Evaluation of GC-MS/MS for determination of PBDEs in fish and shellfish samples

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Introduction

Nowadays electron impact (EI) is usually preferred to negative chemical ionization (NCI) because it permits the use of isotopic dilution for quantification. The limited sensitivity of the EI mode, especially toward highly brominated compounds, is improved when combined to sector high resolution mass spectrometer (HRMS). The major drawbacks of EI-HRMS result from the high cost of the instrument, the requirement of highly qualified staff, and the necessity to perform heavy maintenances on a regular basis to ensure proper operation. For 2 or 3 years, alternatives to HRMS have thus been investigated. Large volume injection[1,2], which allows a substantial decrease of the detection limits of EI-low resolution (LR)MS,, or the use of time-of-flight (TOF)MS[3,4] as detector. Since our first report on tandem in time mass spectrometry (MS/MS) using a quadrupole ion storage (QIST)MS for the determination of PBDEs[5], the method has been developed and improved in several laboratories for biological samples[6,7] and soils[8].

The goal of the present work was to evaluate the previously developed GC-QISTMS/MS technique for the measurement of PBDEs in fish and shellfish samples using an improved automated cleanup procedure. Comparison to GC-HRMS is reported.

Materials and Methods

Salmon steak, whole trout and Spanish mussels were purchased from Belgian supermarket. Trout filet used as reference material was issued from the Norvegian Institute of Public Health (Nydalen, Norway) and assigned levels were obtained from the interlaboratory study "Food 2004". Fat extraction was previously detailed by Focant et al.[9]. Briefly, pressurized liquid extraction (PLE) was performed on freeze-dried products using an ASE™ 200 extractor (Dionex, Sunnyvale, CA, USA) with hexane as solvent. Fat extracts were dried on sodium sulphate prior to lipid content determination using gravimetric analysis. Subsequent clean-up steps were performed on 0.2 g of fat. The multi-analyte purification step previously developed[5] on the Power-Prep™ system (Fluid Management Systems Inc., Waltham, MA, USA) was revised and considerably simplified for PBDE collect only. Smaller disposable column sets were used and were composed of multi-layer silica columns (2 g acid, 1 g base and 0.75 g neutral) and basic alumina (6 g).After a brief column conditioning step, the fat sample diluted in 20 mL of hexane wasloaded on multi-layer silica, then eluted on alumina with 40 mL of hexane and finally collected with 40 mL of a 50:50 hexane:dichloromethane mixture. This fraction was then evaporated down to 150µL under a gentle stream on nitrogen and transferred to a conical GC vial containing 10 µL of nonane used as keeper.

Table 1. Retention times, CID voltages, masses of isolated parent ions and monitored daughter ions.

Window	Time (min)	Congener	Parent ion (m/z)	CID (V)	Daughter ion (m/z)
1	6.78	^{12}C TBDE-47	326 [M-Br_2]	5.0	217/219
	6.78	^{13}C TBDE-47	338 [M-Br_2]	5.0	229/231
2	8.13	^{12}C PeBDE-100	404 [M-Br_2]	5.0	295/297/299
	8.51	^{12}C PeBDE -99	404 [M-Br_2]	5.0	295/297/299
	8.51	^{13}C PeBDE -99	416 [M-Br_2]	5.0	307/309/311
3	9.72	^{12}C HxBDE-154	484 [M+2-Br_2]	5.5	376/378
	9.72	^{13}C HxBDE-154	496 [M+2-Br_2]	5.5	388/390
	10.55	^{12}C HxBDE-153	484 [M+2-Br_2]	5.5	376/378
	10.55	^{13}C HxBDE-153	496 [M+2-Br_2]	5.5	388/390
	11.97	^{13}C HxBDE-139	496 [M+2-Br_2]	5.5	388/390
4	12.08	^{12}C HpBDE-183	564 [M+4-Br_2]	7.0	453/455
	12.08	^{13}C HpBDE-183	576 [M+4-Br_2]	7.0	465/467

Tandem in time mass spectrometry (GC/MS/MS) analyses were performed using a Thermoquest Trace GC 2000 (Milan, Italy) gas chromatograph coupled to a PolarisQ ion trap mass spectrometer (Austin, Tx, USA). The ion trap was set at 225°C, with the transfer line at 300°C. EI mode was used with energy of 70 eV. Table 1 summarises window cutting, masses of parent ions isolated, mass of daughter ions monitored and optimized CID voltages. Analysis carried out by GC-HRMS were performed using a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph and a MAT95XL high-resolution mass spectrometer (Finnigan, Bremen, Germany) working in selected ion monitoring (SIM) at a minimum resolution of 10,000. Both GCs were equipped with a Stx 500 (30m x 0.25 mm x 0.15 µm) capillary column (Restek, Bellefonte, USA).

Results and Discussion

Instrumental limits of detection (LOD_i) and quantification (LOQ_i) gathered in Table 2 for HRMS and QISTMS were defined as the smaller amount giving a signal-to-noise ratio (S/N) greater than 3 and 10, respectively. As expected, high resolution showed lower LOD_i and LOQ_i values, and operating the QISTMS in MS/MS mode instead of SIM improved the sensitivity. This is especially valuable when real samples with higher background and more interferences are injected. The attained sensitivity was better than what has been reported for triple quadrupole mass spectrometer MS/MS[10].

Table 2.LOD_i and LOQ_i for HRMS, QISTMS operating in single ion monitoring (SIM) or in tandem in time mass spectrometry (MS/MS) expressed in pg injected.

	LOD_i (pg)			LOQ_i (pg)		
	HRMS	QISTMS		HRMS	QISTMS	
	SIM	MS/MS	SIM	SIM	MS/MS	SIM
BDE-47	0.05	0.5	2	0.1	2	12
BDE-100	0.05	1	8	0.1	2	15
BDE-99	0.05	1	8	0.1	2	15
BDE-154	0.05	0.5	2	0.5	2	10
BDE-153	0.05	0.5	2	0.5	2	10
BDE-183	0.1	1	10	0.5	2	20

Once purified, fish extracts were injected in triplicates on both HRMS and QISTMS. Table 3 resumes measured mean levels (ng/g fat) and relative standard deviations (RSDs). Non negligible traces of all target congeners were found in procedural method blanks when injected on HRMS whereas only BDE-47, -99 and -100 could be detected by QISTMS/MS. Method LOQ values for these congeners were calculated as the average blank value plus 10 times the standard deviation (SD) of the blanks. For congeners not present in blanks, the LOQ_m was the smaller added concentration in blank subjected to cleanup giving a signal with S/N greater than 10.

Table 3. Mean levels (ng/g fat) of fish extracts (triplicates) on HRMS or MS/MS, relative standard deviation (RSD) between these 3 injections (mentioned between brackets), and LOQ_m values for both MS techniques.

Congeners	Mussel		Trout		Salmon		LOQ	
	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS
BDE-47	5.62	4.71	2.07	1.95	6.07	5.85	3.57	3.56
	(26)	(4)	(13)	(5)	(14)	(2)		
BDE-100	0.61	0.52	0.38	0.45	2.05	1.94	0.89	0.36
	(37)	(4)	(4)	(3)	(11)	(3)		
BDE-99	1.83	1.62	0.24	0.21	0.64	0.70	1.21	1.03
	(6)	(4)	(7)	(8)	(9)	(5)		
BDE-154	0.13	0.12	<LOQ	0.15	1.09	0.98	0.07	0.03
	(21)	(1)	-	(13)	(10)	(5)		
BDE-153	<LOQ	0.04	<LOQ	<LOQ	0.38	0.25	0.07	0.04
	-	(9)	-	-	(24)	(13)		
BDE-183	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.07	0.17
	-	-	-	-	-	-		
Total	8.19	6.24	2.69	2.76	10.22	9.71	5.28	5.01
% lipid	1.9		2.2		14.7			

Levels found by HRMS or MS/MS in fish extracts were similar but RSDs were higher for all congeners when MS/MS was used. LOQ_m values for HRMS or MS/MS technique were very close, demonstrating that analysis of PBDEs in fishes and shellfishes does not necessarily require the use of HRMS, and that QISTMS operating in MS/MS mode is suitable. The $LOQs_m$ were relatively high because of the very small amount of fat that was used for purification.

Levels of PBDEs found in salmons were in the same order of magnitude than those reported from Finnish, Canadian or Japanese markets[11, 12, 13]. On the other hand, mussels and trout filets showed levels really lower than those evaluated by Tittlemieret al.[12] in Canada.

The purification step has been tested on replicate salmon fat extracted by PLE. Levels found in extracts were similar and reasonably low RSDs were obtained (Table 4). Testing demonstrated this cleanup to be reproducible and robust. However, mussel extracts, which were still colorful prior to GC-MS injection, had to undergo an additional purification step. Therefore, collected fractions were evaporated down to 10µL and then eluted with 5mL of hexane on a small Pasteur Pipette filled with 1g of acidic silica.

Table 4. Mean and range recovery percentages of the purification method, and relative standard deviation (RSD) of levels measured on the 3 extracts of salmon run separately.

	Recovery (%)		RSD cleanup
	Mean	Range	(%)
BDE-47	62	[44-88]	4
BDE-100	-	-	10
BDE-99	67	[51-79]	14
BDE-154	60	[49-82]	11
BDE-153	71	[65-79]	10
BDE-183	83	[68-104]	-

Recovery percentages shown on Table 4 seemed to slightly decrease with decrease number of bromine. This may come from the evaporation step which is critical for PBDE analysis. Careful slow evaporation has to be performed when reaching the last microlitres to avoid noticeable drop in recoveries, especially for congeners with low bromination level, which are more volatile.

Reference trout material was used as test material to evaluate the reliability of the whole procedure. Figure 1 shows results obtained from the same purified extract injected in HRMS and in MS/MS, in comparison to assigned values. Results are expressed in ng/g fat for all congeners except for BDE-183 for which levels were in pg/g fat.

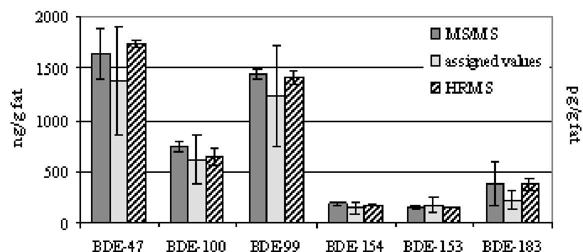

Figure 3. Concentrations (ng/g fat for BDE-47, -100, -99, -154 and -153; pg/g fat for BDE-183) measured in reference trout injected in HRMS and QISTMS/MS, compared to assigned values.

Conclusions

QISTMS operating in MS/MS mode showed to be suitable for the measurement of PBDEs in fishes and shellfishes with good reliability. The simplified automated cleanup only requires 20 min per set of 6 samples and offers good reproducibility and robustness. The method could easily be implemented in small laboratories with limited human and financial resources to allow onsite screening for PBDES in sea products.

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