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Field-portable determination of polychlorinated biphenyls and polynuclear aromatic hydrocarbons in soil using supercritical fluid extraction

Søren Bøwadt, Laurent Mazeas, David J. Miller, Steven B. Hawthorne*

University of North Dakota, Energy and Environmental Research Center, P.O. Box 9018, Grand Forks, North Dakota 58202, USA

Abstract

A single SFE method for field extraction of PCBs and PAHs in soils was developed for use in combination with capillary GC–ECD or FID without the need for cleanup steps after extraction. The method involves 20-min extractions using pure CO_2 at 150°C and 400 bar. GC analysis was performed with 15-min (PCBs) or 22-min (PAHs) temperature programs. The final analytical method was demonstrated in field experiments (with the use of portable power sources) at different waste sites and was shown to give results in excellent agreement with conventional laboratory Soxhlet extraction. The practical determination limits of the method (as concentrations on the 5-g soil samples) without any concentration or cleanup of the SFE extracts was ≈200 ppb of individual PAHs and ≈10 ppb of individual PCBs (≈100 ppb of total PCB as Aroclor 1260). Since the SFE collection solvent was acetone, the extracts were compatible with enzyme-linked immuno-sorbent assay (ELISA). ELISA and GC–ECD determinations of PCBs as total Aroclors agreed within ≈50% when ELISA was performed in the laboratory. However, ELISA failed to yield useable results when performed outdoors under field conditions.

Keywords: Supercritical fluid extraction; Field extraction method; Polychlorinated biphenyls; Polynuclear aromatic hydrocarbons

1. Introduction

Recently, there has been an increase in the need for rapid and reliable field-portable methods for the quantitation of organic pollutants present at waste sites [1]. In many of these waste sites, fast and accurate determination is necessary in order to make appropriate decisions regarding site cleanup and remediation. Although several instruments are now available for field determination [e.g., gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)], there has been a lack of rugged quantitative extraction methods to supply on-site extracts that are suitable for GC analysis without post-extraction clean-up steps.

Conventional extraction methods based on liquid–liquid extractions are not practical in the field because of the time needed to achieve quantitative or near-quantitative extractions, the fragile and elaborate glassware employed, and the large amount of solvents involved [1]. However, in the last few years, supercritical fluid extraction (SFE) has been shown to yield good recoveries for a range of organic pollutants from real environmental samples using short extraction times (20–60 min) and only a minimum of organic solvents [2–4]. There have been multiple reports demonstrating good agreement be-

*Corresponding author. Tel.: +1-701-777-5000; fax: +1-701-777-5181.

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tween SFE and conventional extraction methods, especially for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides [5–8]. Furthermore, the US EPA has recently adopted SFE as the official method for the extraction of total petroleum hydrocarbons (TPHs) and PAHs from environmental matrices, and is presently in the process of adopting a method for the extraction of PCBs and organochlorine pesticides [9]. SFE is, therefore, a natural choice for field extractions because the extraction can be performed quite efficiently with only a minimum of solvents and equipment involved, and can yield real time results that facilitate on-site decisions. Furthermore, SFE using pure CO_2, has often been shown to yield extracts sufficiently clean for direct determination without any labor-intensive and time-consuming clean-up steps, thus minimizing possible mistakes in quantitation (from interfering compounds or sample handling) and simplifying field determinations [10–12].

The compounds chosen for the present study were PAHs and PCBs, since these compounds are very widespread in the environment and can serve as good model compounds for other semi-volatile pollutants like organochlorine pesticides. The goal of this study was to develop a single field portable extraction method which would yield high extraction efficiencies of both PAHs and PCBs, yield extracts that could be analyzed by GC or immunoassay without additional treatment, and use commercially-available instrumentation with only an electrical generator for support. Gas chromatography with flame ionization detector (FID) and electron capture detector (ECD) was chosen as the predominant determination method because of its widespread use, ease of operation, and fast and reliable results. Since enzyme-linked immuno-sorbent assay (ELISA) is presently being promoted for field determinations [13–15], quantitative results for ELISA and from GC analysis were also compared.

2. Materials and methods

2.1. Chemicals

Individual and total PCB standards used in this study were obtained from either Ultra Scientific (North Kingstown, RI) or Accustandard (New Haven, CT) as neat compounds with known purity. Individual PAH standards were obtained from Aldrich as neat compounds with known purity. The solvents used (acetone, methanol, *n*-hexane and dichloromethane) were all 'Optima'-grade obtained from Fisher Scientific (Pittsburgh, PA). SFC grade CO_2 (with 1200 psi helium head-pressure) was obtained from Scott Specialty Gases (Plumsteadville, PA). The filter paper used at both ends of the extraction cells was Whatman Glass Microfiber filters, type GF/B.

2.2. Field sampling

All samples used throughout this study were surface soils containing native (not spiked) PAHs and PCBs. For the developmental studies on PAHs, two railroad bed soils were used. One was collected from a railroad bed which had been abandoned for at least 20 years, the other was from an operational railroad. For PCBs, two soils (PCB Sample 1–2) were collected from storage dumpsters with PCB-contaminated soil at Manitoba Hydro in Winnipeg, Canada, and one sediment (PCB Sample 3) obtained as a gift from Drs. Carl Orazio and Kathy Echols, National Biological Survey, Columbia, MI. All soils were sieved to less than ≈6 mm to remove rocks, sticks and other debris, and homogenized mixing by hand for ≈1 min. No other treatment (such as drying) was performed since only a minimum of sample preparation could be performed in the field.

For the field demonstrations, several separate trips were made in August of 1995, using a standard mini-van with the rear seats removed. No modifications of the van to support the field experiment were needed. A one-day trip included experiments at three individual sites suspected of PAH contamination in and around Grand Forks, ND. The total time required for transportation, instrumental set-up and repacking at each site, and performing triplicate extractions at each site was less than 6 h. The second field trip was performed at the Grand Forks Air Force Base, where samples were collected at two sites suspected of PAH contamination. For these field trips an ISCO SFX 2-10 extractor (ISCO, Lincoln,

NE) with a Model 260D syringe pump was used for SFE, and an HP Model 5980A GC (Hewlett–Packard, Wilmington, DE) with an FID was used for the quantitative determinations of the PAHs. All instruments were fully operational 20–30 min after arrival on the site. The extractions were performed both inside the van and outside on a table in temperatures reaching up to ≈40°C, without major technical problems. The most significant problem encountered was the plugging of two flow restrictors (which were easily replaced in 1–2 min).

On the field trip to Manitoba Hydro in Winnipeg, 6 individual dumpsters containing PCB-contaminated soil were sampled. Each sample was extracted in triplicate (a total of 18 sample extractions). The SFE extracts were then subjected to determination by immunoassay and identical samples were extracted with a soil collection and extraction kit provided with the immunoassay kit in order to compare the extraction efficiency of the two methods. The whole operation at this site took approximately 6 h from the arrival at the actual site until departure. The extraction equipment used on the first field trip was also used on this occasion, however, GC–ECD determinations were performed in the laboratory in Grand Forks to avoid problems associated with crossing an international border carrying a radioactive source (^{63}Ni contained in the ECD). The extractions and immunoassay determinations were performed on a table outside the van in bright sunshine, temperatures up to 29°C, and a wind speed of around 15–20 kilometer per hour without any technical problems (other than the poor performance of the immunoassay, as discussed later).

On all three field demonstrations, Honda-portable power generators were used [a Honda Model EX 1000 (120 V, 1000 W) for the extraction equipment, and a Honda Model EM 3500 SX (120 V or 240 V, 3500 W) for the GC instrument], as sources of electricity in order to be independent of external power supply. No technical problems concerning these generators were encountered.

2.3. Supercritical fluid extraction

The extractor (ISCO SFX 2-10) and the syringe pump (ISCO Model 260D) were chosen because of their small size and simple operation in the field. 5-g samples were packed into plastic extraction cells (provided with the extractor) using a disk of glass filter paper on each end of the extraction cell. Since the extractor can accommodate two samples, all SFE extractions were performed in parallel using pure CO_2 with a dynamic flow-rate of ≈1.5 ml/min (measured as liquid CO_2 at the pump) for each channel.

All extracts were collected by liquid trapping in approximately 10 ml of acetone in a 22-ml glass vial. Internal standards [hexachlorobenzene (HCB) and octachloronaphthalene (OCN) for PCBs, and octahydronaphthalene (OHN) for PAHs] were added after the extraction, and the extract volume was adjusted to 10 ml. For some PAH samples (with individual PAH concentrations below 0.2 ppm), the extracts were concentrated under a gentle stream of clean nitrogen to ≈1.8 ml in order to increase the sensitivity of the method.

2.4. Soxhlet extraction and sonication

For all Soxhlet extractions, ≈5 g of soil were weighed into a cellulose (Whatman 30×80 mm) extraction thimble, and the sample was extracted with 150 ml of methylene chloride for PAHs and 150 ml of a 1:1 mixture (v/v) of acetone and *n*-hexane for PCBs for 24 h with a cycle time of 10–15 min. After the extractions were completed, internal standards (the same as for SFE) were added to the extracts. The solvents were evaporated down to volumes between 2 and 10 ml for the PAH extracts and to 10 ml for the PCB extracts.

In addition to comparing the SFE recoveries to Soxhlet extraction, sonications were performed on most SFE residues (all PAH samples and most PCB samples) in order to provide a second method to verify SFE recoveries. The SFE residues were mixed with 3 g of Na_2SO_4 and 30 ml of methylene chloride for the PAH samples (1:1 mixture (v/v) of acetone and *n*-hexane for PCBs) in a 40-ml vial. After 14 h of sonication, the internal standard was added, the samples were centrifuged and the supernatant was removed. The volume of solvent was then reduced appropriately under a gentle stream of clean nitrogen prior to GC analysis.

2.5. Gas chromatography

PAH extracts were analyzed using an HP Model 5890 GC equipped with a split/splitless injector held at 300°C and an FID held at 320°C. Aliquots (2 μl) of the extracts were injected in the splitless mode (0.2 min) on a 25 m×0.32 mm I.D. (0.17-μm film thickness), 5% phenyl–methyl siloxane HP-5 column. Hydrogen was used as the carrier gas with a constant head pressure of 0.55 bar, giving an initial linear velocity of ≈62 cm/s (at 100°C). The temperature program was the following: initial temperature 100°C, held for 2 min, then increased at a rate of 20 C°/min to 150°C, followed by increase at a rate of 10 C° to 200°C, followed by an increase of 15°C to 320°C, and held for 4 min (a total run time of 21.5 min). Data were collected with an HP Model 3396 integrator. Quantitative measurements of PAHs were performed using peak heights after a 5-point linear calibration curve from gravimetrically prepared standards. Authentic pure compounds were used for the calibration standards for naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene. For the other compounds that were unavailable as standards for this study, the quantitation was based on GC–FID, where the relative response factor was estimated from the standard compounds having the most similar molecular formula and structure. The identity of these compounds was furthermore confirmed by GC–MS based on mass spectra, and retention times of standard PAHs on an HP-5 column.

For PCBs, extracts were analyzed using an HP Model 5890 Series II GC equipped with a split/splitless injector held at 300°C and a ^{63}Ni electron capture detector (ECD) held at 300°C (using pure and dry nitrogen as the make up gas with a flow of 60 ml/min) and an HP 7673A auto injector. Aliquots (1 μl) of the extracts were injected in the split mode (1:30) (splitless for the congener specific analysis) on a 25 m×0.22 mm I.D. (0.10-μm film thickness), 1, 7 - dicarbo-closo-dodecarborane-dimethyl-siloxane HT-5 column (Scientific Glass Engineering, Austin, TX). Hydrogen was used as the carrier gas with a constant head pressure of 1.52 bar, giving an initial linear velocity of ≈77 cm/s (at 100°C). The temperature program was as follows: initial temperature 100°C, held for 1 min, then increased at a rate of 15 C°/min to 280°C and held for 2 min (a total run time of 15 min). This temperature program has previously been shown to yield good separation for the PCBs in question [16]. Quantitative analysis was performed initially as total PCBs in the form of Aroclor 1260 (Aroclor 1254 for PCB Sample 3). This was done because the legislative limit (50 ppm) for remediation of PCB-contaminated soil in North America is based on quantitation as total PCBs [17]. Additional analyses were performed in order to quantify the PCBs as individual congeners, because this method is used for regulative purposes in Europe. In both cases, the temperature program described above was used as were hexachlorobenzene (HCB) and octachloronaphthalene (OCN) as internal standards. For the determination as total Aroclor, the quantitation was performed as the sum of PCB congener areas and calibrated against a 6-point calibration curve (10–500 ppm, gravimetrically diluted from authentic Aroclor mixtures) using the power fit calibration routine provided with the HP Chem 3365 software. For the congener specific analysis, the quantitation was performed after an 8-point calibration curve using the power fit calibration routine. The individual congeners were calibrated in the concentration interval of 1.7 to 1719 pg/μl. Standards were injected after every fifth sample to determine deterioration of the separation.

2.6. ELISA

Determination of PAHs and PCBs with ELISA and the extraction of these compounds with the associated extraction kit was performed according to the instructions obtained from Ohmicron Corporation. Reagent kits and the necessary hardware (magnetic rack and photometric detector) were from the same supplier.

For the extraction of PCBs and PAHs with the ELISA extraction kit, 10-g samples were mixed with an extraction solution (provided with the kit) consisting of 20 ml of methanol containing a soil dispersing agent, and shaken vigorously for at least 60 s. The samples were then left to settle for at least 5 min before filtration (filter provided with the kit). The samples were then diluted appropriately and analyzed either by ELISA or injected on the GC

together with the SFE extracts and calibration standards.

For the actual analysis by ELISA, 200 μl of the extract (sample, standard or control), 250 μl of PCB enzyme conjugate (horseradish peroxidase labeled PCB analog), and 500 μl of a solution of PCB antibody coupled paramagnetic particles were mixed in a test tube, vortexed for 1–2 s, and incubated for 15 min (30 min for PAH) at room temperature. After incubation, the immunoassay complexes were separated on a magnetic rack and washed twice with a washing solution (provided with the kit). 500 μl of a color reagent (consisting of a mixture of hydrogen peroxide and 3,3′,5,5-tetramethyl-benzidine) was added to the test tubes, the mixture was vortexed for 1–2 s and incubated again at room temperature. After 20 min, the reaction was stopped by the addition of 500 μl of stopping solution (0.5 *M* sulfuric acid), and the color intensity determined at 450 nm with a photometric detector (Ohmicron RPA-III). The concentrations were determined using calibration curves obtained with Aroclor 1254 and 1260 standards (at concentrations of 0, 0.25, 1 and 5 ppb) on ln/logit paper provided with the method.

For determination of PCBs in the field samples, a 1:25000 dilution of the sample extracts was needed in order to stay inside the linear range of the immunoassay kit. This was achieved in two steps: 40 μl of the initial SFE extract (collected in 10 ml of acetone) was transferred to 10 ml of pure MeOH, followed by transfer of 250 μl of this solution into 25 ml of diluent obtained with the ELISA kit (containing 50% MeOH and 50% H_2O).

3. Results and discussion

3.1. SFE conditions

In order to enhance the speed of SFE, addition of an organic modifier (e.g., acetone or methanol) to the CO_2 has often been used [3,4,18–20]. Modifier addition, however, also enhances the co-extraction of contaminants to an extent equivalent to Soxhlet extraction (or accelerated solvent extraction) and, therefore, can necessitate clean-up before quantification. Clearly, this would be a disadvantage for a field method where speed and simplicity are first priorities. Earlier, it was shown that pure CO_2, with the use of higher temperatures [5,18,21–23], yields high recoveries of many semi-volatile pollutants, and also yields extracts that are sufficiently clean for direct determination by GC without the need for post-extraction clean-up steps.

Initial SFE conditions were chosen at 150°C (the upper limit of the ISCO extractor) and 400 bar, with a liquid CO_2 flow of 1.5 ml/min. The use of pure CO_2 with higher temperatures has been established for several samples contaminated with PAHs, but has been less well proven for PCBs [5,18–22]. In order to determine the temperature effect at constant pressure (400 bar) on PCB recoveries, extractions were performed on two different soils at two different temperatures (80°C and 150°C). The resulting recovery curves for one of the soils are plotted in Fig. 1 and show that temperature does have a significant effect on the extraction efficiency of PCBs (nearly identical curves were obtained for the other soil tested). At 150°C, quantitative recovery compared to Soxhlet extraction was reached after ≈20 min in contrast to ≈80% recovery achieved after 50 min at 80°C. As a result of these measurements, extraction conditions of 150°C and 400 bar with a flow-rate of 1.5 ml/min was used for all subsequent PAH and PCB extractions.

Because time is a crucial parameter in field measurements, the effect of extraction time versus

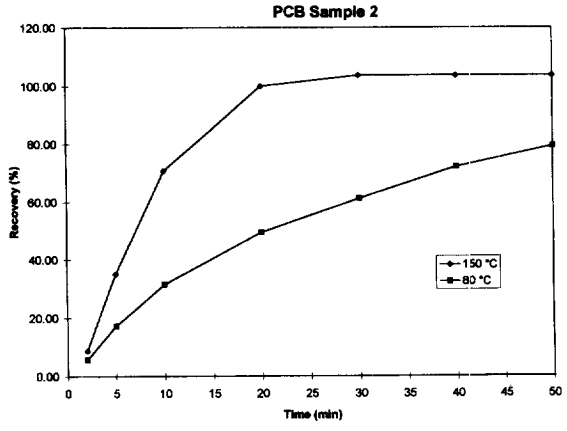

Fig. 1. SFE extraction rates of total PCBs from a PCB-containing soil (≈35 ppm) with two different temperatures (80°C and 150°C) at constant pressure (400 bar). % recovery is based on 24-h Soxhlet extraction.

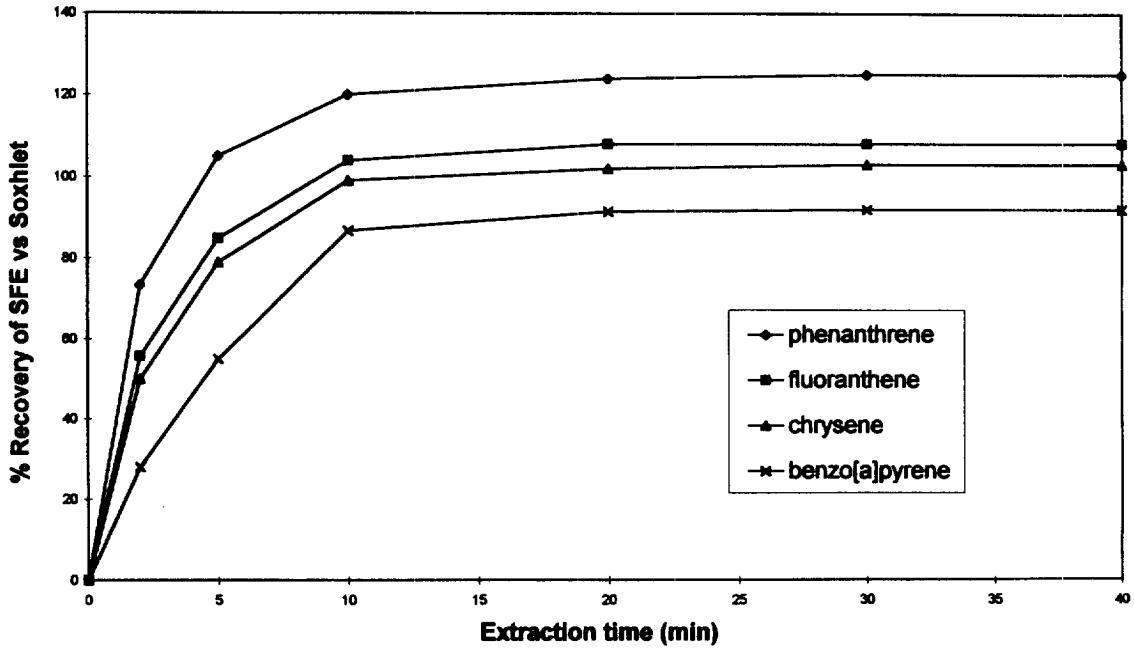

Fig. 2. Extraction rates of representative PAHs from a railroad bed roil (150°C and 400 bar). % recovery is based on 24-h Soxhlet extraction.

recovery was evaluated both for PAHs and PCBs. Fig. 2 shows the extraction profile with time, of four representative PAHs from a railroad bed soil. The results clearly indicate that SFE is completed after 20 min and near-quantitative values for individual PAHs are obtained when compared to Soxhlet extraction, as well as demonstrated by the lack of significant analytes in the sonication extracts of the SFE residues. Since PAH and PCB recoveries were typically >90% after 20 min, all subsequent extractions were performed for 20 min at 400 bar and 150°C.

In order to further verify the recoveries shown in the extraction profiles, three PCB and two PAH samples were extracted for 20 min in triplicate. The PCB data for the three soils (Sample 1–3, ranging from 5–64 ppm of total PCBs) showed quantitative recovery (100% and 98%) when compared with 24-h Soxhlet extraction for Sample 2 and 3, and 89% recovery for Sample 1 (measured as total PCBs). For Sample 1, increasing the extraction time to 30 min yielded quantitative recovery (98% compared to Soxhlet).

The PAH data for the two soils in Table 1 (with a factor of 3 difference in contamination level) also show good recoveries (from 71–114%) when compared with Soxhlet extraction. Sonication (14 h) of the SFE residues confirmed the good recoveries obtained for both samples, with only 5–20% of the high molecular PAHs found in the residue extracts.

It should be noted that the ability of the simple solvent collection system chosen for the SFE extractions to efficiently collect extracted PAHs and PCBs needs to be verified before beginning the extraction studies.

Acetone was chosen as the collection solvent since Langenfeld et al. [24] has previously shown this solvent to yield a quantitative collection of PAHs and PCBs, and because this solvent is tolerated by immunoassay in up to 5% concentration (v/v) in the final diluted extract. Methanol, previously used for collection of PCBs by Lopez-Avila et al. [13], would have been the ideal solvent for immunoassay (tolerated up to 50% in the final extract), but Langenfeld et al. [24] showed this solvent inadequate to assure quantitative collection.

In order to determine collection efficiencies, spiked PAHs and PCBs were extracted from sand using pure CO_2 at 150°C and 400 bar, with a liquid

Table 1
Comparison of 20-min SFE and 24-h Soxhlet extraction for PAHs

Compound	Abandoned rail road soil		Operational rail road soil	
	Soxhlet[a] ppb (% R.S.D)	SFE % Rec (% R.S.D)[b]	Soxhlet[a] ppb (% R.S.D)	SFE % Rec (% R.S.D)[b]
Phenanthrene	200 (12)	105 (13)	390 (41)	114 (12)
Anthracene	150 (5)	80 (19)	1040 (17)	105 (4)
Fluoranthene	700 (4)	95 (13)	1970 (42)	86 (13)
Pyrene	700 (3)	90 (13)	1730 (43)	79 (12)
Benz[*a*]anthracene	260 (8)	92 (14)	620 (41)	83 (31)
Chrysene	950 (18)	72 (14)	2100 (32)	98 (18)
Benzo[*a*]pyrene	300 (23)	76 (13)	630 (26)	80 (4)
Perylene	130 (25)	70 (16)	400 (13)	83 (2)
Benzo[*ghi*]perylene	810 (4)	75 (15)	1050 (16)	82 (10)

[a] Soxhlet extractions were performed for 24 h using 150 ml of methylene chloride. Concentrations and relative standard deviations (% R.S.D) are based on triplicate extractions.
[b] SFE were performed in triplicate with pure CO_2 at 150°C and 400 bar for 20 min. Recovery in % is calculated based on the Soxhlet values. % R.S.D is based on triplicate extractions.

flow of ≈1.5 ml/min for 30 min. As shown in Table 2, the larger volume (10 ml) of acetone was necessary to obtain high collection efficiencies. Table 2 also shows that the recoveries are greatly enhanced (and nearly quantitative) by lowering the temperature of the restrictor to 80°C for both compound types, which demonstrates that the restrictor temperature (using solvent trapping) is a much more important parameter than generally anticipated. This phenomenon could be due to the fact that CO_2 vents as a gas rather than as an aerosol at high restrictor temperatures [25], and is responsible for some of the differences reported in the literature for the collection efficiency of otherwise identical systems. The collection conditions obtained were used throughout the remainder of the study.

3.2. Validation of the method under field conditions

The field method was demonstrated on three separate field trips performed in August, 1995. On two field trips, sites suspected of PAH contamination were sampled using off-line SFE in conjunction with GC–FID and two electrical power generators to provide the power necessary to operate all equipment simultaneously. The trips were performed using a normal van (from which the rear seats had been

Table 2
Effect of collection vial volume and restrictor temperature on collection efficiencies of PAHs and PCBs

Compound	M_r	% Recovery (% R.S.D)[a]		
		7-ml vial with 4 ml of acetone	22-ml vial with 10 ml of acetone	
		Restrictor temp 100°C	Restrictor temp 100°C	Restrictor temp 80°C
Naphthalene	128	46 (16)	75 (11)	100 (3)
Phenanthrene	178	48 (15)	74 (12)	101 (4)
Pyrene	202	48 (15)	75 (12)	103 (5)
Chrysene	228	49 (12)	74 (9)	104 (5)
Benzo[*a*]pyrene	252	48 (17)	75 (11)	95 (8)
Benzo[*ghi*]perylene	276	48 (22)	76 (10)	96 (9)
Aroclor 1260[b]	220–500	73 (3)	71 (4)	93 (3)

[a] Percent recovery and percent relative standard deviations are based on triplicate 30-min extractions at 400 bar and 150°C with pure CO_2 of spiked PAHs and PCBs standards (1 ppm).
[b] Determined as total PCBs. No significant difference between the collection efficiency of individual congeners was observed.

removed) with no additional support. Two samples each of 5-g mechanically mixed and sieved topsoils were extracted simultaneously in 20 min, followed by injection into the GC–FID. All equipment was normally ready for use 20–30 min after arrival on-site, thus, allowing time to collect, sieve, and homogenize the first sample. In these field demonstrations data collection was performed with an integrator that had previously been loaded with calibration data obtained during runs the night before the trip. The integrators generally worked well on the field trips, but they were somewhat sensitive to power surges from the generator, as well as loose wiring connections. We, therefore, tried to run the GC–ECD and GC–FID together with the HP Chem software on a computer under identical conditions. This combination seemed to be a more robust alternative for data collection because it was less susceptible to power surges and had more robust wiring.

The results from four representative samples (out of 7) extracted in triplicate during these two field trips are shown in Table 3. Sample 1 and 2 display quantitative recovery compared to Soxhlet extraction (performed after returning to the laboratory) with SFE recoveries typically ranging from 80 to 120% of the Soxhlet values. PAH sample 3 gave somewhat lower recoveries with the average being ≈81%, while Sample 4 showed recoveries higher than those achieved by 24-h Soxhlet extraction. All in all, the recoveries obtained during the field experiments were very acceptable considering the short extraction times (20 min) using only CO_2, and the field conditions under which they were achieved. The precision of the determinations was also acceptable considering the crude pretreatment of the real samples with the SFE results having equal or slightly lower relative standard deviations than those obtained with triplicate Soxhlet extractions. The linear regression correlation coefficient (r^2) between the results obtained by SFE and Soxhlet extraction was determined to 0.995 (all results shown in Table 3),

Table 3
Comparison of 20-min Field SFE and 24 h Lab Soxhlet for PAHs

Compound	Sample 1		Sample 2		Sample 3		Sample 4	
	Soxhlet[a] ppm (% R.S.D)	SFE,[b] % Rec (% R.S.D)	Soxhlet[a] ppm (% RSD)	SFE,[b] % Rec (% R.S.D)	Soxhlet[a] ppm (% R.S.D)	SFE,[b] % Rec (% R.S.D)	Soxhlet[a] ppm (% R.S.D)	SFE,[b] % Rec (% R.S.D)
Acenaphthylene	0.41 (4)	83 (5)	0.26 (10)	86 (8)	ND[d]	ND	ND	ND
Acenaphthene	3.7 (12)	97 (21)	0.13 (5)	105 (4)	ND	ND	ND	ND
Fluorene	4.3 (23)	102 (19)	0.19 (28)	127 (6)	ND	ND	ND	ND
Phenanthrene	23 (28)	108 (15)	3.5 (8)	102 (5)	0.92 (17)	88 (9)	0.17 (7)	129 (11)
Anthracene	10 (12)	121 (18)	0.81 (5)	124 (9)	0.64 (9)	105 (19)	1.3 (7)	87 (11)
Fluoranthene	46 (10)	101 (10)	11 (4)	78 (2)	3.2 (8)	74 (4)	0.73 (18)	140 (18)
Pyrene	38 (10)	105 (7)	7.5 (5)	84 (2)	2.3 (7)	69 (6)	0.77 (20)	135 (16)
Benz[*a*]anthracene	12 (7)	107 (6)	1.7 (11)	80 (1)	1.1 (3)	65 (1)	0.22 (10)	146 (10)
Chrysene	19 (7)	112 (15)	5.8 (2)	94 (6)	2.2 (6)	76 (15)	0.27 (12)	130 (12)
Benzo[*b*+*k*]fluoranthene[c]	6.0 (13)	99 (16)	3.0 (4)	85 (2)	1.8 (6)	62 (12)	0.91 (4)	106 (16)
Benzo[*a*]pyrene	5.7 (27)	93 (21)	1.9 (3)	79 (7)	0.84 (16)	65 (16)	2.1 (3)	113 (7)
Perylene	1.6 (23)	86 (15)	0.4 (16)	53 (5)	1 (3)	85 (27)	0.42 (22)	118 (3)
Indeno[1,2,3-*cd*]pyrene	2.0 (34)	88 (20)	0.49 (12)	84 (10)	ND	ND	0.47 (29)	106 (21)
Benzo[*ghi*]perylene	1.2 (8)	64 (2)	0.43 (25)	72 (18)	0.2 (13)	85 (13)	0.41 (26)	123 (8)
Dibenzothiophene	2.1 (25)	107 (17)	0.16 (13)	116 (5)	ND	ND	ND	ND
Carbazole	ND	ND	0.97 (6)	135 (5)	0.57 (9)	122 (5)	0.14 (12)	105 (14)

[a] Soxhlet extractions were performed for 24 h using 150 ml of methylene chloride. Concentrations and relative standard deviations are based on triplicate extractions.

[b] SFE were performed in triplicate with pure CO_2 at 150°C and 400 bar for 20 min. Recovery in % is calculated based on the Soxhlet values. Relative standard deviation (% R.S.D) is based on triplicate extractions.

[c] The sum of benzo[*b*]- and benzo[*k*]fluoranthene is reported because they were not adequately resolved by the chromatographic conditions used.

[d] ND = not determined.

thus indicating excellent agreement between the field SFE and laboratory Soxhlet methods.

On the field trip to Manitoba Hydro in Winnipeg, Canada, SFE extractions (all in triplicate) were performed on 6 samples collected from storage dumpsters containing PCB-contaminated soil. Due to the crossing into Canada, it was considered more appropriate to perform the GC–ECD determinations in the home laboratory because the ECD contained a low-grade β-radioactive source. Table 4 shows the determination (as total PCBs) of the SFE extracts obtained on the field trip (Sample 4–6) compared with the Soxhlet extracts from identical samples returned to the laboratory, and compares the SFE and Soxhlet extracts performed in the laboratory during methods development (Sample 1–3). The results clearly show that the SFE method yielded high recoveries whether samples were extracted in the field or in the laboratory. Also, the comparison between the SFE and Soxhlet results show no significant differences, regardless of whether the PCBs were determined as individual congeners (Table 5) or as total PCBs (Table 4).

The comparability between SFE and Soxhlet results is further backed by regression analysis where r^2 is 0.998 (all individual congener results from Table 5) for the congener specific analysis and 0.974 (results from Table 4) for the analysis as total PCBs. The relative standard deviations obtained in the laboratory and field (Table 4) are also similar. A chromatogram of one of the SFE extracts obtained by GC–ECD is shown in Fig. 3. It is well worth noting the clean nature of the chromatogram that is obtained without any clean-up of the SFE extract before injection, while four of the Soxhlet extracts required clean-up over acid silica before GC–ECD. Gravimetric determinations after solvent evaporation also demonstrated that the SFE extracts were relatively free of co-extracted matrix components. For example, Soxhlet yielded 4–90-mg dry residues compared to 0.2–5 mg for SFE for the samples shown in Tables 4 and 5.

3.3. Preliminary evaluation of ELISA for determination of PCBs and PAHs

ELISA is presently being promoted for rapid screening of PCBs and PAHs, as well as other semi-volatile organics in the field, although previous accounts have used this technique only in a controlled environment [13,15,26]. Until now, no publications describe the use of PCB and PAH determinations outdoors. Since commercial ELISA kits designed for soil analysis consist of sample extraction and immunoassay determination kits, the quantitative efficiency of both steps were determined separately.

The quantitative extraction efficiency of the

Table 4
Comparison of extraction methods based on GC determination as total PCBs

Sample	Soxhlet[a] ppm (% R.S.D)	SFE[b] vs. Soxhlet % Rec (% R.S.D)	Methanol ext. vs. Soxhlet[c] % Rec (% R.S.D)
Sample 1[d,f]	64 (8)	89 (9)	54 (4)
Sample 2[d,f]	35 (6)	100 (9)	72 (3)
Sample 3[d,g]	5.3 (4)	98 (1)	73 (7)
Sample 4[e,f]	6.3 (5)	93 (6)	74 (8)
Sample 5[e,f]	4.5 (5)	93 (10)	59 (6)
Sample 6[e,f]	18 (12)	87 (11)	68 (7)

[a] Soxhlet extractions were performed for 24 h using 150 m of 1:1 *n*-hexane:acetone. Concentrations and relative standard deviations were based on triplicate extractions.

[b] SFE were performed in triplicate with pure CO_2 at 150°C and 400 bar for 20 min. Recovery in % is based on the Soxhlet values. Relative standard deviation (% R.S.D) is based on triplicate extractions.

[c] ELISA extraction kit using methanol and a soil dispersing agent, minimum 5 min extraction and 5 min to let the sample settle. Recovery in % is based on the Soxhlet values. % R.S.D is based on triplicate extractions.

[d] SFE performed in the lab.

[e] SFE performed in the field.

[f] Determination of Total PCBs as Aroclor 1260.

[g] Determination of Total PCBs as Aroclor 1254.

Table 5
Comparison of 20-min field SFE and 24-h laboratory Soxhlet for PCBs

PCB No.	Sample 4		Sample 5		Sample 6	
	Soxhlet[a] ppb (% R.S.D)	SFE,[b] % Rec(% R.S.D)	Soxhlet[a] ppb (% R.S.D)	SFE[b] % Rec (% R.S.D)	Soxhlet[a] ppb (% R.S.D)	SFE,[b] % Rec (% R.S.D)
101 (Cl_5)	132 (7)	95 (7)	109 (21)	96 (6)	321 (11)	88 (8)
118 (Cl_5)	42.1 (5)	94 (9)	26.4 (12)	92 (5)	122 (9)	91 (9)
128 (Cl_6)	273 (5)	94 (6)	206 (9)	91 (8)	679 (11)	87 (10)
138 (Cl_6)	499 (5)	95 (8)	322 (9)	92 (4)	1271 (11)	90 (10)
149 (Cl_6)	473 (4)	94 (6)	314 (15)	93 (2)	1180 (11)	87 (8)
153 (Cl_6)	632 (6)	94 (8)	406 (9)	90 (3)	1756 (11)	87 (11)
156 (Cl_6)	90.4 (5)	92 (7)	63.8 (8)	89 (10)	240 (11)	88 (10)
170 (Cl_7)	307 (4)	90 (8)	210 (8)	92 (12)	817 (12)	86 (11)
180 (Cl_7)	746 (5)	89 (8)	533 (10)	85 (14)	2028 (12)	84 (12)

[a] Soxhlet extractions were performed for 24 h using 150 ml of 1:1 *n*-hexane:acetone. Concentrations and relative standard deviations were based on triplicate extractions.

[b] SFE were performed with pure CO_2 at 150°C and 400 bar for 20 min. Recovery in % is based on the Soxhlet values. Relative standard deviation (% R.S.D) is based on triplicate extractions.

ELISA extraction kit can be seen in Table 4 for PCBs and Table 6 for PAHs. For the PCBs, the extraction efficiency was reasonably high, with recoveries around 67% of the recoveries achieved by Soxhlet, although significantly lower than SFE with an average of 93%. For the PAHs, the extraction efficiency was somewhat worse than for the PCBs with a mean of 55% of the recoveries achieved by Soxhlet for the four samples investigated, which is substantially lower than for SFE with an average of 98%.

Determination of PCB and PAH concentrations using ELISA yields a single number for PCBs and PAHs based on the presumption that all components

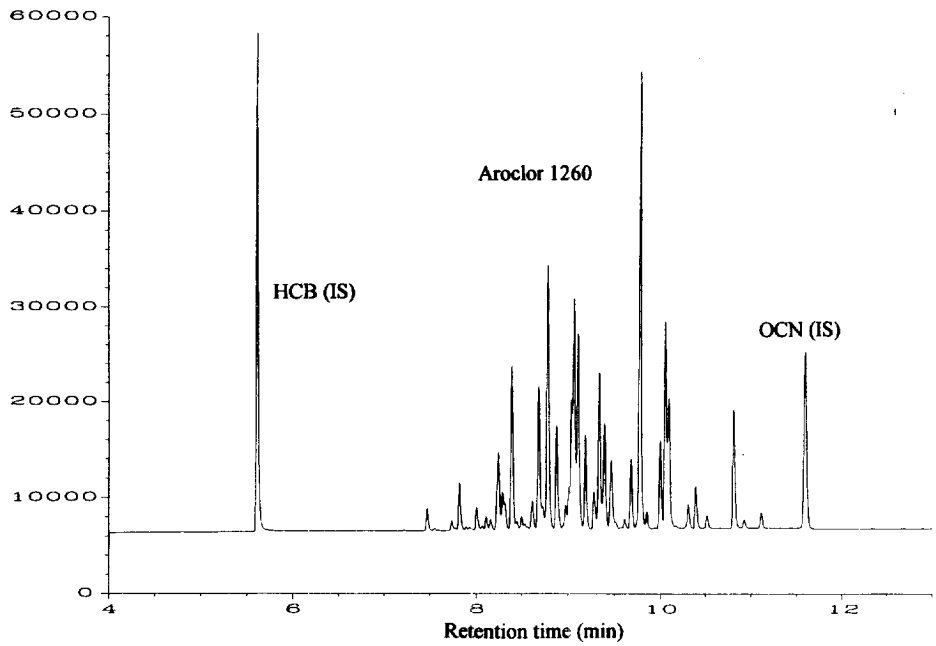

Fig. 3. GC–ECD chromatogram of the SFE extract (without any clean-up) from PCB Sample 6 (≈18 ppm) contaminated with Aroclor 1260.

Table 6
Comparison of extraction methods based on GC determination as total PAHs

Sample	Soxhlet[a] ppm (% R.S.D)	SFE[b] vs. Soxhlet % Rec (% R.S.D)	Methanol ext. vs. Soxhlet[c] % Rec (% R.S.D)
Sample 1	173 (12)	104 (10)	43 (34)
Sample 2	37 (3)	86 (2)	69 (18)
Sample 3	14 (2)	74 (6)	39 (11)
Sample 4	7.8 (1)	115 (9)	68 (21)

[a] Total sum of PAH concentrations (except dibenzothiophene and carbazole) determined by Soxhlet and GC–FID (Table 3).
[b] SFE field extractions (20 min). Recovery in % is based on the Soxhlet values. Relative standard deviation (% R.S.D) is based on the total PAH concentration found in each individual extract (triplicate extraction).
[c] ELISA extraction kit using methanol and a soil dispersing agent, minimum 5 min extraction and 5 min to let the sample settle. Recovery and % R.S.Ds as for SFE.

have the same response. For PCBs, the determinations are performed as 'total Aroclor'. Although each PCB congener and Aroclor mixture will likely have different ELISA responses, it has been reported that Aroclor 1248, 1254, and 1260 have response factors that are very close (±10%), and that Aroclor 1016, 1232, 1242, and 1268, have response factors between 1/2 and 1/3 of the response obtained for Aroclor 1254. Only Aroclor 1221 gave a value of less than 1/10 of Aroclor 1254 [13]. Therefore, ELISA responses for PCBs seem reasonable as long as total Aroclor (not individual speciation) data is sufficient and the test is calibrated with an Aroclor having a similar response to the contaminant PCBs.

In contrast to PCBs, PAHs were not produced as standardized commercial mixtures, and are usually determined as individual compounds. Since the immunological response of the ELISA kit for PAHs was developed against phenanthrene, we determined the relative response of several representative PAHs commonly found in contaminated soils (e.g., from coal tar). As shown in Table 7, phenanthrene and fluoranthene account for the highest ELISA responses, while the higher molecular weight, and generally more mutagenic PAHs show very little ELISA response. Therefore, ELISA determinations will not accurately reflect the concentrations of the higher molecular weight PAHs unless their % composition in each sample is the same. For example, phenanthrene and fluoranthene, which have the highest response factors of the PAHs and, therefore, dominate the response in the ELISA determination, constitute 40%, 40%, 29% and 11% of the total PAHs as listed in Table 4, respectively. For the same samples, benzo[*b*]- and benzo[*k*]fluoranthene, and benzo[*ghi*]perylene (three of the mutagenic PAHs [27] that have virtually no response in the immunoassay determination) constitute 5.3%, 11%, 14% and 23% of the total PAHs, respectively. Because the ELISA kit used in this study has much higher sensitivity towards the dominant non-mutagenic PAHs, the mutagenicity, as well as the total PAH concentration of some samples are likely to be underestimated using this technique.

Because of the large range in relative responses found for PAHs, field determinations of PAHs by ELISA were not performed. However, as discussed above, the relative responses of PCBs as total Aroclors is reasonably constant. Therefore, the PCB

Table 7
ELISA relative response factors of different PAHs.

Compound	Response factor[a]
Naphthalene	<0.002
Phenanthrene	1.00
Anthracene	0.65
Fluoranthene	1.9
Pyrene	0.5
Benz[*a*]anthracene	0.4
Benzo[*a*]pyrene	0.5
Benzo[*k*]fluoranthene	<0.005
Benzo[*ghi*]perylene	<0.002
2-Methyl anthracene	<0.01
1-Methyl phenanthrene	<0.01
Carbazole	<0.01
Dibenzofuran	<0.01
Dibenzothiophene	<0.01

[a] Relative response factors determined by ELISA calibrated with phenanthrene. Results are the average of duplicate determinations which typically agreed with in 10%.

Table 8
Comparison of GC and ELISA for the analysis of SFE field extracts as total PCBs (Aroclor 1260)

Sample	GC–ECD[a] ppm (% R.S.D)	ELISA[a] ppm (% R.S.D)	ELISA vs GC %
Sample 4	5.9 (6)	6.0 (14)	102
Sample 5	4.1 (10)	6.1 (54)	146
Sample 6	16 (11)	7.7 (36)	49
Sample 7	9.1 (17)	7.3 (10)	81
Sample 8	0.74 (9)	0.37 (30)	50
Sample 9	5.5 (6)	4.4 (29)	80

[a] Mean and % R.S.D from determination of 3 sample extracts.

ELISA kit was further tested for the determination of PCBs in SFE extracts. Initial laboratory tests showed comparable results for PCBs with the SFE extracts of the soil samples used for method development, when the extracts were measured as total Aroclor by ELISA and GC–ECD. The immunoassay kit for PCBs was then used on the field trip to Manitoba Hydro where all determinations were performed outdoors on the SFE extracts. Unfortunately, all field determinations with ELISA failed because the color reaction (horseradish-peroxidase enzyme reaction) was strongly overdeveloped (giving rise to a ≈ 10 times overestimation of the PCB concentration in the samples) even though all specifications of the kit were followed in detail. The reason for this failure seemed to be the presence of UV-light in combination with elevated temperatures (bright sunshine and 28–29°C). This was later confirmed in laboratory experiments. Because of this failure, the samples were re-analyzed by ELISA in the laboratory at the same time as the extracts were analyzed by GC–ECD. The results are shown in Table 8. The results of the ELISA determination range from 50 to 146% when compared with GC and the relative standard deviation of the ELISA determination is significantly higher than for GC (see Table 8). The linear regression correlation coefficient (r^2)was only 0.4, which shows very poor agreement between GC–ECD and ELISA.

4. Conclusion

A single SFE method using pure CO_2 at 150°C and 400 bar for 20 min gave good quantitative agreement with 24-h Soxhlet extractions for PAH- and PCB-contaminated soils. The method was reliable when performed under field conditions using a commercially-available extractor with only a portable generator for field support. A laboratory gas chromatograph with an FID detector (GC–FID) also performed well for quantitative determinations of extracted PAHs on portable generator power. Since the time required between arriving on the site and beginning extractions is <30 min, extractions can be performed at several sites per day. ELISA performed under laboratory conditions gave reasonable agreement with GC–ECD for determining total PCBs in SFE extracts, but failed to yield reasonable results under field (outdoor) conditions.

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References

[1] T.L. Francoeur, Environmental Lab, July 1995, p. 27–29.
[2] S. Bøwadt, S.B. Hawthorne, J. Chromatogr. A. 703 (1995) 549–571.
[3] M.E.P. McNally, Anal. Chem. 67 (1995) 308–315.
[4] V. Janda, K.D. Bartle, A.A. Clifford, J. Chromatogr. 642 (1993) 283–299.
[5] S.B. Hawthorne, D.J. Miller, Anal. Chem. 66 (1994) 4005–4012.
[6] S. Bøwadt, B. Johansson, Anal. Chem. 66 (1994) 667–673.
[7] J.L. Snyder, R.L. Grob, M.E. McNally, T.S. Oostdyk, J. Chromatogr. Sci. 31 (1993) 183.
[8] S. Bøwadt, B. Johansson, S. Wunderli, M. Zennegg, L.F. de Alencastro, D. Grandjean, Anal. Chem. 67 (1995) 2424–2430.

[9] a: EPA method 3560, Supercritical Fluid Extraction of Total Recoverable Petroleum Hydrocarbons, SW-846 update III, 1995. b: EPA Method 3561, Supercritical Fluid Extraction of Polynuclear Aromatic Hydro-carbons, SW-846 update III, 1995. c: EPA Method 3562, Supercritical Fluid Extraction of Polychlorinated Biphenyls and Organochlorine Pesticides, scheduled for future update of SW-846.
[10] S. Bøwadt, B. Johansson, F. Pelusio, B.R. Larsen, C. Rovida, J. Chromatogr. A. 662 (1994) 424–433.
[11] F. David, M. Verschuere, P. Sandra, Fresenius' J. Anal. Chem. 344 (1992) 479–485.
[12] S. Bøwadt, B. Johansson, P. Fruekilde, M. Hansen, D. Zilli, B. Larsen, J. de Boer, J. Chromatogr. A. 675 (1994) 189–204.
[13] V. Lopez-Avila, C. Charan, J.M. Van Emon, Environ. Test. Anal. May/June (1994) 34–37.
[14] B. Lesnik, P. Marsden, Environmental Lab, July 1995, p. 16–26.
[15] V. Lopez-Avila, J. Benedicto, C. Charan, R. Young, W.F. Beckert, Environ. Sci. Technol. 29 (1995) 2709–2712.
[16] S. Bøwadt, B. Larsen, J. High Resolut. Chromatogr. 15 (1992) 350–351.
[17] CFR Title 40, Protection of Environment, subpart G-PCB Spill Cleanup Policy (Part 761.120–761.135).
[18] Y. Yang, A. Gharaibeh, S.B. Hawthorne, D.J. Miller, Anal. Chem. 67 (1995) 641–646.
[19] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 67 (1995) 1727.
[20] A. Meyer, W. Kleibohner, J. Chromatogr. A. 657 (1993) 327–335.
[21] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 65 (1993) 338–344.
[22] S. Bøwadt, K. Hartonen, S.B. Hawthorne, D.J. Miller, K.H. Grobecker, Proceedings of Pittcon '95, no. 618, New Orleans, LA, March 1995.
[23] H.B. Lee, T.E. Peart, R.L. Hong-You, D.R. Gere, J. Chromatogr. A. 653 (1993) 83–91.
[24] J.J. Langenfeld, M.D. Burford, S.B. Hawthorne, D.J. Miller, J. Chromatogr. 594 (1992) 297.
[25] M.D. Burford, S.B. Hawthorne, D.J. Miller, T. Braggins, J. Chromatogr. 609 (1992) 321.
[26] K.S. Nam, J.W. King, J. Agric. Food Chem. 42 (1994) 1469.
[27] M.L. Lee, M.V. Novotny, K.D. Bartle, Analytical Chemistry of Polyaromatic Compounds, Academic Press, New York, 1981, Appendix 5.