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Dispersive solid-phase extraction as a simplified clean-up technique for biological sample extracts. Determination of polybrominated diphenyl ethers by gas chromatography–tandem mass spectrometry

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ABSTRACT

Dispersive solid-phase extraction (DSPE) is proposed for the first time as a simplified, fast and low cost clean-up technique of biological sample extracts for polybrominated diphenyl ethers (PBDEs) determination. The combination of a traditional extraction technique, such as ultrasound-assisted leaching (USAL) with DSPE was successfully applied for sample preparation prior to gas chromatography–tandem mass spectrometry (GC–MS/MS) analysis. The analytes were first extracted from 1 g homogenized sample in n-hexane:dichloromethane (8:2) by applying USAL technique and further cleaned-up using DSPE with 0.20 g C_{18}-silica as sorbent material. Different solvent mixtures, sorbent type and amount, and lipid digestion procedures were evaluated in terms of clean-up and extraction efficiency. Under optimum conditions, the method detection limits (MDLs) for PBDEs, calculated as three times the signal-to-noise ratio (S/N) were within the range 9–44 pg g^{-1} wet weight. The calibration graphs were linear within the concentration range of 53–500,000 pg g^{-1}, 66–500,000 pg g^{-1}, 89–500,000 pg g^{-1} and 151–500,000 pg g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively; and the coefficient of determination (r^2) exceeded 0.9992 for all analytes. The proposed methodology was compared with a reference solid-phase extraction technique. The applicability of the methodology for the screening of PBDEs has been demonstrated by analyzing spiked and real samples of biological nature (fish, egg and chicken) with different lipid content as well as reference material (WELL-WMF-01). Recovery values ranged between 75% and 114% and the measured concentrations in certified material showed a reasonable agreement with the certified ones. BDE-47, BDE-100 and BDE-99 were quantified in three of the seven analyzed samples and the concentrations ranged between 91 and 140 pg g^{-1}. In addition, this work is the first description of PBDEs detected in fish of Argentinean environment.

1. Introduction

PBDEs are synthetic compounds used as flame retardant additives into the polymeric mass. As polymer additives, they are not chemically bound to the structure; therefore, PBDEs can leach into the environment and reach animals and humans through their food chain and dust [1,2]. PDBE's persist in the environment and bioaccumulate. Thus, there is an increasing interest in studying biota samples potentially exposed to this type of persistent pollutants [3]. With the aim of the unequivocal identification and determination of PBDEs, highly selective and sensitive analytical techniques such, as capillary gas chromatography (GC) with tandem–mass spectrometry (MS/MS) detection is required for real world applications [4,5]. Sample preparation of biological tissues samples has been recognized as the main bottleneck of the analytical process when trace analytes determination is needed [6]. The complexity of these samples requires efficient extraction, clean-up and preconcentration strategies prior to GC–MS/MS [4]. To this end, several sample preparation techniques including matrix solid-phase dispersion (MSPE) [7,8], pressurized liquid extraction (PLE)

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[9] and microwave assisted extraction (MAE) [10] have been proposed to reduce the use of large solvent volumes that conventional techniques, such as Soxhlet require. Additionally, these extraction techniques require additional clean-up procedures and solvent evaporation steps. One of the most commonly used clean-up technique is solid-phase extraction (SPE) [4]. This technique includes several steps and requires much time and organic solvent volumes than other modern techniques recently reported [11]. In addition, sometimes several packed columns with different sorbents are required to achieve optimum results [12]. To overcome these drawbacks, Anastassiades et al. proposed a rapid and simple clean-up technique for different food and environmental sample extracts (fruit, vegetables, oil, sediment, soil, etc.) named dispersive solid-phase extraction (DSPE) [12]. It is based on the addition of the sorbent material into an extract aliquot to remove the matrix interferences, which is then separated from the extract bulk by centrifugation. In this way, DSPE avoids passing the extract through a SPE column, using a much smaller quantity of sorbent and solvent, saving time and labor. DSPE was included as a novel clean-up technique for the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique. It has been successfully applied to determine several pesticides and other contaminants, such as sulfonamides, polychlorinated biphenyls (PCBs) and PBDEs in diverse type of samples, including food commodities and environmental samples [11,13–17]. The principal advantages of DSPE are its simplicity, repeatability, low cost, speed and wide applicability to different type of samples and analytes. To the best of the authors' knowledge, there is no report about the use of DSPE to clean-up extracts from biological samples containing non-polar analytes, such as PBDEs.

The aim of this work was to develop a simple, fast, inexpensive and robust methodology for the determination of PBDEs in biological samples (fish, egg and chicken) by GC–MS/MS. The proposed methodology includes double step sample preparation technique based on single-phase solvent extraction using USAL, followed by DSPE. Different solvent mixtures, sorbent type and amount, and lipid digestion procedures were evaluated in terms of clean-up and extraction efficiency. The analytical performance of DSPE-GC–MS/MS methodology was evaluated for method detection limits (MDLs), repeatability and linear working range. Validation of the methodology was carried out by analyzing spiked samples and comparing the results with those obtained using a reference SPE clean-up technique. Finally, the optimized methodology was applied for the analysis of different types of biological samples in order to establish the robustness of DSPE-GC–MS/MS for the determination of PBDEs in samples of biological, environmental and food safety interest.

2. Experimental

2.1. Reagents

The standards of polybrominated diphenyl ethers were purchased from Accustandard (New Haven, CT, USA) at 50 mg L^{-1} in isooctane and consisted of: 2,2′,4,4′- tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). Decachloro biphenyl (PCB-209) was used as internal standard (IS), and was purchased from Chem-Lab (Zedelgem, Belgium). The PBDEs standards were stored in the dark at −20 °C. Stock solutions of PBDEs and IS were prepared in methanol at concentration levels of 1 mg L^{-1}. Further dilutions were prepared monthly in methanol and stored in brown bottles at −20 °C.

A Reference material of fish (WELL-WMF-01) with certified concentrations of five PBDEs was obtained from Wellington Laboratories (Ontario, Canada).

Methanol, acetone, n-hexane and sulfuric acid were purchased from Merck (Darmstadt, Germany). Dichloromethane was purchased from Mallinckrodt Baker (Inc. Pillispsburg, NJ, PH, USA). Sorbents (40 μm particle size) for DSPE included neutral silica gel, alumina and florisil purchased from Sigma–Aldrich (Steinheim, Germany), primary secondary amine (PSA) and C_{18}-silica both obtained from Varian (Harbor City, CA, USA). Acidic silica (44% sulfuric acid) was prepared by mixing neutral silica with concentrated sulfuric acid. Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were analytical grade or above.

2.2. Equipment and working conditions

GC–MS/MS analyses were performed on a Varian 3900 gas chromatograph equipped with Varian Saturn 2000 ion trap mass detector (Varian, Walnut Creek, CA, USA). The system was operated by Saturn GC–MS WorkStation v6.4.1 software. The GC column used was VF-5ms (25m × 0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA, USA). The oven temperature program was: 150 °C, held 1 min; ramped 15 °C min^{-1} to 250 °C; ramped 20 °C min^{-1} to a final temperature of 300 °C and held for 10 min. Helium (purity 99,999%) was used as a carrier gas at 1.0 mL min^{-1} flow rate. The injector temperature was set at 300 °C and the injections were performed in the splitless mode. The mass spectrometer was operated in electron impact ionization mode at −70 eV. The trap, manifold and transfer line temperatures were set at 220 °C, 120 °C and 280 °C, respectively. Samples were analyzed in MS/MS mode. Specific MS/MS conditions for each analyte were the same as described in our previous works [11,18]. The peak identification was based on the base peak and the isotopic pattern of the PBDEs congeners. Peak identification and quantification were performed against PCB-209 internal standard.

A 40 kHz and 600 W US-bath with temperature control (Test Lab, Buenos Aires, Argentina) was used for assisting the ultrasound extraction process. Injections into the GC–MS were made using a 5.0-μL Hamilton syringe (Reno, NV, USA).

2.3. Sampling and sample preparation

The studied fish samples were: boga *(Leporinus affinis),* patí *(Luciopimelodus pati),* surubí *(Pseudoplatistoma coruscans)* and moncholo *(Pimelodus albicans).* These species are the most frequently found in *Paraná River*. They were purchased from a *Paraná River's* fisherman, Santa Fe, Argentina. Salmon (*Oncorhynchus tshawytscha*), chicken breast muscle (*Gallus gallus*) and eggs samples were purchased from a local supermarket in Mendoza city, Mendoza, Argentina. The studied samples show a wide range of lipid content. These samples were selected for studying since are included into the regular diet of Argentineans. Before extraction, fish samples were washed and the non-edible parts were removed to obtain clean tissues. The muscle tissue was triturated, homogenized and stored in glass vials in dark at 4 °C before analysis. For method optimization and recovery experiments samples were then spiked with the target PBDEs using methanolic solutions and homogenized as described by Martínez et al. [19]. The fish sample used for method development and optimization was moncholo. It was previously analyzed for the compounds of interest using a reference Soxhlet extraction technique; and none of the studied analytes were detected. The lipid content was determined gravimetrically and percentages of extracted lipids in the tested samples were: 7.8, 6.7, 3.9, 9.2, 8.7, 2.5 and 11.8% for boga, patí, surubí, moncholo, salmon, chicken and egg, respectively.

2.4. USAL–DSPE procedure

USAL: 1 g of homogenized sample was thoroughly dried with 4 g sodium sulfate in a glass mortar to become a fine powder. The powder was placed into a 15 mL glass-centrifuge tube, and 8 mL n-hexane:dichloromethane (8:2) aliquot was added. The mixture was vortexed 10 s, sonicated during 30 min and centrifuged at 3500 rpm (1852.2 g) for 5 min afterwards for separating the supernatant. Then, 5 mL aliquot solvent extract was transferred into a 10 mL clean tube and evaporated to dryness under a gentle stream of nitrogen.

DSPE: Dry extract resulting from USAL step was reconstituted into 500 μL n-hexane containing the IS and 0.20 g C_{18}-silica were added. The tube was then vortexed for 30 s and centrifuged at 3500 rpm (1852.2 g) for 5 min. 1 μL aliquot of the resulting clean extract was injected into GC–MS/MS for analysis.

2.5. USAL-SPE procedure

USAL procedure was the same described above. SPE clean-up was carried out following the procedure described by Covaci et al. [20]. Dry extract resulting from USAL step was reconstituted into 500 μL n-hexane and cleaned-up using a SPE column (7 × 1 cm ID) packed with 4 g acidified silica. PBDEs were eluted with 8 mL n-hexane. The eluent was evaporated to dryness under a gentle stream of nitrogen and the dry extract was reconstituted into 500 μL n-hexane containing the IS prior to the GC–MS/MS analysis.

3. Results and discussion

The fractionation of the target analytes between the extraction phase and lipids remaining after extraction step is the major problem for determining trace levels of organic compounds in biological samples. Lipids and other matrix interferences deteriorate the sensitivity of the instrumental technique by increasing the signal background; therefore, an efficient clean-up step is necessary to overcome this analytical inconvenient. In this sense, several critical variables were considered in the extraction and DSPE clean-up study, including different solvent mixtures, sorbent types and amount, as well as a lipid digestion clean-up. The study and optimization of the above mentioned variables was carried out by modifying one at a time while keeping the remaining constant. Each assay was done by triplicate. The variables optimization was performed by extracting 1 g homogenized dried sample as described in Section 2.4 containing 20 ng g^{-1} of each PBDE with the corresponding solvent and volume of each assay; and cleaning-up the reconstituted extract (500 μL n-hexane with IS) by DSPE with the particular sorbent according to the experiment.

3.1. Optimization of extraction procedure

The original QuEChERS technique is characterized by a single-phase solvent extraction using polar organic solvents and phase separation after salting out and centrifuging the mixture [12]. The principal advantage of the novel procedure is its simplicity and speed. QuEChERS has been applied for extracting analytes with log K_{ow} lower than the log K_{ow} of the studied PBDEs congener (6.81–7.90) [21]. Additionally, these PBDEs have a tendency to be very strongly bound to the sample matrix by interaction with non-polar lipids (triglycerides) [8]. Therefore, it is necessary to count on an extraction technique to disrupt the matrix and efficiently extract the analytes. In this sense, it was interesting to study and compare different extraction techniques including US radiation, vortex and manual shaking. The studies were carried out by extracting 1 g homogenized dried sample, as described in Section 2.4, containing 20 ng g^{-1} of each PBDE with 10 mL n-hexane over 10 min and cleaning-up the reconstituted extract (500 μL n-hexane with IS) by DSPE (0.25 g C_{18}-silica). The results showed that US radiation gives higher relative responses than the others stirring-up techniques. Therefore, USAL was selected as extraction technique for further studies. Additionally, it is interesting to point out that USAL has been already reported for extraction of PCBs and PBDEs from liver and sediments samples, respectively [11,22].

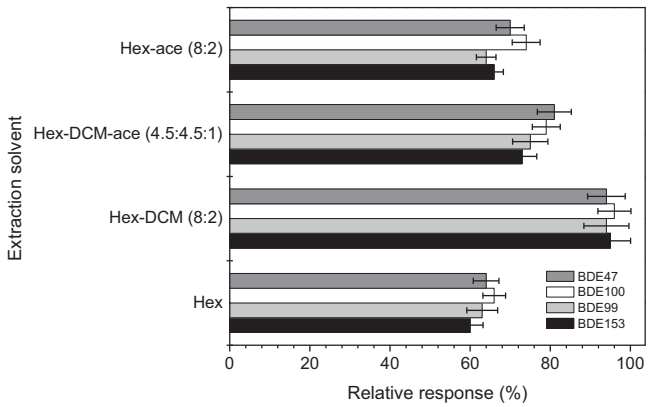

Fig. 1. Extraction solvent effect on the relative response of PBDEs. Extraction conditions: sample mass, 1 g; PBDEs concentration, 20 ng g^{-1}; extraction-solvent, 10 mL; extraction time, 30 min; DSPE, 0.25 g C_{18}-silica and vortexing for 30 s. $n = 3$.

The extraction-solvent is also a critical variable for developing an efficient USAL technique. In this sense, several solvents and solvent mixtures including n-hexane, n-hexane–dichloromethane (8:2), n-hexane–dichloromethane–acetone (4.5:4.5:1) and n-hexane–acetone (8:2) was evaluated in terms of the relative response of the target PBDEs. These solvents and solvents mixtures were selected based on their physicochemical properties and previously reported applications for extraction of non-polar compounds from similar matrices [4,8,9,22]. As can be seen from Fig. 1, n-hexane–dichloromethane (8:2) showed the best results. Although PBDEs affinity for the low polarity solvents increase as the solvent polarity decrease; the solvent efficiency for penetrating into the tissue is lower as its polarity decrease [8]. Therefore, n-hexane was used in combination with dichloromethane to enhance the extraction efficiencies of PBDEs from biological samples [23]. Hexane–acetone (8:2) showed better results than n-hexane alone, but their relative response were lower than n-hexane–dichloromethane (8:2). Additionally, this solvent mixtures reported higher amounts of extracted lipids, which might affect the clean-up and instrumental techniques. This phenomenon could be due to the acetone content of the solvent solution. Acetone is able to denaturalize the protein structure leading to higher polar lipids extraction (phospholipids and cholesterol) [8]. On the other hand, dichloromethane extracts less polar lipids; therefore underestimate the lipids content of the sample. Taking into account these results, n-hexane–dichloromethane (8:2) was selected as extraction solvent for further studies.

The volume of extraction-solvent solution was found important to optimize in order to obtain the highest extraction efficiency and avoid diluting the target analytes using excessive solvent volume. The extraction-solvent volume was studied within a volume range of 5–16 mL. The highest relative responses were obtained for 8 mL solvent solution. Smaller extraction-solvent volume was insufficient to quantitatively extract the target PBDEs. By increasing the solvent solution volume between 8 and 16 mL, relative responses for the analytes remained invariant. However, as the extraction-solvent volume increased higher extract-phase volume was obtained and, thus higher evaporation time was required. Therefore, 8 mL n-hexane–dichloromethane (8:2) was selected to carry out further assays.

The optimization of ultrasound radiation time is also crucial to achieve an efficient USAL procedure. As the extraction-time increased, the bringing of fresh solvent to the surface particles enhances the concentration gradient of the analytes increasing the mass transfer between sample and solvent [11,24]. The US extraction time was defined as the period over which US is continuously applied; and it was varied within the range 0–60 min. It was observed that by increasing the extraction time, the relative responses increased, reaching the maximum value at 30 min, after which remained invariant. Therefore, 30 min was selected as US radiation time for the extraction step to develop further studies.

Since PBDEs are present at trace levels in the studied biological samples USAL extract was evaporated to dryness and reconstituted in 500 μL of n-hexane with IS prior to DSPE stage. Using this evaporation step for concentrating the total extract, the sensitivity was increased without significantly affecting the chromatograms background and the overall time procedure.

3.2. Optimization of clean-up procedure

When an extraction procedure is carry out to extract the target PBDEs from biological samples, many interferences are co-extracted and this fact can affect the determination of the analytes. Although mass spectrometry is a selective detector; the analysis of this type of samples requires an efficient clean-up step in order to reduce the chromatogram background and thus, enhance the methodology sensitivity. Drummond and Watson proposed the use of concentrated sulfuric acid for cleaning-up lipid content of biological extracts prior analytes determination [25]. Therefore, it was of interest to develop and compare an alternative clean-up technique to effectively remove the lipid content from the sample. DSPE was chosen as an alternative clean-up technique. The results achieved without clean-up were compared with two conventional clean-up techniques and the combination of one of them with DSPE. The conventional clean-up techniques were SPE column using the same sorbent material that was tried on DSPE and sulfuric acid digestion. The combination of sulfuric acid digestion and DSPE was the third technique.

For the development of the DSPE technique, different solid sorbents, including florisil, activated silica gel, C_{18}-silica, PSA and neutral alumina were evaluated for the analytes relative responses. The USAL-DSPE procedure was as follows: 5 mL aliquot USAL extract was evaporated to dryness, reconstituted with 500 μL n-hexane with IS, and further cleaned-up with 0.25 g of the selected DSPE sorbent. After sorbent addition, the tube was vortexed and centrifuged. A 1 μL aliquot of the cleaned n-hexane extract was further analyzed by GC–MS/MS. For the clean-up with sulfuric acid, 1 mL of concentrated sulfuric acid (44% w/w) was added to 500 μL reconstituted USAL n-hexane extract with IS. The mixture was shaken for 1 min and centrifuged at 3500 rpm (1852.2 g) for 5 min. A 1 μL aliquot of the cleaned n-hexane extract was analyzed by GC–MS/MS. For the combination of techniques, sulfuric acid and DSPE, 500 μL reconstituted USAL n-hexane extract was cleaned-up by adding 1 mL of concentrated sulfuric acid. The n-hexane phase was further mixed-up with 0.20 g C_{18}-silica and centrifuged as described above, prior to PBDEs determination. The effectiveness of each clean-up technique was evaluated in terms of relative response of the target analytes and background level of the chromatograms. These results were compared against those achieved USAL extract without clean-up. Generally speaking, the achieved results showed that PBDEs' relative responses were higher and chromatograms background lower by applying any of the mentioned clean-up techniques compared to untreated extracts. The study of DSPE sorbents (Fig. 2) shows that all sorbents led to lower chromatographic background and higher relative responses (ca. 20–65%, depending on the sorbent) compared to

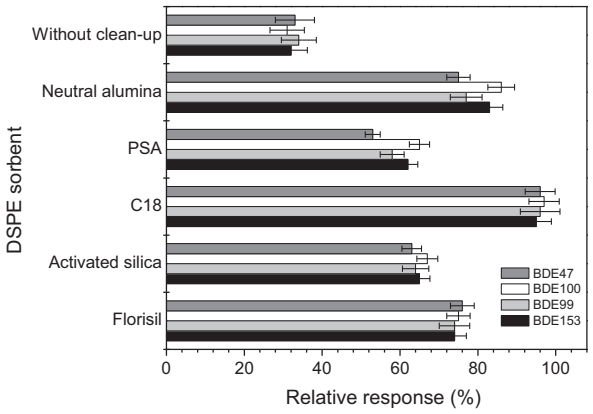

Fig. 2. DSPE sorbent effect on the relative responses of PBDEs. Extraction conditions: sample mass, 1 g; PBDEs concentration, 20 ng g^{-1}; extraction-solvent, 8 mL n-hexane–dichloromethane (8:2); extraction time, 30 min; DSPE, 0.25 g sorbent. $n = 3$.

USAL extract without clean-up. The results showed that higher responses, cleaner chromatograms and mass spectra of the target PBDEs were obtained using C_{18}-silica as DSPE sorbent. These outcomes are due to the fact that the extracted interferences, such as fatty acids, triglycerides, phospholipids and cholesterol, have higher affinity for C_{18}-silica sorbent than for the other DSPE studied sorbents. Therefore, they can be more efficiently removed from the reconstituted extract leading, thus to cleaner chromatograms and mass spectra of the target PBDEs. The observed results were in agreement with those previously reported applications of DSPE in biological samples [13,26–29]. The DSPE sorbent amount was studied within a mass range of 0.05–0.50 g. The procedure was the same as described above. It was observed that by increasing the sorbent amount from 0.05 to 0.20 g, the relative responses increased achieving a maximum at 0.20 g C_{18}-silica. In view of the mentioned results, 0.20 g C_{18}-silica was selected for further studies. By comparing SPE column and DSPE results, DSPE lead to higher relative responses of the analytes (ca. 22–31%) and lower background signals than SPE. Additionally, use of a higher amount of SPE sorbent was also studied and compared with DSPE clean-up (0.20 g C_{18}-silica). Results for 1 g C_{18}-silica SPE column were comparable with those using 0.20 g C_{18}-silica DSPE clean-up; a higher amount did not show significant improvement. These results are expected since in DSPE all sorbent particles interacts equally with the matrix leading to larger sorbent capacity per gram of sorbent [12]. By comparing sulfuric acid digestion against sulfuric acid digestion combined with DSPE, higher relative responses (ca. 38%) and lower background were observed with sulfuric acid digestion combined with DSPE clean-up. The PBDEs analytical responses for sulfuric acid digestion combined with DSPE and DSPE clean-up were comparables. Fig. 3 shows the chromatograms resulting from the four clean-up techniques. DSPE clean-up lead to cleaner chromatograms than sulfuric acid clean-up followed by DSPE. Thus, a better sensitivity is obtained by reducing the chromatograms background and; thus increasing S/N ratio of analytes. DSPE was selected as the clean-up technique to be used because of practical convenience and it better results than others clean-up techniques and their combinations.

3.3. Analytical performance, method validation and comparison with other previously reported methodologies

The extraction efficiencies were established by carrying out successive extractions over the same sample. After performing the first extraction, the upper solvent phase was taken out and the sample was extracted again. Both extract were analyzed separately. The results showed that the analytical responses in

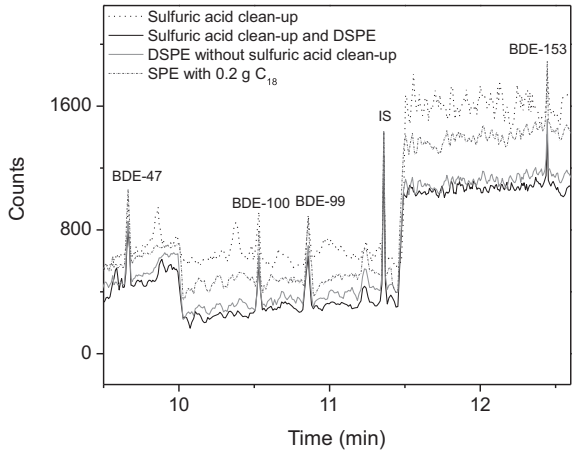

Fig. 3. Effect of different clean-up techniques on the chromatograms background signal (total ion chromatograms for each clean-up). Extraction conditions as Fig. 2. Specific conditions for each assay are explained in the text.

Table 1
DSPE-GC–MS/MS analytical performance for PBDEs determination.

Analyte	RSD%[a,b]	MDL[a,b] (pg g^{-1})	Linear range[a] (pg g^{-1})
BDE-47	8.7	9	53–500,000
BDE-100	10.5	17	66–500,000
BDE-99	9.3	24	89–500,000
BDE-153	10.9	44	151–500,000

Extraction conditions as described in Section 2.4.

[a] 95% confidence interval; $n = 5$

[b] PBDEs concentration for MDLs determination: 65, 75, 100 and 200 pg g^{-1} of BDE 47, 100, 99 and 153 respectively, wet weight.

the second extract were lower than 10%, showing an exhaustive extraction for the proposed technique. The calibration curve was made under optimized conditions using a moncholo sample free of PBDEs spiked at different concentration of target PBDEs prior to extraction. In order to evaluate the matrix effect on the analytical signals, the slopes of the calibration graph of matrix-matched standards and solvent standards were compared. It was observed that the sensitivity decreased for matrix-matched calibration curves. Therefore, quantification was carried out using matrix-matched standards with increased concentrations of target PBDEs. The analytical figures of merits are summarized in Table 1. The MDL of the analytes for extraction of 1 g moncholo sample, calculated as three times the signal-to-noise ratio (S/N = 3), were 9 pg g^{-1}, 17 pg g^{-1}, 24 pg g^{-1} and 44 pg g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively. The precision was evaluated over five replicates resulting RSDs values ≤10.9%. The calibration curves showed a satisfactory linearity within the concentration range: 53–500,000 pg g^{-1}, 66–500,000 pg g^{-1}, 89–500,000 pg g^{-1} and 151–500,000 pg g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively; and the coefficient of correlation (r^2) exceeded 0.9992 for all analytes. The validation of the proposed methodology was carried out by comparison with a previously reported SPE technique [20] (Section 2.4) and by a recovery study over samples with different lipid contents. For comparison with SPE, spiked (200, 250, 400 and 750 pg g^{-1} of BDE-47, BDE-100, BDE-99 and BDE-153, respectively) and none-spiked salmon sample were analyzed using USAL–DSPE–GC–MS/MS and USAL–SPE–GC–MS/MS methodologies (Table 2). Using a two-sample *t*-test at 95% confidence level, it can be concluded that there are no significant differences between recoveries obtained with both techniques ($P > 0.05$). For recoveries experiments, chicken, egg and different fish's species were analyzed separately in triplicate for PBDEs using the proposed DSPE–GC–MS/MS methodology. The recovery study led to a satisfactory robustness achieving recoveries between 75 and 114% (Tables 2 and 3). By analyzing the results showed in Tables 2 and 3 for the analysis of seven different samples with diverse matrices (lipid percentage between 2.5% and 11.8%) it could be observed that there is not a significant difference among the obtained recoveries. Thus, it is possible to conclude that the proposed methodology show a satisfactory robustness for the type of analyzed samples and it could be expected a similar behavior for other samples with similar complexity. The analysis of procedural blanks, corresponding to moncholo sample, demonstrated the absence of contamination problems during sample preparation.

Table 2
Determination of PBDEs in salmon sample using DSPE and SPE followed by GC–MS/MS.

Analyte	DSPE		SPE	
	Base level[a] (ρg g^{-1})	Recovery[b] (%)	Base level[a] (ρg g^{-1})	Recovery[b] (%)
BDE-47	98 ± 12	85 ± 9	95 ± 19	81 ± 11
DE-100	nq	79 ± 6	nq	76 ± 8
BDE-99	93 ± 16	84 ± 8	91 ± 22	83 ± 10
BDE-153	nd	77 ± 8	nd	74 ± 11

Spike level: 200, 250, 400 and 750 pg g^{-1} of BDE-47, BDE-100, BDE-99 and BDE-153, respectively; Extraction conditions for DSPE and SPE as described in Section 2.4 and 2.5.nq: under quantification limit, nd: under detection limit.

[a] Results expressed as $\bar{x} \pm (t \cdot SD)/\sqrt{n}$; $n = 3$; 95% confidence interval; pg g^{-1}.

[b] [(Found − base)/added] × 100.

The advantages of DSPE over conventional SPE clean-up are multifold. DSPE uses smaller quantities of sorbents and solvents. Furthermore, is simple and use inexpensive equipment. Therefore, this technique is beneficial for many laboratories. Additionally, the analytical performance for USAL–DSPE is comparable with other methodologies previously reported for PBDEs determination in fish and biota samples such as MSPD–GC–ECD, PLE-GC–MS/MS and MAE-GC–MS [7,9,10]. DSPE is similar to MSPD; however, the sorbent is added to an aliquot or to the concentrated extract rather than to the original sample as in MSPD. Considering the high cost of the sorbents, the sample size that can be used in MSPD is lim-

Table 3
Concentrations and recovery results of PBDEs in different samples analyzed by DSPE-GC–MS/MS.

Analyte	Boga		Patí		Surubí		Moncholo		Chicken		Egg	
	Base level[a] (ρg g−1)	$R^{a,b}$ (%)	Base level[a] (ρg g^{-1})	$R^{a,b}$ (%)	Base level[a] (ρg g^{-1})	$R^{a,b}$ (%)	Base level[a] (ρg g^{-1})	$R^{a,b}$ (%)	Base level[a] (ρg g^{-1})	$R^{a,b}$ (%)	Base level[a] (ρg g^{-1})	$R^{a,b}$ (%)
BDE-47	nq	84	127 ± 21	107	140 ± 25	106	nd	89	nd	97	nd	79
BDE-100	nd	89	102 ± 20	111	123 ± 24	104	nd	83	nd	104	nd	77
BDE-99	nd	81	118 ± 23	114	nd	95	nd	79	nd	94	nd	76
BDE-153	nd	78	nd	98	nd	89	nd	78	nd	91	nd	75

Spiked concentrations: 200, 250, 400 and 750 pg g^{-1} of BDE-47, BDE-100, BDE-99 and BDE-153, respectively; extraction conditions as described in Section 2.4. nq: under quantification limit.

[a] Results expressed as $\bar{x} \pm (t \cdot SD)/\sqrt{n}$; $n = 3$; 95% confidence interval; pg g^{-1}. nd: under detection limit.

[b] [(Found − base)/added] × 100.

Table 4
Concentrations of PBDEs (ng g^{-1}) in reference material WELL-WMF-01 (freeze-dried fish tissue), $n = 3$ replicates.

Analyte	Certified (conc ± S.D.)	Measured (conc. ± S.D.)
BDE-47	123.2 ± 24.8	87.4 ± 11.8
BDE-100	35.9 ± 14.5	32.2 ± 5.4
BDE-99	37.5 ± 4.2	38.5 ± 5.1
BDE-153	17.0 ± 8.0	16.5 ± 2.3

ited [12]. In the case of PLE and MAE, additional SPE clean-up and solvent evaporation steps are necessary. This fact increase costs, organic solvent wastes and analyst training or attention, reducing the sample throughput of these techniques. DSPE consume smaller amount of sorbent and solvents, furthermore employs simple and inexpensive equipment, making possible their application in the most of the analytical laboratories.

3.4. Analysis of reference material

The accuracy of the proposed methodology was evaluated by analyzing a freeze-dried naturally contaminated fish tissue, with certified concentrations of the studied PBDEs (BDE-47, BDE-100, BDE-99 and BDE-153). Table 4 shows the certified most probable values and 95% confidence intervals for the concentrations of analytes in the reference material, together with corresponding data obtained from three replicate analyses USAL–DSPE–GC–MS/MS. As can be observed, the mean concentrations obtained using the proposed methodology were within the certified 95% confidence intervals for all studied analytes demonstrating the accuracy of DSPE–GC–MS/MS for the determination of studied PBDEs.

3.5. Application to real samples

As can be seen from Tables 2 and 3; surubí, patí, boga and salmon reported detectable levels of PBDEs. The concentration ranged from 91 to 140 pg g^{-1}; however some of the samples reported concentrations between LODs and LOQs. The PBDEs congeners detected were BDE-47, BDE-100 and BDE-99; BDE-153 was not detected in the analyzed samples. Mocholo, chicken and egg samples did not report detectable concentration of studied PBDEs. It could because PBDEs were below the detection limit of the proposed methodology or the analytes were not present in the analyzed samples. The determined concentrations in fish were lower than the reported in fish tissue from mid-continental great rivers of the United States [30]. The PBDEs concentrations determined in salmon samples in the present work were comparable to those reported in Chilean salmon [31] and lower than European salmon [32]. The salmon purchased for the present work was imported from Chile. Hites et al. recently reported that Chilean salmons are lower contaminated with PBDEs than European salmons [32]. Fig. 4a shows the chromatograms of a patí sample spiked with 2 ng g^{-1} PCB 209 and Fig. 4b shows the chromatogram of the same sample spiked with 2 ng g^{-1} PCB 209 and 0.2 ng g^{-1} of target PBDEs.

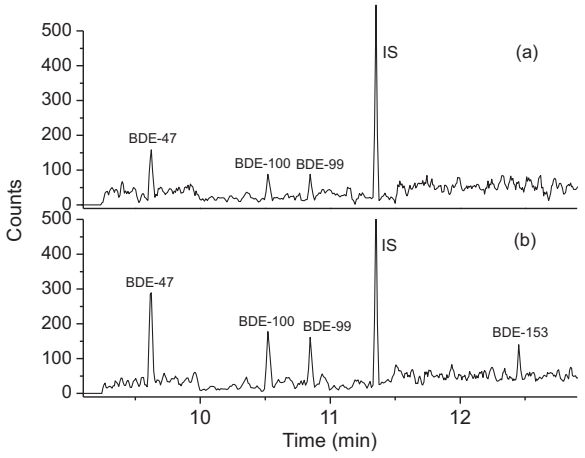

Fig. 4. Analysis of patí sample using DSPE–GC–MS/MS. EIC for 324, 326, 328, 402, 404, 406, 426, 428, 430, 482, 484, 486 and 496 m/z. (a) Patí sample spiked at 2 ng g^{-1} of PCB 209 (b) Patí sample spiked at 2 ng g^{-1} of PCB 209 and 0.2 ng g^{-1} of each PBDE.

4. Conclusions

A low cost, simple and robust extraction and clean-up technique has been proposed as a convenient alternative for sample preparation for determining PBDEs at trace levels in biological samples by GC–MS/MS. The application of DSPE as a novel clean-up for PBDEs determination gave comparable results to those obtained using a reference SPE clean-up technique. However, the proposed technique saves time and requires lower volumes of solvents than SPE, reducing costs and waste. The analysis of reference material showed that the measured concentrations had a reasonable agreement with the certified ones, assessing the accuracy of the proposed methodology. In addition, this work is the first description of PBDEs detected in fish of Argentinean environment; surubí, patí and boga are fishes from *Paraná River*, a mid-continental river and to date there are not evidence about any study of these fishes over Latin-American environment. With the growing needs in identifying emerging toxic chemicals, the results of this work are valuable with the aim to establish probably exposure route to this type of contaminants in development countries.

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