

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BASED ON SOLIDIFICATION OF FLOATING ORGANIC DROPLET TECHNIQUE COMBINED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR PBDES DETERMINATION IN SEDIMENT SAMPLES

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been used extensively over the past two decades as additive flame retardants (FRs) in most types of polymers to prevent ignition and to slow the initial phase of combustion. On the other hand, PBDEs are considered persistent organic pollutants because of their ubiquity, persistence and accumulation in the environment. Its harmful effects on human health and the environment, has led to its inclusion of the Stockholm Convention in 2009.

In the past few years new extraction techniques, especially in the microextraction category, have gained interest for PBDEs determination in biological and environmental samples. Efforts have been placed on the miniaturization of the liquid-liquid extraction procedure by greatly reducing the required organic solvent amount. In this way dispersive L-L microextraction and further solidification of floating organic droplet (DLLME-SFO) technique has been developed and proposed as a new analytical approach for extracting, cleaning up and preconcentrating polybrominated diphenyl ethers (PBDEs) from sediment samples prior gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis. Statistical analysis was used to evaluate the significance of the microextraction factors, and determine which combination leads to the optimum results. The combination of microextraction and chemometrics tools significantly simplify sample processing, and addresses problems related to improvement in detectability and method validation⁶. In the present work, the study and optimization of the DLLME-SFO procedure for determination of PBDEs in sediment samples by GC-MS/MS was carried out through a multivariate approach by using 2^{k-1} factorial and response-surface designs⁴. Desirability function was used to optimize the multiple response criteria based on analytes' peak areas⁴.

Materials and methods

Reagents

The PBDEs standards were purchased from Accustandard (New Haven, CT, USA) and consisted of BDE-47, BDE-99, BDE-100 and BDE-153 in isooctane. Methanol (MeOH), acetonitrile (ACN), acetone, 1-undecanol, 1-dodecanol and 1,10-dichlorodecane were purchased from Merck (Darmstadt, Germany). A 6.15 mol L^{-1} sodium chloride aqueous solution was prepared by dissolving 3.6 g of NaCl (Merck) in 10 mL of ultrapure water. Ultrapure water ($18 \text{ M}\Omega \text{ cm}$) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were of analytical grade or above.

Equipment, software and working conditions

GC-MS/MS analyses were carried out 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 (25m \times 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 250 °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 trap, manifold and transfer line temperatures were set at 220 °C, 50 °C and 280 °C, respectively.

Samples were analyzed in MS/MS 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 (486, 564, 564 and 644 for BDE-47, -100, -99 and -153 respectively) and the base ion was selected as a quantitative ion, while two other ions were used as qualifiers (m/z 324, 326, 328 for BDE-47; m/z 402,404,406 for BDE-100 and -99; m/z 482, 484, 486 for BDE-153).

Experimental design and data analysis were carried out by using the Stat-Ease Design-Expert trial version 7.0.3 software.

DLLME-SFO procedure

An aliquot of 0.5 g sediment sample was placed into a 10 mL glass-centrifuge tube and 1.2 mL MeOH was added. The analytes were leached from the sample by using ultrasonic (US) radiation at 40 °C for 20 min, which were distributed into two cycles 9'22'' long with 1 min break in-between each one. The resulting slurry was centrifuged at 2000 rpm for 10 min, 0.5 mL aliquot of leaching solvent was transferred to a 10 mL empty glass-centrifuge tube and 0.1 mL MeOH (dispersive solvent), 20 mg 1-dodecanol (extracted solvent) and 1 mL 6.15 mol L⁻¹ NaCl were added and mixed up. Afterwards the 4.4 mL, 40 °C ultrapure water was added leading to dispersion of 1-dodecanol droplets into the aqueous bulk. In order to separate the extracting organic phase, the glass-centrifuge tube was then kept into an ice bath for 10 min. An organic droplet was solidified and separated by flotation, which was then transferred to a conical vial for further injection into the gas chromatograph for analysis.

For method development, an aliquot of 0.5 g sediment sample (PBDEs free) was spiked with the target PBDEs using methanolic solutions and homogenized as described by Salgado-Petinal et al.⁵

Quality assurance/quality control

The quantification of PBDEs by GC-MS/MS was accomplished by standards addition method. The limits of detection (LODs), calculated based on three times standard deviation of the background signal (3σ) were 0.03 ng g⁻¹, 0.04 ng g⁻¹, 0.05 ng g⁻¹ and 0.07 ng g⁻¹ for BDE-47, -100, -99 and -153, respectively. The precision of DLLME-SFO-GC-MS/MS evaluated over five replicate, leading RSDs values <9.2 %. The calibration graph was linear with a correlation coefficient of 0.9957 within the concentration range: 0.08–1000 ng g⁻¹ for BDE-47, 0.10–1000 ng g⁻¹ for BDE-100, 0.11–1000 ng g⁻¹ BDE-99 and 0.2–1000 ng g⁻¹ for BDE-153

Results and discussion:

Effect of type dispersive and extraction solvent

Several requirements are considered for choosing the disperser and extraction solvents. Disperser solvents in DLLME should be miscible with water and the extraction solvent. On the other hand, the extraction solvent should not be miscible with water and should also have a melting point higher to the room temperature (≤ 10 -30 °C) in order to succeed the SFO requirements^{2,3}. Additionally, the extraction phase must be compatible with the instrumentation to be used for further analysis. Taking into account these considerations, MeOH, ACN and acetone were assayed as disperser solvents and 1-undecanol, 1-dodecanol and 1,10-dichlorododecane as extraction solvent. The performance of these solvents was studied by adding 0.75 mL of dispersive solvents and 20 mg of extraction solvent to a 0.5 g of sediment containing 60 ng g⁻¹ of each PBDE according with the procedure described above. Best relative responses of PBDEs were achieved utilizing MeOH and 1-dodecanol. Under these conditions a rapid drop formation was achieved, which facilitated its handling and transference from the aqueous bulk. When employing other dispersive or extraction solvents, a decrease in the PBDEs' analytical signal was observed. This could be due to a diffuse formation of the drop, and densities comparable to the water's one. Therefore, MeOH and 1-dodecanol were selected for further studies.

Experimental design

Fractional factorial experimental design (2^{k-1}) was used to evaluate the preliminary significance of the variables that govern each technique, as well as their interactions. Once established the significant variables that influence the leaching and microextraction efficiencies, an optimization procedure was carried out in order to achieve the best extraction yield for the studied PBDEs. In this sense, response surface statistical technique was used to determine the interaction of possible influencing parameters on PBDE extraction by assaying a limited number of planned experiments. For each technique, the experiments were defined by using reduce central composite design (CCD). The ranges of the selected variables were carefully chosen based on prior knowledge about the system under study.

A multiple response criteria using the desirability function was successfully used in order to optimize both procedures. The statistical method proposes a desirability function which includes researcher's priorities and desires on building the optimization procedure. The procedure creates a function for each individual response, and finally obtains a global function D that should be maximized choosing the best conditions of the designed variables.

Optimization of leaching technique

The analyzed factors considered for the fractional factorial experimental design (2^{k-1}) were: US radiation time and mode, temperature and volume of extraction solvent. Table I presents the selected levels for the studied variables. Series of sample solutions were assayed by using 0.5 g of sediment containing 20 ng g⁻¹ of each PBDE and 20 mg of 1-dodecanol. On the other hand, the microextraction procedure conditions were as follows: (A) dispersant solvent volume: 0.10 mL; (B) extracting solvent mass: 22.1 mg; (C) 6.15 mol L⁻¹ NaCl: 1 mL volume; (D) dispersant aqueous bulk volume: 4.40 mL. Peak area and shape were evaluated in each case.

From the variance analysis of the experimental data (peak area) all the variables were shown to be significant ($p < 0.1$), with positive and negative effects¹.

Table I. Screening phase during solvent extraction technique optimization for PBDE determination

Factors and their levels investigated		
Factor	Levels ^a	
	-1	+1
(A) US radiation time (min)	15	30
(B) US extraction mode	Continuo	Mult. step
(C) Leaching temperature (°C)	20	40
(D) Leaching solvent volume (mL)	0.80	1.5

^a-1 and +1: Extreme levels¹

A central composite design was then carried out including 30 experiments. The variables and their ranges considered were: (A) US radiation time (min): 18.75 – 36.41; (B) US radiation steps: 2 – 4; (C) leaching temperature (°C): 27.5 – 42.5; (D) leaching solvent volume (mL): 1.22 – 2.08. Outliers were removed by analyzing the difference between fitted values test (DFFITs)⁴. The model coefficients were calculated by backward multiple regression, and validated by the analysis of variance¹.

Quadratic models are those which better

explain the behavior of the relative response of the analytes under the effect of the studied factors. As could be observed from the statistical parameters corresponding to the fitting for resolution, models are significant ($p < 0.05$) and the lack of fit is not significant ($p > 0.05$).

At 95% confidence level, it was observed that the solvent volume affects the extraction efficiency of the target PBDEs. Temperature and leaching time were not significant; however their interaction affects the leaching efficiency of BDE-100, -99 and -153. The squared of the temperature also affects the leaching efficiency of the studied PBDEs, except PBDE 47; which is affected by the squared of US radiation time. It is important to point out that even though the effect of multiple US radiation steps was significant against continue mode (shown during fractional factorial design), the number of steps required was minimum.

The responses of the four PBDEs (peak area) were simultaneously optimized by using the desirability function. The criterion was followed to maximize the individual responses, all with the same importance. Under the mentioned optimization criteria, the experimental conditions corresponding to one of the maximum in the desirability function ($D=0.762$) were: US radiation time 18'45" min, 1 stop, leaching temperature 40 °C and leaching solvent volume 1.20 mL. The values suggested through the optimization procedure were experimentally corroborated.

Optimization of DLLME-SFO technique

In the screening design of the DLLME-SFO the considered variables includes salting out effect, additional dispersive solvent, dispersive aqueous bulk and extraction solvent mass. The variables and their levels are presented in Table II.

Solvent extraction conditions were fixed at: (A) leaching solvent volume: 1.20 mL; (B) leaching temperature: 40 °C in a US bath; and (C) US radiation steps: two cycles 9'22" long and 1 min break in-between each cycle.

From the variance analysis, the extraction solvent mass was the most significant factor ($p < 0.1$) on the analytical response of the studied PBDEs, followed by interactions between dispersant aqueous bulk volume and extraction solvent mass; salt addition and dispersant aqueous bulk volume; and salt addition and extraction solvent volume. Salt addition showed a positive significant effect on DLLME-SFO.

Table II. Screening phase during the DLLME-SFO optimization for PBDE determination

Factor	Levels ^a	
	-1	+1
(A) Salt addition (mL)	0	1
(B) Additional dispersive solvent (mL)	0	0.5
(C) Dispersive bulk (mL)	2	5
(D) Extracting Solvent (mg)	20	50

^a -1 and +1: Extreme levels¹

(for BDE-47, -99 and -153) and linear (for BDE-100). Moreover, the lack of fit was not significant ($p > 0.05$).

As expected, the p -values showed that at 95% confidence level, the amount of extracting solvent affects the extraction of all PBDEs under study. Leaching temperature only affects the extraction of BDE-153. On the other hand, the interaction of this factor with the extraction solvent mass affects the analytical response of BDE-47 and -99. Moreover, the interaction between leaching temperature and dispersant aqueous bulk volume affects the analytical response of BDE-153. The dispersant aqueous bulk volume directly affects the analytical response of PBDE 100.

The responses of the four PBDEs (peak area) were simultaneously optimized by using the desirability function. The criteria was followed to maximize the individual responses (peak area), all with the same importance. Under the mentioned optimization criteria, the experimental conditions corresponding to one of the maximum in the desirability function ($D = 0.756$) were: (A) dispersant solvent volume: 0.10 mL; (B) extracting solvent mass: 22.1 mg; (C) 6.15 mol L⁻¹ NaCl volume: 1 mL; (D) dispersant aqueous bulk volume: 4.40 mL. The values suggested through the optimization procedure were experimentally corroborated.

Conclusion

DLLME-SFO constitutes a simple and efficient analytical technique for extraction and preconcentration of PBDEs from sediment samples and further analysis by GC-MS/MS. It led to an increment of the analytical methodology sensitivity. Under optimized working conditions, LODs were in the order of nanogram per gram suitable for real world applications with an acceptable precision. DLLME-SFO-GC-MS/MS showed comparable LODs with Soxhlet-GC-MS/MS methodology². However, the proposed methodology requires minimum sample manipulation, organic solvents consumption and increases sample throughput of the analysis. The method performs well achieving good linearity, precision and low detection limits. The robustness of the proposed methodology was proved when the recovery study was carried out over the real samples. The proposed DLLME-SFO-GC-MS/MS methodology can be successfully applied in routine analysis to determine trace levels of PBDEs in sediment samples.

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A central composite design was then carried out including 20 experiments. The ranges of the selected independent variables were: (A) additional dispersive solvent (mL): 0.10 – 0.40; (B) extracting solvent (mg): 22.1 – 43.0; (C) dispersive aqueous bulk (mL) 2.60 – 4.40. Once outliers were removed by analyzing the difference between fitted values test (DFFITS), the model coefficients were calculated by backward multiple regression⁴, and validated by the analysis of variance¹. The models which better explain ($p < 0.05$) the behavior of the relative response of the analytes under the effect of the studied factors were 2-factor interactions (2FI)