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Short communication

Determination of polybrominated diphenyl ethers in marine biological tissues using microwave-assisted extraction

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Abstract

Growing concern on the environmental impact of polybrominated diphenyl ethers (PBDEs) has created the need for rapid and quality assured analytical methods to quantify PBDEs in a spectrum of matrix types. This study presents the first validated method for the quantification of major PBDE congeners (47, 99 and 100) in marine biological tissues using microwave-assisted extraction (MAE). The recovery of polychlorinated biphenyls and various organochlorine pesticides has also been ascertained. Analytical accuracy, precision, limits of detection and cleanup efficiency were evaluated for PBDE congeners, and empirical data justifies the use of MAE for the extraction and analysis of PBDEs in biological matrices. MAE was also compared to Soxhlet extraction efficiency for PBDEs in the standard reference materials SRM2978 and SRM1588a and gave comparable results (<15% variation).

Keywords: Extraction methods; Flame retardants; Food analysis; Polybrominated diphenyl ethers; Polychlorinated biphenyls; Pesticides; Organochlorine compounds

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a major family of brominated flame retardants which are lipophilic, persistent, and toxic to both fauna and humans [1]. The PBDE congeners 47 (2,2′,4,4′-tetra-BDE), 99 (2,2′,4,4′,5-penta-BDE) and 100 (2,2′,4,4′,6-penta-BDE) are dominant in the PBDE profile of numerous environmental matrices, and have been detected in a wide range of marine organisms and seafood types [1]. Quantitative analysis of PBDEs in marine biota tissues has created the need for a rapid and quality assured method.

Published methods for the detection of PBDEs in marine tissue matrices include Soxhlet extraction [2,3], column elution [4,5], and a manual shaking procedure [6]. These methods are both time and solvent consuming, as noted in Table 1. A pressurized liquid extraction technique has also been developed to reduce both time and solvent consumption [7], but at a high capital cost for analytical equipment [8]. Microwave-assisted extraction (MAE) has been successfully applied to the extraction of various persistent organic pollutants (POPs) in marine biological matrices, including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) [9,10]. Vetter [11] reported the use of MAE to extract brominated compounds, including PBDEs form several types of marine biological tissues, but no performance or validation of the procedure was performed. The objective of this study was to determine if the MAE method, which has been previously validated for OCPs and PCBs, can also be applied for PBDE analysis in marine biota.

2. Experimental

2.1. Chemicals

All organic solvents used were pesticide residue analytical grade. PBDE congener 47, 99 and 100 standards,

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Table 1
Reported extraction methods for the determination of PBDEs in marine biological tissues

Extraction technique	Solvent type	Solvent consumption (ml)	Extraction time	Reference
Soxhlet	Hexane–acetone (4:1)	350	6 h	[2]
	Acetone–hexane (1:1)	n.a.	24 h	[3]
Column elution	Methylene chloride	300	n.a.	[4]
	Hexane–methylene chloride (1:1)	250–350	5–7 min	[5]
Manual shaking	Hexane–diethyl ether (3:1)	2100	Three times 10 min	[6]
Pressurized liquid extraction	Methylene chloride	n.a.	Two times 5 min	[7]
MAE	Ethyl acetate–cyclohexane	8	Seven times 5.5 min	[11]

n.a.: non-available data.

as well as OCP standards, were obtained from Accustandard (New Haven, CT, USA). PCB 55 and 61 standards were obtained from Dr Ehrenstorfer (Augsburg, Germany), and other PCB standards were obtained from Accustandard.

2.2. Sample preparation

Muscle tissues of salmon and conger eel, liver tissues of sea bass and the whole soft tissues of green mussel (*Perna viridis*) were used for determining PBDE recovery. Lipid content was determined in tissues gravimetrically after Soxhlet extraction with 150 ml of dichloromethane for 6 h. Lipid content in the tissues was as follows: muscle tissues of conger eel, 1.2%; muscle tissues of salmon, 8.2%; liver tissues of sea bass, 38.5%; and whole soft tissues of green mussel, 3.3%. Tissue moisture content was determined using a Mettler Toledo LJ16 Moisture Analyzer. The moisture content in the tissues was as follows: muscle tissues of conger eel, 78%; muscle tissues of salmon, 67%; liver tissues of sea bass, 48%; and whole soft tissues of the green mussel, 68%. Each tissue type was homogenized in a stainless steel blender, where sample size was selected based on the lipid content of the tissues and the expected concentration in the tissue. Sample size for fish muscles and green mussel tissues was optimized at four grams. As liver tissues usually contain more lipids and higher levels of organic pollutants [1], only 2 g was used. The recovery test was undertaken for target concentrations ranging from 50 to 200 ng/ml in the final extract for each PBDE congener, where this range is representative of congener levels previously reported in biota samples [1]. The spiking solution was allowed to equilibrate with the homogenized tissue sample for two hours prior to extraction. To date, there is no SRM data available for PBDE analysis in marine biological tissues. The analytical method validated in our study was used to quantify PBDEs in mussel tissue SRM2978 and cod liver oil SRM 1588a (NIST, Gaithersburg, MD, USA) and compared to results obtained with conventional Soxhlet extraction. Soxhlet extraction was undertaken on samples using 150 ml of dichloromethane for six hours. Ten procedural blank samples (20 g of Na_2SO_4) spiked with the recovery standards were extracted and analyzed in order to calculate the method detection limit (MDL) for the entire analytical procedure.

2.3. Chemical analysis

Samples of homogenized tissue were ground with sodium sulfate (ratio: 5 g of Na2SO4 per gram of tissue). MAE was performed using a Mars X (CEM, Matthews, NC, USA) oven, with 25 ml of *n*-pentane–DCM (1:1, v/v) as the extraction solvent. The oven was programmed for a temperature increase to 115 °C over a 10 min period which was then maintained for 15 min. Clean-up and pre-concentration steps prior to sample analysis are described in our previous report [12]. Briefly, sample clean-up included the degradation of lipids on a 10 g acid silica gel column followed by gel permeation chromatography (GPC). A typical PBDE analyte elution profile out of the GPC column was determined by the elution of a standard containing both OCPs and PBDEs, and then separately analyzing each 1.9 ml fraction eluted from the column. The analysis and quantification of all analytes was performed using a Shimadzu QP-2010 (Shimadzu Asia-Pacific, Singapore) gas chromatograph coupled with a mass spectrometer (GC–MS). Analytes were separated on a DB-5ms (J&W Scientific, USA) capillary column (30 m length, 0.25 mm i.d.) with a helium flow of 35 cm/min. The GC oven program was as follows: 50 °C held for 1 min, 20 °C/min to 150 °C held for 5 min, 3 °C/min to 250 °C, 10 °C/min to 300 °C held for 10 min. The detector was operated in electron impact ionisation (EI) mode with selected ion monitoring (SIM). Ion masses 326, 486 and 484 were selected for tetra-BDE 47 and masses 406, 404 and 408 for penta-BDE 99 and 100, corresponding to peaks at either M^+ or $M–Br_2^+$. Analyte quantification was performed using a six-point calibration.

3. Results and discussion

3.1. Surrogate recovery

Quantitative results are deemed acceptable provided surrogate compounds are recovered in the range of 70–130%, according to US Environmental Protection Agency (EPA)

Method 1668a [13] for PCB congeners in marine biota tissues. Actual recoveries for PCB 55 and PCB 61 averaged 95 ± 12 and 93 ± 12%, respectively, confirming that there was no unacceptable loss of analytes during the entire analytical procedure.

3.2. Calibration curves and limits of detection

Linear calibration curves were obtained for PBDE congeners 47, 99 and 100 over the 0–200 ng/ml range, with an r^2 coefficient of greater than 0.99. The MDL was calculated as the average of the procedural blank peak area plus three times the standard deviation. The MDL was below 0.1 ng/g of sample for all PBDE congeners and is comparable with other studies using conventional extraction techniques [2].

3.3. Secondary ion ratio

For BDE-47, the secondary ions $m/z = 486$ and 484 represented 84 ± 5 and 55 ± 6% of the quantification ion $m/z = 326$, respectively. For penta-BDEs (99 and 100), the secondary ions $m/z = 404$ and 408 represented 99 ± 10 and 33 ± 13% of the quantification ion $m/z = 406$, respectively. Average sample secondary ion ratios were between 1 and 6% different from the ratio obtained for standards, i.e. substantially lower than the 20% variation recommended by EPA Method 1668a [13] for PCB congener analysis in marine biota tissues.

3.4. Extract clean-up

The analyte elution profile was obtained from the GPC column, where PBDE congeners 47, 99 and 100 were eluted between 18 and 25 ml. This profile is similar to that of OCPs such as DDTs and chlordanes. Hexachlorocychohexanes (HCHs) eluted later at between 20 and 30 ml. Therefore, the first 16 ml fraction, containing lipids, was discarded. Lipids are the main source of interference when performing analysis of trace organic pollutants in biological tissues. A chromatogram showing the quantification ion of the PBDE congeners in a 200 ng/ml mixed standard is shown in Fig. 1a. Chromatograms obtained for SRM1588a and SRM 2978 extracted with MAE are presented in Fig. 1b and c, respectively. The spectra do not show any major interference for either the quantification or confirmation ions (unpublished data). Therefore, the extract clean-up procedure can be considered as effective.

3.5. PBDE recovery in spiked tissues

The accuracy of the method was evaluated by checking the recovery of spiked PBDE-47, 99 and 100 at five concentrations in the four tissue types. The measured concentration for all congeners was linear with respect to spiked concentrations in all tissues ($r^2 > 0.90$). Average analyte recovery

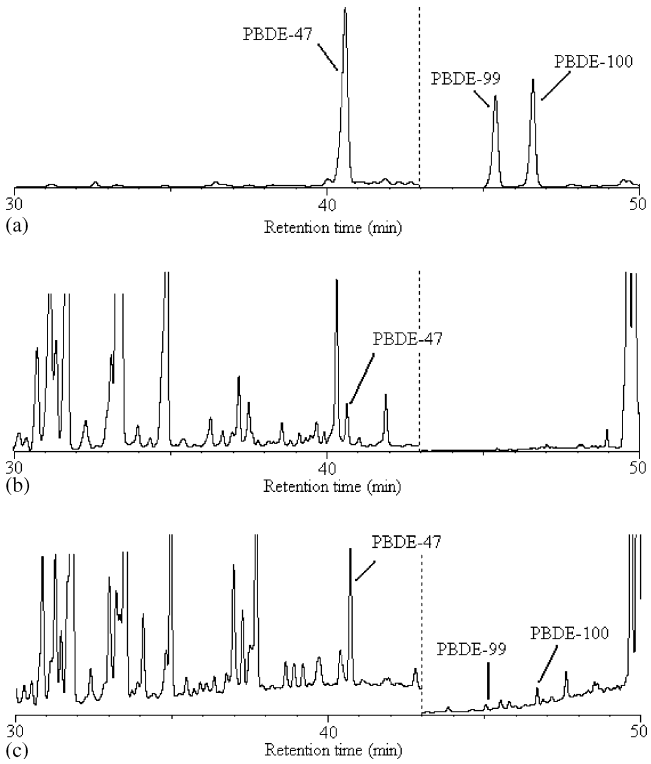

Fig. 1. Comparative chromatograms for the quantification ion of the PBDE congeners (47, 99 and 100) for a 200 ng/ml standard (a) and for SRM 1588a (b) and SRM 2978 (c) extracted using MAE.

was higher for salmon muscle (94 ± 7%) and seabass liver tissues (97 ± 14%) than for conger eel muscle (89 ± 14%) and green mussel tissues (89 ± 7%). Average recovery was slightly higher for congeners 99 (92 ± 11%) and 100 (95 ± 14%) which are penta-brominated, than for the congener 47 (89 ± 8%) which is tetra-brominated. Temperature is a crucial parameter for MAE as it can lead to the degradation or incomplete extraction of analytes [14]. However, the MAE temperature of 115 °C can be deemed as suitable for PBDE extraction as individual congener recovery was high. Other extraction parameters applied, including solvent type, extraction time, and microwave power can also be considered as effective for MAE of PBDEs in the tissue matrices analyzed.

3.6. Analytical precision

The relative standard deviation (R.S.D.) was evaluated at the level corresponding to the medium value of the calibration range (i.e. approximately 100 ng/ml in the final extract). R.S.D. was calculated for seven samples, i.e. three fish muscle tissues, two fish liver tissues and two mussel tissues. The R.S.D. for the seven replicate analyses were 6.8, 13.2 and 11.9%, respectively, for BDE-47, 99 and 100, i.e. substantially lower than a 40% R.S.D. value recommended by EPA Method 1668a [13] for PCB congener analysis in marine biota tissues.

3.7. Comparison of analyte recovery for MAE and Soxhlet extraction

SRM 2978 and SRM 1588a were both analyzed using the MAE and Soxhlet extraction procedures. Recoveries of OCPs and PCBs averaged 83 and 89% for MAE and Soxhlet extraction, respectively. As average recoveries for both OCPs and PCBs were in excess of 80%, analyte recoveries from both methods can be considered as acceptable and comparable. Concentrations of PBDE-47, 99 and 100 were measured in the two SRMs using MAE and Soxhlet extraction. Both extraction methods yielded similar results, with MAE yielding slightly lower concentrations than Soxhlet extraction (less than 15% variation). PBDE-47 was the dominant congener in the two SRMs analysed, reaching 24 ng/g dry mass in SRM 2978 and 21 ng/g wet mass in SRM 1588a.

4. Conclusion

The accuracy and precision of the MAE method, combined with the effective removal of matrix interference, makes possible accurate quantitative analysis of PBDE congeners in marine biological tissues. The extraction solvent volume (25 ml) is substantially lower than that required for conventional Soxhlet extraction and column elution (see Table 1), and the extraction time is reduced from several hours to 25 min. The detection limit of the method is below 0.1 ng/g of sample for all PBDE congeners analysed. No maximum permissible limit for PBDE in food for human consumption is currently available, but a limit of 2 μg/g for PCBs in fish and shellfish is stipulated by the US Food and Drug Administration [15]. If a similar limit were applied to PBDE congeners, then the proposed MAE method is relevant for the rapid, sensitive and quantitative analysis of PBDEs in seafood.

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