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RESEARCH PAPER

Gas chromatography/tandem mass spectrometry method for the simultaneous analysis of 19 brominated compounds in environmental and biological samples

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Abstract A methodology for the simultaneous analysis of eight polybrominated diphenyl ethers (PBDEs); eight methoxylated PBDEs (MeO-PBDEs); and three emerging flame retardants, hexabromobenzene (HBB), pentabromoethyl benzene (PBEB), and decabromodiphenyl ethane (DBDPE) by gas chromatography coupled to tandem mass spectrometry (GC-MS-MS) was developed for two environmental matrices (sediment and sludge) and three biological matrices (fish, dolphin blubber, and bird eggs). The use of selective reaction monitoring (SRM) allows a high selectivity, which is critical in the analysis of complex samples like blubber. Analytical parameters such as linearity, reproducibility, or accuracy were evaluated. Method limits of detection and quantification were evaluated and compared with GC-EI-MS and GC-NCI-MS. Method detection limits were valid for the environmental analysis in all cases, with values between 0.01 and 1.65 ng/g dw for sediment, 0.05 and 2.78 ng/g dw for sludge, 0.04 and 10.6 ng/g lw for fish, 0.01 and 1.11 ng/g lw for dolphin blubber, and 0.03 and 3.20 ng/g lw for bird eggs. The developed method was applied to five samples of each matrix. PBDEs were detected in all samples, while MeO-PBDEs were only detected in dolphin blubber. DBDPE was detected in sediment and sludge.

Keywords Emerging BFRs · GC-MS-MS · MeO-PBDEs · PBDEs

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Introduction

Brominated flame retardants (BFRs) are one of the most used families of flame retardants (FRs), and its demand increases every year. They are used in a wide range of materials such as textiles, furniture, or wire coat materials, where they represent a considerable amount of the total product weight [1]. One of the main BFR families is polybrominated diphenyl ethers (PBDEs) which have been widely used in great amounts for many years. However, this situation has changed and penta- and octa-BDE mixtures are already banned in USA and EU, while the production and usage of deca-BDE formulation is decreasing [2]. Thus, other alternative FRs such as hexabromobenzene (HBB), pentabromoethyl benzene (PBEB), and decabromo diphenyl ethane (DBDPE) have been proposed as an alternative [3]. In contrast to the few studies reporting environmental levels for these new compounds, PBDEs have been found since 1970 [4] in several environmental and biological matrices such as sediment [5], sludge [6], air [7], fish [8], bird eggs [9], or cetaceans [10]. Besides, they have been found in remote places such the Artic, proving their wide-range transport [11].

On the other hand, other brominated compounds naturally produced by sponges or red algae have proved to be present in the environment in similar levels to PBDEs, sometimes even at higher concentrations [12]. Methoxylated PBDEs (MeO-PBDEs) represent an example of these halogenated natural products (HNPs). They have been found in several cetaceans [13] and also in fish [14] around the world.

Brominated compounds were normally analyzed by GC-NCI-MS, which provides a great sensitivity. But, on the other hand, it is a low selectivity technique. Thus, problems related to the coelution of some relevant compounds might occur. The objective of this work was to develop a selective technique for the analysis of these brominated compounds in environmental (sediment and sludge) and biological (fish, dolphin blubber,

and bird egg) matrices by gas chromatography coupled to tandem mass spectrometry (GC-MS-MS). Other works have pointed out the importance of using GC-MS-MS for the analysis of some of these compounds [15–17], but this work is the first which includes such a wide range of matrices and the simultaneous determination of all these compounds. In addition to the selectivity obtained by using MS-MS instead of MS, this technique allows the usage of mass-labeled standards for isotope dilution quantification. Some analytical parameters that are not usually given, such as accuracy, together with recovery values and limits of detection were evaluated.

Materials and methods

Standards and reagents

Method 1614 Surrogate Stock Solution (PAR Solution) containing tri-BDE-28, tetra-BDE-47, penta-BDE-99, penta-BDE-100, hexa-BDE-153, hexa-BDE-154, hepta-BDE-183, and deca-BDE-209; a mixture of MeO-PBDEs containing 5-MeO-BDE-47, 6-MeO-BDE-47, 4-MeO-BDE-49, 2-MeO-BDE-68, 5′-MeO-BDE-99, 5-MeO-BDE-100, 4′-MeO-BDE-101, and 4-MeO-BDE-103; and also HBB, DBDPE, and PBEB were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Method 1614 Labeled Surrogate Stock Solution containing the mass labeled PBDEs (^{13}C-BDE-28, ^{13}C-BDE-47, ^{13}C-BDE-99, ^{13}C-BDE-100, ^{13}C-BDE-154, ^{13}C-BDE-153, ^{13}C-BDE-183, and ^{13}C-BDE-209) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA)

Alumina (0.063–0.2 mm) and copper (<63 μm) were obtained from Merck (Darmstadt, Germany). Al-N and silica cartridges were obtained from Biotage. Dichloromethane and hexane, solvents for organic trace analysis, were purchased from Merck.

Sample collection

Several samples of sediment, sludge, fish, dolphin blubber, and bird egg were analyzed in order to evaluate the performance of the developed methodology in real samples. Among the Llobregat River Basin (Spain), five sediment samples from five different sampling points were collected with a Van Veen drag while five different sludge samples were collected from five different waste water treatment plants (WWTPs). Moreover, five fish samples from different sampling points of the same river were collected by DC electric pulse. Furthermore, dolphin blubber samples from *Tursiops truncatus* were obtained by biopsy sampling in the Gulf of Cadiz, and white stork egg samples that had failed to hatch were collected from Doñana National Park, in southern Spain. All the samples were homogenized and freeze-dried. Sediment and sludge samples were sieved (120 μm). All samples were kept at −20 °C until analysis.

Sample preparation

The sample methodologies used were similar to those previously optimized for the extraction and purification of PBDEs [18–20]. In the case of sediment, selective pressurized liquid extraction (SPLE) was applied. However, this methodology could not be applied for the other matrices since all (sludge, fish, dolphin blubber, and bird egg) require a more complex cleanup and lipid content determination in the case of biota. Normal PLE was used in these cases. Both SPLE and PLE were carried out in an ASE 350 system (Dionex, Sunnywale, CA, USA).

Sediment

Before extraction, 1 g dry weight (dw) was spiked with 5 ng of the mass labeled PBDEs (50 ng for ^{13}C-BDE-209). Samples were kept overnight to equilibrate and were grown with alumina and copper (1:2:2) and loaded into a 22-mL extraction cell previously filled with alumina (6 g). Hexane:dichloromethane (1:1) were used as extraction solvents, with a temperature of 100 °C and a pressure of 1500 psi. Two static cycles of 10 min were made. Extracts were concentrated to incipient dryness and reconstituted in 40 μL of toluene prior to instrument analysis.

Sludge

Before extraction, 1.5 g dw were spiked with 10 ng of ^{13}C-PBDEs and 100 ng of ^{13}C-BDE-209. Samples were grown with copper (1:2) and loaded into the extraction cell. PLE conditions were the same as the ones described before. Resulting extracts were treated with sulphuric acid and then purified with two consecutive solid phase extraction (SPE) since sludge needs a more complex cleanup than the other matrices. The first one was done using silica cartridges (2 g) and the second one using alumina cartridges (5 g). Resulting extracts were concentrated and reconstituted as described before.

Biota samples

Before extraction, 1 g of sample was spiked with 5 ng of ^{13}C-PBDEs and 50 ng of ^{13}C-BDE-209. Samples were kept overnight to equilibrate and then loaded into an 11-mL extraction cell. PLE extractions were done using the same parameters than for sediments. Extracts were evaporated and, after gravimetric determination of the lipid content, redissolved in hexane. Fat was removed with $H_2SO_{4(conc.)}$, and the extracts were

purified with SPE using alumina cartridges (AL-N, 5 g). Resulting extracts were concentrated to 40 μL in toluene.

Instrumental determination

GC-MS-MS analyses were performed on an Agilent Technologies 7890A GC system coupled to a 7000A GC/MS Triple Quadrupole. A DB-5ms capillary column (15 m×0.1 mm i.d., 0.1 μm film thickness) was used for the chromatographic separation with helium as carrier gas. Different temperature ramps were tested. The final gradient started at 140 °C, held for 1 min and then ramped to 310 at 10 °C/min and held for 10 min, for a total run time of 36.5 min.

Two different ionization modes, negative chemical ionization and electron ionization (NCI and EI, respectively), were tested. It is well known that NCI provides a greater sensitivity for halogenated compounds than EI, while on the other hand, EI is a more selective technique. Obtained transitions for PBDEs using NCI-MS-MS did not provide acceptable results in terms of sensitivity. Moreover, the use of EI allows the quantification by isotope dilution, which is far more reliable than using other non-labeled standards. Besides, we compared also EI-MS with EI-MS-MS. The selectivity obtained when using MS-MS is especially important when analyzing complex matrices since there are lot of possible interferences. After comparing the different methodologies, EI-MS-MS was chosen. Selective reaction monitoring (SRM) mode was used with two transitions monitored for each compound. The most intense transitions were used for quantification purposes, and the second ones for confirmation criteria comparing the SRM1/SRM2 ratio calculated for the standards with the ratio found in the samples. Table 1 shows the retention time (Rt) and collision energies (CE) for all the compounds studied. Other MS parameters such as ion source temperature (T), gas flow (GF), injector temperature (IT), and transfer line temperature (LT) were also optimized in order to increase the signal. The optimum values were 300 °C, 2.25 ml/min, 280 °C, and 280 °C, respectively. Ionization energy was set at 70 eV since it is the standard energy used in this technique.

Analytical parameters

Linearity was determined by a six-point calibration curve including all the analytes, with concentrations ranging from 10 to 1000 pg/μL, and the internal standards in a concentration of 100 pg/μL. Standards were prepared in toluene and stored at −20 °C. Repeatability was measured by the relative standard deviation (RSD) of five consecutive injections (intra-assay) and three injections on four different days (inter-assay). Accuracy was measured by the percent deviation (%Dev) of the nominal concentration both for intra- and inter-assays. Both repeatability and accuracy were assessed in three different concentration levels: low level (0.025 ng/μL for PBDEs, MeO-PBDEs, HBB, and PBEB; 0.25 ng/ μL for BDE-209; and 0.06 ng/ μL for DBDPE), medium level (0.2 ng/μL for PBDEs, MeO-PBDEs, HBB, and PBEB; 2 ng/μL for BDE-209; and 0.5 ng/μL for DBDPE);,and high level (1 ng/μL for PBDEs, MeO-PBDEs, HBB, and PBEB; 10 ng/μL for BDE-209; and 2.5 ng/μL for DBDPE).

Instrumental detection limits (IDLs) were determined for each compound as the minimum amount of analyte that gave a signal to noise ratio (S/N) of 3, and the instrumental quantification limits (IQLs) were determined as the minimum amount of analyte that gave a S/N of 10.

Recovery, repeatability, and accuracy were also measured (intra- and inter-assay) for the five matrices studied at three concentration levels by spiking 1 g of sample with 5 ng of PBDEs, MeO-PBDEs, HBB, and PBEB, 50 ng of BDE-209 and 25 ng of DBDPE (low level); 20 ng of PBDEs, MeO-PBDEs, HBB, and PBEB, 200 ng of BDE-209 and 100 ng of DBDPE (medium level); and 50 ng of PBDEs, MeO-PBDEs, HBB, and PBEB, 500 ng of BDE-209 and 250 ng of DBDPE (high level). Five replicates were done for each matrix and level except for dolphin blubber, where due to sample availability only three replicates were made for medium and high levels. Three blank samples were made for each matrix in order to evaluate the presence of these compounds in the matrices used, correcting the value obtained when the contribution was higher than 5 %. However, the contribution never exceeded a 10 % of the total value except for dolphin blubber, which was one of the reasons to only choose medium and high level for this matrix.

Method detection and quantification limits (MDL and MQL, respectively) were calculated using low level points for sediment, sludge, fish, and bird egg, and medium level for dolphin blubber, by the same method used to calculate IDLs and IQLs.

Results and discussion

GC-MS-MS conditions

Two different columns were tested using different temperature programs: a DB-5ms capillary column (15 m×0.1 mm i.d., 0.1 μm film thickness) and a DB-5ms capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness). A 30-m column is used in some studies to achieve a total separation of all the analytes but, on the other hand, BDE-209 is completely lost due to thermal degradation also due to the long retention time in these columns [21]. Since the separation obtained for all the analytes was satisfactory for all the compounds with the temperature program used, the 15-m column was chosen.

Several MS parameters were optimized in order to maximize the signal. The GF was tested modifying its value

Table 1 Retention times, two transitions (SRM_1 and SRM_2), and the collision energies (eV) of each one

	Compound	Rt	SRM_1	CE_1	SRM_2	CE_2
PBDEs	BDE-28	10.8	408>246	25	408>248	25
	BDE-47	12.8	486>326	30	488>328	30
	BDE-100	14.1	406>297	30	564>404	30
	BDE-99	14.6	406>297	30	564>404	30
	BDE-154	15.6	486>377	30	644>484	30
	BDE-153	16.2	486>377	30	644>484	30
	BDE-183	17.7	721>562	30	721>564	30
	BDE-209	23.2	298>220	25	361>280	25
MeO-PBDEs	2-MBDE-68	13.5	516>356	25	516>358	25
	6-MBDE-47	13.8	516>356	25	516>358	25
	5-MBDE-47	14.2	516>356	25	516>358	25
	4-MBDE-99	14.4	516>356	25	516>358	25
	5-MBDE-100	15.1	596>434	30	594>436	30
	4-MBDE-100	15.2	596>434	30	594>436	30
	5-MBDE-99	15.8	596>434	30	594>436	30
	4-MBDE-101	15.9	596>434	30	594>436	30
Emerging BFRs	HBB	17.1	468>308	25	468>310	25
	PBEB	11.1	500>485	30	485>406	30
	DBDPE	24.4	485>406	25	325>165	25

Rt retention times, *CE* collision energies

between 1.25 and 2.5 mL/min (1.25, 1.5, 1.75, 2, 2.25, and 2.5). The optimal value was 2.25 mL/min. ST was tested modifying its value from 150 to 300 °C (150, 200, 250, and 300 °C). The optimal value was set at 250 °C. Higher temperatures caused the degradation of the high brominated compounds such as BDE-209.

The fragmentations of the precursor ion for PBDEs proved to be dependent on the bromination degree. Sánchez-Ávila et al. [15] reported different fragmentation patterns for tri-, tetra-, penta-, hexa-, hepta-, and deca-BDEs. For tri- and tetra-BDEs, parent ions $[M]^+$ and $[M+2]^+$ gave di-brominated product ions ($[M-2Br]^+$ and $[M-2Br+2]^+$, respectively). Penta- and hexa-BDEs had the same parent ions, $[M-2Br]^+$ and $[M+2]^+$, but different product ions. Penta-BDEs gave $[M-COBr]^+$ and $[M-2Br+2]^+$, while hexa-BDEs gave $[M-4Br]^+$ and $[M-2Br+2]^+$. The fragmentation described by Sánchez-Ávila et al. for hepta-BDEs and deca-BDE was the same as for hexa-BDEs. However, these transitions were not optimal for hepta-BDE (BDE-183) and deca-BDE (BDE-209). We found other transitions that gave a much better response: for BDE-183, $[M]^+$ as parent ion gave $[M-2Br]^+$ and $[M-2Br+2]^+$ as product ions and for BDE-209, parent ions $[M-O-8Br]^+$ and $[M-CO-7Br]^+$ gave $[M-O-9Br]^+$ and $[M-CO-8Br]^+$ as product ions. The same transitions were used by Law et al. [22].

Regarding MeO-PBDEs, MeO-tetra-, and MeO-penta-BDEs presented the same pattern. For MeO-tetra-BDEs parent ion $[M+4]^+$ gave $[M-2Br+2]^+$ and $[M-2Br+4]^+$ as product ions, while for MeO-penta-BDEs, parent ion $[M+6]^+$ gave $[M-2Br+2]^+$ and $[M-2Br+4]^+$ as products ions. Despite the fact that other fragmentation patterns have been described for the *ortho*- substituted MeO-PBDEs [23], with $[M-Br-CH3]^+$ as product ion, the most abundant transitions were the ones described before and were considered good enough in terms of selectivity and sensitivity.

Emerging FRs, HBB, PBEB, and DBDPE presented different fragmentation patterns. For HBB, parent ion $[M-Br]^+$ gave $[M-3Br]^+$ and $[M-3Br+2]^+$ as product ions and for PBEB, parent ion $[M+4]^+$ gave $[M-CH3+4]^+$ as product ion and $[M-CH3+4]^+$ was also used as parent ion with $[M-Br-CH3+4]^+$ as product ion. Finally, two product ions were used for DBDPE, $[M-6Br]^+$ and $[M-8Br-5H]^+$, which gave $[M-7Br]^+$ and $[M-10Br-5H]^+$ as product ions.

Analytical parameters

Table 2 shows the instrumental parameters evaluated: linearity, sensitivity, and reproducibility. Regarding linearity, the calibration curve was linear at a range from 10 to 1000 pg/μL, with correlation coefficients (r) between 0.9986 and 0.9999. IDLs ranged from 0.11 to 16.3 injected pg, while IQLs ranged from 0.35 to 54.3 injected pg. For reproducibility, RSD values were lower than 20 % for all the analytes both for inter- and intra-assay experiments and at the three concentration levels. In intra-assay experiments, RSD values ranged from 1.3 to 18 %. Similarly, values in inter-assay experiments ranged from 2.6 to 19 %.

Table 2 Instrumental detection and quantification limits, in injected pg, and reproducibility (relative standard deviation, %) for intra- and interday experiments at the three concentration levels

		R^2	IDL	IQL	Intraday			Interday		
					1	2	3	1	2	3
PBDEs	BDE-28	0.998	0.11	0.35	1.73	3.63	1.75	2.58	3.27	9.55
	BDE-47	0.999	0.18	0.61	4.38	2.32	1.27	7.44	6.25	8.72
	BDE-100	0.996	0.43	1.44	6.34	2.14	4.77	8.71	5.22	8.75
	BDE-99	0.998	0.54	1.81	11.9	4.54	3.75	12.9	7.81	7.29
	BDE-154	0.997	2.27	7.58	5.99	7.40	5.25	5.98	6.62	10.3
	BDE-153	0.997	3.57	11.9	7.32	5.39	8.02	12.4	5.02	7.22
	BDE-183	0.998	12.5	41.7	14.1	9.38	11.4	16.4	14.8	13.8
	BDE-209	0.997	16.3	54.4	7.65	1.45	2.77	7.71	9.37	3.00
MeO-PBDEs	2-MBDE-68	0.999	1.06	3.52	14.3	4.12	8.23	13.1	3.74	7.44
	6-MBDE-47	0.999	0.83	2.78	11.8	3.09	6.79	10.7	2.80	6.28
	5-MBDE-47	0.996	0.47	1.57	11.3	3.52	8.74	11.6	4.18	7.84
	4-MBDE-99	0.996	1.14	3.79	9.09	3.98	7.31	8.85	3.84	6.57
	5-MBDE-100	0.997	3.41	11.4	11.4	8.22	12.4	10.3	8.06	11.7
	4-MBDE-100	0.998	9.38	31.3	17.1	7.22	9.44	15.4	10.6	8.56
	5-MBDE-99	0.998	5.77	19.2	5.60	11.3	11.6	7.93	10.1	10.9
	4-MBDE-101	0.998	4.69	15.6	13.8	10.7	10.3	12.7	11.4	9.49
Emerging BFRs	HBB	0.999	1.32	4.39	6.73	9.25	9.68	6.09	8.29	8.67
	PBEB	0.999	0.90	3.01	8.52	5.07	6.04	8.48	5.34	18.9
	DBDPE	0.996	6.25	20.8	7.14	3.54	18.2	13.2	18.8	15.5

Level 1: 0.025 ng/μL for PBDEs, MeO-PBDEs, HBB, and PBEB; 0.25 ng/ μL for BDE-209; and 0.06 ng/μL for DBDPE.
Level 2: 0.2 ng/μL for PBDEs, MeO-PBDEs, HBB, and PBEB; 2 ng/μL for BDE-209; and 0.5 ng/μL for DBDPE.
Level 3: 1 ng/μL for PBDEs, MeO-PBDEs, HBB, and PBEB; 10 ng/μL for BDE-209; and 2.5 ng/ μL for DBDPE

IDLs instrumental detection limits, *IQLs* instrumental quantification limits, *RSD* relative standard deviation

Recoveries, reproducibility, accuracy, and MDLs and MQLs were calculated for the five different matrices at three different concentration levels (Table 3). As said before, blanks were used to subtract the natural content that samples might had but the values found never surpassed a contribution of 10 % to the total value.

Even though the extraction methodologies used had already proved to provide good recoveries in these matrices, recovery values for these experiments were calculated. Recoveries for PBDEs ranged from 75 to 96 %, from 52 to 67 %, from 57 to 77 %, from 53 to 82 %, and from 57 to 87 % in sediment, sludge, fish, bird egg, and dolphin blubber, respectively. MeO-PBDEs were well recovered as well, with values ranging from 78 to 91 %, from 53 to 68 %, from 51 to 77 %, from 58 to 83 %, and from 70 to 77 % in sediment, sludge, fish, bird egg, and blubber, respectively. Finally, HBB, PBEB, and DBDPE recoveries ranged from 103 to 105 % in sediment, from 52 to 66 % in sludge, from 68 to 80 % in fish, from 70 to 78 % in bird eggs, and from 71 to 76 % in dolphin blubber. As expected, sediment was the matrix which presented the best recovery values and sludge was the matrix which gave the lowest ones.

Regarding intraday assays, RSD values ranged from 0.9 to 7.5 % in sediment, from 1.1 to 18 % in sludge, from 1.1 to 12 % in fish, from 1.81 to 18.0 % in dolphin blubber, and from 2.5 to 11 % in bird egg. For interday assays, RSD values ranged from 1.7 to 17 % in sediment, from 2.7 to 19 % in sludge, from 3.2 to 19 % in fish, from 2.33 to 13.3 % in dolphin blubber, and from 2.0 to 20 % in bird egg.

Regarding the accuracy, expressed as percent deviation (%Dev), values obtained for intra- and inter-assays were satisfactory for all the matrices at the three levels since values of the |%Dev| were always lower than 15 %. In sediment, values ranged from −8.2 to 10 %; in sludge, values ranged from −13 to 13 %; in fish values ranged from −11 to 15 %, while in dolphin blubber, values ranged from −9.12 to 10.7 %. Finally, values in bird egg ranged from −13 to 15 %. As happened for the precision, accuracy values were not statistically different between intra- and inter- assays in most of the cases. This is attributed to the fact that the methodology provides consistent values through time, even though some variations, despite being in acceptable ranges, are quite high. Moreover, not positive or negative values prevailed over the other for the same matrix.

Table 3 Recovery values, method detection and quantification limits for the five matrices studies

		Sediment (ng/g dw)			Sludge (ng/g dw)			Fish (ng/g lw)			Dolphin blubber (ng/g lw)			Bird egg (ng/g lw)		
		R (%)	MDL	MQL	R (%)	MDL	MQL	R (%)	MDL	MQL	R (%)	MDL	MQL	R (%)	MDL	MQL
PBDEs	BDE-28	96	0.01	0.03	65	0.05	0.17	72	0.04	0.12	87	0.01	0.04	82	0.03	0.09
	BDE-47	96	0.01	0.04	67	0.05	0.17	77	0.05	0.18	81	0.01	0.05	72	0.03	0.10
	BDE-100	91	0.02	0.07	65	0.11	0.38	67	0.20	0.67	77	0.06	0.19	67	0.12	0.41
	BDE-99	75	0.03	0.09	61	0.10	0.33	63	0.29	0.97	81	0.03	0.09	73	0.13	0.45
	BDE-154	95	0.08	0.25	65	0.63	2.08	59	0.43	1.42	75	0.21	0.69	57	0.35	1.17
	BDE-153	87	0.12	0.39	59	0.79	2.63	73	0.64	2.13	71	0.13	0.42	53	0.31	1.02
	BDE-183	95	0.39	1.32	59	1.36	4.55	61	3.19	10.6	56	1.39	4.63	61	1.51	5.02
	BDE-209	83	1.65	5.49	51	2.78	9.26	57	10.6	35.4	62	1.11	3.69	57	3.20	10.7
MeO-PBDEs	2-MBDE-68	87	0.07	0.22	62	0.22	0.74	68	1.06	3.54	77	0.09	0.31	58	0.59	1.95
	6-MBDE-47	87	0.05	0.16	60	1.36	4.55	77	0.43	1.42	75	0.24	0.79	67	0.25	0.85
	5-MBDE-47	91	0.03	0.09	53	1.00	3.33	65	0.43	1.42	71	0.06	0.19	65	0.28	0.92
	4-MBDE-99	89	0.07	0.24	53	0.75	2.50	51	2.13	7.09	74	0.16	0.53	61	0.46	1.55
	5-MBDE-100	78	0.13	0.43	56	1.36	4.55	66	1.99	6.64	76	0.31	1.04	76	0.59	1.98
	4-MBDE-100	89	0.38	1.25	68	1.67	5.56	73	2.20	7.33	77	1.59	5.29	83	2.01	6.70
	5-MBDE-99	91	0.16	0.53	55	1.00	3.33	63	3.75	12.5	70	0.47	1.57	63	0.54	1.80
	4-MBDE-101	80	0.22	0.75	64	1.50	5.00	74	3.19	10.6	76	0.80	2.68	73	0.83	2.76
Emerging BFRs	HBB	105	0.03	0.11	66	0.35	1.16	80	0.20	0.67	76	0.06	0.21	75	0.12	0.39
	PBEB	104	0.04	0.14	64	0.56	1.85	70	0.18	0.61	71	0.06	0.19	70	0.14	0.47
	DBDPE	103	0.11	0.37	52	0.94	3.12	58	9.66	32.2	72	1.06	3.52	78	3.54	11.8

R recovery, *MDLs* method detection limits, *MQLs* method quantification limits

Furthermore, MDLs and MQLs are shown in Table 3. The low brominated PBDEs presented better MDLs and MQLs in all cases, while high brominated PBDEs presented the highest values in all the matrices. The same was observed for MeO-PBDEs since the tetra-brominated congeners gave lower values than the penta-brominated congeners. Values obtained for sediment were considerably lower than in sludge, as expected, since sediment is a cleaner matrix. With the exception of BDE-28 and BDE-47, where MDLs in sediment were only up to five times lower, in some cases, the values in sediments were up to 12 times lower than in sludge (i.e., PBEB and HBB). Regarding biological matrices, dolphin blubber and bird egg showed similar MDLs (0.01–1.59 ng/g lw and 0.03–3.54 ng/g lw, respectively), while fish showed slightly higher values (0.04–10.6 ng/g lw). The sensitivity of our developed method was compared with previous published works analyzing PBDEs and MeO-PBDEs by EI-MS-MS. Our IDLs are lower than those reported by Sanchez-Ávila [15] which ranged from 1.5 to 10 injected pg. In this work, BDE-183 and BDE-209, the less sensitive BDE congeners, were not included. So, our IDLs for PBDEs ranged from 0.11 to 3.57 injected pg, which represent an improvement of IDL values up to 13 times less. Similarly, the MDLs they reported for sediment (11 to 44 ng/g dw) were also higher than those obtained in our study (0.008 to 1.68 ng/g dw). On the other hand, our IDLs are similar to those reported by Losada et al. (0.9 to 2.5 injected pg for PBDEs and 0.4 to 1.5 injected pg for MeO-PBDEs). Losada et al. also reported MDLs in whale blubber which were lower than those reported in our study, with values ranging from 0.03 to 0.29 ng/g lw and from 0.05 to 0.18 ng/g lw for MeO-PBDEs and PBDEs, respectively [14].

Due to its instability at the high temperatures, the analysis of BDE-209 by GC-MS-MS represents a challenge since it is difficult to obtain a proper parent ion. Most of the published works do not include this compound in the method. Our IDLs were comparable to those obtained by Law et al. [22], who also included BDE-209 with values ranging from 0.5 to 75 injected pg. Law et al. also reported IDLs for HBB and PBEB, which were similar to those we obtained: 0.9 toward 0.4 and 1.3 toward 1.0 for HBB and PBEB for Law et al. and our methodology, respectively.

EI-MS versus EI-MS-MS

EI-MS and EI-MS-MS were compared in terms of sensitivity. Table 4 shows the MDLs obtained by both methodologies for the several matrices studied spiked at low level, with the exception of dolphin blubber where the medium level was used for the calculations. Since MS-MS provides much better

Table 4 Method detection limits by EI-MS and EI-MS-MS for all the compounds studied

		Sediment (ng/g dw)		Sludge (ng/g dw)		Fish (ng/g lw)		Dolphin blubber (ng/g lw)		Bird egg (ng/g lw)	
		MS	MS-MS	MS	MS-MS	MS	MS-MS	MS	MS-MS	MS	MS-MS
PBDEs	BDE-28	0.06	0.01	0.18	0.05	0.28	0.04	0.11	0.01	0.42	0.03
	BDE-47	0.03	0.01	0.10	0.05	0.32	0.05	0.15	0.01	0.52	0.03
	BDE-100	0.06	0.02	0.44	0.11	0.88	0.20	0.34	0.06	1.37	0.12
	BDE-99	0.06	0.03	0.27	0.10	1.46	0.29	0.43	0.03	1.69	0.13
	BDE-154	0.33	0.08	1.50	0.63	11.2	0.43	0.42	0.21	1.35	0.35
	BDE-153	0.30	0.12	1.76	0.79	11.3	0.64	0.59	0.13	1.33	0.31
	BDE-183	2.54	0.39	–	1.36	4.11	3.19	2.17	1.39	–	1.51
	BDE-209	–	1.65	–	2.78	–	10.6	53.1	1.11	185	3.20
MeO-PBDEs	2-MBDE-68	0.23	0.07	4.11	0.22	15.4	1.06	0.61	0.09	1.53	0.59
	6-MBDE-47	0.16	0.05	1.52	1.36	2.87	0.43	0.53	0.24	0.50	0.25
	5-MBDE-47	0.24	0.03	2.05	1.00	5.25	0.43	0.56	0.06	1.78	0.28
	4-MBDE-99	0.17	0.07	1.89	0.75	5.08	2.13	0.42	0.16	0.92	0.46
	5-MBDE-100	0.54	0.13	5.17	1.36	10.5	1.99	0.44	0.31	1.70	0.59
	4-MBDE-100	0.73	0.38	6.98	1.67	15.0	2.20	0.49	1.59	1.59	2.01
	5-MBDE-99	0.56	0.16	8.88	1.00	18.1	3.75	0.81	0.47	2.77	0.54
	4-MBDE-101	0.76	0.22	8.20	1.50	22.9	3.19	0.53	0.80	2.06	0.83
Emerging BFRs	HBB	–	0.03	–	0.35	3.99	0.20	0.98	0.06	5.64	0.12
	PBEB	0.16	0.04	2.12	0.56	3.54	0.18	0.22	0.06	0.18	0.14
	DBDPE	3.44	0.11	18.5	0.94	72.4	9.66	2.88	1.06	7.35	3.54

MDLs method detection limits

selectivity than MS, the signal to noise (S/N) ratio decreases considerably, providing better MDLs or even allowing the determination of the compound while it was not possible by EI-MS. For instance, BDE-209 could not be determined by EI-MS in sediment, sludge, and fish, while it could be determined by EI-MS-MS. When using EI-MS-MS, MDLs improved considerably: from 2 to 8 times in sediment, from 2 to 18 times in sludge, from 2 to 26 times in fish, from 1.5 to 48 times in dolphin blubber, and from 1.3 to 47 times in bird egg.

Moreover, Fig. 1 shows several chromatograms from real samples of fish and sludge where the difference between EI-MS and EI-MS-MS can be clearly seen. The use of MS-MS allows the correct identification of BDE-100 and BDE-99 due to its higher selectivity, while several unknown peaks appear when using MS. In addition, BDE-209 could not be determined in sludge by MS, whereas the S/N is reduced considerably with the use of MS-MS and thus BDE-209 can be correctly identified and determined.

Application to real samples

The optimized methodology was applied to five different samples for each matrix, as described in section "Sample collection." Different PBDEs were detected in all matrices, while MeO-PBDEs were only detected in dolphin blubber. This fact was expectable since, as explained before, MeO-PBDEs are only found in marine environment. Unfortunately, we could not obtain marine samples of the other matrices. On the other hand, HBB and PBEB were not detected in any sample, whereas DBDPE was only detected in environmental samples (sediment and sludge). Results are summarized in Table 5. Several PBDEs (from tetra-brominated to deca-brominated) were detected in sediments from the Llobregat River Basin, with BDE-209 as the most abundant compound. Total PBDE levels ranged from 2.50 to 48.1 ng/g dw and were slightly higher than the ones that Labandeira et al. reported for the same river in 2007 [19]. On the other hand, Guerra et al. reported higher levels (from 22 to 136 ng/g dw) in sediments from the same river [5], so there is a great variation on the levels depending on the sampling points and year. DBDPE was also detected with levels ranging from not detected (nd) to 30.7 ng/g dw. Kierkegaard et al. detected similar levels (24 ng/g dw) in sediment samples from the Netherlands [24].

Regarding sludge, several PBDEs and DBDPE were detected. Total PBDE levels ranged from nd to 250 ng/g dw and, in the same way than sediments, BDE-209 was the most abundant compound; DBDPE levels ranged from nd to 100 ng/g dw. Our values are clearly lower than those reported by De la Torre et al. (from 58 to 2606 ng/g dw) [25] and Gorga

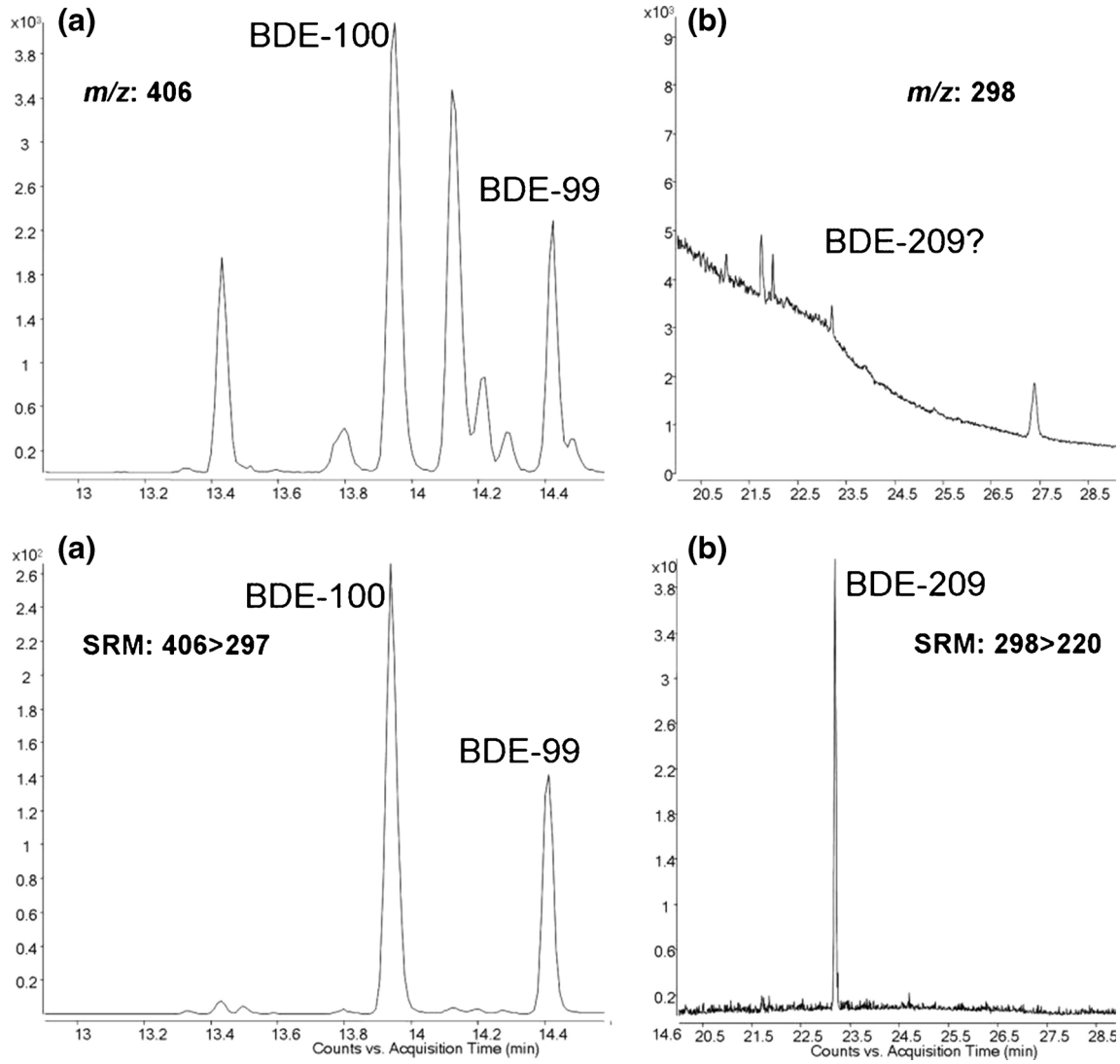

Fig. 1 Comparison between SIM-EI (*up chromatograms*) and SRM-EI (*down chromatograms*) of the same **a** fish and **b** sludge samples

et al. (up to 2303 ng/g dw) [6] in different WWTPs from Spain. On the other hand, our values were similar to ones reported by De la Torre et al., which ranged from 3.24 to 125 ng/g dw [26].

PBDEs were also detected in fish samples. In this case, BDE-47 was the most abundant compound, which is in agreement with other published works. Total PBDE levels ranged from nd to 248 ng/g lw and were in the same range than other study carried out in the Llobregat river by Labandeira et al., who reported PBDE concentrations ranging from 28.8 and 744 ng/g lw [19]. However, since the fish species are different, these results have to be compared with caution. PBDEs have been analyzed in fish worldwide, with a great variability on the levels reported [27].

Table 5 Levels found in sediment and sludge (ng/g dw) and in fish, dolphin blubber, and bird egg (ng/g lw). $N=5$ in all matrices

	Sediment	Sludge	Fish	Blubber	Bird egg
PBDEs	2.51–48.1	nd–250	nd–248	15.5–1350	5.51–40.4
MeO-PBDEs	nd	nd	nd	50.1–1244	nd
HBB	nd	nd	nd	nd	nd
PBEB	nd	nd	nd	nd	nd
DBDPE	nd–30.7	nd–100	nd	nd	nd

nd not detected

Moreover, both PBDEs and MeO-PBDEs were detected in dolphin blubber. BDE-47 was the most abundant PBDE, and 6-MeO-BDE-47 was the most abundant MeO-PBDE. Total PBDE levels ranged from 15.5 to 1350 ng/g lw while total MeO-PBDE levels ranged from 50.1 to 1244 ng/g lw. Dolphins are known to be at the top of the food chain and usually present high PBDE and MeO-PBDE burdens. Recently, Alonso et al. reviewed all the studies reporting PBDEs and MeO-PBDEs around the world [13]. Our results are in the middle of the total range

since there are studies which report concentrations up to 13,000 ng/g lw of PBDEs or MeO-PBDEs.

Finally, PBDEs were also detected in white stork eggs, with levels ranging from 5.51 to 40.4 ng/g lw. BDE-209 was the most abundant compound, which is surprising but in agreement with Muñoz-Aranz et al., who reported the predominance of BDE-209 also in white stork eggs from Doñana National Park [28]. Our values are lower than the ones reported for the same species in the same location, which ranged from 2.92 to 129 ng/g lw [28]. However, these samples were taken in 1999–2000, which could explain this variation. To our knowledge, the higher PBDE values reported for bird eggs were reported for peregrine falcons from the Great Lakes, with values ranging from 530 to 38,000 ng/g lw [9]

Conclusions

An analytical methodology for the simultaneous analysis of eight PBDE congeners (from tri- to deca-BDEs), eight MeO-PBDEs, and three emerging BFRs in two environmental matrices (sediment and sludge) and three biological matrices (fish, dolphin blubber, and bird egg) by GC-EI-MS-MS was developed. The methodology provided MDLs and MQLs adequate for the analysis of these compounds in the environment, and other analytical parameters such as accuracy or precision were also evaluated for all the matrices. Furthermore, differences between NCI and EI, and between EI-MS and EI-MS-MS were studied. Even though NCI is more sensible than EI, the improvement on the selectivity of the EI was considered the main factor to take into account considering the problems that occur in environmental analysis. In addition, EI allows the use of mass labeled standards providing a more reliable quantification. Besides, EI-MS-MS proved to be more sensitive than EI-MS.

The methodology was applied to several samples for each matrix studied. PBDEs were detected in the five matrices, with different levels and congener distributions in environmental and biological samples. DBDPE was only detected in sediment and sludge, while MeO-PBDEs were only detected in the only marine matrix, dolphin blubber.

This methodology allows the reliable determination of these compounds in a wide number of matrices. Important analytical parameters, which are rarely given in other works, were satisfactory and met the requirements for this kind of analysis.

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