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Received May 26, 2016
Revised October 3, 2016
Accepted October 5, 2016

Research Article

Determination of polybrominated diphenyl ethers in milk samples. Development of green extraction coupled techniques for sample preparation

Ultrasound-assisted extraction (UAE), cloud point extraction (CPE), and ultrasound back-extraction (UABE) techniques have been coupled for lixiviation, preconcentration, and cleanup of polybrominated diphenyl ethers (PBDEs) from milk samples for determination by gas chromatography-electron capture detection (GC-ECD). Physicochemical parameters that affect the efficiency of the extraction system were investigated using a design of experiments based on multivariate statistical tools, and considering the sample matrix along the development. The coupling of the leaching step, UAE, enhanced ca. 3.5 times the extraction efficiency of the former sample preparation methodology (CPE-UABE) leading to cleaner sample extracts suitable for GC analysis. Under optimum conditions, the proposed methodology exhibits successful performance in terms of linearity and precision, with recoveries in the range of 68–70% and LODs within the range 0.05–0.5 ng/g dry weight (d.w.). The proposed sample preparation methodology coupled three green analytical techniques. It expands the application frontiers of CPE for the analysis of biological samples by GC. The optimized methodology was used for determination of PBDEs in powder milk samples, from both commercial and human sources.

Keywords:
Cloud point extraction / Experimental design / Milk samples / Polybrominated diphenyl ethers / Ultrasound-assisted extraction DOI 10.1002/elps.201600247

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1 Introduction

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants, usually added to polymers of electronic equipment, plastics, textiles, building materials, carpets, vehicles, and aircraft according to safety regulations. They can easily leach from the polymeric mass to the environment [1], where they can be transported far from their sites of production and use [2]. Due to their physical and chemical properties, these compounds can persist and accumulate in the environment as well as in human and wildlife, magnifying through the food web [3]. Effects on nervous, reproductive, and endocrine systems had been attributed to these compounds [4]. Consequently, increase attention has been directed toward studies on PBDEs exposure and bioaccumulation, as well as biomagnification. Human breast milk is a no-invasive sample, which can be used as an indicator of exposure to PBDEs in the environment; as well as it is directly related to infant PBDE intake [5].

Determining PBDEs in biological samples is often a difficult task and requires highly selective and sensitive instrumentation, due to trace levels of concentration at which the analytes are present [6]. Prior to instrumental analysis, a sample preparation methodology is mandatory. As alternatives to traditional pretreatment techniques, e.g., liquid–liquid extraction (LLE) or solid phase extraction (SPE) green analytical techniques have been used for extraction and preconcentration of PBDEs [7]. The

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Abbreviations: **CCD**, central composite design; **CPE**, cloud point extraction; **LLE**, liquid–liquid extraction; **MFGM**, milk fat globule membrane; **PBDE**, polybrominated diphenyl ethers; **PLE**, pressurized liquid extraction; **SPE**, solid-phase extraction; **SPME**, solid-phase microextraction; **UABE**, ultrasound assisted back-extraction; **UAE**, ultrasound-assisted extraction; **US**, ultrasonic

green approaches not only reduce operator risk and waste generation, but also allow to preserve or enhance the analytical performance of the methodology, leading to efficient, simple, and inexpensive analytical alternatives [7].

Cloud point extraction (CPE) technique was reported for extraction and preconcentration of organic contaminants in environmental (sediments [8, 9]) and biological (human serum [10] and milk [11]) samples prior HPLC-UV and HPLC-FD analysis. Although the reported methodologies were combined with HPLC analysis, they did not achieve a successful cleanup for the analysis of the complex matrix by GC. CPE followed by ultrasound-assisted back-extraction (UABE) prior GC analysis was proposed by our group to extract and isolate PBDEs from water and was assayed for soil samples, with poor results (recovery values ≤ 20%) [12]. Based on these evidences, it was hypothesized that a lixiviation step of the PBDEs from the biological samples prior CPE would improve the performance of the analytical methodology. Thus the combination of three consecutive green extraction techniques were proposed for lixiviation, extraction, clean up, and preconcentration of PBDEs from milk samples before GC-ECD determination. To this aim, four of the most commonly found PBDEs in environment samples were selected as target analytes: 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), and 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). A multivariate approach was used for understanding and optimizing the effect of each variable, as well as their interactions [13], on the analytical response of the target PBDEs. The analytical performance of ultrasound-assisted extraction (UAE)-CPE-UABE-GC-ECD methodology was evaluated in terms of LODs, repeatability, recovery (%), and linear working range. The procedure was applied for the determination of PBDEs in commercial and breast milk samples.

2 Materials and methods

2.1 Reagents

The PBDEs commercial standards at 50 mg/L in isooctane (BDE-47, -99, -100, and -153) were purchased from Accustandard (New Haven, CT, USA) and stored in the dark at –20°C. Further dilutions were prepared weekly in methanol (MeOH) at concentration levels of 1 μg/mL and stored in brown bottles at –20°C. Acetone, toluene, isooctane, and ethyl acetate were purchased from Tedia (Fairfield, OH, USA). Dichloromethane, n-hexane, and MeOH were purchased from J.T. Baker (Center Valley, PA, USA). A 100 g/L aqueous solution of Triton X-114 (Sigma, Milwaukee, WI, USA), and a 359 g/L sodium chloride (NaCl, Sigma) aqueous solution were prepared by dissolving appropriate amounts in ultrapure water. 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 Instrumentation, working conditions, and software

GC–ECD analyses were carried out on a Hewlett-Packard 5890 (Series II) gas chromatograph equipped with an electron capture detector (Hewlett Packard, Avondale, PA, USA) operated at 300°C. The used GC column (DB-5 ms, 30 m × 0.25 mm, 0.25 μm film thickness) was purchased to Agilent Technologies (Santa Clara, CA, USA). Injections were performed in the splitless mode. Nitrogen was used as carrier gas at flow rate of 1.0 mL/min. The injector temperature was 250°C. The temperature program was: 90°C, held 1 min; slope 1: 35°C min^{-1} to 250°C; slope 2: 20°C min^{-1} to a final temperature of 280°C and held for 20 min. Experimental design and data analysis were carried out using the Stat-Ease Design-Expert trial version 7.0.3 software.

2.3 Sampling and sample conditioning

Human and commercial milks were analyzed in this study. Human milk was collected with a breast-pump into a glass container from a volunteer mother of our group (Mendoza, Argentina) and stored at –20°C until analysis. Thirty milliliters of human milk sample was freeze-dried, obtaining 30.5 g dry weight (d.w.) of milk. Commercial powder milks were purchased from a local supermarket (Mendoza, Argentina). For optimization assays, a 0.50 g d.w. aliquot milk sample (PBDEs free) was spiked with a PBDEs mix to get a final concentration of 5 ng/g each targeted PBDE.

2.4 Sample preparation

The analytes were leached from a 0.50 g dried milk sample with 1.75 mL acetone/hexane (30:70) mixture solvent. The process was assisted by ultrasonic (US) waves at room temperature through the application of three cycles 8.2 min long and 1 min break in between. After lixiviation time, the resulting slurry was centrifuged at 3500 rpm (2800 × *g*) for 3 min, and the upper, liquid phase was transferred to a 10 mL empty tube. The leaching procedure was repeated under the same conditions and the two extracts were combined. The leachate was evaporated until dryness under a gentle stream of nitrogen, avoiding over drying the tube. The dry extract was reconstituted with 5 mL DI water, and 0.5 mL 1% w/v Triton X-114 and 0.56 mL 4 % w/v NaCl were added and mixed-up using a vortex stirrer. The centrifuge tube was thermostatized at 55°C for 10 min. Under these conditions, the system reached the cloud point and the coacervate-phase started to separate from the aqueous bulk. The tube was centrifuged at 3500 rpm (2800 × *g*) for 10 min to accelerate the micellar-phase decantation. The centrifuge tube was refrigerated (5°C) during centrifugation. The aqueous supernatant was manually removed. The ultrasound-assisted back-extraction was carried out by adding 90 μL of isooctane to the resulting surfactant-rich phase and sonicated at 40°C for 3 min. One microliter of

the isooctane phase was then injected into the GC-ECD for analysis.

2.5 Experimental design

The optimization of leaching, extraction, and back-extraction steps were studied separately. First, a factorial experimental design (2^k) was used to evaluate the significance of the variables, as well as their interactions that govern each technique on the responses. Working conditions were chosen based on knowledge from preliminary assays [12, 14]. After multiple linear regression and analysis of variance, Pareto charts were used to select the significant variables [13]. Once selected those variables that influence the techniques, a central composite design (CCD) was carried out to identify those conditions that leaded to the maximum analytical responses of PBDEs, and to mathematically predict how the analytical responses were related to the values of the working variable [15]. Outliers were removed by analyzing the difference among fitted values test, due to their disproportionate influence on the predicted response, and thus on the model [13]. Model coefficients were calculated by backward multiple regression and validated by the analysis of variance. Finally, a multiple response criteria, the desirability (*D*) function, was used to find those values that lead the best analytical response for the studied PBDEs [16].

3 Results and discussion

The extraction efficiency of the methodology is conditioned by the mass transfer of the analytes trough the different steps of the proposed approach (UAE-CPE-UABE), as well as by other factors including the analytes availability and compatibility between techniques. Since several techniques are combined in the sample preparation methodology, the study and optimization of the entire analytical methodology is complex. A multivariate statistical approach is used to identify those variables that significantly affect each technique, and to optimize the working conditions and achieve a maximum enhancement factor of the analytical signals within a shorter sample preparation time.

Developing an analytical method using synthetic solutions and testing its further application on real samples generally requires several adjustments for achieving a successful performance. The use of biological matrix all along the process is a convenient choice for avoiding further adjustments when applied to real world analysis. Thus, signal enhancing due to the improvement of the extraction efficiency, as well as satisfactory chromatographic analysis (higher resolution of analytes among them and from the matrix zone, avoid peak tailing, and minimum peak dispersion), were considered. These aspects finally determined the sensitivity and selectivity of the resulting analytical methodology. In this sense each analytical step (UAE, CPE, or UABE) was studied and optimized using PBDE-free commercial milk spiked at 5 ng/g of each PBDE. The influence of the experimental variables on the performance of the proposed sample preparation methodology was investigated and optimized over the relative response of the analytical signal of the target PBDEs.

3.1 Type of leaching solvents for ultrasound-assisted extraction

Based on our previous work, our initial approach was to carried out the CPE technique directly to the milk samples, followed by an UABE with isooctane prior to GC analysis. Similarly with what was observed with soil samples, the recoveries were low, ca. 20% [12], for the four target PBDEs. The low recovery could be due to the complexity of the biological sample matrix. Human milk contains 3–7% lipids, which are emulsified as milk fat globule membranes (MFGM) [17]. On the inner side of the MFGM, there is a layer of unstructured lipoproteins, while the outer layer of the MFGM is composed of a trilaminar membrane consisting of phospholipids, proteins and triacylglicerols with a fluid mosaic structure [17, 18]. Considering the characteristics of the milk samples, it is expected that PBDEs were associated to the triglycerides lipids present in the MFGM due to their non-polar character [19]. UAE technique was chosen as leaching technique, over other traditional extraction techniques, e.g., Soxhlet extraction, since it offers a faster, efficient alternative and requires minimum solvent consumption. Prior to the experimental design, a preliminary study was carried out to define the most suitable leaching solvent for UAE step considering the analytes and matrix particularities. Several organic solvents, generally used for PBDEs extraction, such as hexane, dichloromethane, acetonitrile, acetone, and a combination of them, were evaluated as leaching solvents. The assays were done in triplicate following the analytical methodology described in Section 2.4 and prior to the CPE-UABE-GC using the following conditions: 1 mL 100 g/L Triton X-114, 0.1 mL 359 g/L NaCl, 30 min of extraction time at 70°C; UABE: 250 μL isooctane, 20 min.

The results obtained with different UAE solvents are shown in Fig. 1. The highest relative responses of PBDE were achieved when the mixture of acetone and hexane (30:70) was used for the UAE step. The use of these solvents, in a different proportion, was previously reported for PBDEs extraction from biological tissues [20, 21]. However, there is no report about the optimization of its composition for efficient extraction of PBDEs from biological samples. Even when PBDEs show higher affinity toward solvents with low polarity, such as hexane, their penetration capacity through tissues is less efficient than solvents with higher polarity [22]. On the other hand, acetone is a polar solvent and is able to denaturalize the protein structure of the MFGM present in the milk, thus allowing an easier accessibility of nonpolar solvents. Thus, the mixture of acetone/hexane leads to higher extraction efficiency of nonpolar lipids and PBDE associated to them [12].

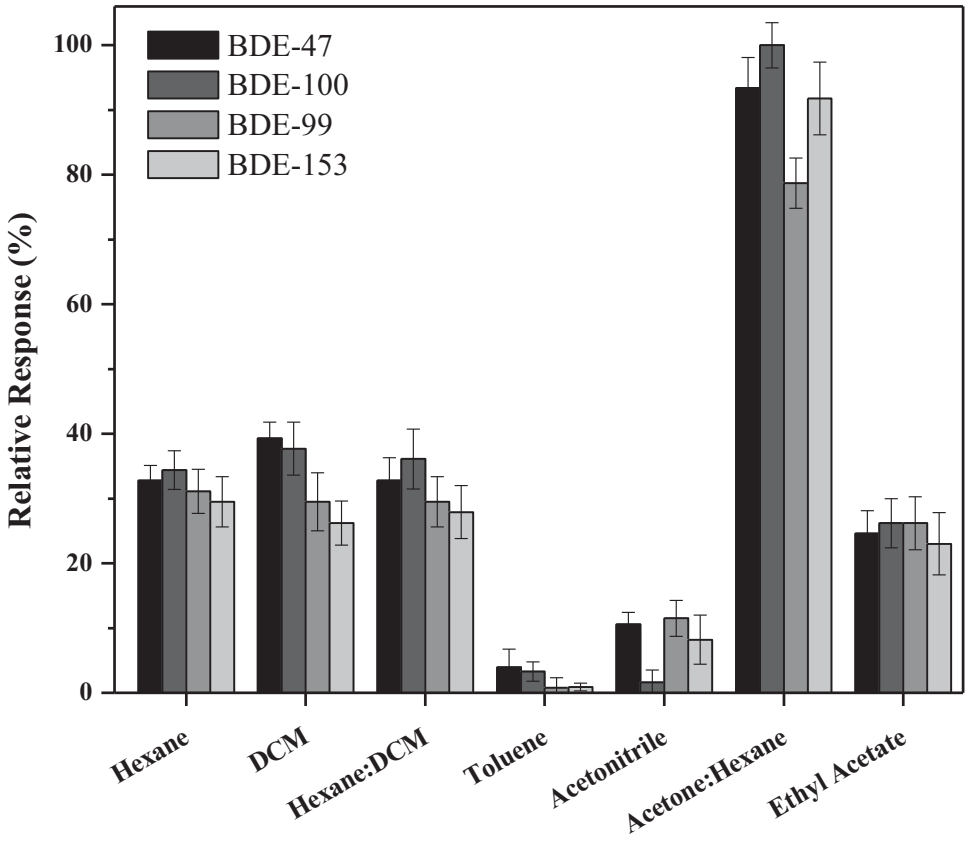

Figure 1. Effect of lixiviation solvents on the relative response of PBDEs. Analytical methodology described in Section 2.4. CPE-UABE conditions: 1 mL 100 g/L Triton X-114, 0.1 mL 359 g/L NaCl 30 min of extraction time at 70°C; UABE: 250 μL isooctane, 20 min.

3.2 UAE technique

Once selected the appropriate combination of solvents for leaching the target PBDE from the milk sample, this extraction step was optimized. Various physical and chemical phenomena including agitation, vibration, pressure, shockwaves, shear forces, microjets, compression and rarefaction, acoustic streaming, cavitation, and radical formation are responsible for US effect of the lixiviation [23]. The main driving force for the extraction effects of sonication at working US frequency (40 kHz) is acoustic cavitation [24]. When ultrasound propagates through any medium, it induces a series of compressions and rarefactions in the molecules of the medium. Such alternating pressure changes cause the formation and, ultimately, the collapse of bubbles in a liquid medium, and it is known as "acoustic cavitation" [24]. The bubble cavitation also disrupts the saturated boundary layer surrounding the particles, thus allowing fresh solvent to reach the particle surface, favoring the mass transference of the analytes to the medium, and thus improving the leaching efficiency of the UAE technique [25]. Besides the sonication time, the schedule of sonication cycles combined with short breaks helps to improve the leaching efficiency [25]. Solvent solution volume, leaching time, number of successive extractions, and the effect of breaks during leaching stage were selected for full factorial experimental design (2^k) to identify those variables, and their interactions that condition the leaching technique. The volume of leaching solvent (acetone/hexane, 30:70) was evaluated at 0.5 and 1 mL. The effect of successive extractions was evaluated considering one and two successive lixiviation steps. Additionally, multiple step cycles of US irradiation, with two stops and 1 min break in-between each cycle, were assayed. In order to make possible a comparison with a continuous mode, the total time of US irradiation was 10 and 30 min. The pH effect was evaluated in previous extraction essays and showed no significance on the analytical signal of target PBDEs. The followed sample preparation methodology for this study was as described in Section 2.4. The CPE-UABE working conditions used during UAE optimization were as follows: 1 mL 100 g/L Triton X-114, 0.1 mL 359 g/L NaCl, 30 min of extraction time at 70°C; UABE: 250 μL iso-octane, 20 min. (Results are shown on Supporting Information Table S1 and Pareto charts in Fig. S1). The variables that showed significance on the analytical response ($p < 0.1$) of the studied PBDEs were volume of leaching solvent and the usage of breaks along the US irradiation; and those interactions among the variables (double and triple interactions). Consequently, two successive extractions with two breaks during leaching time were set for further assays. For optimization, the volume of the leaching solvent (in the range of 0.5–3.0 mL) and leaching time (in the range of 5–45 min) were considered for the CCD analysis (Supporting Information Table S2), consisting in a total of 14 assays. The resulting analytical response for BDE-47 and -100 was best

explained with linear models, while cubic models better explained the analytical responses of BDE-99 and -153. Supporting Information Table S2 shows the statistical parameters corresponding to the fitting for resolution. As can be observed, models were significant ($p < 0.05$) and their lacks of fit were not significant ($p > 0.05$). The *p*-values showed, at 95% confidence level, that the volume of leaching solvent and extraction time conditioned the extraction efficiency of the target PBDEs, and thus, their analytical response. The combination of these variables guarantees an effective denaturalization of the MFGM and efficient extraction of the nonpolar lipids together with the PBDEs. In this sense, the volume of the leaching solvent played an important role wetting the matrix and then extracting the compounds; and the extraction time variable might condition the kinetic of this process. Under the mentioned optimization criteria, the experimental conditions corresponding to one of the maximum in the *D* function ($D = 0.879$, Supporting Information Fig. S2) were: leaching time: 25 min; leaching steps: 3 (two stops, 1 min break in-between each cycle); leaching solvent total volume: 3 mL; successive lixiviation steps: 2. Values suggested through the optimization procedure were experimentally corroborated.

3.3 CPE-UABE techniques

Micelles are in a dynamic equilibrium with monomers, where each monomer can leave the micelle, and may be replaced by another. The time of monomer inside a micelle depends on the type and the structure of the molecule, while the time of leaving of the monomer and its return depends on its diffusion rate. Micelles can be altered by appropriate selection of solution parameters, such as temperature, concentration of the surfactant, as well as the presence, type, and concentration of modifiers in the solution [26]. Therefore, it was worth to consider these variables in the study and optimization of the CPE technique for achieving its maximum efficiency. The use of CPE as sample preparation methodology prior to GC analysis is limited by the risk of column blocking due to the viscosity and non volatility of the resulting surfactant-rich phase. Therefore, after CPE and before GC injection, a supplemental stage, such as UABE [12], is required. CPE and UABE were optimized together, since the extracting phase obtained from CPE is used as donor phase in the UABE technique. The followed sample preparation methodology for this study was as described in Section 2.4. The use of ultrasound and temperature, and a combination of these variables,

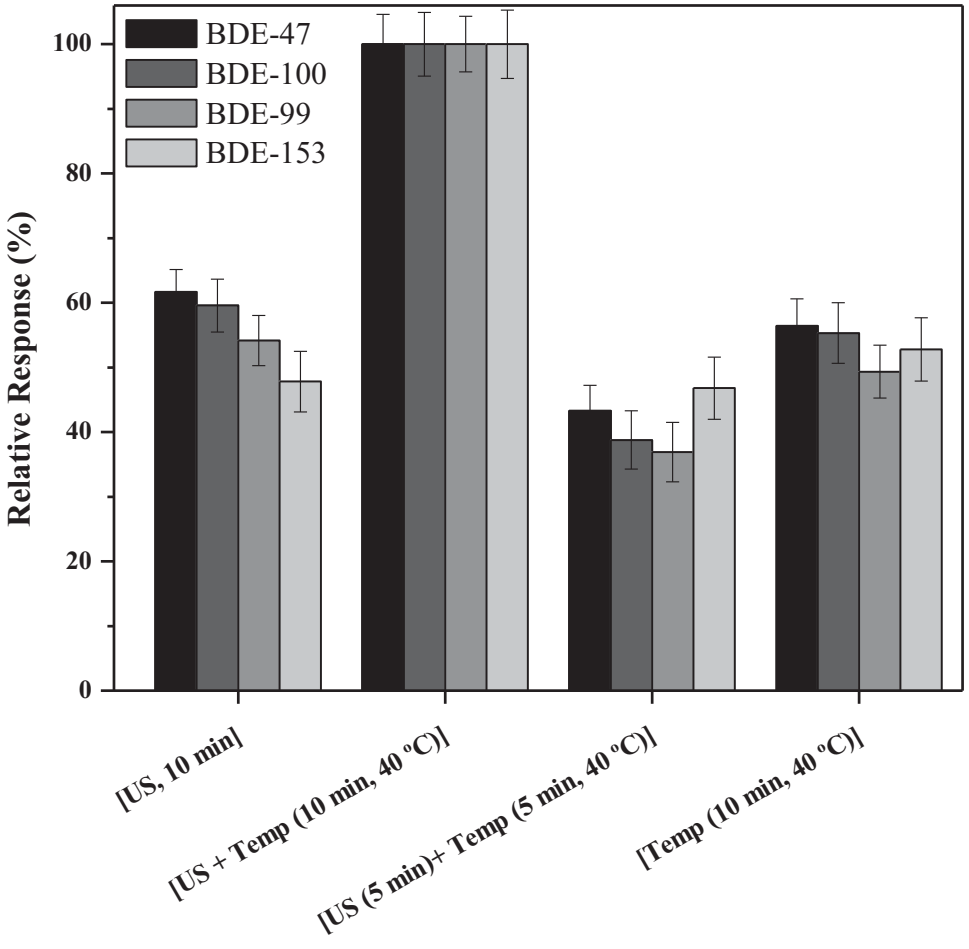

Figure 2. Effect of treatment for back-extraction technique on the relative response of PBDEs. Analytical methodology described in Section 2.4.

were evaluated. As can be seen in Fig. 2, a combination of temperature and ultrasound were needed to achieve the highest analytical response of the target analytes. After selecting the appropriated back-extraction conditions, CPE was optimized together with the established UABE procedure.

Those variables that might influence the extraction and back-extraction of the analytes were selected for an initial screening using fractional factorial design (2^{k-1}). The screening was carried out considering the following conditions: salting out effect using two different types of salts (Na_2SO_4 and NaCl); concentration of surfactant (0.1 and 0.5 mL 100 g/L Triton X-114); working temperature and time needed for extraction (55 and 70°C, and 5 and 30 min, respectively), as well as volume of back-extraction solvent (0.15 and 0.25 mL isooctane) and back-extraction time (3 and 10 min). The working conditions shown in Supporting Information Table S3. Pareto charts (Supporting Information Fig. S3) show the individual variables with significance on the analytical response ($p < 0.1$) of the studied PBDEs. They were the concentration of Triton X-114; addition of salt and type of salt; extraction time; and volume of isooctane for back-extraction; and interactions among the variables. The temperature during extraction did not show to be significant at the evaluated temperature levels, and consequently 55°C was selected for further experiments. Optimization using CCD was carried out considering salt (NaCl) and Triton X-114 concentrations within the range of 0–5% w/v, and 0.3–1% w/v, respectively; extraction time in the range of 5–60 min; and volume of iso-octane in the range of 0.05–3.00 mL, which consisted in a total of 22 assays. The resulting analytical response of the PBDEs under the effect of the studied variables is reported in Supporting Information Table S4. They were best explained by quadratic models for the analyzed PBDEs. Table S4 also shows the statistical parameters corresponding to the fitting for resolution. As can be observed, models were significant ($p < 0.05$) and their lacks of fit were not significant ($p > 0.05$). The *p*-values of the multivariate study showed, at 95% confidence level, that NaCl and Triton X-114 concentrations and the extraction time affect the extraction efficiency of the target PBDEs and thus their analytical response. Surfactant concentration must be above the critical micelle concentration to form an effective micellar phase for efficiently extract the analytes [26]. The salting out effect is based on disfavoring the solvatation of nonpolar analytes in the aqueous medium enhancing thus, its affinity for nonpolar medium, such as the micelles; favoring the extraction efficiency of the technique [23]. The extraction time is related to the kinetic of mass of transference of the analytes from the aqueous solution to micelles [23]. Therefore, these variables were all expected to be significant for the extraction step. The experimental conditions corresponding to one of the maximum in the *D* function ($D = 0.972$, Supporting Information

Table 1. Analytical figures of merits of the proposed methodology and recovery studies in real samples.

A. Analytical figures of merits of the proposed methodology

		BDE-47	BDE-100	BDE-99	BDE-153
Sample consumption (g)	0.5				
Solvent volume (mL)	3.1				
Recovery (%)		70	70	68	69
RSD (%)[a)]		4.1	4.6	4.4	4.9
LOD Method (ng/g d.w.)		0.05	0.13	0.15	0.50
Linear range (ng/g d.w.)		0.08-500	0.18-500	0.20-500	0.70-500

B. Sample analysis and recovery study

	Spiked (ng/g d.w.)	Found (ng/g d.w.)[b)]			
		BDE-47	BDE-100	BDE-99	BDE-153
Human milk (breast)	–	nd	nd	nd	nd
	1	0.92 ± 0.05	0.89 ± 0.04	0.84 ± 0.07	0.80 ± 0.09
	25	24.8 ± 1.22	23.3 ± 1.21	22.5 ± 0.97	21.2 ± 1.53
Commercial milk I[c)]	–	nd	nd	nd	nd
	1	0.90 ± 0.08	0.96 ± 0.05	0.75 ± 0.08	0.82 ± 0.10
	25	23.0 ± 1.08	24.5 ± 1.37	22.3 ± 1.16	21.8 ± 1.70
Commercial milk II[c)]	–	nd	nd	nd	nd
	1	0.95 ± 0.07	0.90 ± 0.04	0.88 ± 0.07	0.79 ± 0.09
	25	23.5 ± 1.22	22.3 ± 1.36	23.0 ± 1.57	20.8 ± 1.33

Extraction condition as described in Section 2.4.
nd: not detectable.
a) 95% confidence interval; $n = 5$.
b) Results expressed as $\pm$ t SD/$\sqrt{n}$; $n = 2$; 95% confidence interval.
c) Commercial powder milk.

Fig. S4A and $D = 0.961$, Supporting Information Fig. S4B) were: 1% w/v Triton X-114, 4% w/v NaCl; 55°C for 10 min as extraction temperature and time, respectively. Since the volume of isooctane did not show a significant influence on the analytical response of the target analytes; it was fixed at 90 μL. Values suggested through the optimization procedure were experimentally corroborated.

The optimized variables of UABE differ from those of UAE, which are expected results. In UAE, the lixiviation was carried out from milk sample, while the back extraction was accomplished from the resulting surfactant-rich phase after CPE. The temperature condition the diffusion rates of the analytes and solvents; as well as, the cavitation phenomena [24, 25]. These phenomena compete between them. Thus, at room temperature the lixiviation of the analytes from the milk MFGM is favored by enhancing the cavitation phenomena. While high temperature (55°C) diminishes the viscosity of the phases, surfactant, and solvent, and enhance the diffusion rate of the analytes.

3.4 Analytical performance and application to real samples

The analytical figures of merits of the proposed methodology and recovery studies are summarized in Table 1. The quantification of PBDEs by GC-EDC was accomplished using external, matrix-matched calibration [27] curves, to avoid matrix effects, within concentration ranges of 0.5–500 ng/g, 0.18–500 ng/g, 0.20–500 ng/g, and 0.70–500 ng/g for BDE-47, BDE-100, BDE-99, and BDE-153, respectively. The calibration curves were built using five points in the mentioned range, considering reported levels of PBDEs in this type of samples. Each analytical sequence included blanks. The calibration curves were applied using linear fit achieving correlation coefficients (r^2) > 0.940. The precision of the methodology was evaluated by intraday RSD of five samples spiked with 5 ng/g d.w., leading to RSDs values < 5%. The optimized methodology was applied for analyzing samples of milk (human and commercial). Neither the commercial samples nor the human samples reported PBDE levels above their LODs. For validation purpose of the analytical methodology, a recovery study of PBDEs at two different concentrations (1 ng/g and 25 ng/g) was carried out. Figure 3 shows the chromatogram of a milk (breast) sample spiked with 1 ng/g of target PBDEs. The test t ($n = 2$, $\alpha = 0.95$) did not show significant differences with the spiked levels. Although the number of reports about levels of PBDEs in environmental and biological samples from Argentina is scarce to make a general conclusion [28–31]; it is useful as indicative of low exposure to these contaminants through diet or environment; which is consistent with the achieved results of the analyzed samples.

Although the sample preparation methodology was proposed for the analysis of PBDEs by GC analysis, it could be feasible to couple it with the LC or CE techniques, with minimal adjustments. Therefore, this sample preparation methodology could be used for analyzing PBDEs and/or other compounds, in human milk samples by LC and CE. It is worth it to consider that the instrumental technique mostly reported for the analysis of PBDEs is GC due to its analytical resolution and senility achievable. These analytes, PBDEs, can be analyzed by LC, however their resolution is lower than by GC [7]. This fact could be critical when several PBDEs are determined in the same run. Other drawback is the low sensitivity of the detectors generally coupled to LC, such as UV or MS. On the other hand, the analysis of PBDEs by CE requires additional strategies since these analytes are

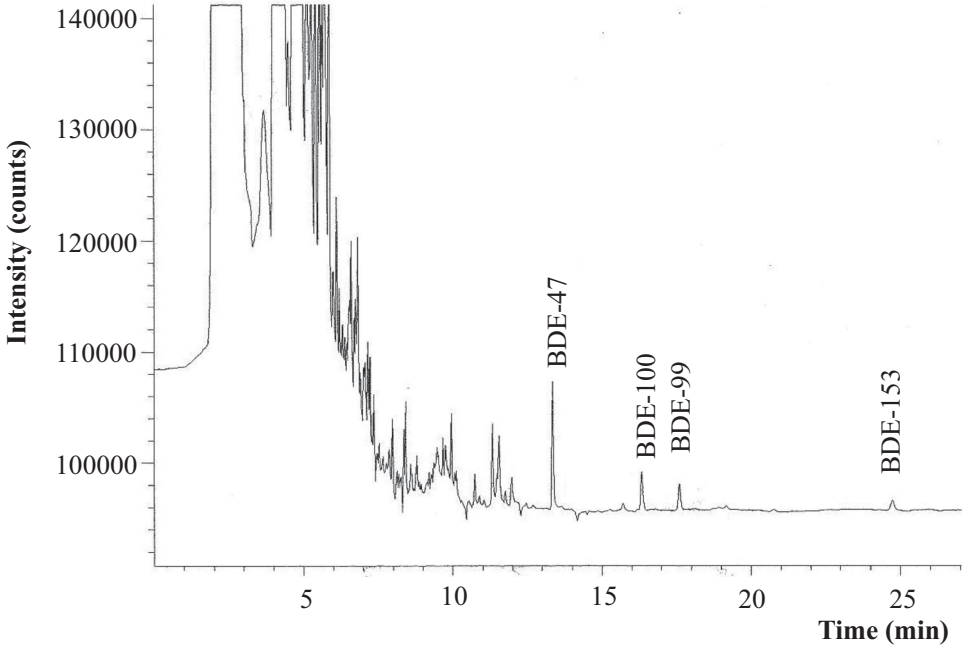

Figure 3. GC-ECD spectra obtained from the analysis of breast milk spiked with 1 ng/g with each PBDE. Methodological conditions were described in Section 2.4.

Table 2. Reported analytical methodologies for determination of PBDEs in milk samples

Instrumental technique	Extraction technique	Sample [g]	Solvent [mL]	Analyzed PBDEs	Lineal range [ng/g]	LOD [ng/g]	Recovery [%]	RSD [%]	Ref.
GC-HRMS	PLE[a)]-H_2SO_4	0.5	22.5	BDE-47, -85, -99, -100, -153, -154	5–300	0.013–0.197	91–103	11–14	[33]
GC-NCI-MS	SPE-PLE	1	113	BDE -47, -49, -99, -100, -153, -154	5–200	0.04–0.05	93–105	3–8	[34]
GC-MS	PLE/ASE[b)]-LLE	5	146	BDE -47,-100, -99, -153	12.9–580.3	0.02–0.14	77–91	2–3	[35]
GC-MS	Sap[c)]-LLE-SPE-DLLME[d)]	10.2	17	BDE -47, -100, -154, 153	20–600	0.012–0.28	78–120	2–11	[6]
GC-ECD	Sap-SPME[e)]	10.2	52	BDE -47, -100, -99, -154, -153	0.027–2.75	3.6–8.6	91–119	7–9	[32]
GC-ECD	UAE-CPE-UABE	0.5	3.1	BDE -47, -100, -99, -153	0.08–500	0.05–0.50	68–70	4–5	Current work

a) PLE, pressurized liquid extraction.
b) ASE, accelerated solvent extraction.
c) Sap, saponification.
d) DLLME, dispersive liquid-liquid-microextraction.
e) SPME, solid-phase microextraction.

nonionizables; however the UV sensitivity is favored due to reminimization of the peaks dispersion [7].

3.5 Comparison with other analytical methodologies

A comparative analysis of the performance of the proposed methodology, UAE-CPE-UABE-GC/ECD, was carried out taking into account bibliographic data remarking the figures of merit of each methodology. Table 2 summarizes analytical methodologies reported in the literature for determining PBDEs in milk samples by using GC. The reported methodologies involve different analytical techniques, and their combination, for sample preparation approaches. Two of the five reports involve a green analytical technique within the sample preparation methodology [6, 32]. The analytical figures of merits of UAE-CPE-UABE-GC-ECD methodology (linear range, RSD%, and sample consumption), were in the same order of magnitude of those previously reported. It resulted simpler and is suitable for batch preparation of biological samples by using simple equipment available in most laboratories. UAE-CPE-UABE requires small volume of organic solvents (ca. 3 mL) unlike traditional PLE, LLE, or SPE, making it a low cost and environmentally friendly methodology. It can be successfully applied for determining four PBDEs (–47, –99, –100, and –153) with a chromatographic run of 25 min.

4 Concluding remarks

The proposed analytical methodology based on the combination of the green analytical techniques (UAE, CPE, and UABE) demonstrated to be an effective approach for biological sample preparation prior GC analysis for PBDEs determination at trace levels. It expands the application frontiers of CPE for the analysis of biological samples by GC. Although the sample preparation methodology was proposed for the analysis of PBDEs by GC analysis, it could be feasible to couple it with other liquid-phase separation techniques, such as LC or CE techniques, with minimal adjustments. The experimental design statistical tool helped to identify and characterize those variables that had more influence on the system, and also their interactions. The resulting sample preparation methodology, UAE-CPE-UABE, is simple and is suitable for batch preparation using equipment available in most laboratories. Under optimized working conditions, LODs were in the order of nanograms per gram, suitable for real world applications. Although no PBDEs were detected in the analyzed samples, this work represents a contribution due to the lack of information about levels of PBDEs in human samples from Argentina.

This research was supported by CONICET and UNCuyo. The projects used for financing this work were from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2012–2429), Organization for Prohibition of Chemical Weapons (L/ICA/ICB/192151/14) and UNCuyo (SeCTyP-UNCuyo; 06/M062).

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