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Automated Clean-up of Various Brominated Compounds: a Breast Milk Example

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1. Introduction

Polybrominated diphenyl ethers (**PBDE**) are almost omnipresent persistent contaminants of the environment and subsequently food and feed. Due to their physico-chemical properties, they have been used as flame retardants. Different to other persistent organic pollutants (POPs) like dioxins and PCBs, overall PBDE concentrations have been increasing within the last years.

Historically, the use of PBDE as flame retardants has tremendously increased since the early 1970s but their commercial production and use of PBDE mixtures has been banned in the EU since 2004 by the EU Restriction of Hazardous Substances (RoHS). In 2008 the use of BDE-209 in electronics was stopped. Although, the ban on PBDE and other brominated flame retardants (BFR) has been in place for some years, the continuous use of brominated substitutes as well as the gradual disposal of old products containing BFRs ensure a significant source of these contaminants for the future years.

The global material cycle inevitably spreads PBDE and other brominated compounds into the environment. This subsequently leads to human consumption and intoxication of liver, thyroid hormone homeostasis, as well as the reproductive and nervous system as mentioned in several mostly medical studies. Moreover, many questions regarding ecotoxicological relevance have not been answered yet and, therewith, strengthen the neccessity of further research on the global impact of PBDEs.

According to the 2011 European Food Safety Authority (EFSA) report milk and dairy products belong to the most contaminated food categories, yet, also baby food contains a vast variety of ingredients of different origins and is under extraordinary control. Although, the eight BDE-congeners are of interest, only four of them derived a benchmark dose by EFSA. As PBDD/Fs, the brominated counterparts of PCDD/F, can be subsequent decomposition products of PBDEs further surveillance is urgently required.

In general, breastmilk studies on organochlorine substances have been regressing. This tendency reflects the widely spread approach: These studies are unnecessary and contraproductive concerning health political ideas of extended breastfeeding.
Because of this reason, the intention of Prof. Frommes project was to start a large campagne for breast milk analysis as instrument of preventive health protection. Besides meaningful dioxin-like PCBs and established "Standard-PCBs", the PBDEs, phthalates, and perflourinated substances form the third pillar as relatively new substances classes.

Swedish breastmilk studies showed a significant increase of flame retardants between 1972 and 1997. Globally, similar study approaches are rare. Thus, a project study on DEXTech Plus was the ideal way to gain more data.

Besides the analytical questions of accuracy and precision, also economic factors e.g. working time, solvent consumptions and consumables are important aspects in daily laboratory work. Hence, a reliable but cheap method covering a wide-range of matrices had to be developed, to analyse a broad variety of PBDE congeners and other brominated compounds simultaneously to PCB, PCDD/F but also PBDD/F compounds.

2. Method Development

The first step of developing an automated sample preparation system used a classical four column set-up (multilayer sulphuric acid column, Florisil® column and two activated carbon columns) to clean-up PBDEs in addition to PCDD/F and PCBs as presented by Bernsmann and colleagues in Madrid 2014. Although, all chlorinated compounds were cleaned-up adequately, the approach showed a rather insufficient performance for brominated molecules.

A replacement of the Florisil® column by aluminium oxide was tested. As the results for brominated substances improved, the aluminium oxide column method marked the starting point to reduce the system technically to a three-column approach as shown in figure 1. This reduction of one column lead to a variety of advantages. Most importantly, high quality results were achieved, yet, the run-time as well as the solvent consumption were minimized. This automatically made the method more cost saving, too.

2.1 Reagents and Materials

- PBDE-standards (EO-5320-A, LGC, Wesel, Germany)
- Basic alumina B for dioxin analysis
- Universal columns for dioxin analysis, alumina clean-up glass columns and carbon clean-up columns (LCTech, Obertaufkirchen, Germany)

Figure 1: Universal, Aluminium-oxide and Carbon columns used for the experiments

2.2 Sample Clean-up

Automated sample clean-up was done by using a DEXTech Plus device (figure 2). The automated clean-up method was originally developed to analyse polychlorinated dioxins and furans (PCDD/Fs) as well as polychlorinated biphenyls (PCBs) in food and feed. Besides mono-ortho- and ndl-PCBs, PBDEs are eluted in fraction 1 by a n-hexane/dichloromethane-mixture (1/1, v/v). (figure 3). Recovery standards were added to extracts for analysing by GC-HRMS [see figures 4 (^{13}C-labelled PBDE-Standard) and 5 (native PBDEs)]. The second fraction containing non-ortho PCBs as well as PCDD/Fs and PBDD/Fs (figure 3) was only partly taken into account for this study.

Figure 2: Automated clean-up system DEXTech Plus

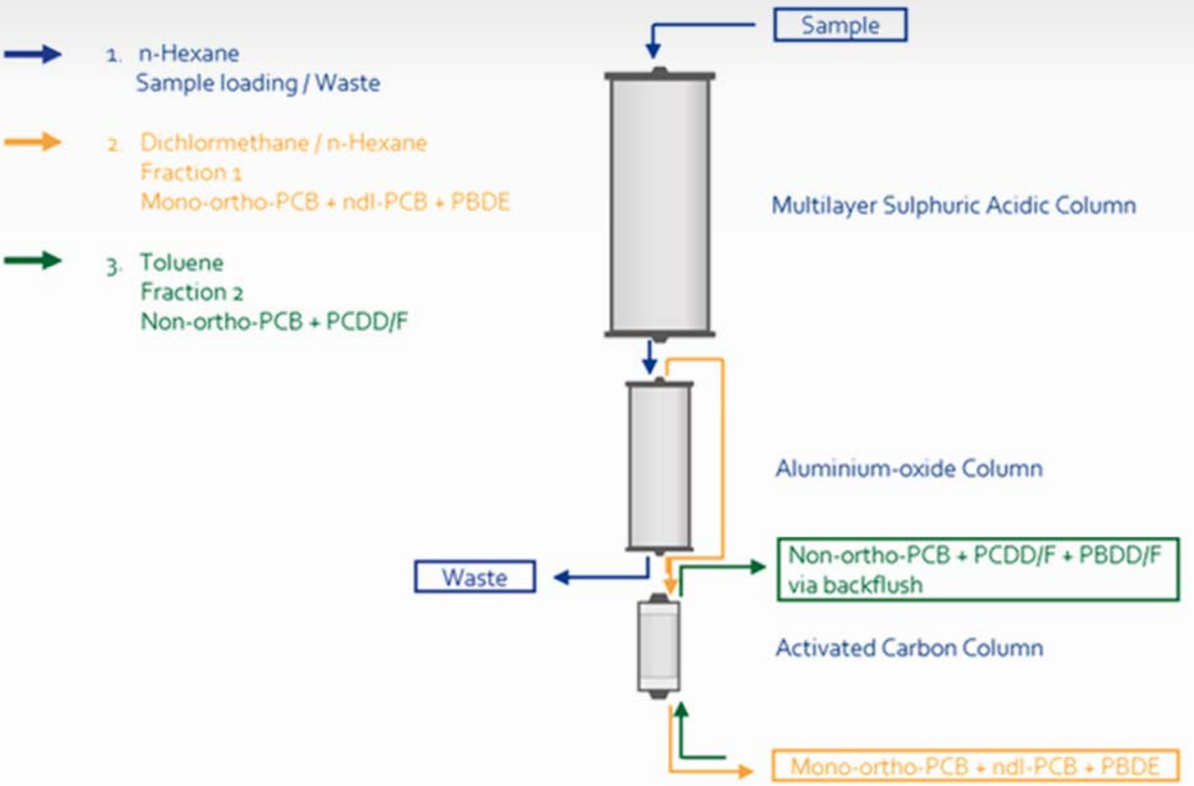

Figure 3: Flowpath of the DEXTech Plus.

The sample is loaded with hexane on the alumina column after lipid degradation on the acidic silica column. Degradation products go to waste while interesting compounds are trapped on the alumina column. Mono-ortho-, non-dioxine-like-PCBs, and PBDEs are separated from the planar compounds and collected as Fraction 1 by a 1:1 mixture of dichloromethane and n-hexane. PCDD/F and coplanar-PCBs are retained on the top of the carbon column and collected in backflush elution with toluene.

2.2.1 Software Protocol

DEXTech Plus provides two default methods for the aluminium oxide column, one using the Universal the second using the SMART column (for samples with > 1.5 g fat content). Additionaly the user can freely parameterise methods and save them in the system.

In this case, the default method with the Universal column (column 1) was used together with the aluminium oxide column (column 2).

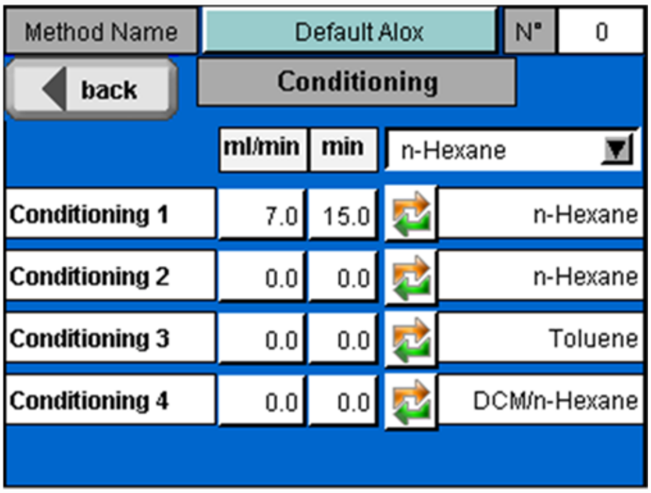

Figure 4: Parameters for the conditioning steps (Default Alox Method)

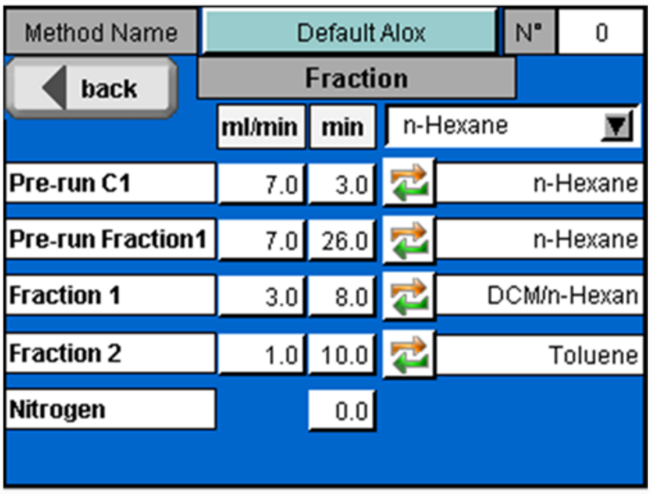

Figure 5: Parameters for the fractioning (Default Alox Method)

2.2.2 Fractions, Process and Solvent Management Parameters

Fractionation:
Fraction 1: ndl-PCBs, mono-ortho PCBs, PBDEs
Fraction 2: non-orthos PCBs, PCDD/Fs, PBDD/Fs

Process time: 65 min.
The process time includes the loading of 10 mL sample as well as the rinsing of the sample vial with 3 x 1 mL solvent.

Solvent consumption:
n-Hexane: 308 mL
n-Hexane /DCM: 24 mL
Toluene: 10 mL
Total: 342 mL

2.3 Instrumentation

A Thermo DFS 2-GC/HRMS in EI+ mode with MID at resolution 10,000 was applied for measuring all fractions, using FC 5311 as internal mass reference. One GC was fitted with two columns (Rtx-Dioxin2, 60 m x 0.25 mm x 0.25 µm to analyse PCDD/Fs, non-ortho-PCBs and PCB-11 as well as DB-5ms, 15 m x 0.2 mm x 0.1 µm for PBDEs and PBDD/Fs). The other GC was used for measuring mono-ortho- and ndl-PCBs with one column (SGE-HT8-PCB, 60 m x 0.25 mm). All separation columns are provided with a 5 m deactivated guard columns with next higher diameter to ensure better evaporation, to avoid contamination of the separation columns and to maintain retention times. The MS-source had the inlet of three columns all the time. If one column is active, both others are in a stand-by-mode with less carrier gas flow. All injectors are PTVs.

PBDEs and PBDD/Fs were separated on a short column (15 m), to reduce risk of on column degradation of higher brominated diphenylethers, with a thin film (0.1 µm) to obtain sufficient separation. The PTV-injector is also essential to avoid degradation especially of higher brominated diphenylethers. As most intensive ion M+ is used for Tri- to Penta-BDE and [M-2Br]+ for Hexa- to Deca-BDE as a function of GC temperature. It must be pointed out that the blank values of PCB-11 have to be subtracted from the measured sample-values.

2.3.1 Quality Control

In general each sample is spiked with $^{13}C12$-labeled internal standard solutions at the beginning of the fat-clean-up. At the end of the sample preparation a $^{13}C12$-labeled recovery standard solutions was added to determine the recoveries for each congener. According to Commission Regulation (EU) 2017/644 [7] recoveries were in the screening range of 30 to 140%, almost all of them in the confirmatory range of 60 to 120%. To ensure correct measure conditions a diluted calibration solution was embedded in every sequence.

2.3.2 LOD/LOQ

LOD level was defined as S/N 3:1 and LOQ as S/N 10:1 and automatically determined by Thermo TargetQuan software.

3. Results

The complete approach was run with various samples and matrices. Besides the results for breastmilk preparation and analysis in this study also bovine milk and infant food was tested in another study (Bernsmann et al., unpublished data) focussing on matrix-specific difficulties and subsequent overall comparability of achieved results. Looking at breast milk, the recoveries of PBDEs we obtained are comparable to the recoveries for PCBs and PBDD/Fs (table 1).

Table 1: Average percentage of recoveries from all internal standard congeners over five breast milk samples from one analytic series determined in each particular DEXTech Plus-fraction.

DEXTech-Fraction	mono-ortho-PCB	recovery [%]	ndl-PCB	recovery [%]	PBDE	recovery [%]	DEXTech-Fraction	PCDD	recovery [%]	PCDF	recovery [%]	PBDD	recovery [%]
1	PCB 123	84	PCB 28	62	BDE-28	57	2	2,3,7,8-TCDD	72	2,3,7,8-TCDF	84	2,3,7,8-TBDD	89
	PCB 118	79	PCB 52	68	BDE-47	72		1,2,3,7,8-PeCDD	69	1,2,3,7,8-PeCDF	73	1,2,3,7,8-PeBDD	82
	PCB 114	83	PCB 101	70	BDE-100	66		1,2,3,4,7,8-HxCDD	84	2,3,4,7,8-PeCDF	77	1,2,3,4,7,8-HxBDD	92
	PCB 105	84	PCB 153	74	BDE-99	70		1,2,3,6,7,8-HxCDD	88	1,2,3,4,7,8-HxCDF	90	1,2,3,6,7,8-HxBDD	92
	PCB 167	82	PCB 138	71	BDE-154	68		1,2,3,4,6,7,8-HpCDD	99	1,2,3,6,7,8-HxCDF	92	1,2,3,4,6,7,8-HpBDD	122
	PCB 156	84	PCB 180	87	BDE-153	75		OCDD	101	2,3,4,6,7,8-HxCDF	90	OBDD	144
	PCB 157	80			BDE-183	98				1,2,3,7,8,9-HxCDF	92		
	PCB 189	90			BDE-197	99				1,2,3,4,6,7,8-HpCDF	93		
					BDE-203	97				1,2,3,4,7,8,9-HpCDF	100		
					BDE-208	82		non-ortho-PCB	recovery [%]	lower chlorinated	recovery [%]	PBDF	recovery [%]
					BDE-207	76		PCB 81	103	PCB 11	40	2,3,7,8-TBDF	82
					BDE-206	97		PCB 77	103			2,3,4,7,8-PeBDF	84
					BDE-209	35		PCB 126	120			1,2,3,4,7,8-HxBDF	91
								PCB 169	134			1,2,3,4,6,7,8-HpBDF	127
												OBDF	26

Moreover, also lower chlorinated PCBs as 3,3-Dichlorobiphenyl (PCB11) were recovered in a range suitable within the screening level. Figures 6 to 7 show distinct peaks for spiked internal standards but also for native congeners. Besides theses sharp chromatograms for PBDEs, the framed HRGC/MS-chromatogram (figure 8) shows excellent results for tetra-, penta-, hexa-, hepta- and octa-brominated dibenzofurans and –dioxins. All three figures proof the cleanliness of the sample fractions after clean-up, thus, successful implementation of the aluminium oxide column into the three column approach of the DEXTech Plus system. In summary, this instrumental and methodical set-up allows to provide a tool for analysis of brominated flame retardants PBDE and their thermal decomposition products PBDD/F in addition to chlorinated compounds PCDD/F, dl- and ndl-PCB for measurement via HRMS.

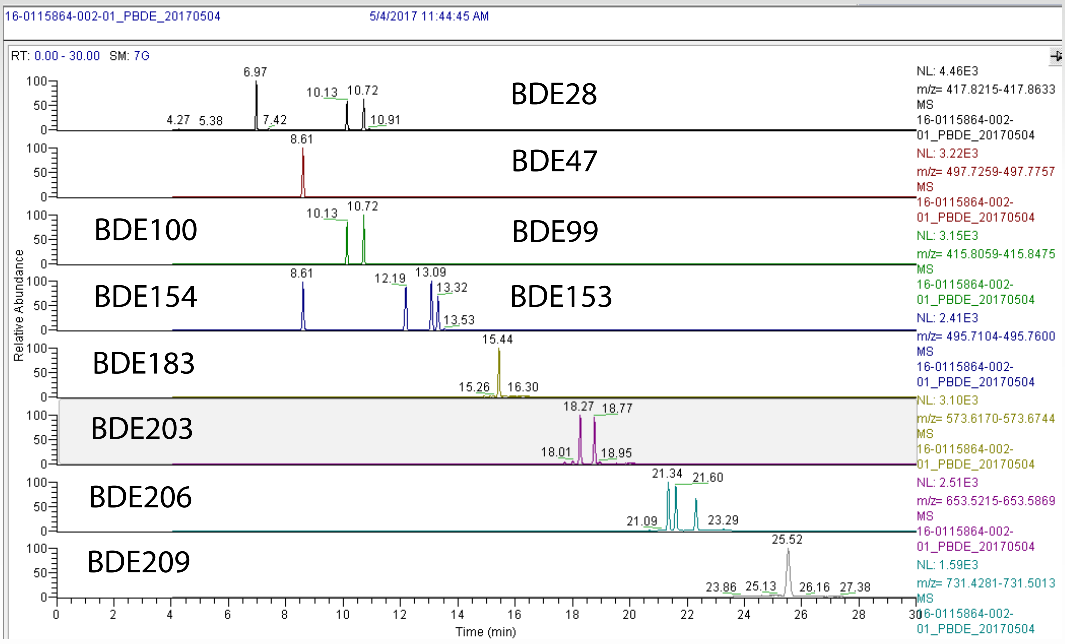

Figure 6: HRMS-chromatogram of internal PBDE-standards

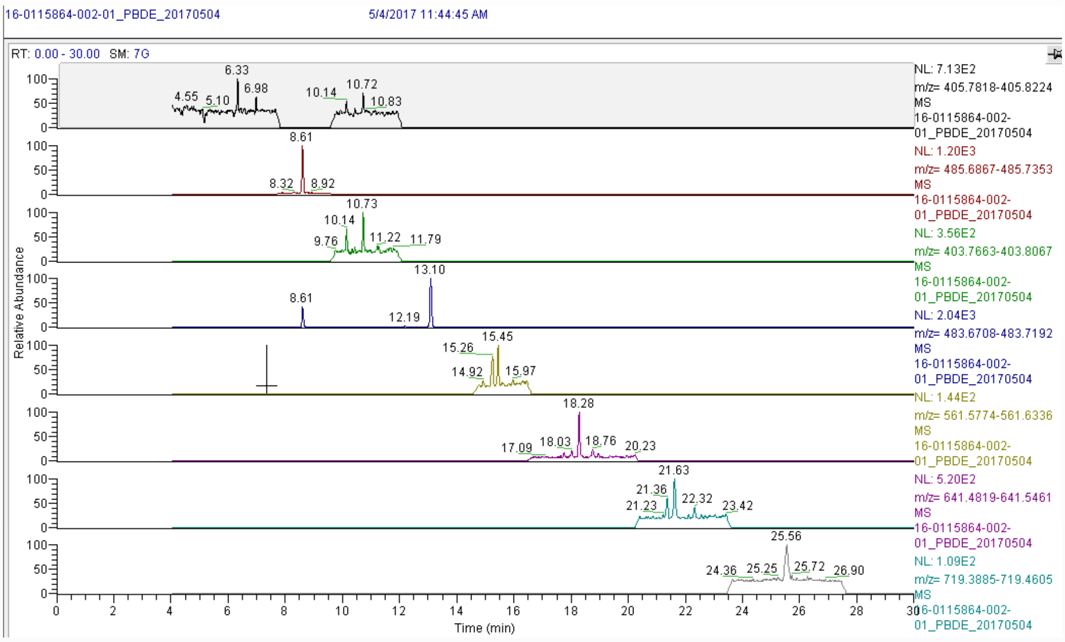

Figure 7: HRMS-chromatogram of native PBDE-congeners in one particular breast milk sample

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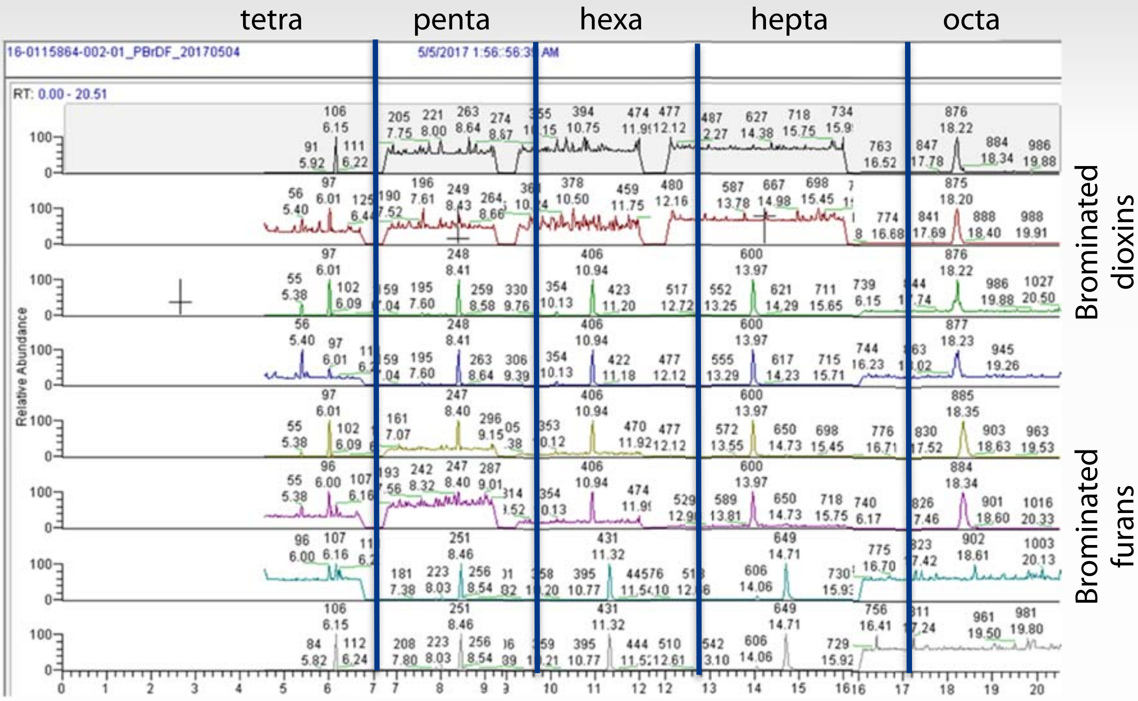

Figure 8: Framed PBDD/F-chromatogram of HRMS-measurement with 5 recorded functions

4. Related Information and References

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