A Novel Cleanup Procedure for Determining Mono- to Deca-BDE in Lipophilic Matrices

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Introduction

Although polybrominated biphenyl ethers (PBDEs) are actively studied as pollutants in the environment and in biological objects for the last 15 years, universally accepted approaches for the PBDE analysis are still lacking.
PBDEs being similar in structure to polychlorinated biphenyls (PCBs), a certain similarity in their analytical chemistry might be expected. However, this is only true for medium-brominated compounds, which are the most popular objects of PBDE analysis [1]. Highly brominated congeners, including deca-BDE, are not always detected, and low-brominated PBDEs even less so. Wherever the feasibility of determining these compounds is discussed in the literature, the authors recognize the problem of recoveries and tend to omit mono-BDEs [2,3]. The problem of deca-BDE can usually be solved by analysis on a short DB-5ht column. Analytical methods for low-brominated congeners are even less developed.
The USEPA 1614 Method has no recoveries criteria for mono- and di-BDEs. The Method E3481 of Ontario Ministry of the Environment and Climate Change allows for the determination of tri- to deca-BDE using the Power-Prep™ automated sample preparation system manufactured by Fluid Management Systems, Inc.
When analyzing a sample of fish meal on a TotalPowerPrep™ system according to the procedure proposed by the manufacturer's official representatives, we obtained 44-77% recovery for medium brominated BDE, there was no monoBDE in the extract, and the recovery for di-BDE did not exceed 15%, with poor reproducibility. Our initial attempts to optimize the elution conditions failed and led to illogical results. A similar problem was described by other FMS customers several years ago [4].
The purpose of this work was to determine the cause of low-brominated PBDEs losses and develop a viable method for determining mono- to decabromobiphenyl ethers in fat-containing food and feedstuff matrices.

Material and methods

The analyses were performed on a Thermo TSQ8000Evo/Trace1310GC triple quad system in MS/MS mode, Thermo TR-5MS column. Heavy brominated BDE analysis and confirmatory test were done on a Waters AutoSpec Premier. $^{13}C_{12}$-labeled and calibration standards (MBDE-MXG, MBDE-ISS-G and BFR-PAR) were purchased from Wellington Laboratories (Ontario, Canada).
The following sorbents were used: Aluminum oxide, basic, Brockman I (Sigma-Aldrich); Aluminum oxide activated, neutral, Brockmann I (Sigma-Aldrich); Aluminum oxide, Type WN-6, Neutral, Activity Grade Super I (Sigma-Aldrich); Florisil (0.150-0.250) (Merck); Florisil PR (Merck); silica gel impregnated by sulfuric acid and potassium silicate were prepared from Silica gel 60 (0.063-0.100 mm) (Merck). Solvents were purchased from various suppliers and tested for critical interferences.

Results and discussion

In our opinion, irreversible sorption of mono- and di-BDE on silica gel or aluminum oxide is less likely to occur than chemical transformations of these substances during sample preparation. If it is true, the basic principle of constructing an analytical method - the absence of chemical reactions between the target substances and the reagents used for their separation from the matrix – could be compromised.

First, we checked the stability of the available isotopically labeled PBDE when passing through sulfuric-acid impregnated silica gel or potassium silicate at room temperature and at 85°C in an ASE 200 (Dionex) extractor. No significant losses were observed.

Next, we tested basic aluminum oxide (4g, activated at 600°C overnight) with sequential elution by hexane (20 ml), hexane:DCM (19:1 v:v, 20 ml) and hexane:DCM (2:3 v:v, 50 ml). Mono- and di-BDEs were lost, other standards were distributed between the last two fractions. These results were even worse than on FMS system.

Then we checked the Florisil PR (2g, 180°C overnight). Using the same elution systems as above, we found the standards present in all fractions, which means that, although there were no signs of the loss of standards, the use of Florisil PR in PBDEs analysis is questionable.

When we used non graded Florisil (activated at 180°C overnight and at 675°C 24 h) with elution by 30 ml hexane, 25 ml hexane:DCM (1:3 v:v) and 40 ml DCM, there was a significant breakthrough of PBDEs to the hexane fraction in the first system and almost complete breakthrough in the second one. Thus, this sorbent is not suitable for PBDEs sorption from the solution, but it can be used to remove fat and other matrix components.

We also tested neutral aluminum oxide, which is not listed by EPA methods for PCBs and PBDEs, but we knew it to be efficient in the PAH analysis. Initial experiments (4 g 400°C overnight) showed excellent results, we did not observe any breakthrough during hexane washing, and all standards were eluted quantitatively with 20 ml hexane:DCM (1:3 v:v). Unfortunately, later the loss of mono- and diBDE was again observed, and even elution with pure toluene or dichloromethane could not solve this problem. We could not find an explanation of this phenomenon, but it disappeared on decreasing the activation temperature to 200°C.

Fractionation of samples is an important tool of fine clean-up, but non-selective sorption is rarely suitable for the quantitative separation of microcomponents from the main matrix components. The combination of chemical and sorption clean-up is usually more effective.

Combining the chemical treatment on Dionex ASE 200 (in-cell clean-up; layers from bottom to top - 1 g Florisil, 1 g Na_2SO_4, 2g K_2SiO_3, 1 g Na_2SO_4, 10 g H_2SO_4/SiO_2, 2 g Na_2SO_4, 1 g Na_2SO_4, 10g H_2SO_4/SiO_2, 1 g Florisil; extraction with ~ 50 ml hexane; 100°C; 5 cycles 2 min) and subsequent fractionation on neutral alumina (4g 400°C) gave a consistently good quality for different fat matrixes. The entire procedure took about 1.5 hours and required less than 100 ml of solvents for ~0,5 g of fat. Recovery for BDE-3 was about 25%, diBDE - 50%, and near 100% for the other congeners. These values are much better than what we got on the FMS, but still did not meet our set goals for the analysis.

We avoided analyte loses and achieved acceptable quality of purification by passing the samples sequentially through columns filled with potassium silicate, non-activated Florisil and neutral alumina. The columns were conditioned with 25 ml of hexane, then 0.5 g of extracted fat in 5 ml of hexane was introduced, washed with 40 ml of hexane, the first two columns were disconnected, the last 15 ml of hexane was washed, and PBDEs eluted by 25 ml hexane:DCM (1:3 v:v). The extract was colorless and could be concentrated to about 10µl without visible signs of fat deposition. Chromatographic peaks did not show distortions characteristic of poor cleaning. However, the calculation of the extraction rates gave paradoxical results: recoveries for BDE-3 and BDE-28 exceeded 130%, dropped to 50-60% for BDE-15 and BDE-99/100/126 and were about 80-95% for the other PBDEs. A total ion chromatogram of this sample obtained using the same GC program contained several large peaks that coeluted with target substances, which could

lead to a local loss of sensitivity. We verified this assumption by passing the same extract through a small layer of H_2SO_4/SiO_2 in a Pasteur pipette (~ 0.1g), after which the recovery was about 90%.

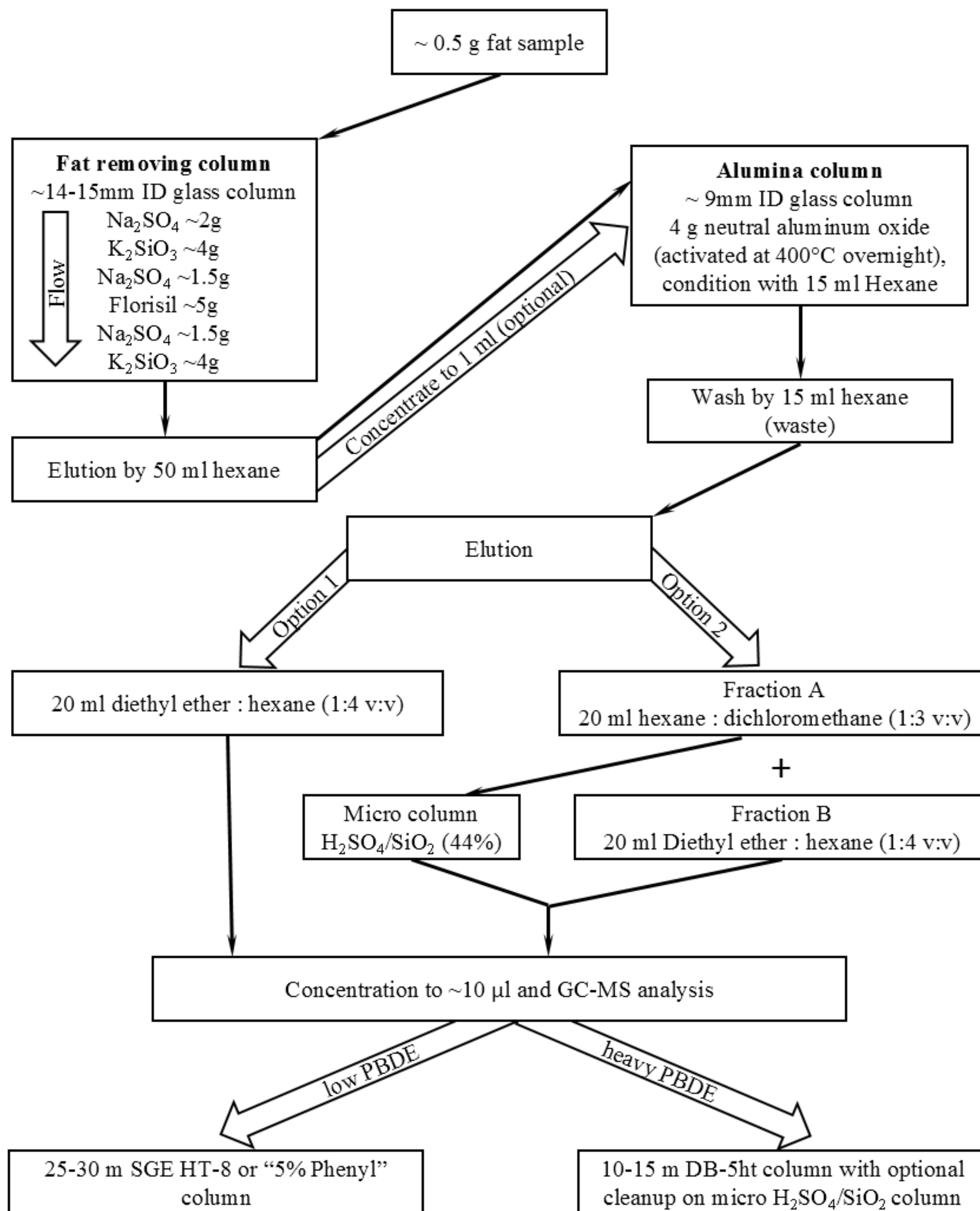

Fig. 1. A flow chart for mono- to deca-BDEs clean-up in the analysis of lipophilic matrices

At the final stage of this study, we found that the observed loss of mono- and di- BDEs on neutral alumina activated at 400°C was not a decomposition, as these substances could be quantitatively eluted by methanol or diethyl ether. Methanol was deemed unsuitable as it dissolves alumina. Finally, a mixture of diethyl ether: hexane (1:4 v:v) was found to provide quantitative elution of PBDEs. Under sequential elution by 20 ml hexane:DCM (1:3 v:v) and 20 ml diethyl ether: hexane (1:4 v:v), the last fraction contained all mono- and di-BDEs and small amounts of tri-BDEs, but much less residual components of the matrix. Thus we get a tool for deeper cleanup. Our final sample preparation method can be realized in glassware with a gravitational solvent flow (Fig. 1) or on custom filled columns for FMS system. Taking into account the columns preparation time, the process times in both cases is similar. The cleanup quality following our method did not significantly differ from that obtained with a FMS system. However, solvents consumption in our method is at least 4 times lower than in the original FMS method, and we obtained at least 80% recovery for all PBDE congeners (table 1).

Table 1: PBDE concentrations and recoveries in fish oil sample

	Aliquot A		Aliquot B		Aliquot C		RSD,%	
	pg/g	*recovery*	pg/g	*recovery*	pg/g	*recovery*	conc.	*recovery*
BDE-3	<DL	*84%*	<DL	*87%*	<DL	*87%*		*1.9*
BDE-15	6.1	*101%*	5.9	*92%*	5.1	*92%*	9.1	*5.6*
BDE-28	69	*108%*	69	*102%*	70	*100%*	0.8	*3.8*
BDE-47	569	*105%*	572	*100%*	574	*98%*	0.4	*3.5*
BDE-79*	82	---	89	---	97	---	8.9	---
BDE-100	158	*97%*	166	*91%*	161	*89%*	2.5	*4.7*
BDE-99	227	*88%*	242	*85%*	223	*83%*	4.5	*3.0*
BDE-126		*102%*		*102%*		*88%*		*8.8*
BDE-154	177	*97%*	189	*98%*	205	*96%*	7.7	*0.9*
BDE-153	56	*121%*	54	*118%*	53	*113%*	2.9	*3.3*
BDE-138*	<DL	---	<DL	---	<DL	---		---
BDE-183	6.1	*115%*	6.1	*98%*	7.0	*93%*	8.8	*11.5*
BDE-197	<DL	*91%*	<DL	*89%*	<DL	*84%*		*3.8*
BDE-207	<DL	*101%*	<DL	*96%*	<DL	*92%*		*4.6*
BDE-206*	<DL	---	<DL	---	<DL	---		---
BDE-209	<DL	*86%*	<DL	*91%*	<DL	*99%*		*6.8*

*) recovery standards

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