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Appraisal of "classic" and "novel" extraction procedure efficiencies for the isolation of polycyclic aromatic hydrocarbons and their derivatives from biotic matrices

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Abstract

In this study the extraction efficiency of pressurized liquid extraction (PLE), employing different extraction solvent mixtures under different extraction conditions, was compared with extraction efficiencies of commonly used procedures, Soxhlet extraction and extraction enhanced by sonication. Spruce needles and fish tissue were selected as test samples. Purification of obtained extracts was carried out by gel permeation chromatography (GPC) employing gel Bio-Beads S-X3. Identification and quantitation of target PAHs was performed by high-performance liquid chromatography with fluorescence detection (HPLC–FLD).

Within optimisation of PLE conditions, temperature of extraction, type of solvent, duration and number of static cycles as well as the influence of sample pre-treatment (drying, homogenisation, etc.) were tested. Comparison of the extraction efficiency of PLE with the efficiencies of the other techniques was done under the optimised conditions, i.e. sample slurry obtained by desiccation with anhydrous sodium sulphate, extracted at 100 °C in 1 cycle lasting 5 min. Hexane:acetone (1:1, v/v) was chosen as the most suitable extraction solvent for isolation of analytes from test samples.

Comparison of mentioned isolation techniques with respect to the amount of co-extracts, procedure blank levels and time and solvent volume demands was also done.
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Keywords: Polycyclic aromatic hydrocarbons; Pressurized liquid extraction; Soxhlet extraction; Extraction enhanced by sonication; Environmental samples

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of environmental carcinogens, levels of which have been often monitored in water, air, soil and food matrices. Many data have been generated in order to control compliance to legislation limits [1–5].

Utilization of needles and/or leaves of "ever green" plants, mosses and other terrestrial biotic matrices as passive samplers for monitoring of air pollution by PAHs has been reported in several studies. Advantages of using vegetation as bioindicators of immission burden, as well as a tool for investigation of contaminants distribution in environmental compartments have been documented [6–11].

Extraction typically represents a critical step in the accurate determination of organic contaminants in environmental matrices. Various extraction techniques employing organic solvents such as methanol, acetone [12,13], chloroform, dichloromethane [14–16], hexane [17] and cyclohexane [18,19] are used for the extraction of PAHs from environmental matrices. Techniques, for isolation of PAHs involving Soxhlet extraction [20,21], extraction enhanced by sonication [22,23] and saponification [24], represent common methods of choice. Nowadays, alternative extraction techniques, pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) [22,25–28] and microwave-assisted solvent extraction (MAE) [27–30], are also employed.

Pressurized liquid extraction represents an alternative extraction technique enabling: (i) reduction of the volume of solvents required for extraction, (ii) improvement of the precision of analyte recovery, (iii) reduction of the extraction

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times, and (iv) reduction of sample preparation costs. To enhance recovery efficiency, extractions are usually performed at elevated temperatures most often within the range of 50–200 °C, for 5–10 min and pressures of 10–15 MPa (to keep the solvents in liquid state). Apart from a few exceptions, many different solvents can be used for PLE. Toluene [31], hexane, hexane–acetone (1:1, v/v) [31,32] and mixture dichloromethane–acetone (1:1, v/v) [27,31] are the most often used extraction solvents for the isolation of PAHs from soil [27,31], sediment [32], moss [33] and needles [33].

The aim of our study was to compare the extraction efficiencies of PLE employing different extraction solvent mixtures under different extraction conditions with the efficiencies of Soxhlet extraction and extraction enhanced by sonication, for isolation of PAHs, methyl derivatives and sulfur heterocycles from environmental matrices (spruce needles) and biological samples (fish tissues).

2. Materials and methods

2.1. Experimental materials

The sample of fish (trout) used for optimisation of the PLE procedure was obtained from the common market of the Czech Republic. Before homogenisation in a blender, the skin, offal and bones were removed. Homogenised sample (3 kg of fish fillets) was stored at −20 °C.

Samples of spruce needles (*Picea abies*) were collected from five different regions of the Czech Republic and fully mixed. These regions were chosen to get an "average" sample with respect to PAH content, wax amount, dry matter, etc.

2.2. Chemicals

Chloroform and acetone (analytical reagent grade, Lachema Brno, Czech Republic) were redistilled in glass before use. Acetonitrile (gradient grade, for chromatography, Merck Germany), hexane (for organic trace analysis, Merck Germany) were used as supplied. Deionised water was obtained from Milli-Q water purification system (Millipore, USA). Anhydrous sodium sulphate (Penta Praha, Czech Republic) was dried at 500 °C for 5 h and then stored in a tightly capped glass bottle.

The standard mixture 1647d of 16 priority PAHs—naphthalene (Naph), acenaphthene (Ace), fluorene (Fln), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (B[*a*]A), chrysene (Chr), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenz[*a*,*h*]anthracene (DB[*ah*]A), benzo[*g*,*h*,*i*]perylene (B[*ghi*]P) and indeno[1,2,3-*cd*]pyrene (I[*cd*]P) dissolved in acetonitrile was supplied by National Institute of Standards and Technology (NIST, USA). Standards of individual PAH derivatives—1-methylnaphthalene (1-MeNaph), dibenzothiophene (DBT), 2-methylanthracene (2-MeAnt), 1-methylpyrene (1-MePyr), 5-methylchrysene (5-MeChr), benzo[*b*]naphtho[2,1-*d*]thiophene (B[*b*]N[*d*]T), benzo[*e*]pyrene (B[*e*]P) and 1-methylchrysene (1-MeChr) dissolved in acetonitrile (10 μg/ml) were supplied by Dr. Ehrenstorfer (Germany). Purity of individual standards was not less than 95%. Working standard solutions were prepared in acetonitrile and stored in refrigerator at 4 °C.

Before use, all glassware was washed with detergent, rinsed with distilled water and acetone and then heated at 200 °C for at least 4 h.

2.3. Equipment

A laboratory blender (Waring blender, 38BL-40, Waring Commercial, USA) was used for homogenization of samples.

A Dionex ASE 100 (Dionex, USA) with stainless steel vessels (33 and 66 ml), an ultrasonic bath Sonorex RK 510 (Bandeline, Germany) and a Soxhlet extractor (Gerhardt, Germany) with cellulose extraction thimbles (Whatman, UK) were used for sample extraction.

An automated gel permeation chromatography (GPC) system consisting of 305 Master pump, fraction collector, automatic regulator of loop XL, microcomputer (software 731 PC via RS232C), dilutor 401C (Gilson, France) and stainless steel column 500 mm × 8 mm i.d. packed with Bio-Beads S-X3, 200–400 mesh (Bio-Rad Laboratories, USA) was used for clean-up of extracts.

An vacuum evaporator (Büchi Rotavapor R-114 a Waterbath B-480, Switzerland) was used for concentration of extracts.

A high-performance liquid chromatographic system (HPLC) Hewlett-Packard 1100 Series composed of quarternary pump system with degasser, autosampler, column thermostat, fluorescence detection (FLD) system (Hewlett-Packard, USA), and a LiChroCART 250–4 (250 mm × 4 mm i.d.) column with the sorbent LiChrospher PAHs (Merck, Germany), was used for PAH analysis.

2.4. Analytical procedures

2.4.1. Isolation

To examine the isolation efficiency of PAHs from fish tissue and spruce needles by Soxhlet extraction and PAHs from spruce needles by sonication, the accredited analytical procedures (EN ISO/IEC 17025) described below were employed.

For Soxhlet extraction, an azeotropic mixture hexane–acetone (1:1, v/v) was used. For sonication enhanced extraction, a hexane–acetone (3:2, v/v) mixture was applied.

The colour intensity of obtained extracts given in Tables 2–5 was classified visually using four points scale.

Procedure blank samples were handled together with extracts of tested material in the same way as real sample. The

values of PAHs determined in blanks were subtracted from obtained results.

2.4.1.1. Soxhlet extraction

The flowing powder consisting of 10 g of homogenized sample (spruce needles or fish tissues) and 5 g of anhydrous sodium sulphate (needles) or 80 g (fish) mixed in a grinding mortar, were placed into the extraction cellulose thimble, covered with glass wool, and inserted into the Soxhlet extractor. Thimbles were preextracted for 2 h with an extraction solvent to obtain lower PAHs procedure blank. Extraction was carried out with 170 ml of hexane–acetone (1:1, v/v) mixture for 6 h (10 cycles/h). The Soxhlet apparatus was covered with an aluminium foil to avoid access of daylight (to prevent the risk of photodegradation). The extraction solvent was then carefully evaporated by rotatory vacuum evaporation at 40 °C just to dryness. Residue after evaporation was determined gravimetrically and quantitatively transferred into a 10-ml volumetric flask by chloroform.

2.4.1.2. Extraction enhanced by sonication ("Sonication")

Ten grams of homogenized sample (spruce needles) were transferred into an Erlenmeyer flask with 50 ml of solvent mixture hexane–acetone (3:2, v/v). The flask was covered with an aluminium foil to avoid access of daylight and placed into an ultrasonic bath for 20 min. The extract was then carefully filtered through a layer of anhydrous sodium sulphate. Extraction was repeated with 30 ml of extraction solvent. Combined filtrates were evaporated by rotatory vacuum evaporation at 40 °C just to dryness. Residue after evaporation was determined gravimetrically and quantitatively transferred into a 10-ml volumetric flask by chloroform.

2.4.1.3. Pressurized liquid extraction (PLE)

The flowing powder consisting of 10 g of homogenized sample (spruce needles or fish tissues) and 5 g of anhydrous sodium sulphate (needles) or 80 g (fish) mixed in a grinding mortar, were transferred into an extraction cell with volume 33 and 66 ml, respectively. Extractions were carried out under different condition settings (extraction solvent, temperature, duration of the static extraction, number of static cycles, and purge time, see Fig. 1) at a constant pressure 10 MPa. Extracts were collected and purged into the extraction vessels. After cooling, filtration through a layer of anhydrous sodium sulphate into 250 ml round bottom flasks followed. The extraction solvent was then evaporated by rotatory vacuum evaporation at 40 °C just to dryness. Residue after evaporation was determined gravimetrically and quantitatively transferred into a 10-ml volumetric flask by chloroform.

2.4.2. Clean-up

The clean-up procedure was carried out for both matrices by gel permeation chromatography employing the Bio-Beads S-X3 gel. The mobile phase (chloroform) flow rate was set at 0.6 ml/min; the volume of sample injected onto the GPC column was 2.5 ml in case of needles and 2 ml for fish. After discarding of the first 15.5 ml of eluate, the next 15.5 ml were collected. The purified extracts were subsequently subjected to concentration by rotatory vacuum evaporation at 40 °C just to dryness. The residue obtained after evaporation of chloroform was dissolved in 0.5 ml of acetonitrile before HPLC–FLD determinative step. This solution was then transferred into a 2 ml amber glass vial.

2.4.3. HPLC determination

The HPLC–FLD analyses were carried out under the following chromatographic conditions: gradient elution (A: acetonitrile, B: water; 0 min: 55% A, 40 min: 100% A, 42 min: 100% A), injection volume 20 μl, column temperature 35 °C. The FLD timetable is shown in Table 1. The external standard method based on peak heights was used for quantitation of PAHs.

Examples of the PAH separation in the standard mixture, spruce needles and fish samples are shown in Figs. 2–4.

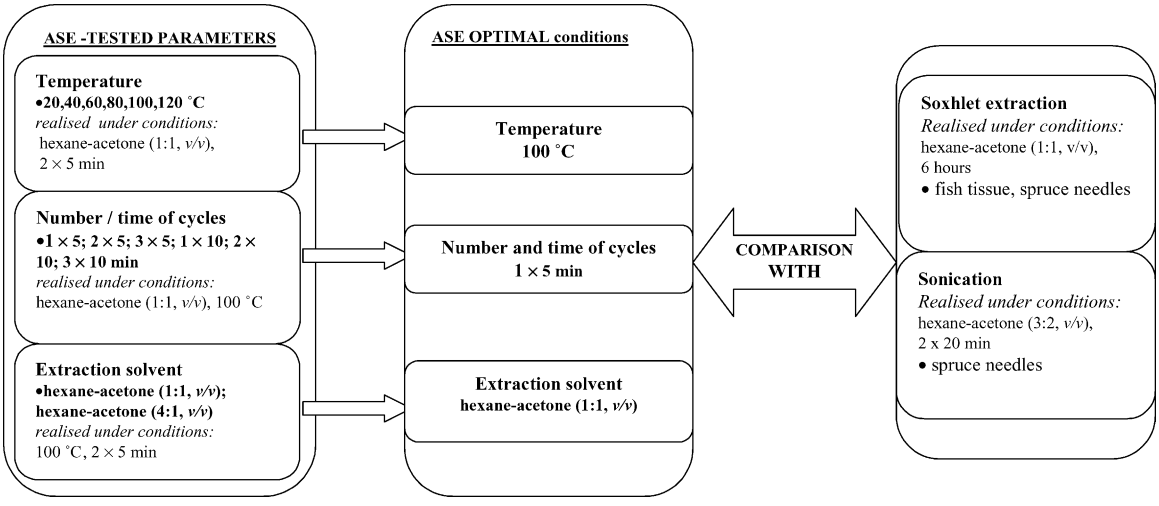

Fig. 1. Overview of testing of the PLE performance: experimental set-up.

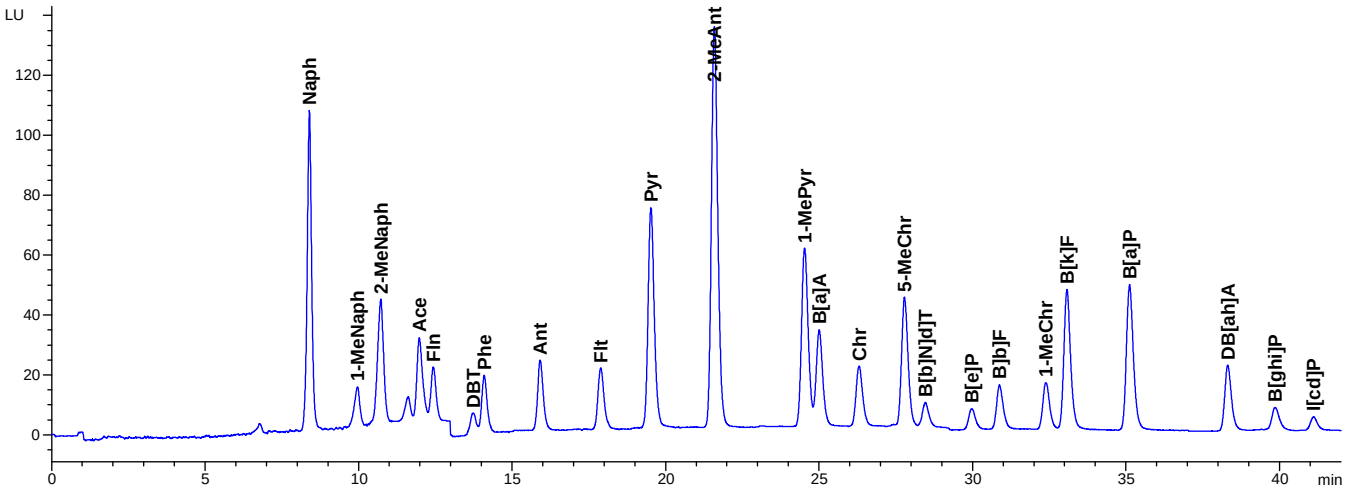

Fig. 2. Chromatogram of standard solution of PAHs and their derivatives ($C = 5$–$80\,\mu g\,l^{-1}$).

Table 1
FLD settings

PAHs	Time window (min)	λ excitation (nm)	λ emission (nm)
Naph, 1-MeNaph, 2-MeNaph	0–1.0	217	338
Ace, Fln	1–10.6	250	341
DBT, Phe	10.6–12.1	240	360
Ant	12.1–14.0	248	405
Flt	14.0–16.0	232	445
Pyr	16.0–17.4	236	389
2-MeAnt	17.4–19.3	250	397
1-MePyr, B[*a*]A, Chr	19.3–21.9	270	401
5-MeChr, B[*b*]*N*[*d*]T	21.9–25.5	270	369
B[*e*]P, B[*b*]F, 1-MeChr, B[*k*]F, B[*a*]P	25.5–27.5	266	425
DB[*ah*]A, B[*ghi*]P	27.5-35.0	295	405
I[1,2,3-*cd*]P	35.0-38.7	248	484

3. Results and discussion

3.1. Optimisation of PLE conditions

As already emphasised, the main assumption for good accuracy of generated data is fine-tuning of the extraction process. In the first part of our study we focused on the optimisation of the PLE settings with the aim to achieve efficient extraction of PAHs from spruce needles. This represents complex matrix containing high fraction of lipid-rich structures, as well as high amounts of plant pigments. Optimised PLE conditions were applied for extraction of desiccated fish samples.

In spite of impossibility to check the true concentration of individual PAHs (no CRM available), evaluation of efficiency of individual experiments was carried out on the relative basis taking the highest result as the reference (100%).

The overall experimental set-up is shown in Fig. 1. Individual experiments are discussed in details in the following paragraphs.

3.1.1. Extraction temperature

Extraction temperature has a significant influence on the diffusion coefficients of solvents, hence the kinetic of extraction process and its overall efficiency is strongly dependent on this parameter. Concentrations of PAHs obtained in individual experiments (extraction temperatures in the range 40–140 °C with 100 °C as reference) by a mixture of hexane–acetone (1:1, v/v) in spruce needles are summarized in Table 2. At lower temperature settings (40 and 60 °C) the extraction efficiency for two- and three-ring PAHs was clearly insufficient. For other target PAHs (four-, five- and six-rings) comparable results within repeatability range of procedure (see Section 3.2) were obtained for temperatures in the range 40–120 °C. Although for some analytes the highest mean values of recovered PAHs were obtained at 140 °C, the selectivity of extraction largely decreased and high amounts of matrice components (pigments and waxes) were contained in crude extract. Considering all the above facts, the temperature 100 °C was chosen as a compromise. It should be noted that apparent low weight of co-extracts obtained for extraction temperatures above 100 °C, i.e. under conditions of rather low extraction selectivity, was due to precipitation of abundant waxes on the walls of extraction vessel when the solution was cooled down. Difficulties with quantitative transfer of the whole crude extract into the flask used for gravimetric determination (residue remaining after evaporation of solvent) were encountered.

3.1.2. Extraction solvent

For the evaluation of the influence of various extraction solvents on the PLE efficiency, both spruce needles and fish samples were extracted with two solvent mixtures differing in polarity, hexane–acetone (4:1, v/v) and hexane–acetone

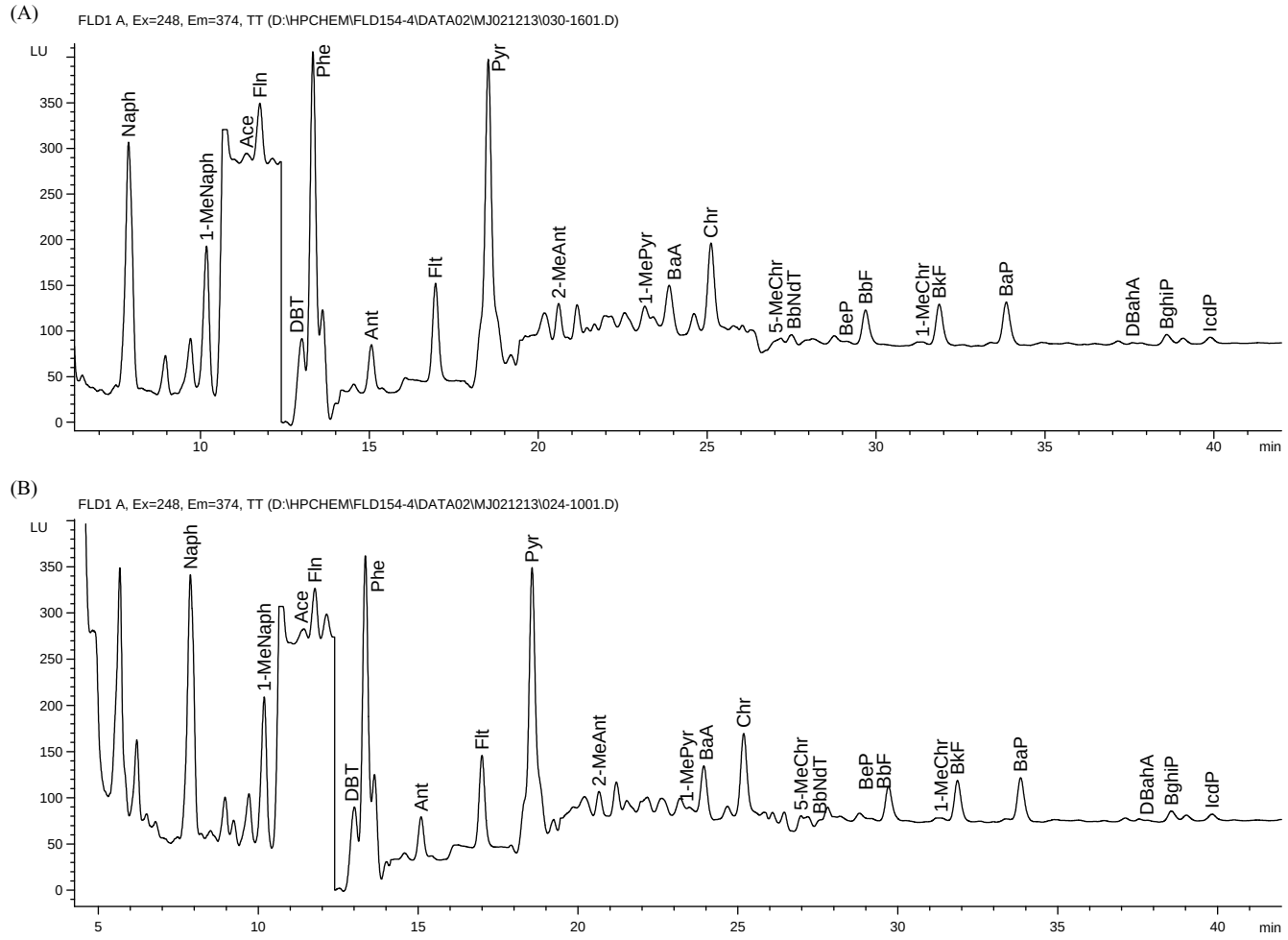

Fig. 3. Chromatogram of *non-ground* spruce needles (A) and *ground* (B) spruce needles extract.

(1:1, v/v). Considering the whole set of target analytes, generally better results were obtained by the latter extraction mixture, see Table 3. Although PAHs, mainly the four-, five- and six-ring representatives of this group, are highly hydrophobic compounds and one would expect higher extraction efficiency for less polar (hexane–acetone (4:1, v/v)) mixture, more efficient penetration of extraction mixture containing higher portion of water miscible solvent (hexane–acetone (1:1, v/v)) into rather hydrophilic matrix (namely fish tissue) was the dominating factor in terms of

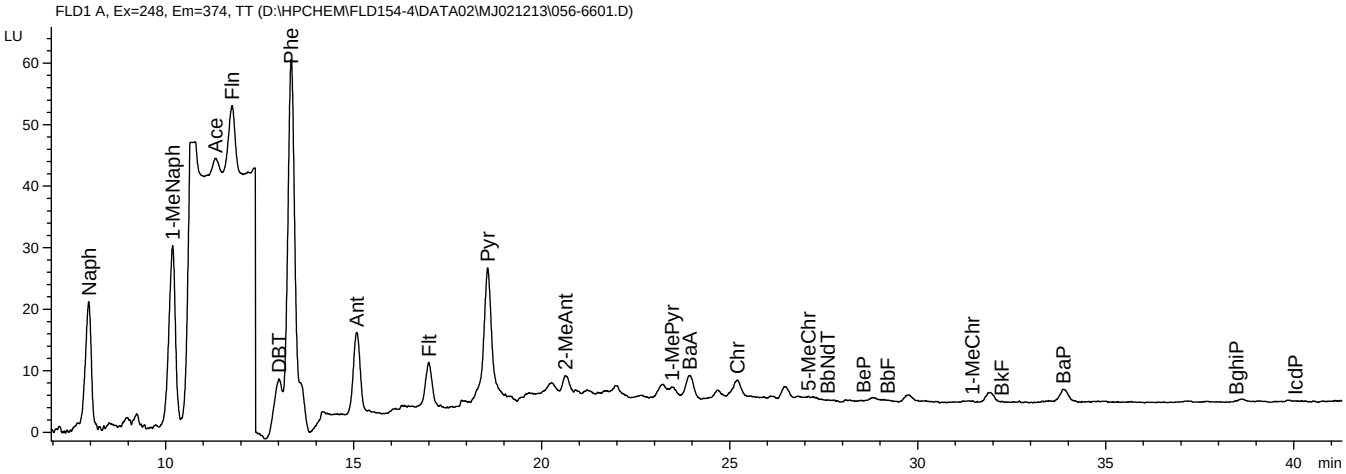

Fig. 4. Chromatogram of fish tissue extract.

Table 2
Comparison of PLE efficiencies for spruce needle PAHs using different extraction temperatures (*extraction conditions*: solvent mixture hexane–acetone (1:1, v/v); static cycle 1 × 5 min; purge time 2 s)

PAHs in spruce needles	Relative efficiencies (%)[a]					Analyte content[b] (μg kg^{-1})
	40 °C	60 °C	80 °C	120 °C	140 °C	100 °C
Naph	43	53	72	67	93	9.07
1-MeNaph	67	107	91	89	106	4.65
Ace	26	40	43	57	81	1.36
Fln	19	46	75	109	112	1.60
DBT	63	88	81	74	117	10.90
Phe	78	97	95	98	117	7.66
Ant	86	93	100	108	119	0.16
Flt	93	104	102	93	104	4.16
Pyr	82	95	96	94	107	3.69
2-MeAnt	92	110	87	87	115	0.18
1-MePyr	78	62	71	85	113	0.19
B[*a*]A	97	93	82	83	109	0.64
Chr	93	104	97	89	95	1.94
5-MeChr	52	49	68	76	115	0.10
B[*b*]N[*d*]T	79	74	86	104	72	1.48
B[*e*]P	108	104	99	88	92	1.21
B[b]F	105	99	96	86	93	1.25
1-MeChr	104	92	108	111	100	0.28
B[*k*]F	104	103	97	87	93	0.51
B[*a*]P	100	102	101	90	103	0.59
DB[*ah*]A	40	50	87	96	104	0.07
B[*ghi*]P	104	96	90	83	98	0.73
I[*cd*]P	96	93	94	83	92	0.78
Residue (%)[c]	1.8	1.7	1.5	1.7	1.3	2.1
Intensity of extracts colour[d]	+	+	+	+++	++++	++

[a] Value obtained at 100 °C was set as 100%.
[b] Content of PAHs was calculated on a fresh weight basis (moisture of sample was 54%).
[c] Amount of co-extracts obtained after evaporation of solvent; percent of sample weight.
[d] "+" corresponds to the least intensive colour of extract, "++++" to the most intensive colour.

recovering incurred PAHs. It should be noted that analogously to our experience, more polar extraction mixture enables better recoveries of hydrophobic POPs. Non-polar solvents do not readily wet the surface of dry sample and are too immiscible with water to be able to penetrate the wet material [34]. The usage of mixture hexane–acetone (1:1, v/v) for extraction of semivolatile organics, OCPs and PCBs is also recomanded by EPA method 3545A [35].

Selectivity of extraction process was another parameter that we evaluated. Relatively increased amount of matrix components contained in spruce needles and fish sample was isolated by hexane–acetone (1:1, v/v) mixture as compared to the less polar mixture (4:1, v/v). The residue after evaporation of the extraction solvent was not completely soluble in the GPC mobile phase (chloroform) and therefore careful filtration of the turbid solution was needed before loading on the GPC column. In spite of this inconvenience, this more polar extraction mixture was preferred over hexane–acetone (4:1, v/v) due to higher recoveries of target analytes obtained.

3.1.3. Duration of extraction and number of static cycles

To obtain information about the influence of the duration of extraction and the number of extraction cycles on PLE extraction efficiency, spruce needle samples were used. The relative efficiencies (1 cycle for 5 min = 100%) for individual experiments are listed in Table 4. High speed and efficiency of the extraction process is documented by very similar results, which were obtained for each combination of duration and number of extractions. To get maximum attainable efficiency of extraction, 1 cycle for 5 min is more or equally sufficient with extraction procedures involving more and/or longer cycles. With increasing the number and duration of extraction cycles, the intensity of extract colour and the amount of co-extracted waxes (precipitated after cooling of extraction solvent) also increased. For that reason, handling with extract (transferring into the flask used for evaporation of solvent) was getting more difficult, similarly as in the case of extraction temperature higher than 100 °C.

3.1.4. Purge time

After finishing of working cycle, the remaining solvent is purged out from the cell by means of intensive stream of nitrogen. Purge time 100 s was recommended in producer's documents. However, because of expectation of some looses of the more volatile PAHs during this process, additionally to purge time 100 s, a much shorter time 2 s, was also tested.

Table 3
Efficiency of PLE using different extraction solvent (*extraction conditions*: static cycle 1 × 5 min; extraction temperature 100 °C; purge time 2 s)

PAHs	Spruce needles		Fish tissue	
	Analyte content[b] ($\mu g\,kg^{-1}$), H–A (1:1)	Relative efficiencies (%)[a], H–A (4:1)	Analyte content[b] ($\mu g\,kg^{-1}$), H–A (1:1)	Relative efficiencies (%)[a], H–A (4:1)
Naph	8.56	85	27.16	112
1-MeNaph	7.19	81	21.23	98
Ace	1.06	105	46.13	106
Fln	1.85	73	68.60	106
DBT	14.57	63	63.86	83
Phe	11.04	73	53.83	103
Ant	0.28	69	7.95	111
Flt	5.22	71	6.30	103
Pyr	4.41	63	3.98	87
2-MeAnt	0.24	67	0.93	105
1-MePyr	0.27	63	0.36	75
B[*a*]A	0.71	86	0.39	62
Chr	2.64	72	0.46	69
5-MeChr	0.30	94	<0.09	–
B[*b*]N[*d*]T	1.52	95	1.18	93
B[*e*]P	1.56	77	0.83	100
B[*b*]F	1.46	80	0.24	79
1-MeChr	0.42	76	0.12	91
B[*k*]F	0.60	80	0.07	86
B[*a*]P	0.68	80	0.66	78
DB[*ah*]A	0.07	74	<0.03	–
B[*ghi*]P	0.84	79	0.40	82
I[*cd*]P	0.86	82	0.22	88
Residue (%)[c]	2.1	1.8	9.1	8.3
Intensity of extracts colour[d]	++	+	++	+

H–A (1:1): hexane–acetone (1:1, v/v); H–A (4:1): hexane–acetone (4:1, v/v).
[a] The H–A (1:1) value was set as 100%.
[b] Content of PAHs was calculated on a fresh weight basis (moisture of sample was 54% for spruce needles and 67% for fish tissue).
[c] Amount of co-extracts obtained after evaporation of solvent; percent of sample weight.
[d] "+" corresponds to the least intensive colour of extract, "++++" to the most intensive colour "<" means less than limit of quantitation.

Nevertheless, data obtained by both purge times were comparable.

3.1.5. Grinding of spruce needles sample before PLE

Efficient penetration of extraction solvent is another general prerequisite for achieving of good recovery of incurred analytes. As shown in Table 5, the grinding of spruce needles prior to extraction did not significantly influence the results as compared to processing of whole needles. The PLE conditions were obviously intensive enough to isolate PAHs from intact needles, moreover the major part of analytes is probably located in the surface layer of sample. The only consequence of sample disintegration was facilitated extraction of plant pigments and other matrix components. However, no increased interferences in HPLC–FLD analysis were encountered (see Fig. 3), probably due to the selectivity of the fluorescence detection.

3.1.6. The trueness of PLE

At present no certified reference material relevant to matrices involved in our study is commercially available. To assess the trueness of our results, fish paste sample, prepared by the Central Science Laboratory (York, UK) within FAPAS (Food Analysis Performance Assessment Scheme), Series 6, Round 10, was used. Obtained results, assigned values, target values for standard deviation and calculated z-scores for tested analytes (i.e. Fln, Flt, B[*b*]F, B[*a*]P and B[*ghi*]P) are summarized in Table 6. As can be seen, our results obtained by PLE, when classified as z-scores, were with one exception even lower than |1| (critical value is |2|), what documents good trueness of our approach for Fln, Flt, B[b]F, B[a]P, B[ghi]P involved in this proficiency test. PAHs containing three-, four-, five- and six aromatic rings (hence substances differing in physical-chemical properties, i.e. volatility, hydrofobity, solubility in water, etc.) were represented in FAPAS. Adopting generic approach, similar behaviour under extraction conditions can be anticipated for other target PAHs. In other words under existing circumstances the use of FAPAS material was the only reasonable way to obtain traceability of our measurement. One may consider spiking of blank matrix by target analytes as a conceivable alternative. To our opinion this approach is not viable since large differences may exist in extractability of incurred residues and those not incorporated into matrix in a natural way [34,36]. Moreover, it is difficult to get blank sample, since traces of PAHs occur practically in all biotic and abiotic samples.

Table 4
Efficiency of PLE of PAHs from spruce needles using different duration of extraction and number of static cycles (*extraction conditions*: solvent mixture hexane–acetone (1:1, v/v); extraction temperature 100 °C; purge time 2 s)

PAHs	Relative efficiencies (%)[a]					Analyte content[b] ($\mu g\,kg^{-1}$), 1 × 5 min
	2 × 5 min	3 × 5 min	1 × 10 min	2 × 10 min	3 × 10 min	
Naph	86	95	80	103	90	10.54
1-MeNaph	75	87	55	51	67	8.42
Ace	76	101	73	109	101	1.80
Fln	86	109	93	100	108	1.87
DBT	82	102	86	90	65	13.27
Phe	77	106	86	93	95	9.95
Ant	73	103	88	94	96	0.22
Flt	88	91	80	99	101	4.71
Pyr	89	94	85	102	101	4.16
2-MeAnt	90	103	79	98	87	0.20
1-MePyr	77	90	73	94	82	0.24
B[*a*]A	79	95	59	96	74	0.81
Chr	89	86	79	96	95	2.19
5-MeChr	64	80	95	92	102	0.26
B[*b*]N[*d*]T	81	87	95	104	62	1.84
B[*e*]P	90	80	78	91	91	1.35
B[*b*]F	92	85	79	98	93	1.36
1-MeChr	75	80	80	86	107	0.38
B[*k*]F	89	84	78	98	93	0.58
B[*a*]P	84	87	78	103	92	0.70
DB[*ah*]A	102	104	88	108	103	0.07
B[*ghi*]P	88	85	76	100	88	0.83
I[*cd*]P	92	88	84	102	93	0.85
Residue (%)[c]	2.1	2.1	2.3	2.5	1.8	2.9
Intensity of extracts colour[d]	++	++	++	+++	++++	++

[a] Value obtained by 1 × 5 min was set as 100%.
[b] Content of PAHs was calculated on a fresh weight basis (moisture of sample was 54%).
[c] Amount of co-extracts obtained after evaporation of solvent; percent of sample weight.
[d] "+" corresponds to the least intensive colour of extract, "++++" to the most intensive colour.

3.2. Comparison of PLE extraction efficiency with Soxhlet extraction and sonication

An average values calculated from five individual experiments/replicates, obtained for extraction efficiency of PAHs from needles and fish tissue by three alternative extraction techniques (Figs. 5 and 6), were subjected to *t*-test, to determine the statistical differences between mean values. PLE was performed under the optimized conditions described above, e.g. extraction by solvent mixture hexane–acetone

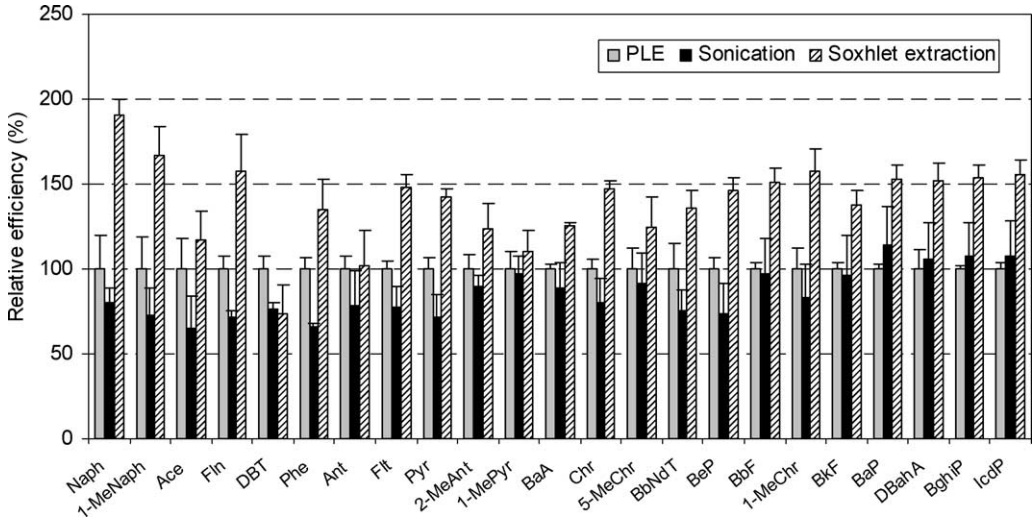

Fig. 5. Comparison of PAHs extraction efficiencies from spruce needles employing alternative isolation techniques (PLE value = 100%), $n = 5$. "Y error bars" represent repeatability of procedure in percent.

Table 5
Influence of grinding of spruce needles on efficiency of PLE (*extraction conditions*: solvent mixture hexane–acetone (1:1, v/v); static cycle 1 × 5 min; extraction temperature 100 °C; purge time 2 s)

PAHs	Ground needles, relative efficiencies (%)[a]	Non-ground needles, analyte content[b] ($\mu g\,kg^{-1}$)
Naph	95	5.55
1-MeNaph	101	2.33
Ace	67	2.27
Fln	95	1.50
DBT	99	9.53
Phe	93	6.31
Ant	95	0.13
Flt	101	3.66
Pyr	10	3.31
2-MeAnt	89	0.12
1-MePyr	87	0.25
B[*a*]A	85	0.62
Chr	100	1.75
5-MeChr	89	0.28
B[*b*]N[*d*]T	84	1.18
B[*e*]P	104	0.95
B[*b*]F	94	1.04
1-MeChr	87	0.24
B[*k*]F	95	0.43
B[*a*]P	94	0.43
DB[*ah*]A	98	0.03
B[*ghi*]P	93	0.56
I[*cd*]P	93	0.66
Residue (%)[c]	1.7	1.3
Intensity of extracts colour[d]	++++++	+

[a] Non-ground needles value was set as 100%.
[b] Content of PAHs was calculated on a fresh weight basis (moisture of sample was 54%).
[c] Amount of co-extracts obtained after evaporation of solvent; percent of sample weight.
[d] More "+" points means more intensive colour of extract.

(1:1, v/v), extraction temperature 100 °C, 1 cycle for 5 min and purge time 2 s. For description of a Soxhlet extraction and an extraction enhanced by sonication see Section 2.4.

The mean values of PAHs content obtained by Soxhlet extraction were higher than those obtained by PLE and for sonication for both matrices. In case of spruce needles, efficiencies of PLE and sonication were comparable except for some of three- and four-ring PAHs for which PLE appeared to be more efficient. Comparison of the five- and six-rings PAH levels obtained from fish tissue was quite difficult because of very low levels of these analytes hence high uncertainty of measurement in test sample.

Rather surprisingly statistically higher results obtained for most of PAHs occurring in spruce needles by Soxhlet extraction as compared to PLE or sonication may suggest further optimisation of the latter procedures for this matrixes needed. However, as shown above all the conceivable parameters were tested. To our opinion one of the major factors that might contribute to obtaining efficient recovery in Soxhlet extraction for this matrix is fairly higher volume of extraction solvent that passes through the matrix (7000 ml compared to 80 ml in case of the 3 × 10 min experiment, see Table 4). Unfortunately, we did not test higher extraction volumes for PLE, nevertheless its increasing means also longer duration extraction process hence losing of one of the major advantage, i.e. the speed of this step. It should be emphasized, that repeatability of extraction carried out by PLE was comparable to that of Soxhlet extraction and therefore PLE results corrected for lower recovery (considering Soxhlet as reference procedure) can be used in practice for monitoring purposes.

Repeatability (expressed as R.S.D., %) of the PLE and Soxhlet extraction procedures was similar; it ranged for individual PAHs from 2 to 13%, for the procedure employing sonication this value was rather higher approaching 20%. A variability of results of PLE determined within optimisation of extraction parameters, run under different intermediate precision conditions, was, in accordance with expectation, rather worse—in the range 11 to 48%. The highest value was obtained for 5-MeChr (39%) occurring in the spruce needles sample at very low levels, close to the limit of quantitation (0.3 μg/kg). Similarly high variability of measurements (48%) was encountered for one of the most volatile PAHs 1-MeNaph. The apparent differences in PAHs content reported in Tables 2–5 for spruce needle, more than to its poor homogeneity, can be attributed to uncertainties of measurement that are common in trace analysis.

Unfortunately, the selectivity of tested extraction techniques was rather low and increased recoveries are in most cases accompanied with higher amounts of matrix components in crude extracts. Expressed as percent of original

Table 6
Evaluation of trueness of PLE optimised procedure: calculation of z-scores for fish paste test material

Analytes	Obtained result[a], x ($\mu g\,kg^{-1}$)	Assigned value[b], X ($\mu g\,kg^{-1}$)	Target value for standard deviation[c], σ ($\mu g\,kg^{-1}$)	Calculated z-score[d], z
Fln	76.49	65.31	14.37	0.78
Flt	39.48	57.40	12.63	−1.42
B[*b*]F	2.92	3.07	0.68	−0.22
B[*a*]P	2.05	2.36	0.52	−0.60
B[*ghi*]P	1.37	1.30	0.29	0.25

[a] Result obtained by our laboratory.
[b] Assigned value (X) was calculated as the robust mean of data submitted by participants after correction for recovery.
[c] Standard deviation (σ) was calculated from results submitted by participants.
[d] $z = (x - X)/\sigma$.

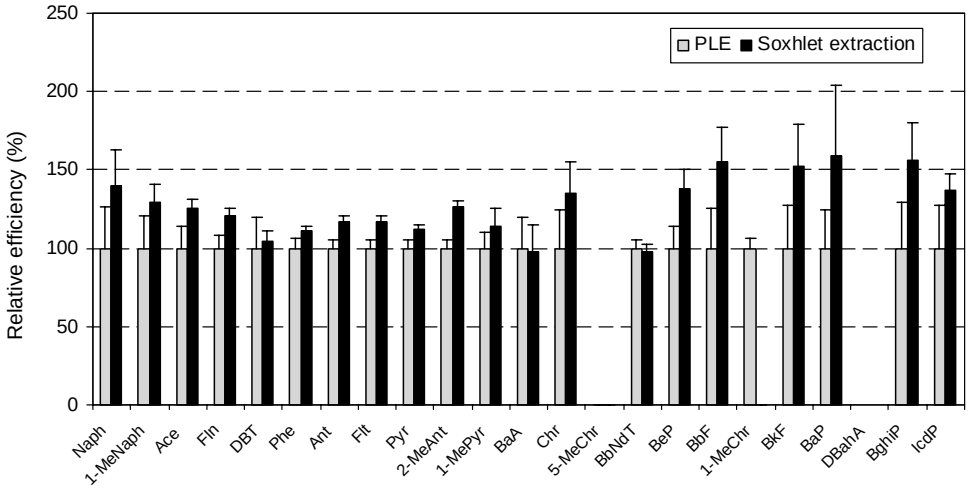

Fig. 6. Comparison of PAHs extraction efficiencies from fish tissue samples employing alternative techniques (PLE value = 100%), $n = 5$. "Y error bars" represent repeatability of procedure in percent.

sample weight, 7% of co-extracts were extracted from spruce needles by Soxhlet extraction, 1.3% by PLE and 0.8% by sonication. In case of fish tissue, Soxhlet extraction was also more efficient with 14.3% of co-extracts to compare with 7.7% obtained by PLE.

As regards blank values, which may cause under certain circumstances serious problems in PAHs analysis, all the tested extraction procedures provided relatively low values. The blank values expressed for average weight of sample as sum of PAHs ranged from 5.7 $\mu g\,kg^{-1}$ for spruce needles to 30 $\mu g\,kg^{-1}$ for fish, with standard deviation 0.79 $\mu g\,kg^{-1}$ and 11 $\mu g\,kg^{-1}$ respectively. The contribution of two- and three-ring PAHs including derivatives to total PAHs level in blank was about 80%, for four-rings it was about 20%; five- and six-ring PAHs were not detected in blank samples.

Besides the quality of generated data, the cost-effectiveness and sample throughput are important criteria to be considered when assessing particular techniques. Although the cost of PLE device is fairly high as compared to equipment used for Soxhlet and/or extraction enhanced by sonication, the time needed for finishing the PLE extraction process is only 18 min that is 25 times shorter than the time-consuming Soxhlet extraction. In addition, the solvent consumption in PLE is lower than those needed for the classic isolation techniques tested in this study, which, due to low costs, are still widely used in many laboratories concerned with analysis of organic contaminants in environmental and biological samples.

4. Conclusions

PLE represents a viable alternative for isolation of PAHs from plant (spruce needles) and animal (fish) matrices. In addition to relatively good recoveries (not significantly different from those obtained by employing classic techniques such as Soxhlet extraction and extraction enhanced by sonication) and repeatability, higher sample throughput and low solvent consumption are the major advantages of PLE.

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