NOVEL CLEAN-UP TECHNIQUE BASED ON DISPERSIVE SOLID-PHASE EXTRACTION FOR THE DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS IN BIOLOGICAL SAMPLES

Fontana AR[1], Martinez LD[2,3], Altamirano, JC[1,4]

[1]Grupo de Investigación y Desarrollo en Química Analítica (QUIANID) (LISAMEN, CCT CONICET – Mendoza), Av. Ruiz Leal S/N, 5500 Mendoza, Argentina; [2]Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Argentina; [3]INQUISAL-CONICET, Departamento de Química Analítica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Argentina; [d]Instituto de Ciencias Básicas, Universidad Nacional de Cuyo, Argentina.

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]. Thus, there is an increasing interest in studying biota samples potentially exposed to this type of persistent organic pollutants. Sample preparation of biological tissues samples has been recognized as the main bottleneck of the analytical process when trace analytes determination is carried out [3]. The complexity of these samples requires efficient extraction, clean-up and preconcentration strategies prior to GC–MS/MS analysis [4]. Recently, Anastassiades et al. proposed a rapid and simple technique for cleaning-up different food and environmental sample extracts named dispersive solid-phase extraction (DSPE) [5]. It is based on the addition of the sorbent material into an extract aliquot, which is then separated from the extract bulk by centrifugation. In this way, DSPE avoids using SPE column and requires a much smaller quantity of sorbent and solvent, saving time and labor. DSPE was included as a novel clean-up for the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique. The principal advantages of DSPE are its simplicity, repeatability, low cost, speed and wide applicability to different type of samples and analytes.

The aim of this work was to develop a simple**,** fast, inexpensive and robust methodology for the determination of PBDEs in biological samples by GC–MS/MS. The proposed technique includes double step sample preparation technique based on single-phase solvent extraction by using ultrasound assisted leaching (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 in terms of method detection limits (MDLs), repeatability and linear working range for each studied analyte. Validation of the methodology was carried out 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). Finally, the optimized methodology was applied for the analysis of different types of biological samples of biological, environmental and food safety interest.

Materials and methods

The standards of polybrominated diphenyl ethers were purchased from Accustandard at 50 mg L^{-1} in isooctane. PCB-209 was used as internal standard (IS), and was purchased from Chem-Lab. A Reference material of fish (WELL-WMF-01) was obtained from Wellington Laboratories. Sorbents (40 µm particle size) for DSPE included neutral silica gel, alumina and florisil were purchased from Sigma–Aldrich, primary secondary amine (PSA) and C_{18}-silica both obtained from Varian. All reagents were analytical grade or above.

GC–MS/MS analyses were performed on a Varian 3900 gas chromatograph equipped with Varian Saturn 2000 ion trap mass detector. The chromatographic and MS/MS conditions for each analyte were the same as described in our previous work [6]. The ions used for identification and quatification of analytes were: 324, 326 and 328 for BDE-47, 402,404 and 406 for BDE-100 and BDE-99; and 482, 484 and 486 for BDE-153.

A 40 kHz and 600W US-bath with temperature control was used for assisting the ultrasound extraction process.

The studied fish samples were: boga *(Leporinus affinis),* patí *(Luciopimelodus pati),* surubí *(Pseudoplatistoma coruscans)* and moncholo *(Pimelodus albicans).* Salmon (*Oncorhynchustshawytscha*), chicken breast muscle (*Gallus gallus*) and eggs samples were purchased from a local supermarket in Mendoza city, Mendoza,

Argentina. The lipid content was determined gravimetrically and percentages of extracted lipids 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 samples, respectively.

USAL-DSPE optimized procedure

USAL: 1 g of homogenized sample was thoroughly dried with 4 g sodium sulphate 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 (1852.2g) 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. A 1 µL aliquot of the resulting clean extract was injected into GC–MS/MS for analysis.

Results and discussion:

Optimization of extraction procedure

Different 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. As can be seen from Figure 1, n-hexane-dichloromethane (8:2) showed the best results. The volume of extraction solvent was studied within a volume range of 5–16 mL. The highest relative responses were obtained for 8 mL solvent mixture.

The optimization of ultrasound radiation time was carried out by varying within the range 0 to 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.

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.

Optimization of clean-up procedure

The extraction of concomitant from biological samples, particularly lipids, could interfere the determination of target analytes. Therefore, it is necessary to remove them by using use an efficient clean-up technique. In this way, it was of interest to develop an alternative clean-up technique based on the advantages of DSPE. 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. The study of DSPE sorbents (*Figure 1*) showed that all sorbents led to lower chromatographic background and higher relative responses of PBDEs (ca. 20-65 %, depending on the sorbent) compared to USAL extract without clean-up. Higher responses, cleaner chromatograms and mass spectra of the analytes were obtained by using 0.20 g C_{18}-silica as DSPE sorbent.

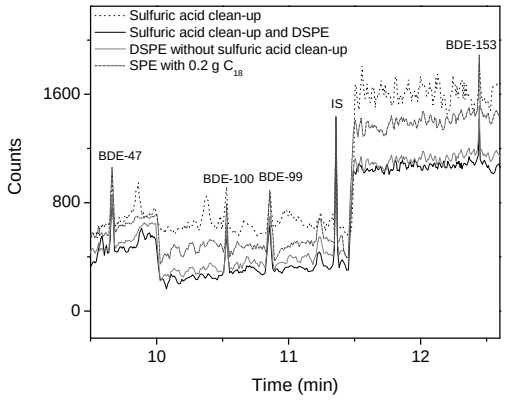

Figure 1: Effect of different clean-up techniques on the chromatograms background signal (total ion chromatograms for each clean-up).

By comparing SPE column (packed with 0.20 g C_{18}-silica) and DSPE results, DSPE lead to higher relative responses of the analytes (ca. 22 - 31 %) and lower background signals than SPE. By comparing sulfuric acid digestion against sulfuric acid digestion combined with DSPE, higher relative responses (ca. 38 %) and lower background were observed for the last approach. The PBDEs analytical responses for sulfuric acid digestion

combined with DSPE and DSPE clean-up were comparables. In this sense, DSPE was selected as the clean-up technique because it showed better results than others clean-up techniques and their studied combinations.

Analytical performance

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 by using matrix-matched standards with increased concentrations of target PBDEs. The analytical figures of merits were summarized in Table 1.

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	53-500000
BDE-100	10.5	17	66-500000
BDE-99	9.3	24	89-500000
BDE-153	10.9	44	151-500000

[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.

Method validation

Validation of the methodology was carried out by analyzing spiked and real samples of biological nature (fish, egg and chicken; lipid percentage between 2.5 to 11.8 %) as well as reference material. The recovery study led to satisfactory results achieving recoveries between 75–114 %. For the reference material 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.

Table 2
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

In the case of analyzed samples, surubí, patí, boga and salmon reported detectable levels of PBDEs ranged from 91-140 pg g^{-1}. The PBDEs congeners detected were BDE-47, BDE-100 and BDE-99. The determined concentrations in fish were lower than the reported in fish tissue from mid-continental great rivers of the United States [7]. The PBDEs concentrations determined in salmon samples were comparable to those reported in Chilean salmon [8] and lower than European salmon [9].

Conclusions

The analytical performance demonstrated appropriated sensitivity, accuracy and precision suitable for determining PBDEs in biological samples by GC–MS/MS. Additionally, the proposed extraction and clean-up technique is a convenient alternative for sample preparation due to its low cost, simplicity and versatility. The analysis of reference material showed reasonable results, assessing the accuracy of the proposed methodology. On the other hand, this work is the first description of PBDEs detected in fish of Argentinean environment. The studied fish species (surubí, patí and boga) are from *Paraná River*, a mid-continental river. Due to the relevance of the results, this work is valuable with the aim to establish probably exposure route to PBDEs in development countries.

Acknowledgements:
This work was supported by CONICET; FONCYT PICT-BID, UNCuyo and UNSL, Argentina; and Eramus Mundus, Italy. Authors also thank to Dr. I.R.P. for his assistance with the reference material.

References:

[1] C.A. de Wit (2002); Chemosphere 46(5): 583.
[2] Y. Wang, G. Jiang, P.K.S. Lam, A. Li (2007); Environ. Int. 33(7): 963.
[3] C. Nerín, J. Salafranca, M. Aznar, R. Batlle (2009); Anal. Bioanal. Chem. 393(3): 809.
[4] A. Covaci, S. Voorspoels, L. Ramos, H. Neels, R. Blust (2007); J. Chromatogr. A 1153(1-2): 145.
[5] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck (2003); J. AOAC Int. 86(2): 412.
[6] A.R. Fontana, N.B. Lana, L.D. Martinez, J.C. Altamirano (2010); Talanta 82(1): 359.

[7] K.A. Blocksom, D.M. Walters, T.M. Jicha, J.M. Lazorchak, T.R. Angradi, D.W. Bolgrien (2010); Sci. Total Environ. 408(5): 1180.
[8] M. Montory, E. Habit, P. Fernandez, J.O. Grimalt, R. Barra (2010); Chemosphere 78(10): 1193.
[9] R.A. Hites, J.A. Foran, S.J. Schwager, B.A. Knuth, M.C. Hamilton, D.O. Carpenter (2004); Environ. Sci. Technol. 38(19): 4945.