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Miniaturised pressurised liquid extraction of polycyclic aromatic hydrocarbons from soil and sediment with subsequent large-volume injection–gas chromatography

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Abstract

Analyte extraction is the main limitation when developing at-line, or on-line, procedures for the preparation of (semi)solid environmental samples. Pressurised liquid extraction (PLE) is an analyte- and matrix-independent technique which provides cleaner extracts than the time-consuming classical procedures. In the study, the practicality of miniaturised PLE performed in a stainless-steel cell, and combined with subsequent large-volume injection (LVI)–GC–MS was studied. As an example, the new system was applied to the determination of polycyclic aromatic hydrocarbons (PAHs) in soils and a sediment. Variables affecting the PLE efficiency, such as pressure and temperature of the extraction solvent and total solvent volume, were studied. Toluene was selected as extraction solvent and a total solvent volume of 100 μl was used for the 10 min static-dynamic PLE of 50-mg samples. Additional clean-up or filtration of the sample extracts was not required. Detection limits using LVI–GC–MS were below 9 ng/g soil for the 13 PAHs more volatile than indeno[1,2,3-*cd*]pyrene in real soil samples and the repeatability of the complete PLE plus LVI–GC–MS method for the analysis of the endogenous PAH was better than 15%. Comparison of PLE and Soxhlet or liquid-partitioning extraction results for the analysis of non-spiked samples showed that the efficiency of PLE is the same or better than for the other two extraction methods assayed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Pressurized liquid extraction; Extraction methods; Soil; Sediments; Polynuclear aromatic hydrocarbons

1. Introduction

Classical methods for the determination of trace pollutants in environmental solid samples are usually laborious and time-consuming multi-step procedures which require much manual handling of the extracts [1,2]. At-line, or on-line, coupling of these early steps of the analytical process is one of the main aims of modern analytical chemistry. Several examples of on-line clean-up procedures can be found in the literature ([3,4] and references therein). However, the analyte extraction itself is usually regarded as the most difficult step when developing completely on-line and/or automated procedures for solid or semi-solid environmental samples.

Because of the low levels at which microcontaminants are generally present in the environment and the variety of the samples, the selected

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extraction technique should be essentially exhaustive [5] and, preferably, easy to standardise. This explains the general preference for techniques such as Soxhlet or Soxtec extraction [6] rather than more selective, but also highly analyte- and/or matrix-dependent, techniques such as supercritical fluid extraction [7]. Microwave-assisted solvent extraction (MASE) and pressurised liquid extraction (PLE) are generally faster, and less analyte- and matrix-dependent and provide cleaner extracts than conventional methods involving heat treatment. These characteristics have caused both techniques, and specifically PLE, to be frequently used as extraction procedures for a variety of environmental applications. However, they are always carried out off-line. The at-line, or on-line, coupling of MASE or PLE with the separation-plus-detection part of the system would require miniaturisation of the extraction devices and, if at all possible, no additional clean-up step. Regarding the latter aspect, PLE has the advantage over MASE that no additional filtration step is required. The acceptation of PLE as a US Environmental Protection Agency (EPA) method [8] can be taken as an additional stimulus to consider this procedure.

In this paper, a laboratory-made miniaturised device for PLE of microcontaminants from solid samples is described. It was used in a combined static-dynamic extraction procedure, which was optimised with regard to organic solvent choice, temperature and pressure, and purging conditions. The performance of the novel set-up, which was combined at-line with gas chromatography–mass spectrometry (GC–MS), was tested for the determination of polycyclic aromatic hydrocarbons (PAHs) in non-spiked soils and sediment. The results were compared with those obtained for the same samples when analysed by more conventional procedures including Soxhlet extraction and liquid partition (LP).

2. Materials and methods

2.1. Chemicals

The 16 EPA PAHs [9,10] were selected as test compounds (see Table 2 below). Two working stock solutions were prepared from individual PAH standards (Sigma–Aldrich, Zwijndrecht, Netherlands and Supelco, Bellefonte, PA, USA) containing 5 μg/ml of each analyte in toluene. These were used for further dilutions and spiking of the samples in the preliminary experiments. One stock solution contained naphthalene and pyrene and was used for the initial PLE optimisation. The second stock solution contained all EPA PAHs, except phenanthrene, and was used for further validation of the method once all the experimental PLE variables had been optimised. In all cases, phenanthrene was used as an internal standard (1 μg/ml in toluene) to evaluate the efficiency of the different extraction methods. [$^{2}H_{10}$]Phenanthrene (98%, MSD Isotopes, Merck Sharp and Dohme, Montreal, Canada) was used as external standard (1 μg/ml in toluene) for the GC–MS analysis. The internal standard was added to the samples just before PLE, Soxhlet extraction or LP. The external standard was added to the final extracts just before the chromatographic analysis. Analytical-reagent grade *n*-hexane and pesticide-residue-grade methanol and toluene were obtained from J.T. Baker (Deventer, Netherlands). *n*-Hexane was glass-distilled prior to use.

An organic and a sandy soil from the Amsterdam region (Netherlands) and a Haringvliet river sediment (Den Bommel, Netherlands) were used as samples. They were air-dried and sieved to 270 mesh. This fraction was used for subsequent studies. Properties of the soil and sediment fractions used were determined by standard methods[1] (Table 1).

2.2. Instrumentation and procedures

A drying cartridge holder previously used for the removal of water from solid-phase extraction (SPE) desorption solvents in on-line SPE–GC [12,13] was modified and adapted for the PLE experiments. The device consists of a heatable 10×3.0 mm I.D. stainless-steel holder which serves as the extraction cell (Fig. 1). The extraction cell was sealed off by a

[1]Soils and sediment pHs were determined (744 pH meter, Metrohm, Herisau, Switzerland) after stirring a sample-quartz-distilled water (1:2, w/v) mixture for 1 min every 15 min during 1 h [11]. The elemental analysis was carried out on a Scintag (Pasadena, CA, USA) XDS 2000 X-ray diffractometer using Cu-K alpha radiation. The percentage of organic matter was determined by using an element analyser (Carlo Erba NA 1500) after removal of the carbonates with HCl.

Table 1
Relevant physico-chemical characteristics of the selected soil and sediment samples

Element	Per cent of the element in		
	Organic soil	Sandy soil	Sediment
Si	70	70	70
Ca	7	7	30
Fe	3	–	35
K	3	3	10
Al	15	–	20
C	11.4	1.3	5.4
H	1.0	0.1	0.4
N	0.6	0.02	0.3
pH_{soil}	6.5	8.3	7.2
pH_{slurry}	7.2	8.2	8.1

5-μm stainless-steel frit (Sigma, Zwijndrecht, Netherlands) at its upper end (in the direction of solvent flow) to prevent clogging of the exit tubing and valve by soil/sediment particles. This frit was never removed during the entire study. No clogging problems of either frit or tubing were observed during 3 months of constant use. Once the sample and the internal standard had been put into the cell,

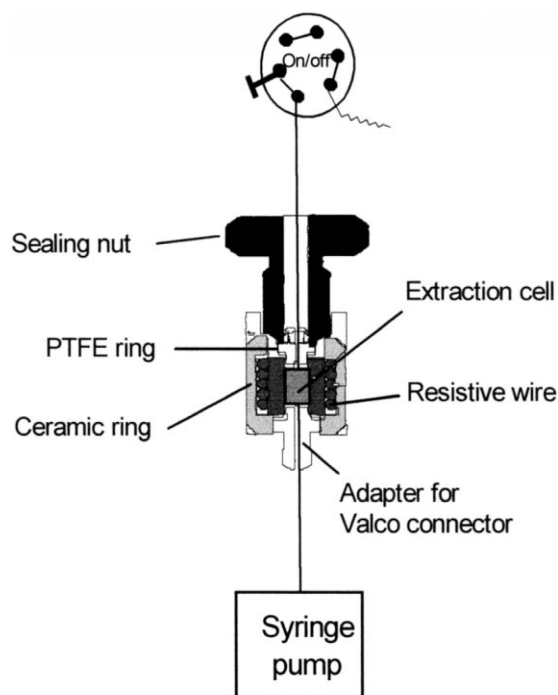

Fig. 1. New device for PLE of solid and semisolid samples.

the lower part was sealed by a laboratory-made manually removable 5-μm stainless-steel screen. Two PTFE rings positioned at the top and bottom ends of the extraction cell allowed to fix it to two adapters for connection to standard Valco nuts and stainless-steel tubing. The two adapters and the cell were pressed together to achieve leak-tightness by tightening a large nut at the top of the cartridge. The extraction cell was surrounded by a stainless-steel ring to which a resistive wire and a thermocouple were attached for heating and temperature control, respectively. Isolation was achieved by a ceramic ring around the stainless-steel ring [13]. The temperature was programmed by defining a start temperature, a temperature rate, a final temperature and a hold time in a controller. The temperature programme was manually started at the beginning of each experiment.

A Phoenix 20 CU syringe pump (Carlo Erba, Milan, Italy) was used to deliver the extraction solvent. The extraction cell was placed between this pump and a 6-port automated Valco valve (Must HP6, Spark Holland, Emmem, Netherlands) for direct control of the pressure in the cell via the pump. All tubing was of stainless-steel. Tubing connected to the extraction cell was 0.13 mm I.D. and tubing leading from the valve port to the vial for sample collection was 0.20 mm O.D. and 0.075 mm I.D. to improve heat dissipation before solvent collection.

In a typical experiment, 50 mg of a spiked sample were weighed into the extraction cell (85% of its total volume) already provided with the stainless-steel frit. The internal standard was added before closing the cell with the stainless-steel screen. Then, the cell was mounted in the device and the selected solvent was pumped to fill the cell and the lines from the pump to the valve. Next, the solvent was pressurised to the selected pressure using the constant pressure mode. Simultaneously, the temperature programme was started to heat both the sample and the extraction solvent. After a preselected static extraction time, the valve was switched to allow the extraction solvent to leave the cell. An additional volume of solvent was briefly then pumped through the cell and the lines (dynamic extraction step) to ensure proper purging of the sample and the lines. Blank samples (Soxhlet-cleaned silica) showed that

no additional clean-up or reconditioning was required between consecutive extractions when using this combined static-dynamic extraction.

The suitability of PLE for PAH extraction was preliminarily evaluated by analysing organic soil samples spiked at six concentration levels of 10–250 ng/g soil. Spiked samples were prepared by adding the proper amount of the PAHs dissolved in methanol to a soil or sediment sample (1:1, w/v). The mixture was homogenised by 2 min shaking and the methanol allowed to evaporate in a fume hood. The analyses were performed 24 h after spiking the samples. Definitive evaluation of the PLE feasibility for PAH extraction was carried out by determining the target compounds in non-spiked samples and by comparison the PLE results with those obtained using more conventional procedures for this kind of analysis, such as Soxhlet extraction and LP.

Because of an intended comparison of the different extraction methods assayed, the analytical conditions in these experiments were initially kept as identical as possible to those used in PLE. Therefore, the finally selected PLE extraction solvent was used in all cases. In the case of Soxhlet extraction, 0.5-g aliquots of the spiked soil in the 10×50 mm thimble were spiked with an amount of internal standard to provide a final concentration per gram of soil or sediment similar to that used in PLE. The sample was then extracted for 6 h with 40 ml of the selected solvent. With LP, 100-mg aliquots of the soil were also spiked with the internal standard to provide a final concentration per gram of soil similar to that used in PLE, and extracted by 10 min shaking with the selected solvent.

All experiments were carried out in triplicate. Extracts from the PLE experiments were coloured but transparent, i.e. they were not cloudy and no precipitate was found in the solutions. They were therefore analysed without any additional clean-up. Because of the intended comparison of the different extraction methodologies, Soxhlet and LP extracts were also analysed without any additional purification.

2.3. LVI–GC–MS

PAHs determination in the collected extracts was carried out by capillary gas chromatography (HP 6890 Series, Hewlett-Packard, Palo Alto, CA, USA) with MS (HP 6890 Series) detection in the selected ion monitoring (SIM) mode. Amounts of 50-μl of the extract were injected in the at-once large volume injection (LVI) mode on a programmed temperature vaporising (PTV) injector (Optic 2, Ai Cambridge, Cambridge, UK) provided with a packed 'A' type liner. GC separation was performed on a Restek XTI-5 capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness). Helium was used as the carrier gas at a column head pressure of 97 KPa. The split flow was 120 ml/min. After solvent elimination, the PTV was heated at 7°C/s from 80 to 300°C. The splitless time was 1.5 min. The column temperature was programmed from 103°C (4.5 min) to 280°C at 12°C/min. The final temperature was held for 12 min.

Identification of the target compounds was based on the simultaneous detection, at the appropriate retention time, of the chromatographic signals corresponding to the two m/z values selected for each congener (see Table 2), and on their ratios being within ±15% of the previously calculated theoretical ratio. Quantification was based on the individual peak areas and the response factor of the individual compounds related to the selected external standard. Recovery of the internal standard (in all cases above 82%) was taken as a control parameter for the efficiency of the proposed extraction procedures. In other words, the PAH levels reported were not corrected for the recovery of the internal standard.

3. Results and discussion

3.1. PTV injection and GC–MS analysis

In the present study, the PTV injector was packed with so-called 'A' type adsorbent, because this material is known to be inert for the present set of target compounds [14,15]. Optimisation of the injection procedure was performed according to a published procedure [16] with special attention for the maximum volume of solvent that can be rapidly injected without flooding the liner and the solvent elimination time.

The advantages of using a PTV over a split/splitless or on-column injection for LVI of relatively

Table 2
Analytical data for the LVI–GC–MS analysis of standard solutions

Compound	Peak No.	t_R (min)	m/z [a]	Regression coefficient [b]	RSD (%) [c]	LOD (ng/ml) [d]
Naphthalene	1	4.14	128/102	0.96	8	0.5
Acenaphthylene	2	7.87	153/152	0.97	6	0.4
Acenaphthene	3	8.30	153/152	0.97	8	0.5
Fluorene	4	9.45	165/166	0.994	1	0.3
Phenanthrene (I.S.) [e]	5	11.51	178/176	0.995	5	0.1
Anthracene	6	11.61	178/176	0.998	8	0.1
Fluoranthene	7	14.04	202/101	0.999	2	0.05
Pyrene	8	14.49	202/101	0.999	2	0.06
Benzo[*a*]anthracene	9	17.03	228/226	0.999	10	0.09
Chrysene	10	17.11	228/226	0.999	10	0.07
Benzo[*b*]fluoranthene	11	19.20	252/250	0.999	2	0.09
Benzo[*k*]fluoranthene	12	19.25	252/250	0.998	2	0.04
Benzo[*a*]pyrene	13	20.05	252/250	0.999	8	0.1
Indeno[1,2,3-*cd*]pyrene	14	23.61	278/276	0.999	9	0.2
Benzo[*ghi*]perylene	15	23.77	278/276	0.999	6	0.3
Dibenzo[*a,h*]anthracene	16	24.60	278/276	0.998	2	0.3
[$^2H_{10}$]Phenanthrene [f]		11.50	188/94	–	–	–

[a] Two most abundant ions.
[b] Regression coefficient of response vs. area plot (see text for range).
[c] $n=3$ at 0.5 ng/ml.
[d] Experimentally determined limit of detection (S/N, 3:1); 50 μl injected.
[e] Internal standard.
[f] External standard.

dirty samples is widely recognised [16–18]. Since non-volatile matrix constituents remain in the liner, GC (pre)column contamination is prevented. On the other hand, the presence of these matrix components in the liner can affect the analyte response [19,20]. In such cases, quantification based on calibration plots constructed using a matrix similar to the sample is recommended [16]. In the present study, differences in analyte responses were also observed after the analysis of real-life samples. However, analysis of standard solutions randomly inserted in the real-sample series revealed that the variations of the response factors invariably were within the range of experimental errors measured for standard solutions analysed on different days during the initial GC–MS calibration (relative standard deviations, RSDs, of less than 10%). That is, deactivation of the liner due to adsorption of non-volatile matrix components did not really affect the results when using the response-factor-based quantification procedure. In addition, no memory effects were observed when analysing pure solvent after a real-sample run. As regards the inertness of the packing material of the liner, it is important to add that over 230 analyses were carried out — with about 100 of these being analyses of real samples — with the same liner. The only problem encountered was some peak tailing observed for the PAHs more volatile than phenanthrene in the final 15 or 20 analyses.

The occurrence of analyte losses due to co-evaporation during the solvent elimination step of the LVI procedure was also studied. Losses were observed for naphthalene which gave a 59% (RSD=8% at 30 ng/ml level; $n=4$) response of that obtained by cold splitless injection. However, satisfactory responses were obtained for the other volatile PAHs, acenaphthylene (83%), acenaphthene (111%), fluorene (106%) and phenanthrene (102%); the RSDs were 4–7% ($n=4$). These percentages were higher than those previously published for similar analyses [16]. For the less volatile PAHs the recoveries after solvent elimination were also quantitative (94–108%) with RSDs of 1–6% ($n=4$).

Because of the complexity of the samples and the exhaustive character of the extraction methods, and also because no additional clean-up of the extracts

was carried out, it was necessary to use MS detection in the SIM mode. The experimental conditions finally proposed in this study allowed proper analyte recognition as well as quantification. However, coelution of chrysene and dibenzo[*a,h*]anthrancene with triphenylene and dibenzo[*a,c*]anthrancene, respectively, in real-life samples cannot be ruled out. For typical standard and sample chromatograms, the reader is referred to Fig. 4 below.

3.2. Optimisation of the PLE parameters

Preliminary experiments were carried out to optimise the main parameters affecting the PLE efficiency. For this study, an organic soil spiked with 75 ng/g soil of naphthalene and pyrene was used.

Firstly, the actual temperature of the soil particles during heating of the extraction cell was determined. A cell holder was completely filled with a soil sample, and a thermocouple manufactured in-house was placed in the centre of the cell. The heater was programmed from 25 to 251°C at a rate of 30°C/min. Every 30 s, the temperature readout on the power supply was compared with the actual temperature of the soil particles. A calibration plot was constructed on the basis of these results and was used to find the time necessary to reach and stabilise the temperatures to be used in subsequent extraction experiments. The maximum temperature of the soil particles was found to be 240°C. More importantly, the device allowed temperatures typically used in PLE experiments [8,21–23] to be reached within a short time: the soil particles reached temperatures of 100 and 150°C in 4 and 6 min, respectively. In other words, the heater was well suited for the present study.

As regards the preferred mode of PLE extraction, a smaller volume of organic solvent may be expected to be required for a static as compared with a dynamic extraction. However, a combined static-(brief) dynamic extraction was considered a better option, in order to ensure the removal of the extraction solvent remaining in the cell and for final washing of the soil and the capillary tubing. The solvent volume required for the dynamic step was carefully optimised to prevent the total volume to become unnecessary large. Preliminary experiments carried out by extracting the organic spiked soil with *n*-hexane at 80°C and 15 MPa for 5 min showed that the first 75 μl of solvent extracted 93 and 91%, respectively, of the amount of naphthalene and pyrene extracted with 300 μl. An additional volume of 25 μl corresponding to a brief dynamic extraction step was added to the initially selected 75 μl as a safety measure which simultaneously guarantees the removal of the extraction solvent from the cell and tubing. No further improvement of the yields was achieved by increasing the amount of *n*-hexane to over 100 μl: the increase was less than 5% for the second 100 μl and less than 2% for the third 100 μl. Therefore, a total solvent volume of 100 μl for the static-plus-dynamic extraction was selected for subsequent experiments.

In agreement with previously published results for ASE (PLE as commercialised by Dionex) [21–23] or laboratory-made PLE [1,24], pressure was found to play no role other than to keep the extraction solvent liquid at the high temperatures used. As an example, results found for naphthalene and pyrene when a spiked soil was extracted with toluene for 10 min at 180°C are shown in Fig. 2A. The reported values were normalised against the recoveries found at 15 MPa. The differences observed for the different pressures were in the range of the experimental errors found at each pressure. A pressure of 15 MPa was selected for further work because (i) at this pressure the solvents studied, *n*-hexane and toluene, were in the liquid state in the range of temperatures used (for details, see below), (ii) with this pressure standard Valvo valves could easily be used, and (iii) it allowed a good control of the elution flow-rate from the extraction cell during the extraction step at 100 μl/min. Because the elution flow-rate is known to have little effect on the experimental results [24], it was not separately optimised. A flow-rate of 100 μl/min was selected because it was low enough to allow (i) the extraction of the target compounds and (ii) the accurate collection of the extraction solvent volume finally selected (100 μl) in an autosampler vial for the subsequent LVI–GC–MS analysis.

The nature of the extraction solvent and the temperature have, for obvious reasons, a profound effect on PLE efficiency [1,22–24]. In this study, *n*-hexane and toluene were tested, which were selected on the basis of their frequent use as extraction solvents for PAH environmental analysis

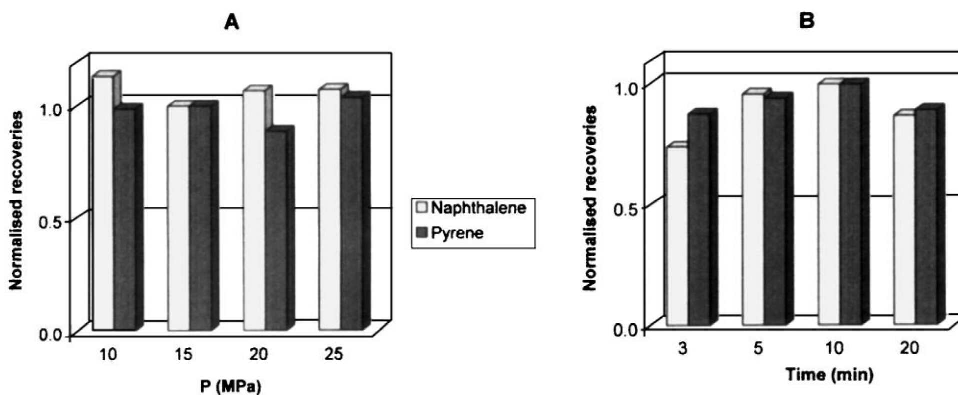

Fig. 2. Influence of (A) extraction pressure and (B) extraction time on the recoveries of naphthalene and pyrene spiked to an organic soil at the 75 ng/g level. The recoveries were normalised against the yields found when the soil was extracted with toluene for 10 min (A) at 180°C and 15.0 MPa, and (B) at 200°C and 15.0 MPa.

[23,25,26]. Temperatures ranging from 70 to 90°C and from 175 to 200°C were used for *n*-hexane and toluene, respectively. In all cases, a system pressure of 15 MPa was used and a static extraction time of 10 min after reaching the selected extraction temperature. In general, the recoveries increased with the extraction temperature for both solvents. For *n*-hexane, the increase of the naphthalene and pyrene yields was 8% and 15%, respectively, when the temperature increased from 70 to 80°C. No further improvement was observed when using a temperature of 90°C. In the case of toluene, the highest yields were obtained at temperatures higher than 190°C. For the rest, toluene was found to be a more efficient extractant than *n*-hexane, especially for the less volatile PAHs: the per cent differences for naphthalene and pyrene extracted were 3 and 29%, respectively. Consequently, toluene (at 200°C) was selected as extraction solvent for subsequent experiments.

As regards the static extraction time, Fig. 2B summarises data on naphthalene and pyrene yields when the spiked soil was extracted with 100 μl of toluene at 200°C and 15 MPa, for static extraction times (after particles reached selected temperature) from 3 to 20 min. The reported values, which were normalised with respect to the 10-min recoveries, show that for both compounds, the recoveries distinctly increased with time from 3 to 10 min, with no further improvement at 20 min. The somewhat low results for the latter experiments (which were repeated several times) may well have been caused by leaking of the solvent vapour upon such prolonged extraction. A static extraction time of 10 min was selected for subsequent experiments.

Finally, it should be added that, in the present set-up, no additional cooling of the transfer lines connecting the extraction cell and the microvials was provided. Even so, the naphthalene and pyrene yields were the same within the experimental errors normally observed when the extraction solvent was collected directly in the microvial or when a small volume of the solvent was previously put into the vial to act as a trap. Obviously, heat exchange of the heated and pressurised extraction solvent with the surrounding air via the 0.20 mm O.D. tubing is rather rapid. This is a distinct advantage of our miniaturised PLE compared with other (large scale) devices, with which cooling of the extraction solvent or collection in a sealed vial was mandatory [1,21–23].

3.3. Analytical data

To evaluate the linearity of the detector responses after LVI–GC–MS, standard solutions containing all 16 PAHs were prepared. The results of this study are summarised in Table 2. For naphthalene, acenaphthylene and acenaphthene responses were linear over the tested range of 0.5–100 ng/ml with regression coefficients better than 0.96 (n=7). For the other PAHs, regression coefficients from 0.994 to 0.999 (n=10) were found in the range 0.05–500 ng/ml.

The repeatability, which was determined by analysing a solution at the 0.5 ng/ml level, was satisfactory with relative standard deviations (RSDs) of 1–10%. The experimentally determined limits of detection (LOD) were 0.3–0.5 ng/ml for the PAHs which are more volatile than phenanthrene, and substantially better, i.e. 0.04–0.1 ng/ml, for most other analytes. The lower values for the very late eluting PAHs are due to tailing of these peaks. All these results showed that reliable quantification should be possible for PAHs at levels as are typically encountered in soil and sediments [16,22,23,25,27] even if only 50 mg of sample are used for an extraction.

The analytical performance of the at-line PLE plus LVI–GC–MS procedure for real-life samples was preliminary evaluated by analysing an organic soil spiked at six different levels (10–250 ng/g soil of each PAH). Three PAHs, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene, were spiked at 10-fold higher levels to evaluate simultaneously if the proposed PLE procedure can also be used for heavily contaminated soils without any further modification. Three separate analyses were carried out for each of the six spiking levels, 24 h after spiking. Relevant analytical data are summarised in Table 3. The total procedure showed good linearity over the whole test range for all target compounds with regression coefficients ranging from 0.95 to 0.99 ($n=6$). The experiments demonstrate that the present procedure is also suitable if individual PAH concentrations are in the 1–2 μg/g range. The repeatability of the whole analytical procedure was evaluated by analysing non-spiked (cf. footnote to Table 3) organic soil as well as soil spiked at the 150 ng/g level. The RSD data, which were essentially the same irrespective of the PAH concentration level, were 10% or better for all PAHs. This result is similar to or better than data reported for similar analyses using ASE and involving larger amounts of sample and solvent [2,23,27]. One may conclude that the proposed PLE plus LVI–GC–MS methodology shows fully satisfactory performance under conditions typically encountered in environmental PAH analysis.

Table 3
Analytical performance of PLE plus LVI–GC–MS for soil samples

Compound	Regression coefficient[a]	Linear range (ng/g soil)[b]	RSD (%)[c]	LOD (ng/g soil)[d]
Naphthalene	0.95	10–250	7	1
Acenaphthylene	0.97	10–250	9	0.8
Acenaphthene	0.98	10–250	5	5
Fluorene	0.99	10–250	10	2
Anthracene	0.97	10–280	9	4
Fluoranthene	0.98	10–425	4	8
Pyrene	0.99	10–350	2	9
Benzo[*a*]anthracene	0.98	10–350	10	4
Chrysene	0.98	10–350	4	1
Benzo[*b*]fluoranthene	0.97	100–1700	5	2
Benzo[*k*]fluoranthene	0.98	100–1250	9	2
Benzo[*a*]pyrene	0.99	100–2200	5	3
Indeno[1,2,3-*cd*]pyrene	0.98	10–325	8	30
Benzo[*ghi*]perylene	0.96	10–320	8	30
Dibenzo[*a,h*]anthracene	0.97	10–300	10	30

[a] For response vs. area plot (see text for conditions).

[b] Dynamic linear range according to calibration lines for PAH-containing spiked soils at the 10–250 ng/g level; (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene spiked at 10-fold higher levels).

[c] Evaluated from three separate analyses of non-spiked organic soil (20–40 ng/g added for naphthalene to fluorene to reach acceptable levels; cf. Fig. 4) and three separate analyses of soil spiked at the 150 ng/g level.

[d] Experimentally determined limit of detection (S/N, 3:1) in organic soil samples; 50 μl injected.

3.4. Method validation and application

To further illustrate the potential of the proposed method, the new set-up was used to extract PAHs from three samples with widely different physico-chemical characteristics, an organic soil, a sandy soil and a sediment. The soils and sediment were analysed both without spiking and after spiking at a realistic level of 75 ng/g soil. In all cases, the performance of the PLE-based procedure was compared with results obtained by LP and Soxhlet extraction of the samples. According to expectations, essentially the same results were obtained with the non-spiked and spiked samples which proves the feasibility of the PLE method for the analysis of the target compounds in real-life non-spiked samples. As an example, Fig. 3 summarises the results for the non-spiked set of samples. The mean concentrations of each PAH as calculated by three separate analyses of each soil or sediment are shown for the LP and Soxhlet extraction procedures, normalised against the corresponding PLE results. Not unexpectedly, closely similar results were found for the target compounds with all three extraction methods when extracting the sandy soil (Fig. 3B). However, for more complex samples, i.e. with higher organic content, LP was generally found to be less efficient than Soxhlet or PLE for the extraction of the endogenous PAHs (Fig. 3A and C). The strong adsorption of the endogenous PAHs to the organic matter of the organic soil and the sediment can be regarded to be responsible for the relatively low LP yields in these cases. On the other hand, the PLE extraction efficiency was found to be similar, or even better (least volatile analytes) than that of Soxhlet extraction with these samples. Similar results were previously reported for spiked and contaminated soils [2,24] and certified sediments [22,23]. However, the differences observed in the present study were larger than those found in the literature with 20–30% improved results for the least volatile PAHs. This demonstrates the practicality of the miniaturised PLE device for the determination of the endogenous PAHs in real-life samples and proves the PLE to be a valuable alternative to solvent and time consuming conventional procedures such as Soxhlet extraction and LP. This was also apparent from the RSD data recorded for the organic soil, which were substantially better for PLE (2–15%; $n=3$) than for LP and Soxhlet extraction (3–35% and 5–27%, respectively).

As an illustration of the GC–MS data obtained, Fig. 4 shows the merged fragmentogram traces obtained for a standard PAH solution, and for the non-spiked organic soil. The quantitative results obtained for all samples can be read from Figs. 3 and 4. In all instances fluoranthene, pyrene, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene, were present in the highest concentrations. The limits of detection in the real-life samples, which are included in Table 3, were less than 9 ng/g soil for all but the three late eluting PAHs for which values of 30 ng/g soil were found. Again, this demonstrates that 50 mg of sample is amply sufficient.

4. Conclusions

The practicality of miniaturised PLE combined at-line with LVI–GC–MS was demonstrated for the trace-level determination of endogenous PAHs in soils and sediment. The favourable conditions inherent to a PLE extraction (closed extraction vessel and extraction solvent at high pressure and temperature), explain the good extraction efficiencies compared with Soxhlet extraction and, much more so, liquid-partitioning. Compared with conventional PLE procedures, the present approach reduces sample volumes to about 50 mg, and solvent consumption to 100 μl rather than 20–200 ml. The reduced solvent volume, together with the use of LVI, allowed the at-line coupling of the extraction and separation-plus-detection steps since no analyte concentration is necessary prior to GC analysis. Even so, the detection limits for a large majority of the target analytes were 1–9 ng/g soil, and analytical performance was fully satisfactory.

As regards the maintenance of the PLE device, no clogging of either frit or tubing were observed during 3 months of constant use. However, due to the relatively high pressures used during extraction, the stainless-steel screen placed on the bottom part of the extraction cell was replaced every 3–4 extractions. (The screen at the top of the extraction cell was

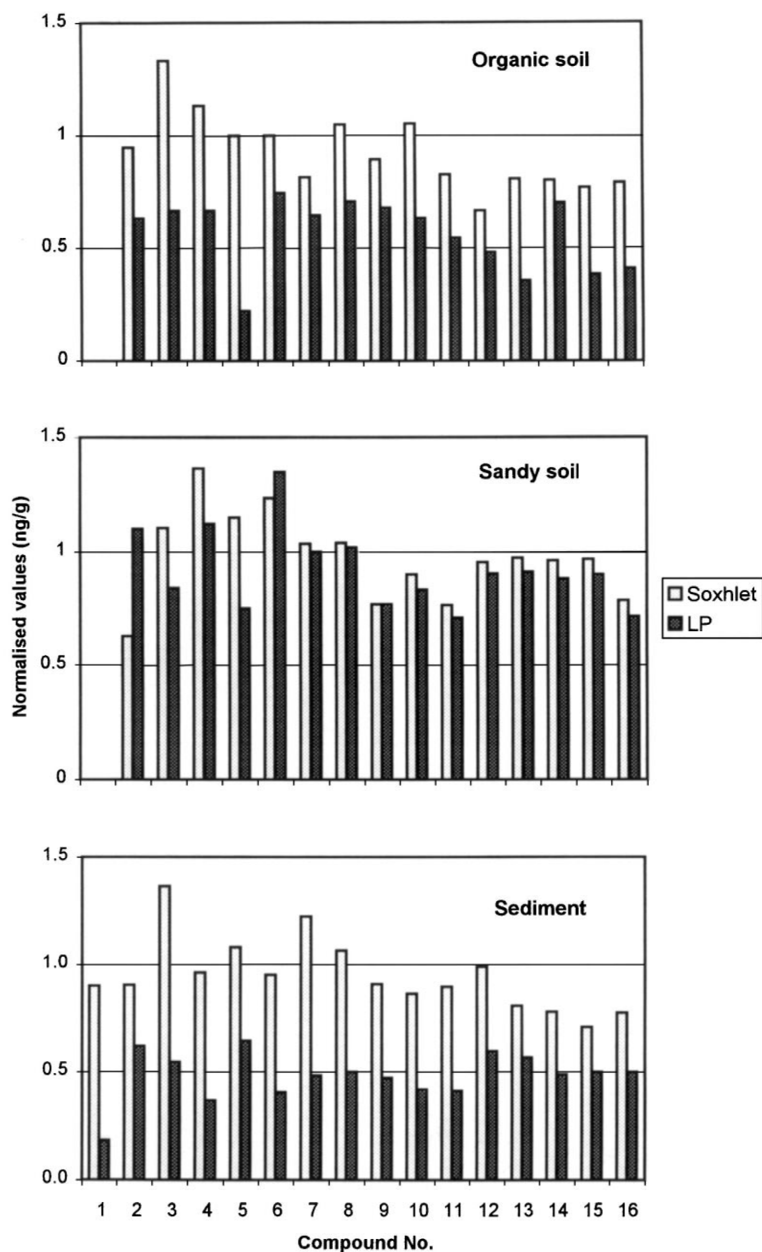

Fig. 3. PAH concentrations determined by LVI–GC–MS in three non-spiked samples after Soxhlet or LP extraction and subsequent normalisation of the values against concentrations determined by 10 min PLE of the samples with 100 μl of toluene at 200°C and 15.0 MPa. The concentrations were calculated from three separate analyses of each sample. See Table 2 for compound numbering.

never replaced.) Memory effects were absent because of the so-called dynamic step which consisted of a brief flush of the cell and capillaries with 25 μl of the extraction solvent. Leaking was only detected — or, at least, suspected — when using extraction times which were much longer than conventionally re-

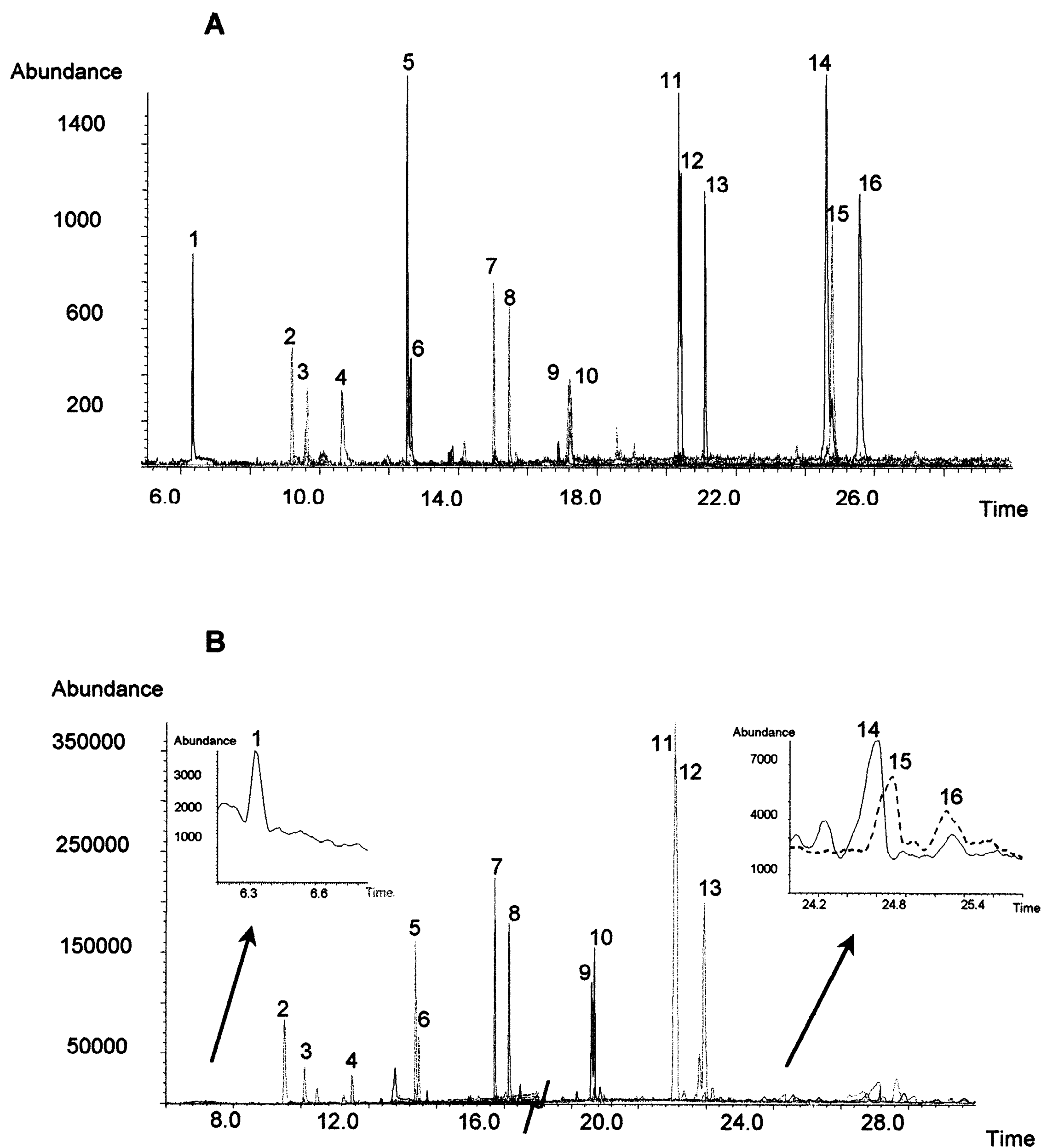

Fig. 4. LVI–GC–MS of (A) standard solution of PAHs (concentration, 5 ng/ml) and (B) PLE extract of a non-spiked organic soil. For selected m/z values and other experimental details, see Table 2 and text. Time scales in min.

quired. Finally, the simple design of the miniaturised PLE device allowed the use of open microvials rather than large sealed vials for collecting the extractant.

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