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Ultrasound-assisted leaching-dispersive solid-phase extraction followed by liquid–liquid microextraction for the determination of polybrominated diphenyl ethers in sediment samples by gas chromatography–tandem mass spectrometry

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ABSTRACT

Ultrasound-assisted leaching-dispersive solid-phase extraction followed by dispersive liquid–liquid microextraction (USAL-DSPE-DLLME) technique has been developed as a new analytical approach for extracting, cleaning up and preconcentrating polybrominated diphenyl ethers (PBDEs) from sediment samples prior gas chromatography–tandem mass spectrometry (GC–MS/MS) analysis. In the first place, PBDEs were leached from sediment samples by using acetone. This extract was cleaned-up by DSPE using activated silica gel as sorbent material. After clean-up, PBDEs were preconcentrated by using DLLME technique. Thus, 1 mL acetone extract (disperser solvent) and 60 μL carbon tetrachloride (extraction solvent) were added to 5 mL ultrapure water and a DLLME technique was applied. Several variables that govern the proposed technique were studied and optimized. Under optimum conditions, the method detection limits (MDLs) of PBDEs calculated as three times the signal-to-noise ratio (S/N) were within the range 0.02–0.06 ng g^{-1}. The relative standard deviations (RSDs) for five replicates were <9.8%. The calibration graphs were linear within the concentration range of 0.07–1000 ng g^{-1} for BDE-47, 0.09–1000 ng g^{-1} for BDE-100, 0.10–1000 ng g^{-1} for BDE-99 and 0.19–1000 ng g^{-1} for BDE-153 and the coefficients of estimation were ≥0.9991. Validation of the methodology was carried out by standard addition method at two concentration levels (0.25 and 1 ng g^{-1}) and by comparing with a reference Soxhlet technique. Recovery values were ≥80%, which showed a satisfactory robustness of the analytical methodology for determination of low PBDEs concentration in sediment samples.

1. Introduction

PBDEs are brominated flame retardants used to protect potentially flammable organic materials by increasing the resistance to ignition and delaying the spread of fire [1,2]. They are added to textile, building material, motor vehicle, electrical and electronic equipment and commercial products [3]. Their distribution throughout the environment and their toxicity exhibits similarities with polychlorinated biphenyls (PCB). PBDEs are additives mixed into polymers and are not chemically bound to the plastic or textiles. Therefore, they may leach from the polymeric product into the environment [4,5]. Once in the environment, PBDEs can be persistent or break down into other chemical forms, depending on particular surrounding conditions. PBDEs have low water solubility and high affinity to particulate matter, which favors their transport and bioaccumulation in hydrophobic mediums of the biota, such as sediments [4]. In this way, they can easily reach animals and humans through their food chain [3,6]. The toxicological concerns about exposure to low PBDEs concentrations focus on their potential to act as hormone disruptors, neurodevelopment toxics and probably carcinogenic agents [7,8].

In the last 10 years, the development of robust analytical methodologies to quantify PBDEs in environmental matrixes has reported a rapid growth. Several analytical approaches for both sample preparation and instrumental analysis have been proposed [9,10]. With the aim of their unequivocal identification and determination, highly selective and sensitive analytical techniques are required. In this sense, the chosen techniques are capillary gas chromatography (GC) with electron-capture (ECD) or mass spectrometry (MS) detection [4,11]. Sediment is one of the major sink of PBDEs in the aquatic environment. Since contaminants can

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be bioavailable in sediment to different aquatic organisms, the study of sediment is an important stage in tracing possible exposure route to aquatic biota [12]. The analysis of sediment samples for PBDEs determination requires highly efficient extraction techniques because the analytes tend to be very strongly bound to the sample matrix. Furthermore, due to the low concentration of the target analytes in sediment samples, it is necessary to count on highly efficient preconcentration techniques for their determination. The extraction of PBDEs from sediment samples has been usually carried out by using conventional Soxhlet extraction [13], solid-phase microextraction (SPME) [14] and microwave-assisted extraction (MAE) [12]. 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 kinetic of the mass-transfer process of the target analytes to the liquid phase. This leads to an increment in the extraction efficiency of the technique in a minimum amount of time [15,16]. The effects of US are primarily related with the cavitation phenomenon, which involves the implosion of bubbles formed in the liquid medium during US application. The bubble implosion generates rapid adiabatic compression of gases and vapours within the bubbles or cavities and, as a consequence, high temperature and pressure are generated. The increased pressure favors penetration of the leachant into the sample matrix and transport between the solid matrix and liquid phase at the interface [15]. Another factor that increases the efficiency of USAL is the presence of free radicals formed through cavitation. In fact, the oxidative energy of radicals created by sonolysis of the solvent dramatically improves the efficiency of leaching [15]. Those phenomenon results in an increment in the solubility of the analytes into the leachant and their diffusivity from the sample matrix to the outer region, which is the limiting step of mass transfer.

Recently, a novel microextraction technique, dispersive liquid–liquid microextraction (DLLME), have been reported for extracting and/or preconcentration target analytes from aqueous samples [17]. DLLME employs a mixture of a high-density non-polar water immiscible solvent (extraction solvent) and a polar water miscible solvent (disperser solvent). The disperser solvent is used for dispersing the extraction solvent as very fine droplets into the aqueous bulk and increase the contact surface with the extraction solvent. An efficient dispersion of extraction solvent favors the mass-transfer process between two immiscible phases. After a short contact time, the dispersed phase is separated by centrifugation and the extracted analytes can be determined by conventional analytical techniques. DLLME have high preconcentration capabilities in a very short time. DLLME have been previously applied mainly for determination of organic compounds from liquids samples. The application of DLLME to solid samples had received minor attention and only fruit, plant and soils samples were studied [18–21]. The main disadvantage of the DLLME is that it is not a selective extraction technique and also fails if phases do not separate even after centrifugation (in the case of heavily contaminated extracts). Thus, in order to overcome this problem it is necessary to include a clean-up stage after the analyte leaching and previous to DLLME technique. Dispersive solid-phase extraction (DSPE) was recently introduced as a rapid and simple technique for clean-up crude extracts of different food and environmental samples [19,22–24]. It is based on the addition of the sorbent material into the extract to remove the matrix concomitants, which is then separated from the extract bulk by centrifugation. The introduced technique was named as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). The use of DSPE after USAL would increase the extraction efficiency of DLLME technique and extend its applicability to sediment samples. To the best of the knowledge of the authors, there is no report about the use of USAL-DSPE-DLLME to extract and preconcentrate PBDEs from sediment samples prior to GC–MS/MS analysis.

The purpose of the present work is to develop a new analytical approach based on USAL-DSPE-DLLME and demonstrate its applicability for extraction and preconcentration PBDEs from sediment samples and further determination by GC–MS/MS. To this aim, four of the most commonly found PBDEs in sediment 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), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). The influence of several factors on the performance of the analytical methodology were studied and optimized over the relative response of the PBDEs. The analytical performance of USAL-DSPE-DLLME-GC–MS/MS methodology was evaluated in terms of method detection limits (MDLs), repeatability and linear working range. The optimized method was evaluated by comparing the results with those found with a reference Soxhlet extraction technique. Moreover, the procedure was applied for the determination of PBDEs in sediment samples of environmental 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 internal standard 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.

Methanol, acetone, acetonitrile, chloroform and trichloroethene were purchased from Merck (Darmstadt, Germany) and carbon tetrachloride was purchased from Sigma–Aldrich (Steinheim, Germany). Sodium chloride was purchased from Merck. 6.15 mol L^{-1} sodium chloride aqueous stock-solution was prepared. Sorbents (40 μm particle size) for DSPE included neutral silica gel purchased from Sigma–Aldrich, primary secondary amine (PSA) and C_{18} both obtained from Varian (Harbor City, CA, USA). 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 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 (25 m × 0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA, USA). The oven temperature program was: 150 °C, held 1 min; rating 15 °C min^{-1} to 250 °C; rating 10 °C min^{-1} to a final temperature of 300 °C and held for 7 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

Table 1
GC–MS/MS experimental parameters.

Analyte	t'_R (min)	m/z range	Parent ion (m/z)	Quantification ions (m/z)	Isolation window (m/z)	Excitation storage level (m/z)	Excitation amplitude (V)
BDE-47	0.82	300–500	486	324+326+328	8	214.6	1.37
BDE-100	0.93	380–580	566	402+404+406	8	250.0	1.60
BDE-99	0.96	380–580	566	402+404+406	8	250.0	1.60
BDE-153	1.11	400–650	644	482+484+486	8	284.5	1.40

t'_R: relative retention times to PCB-209.

conditions for each analyte are listed in Table 1. 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 leaching process. Injections into the GC–MS were made by using a 5.0-μL Hamilton syringe (Reno, NV, USA).

2.3. Sampling and sample preparation

River and lake sediments with different total organic carbon content (%TOC) were analyzed in this study. River sediment was collected upstream to *Mendoza River*, near to *Potrerillos Lake* (Sample 1, TOC: 0.19%) and a second river sample was collected downstream to *Pescara waterway* (Sample 2, TOC: 4.61%) which is an artificial channel destined for industrial waste collection and irrigation. A third sample was collected in Mendoza city (Sample 3, TOC: 2.15%). After collection, samples were dried at 60 °C for 72 h. Dried sediment samples were homogenized using a porcelain mortar and sieved through a 0.3-mm stainless steel sieve. Dried samples were stored in amber bottles in the darkness at room temperature until analysis.

The sediment samples used for method optimization were previously analyzed for the compounds of interest by using a reference Soxhlet extraction technique [25] and none of the studied analytes was detected. These samples were then spiked with the target PBDEs using methanolic solutions and homogenized as described by Salgado-Petinal et al. [14].

2.4. USAL-DSPE-DLLME procedure

A 0.25 ± 0.05 g sediment sample were placed into a 10 mL glass-centrifuge tube, followed by the addition of 1.5 mL acetone. The mixture was sonicated at 35 ± 2 °C in a US bath containing 0.2% of detergent. USAL was carried out in six leaching cycles 5 min long and 1 min break in-between each cycle. The resulting slurry was centrifuged at 3500 rpm (1852.2 × *g*) for 5 min. For DSPE, 1.2 mL aliquot of acetone extract obtained from USAL was transferred to a 10 mL tube and 100 mg of activated silica gel was added, vortexed for 30 s and centrifuged at 3500 rpm (1852.2 × *g*) for 3 min. For DLLME, 1 mL DSPE acetone extract and 60 μL carbon tetrachloride were added to 5 mL ultrapure water placed in a 10 mL glass-centrifuge tube and mixed up for 10 s by handshaking. A cloudy solution was formed due to the dispersion of fine carbon tetrachloride droplets into the aqueous bulk. The centrifuge tube was kept in a thermostatic bath at 35 ± 2 °C for 5 min and then centrifuged at 3500 rpm (1852.2 *g*) for 2 min. After centrifugation, the carbon tetrachloride phase (extraction phase) remained at the bottom of the conical tube. A 1 μL aliquot of extraction phase was injected into GC–MS/MS for analysis.

2.5. Soxhlet extraction

Extraction of PBDEs in sediment samples was performed following the procedure described by Mai et al. [25]. Sample preparation procedure was the same described above. A 5 g sediment sample was extracted with 150 mL hexane–acetone (1:1) for 18 h. In order to remove de elemental sulfur of the sample, 10 g of activated copper granules were added to the extraction flasks. The clean-up of the extract was carried out by passing it through a column (7 cm × 1 cm ID) packed with neutral alumina. PBDEs were then eluted with 30 mL hexane–trichloroethylene (2:1). The eluent was evaporated to dryness under a gentle stream of nitrogen and the residue was redissolved into 200 μL carbon tetrachloride containing the internal standard prior to the GC–MS/MS analysis.

3. Results and discussion

3.1. USAL-DSPE variables

Leaching the analytes from the sample provides a cleaner liquid extracts since some matrix interferences remain in the sediment [15]. However, this extract steel might contain some matrix concomitants that could interfere in the preconcentration technique. Therefore, it is necessary to include a clean-up step after leaching in order to overcome the mentioned analytical inconvenient during the preconcentration stage. Thus, several critical variables should be considered into the USAL-DSPE study and optimization, including leaching solvent type and volume, sorbent type used for DSPE, ultrasonication time and mode, nature of the transmitting liquid and leaching temperature. The study and optimization of the above mentioned variables were performed by modifying one at a time while keeping the remaining constant. A 0.25 g of sediment containing 50 ng g^{-1} of each PBDE was used to perform each assay, which were done by triplicate. The chromatographic peak area was the parameter used to evaluate the influence of those variables on the extraction efficiency of USAL-DSPE-DLLME technique.

3.1.1. Leaching-solvent and volume

The leaching-solvent is critical for developing an efficient USAL-DSPE-DLLME technique and making possible the combination of USAL-DSPE with DLLME. The USAL-solvent must be able to efficiently leach the target analytes from sediment samples and act as disperser solvent in DLLME technique. The selection of disperser solvents in DLLME is based on its water miscibility and its capability to dissolve the DLLME-extraction solvent. Taking into account these considerations, methanol, acetone and acetonitrile were assayed as leaching-solvents. The performance of these solvents was studied by adding 2 mL of each of the solvents mentioned above to a 0.25 g of sediment containing 50 ng g^{-1} of each PBDE. The USAL-DSPE and DLLME procedures were described above. The relative responses of the studied PBDEs using different leaching-solvents are shown in Fig. 1a. The results revealed that the relative responses of PBDEs using acetone are higher than the obtained with methanol and acetonitrile. It could be due to the polarity of acetone, which is lower

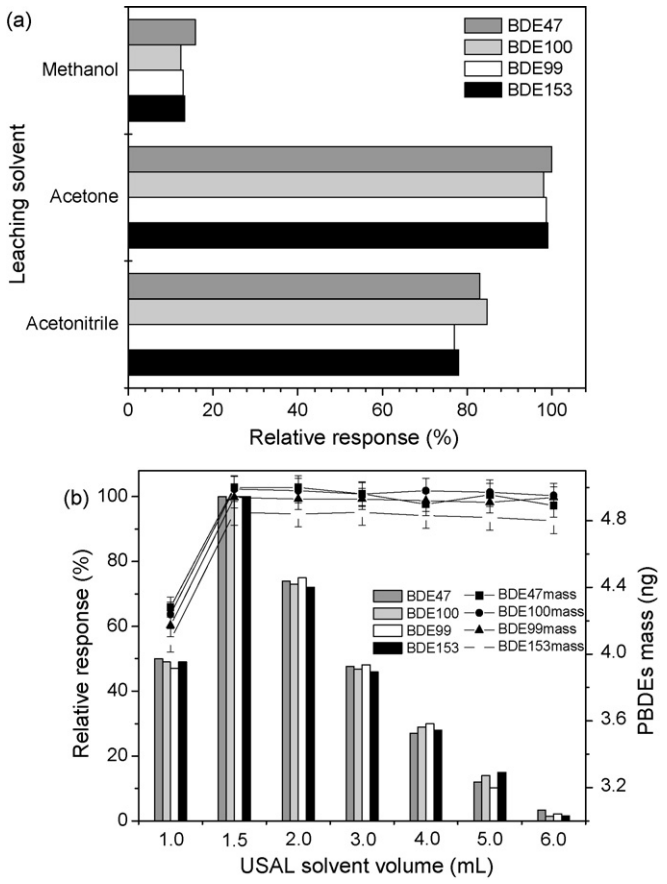

Fig. 1. Study of the leaching solvent on the relative response of PBDEs: (a) leaching solvent type and (b) leaching solvent volume. USAL conditions: temperature, 22 ± 2 °C; US radiation mode, 40 min continuous irradiation; centrifugation time, 5 min; PBDEs concentration: 50 ng g^{-1}; DSPE: 0.15 g C_{18}; DLLME: ultrapure water, 5 mL; USAL extract, 1 mL; extraction solvent volume, 60 μL carbon tetrachloride; DLLME temperature, 22 ± 2 °C; centrifugation time, 2 min.

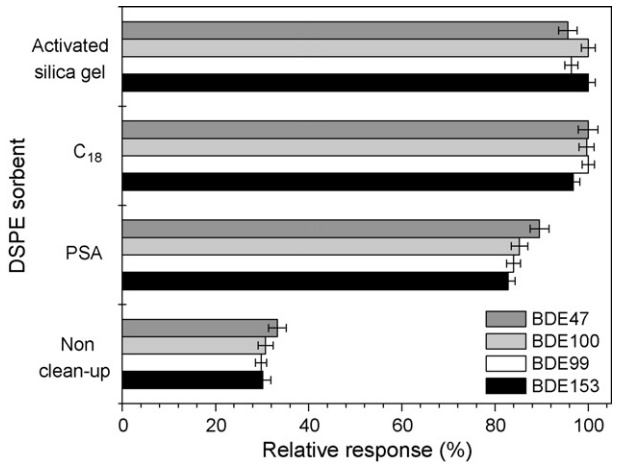

Fig. 2. DSPE sorbent materials effect on the relative response of PBDEs. Extraction conditions as described in Fig. 1. PBDEs concentration: 50 ng g^{-1}. Extraction conditions: leaching-solvent, 1.5 mL acetone. Other conditions as described in Fig. 1.

than acetonitrile and methanol. Thus, the PBDEs affinity for acetone is higher than for the other solvents resulting into higher USAL efficiency. Thus, the leaching-solvent selected for further studies was acetone.

The volume of leaching-solvent was found important to optimize in order to obtain the highest USAL efficiency and the highest relative response of the target analytes. The leaching-solvent volume was studied within a volume range of 1–6 mL. Fig. 1b shows that the greatest relative responses for the target PBDEs were obtained when 1.5 mL acetone volume was used. By increasing the volume of acetone between 1.5 mL and 6 mL, leaching efficiency for the analytes remained invariant. However, as the leaching-volume increased lower relative responses were obtained due to a dilution effect of the analytes into the resulting acetone phase. Leaching-solvent volumes smaller than 1.5 mL lead to small resulting extracts volumes, which were not enough to achieve a stable dispersion into the DLLME stage after DSPE and thus, lower leaching efficiency was observed. This phenomenon leaded to poor relative responses. Consequently, 1.5 mL leaching-solvent volume was selected for further studies.

3.1.2. Dispersive solid-phase extraction sorbent

Sediments samples generally contain a significant amount of organic matter additionally to the target analytes to be extracted. When a leaching procedure is carry out from this type of samples, many concomitants are co-extracted and they can affect the analytes determination. As mentioned above, cleaner extract is mandatory to achieve satisfactory DLLME efficiency. Although mass spectrometry is a selective detector; the analysis of this type of samples requires a clean-up stage in order to reduce the chromatogram background and increment the sensitivity of the methodology. In this sense, a DSPE clean-up was evaluated. The assays were carried out by adding 0.1 g sorbent materials to a 1.2 mL USAL acetone extract. Different solid sorbents, including activated silica gel, C_{18} and PSA were evaluated. It was observed that all sorbents leaded to cleaner chromatograms compared with the USAL extract without clean-up (Fig. 2). Consequently, the relative response for all PBDEs significantly increased (ca. 45%). The highest relative responses were observed when C_{18} or activated silica gel was used as DSPE sorbent. The results suggested that these sorbents absorb more efficiently matrix co-extractive interferences present in sediment extracts leading cleaner chromatograms and mass spectra. On the other hand, PSA is known to exhibit a strong retaining activity for sugars, fatty acids and other organic acids which are rarely present in sediments [22]. Due to the comparable results and lower cost of activated silica gel, the last was selected for further studies.

3.1.3. Leaching time and mode

The optimization of ultrasonication time is crucial to achieve an efficient USAL-DSPE procedure. The US can be applied in two different forms, one is a continuous mode and the other is a cycle mode. As the leaching time increase, the collapsing cavitation bubbles help to disrupt the saturated boundary layer surrounding the particles, bringing fresh solvent to the surface. It favor the mass transfer of the analyte into the solution leading to an enhancement of the leaching efficiency [26]. Additionally, it has been observed that by applying several leaching cycles, the leaching efficiency can be improved depending on the analytes and the type of sample matrix [15]. The ultrasonication time in the continuous mode was defined as the period over which US is continuously applied; and it was varied within the range of 0–60 min. It was observed that by increasing the extraction time, the relative responses increase, reaching the maximum value at 30 min, after which remained invariant. On the other hand, different irradiation cycles, 5–15 min long with 1 min break in-between each cycle, were assayed. In order to make possible a comparison with a continuous mode, the total time of irradiation was 30 min. The USAL-DSPE and DLLME procedures were described above. As can be seen in Fig. 3, the relative response of PBDEs

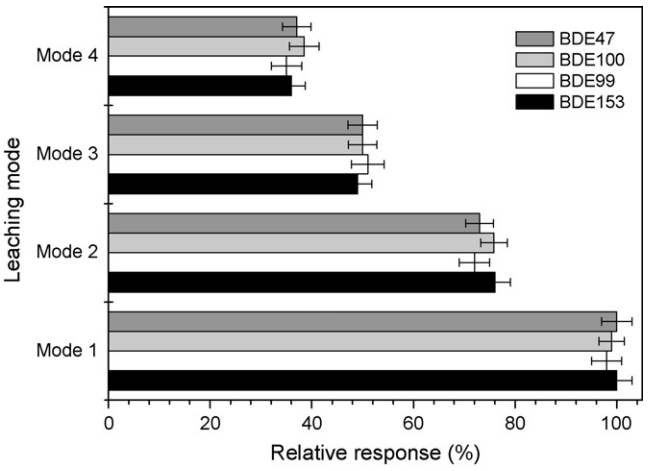

Fig. 3. Ultrasound-assisted mode effect on the relative response of PBDEs. Mode 1: six leaching cycles 5 min long with 1 min break in-between each cycle, Mode 2: three leaching cycles 10 min long with 1 min break in-between each cycle, Mode 3: two leaching cycles 15 min long with 1 min break in-between each cycle, Mode 4: 30 min continuous irradiation. Extraction conditions: DSPE, 0.1 g activated silica gel. Other conditions as described in Fig. 1.

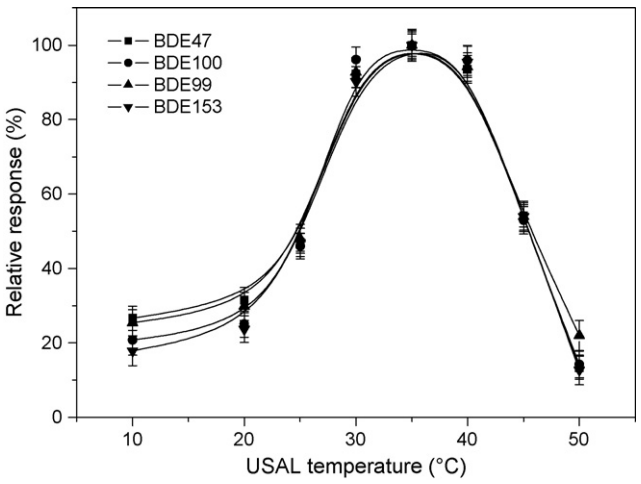

Fig. 4. USAL temperature effect on the relative response of PBDEs. Extraction conditions: leaching solvent volume, 1.5 mL acetone; USAL mode, six leaching cycles 5 min long with 1 min break in-between each cycle; centrifugation time, 5 min; DSPE, 0.1 g activated silica gel; DLLME conditions as described in Fig. 1.

increased as the time of the irradiation cycles decreased, reaching a maximum when six leaching cycles of 5 min long were applied. The best relative responses were achieved by using the cycle USAL-DSPE mode; therefore this was the USAL-mode selected for further studies.

3.1.4. Transmitting liquid nature

The nature of the US transmitting liquid used is frequently omitted in optimization tests, however their influence has been demonstrated by Nascentes et al. [27]. The nature of the transmitting liquid influences the cavitation phenomenon since affects the density and viscosity of the transmitting liquid. These phenomena affects the wave transmission and the leaching efficiency of the technique [15]. Additionally, cavitation induces to the generation of a liquid–gas interface into the transmitting liquid, which can be favored by the addition of a surfactant to the liquid medium [15]. Therefore, it was found interesting to study the influence of the transmitting liquid nature when Genapol X-080 was used as surfactant within the concentration range of 0–1%. The leaching procedure was the one described above. The best relative response for all four PBDEs was observed when 0.2% surfactant was assayed. Surfactant concentrations higher than 0.25% showed lower relative responses. Therefore, 0.2% Genapol X-080 was the selected condition for further studies.

3.1.5. Leaching temperature

Temperature can affect the extraction efficiency of the USAL-DSPE procedure. It affects the solubility of the analytes in the leaching solvent and the cavitation phenomenon, and thus the mass-transfer process efficiency. To determine the optimum leaching temperature, different temperatures ranging from 10 °C to 50 °C were studied (Fig. 4). The USAL-DSPE and DLLME procedures were described above. At low temperatures (<20 °C) low relative response for all PBDEs was observed. By increasing the leaching temperature, the relative responses increased achieving the highest value at 35 °C. At temperatures higher than 35 °C the relative responses decreased. This fact can be attributed to the diminished efficiency of collapsing bubbles at higher temperature. The effects of cavitational collapse are reduced as the bubbles will act as a barrier to sound transmission and dampen the effective ultrasonic energy from the source entering the liquid medium [15,26]. Therefore, 35 °C was selected as leaching temperature.

3.2. DLLME variables

After USAL procedure with DSPE clean-up was obtained an acetone extract containing the target PBDEs. Due to the low concentration of PBDEs in sediment samples it was necessary applied a preconcentration technique, such as DLLME prior to GC–MS/MS. DLLME was possible to carry out due to the type of solvent used in the USAL-DSPE stage. The efficiency of DLLME technique can be affected by several variables: extraction solvent type and volume, extraction temperature and time, and medium ionic strength. The disperser solvent used was acetone since the USAL-DSPE extract was constituted by this solvent. The assays were done by triplicate. The study and optimization of the above mentioned variables were performed by modifying one at a time while keeping the remaining constant.

3.2.1. Extraction solvent and volume

It is desired that the extraction solvent remains at the bottom of the centrifuge tube after phase separation for practical purposes. Additionally, the analyte must have affinity for the extraction solvent in order to efficiently extract it from the aqueous bulk. Moreover, the extraction solvent must be compatible with the analytical instrumentation to be used for the analyte determination. Taking into account these exigencies three organic solvents, including carbon tetrachloride, chloroform and trichloroethene were examined. The density and water solubility values of the selected organic solvents are 1.58 g mL^{-1} and 0.8 mg mL^{-1} (carbon tetrachloride), 1.48 g mL^{-1} and 8 mg mL^{-1} (chloroform) and 1.46 g mL^{-1} and 1.2 mg mL^{-1} (trichloroethene).

Study was carried out by adding 1 mL DSPE extract containing different volumes of extraction solvent to achieve similar volumes (60 μL) of the extraction phase to 5 mL ultrapure water placed in a 10 mL glass-centrifuge tube. The three solvents were able to get disperse into the aqueous bulk and form a biphasic system after centrifugation. Relative responses of the studied PBDEs as function of the extraction solvent are showed in Fig. 5a. All assayed solvents showed a good chromatographic behavior. The results revealed that the relative response of carbon tetrachloride is higher than chloroform and trichloroethene. Chloroform and trichloroethene have higher solubility in water than carbon tetrachloride. On the other hand, carbon tetrachloride has higher density than the remaining studied solvent. The solvent physicochemical properties condi-

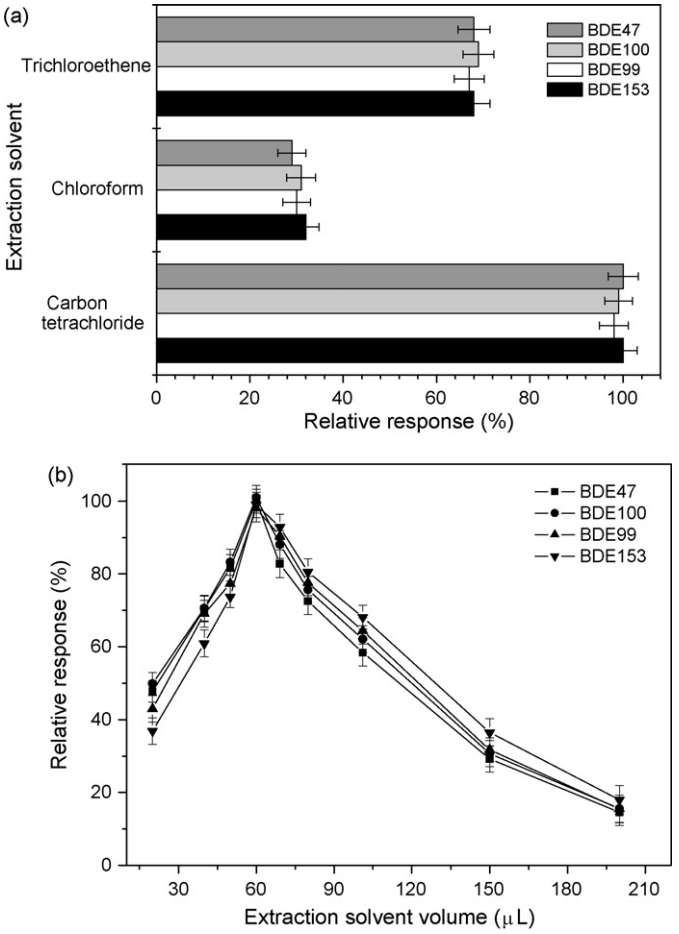

Fig. 5. Study of the DLLME extraction solvent on the relative response of PBDEs: (a) extraction solvent type and (b) extraction solvent volume. Extraction conditions: USAL temperature, 35 ± 2 °C; other USAL-DSPE conditions as described in Fig. 4. DLLME conditions as described in Fig. 1.

tioned the recovery of the extraction phase containing the extracted analytes after centrifugation. This phenomenon leaded to better relative response of the target analytes for carbon tetrachloride. Therefore, carbon tetrachloride was selected as the extraction solvent for further studies.

The volume of extraction solvent was studied within a volume range of 20–200 μL. The aim of this study was to determine the minimum volume of extraction solvent necessary to achieve the highest extraction efficiency and enrichment factor for the proposed preconcentration technique, DLLME. The extraction procedure was the one described above. From Fig. 5b it is possible to observe that by increasing the volume of carbon tetrachloride from 20 μL to 60 μL, the relative responses increases achieving the highest value for 60 μL. The relative response of the PBDEs obtained for smaller volumes were lower since the volume of carbon tetrachloride was insufficient to quantitatively extract the analytes. Higher volumes reported lower relative responses due to a dilution effect of the analytes into the resulting organic phase. Therefore, 60 μL carbon tetrachloride was selected to develop further studies.

3.2.2. Supplementation of disperser solvent volume

Variations of the disperser solvent affect the solubility of the extraction solvent and PBDEs in the aqueous bulk. As the disperser solvent volume increase, the resulting extraction phase diminishes due to an increment of the extraction solvent solubility. On the other hand, the increment of the disperser solvent volume enhance the PBDEs' solubility in water, thus extraction efficiency decreases. Therefore, it was important to determine the optimum volume of acetone that lead to the maximum extraction efficiency of the microextraction system. To determine the influence of the disperser solvent volume on the DLLME efficiency, different volumes of acetone, additionally to the USAL-DSPE extract aliquot, were added. The extra-acetone volume assayed ranged from 0 mL to 1 mL and the extraction procedure was the one described above. Within 0–0.5 mL volume range, the microextraction technique leaded the maximum relative responses for all four PBDEs. For extra-acetone volumes higher than 0.5 mL the relative response of PBDEs decreased due to the increment of the solubility of the PBDEs in water. Therefore, none extra-acetone was added for further studies.

3.2.3. Extraction temperature and time

Temperature can affect the extraction efficiency of the analytical technique. Through it affects the solubility of the analytes and extraction solvent in water, and thus dispersion phenomenon and the mass-transfer process. The extraction temperature effect was studied within a temperature range of 10–80 °C. The extraction procedure was the one described above. At low temperatures (<20 °C) low relative response values were observed. The carbon tetrachloride viscosity increases affecting negatively the dispersion phenomenon. At temperatures lower than 20 °C it was difficult to get a homogeneous dispersion, resulting in a prompt phase separation. Therefore, the mass-transfer process was limited to a short amount of time, leading to poor extraction efficiency, and consequently low relative responses of the PBDEs. In the 25–60 °C temperature range, the dispersion was easily achieved and remained invariant during the entire extraction time. However, the highest relative responses were obtained in the 30–40 °C temperature range. At a temperature higher than 60 °C, the carbon tetrachloride was completely dissolved into the aqueous bulk; therefore it was not possible to achieve a homogeneous dispersion. However, the phase separation was achieved by cooling down the tube and centrifuging it. Within this temperature range the relative responses of the PBDEs decreased notoriously. The increment of the temperature favored the solubility of PBDEs in water [28,29]. Based on this evidences, the working temperature selected for further studies was 35 °C.

Additionally to the temperature, the extraction time is crucial to achieve an efficient DLLME procedure. Due to the efficient dispersion of the extraction phase into the aqueous bulk, extraction phase is infinitely large; therefore the mass-transference phenomenon is widely favored and fast. However, it is important to consider the extraction time necessary to achieve the thermal equilibrium of the extraction system. The extraction time interval was defined as the time elapsed between extraction mixture addition and the end of the heating stage. In this sense, it was study the extraction time within the range of 0–15 min. The extraction procedure was the one described above. It was observed that by increasing

Table 2
USAL-DSPE-DLLME-GC–MS/MS analytical performance for PBDEs determination.

Analyte	RSD%[a,b]	r^2	LOD[a,b] (ng g^{-1})	Linear range[a] (ng g^{-1})
BDE-47	4.7	0.9992	0.02	0.07–1000
BDE-100	7.3	0.9996	0.03	0.09–1000
BDE-99	5.5	0.9996	0.03	0.10–1000
BDE-153	9.8	0.9991	0.06	0.19–1000

Extraction conditions as described in Section 2.4.
[a] 95% confidence interval; $n = 5$.
[b] PBDEs concentration 1 ng g^{-1}, dry weight.

Table 3
Recovery study of PBDEs in different environmental sediment samples.

Analyte	Sediment 1					Sediment 2				Sediment 3				
BDE	Level found	0.25 ng g^{-1} spiked		1 ng g^{-1} spiked		Level found	0.25 ng g^{-1} spiked		1 ng g^{-1} spiked		Level found	0.25 ng g^{-1} spiked		1 ng g^{-1} spiked	
		Found[a]	Recovery[b]	Found[a]	Recovery[b]		Found[a]	Recovery[b]	Found[a]	Recovery[b]		Found[a]	Recovery[b]	Found[a]	Recovery[b]
47	nd	0.21 ± 0.03	84	1.12 ± 0.13	112	0.32 ± 0.04	0.20 ± 0.04	80	0.80 ± 0.09	80	nd	0.23 ± 0.03	92	1.03 ± 0.12	103
100	nd	0.22 ± 0.03	88	1.04 ± 0.19	104	0.22 ± 0.04	0.21 ± 0.04	84	0.91 ± 0.16	91	nd	0.27 ± 0.05	108	0.89 ± 0.16	89
99	nd	0.27 ± 0.04	108	1.08 ± 0.15	108	0.26 ± 0.05	0.20 ± 0.03	80	0.83 ± 0.11	83	nd	0.25 ± 0.03	100	0.91 ± 0.12	91
153	nd	0.24 ± 0.06	96	0.98 ± 0.24	98	0.26 ± 0.06	0.22 ± 0.07	88	0.92 ± 0.22	92	nd	0.26 ± 0.06	104	1.11 ± 0.27	111

Extraction conditions as described in Section 2.4. nd: not detectable.
[a] Results expressed as $\bar{x} \pm t\ SD/\sqrt{n}$; $n = 3$; 95% confidence interval; ng g^{-1}.
[b] [(Found − base)/added] × 100.

the extraction time, the relative response increases, reaching the maximum value at 5 min, after which, remained constant. Therefore, 5 min extraction time was chosen as working conditions for further studies.

Centrifugation was required to break down the dispersion and accelerate the phase-separation process. In this way, different centrifugation times were assayed ranging from 2 min to 15 min at 3500 rpm (1852.2 × *g*). Similar results were achieved within the studied time frame; thus the minimum time (2 min) was selected as the centrifugation time necessary to get a satisfactory biphasic system.

3.3. Analytical performance and comparison with other analytical techniques

The calibration curve was made under optimized conditions using sediment samples free of PBDEs spiked at different concentration of target PBDEs. In order to evaluate the matrix effect on the analytical signals, the slope of the calibration graph of matrix-matched standards was compared with the slope of solvent calibration graph. The sensitivity decreased from pure solvent calibration to matrix-matched calibration curves. Therefore, quantification was carried out by using matrix-matched with increased concentrations of PBDEs.

The analytical figures of merits were summarized in Table 2. The MDL of the analytes for the extraction/preconcentration of 0.25 g sediment sample spiked with 1 ng g^{-1} each target PBDEs, calculated as S/N = 3 of chromatographic peaks were 0.02 ng g^{-1}, 0.03 ng g^{-1}, 0.03 ng g^{-1} and 0.06 ng g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively. The RSDs obtained were ≤9.8%. The calibration curves showed a satisfactory linearity within the concentration range: 0.07–1000 ng g^{-1} for BDE-47, 0.09–1000 ng g^{-1} for BDE-100, 0.10–1000 ng g^{-1} BDE-99 and 0.19–1000 ng g^{-1} for BDE-153; and the coefficient of estimation (r^2) exceeded 0.9991 for all analytes. The MDLs of the analytes extracted by reference Soxhlet technique were 0.04 ng g^{-1}, 0.05 ng g^{-1}, 0.07 ng g^{-1} and 0.08 ng g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153 respectively. Using a two-sample *t*-test at 95% confidence level, it can be concluded that there are no significant differences between MDLs of both methods ($P > 0.05$). Moreover, the MDLs obtained were similar to those reported by other authors using Soxhlet technique [30–32]. On the other hand, it can be remarked the relative merits of each extraction technique. USAL-DSPE-DLLME consumes smaller solvent volumes than Soxhlet technique and the extraction is carried out in shorter time. Furthermore, the inclusion of DSPE clean-up stage leaded into cleaner chromatograms. Additionally, the analytical performance for USAL-DSPE-DLLME-GC–MS/MS is comparable with other methodologies previously reported for PBDEs determination in sediment samples such as SPME and MAE [12,14,33]. In order to validate the analytical methodology, a recovery study of PBDEs at two different concentrations (0.25 ng g^{-1} and 1 ng g^{-1}) was carried out over the real sediment samples. This study led to a satisfactory robustness achieving recoveries ≥80% (Table 3) including samples with different TOC content.

3.4. Application to real samples

USAL-DSPE-DLLME-GC–MS/MS was applied for the determination of BDE-47, BDE-100, BDE-99 and BDE-153 in sediment samples. The sample analysis and recovery study were performed in triplicate (Table 3). As can be seen, sample 2 reported the presence of PBDEs at concentrations of 0.32 ng g^{-1}, 0.22 ng g^{-1}, 0.26 ng g^{-1} and 0.26 ng g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively. The PBDEs concentration in the others analyzed samples was below the detection limit of the proposed methodology. In order to test the performance of the proposed methodology

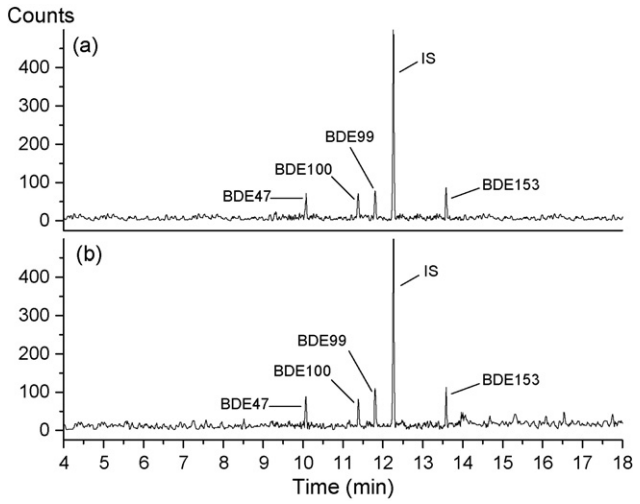

Fig. 6. Analysis of river sediment. EIC for 324, 326, 328, 402, 404, 406, 428, 482, 484, 486 and 496 *m/z*. (a) Sample 2 spiked at 5 ng g^{-1} of PCB 209 analyzed with USAL-DSPE-DLLME-GC–MS/MS. (b) Sample 2 spiked at 5 ng g^{-1} of PCB 209 and analyzed with Soxhlet-GC–MS/MS.

over sediments with naturally incurred PBDEs, the same samples were analyzed with reference Soxhlet extraction technique. PBDEs were detected in sample 2. The concentrations of analytes determined in this sample by reference Soxhlet technique were 0.40 ng g^{-1} (BDE-47), 0.25 ng g^{-1} (BDE-100), 0.31 ng g^{-1} (BDE-99) and 0.29 ng g^{-1} (BDE-153). The PBDEs content in sample 1 and sample 3 were below the detection limit of the Soxhlet-GC–MS/MS methodology. The results of both methodologies were compared side-by-side by using a two-sample *t*-test at 95% confidence level. It was observed that there are no significant differences between the results achieved with of methodologies ($P > 0.05$). Sample 2 was collected near to *Pescara waterway*, which is an artificial waterway that collects urban and industrial waste of the main metropolitan area of Mendoza province. Fig. 6 shows the chromatograms of the same river sediment sample analyzed with USAL-DSPE-DLLME-GC–MS/MS and Soxhlet-GC–MS/MS.

4. Conclusions

The proposed analytical approach based on USAL-DSPE-DLLME is an efficient extraction, clean-up and preconcentration alternative for PBDEs determination at trace levels in sediment samples. The combination of USAL-DSPE leaded to an increment of methodology selectivity and sensitivity; and it explains the DLLME preconcentration capabilities to complex sediment matrixes. Under optimized working conditions, MDLs were in the order of low nanogram per gram suitable for real world applications with an acceptable precision. USAL-DSPE-DLLME-GC–MS/MS showed comparable or lower MDLs with Soxhlet-GC–MS/MS, SPME-GC–MS/MS and MAE-GC–MS/MS methodologies. However, the proposed methodology offers a large time-saving and requires lower volumes of solvents. USAL-DSPE-DLLME employs simple and inexpensive equipment, and it is applicable for most of the analytical laboratories. Furthermore, the developed USAL-DSPE-DLLME provides good linearity, precision and quantitative recoveries. The proposed methodology has been applied for the extraction, clean-up, preconcentration and determination of PBDEs in real sediment samples with satisfactory robustness. One of the samples reported the presence of BDE-47, BDE-100, BDE-99 and BDE-153 and the obtained results were in agreement with those obtained by reference Soxhlet technique. USAL-DSPE-DLLME-GC–MS/MS analysis is appropriate as a potential methodology in routine analysis to determine trace levels of PBDEs in environmental sediments due to their simplicity, ruggedness and cost effectiveness.

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