Journal of Chromatography, 641 (1993) 357–362
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 111

High-speed supercritical fluid extraction method for routine measurement of polycyclic aromatic hydrocarbons in environmental soils with dichloromethane as a static modifier

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(First received December 18th, 1992; revised manuscript received March 22nd, 1993)

ABSTRACT

Supercritical fluid extraction (SFE) modified for the high-speed and efficient extraction of polycyclic aromatic hydrocarbons (PAHs) from polluted soil samples was evaluated and shown to be usable in a routine setting. On starting SFE, a small amount of dichloromethane is added to a chemically dried and cryogenic-ground soil sample. The SFE extract is collected within 15–20 min and the PAHs are determined by HPLC equipped with fluorescence and UV detectors. Within-day and day-to-day reproducibilities were comparable to those obtained after a 4-h sample preparation including liquid–liquid extraction. A good correlation was found between the PAH concentrations measured after modified SFE and liquid–liquid extraction. Recoveries of samples spiked with PAHs were of the order of 100%. In two samples used in a quality control programme, PAH concentrations were similar to those obtained by eleven other laboratories. The modified SFE procedure fulfils the requirements of rapidity, high extraction efficiency and simple performance.

INTRODUCTION

Much progress has been made in the last 10 years in instrumental chromatographic techniques. However, extractions of organic compounds from solids are still performed in traditional ways (liquid–liquid partitioning, Soxhlet extraction, etc.). These extraction techniques are time consuming (5–6 h) for routine applications, require the use of large volumes of mostly toxic organic solvents, and produce substantial amounts of waste solvents, and loss of volatile compounds may occur during evaporation.

Supercritical fluids have physical properties, such as low viscosity, high solute diffusive power and density-linked solvent strength, that make extraction selectivity and automation feasible. Therefore, supercritical fluid extraction (SFE) offers a promising alternative to traditional extraction techniques. Quantitative SFE procedures have already been reported for the extraction of various analytes from different matrices that are difficult to process [1–4]. SFE has been applied to the extraction of soil samples with and without a clean-up procedure [5], to sand samples spiked with nitroaromatic compounds, halo ethers and organochlorine pesticides, and to standard reference soils [6].

Attempts have been made to improve the extraction recoveries of the sixteen polycyclic aromatic hydrocarbons (PAHs) defined according to the US Environmental Protection Agency (EPA) from random samples of different types of

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0021-9673/93/$06.00

environmental soils by combining SFE with mostly dynamic modifiers or alternative extraction fluids [6–9]. Nevertheless, time-consuming and exhaustive extraction procedures are required to achieve an extraction efficiency of 90% [10].

This paper describes the application of SFE in daily routine practice for the extraction of PAHs from environmental soils. The results demonstrate that the addition of small amounts of a "static" modifier, dichloromethane, to the cryogenic-ground sample immediately before SFE extraction is begun is essential to achieve quantitative results. Dichloromethane has the power to penetrate the soil particles and render PAHs soluble in order to obtain quantitative extraction even of high-molecular-mass analytes from soil matrices.

EXPERIMENTAL

Supercritical fluid extraction (SFE)

SFE was performed using special SFE-grade carbon dioxide (Scott Speciality Gases, Breda, Netherlands) on an ISCO (Lincoln, NE, USA) modular SFE Series 2000 system. This consists of a dual solvent-pumping system (Model 260D) for programmable modifier addition or constant solvent delivery and two dual-chamber extraction systems (Model SFX 2-10), operated in the constant-pressure mode. Rapid and efficient SFE of PAHs was obtained with settings of density 0.76 g/ml, pressure 350 atm and temperature 90°C. Flow-rates were controlled by *ca.* 30-cm capillary restrictors (fused-silica tubing of 50 μm I.D.; Chrompack, Middelburg, Netherlands), resulting in pump flow-rates between 2 and 6 ml/min. SFE of PAHs was completed within 15–20 min. The capillary outlet was protected from blockage due to freezing of the extracted water by thermostating the collection vessel at 20°C. Extracted analytes were collected in a 15-ml conical vial (150 × 15 mm) containing 2–5 ml of dichloromethane (organic residue grade).

Liquid–liquid extraction

PAHs were also isolated by means of liquid–liquid partition. Cryogenic-homogenized samples were extracted twice with 100 ml of light petroleum (b.p. 30–60°C), evaporated with a Kuderna Danish apparatus and analysed under the same conditions according to the national standard procedure NEN 5731 [11].

Sample preparation

In order to obtain a representative sample, the sample material was cryogenic-ground and homogenized analogously to the national standard procedure NVN 5730 [11]. A minimum of 100 g of soil sample was chemically dried by adding dry sodium sulphate, chilled under liquid nitrogen, ground and sieved to less than 1-mm particles. Of this homogenate, 5 g were placed in a standard 10-ml sample cartridge. Immediately before SFE was started, 2 ml of dichloromethane was added to the homogenate.

HPLC analyses

HPLC analyses were performed on a Kratos Spectroflow 450 gradient system with serial fluorescence (HP 1046A) and UV detection (Kratos Spectroflow 757) under the following conditions: detection wavelength, fluorescence, 0–9 min, excitation at 275 nm, emission at 348 nm; 9–40 min, excitation at 254 nm, emission, 320 nm cut-off filter; UV absorbance, 230 nm.

The column used was Chromspher 5 PAH, stainless steel (150 × 4.6 mm I.D.), thermostated at 30°C. The column was eluted with an acetonitrile–water gradient at a flow-rate of 1.5 ml/min. The gradient was started with 50% acetonitrile (HPLC reagent grade) for the first 6 min, subsequently linearly programmed to 73% acetonitrile at a rate of 1.4%/min (6–22 min), increased to 100% acetonitrile at a rate of 2.2%/min (22–35 min) and finally held at 100% acetonitrile for the last 5 min.

Blank sample material

Blank sample material was prepared by heating soil originating from a non-contaminated land area at 600°C .

Quality control samples (QC samples)

Samples from interlaboratory quality control programmes were obtained from Wageningen Agricultural University (Netherlands) and used to test the efficiency of the extraction method.

Reference material

A standard reference material solution, SRM 1647B (National Institute of Standards and Technology, Gaithersburg, MD, USA) containing the sixteen EPA chosen priority PAH pollutants at certified concentrations, was diluted to appropriate concentrations in acetonitrile for calibration and spiking purposes. Retene purified by HPLC (ICN Biomedicals, Zoetermeer, Netherlands) was added to all extracts as an internal standard for peak identification (relative retention times).

RESULTS

Parameter settings for SFE such as density, as a consequence of pressure and temperature choice, and carbon dioxide volume (extraction time and flow-rate) were investigated to achieve a high extraction output. The efficiency of SFE in extracting the sixteen PAHs must at least equal that of liquid–liquid extraction. The percentage ratios of the PAHs isolated from actual soil samples by SFE and by liquid–liquid extraction are presented in Fig. 1. The SFE recoveries are poor (*ca.* 30%) for PAHs with molecular masses greater than that of pyrene. The SFE

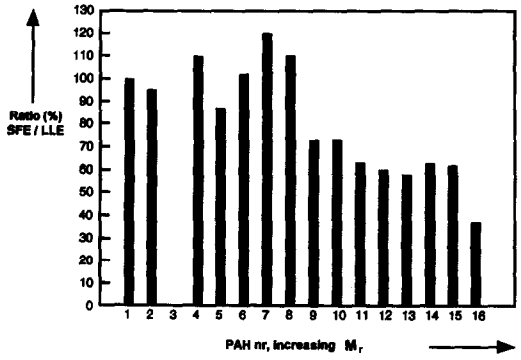

Fig. 1. Percentage ratios of sixteen individual PAHs from actual soil samples ($n = 6$) numbered according to increasing molecular mass (M_r) isolated by means of SFE and liquid–liquid extraction (LLE). 1 = Naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benzo[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = dibenz[*a,h*]anthracene; 15 = benzo[*ghi*]perylene and 16 = indeno[1,2,3-*cd*]pyrene. SFE conditions: carbon dioxide, density 0.77 g/ml; pressure, 270 atm; temperature, 70°C; time, 30 min.

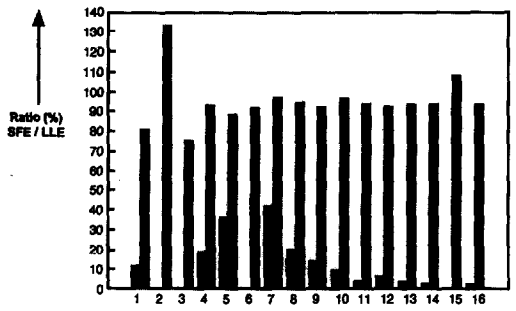

Fig. 2. Percentage ratios of sixteen PAHs determined in a random soil sample by means of HPLC after SFE with (black bars) and without (hatched bars) addition of the static modifier dichloromethane to the pretreated sample. SFE conditions: carbon dioxide, density 0.76 g/ml; pressure, 350 atm; temperature, 90°C; time, 15 min. Compounds as in Fig. 1.

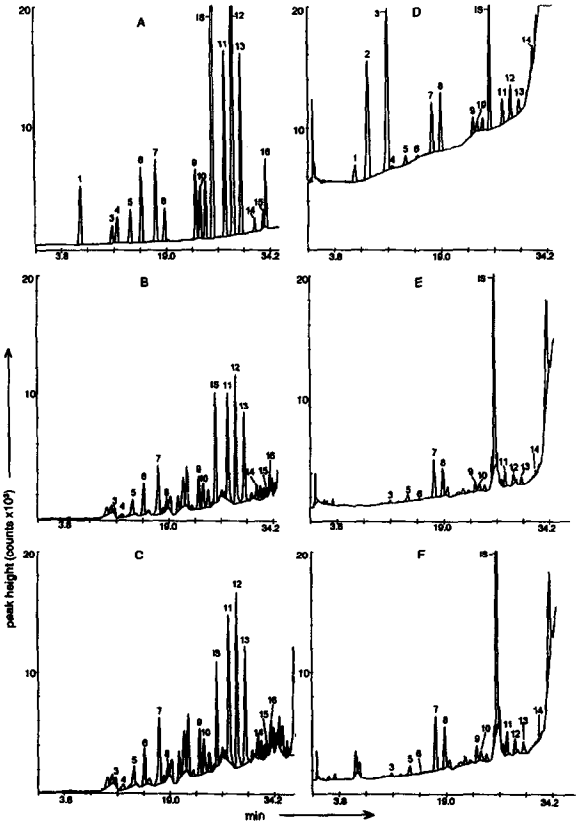

Fig. 3. Chromatograms of PAHs monitored with fluorescence detection from (A) an EPA standard sample, (B) a random soil sample obtained after SFE and (C) after liquid–liquid extraction with (D, E, F) their corresponding UV-monitored chromatograms.

parameter settings were set to values of carbon dioxide density 0.76 g/ml, pressure 350 atm and temperature 90°C. In the SFE procedure the dynamic modifier methanol was added. The results did not show a sufficient improvement, so a static modifier was tested. Dichloromethane was added to the cryogenic-ground sample immediately before SFE was started. Dichloromethane was also introduced as collecting solvent because of its higher solubility capacity than solvents such as acetonitrile or methanol. The SFE procedure could be ended after 15–20 min. Fig. 2 shows the percentage ratios of the 16 PAHs determined by HPLC after SFE with and without addition of dichloromethane as static modifier.

Chromatograms of the PAHs extracted from the EPA standard solution and from a random polluted soil sample are shown in Fig. 3. A good separation of the main PAHs in soil is obtained. The chromatographic patterns obtained after SFE and liquid–liquid extraction are fully comparable. Components isolated by means of SFE and interfering chromatographically with one or more of the individual PAHs are not observed.

Blank soil samples were spiked with PAHs at a level of 0.1 mg/kg. Recoveries of the individual PAHs ranged from 88 to 100% with a relative standard deviation of 2–15%.

The within-day and day-to-day reproducibilities were examined to establish the ruggedness of the SFE extraction procedure. In Table I, the results of the within-day and the day-to-day reproducibilities of PAHs determinations in two random samples are presented. With the exception of the three highest molecular mass PAHs with a low relative chromatographic response, the relative standard deviations of all other components are below 20%.

The PAHs were isolated from five random soil samples by SFE and by liquid–liquid extraction and analysed by HPLC. The PAH concentrations expressed in mg/kg dry mass were correlated. A good correlation was found ($n = 68$; regression coefficient = 0.914, y-axis intercept = 0.094 mg/kg dry mass, correlation coefficient = 0.959).

Two quality control samples from an interlaboratory quality control programme contained PAHs at concentrations of 4 and 10 mg/kg dry mass. The PAHs were isolated from these sam-

TABLE I

WITHIN-DAY AND DAY-TO-DAY REPRODUCIBILITIES OF PAH COMPOUNDS, EXPRESSED IN mg/kg DRY MATERIAL, ESTABLISED IN TWO RANDOM SAMPLES

Compound	Within-day						Day-to-day					
	Sample I ($n = 5$)			Sample II ($n = 5$)			Sample III ($n = 8$)			Sample IV ($n = 9$)		
	x	S.D.	R.S.D. (%)	x	S.D.	R.S.D. (%)	x	S.D.	R.S.D. (%)	x	S.D.	R.S.D. (%)
Naphthalene	0.32	0.06	20	9.2	1.6	17	0.18	0.04	20	7.6	1.5	20
Acenaphthylene	–	–	–	–	–	–	–	–	–	–	–	–
Acenaphthene	–	–	–	–	–	–	–	–	–	–	–	–
Fluorene	0.27	0.06	23	8.2	0.5	6	0.19	0.04	22	10.0	1.9	19
Phenanthrene	0.86	0.16	18	32.8	2.7	8	0.58	0.11	19	34.2	4.9	14
Anthracene	0.22	0.04	16	10.6	0.7	7	0.15	0.02	15	11.0	1.7	15
Fluoranthene	1.44	0 24	17	36.2	3.1	8	1.00	0.16	16	38.1	5.6	15
Pyrene	2.02	0 33	17	33.1	2.3	7	1.16	0.32	28	30.1	5.1	17
Benzo[*a*]anthracene	0.72	0.11	15	13.3	1.3	10	0.47	0.08	17	13.8	2.2	16
Chrysene	0.71	0.11	16	12.6	1.4	11	0.45	0.07	16	12.9	2.4	19
Benzo[*b*]fluoranthene	0.70	0.03	4	14.4	1.9	13	0.58	0.08	15	14.2	2.6	18
Benzo[*k*]fluoranthene	0.21	0 01	6	4.8	0.6	13	0.19	0 03	14	5 0	0.9	27
Benzo[a]pyrene	0.55	0.07	14	8.0	1.1	14	0.40	0.10	24	8.7	2.0	23
Dibenzo[*ah*]anthracene	1.05	0.17	16	23.1	3.5	15	0.56	0.18	33	19.5	5.2	27
Benzo[*ghi*]perylene	0.85	0.11	13	10.9	1.5	14	0.39	0.14	36	9.4	3.2	34
Indeno[1,2,3-*cd*]pyrene	0.23	0.03	14	2.9	0.4	15	0.24	0.04	16	4.1	1.3	33

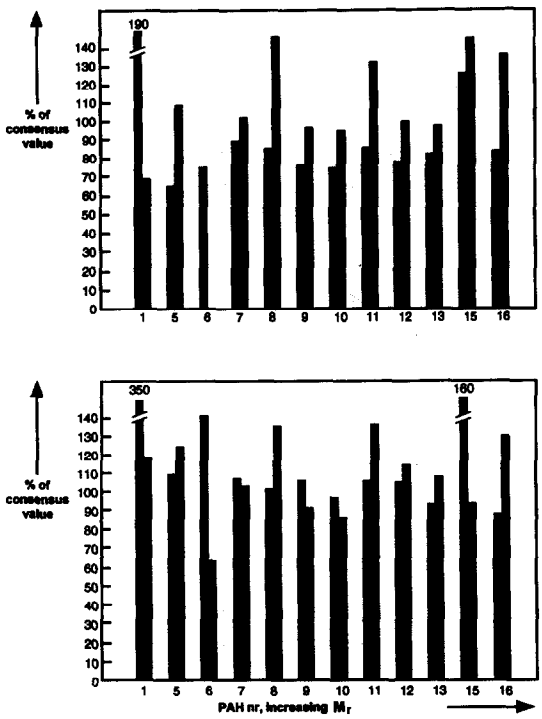

Fig. 4. Individual PAHs isolated from two quality control samples (top: 4 mg/kg dry mass; bottom: 10 mg/kg dry mass) by means of SFE (black bars) and liquid–liquid extraction (hatched bars) and expressed as percentages of the consensus value established by eleven independent laboratories. Compounds as in Fig. 1.

ples by SFE and liquid–liquid extraction. The PAHs were expressed as percentages of the consensus value of the concentrations established by the eleven participating laboratories (Fig. 4). The PAH concentrations measured after isolation by the modified SFE method in the extract are fully comparable with those obtained after a traditional extraction. Naphthalene is extracted even more efficiently by the modified SFE than by liquid–liquid extraction. It is known that loss of naphthalene may occur during evaporation after liquid–liquid extraction.

DISCUSSION

Soil clearance programmes demand new and modern adaptations of laboratory technology. PAH determinations should be capable of being performed rapidly in a routine setting with a low error level. These requirements can only be fulfilled if robust methods for extraction and instrumental measurement (*e.g.*, HPLC) of PAHs are available. HPLC is widely accepted for the determination of individual PAHs. Although highly efficient, the extraction of PAHs still takes many hours and is consequently the rate-limiting step in PAH analyses.

High speed and efficiency in a routine setting might be feasible when SFE is performed under well defined conditions. These conditions include pretreatment of the sample, optimum settings of SFE and the addition and mode of application of a modifier.

Wet sample material can be successfully extracted by SFE provided that it is granular and the water content is less than 40%. However, cryogenic grinding of a soil sample chemically dried with sodium sulphate is recommended. Pretreatment of samples minimizes the matrix effects caused by differences in water content.

The selection of the density and carbon dioxide volume and the choice of the modifier and collection solvent together with the mode of application of the modifier are important factors in the successful isolation of PAHs by SFE. Flow-rates and cell geometry have negligible effects on the extraction efficiency. Inappropriate solvent trapping conditions may result in losses which are wrongly attributed to poor SFE efficiency [12].

In our experiments all sixteen individual EPA PAHs show comparable recoveries, even within the extraction time of 15–20 min. Obtaining a rapid extraction and high recoveries that are independent of the PAH molecular mass is the main problem in all SFE experiments. The extraction of benzo[*a*]pyrene, a high-molecular mass PAH, has been reported to require 2 h of exhaustive extraction (density 0.76 g/l, 350 atm, 90°C) to achieve an extraction recovery of 90% [10]. Also, the matrix may influence the recovery. Actual environmental soils appeared to show interaction forces between analytes and their matrices [7,8], *e.g.*, in diesel fuel contamination of clay-like material [13]. Many modifiers and modes of application have been tried to achieve acceptable recoveries of PAHs over the whole range of molecular masses. Under vari-

able experimental conditions, Lopez-Avila *et al.* [6] used toluene as a modifier. Nevertheless, they found a wide range of recoveries, especially of the high-molecular-mass PAHs.

Addition of methanol to the carbon dioxide improves the recoveries of some PAHs. However, the larger compounds are only partially extracted [14]. The extraction recovery of the high-molecular-mass PAHs can be improved by extending the extraction time, but this is not acceptable when a high-speed extraction is desirable. Owing to its polar character, the dynamic modifier methanol has been shown to extract the drying reagent, sodium sulphate, from the chemically dried samples, causing clogging problems in the capillary restrictor. Hence the use of methanol is very impracticable.

The less polar dichloromethane, added as a static modifier in very small volumes (2 ml), has been shown to give nearly 100% recoveries of the PAHs that are independent of their molecular mass, and within a very short extraction time (15–20 min).

The static modifier dichloromethane has the power to penetrate the soil particles and to render the soil aggregates soluble. In this way, contact between the particles and the extractant is strongly increased. A similar process might occur in the liquid–liquid extraction. This penetration of particles is probably also the explanation for the difference between the extraction yields of PAHs from spiked and actual samples (14). Consequently, in the extraction procedure presented here, hardly any difference was found between the PAH concentrations determined with the two extraction procedures.

SFE also has the advantage of reducing considerably the large volumes of organic extraction fluids needed in liquid–liquid extraction.

Finally, it may be concluded that the modified SFE procedure presented here fulfils all the requirements for use in daily routine practice, *viz.*, simplicity, rapid performance and a high extraction efficiency.

ACKNOWLEDGEMENTS

The authors thank Mrs. A.Ch.M. Cantrijn and Mr. M.J.H. Zodenkamp for their help in preparing the manuscript.

REFERENCES

1 C.A. Thomson and D.J. Chesney, *Anal. Chem.*, 64 (1992) 848.
2 R.M. Smith and M.D. Burford, *J. Chromatogr.*, 600 (1992) 175.
3 W.H. Griest, R.S. Ramsey, C.-H. Ho and W.M. Caldwell, *J. Chromatogr.*, 600 (1992) 273.
4 H.-B. Lee, T.E. Peart and R.L. Hong-You, *J. Chromatogr.*, 605 (1992) 109.
5 B. Wenclawiak, C. Rathmann and A. Tenbie, *Fresenius' J. Anal. Chem.*, 344 (1992) 497.
6 V. Lopez-Avila and W.F. Beckert, in F.V. Bright and M.E.P. McNally (Editors), *Supercritical Fluid Technology —Theoretical and Applied Approaches to Analytical Chemistry* (*ACS Symposium Series*, No. 488), American Chemical Society, Washington, D.C., 1992, Ch. 14, pp. 179–205.
7 J.W. Hills and H.H. Hill, *J. Chromatogr. Sci.*, 31 (1993) 6.
8 S.B. Hawthorne, J.J. Langenfeld, D.J. Miller and M.D. Burford, *Anal. Chem.*, 64 (1992) 1614.
9 T. Paschke, S.B. Hawthorne, D.J. Miller and B. Wenclawiak, *J. Chromatogr.*, 609 (1992) 333.
10 L.J.D. Myer, J.H. Damian, P.B. Lieschеski and J. Tehrani, in F.V. Bright and M.E.P. McNally (Editors), *Supercritical Fluid Technology —Theoretical and Applied Approaches to Analytical Chemistry* (*ACS Symposium Series*, No. 488), American Chemical Society, Washington, D.C., 1992, Ch. 16, pp. 221–236.
11 *NNI Catalogus, Deel 1*, Nederlands Normalisatie Instituut (NNI), Delft, 1992, p. 376.
12 J.J. Langenfeld, M.D. Burford, S.B. Hawthorne and D.J. Miller, *J. Chromatogr.*, 594 (1992) 297.
13 A.P. Emery, S.N. Chesler and W.A. MacCrehan, *J. Chromatogr.*, 606 (1992) 221.
14 S.B. Hawthorne and D.J. Miller, *Anal. Chem.*, 57 (1987) 1705.