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Simple approach based on ultrasound-assisted emulsification-microextraction for determination of polibrominated flame retardants in water samples by gas chromatography–mass spectrometry

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ABSTRACT

A simple, efficient, innovative and environmentally friendly analytical technique was successfully applied for the first time for the extraction and preconcentration of polybrominated diphenyl ethers (PBDEs) from water samples. The PBDEs selected for this work were those most commonly found in the literature in natural water samples: 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). The extracted PBDEs were separated and determined by gas chromatography–mass spectrometry (GC–MS). The extraction/preconcentration technique is based on ultrasound-assisted emulsification-microextraction (USAEME) of a water-immiscible solvent in an aqueous medium. Several variables including, solvent type, extraction time, extraction temperature and matrix modifiers were studied and optimized over the relative response the target analytes. Chloroform was used as extraction solvent in the USAEME technique. Under optimum conditions, the target analytes were quantitatively extracted achieving enrichment factors (EF) higher than 319. The detection limits (LODs) of the analytes for the preconcentration of 10 mL sample volume were within the range 1–2 pg mL^{-1}. The relative standard deviations (RSD) for five replicates at 10 pg mL^{-1} concentration level were <10.3%. The calibration graphs were linear within the concentration range of 5–5000 pg mL^{-1} for BDE-47 and BDE-100; and 5–10,000 pg mL^{-1} for BDE-99 and BDE-153, respectively. The coefficients of estimation were ≥0.9985. Validation of the methodology was performed by standard addition method at two concentration levels (10 and 50 pg mL^{-1}). Recovery values were ≥96%, which showed a successful robustness of the analytical methodology for determination of picogram per milliliter of PBDEs in water samples. Significant quantities of PBDEs were not found in the analyzed samples.

1. Introduction

In the last 50 years polymer industry has notoriously grown, providing polymers with particular properties, which have spread and diversified their applications [1,2]. Due to the flammable character of many polymers it is necessary to add flame retardant (FR) compounds in order to complement safety regulation for their commercialization. Among the most commonly used FR, brominated flame retardants (BFRs) has been the most applied group due to the high efficiency of the bromine atoms to capture free radicals generated during the combustion process [1–3]. Polybrominated diphenyl ethers (PBDEs) are included in this group of compounds. PBDEs have become a ubiquitous analyte of the environment because of their widespread use, and their predisposition to be released from the polymeric mass. 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 [3]. PBDEs have a non-polar character, which favoured their bioaccumulation in hydrophobic mediums in biota, such us humic substances and fat tissues. In this way, they can easily reach animals and humans via their food chain [4,5]. Several epidemiological studies have shown PBDEs to pose health risks [6–9] such as endocrine disruption and adverse neurobehavioral effects. They also act as feasible reproductive toxicants, and probable carcinogens [9].

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As a consequence of the occurrence of PBDEs in the environment, there is a growing interest in developing analytical methods for determining them in these types of samples [10–12]. Sample preparation plays an important role in the determination of PBDEs in environmental samples because of the matrixes complexity and the low concentration of these analytes. Highly selective and sensitive analytical techniques are required for their unequivocal identification and determination. In this way, capillary gas chromatography (GC) with electron-capture (ECD) or mass spectrometry (MS) detection are the chosen techniques for this type of analysis [3]. Since PBDEs concentration levels in natural water samples are regularly low ($\leq$80 ng L^{-1}) it is necessary to count on highly efficient preconcentration techniques for their determination by GC [13–15]. The extraction of PBDEs from environmental water samples has been usually carried out by using conventional liquid–liquid extraction (LLE), solid-phase microextraction (SPME) or stir bar sorptive extraction (SBSE) [16–20]. In the past few years new extraction techniques, especially in the microextraction category, have gained interest. Efforts have been placed on the miniaturization of the LLE extraction procedure by greatly reducing the required organic solvent amount. In this way, Jeannot and Cantwell have developed a liquid-phase microextraction (LPME) technique, which is based on analyte partition between a drop of organic solvent (extraction phase) and the aqueous sample bulk [21]. Microextraction techniques are fast, simple, inexpensive, environmentally friendly and compatible with many analytical instruments [22]. Up to now, several different types of LPME have been developed including, single drop microextraction (SDME) [23], hollow fiber LPME [24], headspace LPME [25] and dynamic LPME [26]. Nevertheless, some drawbacks, such as instability of droplet and relative low precision are often reported [27]. The application of ultrasonic (US) radiation is an efficient tool to facilitates the emulsification phenomenon and accelerate the mass-transfer process between two immiscible phases. This leads to an increment in the extraction efficiency of the technique in a minimum amount of time [28,29]. The most widely accepted mechanism for US-assisted emulsification is based on the cavitation effect. It is based on the implosion bubbles generated by the cavitation phenomenon, which produces intensive shock waves in the surrounding liquid and high-velocity liquid jets. Such microjets can cause droplet disruption in the vicinity of collapsing bubbles and thus, improve emulsification by generating smaller droplet size of the dispersed phase right after disruption [28]. Submicron droplet-size leads to significant enlargement of the contact surface between both immiscible liquids improving the mass-transfer between the phases. The combination of micro-extracting systems and ultrasounds radiation provides an efficient preconcentration technique, such as USAEME for determining analytes at trace level. In fact, this preconcentration technique has been developed by Regueiro et al. [30], who successfully applied it to determine synthetic musk fragrances, phthalate esters and lindane in aqueous samples. They demonstrated that USAEME is an efficient, simple, rapid and non-expensive extraction technique for GC analysis.

The purpose of the present work is to demonstrate that such an innovative and environmentally friendly technique (USAEME) can be successfully applied for extraction and preconcentration of PBDEs from water samples and further determination by GC–MS. To this end, and based on PBDEs relative abundance in environmental samples, four of the most commonly studied PBDEs in this type of samples were selected from the 209 possible congeners: 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). Several factors, including solvent type, extraction time and temperature and matrix modifiers were studied and optimized over the relative response of the PBDEs. The analytical performance of USAEME–GC–MS methodology was evaluated in terms of detection limits (LODs), repeatability and linear working range and also was evaluated the enrichment factor (EF) of the USAEME technique. The type of samples analyzed includes tap, lake and river water.

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, dichloromethane, chloroform and trichloroethylene were purchased from Merck (Darmstadt, Germany) and carbon tetrachloride was purchased from Sigma–Aldrich (Steinheim, Ger-

Table 1
GC–MS parameters and physicochemical properties of target PBDEs.

Analyte	t'_R (min)	Target ion (m/z)	Confirmation ions (m/z)	b.p. (°C)	log K_{ow} [38]
BDE-47	0.80	485.7	483.7, 325.8	396	6.81
BDE-100	0.89	403.7	563.6, 405.7	434	7.24
BDE-99	0.92	403.7	563.6, 405.7	416	7.32
BDE-153	1.08	483.6	643.5, 485.6	453	7.90

t'_R: relative retention times to PCB-209; b.p.: boiling point; log K_{ow}: octanol/water partition coefficient.

Table 2
USAEME–GC–MS analytical performance[a].

PBDE	RSD (%)	EF	Linear range (pg mL^{-1})	r^2	LOD (pg mL^{-1})
BDE-47	8.3	333	5–5000	0.9987	2
BDE-100	9.4	328	5–5000	0.9988	2
BDE-99	9.8	324	5–10,000	0.9984	1
BDE-153	10.4	319	5–10,000	0.9985	1

Extraction conditions: sample volume: 10 mL; extraction solvent: 100 μL CH_3Cl; extraction time, 5 min; centrifugation time: 2 min; extraction temperature: 35 °C.
[a] 95% confidence interval; n = 5.

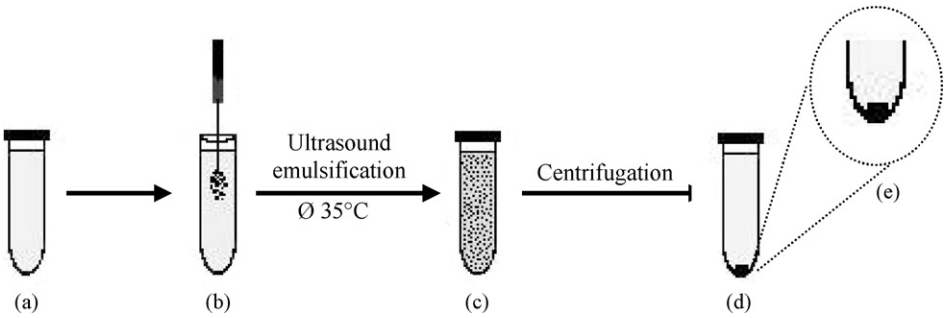

Fig. 1. Schematic diagram of PBDEs preconcentration from water samples by USAEME. (a) Sample solution containing PBDEs, (b) addition of 100 μL of extraction solvent (chloroform) into sample solution, (c) ultrasound-assisted emulsification at 35 °C during 5 min, (d) phase separation after centrifugation, and (e) enlarged view of resulting organic phase (30 μL).

many). Sodium chloride, hydrochloric acid and sodium hydroxide were all purchased from Merck. Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were of analytical grade or above.

2.2. Equipment and working conditions

A 40 kHz and 600 W US-bath with temperature control (Test Lab, Buenos Aires, Argentina) was used for assisting the emulsification process of the micro-extraction technique. The volume of extraction phase was measured using a 250-μL Hamilton syringe (Reno, NV, USA). Injections into the GC–MS were made by using a 5.0-μL Hamilton syringe. 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 (30 m×0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA, USA). The 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 a flow rate of 1.0 mL min^{-1}. 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, 50 °C and 280 °C, respectively. Samples were analyzed in selected ion storage (SIS) 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 performed against PCB-209 internal standard.

2.3. Sampling and sample preparation

For tap water samples collection, domestic water was allowed to run for 20 min and then collected. River water was collected from *Las Tunas River*, *Tupungato* district, and *Cipolleti Lake*, *Lujan de Cuyo* district, both from Mendoza Province. River and lake water samples were both collected at a depth of 20 cm. The total volume of each water sample was 1000 mL. 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.

2.4. USAEME procedure

A 10 mL water sample was placed in a 15-mL glass-centrifuge tube. 500 μL 6.15 mol L^{-1} sodium chloride and 100 μL chloroform were added and mixed. The resulting mix was immersed into an ultrasonic bath for 5 min at 35 ± 2 °C. During the sonication, the solution became turbid due to the dispersion of fine chloroform droplets into the aqueous bulk. The emulsification phenomenon favoured the mass-transfer process of PBDEs from the aqueous bulk to the organic phase. The emulsion was centrifuged at 3500 rpm (1852.2 × *g*) for 2 min in order to disrupt the emulsions and separate both phases (the organic phase remained at the bottom of the conical tube). A 1 μL aliquot of the chloroform phase was removed from the bottom of the centrifuge tube and injected into GC–MS. Fig. 1 shows a scheme of the USAEME procedure.

3. Results and discussion

As it is well known, LLE efficiency can be affected by several working parameters, including type of extraction solvent, extraction solvent volume, sample ionic strength, sample pH, extraction time and temperature as well as centrifugation time. The study and optimization of the above-mentioned variables were performed by modifying one at a time while keeping the remaining constant. A 10 mL aqueous solution containing 1 ng mL^{-1} of each PBDE was used to perform the assays. The chromatographic peak area was the parameter used to evaluate the influence of those variables on the extraction efficiency of USAEME technique.

3.1. Effect of extraction solvent

The extraction solvent is critical for developing an efficient USAEME technique since its physicochemical properties govern the emulsification phenomenon, and consequently, the extraction efficiency. For practical purposes, it is convenient that the extraction solvent remain at the bottom of the centrifuge tube after phase separation. Therefore, the extraction solvent has to be denser than the water and its water immiscible. Moreover, chosen organic solvents must be able to extract compounds of interest and be compatible with the analytical instrumentation to be used. Taking into account these exigencies four organic solvents, including carbon tetrachloride, chloroform, dichloromethane and trichloroethene were examined. The density values of the selected organic solvents are 1.58 g mL^{-1} (carbon tetrachloride), 1.48 g mL^{-1} (chloroform), 1.33 g mL^{-1} (dichloromethane) and 1.46 g mL^{-1} (trichloroethene).

The compatibility of these solvents with the USAEME technique was studied by adding 100 μL of each of the solvents mentioned above to a 10-mL aqueous solution containing 1 ng mL^{-1} of each PBDE. Dichloromethane was completely dissolved in the aqueous solution (water solubility: 13 mg mL^{-1}) therefore, it was not considered in further studies. The remaining solvents (carbon tetrachloride, chloroform and trichloroethene) were able to form an emulsion during sonication, leading a biphasic system after

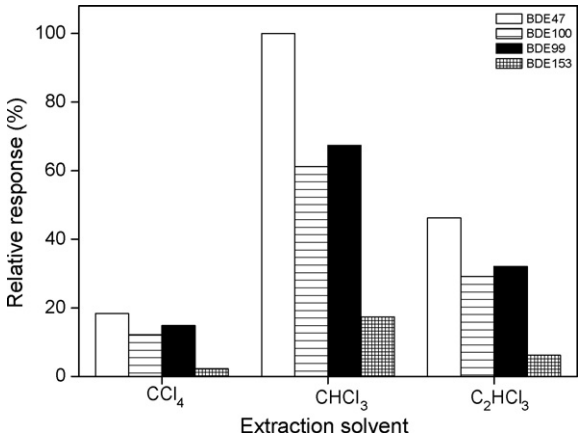

Fig. 2. Extraction solvent type and their extraction efficiency. Extraction conditions: sample volume, 10 mL; extraction solvent volume: 100 μL; extraction time, 5 min; centrifugation time: 2 min; extraction temperature: 35 °C; PBDEs: 1 ng mL^{-1}.

centrifuging the solution. The relative response of the studied PBDEs by using different solvents is shown in Fig. 2. The results revealed that the extraction efficiency of chloroform is higher than trichloroethene and carbon tetrachloride. Therefore, chloroform was selected as the extraction solvent for further studies.

3.2. Effect of extraction solvent volume

The volume of extraction solvent to be added in order to obtain the highest extraction efficiency and the highest relative response of the technique was studied within a volume range of 75–300 μL. Volumes smaller than 75 μL were completely dissolved in the aqueous bulk. The extraction procedure was the one described above. The resulting organic-phase volume was measured by using a 250 μL glass syringe and 1 μL aliquot of this phase was injected and analyzed in the GC–MS. From Fig. 3 it is possible to observe that for 75 μL chloroform, the resulting organic phase volume was 10 μL. The relative response of the PBDEs obtained in this case was lower that 60% sine the volume of chloroform was insufficient to quantitatively extract the analytes. By using 100 μL chloroform it was achieved the highest relative response of the PBDEs. Higher volumes reported lower relative responses due to a dilution effect of the analytes into the resulting organic phase. Therefore, 100 μL chloroform was selected to develop further studies.

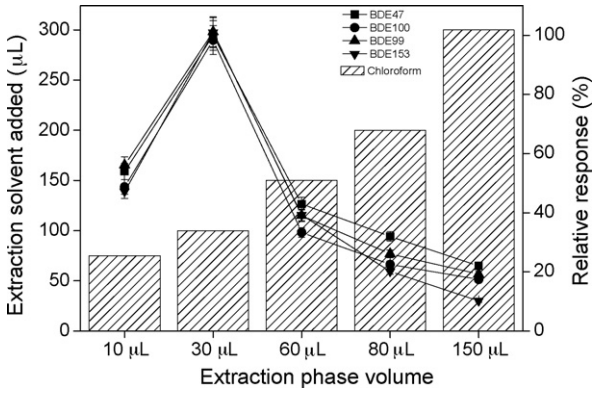

Fig. 3. Correlation between the added chloroform, extraction phase volume and relative response of analytes. Extraction conditions: sample volume: 10 mL; extraction solvent: 100 μL chloroform; extraction time, 5 min; centrifugation time: 2 min; extraction temperature: 35 °C; PBDE: 1 ng mL^{-1}.

3.3. Effect of extraction temperature

Temperature can affect the extraction efficiency of the analytical technique. It affects the analyte and organic solvent solubility in water as well as the emulsification phenomenon. Thus, affects the mass-transfer process. To determine the influence of the extraction temperature 10 mL aqueous solution containing 1 ng mL^{-1} of each PBDE was extracted at different temperatures ranging from 5 °C to 75 °C (Fig. 4a). At low temperatures (<20 °C) low relative response values were observed. The water solubility of PBDEs diminish as the temperatures decrease [31]. However, the chloroform viscosity increases affecting negatively the emulsification phenomenon. At temperatures lower than 20 °C and it was difficult to get an homogeneous emulsion, resulting in a prompt phase separation [32]. Therefore, the mass-transfer process was limited to a short amount of time, leading poor extraction efficiency, and consequently low relative responses of the PBDEs. In the 25–55 °C temperature range, the emulsification was easily achieved and remained invariant during the whole extraction time; however the highest relative response was obtained at 35 °C. At a temperature higher than 55 °C, the chloroform was completely dissolved into the aqueous bulk; therefore it was not possible to achieve a homogeneous emulsion. However, the phase separation was achieved by cooling down the tube and centrifuging it. Within this temperature range the relative response of the PBDEs decreased notoriously. The increment of the temperature favoured the solubility of PBDEs in water. Based on this evidences, the working temperature selected for further studies was 35 °C.

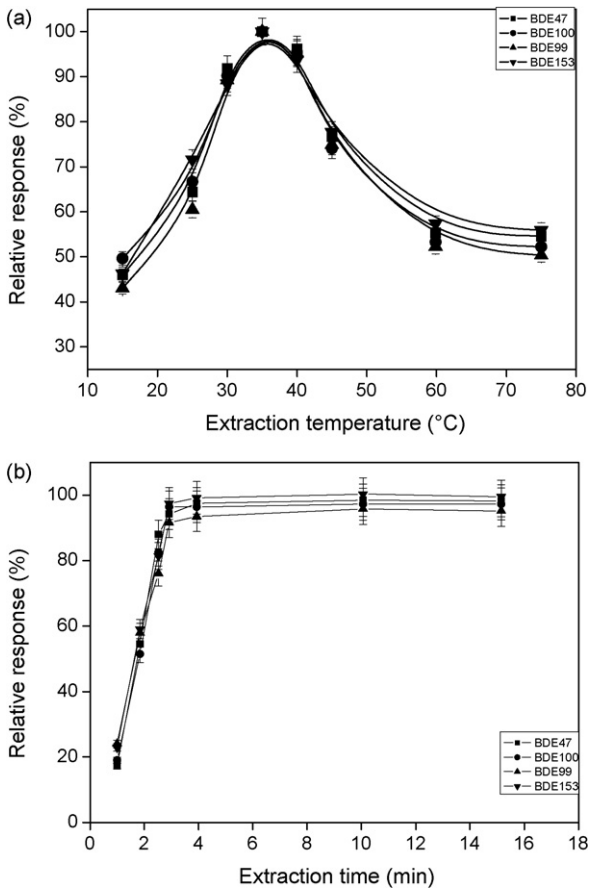

Fig. 4. (a) Extraction temperature effect on the relative response for PBDEs determined by USAEME–GC–MS. (b) Extraction time effect on the relative response for PBDEs determined by USAEME–GC–MS. Extraction conditions as described in Fig. 2.

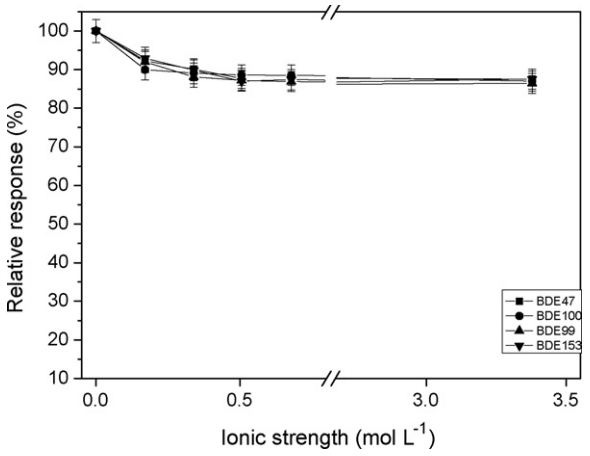

Fig. 5. Ionic strength effect on the relative response for PBDEs determined by USAEME–GC–MS. Extraction conditions as described in Fig. 2.

3.4. Effect of extraction and centrifugation time

Time plays an important role into the emulsification and mass-transfer phenomena. Both phenomena influence the extraction efficiency of the PBDEs, and thus, their relative response. For this reason, extraction time was studied in order to achieve the best relative response in a minimum amount of time. The extraction time interval was defined as the time elapsed between CH_3Cl addition and the end of the sonication stage; and it was varied within the range of 1–15 min. The extraction procedure was the one described above. Fig. 4b shows the relative response of the four PBDEs versus their extraction time. It was observed that by increasing the extraction time, the relative response increases, reaching the maximum value at 4 min, after which, remained constant. Therefore, 5 min sonication time was chosen as working conditions for further studies.

Centrifugation was required to break down the emulsion 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 in the whole time frame studied; thus, the minimum time (2 min) was selected as the centrifugation time necessary to get a satisfactory biphasic system.

3.5. Effect of sonication

Sonication and vigorously stirring were compared as emulsification-assistance. By vigorously stirring the solution for 5 min, the relative responses obtained for the four PBDEs were comparable to that obtained by sonication for 2 min. Sonication stirring produces smaller droplets of organic solvent in the aqueous bulk than vigorous stirring. This significantly enlarges the contact surface of the organic solvent with the aqueous bulk favouring the mass-transfer process of PBDEs into the organic phase. Additionally, the reproducibility of the results using vigorous stirring was worse (>14%) compared with that obtained by sonication stirring.

3.6. Effect of ionic strength and pH

As it is well known, the ionic strength affects the partitioning coefficients of analytes between an aqueous and organic phase. On the other hand, as the ionic strength the medium increases, the viscosity and density increase; diminishing thus, the efficiency of the mass-transfer process and consequently, the extraction efficiency of the technique [30]. Additionally, the ultrasound waves

Table 3
Recovery study of the four PBDEs compound in different water samples.

BDE	Level found	Tap water				Level found	Lake water				Level found	River water			
		10 pg mL^{-1} spiked		50 pg mL^{-1} spiked			10 pg mL^{-1} spiked		50 pg mL^{-1} spiked			10 pg mL^{-1} spiked		50 pg mL^{-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	9.7 ± 2.0	97	48 ± 4	96	nd	10.0 ± 2.0	100	51 ± 4	102	nd	10.3 ± 1.5	103	52 ± 3	104
100	nd	10.5 ± 2.5	105	53 ± 5	106	nd	9.8 ± 1.5	98	52 ± 2	104	nd	10.1 ± 1.5	101	53 ± 3	106
99	nd	10.1 ± 2.0	101	51 ± 5	103	nd	9.7 ± 2.0	97	53 ± 2	106	nd	9.8 ± 2.0	98	53 ± 2	106
153	nd	9.8 ± 2.5	98	48 ± 5	97	nd	9.5 ± 2.5	95	48 ± 5	96	nd	10.1 ± 2.0	101	50 ± 4	100

[a] Results expressed as $\bar{x} \pm \frac{t \cdot SD}{\sqrt{n}}$; $n = 3$; 95% confidence interval, $n = 3$. Extraction conditions: sample volume: 10 mL; extraction solvent: 100 μL CH_3Cl; extraction time, 5 min; centrifugation time: 2 min; extraction temperature: 35 °C. nd: not detectable.
[b] [(Found-base)/added] × 100.

Table 4
Determination of PBDEs in water samples by using different analytical methodologies.

Method	LOD (pg mL^{-1})	LR (pg mL^{-1})	RSD (%)	Extraction time (min)	Sample volume (mL)	References
SPME–GC–MS/MS	0.02–0.20	0.12–205	4–20	30	10	[39]
SPME–GC-ECD	3.6–8.6	28–2,800	7–9	30	10	[17]
DLLME–HPLC-VWD	32–52	0.05–100	3.8–6.3	0.3	5	[40]
USAEME–GC–MS	1–2	5–10,000	8.3–10.4	5	10	This work

SPME–GC–MS/MS: solid-phase microextraction and gas chromatography–tandem mass spectroscopy. SPME–GC-ECD: solid-phase microextraction and gas chromatography–electron capture detection. DLLME–HPLC-VWD: Dispersive liquid–liquid microextraction and high performance liquid chromatography-variable wavelength detection.

can be absorbed and dispersed in a viscous medium as calorific energy; thus, the cavitation process could be withdrawn reducing the emulsification phenomenon [33]. The literature reports discrepancies about the ionic strength effect on the extraction efficiency of PBDEs [34,35]. Therefore, it was found interesting to study the ionic strength effect on the extraction technique within the concentration range of 0–3.4 mol L^{-1} adjusting it with sodium chloride. The best relative response for all four PBDEs was observed when no sodium chloride was added to the extraction solution. As the ionic strength of the medium was increased, their relative response decreased up to a minimum of 90%, after which it remained constant. The results are shown in Fig. 5. It is worth to point out that even at high ionic strength values, the micro-extraction technique reported reproducible results. The ionic strength values of water samples can vary depending on the type of sample. Values commonly found for tap, river and lake waters of this region of Argentina are within the range of 0.01–1.5 mol L^{-1}. Therefore, since the variability of the ionic strength of real samples could be considerable high, a compromise situation was chosen to ensure comparable responses for all PBDEs in real samples and external calibration curve. In this sense, 500 μL 6.15 mol L^{-1} sodium chloride were added to the standards of the calibration curve and samples.

The effect of the sample pH was investigated within the pH range of 2–12 adjusting it by addition of hydrochloric acid or sodium hydroxide solutions. No significant changes in the extraction efficiency were observed for any of the PBDEs studied, which is the expected performance due to the molecular structure of the analytes. Similar results were observed by other authors [18].

3.7. Analytical performance

The analytical figures of merits were summarized in Table 2. Extraction efficiency higher than 99.9% was achieved when the procedure was carried out under optimum conditions. The enrichment factor was obtained from the ratio of the calibration curve slopes for each PBDE with and without the preconcentration step. The LODs of the analytes for the preconcentration of 10 ml sample volume, calculated as three times the signal-to-noise ratio (S/N = 3), were 2 pg mL^{-1}, 2 pg mL^{-1}, 1 pg mL^{-1}, 1 pg mL^{-1} for BDE-47, BDE-100, BDE-99 and BDE-153, respectively. The precision of USAEME-GC–MS was evaluated over five replicate, resulting RSDs ≤ 10.4%. The calibration curves showed a satisfactory linearity within the concentration range of 5–5000 pg mL^{-1} for BDE-47 and BDE-100; and 5–10,000 pg mL^{-1} for BDE-99 and BDE-153, respectively. Furthermore, the coefficient of estimation (r^2) exceeded 0.9984 for all analytes. In order to validate the analytical methodology, a recovery study of the four PBDEs at two different concentration levels (10 pg mL^{-1} and 50 pg mL^{-1}) was carried out over the real water samples. This study led to a satisfactory robustness achieving recoveries ≥95% (Table 3).

In order to evaluate the number of samples that it is possible 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 according to the extraction procedure described above and analyzed as follows. 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 Fang et al. [36]. Therefore, the sample preparation was performed within the same day of the samples analysis.

3.8. Application to real samples

USAEME-GC–MS was applied for the determination of BDE-47, BDE-99, BDE-100 and BDE-153 in water samples, including tap, lake and river waters as well as the water used by the City of Mendoza for irrigation. The samples were collected and immediately analyzed as described above. The partitioning of pollutants between water and humic organic matter (HOM) is based on reversible interactions, which may be specific or hydrophobic in nature [37]. The analyzed samples presented low dissolved organic-matter content (<4 mg L^{-1}) and the recovery values were satisfactory (≥96%). Therefore, it was assumed that by applying this extraction technique (liquid–liquid extraction) it was possible to determine the total PBDEs concentration, which includes the freely dissolved PBDEs portion and the one that is reversibly linked to a pseudophase HOM. Since no matrix effects were observed, even in the most complex samples, quantification could be performed by external calibration using PBDEs standard solutions prepared in chloroform spiked with PCB 209 (IS) 1 ng mL^{-1}. The sample results and the recovery study were performed in triplicate (Table 3). Although different types of water samples were analyzed, PBDEs were found in none of them. Fig. 6a shows the chromatogram of a river water sample spiked with 10 ng mL^{-1} PCB 209 and Fig. 6b shows the chromatogram of the same sam-

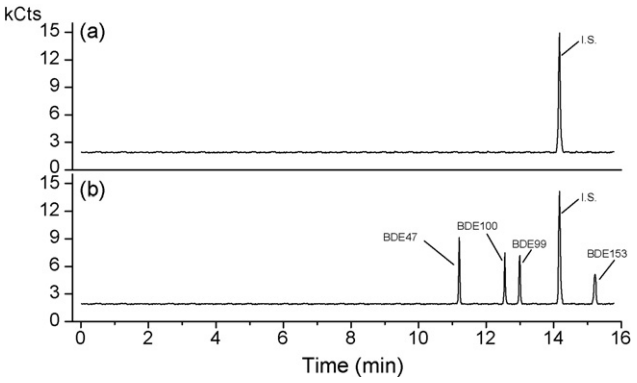

Fig. 6. Analysis of river water. EIC for *m/z* 325.8, 405.7, 483.7, 485.6, 563.6, and 643.5. (a) Sample spiked at 10 ng mL^{-1} of PCB 209 and (b) Sample spiked with 10 ng mL^{-1} PCB 209 and 50 pg mL^{-1} of each PBDE.

ple spiked with 10 ng mL^{-1} PCB 209 and 50 pg mL^{-1} of target PBDEs.

3.9. Comparison of USAEME–GC–MS with other analytical methodologies

The analytical performance of USAEME–GC–MS for PBDEs determination in water samples was compared with other analytical methodologies previously reported (Table 4). It can be observed that the analytical performance for USAEME–GC–MS is comparable with methodologies previously used for PBDEs determination. Only SPME–GC–MS/MS showed lower LODs than USAEME but the mean RSDs values were higher. However, USAEME analytical technique is not time consuming and the GC–MS is more accessible for a wide range of laboratories.

4. Conclusions

USAEME is an efficient microextraction technique based on the emulsion phenomenon induced by sonication, which was satisfactorily applied for the determination of PBDEs at trace levels by GC–MS. Under optimized working conditions, high EF were obtained from the target analytes allowing to reach detection limits in the order of low picogram per milliliter with an acceptable precision. The robustness of the methodology was proved by the recovery study carried out over the real samples not showing matrix effects, even in the most complex samples. This fact allowed performing the quantification by using the external standard prepared in chloroform and contributed to simplify the PBDEs determination routine improving the sample throughput of the analytical methodology. Its simplicity and swiftness make it a convenient alternative for trace analysis by GC. All these results disclosed that USAEME is a sensitive, rapid and reproducible technique. Additionally, it is important to point out that USAEME is a low organic solvent consuming extraction technique, which turns it into a low cost and environmentally friendly technique. USAEME was finally applied to the analysis of several real water samples including tap water, lake water and river water; none of them reported the presence of PBDEs.

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