SIMULTANEOUS SELECTIVE PRESSURISED LIQUID EXTRACTION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYLS ETHERS FROM FEEDSTUFFS

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Introduction

Polybrominated diphenyl ethers (PBDEs) belong to the category of emerging pollutants. However, they have already been recognised as worldwide distributed contaminants and there is an increasing interest for determining their concentration levels in environmental samples. Because of the non-polar and lipophilic character of PBDEs, in many instances, sample preparation methods similar to those previously validated for close related microcontaminants, such as polychlorinated biphenyls (PCBs) and/or polychlorinated dibenzo-*p*-dioxins and furans, have been used for the (typically simultaneous) extraction and clean-up of PBDEs and, if required, final fractionation of the investigated families of pollutants when dealing with complex fat containing matrices [1-3]. In general, these analytical methods are expensive in terms of solvent, sorbent and time consumption, usually involve much manipulation of the extracts and, because of the lack of method reoptimisation, in some cases, have resulted in rather unsatisfactory results for the more brominated congeners, i.e. hepta- to deca-BDEs.

Pressurised liquid extraction (PLE) is a relatively analyte- and matrix-independent technique which provides cleaner extracts than the time-consuming classical procedures used for extraction of PCBs and PBDEs from fat-containing complex matrices, such as animal feedstuffs. Careful optimisation of the parameters affecting the efficiency and selectivity of the PLE process, combined with an appropriate *in-cell* clean-up strategy, has been demonstrated to be a valuable analytical approach able to generate clean extracts ready for instrumental analysis [2,4]. Nevertheless, up to now, amounts of solvent and sorbents in the range of those of classical methods have been used when this type of strategy has been evaluated for the determination of PCBs and PBDEs in fatty samples, including animal feedstuffs for which a rather limited number of methodologies have been reported [1-2].

This paper describes a new miniaturised PLE-based method for fast simultaneous determination of PCBs and PBDEs in feedstuff samples. Once optimised, the analytical procedure allowed the exhaustive extraction of the analytes from the sample and the clean-up of the extracts to be performed in a single step with a minimum consumption of solvent, sorbents and time. The performance of the analytical procedure developed, which was combined at-line with gas chromatography–micro electron capture detection (GC–microECD) for PCB analysis and with gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS) for PBDE determination, was tested for the simultaneous determination of PCBs and PBDEs in a non-spiked aquiculture potato-based feed sample. The results were compared with those obtained when the same sample was prepared according to a more conventional procedure previously validated in our laboratory [5]. The developed method has been applied to the analysis of selected PCB and PBDE congeners in a variety of non-commercial and commercial aquiculture feed matrices.

Materials and Methods

All solvents used were pestipur quality and were purchased from SDS (Peypin, France), except *n*-hexane (Merck, Darmstadt, Germany). Sulphuric acid was pro analysis quality (Merck). Anhydrous sodium sulphate was obtained from J.T. Baker (Deventer, The Netherlands) and Silica gel 60 from Merck.

The 23 PCB congeners studied (see Table 1 below) were selected because of their toxicity and relative abundance in environmental samples. A working stock solution was prepared from individual PCB standards (Ehrenstorfer, Augsburg, Germany) containing 1000 pg/µl of each compound in isooctane. This solution was used for further dilution. 1,2,3,4-Tetrachloronaphtalene (TCN, Ehrenstorfer) and PCB 209 were used as external standards for PCB determination by GC–microECD and added to the final extracts just before the chromatographic analysis. Labelled standards of the 13 most toxic congeners were added to the extracts

before confirmation by GC with ion trap tandem mass spectrometry, GC–ITD(MS/MS) [6].

15 PBDE congeners (see Table 2 below) containing from three to ten bromo-substitutions were selected among those frequently detected in environment samples and included in the present study. A working stock solution was also prepared from individual PBDE standards (Ehrenstorfer) containing 1000 pg/µl of each compound in isooctane and used for further dilution. $^{13}C_{12}$-Labelled PBDE 139 standard was added before GC–NCI-MS analysis [7].

The feed samples investigated included two (under development) vegetal-based feed for aquiculture use (20% of fat, w/w) provided by a commercial company, three standard aquiculture feeds (26-28% fat content, w/w) and a commercial feed for cold water fishes (5% fat, w/w) purchased from a supermarket in Madrid (Spain). All samples were conserved under a dried atmosphere and protected from light until analysed.

After optimisation of the different parameters affecting the efficiency of the simultaneous extraction and clean-up procedure proposed, namely the type and amount of sorbent used for dispersion of the sample and for subsequent fat removal, the nature and volume of the extraction solvent, the extraction temperature and the number of static extraction cycles, a typical experiment consisted on the dispersion of a representative portion of the feed sample, ca. 1.0 g, on similar amounts of Na_2SO_4 and silica modified with 44% (w/w) sulphuric acid (SiO_2-H_2SO_4). After blending and homogenisation in a mortar using a pestle, 0.750 g of this mixture, corresponding to 0.250 g of sample, were packed in a stainless steel extraction cell on top of a layer of SiO_2-HSO_4 packed between layers of activated neutral SiO_2. The cell was then installed in a miniaturised home-made PLE system [8] and the selected extraction solvent was pumped at 0.4 mL/min to fill the extraction cell and lines. After pressurisation at 10.5 MPa and heating at 50ºC, a first static PLE was performed for 7 min. Afterwards, the solvent, *n*-hexane, was completely replaced by a 1:1 (v/v) mixture of *n*-hexane:dichloromethane and a second 7 min static PLE was carried out. Finally, some fresh solvent was eluted through the column to ensure proper purging of the sample, the clean-up sorbents and the lines. The eluates from both PLE cycles were jointly collected, concentrated under a gentle nitrogen current and subjected to instrumental analysis by the corresponding technique. Procedure blanks were prepared following the same procedure as for feedstuffs but without sample. No background interference was found to be introduced by the methodology proposed.

Definitive evaluation of the combined matrix solid-phase dispersion (MSPD) plus PLE with *in-cell* purification procedure arrangement proposed for simultaneous PCB and PBDE determination was carried out by determination of the target compounds in the test non-spiked feed sample and subsequent comparison the results obtained with those found using a more conventional procedure for this kind of analysis based on MSPD of the sample and *off-line* fat removal with SiO_2-H_2SO_4 plus activated SiO_2 described elsewhere [5]. Otherwise specified, all experiments were carried out in triplicate.

Determination of the selected PCBs in the final extracts was performed by GC (HP 6890 Series, Hewlett-Packard, Palo Alto, CA, USA) with micro-ECD. Samples were injected in the hot splitless mode (1 µl, 270ºC, splitless time 1.0 min) in a capillary BPX-5 column (60 m, 0.25 mm i.d., 0.25 µm film thickness) purchased from SGE (Melbourne, Australia). The column temperature was programmed from 80ºC (2 min) to 250ºC (50 min) at a rate of 30ºC/min and then to 270ºC (10 min) at 5ºC/min. Nitrogen was used as carrier gas (constant flow, 1.5 mL/min) and as make-up gas 30 mL/min. The detector temperature was set at 300ºC. Confirmation of the individual PCB congeners investigated was carried out in a GC (CP-3800, Varian, CA, USA) equipped with an ion trap MS detector (Saturn 2000, Varian) working in the MS/MS mode under the experimental conditions described elsewhere [6].

Determination of the tested PBDEs in the concentrated extracts was performed by GC (HP 6890 Series, Hewlett-Packard, Palo Alto, CA) with quadrupole MS after NCI and working in the selected ion monitoring (SIM) mode [7]. The source and transfer lines temperatures were set at 300ºC and 150ºC, respectively. Chromatographic conditions were carefully optimised to avoid PBDE 209 degradation during the analysis. Thereby, samples were injected in the hot splitless mode (1 µl, 270ºC, pulsed splitless time 4.0 min, P pulse, 5 psi) in a capillary DB-5 column (15 m, 0.20 mm i.d., 0.20 µm film thickness) purchased from J&W Scientific (USA). The column temperature was programmed from 120ºC (4.2 min) to 200ºC at a rate of

30ºC/min, then to 275ºC at 5ºC/min, then to 300ºC (10 min) at 40ºC/min and then to 310ºC (2 min) at 10ºC/min. Helium was used as carrier gas (constant flow, 1.5 mL/min).

Results and discussion

A previously validated method for miniaturised selective PLE of PCBs from fatty food matrices [4] has now been modified and adapted for the simultaneous determination of two relevant organochlorinated families of persistent pollutants, PCBs and PBDEs, in aquiculture feed matrices. Variables affecting the efficiency of the PLE, such as nature, pressure and temperature of the extraction solvent(-s), total solvent(-s) volume and extraction time; and fat removal, such as amount of silica modified with sulphuric acid used for dispersion of the sample and final purification of the extracts, have been evaluated and optimised.

Experiments were carried out to systematically reduce the amount of sample, and consequently those of SiO_2 and Na_2SO_4, required to disperse the feed tested from the 5-10 g involved in conventional procedures to the finally 0.250 g used in the present study while allowing a reliable determination of the endogenous PCBs and PBDEs selected. One of the main experimental parameters affecting the efficiency of the PLE process is the solvent nature. *n*-Hexane has been demonstrated to provide quantitative recoveries during PLE of PCBs from fat-rich matrices [4]. However, the use of a more selective extraction solvent was advisable to ensure simultaneous efficient displacement of PBDEs from these highly sorptive samples [1] with minimum solvent consumption. After some preliminary experiments involving several solvent mixtures, *n*-hexane:dichloromethane (1:1, v/v) was selected as the most convenient extraction solvent for exhaustive PLE of the most highly brominated BDEs without affecting the efficiency of the *in-cell* fat removal. Using *n*-hexane as extraction solvent during the first PLE cycle resulted in a PCB-enriched fraction. However, some less brominated BDEs were found to partially elute in this fraction. Consequently, both PLE fractions were jointly collected. The selectivity offered by the separation-plus-detection techniques selected for subsequent instrumental analysis of the extract prevented from interference among analytes belonging to the two studied families. The amounts of activated silica and SiO_2-H_2SO_4 (44%, w/w) to be packed in the extraction cell to simultaneously ensure efficient fat removal irrespective of the lipidic content of the feed matrix and minimum sorbent(-s) and solvent consumption were also investigated. Assays were also conducted to optimise other experimental parameters affecting the efficiency of the process, such as extraction temperature, static extraction time, number of static PLE cycles, and dynamic removal of the extraction solvent from the cell and PLE lines. Once optimised, the developed selective PLE method provided quantitative recoveries of the endogenous PCBs and PBDEs and complete fat elimination in a single step using *n*–hexane and *n*–hexane:dichloromethane at 50ºC as extraction solvents. A total solvent consumption of 8 mL was required for the two consecutive 7 min static PLE of 250–mg samples and complete sample preparation was achieved in only 30 min. Additional clean–up of the collected extracts was not required.

Satisfactory recoveries (except for a few exceptions, in the range 70-121%) and a repeatability better than 20% were obtained for all target compounds at the two assayed spiking levels, 4 and 0.4 ng/g sample, for the complete PLE plus GC–micro-ECD (for PCBs) or GC–NCI-qMS (for PBDEs) methods proposed. Results obtained for the endogenous PCB and PBDE congeners in the selected test feed matrices proved that accurate determination of the target analytes was possible even if an as small amount of sample as 250 mg was used, with limits of detection in general in the 0.01-0.06 ng/g sample for PCBs and ranging from 0.002-0.01 ng/g sample for PBDEs (as calculated for real-life matrices). These results illustrated the feasibility of the method proposed for fast and accurate determination of PCBs and PBDEs in fatty feed samples.

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Table 1. Recoveries (%) and relative standard deviations (RSDs) calculated for the studied PCB congeners at the two investigated spiking levels of 0.4 and 4 ng/g sample (n=3).

	Recovery (RSD)	
PCB No	0.4 ng/g	4 ng/g
PCB 28	67 (8)	72 (13)
PCB 52	99 (15)	65 (15)
PCB 95	92 (5)	60 (10)
PCB 101	97 (7)	61 (19)
PCB 77	75 (6)	68 (20)
PCB 149	110 (4)	62 (17)
PCB 123	61 (15)	96 (6)
PCB 118	86 (6)	82 (8)
PCB 114	123 (18)	58 (13)
PCB 153	118 (3)	72 (8)
PCB 132	75 (18)	83 (7)
PCB 105	88 (13)	99 (9)
PCB 138	103 (1)	85 (10)
PCB 126	47 (12)	78 (13)
PCB 183	130 (2)	88 (9)
PCB 167	80 (14)	85 (10)
PCB 156	71 (13)	92 (8)
PCB 157	71 (15)	31 (11)
PCB 180	87 (7)	121 (11)
PCB 169	68 (9)	110 (9)
PCB 170	91 (11)	99 (7)
PCB 189	81 (11)	112 (8)
PCB 194	90 (3)	94 (10)

Table 2. Recoveries (%) and RSDs calculated for the studied PBDE congeners at the two investigated spiking levels of 0.4 and 4 ng/g sample (n=3).

	Recovery (RSD)	
PBDE No	0.4 ng/g	4 ng/g
PBDE 17	98 (13)	107 (5)
PBDE 28	98 (12)	114 (6)
PBDE 47	110 (11)	111 (9)
PBDE 66	95 (7)	109 (8)
PBDE 100	114 (17)	102 (9)
PBDE 99	107 (13)	104 (11)
PBDE 85	90 (8)	99 (15)
PBDE 154	92 (20)	100 (13)
PBDE 156	95 (12)	93 (17)
PBDE 184	105 (13)	90 (16)
PBDE 183	96 (9)	92 (15)
PBDE 191	97 (6)	90 (15)
PBDE 197	103 (7)	93 (13)
PBDE 196	103 (9)	86 (18)
PBDE 209	145 (20)	100 (27)