SIMPLIFYING MULTI-RESIDUE ANALYSIS OF FLAME RETARDANTS

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Introduction

To decrease flammability, flame retardants (FRs) are frequently added to consumer products ranging from upholstered furniture to textiles, electronics and building materials. Unfortunately, most of these compounds have a negative impact on the environment and a potential risk for animal and human health. Although FRs have been investigated for more than 15 years, our understanding of their environmental fate and toxic effects still remains limited.

In order to address these issues, it is important to employ sensitive and selective analytical methods (e.g. GC-MS or LC-MS) which allow the simultaneous determination of various classes of FRs, such as brominated (BFRs), organophosphorous (OPFRs) and chlorinated FRs. Still, the routine analysis suffers due to the complexity of the matrices (e.g. dust) and moreover, several target compounds may coelute (e.g. TBBP-A with BDE-153 or the degradation products of HBCD with BDE-49 and BDE-99)[1].

This study is part of the INFLAME Marie Curie Initial Training Network, an EU funded project. The aim of this work was to develop a fractionation procedure that would reduce the sample complexity, prevent coelutions and thus facilitate FR analysis. This was done by dividing the target compounds (FRs) in several fractions according to their polarity. A combination of ultrasonic-assisted extraction (UAE)[2] and solid phase extraction (SPE) was applied for household dust. FRs were eluted from the SPE cartridge by using different solvents of increasing polarity. In this way, PBDEs were separated from HBCD and TBBP-A, which in turn were separated from OPFRs, eliminating coelutions and allowing for the simultaneous determination of these classes of FRs.

Materials and methods

All solvents used during analysis were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, *n*-butyl chloride, ethyl acetate, methanol and *iso*-octane were purchased from Merck (Darmstadt, Germany). Supelclean™ ENVI™- Florisil® SPE cartridges (500 mg/3mL) were purchased from Supelco (Bellefonte, PA, USA), Bond Elut-Si from Agilent (Santa Clara, CA, USA) and Oasis HLB from Waters (Milford, MA, USA). Empty polypropylene filtration tubes (3 mL) SPE cartridges were also purchased from Supelco and Aluminium oxide 60 from Merck (active basic, activity stage I, particle size 0.063-0.2 mm).

Standards of BDE 28, 47, 66, 85, 99, 100, 153, 154, 183 and 209, α-HBCD, β-HBCD, γ-HBCD, BTBPE, DBDPE, HCDBCO, TBB, TBPH, HBB, TBBPA-dbpe, TBBPA, TBECH isomers, ATE, BATE, DPTE, TBCO isomers, OBIND, dechlorane plus (DP) isomers were purchased from Wellington Laboratories (Guelph, ON, Canada). Standards of PBBs were purchased from Dr. Ehrenstorfer (Augsburg, Germany). BDE 77 was obtained from AccuStandard Inc. (New Haven, CT, USA). Standards of tri-isobutyl phosphate (TiBP), tri-n-butyl phosphate (TnBP), triphenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), ethyl-hexyl-diphenyl phosphate (EHDPhP), triscresyl phosphate (TCP, mixture of 4 isomers) and tris(1,3-dichloropropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). Tris(2-butoxyethyl) phosphate (TBEP) were purchased from Sigma Aldrich. Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBEP (>94%). SRM 2585 was purchased from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

The extraction method used was based on the method described by van den Eede et al. (2011)[3]. For method development, a dust sample collected from a carpenter shop containing all the major classes of FRs and a mixture of standards were used. The solvent mixture employed in the extraction was hexane/acetone (3:1, *v/v*). The process consisted of consecutive steps of vortexing (1 min) and ultrasonication (5 min) with 2 mL of the aforementioned solvent mixture. This cycle was repeated 3 times and after each cycle, the supernatant was transferred to a clean tube. The extracts were evaporated to near dryness using a gentle nitrogen stream and the solvent was exchanged to hexane (1 mL).

No destructive clean-up method was applied to insure that no analytes of interest are degraded. The PBDEs and non-polar NBFRs were eluted with 8 mL of hexane, the HBCDs, TBBP-A, TBPH and related compounds with 8 mL of *n*-butyl chloride, the OPFRs with 8 mL of ethyl acetate and compounds more polar than the OPFRs with 8 mL of methanol.

The fractions obtained were injected on a GC-MS system (Agilent 6890 GC coupled to an Agilent 5973 MS), operated in electron capture negative ionization (ECNI) mode. The GC system was equipped with a programmable-temperature vaporizer inlet (PTV) which was run in the pulsed splitless mode. One µL of extract was injected on a DB-5 column (15 m × 0.25 mm × 0.10 µm). The GC temperature program was 90 °C, hold 1.5 min, ramp 10 °C/min to 300 °C, hold 4 min, ramp 40 °C/min to 310 °C, hold 15 min. Helium (purity 5.9) was used as a carrier gas with a ramped flow. Methane was used as moderating gas (purity 4.5). The initial flow was 1 mL/min (for 19 min), and then ramp 10 mL/min to 2 mL/min. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

Each fraction was also injected in an identical GC-MS system operated in the electron ionisation (EI) mode. The PTV was run in the pulsed splitless mode. One µL of extract was injected on a SGE-HT8 column (25 m × 0.22 mm × 0.25 µm). The GC temperature program was 90 °C, hold 1.50 min, ramp 10 °C/min to 310 °C, hold 20 min. Helium (purity 5.9) was used as a carrier gas with a constant flow (1 mL/min). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

Results and discussion

Development and optimisation. To determine which sorbent is most adequate for obtaining clear-cut fractions, the same dust sample (containing all the major classes of FRs) was extracted, fractionated using different sorbent types and injected in both the aforementioned instruments. The chromatograms of the individual fractions were analysed and the FR compounds detected were logged. On this basis, a comparison between different sorbents was done and the most adequate one was determined to be underivatised silica (Table 1), with a particle size of 40 µm and an average pore diameter of 60 $Å^2$.

Table 1: Fitness for purpose of different sorbent types

Sorbent	Signal/noise in chromatograms	Clear-cut fractions	Unwanted coelutions	Other issues	Fitness for purpose
Florisil	+++	No	Yes	HBCDs in two fractions	+
Silica	++	Yes	No	-	+++
Alumina	++	No	No	TPhP present in n-BC fraction	++
Oasis HLB	++	No	Yes	HBCD degradation	-

To get a better idea about what compounds are to be expected in which fractions, two mixtures of standards were prepared. The first mixture contained lower PBDEs (tri-hepta), HBCDs, TBBP-A, MeTBBP-A, α,β-TBCO, OBIND, α,β-TBECH, syn-DP, anti-DP, HBB, BTBPE, DBDPE and BDE-209. The second mixture contained PBBs (153, 155, 103, 80, 209), ATE, BATE, DPTE, TBA, γ,δ-TBECH, OPFRs, BTBPE, HCDBCO, TBB, TBPH and TBBPA-BDPE. These mixtures were fractionated on Silica SPE and logged for future reference (Table 2).

Table 2: Compounds per fraction

Fraction Nr.	Elution solvent	Compounds
1	Hexane	PBDEs, PBBs, α,β-TBCO, HBB, BTBPE*, syn,anti-DP, OBIND, DBDPE, TBA, ATE, DPTE, TBB, HCDBCO
2	*n*-Butyl Chloride	HBCDs and breakdown compounds, TBBP-A, MeTBBP-A, TBBPA-DBPE, TBPH
3	Ethyl Acetate	OPFRs including TDBPP

4	Methanol	None of the compounds in the standard mixtures; normally compounds more polar than the OPFRs

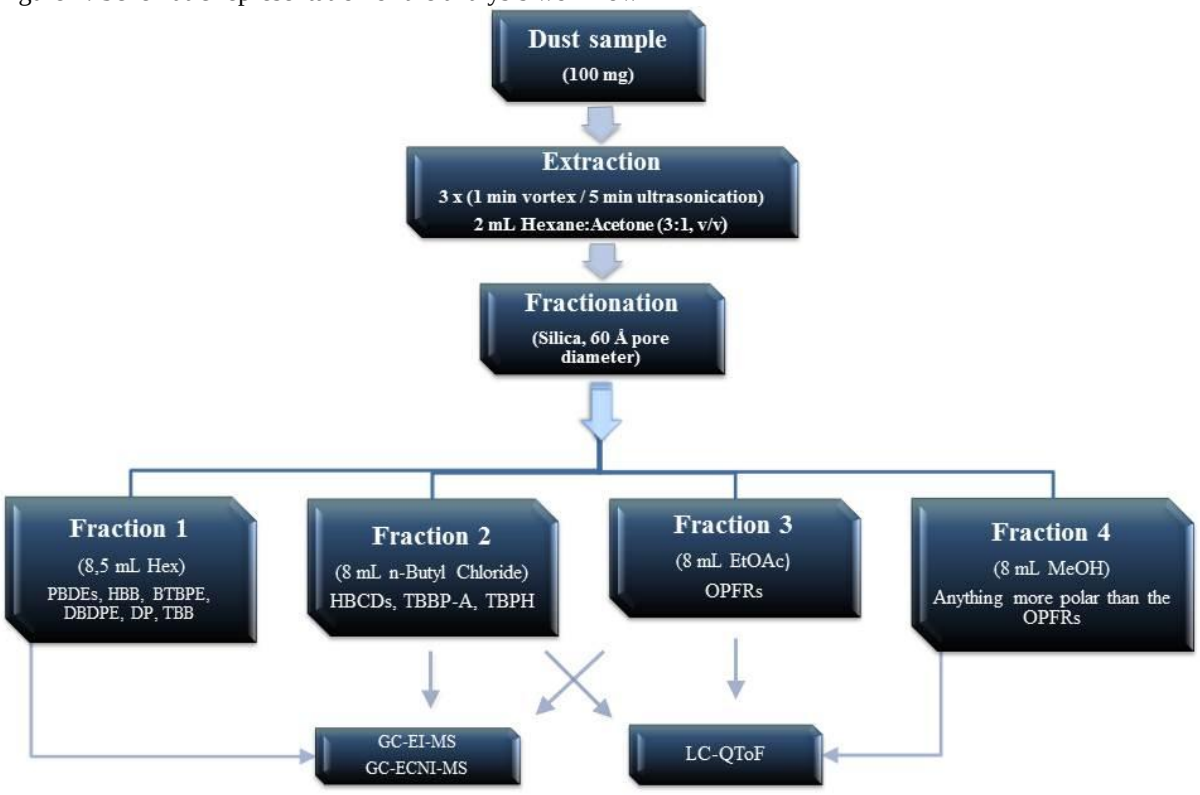
*A small part of the BTBPE is in the *n*-Butyl Chloride fraction. This can be easily corrected by increasing the hexane elution volume by 0.5 mL.

The only compound that is not entirely in one of the fractions is TBECH, which is found in both the hexane fraction and in the n-Butyl Chloride fraction.

Fractionation of SRM 2585. For the purpose of confirming the distribution of compounds for each fraction, the NIST SRM 2585 reference material for organic contaminants in house dust was analysed using the developed procedure. The same solvents and volumes were used for the elutions from the SPE cartridge.
The reference material used is not certified for NBFRs or OPFRs, but in other publications[3,5] these compounds have been reported and quantified. Upon analysis of the obtained chromatograms, it was found that the distribution of the analytes in the fractions was consistent with our previous observations from the method development stages.

When applying this procedure to generic samples, all but the non-polar fraction are also injected on a LC instrument (Figure 1) – a LC-QToF to screen for and identify unknown compounds or a LC-MS/MS for the quantification of known analytes. Through fractionation, the sample complexity is significantly decreased and the compounds fall into known intervals of polarity. This further information can prove very valuable in identifying unknown compounds or at the very least narrowing down the number of possibilities.

Figure 1: Schematic representation of the analysis workflow



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