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Pressurized liquid extraction in determination of polychlorinated biphenyls and organochlorine pesticides in fish samples

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Abstract

Pressurized liquid extraction (PLE) is a relatively new technique applicable for the extraction of persistent organic pollutants from various matrices. The main advantages of this method are short time and low consumption of extraction solvent. The effects of various operational parameters (i.e. temperature of extraction, number of static cycles and extraction solvent mixtures) on the PLE efficiency were investigated in this study. Fish muscle tissue containing 3.2% (w/w) lipids and native polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and other related compounds was used for testing. Purification of crude extracts was carried out by gel permeation chromatography employing Bio-Beads S-X3. Identification and quantitation of target indicator PCBs and OCPs was performed by high-resolution gas chromatography (HRGC) with two parallel electron-capture detectors (ECDs). Results obtained by the optimized PLE procedure were compared with conventional Soxhlet extraction (the same extraction solvent mixtures hexane–dichloromethane (1:1 v/v) and hexane–acetone (4:1 v/v) were used). The recoveries obtained by PLE operated at 90–120 °C were either comparable to "classic" Soxhlet extraction (for higher-chlorinated PCB congeners and DDT group) or even better (for lower chlorinated analytes). The highest recoveries were obtained for three static 5 min extraction cycles.

Keywords: Polychlorinated biphenyls; Organochlorine pesticides; Accelerated solvent extraction; Soxhlet extraction; Environmental samples

1. Introduction

Persistent organic pollutants (POPs) are chemicals that may persist for a long period of time in the environment. POPs include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) such as 1,2,3,4,5,6-hexachlorocyclohexane (HCH) mixed isomers, 1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane (DDT) and its degradation products DDE and DDD, chlorobenzenes, and several other anthropogenic compounds. POPs are already strictly regulated and most of them are not currently in production. These chemicals are prone to long-range transport through the upper levels of atmosphere and can be deposited 1000 miles away from the pollution source. Through atmospheric deposition [1] and river inputs [2] they have spread to all aquatic environments. POPs are characterized by being lipophilic (high octanol–water partition coefficient K_{ow}) and hydrophobic. Due to the persistence and lipophilic properties of the POPs, they are able to accumulate in the ecosystem. In addition to carcinogenetic/mutagenic potential they may cause toxic effects on animal reproduction, development, and immunological function.

The analyses of PCBs and OCPs play an important role in the monitoring of environmental contamination. Information needed for regulatory purpose as well as ecotoxicological risk assessments is obtained in this way. It should be noted that a good accuracy of generated data is a critical aspect in any decision-making process.

Determination of PCBs and OCPs in biotic samples commonly consists of three steps: (i) extraction, (ii) purification/fractionation of crude extract and (iii) chromatographic separation, identification/quantitation. Although selective extraction of organics appears to be an attractive option, different types of binding of analytes on to adsorption sites or their interactions with matrix require an exhaustive technique to recover the maximum amount of target analytes from the substrate. Generally, low selectivity of extraction method yields considerable amounts of undesirable

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co-extractives (e.g. lipids, pigments) which must be removed before the final GC determination. Lipid separation is usually performed by gel permeation chromatography (e.g. Bio-Beads) or adsorption chromatography (Florisil, silica gel, alumina, etc.) [3–5].

The most common procedure employed for the extraction of non-polar and semi-polar trace organics compounds (e.g. PCBs and OCPs) from a wide variety of matrices such as sediments, soils, animal and plant tissues is Soxhlet extraction [6–10]. This procedure is carried out with non-polar and/or semi-polar solvents (pentane, hexane, dichloromethane, acetone, and diethyl ether) or their mixtures advantageously in azeotrophic ratio. It should be noted that the Soxhlet method requires large volumes of highly purified organic solvents and a relatively long time for completing total extraction of all analytes. In case of high-moisture samples their thorough desiccation is needed to enable good penetration of solvent into the sample matrix. In addition, some volatile compounds may be lost unless efficient condensers are used [11].

The other very simple isolation technique is batch extraction enhanced by sonication. "Dirty" extracts (containing a lot of co-extracted matrix components) are typically obtained by both the above-mentioned techniques. In the last decade, alternative extraction techniques enabling reduction of the volume of extraction solvents and saving the extraction time have been searched. Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and/or pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) represent recent techniques meeting at least some of these requirements [12–17].

The latter one, PLE uses conventional liquid solvents at elevated pressures (10–15 MPa) and temperatures (50–200 °C) for short time periods (5–10 min) to extract solid samples quickly and with much less solvent than conventional techniques [18–21]. Nevertheless, samples with high-moisture content require desiccation before the extraction step. Hexane, hexane–dichloromethane (1:1 v/v), hexane–acetone (1:1 v/v) and toluene are extraction solvents often used for the isolation of PCBs and OCPs from abiotic (sediment, soil, sludge, urban dust) or biotic samples (oyster, mussel) [21–25].

The aim of this study was: (i) to optimize extraction efficiency of PLE employing different extraction solvent mixtures under the different extraction conditions and (ii) to compare performance characteristics of this novel technique with conventional Soxhlet extraction.

2. Experimental

2.1. Sample material

The fish filets homogenate (skin removed) prepared from chub (*Leuciscus cephalus*) containing "native" levels of PCBs and OCPs was used for the testing of individual operational PLE parameters. This material is commonly used in our laboratory as internal reference material in traceability to CRM 350 and CRM 430 (BCR, Belgium). The average lipid content of this material is 3.16%. The fish tissue was chosen because fish products are considered as significant source of PCBs in the diet. Some fish species are commonly used in the environmental monitoring of these compounds.

2.2. Chemicals

The mixture of indicator PCBs (IUPAC numbers 28, 52, 101, 118, 138, 153 and 180) in isooctane (trimethylpentane) and standards (solids) of organochlorine pesticides (hexachlorobenzene (HCB), α-, β- and γ-isomers of hexachlorocyclohexane, octachlorostyrene (OCS), DDT and its degradation products DDE and DDD) used in this study were obtained from Dr. Ehrenstorfer (Germany). The purity of individual standards was higher than 96%. Working standard solutions were prepared in isooctane and stored in the refrigerator (5 °C).

The solvents used (*n*-hexane, dichloromethane, isooctane, cyclohexane) were all supplied by Merck (Germany). Ethyl acetate was supplied by Scharlau (Spain). All these solvents were "organic trace analysis" grade. Acetone was obtained from Lachema (Brno, Czech Republic) and redistilled before use. Anhydrous sodium sulphate obtained from Penta Chrudim was heated at 500 °C for 5 h and then stored in the desiccators before use. Styrene–divinylbenzene gel (Bio-Beads S-X3, 200–400 mesh) was purchased from Bio-Rad (USA). Sulphuric acid (98%) was obtained from Merck.

2.3. Equipment

A Dionex ASE 100 system (Dionex, USA) with stainless steel vessels (66 ml) and Soxhlet extractor (Gerhardt 173200 EV, Germany) with cellulose extraction thimbles (Whatman, UK) were used for realization of the extraction step.

An automated gel permeation chromatography (GPC) system consisting of 305 MASTER pump, fraction collector, automatic regulator of loop XLI, 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) was used for the clean-up of extracts.

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

A Hewlett-Packard 5890 Series II gas chromatograph equipped with electronic pressure control (EPC), split/splitless injector, two parallel ^{63}Ni electron-capture detection (ECD) systems and two parallel columns possessing different selectivity (DB-5 and DB-17, both J&W Scientific, USA) were employed for the analyses of PCBs and OCPs.

2.4. Analytical method

2.4.1. Isolation

Two alternative procedures described below were used for isolation of analytes.

2.4.1.1. Soxhlet extraction. Samples with high-moisture content have to be desiccated before the extraction step to enable good penetration of solvent into the sample matrix. The 10 g fish homogenate were mixed with 70 g anhydrous sodium sulphate to form a flowing powder. This sample was then transferred into a cellulose extraction thimble and stored in desiccators for 12 h. After this time it was inserted into a Soxhlet apparatus and extracted for 8 h (7 cycles/h). The 170 ml of solvent mixture, either hexane–dichloromethane (1:1 v/v) or hexane–acetone (4:1 v/v), were used as an extraction solvent. The crude extracts were carefully evaporated by rotary vacuum evaporator at 40 °C and the rest of solvent was removed by a gentle nitrogen stream. The lipid content was determined gravimetrically. Extracted lipids were dissolved in 4.5 ml of cyclohexane–ethyl acetate (1:1 v/v) including PCB congener No. 112 in a concentration of 5 ng/ml as an internal standard (to our experience, this congener never occurs in fish samples above detection limits).

2.4.1.2. PLE. The sample preparation procedure was carried out in the same way as in the case of Soxhlet extraction, including desiccation. The flowing powder was placed into the extraction cell. The remaining volume of the cell was completely filled with anhydrous sodium sulphate. The sample cell was loaded into the ASE 100 system. Hexane–dichloromethane (1:1 v/v) and hexane–acetone (4:1 v/v) were used for testing of extraction solvents. The experimental set-up of PLE testing is shown in Fig. 1. The pressure 10 MPa, flush volume 60% of the extraction cell volume (the volume of fresh extraction solvent mixture used for flushing of the extraction cell after static extraction, i.e. about 40 ml) and purge time (N_2) 5 s were used for all PLE experiments. Processing of obtained crude extracts was identical as for Soxhlet extraction.

2.4.2. Clean-up

A clean-up of crude extracts was carried out by GPC employing Bio-Beads S-X3. Cyclohexane–ethyl acetate

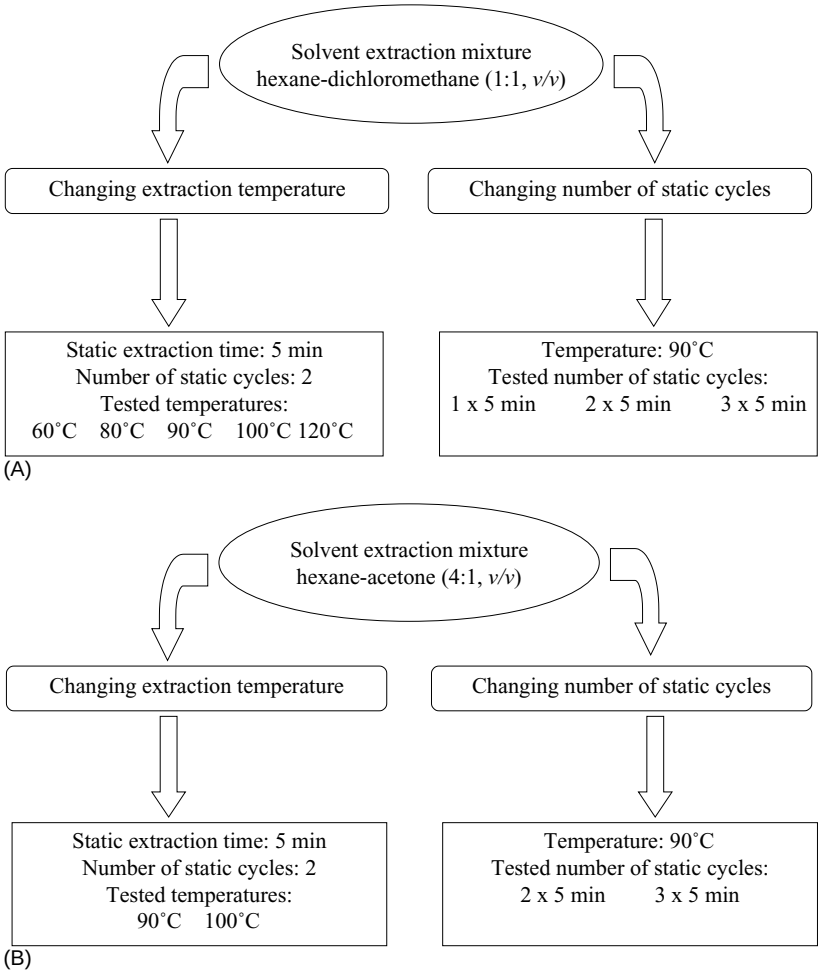

Fig. 1. Experimental set-up: optimization of operational parameters for both tested extraction solvent mixtures: (A) hexane–dichloromethane (1:1 v/v) and (B) hexane–acetone (4:1 v/v).

(1:1 v/v) was used as mobile phase; the flow rate was 0.6 ml/min; injection volume 2 ml. The fraction corresponding to the elution volume of 14.5–28.0 ml was collected. The eluate was evaporated by rotary vacuum evaporator at 40 °C and the rest of solvent was carefully removed by a gentle stream of nitrogen. The residue after evaporation was dissolved in 1 ml of isooctane and treated with concentrated sulphuric acid (1 ml) to remove potentially remaining lipids. Aliquot of upper isooctane layer was transferred into a glass vial for further GC analysis.

2.4.3. GC/ECD determination

HRGC/2ECD was used for analysis of PCBs and OCPs. GC conditions are summarized in Table 1. Identification of analytes was carried out by comparison of retention times in chromatogram with that of PCBs and OCPs standards. Quantitation of target compounds was performed by multi-level calibration.

Table 1
GC conditions used for indicator PCBs and OCPs analysis

Parameter	Description
Column types	DB-5 (5% phenyl–methylpolysiloxane), DB-17 (50% phenyl–methylpolysiloxane)
Column size	60 m × 0.25 mm I.D., 0.25 μm (both)
Injector temperature	250 °C
Detector temperatures	300 °C
Oven temperature program	60 °C (2.5 min), 30 °C/min to 220 °C, 0.5 °C/min to 240 °C, 2.5 °C/min to 280 °C (10 min)
Splitless period	2.0 min
Carrier gas	Helium
Inlet pressure program	Constant flow 1.7 ml/min, i.e. 207 kPa (60 °C)
Linear velocity of carrier gas	29.3 cm/s
Injected sample volume	1 μl
Data processing software	HP GC ChemStation Rev.A.06.03 (509)

3. Results and discussion

3.1. Optimization of PLE parameters

Although several studies concerned with application of PLE for the isolation of PCBs and/or OCPs from biotic samples have been published, none of them focused on a comparison of this technique with conventional approaches. In this first part of experiments the attention was paid to a critical assessment of the influence of various parameters on PLE performance and selection of optimal ones. Various extraction temperatures together with different number of static cycles were tested. The extraction efficiency of two solvent

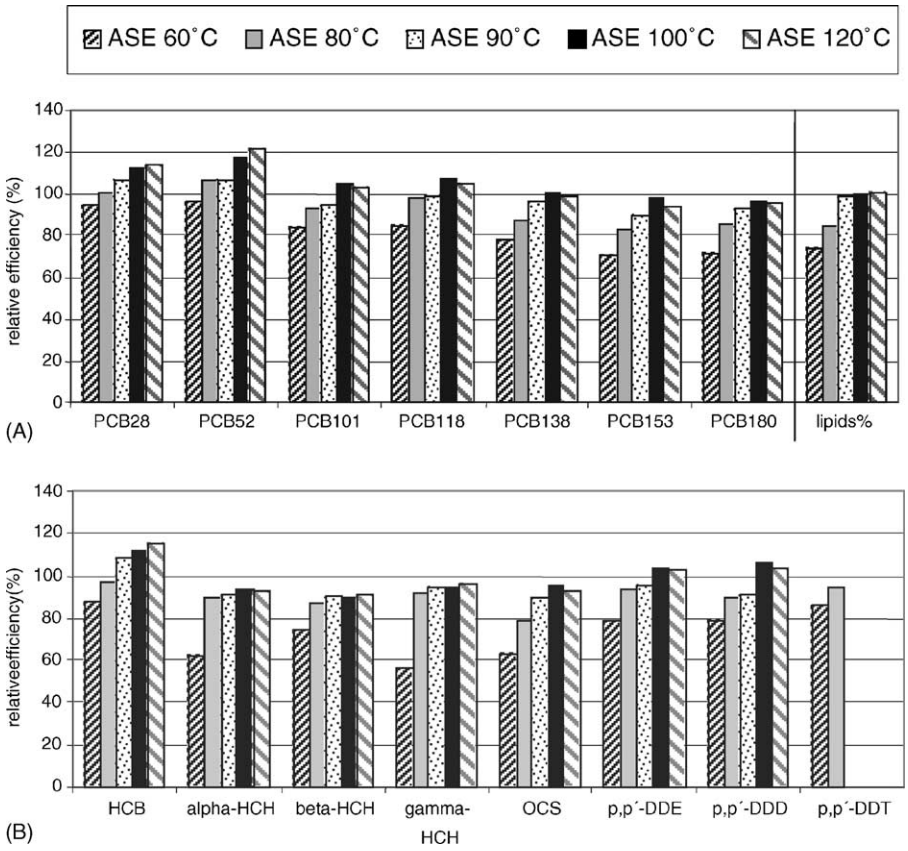

Fig. 2. The effect of extraction temperature on the extraction efficiency of PLE for: (A) indicator PCBs, lipids and (B) OCPs; extraction solvent hexane–dichloromethane (1:1 v/v) (Soxhlet extraction with hexane–dichloromethane was set at 100%).

mixtures (hexane–dichloromethane and hexane–acetone) was compared as well. The data obtained under tested parameters were compared with those achieved by Soxhlet extraction that is commonly used in one laboratory for isolation of halogenated POPs from both biotic and abiotic matrices.

All experiments were designed to eliminate random errors by means of replicates. Each result shown in bar graphs (Figs. 2–4) represents the average value calculated from at least five individual experiments/replicates and subjected to *t*-test to determine the statistical differences between mean values. Typical relative standard deviations (RSD, %) are stated in Table 2 and Fig. 4. These values are very similar in all experiments and depend rather on the concentration level of analyte than on the technique or solvent mixture used (in fact, the *F*-test did not reveal any difference at all).

3.1.1. Influence of extraction temperature

The extraction temperature via changing diffusion coefficient has a distinct influence on the extraction kinetics and therefore also on overall recoveries [26]. In general, increased temperature can disrupt the strong solute–matrix interactions caused by Van der Waals forces, hydrogen bonding or dipole attractions of the solute molecules and active sites in the matrix. Higher temperatures also decrease the viscosity of solvents, thus allow their better penetration into matrix particles and enhance extraction efficiency [21]. Fig. 2 shows the effect of temperature setting on the extraction efficiency of PLE for individual analytes from fish homogenate. Experiments were carried out at constant pressure (10 MPa). The most pronounced increase of PLE efficiency was observed in the temperature range 60–90 °C, further increasing of this parameter did not result in significantly elevated recoveries. The extraction efficiency of PLE for higher-chlorinated PCB congeners (Nos. 101, 118, 138, 153, 180) and *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT in the range 90–120 °C was comparable to "classic" Soxhlet extraction; for lower chlorinated PCBs and HCB it was even higher. On the other hand, for isomers of HCH and OCS slightly lower results were obtained with PLE as compared to Soxhlet extraction. It should be noted that only very low levels of these analytes were contained in examined fish samples hence the values of uncertainty of measurement were relatively high.

3.1.2. Influence of number of static cycles

The number of extraction cycles is another important parameter for achieving quantitative extraction. Björklund et al. [27] showed the importance of this parameter in study concerned with extraction of PCBs from sediments with different particle sizes. While the target analytes were quantitatively extracted with hexane–acetone mixture from materials with small differences in particle size using a

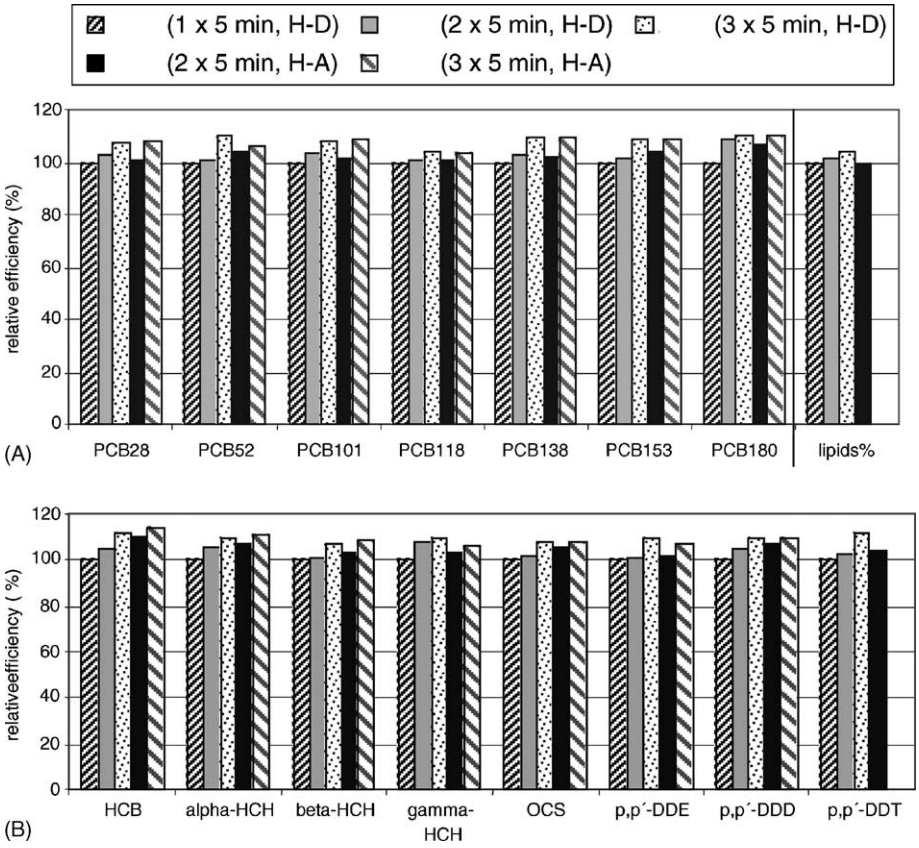

Fig. 3. Influence of number of static cycles on the extraction efficiency of PLE for: (A) indicator PCBs, lipids and (B) OCPs (number 1 × 5 min was set-up as 100%); H–D: hexane–dichloromethane; H–A: hexane–acetone.

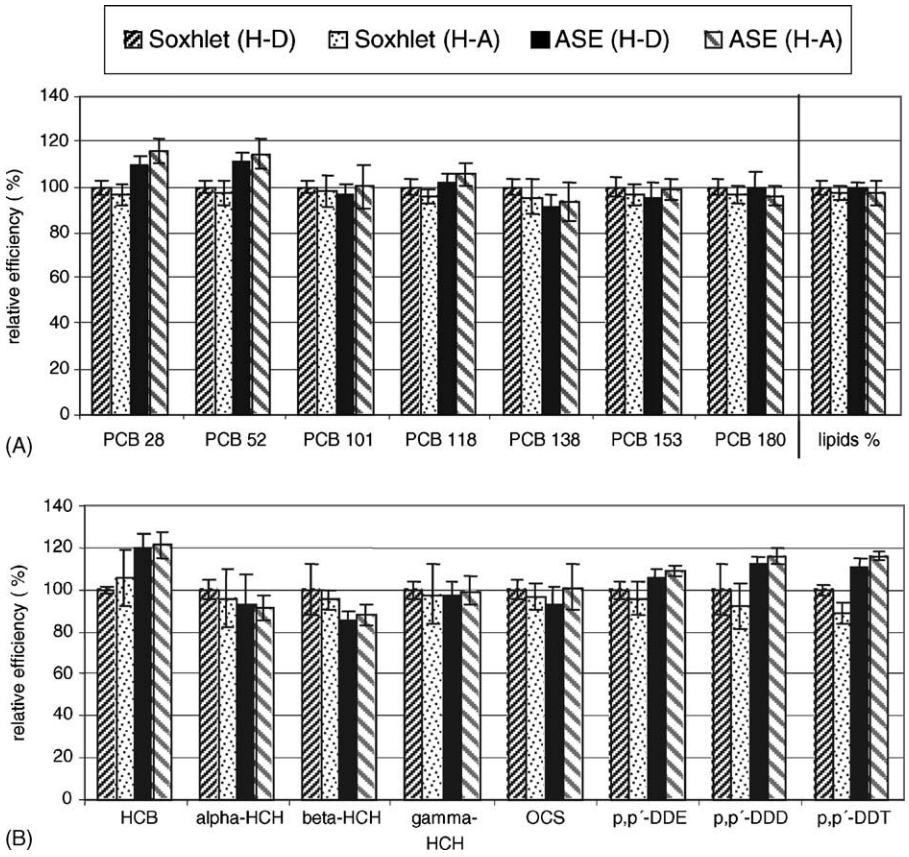

Fig. 4. Comparison of relative efficiency of optimized ASE and Soxhlet extraction: two tested extraction solvent mixtures (values obtained by Soxhlet extraction with hexane–dichloromethane were set-up as 100%): (A) PCBs and lipids and (B) OCPs; error bars represent standard deviation of results ($n = 5$).

Table 2
Contents of PCBs and OCPs ($\mu g\,kg^{-1}$ fresh tissue) and repeatability of measurements ($n = 5$) obtained by procedures involving: (i) PLE and (ii) Soxhlet extraction (H–D: hexane–dichloromethane; H–A: hexane–acetone); RSD: relative standard deviation (%)

Analyte	Soxhlet, H–D (1:1)		PLE, H–D (1:1)		Soxhlet, H–A (4:1)		PLE, H–A (4:1)	
	$\mu g\,kg^{-1}$	RSD (%)	$\mu g\,kg^{-1}$	RSD (%)	$\mu g\,kg^{-1}$	RSD (%)	$\mu g\,kg^{-1}$	RSD (%)
PCB 28	41	3	45	4	40	5	48	6
PCB 52	39	3	43	4	38	5	48	7
PCB 101	24	3	22	4	23	7	24	10
PCB 118	18	4	19	5	17	3	19	5
PCB 138	47	3	43	5	45	8	44	8
PCB 153	101	4	98	7	99	5	102	5
PCB 180	57	3	57	8	55	4	55	4
HCB	8	2	9	6	8	14	10	6
α-HCH	0.8	5	0.8	14	0.8	14	0.8	9
β-HCH	1.0	12	0.9	4	0.8	5.	0.9	5
γ-HCH	0.7	4	0.6	7	0.7	14	0.7	7
OCS	0.3	5	0.3	9	0.3	6	0.3	11
p,p′-DDE	68	4	72	4	65	8	74	2
p,p′-DDD	8	12	9	3	7	11	10	3
p,p′-DDT	4	3	4	5	4	5	5	2

single 5 min extraction step (less than 1% PCBs of total content were found in the second extract), other very inhomogeneous sediments retained as much as 14% of some congeners, which were then completely released during a second 5 min step. Poop et al. [28] also emphasized the importance of application of repeated extraction cycles for efficient isolation of OCPs from soil. In general, many "optimal" extraction set-ups/solvent mixtures can be found in literature [21,24,27–30]. In our study five experiments differing in the number of extraction cycles and used

solvents were employed. The results are summarized in Fig. 3. Negligible differences were found between one and two static cycles. On average, three static cycles provided maximum recoveries for all target analytes and for both tested solvent mixtures but the differences between two and three static cycles were not statistical significant. It should be noted that three static cycles prolong the total extraction time (as well as the solvent consumption) without any significant effect. Thus, two cycles offer a practical solution.

The consumption of extraction solvent depends on both the number of extraction cycles and the portion of fresh solvent used to rinse the cell after the static extraction step. The total solvent volume for one, two and three static cycles was 70, 85, and 105 ml, respectively, which is significantly less as compared with common Soxhlet technique.

3.1.3. Influence of extraction solvent mixtures

In general, physico-chemical properties such as boiling point, polarity, specific density (influences a penetration into the sample matrix) as well toxicity (makes a workplace hazard) are facts considered when choosing extraction solvent [25]. For evaluation of the extraction solvents on PLE efficiency, fish samples were extracted by solvent mixtures hexane–dichloromethane (1:1 v/v) and hexane–acetone (4:1 v/v). Considering the recoveries of target analytes, regardless the solvent mixture used for extraction, no statistically significant differences were found between the results obtained by optimized PLE (extraction temperature 100 °C, number of static cycles 2 × 5 min) and those obtained by Soxhlet extraction, see Fig. 4. The same results were obtained for extracted lipids. This fact is very important since the concentrations of lipophilic contaminants are often standardized to the lipid content.

Separation of PCBs from co-extracted lipids is necessary to obtain extracts that can be analysed by GC. Using PLE, selective extraction of PCBs can be achieved by mixing the sample with suitable sorbent retaining lipids. Alumina and/or silica gel impregnated by sulphuric acid can be used for this purpose. Decrease of turbidity as well as the reduced content of matrix pigments in crude extracts obtained by PLE could be affected by optimization of polarity of used extraction solvent mixture. In this particular case, the polarity parameter of hexane–dichloromethane ($P' = 1.6$) was slightly higher in comparison with hexane–acetone ($P' = 1.1$) [31].

3.1.4. Repeatability of PLE and Soxhlet extraction

Five replicate extractions of fish samples were performed employing both tested extraction solvent mixtures to calculate repeatability of PLE and Soxhlet extraction (expressed as RSD, %). The results are shown in Table 2. The repeatability of PLE ranged from 3 to 14% for all the target analytes, which was comparable to performance of conventional Soxhlet extraction procedure. Generally, the worst repeatability was obtained for analytes occurring in real samples at very low concentration levels such as HCH isomers and OCS, without regard to the extraction technique used.

Laboratory throughput is an important issue in any routine laboratory. The total extraction time needed in our experiments for one, two and three static cycles was 11, 17, and 24 min, respectively. Using the ASE 300 device (designed for high sample capacity), automated extraction of batch of 12 samples last approximately 3.5 h using two extraction static cycles. It is a relatively short time in comparison with the Soxhlet technique, which requires approximately 8 h for the efficient extraction of organochlorine pollutants from biotic samples.

In addition, the estimated cost per sample for "classic" Soxhlet extraction is about US$ 27 comparing to US$ 14 for PLE. This cost is based on the assumption of processing of 2000 samples per year; appropriate laboratory equipment (including its depreciation) personnel costs and consumables were considered.

4. Conclusions

The results generated in this study document that for extraction of indicator PCBs and some organochlorine pesticides from fish matrix the performance characteristics of PLE are essentially equivalent or even better than those of classic Soxhlet procedure. The efficiency of extraction for some more volatile (HCB) as well as semi-volatile compounds (PCB 28, 52) obtained by PLE was slightly higher as compared to the Soxhlet extraction. The main advantages of PLE were low consumption of extraction solvents and short time needed for the realization of extraction step (average solvent consumption was 85 ml and extraction time was 17 min per sample while to 170 ml and 8 h in case of Soxhlet). However, the purchase cost of this equipment is much higher compared to common Soxhlet or batch extraction enhanced by sonication. Another limitation of PLE application is the maximum amount of sample that can be placed into the extraction thimble under experimental condition we used, only 10 g sample (flowing powder obtained by other addition of sodium sulphate). This might be drawback namely in case of analysis of sample with very low levels of target analytes.

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