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Ultrasound leaching–dispersive liquid–liquid microextraction based on solidification of floating organic droplet for determination of polybrominated diphenyl ethers in sediment samples by gas chromatography–tandem mass spectrometry

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ABSTRACT

Ultrasound leaching–dispersive liquid–liquid microextraction using solidification of floating organic droplet (USL–DLLME-SFO) technique is proposed for extraction and isolation of polybrominated diphenyl ethers (PBDEs) from sediment and further determination by gas chromatography–tandem mass spectrometry (GC–MS/MS). Parameters that affect the efficiency of the procedure were investigated by a full factorial (2^k) screening design. Variables showing significant effects on the analytical responses were considered within a further central composite design (CCD). The optimization assays have led to following protocol: ultrasound assisted lixiviation of 1 g sediment was carried out by using 1.2 mL MeOH. Further, the analytes were isolated from 0.4 mL of the extract using the DLLME-SFO technique. The microextraction was performed using 0.1 mL MeOH, 22 mg 1-dodecanol, 1 mL NaCl solution 6.15 M and 4.4 mL ultrapure water as dispersive and extracting solvents, medium ionic strength and dispersant bulk, respectively. Under optimum conditions, the method exhibits good performance in terms of linearity and precision (RSD < 9.2%), with recoveries above 71% and limits of detection (LODs) within the range 0.5–1.8 pg g^{-1} dry weight (d.w.). Method validation was demonstrated through the analysis of environmental sediment samples in which PBDEs were detected and quantified. The presence of BDE-47, -100, -99 and -153 was reported within the concentration range of <LOD to 29 pg g^{-1} d.w. The proposed methodology constitutes a suitable approach for the analysis of PBDEs in complex solid samples requires minimum organic solvents consumption, sample manipulation, and increases sample throughput.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) were extensively used over the past two decades as brominated flame retardants (BFRs) in electronic equipment, plastics, textiles, building materials, carpets, vehicles, and aircrafts [1]. Due to their additive role in the polymers, they can be released into the environment during their usage time, as well as after final disposal of the products, including stock pile leaching, industrial waste or accidental leaking [2]. Once in the environment, they can be transported through atmosphere or water streams, deposited by wet or dry fluxes and can accumulate into the ecosystems far from their production and use sites. As a consequence, PBDEs are detected in environmental samples including water, particulate matter, sediments, sludge, and effluents [3]. For PBDEs, identification and determination in environmental

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samples, highly selective and sensitive instrumentation is required because of the matrix complexity. In this sense, several GC/MS techniques based on electron ionization (EI) and/or electron capture negative ionization (ECNI) were proposed [4]. The extraction and isolation of analytes from the solid matrices is usually carried out by using conventional Soxhlet extraction [5], solid-phase microextraction (SPME) [6] and microwave-assisted extraction (MAE) [7]. Room temperature lixiviation is another alternative for extracting PBDEs from sediment samples. It can be assisted by auxiliary energies such as ultrasonic (US) radiation in order to favor the kinetics of the mass-transfer process of the target analytes to the liquid phase [8]. This phenomenon results in an increment in the solubility of the analytes into the liquid phase and their diffusivity from the sample matrix to the outer region, which is the limiting step of mass transfer.

In the last few years, new extraction techniques, especially microextraction ones, have witnessed incessant growth. Mainly, because reduction of solvent quantities used in sample preparation is one of the leading objectives under the new paradigm of the green chemistry. Rezaee et al. developed a dispersive liquid–liquid microextraction (DLLME) in 2006 [9]. Briefly, this technique combines a nonpolar solvent (extraction solvent) with a polar one (dispersive solvent), to efficiently disperse the first within the aqueous bulk, thus improving the efficiency of the microextraction technique [10].

A solid floating organic droplet (SFO) was recently introduced for determination of analytes such as estrogens, 2-pyrazoline and fat-soluble vitamins [11–17]. The technique uses an extraction solvent less dense than water and with a melting point near or below room temperature (10–30 °C). As a consequence, the solvent can be solidified by decreasing the operating temperature, resulting in the formation of a droplet of solvent floating after centrifugation [18]. DLLME-SFO was successfully applied to the extraction of organic compound such as polychlorinated biphenyls [3] and organochlorine pesticides [3,19]. DLLME-SFO was also reported for the determination of BDE-209 in sediment samples by using HPLC–UV [20]. However, the sensitivity and selectivity of the methodology was not suitable for the analysis of PBDE congeners with low number of Br atoms. To the best of the authors' knowledge, there are no reports on the use of DLLME-SFO-GC–MS/MS after ultrasound leaching (USL) for the analysis of PBDEs congeners in sediment samples.

In this paper, the USL–DLLME-SFO technique is proposed for the extraction and isolation of PBDEs from sediment, while determination is achieved by GC–MS/MS analysis. To this aim, four PBDEs commonly found in environment samples were selected as target analytes: 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), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). The influence of several variables that condition the performance of the leaching and microextraction techniques was studied and optimized over the relative response of the PBDEs. A multivariate approach with a minimum number of experimental runs [21] was used for optimizing and understanding the effect of each variable, as well as their interactions, on the analytical response of the target PBDEs. After employing full factorial and central composite designs (CCD), a desirability function (D) was used in order to optimize the multiple response criteria based on peak areas of the analytes [21]. The analytical performance of USL–DLLME-SFO-GC–MS/MS methodology was evaluated in terms of LODs, repeatability, recovery (%), and linear working range. The procedure was applied for the determination of PBDEs in sediment samples from Potrerillos reservoir, Mendoza, Argentina.

2. Experimental

2.1. Reagents

The PBDE standards were purchased from Accustandard (New Haven, CT, USA) at 50 mg L^{-1} in iso-octane and consisted of BDE-47, BDE-99, BDE-100, and BDE-153, which were stored at −20 °C. Further dilutions were prepared weekly in methanol (MeOH) at concentration levels of 1 μg mL^{-1} and stored in brown bottles at −20 °C. BDE-77 and BDE-128 (200 ng mL^{-1}) were used as internal standards (IS). Acetone, acetonitrile (ACN), MeOH, 1-undecanol, 1-dodecanol, 1,10-dichlorodecane, iso-octane, and sodium chloride (NaCl) were purchased from Merck (Darmstadt, Germany). A NaCl aqueous solution (6.15 M) was prepared by dissolving 3.6 g NaCl in 10 mL of ultrapure water. Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). Conical centrifuge tubes (10 mL), vials (1500 μL) and conical inserts (300 μL) were purchased from Fisher (Scientific, Pittsburg, USA). All reagents were of analytical grade or above.

2.2. Instrumentation, working conditions and software

GC–MS/MS analyses were carried out on a Varian 3900 gas chromatograph equipped with Varian Saturn 2000 ion trap mass detector (40–670 Da; Varian, Walnut Creek, CA, USA) and operated by Saturn GC–MS WorkStation v6.4.1 software. The GC column used was VF-5 ms (25 m × 0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA, USA). Helium 5.0 was used as a carrier gas at flow rate of 1.0 mL min^{-1}. The temperature program was: 150 °C, held 1 min; slope 1: 15 °C min^{-1} to 250 °C; slope 2: 10 °C min^{-1} to a final temperature of 300 °C and held for 7 min. The injector temperature was set at 250 °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, 50 °C, and 280 °C, respectively. Samples were analyzed in MS/MS mode. Specific MS/MS conditions were previously described [10]. The mass fragments for monitoring BDE-77 and BDE-128 ISs were 324 and 484, respectively. The peak identification was based on the base peak and the isotopic pattern of the PBDEs congeners. Peak identification and quantification were carried out against IS. Decabromodiphenyl ether (BDE-209) could not be determined due to mass range limitation of the mass spectrometer.

A 40 kHz and 600 W US-bath with temperature control (Test Lab, Buenos Aires, Argentina) was used for assisting on the leaching and extraction process. Experimental design and data analysis were carried out by using the Stat-Ease Design-Expert trial version 7.0.3 software.

2.3. Sampling and sample conditioning

Lake sediment samples from Potrerillos reservoir, Mendoza, Argentina (32°58′13″S, 69°09′13″W) were analyzed in this study. One kilogram sediment was taken per sampling point by using a Van Veen grab. The samples were collected in brown flasks, transported at 4 °C to the laboratory and stored at −20 °C until analysis. Before sample preparation, each sample was thawed and homogenized. Afterward, an aliquot was dried at 40 °C and finally sieved using a 425 μm mesh size.

2.4. Sample preparation

The analytes were leached from 1 g dried sample aliquot with 1.2 mL MeOH (leaching solvent). The process was assisted by US at 40 ± 2 °C on two cycles of 9.2 min with 1 min break in-between. The

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resulting slurry was centrifuged at 2000 rpm for 10 min, and 0.4 mL aliquot of liquid phase was transferred to a 10 mL empty tube. Afterwards, 0.1 mL MeOH (dispersive solvent), 22 mg (~26.5 μL, 20 °C) 1-dodecanol (extractant solvent) and 1 mL of NaCl 6.15 M were added to the tube and vortex mixed. In order to induce a fine dispersion and subsequent efficient separation of the extractant phase, 4.4 mL 40 °C ultrapure water was slowly added against the tube wall. It was observed that warm water facilitated the consolidation and formation of a single organic drop. This was not observed when colder water was added. Solidification of the extractant phase and separation by floating was achieved by cooling the tube into an ice bath for 10 min. The solidified organic droplet was collected with a microspatula and transferred to a conical vial. Before GC analysis, the extractant droplet was melt at room temperature and 3 μL of iso-octane was added and vortex mixed, in order to improve the chromatographic performance [22]. The final volume achieved was 20 μL. One microliter aliquot was finally analyzed.

3. Results and discussion

The extraction efficiency of the USL–DLLME-SFO technique is conditioned by complex mass transfer processes of the leaching and microextraction steps, as well as by other factors including the analytes availability, matrix loading, and compatibility among techniques. These factors can be limited by carefully selecting the working conditions that favor mainly the mass balance of the analytes of interest or minimized undesired ones. Considering the particularities of the leaching and microextraction steps, as well as their interconnection, the study of the total sample preparation process is complex. Therefore, a multivariate statistical approach was proposed to identify variables and interactions that principally affect each step, as well as to optimize the working conditions, in order to achieve a maximum enhancement factor of the analytical signals within a shorter sample preparation time. The studied variables were: solvent type and volume, temperature, mixing assistance (mode and time required), and salting out effect. The relative chromatographic peak area was used as analytical signal for the multivariate study. To carry out the assays, a PBDE-free sediment sample was divided into 1 g aliquots and spiked to get a final concentration of 5 ng g^{-1} each target PBDE and IS, respectively.

Prior to the experimental design, a preliminary study was carried out to define the most appropriate dispersive and extraction solvents for DLLME-SFO. Furthermore, in order to simplify the pattern, each step (leaching or microextraction) was separately analyzed, leaving the other unchanged.

3.1. Type of dispersive and extraction solvents in USL–DLLME-SFO

Several requirements were considered for choosing the dispersive and extraction solvents. Dispersive solvents in DLLME should be miscible with both water and the extraction solvent, in order to efficiently spread the extraction solvent within the aqueous bulk and thus favor the mass transfer process [10]. On the other hand, the extraction solvent should not be miscible with water. Furthermore, for the SFO technique, the extraction solvent should have a melting point at near or below room temperature and a density lower than water, in order to solidify and easily separate the floating extractant phase into small droplets [10,16]. Additionally, the resulting extraction phase must be compatible with the analytical instrumentation used for analysis (e.g. GC–MS). Taking into account these considerations, MeOH, acetone, and ACN were tested as leaching and dispersive solvents; while 1-undecanol, 1-dodecanol, and 1,10-dichlorodecane were evaluated as extraction solvents. Physico-chemical properties of the proposed extraction solvents, such as density, water solubility, boiling and melting points, as well as vapor expansion volumes, are shown in Table S1 of the Supplementary Data section. The extraction solvents have a melting point close to room temperature, densities below 1 g mL^{-1} and low water solubility. Furthermore, 1-undecanol, 1-dodecanol, and 1,10-dichlorodecane show a lower expansion volume than other GC solvents, such as iso-octane, at the injection temperature. This can avoid the backflush phenomenon during the injections.

The relative responses of the studied PBDEs using different dispersive and extraction solvents are shown in Fig. 1. The highest

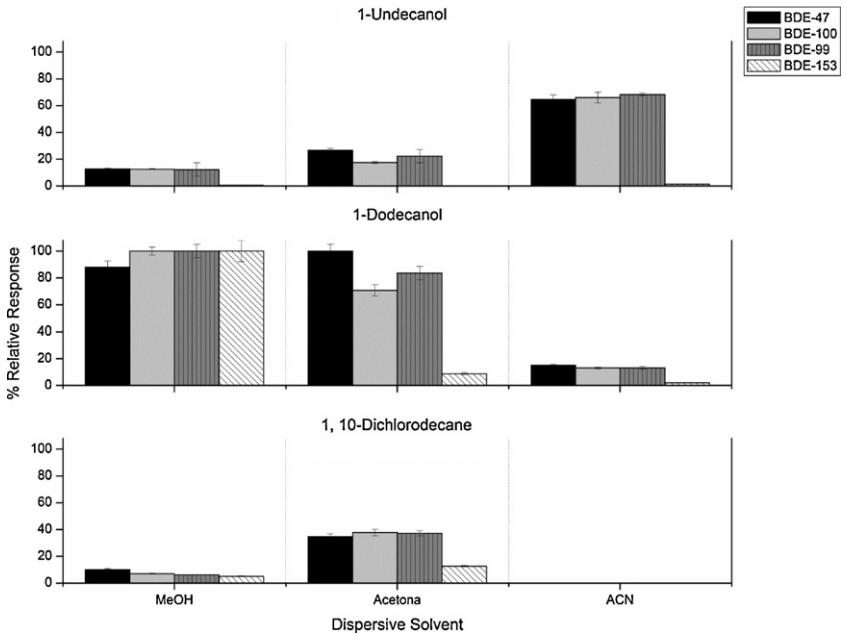

Fig. 1. Effect of dispersive and extraction solvents on the relative analytical response (and their error bars) of the studied PBDEs. USL condition: leaching solvent volume, 2 mL (MeOH; acetone and ACN); US radiation time, 30 min continuous irradiation; temperature, 30 ± 2 °C; PBDEs concentration 5 ng g^{-1}; centrifugation time, 10 min; DLLME-SFO condition: leaching extract 0.4 mL (MeOH, acetone and ACN); extraction solvent mass, 20 mg (1-undecanol, 1-dodecanol and 1,10-dichlorodecane); dispersant bulk volume, 4 mL ultrapure water; ice bath, 10 min.

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relative responses of PBDEs were achieved when the combination of MeOH and 1-dodecanol was used for the DLLME-SFO step. Since no significant differences on the chromatographic performance were observed when different solvents were used for leaching or extraction, these results could be due to practical aspects. In this sense, it is worth to mention that this solvent combination (MeOH and 1-dodecanol) led to an efficient separation of phases, due to an easy formation of a well-defined floating organic droplet. For the other solvent combinations, a diffuse formation of the droplet did not allow an efficient phase separation, thus inducing poor analytical results. Therefore, MeOH and 1-dodecanol were selected for further studies.

3.2. Experimental design

A full factorial experimental design (2^k) was used to evaluate the significance of the experimental variables, as well as their interactions, on the analytical response. After multiple linear regression and analysis of variance, a Pareto chart was used to select significant variables [21]. Once the variables that influence the leaching and microextraction techniques were established, a CCD was carried out. The latter study allowed to identify those conditions that leaded to the maximum analytical responses of PBDEs, and also to mathematically predict how an analytical response is related to the values of various working variables [23]. The working ranges of the selected variables were carefully chosen based on prior knowledge about the system under study and preliminary assays [10]. After experimental procedures, outliers were removed by analyzing the difference among fitted values test (DFFITS), due to their disproportionate influence on the predicted response, and thus on the model [21]. Afterward, the model coefficients were calculated by backward multiple regression and then validated by the analysis of variance [24]. Finally, a multiple response criteria, the desirability (D) function, was successfully used in order to define the values that lead the best analytical response for the studied PBDEs. The D function also includes researcher's priorities and desires on building the optimization procedure. The procedure creates a function for each individual analytical response, and finally obtains a global D function that should be maximized by choosing the best conditions of the designed variables.

3.3. USL technique

It is known that USL technique is conditioned by several variables including US-radiation intensity, application mode (one or several US-radiation steps separated by short time breaks), as well as the composition of the leaching medium and its temperature [10,25,26]. The phenomenon of cavitation affects both processes: mechanical erosion and partition of particles. On the other hand, the remaining variables influence the physico-chemical properties of the analytes and the leaching medium. Their appropriate adjustment and combination may lead to a successful leaching efficiency. Thus, the effect of their individual and combined influence on the analytical response was study on the analytical response of the methodology.

These selected variables and the assayed levels are presented in Fig. 2. The sample preparation procedure was as described in Section 2.4 and the particular DLLME-SFO working conditions were 0.4 mL MeOH as leaching extract; 20 mg 1-dodecanol as extraction solvent; and 4 mL 40 °C ultrapure water as dispersant aqueous bulk. In this first stage, US radiation time variable was evaluated at 15 and 30 min. Additionally, multiple step cycles, with 3 stops of 1 min break in-between each cycle, were tested. In order to make possible a comparison with a continuous mode, the total time of US irradiation was 15 and 30 min. MeOH was chosen as leaching solvent according with the experimental optimization described in

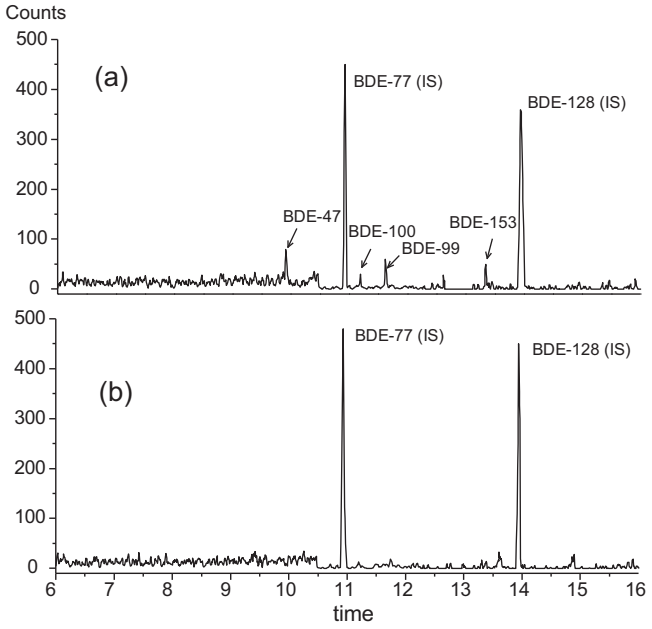

Fig. 2. GC–MS/MS chromatograms of sediment. EIC for 324, 326, 328, 402, 404, 406, 482, 484 and 486 m/z. (a) Sample M4 and (b) procedural blank (IS 5 ng g^{-1}, respectively) of USL–DLLME-SFO-GC–MS/MS.

Section 3.1. The studied leaching temperatures were 20 °C and 40 °C; and the leaching solvent volumes were 0.8 mL and 1.5 mL MeOH. In order to compare the results of the leaching solvent volume, 0.7 mL MeOH was added to the smaller volume after finishing the leaching stage and prior taking the aliquot for DLLME-SFO.

As it can be observed in Fig. S1 of the Supplementary Data section (Pareto Charts USL study), the individual variables that showed significance on the analytical response ($p < 0.1$) of the studied PBDEs were leaching the solvent volume (D) and US radiation time (A). Although the remaining individual variables did not show to be significant, their interactions did. The significant interactions ($p < 0.1$) were mainly: (1) US radiation time and US extraction mode (AB); (2) US radiation time and leaching temperature (AC); (3) US radiation time and leaching solvent volume (AD); and (4) US extraction mode and leaching temperature (BC). As a consequence, all variables were selected and further considered for the CCD analysis. The working ranges selected for the CCD of the US radiation time (I), US radiation steps (II), leaching temperature (III), and leaching solvent volume (IV), are shown in Table S2 of the Supplementary Data section. The CCD was carried out based on 30 assayed sketches. The resulting behavior of the analytical response of the PBDEs under the effect of the studied variables was best explained by quadratic models. Table S2 also showed the statistical parameters corresponding to the fitting for resolution. Models were significant ($p < 0.05$) and their lacks of fit were not significant ($p > 0.05$). The p-values showed, at 95% confidence level, that the leaching solvent volume and temperature affected, in general, the extraction efficiency of the target PBDEs and thus their analytical response. These results were expected due to the influence of temperature on the solubility of analytes and diffusion coefficients involved in this system. On the other hand, leaching solvent volume might condition solubility of the analytes and additional matrix concomitants substances. It is worth to notice that for BDE-47, the leaching temperature does not affect its analytical response; however, the US radiation time does. The effect of temperature was not significant for BDE-47 due to its higher solubility in the solvent leaching. However, the US radiation time is important to break up the particles of sediment and improve the contact surfaces for leaching. Also, this variable may favor the

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diffusion of solvent through pores of the particles and therefore reach locations difficult to access. Furthermore, it was observed that the interaction of these two variables (US radiation time and leaching temperature) significantly affects the leaching efficiency for BDE-100, -99 and -153. These results were also understandable due to their lower solubility into the leaching solvent compared with BDE-47. Comparable observations were reported previously when univariate analysis was used [10]. However, it was observed that although the effect of multiple US radiation steps was significant, the number of steps was not significant [10]. In this sense, the role of experimental design was important since it allowed observing the impact of the variables interaction on the leaching system.

After CCD, the *D* function was used for simultaneously maximize the individual responses of the four PBDEs, all with the same importance. Under the mentioned optimization criteria, the experimental conditions corresponding to one of the maximum in the *D* function ($D = 0.762$) were: US radiation time: 18.4 min; US radiation steps: 2 (one stop, 1 min break in-between each cycle); leaching temperature: 40 °C; leaching solvent volume: 1.2 mL. The values suggested through the optimization procedure were experimentally corroborated.

3.4. DLLME-SFO technique

As reported [19], the extracting solvent mass conditions the efficiency of DLLME-SFO. It refers to the minimum mass that lead to the maximum extraction efficiency and analytical signal enhancement. The selecting criterion depends mainly on the physico-chemical characteristics of the target analytes, although sample matrix (type and load) can also influence it. In our case, the matrix load was not significant, since DLLME-SFO was carried out over an aliquot of supernatant phase obtained after USL of the sediment. Since the supernatant aliquot was colorless and clear, it was considered that only MeOH-soluble components of the sediment would be carried to the DLLME-SFO stage. Furthermore, the solubilization thereof in dispersant aqueous bulk contributed to its dilution and therefore decreased the matrix load on the resulting extraction phase.

The extraction efficiency of DLLME-SFO is also conditioned by the volume of dispersive solvent, which affects the solubility of the extraction solvent and concomitant PBDE matrix in the aqueous mass [10]. Since an aliquot of the USL supernatant was used for further isolate the analytes by DLLME-SFO, a basal MeOH volume is considered for our microextraction system. However, it is important to evaluate whether additional volume is required to maximize the extraction efficiency of DLLME-SFO.

It is clear that the dispersant aqueous bulk where DLLME-SFO is carried out plays a key role on the extraction efficiency of the technique. Additionally to the previous considerations, the aqueous bulk conditions the solubility of solvents, analytes and matrix concomitant substances, as well as droplet formation and phase separation [16]. The dispersive solvent required for DLLME is acting as medium modifier, affecting the mentioned solubility into the aqueous bulk. However, pH and salt addition can also affect the solubility and thus, the extraction efficiency of the DLLME technique [16]. In our case, due to the physico-chemical characteristic of the analytes, only the salt concentration of the dispersant aqueous bulk would affect the DLLME-SFO efficiency. On the other hand, as the salt concentration of the medium (aqueous bulk) increases, its viscosity also increases; diminishing thus the efficiency of the mass-transfer process and consequently, the extraction efficiency of the technique.

In order to identify the variables that influence the DLLME-SFO technique, the salting out effect, additional dispersive solvent volume, dispersive bulk volume, and extraction solvent mass were considered for assaying and analysis by Pareto Chart. These selected variables and their levels are presented in Fig. 3. The sample preparation and USL procedures followed in this study were previously described (Sections 2.4 and 3.3, respectively). The microextraction working conditions were as follow.

The statistical analysis of the resulting analytical signals by Pareto Charts is shown in Fig. S2 of the Supplementary Data section. As it can be observed, extracting solvent mass (*D*) and salt addition (*A*) were significant on the analytical response of the studied PBDEs. It is worth to mention that the effect of the extracting solvent mass variable was the most significant ($p < 0.1$). Salt addition showed a significant positive effect on the analytical responses of all the studied analytes, and therefore on the DLLME-SFO efficiency. Although the remaining individual variables did not appear to be significant, their interactions did. The significant interactions were: (1) additional dispersive solvent and dispersant bulk volume (*BC*); (2) salt addition and extracting solvent (*AD*); (3) salt addition and dispersant bulk volume (*AC*); and (4) additional dispersive solvent and extracting solvent (*BD*). As a consequence, all variables were selected and considered for the CCD study. The working ranges selected for the CCD of the additional dispersive solvent (I), extracting solvent (II), and dispersive bulk (III), are shown in Table S3 of the Supplementary Data. Salt addition was considered as a fixed variable, since it was significant independently from the added volume of NaCl saturated solution. The CCD was carried out based on 20 assayed sketches. The resulting behavior of the analytical response of the PBDEs under the effect of the studied variables was better explained by 2-factor interactions (2FI) and linear models ($p < 0.05$). Table S3 also shows the statistical parameters corresponding to the fitting for resolution, where the lack of fit is not significant ($p > 0.05$). From this analysis, it is confirmed that the extracting solvent mass variable affects considerably the analytical response of all studied PBDEs. The others individual variables that impact on the analytical performance are additional MeOH and dispersant bulk volume, but they only seems to affect BDE-153 and BDE-100, respectively. Analytical responses of BDE-47 and BDE-99 were affected by the combination of additional MeOH with the extraction solvent mass variables. On the other hand, the interaction between additional MeOH and dispersant bulk volume also affects the analytical response of BDE-153.

These results show that MeOH plays an important role in the performance of the analytical methodology. In addition to its role as a dispersing solvent, MeOH has a strong influence as organic modifier for aqueous extraction medium. In this way, it alters the polarity of the aqueous medium and therefore, the affinity of the analytes for it. Interestingly, each of the analytes showed different behavior against this situation, which were conditioned by their particular polarities. It is important to highlight that, due to the complexity of the system; this analysis would not be possible without using a multivariate statistical tool.

The *D* function was selected in order to simultaneously maximize the individual responses of the four PBDE congeners, all with the same importance. Under the mentioned optimization criteria (see Section 3.2), the experimental conditions corresponding to one of the maximum of the *D* function ($D = 0.756$) were: additional dispersive solvent: 0.10 mL; extracting solvent mass: 22 mg; dispersant bulk volume: 4.4 mL; volume of 6.15 M NaCl: 1 mL. The values suggested through the optimization procedure were experimentally corroborated.

3.5. Analytical performance and comparison whit other analytical techniques

Developing analytical method using synthetic solution and further application on real samples generally require severe adjustments. This could lead to a new method different from the former one. The use of multivariate statistical tool and real matrix all along

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Table 1
Analytical figures of merits of the proposed methodology for PBDEs determination in sediment samples PAPER.

PBDE	r^2	RSD (%)[a]	LOQ method (pg g^{-1})[b]	Linear range (pg g^{-1}) [a]
BDE-47	0.954	3.5	1.7	2–1000
BDE-100	0.992	4.9	6.0	6–1000
BDE-99	0.986	7.8	5.0	5–1000
BDE-153	0.974	9.2	4.3	5–1000

Extraction condition as described in Section 2.4.
[a] 95% confidence interval; $n = 3$.
[b] Dry weight.

the process is a convenient choice for characterizing and optimizing the analytical methodology that is been developed. Thus, the particular factor of a real matrix, as well as all operative variables that condition the analytical responses of the analytes are evaluated together, in addition to individually. In this sense, not only the signal enhancing due to the improvement of the extraction efficiency is important, but also the matrix load minimization, which lead to a successful chromatographic analysis. These aspects are what finally determine the sensitivity and selectivity of the resulting analytical methodology.

The analytical figures of merits of the proposed methodology are summarized in Table 1. The quantification of PBDEs by GC–MS/MS was accomplished by internal standard method. Each analytical sequence included quality control standards (BDE-77 and -128) and blanks to monitor background levels and possible carryover between samples. The calibration curves were applied using linear fit and showed a satisfactory linearity within the concentration range of 2–1000 pg g^{-1} for BDE-47, 6–1000 pg g^{-1} for BDE-100, 5–1000 pg g^{-1} for BDE-99, and 5–1000 pg g^{-1} for BDE-153, with correlation coefficients (r^2) higher than 0.954. The precision of USL–DLLME-SFO-GC–MS/MS was evaluated over five replicates at 15 and 500 pg g^{-1}, leading to RSD values < 9.2%. In order to validate the analytical methodology, a recovery study of PBDEs at different concentrations (250 pg g^{-1} and 500 pg g^{-1}) was carried out over the real sediment samples. The data demonstrated a good recovery in the range of 71–104% (Table 2). Additionally, the studied PBDEs did not show a significant shift in the retention time and/or sensitivity in the chromatographic analysis. Fig. 2 showing sample and blank procedure chromatograms was included.

A comparative study of the analytical performance of the proposed methodology was carried out taking into account the experimental assays and bibliographic data remarking the relative merits of each methodology. In this sense, the LODs resulting from the Soxhlet-GC–MS/MS methodology were significantly higher than those achieved by the proposed methodology (Table 3).

Other aspects worth mentioning with respect to USL–DLLME-SFO include limited use of toxic solvents, as well as simple and rapid procedure, without requiring advanced equipment or materials. By comparing against other analytical methodologies reported for the determination of PBDEs in sediment and sludge samples (Table 3), it is possible to emphasize other technical aspects, in addition to the improved LODs. In this regard, two interesting approaches using liquid chromatography was reported. These methods have some

Table 2
Recovery study of PBDEs in sediment samples from Potrerillos Reservoir, Mendoza, Argentina.

Sample	Level found (pg g^{-1} d.w.)	250 pg g^{-1} spiked		500 pg g^{-1} spiked	
		Found[a]	Recovery[b]	Found[a]	Recovery[b]
M1					
BDE-47	n.d.	203 ± 21	81	425 ± 11	85
BDE-100	n.d.	201 ± 18	80	405 ± 12	81
BDE-99	n.d.	198 ± 13	79	372 ± 6	74
BDE-153	<LOQ	181 ± 7	72	379 ± 3	75
M2					
BDE-47	6	219 ± 28	85	455 ± 5	89
BDE-100	n.d.	240 ± 11	96	520 ± 10	104
BDE-99	<LOQ	190 ± 15	75	410 ± 9	81
BDE-153	14	197 ± 9	73	431 ± 1.3	83
M3					
BDE-47	8	223 ± 25	86	461 ± 17	90
BDE-100	<LOQ	228 ± 21	90	473 ± 6	94
BDE-99	<LOQ	225 ± 15	88	448 ± 18	89
BDE-153	10	198 ± 11	75	426 ± 10	83
M4					
BDE-47	20	202 ± 21	73	415 ± 13	79
BDE-100	<LOQ	207 ± 22	82	421 ± 7	84
BDE-99	22	218 ± 15	78	432 ± 8	82
BDE-153	15	192 ± 17	71	411 ± 12	79
M5					
BDE-47	14	223 ± 21	84	451 ± 13	87
BDE-100	n.d.	195 ± 12	78	405 ± 5	81
BDE-99	<LOQ	213 ± 21	84	413 ± 4	82
BDE-153	6	206 ± 12	80	424 ± 9	83
M6					
BDE-47	18	235 ± 23	87	472 ± 5	91
BDE-100	n.d.	215 ± 32	86	425 ± 11	85
BDE-99	<LOQ	200 ± 19	79	438 ± 9	87
BDE-153	<LOQ	216 ± 25	86	409 ± 12	81
M7					
BDE-47	29	226 ± 25	79	436 ± 12	81
BDE-100	n.d.	232 ± 19	93	450 ± 12	90
BDE-99	8	249 ± 29	96	513 ± 8	101
BDE-153	<LOQ	226 ± 18	90	459 ± 10	91

n.d.: not detectable; LOQ: limit of quantification.
[a] Results expressed as mean ± SD; 95% confidence interval; pg g^{-1} d.w.
[b] [(Found − base)/added] × 100. Base concentrations of individual compounds below the LOQ were substituted with a value equal to (1/2) LOQ.

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Table 3
Comparison of USL–DLLME-SFO-GC–MS/MS with other analytical method previously reported for PBDE determination in similar samples.

Method	Congeners	Sample (g)	LOD (pg g^{-1})	RSD (%)	Recovery (%)	Reference
SE–HS-SPME-GC–MS–MS[a]	BDE-47, -100, -99, -85, -154, -153	Sewage sludge (2 g)	10–1200	2.4–14	96–138	[25]
HS-SPME-GC–MS/MS	BDE-47, -100, -99, -85, -154, -153	Sediment (0.5 g)	5.2–214	1.1–16	82–104	[26]
UPLC–APCI/MS/MS[b]	BDE-47, -99, -100, 153, -154, -183, -196, -197, -203, -205, -209	Sewage sludge (5 g)	3.6–390	–	65–112	[23]
DLLME-SFO-HPLC–UV	BDE-209	Sediment (0.1 g)	2.3	2.1–3.7	98–104	[16]
USAL–DSPE–DLLME-GC–MS/MS[c]	BDE-47, -100, -99, -153	Sediment (0.25 g)	20–60	4.7–9.8	80–112	[6]
Soxhlet-GC–MS/MS	BDE-47, -100, -99, -153	Sediment (5 g)	40–80	–	–	[6]
USL–DLLME-SFO-GC–MS/MS	BDE-47, -100, -99, -153	Sediment (1 g)	0.5–1.8	3.5–9.2	73–104	Proposed method

[a] SE–HS-SPME: solvent extraction + head space-solid phase microextraction.
[b] UPLC–APCI: ultrahigh pressure liquid chromatography–atmospheric pressure chemical ionization.
[c] USAL–DSPE: ultrasound-assisted leaching–dispersive solid-phase extraction.

limitations: while one of the proposed methodologies requires expensive analytical instrumentation (UPLC–APCI/MS/MS), the second one was proposed for the determination of only one of the PBDE congeners (BDE-209) [20,26]. Moreover, even though solid phase microextraction (SPME) is extensively employed as an extraction and preconcentration technique, it requires long periods of stabilization, while the adsorbent microfiber has a limited life. In comparison to USAL–DSPE–DLLME, the proposed method could be a viable alternative for the determination of PBDEs in sediment samples by GC–MS/MS, since it shows comparable advantages, such as minimum sample manipulation, and lower organic solvents and time consumption.

3.6. Application to real samples

The newly optimized USL–DLLME-SFO-GC–MS/MS method was applied for the determination of PBDEs in sediment samples from Potrerillos Reservoir (32°58′13″S, 69°09′13″W), located in the north of Mendoza province, Argentina. It is a regulatory dike of the Mendoza River, which is use for irrigation, population, and factory consumer. This water reservoir receives inputs from the mountainous region located upstream and includes rural and suburban activities. Sediment samples were collected according with the procedure descript above (Section 2.3). The same samples were analyzed by the standard addition method. The sample analysis and recovery study were performed in triplicate. The PBDEs concentration in the samples were between not detected to 29 pg g^{-1} d.w. (Table 2). BDE-47 was the most abundant congener followed by BDE-153. BDE-99 was detected in 28% of the analyzed samples, while BDE-100 was below LOQ in all samples.

4. Conclusions

The proposed analytical methodology based on USL–DLLME-SFO technique is an efficient alternative for PBDEs determination at trace levels in sediment samples. It is important to highlight that experimental design helped to identify and characterize the variables and their interactions that govern the system. This statistical tool also allowed reaching optimum working conditions with a minimum number of analytical assays. Under optimized working conditions, LODs of USL–DLLME-SFO-GC–MS/MS were in the order of pg per g, with an acceptable precision. Therefore, the proposed methodology can be successfully applied in routine analysis to determine trace levels of PBDEs in sediment samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2013.02.027.

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