# Application of experimental design approach to the optimization of supercritical fluid extraction of polychlorinated biphenyls and polycyclic aromatic hydrocarbons

Inma Fernández, Jordi Dachs, Josep M. Bayona*

*Department of Enviromental Chemistry, CID-CSIC, Jordi Girona Salgado 18-26, E-08034 Barcelona, Spain*

## Abstract

Extraction variables (pressure and temperature) were optimized by using a central composite experimental design strategy for the supercritical fluid extraction (SFE) of incurred polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from sediment using 100% $CO_2$ and $CO_2$ containing 2% and 10% (v/v) of MeOH. Under the optimum SFE extraction conditions, the recoveries for total PCBs and PAHs were higher than with Soxhlet extraction (15%). The lower precision obtained by SFE vs. Soxhlet extraction (R.S.D. = 9.0 and 3.7%, respectively) was accounted for by the higher number of chromatographic determinations, since SFE extracts were recovered in three fractions.

## 1. Introduction

There is great interest in the application of supercritical fluid extraction (SFE) to environmental analysis owing to the faster mass transfer and less solvent usage than with conventional methods of sample preparation [1-4]. Unfortunately, the selection of the operating conditions in SFE is still an area of active research that is characterized by much trial and error. At present there are few models that can predict a priori the optimum SFE conditions [5,6].

Although most of the reported SFE methods have been optimized by using one variable at a time, this approach assumes no interaction between variables, which can lead to biased results. The potential of statistical approaches combining experimental design with multilinear regression analysis has been illustrated in only a few applications of SFE to environmental samples. Lopez-Avila et al. [7] used a fractional experimental design for the determination of the main effects of seven variables on the extraction efficiency of organochlorine and organophosphorus pesticides. A similar approach has been pursued for the evaluation of several extraction variables for the recovery of organotin from soil and sediment [8,9]. By combining experimental design of a reduced number of variables with multilinear regression, optimum extraction conditions can be achieved [10,11]. This approach has not yet been applied to the SFE of naturally contaminated samples.

Supercritical carbon dioxide is less efficient than other more polar fluids (e.g., $N_2O$, $CHClF_2$) for the SFE of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from environmental samples

* Corresponding author.

[12–14]. Unfortunately, the oxidant properties of $N_2O$ restrict its application to matrices with a low organic matter content [15] and chlorodifluoromethane and other CFCs will soon be banned as a consequence of international agreements. Langenfeld et al. [16] have reported a significant improvement in the extraction of PCBs and PAHs from environmental samples at very high temperatures (200°C) which are not attainable with commercially available instrumentation. Furthermore, subcritical and supercritical water have shown some potential for the extraction of hydrophobic contaminants but their routine application requires dedicated instrumentation [17]. Accordingly, up to now, carbon dioxide modified with methanol or toluene [18–20] has been the best choice for the extraction of PCBs and PAHs from different matrices owing to their effectiveness. Methanol was chosen as a carbon dioxide modifier in this study because of its well known phase behaviour and lower boiling point than other modifiers previously reported (e.g., toluene).

The objectives of this work were (i) optimization of pressure and temperature for the extraction of PCBs and PAHs from a naturally contaminated sediment, (ii) to evaluate the possibility of fractionation according to chemical classes by increasing the fluid polarity and (iii) to compare precision and accuracy between Soxhlet extraction and SFE for the determination of PCBs and PAHs in sediment.

## 2. Experimental

### 2.1. Materials and reagents

A sediment sample was collected in the Rhône Estuary at a 25 m water column depth by using a Van Veen grab. The sample was immediately frozen at −20°C onboard, freeze-dried and sieved (120 μm). Pesticide-grade methanol, acetone, 25% hydrochloric acid and isooctane were obtained from Merck (Darmstadt, Germany), pesticide-grade n-hexane and dichloromethane from Baker (Deventer, Netherlands) and per-

deuterated pyrene from CIL (Woburn, MA, USA). Neutral alumina (70–230 mesh) was obtained from Merck and was Soxhlet extracted with dichloromethane–methanol (2:1) prior to use and activated at 120°C without any further treatment. Carbon dioxide (99.995%) was purchased from Liquid Carbonic Española (Barcelona, Spain). Standard PAHs and octachloronaphthalene were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and PCBs from Promochem (Wesel, Germany). Fused-silica tubing used as a linear restrictor was obtained from MicroQuartz (Munich, Germany).

### 2.2. Extraction procedures

#### SFE

A dual-syringe pump (SFC 3000; Fisons, Milan, Italy) was used for the delivery of carbon dioxide and modifier as described elsewhere [8]. A 1.4-g amount of freeze-dried sediment was transferred into a 1.67-ml extraction cell (Dionex, Sunnyvale, CA, USA). The restrictor temperature was held at 150°C. The flow-rate as liquid was adjusted by a fused-silica linear restrictor (10 cm × 50 μm I.D.) at ca. 1 ml/min measured from the LCD of the pump. The total volume of fluid used per extraction was 15 ml. The extract was collected in 10 ml of n-hexane at 0°C containing perdeuterated pyrene and octachloronaphthalene as internal standards (spiking level 250 and 5 ng, respectively). Three different extraction agents were used: (a) neat carbon dioxide (fraction I), (b) 2% (v/v) methanol in carbon dioxide (fraction II) and (c) 10% (v/v) methanol in carbon dioxide (fraction III).

#### Soxhlet

A 2-g amount of freeze-dried sediment were extracted with dichloromethane–methanol (2:1) for 20 h. The sample was spiked with the internal standards directly in the extraction thimble. The recovered extracts were concentrated to a small volume by rotary evaporation and to dryness under a gentle stream of nitrogen.

## 2.3. Analytical determinations

Whereas SFE extracts were analysed without any further clean-up, the Soxhlet extracts required column fractionation with 2 g of neutral alumina by using a 35 cm × 0.9 cm I.D. glass column. The following fractions were collected: (I) 6 ml of *n*-hexane (PCBs) and (II) 6 ml of hexane–dichloromethane (2:1) (PAHs). The PCB-containing fraction was treated with activated elemental copper to remove sulphur prior to determination by capillary gas chromatography with electron-capture detection (cGC–ECD).

### PCBs

PCBs were determined by cGC–ECD using a Hewlett-Packard Model 5890 instrument equipped with an autosampler (HP 7673A). The injection port and detector temperatures were held at 280 and 310°C, respectively. Injection was performed in the splitless mode by activating the purge valve after 35 s of injection. Helium and nitrogen were used as carrier (30 cm/s) and make-up gases (30 ml/min), respectively. The analytical column was DB-5 (30 m × 0.25 mm I.D.) with film thickness 0.25 μm. The column temperature was programmed from 60 to 130°C at 15°C/min and then to 300°C at 6°C/min, maintaining the final temperature for 15 min.

### PAHs

The determination of PAHs was accomplished by cGC–MS using a Fisons MD 800 instrument in the electron impact (EI) mode (70 eV). The injector, transfer line and ion source temperatures were held at 280, 280 and 200°C, respectively. The column temperature was programmed from 90 to 150°C at 15°C/min and then to 310°C at 4°C/min, holding the final temperature for 10 min. Scans were acquired from 50 to 500 u every 0.9 s. Other analytical conditions were similar to those described for PCB determination. Peak integration was performed by VG Lab-Base from the mass fragmentograms of the molecular ions (*m/z* 184, 178, 192, 198, 202, 206, 234, 228, 252, 276 and 278).

## 2.4. Quantification

The external standard method was used with the sixteen PAHs included in the EPA priority pollutant list and six PCB congeners (IUPAC Nos. 28, 52, 101, 153, 138 and 180) as calibrants. The results were corrected for recovery. Extraction blanks were obtained during the analytical procedure. Precision studies were performed with at least four independent replicates.

## 2.5. Statistical optimization

Pressure and temperature were optimized by using a hybrid experimental design for fraction I and the sum of fractions I, II and III. Fig. 1 shows the values of the variables used in the optimization. The parametric equation tested contains the following terms:

$$R = aP + bT + cP^2 + dT^2 + ePT + f \qquad (1)$$

where $R$ is the response (amount extracted), $a$, $b$, $c$, $d$ and $e$ are the parametric coefficients, $f$ is the intercept and $P$ is pressure and $T$ is temperature. Experiments were carried out in a random order for each mobile phase composition to minimize the bias effect (Fig. 1). Multilinear least-squares regression was used to calculate the parametric coefficients $a$, $b$, $c$, $d$ and $e$. Significance was determined by a *t*-test with a confidence level of 0.95 ($P < 0.005$).
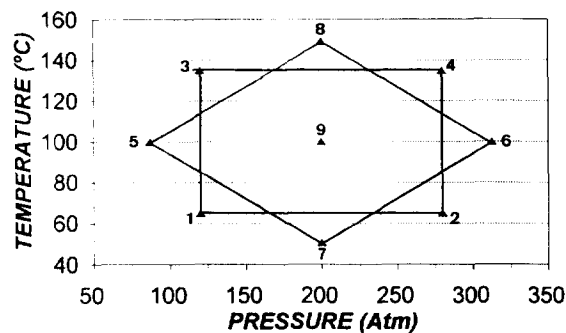


Fig. 1. Extraction conditions used in the experimental design. The experimental conditions are numbered according to the order in which they were applied in experiments.

## 3. Results and discussion

Quantitative SFE of environmental samples involves three steps: (i) partitioning of the analytes from the sample to the extraction fluid, (ii) removal from the extraction vessel and (iii) collection of the extracted analytes [21]. All of them must be optimized to obtain the highest extraction efficiency. The last step is the best understood and several collection techniques have proved to be effective for a broad variety of compounds and fluids [22,23]. Solvent collection with a large volume at low temperature was effective for the collection of volatile two- and three-ring PAHs [18]. Therefore, we used *n*-hexane as the collection solvent because it allows direct injection in GC and all the analytes of interest are soluble in it.

We focused on the optimization of the first and second steps for the SFE method development for PCBs and PAHs from a naturally contaminated marine sediment in order to consider the analyte–matrix interaction in the optimization process. The feasibility of chemical class fractionation was also evaluated by increasing the extractant agent polarity [0–10% (v/v) MeOH in $CO_2$]. Accordingly, three fractions of increasing polarity from 100% carbon dioxide to 10% MeOH were obtained in a sequential order. For every fraction a total of nine extraction con-

ditions of pressure and temperature were evaluated (Fig. 1).

### 3.1. Optimization of pressure and temperature

*PCBs*

The parametric coefficients of Eq. 1 calculated for every PCB congener with a high significance are listed in Table 1. Usually most of congeners exhibited high correlations ($R^2 = 62–92\%$), except those of lowest molecular mass (IUPAC Nos. 28 + 31 and 52), which are not reported. Taking into consideration the total of six congeners determined, a significant correlation was also found, evidencing the major contribution to the total mass of the highest molecular mass components. It is interesting that temperature was significant for most of the PCB congeners but pressure only for the less chlorinated congeners. Further, no first-order interactions were found between pressure and temperature. Fig. 2 shows the response surface and contour map for the sum of six PCB congeners showing a maximum located at 100°C and 200 atm. These results demonstrate that solubility is the prevalent extraction parameter reaching the highest extraction efficiency when the solubility parameter of the fluid is close to the compounds of interest [24]. Lee and Peart [25] reported a similar SFE procedure for the extraction of PCBs from

Table 1
Parametric coefficients of Eq. 1 for PCBs according to the extraction fluids and their statistical significance

| IUPAC No. | Fraction[a] | *a* | *b* | *c* | *d* | *e* | $r^2$ (%) | *P* |
|---|---|---|---|---|---|---|---|---|
| 101 | I | −1.4 | −1.4 | n.s. | −1.9 | n.s[b] | 61.6 | 0.008 |
| 101 | I–III | −1.4 | 1.2 | −1.9 | −3.0 | −1.3 | 93.0 | 0.000 |
| 153 | I | n.s. | 2.6 | −6.5 | −6.2 | n.s. | 88.0 | 0.000 |
| 153 | I–III | −2.6 | 2.9 | −7.9 | −8.3 | n.s. | 90.3 | 0.000 |
| 138 | I | n.s. | 2.4 | −5.5 | −4.3 | n.s. | 92.9 | 0.000 |
| 138 | I–III | n.s. | 2.4 | −7.7 | −6.6 | n.s. | 73.8 | 0.001 |
| 180 | I | n.s. | 1.8 | −5.5 | −5.2 | n.s. | 86.8 | 0.000 |
| 180 | I–III | n.s. | n.s. | −7.0 | −6.7 | n.s. | 81.1 | 0.000 |
| ΣPCB congeners | I | n.s. | 9.7 | −18.8 | −19.2 | n.s. | 93.2 | 0.000 |
| ΣPCB congeners | I–III | n.s. | 9.8 | −25.0 | −25.9 | n.s. | 84.7 | 0.000 |

[a] Fraction I, 100% $CO_2$; II, 2% MeOH in $CO_2$; III, 10% MeOH in $CO_2$.
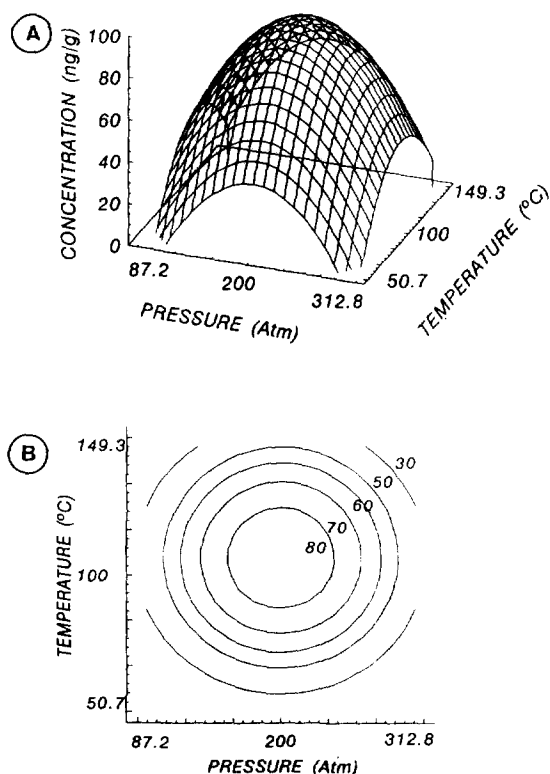[b] n.s.: Not statistically significant.

Fig. 2. (A) Response surface and (B) contour map for total PCB congeners extracted from fractions I–III, studying the effects of pressure and temperature on the extracted amount.

sediment by using neat $CO_2$ but the sample was extracted with a significant moisture content (11–50%). The use of methanol as carbon dioxide modifier clearly has benefits from the sample handling point of view.

## PAHs

Parametric coefficients of Eq. 1 were calculated for volatile, semivolatile and total PAHs (Table 2). A significant correlation was found for all the groups evaluated. The significant variables were pressure, temperature and their mutual first-order interaction. Surface response and contour map plots were obtained for total PAHs (Fig. 3). Recovery increases with increase in pressure (80–340 atm) and temperature (50–150°C). These results indicate that PAHs have a stronger interaction with the matrix and higher temperatures are needed to release them from naturally incurred samples. The optimum extraction conditions found in this work are comparable to those reported before for PAHs from a marine sediment with certified values using toluene as $CO_2$ modifier [19].

### 3.2. Chemical class fractionation

Selectivity of SFE for different chemical classes has been reported for PCBs from lipids [26] and hydrocarbons from petroleum source rocks [27] by optimizing the pressure and temperature. In this work, we evaluated the extraction selectivity for PCBs and PAHs by increasing the polarity of the extractant performing a sequential extraction. Only low-chlorinated PCBs (IUPAC Nos. 28 and 52) can be quantitatively extracted with 100% $CO_2$ (fraction I), with the remaining (IUPAC Nos. 101, 153, 138

Table 2
Parametric coefficients of Eq. 1 for PAHs according to the extraction fluid and their statistical significance

| Compounds | Fraction[a] | a | b | c | d | e | $r^2$ (%) | P |
|---|---|---|---|---|---|---|---|---|
| Volatile | I | 96 | 109 | −64 | n.s.[b] | 96 | 91 | 0.001 |
| Volatile | I–III | 67 | 72 | −105 | n.s. | 108 | 95 | 0.002 |
| Semivolatile | I | 40 | n.s. | −24 | n.s. | 29 | 107 | 0.004 |
| Semivolatile | I–III | 74 | −64 | −75 | n.s. | 97 | 83 | 0.004 |
| Total | I | 139 | 110 | −87 | n.s. | 126 | 94 | 0.000 |
| Total | I–III | 180 | n.s. | −127 | n.s. | 232 | 82 | 0.002 |

[a] Fraction I, 100% $CO_2$; II, 2% MeOH in $CO_2$; III, 10% MeOH in $CO_2$.
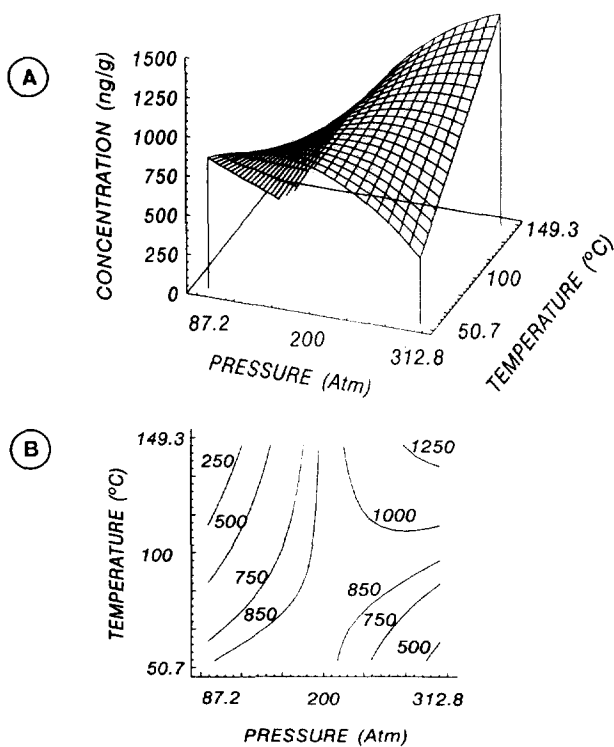[b] n.s.: Not statistically significant.

Fig. 3. (A) Response surface and (B) contour map for total PAHs recovered from fractions I–III, studying the effects of pressure and temperature on the extracted amount.

### 3.3. Intercomparison between Soxhlet extraction and SFE

#### PCBs

The individual PCB congener concentrations are reported in Table 3. SFE exhibited a 15% higher recovery for total PCBs in comparison with Soxhlet extraction, which can be accounted for by the highly chlorinated congeners (IUPAC Nos. 153, 138 and 180). The lower reproducibility of SFE than Soxhlet extraction could be attributable to the fact that in the former extraction procedure, PCB determination was carried out in three fractions, thus decreasing the reproducibility.

#### PAHs

Individual PAH concentrations obtained by SFE and Soxhlet extraction are reported in Table 4. The extraction efficiency of SFE was 17% higher than that of Soxhlet extraction, which was apparently due to the high-molecular-mass PAHs (100%). The SFE reproducibility was also lower than that of Soxhlet extraction (8.3 and 4.3%, respectively), which could be attributable to the same arguments as mentioned above for PCBs.

and 180) spread over all fractions accounting for 18% of total PCBs (Fig. 4A).

An increased selectivity according to ring number was found for PAHs. While the three- and four-ring PAHs (i.e., dibenzothiophene, phenanthrene, anthracene, methyldibenzothiophenes, methylphenanthrenes, dimethylphenanthrenes, fluoranthene and pyrene) are almost quantitatively extracted with carbon dioxide, the six-ring compounds (indeno[1,2,3-cd]pyrene, benzo[ghi]perylene and dibenz[a,h]anthracene) were extracted mostly in fraction III (10% MeOH in $CO_2$). On the other hand, the four- and five-ring PAHs spread over fractions I–III (Fig. 4B). These results demonstrate that chemical class fractionation under SFE conditions is not feasible.

### 4. Conclusions

The usefulness of a central composite experimental design was demonstrated, allowing the optimization of pressure and temperature for the SFE of PCBs and PAHs from a naturally contaminated sediment. A polynomial six-term parametric equation exhibited a significantly high correlation for most of individual PCB congeners (IUPAC Nos. 101, 153, 138 and 180) and PAHs. Pressure and temperature were significant for both PAHs and PCBs and a first-order interaction between the optimized variables was obtained for the former class of compounds. These results indicate that optimization strategies using one variable at a time can lead to biased results.
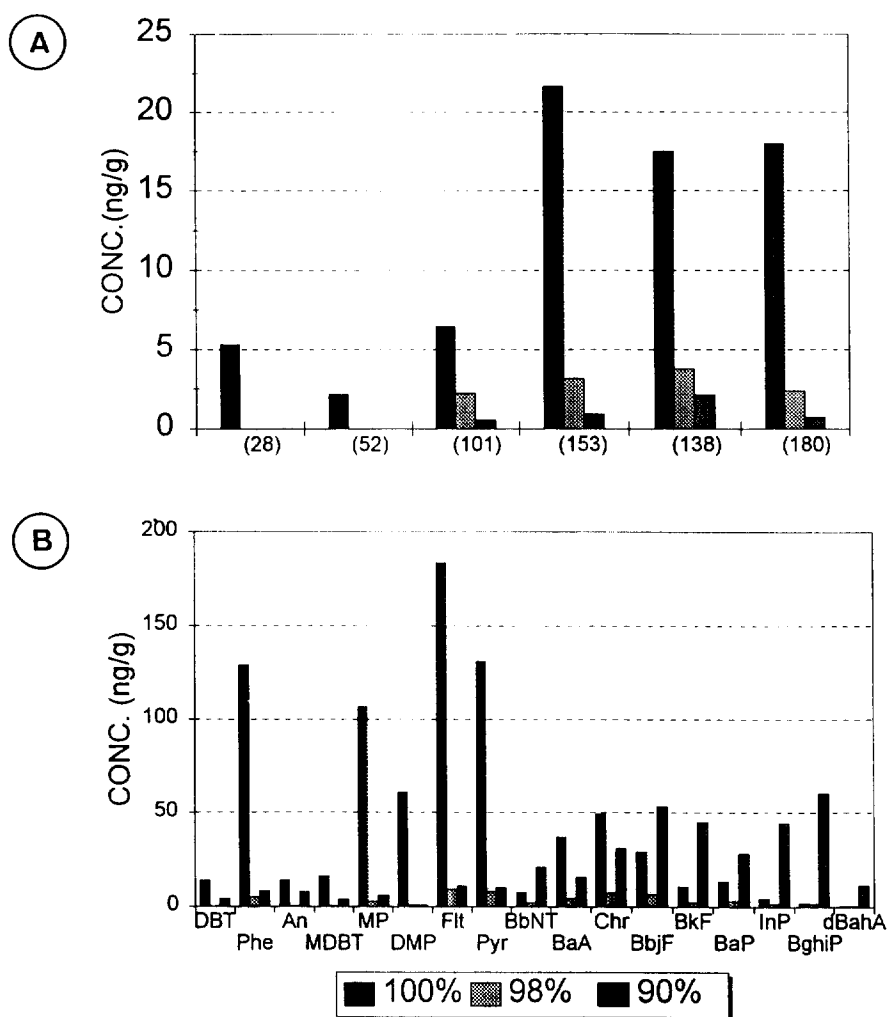
Fig. 4. Extraction selectivity for (A) individual PCB congeners and (B) PAHs using 100% $CO_2$ and 2% MeOH and 10% MeOH in $CO_2$. Compounds: DBT = dibenzothiophene; Phe = phenanthrene; An = anthracene; MDBT = methyldibenzothiophenes; MP = methylphenanthrenes; DMP = dimethylphenanthrenes; Flt = fluoranthene; Pyr = pyrene; BbNT = benzonaphthothiophenes; BaA = benz[a]anthracene; Chr = chrysene; BbjF = benzo[j]fluoranthene; BkF = benzo[k]fluoroanthene; BaP = benzo[a]pyrene; InP = indeno[1,2,3-cd]pyrene; BgiP = benzo[ghi]perylene; dBahA = dibenz[a,h]anthracene.

Optimum SFE conditions for PCBs are dependent on fluid solubility, reaching a maximum at 200 atm and 100°C. On the other hand, the recoveries of PAHs improved with increase in temperature (50–150°C) and pressure (87–313 atm), which could be accounted for by a significant matrix interaction.

Sequential extractions by increasing the fluid polarity from 100% (v/v) carbon dioxide to 10% (v/v) methanol demonstrate that the extraction selectivity depends on the analyte solubility and its mass transfer from matrix to fluid. In this regard only the low-molecular-mass PCBs can be quantitatively extracted with carbon dioxide and the other classes of compounds are spread all over the fractions, except the high-molecular-

Table 3
Intercomparison of extraction accuracy and precision between SFE and Soxhlet extraction for the determination of PCBs

| IUPAC No. | SFE | | | Soxhlet extraction | | |
|---|---|---|---|---|---|---|
| | Concentration (ng/g) | S.D.[a] | R.S.D[a] (%) | Concentration (ng/g) | S.D.[a] | R.S.D.[a] (%) |
| 28 + 31 | 7.7 | | | 8.9 | | |
| 52 | 2.0 | | | 4.3 | | |
| 101 | 8.6 | | | 7.3 | | |
| 153 | 25.4 | | | 19.1 | | |
| 138 | 26.6 | | | 21.1 | | |
| 180 | 23.0 | | | 15.1 | | |
| Σcongeners | 87.1 | 7.8 | 9.0 | 75.8 | 2.8 | 3.7 |

[a] $n = 4$.

mass PAHs, which are entirely extracted in the 10% MeOH fraction. Consequently, chemical class fractionation in SFE is not feasible under the extraction conditions used in this work.

## Acknowledgements

Table 4
Comparison of extraction accuracy and precision between SFE and Soxhlet extraction for the determination of PAHs

| Compound | SFE | | | Soxhlet extraction | | |
|---|---|---|---|---|---|---|
| | Concentration (ng/g) | S.D.[a] | R.S.D.[a] (%) | Concentration ng/g | S.D.[a] | R.S.D.[a] (%) |
| Dibenzothiophene | 18.2 | | | 5.4 | | |
| Phenanthrene | 142.5 | | | 99.5 | | |
| Anthracene | 22.1 | | | 9.3 | | |
| $\Sigma C_1$-dibenzothiophene | 20.7 | | | 8.7 | | |
| $\Sigma C_1$-phenanthrene | 115.7 | | | 52.6 | | |
| $\Sigma C_2$-phenanthrene | 63.0 | | | 45.4 | | |
| Fluoranthene | 204.2 | | | 212.1 | | |
| Pyrene | 149.5 | | | 174.3 | | |
| ΣBenzothiophenes | 30.4 | | | 21.3 | | |
| Benz[a]anthracene | 56.9 | | | 60.2 | | |
| Chrysene | 88.0 | | | 115.6 | | |
| Benzo[b + j]fluoranthenes | 89.2 | | | 87.2 | | |
| Benzo[k]fluoranthene | 58.2 | | | 55.6 | | |
| Benzo[a]pyrene | 44.5 | | | 33.4 | | |
| Indeno[1,2,3-cd]pyrene | 50.7 | | | 32.4 | | |
| Benzo[ghi]perylene | 64.4 | | | 23.5 | | |
| Dibenz[a,h]anthracene | 12.1 | | | 7.1 | | |
| Total | 1230.4 | 102.4 | 8.3 | 1043.4 | 44.5 | 4.3 |

[a] $n = 5$.

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