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Optimization of the determination of polybrominated diphenyl ethers in human serum using solid-phase extraction and gas chromatography-electron capture negative ionization mass spectrometry

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Abstract

A simple, rapid, sensitive and reproducible method based on solid-phase extraction (SPE) and acidified silica clean-up was developed for the measurement of 12 polybrominated diphenyl ethers (PBDEs), including BDE 209, and 2,2′,4,4′,5,5′-hexabromobiphenyl (BB 153) in human serum. Several solid-phase sorbents (Empore^{TM} C_{18}, Isolute Phenyl, Isolute ENV+ and OASIS^{TM} HLB) were tested and it was found that OASIS^{TM} HLB (500 mg) gives the highest absolute recoveries (between 64% and 95%, R.S.D. < 17%, $n = 3$) for all tested analytes and internal standards. Removal of co-extracted biogenic materials was performed using a 6 ml disposable cartridge containing (from bottom to top) silica impregnated with sulphuric acid, activated silica and anhydrous sodium sulphate. PBDEs and BB 153 were quantified using a gas chromatograph coupled with a mass spectrometer (MS) operated in electron-capture negative ionization mode. The method limits of quantification (LOQ) ranged between 0.2 and 25 pg/ml serum (0.1 and 4 ng/g lipid weight). LOQs were dependent on the analyte levels in procedural blanks which resulted in the highest LOQs for PBDE congeners found in higher concentrations in blanks (e.g. BDE 47, 99 and 209). The use of OASIS^{TM} HLB SPE cartridge allowed a good method repeatability (within- and between-day precision < 12% for all congeners, except for BDE 209 < 17%, $n = 3$). The method was applied to serum samples from a random Belgian population. The obtained results were within the range of PBDE levels in other non-exposed population from Europe.

Keywords: Polybrominated diphenyl ethers; Human serum; Solid-phase extraction

1. Introduction

Brominated flame retardants (BFRs) and, in particular polybrominated diphenyl ethers (PBDEs), have been identified as new environmental contaminants with global distribution as shown by their identification in both aquatic and terrestrial compartments in Europe [1,2], North America [1,3] and Asia [4]. Although banned for use for several decades, polybrominated biphenyls (PBBs) may still be found in various environmental compartments [5]. PBDEs have been shown to act as hormone disruptors, neuro-developmental toxicants and, in some cases, carcinogenic agents. This has raised high concerns about exposure to even low environmental concentrations [6]. Although increasing levels in the last decades have been found in human milk from Sweden [7] and human serum from Norway [8], human exposure to PBDEs in Europe is still at low levels [9]. Therefore, there is a need for sensitive, yet simple methods, ready to be applied in epidemiological studies involving PBDEs.

Most methods developed in the past for the determination of PBDEs in human serum employed liquid–liquid extraction (LLE) [10–12] which has the disadvantages of being laborious. Recent methods take advantage of solid-phase extraction (SPE) techniques and several sorbents have been already tested [13–15]. The SPE techniques have several advantages over the LLE procedures, such as reduced solvent consumption and processing time, possibility of miniaturization, high reproducibility and parallel sample preparation. However, none of the previous

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articles offer a comparison between the efficiencies of various sorbents.

The aim of this study is to set up a simple, rapid, sensitive and reproducible method using SPE for the determination of 12 major PBDE congeners and BB 153 in human serum. The list of PBDE congeners includes BDE 209, the principal component of the sole PBDE technical mixture presently used in Europe [16]. Due to relatively high analytical background of BDE 209 [13,14], this congener was not measured or not reported in most studies on the PBDE levels in human serum. Additionally, different SPE sorbents were compared with regard to their ability to yield high recoveries of PBDEs.

2. Experimental

2.1. Reagents, materials and solutions

PBDE congeners 28, 47, 49, 66, 85, 99, 100, 138, 153, 154, 183 and 209 (nomenclature and structure in Ref. [17] and Table 1) were purchased from Wellington Laboratories (Guelph, ON, Canada). ^{13}C-BDE 209 was also purchased from Wellington Laboratories and had ten bromine atoms and twelve ^{13}C atoms, with an isotopic purity of 99%. Brominated biphenyl (BB) 153 was from Accustandard (New Haven, CT, USA). BDE 104 and BDE 140 were used as internal standards (IS), while BDE 139 was used as recovery standard (RS) and were also obtained from Accustandard. They were shown not to co-elute with the analytes of interest and has similar degree of bromination to the predominant analytes found in human serum. All standard solutions were prepared in *iso*-octane. Acetone, *n*-hexane (Hex), dichloromethane (DCM), methanol (MeOH) and *iso*-octane were of pesticide grade (Merck, Darmstadt, Germany). Concentrated sulphuric acid (98%) and formic acid (99%) were from Merck. Anhydrous sodium sulphate (Na_2SO_4) and silica gel (Merck) were washed with Hex and used after heating overnight at 150 °C. The acidified silica gel (44% acid, w/w) was prepared as previously described [18]. All glassware was washed with detergent, rinsed with water and dried at 150 °C. Prior to use, all glassware was rinsed with Hex.

A Positive Pressure Manifold (3 M Company, St. Paul, MN, USA) was used for the extraction and clean-up. The following SPE cartridges were tested: 10 mm/6 ml C_{18} Empore^{TM} extraction disk cartridges (3 M Company), 500 mg/6 ml Oasis^{TM} HLB cartridges (Waters, Milford, MA, USA), 500 mg/10 ml Isolute Phenyl cartridges and 500 mg/6 ml Isolute Env+ cartridges (International Sorbent Technology, Hengoed, UK). Empty polypropylene columns for clean-up (6 ml) were from Supelco (Bellefonte, PA, USA).

2.2. Serum samples and lipid determination

Pooled serum used for the development and validation of the analytical method was obtained in 2002 from the Blood Transfusion Centre of the University Hospital of Antwerp (Belgium) and it was a composite from approximately 100 individual serum samples. Individual serum and pooled umbilical cord serum samples were available from other projects which took place between 1999 and 2004 in Belgium. Total cholesterol (CHOL) and triglycerides (TG) were determined enzymatically in a separate aliquot of serum at a clinical laboratory. Total lipids (TL) were calculated as described by Covaci et al. [19] using the following formula: TL (g/l) = 1.12 × CHOL + 1.33 × TG + 1.48. Therefore, the concentrations of PBDEs in individual samples were also reported as ng/g lipid weight.

2.3. Extraction and clean-up

The procedure for extraction and clean-up of PBDEs from human serum was used with major modifications from the method described by Covaci and Schepens [18] for the determination of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in human serum. The modifications consisted in the use of higher volumes for elution from the SPE cartridges

Table 1
Absolute recoveries and relative standard deviation (in parentheses) of target analytes and internal standards using different SPE cartridges

Analyte	Structure	C_{18} Empore^{TM}	Isolute-phenyl	Isolute-ENV+	OASIS^{TM} HLB
BDE 28	2,4,4′-Tribromodiphenyl ether	47 (4)	20 (17)	49 (13)	84 (10)
BDE 47	2,2′,4,4′-Tetrabromodiphenyl ether	38 (4)	15 (16)	54 (22)	88 (9)
BDE 49	2,2′,4,5′-Tetrabromodiphenyl ether	41 (4)	16 (16)	56 (18)	84 (6)
BDE 66	2,3′,4,4′-Tetrabromodiphenyl ether	38 (4)	13 (15)	53 (20)	85 (7)
BDE 85	2,2′,3,4,4′-Pentabromodiphenyl ether	35 (2)	12 (14)	58 (12)	92 (5)
BDE 99	2,2′,4,4′,5-Pentabromodiphenyl ether	31 (5)	11 (13)	50 (2)	88 (6)
BDE 100	2,2′,4,4′,6-Pentabromodiphenyl ether	31 (3)	11 (15)	46 (7)	84 (5)
BDE 138	2,2′,3,4,4′,5′-Hexabromodiphenyl ether	27 (2)	10 (13)	54 (12)	86 (6)
BDE 153	2,2′,4,4′,5,5′-Hexabromodiphenyl ether	22 (4)	8 (16)	44 (9)	82 (4)
BDE 154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether	24 (4)	8 (17)	43 (14)	74 (9)
BDE 183	2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether	18 (4)	7 (16)	38 (11)	66 (12)
BB 153	2,2′,4,4′,5,5′-Hexabromobiphenyl	24 (4)	10 (15)	46 (11)	81 (3)
BDE 104 (IS1)	2,2′,4,6,6′-Pentabromodiphenyl ether	38 (6)	17 (18)	46 (19)	84 (8)
BDE 140 (IS2)	2,2′,3,4,4′,6′-Hexabromodiphenyl ether	29 (8)	10 (19)	45 (18)	95 (5)
^{13}C-BDE 209 (IS3)	2,2′,3,3′,4,4′,5,5′,6,6′-Decabromodiphenyl ether ($^{13}C_{12}$)	22 (28)	8 (50)	28 (13)	64 (17)

Recoveries are given as percentages (%). Each experiment was done in triplicate.

(other than Empore^{TM} C_{18} disks) and an additional layer of activated silica in the clean-up cartridge.

All samples were first thawed and then homogenised by shaking the serum for 2 min. Internal standards (500 pg BDE 104, BDE 140 and ^{13}C-BDE 209) were added to a glass tube and the solvent was evaporated. The ISs were redissolved in 100 μl acetone, vortexed, after which 5 ml serum was added, then vortexed and finally sonicated for 20 min. The spiked serum was kept overnight at +4 °C.

The next day, the serum was mixed with 2 ml formic acid and 3 ml water and sonicated for 20 min. Prior to sample application, the SPE cartridges were washed with 5 ml DCM and activated with 5 ml MeOH and 5 ml water. After sample loading at a low positive pressure of 2–4 psi, the SPE cartridges were rinsed with 3 ml water. The sorbent bed was dried thoroughly under a nitrogen stream at 20 psi positive pressure (10 min) and by centrifugation (15 min, 4000 rpm). The SPE cartridges were eluted with 3 × 3 ml DCM in a separate tube and the eluate was concentrated to ∼1 ml under nitrogen.

An empty cartridge (6 ml) filled (from bottom to top) with 2 g of acid silica, 200 mg activated silica (freshly activated for 2 h at 200 °C) and 500 mg Na_2SO_4 was pre-washed with 5 ml DCM. The concentrated eluate obtained from the SPE cartridge was then loaded on the clean-up cartridge and the analytes were eluted with 8 ml DCM. The final eluate was concentrated under a gentle nitrogen stream at room temperature until dryness and resolubilised in 60 μl *iso*-octane. Recovery standard (40 μl BDE 139 with a concentration of 10 pg/μl) was added, the mixture was vortexed and transferred to a vial for GC analysis.

As procedural blank, 5 ml of water in place of serum sample was subjected to the same procedure. The value of each analyte in the procedural blank was subtracted from values found in the serum samples.

2.4. Instrumentation

An 6890 Agilent (Palo Alto, CA, USA) gas chromatograph (GC) coupled to a 5973 mass spectrometer operated in electron-capture negative ionization (ECNI) was equipped with a 12 m × 0.18 mm × 0.10 μm AT-5 (5% phenyl dimethylpolysiloxane) capillary column (Alltech, Lokeren, Belgium). The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 ml/min). One μl extract was injected in solvent vent mode (injector initial temperature 90 °C, kept for 0.05 min, then raised with 700 °C/min to 295 °C, kept for 18 min, vent flow 100 ml/min, vent time 0.03 min, purge time 1.5 min). The temperature of the AT-5 column was programmed from 90 °C, kept for 1.50 min, then raised with 30 °C/min to 200 °C, then with 5 °C/min to 275 °C and finally with 40 °C/min to 300 °C, and kept for 5 min. The following ions were monitored during the entire run: $m/z = 79$ (bromine trace) and 81, $m/z = 485/487$ and 495/497, corresponding to the ion $[C_6Br_5O]^-$ obtained by fragmentation of BDE 209 and ^{13}C-BDE 209, respectively [20]. Dwell times were set at 40 ms.

For the determination of BB 153, the GC/ECNI-MS was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 (1,7-dicarba-closo-dodecarborane 8% phenyl methyl siloxane) capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures, together with injection parameters were as described above. The temperature of the HT-8 column was programmed from 90 °C, kept for 1.50 min, then raised with 30 °C/min to 200 °C, then with 5 °C/min to 300 °C, and kept for 20 min. Bromine ions $m/z = 79$ and 81 were monitored during the entire run. Dwell times were set at 40 ms.

2.5. Recovery experiments

For adsorbent selection, four different cartridges were tested (see Section 2.1). For each type of adsorbent, three spiked and two non-spiked pooled serum samples, together with two procedural blanks, were used to assess the recoveries of analytes and internal standards. The spiking level was 80 pg/ml serum for all PBDEs except BDE 209 spiked at 250 ng/ml serum. The extraction and clean-up procedures were as described in Section 2.3, except for experiments conducted on C_{18} Empore^{TM} extraction disk cartridges for which the procedure described by Covaci and Schepens [18] was used. To assess between-day precision and accuracy, experiments on the OASIS^{TM} HLB cartridge were performed in three replicates during 3 days. The absolute recovery was determined using external calibration with BDE 139 as RS, while the recovery relative to IS (BDE 104 and BDE 140) was calculated using internal standard calibration. The tri- (BDE 28), tetra- (BDE 47, 49, 66) to penta- (BDE 85, 99, 100) congeners were calculated based on BDE 104, while hexa- (BDE 138, 153, 154), hepta- (BDE 183) congeners and BB 153 were calculated based on BDE 140. BDE 209 was calculated based on ^{13}C-BDE 209.

2.6. Method validation

Five-points calibration curves were created for the quantification and high correlation coefficients ($R^2 > 0.998$) were obtained for the tested interval (0.5–1000 pg/ml). The ratios between peak areas of analytes and the corresponding ISs or RS were plotted against the corresponding concentration ratios using inverse square of concentration-weighted linear regressions. Quantification was based on the sum of ions 79 and 81 for all analytes and IS, except for BDE 209 and ^{13}C-BDE 209 for which ions m/z 487 and 495 were used. The identification of PBDEs was based on their relative retention times to the IS used for quantification, ion chromatograms and intensity ratios of the monitored ions (for BDE 209). At least two procedural blanks were included with each sample batch and values obtained for the serum samples were blank-corrected. All individual samples were processed in one batch together with two procedural blanks and the pooled serum used for method validation.

2.7. Quality assurance

While our laboratory regularly participates with good results to the Arctic Monitoring and Assessment Programme (AMAP) ring test for PCBs and OCPs in humans serum, organised by the Toxicological Centre of Québec (Canada), there are no such

programs for the determination of PBDEs in human matrices. However, regular participation to the QUASIMEME proficiency exercises for PBDEs in environmental samples ensures a sufficient knowledge on the analysis of PBDEs. A variation of less than 15% from mean values obtained from 42 participating laboratories was obtained during the second interlaboratory study on PBDEs, where a human milk sample processed through a similar SPE method, was included [21].

3. Results and discussion

3.1. General considerations

The use of the SPE procedure replaced the LLE step and implicitly allowed a reduced solvent consumption together with parallel processing of a higher number of samples. The tested SPE adsorbents had a high hydrophobic character needed for the successful retention of highly lipophilic compounds, such as PBDEs. In the present method, the hydrophilic co-extractables present in serum were removed by SPE, followed by the elimination of hydrophobic compounds (mainly lipids) by normal phase clean-up on acidified silica gel.

The activated silica layer on the top of the clean-up cartridge was essential for a good performance of the clean-up step and allowed an efficient retention of polar biogenic material. The activated silica acted as trapping layer for cholesterol and prevented the conversion of cholesterol to the acid-resistant cholestene in the acidified silica layer [14,22].

The short AT-5 capillary column offered sufficient resolution for the baseline separation of the investigated PBDE congeners (Fig. 1). Furthermore, no degradation of BDE 209 was observed due to a short run time (<18 min). However, this type of stationary phase does not allow a separation between BDE 154 and BB 153, which is achievable on a HT-8 stationary phase (Fig. 2).

3.2. Recovery

The absolute recoveries of the analytes and ISs calculated based on the RS are shown in Table 1. For BDE 209, to exclude variations from inconsistent values in procedural blanks and non-spiked serum samples, only the recovery of ^{13}C-BDE 209 was evaluated. The lowest recoveries were observed on the Isolute Phenyl, followed by C_{18} Empore^{TM} and Isolute-ENV+, while the highest recoveries were obtained on the OASIS^{TM} HLB cartridge (Table 1). This is probably due to a higher hydrophobic character associated with an increased retention capability through hydrophilic interactions between the sorbent and analytes. On the OASIS^{TM} HLB cartridge, the absolute recoveries of most PBDE congeners and ISs were in acceptable range (Table 1), while the lowest recoveries (between 64% and 74%) were observed for ^{13}C-BDE 209 and BDE 183, followed by BDE 154. The lower recoveries observed for higher brominated PBDE congeners are probably due to poor desorption from the SPE cartridge because of high lipophilicity of these compounds and/or strong π–π interactions with the sorbent. More polar solvents (e.g. acetone or methanol) could not be used for the elution of PBDEs from the SPE cartridge due to their incompatibility with the subsequent acid silica clean-up. Similar recoveries on OASIS^{TM} HLB with those observed in the present study were obtained by Sjödin et al. [14] for which mean recoveries of the ^{13}C-labelled ISs ranged from 69% to 95% for the PBDEs (except BDE 209) and BB 153.

The recoveries of analytes relative to the ISs used for their quantification on the four tested sorbents are given in Table 2. For C_{18} Empore^{TM}, Isolute Phenyl, and Isolute-ENV+, a strong

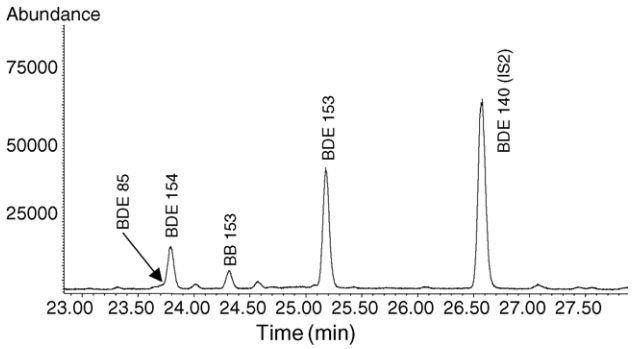

Fig. 2. Partial chromatogram (ions *m/z* 79 and 81 monitored) of a spiked serum on HT-8 (25 m × 0.22 mm × 0.25 μm) showing baseline separation of BDE 154 (1.5 pg/ml) and BB 153 (0.8 pg/ml).

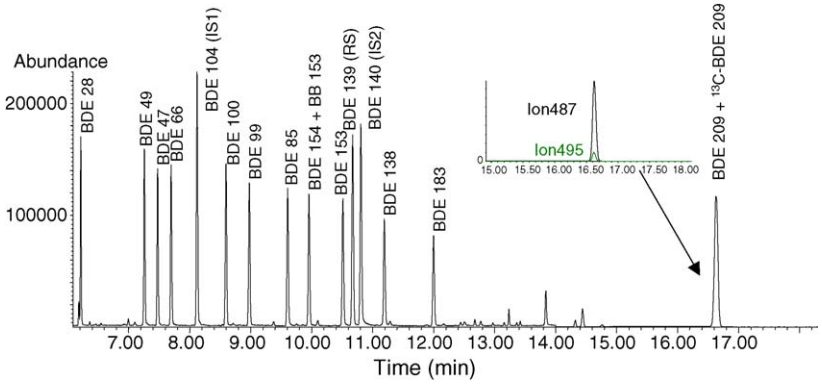

Fig. 1. Total ion chromatogram (ions *m/z* 79 and 81 together) on AT-5 (12 m × 0.18 mm × 0.10 μm) of a spiked serum sample after SPE and clean-up. The serum sample was spiked with 80 pg/ml serum of all analytes except BDE 209, which was spiked with 250 pg/ml. Ions *m/z* 487 and 495 were monitored for BDE 209 and ^{13}C-BDE 209, respectively.

Table 2
Relative recoveries and relative standard deviation (in parentheses) of target analytes based on internal standard calibration using different SPE cartridges

Analyte	C_{18} Empore™	Isolute-phenyl	Isolute-ENV+	OASIS™ HLB
BDE 28	153 (4)	156 (5)	114 (7)	96 (9)
BDE 47	141 (1)	143 (10)	140 (15)	109 (3)
BDE 49	130 (3)	124 (3)	131 (12)	95 (4)
BDE 66	121 (2)	101 (5)	123 (15)	95 (3)
BDE 85	111 (3)	100 (6)	135 (6)	106 (11)
BDE 99	103 (1)	91 (12)	119 (6)	102 (4)
BDE 100	98 (1)	84 (8)	105 (1)	95 (5)
BDE 138	106 (6)	99 (11)	130 (3)	94 (5)
BDE 153	99 (5)	90 (12)	115 (1)	88 (6)
BDE 154	100 (8)	97 (9)	108 (4)	81 (5)
BDE 183	67 (3)	65 (15)	88 (2)	68 (8)
BDE 209	104 (5)	110 (10)	97 (6)	98 (6)
BB 153	95 (5)	94 (9)	112 (3)	90 (5)

BDE 28, 47, 49, 66, 85, 99 and 100 were calculated based on BDE 104, while BDE 138, 154, 153, 183, together with BB 153 were calculated based on BDE 140. Recoveries are given as percentages (%). Each experiment was done in triplicate.

tendency of decreasing recoveries with the increasing number of bromine atoms was observed (Tables 1 and 2). Such trend was less evident for the OASIS™ HLB, for which only BDE 153, BDE 154 and BDE 183 presented lower recoveries than its corresponding IS (Table 2). To compensate for the difference in the absolute recoveries of each congener and its corresponding IS (BDE 140), the concentrations of BDE 153, BDE 154 and BDE 183 measured in individual samples were corrected with 10%, 20% and 30%, respectively.

3.3. Procedural blanks and limits of quantification

A low level of contamination of procedural blanks has been obtained by minimizing the number of steps and glassware used for the analysis. Only BDE 47, 99 and 209 were detected at higher, but consistent (R.S.D. < 25%) levels in the procedural blanks. For these PBDE congeners, the limit of quantification (LOQ) was dependent on the procedural blanks and was calculated as 3 × S.D. of the blank value [23]. For the remaining PBDE congeners and BB 153, which were not detected or at very low levels in the procedural blanks, the LOQs were calculated in the spiked serum samples on the basis of a signal-to-noise ratio (S/N) of 10. For calculation of concentrations in the samples, the value of each PBDE congener in the procedural blank was subtracted from the corresponding value in the sample and the resulting value was compared to the LOQ calculated for each congener. LOQs ranged between 0.2 and 1.5 pg/ml for BB 153 and all PBDEs except BDE 209, for which LOQ was 25 pg/ml, and were in the range of LOQ values previously reported for PBDEs [13]. Expressed on a lipid weight basis, the LOQs ranged between 0.1 and 4 ng/g lipid weight (the latter corresponding to BDE 209).

Table 3
Within- and between-day precision and accuracy of the method developed for the determination of PBDEs and BB 153 in human serum which includes OASIS™ HLB SPE and multilayer clean-up cartridge

Analyte	Within-day precision (%)	Between-day precision (%)	Accuracy (%)
BDE 28	5	10	−4
BDE 47	5	9	+9
BDE 49	2	6	−5
BDE 66	3	7	−5
BDE 85	2	5	+6
BDE 99	4	6	+2
BDE 100	3	5	−5
BDE 138	6	6	+6
BDE 153	4	4	−11
BDE 154	4	9	−18
BDE 183	10	12	−32
BB 153	3	3	−10
BDE 104 (IS1)	7	8	−16
BDE 140 (IS2)	5	5	−5
^{13}C-BDE 209 (IS3)	15	17	−36

Validation was done at single fortification level 80 pg/ml serum for all PBDEs, except BDE 209 spiked at 250 ng/ml serum. Each experiment was done in triplicate.

Table 4
Linear regression equations and correlation coefficients for calibration curves of each PBDE congener

Analyte	Linear regression equation	Correlation coefficient (R^2)
BDE 28	$y = 0.90 \times x - 9.7 \times 10^{-4}$	1.000
BDE 47	$y = 0.87 \times x + 1.0 \times 10^{-3}$	0.999
BDE 49	$y = 1.22 \times x + 2.5 \times 10^{-4}$	0.998
BDE 66	$y = 1.00 \times x - 5.4 \times 10^{-4}$	0.998
BDE 85	$y = 0.82 \times x + 1.9 \times 10^{-3}$	0.999
BDE 99	$y = 0.89 \times x + 9.8 \times 10^{-4}$	0.998
BDE 100	$y = 1.05 \times x + 2.3 \times 10^{-3}$	0.999
BDE 138	$y = 0.90 \times x + 6.4 \times 10^{-4}$	0.999
BDE 153	$y = 1.11 \times x - 1.4 \times 10^{-3}$	0.999
BDE 154	$y = 1.24 \times x - 1.0 \times 10^{-3}$	0.999
BDE 183	$y = 0.92 \times x + 1.1 \times 10^{-3}$	0.998
BDE 209	$y = 1.24 \times x + 5.2 \times 10^{-3}$	0.999
BB 153	$y = 1.20 \times x + 2.9 \times 10^{-3}$	0.998

Five-points calibration curves (0.5–1000 pg/ml) were created as the ratios between peak areas of analytes and the corresponding internal standards plotted against the corresponding concentration ratios.

Table 5
Concentrations of PBDE congeners and BB 153 in 4 pooled cord serum and 11 individual serum samples from Belgium (1999–2004)

	Cord 1	Cord 2	Cord 3	Cord 4	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5	Serum 6	Serum 7	Serum 8	Serum 9	Serum 10	Serum 11
Lipids (g/l)	2.63	2.62	2.60	2.60	4.19	6.45	5.11	6.11	7.02	7.24	5.60	7.80	6.11	6.34	5.77
Serum (pg/ml)															
BDE 28	<0.2	<0.2	<0.2	<0.2	0.5	<0.2	0.8	0.3	<0.2	0.5	0.3	2.5	0.8	0.4	0.4
BDE 47	3.6	3.3	2.9	3.2	12.9	3.2	10.4	3.4	1.7	8.5	7.7	5.1	5.2	8.6	10.6
BDE 100	0.3	0.3	0.4	0.5	1.5	1.7	1.7	1.1	0.5	1.3	1.0	1.9	11.6	1.7	1.9
BDE 99	1.7	<0.9	1.7	<0.9	4.3	<1.3	2.8	<1.3	1.3	2.1	1.6	<1.3	1.5	2.6	3.2
BDE 153	1.6	1.2	2.2	1.6	6.5	9.1	10.7	17.3	6.9	10.0	7.6	8.9	18.8	10.6	9.1
BDE 183	<0.3	<0.3	<0.3	<0.3	0.9	<0.5	1.1	<0.5	<0.5	1.3	1.3	0.9	1.9	2.6	1.5
Sum PBDEs[a]	7.5	5.5	7.4	6.0	35.5	15.1	27.5	23.1	10.8	23.7	19.6	20.0	39.8	26.5	26.6
BDE 209	56	122	72	74	64	55	135	58	28	26	62	61	94	107	191
BB 153	<0.3	<0.3	<0.3	<0.3	1.6	3.9	5.0	1.5	4.3	3.0	3.9	5.8	10.0	7.5	4.2
Lipid weight (ng/g)															
BDE 28	<0.10	<0.10	<0.10	<0.10	0.12	<0.10	0.15	<0.10	<0.10	<0.10	<0.10	0.32	0.13	<0.10	<0.10
BDE 47	1.38	1.26	1.11	1.22	3.07	0.50	2.03	0.56	<0.40	1.17	1.38	0.65	0.85	1.36	1.83
BDE 100	0.13	0.10	0.15	0.19	0.35	0.27	0.34	0.18	<0.10	0.18	0.18	0.25	1.90	0.27	0.33
BDE 99	0.64	<0.40	0.65	<0.40	1.02	<0.40	0.56	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	0.40	0.55
BDE 153	0.62	0.46	0.84	0.61	1.55	1.42	2.09	2.83	0.99	1.38	1.36	1.15	3.07	1.68	1.57
BDE 183	<0.20	<0.20	<0.20	<0.20	0.21	<0.20	0.21	<0.20	<0.20	<0.20	0.22	<0.20	0.30	0.41	0.27
Sum PBDEs[a]	2.88	2.10	2.87	2.31	6.32	2.35	5.37	3.78	1.55	3.27	3.49	2.56	6.50	4.19	4.61
BDE 209	22.5	46.5	27.5	28.6	15.4	8.6	26.5	9.5	4.0	3.6	11.1	7.8	15.4	16.9	33.1
BB 153	<0.15	<0.15	<0.15	<0.15	0.38	0.60	0.97	0.25	0.61	0.41	0.69	0.75	1.63	1.18	0.73

To facilitate comparison with other studies, the results were expressed in pg/ml and ng/g lipid weight. For calculation of the sum, concentrations below LOQ were set to 1/2LOQ.

[a] All congeners except BDE 209.

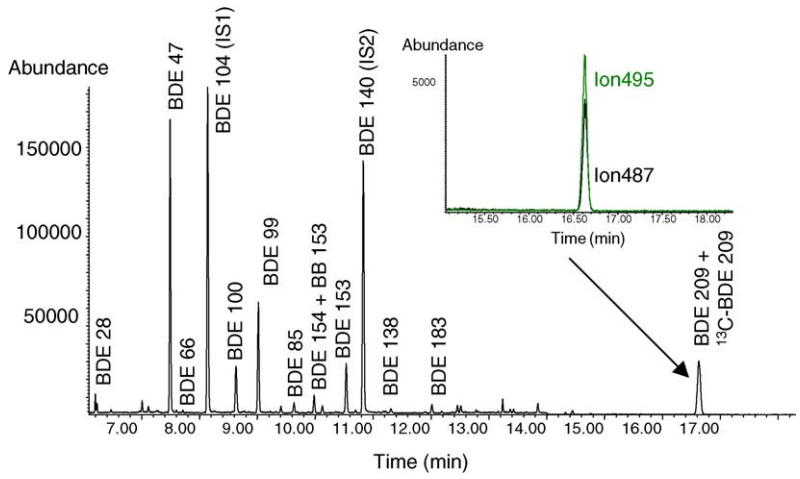

Fig. 3. Total ion chromatogram (ions *m/z* 79 and 81 together) on AT-5 (12 m × 0.18 mm × 0.10 μm) of a non-spiked pooled serum sample after SPE and clean-up. Note that BDE 154 and BB 153 co-elute on AT-5. Ions *m/z* 487 and 495 were monitored for BDE 209 and ^{13}C-BDE 209, respectively.

3.4. Validation

The precision was established by repeated determinations ($n = 3$) using serum spiked with 80 pg/ml for all PBDE congeners except BDE 209 spiked at 250 ng/ml serum. The precision was expressed as the relative standard deviation (R.S.D.). The use of OASIS^{TM} HLB SPE cartridge allowed a good repeatability with a within- and between-day precision of less than 10% and 12%, respectively, except for BDE 209 for which precision was 15% and 17%, respectively (Table 3). The accuracy was estimated by analysing the pooled serum samples ($n = 3$) spiked at a concentration level of 80 pg/ml serum. The values of PBDE congeners in the non-fortified serum sample were subtracted in each case. The highest deviations from were observed for ^{13}C-BDE 209, followed by BDE 183, BDE 154 and BDE 153 (Table 3). High correlation coefficients ($R^2 > 0.998$) were obtained for the inverse square of concentration-weighted linear regressions constructed for each analyte in the concentration range expected in human serum samples (0.5–1000 pg/ml). The regression equations for individual PBDE congeners are presented in Table 4 and it can be seen that the variation interval of the slopes had a very narrow range (between 0.82 and 1.24).

3.5. Analysis of individual human serum samples

The method has been used for the determination of PBDEs and BB 153 in serum samples collected from Belgian population. The results expressed in pg/ml serum as well as ng/g lipid weight are presented in Table 5 and a typical chromatogram obtained for sample no. 1 is given in Fig. 3. In all samples, some PBDE congeners were under LOQ (BDE 49 and 66 < 0.2 pg/ml, BDE 85, 154 and 138 < 0.3 pg/ml.

The measured levels of PBDEs (except BDE 209) in the Belgian serum specimens were in the same range with levels measured in Belgian human serum [24], human adipose tissue [25] or human milk [26]. They are also in the same range as previously reported PBDE levels in serum samples from Norway [8], Sweden [7,10], The Netherlands [27], but lower than levels reported from USA [28].

The presented method for the determination of PBDEs in human serum using SPE and GC/ECNI-MS is fast, simple, consumes lower amounts of solvents, demands less equipment and presents a low risk of sample contamination compared to traditional methods based on LLE. Up to 24 samples can be prepared within a day by one analyst. The method shows sufficient sensitivity and provides repeatable quantification in a wide concentration range for all investigated analytes.

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