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Mini-round-robin study of a supercritical fluid extraction method for polynuclear aromatic hydrocarbons in soils with dichloromethane as a static modifier

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Abstract

A mini-round-robin study of a supercritical fluid extraction (SFE) method for polynuclear aromatic hydrocarbons (PAHs) from soil samples was conducted. Three laboratories participated in the study, and each laboratory extracted three real-world samples in triplicate. The cryogenically milled samples were extracted at 350 atm (1 atm = 101 325 Pa) and 90°C for 20 min in the dynamic mode using supercritical carbon dioxide at a flow-rate of 1 to 1.5 ml/min and the extracted material was collected in 10 ml dichloromethane, which was then subjected to silica chromatography. The SFE method accuracy (percent recovery) was determined relative to the sonication extraction since the true levels of PAHs in these samples are not known. The PAHs were recovered quantitatively (recovery > 80%) by SFE when present at concentrations of 1 mg/kg or higher. The interlaboratory method precisions (overall R.S.D.s) appear to be concentration-dependent; at concentrations above 1 mg/kg, they were 27% or lower; at concentrations below 1 mg/kg, they ranged from 19 to 80%. From these results, we concluded that the method appears quite rugged, and the interlaboratory data compare well with other SFE interlaboratory studies.

1. Introduction

The extraction of organic pollutants from environmental matrices is a crucial step in their determination. The technique chosen for sample extraction should, to the extent possible, yield quantitative recoveries of the target analyte(s), be selective, not generate large volumes of waste solvents, require few steps in sample and extract handling, and be inexpensive. One such technique that has generated much interest in the last few years is supercritical fluid extraction (SFE).

The purpose of our study was to select one of the SFE methods that were published in the literature, which appears promising to work on all commercial SFE systems, and to evaluate it. Of the 10 literature references that deal specifically with the extraction of polynuclear aromatic

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hydrocarbons (PAHs) by SFE [1–10], we selected four promising methods [1–4] for closer scrutiny. A brief description of each method follows.

In the method by Dankers *et al.* [1], which we evaluated in this mini-round-robin study, a cryogenically milled sample (5 g) is extracted with supercritical carbon dioxide at 350 atm (1 atm = 101 325 Pa) and 90°C for 20 min (dynamic). Dichloromethane (2 ml) is added as a static modifier to the sample immediately prior to extraction, and the extracted material is collected in dichloromethane and is then analyzed by GC–MS.

The procedure by Lee *et al.* [2] uses three consecutive extractions. The sample (1 g plus 0.5 ml water), to which has been added 500 μl of methanol–dichloromethane (1:1), is first extracted with supercritical carbon dioxide at 336 atm for 7 min (2 min static, 5 min dynamic) at 120°C, and a flow-rate of 4 ml/min (as liquid). The PAHs are collected on a C_{18}-bonded silica trap held at 15°C and are rinsed from the trap with either 1.5 ml isooctane–dichloromethane (1:3) for GC–MS analysis or with 1.5 ml tetrahydrofuran–acetonitrile (1:1) for HPLC analysis. The extraction is continued at 336 atm and 120°C with carbon dioxide modified with 1% methanol and 4% dichloromethane at 2 ml/min for 31 min (1 min static, 30 min dynamic), and then with carbon dioxide alone for 2.5 min at 4 ml/min. After extraction, the trap is rinsed with an additional 1.5 ml and then 1.2 ml of the solvent (indicated above) for GC–MS or HPLC analysis.

The method reported by Gere *et al.* [3] also uses three steps. In step 1, extraction is performed with supercritical carbon dioxide at 119 atm and 120°C (2 min static, 10 min dynamic) at a flow-rate of 2 ml/min; the extracted material is collected on a C_{18}-bonded silica trap held at 5°C and is subsequently rinsed from the trap with 0.8 ml of tetrahydrofuran–acetonitrile (1:1). The extraction is then continued at 333 atm and 120°C with carbon dioxide–methanol–water (95:1:4) (1 min static, 30 min dynamic) at a flow-rate of 4 ml/min. During step 2 of the extraction, the trap temperature is 80°C (to prevent modifier from condensing onto the trap), and the nozzle temperature is kept at 45°C. In step 3 of the extraction, pressure and temperature remain the same as in step 2, but the fluid used is carbon dioxide. The material collected on the trap is rinsed off with 0.8 ml of tetrahydrofuran–acetonitrile (1:1) and analyzed by HPLC. To make the SFE method compatible with GC analysis, Gere *et al.* recommend using carbon dioxide–methanol–dichloromethane (95:1:4) in step 2 and rinsing the extracted material from the trap with methanol–dichloromethane (50:50).

Levy *et al.* [4] reported experiments performed at 75°C and three pressures (250, 350 and 450 atm) and at 475 atm and three temperatures (40, 100 and 150°C) and concluded that the highest extraction efficiencies for PAHs were achieved at 450 atm and 150°C. However, this method has not yet been sufficiently validated with real-world samples and, thus, was not considered in our study.

Refs. 5–10 discuss applications that deal with extraction of PAHs from various environmental matrices by SFE, but because they have not been fully optimized or validated, they did not appear to be relevant to this study.

Following the literature review, we concluded that the method of Dankers *et al.* would work on any of the commercial SFE systems and, thus, we subjected it to the mini-round-robin study.

2. Experimental

2.1. Materials

Analytical reference standards of the 16 PAHs were obtained as a composite solution in dichloromethane–benzene (50:50) (concentration 2 mg/ml per compound) from Supelco (Bellefonte, PA, USA). Purities were stated to be higher than 98.2%. Working calibration standards at 1, 5, 10, 25, 50 and 100 ng/μl were prepared by dilution of the composite stock solution with dichloromethane. Five deuterated compounds ([$^2H_8$]naphthalene, [$^2H_{10}$]acenaphthene, [$^2H_{10}$]phenanthrene, [$^2H_{12}$]chrysene and [$^2H_{12}$]perylene) were used as internal standards; they were also obtained as a composite stock solution in dichloromethane (concentration 2

mg/ml per compound, purity > 99%). Of this composite stock solution, 20 μl were spiked into every standard, and 10 μl of the composite stock solution were spiked into every sample extract immediately prior to GC–MS analysis (note that the sample extracts were concentrated to 0.5 ml).

The three samples used in this study, identified as samples A, B and C, were non-spiked, real-world soil samples, randomly chosen from samples analyzed at the BCO Centre for Research, Breda, Netherlands. Sample A was a sandy material from a polluted industrial site with 85% dry residue, sample B was a non-polluted soil with 87% dry residue and sample C was a highly contaminated soil sample from a polluted industrial site with 94% dry residue. Each sample was subjected to cryogenic milling before extraction (done at laboratory 1) as follows: 100 g anhydrous sodium sulfate, cooled to 4°C and contained in a 500-ml polyethylene bottle, and 100 g sample were mixed in this bottle with a spatula. The polyethylene bottle was then placed in a Dewar flask filled with liquid nitrogen; after 10 min the contents of the bottle were transferred to a stainless-steel cryogenic homogenizer (Model 300A; ProScientific, Monroe, CT, USA) and mixed for approximately 30 s. After a brief shake, the grinding was repeated for an additional 30 s. The milled sample was sieved through a 1-mm mesh size sieve and was then split into two 20-g portions and one 60-g portion. One 20-g portion was kept by laboratory 1, the other 20-g portion was sent to laboratory 3 and the 60-g portion was sent to laboratory 2 (this laboratory extracted the three samples in parallel by SFE and sonication extraction).

SFE-grade carbon dioxide (Air Products, Allentown, PA, USA) was used for extraction by laboratories 1 and 2. Laboratory 3 used supercritical fluid chromatography-grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, USA).

2.2. SFE procedure

All extractions were performed with an Isco (Lincoln, NE, USA) dual-chamber extraction module (Model SFX 2-10) and Isco Model 260D pump operated in the constant-pressure mode. The extraction conditions were 350 atm, 90°C, 20 min dynamic. For laboratories 1 and 2, the flow-rate of the carbon dioxide was controlled by a stainless-steel capillary (37 cm × 50 μm I.D.) and was approximately 1.5 ml/min (as liquid). Laboratory 3 used a variable restrictor (prototype from Isco) and reported a flow-rate of approximately 1 ml/min. To prevent plugging during extraction, the restrictor was heated at 100°C (except laboratory 3 at 60°C), and the collection vial (initially filled with 10 ml dichloromethane) was kept in a small beaker with water at 30°C. A 10-ml disposable extraction cartridge was used to extract a 5-g milled sample. Immediately prior to extraction, 2 ml dichloromethane were added to the sample directly in the extraction vessel. The cartridge was first pressurized to 350 atm before the outlet valve of the extractor was opened to avoid immediate removal of dichloromethane by the extraction fluid. The reader is cautioned that if full pressurization of the extraction cartridge is not reached before the outlet valve is opened, recoveries could be much lower that those reported here since the modifier does not contact the sample at full pressure.

2.3. Sonication extraction

Extractions using a sonic probe (Sonifier 450; Branson Ultrasonics, Danbury, CT, USA) were performed with 30-g portions of each milled sample mixed with 60 g anhydrous sodium sulfate. The resulting mixtures were sonicated for 3 min at 50% power (output setting 10) with 100 ml dichloromethane–acetone (1:1) and then decanted; the extraction was repeated twice with 100 ml fresh solvent. The decanted extracts were filtered through Whatman 31 filter paper and combined, the solvent was exchanged to hexane, and the hexane solution was concentrated to 1 ml. A silica gel procedure using 1.8 g silica gel (80–150 μm mesh; EM Science, Gibbstown, NJ, USA), activated for 16 h at 130°C prior to use, was used to clean up the extracts. The first fraction that was eluted with 10 ml hexane was discarded. PAHs were then eluted from the silica gel column with 10 ml hexane–dichloromethane

(60:40). This fraction was concentrated to 0.5 ml. The silica procedure was verified prior to use to ensure that quantitative recoveries (>90%) of the target compounds were obtained under these conditions.

2.4. GC–MS analyses

All GC–MS analyses were performed by laboratory 2 on a Hewlett-Packard (Wilmington, DE, USA) 5890 Series II gas chromatograph interfaced to a 5971A mass-selective detector and a Hewlett-Packard DOS Chemstation. The column used was a Supelco PTE-5 fused-silica capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness). The column temperature was held at 75°C for 3 min, then programmed at 12°C/min to a final temperature of 300°C. Helium at a linear velocity of 39 cm/s was used as carrier gas. The injector temperature was held at 250°C, the transfer line temperature at 280°C and the ion source at 188°C. The mass spectrometer was scanned from 40 to 500 u at a rate of 1.6 s/scan. All 1-μl injections were performed in the splitless mode (splitless time 1 min). Quantitation was performed using internal standard calibration.

2.5. Quality control procedures

The GC–MS analyses were performed according to method 8270 of the US Environmental Protection Agency (EPA) [11] for semivolatile organics. To ensure the quality of the data generated, the following quality control procedures were implemented: (1) all extracts were analyzed by one laboratory (laboratory 2); (2) SFE system blanks were performed by each laboratory; no PAHs were detected in these blanks; (3) the GC–MS system was tuned to meet the decafluorotriphenylphosphine (DFTPP) specifications; (4) a six-level calibration (using standards at 1, 5, 10, 25, 50 and 100 ng/μl) was performed daily, during sample analysis; when the response factors did not meet the criteria specified in EPA Method 8270, then either the multilevel calibration was repeated or fresh standards were prepared; (5) five internal standards ([$^2H_8$]naphthalene, [$^2H_{10}$]acenaphthene, [$^2H_{10}$]phenanthrene, [$^2H_{12}$]chrysene and [$^2H_{12}$]perylene) were spiked into every sample extract immediately prior to GC–MS analysis; the areas of the quantitation ions of the five internal standards were monitored during every 12-h period to ensure that they were within −50/ + 100% of the corresponding areas established for the mid-level calibration standard; any sample extracts for which the internal standards fell outside the quality control criteria were reanalyzed; (6) a GC–MS column blank was performed before any batch of sample extracts was analyzed to ensure the cleanliness of the system; (7) sample extracts that were found to contain concentrations in excess of 100 ng/μl were diluted and reanalyzed; and (8) compounds known to be present in the sample extracts (from previous data acquired on that particular sample) but not detected by the automated processing routines were searched for manually.

3. Results and discussion

3.1. Method accuracy

Tables 1–3 present the concentrations of the 16 compounds found in the three soils by sonication extraction–GC–MS and the recoveries using SFE–GC–MS. The SFE data are presented by laboratory as the individual average recoveries (method accuracy) and R.S.D.s (method precision), and the overall method accuracy and precision. The SFE recoveries were determined relative to the sonication data since the sonication method is an approved EPA procedure (EPA method 3550 [12]).

For sample A (Table 1), which was known to be highly polluted, the SFE recoveries looked almost as good as one might expect from a freshly spiked sample. From the 45 values (3 laboratories × 15 compounds) reported in Table 1 as average recoveries for the three laboratories, there was one value below 80% (for benzo[*ghi*]perylene), and three values were exceeding 120% (for naphthalene); the remainder of

Table 1
SFE method performance for sample A

Compound	Concentration by sonication (mg/kg)[a]	Laboratory 1[b]		Laboratory 2[b]		Laboratory 3[b]		Overall average recovery (%)	Overall R.S.D. (%)
		Average recovery (%)	R.S.D (%)	Average recovery (%)	R.S.D. (%)	Average recovery (%)	R.S.D. (%)		
Naphthalene	0.16	158	61	131	50	125	30	138	48
Acenaphthylene	1.79	93.1	6.0	93.8	0.6	86.4	5.9	91.1	5.8
Acenaphthene	0.42	103	16	94.0	33	79.9	11	92.4	23
Fluorene	2.00	103	7.4	98.4	8.0	87.3	4.2	96.4	9.6
Phenanthrene	28.8	102	15	103	11	96.8	7.6	101	11
Anthracene	4.33	105	6.4	97.6	8.5	94.5	1.1	98.9	7.2
Fluoranthene	44.7	102	16	101	11	96.5	8.5	99.9	11
Pyrene	35.4	103	16	98.6	9.5	94.4	8.6	98.8	12
Benzo[*a*]anthracene	13.2	107	2.6	98.5	1.3	99.5	4.3	102	4.7
Chrysene	15.4	105	2.6	96.6	1.7	97.3	3.1	99.5	4.5
Benzo[*b* + *k*]fluoranthene[c]	22.6	108	1.1	97.1	2.0	104	3.8	103	5.0
Benzo[*a*]pyrene	13.2	107	1.2	98.2	1.3	101	4.2	102	4.5
Indeno[1,2,3-*cd*]pyrene	8.44	99.7	3.7	103	2.7	82.1	5.5	94.9	11
Dibenzo[*a*,*h*]anthracene	2.59	95.0	2.1	97.1	5.1	86.7	5.8	92.9	6.5
Benzo[*ghi*]perylene	9.06	81.2	5.0	100	3.3	74.9	6.3	85.4	14

[a] Single determination.
[b] The number of replicates was four. The SFE recoveries were determined relative to the sonication data.
[c] Benzo[*b*]fluoranthene and benzo[*k*]fluoranthene could not be chromatographically resolved, so the results reported are the sums of the concentrations of both compounds or the total recoveries for both compounds.

Table 2
SFE method performance for sample B

Compound	Concentration by sonication (mg/kg)[a]	Laboratory 1[b]		Laboratory 2[b]		Laboratory 3[b]		Overall average recovery (%)	Overall R.S.D. (%)
		Average recovery (%)	R.S.D (%)	Average recovery (%)	R.S.D. (%)	Average recovery (%)	R.S.D. (%)		
Phenanthrene	0.22	56.6	31	49.8	10	52.7	8.1	53.0	19
Anthracene	0.04	50.1	37	43.0	18	50.0	13	47.7	24
Fluoranthene	0.36	69.8	31	48.7	10	50.7	8.0	56.4	27
Pyrene	0.23	72.7	40	46.5	15	52.0	13	57.1	35
Benzo[*a*]anthracene	0.09	80.1	47	49.8	19	57.0	18	62.3	40
Chrysene	0.15	62.1	37	48.5	13	45.7	18	52.1	29
Benzo[*b* + *k*]fluoranthene[c]	0.13	87.8	58	47.5	25	55.7	24	63.7	53
Benzo[*a*]pyrene	0.04	170	69	61.6	28	84.2	40	105	77

[a] Single determination. The other PAH compounds reported in Tables 1 and 3 were not detected in this sample.
[b] The number of replicates was four. The SFE recoveries were determined relative to the sonication data.
[c] Benzo[*b*]fluoranthene and benzo[*k*]fluoranthene could not be chromatographically resolved, so the results reported are the sums of the concentrations of both compounds or the total recoveries for both compounds.

Table 3
SFE method performance for sample C

Compound	Concentration by sonication (mg/kg)[a]	Laboratory 1[b]		Laboratory 2[b]		Laboratory 3[b]		Overall average recovery (%)	Overall R.S.D. (%)
		Average recovery (%)	R.S.D (%)	Average recovery (%)	R.S.D. (%)	Average recovery (%)	R.S.D. (%)		
Naphthalene	6.49	106	32	126	22	89.1	18	107	27
Acenaphthylene	2.33	75.7	10	90.1	5.3	84.7	6.9	83.5	10
Acenaphthene	0.77	106	7.2	94.2	8.4	92.9	5.9	97.7	9.2
Fluorene	3.39	106	14	98.9	6.6	100	10	102	10
Phenanthrene	23.7	97.4	15	121	7.3	131	23	117	20
Anthracene	5.08	88.8	14	93.2	6.4	92.0	7.8	91.3	9.1
Fluoranthene	31.2	98.1	11	122	10	122	25	114	19
Pyrene	21.9	104	11	121	8.2	124	24	116	17
Benzo[*a*]anthracene	10.7	95.9	8.9	88.1	3.7	87.3	7.6	90.4	7.9
Chrysene	13.9	100	8.7	92.5	6.5	89.1	9.0	94.0	9.0
Benzo[*b* + *k*]fluoranthene[c]	24.2	96.0	11	90.6	6.1	99.5	12	95.4	10
Benzo[*a*]pyrene	10.0	98.3	9.0	93.1	5.9	88.0	9.1	93.1	8.8
Indeno[1,2,3-*cd*]pyrene	5.29	73.2	2.2	85.3	8.0	60.5	11	73.0	16
Dibenzo[*a*,*h*]anthracene	1.83	71.9	4.6	90.0	4.7	64.3	11	75.4	16
Benzo[*ghi*]perylene	5.18	55.0	5.1	82.0	8.2	54.7	13	63.9	22

[a] Single determination.
[b] The number of replicates was four. The SFE recoveries were determined relative to the sonication data.
[c] Benzo[*b*]fluoranthene and benzo[*k*]fluoranthene could not be chromatographically resolved, so the results reported are the sums of the concentrations of both compounds or the total recoveries for both compounds.

the individual average recoveries ranged from 80 to 108%. The overall average recoveries ranged from 85.4 to 138%. The high recovery of naphthalene by SFE may be a consequence of the higher losses during sonication extraction because of the heat released during the 3-min sonication in an open vessel.

For sample B (Table 2), which was known to be relatively clean, the individual average recoveries were low, ranging from 43.0 to 87.8% (excluding two values over 100% for benzo[*a*]pyrene); the overall average recoveries ranged from 47.7 to 105%. This is not surprising since the levels that we detected by GC–MS were at or below 1 ng/μl, where the precision of the measurement was approximately ± 20%.

For sample C (Table 3), which was also a highly polluted soil, the individual average recoveries ranged from 54.7 to 131%. From the 45 values given in Table 3 for the individual average recoveries, seven values were below 80% and seven were above 120%. The overall average recoveries ranged from 63.9 to 117%, with three values being below 80%. The three compounds for which we had low but still reasonable recoveries were indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene.

3.2. Method precision

Method precision is reported (Tables 1–3) for each compound as the R.S.D. for each laboratory and also as the overall R.S.D. for the three laboratories. In general, as expected, the R.S.D.s for the individual laboratories were lower than the overall R.S.D.s, and the lower the concentration of the target analyte was in the sample, the higher was the R.S.D. For example, for sample A, the individual R.S.D.s were below 10% for most compounds (specifically, from the 45 values reported in Table 2 for R.S.D., 34 values were below 10%), whereas only half of the overall R.S.D.s were below 10%. In the case of naphthalene, which had the lowest concentration in this sample, the individual R.S.D.s were 61, 50 and 30% for laboratories 1, 2 and 3, respectively; these values were significantly higher than the R.S.D.s for the other compounds. Acenaphthene also exhibits a high R.S.D., and its concentration is almost two orders of magnitude lower than some of the other compounds present in that sample.

4. Conclusions

The results of this mini-round-robin study, in which three laboratories participated, indicate that PAHs can be extracted with better than 80% recoveries by SFE when present at concentrations of 1 mg/kg or higher. At lower concentrations (<0.2 mg/kg), average recoveries ranged from 48 to 105%, with most values in the range of 50 to 60%. The interlaboratory method precisions (overall R.S.D.s) also appear to be concentration dependent; at concentrations above 1 mg/kg, they were 27% or lower; at concentrations below 1 mg/kg, they ranged from 19 to 80%. We correlated the results obtained by SFE for samples A and C with those obtained by sonication extraction with dichloromethane–acetone (1:1) (the SFE data were plotted on the *y*-axis) and found excellent correlation between SFE and sonication extraction (the slopes of the regression lines were 1.01 for sample A and 1.16 for sample C; the correlation coefficients were 0.999 and 0.991).

This study addressed the performance of the method of Dankers *et al.* [1] with a very limited number of samples and only three laboratories using the same type of SFE system. Nonetheless, the method appears to be quite rugged, and the interlaboratory data compare well with those from the interlaboratory study of the SFE method for petroleum hydrocarbons [13].

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References

[1] J. Dankers, M. Groenenboom, L.H.A. Scholtis and C. van der Heiden, *J. Chromatogr.*, 641 (1993) 357.

[2] H.-B. Lee, T.E. Peart, R.L. Hong-You and D.L. Gere, *J. Chromatogr.*, 653 (1993) 83.

[3] D.R. Gere, C.R. Knipe, P. Castelli, L.G. Randall Frank, H. Schulenberg-Schell, R. Schuster, L. Doherty, J. Orolin and H.-B. Lee, *J. Chromatogr. Sci.*, 31 (1993) 246.

[4] J.M. Levy, L.A. Dolata and R.M. Ravey, *J. Chromatogr. Sci.*, in press (1993).

[5] M.M. Schantz and S.N. Chester, *J. Chromatogr.*, 363 (1986) 397.

[6] S.B. Hawthorne and D.J. Miller, *J. Chromatogr. Sci.*, 24 (1986) 258.

[7] S.B. Hawthorne and D.J. Miller, *Anal. Chem.*, 59 (1987) 1705.

[8] B.W. Wright, C.W. Wright, R.W. Gale and R.D. Smith, *Anal. Chem.*, 59 (1987) 38.

[9] S.B. Hawthorne, D.J. Miller and J.J. Langenfeld, *J. Chromatogr. Sci.*, 28 (1990) 2.

[10] V. Lopez-Avila, N.S. Dodhiwala and W.F. Beckert, *J. Chromatogr. Sci.*, 28 (1990) 468.

[11] *Test Methods for Evaluating Solid Waste, Physical/ Chemical Methods, SW-846*, US Environmental Protection Agency, Office of Solid Waste, Washington, D.C., 3rd ed., 1991, method 8270.

[12] *Test Methods for Evaluating Solid Waste, Physical/ Chemical Methods, SW-846*, US Environmental Protection Agency, Office of Solid Waste, Washington, D.C., 3rd ed., 1991, method 3550.

[13] V. Lopez-Avila, R. Young, R. Kim and W.F. Beckert, *J. AOAC Int.*, 76 (1993) 555.