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Short communication

Multi-residue screening of chlorinated and brominated compounds from aquaculture samples using matrix solid-phase dispersion—gas chromatography–mass spectrometry

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Abstract

An effective multiresidual method for the trace analysis of fifteen compounds from a diverse group of pesticides, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyl (PCBs) and polybrominated biphenyl (PBBs) in aquaculture feed is described. The analytical procedure is based on the matrix solid-phase dispersion (MSPD) of feed sample and subsequent elution with hexane. The MSPD process was evaluated using an asymmetrical experimental design $2^3 3^2//9$. Factors such as C_{18} sorbent amount, kind of adsorbents, solvent volume and elution mode were considered. The results suggest that the operational MSPD conditions are elution with pressure, 1 g of C_{18}, basic alumina as adsorbent and 30 mL of hexane. The overall method including MSPD procedure and GC coupled to mass spectrometry (MS/MS) has been applied to several samples of aquaculture feed and marine species. Precision and accuracy of the analytical method were determined using the reference material from the International Atomic Energy Agency (IAEA-406), showing a good agreement to the referenced values.

Keywords: Polyhalogenated compounds; Matrix solid-phase dispersion; Gas-chromatography coupled to mass spectrometry; Aquaculture feed

1. Introduction

Aquaculture is a relatively new and rapidly growing activity in the European Union. The aquaculture production in Galicia (Spain) (essentially: mussel, clam, turbot and salmon) represents about 35% of the total production of the European fisheries sector.

The determination of halogenated compounds in aquaculture feed and fish and the identification of the original sources of the contamination is, therefore, important for dietary exposure assessment and the protection of public health, particularly in view of the increasing availability of the consumer to farmed fish [1–4]. Recently, the significance of human exposure to halogenated compounds has been the subject of extensive discussions by the European Union to establish of daily intake limits [1,5,6].

Many extraction procedures of these compounds require extensive sample preparation, multiple extractions, extract purification, and concentration before chromatographic analysis [7–10].

MSPD allows the extraction and clean-up in a single step using adsorbents, reduces analyst time, increases sample throughput and provides a more solventless approach [11–14].

Applications of MSPD in food analysis using different types of sorbents as aluminium oxide, Florisil or octadecylsilyl-derivatized silica (ODS) were studied [15,16].

The aim of this work was to present an approach based on MSPD for various contaminant residues from animal tissues to achieve sufficient selectivity in one direct extraction-cleanup step by the combination of different adsorbents. The MSPD–GC–MS/MS method was developed for the isolation and determination of a diverse group of fifteen compounds, pesticides, PBDEs, PCBs and PBBs,

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in aquaculture feed and fish. Experimental design methodology (an asymmetric matrix $2^3 3^2//9$) was used to evaluate the influence of four MSPD parameters. The analytical method was validated using the IAEA 406 reference material.

2. Experimental

2.1. Reagents and standards

Sulphuric acid, 96 %, isooctane, Silica gel 60 Å pore size (0.040–0.063 mm, 230–400 mesh), HPLC-grade methanol, and ethanol, 99.9% were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulphate (99%) was obtained from BDH (Poole, UK). Pesticide *n*-hexane grade (α-benzene hexachloride (α-BHC), 1000 μg/mL in MeOH; γ-benzene hexachloride (γ-BHC), 1000 μg/mL in MeOH; heptachlor, 1000 μg/mL in methanol and 4,4′-DDT, 98.4% as solid) were supplied by Supelco (Bellefonte, P.A., USA). Mixture of PCBs at 10 μg/mL in ethanol: 2,6-dichlorobiphenyl (PCB-10, 100%), 2,4,4′-trichlorobiphenyl (PCB-28, 100%), 2,2′,5,5′-tetrachlorobiphenyl (PCB-52, 100%), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB-138, 100%), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153, 100%) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB-180, 100%) was obtained from Supelco (Bellefonte, P.A., USA). Mixture of PBDEs at 10 μg/mL in cyclohexane: 2,2′,4,4′-tetrabromodiphenyl ether (PBDE-47, 42.5%), 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE-99, 10.9%), 2,2′,4,4′,6-pentabromodiphenyl ether (PBDE-100, 39.3%), was supplied by Dr. Ehrenstorfer (Augsburg, Germany). 4,4′-Dibromobiphenyl (PBB-15, 99.8%) and 2,2′,4,5′-tetrabromobiphenyl (PBB-49, 97%) as solid was purchased from Supelco (Bellefonte, P.A., USA). Isooctane stock solutions of PBBs and PBDEs were prepared. Mixture of [^{13}C] labelled PCBs, 5 μg/mL in nonane: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180 and PCB-209, was supplied by Cambridge Isotope Laboratories (Andover, MA, USA).

Aluminium oxide activated basic (150 mesh), Florisil (60–100 mesh) and ODS, octadecilsilane, C_{18} (9–12% carbon loading) were obtained by Aldrich (Steinheim, Germany).

A stock standard solution (400 ng/mL) was used to prepare the working standard solutions by dilution, except PBDEs that were used as total concentration.

2.2. Instrumentation

The analysis of the extracts to evaluate and optimize the MSPD procedure was performed using GC-ECD, on a Hewlett-Packard (Avondale, PA, USA) 5890A Series II gas chromatograph equipped with an electron capture detector and a Hewlett-Packard HP-1079 automatic injector and split–splitless capillary injection port. Instrumental conditions were described in detail by Rodil et al. [17].

A Varian (Walnut Creek, CA, USA) 3900 gas chromatograph equipped with an ion trap mass detector Varian Saturn 2100T mass spectrometer was used. Gas chromatography was carried out on a 30 m × 0.25 mm i.d. HP-5 ms (5% polydiphenylsiloxane) (Agilent Technologies, USA) fused-silica column (0.25 μm film thickness). Split flow was set at 50 mL/min. The initial temperature was 70 °C, held for 2 min; ramped at 20 °C/min up to 170 °C, and held for 2 min; a second rate at 4 °C/min up to 250 °C; a third rate at 10 °C/min up to 300 °C and held for 5 min. Helium (purity 99.999%) was employed as carrier gas with a constant column flow of 1 mL/min. The mass spectrometer was operated in electron ionization mode at 70 eV. The mass range scanned from 90 to 650 *m/z* at 0.80 s/scan for full-scan mode. The trap, manifold and transfer line temperatures were maintained at 250, 50 and 280 °C, respectively. General parameters were as follows: multiplier offset +100, emission current 90 μA, AGC target value 2000 counts. For MS/MS, all compounds were analyzed using a resonant waveform type. Specific MS/MS conditions for each analyte were listed previously [18]. Quantitation was accomplished by relative areas versus [^{13}C] PCBs used as internal standards, which was added just before the MSPD process.

2.3. Sample preparation

Screening experiments were carried out on turbot feed spiked with 200 μL of pesticides, PBDEs and PCBs 10 μg/mL solution mixture, 135 μL of PBB-49, 15 μg/mL solution and 90 μL of PBB-15, 22 μg/mL solution.

Basic alumina, silica gel and Florisil were stored in a sealed bottle until analysis. Sixty grams acidic silica gel was prepared adding 40 g concentrated sulphuric acid (H_2SO_4).

One hundred grams spiked turbot feed with a final concentration of 19 ng/g of each compound (PBDEs were used as total concentration) was used in the evaluation of MSPD. 1.5 g of spiked sample and 1 g of C_{18} were placed in a glass mortar. A syringe barrel, containing a frit at the bottom, was filled (from bottom to top) with 2 g acidic silica, 1.5 g alumina (as clean-up adsorbents) and, the homogenized matrix, in sandwich mode using another frit at the top of the column as a retainer. Then the column was compressed to 8 mL with a syringe plunger to eliminate voids and channelling. The compounds studied were eluted with 30 mL hexane. The elution sequence applied to the MSPD columns varies with the analytical needs and the chemical characteristics of the studied analytes. We have begun preliminary experiments with a non polar solvent, such as hexane, due the high liposolubility of the polyhalogenated compounds studied. The results obtained have provided satisfactory recoveries and we have thought that it was not necessary to assayed other solvents. The eluate obtained was concentrated to 0.5 mL in a Turbo-Vap II Station (Zymark, Hopkinton, MA, USA) and finally to dryness by nitrogen blowdown concentrator. The residue

was redissolved in 200 μL hexane in conical glass inserts for 2 mL GC vials obtained from Supelco (Bellefonte, P.A., USA).

Real world samples of fish feed, turbot, and shellfish (clam, mussel and cockle) from aquaculture activities were freeze-dried. The shellfish samples were taken from farmed industry of Galician coast. Different fish feed samples analyzed are commercially available and turbot samples were achieved at local market. We also analyzed a certified reference material, IAEA 406 obtained from the International Atomic energy Agency (Vienna, Austria).

2.4. Experimental design approach

An asymmetrical screening design $2^3 3^2 // 3^2$ derived by "collapsing A" from the $3^4 // 3^2$ symmetrical design [19], was used to establish the relatively influence of four considered factors: sorbent amount (octadecyl silane phase, C_{18}, studied at three levels, 0.5, 1 and 2 g; basic alumina or Florisil as adsorbent; solvent volume (*n*-hexane) studied at three levels, 10, 20 and 30 mL; and elution mode (gravitational flow or flow by pressure generated manually pushing a syringe plunger 1 cm/5 min), using nine experiments. The order of running experiments was randomized to eliminate possible bias.

The experimental design was generated and all analytical treatments were supported by the software NemrodW 2000 [20].

3. Results and discussion

3.1. Clean-up

Methods for some biological tissues require additional clean-up steps based on two different steps [7] or one step [18,21]. Sample extraction and clean-up are carried out in the same step with good recovery and reproducibility for MSPD method [8].

In a preliminary study, silica gel, alumina and Florisil were used to interference removal in a supercritical fluid extraction (SFE) procedure [17]. In this paper, different MSPD column for direct in-line sample clean-up were evaluated using similar adsorbents. In our case, acidified silica gel was placed at the bottom of the MSPD column because basic compounds are retained more strongly on mildly acidic silica [7]. We therefore tested another adsorbent together with silica gel (alumina basic or Florisil), both evaluated by experimental design.

3.2. Optimization of MSPD procedure

The data obtained in each run of the screening design for each compound were evaluated by ANOVA at 5% significance level and by different statistical approaches [22].

Elution by pressure and the use of alumina as adsorbent were statistically significant for the extraction of PBB-49. We have observed that PBB-15 and PBDE-100 were better extracted using elution without pressure. Pesticides α-BHC, β-BHC and heptachlor presented higher recoveries when Florisil was used as adsorbent. So, it is necessary to find the experimental MSPD conditions where the different responses (recoveries) are most satisfactory, overall.

In general terms, the MSPD recoveries were more effective when elution was by pressure, 1 g of C_{18} and 30 mL of hexane were used. It appeared that the kind of adsorbent it is not clear for all the analytes studied because of their inherent chemical differences. Then, $n = 5$ experiences were carried out using Florisil or basic alumina as the adsorbent whereas the others factors were fixed at the values referred above. Basic alumina provides better recoveries for all the compounds (results not shown).

A later study reported a decrease in efficiency of MSPD (recoveries lower than 100%) when 1 g anhydrous sodium sulphate was blended into 1.5 g feed sample plus 1 g C_{18} to yield a dry homogeneous material (results not shown).

The difference in MSPD recovery was next studied changing the order of adsorbents for the packed column (1.5 g alumina was placed before 2 g acidic silica from the bottom of the syringe barrel, then the homogeneous mixture of 1.5 g feed sample and 1 g C_{18} was added and, finally, a frit was placed at the top of the column). The majority of polyhalogenated compounds present recoveries higher than 100% due the removal of co-extracted interferences.

The final operational conditions were elution by pressure, alumina as adsorbent, 1 g of C_{18} and 30 mL of hexane.

3.3. Performance of the analytical methods

3.3.1. GC-ECD

Linearity of the GC-ECD method was tested with standard mixtures at four concentration levels ($n = 3$) in the range 50–400 ng/mL for each compound. Correlation coefficients above 0.995 were obtained for all of the compounds. Relative standard deviations (R.S.D.%) for $n = 5$ consecutive injections of a standard, containing all species at the 100 ng/mL level (precision within days), ranged from 1.1 to 7.7 were obtained. Quantification limits (for a signal-to-noise of 10) of the analytical procedure applied to spiked turbot feed samples, were obtained ranged 0.1–7.1 ng/g. Once the MSPD optimal conditions were chosen, the method was tested for recovery employing spiked samples of turbot feed. The results obtained for $n = 5$ were ranged between 70 and 96% except for α-HBC which provided only 50% of recovery. Blanks were made to verify the absence of tested compounds.

However, it should be noted that GC-ECD system can not resolve all compounds (PCB 180 and PBDE 47), due to the similar chromatographic behaviour of these kinds of compounds. This has led to changes in the detector to achieve a complete separation, identification and quantification of pes-

Table 1
Performance and validation of the GC–MS/MS method for polyhalogenated compounds in feed aquaculture samples using MSPD extraction

Compound	Correlation coefficient ($n = 3$)	Repeatability (R.S.D., %) ($n = 6$)	Method LOD (ng/g)	Method LOQ (ng/g)	IAEA 406 referenced value (ng/g)	IAEA 406 obtained value (ng/g)[a] ($n = 4$)	Referenced confidence interval (ng/g)
PCB-10	0.999	2	0.02	0.06	–	–	–
α-BHC	0.998	2	0.09	0.3	0.79	0.28 ± 0.03	0.23–1.7
γ-BHC	0.999	2	0.1	0.4	0.27	0.72 ± 0.09	0.11–0.80
PCB-28	0.999	1	0.2	0.5	0.57	1.06 ± 0.06	0.43–1.3
Heptachlor	0.997	1	0.2	0.8	0.32	n.d.	0.23–0.46
PCB-52	0.998	3	0.03	0.1	1.30	1.53 ± 0.05	1.0–2.2
PBB-15	0.995	3	0.3	1.1			
PCB-153	0.998	2	0.1	0.3	3.70	3.71 ± 0.18	2.9–6.0
PBB 49	0.995	7	0.2	0.6			
4,4′-DDT	0.995	5	0.3	1.0	3.00	2.22 ± 0.09	1.8–5.6
PCB-138	0.998	2	0.04	0.1	4.00	3.34 ± 0.10	2.5–6.3
PCB-180	0.996	5	0.1	0.5	1.20	1.16 ± 0.14	1.0–1.2
PBDE-47	0.995	5	0.9	3.1	–	–	–
PBDE-100	0.995	9	0.3	1.0	–	–	–
PBDE-99	0.995	7	0.3	1.1	–	–	–

[a] Mean value ± S.D.

ticides, PCBs, PBBs and PBDEs must be accomplished by GC–MS/MS.

3.3.2. GC–MS/MS

The overall analytical procedure (MSPD–GC–MS/MS) was tested for linearity in the range 10–400 ng/mL, recovery of fortified feed samples at the spiked values given in Section 2, detection and quantification limits (obtained when the signal was 3 or 10 times the background noise in the chromatogram at the lowest concentration assayed) and chromatographic repeatability (Table 1). Concentrations of polyhalogenated species were determined using a linearity range between 10 and 400 ng/mL and internal standard method (50 ng/mL of mixture of [^{13}C] labelled PCBs). Repeatability of the MSPD–GC–MS/MS method was studied using a 100 ng/mL standard solution ($n = 6$). Detection limits and quantification limits were below 0.4 and 1.2 ng/g, respectively, except for PBDE-47. The accuracy of the proposed method was tested and validated by the analysis of a multispecies biological reference material (IAEA 406). The obtained concentrations agree well with referenced values and all of them are inside the confidence interval for IAEA 406, as can be seen in Table 1.

3.4. Application to real samples

The optimized procedure was applied to the analysis of several real samples of different origin: aquaculture fish feeds, cultured marine species such as cockle, clam, and mussel. Table 2 shows the results obtained. All the samples analysed contained appreciable amounts of PCB-153 and PCB-138. PCB-52 was only not detected from turbot feed (sample 3) and PCB-180 was only not detected from small turbot feed (sample 5). These findings are in concordance with other authors' data showing that higher chlorinated PCBs congeners are the most frequently detected in biological samples [23,24]. The highest concentrations corresponded to 4,4′-DDT from clam and mussel samples. In addition, 4,4′-DDT was not detected from trout feed (samples 1 and 2). Only a polybrominated compound, PBDE-47, was found from sample 4 which correspond to large turbot feed. Fig. 1 shows the selected extracted ion GC–MS/MS

Table 2
Concentrations (ng/g) of polyhalogenated compounds detected in real samples of aquaculture fish feed and cultured marine species ($n = 3$)

Compound	Sample 1 (large trout feed)	Sample 2 (small trout feed)	Sample 3 (turbot feed)	Sample 4 (large turbot feed)	Sample 5 (small turbot feed)	Sample 6 (cockle)	Sample 7 (clam)	Sample 8 (mussel)
PCB-10	n.d.	n.d.	n.d.	1.0	n.d.	n.d.	0.2	0.1
γ-BHC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.2	9.4
PCB-28	n.d.	1.0	n.d.	2.4	n.d.	n.d.	3.8	2.6
PCB-52	0.2	0.6	n.d.	10.2	0.9	5.1	11.4	16.2
PCB-153	5.1	3.1	1.2	3.4	2.4	4.2	10.9	22.3
4,4′-DDT	n.d.	n.d.	0.9	1.8	1.5	3.7	166.6	28.6
PCB-138	3.1	2.5	1.0	3.2	1.7	2.4	7.3	13.5
PCB-180	1.6	1.1	0.4	1.0	n.d.	0.6	1.5	1.1
PBDE-47	n.d.	n.d.	n.d.	1.3	n.d.	n.d.	n.d.	n.d.

n.d. = not detected.

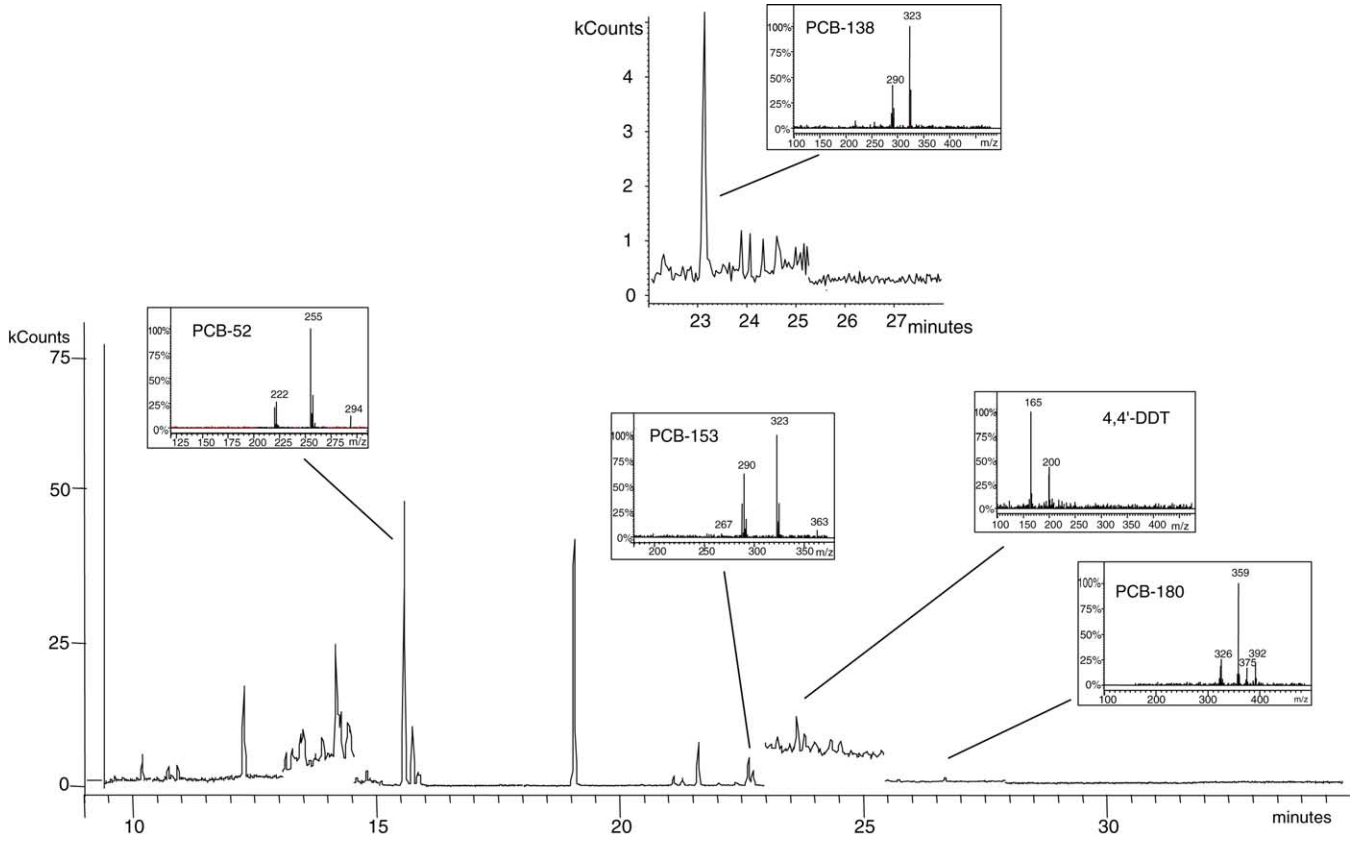

Fig. 1. GC–MS/MS chromatograms for a cockle analyzed and MS/MS spectra of the detected compounds.

chromatograms obtained after the application of the procedures developed to the analysis of a cockle sample. The spectrum of each peak obtained by MS/MS confirms the identity of the polyhalogenated compounds detected in the sample.

4. Conclusions

A method based on MSPD–GC–MS/MS for the trace analysis of fifteen polychlorinated and polybrominated compounds from aquaculture feed and cultured marine species has been developed. Aquaculture samples are difficult samples to work with because of the relatively large and varying amounts of biological substances present (minerals, lipids, acids, etc.). However, the MSPD procedure did not require additional clean-up steps because of the addition of adsorbents for interference removal on the extraction of the column to minimize the sample handling. The procedure was simple and rapid and required only small samples and volumes of solvent. Less solvent waste supports in general efforts to decrease environmental pollution. Whether to protect the human food supply or to monitor environmental contamination, all regulatory agencies involved in isolating and detecting chemical residues in fish feed and aquatic species could benefit from screening protocols with this type of extraction process.

In addition, using the method of GC–MS/MS developed for multi-residue screening in the aquaculture industry, adequate separation, confirmation, and determination of many important compounds was achieved in a relatively short time.

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