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Suitability of tandem-in-time mass spectrometry for polybrominated diphenylether measurement in fish and shellfish samples: Comparison with high resolution mass spectrometry

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Abstract

The first part of the present study focused on the development of an alternative automated sample preparation method for the measurement of selected polybrominated diphenyl ethers (PBDEs) in fish and shellfish. A previously developed automated method has been further optimized and simplified to decrease blank levels and cost. Sorbent and solvent quantities have been significantly reduced without altering extract quality. The second part of the study consisted of comparing tandem-in-time quadrupole ion storage mass spectrometry (QISTMS/MS) to high resolution mass spectrometry (HRMS) for the isotope dilution (ID) measurement step after gas chromatography (GC) separation. Both mass spectrometric methods performed similarly in terms of accuracy but better precision was observed for HRMS. Although better sensitivity can be attained with the high resolution sector instrument, method limits of quantification (mLOQs) were very similar for both approaches as they were dependent on the procedural blanks levels. The mLOQ values ranged between 0.04 and 3.56 ng/g fat, depending on the congener. They allowed the unambiguous identification and quantification of all target analytes, except for BDE-183, in most considered fish extracts. An analytical procedure based on rapid automated sample preparation and QISTMS/MS appeared to be suitable for the measurement of PBDEs in fish and shellfish speciment under quality assurance/quality control (QA/QC) criteria.
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Keywords: PBDEs; Sample preparation; EI-MS/MS; QISTMS; EI-HRMS; Fish

1. Introduction

Since 1999, the number of research papers dedicated to environmental levels and to analytical methodologies for the measurement of polybrominated diphenylethers (PBDEs) has drastically increased. This confirmed the growing concern towards these pollutants. As knowledge was growing, better understanding of their environmental and human health hazards conducted to the phasing out of specific commercial mixtures in the European Communities [1]. On the human exposure aspect, although other exposure routes are also of concern [2], recent studies on PBDE levels in food [3,4] demonstrated that fish consumption significantly contributed to human exposure through food consumption.

In the early days, as no labelled internal standard solutions were readily available, the measurement of PBDEs was carried out by gas chromatography (GC) coupled to electron capture detector (ECD) or to low resolution mass spectrometry (LRMS) operated in negative chemical ionisation (NCI) mode. Both detectors demonstrated adequate sensitivity for the detection of these compounds, but they showed a lack of selectivity. Because ECD responds to halogenated chemicals without distinction, and NCI does with all brominated compounds, efforts have to be considered to reduce the number of potential interfering analytes in the same extract prior detection. Moreover, both detectors are not compatible with quantification based on isotope dilution (ID).

The improving commercial availability of ^{13}C-labelled internal standards now permits the use of ID electron ionization (EI) MS for accurate identification and quantification of PBDEs. The limited efficiency of the ionisation mode unfortunately yields to a diminished sensitivity, especially toward highly brominated compounds. This is offset when combined to sector high resolution mass spectrometers (HRMS) operated in selected ion

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monitoring (SIM) mode. The major drawbacks of EI-HRMS result from its high cost, the requirement of highly qualified personal and the necessity to perform heavy maintenances on a regular basis to ensure proper functioning. Recently, alternatives have been proposed and studied to offer a substitute to the use of HRMS while maintaining the use of EI and ID. Large volume injection, based on programmable temperature vaporization (PTV) injectors, allows to decrease substantially the detection limits of EI-LRMS [5,6]. The use of time-of-flight (TOF) MS also showed good capability to measure PBDEs in various matrices [7–9]. In 2002, we introduced the use of tandem in time mass spectrometry (MS/MS) on a quadrupole ion storage mass spectrometer (QISTMS) for the measurement of PBDEs [10] and further validated it for biological high lipid content sample analysis [11]. Since then, this approach has been repeated and improved in several laboratories for the analysis of biological samples [12,13], soil samples [14], or aqueous samples using solid-phase microextraction (SPME) [15] and matrix solid-phase dispersion (MSPD) [16].

Previously, as part of our ongoing multi-analyte measurement strategy, we reported on an automated sample preparation procedure for the comprehensive determination of polychlorodibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorobiphenyls (PCBs), and PBDEs [11] in high fat content matrices. This was based on the use of a well-established instrument that was already known for its suitability to prepare extracts from environmental and biological matrices for PCDD/F measurement [17–21]. For high fat content matrices, due to the low background levels of the target toxicants, a fat sample size of 4–6 g is required to ensure PCDD/F levels above the method limits of quantification (mLOQs). Therefore, the typical set of disposable columns (a multilayer 4 g acid, 2 g base and 1.5 g neutral silica column, an 8 g basic alumina, and a 2 g PX-21 carbon dispersed on Celite) has to be preceded by a high capacity 50 g acidic silica disposable column to ensure degradation of most of the lipid prior fractionation. This resulted in the use of both large sorbent and solvent quantities that were responsible for relatively high blank levels for some PCBs and PBDEs for which reduced method LOQs were obtained. As not all laboratories have to measure PCDD/Fs and PCBs with the PBDEs, we decided to develop a parallel approach dedicated to PBDEs only.

The goal of the present study was to apply the previously developed GC–IDQISTMS/MS method for the measurement of selected PBDEs in fish and shellfish samples and to compare it to GC–IDHRMS, the usual reference tool for trace analysis. Additionally, based on previously reported data [11], a specific automated sample preparation procedure was also developed and tested for this comparison exercise.

2. Experimental

2.1. Reagents and standards

Hexane and dichloromethane are Pestanal® reagents (Promochem, Molsheim, France). Nonane puriss p.a. standard for GC was purchased from Fluka (Steinheim, Germany). Anhydrous sodium sulfate was from Acros Organics (Geel, Belgium). Liquid nitrogen was purchased at Air Liquide (Liege, Belgium). The ^{13}C-labelled internal standard (MBDE-MXC) and the native (BDE-MXC) solutions were from Wellington Laboratories (Ontario, Canada), compositions and concentrations were previously listed [11]. The MBDE-139 MXC ^{13}C-labelled internal standard solution (Wellington Labs) contains 2,2′,3,4,4′,6-HexaBDE (PBDE-139) at concentration level of 50 ng/μl and was used to calculate recovery rates.

2.2. Samples

Salmon steak, whole trout and Spanish mussels were purchased from a Belgian supermarket. A cod liver oil was from the Norvegian Institute of Public Health (N.I.P.H., Nydalen, Norway) as part of the interlaboratory round test "Food 2005". Trout filet, used as reference material, also originated from the N.I.P.H., and certified levels were obtained from the interlaboratory study "Food 2004" excercise.

2.3. Analytical method

Skin and fish bones were removed from salmon and trout, analyses were performed on the enable part of the muscles. Mussels were separated from their shell. Fleshes were homogenised and frozen under liquid nitrogen before freeze-drying for 48 h. The freeze-dried products were ground in order to obtain a fine powder. Fat extraction was previously detailed by Focant et al. [22]. Briefly, pressurised liquid extraction (PLE) was performed on the dried powder using an ASE™ 200 extractor (Dionex, Sunnyvale, CA, USA) with hexane as extraction solvent. Fat extracts were dried on sodium sulfate prior to lipid content determination using gravimetric analysis. Internal standard was added to aliquots of 0.2 g of fat prior to subsequent clean-up steps. A previously developed automated multi-analyte purification procedure [11] using the Power-Prep™ system (Fluid Management Systems Inc., Waltham, MA, USA) was revised and considerably simplified to accomodate PBDE only. Recovery standard was added to extract after final evaporation step.

2.4. Instrumental analysis

Gas chromatography quadrupole ion storage tandem in time mass spectrometry (GC–QISTMS/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 temperature was set to 225 °C, with the transfer line at 300 °C. Electron ionization (EI) was used as the ionisation mode, with an energy of 70 eV. Table 1 summarises the acquisition window definition, masses of precursor and product ions that are monitored, and the optimized collision induced dissociation (CID) voltages.

Analysis performed by GC–HRMS used a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph and a MAT95XL high resolution mass spectrometer (ThermoFinnigan, Bremen, Germany) working in select ion monitoring (SIM) at a minimum resolution of 8000. Masses of FC-5311 (perfluo-

Table 1
Major acquisition parameters for QISTMS/MS and HRMS

Windows	Congeners	MS/MS			HRMS		
		Parent ions (*m/z*)	CID (V)	Daughter ions (*m/z*)	Ions monitored (*m/z*)		Isotope ratios
w 1	Tri-BDE						
4–6.5 (min)	[^{12}C] BDE-28	406 [M]	4.0	246/248	405.8026 [M + 2]	407.8006 [M + 4]	0.986
	[^{13}C] BDE-28	418 [M]	4.0	258/260	417.8429 [M + 2]	419.8409 [M + 4]	0.986
w 2	Tetra-BDE						
6.5–8 (min)	[^{12}C] BDE-47	326 [M − Br_2 + 2]	5.0	217/219	485.7111 [M + 4]	483.7131 [M + 2]	0.678
	[^{13}C] BDE-47	338 [M − Br_2 + 2]	5.0	229/231	497.7513 [M + 4]	495.7733 [M + 2]	0.678
w 3	Penta-BDE						
8–9.3 (min)	[^{12}C] BDE-100	404 [M − Br_2 + 2]	5.0	295/297/299	563.6215 [M + 4]	565.6195 [M + 6]	0.984
	[^{12}C] BDE-99	404 [M − Br_2 + 2]	5.0	295/297/299	563.6215 [M + 4]	565.6195 [M + 6]	0.984
	[^{13}C] BDE-99	416 [M − Br_2 + 2]	5.0	307/309/311	575.6618 [M + 4]	577.6598 [M + 6]	0.984
w 4	Hexa-BDE						
9.3–12 (min)	[^{12}C] BDE-154	484 [M − Br_2 + 2]	5.5	376/378	485.7111 [M − Br_2 + 4]	483.71 [M − Br_2 + 2]	0.670
	[^{13}C] BDE-154	496 [M − Br_2 + 2]	5.5	388/390	497.7513 [M − Br_2 + 4]	495.773 [M − Br_2 + 2]	0.670
	[^{12}C] BDE-153	484 [M − Br_2 + 2]	5.5	376/378	485.7111 [M − Br_2 + 4]	483.71 [M − Br_2 + 2]	0.670
	[^{13}C] BDE-153	496 [M − Br_2 + 2]	5.5	388/390	497.7513 [M − Br_2 + 4]	495.773 [M − Br_2 + 2]	0.670
	[^{13}C] BDE-139	496 [M − Br_2 + 2]	5.5	388/390	497.7513 [M − Br_2 + 4]	495.773 [M − Br_2 + 2]	0.670
w 5	Hepta-BDE						
12–18 (min)	[^{12}C] BDE-183	562 [M + 4 − Br_2]	6.0	453/455	561.606 [M − Br_2 + 4]	563.604 [M − Br_2 + 6]	0.995
	[^{13}C] BDE-183	574 [M + 4 − Br_2]	6.0	465/467	573.6462 [M − Br_2 + 4]	575.6442 [M − Br_2 + 6]	0.995

rophenantrene) were used as lock masses. The different recorded masses are reported in Table 1. Electron energy was set to 60 eV and source temperature at 270 °C. Dwell times for native and labelled congeners were 141.9 and 46.4 ms, respectively.

Both chromatographs were equipped with a Stx-500^{TM} (40 m × 0.18 mm I.D. × 0.20 μm df) capillary column (Restek, Bellefonte, PA, USA). The GC temperature program was optimised as follow: splitless injection of 1 μl at 140 °C, splitless time of 2 min, initial oven temperature of 140 °C held for 1.5 min, and increased at 50 °C/min to 260 °C, then increased at 5 °C/min to 290 °C, finally increased at 25 °C/min to 325 °C and held for 10 min. Helium (N60, Air Liquide, France) was used as the carrier gas.

3. Results and discussion

3.1. Sample preparation

As PBDE measurement in biological matrices requires smaller fat quantities than for PCDD/F determination, portions of 0.2 g of extracted fat were processed and cleaned-up. It was thus not necessary to use large quantities of acidic silica for the removal of large lipid quantities anymore. Additionally, as the isolation of dioxins and furans was not part of the task, the carbon column responsible for the fractionation of the planar species from the non-planar species was removed and a simple automated procedure was developed based on silica and alumina only. Small sizes disposable columns were designed and tested to further reduce sorbent and solvent quantities. They consisted in multilayer silica (2 g acid, 1 g basic, and 0.75 g neutral) and basic alumina (4 g) Teflon disposable columns. Consequently, the clean-up consisted in three major steps: the conditioning of the columns, the load of the fat extract, and the elution of PBDEs after lipid removal and isolation of interfering compounds. Fig. 1 describes the sequence of events and the related solvent consumptions for both the multi-analyte procedure [11] and the new PBDE procedure. The total run for the PBDE procedure is 20 min including conditioning steps. As comparison, the multi-analyte run time was 85 min. The 40 ml fraction of hexane-dichloromethane 50:50 that contains the PBDEs is evaporated down to 150 μl under a gentle stream on nitrogen and then transferred to a conical GC vial containing 10 μl of nonane used as keeper. The evaporation-transfer step requires no more than 15 min.

This automated clean-up was tested in terms of recovery rates, repeatability and robustness. For this purpose, fat extracted by PLE from raw salmon muscle tissues was split in three subsamples to undergo individual purification. Additionally, five procedural blanks were run following the same new procedure. Table 2 summarises congener specific recovery rate data for all matrices and relative standard deviation (RSD) for multiplicate salmon extracts, as well as measured average blank levels in procedural blanks. Limits of quantification of the whole method (mLOQ) were also reported but will be discussed later. All measurements were carried out on the QISTMS in tandem in time MS mode. Recovery rate on BDE-100 was not mentioned in Table 2 because as internal standard solution did not contain this specific labelled congener, recovery could not be evaluated.

Recovery rates appeared to be slightly lower with decreasing bromination level. This may come from the higher volatility of lower brominated congeners, leading to a non-negligible lost during the evaporation step. Careful slow evaporation was therefore performed when reaching the last microlitres to reduce this noticeable drop in recoveries. RSD on salmon triplicate levels

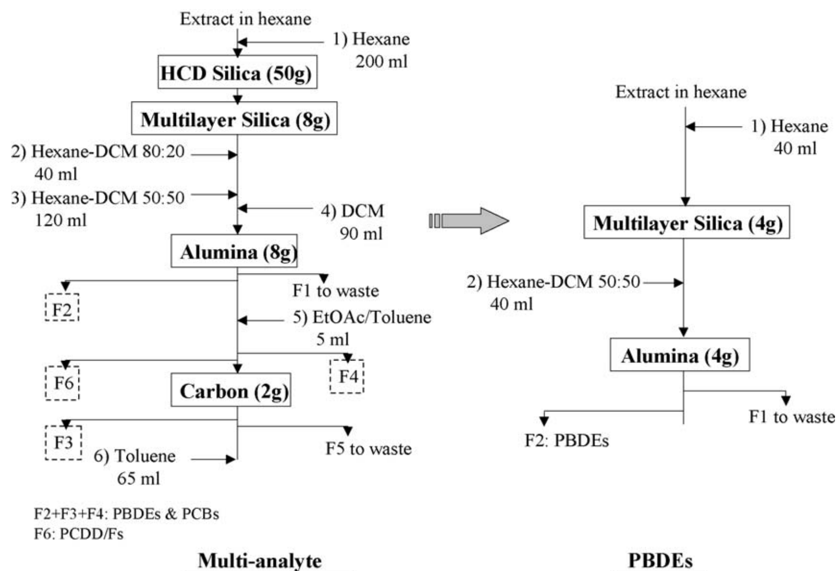

Fig. 1. Schemes of the previous developed multi-analytes clean-up (left) and the new PBDE dedicated procedure (right).

did not exceed 15%, demonstrating the good repeatability of the procedure. Moreover, this deviation would likely be due to the variation of the spectrometer answer rather than to the sample prep. This variation will be discussed in the next section. Traces of PBDE-28, -47, -99 and -100 were detected in blanks, in non-negligible levels for PBDE-47 and PBDE-99. These detected amounts were lower than those observed by Huwe et al. [23] for PBDE determination in chickens, but were higher than those reported by Päpke et al. [24] for fish analysis.

The robustness of the clean-up method has been tested by processing several types of fish or shellfish matrices. Except in the case of mussel extracts, all final 10 μl extracts appeared to be sufficiently clean to be injected without any noticeable GC phase alteration effect nor ion suppression effect in the MS domain. The level of interferences was kept to a satisfactory low level permitting unequivocal peak assignment and integration, as shown on Fig. 2 for a salmon extract. Mussel extracts were the exception, and as they were still colorful prior to GC–MS injection, they had to undergo an additional purification step. Therefore, collected fractions were evaporated down to 10 μl and then eluted with 5 ml of dichloromethane on a small Pasteur Pipette filled with 1 g of acidic silica. This quick manual step ensured good quality of mussel extracts without the need of modifying the automated procedure (that was efficient for all other matrices) that remained a fast procedure. This was because we wanted to keep to capability of processing all types of matrices in parallel. In the case of a laboratory processing mussels only, one can easily scale up the silica column size to avoid the manual step.

3.2. Mass spectrometric measurement

As it was presented by Restek as an ideal phase for separation of brominated flame retardants, co-planar PCB congeners and other analytes with high boiling temperatures, the Stx-500 column was used for gas chromatographic separation prior to MS detection [25]. The GC run was optimized to be as short as possible, and allow the complete separation of the seven target congeners. Analysis was focused only on these target compounds as they used to be the ones mainly found in aquatic samples. This was achieved with a GC program of 23 min (13 min separation time + 10 min column cleaning). Stx-500 was also compared to a classical Rtx®-5Sil-MS column (30 m × 0.25 mm I.D. × 0.25 μm df) using the same optimized

Table 2
Mean and range recoveries for all matrices, relative standard deviation (RSD) on salmon levels purified in triplicate, traces measured in procedural blanks (pg) and subsequent method limits of quantification (mLOQ)

	Recovery (%)		RSD on salmon level (%)	Solvent blanks (pg)	mLOQ (ng/g fat)	
	Mean	Range			MS/MS	HRMS
BDE-28	53	[33–93]	<LOQ	8	0.18	0.10
BDE-47	62	[44–88]	4	254	3.57	3.56
BDE-100	–	–	10	45	0.89	0.36
BDE-99	67	[51–79]	14	28	1.21	1.03
BDE-154	60	[49–82]	11	nd	0.07	0.03
BDE-153	71	[65–79]	10	nd	0.07	0.04
BDE-183	83	[68–104]	<LOQ	nd	0.07	0.17

"nd": non detected; "<LOQ": the two levels measured were below the limit of quantification and therefore, RSD could not be calculated.

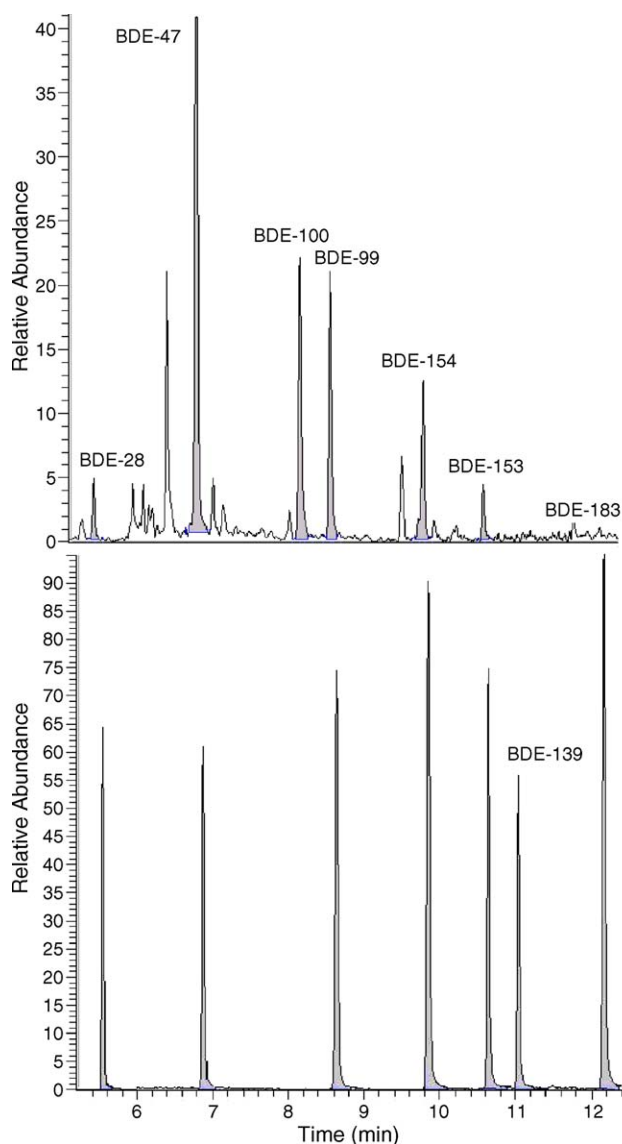

Fig. 2. Chromatograms of native (A) and ^{13}C-labelled (B) congeners of a real salmon extract injected in GC–MS/MS. BDE-139 was used for recovery calculation.

GC program. Chromatograms obtained with both columns on the same calibration solution (1 ng/μl) are shown in Fig. 3. As it can be seen on these chromatograms, a better separation between the pentabrominated congeners BDE-100 and -119 was obtained with the Rtx®-5Sil-MS column compared to the Stx-500. Nevertheless, BDE-119 present in the calibration solutions used to not be found in most real samples, and especially in aquatic matrices. An advantage of the Stx-500 column was the greater isolation of BDE-47 from other closely eluting congeners.

Principal MS/MS parameters are listed in Table 1. They result from further optimization of our previously reported data [11]. Especially for BDE-28, selected parameters were quite different. For this congener, although it did not produce the most intense signal, the molecular ion was chosen as the parent ion instead of the $[M - Br_2]$ fragment. This choice was guided by the fact that fragment masses resulting from the CID of the $[M - Br_2]$ parent fragment were at the low end of the mass range and, consequently, difficult to monitor in the permanent background signal noise resulting from GC column bleed and potential matrix interferences. This phenomenon, which did not significantly appear while optimization on standard solution was taking place, was markedly observed when real sample matrices were considered.

Limits of detection of QISTMS and HRMS have been estimated to compare instrumental sensitivity of both MS types. HRMS worked in selected ion monitoring (SIM) at a minimum resolution of 8000, while QISTMS was tested in both SIM mode and in MS/MS mode in order to explore the possible gain of sensitivity offered by the later mode. In single dimension MS, both parent ions and fragment ions resulting from the loss of a dibromine molecule were recorded for each homologue to achieve the best practical possible sensitivity. Instrumental limits of detection (iLOD) and quantification (iLOQ) were defined as the smallest amount injected giving a peak with a signal-to-noise ratio (S/N) greater than 3 and 10, respectively. These values for HRMS, LRMS (SIM) and MS/MS for 1 μl injection are gathered in Table 3. Operating QISTMS in MS/MS mode instead of SIM mode enabled to decrease iLOD and iLOQ, to as much as a factor of 10. Such an improvement is especially valuable when complex real samples characterized by extracts containing high background and interference levels are injected. The working sensitivity was better than what has been reported for triple quadrupole mass spectrometer MS/MS [26], but still remained from 10- to 20-fold lower than what was attained with EI HRMS.

As non-negligible traces of most congeners were found in procedural method blanks, their mLOQ (Table 2) were calculated as the average measured blank value plus 10 times the associated standard deviation (SD). For congeners not present in the method blanks, the mLOQ were equal to the smaller added quantity spiked in a solvent sample subjected to the entire sample preparation procedure that gave a signal with S/N greater than 10. Whereas all target congeners were found in procedural method blanks when injected on HRMS, only BDE-47, -99 and -100 has been detected by QISTMS/MS. However, mLOQ values for HRMS or MS/MS technique were very close, demonstrating that even if instrumental sensitivity of high resolution is definitely higher, analysis of PBDEs in fishes and

Table 3
Instrumental limits of detection and quantification (iLOD and iLOQ), defined as the smaller amount injected in 1 μl giving a peak with a S/N greater than 3 and 10, respectively, evaluated for HRMS and QISTMS (in SIM and MS/MS mode)

	iLOD (pg)			iLOQ (pg)		
	HRMS	QISTMS		HRMS	QISTMS	
	SIM	MS/MS	SIM	SIM	MS/MS	SIM
BDE-28	0.05	1	8	0.1	5	20
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

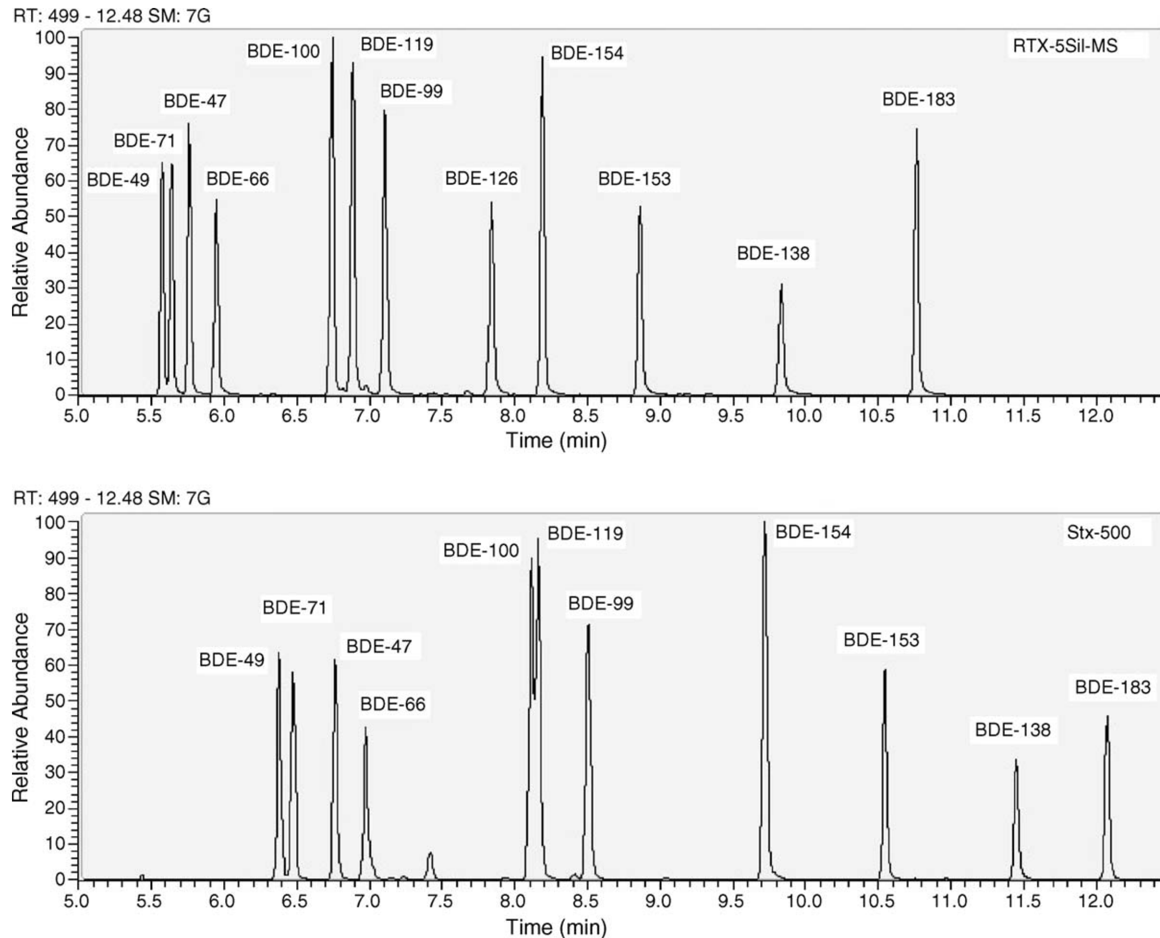

Fig. 3. Calibration solution (1 ng/μl) injected on the Rtx-5Sil-MS capillary column (top chromatogram) and on the Stx-500 capillary column (bottom chromatogram) using similar GC temperature programs.

shellfishes does not necessarily require its use. QISTMS operating in MS/MS mode is therefore suitable. The mLOQs were relatively high for BDE-47 and -99, because amounts found in blank were divided by the very small amount of fat that was used for purification (0.2 g), in order to be expressed on lipid weight basis. Nevertheless, whatever matrix considered, most congeners showed levels higher than their corresponding mLOQ value, except BDE-183 which could never be quantified

Table 4
Mean levels ($n = 3$) measured on real sample extracts with both HRMS and MS/MS

Congeners	Mussel		Trout		Salmon		Cod liver oil		RSD range (%)	
	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS	MS/MS	HRMS
Tri-BDE										
BDE-28	<LOQ	0.11	<LOQ	<LOQ	0.44	0.39	0.45	0.30	[0–8]	[3–19]
Tetra-BDE										
BDE-47	5.62	4.71	2.07	1.95	6.07	5.85	5.90	5.92	[11–26]	[1–5]
Penta-BDE										
BDE-100	0.61	0.52	0.38	0.45	2.05	1.94	0.88	0.82	[4–37]	[3–13]
BDE-99	1.83	1.62	0.24	0.21	0.64	0.70	<LOQ	<LOQ	[6–9]	[5–8]
Hexa-BDE										
BDE-154	0.13	0.12	<LOQ	0.15	1.09	0.98	0.46	0.42	[4–21]	[1–13]
BDE-153	<LOQ	0.04	<LOQ	<LOQ	0.38	0.25	<LOQ	<LOQ	[0–24]	[9–13]
Hepta-BDE										
BDE-183	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	–	–
Total	8.19	7.11	2.69	2.76	10.66	10.10	7.69	7.46		

in our samples as it can be seen in Table 4. This table shows for both MS types the measured mean levels expressed in ng/g fat as well as relative standard deviation ranges in real samples extracts. These real samples were extracted and purified using the simplified automated clean-up described above, and each single cleaned extract was injected in triplicate in both GC–HRMS and GC–MS/MS. BDE-153 levels appears to be very low and could only be quantified in salmon extract. Note that method blank levels have been subtracted from sample levels after comparison between mLOQs and values obtained from MS measurement. This approach seemed to be the most logical as mLOQs already take into account the mean blank levels.

Levels measured by HRMS and MS/MS in fish extracts were similar. Differences between both methods equal to 2.5, 5.5 and 3% for trout, salmon and cod liver oil, respectively. A 15% difference was observed in the case of the more delicate mussel extracts. Regarding the precision, RSDs appeared to be markedly higher when the low resolution MS instrument was used. This was already previously observed for dioxin analysis when QISTMS [27], and TOFMS [28] were compared to HRMS in other studies. A potential explanation to this phenomenon could be the lower intrinsic sensitivity of the instrument. As a peak area for a same concentration is lower when using QISTMS, a larger relative deviation can be expected when repeated peak integrations are performed.

Reference trout material was used as test material to evaluate the reliability of the whole procedure. Fig. 4 shows results obtained from the same purified extract injected in triplicate on both GC–HRMS and GC–MS/MS. Those data are compared to the assigned values that resulted from an international intercalibration exercise. Results are expressed in ng/g fat for all congeners except for BDE-183 for which levels were in pg/g fat. The concentration of BDE-28 was multiplied by a factor of 100 to facilitate readability of the figure.

Levels measured by GC–HRMS and GC–MS/MS were very similar with higher RSD for QISTMS. Compared to assigned values, the measured concentrations were in the upper range observed by the interlaboratory participants. These slightly higher measured levels would likely be related to the sample preparation step rather than to the mass analyser used. As our levels were corrected from residual laboratory contamination by subtracting blank levels, purification step would not be involved in the little overestimation. On the other hand, the extraction was

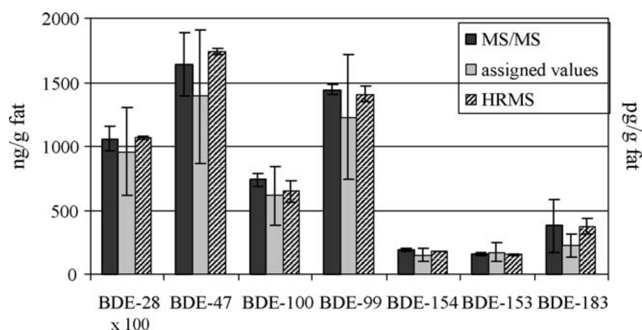

Fig. 4. Levels measured on reference trout filet samples using GC–HRMS and GC–MS/MS compared to assigned values (interlaboratory study).

performed by PLE that has been reported to be one of the most efficient technique for contaminant extraction [29,30]. This may partially explain differences compared to the consensus value of the intercalibration that is based on other extraction procedures, which included more conventional extraction steps.

4. Conclusion

QISTMS operating in MS/MS mode showed to be suitable for the measurement of selected PBDEs in fishes and shellfishes with good reliability, but provided higher standard deviations than high resolution mass spectrometer. Instrumental limits of detection and quantification were higher for QISTMS but LOQs of the whole method were comparable independently to the detector used. This demonstrates that PBDE analysis in fishes and shellfishes does not necessarily require HRMS. The simplified automated clean-up has been demonstrated to be robust, repeatable and accurate, producing good recovery rates. Low adsorbent and solvent quantities are consumed, and only 20 min per set of six samples are required. 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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