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Method development for the simultaneous determination of polybrominated, polychlorinated, mixed polybrominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans, polychlorinated biphenyls and polybrominated diphenyl ethers in fish

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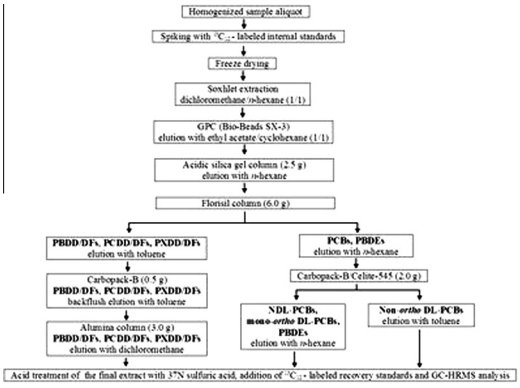
HIGHLIGHTS

- The procedure for simultaneous determination of five groups of POPs is presented.
- The method provides the analysis of PBDD/DFs and PXDD/DFs.
- Validation results for POP groups are presented.
- The methodology was used for analysis of Baltic wild salmon samples.

GRAPHICAL ABSTRACT



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ABSTRACT

An analytical methodology was developed for simultaneous determination of five groups of persistent organic pollutants (POPs) including polybrominated, polychlorinated and mixed brominated–chlorinated dibenzo-*p*-dioxins and dibenzofurans (PBDD/DFs, PCDD/DFs and PXDD/DFs, respectively), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish samples. The presented analytical approach was based on well established and robust method for determination of PCDD/DFs and PCBs, which was augmented with gas chromatography coupled to high resolution mass spectrometry (GC–HRMS) to include PBDEs, as well as poorly investigated PBDD/DFs and PXDD/DFs at toxicologically significant levels. Intensive clean-up and fractionation procedures in combination with optimized instrumental parameters provided reliable detection and quantification of these compounds. The application of $^{13}C_{12}$-labeled surrogates of analyzed compounds allowed the internal standardization and accurate measurement of selected contaminants. The developed procedure was validated while taking into account the Commission Regulation (EU) 252/2012 requirements for PCDD/DFs and PCBs, and was used to measure the occurrence of priority POPs in Baltic wild salmon.

1. Introduction

Various groups of halogenated POPs such as polyhalogenated dibenzo-*p*-dioxins and dibenzofurans including chlorinated

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(PCDD/DFs), brominated (PBDD/DFs), and mixed brominated/chlorinated (PXDD/DFs) substituted congeners, as well as polyhalogenated biphenyls comprising polychlorinated (PCBs) and mixed brominated/chlorinated (PXBs) derivatives, as well as polychlorinated naphtalenes (PCNs) are known xenobiotics of high toxicological significance with an aryl hydrocarbon (Ah) receptor mediated mechanism of toxicity (Falandysz, 2003; Falandysz et al., 2012; Van den Berg et al., 2006, 2013). The binding of these compounds to the Ah receptor defines a so-called dioxin-like toxicity and results in carcinogenicity, teratogenicity, immunotoxicity, embriotoxicity and other adverse effects (WHO, 1998). While PBDD/DFs, PCDD/DFs and PXDD/DFs have not been produced intentionally and originate merely as byproducts during incineration of halogen containing waste, in metallurgical industries and manufacturing of brominated flame retardants (BFRs) (Buekens et al., 2001; Ren et al., 2011) crude PCB mixtures were widely used as capacitor oils, lubricants and oil additives, and were thus introduced into environment. Another group of global environmental contaminants – polybrominated diphenyl ethers (PBDEs) represents mass produced BFRs incorporated into various commercial materials such as plastics, rubbers, textiles, paints and electronic components. Discarding of such materials has resulted in release of PBDEs into the environment (Hutzinger and Thoma, 1987). Although the long-term environmental effects of these compounds are not well established (De Wit, 2002), some literature sources reported moderate dioxin-like activity of PBDEs towards human estrogen receptors (Meerts et al., 2001; Behnisch et al., 2003). Even though the production of PBDE and PCB formulations and their commercial availability was banned in European Union (Council Directive 76/769/EEC, 1976; Council Directive 2003/11/EC, 2003; Court Proceeding 2008/c116/02, 2008), these compounds are ubiquitous and found in air, water, soil, sediments, fish, birds, mammals and people. All these groups of contaminants are referred to as POPs and have similar structures, physical and chemical properties. Due to their lipophilicity, persistence and bioaccumulative properties, POPs tend to migrate through the food chain and present a high risk of adverse effects in humans.

Fish and fish products are recommended sources of valuable nutrients, such as omega-3 polyunsaturated fatty acids, but are also known to be likely contaminated with POPs and serving as a significant pathway in the human dietary intake of these contaminants (Smith and Gangolli, 2002). Although a few studies over last two decades have been dedicated to analysis of POPs, such as PCDD/DFs, PCBs and PBDEs in fish, only isolated references regarding the analysis of PBDD/DFs and PXDD/DFs are available (Fernandes et al., 2008, 2011). The main reasons for the lack of data on these compounds are analytical difficulties due to the thermal degradation of analytes during a gas chromatography analysis (especially for PBDD/DFs) and limited availability of reference standards (Hagberg, 2009). Few literature references have demonstrated the presence of PBDD/DFs and PXDD/DFs in biota, food products, environmental samples and human adipose tissue (Fernandes et al., 2008, 2009a,b, 2011; Ericson Jogsten et al., 2010; Haglund et al., 2005; Myers et al., 2012; Van den Berg et al., 2006, 2013), and more detailed information on the occurrence of these contaminants is needed for understanding the potential risks to human health. Methodologies reported in the scientific literature on the analysis of PBDD/DFs, PCDD/DFs, PXDD/DFs, PBDEs and PCBs involve several individual sample preparation procedures optimized for targeted contaminant groups, followed by instrumental analysis and often lack the necessary selectivity and sensitivity, especially for organobromine compounds. Therefore, the elaboration of reliable complex methodologies which are capable to cover simultaneously different classes of priority POPs , including organobromines such as PBDD/Fs and PXDD/Fs and chlorinated analogs, is of great significance in terms of providing more objective information on contamination status of the analyzed samples.

This study describes an efficient and reliable analytical procedure for the simultaneous analysis of five target groups of compounds from a single sample extraction and gel permeation chromatography clean-up, followed by combining multiple column chromatography steps and detection with GC–HRMS, and presents results of routine application of the elaborated method. The developed methodology was based on the analytical approach for determination of PCDD/DFs and PCBs routinely used for fish samples (Zacs et al., 2012) with modifications in fractionation procedure and instrumental analysis to optimize the performance for organobromines. However, there were no specific requirements regarding the analytical procedures for determination of PBDD/DFs and PXDD/DFs, according to the criteria of Commission Regulation (EU) No 252/2012 (EU No 252/2012, 2012) elaborated for PCDD/DFs and dioxin-like PCBs (DL-PCBs).

2. Experimental

2.1. Chemicals and materials

All the solvents used were at least of pesticide purity grade. Hexane, toluene, dichloromethane, cyclohexane, and ethyl acetate were purchased from Lab-Scan (Glivice, Poland); silica gel (Kieselgel 60, 0.063–0.200 mm), Florisil, Celite-545, and Carbopack B were from Sigma–Aldrich Chemie GmbH (Buchs, Switzerland). Alumina (basic, 50–200 μm), sulfuric acid and sodium sulfate were obtained from Acros (New Jersey, USA). The native and isotopically labeled standards for analyzed contaminants were purchased either from Cambridge Isotope Laboratory (CIL), Inc. (MA, USA) or from Wellington Laboratories (Ontario, Canada).

The following five groups of persistent organic pollutants were analyzed (for compounds given in bold, $^{13}C_{12}$-labeled surrogates were available and were used as internal or recovery standards):

(1) *Tetra-octa polychlorinated dioxins and furans (PCDD/DFs):* **2,3,7,8-TetraCDF, 1,2,3,7,8-PentaCDF, 2,3,4,7,8-PentaCDF, 1,2,3,4,7,8-HexaCDF, 1,2,3,6,7,8-HexaCDF, 2,3,4,6,7,8-HexaCDF, 1,2,3,7,8,9-HexaCDF, 1,2,3,4,6,7,8-HeptaCDF, 1,2,3,4,7,8,9-HeptaCDF, OctaCDF, 2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD, 1,2,3,4,7,8-HexaCDD, 1,2,3,6,7,8-HexaCDD, 1,2,3,7,8,9-HexaCDD, 1,2,3,4,6,7,8-HeptaCDD, OctaCDD**

(2) *Tri-hepta polychlorinated biphenyls (PCBs):* IUPAC numbers 18, **28**, 33, 47, 49, 51, **52**, 60, 66, 74, **77**, **81**, 99, **101**, **105**, 110, **114**, **118**, **123**, **126**, **138**, 153, **156**, **157**, **167**, **169**, 180, **189**

(3) Di-deca polybrominated diphenyl ethers (PBDEs): IUPAC numbers 7, **15**, 17, **28**, **47**, 49, 66, 71, 77, 85, **99**, 100, 119, 126, **138**, **153**, **154**, 155, 166, 181, **183**, 190, 203, **204**, 205, 206, **207**, **209**

(4) *Tetra-octa polybrominated dioxins and furans (PBDD/DFs):* **2,3,7,8-TetraBDF, 1,2,3,7,8-PentaBDF, 2,3,4,7,8-PentaBDF, 1,2,3,4,7,8-HexaBDF, 1,2,3,4,6,7,8-HeptaBDF, OctaBDF, 2,3,7,8-TetraBDD, 1,2,3,7,8-PentaBDD, 1,2,3,4,7,8-HexaBDD, 1,2,3,6,7,8-HexaBDD, 1,2,3,7,8,9-HexaBDD, 1,2,3,4,6,7,8-HeptaBDD, OctaBDD**

(5) *Tetra-octa mixed bromo-chloro polyhalogenated dioxins and furans (PXDD/DFs):* 3-B-2,7,8-TriCDF, 2-B-3,7,8-TriCDD, **2,3-DiB-7,8-DiCDD**, 1-B-2,3,7,8-TetraCDF, **1-B-2,3,7,8-TetraCDD**, 2-B-1,3,7,8-TetraCDD, 2-B-3,6,7,8,9-PentaCDD, 1-B-2,3,6,7,8,9-HexaCDD, 1-B-2,3,4,6,7,8,9-HeptaCDD

2.2. Analyzed Baltic wild salmon samples

Ten Baltic wild salmon specimens of various age, length and weight were caught during the spawning period in September

2013 from the Daugava river. After individual laboratory codes were given, the length and weight of all specimens was measured, and the gender was determined. Age of the fish was determined according to squama characteristics. For this study, 5 male and 5 female specimens were collected. Average length and weight of female salmons was 74 cm (range from 70 to 80 cm) and 5.5 kg (range from 4.9 to 6.0 kg), and the female fish had an age of 2–3 years. Characteristics of male salmon were as follows: average length 58 cm (range from 53 to 65 cm) and an average weight of 2.5 kg (range from 1.8 to 3.8 kg). The male specimens were 1–2 years old with the most common age of 1 year. After the sample pretreatment the specimens were dissected, the fillets were isolated and homogenized using a food blender (Kenwood FP101T, Kenwood Ltd., UK), and the homogenates were packed into polyethylene bags and stored at −18 °C until analysis.

2.3. Sample preparation and clean-up

Sample aliquot was spiked in a porcelain dish with 500 μL of $^{13}C_{12}$-labeled POP congener mixture solution diluted with toluene to a final concentration of 0.1–1.5 pg μL^{-1} for PBDD/DFs, PCDD/DFs and PXDD/DFs, to 1.0 pg μL^{-1} for dioxin-like mono-*ortho* and non-*ortho* PCBs, to 2.0 pg μL^{-1} for non-dioxin-like PCBs and to 1.0–5.0 pg μL^{-1} for PBDEs. Weight of the sample aliquot was dependent on the species of the fish and lipid content in the sample, and usually was equivalent to not more than 8 g of lipids. After equilibration for 1 h, the sample was freeze dried for 48 h and the lipids were extracted using Soxhlet extraction with dichloromethane/*n*-hexane (1:1, v/v) mixture with extraction time at least 16 h. The extract was filled into pre-weighed round-bottom flask and the solvent was removed using rotary evaporator at <30 °C. The lipid content was determined gravimetrically according to EPA-1613 method (US Environmental Protection Agency, 1994). High molecular substances were removed by gel permeation chromatography (GPC) using O-I-Analytical system (College Station, Texas, USA) consisting of HPLC pump, autosampler and fraction collector. The system was equipped with a glass column (42 × 2.8 cm) filled with Bio-Beads SX-3 (Bio-Rad, Philadelphia, USA) stationary phase and eluted with cyclohexane/ethyl acetate (1:1, v/v) mobile phase at a flow rate of 5 mL min^{-1}. The number of injections per sample depended on the lipid weight taken for analysis divided by the GPC column loading capacity of up to 1.6 g of fat. The automated GPC program was as follows: dump time 0–25 min, collection time 25–60 min. After the addition of 50 μL of *n*-dodecane as "keeper" solvent, the eluate was concentrated by rotary evaporation. The pre-purified sample was placed on top of a glass column (25 × 1.2 cm) filled with silica gel containing 50% sulfuric acid for degradation of remaining lipids (the weight of the acidic silica gel depended on the quantity of remaining fat in the extract and was not less than 2.5 g). The analytes were eluted with 1.0 mL of toluene and subsequently with 25 mL of *n*-hexane, and the eluate volume was reduced by rotary evaporation to 0.5 mL, consisting mainly of toluene. Target compounds were separated using a glass column (25 × 1.2 cm) filled with 6.0 g of Florisil deactivated with 3% water: first the fraction of the PBDEs and PCBs was eluted with 80 mL of *n*-hexane, followed by 120 mL toluene for elution of the PBDD/DF, PCDD/DF and PXDD/DF fraction. After solvent removal and concentration of each of the two fractions by rotary evaporation to 1.0 mL, additional clean-up and separation steps were performed in an automated manner using Waters (Milford, USA) preparative chromatography system consisting of Controller 600, Autosampler 717 plus and Fraction collector III. For the PBDD/DFs, PCDD/DFs and PXDD/DFs a glass column (25 × 1.0 cm) filled with 0.5 g of Carbopack B was used. After application of the sample extract, the column was washed with *n*-hexane/dichloromethane (65:35, v/v) mixture at a rate of 1.5 mL min^{-1} for 20 min and the PBDD/DFs, PCDD/DFs, and PXDD/DFs were eluted with toluene in the back flush mode at a flow rate of 2.5 mL min^{-1} for 40 min. PBDEs, dioxin-like and non-dioxin-like PCBs were separated in direct flow using a glass column (25 × 1.0 cm) filled with 2.0 g of a 1:1 (w/w) mixture of Carbopack B and Celite-545; PBDEs, mono-*ortho* PCBs and non-dioxin like PCBs were eluted with *n*-hexane at a flow of 2.0 mL min^{-1} within 25 min, the non-*ortho* PCBs subsequently by elution with toluene at a flow rate of 2.5 mL min^{-1} within 30 min. Finally, to remove potentially remaining interferants such as PBDEs and PCBs from the fraction containing PBDD/DFs and PXDD/DFs, the fraction was purified using glass column filled with 3.0 g of basic alumina (activated at 450 °C for 16 h) as follows: the fraction containing PBDD/DFs, PCDD/DFs, and PXDD/DFs was evaporated on rotary evaporator and the solvent was exchanged to *n*-hexane without traces of toluene; after addition of the *n*-hexane extract to column, the column was rinsed with 20 mL of *n*-hexane/dichloromethane (90:10, v/v) mixture to waste and analytes of interest were collected with a 60 mL fraction of dichloromethane. After rotary evaporation to about 150–200 μL all three extracts were transferred to 2 mL chromatographic vials, treated with 37 N sulfuric acid (30 μL) and mixed using a vortex mixer (Barnstead International, Type 37600 Mixer, Dubuque, USA). The mixture was allowed to stand for 20 min and centrifuged at 3000 rpm at 10 °C using Falcon 6/300 bench top centrifuge (MSE, London, UK) to separate the acid and organic layers. The acidic bottom layer was discarded and the organic layer was evaporated with addition of recovery standard solutions in *n*-nonane. As recovery standards, 1,2,3,7,8-PentaBDF and 1,2,3,7,8,9-HexaBDD were used for PBDD/DFs, while $^{13}C_{12}$-1,2,3,4-TetraCDD and $^{13}C_{12}$-1,2,3,7,8,9-HexaCDD were used for PCDD/DFs and PXDD/DFs. For PBDE, mono-*ortho* PCB and non-dioxin-like PCB fraction, the $^{13}C_{12}$ PBDE 138 $^{13}C_{12}$ PCB 101, and $^{13}C_{12}$ PCB 138 surrogates were added as recovery standards, while for non-*ortho* PCB fraction the recovery standards were $^{13}C_{12}$ PCB 101 and $^{13}C_{12}$ PCB 138. The final volume of the PBDD/DF, PCDD/DF and PXDD/DFs fraction was 10 μL. The fraction containing PBDEs, mono-*ortho* PCBs and non-dioxin-like PCBs was evaporated to 50 μL. Non-*ortho* PCB fraction was also reduced to 50 μL. An overall diagram of clean-up procedure is shown in Fig. 1.

2.4. Instrumental analysis and quantification by GC-HRMS

All analyte groups were analyzed by GC–HRMS. The measurements were performed on Micromass Autospec Premier high resolution mass spectrometer (Milford, USA) coupled with Agilent 6890 N gas chromatograph (Santa Clara, USA). Aliquots of 1 μL of the final sample extracts and calibration solutions were introduced into GC–HRMS system equipped with a silica capillary column and a split/splitless injector operated in splitless mode. The transfer line from the GC to the HRMS and the ion source temperatures were kept at 280 °C. Ion source was operated in the positive electron impact mode (EI^+) with electron energy of 36 eV and a trap current of 600 μA. The resolution of the mass spectrometer was better than 10 000 (at 10% peak valley) for all analyte groups with the exception of PXDD/DFs, for which resolution was in the range of 13 500–15 000 (at 10% peak valley). Mass calibration for all analyzed compounds was obtained at acceleration voltage of 7.5 kV. Table 1 presents the GC conditions used for selected POP groups.

All analyte groups were determined by selected ion monitoring (SIM) using the two most abundant ions of the respective molecular ion cluster of both the native and the $^{13}C_{12}$-labeled surrogates. The run was time segmented and SIM descriptors changed according to elution times for analytes of interest. Quantification was carried out using isotope dilution method applying the $^{13}C_{12}$-labeled surrogates as internal standards. Masslynx™ software was used

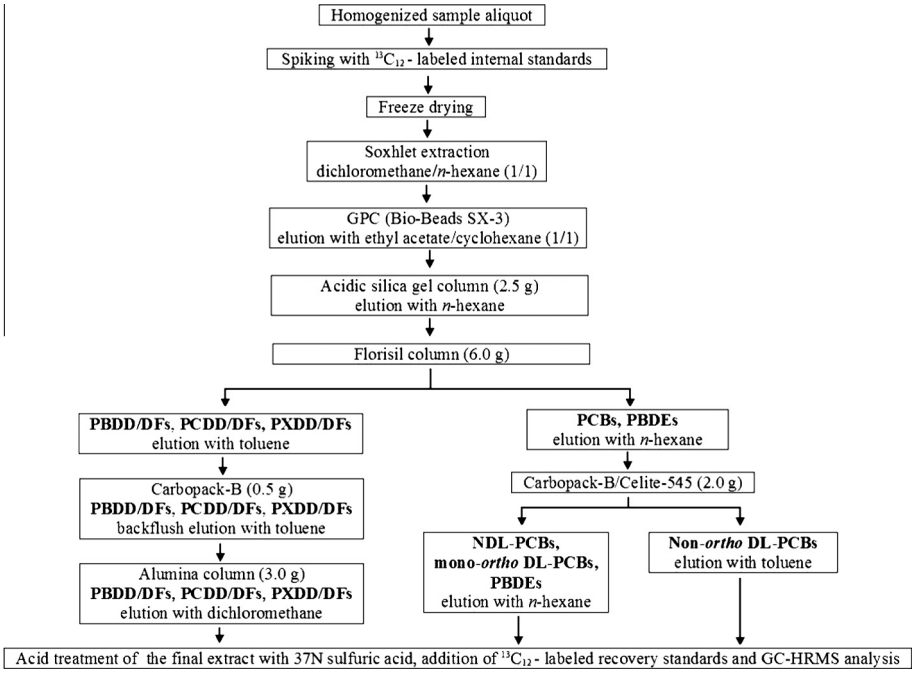

Fig. 1. Sample preparation flow diagram for determination of PBDD/DFs, PCDD/DFs, PXDD/DFs, PBDEs and PCBs in fish samples.

Table 1
GC conditions for the analysis of target compounds.

PCDD/DFs	
Column	ZB-5MS, 60 m, 0.25 mm i.d., 0.25 μm film thickness (Phenomenex, Torrance, USA)
Temperature program	140 °C (held for 3 min), 15 °C min^{-1} to 200 °C, 3 °C min^{-1} to 235 °C (held for 15 min), 4 °C min^{-1} to 300 °C (held for 12 min)
Injection temperature	280 °C
Carrier gas	Helium at flow rate of 1.0 mL min^{-1}
PCBs	
Column	ZB-5MS, 60 m, 0.25 mm i.d., 0.25 μm film thickness (Phenomenex, Torrance, USA)
Temperature program	75 °C (held for 2 min), 15 °C min^{-1} to 150 °C, 2.5 °C min^{-1} to 290 °C (held for 1 min)
Injection temperature	270 °C
Carrier gas	Helium at flow rate of 1.0 mL min^{-1}
PBDD/DFs	
Column	DB-5MS, 18 m, 0.25 mm i.d., 0.10 μm film thickness (J&W Scientific, Folsom, USA)
Temperature program	100 °C (held for 4 min), 40 °C min^{-1} to 200 °C (held for 3.5 min), 10 °C min^{-1} to 320 °C (held for 2.5 min),
Injection temperature	260 °C
Carrier gas	Helium at flow rate of 1.0 mL min^{-1}
PXDD/DFs	
Column	DB-5MS, 30 m, 0.25 mm i.d., 0.10 μm film thickness (J&W Scientific, Folsom, USA)
Temperature program	120 °C (held for 2 min), 20 °C min^{-1} to 240 °C, 5 °C min^{-1} to 320 °C (held for 4 min),
Injection temperature	250 °C
Carrier gas	Helium at flow rate of 1.0 mL min^{-1}
Di-hepta BDEs	
Column	DB-5MS, 30 m, 0.25 mm i.d., 0.10 μm film thickness (J&W Scientific, Folsom, USA)
Temperature program	100 °C (held for 3 min), 5 °C min^{-1} to 320 °C (held for 15 min)
Injection temperature	250 °C
Carrier gas	Helium at flow rate of 1.0 mL min^{-1}
Octa-deca BDEs	
Column	DB-5MS, 15 m, 0.25 mm i.d., 0.10 μm film thickness (J&W Scientific, Folsom, USA)
Temperature program	100 °C (held for 3.5 min), 40 °C min^{-1} to 200 °C (held for 4 min), 10 °C min^{-1} to 320 °C (held for 3 min)
Injection temperature	280 °C
Carrier gas	Helium at flow rate of 1.0 mL min^{-1}

for raw data interpretation and for targeting/quantification of selected contaminants.

2.5. Quality assurance and quality control (QA/QC)

Before use, all the glassware was solvent washed to remove possible background contamination. All stages of analytical procedure, including sample extraction, purification and handling of the final extracts were performed under "UV-protect" conditions (e.g. using amber glassware or wrapping the glassware with aluminum foil). To evaluate extraction and clean-up steps, each sample was spiked before the analytical procedure with $^{13}C_{12}$-labeled congeners which were used as internal standards. Recovery standards added after clean-up procedure were used for internal standard recovery control and calculation. Quality control criteria for the positive identification of analytes of interest included the retention time of the native compounds within a window of +3 to 0 s compared to the corresponding ^{13}C-labeled isomer, chromatographic

peak signal-to-noise ratio greater than three and the isotope ratio of the two monitored ions within ±15% of the theoretical value. Five point calibration curves were used for quantification of congener concentrations in each sample run. At the end of the analytical run, a reference standard solution was analyzed to check the system performance and calibration validity. Linearity of the calibration curves was checked with relative response factors (RRFs). Procedural blanks and quality control samples were analyzed in each sample sequence, consisting of not more than 8 samples. To control the ongoing precision and recovery of the PBDD/DFs and PXDD/DFs, quality control samples were spiked with analytes of interest. To reveal the performance in analysis of persistent organic pollutants, laboratory participates on a regular basis in international proficiency tests on determination of PCDD/DFs and PCBs in food and feed matrices.

3. Results and discussion

3.1. Clean-up and fractionation

Because of the structural similarity of PBDD/DFs and PXDD/DFs to PCDD/DFs, for which a robust method has been successfully used over the course of many years for food and feed matrices (Zacs et al., 2012, 2013a), a similar analytical approach with modifications to better suit PBDD/DF and PXDD/DF analysis was used. Taking into account the typically low levels (ppt or sub-ppt) at which PBDD/DFs and PXDD/DFs are represented in food products and environmental matrices, relatively high sample aliquots were taken for analysis with a concentration factor up to 10 000 to provide better sensitivity for PBDD/DFs and PXDD/DFs. The most commonly used procedures to remove the bulk of high molecular compounds from the samples of animal origin are destructive (acid/base treatment) and nondestructive methods (size exclusion chromatography (e.g. GPC), dialysis through the semi-permeable membranes) (Van Bavel et al., 1996). Although under the conditions of well established analytical procedures for POPs destructive methods give reliable results, there are some potential risks. On one hand, the acid treatment of large sample amounts with high fat content leads to a risk of loosing analytes of interest by adsorption on the treated carbonaceous matrix. On the other hand, sample digestion with alkali could cause the breakdown of highly halogenated compounds resulting in formation of respective congeners with lower halogenation pattern (Ryan et al., 1989). Moreover, the acid treatment alone is not effective for some types of matrices (e. g. materials of plant origin), due to the presence of waxes, which are relatively stable to hydrolysis. To prevent the possible risks related to the usage of destructive lipid elimination methods, gel permeation chromatography was used to remove the bulk (up to 95%) of high molecular compounds from the sample. Furthermore, GPC presented several advantages: it was flexible and robust; a column could be used repetitively without regeneration and could be easily operated in automated manner. Additionally an acidic silica column was used for the degradation of the remaining part of lipids, as well as for retaining the polar matrix components.

As it was reported previously (Donelly and Sovocol, 1990, Donnelly et al., 1987; Ebert et al., 1999; Fernandes et al., 2011), the separation of PBDEs and PCBs from PBDD/DFs and PXDD/Fs has been of great concern to prevent the mass spectrometric interference by production of similar fragments during the ionization. Typically, fish is the matrix most contaminated with PCBs and PBDEs among the food products and the latter compounds reach ppb or even higher concentrations on fresh weight basis (Szlinder-Richert et al., 2010; Zacs et al., 2012, 2013b). Due to the significant difference of concentration profiles of target compound groups in the samples and the ability of some compounds to affect or interfere with instrumental analysis, these groups should be eliminated from the extracts as completely as possible during the clean-up procedure, thus extensive sample fractionation should be used. In our study an elaborate clean-up procedure with three-step fractionation for the fraction containing PBDD/DFs and PXDD/DFs was used in order to provide a more complete exclusion of possible interferants. The first fractionation stage involved a Florisil column chromatography, taking advantage of the almost complete separation of PCBs and PBDEs from PBDD/DFs, PCDD/DFs and PXDD/DFs (Ebert et al., 1999; Kotz, 2006). Although the Florisil column was capable of separating up to 99.5% of PBDEs from the sample extract, taking into account the differences in typical levels of PBDEs and PBDD/DFs (ppb and ppt or sub-ppt, respectively), the remaining 0.5% of PBDEs could provide undesirable GC–MS interference. Due to the different retention characteristics of planar and non-planar aromatic molecules on activated carbon, based on different interactions between the π-electrons of the aromatic molecules and π-electrons of the carbon graphite structure, carbon column chromatography was suggested for the separation of PCBs and PBDEs from PBDD/DFs and PXDD/DFs (Fernandes et al., 2008, 2011; Hagberg, 2009; Wang et al., 2007), and it was applied in this study in automated manner as a second fractionation step. Polyhalogenated dioxins and furans have planar aromatic structures and tend to interact stronger with the carbon surface in comparison to the mono- to tetra-*ortho*-substituted PCBs and PBDEs, which have restricted rotation around the phenyl–phenyl bond and remain relatively non-planar. This results in weak or intermediate retention of PCBs and PBDEs on activated carbon and by using moderately polar solvent mixtures (e.g., hexane/dichloromethane) it is possible to isolate these potential interferants from the extract. The final purification stage utilize a basic alumina column to exclude the possible remaining mass interferants, and this step is particularly useful in analysis of matrices with high PCB and PBDE contamination (e.g., Baltic wild salmon and other fish from the Baltic Sea).

Due to the fact that non-*ortho* PCBs can attain a planar configuration, these compounds are more toxic in comparison with *ortho* PCBs and PBDEs (Van den Berg et al., 2006). Taking into account the toxicological significance of non-*ortho* PCBs and the occurrence of these compounds in the samples at lower concentrations compared to other PCBs, this group of contaminants was isolated as an individual fraction using carbon column, to improve the sensitivity and robustness of the method.

3.2. Gas chromatography – mass spectrometry

Although the increase in the halogenation degree of dibenzo-*p*-dioxins and furans is linked to a decrease in their potential toxicity, some of the PBDD/DFs with high degree of bromination (e.g., hepta- and octa- brominated congeners) might be of high toxicological concern. Unfortunately, in most studies data on highly brominated congeners is sparse. Although the sensitivity of reported GC–HRMS methods for analysis of PBDD/DF congeners with low degree of substitution, such as di- to penta-brominated dioxins and furans, is comparable to the methods reported for chlorinated analogs, the analysis of highly brominated components is often complicated, resulting in increased LOQs for these toxicologically significant compounds. Due to the thermal lability of highly brominated congeners, the transit time through the GC column becomes critical for these compounds. To avoid thermal degradation problems during the GC separation of brominated POPs, such as PBDD/DFs and PBDEs, shorter columns with a thinner internal coating are more preferable (Stapleton, 2006; Covaci et al., 2007; Hagberg, 2009). We used an 18 m GC column with phase coating of 0.10 μm that offered both adequate chromatographic resolution and sensitivity for PBDD/DFs. Taking into account the possibility of thermal degradation of PXDD/DFs, especially at high degree of

halogenation, the latter were checked by application of two columns with different lengths and phase loadings (a 60 m column with phase loading of 0.25 μm and 30 m column with phase loading of 0.10 μm). As was expected, the 30 m column with film thickness of 0.10 μm provided much better sensitivity for hepta- and octa-halogenated PXDDs without significant loss of chromatographic resolution for low halogenated congeners. For chromatographic separation of PBDEs, where thermal degradation was of great concern during the GC–MS analysis (Stapleton, 2006; Covaci et al., 2007), columns with phase loading of 0.10 μm were used to improve the sensitivity.

In spite of the extensive multistep sample clean-up and fractionation, the possible presence of mass interferants (e.g., PBDEs and PCBs) in the final PBDD/DF and PXDD/DF extracts could not be excluded. During the analysis of PBDD/DFs, possible thermal degradation of potentially present PBDEs and debromination with release of two bromine atoms can be expected, resulting in the formation of intensive fragment peaks with the same number of bromines and molecular weight as the PBDF congeners (Hagberg, 2009). To prevent the false-positive identification of PBDFs, simultaneous monitoring of the fragment ions of PBDEs with one and two extra bromines in comparison with PBDFs of interest was carried out and no signals with the retention times corresponding to the analytes of interest were found. Taking into account previous studies (Huang et al., 1992; Fernandes et al., 2011), the corresponding ions for PXDD/DFs were chosen in the way to prevent the overlapping of the analyte ions with the main mass interferants (PBDEs and PCBs) as much as possible. In addition, higher mass resolution (13 500–15 000) was used during the analyses of PXDD/DFs. Within the scope of this study we chose $[M]^+$ fragments for GC–MS determination of di- to hepta-BDEs, although for octa- to deca-BDEs we used $[M\text{-}2Br]^+$ type ions because of the higher peak intensity in the mass spectrum compared to ions from $[M]^+$ ion cluster (Wang et al., 2005). The selected mass descriptors for measurement of PBDD/DFs, PBDEs and PXDD/DFs are overviewed in the supporting information. Specific *m/z* fragments for the determination of PCDD/DFs and PCBs were selected according to the standard methods US EPA 1613 and US EPA 1668A (US Environmental Protection Agency, 1994, 1999).

3.3. Validation of the method

Since there were no specific requirements or guidelines for the validation of analytical procedures for determination of PBDD/DFs and PXDD/DFs, a validation protocol based on the criteria of Commission Regulation (EU) No 252/2012 was used (EU No 252/2012, 2012). The validation study was performed in terms of recovery, precision and limit of quantification. The experiments were carried out at two spiking levels with six parallel samples and each fortification level was repeated on two different days. The precision, repeatability and recovery were calculated for fish oil spiked with native compounds. The detailed validation results are summarized in Table 2. For reproducibility estimation of the developed method within our laboratory, in-house prepared reference material (freeze-dried salmon tissue) was used, which was naturally contaminated with PCDD/DFs, PCBs and PBDEs and was fortified with native PBDD/DFs and PXDD/DFs. Repeated analyses of this material were performed in different routine sample sequences. The results showed good consistency at selected fortification levels. Reproducibility for selected compounds appeared to be similar to that found for PCDD/DFs and PCBs in routine analyses. There were no proficiency tests or certified reference materials for PBDD/DFs and PXDD/DFs, but in order to confirm the performance in persistent organic pollutant analysis, our laboratory successfully participated in international proficiency tests for determination of PCDD/DFs and PCBs in food and feed matrices organized by European Union Reference Laboratory for Dioxins and PCBs in Feed and Food, Freiburg, Germany.

In spite of multi-stage sample preparation and fractionation, typical quantitative recoveries of used $^{13}C_{12}$-labeled internal standards were in the range 60–110% with exception of $^{13}C_{12}$-labeled octa-brominated and octa-chlorinated dibenzo-*p*-dioxins and furans and octa- through deca-brominated diphenyl ethers, for which typical recoveries were in the range of 30–50%. The reagent blanks were free of PBDD/DFs and PXDD/DFs. Some insignificant concentrations of PCDD/DF, PBDE and PCB congeners were usually found in the reagent blank extracts and the calculated concentrations in the samples were subjected to correction. The limits of quantification (LOQ) of selected POPs for the developed analytical procedure were calculated on the basis of investigated unspiked matrix. As it was anticipated, the sensitivity of the method decreased with higher degree of halogen substitution in the molecules of investigated compounds. This phenomenon was particularly emphasized for PBDD/DFs and, to a lesser extent, for PXDD/DFs, as expected from the thermal lability of highly halogenated organobromines (Hanari et al., 2006; Hagberg, 2009). The sensitivity of our method for PBDD/DFs and PXDD/DFs was in the range of 0.03–1.61 pg g^{-1} fat, which was sufficient for determination of these contaminants in fish, while taking into account the toxicological properties and typical distribution of these compounds in aquatic biota.

Table 2
Validation results for selected POP groups.

Congener group	Linearity of measurement, pg	LOQ [a], pg g^{-1} product	1st spiking level				2nd spiking level			
			Spiking level[a], pg g^{-1} fat	Recovery (n = 2)[b], %	Intra-day precision, (n = 2)[c], %	Inter-day precision (n = 2)[d], %	Spiking level[a], pg g^{-1} fat	Recovery (n = 2)[b], %	Intra-day precision, (n = 2)[c], %	Inter-day precision (n = 2)[d], %
PBDD/DFs	0.05–30	0.04–1.61	0.53–4.53	85.8–116.7	4.7–23.3	4.3–23.7	1.07–8.87	80.6–116.5	3.2–18.0	3.3–17.4
PCDD/DFs	0.05–200	0.02–0.11	0.25–2.50	90.2–119.9	2.6–12.5	3.2–14.0	0.50–5.00	93.7–119.1	1.6–9.6	2.1–10.1
PXDD/DFs	0.05–3.75	0.03–0.10	0.33–1.33	91.6–105.9	7.0–12.3	8.3–14.2	0.67–2.67	89.2–114.3	3.2–15.1	3.7–14.4
DL-PCBs	0.10–200	0.17–0.34	100	93.6–115.1	2.0–8.2	3.5–8.6	200	93.4–114.2	1.9–3.8	2.5–5.1
NDL-PCBs	1.00–700	0.25–0.65	625–937	81.3–119.7	4.4–8.7	5.9–9.2	1250–1875	83.5–120.4	5.6–9.3	5.8–10.4
PBDEs	0.50–500	0.11–2.17	188–938	75.2–122.8	3.1–9.7	3.9–11.0	375–1875	78.5–120.4	4.9–9. 6	5.5–9.8

[a] Parameters differ for individual congeners depending on halogenation degree.
[b] Recovery range (%) for selected contaminant group for corresponding fortification level calculated from the data obtained on two different days.
[c] Intraday precision range (%) for selected contaminant group for corresponding fortification level calculated from the data obtained on two different days.
[d] Interday precision range (%) for selected contaminant group for corresponding fortification level calculated from the data obtained on two different days.

Table 3
Occurrence levels of selected POP groups in Baltic wild salmon samples calculated on fresh weight basis (values are given with three significant digits).

Parameter	Minimum	Maximum	Average[a]
Length, cm	53	80	66
Age, years	1	3	2
Weight, kg	1.8	6.0	4.0
Fat content, g 100 g^{-1} of sample	2.9	5.4	4.3
PCDD/DFs (pg g^{-1})			
2,3,7,8-TetraCDF	3.67	10.6	6.90
1,2,3,7,8-PentaCDF	0.39	1.26	0.81
2,3,4,7,8-PentaCDF	3.00	8.06	5.24
1,2,3,4,7,8-HexaCDF	0.04	0.19	0.11
1,2,3,6,7,8-HexaCDF	0.07	0.29	0.17
2,3,4,6,7,8-HexaCDF	0.08	0.25	0.15
1,2,3,7,8,9-HexaCDF	<0.003	0.02	0.01
1,2,3,4,6,7,8-HeptaCDF	0.01	0.03	0.02
1,2,3,4,7,8,9-HeptaCDF	<0.003	0.01	0.01
OctaCDF	<0.002	0.02	0.01
2,3,7,8-TetraCDD	0.22	0.56	0.38
12378-PentaCDD	0.40	1.03	0.70
1,2,3,4,7,8-HexaCDD	0.01	0.06	0.03
1,2,3,6,7,8-HexaCDD	0.15	0.43	0.28
1,2,3,7,8,9-HexaCDD	0.01	0.05	0.03
1,2,3,4,6,7,8-HeptaCDD	0.01	0.04	0.02
OctaCDD	0.01	0.05	0.02
WHO(2005)-PCDD/F-TEQ (LB)	1.93	5.01	3.44
WHO(2005)-PCDD/F-TEQ (UB)	1.93	5.01	3.44
PBDD/DFs (pg g^{-1})			
2,3,7,8-TetraBDF	0.16	0.61	0.34
1,2,3,7,8-PentaBDF	<0.02	0.65	0.17
2,3,4,7,8-PentaBDF	0.03	0.27	0.11
1,2,3,4,7,8-HexaBDF	0.11	1.23	0.42
1,2,3,4,6,7,8-HeptaBDF	0.44	3.67	1.47
OctaBDF	0.38	1.37	0.66
2,3,7,8-TetraBDD	<0.01	<0.03	<0.01
1,2,3,7,8-PentaBDD	<0.004	<0.01	<0.01
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	<0.01	0.01	0.01
1,2,3,7,8,9-HexaBDD	<0.004	<0.02	<0.01
1,2,3,4,6,7,8-HeptaBDD	0.04	0.15	0.09
OctaBDD	<0.03	<1.33	<0.23
WHO(2005)-PBDD/F-TEQ (LB)	0.04	0.32	0.13
WHO(2005)-PBDD/F-TEQ (UB)	0.06	0.34	0.16
PXDD/DFs (pg g^{-1})			
3-Br-2,7,8-TriClDF	0.01	0.03	0.02
2-Br-3,7,8-TriClDD	<0.003	<0.01	<0.01
2,3-DiBr-7,8-DiClDD	<0.003	<0.01	<0.01
1-Br-2,3,7,8-TetraClDF	<0.003	<0.01	<0.01
1-Br-2,3,7,8-TetraClDD	<0.002	<0.01	<0.01
2-Br-1,3,7,8-TetraClDD	<0.002	<0.01	<0.01
2-Br-3,6,7,8,9-PentaClDD	<0.001	<0.01	<0.003
1-Br-2,3,6,7,8,9-HexaClDD	<0.002	<0.01	<0.004
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.004	<0.02	<0.01
WHO(2005)-PXDD/F-TEQ (LB)	0.001	0.003	0.002
WHO(2005)-PXDD/F-TEQ (UB)	0.01	0.04	0.02
DL-PCBs (pg g^{-1})			
2′,3,4,4′,5-PentaCB (#123)	110	291	194
2,3′,4,4′,5-PentaCB (#118)	3660	7620	5700
2,3,4,4′,5-PentaCB (#114)	76.5	148	113
2,3,3′,4,4′-PentaCB (#105)	1450	3000	2220
2,3′,4,4′,5,5′-HexaCB (#167)	262	626	458
2,3,3′,4,4′,5-HexaCB (#156)	527	1190	889
Column continued			
2,3,3′,4,4′,5′-HexaCB (#157)	13.6	297	196
2,3,3′,4,4′,5,5′-HeptaCB (#189)	44.9	111	80.6
3,4,4′,5-TetraCB (#81)	1.51	3.24	2.27
3,3′,4,4′-TetraCB (#77)	59.0	146	102
3,3′,4,4′,5-PentaCB (#126)	18.7	50.4	34.5
3,3′,4,4′,5,5′-HexaCB (#169)	5.15	14.2	9.35
WHO(2005)-PCB-TEQ (LB)	2.22	5.79	4.04
WHO(2005)-PCB-TEQ (UB)	2.22	5.79	4.04
NDL-PCBs (ng g^{-1})			
2,2′,5-TriCB (#18)	0.19	0.35	0.27
2,2,4′-TriCB (#28)	0.99	1.94	1.47
2′,3,4-TriCB (#33)	0.11	0.20	0.15
2,2′,4,4′-TetraCB (#47)	0.27	0.70	0.48
2,2′,4,5′-TetraCB (#49)	0.38	1.10	0.70
2,2′,4,6′-TetraCB (#51)	0.01	0.03	0.02
2,2′,5,5′-TetraCB (#52)	0.85	2.33	1.52
2,3,4,4′-TetraCB (#60)	0.30	0.61	0.45
2,3′,4,4′-TetraCB (#66)	0.76	1.58	1.16
2,4,4′,5-TetraCB (#74)	0.47	1.00	0.73
2,2′,4,4′,5-PentaCB (#99)	1.46	3.19	2.40
2,2′,4,5,5′-PentaCB (#101)	2.46	5.95	4.27
2,3,3′,4′,6-PentaCB (#110)	1.90	4.25	3.23
2,2′,3,4,4′,5′-HexaCB (#138)	5.89	13.9	9.41
2,2′,4,4′,5,5′-HexaCB (#153)	7.85	17.0	12.2
2,2′,3,4,4′,5,5′-HeptaCB (#180)	2.60	5.31	4.09
∑NDL-PCB (LB)	26.7	58.8	42.5
∑NDL-PCB (UB)	26.7	58.8	42.5
PBDEs (ng g^{-1})			
2,4-DiBDE (#7)	<0.001	0.01	0.002
4,4-DiBDE (#15)	0.002	0.01	0.003
2,2′,4-TriBDE (#17)	0.01	0.04	0.02
2,4,4′-TriBDE (#28)	0.02	0.08	0.05
2,2′,4,4′-TetraBDE (#47)	0.66	1.87	1.20
2,2′,4,5′-TetraBDE (#49)	0.03	0.54	0.31
2,3′,4,4′-TetraBDE (#66)	0.02	0.09	0.06
2,3′,4′,6-TetraBDE (#71)	<0.0001	<0.0004	<0.0002
3,3′,4,4′-TetraBDE (#77)	0.01	0.02	0.01
2,2′,3,4,4′-PentaBDE (#85)	0.003	0.01	0.01
2,2′,4,4′,5-PentaBDE (#99)	0.08	0.33	0.19
2,2′,4,4′,6-PentaBDE (#100)	0.11	0.34	0.22
2,3′,4,4′,6-PentaBDE (#119)	0.03	0.11	0.07
3,3′,4,4′,5-PentaBDE (#126)	<0.0003	0.001	0.001
2,2′,3,4,4′,5′-HexaBDE (#138)	0.001	0.004	0.002
2,2′,4,4′,5,5′-HexaBDE (#153)	0.04	0.14	0.08
2,2′,4,4′,5,6′-HexaBDE (#154)	0.09	0.26	0.17
2,2′,4,4′,6,6′-HexaBDE (#155)	0.04	0.13	0.09
2,3,4,4′,5,6-HexaBDE (#166)	0.0003	0.001	0.001
2,2′,3,4,4′,5,6′-HeptaBDE (#181)	0.001	0.003	0.001
2,2′,3,4,4′,5′,6-HeptaBDE (#183)	0.002	0.01	0.01
2,3,3′,4,4′,5,6-HeptaBDE (#190)	0.0004	0.002	0.001
2,2′,3,4,4′,5,5′,6-OctaBDE (#203)	0.001	0.02	0.01
2,3,3′,4,4′,5,5′,6-OctaBDE (#205)	<0.001	0.001	0.001
2,2′,3,3′,4,4′,5,5′,6-NonaBDE (#206)	0.003	0.01	0.01
2,2′,3,3′,4,4′,5,6,6′-NonaBDE (#207)	0.01	0.05	0.03
2,2′,3,3′,4,4′,5,5′,6,6′-DecaBDE (#209)	0.06	0.31	0.16
∑PBDE (LB)	1.49	3.98	2.70
∑PBDE (UB)	1.49	4.00	2.70

[a] Where it was possible, average values were calculated taking into account only results which were above the LOQ level. For congeners which were not found above the LOQ in any of samples, average LOQ values are given.

3.4. Application of the procedure to fish samples

The developed methodology was used in a study of ten Baltic wild salmon samples collected in Daugava River in September 2013 for the quantitative determination of selected persistent organic pollutants. As it was reported earlier, wild salmon collected in the Baltic Sea is highly contaminated with dioxin-like compounds, such as PCDD/DFs, PCBs and PBDEs (Szlinder-Richert et al., 2010; Zacs et al., 2012, 2013b). Taking into account the bioaccumulative properties and formation mechanisms of PBDD/DFs and PXDD/DFs, the presence of these compounds in Baltic wild salmon tissues might be expected. Due to the lack of analytical standards, from the wide range of possible bromo-, and particularly mixed bromo-chloro-substituted congeners in 2,3,7,8 positions, only selected PBDD/DFs and PXDD/DFs were analyzed. The overview of the composition of selected POPs in these samples is summarized in Table 3. For the 17 PCDD/DFs and 12 DL-PCBs, which had toxic equivalency factors (TEFs) set by the World Health Organization (WHO), the rounded result of each congener was multiplied by the corresponding TEF (Van den Berg et al., 2006),

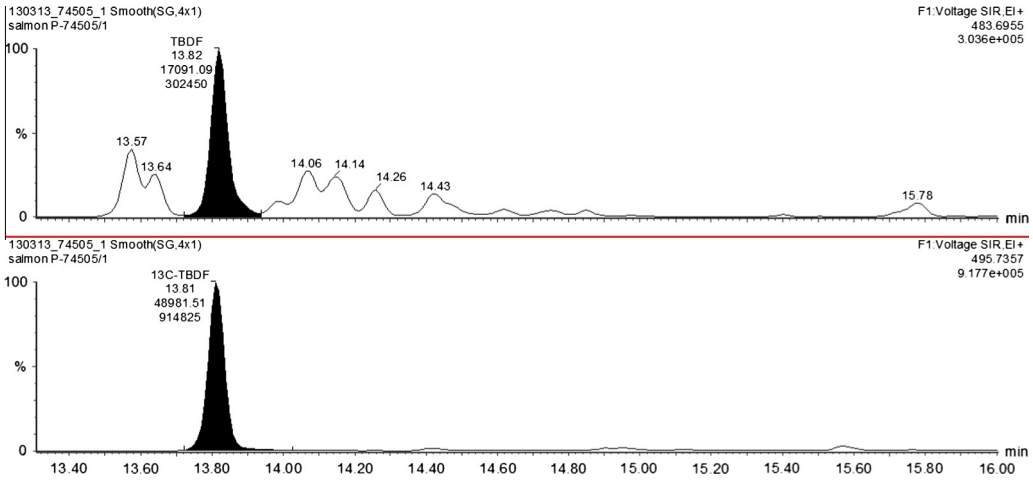

Fig. 2. Typical chromatogram of 2,3,7,8-TetraBDF in Baltic wild salmon sample with the corresponding internal standard.

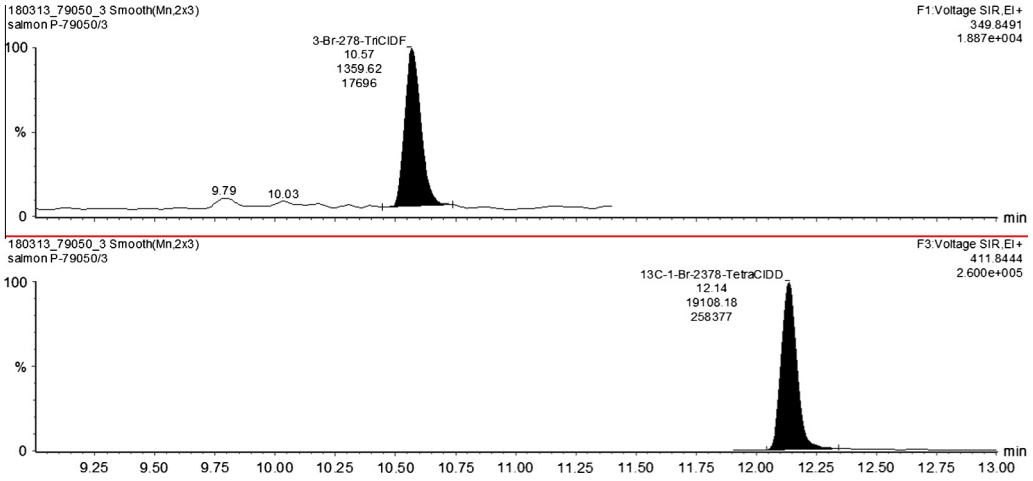

Fig. 3. Typical chromatogram of 3-Br-2,7,8-TriCDF in Baltic wild salmon sample with the corresponding internal standard.

and TEQs were calculated by summarizing the obtained values for selected POP groups. Because the TEFs have not been accepted for PBDD/DFs and PXDD/DFs, for tentative estimation of the possible toxicity risks of salmon tissue caused by the presence of these compounds, the TEQs for these contaminant groups were estimated using the corresponding TEFs of chlorinated analogs. Results were expressed as upperbound (UB) and lowerbound (LB) values. The obtained data confirms the presence of selected less studied organobromines in all salmon samples and are in good agreement with data reported in our previous study on Baltic wild salmon samples (Zacs et al., 2013b). A wide range of PBDD/DFs was detected in salmon tissue with prevalence of PBDFs, while only one congener among the analyzed PXDD/DFs showed quantifiable levels. Nevertheless, during the analysis of wild salmon samples it was not possible to identify other intense peaks on the SIM chromatograms corresponding to analyzed PBDD/DFs and PXDD/DFs, for which the quality control criteria of positive identification regarding the isotope ratio of ±15% would be fulfilled. Taking into account the selectivity of used methodology it was quite possible that these chromatographic peaks corresponded to other non-targeted PBDD/DF and PXDD/DF congeners present in the samples. Chromatograms of 2,3,7,8-TetraBDF and 3-Br-2,7,8-TriCDF in Baltic wild salmon tissue with the corresponding internal standards are shown in Figs. 2 and 3.

4. Conclusions

This study has introduced an analytical procedure for simultaneous determination of 95 compounds representing five groups of priority POPs including PBDD/DFs, PCDD/DFs, PXDD/DFs, PBDEs and PCBs. Multistage clean-up and fractionation procedure and detection with gas chromatography coupled to high resolution mass spectrometry ensure detection of the PBDD/DFs and PXDD/DFs at toxicologically significant levels. An optimized fractionation procedure involves a series of column chromatography steps with Florisil, activated carbon and basic alumina, providing more complete removal of non-planar interferants such as PBDEs and PCBs from the fractions containing PBDD/DFs and PXDD/DFs, thus significantly increasing the selectivity of the method for these compounds. The developed method was validated according to the requirements for routine analysis of PCDD/DFs and PCBs. The method has been applied to the analysis of wild salmon samples collected from the Baltic Sea, and the presence of less studied PBDD/DFs and PXDD/DFs was confirmed with prevalence of polyhalogenated dibenzofurans.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.06.032.

References

Behnisch, P.A., Hosoe, K., Sakai, S., 2003. Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environ. Int. 29, 861–877.

Buekens, A., Stieglitz, L., Hell, K., Huang, H., Segers, P., 2001. Dioxins from thermal and metallurgical processes: recent studies for the iron and steel industry. Chemosphere 42, 729–735.

Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006, Off. J. Eur. Commun. L 84, 1-22.

Council Directive 2003/11/EC of 6 February 2003 amending for the 24th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). Off. J. Eur. Commun. L 42/45.

Council Directive 76/769/EEC of 27 July 1976 on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations. Off. J. Eur. Commun. L 262, pp. 201–203.

Court Proceeding 2008/c116/02, 2008. Off. J. Eur. Commun. C 116/2.

Covaci, A., Voorspoels, S., Ramos, L., Neels, H., Blust, R., 2007. Recent developments in the analysis of brominated flame retardants and brominated natural compounds. J. Chromatogr. A 1153, 145–171.

De Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.

Donelly, J.R., Sovocol, G.W., 1990. Mass spectral characteristics of bromochlorinated dibenzo-p-dioxins and dibenzofurans. Chemosphere 20, 295–300.

Donnelly, J.R., Munslow, W.D., Vonnahme, T.L., Nunn, N.J., Hedin, C.M., Sovocool, G.W., Mitchum, R.K., 1987. The chemistry and mass spectrometry of brominated dibenzo-p-dioxins and dibenzofurans. Biomed. Environ. Mass Spectrom. 14, 465–472.

Ebert, J., Lorenz, W., Bahadir, M., 1999. Optimization of the analytical performance of polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/F). Chemosphere 39, 977–986.

Ericson Jogsten, I., Hagberg, J., Lindstrom, G., van Bavel, B., 2010. Analysis of POPs in human samples reveal a contribution of brominated dioxin of up to 15% of the total dioxin TEQ. Chemosphere 78, 113–120.

Falandysz, J., 2003. Chloronaphthalenes as food-chain contaminants: a review. Food Addit. Contam. 20, 995–1014.

Falandysz, J., Rose, M., Fernandes, A.R., 2012. Mixed poly-brominated/chlorinated biphenyls (PXBs): widespread food and environmental contaminants. Environ. Int. 44, 118–127.

Fernandes, A., Dicks, P., Mortimer, D., Gem, M., Smith, M.F., White, S., Rose, M., 2008. Brominated and chlorinated dioxins, PCBs and brominated flame retardants in Scottish shellfish: methodology, occurrence and human dietary exposure. Mol. Nutr. Food. Res. 52, 238–249.

Fernandes, A., Mortimer, D., Dicks, P., Gem, M., Smith, F., Rose, M., 2009a. Brominated dioxins (PBDD/Fs) and PBDEs in marine shellfish in the UK. Food Addit. Contam. A 26, 918–927.

Fernandes, A.R., Tlustos, C., Smith, F., Carr, M., Petch, R., Rose, M., 2009b. Polybrominated diphenylethers (PBDEs) and brominated dioxins (PBDD/Fs) in Irish food of animal origin. Food Addit. Contam. B 2, 86–94.

Fernandes, A.R., Rose, M., Mortimer, D., Carr, M., Panton, S., Smith, F., 2011. Mixed brominated/chlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls: Simultaneous congener-selective determination in food. J. Chromatogr. A 1218, 9279–9287.

Hagberg, J., 2009. Analysis of brominated dioxins and furans by high resolution gas chromatography/high resolution mass spectrometry. J. Chromatogr. A 1216, 376–384.

Haglund, P., Lindkvist, K., Malvarn, A., Wiberg, K., Bignert, A., Nakano, T., Apslund, L., 2005. High levels of potentially biogenic dibromo and tribromo dibenzo-p-dioxins in Swedish fish. Organohal. Comp. 67, 1267–1270.

Hanari, N., Kannan, K., Miyake, Y., Okazawa, T., Kodavanti, P.R.S., Aldous, K.M., Yamashita, N., 2006. Occurrence of Polybrominated Biphenyls, Polybrominated Dibenzo-p-dioxins, and Polybrominated Dibenzofurans as Impurities in Commercial Polybrominated Diphenyl Ether Mixtures. Environ. Sci. Tech. 40, 4400–4405.

Huang, L.Q., Paiva, A., Tong, H., Monson, S.J., Gross, M.L., 1992. Application of gas chromatography high-resolution mass spectrometry to the determination of trace monobromopolychlorodibenzo-p-dioxins in environmental samples. J. Am. Soc. Mass Spectrom. 3, 248–259.

Hutzinger, O., Thoma, H., 1987. Polybrominated dibenzo-p-dioxins and dibenzofurans: the flame retardant issue. Chemosphere 16, 1877–1880.

Kotz, A., 2006. Bestimmung von bromierten und gemischt bromiert-chlorierten unweltkontaminanten in biologischen matrizes mit HRGC–HRMS, Ph.D. thesis, Faculty of Science, University of Basel, Basel, Switzerland.

Meerts, I.A.T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J.G., Van der Burg, B., Brouwer, A., 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. Environ. Health Perspect. 109, 399–407.

Myers, A.L., Mabury, S.A., Reiner, E.J., 2012. Analysis of mixed halogenated dibenzo-p-dioxins and dibenzofurans (PXDD/PXDFs) in soil by gas chromatography tandem mass spectrometry (GC–MS/MS). Chemosphere 87, 1063–1069.

Ren, M., Peng, P., Cai, Y., Chen, D., Zhou, L., Chen, P., Hu, J., 2011. PBDD/F impurities in some commercial deca-BDE. Environ. Pollut. 159, 1375–1380.

Ryan, J.J., Lizotte, R., Panopio, L.G., Lau, B.P.-Y., 1989. The effect of strong alkali on the determination of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs). Chemosphere 18, 149–154.

Smith, A.G., Gangolli, S.D., 2002. Organochlorine chemicals in seafood: occurrence and health concerns. Food Chem. Toxicol. 40, 767–779.

Stapleton, H.M., 2006. Instrumental methods and challenges in quantifying polybrominated diphenyl ethers in environmental extracts: a review. Anal. Bioanal. Chem. 386, 807–817.

Szlinder-Richert, J., Barska, I., Usydus, Z., Grabic, R., 2010. Polybrominated diphenyl ethers (PBDEs) in selected fish species from the southern Baltic Sea. Chemosphere 78, 695–700.

US Environmental Protection Agency, 1994. Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, Method 1613.

US Environmental Protection Agency, 1999. Chlorinated Biphenyl Congeners in Water, Soil, Sediment and Tissue by HRGC/HRMS, Method 1668A.

Van Bavel, B., Näf, C., Bergqvist, P.-A., Broman, D., Lundgren, K., Papakosta, O., Rolff, C., Strandberg, B., Zebühr, Y., Zook, D., Rappe, C., 1996. Levels of PCBs in the aquatic environment of the Gulf of Bothnia: Benthic species and sediments. Mar. Pollut. Bull. 32, 210–218.

Van den Berg, M., Birnbaum, L., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.

Van den Berg, M., Denison, M.S., Birnbaum, L.S., De Vito, M.J., Fiedler, H., Falandysz, J., Rose, M., Schrenk, D., Safe, S., Tohyama, C., 2013. Polybrominated dibenzo-p-dioxins, dibenzofurans, and biphenyls: inclusion in the toxicity equivalency factor concept for dioxin-like compounds. Toxicol. Sci. 133, 197–208.

Wang, D., Chai, Z., Jiang, G., Wong, M.H., Wong, W.K., 2005. Gas chromatography/ion trap mass spectrometry applied for the determination of polybrominated diphenyl ethers in soil. Rapid Commun. Mass Spectrom. 19, 83–89.

Wang, D., Jiang, G., Chai, Z., 2007. Method development for the analysis of polybrominated dibenzo-p-dioxins, dibenzofurans and diphenyl ethers in sediment samples. Talanta 72, 668–674.

WHO, 1998. Polybrominated Dibenzo-p-dioxins and Dibenzofurans. Environmental Health Criteria, p. 205.

Zacs, D., Bartkevics, V., Frank, H., 2012. Levels and congener profiles of PCDD/Fs and dioxin-like PCBs in Baltic wild salmon (Salmo salar). Toxicol. Environ. Chem. 94, 1502–1510.

Zacs, D., Bartkevics, V., Viksna, A., 2013a. Content of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in fish from Latvian lakes. Chemosphere 91, 179–186.

Zacs, D., Rjabova, J., Bartkevics, V., 2013b. Occurrence of Brominated Persistent Organic Pollutants (PBDD/DFs, PXDD/DFs, and PBDEs) in Baltic Wild Salmon (Salmo salar) and Correlation with PCDD/DFs and PCBs. Environ. Sci. Tech. 47, 9478–9486.