COMPREHENSIVE ANALYSIS OF PBDEs, PCDDs, PCDFs AND PCBs

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Introduction

Nowadays, the list of man-made chemicals that need to be monitored in environment is still extending and laboratories have to offer the capability to analyse a growing number of various target compounds. Polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs) and polychlorobiphenyls (PCBs) are probably the most well-known contaminants belonging to this class of targeted molecules but other halogenated chemicals, such as polybrominated diphenyl ethers (PBDEs), are a cause of growing concern. In the prospect of developing an analytical strategy covering a wider range of compounds, we investigated the extension of a well-established method, dedicated to PCDD/Fs and PCBs analysis in foodstuffs, to PBDEs.
We therefore developed a single comprehensive automated sample preparation step followed by a multiple injection procedure using a quadrupole ion storage mass spectrometer (QISTMS) performing in the tandem mode for the measurement of PCDD/Fs, PCBs, and PBDEs in foodstuffs.

Methods and Materials

Automated sample preparation

Purification steps were based on the Power-Prep™ automated multi-column cleanup system (FMS Inc., Waltham, MA, USA). This system uses disposable multi-layer silica columns, basic alumina and PX-21 carbon columns in order to separate analytes from matrix interferences. The system configuration allows the collection of different fractions during the purification.

Instrumental analysis

Analyses were conducted on a ThermoQuest Trace GC PolarisQ QISTMS (Austin, Tx, USA) equipped with a Rtx 5-MS (40m x 0.18 mm x 0.18 µm) capillary column (Restek, Evry, France). The PBDEs Analytical Standard Solution EO-4980 was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and contains 40 native congeners from mono- to heptaBDEs and 5 ^{13}C-labelled PBDEs (-47,-77,-99,-100 and -126). The MBDE-MXC ^{13}C-labelled internal standard solution was from Wellington Laboratories (Ontario, Canada) and contains mono- (3), di- (15), tri- (28), tetra- (47), penta- (99), hexa- (153 and 154) and heptaBDE (183).

Results and Discussion

1. Optimisation of the QISTMS

Analysis of PCDD/Fs, PCBs and PBDEs were carried out on separate injections. Measurements of PCDD/Fs and PCBs using, respectively, PTVLV-GC/MS/MS and GC/MS/MS have already been optimised using QISTMS in one of our previous studies[1]. It was demonstrated that the use of

PTVLV injection allowed to achieve LOD values in the range of 0.1-0.2 pg/g fat for PCDDs and PCDFs.

Electron impact (EI) was chosen as ionisation mode in spite of its poor sensitivity towards PBDEs (compared to NCI) in order to perform isotopic dilution and improve the accuracy of the measurements. The efficiency of this ionisation mode is however dependent of the bromination level and is less sensitive when the number of bromine increases, making heptaBDE detection very difficult at low concentration[2]. As described in several studies on EI of PBDEs, spectra are dominated by $M^{+\cdot}$ and $[M-Br_2]^{+\cdot}$ species for low and high degrees of bromination, respectively[2,3,4,5].

Differences however appeared between congeners containing the same number of bromine. $M^{+\cdot}$ species provided the most intense peak for diBDEs, except for PBDE-7, PBDE-8 and PBDE-10, for which $[M-Br_2]^{+\cdot}$ was the predominant peak. For tri- to hexaBDE, 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 do not have bromine atoms in the *ortho* position. This was consistent with observations of Alaee et al.[6] and Marsh et al.[7], who reported that bromine *ortho* substitution favoured the formation of the $[M-Br_2]^{+\cdot}$ ion over the $M^{+\cdot}$ ion. Whatever the electron energy (tests were carried out at 30, 40, 50, 60 and 70eV) or the temperature source (220 or 250°C) no significant difference was found in the fragmentation process nor in the intensity of the signal.

Once parent ions were isolated in the trap, they were fragmented by collision-induced dissociation (CID), producing daughter ions characteristic of target molecules. Again, the fragmentation of the selected ions was congener dependent. For tetra-, penta- and hexaBDEs, $[M-COBr]^{+\cdot}$ was the main fragment, although non-*ortho* substitued congeners (TBDE-77 and PeDBE-126) were mainly fragmented with loss of Br_2 (such as PCBs lose Cl_2). This is similar to dioxins that have characteristic daughter ions resulting from the loss of –$COCl$[8]. An opposite trend was observed for lower degree of bromination. Di- and triBDEs lost the last bromine during the exciting step, except for DiBDE-11, -12, -13, -15 and TriBDE-35 and -37 (which have no bromine atom on the *ortho* positions) showing more intense peaks in mass spectra corresponding to loss of -$COBr^{\cdot}$. Table 1 shows the optimized acquisition parameters.

Table 1 Acquisition parameters

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

2. Evaluation of the analytical procedure

Linearity, repeatability and reproducibility have been evaluated and the quite satisfactory results obtained demonstrated the suitability of the combination EI-MS/MS for determination of PBDEs. Instrumental LODs were defined as the smaller amount giving a S/N>3. These LODs ranged between 0.5 to 3 pg depending on congeners. This is slightly less sensitive than HRMS[3], but at least as good as EI-LRMS[5], especially for higher bromination degree. They are quite close to what can be achieved in NCI mode, making EI-MS/MS useful regarding levels of PBDEs reported in the environment.

3. Optimisation of the purification step

For the isolation of PCDD/Fs and cPCBs in foodstuffs, the automated Power-Prep™ system has already proven its efficiency[9]. More recently, the procedure has been extended to 14 additional PCBs (mono-ortho-PCBs and marker PCBs)[10]. The present study enlarges this cleanup to PBDEs after optimisation of solvent types and flows responsible for the fractionation in sub-groups. The column set consists in the succession of 3 different types of sorbents (multi-layer silica, basic alumina and PX-21 carbon). Figure 1 depicts the scheme of the optimised clean-up procedure and Table 2 shows the entire elution pattern for the multi group isolation of selected PBDEs, PCBs, PCDDs and PCDFs. Due to the high selectivity of the EI-MS/MS method, PCBs and PBDEs did not have to be separated from each other. This allowed the simultaneous collect of both families of chemicals in the same fraction.

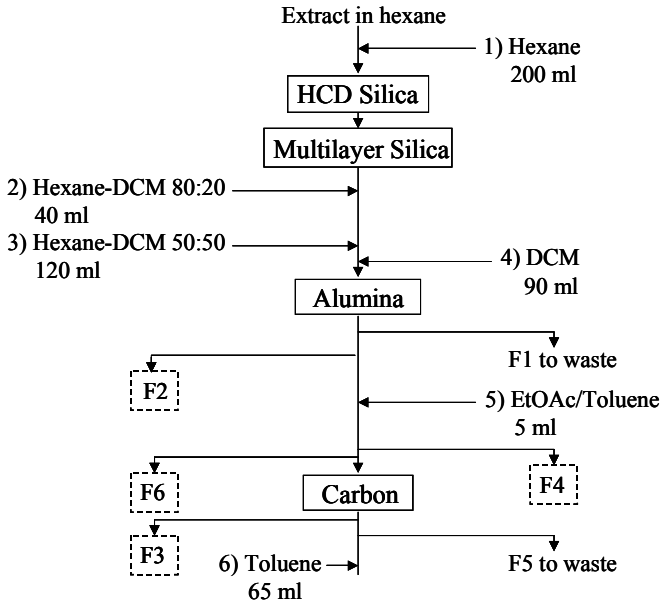

Figure 1 Multi-group fractionation scheme for the automated clean-up using disposable columns.

Although all PCDD/F and PCB ^{13}C labels are available, few were available for PBDEs at the time of the study. A single labelled compound was thus used to quantify several congeners with the same degree of bromination. This generated some troubles for 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 $^{13}C_{12}$-PBDE-28 (with adequate RRF).

4. Evaluation of the whole procedure

In order to evaluate the method, "home-made" QC samples (fortified beef fat) were purified and analysed using our multi-analyte method in triplicate. Mean levels obtained are shown in Table 3. In the absence of a certified reference material, accuracy was estimated regarding values measured in the QC samples towards what expected following spike levels. Repeatability of the procedure was tested by calculating relative standard deviation (RSD) between the 3 analysis. Keeping in mind that samples were home-made fortified fat and not certified reference materials, accuracy was fairly good excepted for some of the pentaBDE congeners. This was probably the result of the fractionation trouble mentioned above and the lack of an appropriate internal standard. Nevertheless, BDE-28, -47, -66, -99, -100, -153, -154, which are the congeners that are found most predominantly in abiotic and biological samples, i.e.[11], were accurately measured.

Conclusions

This simple, time and resource saving strategy allows to incorporate analysis of a broad range of PBDEs to classical PCDD/F and PCB analysis. Purification and isolation of 25 PBDEs, from di- to hexabrominated congeners, the 12 non- and mono-*ortho* PCBs, the 7 congeners of Arolcor 1260, the 17 PCDDs and PCDFs are performed within a single and automated clean-up. Final determinations are carried out on a single GC ion trap mass spectrometer, in 4 separate injections. This method could eventually be extended to automated and integrated extraction and clean-up of biological fluids and solids, as already reported for PCDD/Fs and PCBs[12,13].

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Table 2 Multi-group fractionation and recovery rates for the 65 target analytes.

Congeners	80/20 F2	50/50 F3	DCM F4	Tolu F6	Recov. %	Congeners	80/20 F2	50/50 F3	DCM F4	Tolu F6	Recov. %
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

Table 3 Congener-specific data (pg/g fat) for 'in-house' beef QV=C pool (n=3).

Congeners	Levels in QC pg/g fat	RSD (%)	Accuracy (%)	Congeners	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-HpCDI	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-HpCDI	2.55	7	128
PeCB-114	396	7	99	1,2,3,4,7,8,9-HpCDI	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				