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Determination of polybrominated diphenyl ethers in water and soil samples by cloud point extraction-ultrasound-assisted back-extraction-gas chromatography–mass spectrometry

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ABSTRACT

A novel and efficient analytical methodology is proposed for extracting and preconcentrating polybrominated diphenyl ethers (PBDEs) from samples of environmental interest prior gas chromatography–mass spectrometry (GC–MS) analysis. It is based on the induction of micellar organized medium by using a non-ionic surfactant (Triton X-114) to extract the target PBDEs. To enable coupling the efficient extracting technique with GC analysis, ultrasound-assisted back-extraction (UABE) into an organic solvent was required. Several factors, including surfactant type and concentration, equilibration temperature and time, ionic strength, pH and buffers nature and concentration were studied and optimized over the extraction efficiency of the proposed technique. Under optimal experimental conditions, the target analytes were quantitatively extracted achieving an enrichment factor of 250 when 10 mL aliquot of ultrapure water spiked with PBDE-standard mixture (10 pg mL^{-1} each PBDE) was extracted. Method detection limits (MDLs) calculated with aqueous PBDEs solutions as three times the signal-to-noise ratio (S/N), ranged from 1 to 2 pg mL^{-1} with RSDs values $\leq$8.5% (n = 5). The coefficients of estimation of the calibration curves obtained following the proposed methodology were $\geq$0.9987 and linear range of all PBDEs was 4–150 pg mL^{-1}. The proposed methodology was validated by carrying out a recovery study by spiking the samples at two different concentration levels of PBDEs (10 and 50 pg mL^{-1} for waters samples). Recoveries values in the range of 96–106% for water samples were obtained showing satisfactory robustness of the method for analyzing PBDEs in water samples. The proposed methodology was applied for the analysis of PBDEs: 2,2′,4,4′-tetraBDE (BDE-47), 2,2′,4,4,5-pentaBDE (BDE-99), 2,2′,4,4,6-pentaBDE (BDE-100) and 2,2,4,4′,5,5′-hexaBDE (BDE-153) in water samples, including drinking, lake, river water and soil samples. Significant quantities of PBDEs were not found in the analyzed samples.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are extensively used as flame retardants (FR) in various polymers such as plastics, textiles, electronic circuitry and other materials to prevent fires [1,2]. Some brominated flame retardants are additives mixed into polymers and are not chemically bound to the plastic or textiles. Therefore may separate or leach from the surface of their product applications into the environment when conditions are favorable [2]. Once in the environment, PBDEs can be very persistent or break down into other forms, depending on surrounding conditions [3]. Furthermore, their concentration levels found in global environment as well as in human and other biota samples have rapidly increased in the last three decades [4,5]. PBDEs are structurally similar to other environmental pollutants, such as dioxins, PCBs and PBBs. PBDEs are persistent, have low water solubility, high binding affinity to particles and a tendency to accumulate in soils, sediments and fat tissues. In this way, they can easily reach animals and humans via their food chain [6,7].

The number of analytical methodologies for determining PBDEs in environmental and biological samples has increased rapidly within the last few years [1,8,9]. Most of them are based on established methods for chlorinated pollutants. The analytical methodologies for determining environmental persistent pollutants, such as PBDEs, require highly sensitive and selective analytical technique for unequivocal identification and determina-

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tion of the target analytes. In this way, capillary gas chromatography (GC) with electron-capture detection (ECD) or mass spectrometry (MS) detection are the chosen techniques for this type of analysis [9,10]. Since PBDEs concentration levels in natural water and soil samples are typically low (water sample: <11 ng L^{-1}; soil sample: <5000 ng g^{-1}) [11–16], it is necessary to count on highly efficient preconcentration techniques for their determination by GC–MS. The extraction of PBDEs from environmental water samples has been carried out by using conventional liquid–liquid extraction (LLE), solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE) [8,17–20]. Cloud point extraction (CPE) is an efficient extraction technique based on micellar organized media, which, under particular physicochemical conditions, is *in situ* separated from the aqueous bulk, extracting the analytes of interest. CPE is a convenient alternative to conventional liquid–liquid extraction techniques due to its preconcentration capabilities for solubilizing different types of analytes. CPE have been used for efficiently preconcentrate metallic, organic and organo-metallic analytes prior their determination by several techniques [21]. The affinity of the analytes for the micelles is based on electrostatic and/or hydrophobic interactions [22]. Moreover, CPE technique is a low cost and simple operational procedure; and it is an environmentally friendly technique. The cloud point of a non-ionic surfactants aqueous-solution is the temperature at which the solution becomes turbid. It is due to a decrement of the amphiphile water solubility and to the sharp increase in the micelle aggregation number [22]. This arrangement favored the analytes affinity for the micelles. The cloud point phenomenon occurs in a narrow temperature range and depends on the nature of the amphiphile and its concentration. Above the cloud point temperature the solution experiences a phase separation of two isotropic phases. One of them is the "coacervate-phase" (surfactant-rich phase) in which the analytes are extracted; and the other one is the "aqueous phase" [21,23]. Coacervate-phase volume is typically small (<100 μL) and it decants into the bottom of the centrifuge tube, while the aqueous bulk remains on the top of it. CPE has been used prior several separative techniques such as HPLC and capillary electrophoresis [24], as well as non-separative one including flow injection analysis coupled to AAS and ICP-OES [25]. However, the use of CPE prior GC analysis has not been widely developed due to the nature of non-ionic surfactants, which is characterized by its high viscosity and low volatility.

To the best knowledge of the authors, only few works applied CPE as a preconcentration technique prior to GC analysis [26–30] but no one applied this technique for determining PBDEs in water and soil samples. In this work, a CPE technique for determining PBDEs in water and soil samples by GC–MS was developed. After extracting the target analytes from the aqueous bulk into the coacervate-phase, the analytes were ultrasound-assisted back-extracted into isooctane. 1 μL of the resulting isooctane phase was analyzed by GC–MS without the need of any supplemental clean-up. The analytical performance of the proposed method was evaluated in terms of detection limits (LODs), repeatability and linear working range. Moreover, the procedure was applied for the determination of PBDEs in drinking, lake, river water and soil samples and its robustness was evaluated in terms of recovery factors (RF%). It has been shown that the proposed methodology efficiently extracts and preconcentrates PBDEs and can be safely used for GC analysis.

2. Experimental

2.1. Reagents

The standards of PBDEs were purchased from Accustandard (New Haven, CT, USA) 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). The physicochemical properties of these four PBDEs are given in Table 1. Decachloro biphenyl (PCB-209) was used as internal standard (IS), and was purchased from Dr. Ehrenstorfer (Augsburg, Germany). 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 μg mL^{-1}. Further dilutions were prepared monthly in methanol and stored in brown bottles at −20 °C.

Methanol, ethanol, dichloromethane, chloroform, hexane, ether and isooctane were purchased from Merck (Darmstadt, Germany). Triton X-114 and Triton X-100 were purchased from Sigma–Aldrich (Steinheim, Germany) and used without further purification. PONPE 7.5 was purchased from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). 100 g L^{-1} aqueous stock solution of each non-ionic surfactant was prepared. Citric acid and sodium citrate were all from Mallinckrodt Chemical Works (New York, Los Angeles, St. Louis, USA). Sodium chloride, sodium hydroxide, sodium tetraborate, potasic phosphate, acetic acid and sodium acetate were all from Merck. The buffers solutions were prepared with ultrapure water and the final concentrations were as follows: citrate (0.1 mol L^{-1}, pH 5.6), acetate (0.03 mol L^{-1}, pH 4.0), phosphate (0.01 mol L^{-1}, pH 7.0) and tetraborate (0.05 mol L^{-1}, pH 9.22). Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were analytical of grade or above.

2.2. Equipment and working conditions

A 40 kHz and 600 W US-bath (Test Lab, Buenos Aires, Argentina) was used for assisting the back-extraction process. The volume of coacervate-phase was measured using a 250-μL Hamilton glass syringe (Reno, NV, USA). Injections into the GC–MS were made by using a 5.0-μL Hamilton glass syringe Hamilton. GC–MS analyses were carried out on a Clarus 500 gas chromatograph equipped with Clarus 500 single-quadrupole mass spectrometer detector (PerkinElmer, Shelton, CT, USA). The GC column used was an Elite 5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness PerkinElmer, Shelton, CT, USA). The temperature program of the GC oven was the following one: 150 °C, held 1 min; rating 40 °C min^{-1} to 250 °C; rating 10 °C min^{-1} to a final temperature of 300 °C and held for 7 min. The injector temperature was set at 300 °C and the injections were carried out in the splitless mode. The mass spectrometer was operated in electron impact ionization mode at 70 eV. The transfer line, ion source and quadrupole analyzer temperatures were maintained at 300, 260 and 150 °C, respectively. Samples were analyzed in single ion recording (SIR) mode. The peak identification was based on the base peak and the isotopic pattern of the PBDEs congeners. Specific ions were selected for each PBDE congener and the base ion was selected as a quantitative ion, while two other ions were used as qualifiers (Table 1). Peak identification and quantification were carried out against PCB-209 IS. Neither PBDEs nor IS showed interfering peak from the surfactant (Fig. 5).

2.3. Sampling and sample preparation

For tap water samples collection, domestic water was allowed to run for 20 min and then it was collected. River and lake water samples were collected from *Las Tunas River* and *Cipolleti Lake* of Mendoza, Argentina, respectively; at a depth of 20 cm. 1000 mL water samples aliquots were collected. All samples were collected free of air bubbles in amber glass containers and carried to the laboratory in cooled boxes. Once in the laboratory, samples were filtered through 0.22 μm pore size membrane filters and analyzed within 24 h. The surface soil samples were taken from rural/remote

Table 1
GC–MS-SIR parameters for PBDE determination.

Analytes	t_R' (min)	Target ion (m/z)	Confirmation ions (m/z)	b.p. (°C)	log K_{ow} [31]
BDE-47	0.64	486	484, 326	396	6.81
BDE-100	0.80	404	564, 406	434	7.24
BDE-99	0.84	404	564, 406	416	7.32
BDE-153	1.09	484	644, 486	453	7.90

t_R': relative retention times to PCB-209. b.p.: boiling point. log K_{ow}: octanol/water partition coefficient.

sites in Mendoza province (i.e., away from cities, roads, or other human activity). Samples were dried, homogenized in a porcelain mortar, sieved through a 0.3-mm stainless steel sieve, and stored in darkness at room temperature until analysis.

2.4. Cloud point extraction and ultrasound-assisted back-extraction procedures

2.4.1. Water analysis

A 10 mL water sample aliquot was placed into a 15-mL glass-centrifuge tube. 40 μL Triton X-114 100 g L^{-1}, 400 μL 6.15 mol L^{-1} NaCl and 500 μL 0.1 mol L^{-1} citrate buffer pH 5.6 were subsequently added and mixed-up. The centrifuge tube was thermostatized at 80 °C for 7 min. Under these conditions, the system reached easily the cloud point and the coacervate-phase started to get separated from the aqueous bulk. The tube was centrifuged at 3500 rpm (1852.2 × g) for 5 min to accelerate the coacervate-phase decantation. In order to increase the coacervate-phase viscosity and extract easily the aqueous supernatant, the centrifuge tube was placed into an ice bath for 3 min. The ultrasound-assisted back-extraction was carried out by adding 50 μL of isooctane into the resulting coacervate-phase and sonicating the system for 5 min. Again, two phases were formed: the coacervate-phase and the isooctane one. This time the analytes remained in the isooctane phase, which result on the top of the coacervate-phase one. A 1 μL of the isooctane phase was injected and analyzed into the GC–MS.

2.4.2. Soil samples

A 0.5 ± 0.1 g sample were placed into a 15 mL glass-centrifuge tube, followed by the addition of 10 mL of doubly distilled water. The mixture was sonicated during 1 h. The resulting slurry was extracted following the CPE procedure described above. After centrifugation, the non-dissolved soil remained at the button of the tube and the coacervate-phase on the top of it. After extracting the aqueous bulk, the coacervate-phase was back-extracted by adding 50 μL of isooctane and sonicating by 5 min. Although the resulting isooctane phase did not show any particulate material within its bulk, it was filtered through 0.22 μm pore size membrane filters to avoid any clog in the GC injector. Finally, 1 μL of the filtered isooctane phase was injected and analyzed into the GC–MS.

3. Results and discussion

CPE process can be altered by modifying different variables such as sample pH, surfactant concentration, equilibration time and temperature, matrix modifiers and ionic strength. Therefore, to achieve the greater extraction efficiency of the four PBDEs from the aqueous bulk, these variables needed to be studied and optimized in order to establish the working conditions. These studies were carried out by modifying one of them at the time keeping the remaining one constant. 10 mL PBDE standard-mix (each PBDE 1 ng mL^{-1}) was used to perform the assays, which were done by triplicate. The chromatographic peak area was used to evaluate the system extraction efficiency under different experimental conditions.

3.1. pH and buffer effects

CPE was not previously applied for PBDE extraction; therefore there was no evidence on the effect of pH on CPE extraction efficiency for the studied analytes. However, some authors found no pH effect on the extraction efficiency when other extraction techniques, such as SPME [18], were applied for extraction of PBDEs. The pH study was carried out by adjusting the pH of the extraction solution (10 mL 1 ng mL^{-1} each PBDE) with hydrochloric acid and sodium hydroxide, respectively. The pH was measured into the initial aqueous solution before carrying out the extraction; and into the supernatant, after carrying out the CPE. No significant differences were found between the pH values and no changes were observed on the CPE efficiency when the extraction was carried out within the pH range: 2–12. This can be attributed to the fact that neither PBDEs chemical properties nor micellar organization system is affected by the pH [22]. Four different buffers, including citrate (pH 5.6), acetate (pH 4.0), phosphate (pH 7.0) and tetraborate (pH 9.22), were evaluated. As can be seen from Fig. 1, when no buffer was added to the CPE of the four target analytes, the relative response was lower than when some buffer solution was added. For the studied buffers, citrate (pH: 5.6) was the one that reported the higher relative response. This might be attributed to the organic nature of this buffer, which could modify the micelles structure, and thus the PBDEs affinity for them. Additionally to the buffer nature, buffer concentration was study within a concentration range of 2.5×10^{-3} to 0.5 mol L^{-1}. It was observed that for 4×10^{-3} mol L^{-1} the extraction technique lead the greatest relative response for all PBDEs. For concentrations higher than 4×10^{-3} mol L^{-1}, the analytical response remained invariant. Therefore, in order to work under the best condition, but enough far from the edge of the curve, a concentration higher than 4×10^{-3} mol L^{-1} (5×10^{-3} mol L^{-1}) was chosen for further studies to assure reproducible results. The pH

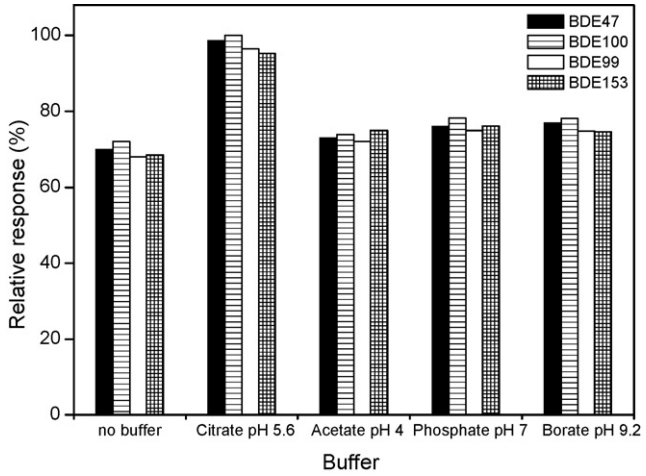

Fig. 1. Buffer type effect on the relative response of target PBDEs. Extraction conditions: 10 mL sample, 1 ng mL^{-1} each PBDE; 400 μL 6.15 mol L^{-1} NaCl; 500 μL of each buffer; 40 μL 100 g L^{-1} Triton X-114; equilibration temperature and time: 80 °C, 10 min; centrifugation time: 5 min; ultrasound-assisted back-extraction: 50 μL isooctane, 5 min.

of all standard and sample solutions was adjusted to 5.6 by adding 500 μL 0.1 M citrate buffer solution.

3.2. Surfactant type and concentration

Three non-ionic surfactants were studied to carry out the CPE of the target PBDEs: Triton X-100, Triton X-114 and PONPE-7.5. The cloud points of Triton X-100, Triton X-114 and PONPE-7.5 are about 65, 24 and 15 °C, respectively [25,29]. The CPE of the target PBDEs was carried out at 80 °C. It is well known that the higher enrichment factors are generally achieved when the CPE process is carried out at equilibration temperatures well above the cloud point temperature of the system [22]. Although all of these micellar systems achieved the cloud point, it was possible to evaluate the extraction efficiency only for Triton X-100 and Triton X-114. By using PONPE-7.5 and 150 μL isooctane it was not possible to achieve the phase separation in the ultrasound-assisted back-extraction stage. A stable emulsion was formed due to the partial solubility of coacervate-phase into the isooctane one, which was not compatible with GC–MS analysis. Triton X-100 and Triton X-114 lead quantitative extractions and their resulting coacervate-phase volumes were 30 and 60 μL, respectively. The isooctane volume required for quantitatively back-extract the analytes and achieve the maximum enrichment factor was studied within 20–200 μL range. It was observed that a minimum volume equal to the coacervate-phase one was required to avoid forming a stable emulsion and achieve a quantitative extraction of the analytes. Larger volumes leaded into a decrement of the analytical response due to subsequent dilutions. 50 and 100 μL isooctane was required to quantitatively back-extract the target PBDEs and efficiently separates the resulting phases, when Triton X-114 and Triton X-100, respectively. Therefore, due to the experimental and analytical convenience, Triton X-114 was chosen for further studies.

As it is well known, surfactant concentration above the critical micellar concentration is required to achieve the cloud point of the system [22]. The critical micelle concentration is defined as the concentration of surfactants above which micelles are spontaneously formed. The surfactant concentration should lead the greater extraction efficiency of the technique. However, it is important to consider that the volume ratio between the aqueous bulk and the coacervate-phase increases as decreases the surfactant concentration [21]. The surfactant concentration study was carried out within the range 0.25–2.00 g L^{-1} Triton X-114. As can be observed from Fig. 2(a), the greater relative response for the target PBDEs was achieved for the concentration range: 0.25–0.50 g L^{-1}. Excessive surfactant concentration decreased the enrichment factor and made the back-extraction process unpractical. Smaller concentrations than 0.25 g L^{-1} lead to coacervate-phase volumes <20 μL, which induced to imprecise separation of the supernatant affecting the reproducibility of the technique. Therefore, a compromised situation should be reached in order to achieve the greater extraction efficiency and enrichment factor of the system, as well as a satisfactory reproducibility of the results. 0.4 g L^{-1} Triton X-114 was selected as optimum for PBDEs-related CPE procedure.

3.3. Effect of the ionic strength

It is well known that the ionic strength of the aqueous medium can affect the phase-separation process of micellar systems based on non-ionic surfactant [22]. As the ionic strength is increased, the micelle size and the aggregation number are increased; but the critical micellar concentration remains constant [21]. In addition, the affinity of non-polar analytes for the heart of the micelles is strengthened and the resulting extraction efficiency values of the CPE technique are enhanced. However, discrepancies have been reported about the salting out effect on the extraction efficiency

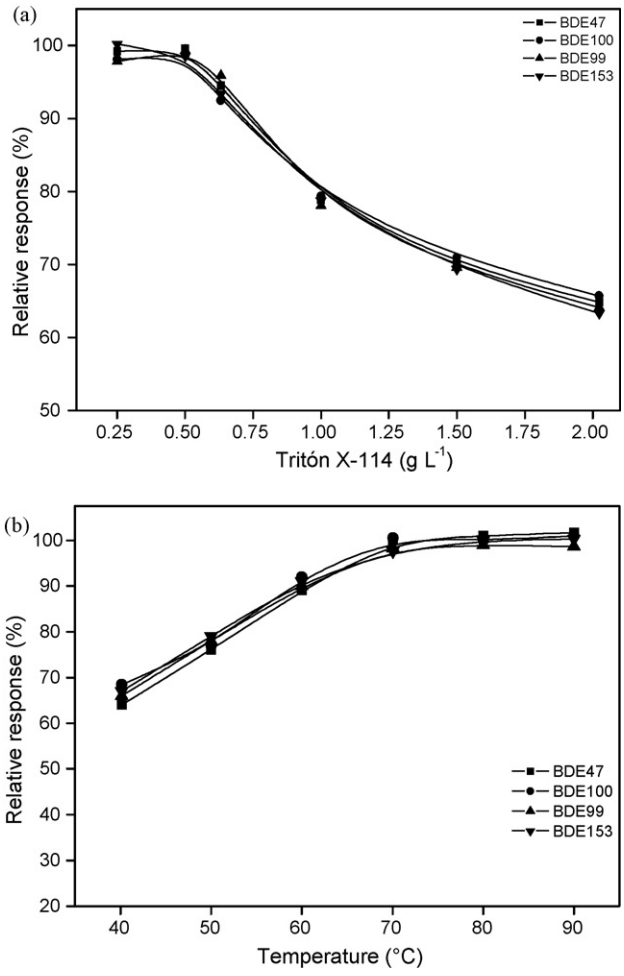

Fig. 2. (a) Effect of surfactant concentration on the relative response of PBDEs. (b) Effect of equilibration temperature on the relative response of PBDEs. Extraction conditions: 10 mL sample, 1 ng mL^{-1} each PBDE; 400 μL 6.15 mol L^{-1} NaCl; 500 μL 0.1 mol L^{-1} citrate buffer pH 5.6; 40 μL 100 g L^{-1} Triton X-114; equilibration temperature and time: 80 °C, 10 min; centrifugation time: 5 min; ultrasound-assisted back-extraction: 50 μL isooctane, 5 min.

of this type of analytes when other extraction techniques, such as SPME, SBSE and DLLME were carried out [19,31,32]. In this work, the ionic strength study was carried out by adding different volumes of 6.15 mol L^{-1} sodium chloride to the extraction system. The assays ionic strength values were within the range: 0.00–3.40 mol L^{-1}. It was observed that ionic strength values higher than 0.4 mol L^{-1} favored the phase separation of the system and enhanced the enrichment factor of the technique. For ionic strength values higher than 0.4 mol L^{-1} the relative response of PBDEs remained invariant. Therefore, 400 μL 6.15 mol L^{-1} NaCl was added to the CPE for further studies.

3.4. Effect of equilibration temperature and time

Equilibration time and temperature play important roles in the CPE performance. Those variables govern the micelles dehydration process, which is desirable to achieve smaller coacervate-phase volumes, and also to accelerate the phase-separation process. It is reported that by increasing the equilibration temperature or time is favored the micelles dehydration phenomenon, and thus smaller coacervate-phase volumes are achieved [21]. By increasing the extraction temperature, also diminished the aqueous solubility of the micelles [22]. As it was mentioned above, smaller coacervate-phase volumes were desired since they favored the

ultrasound-assisted back-extraction process and enhanced the enrichment factor of the CPE technique. On the other hand, faster sample preparation procedures were preferred in order to increase the sample throughput of the technique. Therefore, two different studies were carried out. In the first one, the equilibration time was kept constant at 13 min meanwhile the equilibration temperature was varied between 40 and 90 °C. The results are shown in Fig. 2(b). An increment in the relative response of the analytical signal was observed for the temperature range: 40–70 °C. After 70 °C the relative response of the PBDEs remained invariant. Therefore, a working equilibration temperature of 80 °C was chosen for further studies. Afterwards, the equilibration temperature was set at 80 °C and different equilibration times ranging from 3 to 20 min were assayed. No significant changes in the relative response of the analytical signals were observed after 5 min. Therefore 7 min was selected as equilibration time in this work.

3.5. Ultrasound-assisted back-extraction

Due to the high viscosity and low volatility of the surfactant-rich phase, it cannot be injected directly into the GC. Therefore, after CPE extraction and before the injection, a supplemental stage was required in order to avoid clogging the injector and deteriorate the column. Ultrasound-assisted back-extraction was selected as a suitable approach for coupling CPE to GC–MS.

3.5.1. Effect of solvent

Different water-immiscible solvents (hexane, isooctane, chloroform, ether and dichloromethane) were studied in order to evaluate their back-extraction efficiencies for extracting the target analytes from of coacervate-phase. The study was carried out by adding 50 μL of the studied solvent to the coacervate-phase and sonicating the resulting mix for 7 min. When chloroform or dichloromethane were added, they were completely solubilized into the coacervate-phase. Therefore, they were not further used. On the other hand, the three remaining solvents formed two-phase systems. The relative response of the systems revealed that the extraction efficiency of isooctane is higher than hexane and ether. Thereby, isooctane was selected as the back-extracting solvent for further studies. The isooctane volume was investigated within the range: 25–400 μL with a view to recover the target PBDEs from the coacervate-phase yielding the highest enrichment factor with the minimum solvent consumption. Fig. 3 shows that the greater relative response for the target PBDEs were obtained when 50 μL isooctane were used. When 25 μL aliquot of isooctane was used, a stable emulsion with the coacervate-phase was formed. Volumes larger than 50 μL result in a gradual decrease of the relative response of the analytes due to subsequent dilution. The resulting isooctane volume after the ultrasound-assisted back-extraction stage was 40 μL. Therefore; 50 μL of isooctane were selected to develop further studies.

3.5.2. Effect of sonication time

It is the ultrasound-assisted back-extraction makes possible coupling CPE to GC–MS; and is governed by the ultrasound-source power and the sonication time. Since it was not possible to vary the ultrasound-source power, only the sonication time was studied with in the range of 1–15 min. As can be seen in Fig. 4, the relative response of the analytes increased as the time increase, reaching a maximum at 4 min. No significant increments were observed when longer periods of time were assayed. Thus, 5 min were chosen as the ultrasound-assisted back-extraction time for further studies.

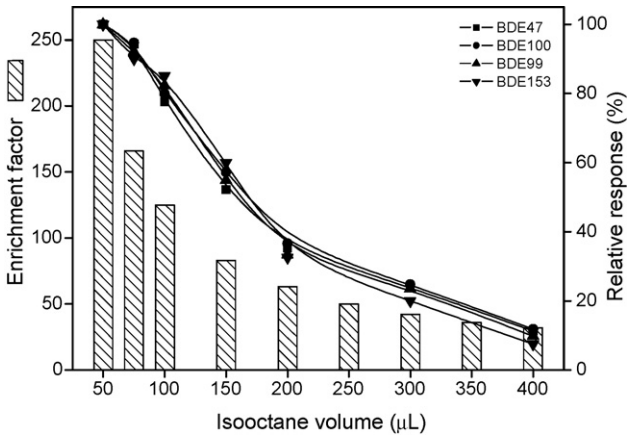

Fig. 3. Correlation between the isooctane added, enrichment factor and relative response of analytes. Extraction conditions as described in Fig. 2.

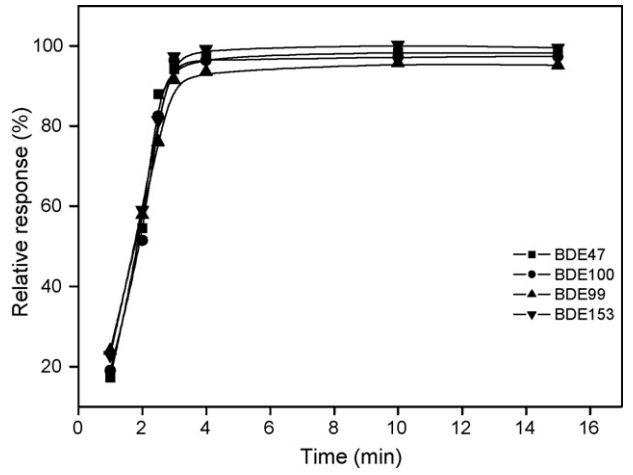

Fig. 4. Effect of ultrasound-assisted back-extraction time on the relative response for PBDEs. Extraction conditions as described in Fig. 2.

3.6. Analytical performance

The analytical figures of merits of the proposed methodology were summarized in Table 2. For an aqueous sample volume of 10 mL, the achieved extraction efficiency was >99.9% when the procedure was carried out under optimum conditions. The obtained EF for a sample volume of 10 mL and a resulting isooctane phase volume of 40 μL was 250. EF was calculated as the ratio between

Table 2
Analytical performance of the CPE-UABE-GC–MS method[a].

PBDEs	Water sample				Soil sample			
	RSD (%)	Linear range (pg mL^{-1})	r^2	LOD (pg mL^{-1})	RSD (%)	Linear range (ng g^{-1})	r^2	LOD (ng g^{-1})
BDE-47	4.2	5–150	0.9989	1.2	5.0	4–5000	0.9984	1.0
BDE-100	6.2	4–150	0.9987	1.0	6.6	12–5000	0.9990	3.7
BDE-99	6.9	6–150	0.9992	1.5	7.2	5–5000	0.9993	1.5
BDE-153	8.3	7–150	0.9994	2.0	8.5	5–5000	0.9992	1.5

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

the initial aqueous volume and the resulting isooctane one after the CPE-UABE procedure. The MDL of the analytes for the preconcentration of 10 mL sample volume, calculated as three times the signal-to-noise ratio (S/N = 3), were 1, 1, 2 and 2 pg mL^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively. The precision of CPE-UABE-GC–MS was evaluated over five replicate, resulting RSDs ≤8.3%. The calibration curves showed a satisfactory linearity within the concentration range: 5–150 pg mL^{-1} for BDE-47, 4–150 pg mL^{-1} for BDE-100, 6–150 pg mL^{-1} for BDE-99 and 7–150 pg mL^{-1} for BDE-153; and the coefficient of estimation (r^2) exceeded 0.9987 for all analytes. In order to validate the proposed methodology, a recovery study of the four PBDEs at two different concentration levels (10 and 50 pg mL^{-1}) was carried out over the real water samples. The recovery studies showed satisfactory robustness leading recovery ≥96% (Table 3). The developed CPE-UABE-GC–MS method was also applied to soil samples free of PBDEs. 0.5 g aliquots of this sample was spiked with individual standards of the target analytes and analyzed as described above. The achieved MDLs for the analytes were 1, 4, 2 and 2 ng g^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively. The RSDs obtained in soil samples were ≤9.5%. The calibration curves showed a satisfactory linearity within the concentration range: 4–5000 ng g^{-1} for BDE-47, 12–5000 ng g^{-1} for BDE-100, 5–5000 ng g^{-1} for BDE-99 and 5–5000 ng g^{-1} for BDE-153; and the coefficient of estimation (r^2) exceeded 0.9971 for all analytes. However, the recovery values obtained when a recovery study was carried out at two concentration levels (25 and 100 ng g^{-1}) was ca. 20%.

In order to evaluate the number of samples feasible to prepare and analyze without suffering any degradation of the analytes, 10 mL of aqueous solution containing 1 ng mL^{-1} of each PBDE was extracted as described above. The organic phase containing the extracted PBDEs was analyzed in consecutive injections during a 24-h period of time and the relative areas of each PBDE were compared. It was observed that up to 10 h, no significant changes in the relative peak areas were detected (≤10%). The relative peak areas analyzed after 24 h showed a 50% of retreat. The signal deterioration could be due to photo-degradation of PBDEs in the organic medium which is dependent on the degree of bromination. Similar results were already reported by other authors [33–35]. Therefore, the sample preparation was performed within the same day of the samples analysis.

3.7. Application to real samples

CPE-UABE-GC–MS was applied for the determination of BDE-47, BDE-99, BDE-100 and BDE-153 in three environmental water samples including, drinking water, lake water, river water and two soil samples. The samples were collected and immediately analyzed as described above. No matrix effects were observed even in the most complex samples, therefore quantification could be carried out by external calibration using PBDEs standard solutions prepared in isooctane spiked with 1 ng mL^{-1} PCB 209. The sample results and the recovery study were carried out in triplicate. For water samples satisfactory recoveries (>96%) were obtained, showing CPE-UABE-GC–MS as a robust methodology for determining PBDEs in such samples (Table 3). Soil samples lead recoveries values ca. 20%. However, the method showed an acceptable precision and this notion presents an opportunity for further analytical developments in this type of environmental samples. The PBDEs contents in the analyzed samples were below the detection limit of the proposed methodology. Fig. 5b shows the chromatogram of a lake water sample spiked with 1 ng mL^{-1} PCB 209, Fig. 5c shows the chromatogram of the same sample spiked with 1 ng mL^{-1} PCB 209 and 80 pg mL^{-1} of target PBDEs and Fig. 5d shows the chromatogram of a soil sample spiked with 500 ng g^{-1} PCB 209 and 50 ng g^{-1} of target PBDEs.

Table 3
Method validation.

PBDEs	Drinking water						Lake water						River water					
	Level found	10 pg mL^{-1} spiked		50 pg mL^{-1} spiked			Level found	10 pg mL^{-1} spiked		50 pg mL^{-1} spiked			Level found	10 pg mL^{-1} spiked		50 pg mL^{-1} spiked	
		Found[a] (pg mL^{-1})	Recovery[b] (%)	Found[a] (pg mL^{-1})	Recovery[b] (%)			Found[a] (pg mL^{-1})	Recovery[b] (%)	Found[a] (pg mL^{-1})	Recovery[b] (%)			Found[a] (pg mL^{-1})	Recovery[b] (%)	Found[a] (pg mL^{-1})	Recovery[b] (%)
BDE-47	nd	9.9 ± 1.0	99	49.0 ± 5.1	98		nd	10.6 ± 1.1	106	52.5 ± 5.5	105		nd	10.8 ± 1.1	108	53.0 ± 5.5	106	
BDE-100	nd	10.9 ± 1.7	109	52.0 ± 8.0	104		nd	9.8 ± 1.5	98	51.0 ± 7.8	102		nd	10.3 ± 1.6	103	52.0 ± 8.0	104	
BDE-99	nd	10.2 ± 1.7	102	49.5 ± 8.5	99		nd	9.7 ± 1.7	97	53.5 ± 9.2	107		nd	9.7 ± 1.7	97	51.0 ± 8.7	102	
BDE-153	nd	9.7 ± 2.0	97	48.0 ± 9.9	96		nd	9.9 ± 2.0	99	48.5 ± 9.9	97		nd	10.4 ± 2.1	104	48.5 ± 9.9	97	

Extraction conditions: 10 mL sample, 1 ng mL^{-1} each PBDE; 300 μL 6.15 mol L^{-1} NaCl; 40 μL 100 g L^{-1} Triton X-114; 0.5 mL 0.1 mol L^{-1} citrate buffer pH 5.6; equilibration temperature and time: 80 °C, 7 min, respectively; centrifugation time: 2 min; ultrasound-assisted back-extraction: 50 μL isooctane, 5 min. nd: not detectable.

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

[b] [(found − base)/added] × 100.

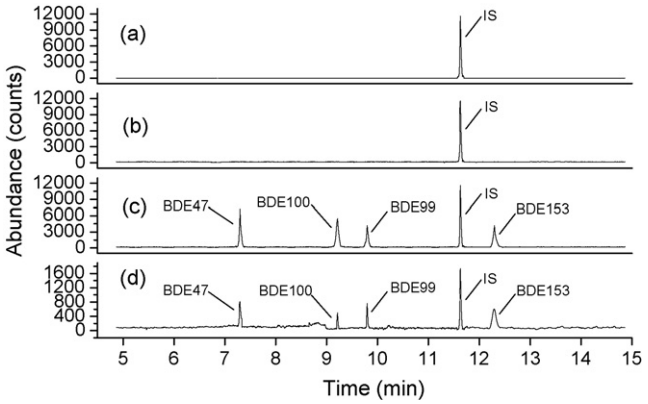

Fig. 5. SIR for *m*/*z* 326, 406, 484, 486, 564 and 644. Analysis of lake water and soil samples. (a) Blank spiked at 1 ng mL^{-1} of PCB 209 (b) Sample spiked at 1 ng mL^{-1} of PCB 209 and (c) Sample spiked with 1 ng mL^{-1} PCB 209 and 80 pg mL^{-1} of each PBDE. (d) Soil sample spiked with 500 ng g^{-1} PCB 209 and 50 ng g^{-1} of target PBDEs. Extraction conditions as described in Fig. 2.

3.8. Comparison of CPE-UABE-GC–MS with other analytical methodologies

The analytical performance of CPE-UABE-GC–MS for PBDEs determination in water samples was compared with other analytical techniques previously reported (Fig. 6). It can be observed that the analytical performance for CPE-UABE-GC–MS is comparable with the commonly used techniques for PBDEs determination. Only SPME-GC–MS/MS showed lower LODs than the CPE-UABE-GC–MS, but the mean RSDs values were higher. CPE employs simple and inexpensive equipment so it is applicable for most of the analytical laboratories. Moreover, the extraction equilibrium is established within a few minutes. All these results disclosed that CPE-UABE-GC–MS is a sensitive, rapid, versatile and reproducible technique. Additionally, it is important to point out that this methodology is a low organic solvent consuming extraction technique, which turns it into a low cost and environmentally friendly technique.

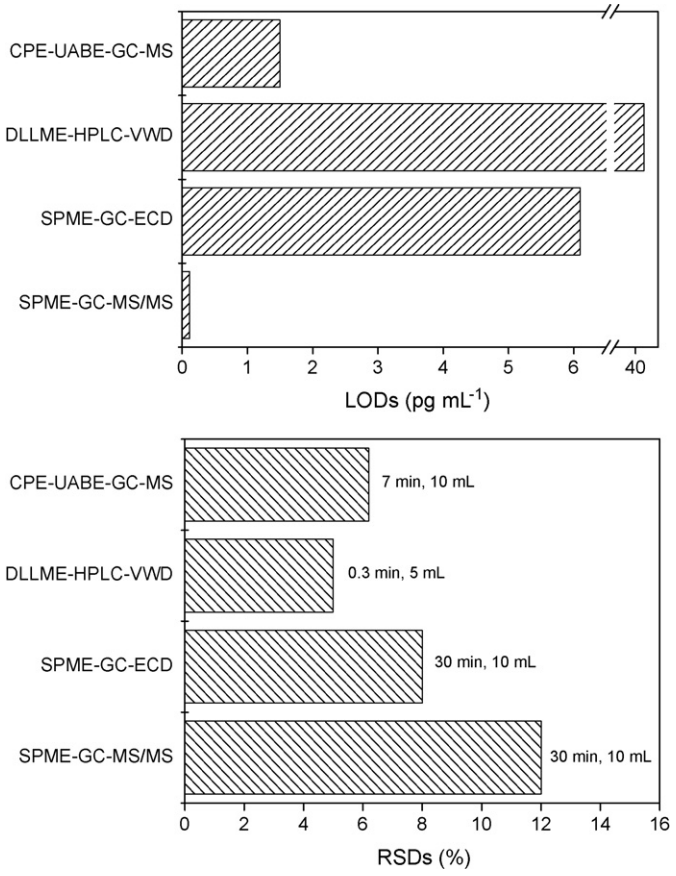

Fig. 6. Comparison of CPE with SPME and DLLME for determination of PBDEs in water samples. SPME-GC–MS/MS: solid-phase microextraction and gas chromatography–tandem mass spectroscopy [31]. SPME-GC-ECD: solid-phase microextraction and gas chromatography-electron capture detection [17]. DLLME-HPLC-VWD: dispersive liquid–liquid microextraction and high performance liquid chromatography-variable wavelength detection [32].

4. Conclusions

The application of the proposed analytical methodology based on CPE proved to be effective for the extraction and preconcentration of PBDEs at trace levels in samples of environmental interest. Under optimized working conditions, high EF were obtained for the target analytes allowing reaching detection limits suitable for real world applications with an acceptable precision. The back-extracted analytes were introduced to GC–MS successfully without declining the separation efficiency of the capillary column. An important aspect of the proposed methodology to point out is the low organic solvent consumption, which turns it into a low cost and environmentally friendly technique. The robustness of the proposed methodology was proved when the recovery study was carried out over the real samples; no matrix effect was observed for quantification even in the most complex samples. This fact allowed performing the quantification by using external standard prepared in isooctane and contributed to simplify the sample analysis. It improved the sample throughput of this methodology compared with other more traditional techniques, such as SPME. The proposed methodology was successfully applied for the analysis of PBDEs in soil samples; however, it is evident more efforts need to be invested in this analytical field in order develop new microextraction technique for environmental samples analysis, including soil. The proposed CPE-UABE-GC–MS analysis is well suited as a potential method in routine analysis to determine trace levels of PBDEs in environmental matrices.

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