Multi-residue Analysis of Phosphate and Brominated Flame Retardants in Food Matrices Using Ultrasonication and vacuum assisted extraction (UVAE) and GC-MS

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Introduction

Flame retardants (FRs) such as polybrominated diphenyl ethers (PBDEs), have been used for decades as additives to reduce the fire risk of furniture, electronics, foams, building materials, vehicles, textiles etc. Recent bans and restrictions in the use of PBDEs have led to the increased use of emerging FRs (EFRs) and organophosphate FRs (PFRs). EFRs and PFRs has been found in environment, such as air, dust, soil and sediment[1], but limited studies have investigated the contamination of FRs, especially PFRs, in biota or food matrices. Food matrices are complex. Lipids and pigments are two major interferences, which could introduce high signal background or even damage the instruments. Beside, PFRs and some EFRs (e.g. TBPH and TBB) cannot be used with strong acid or base clean-up, which is the traditional method for persistent organic pollutants (POPs). Recently, QuEChERS method was introduced in food analysis, due to its simple procedure and its high efficiency for lipid removal[2,3]. However, it is not an excellent clean-up method, since pigments and lipids may still be present in the final extract. Some studies[2,3] report on the analysis of FRs and other organic pollutants, but few of them have achieved a good clean-up for samples rich in lipids and pigments. Here, we present a new method for the analysis of FRs in various food matrices that are rich in lipids and pigments.

Materials and methods

Chemicals and materials

Standards of BDE 28, 47, 99, 100, 153, 154, 183 and 209, BTBPE, DBDPE, DP (syn- and anti- isomers), HCDBCO, TBB, TBPH and isotope labelled internal standards (IS) BDE 209, TBPH, and TBB were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 and 128 (IS) were obtained from AccuStandard Inc. (New Haven, CT, USA). ^{13}C-syn and anti-DP (IS) were purchased from Cambridge Isotope Laboratories (Andover, MA, US). Standards of TCP (mixtures of 4 isomers), TEHP, EHDPP, tri-n-propyl phosphate (TnPP), tri-n-butyl phosphate (TnBP), triphenyl phosphate (TPP), tris(2-chloroethyl) phosphate (TCEP), tricresyl phosphate (TCP, mixture of 4 isomers), and tris(1,3-dichloropropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). Triamyl phosphate (TAP; IS) was purchased from TCI Europe (Zwijndrecht, Belgium). Labeled TPP-D15, TDCPP-D15 and TCEP-D12 (IS) were custom synthesized. Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). DSC-18 sorbent, Z-SEP sorbent and Supelclean™ ENVI™-Florisil® cartridges (500 mg, 3mL) were purchased from Supelco (Bellefonte, PA, USA). Aminopropyl silica (APS) cartridges (500 mg, 3 mL) were purchased from Agilent (Santa Clara, CA, USA). Silica gel, anhydrous sodium sulfate (Mg_2SO_4), and concentrated sulfuric acid (H_2SO_4, 98%) were purchased from Merck (Darmstadt, Germany).

Sample collection and pretreatment

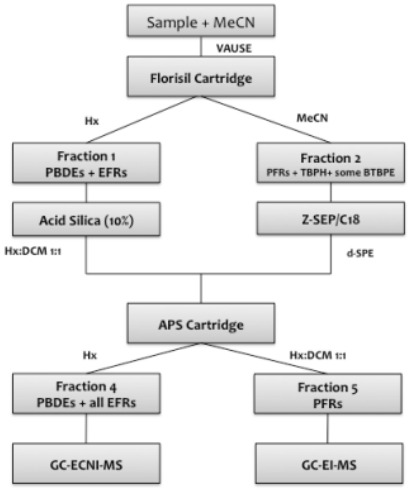
Various types of food samples, including seafood, meats and eggs, were purchased in Belgian supermarkets. The edible part of each sample was homogenized individually with a blender and then lyophilized. Dried samples were further grounded and stored under -20 ^{o}C till analysis.

Ultrasonication and vacuum assisted extraction (UVAE)

The flow chart below illustrates the entire sample treatment procedure and more details are given in the sections below. The extraction was developed based on the concept of unbuffered QuEChERS. In brief, 2.5 g of different types of freeze-dried food samples were added into a 15 ml polypropylenetube with screw cap. Beef, chicken eggs and salmon were used as spiking matrices for recovery tests. Each sample was spiked with known amounts of IS mixture. For the recovery tests, standards were also spiked at QL and QH levels (QH = 3xQL). After 15

min, 5 ml of MeCN:toluene 9:1 mixture was added. The tube was vortexed for 20 s, and then ultrasonicated for 1 h. The phase separation was performed on a 12-port Visiprep^{TM} vacuum manifold (Sigma–Aldrich, St. Louis, MO, USA), on which empty SPE cartridges (25 mL) were connected. The empty cartridge had a frit at the bottom and with a thin layer of $MgSO_4$ on top (about 2 g). Sample-solvent mixture was load into the cartridge, and tightly pressed by another frit on top. Finally, the extract was pump out under vacuum and collected in a glass test tube.

Clean-up and fractionation



The extract was concentrated to nearly dryness, reconstituted with 2 ml of hexane, and then loaded on a Florisil® cartridge (pre-conditioned with 8 mL of hexane and 6 mL of ethyl acetate). Fractionation was achieved with 8 ml of hexane (F1) and 5 ml MeCN (F2). F1 was concentrated to approximate 1 mL and loaded on 2 g of 10% acid silica (AS10%, pre-cleaned with 10 ml hexane). 10 ml of hexane:DCM 1:1 mixture was used to elute all target compounds from acid silica (F3). F2 was concentrated to 2.5 mL, added with 200 mg DSC18/Z-SEP mixture. After performing the dispersive SPE, F2 was centrifuged and the supernatant was combined with F3. 1 mL of MeCN was added to the DSC18/ Z-SEP sorbent for washing and also added into the combined solution (CS). CS was concentrated to near dryness, reconstituted with 2 ml hexane and loaded on an aminopropyl silica cartridge (pre-conditioned with 10 mL hexane:DCM 1:1). Final, fractionation was achieved with 10 mL hexane (F4) and 10 mL hexane:DCM 1:1 (F5). F4 and F5 were concentrated to nearly dryness, and reconstituted with 100 µL iso-octane and 100 µL iso-octane:ethyl acetate 1:1, respectively. All samples were transferred to injection vials and stored under -20 ^{o}C for 1 h before analysis, in order to check if any sample has residual lipids. F4 was injected on GC-ECNI-MS to analyze PBDEs and EFRs, while F5 was injected on GC-EI-MS to analyze PFRs.

Instrumental analysis

The instrumental analysis method used in this study was slightly modified from van den Eede et al. [1]

GC-ECNI-MS: Analysis of F4, containing PBDEs and EFRs, was performed with an Agilent 6890 GC coupled to an Agilent 5973 MS operated in electron capture negative ionization (ECNI) mode. The GC system was equipped with electronic pressure control and a programmable-temperature vaporizer (PTV). 1 µL of cleaned extract were injected on a DB-5 column (15 m × 0.25 mm × 0.10 um), with a deactivated retention gap from (Agilent, 1.0 m × 0.22 mm) connected in front, using solvent vent injection. The GC temperature program was 90°C, hold 1 min, ramp 20°C/min to 200°C, hold 1 min, ramp 5°C/min to 220°C, hold 0.5 min, ramp 5°C/min to 250°C, hold 0.5 min, ramp 14°C/min to 310°C, hold 7 min. The mass spectrometer was operated in selected ion monitoring (SIM) mode and 9 PBDE and 7 EFRs were quantified.

GC-EI-MS: PFRs (F5) was analyzed using an Agilent 6890 GC coupled to an Agilent 5973 MS operated in electron impact ionization (EI) mode. The GC system was equipped with electronic pressure control and PTV. A deactivated retention gap from Agilent (1.0 m × 0.22 mm) was used in front of the HT-8 column (25 m × 0.22 mm × 0.25 um). 1 µL of purified extract was injected using cold splitless injection. The GC temperature program was 90°C, hold 1.25 min, ramp 10°C/min to 240°C, ramp 20°C/min to 310°C, hold 16 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The mass spectrometer was run in SIM mode and TEHP, TnPP, TnBP, TCEP, TCPP, EHDPP, TPP, TDCPP and TCP were analyzed.

Results and discussion

Instrumental method optimization

The instrumental method was optimized from the method developed by van den Eede at al.[1] Guard columns were applied to prevent lipid residues being injected into the instrument. Results showed that guard columns could reduce the sensitivity lost during sequences and prevent column damage caused by injecting insufficiently

cleaned samples. On GC-ECNI-MS, the temperature program was also modified to allow separation between BDE99 and ^{13}C-TBB (both producing ions with m/z 79 and 81). Some marine fishes contain high level of 2'-MeO-BDE68 and 6'-MeO-BDE47, which co-eluted with BDE77 (IS) in the original ramp[1]. The optimized program could split the co-eluted peaks into individual peaks.

Extraction and clean-up optimization

The UVAE, a new extraction method, was developed based on the concept of QuEChERS. Like many QuEChERS studies, UVAE used MeCN as solvent. But 10% of toluene was also added in order to improve the recovery of less polar compounds, such as PBDEs. Instead of centrifugation, a vacuum manifold was used to separate the solvent from the solid phase. In this way, the waste factor of UVAE could be reduced to < 10%, while waste factor was usually > 50% for normal QuEChERS. Thus, less solvent and sample are required for UVAE. As a result, fewer lipids could be extracted without sacrificing the recovery of target compounds. Less time was consumed on concentrating MeCN, as well.

In QuEChERS, a dispersive SPE step is applied to further remove lipid residues after extraction. However, when analyzing samples with high lipid contents, this step cannot completely remove the lipids from the extract, unless large amounts of sorbent are used. Sorbents not only bond lipids, but also other compounds, like pigments. In our method, the extract was first fractionated on Florisil cartridge and then F1 and F2 were further cleaned with AS10% and d-SPE, respectively. PBDEs and EFRs (in F1) are resistant to acid and major part of lipids elute with them, so AS10% could well clean-up this fraction. Since TBPH and PFRs (in F2) are not acid resistant, Z-SPE/DSC18 mixture could efficiently remove the minor part of lipids that eluted in F2. As TBPH and part of BTBPE eluted in F2, van den Eede et al[1] injected F2 on GC-ECNI-MS to quantify them, but this could bring large errors during reinjection of F2 and summing of the BTBPE amount. The cleaned F1 and F2 were combined together and re-fractionated on APS cartridge. As a result, all PBDEs and EFRs were in one fraction (F4), while all PFRs were eluted together (F5). The re-injection of F5 on GC-ECNI-MS was thus avoided. Our pre-treatment method preformed a good clean-up and efficient extraction, as well as a universal applicability on various food matrices. However, the new method has several steps, especially solvent exchange steps, so more system errors may be introduced during sample preparation comparing with the method of van den Eede et al[1].

Spiking tests

In order to develop a universal method for analyzing FRs in food matrices, three types of food (beef, egg and salmon) were selected for recovery tests. All of them contain high percentage of lipids after freeze drying (beef 18%, egg 20% and salmon 38% by dry weight) and are rich in pigments. Our method showed the best clean-up result among published studies so far. Table 1 show the recoveries of FRs in three types of matrices. Relative recoveries were calculated based on the injection of a standard solution with the same concentration compared to the Qlow and Qhigh spiked samples. Accuracy was generally acceptable and ranged between 72 and 125%, with RSD <18% (see table 1). The recoveries of few compounds were slightly out of acceptable range, of which possible explanation could be lack of appropriate IS (e.g. for EHDPP and BDE28), high background peak interference (like TEHP), or high detection limit (like TCP). PBDEs and EFRs had better reproducibility than PFRs, due to the AS10% performed a perfect clean-up on F1, but d-SPE didn't perform a thorough clean-up on F2. Recoveries of different matrices also showed the universal applicability of our method on various food matrices. Distinct recoveries of a few compounds, such as TnPP and EHDPP, could be observed among matrices, indicating that matrix effects remain affecting the recoveries for a certain extent, especially for PFRs. A possible reason could be the d-SPE could not control the matrix effect as good as AS10%.

References:

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3. Kalachova K, Pulkrabova J, Cajka T, Drabova L, Stupak M, Hajslova J (2013); *Anal. Bioanal. Chem.* 405: 7803-15

Table 1Recoveries of different FRs in different matrices

Target Compounds	IS	Low Spike Recoveries (%)							High Spike Recoveries							MLOQ ng/g dw
		Spike (ng)	Beef (n=4)		Egg (n=4)		Salmon (n=3)		Spike (ng)	Beef (n=4)		Egg (n=4)		Salmon (n=3)		
			Rec	RSD	Rec	RSD	Rec	RSD		Rec	RSD	Rec	RSD	Rec	RSD	
BDE 28	BDE 77	1	120	1	143	3	144	4	3	124	4	144	4	137	7	0.008
BDE 47	BDE 77	1	100	2	111	3	113	21	3	104	3	111	5	112	3	0.016
BDE 66	BDE 77	1	97	5	94	4	100	7	3	99	2	97	2	101	3	0.016
BDE 100	BDE 77	1	96	3	94	8	89	10	3	97	3	91	3	94	6	0.016
BDE 99	BDE 77	1	92	2	89	4	82	4	3	92	3	87	1	86	4	0.016
HCDBCO	BDE 77	2.5	93	5	97	17	94	11	7.5	93	10	91	12	103	5	0.077
BDE 85	BDE 77	1	98	4	97	11	94	5	3	100	4	95	5	100	4	0.016
BDE 154	BDE 128	1	107	5	93	2	99	7	3	106	0	95	3	105	9	0.020
BDE 153	BDE 128	1	103	6	96	2	100	6	3	102	2	93	2	105	8	0.020
BDE 183	BDE 128	1	111	5	93	6	94	6	3	109	0	96	3	103	5	0.020
BTBPE	BDE 128	2.5	79	1	74	3	69	5	7.5	95	5	79	7	72	7	0.034
TBB	$^{13}C_6$-TBB-D_{17}	2.5	84	5	87	9	92	13	7.5	94	10	88	2	94	11	0.200
TBPH	$^{13}C_6$-TBPH-D_{34}	2.5	122	5	113	11	105	16	7.5	111	5	105	10	106	10	0.036
s-DP	$^{13}C_{10}$-s-DP	2	99	12	112	16	89	9	6	105	4	99	5	92	7	0.063
a-DP	$^{13}C_{10}$-a-DP	2	102	10	113	9	110	1	6	100	5	106	5	105	3	0.033
BDE 209	$^{13}C_{12}$-BDE 209	5	83	5	92	22	87	7	15	72	3	76	6	78	10	1.2
TEHP	TAP	10	92	10	81	12	87	1	30	105	4	90	6	91	12	3.6
TnPP	TAP	10	58	14	74	19	78	6	30	59	13	81	4	77	14	2.0
TnBP	TAP	10	89	9	85	15	110	13	30	95	3	96	2	101	5	3.7
TCEP	TCEP-D_{12}	10	110	9	121	11	101	23	30	105	5	119	13	105	4	1.7
TPhP	TPhP-D_{15}	10	104	7	93	9	89	19	30	106	2	107	6	96	3	2.4
TDCPP	TDCPP-D_{15}	10	107	11	91	9	111	7	30	109	4	109	9	107	3	2.5
Total TCPP	TDCPP-D_{15}	10	91	15	107	2	99	14	30	79	21	96	6	98	8	2.5
EHDPP	TDCPP-D_{15}	10	69	18	62	18	67	24	30	86	22	61	8	66	12	1.4
Total TCP	TDCPP-D_{15}	10	73	3	65	9	85	12	30	74	22	64	5	71	7	3.6