranthene; B[a]P = benzo[a]pyrene; I[cd]P = indeno[1,2,3-cd]pyrene. The results are presented in comparison with those of the conventional Soxhlet extraction with n-hexane–acetone (100% line). Each extraction was performed in triplicate at 400 atm for 75 min.

equilibration of the system was obtained by maintaining the loaded extraction cells at 120°C for 15 min prior to dynamic extractions that were carried out at 400 atm and 100 or 140°C for 75 min. The best results were obtained at 140°C (Fig. 4). Compared with SFE with pure CO_2, the discrepancy between the SFE and Soxhlet extraction results decreased and for most of the PAHs, SFE with toluene-modified CO_2 yielded even better results than the conventional method. However, the extraction efficiency of SFE was less than that from Soxhlet extraction for PAHs with higher molecular masses.

Replacing CO_2 with the more polar N_2O

should increase the SFE efficiency [2]. Because of this, extractions were performed at 100 and 140°C with toluene-modified nitrous oxide. In contrast to SFEs with toluene-modified CO_2, extractions at 100°C in most instances yielded results comparable to or even better than those which were obtained at 140°C. For safety reasons, subsequent extractions were performed with toluene-modified CO_2, as the results (Fig. 4) did not differ significantly from those obtained with CO_2 at 140°C.

To evaluate the influence of the liquid–solid trap on the collection efficiency, SFEs were performed with the following collection systems:
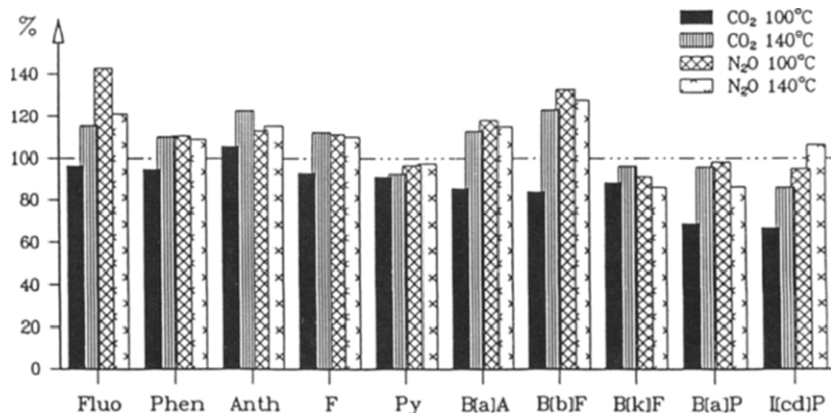

Fig. 4. Results of SFEs with toluene-modified carbon dioxide and nitrous oxide. Abbreviations as in Fig. 3. The results are presented in comparison with those of the conventional Soxhlet extraction with n-hexane–acetone (100% line). SFE parameters: $V_{toluene} = 1$ ml; $T_{0(equilibration)} = 120°C$; $t_0 = 15$ min; $p = 400$ atm; $t = 75$ min. Analyte collection was performed with light petroleum–silica gel traps. Each extraction was performed in triplicate.

TABLE II

COMPARISON BETWEEN ANALYTE COLLECTION VIA LIQUID–SOLID TRAPPING AND COLLECTION IN PURE ORGANIC SOLVENTS

Systems: 1 = pure n-hexane; 2 = silica gel–n-hexane–dichloromethane; 3 = silica gel–alumina–n-hexane–dichloromethane; 4 = Florisil–n-hexane–dichloromethane; 5 = pure light petroleum; 6 = silica gel–light petroleum–toluene. SFE parameters: $V_{toluene}$ = 1 ml; $T_{0(equilibration)}$ = 120°C; t_0 = 15 min; $T_{extraction}$ = 140°C; p = 400 atm; t = 75 min. Each extraction was performed in triplicate.

PAH	$\bar{x} \pm \sigma$ (μg/g)					
	System 1	System 2	System 3	System 4	System 5	System 6
Fluorene	6.3 ± 0.5	7.1 ± 0.3	6.8 ± 0.5	6.8 ± 0.8	6.1 ± 0.5	7.2 ± 0.6
Phenanthrene	74.8 ± 6.0	87.3 ± 1.1	84.5 ± 1.7	82.9 ± 6.4	75.7 ± 1.7	87.7 ± 1.7
Anthracene	8.7 ± 0.9	9.9 ± 0.3	9.8 ± 0.5	9.8 ± 0.8	8.9 ± 0.2	9.7 ± 0.1
Fluoranthene	58.2 ± 4.3	69.0 ± 1.2	67.9 ± 2.2	67.2 ± 3.8	60.1 ± 1.7	70.7 ± 1.2
Pyrene	37.7 ± 3.1	44.4 ± 1.5	42.6 ± 1.5	42.1 ± 3.7	38.2 ± 1.6	43.3 ± 1.5
Benz[a]anthracene	9.7 ± 1.9	10.4 ± 0.5	10.0 ± 0.4	9.5 ± 0.7	9.0 ± 0.5	10.4 ± 0.3
Benzo[b]fluoranthene	7.4 ± 1.0	9.0 ± 0.9	7.2 ± 0.7	7.7 ± 0.9	7.2 ± 0.6	7.6 ± 0.4
Benzo[k]fluoranthene	2.9 ± 0.3	3.2 ± 0.3	2.5 ± 0.3	3.1 ± 0.3	3.4 ± 0.3	3.7 ± 0.4
Benzo[a]pyrene	3.8 ± 0.3	4.1 ± 0.4	4.0 ± 0.2	4.5 ± 1.4	4.2 ± 0.7	4.6 ± 0.7
Indeno[1,2,3-cd]pyrene	2.6 ± 0.5	3.4 ± 0.4	2.4 ± 0.7	2.6 ± 0.5	3.0 ± 1.0	3.3 ± 0.4

(1) pure n-hexane; (2) silica gel–n-hexane; (3) silica gel–alumina–n-hexane; (4) Florisil–n-hexane; (5) pure light petroleum; (6) light petroleum–silica gel. Further treatment of the collection columns and solvents was carried out as described above (see clean-up procedures). Solvent exchange steps were not necessary as the solvent chosen for the clean-up (light petroleum or n-hexane) was already used as the collection solvent. Following procedures (1) and (5), clean-up was performed on silica gel with either n-hexane–dichloromethane (clean-up procedure 2) or light petroleum–toluene (clean-up procedure 1). With the liquid–solid traps, efficient analyte collection and clean-up were obtained in one step.

The results are presented in Table II. Analyte collection in pure n-hexane and light petroleum yielded similar results but these conventional methods were up to 50% less efficient than collection with any of the liquid–solid combinations. Langenfeld et al. [8] have reported that analyte collection in pure acetone yielded better results than pure n-hexane. However, we were not able to confirm this observation in a previous study [4]. The best recoveries were obtained with the light petroleum–silica gel traps. These results indicate that evaporation losses strongly influence the collection efficiency and that poor recoveries should not only be attributed to poor extraction efficiencies.

If our results are compared with those of other workers (Table III), the procedure presented here was more efficient for extractions of PAHs from marine sediment HS-3. The certified values and our SFE and Soxhlet extraction results are given in Table IV. There is no significant difference between SFE with toluene-modified fluids (CO_2 or N_2O) and conventional Soxhlet extraction with either n-hexane–acetone or toluene, but the certified values were not achieved for all compounds. However, these results are difficult to interpret because different methods may yield varying results when applied to one sample [19], and no information about the analytical procedure used for certification of HS-3 was available.

CONCLUSIONS

PAHs were extracted with supercritical carbon dioxide and nitrous oxide from the marine sediment HS-3 which was pretreated with hydrochloric acid in order to reduce interactions with the

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TABLE III

COMPARISON OF RESULTS WITH THOSE OBTAINED BY OTHER WORKERS

PAH	Concentration (μg/g)			
	SFE with pure CO_2		SFE with modified CO_2	
	This work[a]	Literature [11][b]	This work[c]	Literature [11][d]
Fluorene	6.4	5.9	7.2	4.8
Phenanthrene	77.5	46.5	87.7	52.0
Anthracene	10.2	5.0	12.0	7.0
Fluoranthene	58.6	31.5	70.7	45.0
Pyrene	41.0	18.5	43.3	25.0
Benzo[a]anthracene	7.8	5.9	10.4	10.0
Benzo[b]fluoranthene	5.0	2.4	7.6	5.2
Benzo[k]fluoranthene	3.5	2.6	3.7	5.7
Benzo[a]pyrene	3.2	1.1	4.6	3.7
Indeno[1,2,3-cd]pyrene	1.9	0.4	3.3	2.0

[a] $T = 140$°C; $p = 400$ atm; $t = 75$ min; HS-3 was pretreated with hydrochloric acid as described and extracted in triplicate with pure CO_2.
[b] $T = 70$°C; $p = 400$ atm; $t = 25$ min; HS-3 was extracted in duplicate.
[c] $T = 140$°C; $p = 400$ atm; $t = 75$ min; HS-3 was pretreated with hydrochloric acid and extracted in triplicate with CO_2 that was modified with 1 ml of toluene.
[d] CO_2 was modified with 10% of methanol.

matrix. The extraction temperature strongly influences the SFE efficiency and the best results were obtained with toluene-modified fluids at 140°C.

For safety reasons, CO_2 was used for most of the experiments in this study, especially as the use of nitrous oxide did not increase the extraction efficiency as much as described by other

TABLE IV

RESULTS OF THE OPTIMIZED SFE PROCEDURE AND THE SOXHLET EXTRACTIONS

PAH	$\bar{x} \pm \sigma$ (μg/g)				
	Certified values	SFE with toluene-modified CO_2[a]	SFE with toluene-modified N_2O[a]	Soxhlet extraction with toluene	Soxhlet extraction with n-hexane–acetone
Fluorene	13.6 ± 3.1	7.2 ± 0.6	7.5 ± 0.5	6.6 ± 0.2	6.2 ± 1.0
Phenanthrene	85 ± 20	88 ± 2	87 ± 5	78.4 ± 1.5	79.6 ± 0.5
Anthracene	13.4 ± 0.5	9.7 ± 0.1	9.1 ± 0.6	9.4 ± 0.7	7.9 ± 0.5
Fluoranthene	60 ± 9	71 ± 1	69 ± 4	62 ± 2	63 ± 1
Pyrene	39 ± 9	43 ± 2	46 ± 2	48 ± 2	47 ± 0.3
Benz[a]anthracene	14.6 ± 2.0	10.4 ± 0.3	10.6 ± 0.8	9.0 ± 0.3	9.2 ± 0.3
Benzo[b]fluoranthene	7.7 ± 1.2	7.6 ± 0.4	7.9 ± 0.6	9.1 ± 0.3	6.2 ± 0.2
Benzo[k]fluoranthene	2.8 ± 2.0	3.7 ± 0.4	3.3 ± 0.3	3.2 ± 0.1	3.8 ± 0.5
Benzo[a]pyrene	7.4 ± 3.6	4.6 ± 0.7	4.1 ± 0.4	5.8 ± 0.3	4.8 ± 0.4
Indeno[1,2,3-cd]pyrene	5.4 ± 1.3	3.3 ± 0.4	4.0 ± 0.4	4.2 ± 0.5	3.8 ± 0.1

[a] SFE parameters: $V_{toluene} = 1$ ml; $T_{0(equilibration)} = 120$°C; $t_0 = 15$ min; $T_{extraction} = 140$°C; $p = 400$ atm; $t = 75$ min. Each extraction was performed in triplicate.

workers. The loaded extraction cells were heated at 120°C for 15 min prior to extraction at 400 atm and 140°C for 75 min. Collection of the extracted analytes was carried out with liquid–solid traps (mostly with light petroleum–silica gel traps), which were more efficient than analyte collection in pure solvents, a fact which confirms that poor analyte recoveries are often caused by incomplete analyte collection due to aerosol losses. Further clean-up steps were unnecessary because a clean-up system was already used for the analyte collection. The advantage of the liquid–solid traps over analyte trapping via solid sorbents was their applicability for extractions with modified carbon dioxide, because washed out analytes are collected in the solvent. The results of our SFE procedure did not differ significantly from those of the conventional Soxhlet extraction with either toluene or n-hexane–acetone, but none of these methods reached the certified values for all compounds.

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