Novel Approaches to Determination of PAHs and Halogenated POPs in Canned Fish

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Abstract

Drábová L., Pulkrabová J., Kalachová K., Hradecký J., Suchanová M., Tomaniová M., Kocourek V., Hajšlová J. (2011): **Novel approaches to determination of PAHs and halogenated POPs in canned fish**. Czech J. Food Sci., **29**: 498–507.

A simple method is described for simultaneous isolation of 7 indicator polychlorinated biphenyls (PCBs), 10 polybrominated diphenyl ethers (PBDEs), 22 organochlorine pesticides (OCPs), and 16 polycyclic aromatic hydrocarbons (16 EU PAHs). The sample preparation procedure, including a pressurised liquid extraction (PLE) followed by gel permeation chromatography (GPC) for the selective isolation of the target compounds, was optimised and validated. For the final identification/quantitation of the target PCBs, PBDEs, OCPs, and PAHs, gas chromatography (GC) coupled to a high speed time-of-flight mass spectrometer (TOF MS) was used. The performance characteristics of the procedure were assessed including the recoveries (86–118% for PCBs, 73–113% for PBDEs, 71–113% for OCPs, and 85–111% for PAHs), repeatabilities (3–12% PCBs, 3–9% PBDEs, 1–11% OCPs and 3–10% PAHs), and limits of quantitation (LOQs – 0.5 µg/kg PCBs, 0.1–0.3 µg/kg PBDEs, 0.1–0.5 µg/kg OCPs, and 0.03–0.1 µg/kg PAHs). Within the follow-up study, this method will be used for the monitoring of contamination of canned fish and sea food products available at the Czech market.

Keywords: PAHs; POPs; canned fish; GC-TOF MS

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs) represent ubiquitous pollutants of various environmental compartments. Among the possible routes of human exposure, such as inhalation of air, dermal absorption, and/or dietary intake, the consumption of contaminated food is the major source of these contaminants. On this account, reliable testing methods enabling the effective control of these contaminants in both environmental and food matrices are required (Usydus *et al.* 2009).

Several techniques employing different approaches for the extraction, clean-up, and detection have been described for the analysis of persistent organic pollutants (POPs) and PAHs in fish and fish products (Rodil *et al* . 2007). Common difficulties associated with the determination of the above mentioned food contaminants in such complex matrices are (ultra) trace concentrations and potential interferences.

Supported by the 7th FP EC research project CONFFIDENCE "Contaminants in food and feed: Inexpensive detection for control of exposure" No. FP7-211326-CP and by the Ministry of Education, Youth and Sports of the Czech Republic, Project MSM 604637305.

Most of the procedures widely used for the isolation of lipophilic organic compounds are based on the extraction of the respective samples in the Soxhlet apparatus (Booij & Van den Berg 1994; Vives & Grimalt 2002; Jánská *et al.* 2004; Suchan *et al.* 2004). To increase the laboratory throughput and reduce the required workload, automated procedures enabling a short extraction time, such as supercritical fluid extraction (SFE) (Antunes & Bernardo-Gil 2003; Rodil *et al.* 2005, 2007; García-Rodríguez *et al.* 2008), microwave assisted extraction (MAE) (Bayen *et al.* 2004; Pena *et al.* 2006; Barriada-Pereira *et al.* 2008), and pressurised liquid extraction (PLE) (Suchan *et al.* 2004; Liguori *et al.* 2006; Losada *et al.* 2009) have been reported in many studies. Moreover, the efficiency of PLE can be increased by the addition of a purification phase into the PLE cell which allows a pre-purification step resulting in a higher method selectivity (Björklund *et al.* 2001; Gómez-Ariza *et al.* 2002; Sporring & Björklund 2004; Lund *et al.* 2009).

Purification of crude extracts using gel permeation chromatography on Bio Beads® S-X3 or Envirosep ABC as the stationary phase combined with dichloromethane, dichloromethane-hexane, trichloromethane or other mobile phases (Weichbrodt *et al.* 2000; Navarro *et al.* 2006) is a frequently used approach. Nevertheless, a solid phase extraction (SPE) on silica gel or Florisil® combined/together with dichloromethane-hexane, *n*-hexane-toluene, or other mobile phases can be considered as an effective alternative.

PCBs, OCPs, PBDEs, and PAHs are usually analysed by gas chromatography coupled to mass spectrometry (GC-MS) using single quadrupole instruments (Rodil *et al.* 2005; Yurchenko & Mölder 2005; Poster *et al.* 2006; van Leeuwen & de Boer 2008; Lund *et al.* 2009). Gas chromatography-tandem mass spectrometry (GC-MS/MS) (Verenitch *et al.* 2007; Fernández-González *et al.* 2008; Losada *et al.* 2009; Nácher-Mestre *et al.* 2009) and gas chromatography with time-of-flight mass spectrometry (GC-TOFMS) (Čajka *et al.* 2005; Poster *et al.* 2006; Nácher-Mestre *et al.* 2009) have been also recently applied. For the determination of PAHs in food matrices, high performance liquid chromatography employed with fluorescence detection (HPLC-FLD) is often used in routine practice (Jánská *et al.* 2004, 2006; Ciecierska & Obiedzinski 2007).

Nowadays, due to the growing need for the control of many groups of contaminants in food, it is necessary to have fast and reliable methods which allow a high sample laboratory throughput. Moreover, ecological and economical aspects force modern laboratories to employ fast and reliable methods enabling simultaneous determination of various groups of contaminants.

The aim of this study was to optimise and validate an efficient extraction procedure for simultaneous determination of PCBs, PBDEs, OCPs, and PAHs in canned fish and sea food products that represent widely consumed protein and lipid rich food commodities.

MATERIAL AND METHODS

Sample materials. A sample of canned smoked sprats (originated in Poland) obtained from the Czech retail market was used for the method optimisation and validation. The whole content of the can was homogenised and this testing material was stored at a temperature below –12°C in a freezer. The verification of the optimised procedure was performed using the Standard Reference Material SRM 2977 Mussel Tissue (PAHs, PCBs, OCPs) and Standard Reference material SRM 1947 Lake Michigan fish tissue (PCBs, OCPs, PBDEs, elements, fatty acids) (NIST, Boulder, USA).

Chemicals. Cyclohexane, *n*-hexane, isooctane (SupraSolv® quality; Merck, Darmstadt, Germany), toluene (Merck, Darmstadt, Germany), ethyl acetate, and dichloromethane (for GC residue analysis; Scharlau, Sentmenat, Spain) were used as supplied. Anhydrous magnesium sulphate (pro analysis; Penta, Prague, Czech Republic) was heated at 600°C for 5 h and then stored before the use in a tightly capped glass bottle. Styrene-divinylbenzene gel (Bio-Beads® S-X3, 200–400 mesh) was purchased from Bio-Rad Laboratories (Hercules California, USA).

Standard mixture PAH Mix 9 of 16 priority PAHs – acenaphthene (AC), acenaphthylene (ACL), anthracene (AN), benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbFA), benzo[*k*]fluoranthene (BkFA), benzo[*g,h,i*]perylene (BghiP), dibenz[*a,h*]anthracene (DBahA), fluoranthene (FA), fluorene (FL), chrysene (CHR), indeno[1,2,3-*cd*]pyrene (IP), naphthalene (NA), phenanthrene (PHE) and pyrene (PY) dissolved in cyclohexane, and standards of individual PAHs

– benzo[*c*]fluorene (BcFL), cyclopenta[*cd*]pyrene (CPP), 5-methylchrysene (5-MC), benzo[*j*]-fluoranthene (BjFA), dibenzo[*a,l*]pyrene (DBalP), dibenzo[*a,e*]pyrene (DBaeP), dibenzo[*a,i*]pyrene (DBaiP), dibenzo[*a,h*]pyrene (DBahP) dissolved in cyclohexane were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purity of the individual standards was not less than 95%. Certified standard solution of ^{13}C labelled PAHs in nonane, US EPA 16 PAH Coctail (AC-$^{13}C_6$, ACL-$^{13}C_6$, AN-$^{13}C_6$, BaA-$^{13}C_6$, BaP-$^{13}C_4$, BbFA-$^{13}C_6$, BkFA-$^{13}C_6$, BghiP-$^{13}C_{12}$, DBahA-$^{13}C_6$, FA-$^{13}C_6$ FL-$^{13}C_6$, CHR-$^{13}C_6$, IP-$^{13}C_6$, NA-$^{13}C_6$, PHE-$^{13}C_6$, PY-$^{13}C_3$) was supplied by Cerilliant (New Zealand). Certified standard of DBaiP-$^{13}C_{12}$ and DBaeP-$^{13}C_6$ in nonane was supplied by Cambridge Isotope Laboratories Inc. (Andover, USA).

Standard mixture PCB Mix 3 of indicator PCBs (IUPAC Nos. 28, 52, 101, 118, 138, 153, and 180) dissolved in isooctane and solid standards of OCPs [hexachlorobenzene (HCB), α-, β-, γ-isomers of hexachlorocyclohexane (HCH), *o,p'*-DDT, *p,p'*-DDT and their degradation products *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE and *p,p'*-DDE, α-, β-endosulfan, endosulfan sulfate, endrin, dieldrin, aldrin, *cis-*, *trans-*isomers of heptachlor epoxide (HEPO), *cis-*, *trans-*isomers of chlordane, oxy-chlordane, heptachlor], and PCB 112 were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purity of the individual standards was not less than 98%. Standard 13C-PCB 77 was supplied by Cambridge Isotope Laboratories (Andover, USA).

The individual standard solutions of PBDEs congeners (IUPAC Nos 28, 37, 47, 49, 66, 85, 99, 100, 153, 154, and 183) dissolved in nonane were supplied by Wellington Laboratories (Guelph, Ontario, Canada). The purity of the individual standards was not less than 98%. The working standard solutions of PAHs were prepared in toluene, the standard solutions of PCBs, PBDEs and OCPs were prepared in isooctane and stored at –12°C.

Analytical method

Isolation. The whole content of the can containing fish and oil was homogenised in a beaker using an Ultra-Turrax homogenizer (IKA, Königswinter Germany). The PLE was used for the extraction of the target compounds. The flowing powder consisting of 5 g of the homogenised sample and 25 g of anhydrous magnesium sulphate, mixed in a grinding mortar, was transferred into a 33 ml extraction cell of the Dionex ASE 300 system (Accelerated Solvent Extractor, Dionex, USA). The extraction was carried out at a constant pressure 1500 psi (10.34 MPa) under various experimental conditions (extraction solvent, extraction temperature, number of static cycles) to obtain optimal settings for the isolation procedure step (Figure 1). After finalisation of the extraction process, the extracts were collected and transferred into the extraction vessels. The solvent was then evaporated in a rotary vacuum evaporator just to reach dryness. The residue after evaporation was transferred into a 10 ml volumetric flask with a mixture of cyclohexane-dichloromethane (1:1, v/v) including PCB 112 at a concentration of 5 ng/ml as an internal standard.

Clean-up. A clean-up of the crude extract was carried out by an automated gel permeation chro-

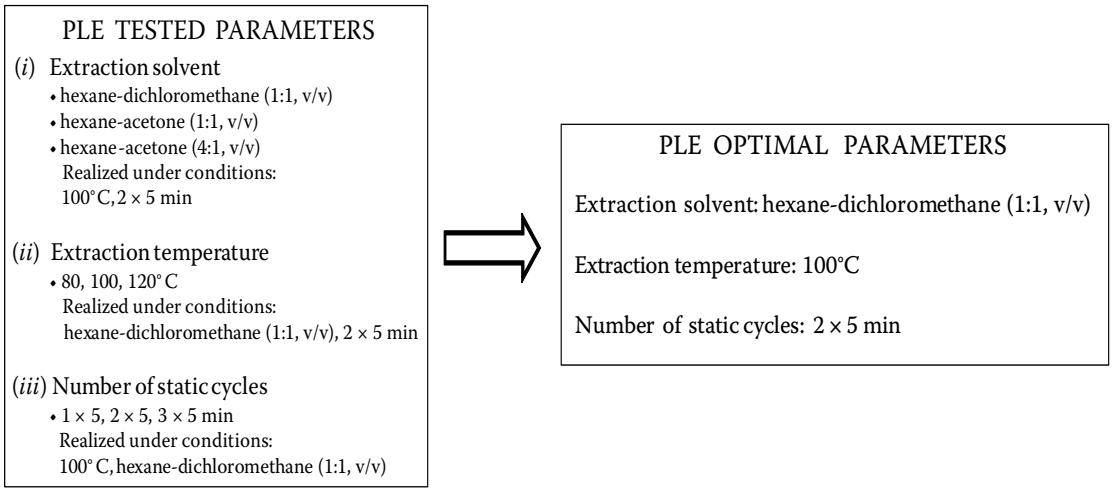

Figure 1. Experimental set-up: optimization of parameters for PLE extraction step

matography (GPC) system (consisting of 305 MASTER pump, fraction collector, automatic regulator of loop XL, microcomputer (software 731 PC via RS232C), dilutor 401C (Gilson, France)) on the Bio-Beads® S-X3 gel (glass column 500 mm × 10 mm *i.d*). Cyclohexane-dichloromethane (1:1, v/v) was used as the mobile phase, with the constant flow rate of 1 ml/min and injection volume of 2 ml. The fraction corresponding to the elution volume of 20–45 ml was collected. The eluate was then evaporated in a rotary vacuum evaporator at 40°C, followed by a gentle stream of nitrogen just to reach dryness. The residue was dissolved in 0.5 ml of isooctane with PBDE 37, labelled ^{13}C-PCB 77 and ^{13}C-PAHs, and transferred into a glass vial for the subsequent GC analysis.

GC-TOF MS analysis. All experiments were performed using an Agilent 6890N GC system (Agilent Technologies, Santa Clara, USA) coupled to a Pegasus III (LECO Corp., St. Joseph, USA) high-speed time-of-flight mass spectrometer (GC-TOFMS) operated in the electron ionisation mode (EI) that allowed the identification and quantification of all target PCBs, PBDEs, OCPs, and PAHs within a single analytical run. The target analytes were separated on BPX-50 capillary column (30 m × 0.25 mm *i.d*. × 0.25 µm film thickness) (SGE Analytical Science, Melbourne, Australia). The GC conditions were as follows: oven temperature programme 80°C (4.3 min), @ 30°C/min to 220°C, @ 2°C/min to 240°C and @ 10°C/min to 340°C (held for 15 min); carrier gas helium with a ramped flow 1.3 ml/min (held for 19 min), @ 50 ml/min to 2 ml/min (held for 16 min); PTV injection in the solvent vent mode (split/splitless injector and a MPS 2 autosampler; Gerstel, Mülhe-

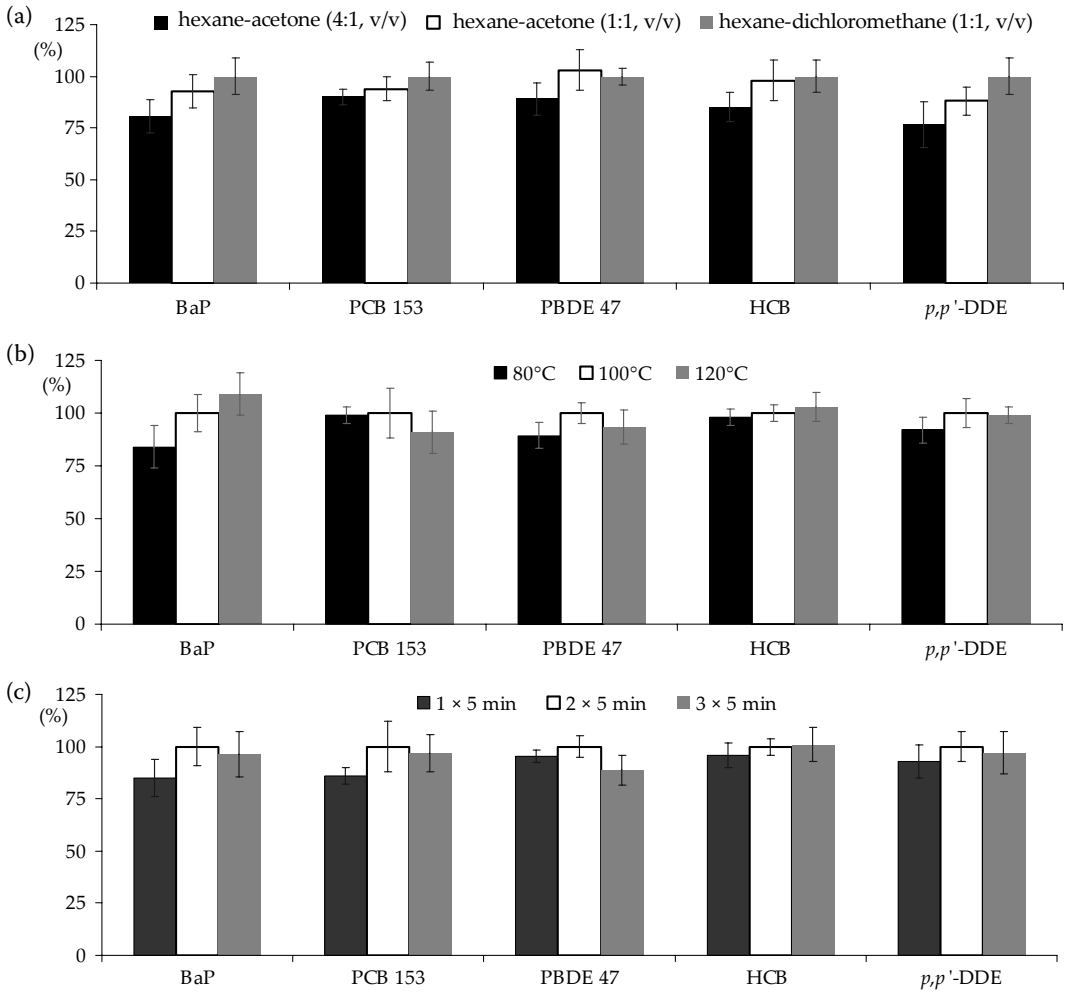

Figure 2. The effect of (a) extraction solvent mixture, (b) extraction temperature and (c) number of extraction cycles on the extraction efficiency of PLE for the most important representatives of PAHs, PCBs, PBDEs, and OCPs (BaP, PCB 153, PBDE 47, HCB, and *p,p*'-DDE). Optimised conditions shown in Figure 1. The results in the graphs represent mean values calculated from six replicates (n = 6)

im an der Ruhr, Germany); injected volume 1 × 8 µl; vent time 2.3 min; vent flow 50 ml/min; vent pressure 50 psi; initial temperature 50°C (2.3 min); inlet heating rate 400°C/min; final inlet temperature 300°C.

The MS detector was operated under the following conditions: mass range *m/z* 45–750; ion source temperature 250°C; transfer line temperature 280°C; detector voltage 1950 V; acquisition rate 3 spectra/s. The ChromaTOF 4.24 software (LECO Corp., St. Joseph, USA) was used for the data processing.

RESULTS AND DISCUSSION

Optimisation of PLE conditions

Within the first step of this study, several parameters of the PLE procedure were optimised: (*i*) extraction solvent, (*ii*) extraction temperature, and (*iii*) number of static extraction cycles. The default conditions (pressure 1500 psi, flush volume 60% of the extraction cell volume (the volume of the extraction solvent mixture used for flushing the extraction cell after the static extraction) and purge time (purge gas nitrogen, 60 s) were used in all PLE experiments. An overview of the experimental set-up applied for the optimisation process is illustrated in Figure 1. For the method development, spiked homogenates prepared from smoked sprats in oil were used. The spiking levels were 20 µg/kg for PCBs and OCPs, 10 µg/kg for PBDEs, and 5 µg/kg for PAHs, respectively. All experiments were designed to minimise the random errors by means of replicates (n = 6).

The influence of the individual parameters on the extraction efficiency is shown in Figure 2. To simplify the presentation of the data obtained, only some representatives of the respective contaminants were selected for this purpose: BaP has been up to now the only regulated PAH in fish samples and *p,p*'-DDE, HCB, PCB 153, PBDE 47 are typically the most abundant organohalogenated contaminants occurring in this type of matrix (Ackerman *et al.* 2008; Usydus *et al.* 2009; Szlinder-Richert *et al.* 2010).

In the PLE, the polarity of the extraction solvent is one of the key factors affecting the recovery of the target analytes isolation. For this reason, solvent mixtures of hexane-acetone (1:1, v/v), hexane-acetone (4:1, v/v), and hexane-dichloromethane (1:1, v/v) were compared in our study. These extraction solvents were selected as they have been frequently used for the isolation of POPs and PAHs from biotic samples (e.g. fish and mussels) using PLE (Suchan *et al.* 2004; Schantz 2006). The settings for these initial experiments (extraction temperature 100°C, static extraction time 5 min, two static extraction cycles) were based on the recently published data on the isolation of target POPs and PAHs in biotic matrices (Jánská *et al.* 2004; Suchan *et al.* 2004). As demonstrated in Figure 2a, the extraction mixture of hexane-dichloromethane (1:1, v/v) provided the highest recoveries for most of the compounds studied and thus was used as the extraction solvent in all following experiments.

The extraction temperature is a critical parameter for PLE because it greatly improves the extraction efficiency by increasing the solubility of the analyte in the solvent, improving mass transfer from the matrix to the solvent and disrupting analyte-matrix interaction (Kania-Korwel *et al.* 2008). Within our study, the influence of three different extraction temperatures (80°C, 100°C, and 120°C) was assessed. As shown in Figure 2b, no statistically significant differences between the recoveries of the target analytes using different temperatures were obtained. Based on these results, the temperature of 100°C was used for further investigations.

The number of the extraction cycles is the last important parameter for achieving satisfactory recoveries. In our study, three experiments differing in the number of extraction cycles (1 × 5 min, 2 × 5 min, and 3 × 5 min) were performed. The recovery obtained with one static extraction cycle was slightly lower compared to two and three static cycles (Figure 2c). For further experiments, 2 × 5 min extraction cycles were used to speed up the isolation procedure as well as to lower the volume of the extraction solvent.

Validation of the extraction method

In order to verify the accuracy of the optimised isolation procedure employing the extraction with hexane-dichloromethane solvent mixture (1:1, v/v), temperature 100°C, and 2 × 5 min extraction cycles, recovery and repeatability experiments were carried out. Samples of smoked fish spiked with the target compounds at two concentration levels were used and six replicates were performed at each spiking level. Each batch of samples included a procedure (reagent) blank. The spiking level I (5 µg/kg)

Table 1. Accuracy data of PCBs, PBDEs, OCPs and PAHs spiked at two levels in smoked sprats sample

Analyte	Spiking level I[a]		Spiking level II[b]		LOQ (µg/kg)
	R (%)	*RSD* (%)	*R* (%)	*RSD* (%)	
α-HCH	89	4	94	2	0.1
β-HCH	104	6	108	11	0.1
γ-HCH	77	6	88	5	0.1
Hexachlorobenzene	107	3	87	10	0.1
Heptachlor	82	8	79	2	0.3
trans (A)-HEPO	82	6	83	4	0.3
cis (B)-HEPO	72	5	71	2	0.3
Aldrin	77	5	80	2	0.3
Dieldrin	85	4	93	3	0.5
cis-Chlordane (alpha)	90	3	108	3	0.5
trans-Chlordane (gamma)	78	6	92	1	0.5
oxy-Chlordane	92	2	88	2	0.5
α-Endosulfan	74	4	74	3	0.5
β-Endosulfan	94	4	96	2	0.5
Endosulfan-sulfate	86	4	92	3	0.5
Endrin	93	4	104	6	0.5
p,o'-DDE	95	5	97	5	0.5
p,p'-DDE	109	3	95	3	0.5
o,p'-DDD	93	5	103	4	0.5
p,p'-DDD	111	5	113	2	0.5
o,p'-DDT	94	4	113	3	0.5
p,p'-DDT	89	5	97	1	0.5
PCB 28	100	5	115	3	0.5
PCB 52	77	6	92	5	0.5
PCB 101	100	12	109	3	0.5
PCB 118	86	8	109	4	0.5
PCB 153	93	4	115	4	0.5
PCB 138	90	7	118	6	0.5
PCB 180	74	5	94	4	0.5
PBDE 28	113	8	99	9	0.1
PBDE 49	83	3	95	9	0.1
PBDE 47	96	7	100	6	0.1
PBDE 66	79	7	78	9	0.1
PBDE 85	87	3	80	7	0.1
PBDE 99	78	3	73	6	0.1
PBDE 100	76	4	76	6	0.1
PBDE 153	92	3	82	7	0.1
PBDE 154	74	4	73	8	0.1
PBDE 183	89	8	90	7	0.3
BcFL	88	8	90	10	0.05
BaA	111	3	98	5	0.03
CHR	102	7	101	8	0.03
CPP	97	8	93	7	0.03
5 MC	103	4	107	4	0.05
BbFA+BkFA	89	5	101	3	0.03
BjFA	106	4	97	5	0.03
BaP	97	5	85	8	0.03
DBahA	92	5	95	3	0.03
IP	96	5	93	5	0.03
BghiP	101	4	94	4	0.03
DBalP	98	4	95	5	0.1
DBaeP	100	3	93	3	0.1
DBaiP	87	8	89	8	0.1
DBahP	93	7	100	4	0.1

R (%) = mean value recovery (n = 6); *RSD* (%) relative standard deviation; LOQ = limit of quantitation

[a]20 µg/kg PCBs, OCPs, 5 µg/kg of each PAHs and 10 µg/kg PBDEs added in the spiked sample

[b]10 µg/kg PCBs, OCPs, 1 µg/kg of each PAHs and 5 µg/kg PBDEs added in the spiked sample

for PAHs corresponds to maximum legislation limit for BaP in smoked fish samples. The level II (1 µg/kg) was set as a half of maximum legislation limit for non-smoked fish products. The spiking levels for PBDEs, PCBs, and OCPs (level I: 20 µg/kg for PCBs and OCPs, 10 µg/kg for PBDEs; level II: 10 µg/kg for PCBs and OCPs, 5 µg/kg for PBDEs) were chosen based on the expected levels of these contaminants in the analysed fish tissue. The recoveries are reported as mean values (*R*, *%*) with the corresponding relative standard deviations (*RSD*, %) in Table 1.

The recovery for the spiking level I varied in the range of 74–113%, 74–100%, 72–111%, and 87–111% for PBDEs, PCBs, OCPs, and PAHs, respectively. For the spiking level II, the recoveries were in the range 73–100%, 92–118%, 71–108%, and 85–107% for PBDEs, PCBs, OCPs, and PAHs, respectively. The lower recoveries obtained in the validation study for some OCPs were probably due to the loss of volatile analytes during the solvent evaporation.

The repeatability of the procedure, expressed as the relative standard deviation *(RSD, %)* of spiked fish samples replicates (n = 6), was satisfactory for all target analytes. In general, *RSD*s for most of the analytes did not exceed 10% and were in the range of 3–8% for PBDEs, 4–12% for PCBs, 2–8% for OCPs, and 3–8% for PAHs for the spiking level I, and in the range of 6–9%, 3–6%, 1–11%, and 3–10% for PBDEs, PCBs, OCPs, and PAHs, respectively, for level II (Table 1).

Trueness of results

The trueness of the final method was tested by analysing two standard reference materials (SRMs),

Table 2. Results obtained by analysis of the standard reference material "SRM 2977 mussel tissue"

	Obtained value (µg/kg)	Certified value and expanded uncertainty (µg/kg)	Calculated En-score*
BaA	19.42	20.34 ± 0.78	–0.3
BbFA+BkFA	10.86	11.01 ± 0.28	–0.1
BaP	7.89	8.35 ± 0.72	–0.3
BghiP	9.03	9.53 ± 0.43	–0.4
IP	3.96	4.84 ± 0.81	–0.8
DBahA	1.22	1.41 ± 0.19	–0.7
CHR	51.57	49 ± 2	0.2
BjFA	4.38	4.6 ± 0.2	–0.3
BkFA	3.17	4 ± 1	–0.8
PCB 28	4.97	5.37 ± 0.44	–0.3
PCB 52	8.23	8.37 ± 0.54	–0.1
PCB 101	10.12	11.2 ± 1.2	–0.6
PCB 118	9.36	10.5 ± 1.0	–0.6
PCB 138	15.32	16.6 ± 1.6	–0.3
PCB 153	13.23	14.1 ± 1.0	–0.4
PCB 180	6.24	6.79 ± 0.67	–0.5
cis-Chlordane	1.31	1.42 ± 0.13	–0.5
Dieldrin	5.46	6.04 ± 0.52	–0.7
p,p'-DDE	10.86	12.5 ± 1.6	–0.7
o,p'-DDD	3.05	3.32 ± 0.29	–0.6
p,p'-DDD	3.86	4.3 ± 0.38	–0.5
p,p'-DDT	1.13	1.28 ± 0.18	–0.7

*|En| ≤ 1 – the result is satisfactory and the trueness was proved; En-score – provides a measure how closely an obtained result agrees with the certified value, considering uncertainties of both the test result and certified value; En-score is based on a 95% coverage probability for the expanded uncertainty

Table 3. Results from the analysis of the certified reference material "SRM 1947 fish tissue"

	Obtained value (μg/kg)	Certified value and expanded uncertainty (μg/kg)	Calculated En-score*
PBDE 28	1.9	2.3 ± 0.5	–0.6
PBDE 47	64.5	73.3 ± 2.9	–0.7
PBDE 49	3.6	4 ± 0.1	–0.4
PBDE 66	1.6	1.9 ± 0.1	–0.5
PBDE 85	N/A	N/A	N/A
PBDE 99	15.8	19.2 ± 0.8	–0.8
PBDE 100	14.1	17.1 ± 0.6	–0.7
PBDE 153	3.1	3.8 ± 0.0	–0.8
PBDE 154	5.7	6.9 ± 0.5	–0.6
PBDE 183	N/A	N/A	N/A
PCB 28	12.9	14.1 ± 1	–0.5
PCB 52	32.1	36.4 ± 4.3	–0.4
PCB 101	82.4	90.8 ± 0.3	–0.3
PCB 118	103	112 ± 6.0	–0.3
PCB 138	135.5	162 ± 6.9	–0.8
PCB 153	181.8	201 ± 3.0	–0.8
PCB 180	70.3	80.8 ± 5.0	–0.4

*$|En| \leq 1$ – the result is satisfactory and the trueness was proved; En-score – provides a measure how closely an obtained result agrees with the certified value, considering uncertainties of both the test result and certified value; En-score is based on a 95% coverage probability for the expanded uncertainty; N/A – not available

namely mussel tissue SRM 2977 for PAHs, PCBs, and OCPs, and SRM 1947 Lake Michigan fish tissue for PCBs, OCPs, and PBDEs. The obtained results, certified values, and calculated En-scores for the analytes tested are summarised in Tables 2 and 3. As can be seen, our results obtained by optimised PLE method, when classified as En-scores, were satisfactory both for the mussel tissue material (SRM 2977) and for fish tissue material (SRM 1947): $|En| \leq 1$ for all analytes. Despite the satisfactory trueness of the experimental results for each individual compound having been well documented, certain negative bias can be detected as nearly all En-score values were slightly negative. As the repeatability was quite good, the correction for the recovery could be considered to eliminate the potential bias.

CONCLUSIONS

The pressurised liquid extraction (PLE), with the binary hexane-dichloromethane solvent mixture (1:1, v/v), applying the extraction temperature (100°C) and two static five minutes extraction cycles, allows an efficient simultaneous extraction of PCBs, OCPs, PBDEs, and PAHs from fish and related types of biotic matrices. The time needed for the completion of the optimised PLE extraction process was only 20 min, which is 25 times shorter than the time-consuming "classic" Soxhlet extraction; the amount of the solvent was reduced from 170 ml to 45 ml when using PLE. In this way both an increased sample throughput and an improved cost-effectiveness were achieved. The method met the performance characteristics required (i.e. recoveries in the range of 70–120% and repeatabilities ≤ 20%) (Document No. SANCO/10684/2009). In the follow-up project, the set of 54 samples of smoked and non-smoked fish and sea food will be examined using this new method.

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Received for publication October 20, 2010
Accepted after corrections March 28, 2011

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