Clean-up method for the detection of polybrominated diphenylethers (PBDE) in food and human biomonitoring samples

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Introduction

Brominated flame retardants (BFRs) are organic compounds, which are added to products in order to reduce the combustibility of the material. BFRs are applied in diverse consumer goods (e.g. electronic devices, furniture, etc.). As polybromianted diphenylethers (PBDEs) can be released e.g. by leaching and consumer goods are disposed of at the end of their lifecycle, these agents may contaminate environment and enter the food chain over time.

A main class of flame retardants are PBDEs which are persistent and lipophilic, and therefore bioaccumulative. Acute toxicity of PBDEs is low but they are suspected to cause neurological deficits and disorders of hormonal system [1]. PBDEs have an ecotoxicological potential and are detected in various environmental compartments [2].

According to the Recommendation 2014/118/EU member states should monitor brominated flame retardants in food [3].

Material and Method

Chemicals and Standards: Solvents and PBDE-Standards (EO-5320-A) was purchased at LGC (Wesel, Germany). Basic aluminia B for dioxin analysis (Cat.No. 04569) was delivered by MP Biomedicals (Eschwege, Germany). Colums for DEXTechTM device (Silica gel with sulfuric acid, florisil and active carbon) were provided by LCTech (Dorfen, Germany).

Extraction: Due to their lipophilicity the determination of PBDE besides PCDD/F and PCB started with fat extraction. For matrices like fish it could be done quite unproblematic with cold extraction by organic solvents. For this purpose, freeze-dried fish was mixed with sodium sulfate, filled in a column and extracted using hexane/acetone (2:1, v:v) In human plasma fat is embedded in lipoprotein particles, thus particles have to be broken up to get fat fraction. For this purpose, solvent extraction under pressure and elevated temperature (PSE; Buchi Speed Extractor^{TM}) was used successfully [4]. Due to the thermal instability, especially of higher brominated diphenylethers, attention has to be payed to the recovery rates, in particular for the widely used deca-BDE. Freeze-dried plasma sample was mixed with Kieselguhr and filled in the extraction cells. After addition of internal standards, two extraction cycles of 5 min hold time were carried out at 100 °C and 100 bar. Extracts were concentrated using rotary evaporator and concluding nitrogen flow.

Clean-up: Automated sample preparation (fish and plasma) was done by using DEXTech^{TM} device (see figure 1). The automated clean-up method was originally developed for polychlorinated dioxins and furanes (PCDD/Fs) as well as polychlorinated biphenyls (PCBs) [5]. Besides mono-ortho- and ndl-PCBs PBDEs are eluted in pear shape flask 1, wherefore this fraction was analyzed for PBDEs. However, severe signal suppression, in particular for higher brominated diphenylethers, was observed (see figures 2a ($^{13}C_{12}$-labelled PBDE-Standard) and 2b (native PBDE)). Therefore, this fraction of the DEXTech^{TM}-clean-up was subsequently purified manually through an alumina column. Highly concentrated mono-ortho-

and ndl-PCBs were separated by n-Hexane/Dichloromethane-mixture (98/2, v/v) and by second step PBDEs were eluated with n-Hexane/Dichloromethane-mixture (1/1, v/v) from the alumina column (see figures 3a ($^{13}C_{12}$-labelled PBDE-Standard) and 3b (native PBDE)). In both undermost lines of the mass tracks a singular peak of decaBDE is visible.

Recovery standards were added to extracs for analyzing by GC-HRMS.

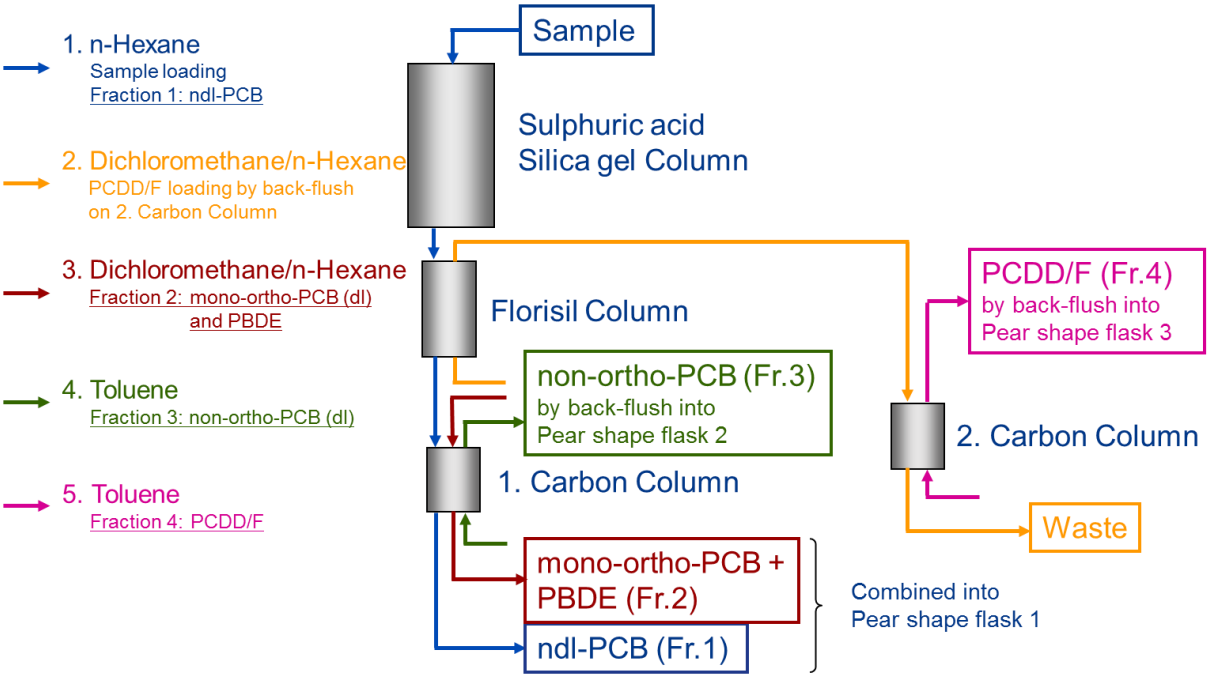

Figure 1: Flow Scheme Clean-up by DEXTech^{TM} device

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 (VF-5ms, 60 m x 0,25 mm x 0,25 µm to analyse PCDD/Fs and non-ortho-PCBs and DB-5ms, 15 m x 0,2 mm x 0,1 µm for PBDEs). The other GC is used for measuring mono-ortho- and ndl-PCBs with one column (SGE-HT8-PCB, 60 m x 0,25 mm). 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.

Quantification: PBDEs were separated on a short column (15 m), to reduce risk of on column degradation of higher brominated diphenylethers, with 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 $[M\text{-}2Br]^+$ for Hexa- to Deca-BDE as a function of GC temperature [6]. It must be pointed out that the blank values of the individual BDEs, in particular the highly concentrated deca-BDE have to be substracted from the measured sample-values.

Quality control: In general each sample is spiked with $^{13}C_{12}$-labeled internal standard solutions at the beginning of the accelerate solvent extraction (blood serum) or clean-up (fat). At the end of the clean-up a $^{13}C_{12}$-labeled recovery standard solutions were added to determine the recoveries for each congenere. According to Commission Regulation (EU) No 589/2014 [7] recoveries were in the range of 50 to 125%. To ensure correct measure conditions a diluted calibration solution was embedded in every sequence.

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

Results and discussion

Combination of automated clean-up and manually alumina column resulted in adequate clean extracts which could be properly analyzed for PBDEs (see figures 3a, $^{13}C_{12}$-labelled PBDE and 3b, native PBDE). Comparison of internal standards of sample extracts and calibration solutions showed further that the degradation, especially of the thermo-labile deca-BDE (degradation to nona- and octa-BDE), was not as pronounced as suspected. The detection of the only low content of degradation products compared to the much more concentrated deca-BDE illustrated the robustness of this method for the determination of PBDEs. Attention should be paid to the non insignificant blank values of the individual BDEs, in particular the highly concentrated deca-BDE. To receive the real PBDE-concentrations the blank-values have to be substracted from the measured sample-values. For a better presentation of the total PBDE-burden the individual concentrations, measured in ng/g fat, are summarized to a sum concentration.

References

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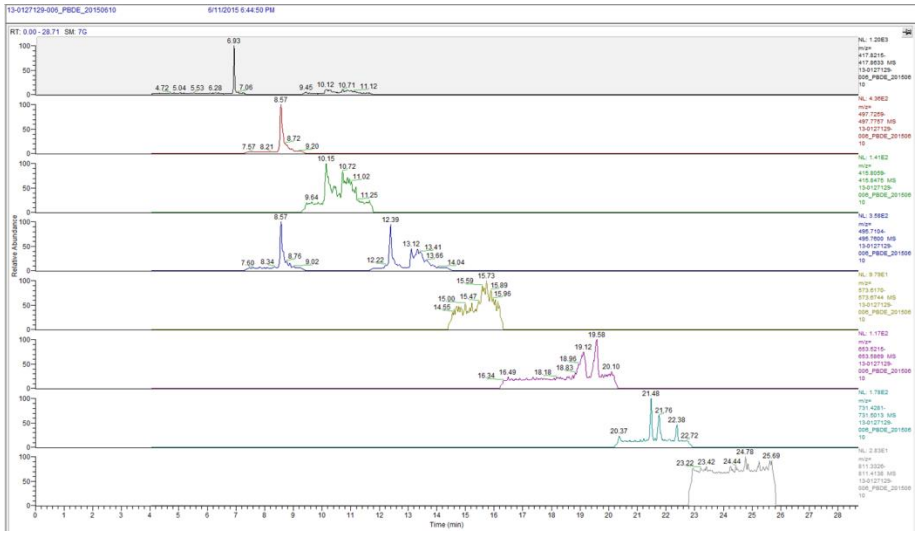

Figure 2a: **DEXTech-pear shape flask-1**_$^{13}C_{12}$-PBDE-Standard

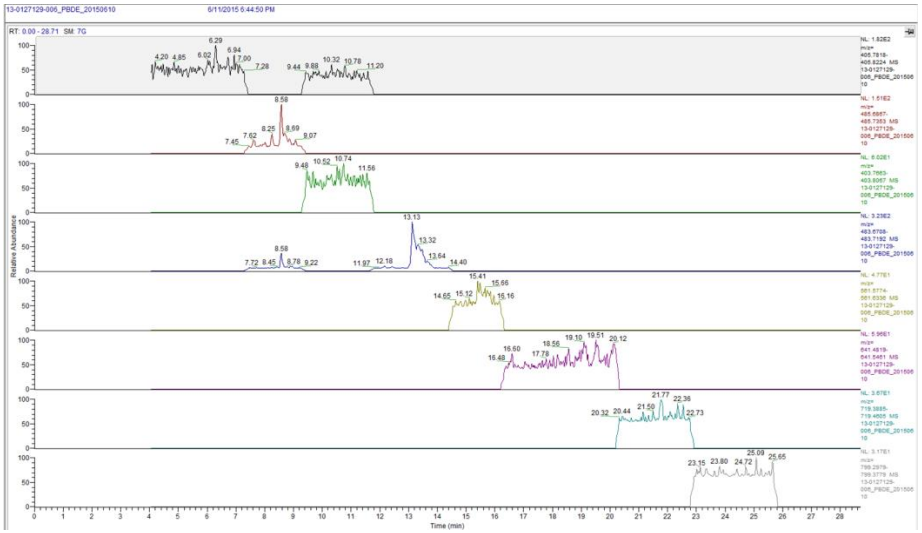

Figure 2b: **DEXTech-pear shape flask-1**_native-PBDE

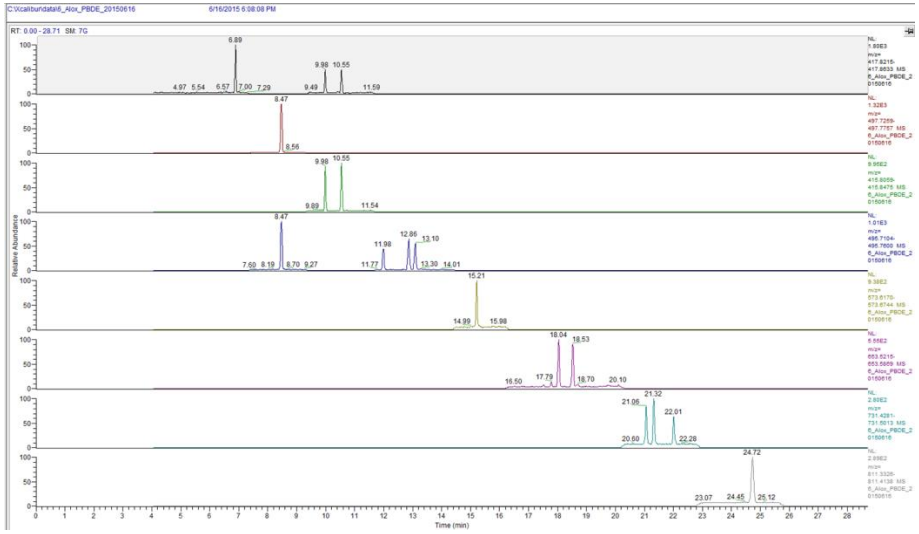

Figure 3a: $^{13}C_{12}$-PBDE-Standard after **Separation on aluminia column**

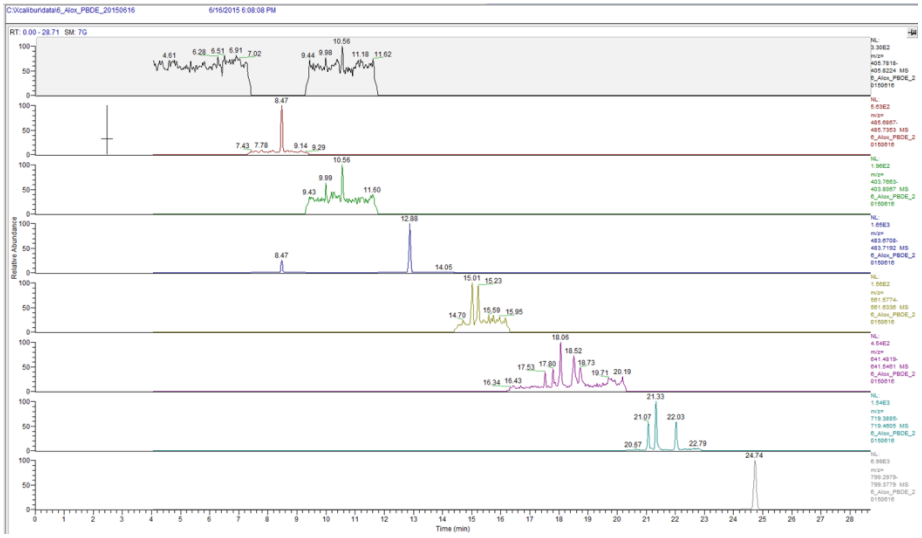

Figure 3b: native-PBDE after **Separation on aluminia column**