



# Simplified and rapid determination of polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in fish and shrimps integrated into a single method

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## ABSTRACT

In this study, a new rapid and flexible method for the simultaneous determination of 18 key representatives of polychlorinated biphenyls (PCBs), 7 polybrominated diphenyl ethers (PBDEs), and 32 polycyclic aromatic hydrocarbons (PAHs) in fish and shrimps by gas chromatography coupled to mass spectrometry (GC–MS) was developed and validated. A substantial simplification of sample processing prior to quantification step was achieved: after addition of water to homogenized sample, transfer of hydrophobic analytes into ethyl acetate was supported by added inorganic salts. Bulk fat, contained in crude organic extract obtained by partition, was subsequently removed on a silica minicolumn. This approach enabled to process six samples in less than 1 h; moreover, the volume of an extraction solvent and consumption of other chemicals can be significantly reduced compared to, e.g., traditional Soxhlet extraction followed by gel permeation chromatography. The recoveries of target analytes were in the range of 73–120% even at the lowest spiking level ( $1 \mu\text{g kg}^{-1}$ ), repeatabilities (relative standard deviations, RSDs) ranged from 1 to 20%. Under optimized GC–MS conditions (time-of-flight mass analyzer, TOF), the limits of quantification (LOQs) were as follows: PCBs  $0.1\text{--}0.5 \mu\text{g kg}^{-1}$ , PBDEs  $0.5 \mu\text{g kg}^{-1}$ , and PAHs  $0.05\text{--}0.25 \mu\text{g kg}^{-1}$ . Ambient mass spectrometry employing a direct analysis in real time (DART) ion source was shown as an effective tool for fat control in extract, which is needed during the method development and examination of unknown samples prior to the analysis. Further extension of a method scope by other similar analytes is easily possible.

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## 1. Introduction

Polychlorinated biphenyls (PCBs), polybrominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs) represent the major groups of ubiquitous environmental pollutants that might be transferred into the food chains. Recently, in response to the food business operators' and consumers' concern on their food and feed safety, the European Food Safety Authority (EFSA) has raised the request for an up-dating of occurrence data in order to review its scientific opinion on the dietary exposure and health risks posed by these chemicals to humans [1–5].

With regard to the urgent need to collect a large set of reliable data in a short time, a European research project called CONFIDENCE [6] was established to support respective food safety issues through the development and validation of screening tools, which are simple, inexpensive, rapid, and able to detect (if possible

simultaneously) the above priority pollutants (and other similar harmful chemicals). To fulfill above-mentioned tasks, availability of relevant analytical tools is important for enabling the control of effectiveness of applied measures aimed at prevention/reduction contamination and for assurance of a flexible response to the Rapid Alert System for Food and Feed (RASFF) emergencies [7].

A wide range of group-specific analytical methods has been developed for the analysis of PCBs, PBDEs, and PAHs in food matrices. With regards to similarities in physico-chemical properties of these chemicals (e.g., hydrophobicity, relatively good thermal stability, semivolatility), some steps in various “traditional” analytical methods are almost identical. Nevertheless, until now, any uniform analytical flow-chart encompassing all three groups of these target analyte groups has not been introduced into a routine practice [8,9]. The diversity, as well as the overlaps, of existing analytical strategies is illustrated in Fig. 1 [8–23]. Various modes of non-selective extraction of analytes by semi-/non-polar solvents or their mixtures are typically employed. The main drawback of the most often used approach – Soxhlet extraction, is not only high solvents consumption but also time demands [8,9,12]. These parameters can be significantly reduced by using a number of

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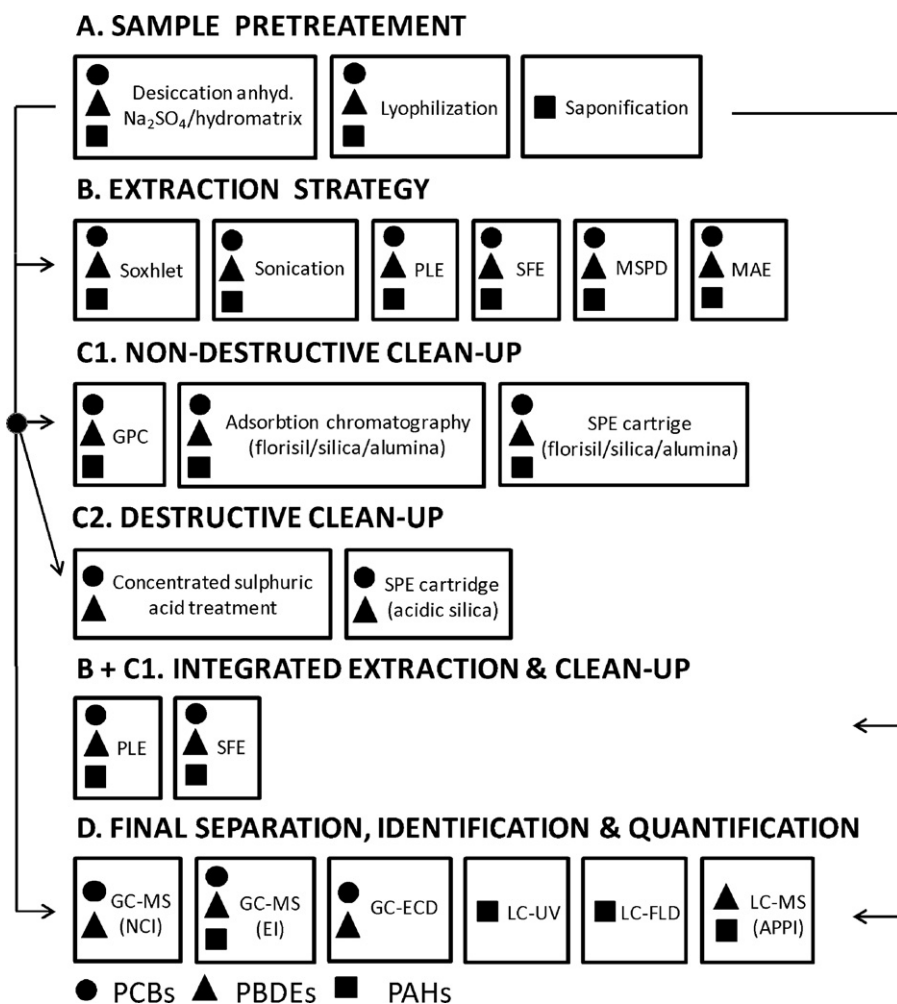


Fig. 1. Simplified overview of the most common analytical flow charts used for determination of PCBs, PBDEs and PAHs [8–23].

alternative semi-automated sample preparation techniques such as microwave assisted extraction (MAE), pressurized liquid extraction (PLE), and super-critical fluid extraction (SFE). When a suitable sorbent is added to a desiccated sample, the latter two techniques, PLE and SFE, may benefit, after careful optimization of operation conditions, from the possibility to integrate both extraction and clean-up into one step [8,9,12–21].

In many cases, bulky lipid co-extracts have to be eliminated prior to the determinative step, unless their saponification is not carried out like in older methods for the analysis of PAHs [10,11]. Conventional clean-up strategies typically include either semi-automated solid-phase extraction (SPE) on cartridges with alumina, florisil, silica (and/or their combinations), [22] or gel permeation chromatography (GPC) [8,9,12]. Destructive clean-up techniques comprise, for instance, sulfuric acid treatment. However, the main limitation of these procedures is that some analytes (e.g., PAHs) are degraded under conditions of lipid removal employing strong acids [8,9,12,13,15,16,23].

Recently, the use of another challenging procedure based on so called QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach, originally developed for the analysis of multiple pesticide residues in high moisture low fat matrices [24,25], has been successfully tested in the analysis of PAHs in contaminated fish [26]. Although possible use of QuEChERS for the determination of some halogenated persistent POPs (e.g., DDT) has been discussed, no peer reviewed paper reported the validated procedure for PCBs or BFRs.

PCBs, PBDEs, and PAHs are semivolatile compounds amenable to gas chromatography (GC). Nowadays, this separation technique hyphenated to mass spectrometry (MS) represents the most preferred option for their identification/quantification in food matrices [8,9,12]. In addition to this approach, reversed phase high performance liquid chromatography (RP-HPLC) with fluorescence detection (FLD) is commonly employed in case of PAHs, however the scope is rather limited since some analytes (e.g., cyclopenta[cd]pyrene and benzo[*j*]fluoranthene) are not fluorescent [11,27].

The main aim of the presented study was to develop and validate an integral sample preparation procedure with reduced requirements for sample amount, extraction solvents, and expensive laboratory equipment. For a fast and flexible determination of PCBs, PBDEs, and PAHs in fish/shrimps extracts within a single analytical run GC–MS employing a time-of-flight mass analyzer (TOF) was selected.

## 2. Experimental

### 2.1. Standards

Three groups of certified standards, 7 major PBDEs, 18 major and dioxin-like PCBs, and 32 PAHs were used within this validation study.

Individual PBDE congeners – 28, 47, 99, 100, 153, 154, and 183 (all with declared purity >98%) were supplied by Wellington

Laboratories (Guelph, Ontario, Canada). PCB standards 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189 (all with declared purity >97%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Calibration solutions (stored at  $-12^{\circ}\text{C}$ ) were prepared in isooctane; they contained all PCBs and PBDEs mentioned above at concentration levels 0.05, 0.1, 0.5, 1, 5, 10, 50, and  $100\text{ ng mL}^{-1}$ . Standards  $^{13}\text{C}$ -PCB 101, 77 and PBDE 37, 77 were supplied by Cambridge Isotope Laboratories (Andover, MA, USA) and Wellington Laboratories (Guelph, Ontario, Canada), respectively. The standard reference material Lake Michigan Fish Tissue, SRM 1947 ( $10.4 \pm 0.5\%$  (w/w) of fat), for selected PCBs, organochlorinated pesticides (OCPs), and PBDEs, was supplied by NIST (Gaithersburg, MD, USA).

The certified standards of individual PAHs: acenaphthene (AC), acenaphthylene (ACL), anthracene (AN), benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbFA), benzo[*c*]fluorene (BcFA), benzo[*j*]fluoranthene (BjFA), benzo[*k*]fluoranthene (BkFA), benzo[*ghi*]perylene (BghiP), chrysene (CHR), cyclopenta[*cd*]pyrene (CPP), dibenz[*ah*]anthracene (DBaA), dibenzo[*ae*]pyrene (DBaE), dibenzo[*ah*]pyrene (DBaH), dibenzo[*ai*]pyrene (DBaI), dibenzo[*al*]pyrene (DBaL), dibenzothiophene (DBT), fluoranthene (FA), fluorene (FL), indeno[1,2,3-*cd*]pyrene (IP), naphthalene (NA), phenanthrene (PHE), pyrene (PY), 2-methylanthracene (2MA), 1-methylchrysene (1MC), 3-methylchrysene (3MC), 5-methylchrysene (5MC), 1-methylnaphthalene (1MN), 2-methylnaphthalene (2MN), 1-methylphenanthrene (1MPH), and 1-methylpyrene (1MP) (all with declared purity >98%) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The certified standard solution of labeled PAHs used for quantification of target PAHs – US EPA 16 PAH Cocktail ( $^{13}\text{C}_6$ -AC,  $^{13}\text{C}_6$ -ACL,  $^{13}\text{C}_6$ -AN,  $^{13}\text{C}_6$ -BaA,  $^{13}\text{C}_4$ -BaP,  $^{13}\text{C}_6$ -BbFA,  $^{13}\text{C}_6$ -BkFA,  $^{13}\text{C}_{12}$ -BghiP,  $^{13}\text{C}_6$ -DBaA,  $^{13}\text{C}_6$ -FA,  $^{13}\text{C}_6$ -CHR,  $^{13}\text{C}_6$ -IP,  $^{13}\text{C}_6$ -NA,  $^{13}\text{C}_6$ -PHE,  $^{13}\text{C}_3$ -PY) was purchased from Cerilliant (Round Rock, TX, USA). Certified standards of  $^{13}\text{C}_{12}$ -DBaI and  $^{13}\text{C}_6$ -DBaE were supplied by Cambridge Isotope Laboratories Inc. Calibration solution (levels) were prepared as for PCBs and PBDEs in isooctane and stored at  $-12^{\circ}\text{C}$ . The standard reference material of mussel tissue, SRM 1974b (fat content not provided), for selected PAHs, PCBs and OCPs, was supplied by NIST.

## 2.2. Chemicals, reagents and other material

*n*-Hexane, dichloromethane, and isooctane were supplied by Merck (Darmstadt, Germany). Ethyl acetate and acetonitrile were purchased from Sigma–Aldrich (Steinheim, Germany). All solvents were of analytical grade. Silica (0.063–0.200 mm) supplied by Merck was activated by heating at  $180^{\circ}\text{C}$  for 5 h than deactivated by adding 2% of deionized water, shaking for 3 h and finally stored in a desiccator for 16 h before use. Magnesium sulfate and sodium chloride for the QuEChERS-like extraction were delivered from Sigma–Aldrich and Lach-ner (Neratovice, Czech Republic), respectively. Bondesil-C18 (40UM) was provided by Agilent Technologies (Palo Alto, CA, USA). A Pasteur pipette (D812, 230 mm length) and a glass wool were received from Poulten & Graf GmbH (Wertheim, Germany) and Merck, respectively. Glass column (1 cm i.d.) for adsorption chromatography was obtained from Merci (Brno, Czech Republic).

## 2.3. Instruments

A tissue grinder Waring blender (model 38BL40) was supplied by Waring (Torrington, CT, USA). A rotary vacuum evaporator Buchi Rotavapor R-114 and R-200 with a heating bath were obtained from Buchi Rotavapor (Flawil, Switzerland). A centrifugal machine Rotina 35R was supplied by Hettich Zentrifugen (Tuttlingen, Germany). For the clean-up efficiency experiments a system

consisting of a direct analysis in real time (DART) ion source (IonSense, Saugus, MA, USA), an AccuTOF LP high-resolution time-of-flight mass spectrometer (JEOL (Europe), SAS, Croissy sur Seine, France), and an HTC PAL autosampler AutoDART-96 (Leap Technologies, Carrboro, NC, USA), was used.

All GC–MS experiments were performed using a gas chromatograph Agilent 6890N (Agilent Technologies) coupled to a high-speed time-of-flight mass spectrometer Pegasus III (LECO Corp., St Joseph, MI, USA) operated in an electron ionization mode (EI). The GC system was equipped with an electronic pressure control (EPC), a programmable temperature vaporization (PTV) injector and an MPS 2 autosampler (Gerstel, Germany). A multi baffled deactivated PTV liner (ID 1.8 mm, volume  $150\ \mu\text{L}$ ) was supplied by Agilent. Capillary columns BPX-50 ( $30\text{ m} \times 0.25\text{ mm i.d.} \times 0.25\ \mu\text{m}$  film thickness) and BPX-5 ( $30\text{ m} \times 0.25\text{ mm i.d.} \times 0.25\ \mu\text{m}$  film thickness) were obtained from SGE (Austin, TX, USA). The ChromaTOF 4.24 software (LECO Corp.) was used for data processing.

## 2.4. Samples

Two different fish (skin-free fillets) and shrimps samples were used for experiments. Trout, salmon and shrimps from the Czech retail market previously tested for the presence of PCBs, PBDEs, and PAHs were used for the quality assurance/quality control (QA/QC) experiments. The average lipid content of these materials was as follows: trout 1.6% (w/w) fat, salmon 14% (w/w) fat, shrimps 0.4% (w/w) fat. All samples were kept at  $-18^{\circ}\text{C}$  after pooling and homogenization.

## 2.5. Partition based sample preparation

### 2.5.1. Acetonitrile partitioning with dSPE (QuEChERS)

In the first step, an original QuEChERS extraction with acetonitrile followed by liquid–liquid partition and dispersive solid phase extraction with (i) primary–secondary amine (PSA) and (ii) PSA + C18 was tested [24,25,28].

### 2.5.2. Acetonitrile or ethyl acetate partitioning followed by silica minicolumn clean-up

An amount of 10 ng of BDE 37 and  $^{13}\text{C}$ -CB 77 used as surrogates were added to 10 g of fish muscle tissue homogenate prior to the extraction. Subsequently, 5 mL of distilled water were added (in case of ethyl acetate also 0 and 10 mL were tested) and shaken vigorously with 10 mL of acetonitrile or ethyl acetate in a polypropylene tube for 1 min. Then 4 g of magnesium sulfate and 2 g of sodium chloride were added to the extract. The tube was shaken for another 1 min, centrifuged (5 min at 11,000 rpm), and an aliquot of 5 mL was removed from the upper organic phase. Solvent (5 mL) was evaporated to the last drop and its residues were carefully eliminated under the gentle stream of nitrogen.

### 2.5.3. SPE minicolumn clean-up

According to the fat content of the analyzed fish (for the fat determination, see Section 2.7) two size of the handmade silica minicolumn were used: (i) Pasteur pipette filled with glass wool, 1 g of silica, and a thin layer of sodium sulfate for fish with fat content up to 2%; (ii) glass column filled with glass wool, 5 g of silica, and a thin layer of sodium sulfate for fish with fat content higher than 2% (up to 0.8 g of fat can be loaded on 5 g column).

At first, an evaporated extract was re-dissolved in 1 mL of *n*-hexane and purified using the handmade silica minicolumn. The column was conditioned with (i) 6 mL or (ii) 18 mL of the elution solvent (*n*-hexane:dichloromethane (3:1, v/v)) followed by (i) 4 mL or (ii) 12 mL of *n*-hexane. Then the extract was applied onto the minicolumn and analytes were eluted with (i)

10 mL or (ii) 30 mL of a mixture of *n*-hexane:dichloromethane (3:1, v/v). Secondly, the evaporated extract was re-dissolved in 1 mL of *n*-hexane:dichloromethane (3:1, v/v). The column was conditioned with *n*-hexane:dichloromethane (1:1, v/v) followed by *n*-hexane:dichloromethane (3:1, v/v) and analytes were eluted by *n*-hexane:dichloromethane (1:1, v/v). The volumes of solvents were the same as in the first case. Collected eluates were carefully evaporated using a vacuum rotary evaporator and the residual solvents were removed under the gentle stream of nitrogen. Residues were finally re-dissolved in 0.5 mL of isooctane containing BDE 77 (5 ng mL<sup>-1</sup>), <sup>13</sup>C-CB 101 (40 ng mL<sup>-1</sup>), and <sup>13</sup>C-PAHs (2 ng mL<sup>-1</sup>) used as syringe standards.

## 2.6. GC–TOFMS analysis

All experiments were performed using an Agilent 6890N GC system coupled to a Pegasus III high-speed time-of-flight mass spectrometer (GC–TOFMS) operated in an electron ionization mode (EI) that allowed identification and quantification of all target PCBs, PBDEs, and PAHs within a single analytical run. Target analytes were separated on a BPX-50 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). A volume of 8 μL was injected using PTV injection in a solvent vent mode (vent time: 2.3 min; vent flow: 50 mL min<sup>-1</sup>; vent pressure: 50 psi) with initial temperature 50 °C (2.3 min); inlet heating velocity: 400 °C s<sup>-1</sup> and final inlet temperature: 300 °C. Injection speed was 10 μL s<sup>-1</sup> with initial temperature 50 °C (2.3 min); inlet heating velocity: 400 °C s<sup>-1</sup> and final inlet temperature: 300 °C. Injection speed was 10 μL s<sup>-1</sup>. Helium was used as a carrier gas using ramped flow 1.3 mL min<sup>-1</sup> (19 min) increased at a rate 50 mL min<sup>-1</sup> up to 2 mL min<sup>-1</sup> (16 min). A GC oven temperature program was as follows: 80 °C (4.3 min), 30 °C min<sup>-1</sup> to 220 °C, 2 °C min<sup>-1</sup> to 240 °C, and 10 °C min<sup>-1</sup> to 340 °C (15 min).

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<sup>-1</sup>. The ions (*m/z*) selected for quantification and confirmation are shown in Table S-1 (Supplementary data).

Quantification of all target analytes according to their height was performed using an eight point calibration. For elimination of potential injection inaccuracies syringe standards were used as follows: BDE 77 for all PBDEs, <sup>13</sup>C-CB 101 for all PCBs and corresponding <sup>13</sup>C-labeled analogues for PAHs. For those PAHs that do not have their own <sup>13</sup>C-labeled standard following <sup>13</sup>C-PAHs were used for the quantification: <sup>13</sup>C<sub>4</sub>-BaP for B<sub>j</sub>F<sub>A</sub>, <sup>13</sup>C<sub>12</sub>-DBaIP for DBaHP and DBaIP, <sup>13</sup>C<sub>6</sub>-AN for 2MA, <sup>13</sup>C<sub>6</sub>-CHR for DBT, CPP, 1MC, 3MC and 5MC, <sup>13</sup>C<sub>6</sub>-NA for 1MN, 2MN, <sup>13</sup>C<sub>6</sub>-PHE for 1MPH, <sup>13</sup>C<sub>3</sub>-PY for 1MPY, and <sup>13</sup>C<sub>6</sub>-FA for BcFA.

## 2.7. DART–TOFMS

The operating conditions of a DART ion source were as follows: positive ion mode; helium flow: 4.0 L min<sup>-1</sup>; gas beam temperature: 350 °C; discharge needle voltage: 3.0 kV; perforated and grid electrode potentials: +150 and +250 V, respectively. Conditions of TOFMS: cone voltage: +20 V; peaks voltage: 1000 V; monitored mass range: *m/z* 50–1100; acquisition rate: 2 spectra s<sup>-1</sup>; mass resolving power: approx. 6000 FWHM (full width at half maximum). Sample introductions were carried out automatically using Dip-it samplers (IonSense, Saugus, MA, USA). The sampling glass rod was immersed for 1 s into the sample hole of a deepwell micro-plate (Life Systems Design, Merenschwand, Switzerland) containing approx. 300 μL of respective sample, and transferred to the optimized position in front of the DART gun exit. The sample was then desorbed from the glass rod surface within 30 s (monitoring of TAG profiles during optimization of the clean-up strategy) or 5 s (determination of TAG content), while the spectral data were recorded. To improve the sensitivity of detection of TAGs, 2 mL vial containing 25% (w/w) aqueous ammonia solution (dopant) was

placed 4.5 mm below the ion source exit to produce ammoniated ions.

For the monitoring of TAG profiles during optimization of the clean-up strategy (see Section 3.1) the obtained extracts (see Section 2.5) were evaporated and re-dissolved in 1 mL of hexane (solvent compatible with subsequent purification using SPE mini-columns).

For the rapid determination of TAG content, the ethyl acetate extract obtained during partition based liquid extraction (see Section 2.5.2) was 5000-times diluted with ethyl acetate. After that, a volume of 20 μL of azoxystrobin (25 μg mL<sup>-1</sup>), used as an internal standard, was added to 980 μL of diluted extract followed by immediate DART–TOFMS analysis. The calibration graph was constructed by plotting known content of TAGs in the salmon samples (1, 2, 5, 10, 25, and 50% of lipids) (*x*-axis) determined gravimetrically against a peak area ratio of the TAGs/azoxystrobin response (*y*-axis). In the case of azoxystrobin, protonated molecule [M+H]<sup>+</sup> with *m/z* 404.12 was monitored, while for TAGs, the adduct ions [M+NH<sub>4</sub>]<sup>+</sup> in the range *m/z* 840–1020 were used.

To perform a mass drift compensation for accurate mass measurements, a polyethylene glycol (average relative molecular weight 600, Sigma–Aldrich, Steinheim, Germany) 200 μg mL<sup>-1</sup> solution in methanol, was introduced manually at the end of analysis run.

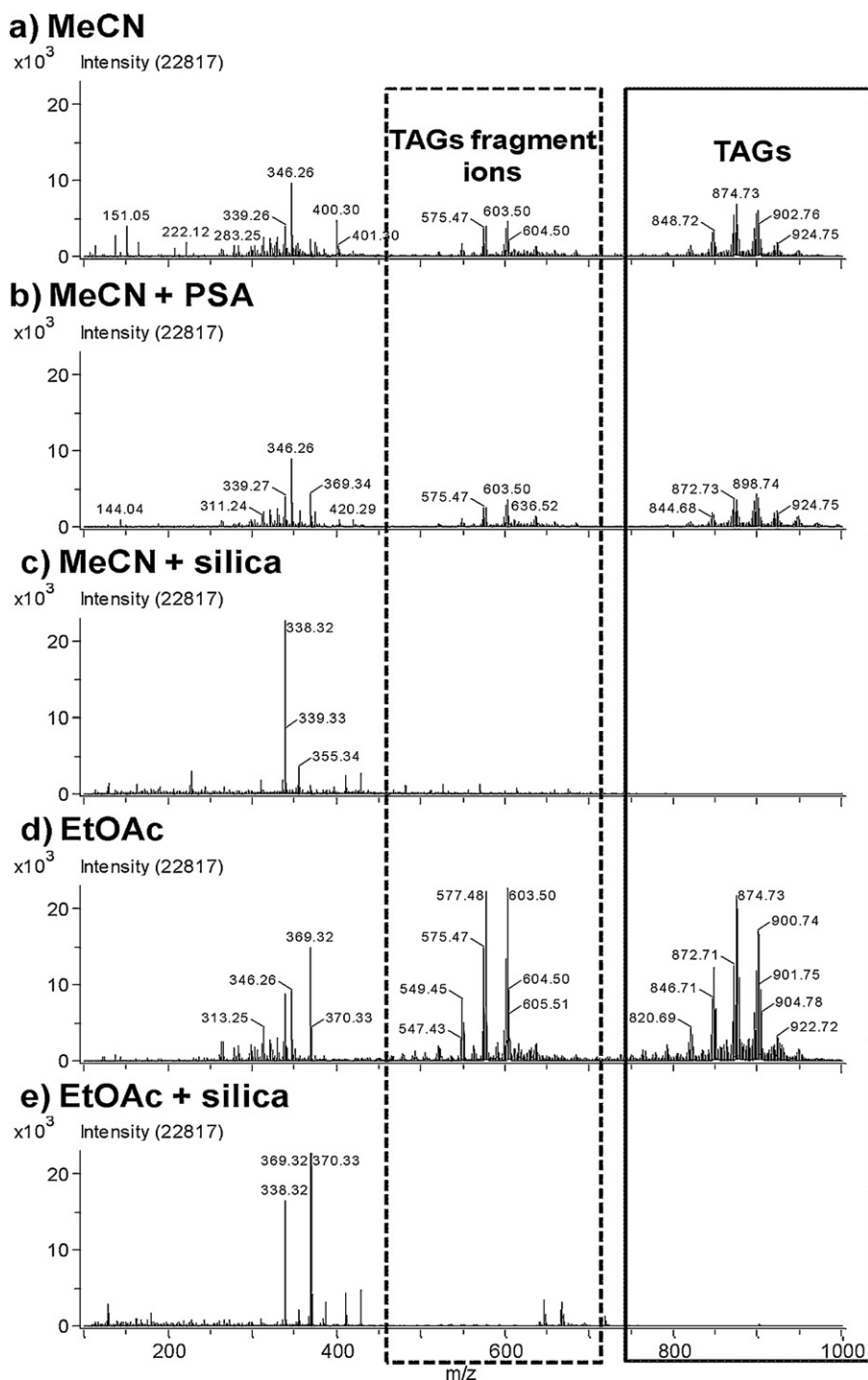
The Mass Center software version 1.3 (2006) (JEOL, Tokyo, Japan) was used for data processing. Mass spectral data were obtained by averaging of the mass spectra recorded during the exposure of the sample to the DART gas beam; background ions were subtracted and a mass drift was corrected.

## 3. Results and discussion

As mentioned in Section 1, several analytical procedures, each dedicated to a specific analytes group, have been routinely used in control laboratories to determine environmental pollutants in a particular food sample. As a challenging, simple and cost saving alternative to existing sample preparation approaches, QuEChERS (Quick, Easy, Cheap, Efficient, and Rugged), procedure widely used in pesticide multiresidue analysis, was tested in our study. The final objective was to develop an integral method for rapid control of several groups of pollutants in fish/shrimps, within a single run [6]. When planning our experiments, we presumed that QuEChERS might be applicable for the isolation of PCBs, PBDEs and PAHs, since effective isolation of pesticides of a wide polarity range, including lipophilic ones with relatively high *K*<sub>ow</sub> values (comparable to those of our target analytes), can be achieved from various food matrices [28,29]. Until now, the only one study using QuEChERS in the field similar to ours was focused (only) on PAHs [26]. Since, in this particular case, HPLC–FLD method was used for analytes separation/detection, the compatibility of QuEChERS sample preparation procedure with GC–MS analysis of PAHs (and other POPs) employed in this study could be hardly assessed.

For a rapid and flexible control of the amount of fat in analyzed fish/shrimp samples as well as for checking the transfer of matrix co-extracts (mainly lipids) across sample processing steps, an innovative technique, ambient mass spectrometry employing a DART (direct analysis in real time) ion source coupled to a time-of-flight mass spectrometer (TOFMS) was introduced. Compared to, e.g., traditionally use GC coupled to a flame ionization detector (FID), no special sample preparation is needed prior to the analysis and verification of lipid content could be done immediately.

A large set of experiments was carried out within this study; the phases of method development and its validation are described in the following sections.



**Fig. 2.** Optimization of partition-based sample processing documented by DART-MS technique – spectral fingerprints (positive ions): (a) MeCN = crude acetonitrile extract, (b) MeCN + PSA = acetonitrile extract purified using dispersive SPE with PSA, (c) MeCN + silica = acetonitrile extract purified using a silica minicolumn, (d) EtOAc = crude ethyl acetate extract, (e) EtOAc + silica = ethyl acetate extract purified using a silica mini-column (*TAGs* – triacylglycerides).

### 3.1. QuEChERS-like extraction

In the first part of experiments, the ‘original’ QuEChERS procedure consisting of (i) acetonitrile extraction, (ii) liquid partition induced by inorganic salts, and (iii) clean-up of crude acetonitrile extract by dispersive SPE employing PSA, was tested. As documented using a quick check by DART-TOFMS, very small reduction of lipid content in acetonitrile extract was achieved by dispersive PSA sorbent (see Fig. 2). Subsequently, PSA with C18 sorbent, applied in the pesticides analysis for low fatty food, were used for

the clean-up of crude acetonitrile extract. The addition of C18 led to a lower residual fat in the final extract, but not enough low for the GC-EL-TOFMS analysis, especially of early eluting PAHs. A bulk of coextracts that occurs in this part of chromatogram make impossible to integrate peaks of respective PAHs. Moreover, low recoveries of other PAHs were achieved, since the possible sorption of non-polar PAHs on C18 could appear. On this account, the ‘original’ QuEChERS sample preparation approach was found to be incompatible with the follow-up GC-MS analysis of fish/shrimps extracts. Another clean-up strategy had to be searched to avoid

deterioration of chromatographic performance. A minicolumn packed with silica, a commonly used sorbent for clean-up in trace-analysis, was selected as the best option for removing co-extracted lipids from a crude acetonitrile extract (florisil was also tested, however, the affinity of PAHs was rather higher making their separation from lipids more difficult). Prior to the sample loading onto the silica minicolumn, a crude acetonitrile extract had to be evaporated and then the residue (equivalent 5 g of original matrix) was transferred into 1 mL of hexane. When analyzing shrimps and trout (fat content 1.6%, w/w) spiked by three groups of target analytes at level  $1 \mu\text{g kg}^{-1}$ , recoveries of target analytes were in the range of 48–103%. The lowest values were obtained for some PBDE congeners and PAHs (for instance 46% for BDE 183 and 56% for BcFA). Drop of recoveries was encountered when fatty fish such as salmon was taken for analysis. Similarly, a strong negative relationship between the fat content in analyzed matrix and recoveries of non-polar analytes was observed in other studies investigating the QuEChERS scope [24,28]. It is assumed that partition of lipophilic contaminants between lipid phase and relatively weak extraction solvent plays a role. Another parameter influencing analytes recovery was moisture content in respective matrix. More detailed investigation was performed within experiments described below.

### 3.2. Ethyl acetate-based extraction

Not only fat content-dependence of extraction recoveries, but also time demands of an evaporation step, toxicity (workplace hazard) and relatively high cost [24,30], led us to replace acetonitrile by another extraction solvent. Finally, ethyl acetate was selected as it has a better capability to penetrate into the high moisture matrix such as fish/shrimps and thus enabling (by support of strong shaking) effective isolation of non-polar analytes [28,30–34]. As expected, the higher amount of lipids was extracted by ethyl acetate compared to acetonitrile. The same clean-up approach, fractionation on a silica minicolumn was used here after solvent exchange. However, because of its limited capacity (ca. 0.1 g of fat/1 g silica, what corresponds to 5 g of fish with max fatness 2%, w/w), either more sorbent, two consecutively used columns or a reduced sample equivalent loaded onto a minicolumn had to be used to achieve required limits of quantification. Again, a DART-TOFMS technique was used for rapid optimization of a clean-up procedure and its usefulness is well illustrated in Fig. 2. As shown here, regardless it was an acetonitrile or ethyl acetate extract, practically no lipids were contained in analytes fraction (*n*-hexane:dichloromethane, 3:1, v/v, eluate). All these troublesome matrix co-extracts were effectively retained on the silica sorbent. When testing the second condition/elution conditions (with more polar elution mixture – *n*-hexane:dichloromethane, 1:1, v/v) more than 60% of fat applied onto the minicolumn was transferred into the final extract and thus this set up was denied. Higher recoveries were achieved by ethyl acetate-based extraction, when analyzing fish spiked at a level of  $1 \mu\text{g kg}^{-1}$ , compared to those achieved by acetonitrile. Further increase of extraction efficiency was obtained by addition of water to matrix prior to extraction. The best mean recoveries, around 100% (in particular case 104, 106 and 99%, for target PCBs, PBDEs, and PAHs, respectively), were obtained when 5 mL of water were added to 10 g of fish homogenate. Addition of 10 mL led to comparable recoveries but slightly worst repeatabilities. Moisture content in the sample should always be taken into consideration prior to establishing the final sample processing protocol. Generally, dry matter varies in seafood/fish tissues in a wide range, it might be as low as 17% (w/w) in oysters and mussels, in common fish it is around 25% (w/w). As far as water losses occur during heat processing of (e.g., during smoking), it can be as high as 49% (w/w, smoked eel) [35]. In any case, ethyl acetate:water ratio should be adjusted at least to 1:1 (slightly higher amount of water

does not negatively affect the analysis). A similar strategy is in fact applied in the 'original' QuEChERS procedure: the addition of water to achieve a total of 10 g of water, when 10 g of sample and 10 mL of acetonitrile is employed, is required when dealing with samples containing between 25 and 80% (e.g., bananas) [24,25].

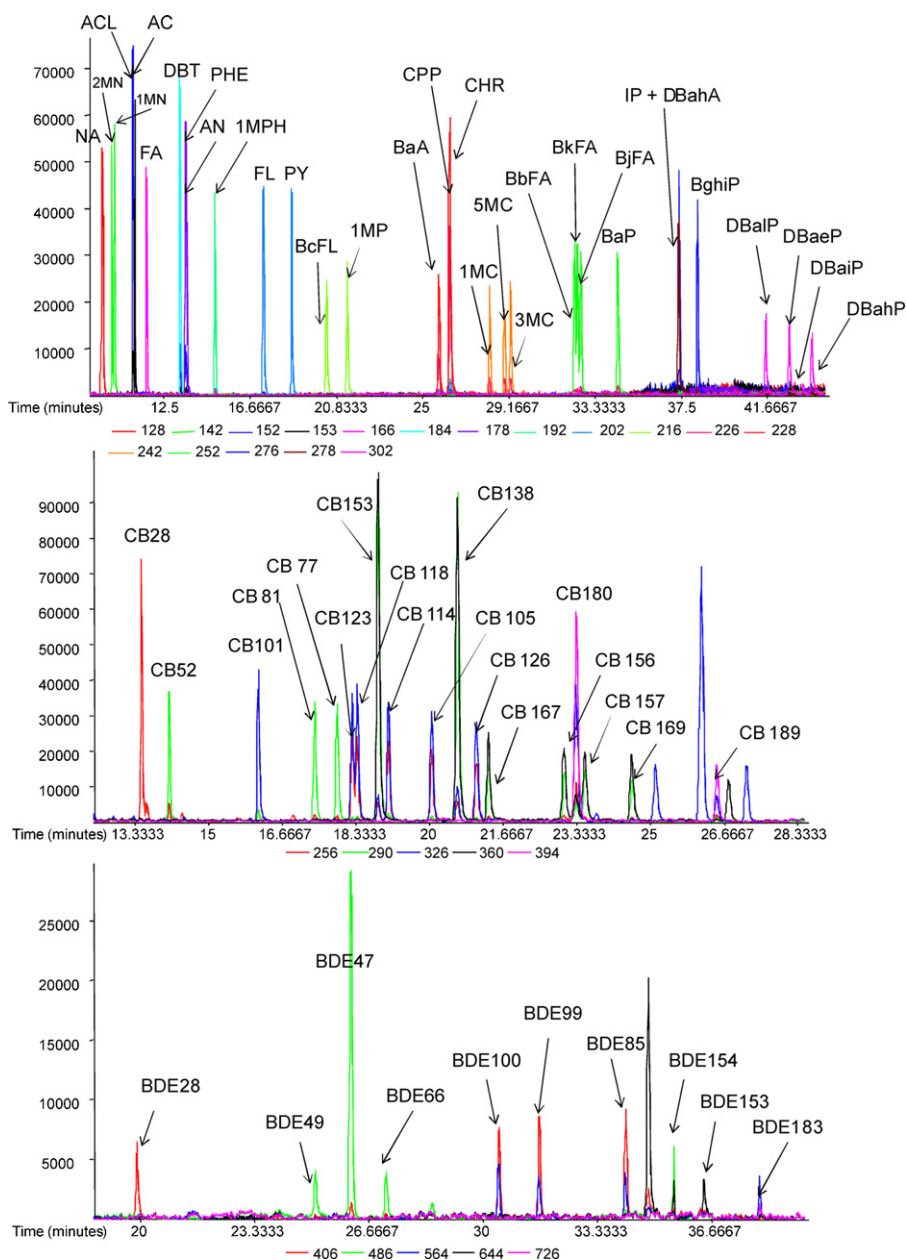
### 3.3. GC-EI-TOFMS conditions

As mentioned in Section 1, GC coupled to MS represents a 'gold standard' in the determination of PCBs, PBDEs, and PAHs. In our study, for optimization purpose, we use a fast time-of-flight (TOF) detector which enables, if needed, post run data mining on the presence of other contaminants than those on the original target list. Widely used BPX-5 and BPX-50 capillaries with a high upper temperature limit (more than  $360^\circ\text{C}$ ) needed for elution of high molecular weight PAHs were used for one-dimensional separation experiments. While all target PCBs and PBDEs were well resolved, the attention had to be focused on separation of three 'critical' groups of isomeric PAHs: (i) BaA, CPP, and CHR; (ii) BfFA, BkFA, and BbFA, and (iii) DBaHA, IP, and BghiP. Although none of the two tested columns allowed a baseline separation of all target PAHs, BPX-50 showed better ability to separate the second group of PAHs. Fig. 3 shows an example of the chromatogram of PCBs, PBDEs, and PAHs obtained by analysis of fresh fish muscle tissue spiked at  $5 \mu\text{g kg}^{-1}$ .

### 3.4. Method validation

Using the ethyl acetate-based extraction characterized above and followed by the optimized GC-TOFMS method, spiked trout (lean fish), salmon (fatty fish) and shrimps were analyzed to demonstrate method performance. The overview of validation data (recovery, repeatability and LOQ) obtained on these matrices are shown in Tables S-2, S-3, and S-4 (Supplementary data), respectively. The LOQ was defined as the lowest concentration of each analyte in matrix at which the quantification and confirmations are intensive enough to meet pre-defined criteria (SANCO document No. 10684/2009 [36]). Based on preliminary GC-TOFMS measurements using matrix samples contaminated at low concentrations, the LOQs were as follows: PCBs:  $0.1\text{--}0.5 \mu\text{g kg}^{-1}$ , PBDEs:  $0.5 \mu\text{g kg}^{-1}$ , and PAHs:  $0.05\text{--}0.25 \mu\text{g kg}^{-1}$  (the highest values for the late eluting dibenzopyrenes).

Together with each batch of samples, the procedural blank (i.e., sample prepared in a common way, but without the use of test matrix) was prepared. The recoveries (%) and repeatabilities of the measurement (expressed as RSD, %) were calculated from six replicate analyses of 'blank' trout, salmon and shrimps (fish with minimal background contamination was selected) fortified 15 min prior to the extraction with a mixture of target analytes at two concentration levels (for most target PCBs, PBDEs, and all PAHs, level 1 was  $1 \mu\text{g kg}^{-1}$ , level 2 was  $5 \mu\text{g kg}^{-1}$ ). Since the levels of major PCBs, congeners 138, 153, 180 and PBDE 47 in 'blank' samples were higher than  $1 \mu\text{g kg}^{-1}$ , the concentration levels of 5 and  $25 \mu\text{g kg}^{-1}$  were applied in case of these four analytes. For all matrices, the recoveries (%) and RSD (%) were in the range: 74–120% (RSD 2–20%) for PCBs, 79–120% (RSD 3–12%) for PBDEs, and 73–109% (RSD 1–15%) for PAHs. The key performance characteristics documented through the validation protocol met the criteria applied in the European Union in food contaminants control (SANCO document No. 10684/2009 [36] originally designed for pesticide residue analysis but commonly applied also for other organic food contaminants): recoveries in the range 70–120% and the repeatability less than 20%. Moreover for BaP the methods' performance criteria were also tested against commission regulation (EC) No 333/2007 (LOQ  $< 0.9 \mu\text{g kg}^{-1}$ , recovery 50–120%, precision expressed as HORRAT<sub>r</sub> less than 2) [37].



**Fig. 3.** An example of chromatogram of fish muscle tissue spiked with PCBs, PBDEs, and PAHs at  $5 \mu\text{g kg}^{-1}$  (major PCBs 138, 153 and 180 and PBDE 47 at  $25 \mu\text{g kg}^{-1}$ ).

The trueness of the method was demonstrated by using the US National Institute of Standards and Technology (NIST) SRM 1947 Lake Michigan Fish Tissue reference material for selected PCBs and PBDEs and SRM 1974b Mussel Tissue for PAHs and PCBs; see Tables S-5 and S-6 (Supplementary data) for the obtained results. The determined values for all analytes were in accordance with certified/reference values except for CB28 and 2MN in SRM1947b for which the determined values were slightly lower compared to those stated in the certificate. Although other PCBs and OCPs were not primarily included in the study (the list of analytes was pre-defined in the Description of work of the European project CONFIDENCE [6]), several of them (e.g., *o,p'*-DDE, *p,p'*-DDE, *p,p'*-DDD) were identified in both reference materials, as the GC–TOFMS technique applied for the determination of all target compounds allows also non-target screening of other potentially present contaminants. However, since these analytes were not included into the validation process of the entire method, the identification and eventual quantification of these compounds

can be taken only as a preliminary, further confirmation is needed.

#### 4. Conclusions

To our best knowledge, this is a very first study introducing fast and simple sample preparation procedure enabling simultaneous determination of PCBs, PBDEs, and PAHs in fish tissue and/or shrimps, within a single GC–MS run. The features of a new analytical strategy together with benefits resulting from its application are summarized in the following points:

- (i) Using the newly developed procedure based on ethyl acetate–aqueous sample suspension partition step followed by the SPE minicolumn silica clean-up the laboratory throughput can be fairly increased; up to 6 samples can be prepared in less than 1 h compared to several hours needed for Soxhlet

extraction followed by the relevant clean-up technique, typically gel permeation chromatography, GPC.

- (ii) In addition to time saving, also the volume of extraction solvents is significantly reduced when applying the new sample processing strategy, thus not only cost reduction but also the environment protection is achieved.
- (iii) Ambient mass spectrometry employing a DART ion source was demonstrated to be a very efficient tool for the fast determination of lipids and other ionizable impurities that can be controlled immediately.
- (iv) The recoveries of all target analytes were between 73 and 120% and the repeatability of the measurements was less than 20%, even at the lowest spiking level ( $1 \mu\text{g kg}^{-1}$ ). LOQs achieved by TOF mass analyzer were in the range of  $0.05\text{--}0.5 \mu\text{g kg}^{-1}$ . Further decrease of LOQs together with improved confirmation capability might be obtained by comprehensive two-dimensional GC  $\times$  GC which offers re-focusing of the peaks and better chromatographic resolution.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2011.09.016.

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1 Simplified and rapid determination of polychlorinated  
2 biphenyls, polybrominated diphenyl ethers, and  
3 polycyclic aromatic hydrocarbons in fish and shrimps  
4 integrated into a single method

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12 **Table S-1 (Supplementary data).** Ions selected for quantification and confirmation of target PCBs,  
 13 PBDEs, PAHs, and their <sup>13</sup>C-labelled analogues.

	Analytes	Quantification ions ( <i>m/z</i> )	Confirmation ions ( <i>m/z</i> )
PCBs	triCB – 28	256	258
	tetraCB – 52, 77, 81	290	292
	pentaCB – 105, 101, 114, 118, 123, 126	326	324, 328
	hexaCB – 138, 153, 156, 157, 167, 169	360	358, 362
	heptaCB – 180, 189	394	392, 396
PBDEs	triBDE – 28	406	246, 408
	tetraBDE – 47	486	326, 484
	pentaBDE – 99, 100	564	404, 566
	hexaBDE – 153, 154	644	484, 642
	heptaBDE – 183	726	641, 724
PAHs	NA	127	128
	1MN, 2MN	115	142
	ACL	151	152
	AC	152	153
	FA	165	166
	DBT	139	184
	PHE, AN	177	178
	1MPH, 2MA	189	192
	FL, PY	200	202
	BcFL, 1MP	215	216
	CPP	228	226
	BaA, CHR	226	228
	5MC, 3MC, 1MC	241	242
	BbFA, BkFA, BjFA, BaP	253	252
	IP, BghiP	277	276
	DBahA	276	278
	DBalP, DBaeP, DBaiP, DBahP	303	302
	<sup>13</sup> C- PAHs	<sup>13</sup> C <sub>6</sub> -NA	136
<sup>13</sup> C <sub>6</sub> -ACL		157	158
<sup>13</sup> C <sub>6</sub> -AC		160	159
<sup>13</sup> C <sub>6</sub> -FL		171	172
<sup>13</sup> C <sub>6</sub> -PHE, <sup>13</sup> C <sub>6</sub> -AN		183	184
<sup>13</sup> C <sub>6</sub> -FA		205	208
<sup>13</sup> C <sub>6</sub> -PY		208	205
<sup>13</sup> C <sub>6</sub> -BaA, <sup>13</sup> C <sub>6</sub> -CHR		232	234
<sup>13</sup> C <sub>6</sub> -BbFA, <sup>13</sup> C <sub>6</sub> -BkFA		259	258
<sup>13</sup> C <sub>4</sub> -BaP		257	256
<sup>13</sup> C <sub>6</sub> -IP		283	282
<sup>13</sup> C <sub>6</sub> -DBahA		282	284
<sup>13</sup> C <sub>12</sub> -BghiP		289	288
<sup>13</sup> C <sub>6</sub> -DBaeP		307	308
<sup>13</sup> C <sub>12</sub> -DBaiP		313	314

14 **Table S-2 (Supplementary data).** Recoveries (%) and repeatabilities (expressed as relative standard  
 15 deviations, RSD, %) calculated from six replicates of trout (1.6% of fat) spiked with target analytes at  
 16 two concentration levels (Level 1—1  $\mu\text{g kg}^{-1}$  for 3 major PCBs, 8 mono-*ortho* PCBs, 4 non-*ortho* PCBs,  
 17 6 PBDEs, and 32 PAHs and 5  $\mu\text{g kg}^{-1}$  for major PCBs 138, 153 and 180, and PBDE 47; Level 2—  
 18 5  $\mu\text{g kg}^{-1}$  for 3 major PCBs, 8 mono-*ortho* PCBs, 4 non-*ortho* PCBs, 6 PBDEs and 32 PAHs  
 19 and 25  $\mu\text{g kg}^{-1}$  for major PCBs 138, 153 and 180, and PBDE 47).

Analytes	Level 1		Level 2		LOQ ( $\mu\text{g kg}^{-1}$ )	Analytes	Level 1		Level 2		LOQ ( $\mu\text{g kg}^{-1}$ )	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)			Rec (%)	RSD (%)	Rec (%)	RSD (%)		
Mono- <i>ortho</i> PCBs	PCB 105	113	9	108	11	0.1	NA	73	13	75	11	0.1
	PCB 114	99	18	100	6	0.1	AC	78	11	78	5	0.1
	PCB 118	95	9	87	13	0.1	ACL	78	11	78	5	0.1
	PCB 123	85	14	79	7	0.1	FL	83	9	80	6	0.05
	PCB 156	96	9	77	11	0.1	PHE	79	6	82	3	0.05
	PCB 157	91	8	95	9	0.1	AN	81	7	84	4	0.05
	PCB 167	75	10	76	10	0.1	FA	86	7	92	6	0.05
	PCB 189	106	16	93	15	0.5	PY	79	10	86	9	0.05
Major PCBs	PCB 28	89	10	86	5	0.1	BaA	82	2	88	6	0.05
	PCB 52	93	7	101	9	0.1	CHR	89	6	91	6	0.05
	PCB 101	98	3	93	3	0.1	BbFA	84	4	86	7	0.05
	PCB 138	82	8	78	11	0.1	BkFA	85	5	90	4	0.05
	PCB 153	96	5	74	10	0.1	BaP	97	6	96	4	0.05
	PCB 180	84	11	77	8	0.5	DBahA	94	6	95	5	0.05
Non- <i>ortho</i> PCBs	PCB 77	88	7	87	6	0.25	BghiP	96	6	94	4	0.05
	PCB 81	91	4	84	5	0.25	IP	95	4	91	6	0.05
	PCB 126	77	5	74	11	0.1	BcFL	76	6	85	2	0.05
	PCB 169	105	10	101	11	0.1	CPP	83	7	89	5	0.05
PBDEs	PBDE 28	82	8	87	7	0.5	5MC	79	6	78	6	0.05
	PBDE 47	86	9	93	7	0.5	BjFA	85	4	92	4	0.05
	PBDE 99	97	7	95	5	0.5	DBalP	90	9	92	6	0.25
	PBDE 100	98	8	107	6	0.5	DBaeP	85	3	86	2	0.25
	PBDE 153	95	7	95	6	0.5	DBaiP	83	4	85	6	0.25
	PBDE 154	95	8	94	5	0.5	DBahP	83	3	86	3	0.25
	PBDE 183	93	6	94	7	0.5	1MN	85	3	79	9	0.1
							2MN	98	5	82	6	0.1
						1MPH	107	4	98	5	0.1	
						2MA	84	5	83	7	0.1	
						1MP	92	8	83	7	0.1	
						1MC	96	5	92	4	0.1	
						3MC	87	4	87	9	0.1	
						DBT	86	3	86	7	0.1	

20 **Table S-3 (Supplementary data).** Recoveries (%) and repeatabilities (expressed as relative standard  
 21 deviations, RSD, %) calculated from six replicates of salmon (14% of fat) spiked with target analytes at  
 22 two concentration levels (Level 1—1  $\mu\text{g kg}^{-1}$  for 3 major PCBs, 8 mono-*ortho* PCBs, 4 non-*ortho* PCBs,  
 23 6 PBDEs, and 32 PAHs and 5  $\mu\text{g kg}^{-1}$  for major PCBs 138, 153 and 180, and PBDE 47; Level 2—  
 24 5  $\mu\text{g kg}^{-1}$  for 3 major PCBs, 8 mono-*ortho* PCBs, 4 non-*ortho* PCBs, 6 PBDEs, and 32 PAHs  
 25 and 25  $\mu\text{g kg}^{-1}$  for major PCBs 138, 153 and 180, and PBDE 47).

Analytes	Level 1		Level 2		LOQ ( $\mu\text{g kg}^{-1}$ )	Analytes	Level 1		Level 2		LOQ ( $\mu\text{g kg}^{-1}$ )	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)			Rec (%)	RSD (%)				
Mono- <i>ortho</i> PCBs	PCB 105	93	6	100	5	0.1	NA	78	8	76	7	0.1
	PCB 114	97	6	104	4	0.1	AC	81	7	79	9	0.1
	PCB 118	88	7	99	4	0.1	ACL	78	10	80	5	0.1
	PCB 123	98	6	106	13	0.1	FL	85	6	84	4	0.05
	PCB 156	88	6	91	5	0.1	PHE	86	5	91	5	0.05
	PCB 157	84	5	90	3	0.1	AN	85	3	82	4	0.05
	PCB 167	92	4	103	2	0.1	FA	88	4	91	2	0.05
	PCB 189	87	5	93	3	0.5	PY	90	3	85	6	0.05
Major PCBs	PCB 28	105	4	111	11	0.1	BaA	97	4	83	3	0.05
	PCB 52	120	9	110	6	0.1	CHR	104	3	88	2	0.05
	PCB 101	107	19	110	3	0.1	BbFA	85	3	83	3	0.05
	PCB 138	98	5	105	3	0.1	BkFA	97	5	85	3	0.05
	PCB 153	97	4	103	3	0.1	BaP	92	3	87	2	0.05
	PCB 180	86	4	94	3	0.5	DBahA	82	2	89	3	0.05
Non- <i>ortho</i> PCBs	PCB 77	95	9	104	9	0.25	BghiP	101	1	87	3	0.05
	PCB 81	102	7	106	6	0.25	IP	87	12	82	3	0.05
	PCB 126	87	3	96	5	0.1	BcFL	103	4	85	6	0.05
	PCB 169	83	5	94	3	0.1	CPP	86	5	92	5	0.05
PBDEs	PBDE 28	92	11	97	8	0.5	5MC	86	2	91	3	0.05
	PBDE 47	94	5	96	6	0.5	BjFA	97	4	94	3	0.05
	PBDE 99	100	9	102	5	0.5	DBalP	93	4	87	6	0.25
	PBDE 100	89	6	93	8	0.5	DBaeP	92	3	87	7	0.25
	PBDE 153	90	10	81	10	0.5	DBaiP	96	3	85	3	0.25
	PBDE 154	97	8	90	9	0.5	DBahP	81	15	83	3	0.25
	PBDE 183	96	12	99	3	0.5	1MN	78	12	77	9	0.1
							2MN	80	10	80	7	0.1
						1MPH	85	6	89	6	0.1	
						2MA	79	4	85	3	0.1	
						1MP	88	4	82	4	0.1	
						1MC	91	5	92	4	0.1	
						3MC	89	2	86	3	0.1	
						DBT	90	5	93	4	0.1	

26 **Table S-4 (Supplementary data).** Recoveries (%) and repeatabilities (expressed as relative standard  
 27 deviations, RSD, %) calculated from six replicates of shrimps (0.4% of fat) spiked with target analytes  
 28 at two concentration levels (Level 1—1  $\mu\text{g kg}^{-1}$  for 3 major PCBs, 8 mono-*ortho* PCBs, 4 non-*ortho*  
 29 PCBs, 6 PBDEs, and 32 PAHs and 5  $\mu\text{g kg}^{-1}$  for major PCBs 138, 153 and 180, and PBDE 47; Level  
 30 2—5  $\mu\text{g kg}^{-1}$  for 3 major PCBs, 8 mono-*ortho* PCBs, 4 non-*ortho* PCBs, 6 PBDEs, and 32 PAHs  
 31 and 25  $\mu\text{g kg}^{-1}$  for major PCBs 138, 153 and 180, and PBDE 47).

Analytes	Level 1		Level 2		LOQ ( $\mu\text{g kg}^{-1}$ )	Analytes	Level 1		Level 2		LOQ ( $\mu\text{g kg}^{-1}$ )	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)			Rec (%)	RSD (%)	Rec (%)	RSD (%)		
Mono- <i>ortho</i> PCBs	PCB 105	98	12	97	11	0.1	NA	78	9	77	8	0.1
	PCB 114	96	10	97	8	0.1	AC	79	6	86	6	0.1
	PCB 118	101	6	99	8	0.1	ACL	85	8	89	6	0.1
	PCB 123	94	9	97	9	0.1	FL	83	7	81	5	0.05
	PCB 156	98	9	98	11	0.1	PHE	79	9	84	4	0.05
	PCB 157	105	20	91	7	0.1	AN	78	7	79	2	0.05
	PCB 167	100	8	101	11	0.1	FA	97	6	108	7	0.05
	PCB 189	96	9	93	7	0.5	PY	85	7	82	4	0.05
Major PCBs	PCB 28	86	9	93	5	0.1	BaA	86	5	88	3	0.05
	PCB 52	94	7	104	8	0.1	CHR	86	7	89	3	0.05
	PCB 101	119	14	120	9	0.1	BbFA	88	8	90	4	0.05
	PCB 138	99	9	100	9	0.1	BkFA	91	7	93	3	0.05
	PCB 153	98	11	93	7	0.1	BaP	90	7	92	3	0.05
	PCB 180	96	5	97	8	0.5	DBahA	91	6	92	3	0.05
Non- <i>ortho</i> PCBs	PCB 77	102	12	97	9	0.25	BghiP	91	6	94	3	0.05
	PCB 81	100	12	97	10	0.25	IP	92	7	95	4	0.05
	PCB 126	99	10	98	12	0.1	BcFL	92	10	94	5	0.05
	PCB 169	97	9	96	13	0.1	CPP	91	4	95	3	0.05
PBDEs	PBDE 28	112	10	109	11	0.5	5MC	86	6	89	5	0.05
	PBDE 47	102	8	80	4	0.5	BjFA	90	6	87	9	0.05
	PBDE 99	107	8	79	6	0.5	DBalP	89	3	91	6	0.25
	PBDE 100	110	8	105	5	0.5	DBaeP	90	6	89	4	0.25
	PBDE 153	115	7	99	4	0.5	DBaiP	85	8	95	7	0.25
	PBDE 154	120	11	99	10	0.5	DBahP	89	3	88	3	0.25
	PBDE 183	99	7	112	5	0.5	1MN	83	14	69	6	0.1
							2MN	109	15	80	6	0.1
						1MPH	101	13	100	3	0.1	
						2MA	73	9	86	5	0.1	
						1MP	87	11	86	7	0.1	
						1MC	91	10	92	4	0.1	
						3MC	88	10	91	4	0.1	
						DBT	83	3	91	5	0.1	

32 **Table S-5 (Supplementary data).** Verification of trueness of generated data: Analysis of selected PCBs, PBDEs and PAHs in standard reference  
 33 material – Lake Michigan fish tissue (SRM 1947, NIST, USA).

Analytes		Determined value ( $\mu\text{g kg}^{-1}$ )	Certified/reference value ( $\mu\text{g kg}^{-1}$ )	Agreement Yes/No	Analytes		Determined value ( $\mu\text{g kg}^{-1}$ )	Certified/reference value ( $\mu\text{g kg}^{-1}$ )	Agreement <sup>34</sup> Yes/No
Mono-ortho PCBs	PCB 105	53.2 ± 4.8	50.3 ± 3.7	Yes	PAHs	NA	1.25 ± 0.20	N/A	N/A
	PCB 114	11.4 ± 2.1	N/A	N/A		AC	<LOQ	N/A	N/A
	PCB 118	115 ± 10	112 ± 6	Yes		ACL	<LOQ	N/A	N/A
	PCB 123	24.1 ± 3.4	N/A	N/A		FL	n.d	N/A	N/A
	PCB 156	14.6 ± 0.5	13.3 ± 0.9	Yes		PHE	n.d	N/A	N/A
	PCB 157	3.8 ± 0.3	4.1 ± 0.8	Yes		AN	n.d	N/A	N/A
	PCB 167	8.6 ± 0.9	N/A	N/A		FA	n.d	N/A	N/A
	PCB 189	3.5 ± 0.6	N/A	N/A		PY	n.d	N/A	N/A
Major PCBs	PCB 28	13.4 ± 1.3	14.1 ± 1.0	Yes		BaA	n.d	N/A	N/A
	PCB 52	36.4 ± 2.5	36.4 ± 4.3	Yes		CHR	n.d	N/A	N/A
	PCB 101	89.4 ± 2.7	90.8 ± 0.3	Yes		BbFA	n.d	N/A	N/A
	PCB 138	167 ± 13	162 ± 7	Yes		BkFA	n.d	N/A	N/A
	PCB 153	204 ± 10	201 ± 3	Yes		BaP	n.d	N/A	N/A
	PCB 180	83.3 ± 9.2	80.8 ± 5.0	Yes		DBahA	n.d	N/A	N/A
Non-ortho PCBs	PCB 77	2.8 ± 0.2	N/A	N/A		BghiP	n.d	N/A	N/A
	PCB 81	n.d.	N/A	N/A		IP	n.d	N/A	N/A
	PCB 126	0.9 ± 0.05	N/A	N/A		BcFL	n.d	N/A	N/A
	PCB 169	1.4 ± 0.14	N/A	N/A		CPP	n.d	N/A	N/A
PBDEs	PBDE 28	2.11 ± 0.17	2.26 ± 0.46 <sup>1</sup>	Yes		5MC	n.d	N/A	N/A
	PBDE 47	70.7 ± 6.4	73.3 ± 2.9	Yes		BjFA	n.d	N/A	N/A
	PBDE 99	18.4 ± 1.3	19.2 ± 0.8	Yes		DBalP	n.d	N/A	N/A
	PBDE 100	17.7 ± 1.4	17.1 ± 0.6	Yes		DBaeP	n.d	N/A	N/A
	PBDE 153	3.8 ± 0.3	3.8 ± 0.04	Yes		DBaiP	n.d	N/A	N/A
	PBDE 154	6.3 ± 0.5	6.9 ± 0.5	Yes		DBahP	n.d	N/A	N/A
	PBDE 183	<LOQ	N/A	N/A		1MN	<LOQ	N/A	N/A
				2MN		<LOQ	N/A	N/A	
				1MPH		n.d	N/A	N/A	
				2MA		n.d	N/A	N/A	
				1MP	n.d	N/A	N/A		
				1MC	n.d	N/A	N/A		
				3MC	n.d	N/A	N/A		
				DBT	n.d	N/A	N/A		

<sup>1</sup> certified value is a sum of PBDE 28 and 33

35 **Table S-6 (Supplementary data).** Verification of trueness of generated data: Analysis of selected PCBs, PBDEs and PAHs in standard reference  
 36 material – Mussel Tissue (SRM 1974b, NIST, USA).

	Analytes	Determined value ( $\mu\text{g kg}^{-1}$ )	Certified/reference value ( $\mu\text{g kg}^{-1}$ )	Agreement Yes/No		Analytes	Determined value ( $\mu\text{g kg}^{-1}$ )	Certified/reference value ( $\mu\text{g kg}^{-1}$ )	Agreement <sup>37</sup> Yes/No
Mono-ortho PCBs	PCB 105	3.58 ± 0.32	4.00 ± 0.18	Yes	PAHs	NA	2.46 ± 0.82	2.43 ± 0.12	Yes
	PCB 114	0.41 ± 0.07	N/A	N/A		AC	0.33 ± 0.04	0.274 ± 0.054	No
	PCB 118	8.92 ± 0.80	10.3 ± 0.4	No		ACL	0.50 ± 0.09	0.48 ± 0.12	Yes
	PCB 123	0.90 ± 0.13	N/A	N/A		FL	0.51 ± 0.09	0.494 ± 0.036	Yes
	PCB 156	0.70 ± 0.06	0.718 ± 0.080	Yes		PHE	2.46 ± 0.60	2.58 ± 0.11	Yes
	PCB 157	0.26 ± 0.02	0.236 ± 0.024	Yes		AN	1.93 ± 0.29	0.527 ± 0.071	No
	PCB 167	0.31 ± 0.03	N/A	N/A		FA	17.7 ± 3.8	17.1 ± 0.7	Yes
	PCB 189	<LOQ	N/A	N/A		PY	18.0 ± 3.3	18.04 ± 0.6	Yes
Major PCBs	PCB 28	5.48 ± 0.55	3.43 ± 0.25	No		BaA	4.26 ± 0.52	4.74 ± 0.53	Yes
	PCB 52	5.78 ± 0.40	6.26 ± 0.37	Yes		CHR	6.06 ± 1.10	6.3 ± 1.0	Yes
	PCB 101	10.7 ± 0.32	10.7 ± 1.1	Yes		BbFA	5.98 ± 1.09	6.46 ± 0.59	Yes
	PCB 138	9.32 ± 0.75	9.2 ± 1.4	Yes		BkFA	3.06 ± 0.46	3.16 ± 0.18	Yes
	PCB 153	13.1 ± 0.7	12.3 ± 0.8	Yes		BaP	2.77 ± 0.34	2.80 ± 0.38	Yes
	PCB 180	0.97 ± 0.11	1.17 ± 0.10	Yes		DBahA	0.34 ± 0.05	0.327 ± 0.031	Yes
Nonortho PCBs	PCB 77	0.59 ± 0.04	N/A	N/A		BghiP	3.22 ± 0.49	3.12 ± 0.33	Yes
	PCB 81	n.d.	N/A	N/A		IP	2.09 ± 0.38	2.14 ± 0.11	Yes
	PCB 126	<LOQ	N/A	N/A		BcFL	n.d.	N/A	N/A
	PCB 169	n.d.	N/A	N/A		CPP	1.02 ± 0.08	N/A	N/A
PBDEs	PBDE 28	<LOQ	N/A	N/A		5MC	n.d.	N/A	N/A
	PBDE 47	2.02 ± 0.18	N/A	N/A		BjFA	2.86 ± 0.29	2.99 ± 0.29	Yes
	PBDE 99	0.90 ± 0.06	N/A	N/A		DBaIP	n.d.	N/A	N/A
	PBDE 100	0.57 ± 0.05	N/A	N/A		DBaeP	n.d.	N/A	N/A
	PBDE 153	<LOQ	N/A	N/A		DBaiP	n.d.	N/A	N/A
	PBDE 154	<LOQ	N/A	N/A		DBahP	n.d.	N/A	N/A
	PBDE 183	n.d.	N/A	N/A		1MN	0.32 ± 0.09	0.614 ± 0.050	No
						2MN	2.15 ± 0.22	1.25 ± 0.09	No
				1MPH		1.00 ± 0.15	0.98 ± 0.13	Yes	
				2MA		0.23 ± 0.03	0.232 ± 0.004	Yes	
				1MP	n.d.	N/A	N/A		
				1MC	n.d.	N/A	N/A		
				3MC	1.14 ± 0.17	N/A	N/A		
				DBT	n.d.	N/A	N/A		

Note: Yes/No – result is/not in agreement with the certified value

N/A – not available

n.d. – not detected

<LOQ – below limit of quantification