CLEAN-UP METHOD FOR DETERMINATION OF PBDES, HBCDS AND EMERGING BFRS IN DUST

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Introduction

Brominated flame retardants (BFRs) are used in a wide variety of products to enhance fire resistancy. Many BFRs are only physically mixed[1] into the material, and due to the lack of covalent bonds between the FR and the polymer, the release of these compounds into the environment is evident. PBDEs, a group of BFRs, have been used as three different mixtures, two of which (penta- and octaBDEs) were banned by the European Union in 2004[2]. However, the use of decaBDE is not widely restricted and it is still produced and distributed in the environment. The restricted PBDEs are being replaced by other FR chemicals by the industry. Hexabromocyclododecane (HBCD), 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE) and decabromodiphenyl ethane (DBDPE) have been detected in various biota and environmental matrices indicating their increasing use[3]. Two emerging BFRs, (2-ethylhexyl) tetrabromobenzoate (TBB) and bis-(2-ethylhexyl) tetrabromophthalate (TBPH) are the major components in the FR mixture Firemaster 550 (FM 550), and have recently been detected in house dust in the U.S.[4] and in marine mammals from Hong Kong, China[5]. The objective of this study was to develop an analytical method for determination of all the above mentioned BFRs (PBDEs, HBCD, BTBPE, DBDPE, TBB and TBPH) in dust samples. TBB and TBPH are sensitive to breakdown in acid and thus the effective and easy clean-up procedure using concentrated sulphuric acid cannot be applied. Other clean-up methods such as gel permeation chromatography (GPC) are laborious and require large amounts of solvents enhancing the risk of blank problems and thus higher detection limits. The clean-up method developed in this study enables the determination of all analytes in the same sample extract using reasonable amounts of solvents. After extraction, the sample is separated into three fractions that are subsequently cleaned up individually. Two of the fractions are analysed on GC/MS and one on LC/MS.

Materials and methods

Recovery studies

The method recovery was determined by spiking standard reference material (SRM 2585), "Organic Contaminants in House Dust" (NIST, Gaithersburg, USA) with high and low concentration levels of the emerging BFRs (TBB, TBPH, BTBPE and DBDPE). Recovery was determined for five aliquots each of the low and high spiked SRM 2585. Three aliquots of an inert material (Bulk Isolute Sorbent, Isolute HM-N, International Sorbent Technology Ltd, UK, prewashed with dichloromethane) were spiked at one level to test for matrix effects. Five unspiked dust samples were also analysed to quantify existing levels of these compounds in the SRM material. All samples were fortified with ^{13}C-labelled internal standards (BDE-183, -197, -207, -209, BTBPE, α -HBCD, β -HBCD and γ -HBCD) to quantify the levels of the different BFRs.
Two laboratory blanks were processed together with the samples.

Extraction

Sample extraction was performed according to Björklund et al.[6] In short, about 10 mg of each sample was weighed in a 18 ml glass test tube and internal standards were added. The samples were extracted twice with 18 ml DCM in an ultrasonic bath for 30 min. The extracts were combined and evaporated to 1 ml in a vacuum evaporator, and the solvent was changed to *n*-hexane.

SPE clean-up

The clean-up procedure is shown in Figure 1. The extracts were applied to prewashed SPE columns packed with 2 g silica (deactivated with 2.5 % H_2O) and 1 g Na_2SO_4. The analytes were eluted in three fractions: PBDEs and DBDPE with 30 ml *n*-hexane (I), TBB, TBPH and BTBPE with 10 ml 5 % diethyl ether (DEE) in *n*-hexane (II) and HBCDs with 10 ml 50 % DEE in *n*-hexane (III). Fractions I and III were evaporated to 2 ml (solvent changed to *n*-hexane in fraction III). 4-6 ml H_2SO_4 were added to fractions I and III, the test tubes were gently rocked 20 times and the phases separated by centrifugation. Fraction II was evaporated to 0.5 ml, solvent

changed to *n*-hexane and applied on prewashed aminopropyl (NH_2) columns (0.5 g). TBB, TBPH and BTBPE were eluted with 12 ml *n*-hexane. Fractions I and II were evaporated to ~500 µl under a gentle stream of nitrogen and transferred to GC vials with 50 µl recovery standard (^{13}C-CB-180, 21 pg/µl) and the volume adjusted to 50 µl. The solvent in fraction III was changed to acetonitrile, evaporated to ~250 µl and transferred to LC vials with 50 µl recovery standard (d_{18}-β-HBCD, 12 pg/µl).

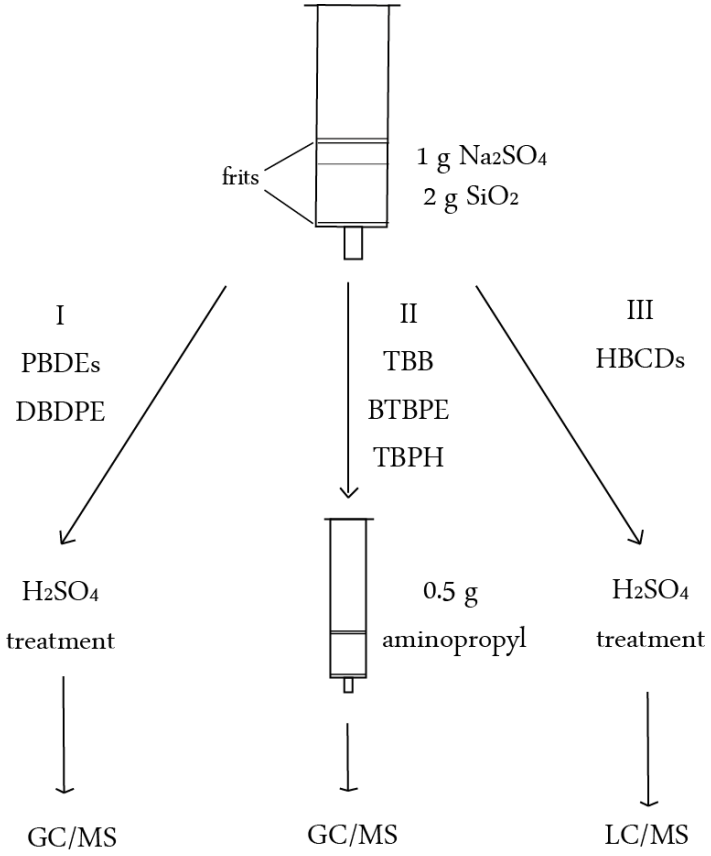

Figure 1. The clean-up procedure for dust samples

Instrumental analysis

GC/MS

Fractions I and II were injected (1 µl) into a Trace GC Ultra coupled to a DSQ II MS (both Thermo Scientific, Waltham, USA) to determine PBDEs, TBB, TBPH, BTBPE and DBDPE. The GC was equipped with programmable temperature vaporiser (PTV) injector and DB-5MS fused silica columns (J&W Scientific, Folsom, CA, USA, 0.25 mm inner diameter, 0.1 µm film thickness), 15 m for the analysis of octa-decaBDEs, DBDPE, TBB, TBPH and BTBPE, and 30 m for other PBDEs. Helium (purity 4.6, Aga, Lidingö, Sweden) was used as the carrier gas (1.5 ml/min). Electron capture negative ionisation (ECNI) with ammonia (purity 5.0, Aga) as moderating gas (5.0 ml/min) was used and the MS was operated in single ion monitoring (SIM) mode recording the bromide ions (m/z 79,81) and different higher mass fragment ions for the different analytes (Table1).

Table 1. Ions (m/z) recorded in the instrumental analyses

GC/ECNI-MS	m/z	UPLC/ESI-MS	m/z	
			parent ions	daughter ions
DBDPE	79, 81	native HBCDs	639, 641, 643	79, 81
TBB	357, 359, 469, 471	^{13}C-HBCDs	651	79, 81
BTBPE	249, 251, 253	d_{18}-HBCD	660	79, 81
TBPH	384, 386			
^{13}C-BTBPE	257, 259			
^{13}C-BDE-183 & 197	415, 417			
^{13}C-BDE-207 & -209	495, 497			

UPLC/MS

Fraction III was injected into an ultra performance liquid chromatograph (ACQUITY™ UltraPerformance LC) coupled to tandem-quadrupole mass spectrometer (Xevo™ TQ-S) to determine the three major stereoisomers of HBCDs (α-, β-, and γ-HBCD). The separation was performed on a UPLC column (ACQUITY UPLC® HSS C18; 1.8 µm; 2.1x100 mm), coupled to a pre-column (ACQUITY UPLC™ HSS C18; 1.8 µm VanGuard™; 2.1 x 5mm) with a mobile phase linear gradient from 78:22 to 93:7 methanol:H_2O. The UPLC/MS instrument and columns used were from Waters (Milford, USA). Electrospray ionisation (ESI) in negative mode was applied for the ionisation of the analytes and the MS was run in multiple reaction monitoring mode (MRM) (Table 1).

Results and discussion:

The method enables analysis of different BFRs in the same sample providing very good recoveries for all the BFRs studied. The recoveries of ^{13}C-labelled internal standards are shown in Table 2, and no differences were indicated between the inert sorbent material and SRM dust.

Table 2. The method recoveries of ^{13}C-labelled BFRs (%)

	sorbent material (n = 3)		SRM 2585 (n = 15)	
	mean	sd	mean	sd
^{13}C-BDE-183	107	11	110	13
^{13}C-BDE-197	106	6	102	8
^{13}C-BDE-207	107	7	103	8
^{13}C-BDE-209	98	5	113	9
^{13}C-BTBPE	92	10	94	12
^{13}C-α-HBCD	76	9	76	13
^{13}C-β-HBCD	112	10	120	21
^{13}C-γ-HBCD	103	13	114	25

The recoveries of the emerging BFRs lacking available ^{13}C-labelled standards are shown in Table 3. The low spiking level of TBPH was too low in comparison to the already existing TBPH level in the SRM. Thus the recovery of TBPH is reported based on only the high spiking level. The recovery of DBDPE in relation to ^{13}C-BDE-209 was close to 100 %, indicating that ^{13}C-BDE-209 is a suitable internal standard for DBDPE.

Table 3. The method recoveries of emerging BFRs lacking ^{13}C-labelled standards (%)

	spiked sorbent material (n = 3)		spiked SRM 2585 (n = 10)	
	mean	sd	mean	sd
TBB	86	2	85	7
TBPH	72	5	60*	5*
DBDPE	108	10	118	19

* only high spiking level (n = 5)

The emerging BFRs and HBCD isomers were also quantified in unspiked SRM 2585 in order to correct for background concentrations in the recovery study. The concentrations of TBB, TBPH and BTBPE found in this study (Table 4) are higher than those previously reported by Stapleton et al[4]. The α- and β-HBCD concentrations were similar to those found by Abdallah et al[7] and Schantz et el[8], but deviated considerably between replicates (Table 5), with relative standard deviations (rsds) of 65, 60, and 31% for α-, β- and γ-HBCD, respectively. The γ-HBCD concentration found in this study is 4 times lower than the levels Abdallah et al[7] and Schantz et al[8] reported. The method recoveries of the ^{13}C-HBCDs were high (Table 2), with fairly low deviations (rsds 17, 17 and 22% for α-, β- and γ-HBCD, respectively). Although the material has been shown to be fairly homogeneous for PBDEs[7], this raises a question about the homogeneity of the SRM 2585 material for the HBCDs. An inhomogeneity in the material combined with the small sample intake (about 10 mg dust) could possibly explain the high deviations for the HBCDs. The HBCD concentrations in the SRM material should be determined again using larger sample intake, and the suitability of SRM 2585 as quality control sample for HBCD analysis should be further examined.

Table 4. Concentrations (ng/g) of emerging BFRs in SRM 2585 (n = 5)

	mean	sd
TBB	36	2
BTBPE	36	5
TBPH	1359	102
DBDPE	n.d.	-

Table 5. Concentrations (ng/g) of α-, β- and γ-HBCD in SRM 2585 (n = 5)

	mean	sd
α-HBCD	17	11
β-HBCD	5	3
γ-HBCD	29	9
total HBCD	51	18

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