Simultaneous extraction of PCDDs/PCDFs, PCBs and PBDEs – Extension of a sample preparation method for determination of PCDDs/PCDFs

Cathrine Thomsen[1], Tove Nicolaysen[1], Sharon L Broadwell[1], Line S Haug[1], Georg Becher[1]

[1]Norwegian Institute of Public Health, Oslo

Introduction

Due to emission controls and regulatory measures, the levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/PCDFs) and polychlorinated biphenyls (PCBs) have been steadily decreasing in the environment[1] and in human samples[2,3] the last decades. Nevertheless, the exposure of general populations is still considered to be high and many individuals may have a dietary intake above the established tolerable daily intake[4]. During the recent years, several brominated flame retardants (BFRs) and especially the polybrominated diphenyl ethers (PBDEs) have been shown to be potential persistent organic pollutants (POPs)[5]. In contrast to PCDDs/PCDFs and PCBs, levels of BFRs seem to be increasing in several environmental compartments[5,6]. Thus it is of great importance to obtain information on levels of both PCDDs/PCDFs, PCBs and BFRs.

Traditionally, PCDDs/PCDFs have been extracted together with the non-ortho PCBs, while extracts of other POPs and PCBs have been prepared separately. Recently, efficient automated methods preparing PCDDs/PCDFs and PCBs extracts at the same time, have been described[7,8]. A simultaneous sample preparation is advantageous in cases where limited amounts of sample is available, e.g. when analysing human milk or blood, and assures comparable results since the different POPs are determined in exactly the same sample aliquot. Also, due to the low concentration of PCDDs/PCDFs and non-ortho PCBs usually present, a relatively large amount of sample is applied for the extraction, which leads to the possibility of detecting other POPs that are normally not found. We present here a simple and inexpensive extension of our sample preparation method used for determination of PCDDs/PCDFs and non-ortho PCBs that leads to inclusion of both ortho PCBs and PBDEs.

Materials and methods

Chemicals

The standard solutions of PCBs and BFRs were purchased from Wellington Laboratories (Guelph, Ontario, Canada), CIL (Andover, MA) or AccuStandard (New Haven, CT). All solvents used were of pesticide grade from sds (Peypin, France) and the concentrated sulphuric acid (p.a. quality), sodium sulphate and silica gel (60) were from Merck (Darmstadt, Germany).

GC-MS

A HP (Avondale, PA, USA) 6890 gas chromatograph was used and the separations were performed on a HP-5 MS column (30 m x 0.25 mm ID, 0.25 µm film thickness) connected to a deactivated retention gap of 1.5 m x 0.32 mm ID (J & W Scientific, Folsom, CA, USA). The injector temperature was 290°C and samples of 1µL were injected in pulsed splitless mode. Helium was used as carrier gas and separation was performed at a constant flow of 1.2 mL/min. The temperature program for the BFRs were 90°C for 1 min, then raised by 20°C/min to 190°C, 5 °C/min to 230°C, 1 °C/min to 235°C, 3.5 °C/min to 250°C and finally 30°C/min to 325°C, which was held for 4 min. The PCBs were separated using a temperature program of 90°C for 1 min, then raised by 20°C/min to 190°C, 3 °C/min to 230°C, 1 °C/min to 235°C, 3.5 °C/min to 250°C and finally 30°C/min to 325°C, which was held for 4 min.

The mass spectrometer, a HP 5973 MSD with chemical ionisation (CI) option, was operated in the electron capture mode with methane as buffer gas. The brominated compounds were monitored at *m/z* 79/81. The PCBs were monitored at two isotopes of the molecular ion, except for CB-18, CB-66, CB-74, CB-99 and CB-110 for which two masses of the chlorine ion were measured as well (m/z 35 and 37). Identification was based on retention time and isotope abundance ratio. The temperature of the ion source was 250°C and 150°C when detecting BFRs and PCBs, respectively.

Method development

The sample preparation method for extraction of PCDDs/PCDFs and non-ortho PCBs is based on the method described by Smith et al.[9]. In brief, the sample is mixed with sodium sulphate, added internal standards and packed on a multi-layer column containing silica gel and potassium silicate. The compounds are dynamically extracted from the matrix by a 1:1 mixture of cyclohexane and dichloromethane, which are further passed through a column of activated carbon in line. The planar PCDDs/PCDFs and non-ortho PCBs are retained on the carbon column, while other compounds as well as matrix constituents are led to waste. Normally a volume of 550 mL cyclohexane-dichloromethane is used. In the first part of the method development this volume was collected in aliquots of 50 mL, which were further cleaned and analysed separately, to examine the elution profile for the PCBs, BFRs and the lipids in the sample. This experiment was performed in triplicate. The lipids were determined gravimetrically. Next, the clean-up of this extract was optimised using different amounts of sulphuric acid-silica gel (1:3) and different solvent mixtures for elution. For the method development samples of 10 g of soy oil or cod liver oil were used.

Resulting method

The first 200-250 mL of cyclohexane-dichloromethane eluted from the multi-layer column through the carbon column was collected and reduced to about 100 mL, before application on a column of sulphuric acid-silica gel (10 mL+ 30 g), which had been conditioned with heptane. The eluent was collected immediately and the column eluted with additionally 50 mL heptane followed by 75 mL

of dichloromethane-heptane (1:3). The volume was reduced to about 1 mL, before a second clean-up on a column of sulphuric acid-silica gel (2 mL + 6 g), which was eluted by 20 mL of dichloromthane-heptane (1:3). Eventually, the resulting extract was transformed to toluene and reduced to about 150 μL, before the recovery standard (CB-207) was added.

Method validation

The method was validated for the following analytes: BDE 28, 37, 47, 85, 99, 100, 119, 138, 153, 154, 183 and CB 18, 28, 52, 66, 74, 99, 101, 105, 110, 114, 118, 123, 128, 138, 153, 156, 157, 167, 170, 180, 183, 187, 194 and 209. The following compounds were evaluated as internal standards: BDE 18, 51, 77, 103, 156, 181 for the PBDEs and C-13 labelled CB 28, 52, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, 194 and 209 for the PCBs. The recovery and accuracy (recovery relative to the internal standard) of the method were assessed by spiking aliquots of 10 g soy oil at four levels being 50, 500, 5000 and 50000 pg of both the PBDEs and the PCBs. Replicates of 4, 5, 4 and 1 were performed at the four levels, respectively. Samples only added internal standards and procedural blanks were also prepared. The background levels were corrected for in the calculations. In addition, samples from three interlaboratory comparison studies were analysed[10-12].

Results and discussion

In Figure 1, the elution profile from the multi-layer column is shown for the lipids, CB-153 and BDE-47 as an example. Above 80% of the total eluted amount of CB-153 and BDE-47 and about 50% of the fat, were eluted in the first 150 mL. An almost similar profile were observed for all compounds and at 250 mL, above 90% of the PCBs and PBDEs, and about 80% of the lipids had been eluted from the multi-layer column. This volume was thus considered to be acceptable in order to get a high recovery of the analytes.

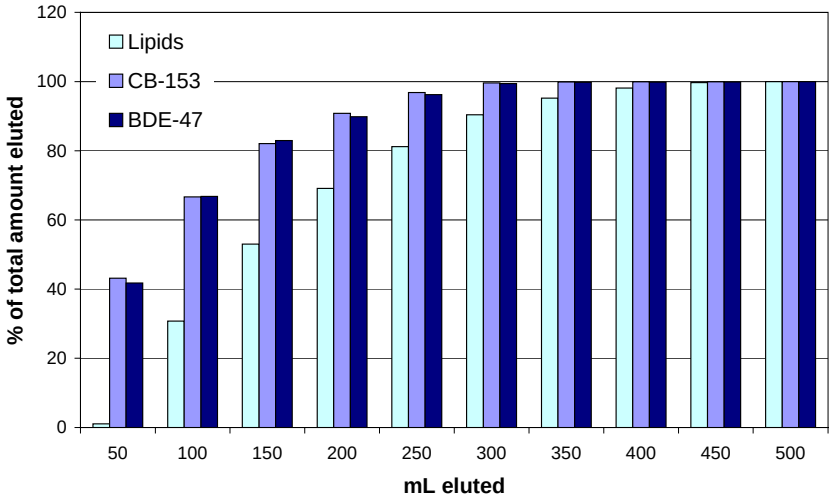

Figure 1. Elution profile of the lipids, CB-153 and BDE-47.

The absolute recoveries of the compounds were calculated using CB-207 as recovery standard, and the accuracy was assessed by the recovery relative to the internal standard. As can be seen from Table 1, all compounds had a recovery above 65% except BDE-183, and the accuracy was above 80% for all compounds. At a spiking level of 5000 pg, most of the compounds had a relative standard deviation (RSD) below 25%. This variation is, in our opinion, acceptable for a manual sample preparation method at this relatively low concentration (0.5 ng/g lipids).

When using electron capture MS (ECMS) as detection mode, the response of the molecular ion of PCBs with few chlorine substituents is low. Thus CB-18, CB-28 and CB-52 could not be detected at the lowest spiking levels, and the response of their corresponding internal standard were below the limit of detection (LOD) at the chosen concentration (1000 pg). Adding a larger amount of these internal standards or using another 13C-CB as internal standard might solve this problem partly. However, the possibility of quantifying these compounds in low contaminated samples using ECMS is nevertheless limited. Hexabromocyclododecane (HBCD) and the decabrominated BDE-209 were also included in the study, but due to the lack of a suitable internal standard, the quantification of these compounds is at present only considered to be semi quantitative.

Table 1. The mean recovery and mean accuracy (recovery with respect to the internal standard) of the PBDEs and PCBs of up to14 samples of 10 g soy oil spiked at four levels (see footnote a). The relative standard deviation (RSD) of the four replicates at spiking level 5000 pg is shown.

Compound	Internal standard	Recovery		Accuracy	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
BDE-28	BDE-18	98	17.3	81	2.8
BDE-37	BDE-18	104	19.0	94	2.0
BDE-47[a]	BDE-51	83	18.4	80	6.2
BDE-85	BDE-103	100	16.7	116	16.0
BDE-99[a]	BDE-103	92	15.9	104	16.2
BDE-100	BDE-103	88	16.8	104	23.6
BDE-119	BDE-103	90	16.6	104	16.6
BDE-138	BDE-156	66	26.5	100	24.4
BDE-153	BDE-103	81	20.4	94	17.4
BDE-154[a]	BDE-103	88	17.4	97	15.7
BDE-183	BDE-181	35	34.4	110	21.8
CB-18[b]	13C-CB-28	104			
CB-28[b]	13C-CB-28	103			
CB-52[b]	13C-CB-52	118			
CB-66[a]	13C-CB-101	84	21.6	102	37.0
CB-74[a]	13C-CB-101	73	19.7	98	3.4
CB-99[a]	13C-CB-101	71	17.8	87	4.2
CB-101[a]	13C-CB-101	83	26.0	119	15.0
CB-105[a]	13C-CB-105	70	20.0	95	1.6
CB-110[a]	13C-CB-101	78	23.8	114	11.7
CB-114	13C-CB-114	69	19.3	91	1.0
CB-118[a]	13C-CB-118	67	21.8	100	6.8
CB-123[a]	13C-CB-123	69	19.3	91	2.5
CB-128[a]	13C-CB-167	57	30.4	89	30.7
CB-138[a]	13C-CB-138	89	28.2	111	12.9
CB-153[a]	13C-CB-153	89	27.9	105	13.2
CB-156	13C-CB-156	72	18.7	97	0.8
CB-157	13C-CB-157	66	18.5	93	0.3
CB-167	13C-CB-167	68	18.3	84	20.1
CB-170	13C-CB-170	85	18.2	96	0.8
CB-180[a]	13C-CB-180	82	18.8	100	2.7
CB-183[a]	13C-CB-180	74	17.3	96	1.7
CB-187[a]	13C-CB-180	79	18.6	100	3.7
CB-189	13C-CB-189	80	18.1	95	0.9
CB-194	13C-CB-194	79	20.0	99	24.4
CB-209	13C-CB-209	89	13.5	98	0.5

[a]This compound were not evaluated at the lowest spiking level because the added amount was too small to be distinguished from the amount already present in the soy oil.
[b]Due to low MS response of the molecular ion, the recovery could only be calculated at the highest spiking level. The responses of the corresponding internal standards were below the LOD and the accuracy could not be assessed.

To further assess the quality of this method, three samples from interlaboratory comparison studies were analysed. For the samples of breast milk and egg yolk, assigned values for the mono-ortho PCBs were available, and the concentration of five PBDEs had been established in the turkey sample[10-12]. For all the mono-ortho PCBs but CB-114, the deviations from the assigned value were below ± 20% in both samples (Table 2). The elevated concentration observed for CB-114 are most probably due to a co-eluting compound, possibly CB-122. This will be investigated further by application of a different GC column. For the PBDEs, the deviations from the assigned value were below ± 22%, except for BDE-183. The assigned value for this compound was, however, only regarded as indicative because the coefficient of variation (CV) was above 70%.

Table 2. **Percentage deviation from the assigned values from interlaboratory comparison studies.**

Compound	Breast milk[a] (% deviation)	Egg yolk[b] (% deviation)	Compound	Turkey[c] (% deviation)
CB-105	3.1	3.0	**BDE-47**	5.7
CB-114	103.6	101.7	**BDE-99**	21.2
CB-118	15.4	12.6	**BDE-100**	-20.4
CB-123	<LOD	<LOD	**BDE-153**	-8.4
CB-156	11.1	9.9	**BDE-154**	8.2
CB-157	2.3	11.1	**BDE-183**	-76.5
CB-167	-8.2	-10.9		
CB-189	15.9	19.3		

[a]Interlaboratory comparison on dioxins in food 2001[10]
[b]Interlaboratory comparison on dioxins in food 2002[11]
[c]The first FIRE intercomparison study[12]

Up to now, we have used this method for determination of the full spectre of compounds in breast milk and several different foodstuff, such as egg, cheese, butter, vegetable oils and meat of chicken, sheep, reindeer, beef, pork. No practical problems have occurred, but for some matrixes an additional clean-up on sulphuric acid-silica has been required.

To summarise, this simple extension of our existing sample preparation method for analysis of PCDDs/PCDFs and non-ortho PCBs has been shown to be suitable for determination of PCBs and PBDEs. The procedure is applicable for sample sizes containing up to 10 g fat. The approach of determining several POPs from the same sample is especially useful in cases when limited sample is available. Also, comparable concentrations on lipid weight basis are assured, and problems due to inhomogeneity of sample aliquots are avoided.

References

1 Baker J.I. and Hites R.A. (2000) Environ. Sci. Technol. 34, 2887.
2 Becher G., Haug L.S., Nicolaysen T., Polder A. and Skaare J.U. (2002) Organohalogen Comp. 56, 325.
3 Norén K. and Meironyté D. (2000) Chemosphere 40, 111.
4 Freijer J.I., Hoogerbrugge R., van Klaveren J.D., Traag W.A., Hoogenboom L.A.P. and Liem A.K.D. (2001) RIVM report 639102 022, National Institute for Public Health and the Environment, Bilthoven, The Netherlands, p. 109.
5 de Wit C.A. (2002) Chemosphere 46, 583.
6 Hites R.A. (2004) Environ. Sci. Technol. 38, 945.
7 Sporring S., Wiberg K., Björklund E. and Haglund P. (2003) Organohalogen Comp. 60, 1.
8 Pirard C., Focant J.F. and De Pauw E. (2002) Anal. Bioanal. Chem. 372, 373.
9 Smith M., Stalling D.L., Johnson J.L. (1984) Anal. Chem. 56, 1830.
10 Becher G., Lindström G., Nicolaysen T., Thomsen C. (2001) Interlaboratory comparison on dioxins in food. Report 2001:4, National Institute of Public Health, Oslo, Norway, ISBN 82-7364-178-3
11 Haug L.S., Nicolaysen T., Thomsen C., Frøshaug M., Becher G. (2002) Interlaboratory comparison on dioxins in food 2002. Report 2002:4, Norwegian Institute of Public Health, Oslo, Norway, ISBN 82-8082-016-7
12 Draft report on the first FIRE intercomparison study on brominated flame retardants, 2004.