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New strategy for comprehensive analysis of polybrominated diphenyl ethers, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls by gas chromatography coupled with mass spectrometry

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Abstract

A strategy for determination of polybrominated diphenyl ethers, polychlorinated dibenzodioxins, polychlorinated dibenzofurans and polychlorinated biphenyls on fatty matrices has been established. After extraction, the proposed method allows the purification and the fractionation of all target groups of compounds in a simple multi-step automated clean-up. Furthermore, their subsequent analysis is carried out using a single benchtop mass spectrometer, in four separate injections. Required sensitivity considering levels found in the environment is attained using electron impact ionisation followed by tandem in time mass spectrometry. The whole method has been evaluated on standard solution and quality control samples consisting of fortified beef fat. Sensitivity, selectivity, accuracy and repeatability were tested with satisfactory results.

Keywords: Polybrominated diphenyl ethers; Polychlorinated dibenzodioxins; Polychlorinated dibenzofurans; Polychlorinated biphenyls; Organochlorine compounds; Organobromine compounds; Halogenated compounds

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are man-made chemicals used as additive flame retardants. They are incorporated into polymers in order to inhibit or slow down both ignition and rate of combustion. They find their major applications as incorporated chemicals in various plastics, textiles, wire, cable insulation, electrical/electronic connectors and other everyday products. The concern regarding these compounds is that they can later migrate out of the finished products and be released into the environment [1]. Like polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), they are persistent and lipophilic compounds and therefore have a tendency to bioaccumulate through the trophic pyramid and reach human via the food chain [1,2].

In 1981, Andersson and Blomkvist were among the first to report the presence of these organohalogen compounds in Swedish fish samples [3]. Twenty years later, still in Sweden, Norén and Meironyté reported a drastic increase of these com-

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pounds in human breast milk, incontestable evidence that humans are currently exposed to these kinds of compounds [4]. Conversely to what is observed for most anthropogenic organic pollutants, levels of PBDEs found in the environment have incessantly increased in the last 20 years world-wide, sometimes being higher than PCB levels [2,5–9]. Nowadays, occurrence of PBDEs are a cause of growing concern, and more and more laboratories have to offer the capability to analyse this class of contaminant, which has begun to be included in monitoring programs. Therefore, a rapid, reliable, time- and resource-saving analysis method is needed.

Analysis of PBDEs in biological samples usually consists of a lipid extraction step using combination of polar and non-polar solvent. Most frequently, Soxhlet or pressurised liquid extraction (PLE) are used and, to a lesser extent and as for PCDD/Fs and PCBs, supercritical fluid extraction (SFE) can be also used [2,6,10]. Further clean-up is currently performed using gel permeation chromatography or sulfuric acidic treatment to remove lipids before additional adsorption chromatography to eliminate co-extracted substances [6,10,11]. The final determination is generally carried out by gas chromatography (GC) coupled with mass spectrometry (MS) or electron-capture detection (ECD), the latter being much less selective [10]. Negative chemical ionisation (NCI) is the most often used ionisation mode for determination of brominated compounds by MS. Such a technique presents a higher sensitivity than electron impact (EI), but is less selective, since only bromine can be monitored, and is less accurate since it does not allow the quantification by internal standard (^{13}C-labelled PBDEs) [10,12,13]. EI however usually requires high-resolution mass spectrometry (HRMS) to overcome the lack of sensitivity of the ionisation mode, whereas low-resolution mass spectrometry (LRMS) is sufficient when based on NCI. From a competitive point of view, comparisons between EI and NCI have already been realised and similar suitability was found regarding determination of PBDEs [13–15]. Concerning EI, even if HRMS provides a high degree of sensitivity and selectivity, it is not free from interferences. For instance, PBDE-47 uses to co-elute with PCB-180 on a classical 30 m DB-5 type column and this detector cannot resolve them in normal operating conditions [14]. This requires a very highly selective purification method to eliminate these interferences. Nowadays analysis using EI of PBDEs in solution containing PCBs is not conceivable using single-dimensional GC.

The aim of the present study was to elaborate a strategy for the analysis of a broad range of organohalogen compounds, including, on one hand, brominated compounds (PBDEs), and on the other hand, chlorinated compounds such as PCDDs, PCDFs and PCBs. Experienced in the use of automated clean-up systems for PCDD/F and PCB purifications, we decided to investigate the potentiality to extend our current methods to PBDEs without excessive changes.

As we had previously reported on the suitability of quadruple ion storage mass spectrometry (QISMS) for PCDD/F and PCB analysis using EI-MS–MS [16,17], we decided to maintain the EI mode in conjunction with MS–MS for PBDE analysis. This combination would avoid interferences such as those mentioned above and would allow determination of PBDEs in solutions holding other organohalogen compounds such as PCBs. To our knowledge, there are to date no results available on the analysis of PBDEs by EI-MS–MS. It should be noted that decabrominated diphenyl ether which is the most important commercial product with regard to the production and use among the brominated diphenyl ethers (mono- to deca-), will be not investigated due to the considerable difficulties in its analysis. This highly brominated compound actually requires a specific GC column owing to its very long retention time [2,18,19]. Nevertheless, although DeBDE used to be abundant in environmental samples, very low levels were found in biological samples probably due to the very poor bioavailability of this kind of highly halogenated compound [2,11,18].

2. Experimental

2.1. Chemicals and reagents

Hexane, toluene, ethyl acetate and dichloromethane are Pestanal reagents (Riedel-de Haën, Seelze, Germany). Nonane (puriss analytical-reagent grade standard for GC) was purchased from Fluka (Steinheim, Germany). Anhydrous sodium sulfate

was Baker analysed (J.T. Baker, Deventer, The Netherlands). Liquid nitrogen was purchased at Air Liquide (Liege, Belgium). The $^{13}C_{12}$-labelled internal standard solution containing dioxins, furans and coplanar PCBs was from Cambridge Isotope Labs. (Andover, MS, USA). This EDF-4144 internal standard solution used for isotopic dilution contains 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, OCDF, 3,3′,4,4′-TCB (PCB-77) (PCB numbering following Ballschmiter and Zell rules [20]), 3,4,4′,5-TCB (PCB-81), 3,3′,4,4′,5-PeCB (PCB-126) and 3,3′,4,4′,5,5′-HxCB (PCB-169) with concentrations ranging from 24 to 125 pg/μl in nonane [21]. The recovery standard solution EDF-4145 (Cambridge Isotope Labs.) contains [$^{13}C_{12}$]3,3′,5,5′-TCB (PCB-80) at 48 pg/μl, [$^{13}C_{12}$]1,2,3,4,7,8,9-HpCDF at 62.5 pg/μl and [$^{13}C_6$]1,2,3,4-TCDD at 25 pg/μl in nonane. Multianalyte calibration solutions (EDF-4143, Cambridge Isotope Labs.) were used to calculate the relative response factors (RRFs) for each congener [21].

The $^{13}C_{12}$-labelled internal standard solution containing non- and mono-*ortho* PCBs was from Wellington Labs. (Ontario, Canada). This WP-LCS internal standard solution contains 3,3′,4,4′-TCB (PCB-77), 3,4,4′,5-TCB (PCB-81), 2,3,3′,4,4′-PeCB (PCB-105), 2,3,4,4′,5-PeCB (PCB-114), 2,3′,4,4′,5-PeCB (PCB-118), 2′,3,4,4′,5-PeCB (PCB-123), 3,3′,4,4′,5-PeCB (PCB-126), 2,3,3′,4,4′,5-HxCB (PCB-156), 2,3,3′,4,4′,5′-HxCB (PCB-157), 2,3′,4,4′,5,5′-HxCB (PCB-167), 3,3′,4,4′,5,5′-HxCB (PCB-169) and 2,3,3′,4,4′,5,5′-HpCB (PCB-189) at a concentration level of 1 ng/μl. The MBP-MXE (Aroclor 1260 congeners) internal standard solution (Wellington Labs.) contains 2,4,4′-TriCB (PCB-28), 2,2′,5,5′-TCB (PCB-52), 2,2′,4,5,5′-PeCB (PCB-101), 2,2′,3,4,4′,5-HxCB (PCB-138), 2,2′,4,4′,5,5′ HxCB (PCB-153), 2,2′,3,4,4′,5,5′-HpCB (PCB-180) and decachlorobiphenyl (PCB-209) at a concentration level of 5 ng/μl.

The PBDE analytical standard solution EO-4980 (Cambridge Isotope Labs.) contains 40 native congeners from mono- to heptabrominated diphenyl ethers and five ^{13}C-labelled PBDEs in nonane. Concentrations of each congener of EO-4980 solution are listed in Table 1 (PBDE numbering following Ballschmiter and Zell rules [20] as for PCBs).

The BDE-MXC native solution (Wellington Labs.) contains 4-MonoBDE (PBDE-3), 2,4-DiBDE (PBDE-7), 4,4′-DiBDE (PBDE-15), 2,2′,4′-TriBDE (PBDE-17), 2,4,4′-TriBDE (PBDE-28), 2,2′,4,4′-TetraBDE (PBDE-47), 2,2′,4,5′-TetraBDE (PBDE-49), 2,3′,4,4′-TetraBDE (PBDE-66), 2,3′,4′,6-TetraBDE (PBDE-71), 3,3′,4,4′-TetraBDE (PBDE-77), 2,2′,3,4,4′-PentaBDE (PBDE-85), 2,2′,4,4′,5-PentaBDE (PBDE-99), 2,2′,4,4′,6-PentaBDE (PBDE-100), 2,3′,4,4′,6-PentaBDE (PBDE-119), 3,3′,4,4′,5-PentaBDE (PBDE-126), 2,2′,3,4,4′,5′-HexaBDE (PBDE-138), 2,2′,4,4′,5,5′-HexaBDE (PBDE-153), 2,2′,4,4′,5,6′-HexaBDE (PBDE-154), 2,2′,3,4,4′,5′,6-HeptaBDE (PBDE-183) and decabromodiphenyl ether (PBDE-209), all these 20 congeners are found at a concentration level of 2 ng/μl.

The MBDE-MXC ^{13}C-labelled internal standard solution was from Wellington Labs. and contains 4-MonoBDE (PBDE-3), 4,4′-DiBDE (PBDE-15), 2,4,4′-TriBDE (PBDE-28), 2,2′,4,4′-TetraBDE (PBDE-47), 2,2′,4,4′,5-PentaBDE (PBDE-99), 2,2′,4,4′,5,5′-HexaBDE (PBDE-153), 2,2′,4,4′,5,6′-HexaBDE (PBDE-154) and 2,2′,3,4,4′,5′,6-HeptaBDE (PBDE-183) at a concentration level of 5 ng/μl. The MBDE-139 MXC ^{13}C-labelled internal standard solution (Wellington Labs.) contains 2,2′,3,4,4′,6-HexaBDE (PBDE-139) at a concentration level of 50 ng/μl.

2.2. Samples

Studies of the elution of the different compounds were performed with $^{13}C_{12}$-labelled internal standard solution for PCBs and dioxins, and with the analytical standard solution EO-4980 for elution of PBDEs, each of those diluted in hexane. In order to evaluate the accuracy of the multi-analyte method, analyses were carried out on ‘‘laboratory-made’’ quality controls (QCs), which were commercially available fortified beef fat. These QC samples contained 40.4 pg/g fat (5.3 pg TEQ/g fat) for the 17 PCDD/Fs, 160 pg/g fat (6 pg TEQ/g fat) for the four coplanar PCBs, 4.9 ng/g fat (1 pg TEQ/g fat) for the eight mono-*ortho* PCBs, 18 ng/g for Aroclor

Table 1
PBDE analytical standard solution EO-4980

Congener	No.	Concentration (pg/μl)	Congener	No.	Concentration (pg/μl)
Unlabelled BDE			3,3′,4,4′-TetraBDE	77	100
			2,2′,3,4,4′-PentaBDE	85	150
2-MonoBDE	1	100	2,2′,4,4′,5-PentaBDE	99	150
3-MonoBDE	2	100	2,2′,4,4′,6-PentaBDE	100	150
4-MonoBDE	3	100	2,3,3′,4,4′-PentaBDE	105	150
2,4-DiBDE	7	100	2,3,4,5,6-PentaBDE	116	150
2,4′-DiBDE	8	100	2,3′,4,4′,6-PentaBDE	119	150
2,6-DiBDE	10	100	3,3′,4,4′,5-PentaBDE	126	150
3,3′-DiBDE	11	100	2,2′,3,4,4′,5′-HexaBDE	138	200
3,4-DiBDE	12	100	2,2′,3,4,4′,6′-HexaBDE	140	200
3,4′-DiBDE	13	100	2,2′,4,4′,5,5′-HexaBDE	153	200
4,4′-DiBDE	15	100	2,2′,4,4′,5,6′-HexaBDE	154	200
2,2′,4-TriBDE	17	100	2,2′,4,4′,6,6′-HexaBDE	155	200
2,3′,4-TriBDE	25	100	2,3,4,4′,5,6-HexaBDE	166	200
2,4,4′-TriBDE	28	100	2,2′,3,4,4′,5,6-HeptaBDE	181	250
2,4,6-TriBDE	30	100	2,2′,3,4,4′,5′,6-HeptaBDE	183	250
2,4′,6-TriBDE	32	100	2,3,3′,4,4′,5,6-HeptaBDE	190	250
2′,3,4-TriBDE	33	100	*^{13}C-Labelled BDE*		
3,3′,4-TriBDE	35	100			
3,4,4′-TriBDE	37	100	2,2′,4,4′-TetraBDE ($^{13}C_{12}$, 99%)	47	100
2,2′,4,4′-TetraBDE	47	100	3,3′,4,4′-TetraBDE ($^{13}C_{12}$, 99%)	77	100
2,2′,4,5′-TetraBDE	49	100	2,2′,4,4′,5-PentaBDE ($^{13}C_{12}$, 99%)	99	150
2,3′,4,4′-TetraBDE	66	100	2,2′,4,4′,6-PentaBDE ($^{13}C_{12}$, 99%)	100	150
2,3′,4′,6-TetraBDE	71	100	3,3′,4,4′,5-PentaBDE ($^{13}C_{12}$, 99%)	126	150
2,4,4′,6-TetraBDE	75	100			

1260 PCBs [22], 100 pg/g for each tetraBDE, 150 pg/g for each pentaBDE and 200 pg/g for each hexaBDE.

2.3. Sample preparation

Purification steps were based on Power-Prep, a commercially available automated multi-column clean-up system (Fluid Management Systems, Waltham, MA, USA). This system has been previously described [21–25]. Briefly, the automated clean-up system is composed of a valve drive module connected to a pump module responsible for the solvent flow in the valve module. The programming of solvent volumes, types, flow-rates and directions is carried out in software operating under Windows. This system uses disposable multi-layer silica columns (4 g acid, 2 g base and 1.5 g neutral), basic alumina (8 g) and PX-21 (2 g) carbon columns in order to separate analytes of interest from matrix interferences following a previously reported strategy [26]. When an extract contains more than 1 g of fat, high-capacity disposable silica (HCDS) columns are added before the classical silica column. Details on the efficiency of these columns are described elsewhere [21]. The configuration of the system allows the operator to collect different fractions at different steps of the purification. Collected fractions can be further concentrated and analysed by GC–MS.

2.4. Instrumental analysis

All analyses were performed by tandem in time mass spectrometry (GC–MS–MS) using a ThermoQuest Trace GC PolarisQ ion trap mass spectrometer (Austin, TX, USA) and a Hewlett-Packard (Palo Alto, CA, USA) 6890 Series gas chromatograph, the latter equipped with an Rtx 5-MS (40 m×0.18 mm, 0.20 μm) capillary column (Restek, Evry, France). The ion trap was set at 250 °C, with the transfer line at 300 °C. Electron impact was used as the ionisation mode, with an energy of 70 eV.

Analysis of PCDD/Fs, PCBs and PBDEs were carried out following separate injections.

2.4.1. Dioxins and furans

Determination of dioxins in GC–MS–MS was already optimised using programmable temperature vaporisation-large volume (PTV-LV) injections in order to increase sensitivity and decrease limits of quantification (LOQs). Details are described elsewhere [17].

2.4.2. PCBs

GC conditions were optimised to separate the 12 non- and mono-*ortho* PCBs and the seven marker PCBs as follows: splitless injection of 1 μl at 140 °C, initial oven temperature of 140 °C for 1 min, then increased at 25 °C/min to 180 °C held for 1 min, then increased at 2 °C/min to 210 °C held for 8 min, finally increased at 3 °C/min to 280 °C and held for 2 min. He (N60, Air Liquide, France) was used as the carrier gas.

2.4.3. PBDEs

The GC temperature program for the separation of PBDEs, from mono- to hexabrominated homologues was optimised as follows: splitless injection of 1 μl at 140 °C, initial oven temperature of 140 °C for 1.5 min, then increased at 20 °C/min to 220 °C held for 1 min, then increased at 2 °C/min to 280 °C, finally increased at 30 °C/min to 300 °C and held for 10 min. He (N60, Air Liquide) was used as the carrier gas.

Quantification was performed using internal standards and the isotopic dilution technique. The labelled compounds were added to the fat after the extraction step. Recoveries were calculated using the recovery standard solution EDF-4145 for PCDD/Fs and PCBs, which was added to the reconstituted nonane extracts prior to the injection onto the GC system. The standard solution MBDE-139 MXC was used to calculate the recoveries of PBDEs.

3. Results and discussion

3.1. Optimisation of mass spectrometry parameters

The analysis of PCDD/Fs and PCBs using PTVLV–GC–MS–MS and GC–MS–MS, respectively, has already been optimised using ion trap mass spectrometry in one of our previous studies [17]. It was demonstrated that use of large volume injection allowed one to achieve an LOQ between 0.1 and 0.2 pg/g fat for all dioxin and furan congeners. The present study does not discuss these results further.

3.1.1. Ionisation

EI was chosen as the ionisation mode in order to allow the use of isotopic dilution as quantification method and therefore make the analysis more accurate. In most studies operating with mass spectrometry based on electron impact, the electron energy was either 70 eV [12,27–29] or 30–40 eV [13,15,30,31]. Knowing that electron energy depends on mass spectrometer characteristics, especially on source geometry, optimisations were carried out between 30 and 70 eV in the full-scan mode. The nature of ions produced during ionisation of PBDEs by EI is known to be dependent on the level of bromination. This ionisation mode has thus been demonstrated to be less sensitive when the number of bromines increases, making heptaBDE detection very difficult at low concentrations [12]. Most studies dedicated to the determination of PBDEs by EI-MS described that spectra obtained after electron impact are dominated by M^+ and $[M\text{-}Br_2]^+$ species for lowest degrees of bromination and higher brominated levels, respectively [12–15,27,30]. Similar trends were observed in the present study, but differences between congeners of a same family have however been observed. M^+ species provided the most intense peak for dibrominated congeners except for PBDE-7, PBDE-8 and PBDE-10, for which $[M\text{-}Br_2]^+$ gave the predominant peak. For tri- to hexaBDEs, loss of Br_2 led to the most abundant ion with the exception of triBDE-35 and -37, tetraBDE-77 and pentaBDE-126 for which ionisation mainly yielded to their molecular ion. A common characteristic of these congeners is that they have actually no bromine atom in the *ortho* position. This was consistent with observations of Alaee et al. [28] and Marsh et al. [32], who reported that bromine *ortho* substitution favoured the formation of the $[M\text{-}Br_2]^+$ species over the M^+ species. Whatever the electron energy (tests were carried out at 30, 40, 50, 60 and 70 eV) no significant difference was found in frag-

mentation process during ionisation or in the intensity of signal when current was reconstructed with adequate ion masses. Changes in the temperature source had only minor influence on the loss of Br_2 (220 or 250 °C) and on the ionisation efficiency. For greater convenience, the ion source was been set at 250 °C and 70 eV was kept as the electron energy. Table 2 summarises the mass of parent ions that were isolated for each congener.

3.1.2. Fragmentation

Once parent ions were isolated in the trap, they were fragmented by collision-induced dissociation (CID) producing daughter ions characteristic of target molecules. Again, fragmentation of selected ion was congener dependent. Fig. 1 shows mass spectra obtained for TBDE-47, TBDE-77, PeBDE-99 and PeBDE-126 after optimisation of CID voltages. Excitation time and energy of excitation parameters were default ones.

For tetra-, penta- and hexabrominated compounds, non-*ortho* substituted congeners (TBDE-77 and PeDBE-126) were fragmented with loss of Br_2 (such as PCBs lose Cl_2) yielding the main ion clusters and with loss of –COBr in a lesser extent. For other congeners, $[M\text{-}COBr]^+$ was the main fragment and breakage of a single Br bound gave a signal of lower intensity. This behaviour is similar to dioxins which have characteristic daughter ions resulting from the loss of –COCl.

An opposite trend was observed for lower degree of bromination. Di- and tribrominated congeners lost the last bromine atom during the exciting step, except for DiBDE-11, -12 & 13, -15 and TriBDE-35 and -37 (which have no bromine atom in the *ortho* positions) showing more intense peaks in mass spectra corresponding to loss of –COBr. Optimised CID voltage as well as daughter ions monitored are gathered in Table 2.

3.2. Evaluation of the analytical procedure

3.2.1. Linearity

A five-point calibration curve was determined for the PBDEs of solution EO-4980 excepted for hepta-BDEs. Linearity was observed for a range of between 1 and 75 pg for di-, tri- and tetrabrominated congeners, between 1.5 and 112 pg for pentabrominated, between 2 and 150 pg for hexabrominated, with satisfactory correlation coefficients. The RRF varied in a broad range of values depending on the congener. This was probably mainly due to the few ^{13}C congeners available. Table 3 presents the RRFs for all congeners, correlation coefficients (R^2) and the range of concentration where linearity were observed.

Table 2
CID voltages, monitored parent ions and daughter ions for each congener

Homologue	Congener		Isolated parent ion (m/z)	CID voltage (V)	Isolated daughter ions (m/z)
DiBDEs	10, 7, 11, 8, 12&13, 15	^{12}C	328 [M+2]	3.75	168, 219/221
	15	^{13}C	340 [M+2]	3.75	180
TriBDEs	30, 32, 17&25, 28&33	^{12}C	246 [M-Br_2]	4.0	167
	28	^{13}C	258 [M-Br_2]	4.0	179
	35, 37	^{12}C	406 [M+2]	4.0	246/248, 297/299/301
TBDEs	75, 71, 49, 47, 66	^{12}C	326 [M+2-Br_2]	5.0	217/219, 245/247
	47	^{13}C	338 [M+2-Br_2]	5.0	229/231, 257/259
	77	^{12}C	486 [M+4]	4.5	324/326/328, 377/379
PeBDEs	100, 119, 99, 116, 85	^{12}C	404 [M+2-Br_2]	5.0	295/297/299, 325/327
	99	^{13}C	416 [M+2-Br_2]	5.0	337/339, 307/309/311
	126	^{12}C	564 [M+4]	4.5	404/406, 325
HxBDEs	154, 153, 140, 138, 166	^{12}C	484 [M+4-Br_2]	5.5	376/378, 403/405
	154, 153	^{13}C	496 [M+4-Br_2]	5.5	388/390, 415/417

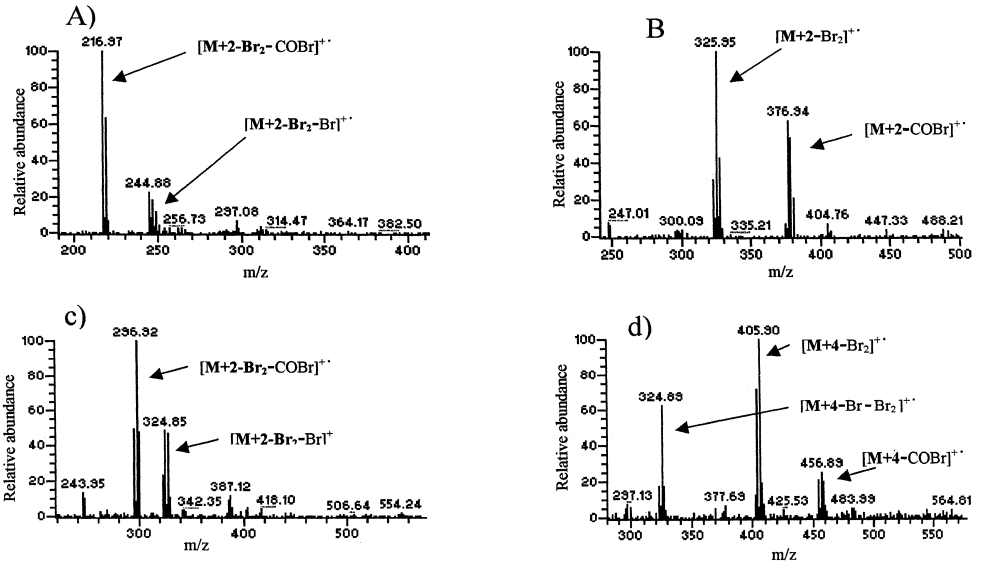

Fig. 1. Mass spectra obtained after CID voltage optimisation of: (A) TBDE-47, parent ion was [M+2-Br_2] (*m*/*z* 326), (B) TBDE-77, parent ion was [M+4] (*m*/*z* 486), (C) PeBDE-99, parent ion was [M+2-Br_2] (*m*/*z* 404), (D) PeBDE-126, parent ion was [M+4] (*m*/*z* 564).

3.2.2. Repeatability and reproducibility

Repeatability (short-term standard deviation) and reproducibility (long-term standard deviation) were evaluated for our analytical method.

Mixtures of native PBDEs (EO-4980) and MBDE-MXC ^{13}C-labelled internal standard solution were injected 10 times consecutively and relative standard deviations (RSDs) between amounts obtained were calculated. These RSD values, which are gathered in Table 3, did not exceed 10%, accounting for the good repeatability of the mass spectrometer response in the EI-MS–MS mode. There was no evidence that RSD increased with increased degree of bromination as it was previously observed in another study using EI-MS [15].

Reproducibility was tested by establishing 10 calibration curves with the BDE-MXC native solution and the MBDE-MXC ^{13}C-labelled internal standard solution at different moments, under different conditions, with different operators. The RSDs of the RRFs found for each calibration and for each congener were evaluated and are reported in Table 3. The BDE-MXC native solution does not contain as many congeners as the EO-4980 solution, data on reproducibility are missing for some congeners. Standard deviation thus slightly increased especially for HxBDE-138, TBDE-77 and PeBDE-126, but remained lower than 18% for latter two. RSDs for other congeners ranged between 3 and 12%.

3.2.3. Limits of detection

Limits of detection (LODs) of the mass spectrometer were defined as the smaller amount giving a signal-to-noise ratio greater than 3. These LODs ranged between 0.5 and 3 pg depending on the congener. Tandem in time mass spectrometry remained slightly less sensitive than HRMS [13], but allowed one to distinctly decrease the LOD compared to EI-LRMS [15], especially for higher bromination degree, and nearly achieved the offset of the lower sensitivity of EI compared to the NCI mode. Regarding levels of PBDEs reported in the environment, MS–MS can really compete with both methods usually used.

3.3. Optimisation of the purification step

In the dioxin purification procedure, the automated Power-Prep system has already proven its efficiency for various types of matrices such as environmental [23], biological [24] and food-type [25]. A previous study has extended this multi-step clean-up to 14

Table 3
Concentration range where linearity was tested, relative response factor (RRF), correlation coefficient (R^2), short-term relative standard deviation (repeatability) and long-term relative standard deviation (reproducibility) for PBDEs

	Concentration range (pg/μl)	RRF	R^2	Repeatability, RSD (%)	Reproducibility, RSD (%)
DiBDE-10	1–75	0.996	0.997	5.9	–
DiBDE-7	1–75	0.886	0.997	3.1	3.5
DiBDE-11	1–75	2.014	0.994	2.7	–
DiBDE-8	1–75	1.015	0.992	2.2	–
DiBDE-13, -12	1–75	2.217	0.991	1.7	–
DiBDE-15	1–75	0.979	0.996	6.3	9.9
TriBDE-30	1–75	0.718	0.996	5.4	–
TriBDE-32	1–75	0.366	0.993	4.3	–
TriBDE-17, -25	1–75	1.495	0.999	5.2	12.1
TriBDE-28, -33	1–75	1.380	0.990	3.4	12.3
TriBDE-35	1–75	6.407	0.995	4.5	–
TriBDE-37	1–75	3.876	0.995	4.5	–
TBDE-75	1–75	0.724	0.991	4.1	–
TBDE-71	1–75	0.802	0.992	2.2	3.2
TBDE-49	1–75	0.789	0.991	4.8	9.8
TBDE-47	1–75	0.888	0.993	1.1	3.6
TBDE-66	1–75	0.631	0.992	6.5	8.1
TBDE-77	1–75	0.086	0.993	9.5	17.8
PeBDE-100	1.5–112	3.244	0.994	1.9	3.9
PeBDE-119	1.5–112	2.148	0.993	2.2	2.4
PeBDE-99	1.5–112	2.348	0.992	1.2	4.2
PeBDE-116	1.5–112	1.892	0.990	4.1	–
PeBDE-85	1.5–112	1.453	0.988	5.0	11.2
PeBDE-126	1.5–112	0.117	0.994	8.4	17.6
PeBDE-105	1.5–112	0.119	0.991	3.2	–
HxBDE-154	2–150	0.305	0.993	3.0	7.0
HxBDE-153	2–150	0.357	0.994	3.1	7.4
HxBDE-140	2–150	0.158	0.993	1.3	–
HxBDE-138	2–150	0.105	0.993	9.7	14.6
HxBDE-166	2–150	0.052	0.997	2.6	–

PCBs using the same sequence of events constituting the program for dioxin isolation [22]. The drawback of this strategy was the quite high volume of solvent used for PCB purification, yielding quite high blank levels and therefore to quite high LOQs. The present study enlarges this clean-up to brominated compounds such as PBDEs with optimisation of type and quantities of solvent used to isolate the different compounds carried out in order to produce as low LOQs as possible.

Details of the clean-up scheme for dioxin analysis on the Power-Prep have been already extensively described [21–25]. It consists of a succession of three different types of columns (HCDS and multilayer silica, basic alumina and PX-21 carbon). Once extracted fat was loaded onto the silica columns, hexane (200 ml) was used to elute compounds from the silica through the alumina column. A mixture (60 ml) of hexane–dichloromethane (98:2) was then applied onto the alumina column where some less-polar PCBs began to elute and were collected. The remaining PCBs and dioxins were eluted from alumina with 120 ml of hexane–dichloromethane (50:50) through the carbon column on which dioxins and other coplanar compounds such as non-*ortho* PCBs (also called c-PCBs) were trapped, while other non-planar PCBs passed through it and were collected. This fraction was pooled to the ‘‘98:2 fraction’’ and constituted the PCB fraction. An ethyl acetate–toluene (50:50) mixture (5 ml) was further applied to the carbon column in the forward direction for additional clean-up. Afterwards, 65 ml of toluene

was applied to the carbon column in the backflush direction for the elution of dioxins and non-*ortho* PCBs.

Since most of the fractionation process occurs on the alumina column, depending on the solvent mixture used, the isolation of any other related compounds such as PBDEs should be studied at that level. In that optic, the elution pattern of PCDD/Fs, PCBs and PBDEs has been investigated on alumina using hexane–dichloromethane (50:5) as solvent. The elution pattern is presented in Fig. 2.

It shows that, under these conditions, alumina was not able to provide a good separation between the different analytes. Only carbon appeared to be suited for the partition between planar and non-planar compounds. The use of carbon column however confronted us with a problem. While c-PCBs were strongly retained on carbon column, some other PCBs, mainly mono-*ortho* congeners, passed through this column but were significantly slowed down. Large quantities of solvent were thus required to collect them quantitatively, resulting in high LOQ values, as reported in our previous PCB determination study.

The only way to avoid the use of such quantities of solvent was to modify the sequence of events such as the PCBs would be isolated prior the carbon column. An adequate solvent mixture was therefore needed to selectively desorb PCBs from alumina without affecting dioxins. Due to the high selectivity of analytical method, PCBs and PBDEs did not have to be separated from each other. This allowed the simultaneous collection of both families of chemicals in the same fraction. The only condition was to be able to do so quantitatively. The solvent mixture that appeared to be the more efficient to elute, specifically those from alumina, was a solution of 20% dichloromethane hexane. Fig. 3 depicts the scheme of the optimised clean-up procedure and Table 4 shows the entire elution pattern for the multi group isolation of PBDEs, PCBs, PCDDs and PCDFs.

If for PCDD/F and PCB analysis, each congener can be quantified with its corresponding mass-labelled compound, PBDE congener quantification has to be performed using the few commercially available labelled compounds. Since the internal standard solution contains only some of the congeners, a single labelled compound quantified several congeners with the same degree of bromination. For the present method, this generated difficulties in the analysis of PBDEs which were not collected in the same fraction as the labelled compound used for their quantification. This was the case for some tetraBDEs (-49, -66, -75 and -77) and some pentaBDEs (-116, -105 and -126). Measurement of these were therefore performed using the tribrominated

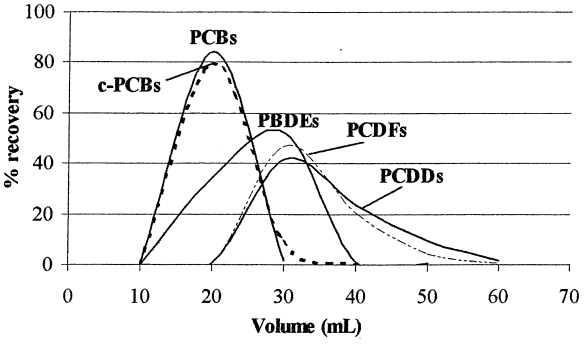

Fig. 2. Percentage of recovery for PCBs (with a distinction between planar and non-planar congeners), PBDEs, PCDDs and PCDFs when hexane–dichloromethane (50:50) is used to elute the alumina column (PBDEs which were not eluted with this mixture of solvent were not taken into account).

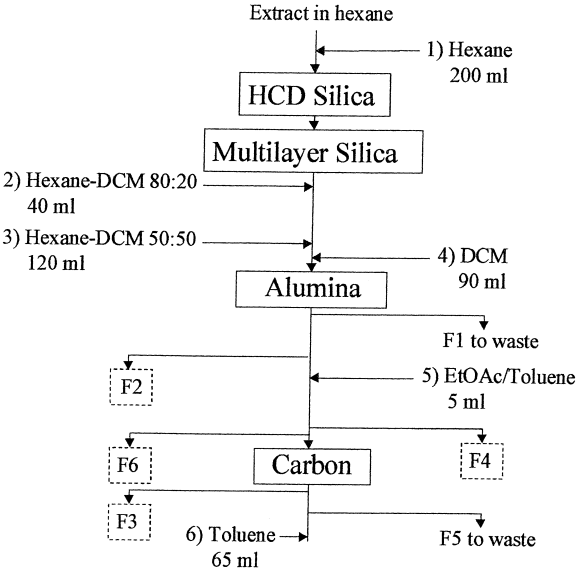

Fig. 3. Scheme of fraction collections for the automated clean-up and multi-group fractionation using multi-layer silica, basic alumina and PX-21 carbon disposable columns.

Table 4
Multi-group fractionation and recovery (%) for the 65 target analytes

Congener	80:20 (F2)	50:50 (F3)	DCM (F4)	Toluene (F6)	Recovery (%)	Congener	80:20 (F2)	50:50 (F3)	DCM (F4)	Toluene (F6)	Recovery (%)
PBDEs						*PCBs*					
DiBDEs						Non-*ortho*					
10		x			40	TCB-77	x				81
7		x			49	TCB-81	x				85
11			x		92	PeCB-126	x				57
8			x		89	HxCB-169	x				53
13–12			x		75	Mono-*ortho*					
15			x		91	PeCB-123	x				100
TriBDEs						PeCB-118	x				104
30		x			47	PeCB-114	x				108
32		x			72	PeCB-105	x				57
17–25		x			88	HxCB-167	x				101
28–33		x			67	HxCB-156	x				102
35		x	x		59	HxCB-157	x				98
37		x	x		56	HpCB-189	x				66
TBDEs						Aroclor 1260					
75		x			72	TriCB-28	x				65
71	x				60	TCB-52	x				65
49		x			59	PeCB-101	x				97
47	x				79	HxCB-153	x				63
66		x			58	HxCB-138	x				94
77		x			93	HpCB-180	x				81
PeBDEs						*PCDD/Fs*					
100	x				66	2,3,7,8-TCDD				x	88
119	x				59	1,2,3,7,8-PeCDD				x	88
99	x				77	1,2,3,4,7,8-HxCDD				x	60
116		x			60	1,2,3,6,7,8-HxCDD				x	62
85	x				62	1,2,3,7,8,9-HxCDD				x	70
126		x			92	1,2,3,4,6,7,8-HpCDD				x	86
105		x			91	OCDD				x	84
HxBDEs						2,3,7,8-TCDF				x	88
154	x				77	1,2,3,7,8-PeCDF				x	84
153	x				62	2,3,4,7,8-PeCDF				x	82
140	x				61	1,2,3,4,7,8-HxCDF				x	61
138	x				67	1,2,3,6,7,8-HxCDF				x	65
166		x			64	1,2,3,7,8,9-HxCDF				x	67
						2,3,4,6,7,8-HxCDF				x	64
						1,2,3,4,6,7,8-HpCDF				x	84
						1,2,3,4,7,8,9-HpCDF				x	100
						OCDF				x	79

[$^{13}C_{12}$]PBDE-28 (with adequate RRF). However, this resulted in a loss of accuracy for quantification of these congeners. This was the price to pay for LOQ reduction for PCB analysis.

3.4. Evaluation of the whole procedure

3.4.1. LODs and LOQs

LODs and LOQs were evaluated using average method blank values. LOD values for congeners present in the blank procedural method were calculated as the average blank value plus three times the standard deviation (SD) of the blank. LOQs were defined as the average blank value plus 10 times the SD. For congeners not found in blank, the LOD was the values determined for sensitivity of the mass spectrometer, that is values of smaller added concentration giving a signal with a signal-to-noise ratio (S/N) greater than 3. Table 5 shows LODs and LOQs for the few congeners found in procedural

Table 5
LODs and LOQs for congeners found in method procedural blanks ($n=5$) with the previous reported clean-up and with the new strategy

Congener	Old strategy		New strategy	
	LOD	LOQ	LOD	LOQ
DiBDE-10	–	–	6	17
TriBDE-35	–	–	5	13
TBDE-47	–	–	64	123
PeBDE-99	–	–	39	69
HxBDE-154	–	–	8	25
HxBDE-153	–	–	17	50
TriCB-28	16.31	34.03	2.38	6.01
TCB-52	14.90	32.61	1.78	4.00
PeCB-101	8.60	18.47	0.83	1.98
PeCB-118	2.80	5.78	0.16	0.30
HxCB-153	4.52	9.47	0.25	0.44
PeCB-105	1.18	2.68	0.03	0.06
HxCB-138	7.66	17.95	0.41	0.72
HxCB-156	0.40	0.98	0.02	0.06
HpCB-180	1.95	4.66	0.22	0.63
HpCB-189	2.36	6.56	2.73	8.20

Data are expressed in pg/g of fat for PBDEs and ng/g of fat for PCBs.

blanks. Several studies have observed significant amount of PBDEs in laboratory blanks [27,33,34], predominantly PBDE-47 and PBDE-99, and more occasionally PBDE-100, PBDE-153 and PBDE-154. Similar trends were observed for our method blanks, even after implementation of actions to minimise laboratory contamination, such as regular silanisation of the few pieces of glassware used.

Table 5 also shows LODs and LOQs for PCBs with this new strategy compared to the previous one. The limits of detection and quantification obtained with this extended clean-up are between 5 and 10-times lower than in our previous study. They are now widely below the norm imposed by BELTEST, the Belgian accreditation body, which fixed LODs and LOQs at, respectively, 5 ng/g and 10 ng/g for each congener of the Aroclor 1260. LOQs for PBDE congeners are definitely lower than level usually found in the environment, which used to range from 0.5 ng/g fat in Swedish human breast milk (for PBDE-100) to up than 5000 ng/g fat in marine mammals [2].

3.4.2. Repeatability and accuracy

In order to evaluate our method, QC samples were purified and analysed using our multi-analyte method in triplicate. Mean levels obtained are gathered in Table 6. In the absence of a certified reference material, accuracy was estimated regarding values measured in the samples towards these expected following spike levels. Repeatability of the procedure was tested by calculating the RSD between the three analyses.

Accuracy ranged between 91 and 122% for PBDEs excepted for PBDE-32 with a very high value of 140%. Results for most of the pentabrominated congeners were not reported due to very incoherent values, with accuracies ranging between 25 and 75% and RSDs reaching sometimes up to 96%. These incoherences were probably the result of the fractionation trouble mentioned above and the lack of an appropriate internal standard. Accuracy for PCB and PCDD/F determination was fairly good, keeping in mind that samples were laboratory-made fortified fat and not certified reference materials. Acceptable repeatability was observed with RSDs being lower than 20% for all compounds except for TBDE-77 and TCDD for which values were slightly higher. PBDE-28, -47, -66, -99, -100, -153, -154, which are the congeners that were found most predominantly in abiotic or biological samples [1,2,11], i.e., are usually monitored in epidemiological studies, such as in breast milk [4,30,35], blood [36] or fish [28,37]. They thus require special attention during the analytical procedure. As shown in Table 6, this method produced really reliable results for these particular compounds, with accuracies ranging from 97 to 112% and SDs below 10%.

4. Conclusion

This simple, time- and resource-saving strategy allows to one incorporate analysis of a broad range of PBDEs with classical dioxin and PCB analysis. Purification and isolation of 25 PBDEs, from di- to hexabrominated congeners, the 12 non- and mono-*ortho* PCBs, the seven congeners of Arolcor 1260, the 17 PCDDs and PCDFs are performed with a single and automated clean-up, while final determinations are carried out on a single GC ion trap mass spectrometer, in four separate injections.

The combination of EI and tandem in time mass spectrometry offers the accuracy of EI-HRMS, in

Table 6
Congener-specific data in pg/g fat for the laboratory-made quality control ($n=3$)

Congener	Levels in QC (pg/g fat)	RSD (%)	Accuracy (%)	Congener	Levels in QC (pg/g fat)	RSD (%)	Accuracy (%)
PBDEs				Aroclor 1260			
DiBDE-10	121	15	121	TriCB-28	2117	6	96
DiBDE-7	101	11	101	TCB-52	2654	8	121
DiBDE-11	108	20	108	PeCB-101	2127	4	97
DiBDE-8	113	7	113	HxCB-153	2039	8	93
DiBDE-13&12	107	7	107	HxCB-138	2432	4	111
DiBDE-15	94	3	94	HpCB-180	2771	1	104
TriBDE-30	94	15	94	c-PCBs			
TriBDE-32	140	13	140	TCB-77	11.3	15	113
TriBDE-17&25	105	2	105	TCB-81	10.6	11	106
TriBDE-28&33	97	10	97	PeCB-126	63.4	4	127
TriBDE-35	122	7	122	HxCB-169	107.1	1	107
TriBDE-37	118	1	118	*Dioxins*			
TBDE-75	103	3	103	PCDDs			
TBDE-71	112	12	112	2,3,7,8-TCDD	0.41	29	103
TBDE-49	99	6	99	1,2,3,7,8-PeCDD	1.92	17	96
TBDE-47	112	10	112	1,2,3,4,7,8-HxCDD	1.83	7	92
TBDE-66	112	1	112	1,2,3,6,7,8-HxCDD	2.22	14	111
TBDE-77	119	27	119	1,2,3,7,8,9-HxCDD	1.81	2	90
PeBDE-100	147	3	98	1,2,3,4,6,7,8-HpCDD	2.28	19	114
PeBDE-99	148	20	98	OCDD	4.20	15	105
PeBDE-116	137	6	91	PCDFs			
HxBDE-154	211	8	106	2,3,7,8-TCDF	0.42	18	105
HxBDE-153	213	12	106	1,2,3,7,8-PeCDF	2.60	4	130
HxBDE-140	205	15	102	2,3,4,7,8-PeCDF	2.26	12	113
HxBDE-138	209	6	104	1,2,3,4,7,8-HxCDF	1.96	19	98
PCBs				1,2,3,6,7,8-HxCDF	1.95	8	98
Mono-*ortho*				1,2,3,7,8,9-HxCDF	2.26	3	113
PeCB-123	325	1	81	2,3,4,6,7,8-HxCDF	1.87	8	94
PeCB-118	2079	4	95	1,2,3,4,6,7,8-HpCDF	2.55	7	128
PeCB-114	396	7	99	1,2,3,4,7,8,9-HpCDF	2.25	10	112
PeCB-105	451	2	113	OCDF	4.17	7	104
HxCB-167	364	5	91				
HxCB-156	362	0	90				
HxCB-157	465	5	116				
HpCB-189	80	19	90				

allowing the quantification by isotopic dilution, and even a higher degree of selectivity achieving to separate isobaric ions such as PBDE-47 and PCB-180 which were not resolved under normal operating conditions with HRMS. On the other hand, MS–MS has advantages of NCI-LRMS in being a user-friendly and low-cost instrument. Working in tandem mass spectrometry increases sensitivity and offsets the use of EI as ionization mode, nearly competing with NCI-LRMS for limits of detection. Spectrometer response has proven to be repeatable, reproducible and linear in the studied concentration range. The whole method has been demonstrated to be robust, repeatable and fairly accurate, producing good recovery rates, especially for PBDE-28, -47, -66, -99, -100, -153 and -154 which are the most relevant congeners. Limits of detection and quantification for PCB analysis were drastically reduced compared to our previous study and are now widely below the values fixed by accreditation body.

This method could be easily extended to biological fluids such as milk and serum using automated on-line (integrated) solid-phase extraction which was already optimised in our laboratory [38]. This will

allow one to make epidemiological studies on a wide range of compounds more efficiently.

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