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Automated solid-phase extraction for the determination of polybrominated diphenyl ethers and polychlorinated biphenyls in serum—application on archived Norwegian samples from 1977 to 2003

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Abstract

An analytical method comprised of automated solid-phase extraction and determination using gas chromatography mass spectrometry (single quadrupole) has been developed for the determination of 12 polybrominated diphenyl ethers (PBDEs), 26 polychlorinated biphenyls (PCBs), two organochlorine compounds (OCs) (hexachlorobenzene and octachlorostyrene) and two brominated phenols (pentabromophenol, and tetrabromobisphenol-A (TBBP-A)). The analytes were extracted using a sorbent of polystyrene-divinylbenzene and an additional clean-up was performed on a sulphuric acid–silica column to remove lipids. The method has been validated by spiking horse serum at five levels. The mean accuracy given as recovery relative to internal standards was 95%, 99%, 93% and 109% for the PBDEs PCBs, OCs and brominated phenols, respectively. The mean repeatability given as RSDs was respectively 6.9%, 8.7%, 7.5% and 15%. Estimated limits of detection (S/N = 3) were in the range 0.2–1.8 pg/g serum for the PBDEs and phenols, and from 0.1 pg/g to 56 pg/g serum for the PCBs and OCs. The validated method has been used to investigate the levels of PBDEs and PCBs in 21 pooled serum samples from the general Norwegian population. In serum from men (age 40–50 years) the sum of seven PBDE congeners (IUPAC No. 28, 47, 99, 100, 153, 154 and 183) increased from 1977 (0.5 ng/g lipids) to 1998 (4.8 ng/g lipids). From 1999 to 2003 the concentration of PBDEs seems to have stabilised. On the other hand, the sum of five PCBs (IUPAC No. 101, 118, 138, 153 and 180) in these samples decreased steadily from 1977 (666 ng/g lipids) to 2003 (176 ng/g lipids). Tetrabromobisphenol-A and BDE-209 were detected in almost all samples, but no similar temporal trends to that seen for the PBDEs were observed for these compounds, which might be due to the short half-lives of these brominated flame retardants (FR) in humans.

Keywords: PBDEs; PCBs; Automated solid-phase extraction; Method development; Validation; Human serum levels

1. Introduction

Flame retardants (FR) are chemicals that are added to materials during or after manufacture, to prevent or reduce the development of a fire. Worldwide, the total production of flame retardants was estimated to be 600,000 tonnes in 1992 [1]. The total market demand of brominated flame retardants (BFRs) in 2001 was 203,790 tonnes [2]. Tetrabromobisphenol-A (TBBP-A) is produced in the largest amounts, followed by polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) [2]. There is no domestic production of BFRs in Norway.

Flame retardants may be either chemically bound to (reactive) or mixed with (additive) polymeric materials. TBBP-A is used both reactively (90%) and additively, while the PBDEs are only used additively due to the lack of binding sites in the molecules. The primary use of TBBP-A is as a reactive intermediate in the manufacture of flame-retarded epoxy and polycarbonate resins, accounting for approximately 90% of all TBBP-A used. Other phenolic compounds used as FRs are 2,4,6-tribromophenol (TriBP) and pentabromophenol (PeBP), which are used in epoxy resins, phenolic resins and as intermediates for polyester resins [3]. There are theoretically 209 PBDE congeners, with one to ten bromine substituents. The congeners are numbered from 1 to 209 using the system established for PCBs [4]. PBDEs are incorporated in acrylonitrile-butadiene-styrene (ABS), high-impact polystyrene (HIPS), flexible polyurethane foams and textile coatings. These different types of flame retarded polymer

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products are present in a number of consumer products, such as computers, household appliances, electronics and TV-sets. PBDEs are also incorporated into products as paints, circuit boards, floor mats, carpets and furniture.

PCBs have been used commercially since 1930 as dielectric and heat-exchange fluids and in a variety of other applications [5]. In recent years, the production of PCBs have been phased out [5]. Current sources of PCB release include volatilisation from landfills, sewage sludge, spills, dredge spoils and improper (or illegal) disposal.

The toxicity of PCBs and BFRs has been reviewed [5–7]. Both PCBs and PBDEs have the potential to cause negative effects on human health, and estrogenic and antiestrogenic activity [8], neurotoxic effects [9] and effects on the thyroid hormone system [10,11] have been shown.

PBDEs and PCBs have been shown to be ubiquitous environmental pollutants, and are presently considered as persistent organic pollutants (POPs). Their levels in humans and the environment have been extensively reviewed [5,12–14]. While the levels of PCBs have been reported to decrease over the years [15,16], a study of PBDEs in breast milk from Swedish women showed an increase in the PBDE levels from 1972 to 1998, after which the levels seem to decrease [17]. Increasing levels of PBDEs have also been reported in serum [18]. However, there is still need for thorough studies investigating both geographic and temporal trends and also analyses of large scale sample series to reveal exposure pathways, vulnerable groups of high exposure and links to possible health effects.

Analytical methods used for determination of PBDEs are often very similar to those of PCBs, and have recently been reviewed [19]. The separation from biological and environmental matrix was earlier typically performed by extraction with organic solvents as in liquid–liquid extraction (LLE) [20]. More recently methods utilising solid-phase extractions (SPE) have been applied and several sorbents have been tested and successfully used for extraction of BFRs from breast milk and serum [21–26]. SPE has the advantages of reduced solvent consumption and processing time, high reproducibility, and ease of miniaturisation and automation compared to LLE techniques. Chromatography (e.g. on gel permeation, silica gel, Florisil, activated carbon) is often used to remove matrix components. Lipids can be removed by destructive methods such as treatment with concentrated sulphuric acid, or by non-destructive methods such as gel permeation. The separation of BFRs and PCBs are accomplished by GC techniques. Long capillary columns are normally used, but the higher brominated PBDEs are often separated on shorter columns because of susceptibility of thermal degradation. The most common approach for identification and quantification is to use MS techniques, in either electron ionisation (EI) or electron capture negative ionisation (ECNI) mode, or to use high resolution MS (HRMS).

The main objective of this study was to develop a fast, selective and sensitive method for determination of halogenated organic pollutants in human serum, to be able to perform large-scale analysis series efficiently. This was achieved by modifying a previously described method based on manual SPE and on-column lipid decomposition [25,27], and transferring it to an automated unit. The second objective was to use this method to study the temporal trends and the role of age of POPs in pooled serum samples from the general Norwegian population.

2. Experimental

2.1. Materials and reagents

An overview of analytes, their abbreviation, respective I.S. and manufacturer is given in Table 1. The purity of the BFR and PCB standard solutions was $\geq$ 98% and $\geq$ 99%, respectively.

All solvents were of pesticide grade, and were purchased from LabScan (Dublin, Ireland). Sulphuric acid (H_2SO_4) and formic acid of analytical grade, Silica gel 60 (0.063–0.200 mm) for column chromatography and sodium sulphate (Na_2SO_4) for organic trace analysis was obtained from Merck (Darmstadt, Germany). Ethanol was obtained from Arcus (Oslo, Norway). *N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald) was purchased from Aldrich (Milwaukee, WI, USA). Water was purified using an Elga Option 4 Water Purifier device (Elga, Bucks, UK).

All glassware was washed in 2.5% RBS 25 foaming cleaner (Chemical Products, Brussels, Belgium) rinsed with distilled water, and subsequently heated at 450 °C for 4 h (volumetric equipment was not heated).

2.2. SPE columns and equipment

Oasis® HLB custom-made solid-phase extraction cartridges (540 mg/3 ml) were purchased from Waters Corporation (Milford, MA, USA). For preliminary method development, Isolute 101 (200 mg/3 ml) obtained from International Sorbent Technology (Mid Glamorgan, UK) and Strata-X (60 mg/3 ml) from Phenomenex (Torrance, CA, USA) were used. More information on the structures of these adsorbents is described in Liane [28].

The columns for additional clean-up were prepared using empty SPE tubes of polyethylene from Sigma–Aldrich (St. Louis, MO, USA). Silica gel 60 rinsed with methanol and dichloromethane was activated overnight at 130 °C and mixed with concentrated sulphuric acid in the ratio 3:1 (w/v). The sodium sulphate was dried at 600 °C overnight. Sulphuric acid–silica (1.6 g) and sodium sulphate (1.0 g) were then filled into the SPE tubes in 4 layers, starting with sulphuric acid–silica and ending with sodium sulphate.

The solid-phase extraction and clean-up was performed using an ASPEC XL4 from Gilson (Middleton, WI, USA). ASPEC XL4 is a rapid and robust 4-channel system, each channel being equipped with its own syringe pump and needle. Four 2-way communal solvent ports permit the method to select from 8 solvent bottles by switching the ports. In addition solvents placed in tubes on the sample tray can be used.

2.3. Samples

2.3.1. Samples for validation and quality control

For method validation, horse serum from Sigma–Aldrich (H-1270) was used because we expected the content of the analytes to be lower in horse serum compared to human serum. The serum

Table 1
An overview of analytes, their abbreviation (abbr.), respective I.S. and manufacturer given as superscript

Analyte	Abbr.	I.S.	Analyte	Abbr.	I.S.
Hexachlorobenzene[a]	HCB	^{13}C-HCB	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl[b]	CB-180	^{13}C-CB-180[a]
2,2′,5-Trichlorobiphenyl[b]	CB-18	^{13}C-CB-28[a]	2,2′,3,4,4′,5′,6-Heptachlorobiphenyl[b]	CB-183	^{13}C-CB-180[a]
2,4,4′-Trichlorobipenyl[b]	CB-28	^{13}C-CB-28[a]	2,2′,3,4′,5,5′,6-Heptachlorobiphenyl[b]	CB-187	^{13}C-CB-180[a]
2,2′,5,5′-Tetrachlorobiphenyl[b]	CB-52	^{13}C-CB-52[a]	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl[b]	CB-189	^{13}C-CB-189[a]
Octachlorostyrene[a]	OCS	^{13}C-CB-101[a]	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl[b]	CB-194	^{13}C-CB-194[a]
2,3′,4,4′-Tetrachlorobipenyl[b]	CB-66	^{13}C-CB-101[a]	2,2′,3,3′,4,4′,5,6,6′-Nonachlorobiphenyl[b]	CB-207	Used as recovery standard
2,4,4′,5-Tetrachlorobiphenyl[b]	CB-74	^{13}C-CB-101[a]	Decachlorobiphenyl[b]	CB-209	^{13}C-CB-209[a]
3,4,4′,5-Tetrachlorobiphenyl[b]	CB-81	^{13}C-CB-101[a]	2,4,4′-Tribromdiphenyl ether[a]	BDE-28	BDE-18 (2,2′,5-Tribromodiphenyl ether)[b]
2,2′,4,4′,5-Pentachlorobiphenyl[b]	CB-99	^{13}C-CB-101[a]	3,4,4′-Tribromodiphenyl ether[a]	BDE-37	BDE-18 (2,2′,5-Tribromodiphenyl ether)[b]
2,2′,4,5,5′-Pentachlorobiphenyl[b]	CB-101	^{13}C-CB-101[a]	2,2′,4,4′-Tetrabromodiphenyl ether[a]	BDE-47	BDE-51 (2,2′,4,6′-Tetrabromodiphenyl ether)[b]
2,3,3′,4′,6-Pentachlorobiphenyl[b]	CB-110	^{13}C-CB-101[a]	2,2′,4,4′,5-Pentabromodiphenyl ether[a]	BDE-99	BDE-103 (2,2′,4,5′,6-Pentabromodiphenyl ether)[b]
2,3,3′,4,4′-Pentachlorobiphenyl[b]	CB-105	^{13}C-CB-105[a]	2,2′,3,4,4′-Pentabromodiphenyl ether[a]	BDE-85	BDE-103 (2,2′,4,5′,6-Pentabromodiphenyl ether)[b]
2,3,4,4′,5-Pentachlorobiphenyl[b]	CB-114	^{13}C-CB-114[a]	2,2′,4,4′,6-Pentabromodiphenyl ether[c]	BDE-100	BDE-103 (2,2′,4,5′,6-Pentabromodiphenyl ether)[b]
2,3′,4,4′,5-Pentachlorobiphenyl[b]	CB-118	^{13}C-CB-118[a]	2,3′,4,4′,6-Pentabromodiphenyl ether[a]	BDE-119	BDE-103 (2,2′,4,5′,6-Pentabromodiphenyl ether)[b]
2′,3,4,4′,5-Pentachlorobipenyl[b]	CB-123	^{13}C-CB-123[a]	2,2′,4,4′,5,5′-Hexabromodiphenyl ether[a]	BDE-153	BDE-103 (2,2′,4,5′,6-Pentabromodiphenyl ether)[b]
2,2′,3,4,4′,5′-Hexachlorobiphenyl[b]	CB-138	^{13}C-CB-138[a]	2,2′,4,4′,5,6′-Hexabromodipenyl ether[a]	BDE-154	BDE-103 (2,2′,4,5′,6-Pentabromodiphenyl ether)[b]
2,2′,4,4′,5,5′-Hexachlorobipenyl[b]	CB-153	^{13}C-CB-153[a]	2,2′,3,4,4′,5′-Hexabromodiphenyl[b]	BDE-138	BDE-156 (2,3,3′,4,4′,5-Hexabromodiphenyl ether)[c]
2,3,3′,4,4′,5-Hexachlorobiphenyl[b]	CB-156	^{13}C-CB-156[a]	2,2′,3,4,4′,5′,6-Heptabromodipenyl ether[a]	BDE-183	BDE-181(2,2′,3,4,4′,5,6-Heptabromodiphenyl ether)[a]
2,3,3′,4,4′,5′-Hexachlorobiphenyl[b]	CB-157	^{13}C-CB-157[a]	Decabromodiphenyl ether[a]	BDE-209	^{13}C-BDE-209[c]
2,2′,3,3′,4,4′-Hexachlorobiphenyl[b]	CB-128	^{13}C-CB-167[a]	2,4,6-Tribromophenol[d]	TriBP	TBCr (tetrabromo-*o*-cresol)[d]
2,3′,4,4′,5,5′-Hexachlorobiphenyl[b]	CB-167	^{13}C-CB-167[a]	Pentabromophenol[e]	PeBP	TBCr (tetrabromo-*o*-cresol)[d]
2,2′,3,3′,4,4′,5-Heptachlorobiphenyl[b]	CB-170	^{13}C-CB-170[a]	Tetrabromobisphenol-A[a]	TBBP-A	CtriBBP-A (chlorotribromobisphenol A)[f]

[a] Cambridge Isotope Laboratories (Andover, MA, USA).
[b] AccuStandard Inc. (New Haven, CT, USA).
[c] Wellington Laboratories (Guelph, Ontario, Canada).
[d] Aldrich (Milwaukee, WI, USA).
[e] Acros (Geel, Belgium).
[f] Gift from the Wallenberg Laboratory (University of Stockholm).

was homogenised by sonication in a Branson 2510E-MT ultrasonic cleaner for 5 min. The serum was then divided into ten sub-samples of 50 ml each, and stored at −18 °C until analysis. As procedural blanks five millilitres of purified, sterilised water containing 0.9% sodium chloride was used. As control samples for the PCBs, three samples from an interlaboratory comparison study administrated by Institute national de santé publique du Québec, Arctic Monitoring and Assessment Programme (AMAP) was used. These samples were pooled human serum spiked with PCBs at different levels.

2.3.2. Sample pools from Norway

A study was performed on two series of pooled serum samples from a local bio-bank at the Norwegian Institute of Public Health. The serum had been sampled from different county hospitals in Norway yearly since 1975 and stored at −20 °C. The first series was restricted to men in the age group 40–50 years, to limit variation of body burden with gender and age. Five hundred microlitres of serum from each individual ($n \sim 20$) was pooled and stored at −20 °C. In the 1997 pool, serum from 14 individuals was sampled because of limited sample volume available from this year. Serum samples from 2002 were chosen for study on BFRs and PCBs levels in groups with different age and gender. In the serum bank the individuals had been divided into eight age groups, serum from 20 individuals was pooled in each age group. For the 0–4 years old where limited sample volume was available, only 250 μl was taken from each individual, 29 individuals in total. The lipids were determined enzymatically at The National Hospital of Norway (Oslo, Norway) and the total lipid content of the samples calculated according to the method described by Grimvall et al. [29].

2.4. Preparation of standard solutions

Stock solutions and dilutions from stock solutions were made volumetrically. Mixtures of analytes were made at eight levels for PBDEs and phenols in a concentration range 0.1–40 pg/μl for all PBDEs except BDE-209, which were in the concentration range 1–400 pg/μl. For the phenols the concentrations were in the range 0.05–29 pg TriBP/μl, 0.1–58 pg PeBP/μl and 0.2–116 pg TBBP-A/μl. Analyte mixtures of PCBs were made at twelve levels in a concentration range 0.1–490 pg/μl (0.1–500 pg/μl for mono-ortho PCBs). The PCB mixture also contained the following ^{13}C internal standards: HCB, CB-28, CB-52, CB-101, CB-123, CB-118, CB-114, CB-153, CB-105, CB-138, CB-167, CB-180, CB-156, CB-157, CB-170, CB-189, CB-194 and CB-209 with concentration 10 pg/μl for all except HCB which was 15 pg/μl.

The internal standard solution for PBDE consisted of BDE-18, BDE-51, BDE-103, BDE-156, BDE-181 and ^{13}C-BDE-209 with concentration 2.5 pg/μl for all compounds except ^{13}C-BDE-209 which was 25 pg/μl. I.S. for phenols consisted of 1 pg/μl TBCr and 3 pg/μl CtriBBP-A. Two separate solutions of I.S. were made for PCBs, one for mono-ortho PCBs with concentration 5 pg/μl and the following ^{13}C congeners; CB-105, CB-114, CB-118, CB-123, CB-156, CB-157, CB-167 and CB-189 and another containing the following ^{13}C congeners: HCB, CB-28, CB-52, CB-101, CB-153, CB-138, CB-180, CB-170, CB-194 and CB-209 with concentration 20 pg/μl. As recovery standard, CB-207 was used with a concentration of 8.75 pg/μl.

The BFR standards (PBDEs and phenols) used for calibration were prepared by mixing 30 μl standard solution with 30 μl internal standard solution and 15 μl recovery standard. The PCB standards were prepared by mixing 30 μl standard solution (with I.S.) with 15 μl recovery standard.

2.5. Sample preparation

2.5.1. Homogenisation and dilution

The frozen serum samples were thawed in a refrigerator at 4 °C overnight and brought to ambient temperature. Approximately 5 g of each serum sample were weighed into separate tubes (15 ml with screw cap) and added I.S. and spiking solution in the validation experiment. The samples were then whirlmixed, sonicated for 5 min and placed in a refrigerator overnight to equilibrate. After bringing the samples to ambient temperature and whirlmixing for 5 min, 5 ml of formic acid/2-propanol (4:1, v/v) were added to denaturate the proteins. The samples were again sonicated for 5 min and stored in a dark place for 50 min. Finally 5 ml of water/2-propanol (4:1, v/v) was added to each tube to dilute the sample, followed by another 5 min of sonication.

2.5.2. Solid-phase extraction and clean-up

The solid-phase extraction was performed on the ASPEC XL4, and the procedure is outlined in Table 2. To eliminate impurities in the column material, the columns were pre-washed with methanol and dichloromethane/methanol (7:3, v/v). The columns were then conditioned with methanol and water/methanol (19:1, v/v) before the diluted sample was applied. The sample tubes were washed with water/methanol (19:1, v/v) to remove residual serum, which was then added to the columns. The columns were then washed with 2-propanol and dried for 45 min with nitrogen (99.999%, Aga). Three times during the drying process 20 μl of methanol/water (7:3, v/v) were added to facilitate extrusion of water from the micropores of the sorbent material. After drying, the analytes were eluted with dichloromethane/methanol (7:3, v/v). The eluates were evaporated to a few microlitres at 40 °C in a TurboVap LV (Zymark, Hopkinton, MA, USA) under a gentle stream of nitrogen (99.999%, Aga) and redissolved in 3.5 ml *n*-heptane/dichloromethane (3:1, v/v), before clean-up on sulphuric acid–silica columns. These columns were rinsed with heptane to remove air bubbles, and conditioned with *n*-heptane/dichloromethane (3:1, v/v). The redissolved extracts were applied and the eluate immediately collected in a glass tube. The initial tube was washed with *n*-heptane/dichloromethane (3:1, v/v), and then applied to the column. The columns were eluted with another portion of *n*-heptane/dichloromethane (3:1, v/v).

2.5.3. Derivatisation

The clean extracts eluted from the sulphuric acid–silica columns contain all the analytes. To be able to identify the presence of native methylated derivatives of the phenols (i.e.

Table 2
Procedures for extraction and clean-up performed on the ASPEC

Step	Process	Application	Flow (ml/min)
SPE using Oasis HLB columns			
1	Prewash	3 ml methanol	1.5
2	Prewash	3 ml dichloromethane/methanol (7:3, v/v)	1.5
3	Condition	5 ml methanol	1.5
4	Condition	5 ml water/methanol (19:1, v/v)	1.5
5	Load	15 ml diluted serum sample	0.4
6	Load	5 ml water/methanol (19:1, v/v)	0.4
7	Wash	10 ml water/2-propanol (19:1, v/v)	1.5
8	Dry	1.0 bar N_2 for 45 min	
9	Dry	3 × 20 μl methanol/water (7:3, v/v)	
10	Elute	12 ml dichloromethane/methanol (7:3, v/v)	0.4
Clean-up using sulphuric acid–silica columns			
1	Wash	10 ml *n*-heptane	1.0
2	Condition	3.0 ml *n*-heptane/dichloromethane (3:1, v/v)	1.0
3	Load and collect	3.7 ml sample	0.4
4	Load and collect	2.2 ml *n*-heptane/dichloromethane (3:1, v/v)	0.4
5	Elute	4.0 ml *n*-heptane/dichloromethane (3:1, v/v)	0.4

1,3,5-tribromo-2-phenoxybenzene, 1,3,4,5,6-pentabromo-2-phenoxybenzene and tetrabromobisphenol A dimethyl ether), the extract was analysed prior to derivatisation. Subsequently, the phenolic compounds were methylated by derivatisation of the hydroxy groups using diazomethane, synthesised by base-catalysed decomposition of *N*-methyl-*N*-nitroso-*p*-toluensulfonamid (diazald) [30]. The sample extracts were concentrated under a gentle stream of nitrogen to about 30 μl, added 25 μl of diazomethane, whirlmixed and left in a dark place at ambient temperature for 30 min. The excess of derivatisation reagent was evaporated by heating at 40 °C for approximately 15 min (or until the yellow colour disappeared).

2.6. Instrumentation and analysis

The chromatographic separation was performed on an HP (Avondale, PA, USA) 6890 GC equipped with an HP 7683 autosampler. The HP ChemStation B.02.05 and D.01.02 softwares operated the system. DB-5MS (5% phenyl 95% dimethyl polysilxane) columns with I.D. 0.25 mm from Agilent Technologies Inc. (CA, USA) were used. The column length was 60 m for determination of the PCBs, 25 m for PBDEs and phenols and 15 m for BDE-209. The film thickness was 0.25 μm except for BDE-209 (0.10 μm). A deactivated retention gap of 1.5 m × 0.32 mm I.D. fused silica (Agilent) was used in front of all the columns. Samples of 2 μl were injected at 290 °C in pulsed splittless mode with a pulsed pressure of 3.79 bar for 1.5 min. Helium (99.998%, Aga) was used as a carrier gas, and the flow was constant at 1.2 ml/min. The PCBs were separated using the following temperature program: initial column temperature 100 °C held for 1 min, 30 °C/min to 190 °C held for 1 min, 2 °C/min to 250 °C, 5 °C/min to 300 °C and finally 20 °C/min to 325 °C, which was held for 1 min. The temperature program used for the PBDEs and phenols has been described earlier [22], however a slightly slower temperature ramp was used for determination of BDE-209: initial column temperature 90 °C held for 1 min, 20 °C/min to 190 °C, 4 °C/min to 230 °C, 1 °C/min to 235 °C, 3.5 °C/min to 250 °C and finally 30 °C/min to 325 °C, which was held for 4 min.

The MS, an HP 5973 MSD was operated in electron capture negative ionisation mode (ECNI) for the determination of PBDEs, phenols and PCBs. In addition, four of the PCBs and HCB were determined in electron impact mode (EI), because of the minor abundance and low response of the molecular ions in CI-mode for these compounds. Methane (99.99%, Aga) was used as buffer gas in the ECNI mode. The ion source was held at a temperature of respectively 250 °C, 200 °C and 150 °C for the PBDEs/phenols, BDE-209 and PCBs (230 °C for PCBs in EI mode). The temperature of the GC–MS interface was 300 °C and the electron energy about 120 eV in ECNI mode and 69.9 eV in EI mode.

The analytes were identified by comparing their retention time in the samples with their respective retention time in the calibration standards. In ECNI mode, the brominated compounds were monitored at *m/z* 79.0 and 81.0. BDE-209 was monitored at *m/z* 484.6 and 486.6 and their abundance ratio was used for confirmation. The PCBs were monitored at two isotope masses of their molecular ions, i.e. *m/z* 256.0/258.0 (tri-CB), *m/z* 289.9/291.9 (tetra-CB), *m/z* 325.9/327.9 (penta-CB), *m/z* 359.8/361.8 (hexa-CB), *m/z* 393.8/395.8 (hepta-CB), *m/z* 427.8/429.8 (octa-CB), *m/z* 463.7 (nona-CB, recovery standard) and *m/z* 497.7/499.7 for (deca-CB). OCS was detected at 308.0. In EI mode HCB was monitored at *m/z* 283.8/285.8, CB-18 and CB-28 at *m/z* 256.0/258.0 and CB-52 and CB-66 at *m/z* 219.9/221.9/289.9/291.9. All compounds were quantified using peak areas of the measured ions obtained by integration in the HP ChemStation software and internal standard calibration. The isotope abundance ratio was used for identification.

2.7. Method validation

The presented method was validated according to guidelines from Tønseth and Døhl [31] and the International Conference of Harmonisation [32]. Seventeen horse serum samples were spiked at five levels in the range 1.2–120 pg PBDEs/g serum, 12–1200 pg BDE-209/g serum, 1.2–120 pg PeBP/g serum, 2.4–240 pg TBBP-A/g serum and 3–300 pg PCBs/g serum. A solvent exchange to ethanol was performed on the spiking solutions prior to the sample preparation, to reduce the volume of non-polar organic solvents applied on the SPE, which may cause low retention of lipophilic compounds. Each level was prepared in four replicates except level four which consisted of one repli-

cate. Four additional samples were extracted, three of them added I.S. The fourth non-spiked sample was used to verify that the horse serum did not contain any native concentrations of the I.S. In addition three procedural blanks were extracted. The samples were extracted in random order, in three batches over a period of two weeks.

The intermediate precision was investigated after two months by spiking four horse serum samples at an intermediate concentration level.

3. Results and discussion

3.1. General considerations

The automated sample preparation method presented here is a further development of our manual SPE method published earlier [27] leading to an increased sample throughput. Up to 24 serum samples can be extracted during 24 h, and 96 samples can be cleaned-up within the same time period. The ASPEC is designed to minimise carry-over of analytes, and the background levels found in this study were comparable with levels found in procedural blanks earlier [33].

During the method development three adsorbents were evaluated for extraction of the POPs from serum, i.e. Isolute 101, Oasis® HLB and Strata-X. Different solvents and solvent mixtures, volumes of eluent and amount of sorbent were tested. The highest absolute recoveries were obtained using the Strata-X column, followed by Oasis® HLB and Isolute 101. However, pressure problems were often encountered when performing extractions of serum using Strata-X columns due to the relative small particle and pore size of this sorbent, thus Oasis® HLB was chosen for validation and further use. Details on these experiments are described elsewhere [28].

In the second clean-up step columns containing sulphuric acid–silica (1.6 g) and sodium sulphate (1.0 g) were used. When splitting the sorbents into four alternating layers, the lipids were removed more efficiently than a one-layer column, i.e. 0.2 g lipids versus 0.15 g lipids. The use of more acidic sulphuric acid–silica was not found to be more efficient.

3.2. Method validation

3.2.1. Evaluation of internal standards

All PCBs were quantified using a ^{13}C-I.S. For the PBDEs this was not an option since the MS was operated in ECNI mode, so several BDE congeners were investigated as potential I.S. When two or more I.S. were found equally suited with respect to accurate results, the I.S. was chosen based on reproducibility, closeness in retention time and degree of bromination (Table 1). Prior to the study, a thorough literature search on BDE congeners found in different biological as well as human samples was performed, to make sure none of the evaluated BDE I.S. were likely to be present in the samples. We have also looked carefully into samples from our earlier studies where only two I.S. were used, and none of the I.S. evaluated above were found to be present [18,34].

3.2.2. Linearity

3.2.2.1. Calibration linearity. The linearity of the calibration curves was examined by the correlation coefficient, *r*. The area ratio between the analyte and I.S. was plotted against the corresponding concentration ratio. The standards were injected three times during an analysis sequence. The first and last injection was used in the calibration curve. The second injection was used to examine the chromatographic performance and stability of mass spectrometric response. Eight levels were used in the calibration curves for PBDEs and phenols, for the PCBs ten levels were used (see section 2.4 for range). Linear fit was used in all calibration curves except for the pesticide HCB and the phenols in the high concentration range, where a quadratic fit was used. All correlation values were in the range 0.989–1.000, with the lowest value accounting for the phenol PeBP.

3.2.2.2. Method linearity. The linearity of the method was examined by plotting the concentration of POPs found in the spiked samples against the concentration added. The native concentration of POPs in the horse serum was calculated, and this value was subtracted from the concentrations found in the spiked samples. Both the results from the initial validation and the second round when investigating the intermediate precision were incorporated when evaluating the linearity. As can be seen from the regression results presented in Table 3, the correlation coefficient were >0.99 for all compounds, and the variation interval of the slopes were narrow (between 0.62 and 1.17). Because of high native concentrations of some of the PCBs in the horse serum, the lowest levels could not be accurately quantified, and the regression data for these compounds are therefore not listed in Table 3.

3.2.3. Detection limits

The detection limits (LOD) of the different POPs, presented in Table 3, were estimated by extrapolating the concentration that would result in a S/N of 3. The S/N ratio was measured at the lowest spiked level in the validation study for all compounds except for the PCBs that were found to be present in high concentrations in the horse serum. For those, the concentration found in non-spiked horse serum and the corresponding S/N ratio were used. Using this approach, the contribution from the procedural blank is also taken into consideration. A non-spiked horse serum sample was also used for PCBs detected in EI mode, and these estimated LODs were from 14 pg/g to 56 pg/g serum.

3.2.4. Accuracy, precision and absolute recovery

The range, mean and RSD of the accuracy determined as recovery relative to the I.S. at the five spiking levels are presented in Table 4. Corrections were made for the native concentrations in the horse serum and contribution from the surroundings. The overall mean accuracy of the PBDEs was 95%, with a mean RSD of 6.9%. The main PBDE congeners found in the horse serum were BDE-47, BDE-99 and BDE-209, resulting in high RSDs for these compounds at low spiking levels. The overall mean accuracy of the PCBs was 99% and the mean RSD 8.7%. Some of the PCBs were also found in relatively high concentrations in the horse serum. Similarly, the mean accuracy of

Table 3
Linear regression data given as the slope (*a*), intercept (*b*) and correlation coefficient (*r*) obtained from the spiked validation samples ($n = 21$). Also presented is the estimated LOD in pg/g serum obtained in ECNI mode

Compounds	*a*	*b*	*r*	LOD	Compounds	*a*	*b*	*r*	LOD
BDE-28	0.8303	4.7361	0.9993	0.5	CB-105	1.0202	−0.7571	1.0000	0.7
BDE-37	0.7657	5.0026	0.9996	0.6	CB-110				5.9
BDE-47	0.6192	9.4487	0.9968	1.8	CB-114	0.9897	2.1158	0.9999	0.2
BDE-85	0.9190	2.7468	0.9999	0.3	CB-118				0.3
BDE-99	0.8101	4.1520	0.9999	0.5	CB-123	1.0042	−3.4690	0.9996	0.3
BDE-100	0.7857	4.6235	0.9998	0.6	CB-128	0.9542	−1.8163	1.0000	1.2
BDE-119	0.8141	4.2840	0.9994	0.7	CB-138				0.3
BDE-138	0.8554	3.5654	0.9997	0.3	CB-153				0.4
BDE-153	0.9207	0.3592	0.9999	0.2	CB-156	0.9934	3.4345	1.0000	0.2
BDE-154	0.9005	1.3140	1.0000	0.3	CB-157	0.9932	0.7285	1.0000	0.1
BDE-183	0.9904	−2.3724	0.9998	0.6	CB-167	0.9929	2.5612	1.0000	0.1
BDE-209	0.9171	79.6490	0.9995	0.9	CB-170	1.0416	−1.6148	1.0000	0.1
PeBP	1.1295	−4.6157	0.9994	0.7	CB-180	1.0212	−3.4718	0.9999	0.1
TBBP-A	1.1749	−4.2801	0.9998	1.1	CB-183	0.8828	8.4833	0.9999	0.1
OCS	0.9844	−1.5305	1.0000	0.5	CB-187	0.8771	8.3379	0.9999	0.1
CB-74				21	CB-189	1.0146	0.4028	0.9999	0.2
CB-81	1.0195	10.5710	0.9995	0.3	CB-194	1.0195	−1.6568	1.0000	0.1
CB-99				8.2	CB-209	1.0110	−1.2677	1.0000	0.1
CB-101				9.2					

the organochlorine compounds HCB and OCS and the phenols were acceptable, 93% and 109%, respectively. TriBP were also investigated in the validation, but high levels of this compound in the horse serum and procedural blanks made the quantification difficult.

The absolute recovery of the I.S. was calculated against a GC–MS recovery standard (CB-207) added to the validation samples ($n = 17$). The absolute recovery of the I.S. used for PBDEs were in the range 64–89% with an overall mean recovery of 79%. The overall RSD was 9%. TBCr and CtriBBP-A used as I.S. for the phenols had recoveries of 64% and 53%, respectively, with RSD of 8% (TBCr) and 35% (CtriBBP-A). The I.S. used for the PCBs and HCB had recoveries in the range 37–82%, with an overall mean recovery of 63% (RSD 9%). The most volatile PCBs and HCB account for the lowest recoveries. This is probably due to evaporation losses during the solvent exchange performed on the spiking solutions. Thus solvent exchange was omitted in the following application and higher absolute recoveries were obtained as expected.

3.2.5. Intermediate precision

Two months after the initial validation, four new horse serum samples were spiked at medium level, extracted and analysed as described in the experimental section. The POPs were only analysed in CI mode. The accuracy and repeatability were calculated and the results were comparable with the previous ones. For the PBDEs and the PCBs the RSD within each of the two data sets were not higher then the total RSD (both data sets included), which shows that the intermediate precision is satisfactory. The total RSD for the two data sets were in the range 5–11% for the individual PBDEs and 0.6–25% for the PCBs. For PeBP and TBBP-A the RSD within the two data sets were slightly higher then the total RSD. The total RSD for PeBP and TBBP-A were 24% and 21%, respectively.

3.2.6. Selectivity and retention stability

Throughout the chromatographic analysis of the validation samples, the compounds' retention time was highly stable and within ±0.1 min. All compounds were baseline separated except CB-138, which were eluted between two other chlorinated compounds, most likely CB-163 and CB-164. Only minor interferences were observed from matrix components. A chromatogram of one of the human serum samples from the application is shown in Fig. 1.

3.3. Analysis of pooled serum samples

3.3.1. Quality control

The method described above was used for the analysis of BFRs and PCBs in 21 pooled serum samples from the general population of Norway. In this study, the mean recoveries of the I.S. were 77%, 60% and 75% for the PBDEs, PCBs and CtriBBP-A, respectively. These recoveries were in close agreement with the results from the validation, except for CtriBBP-A where a higher recovery was obtained. The absolute recovery of all I.S. was above 40% in all samples, which were considered satisfactory. TBCr used as I.S. for TriBP and PeBP could not be correctly quantified because of a co-eluting compound.

To assess the quality of the quantification in this study, samples form an interlaboratory comparison were extracted and analysed. Five PCB congeners (CB-118, CB-138, CB-153, CB-170 and CB-180) were determined, and the results compared with the assigned values from the interlaboratory comparison study. The accuracy of the control samples was in the range 83–111%. Four procedural blanks were included when analysing the archived serum samples. BDE-47, BDE-99, BDE-209, CB-101, CB-118 and CB-153 were detected in all samples, and the limit of quantification (LOQ) was set to three times the blank concentration for these compounds. The LOQ for the other PBDEs and TBBP-A were 0.1 ng/g lipids, which is based on the

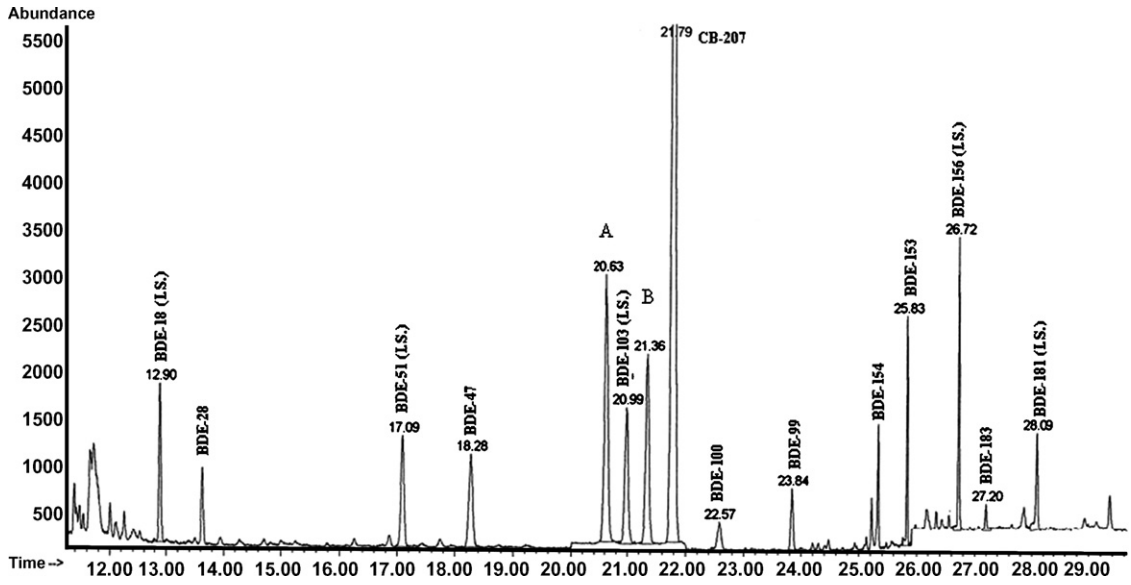

Fig. 1. Total ion chromatogram of the PBDEs of a pooled serum sample from men of age 40–50 years from 1998. Peak A and B are I.S. previously used.

lowest level in the calibration curve. Correspondingly the LOQ for the PCBs were 0.6 ng/g lipids. For calculation of the sum of PBDEs and PCBs the compounds detected <LOQ has been assigned the value ½ LOQ.

3.3.2. Trend studies

The serum concentration of seven PBDE congeners (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183) in pooled serum from men aged 40–50 years sampled from 1977 to 2003 are presented in Fig. 2. The objective of this study was to complete and extend a previous study on time trends of PBDEs in Norwegian pooled serum samples [18], whose results have been incorporated in the figure. The serum concentrations of the 7 PBDEs increased by a factor of about ten from 1977 (0.5 ng/g lipids) to 1998 (4.8 ng/g lipids), but seem to level out or decrease slightly after 1998 with the concentration varying between 3.8 ng/g lipids and 4.6 ng/g lipids (Fig. 2). This is in close agreement with recent trends observed in Swedish breast milk [17]. The temporal trend of the sum of seven PBDEs in the pooled serum from the present study are in accordance with the levels found in the previous study, except for the pools from 1991 and 2002, which were considerably higher than expected from results of preceding and following years. This was surprising as the pools contained at least 20 individual samples, and reasonably averaged levels were expected. However, in a study on 151 Norwegian breast milk samples we found that 5% of the samples had a sum of seven PBDEs exceeding five times the median [35]. In Table 5, the individual PBDE and PCB serum levels in the 21 analysed pools are presented. For samples obtained from different age groups in 2002, the mean of sum of seven PBDEs is 3.8 ng/g lipids (serum from the youngest group excluded) and 3.3 ng/g lipids in men of age 25–59 years. It is thus reasonable to suggest that the pool in the trend study contains serum from one or several persons with high PBDE level. Therefore this pool might not be representative for the average serum level in 2002. No duplicate of the pool from 1991 has been analysed.

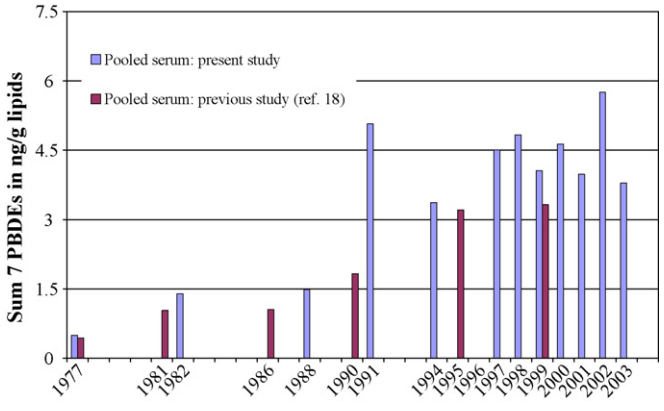

Fig. 2. The concentration of the sum of seven PBDEs (BDE 28, 47, 99, 100, 153, 154 and 183) in ng/g lipids in pooled serum samples from 40 to 50 year old Norwegian men sampled from 1977 to 2003. The two samples from 1977 are from the same pool, but the two samples from 1999 are from different pools.

The main PBDE congeners in all samples were BDE-47, BDE-99 and BDE-153. Closer studies revealed an increase in the relative amount of BDE-153 from the early 1990s, where the contribution from BDE-153 was 18% of sum seven PBDE, to 2003 where the contribution was 38%. In the most recent serum pools, relative levels of BDE-47 and BDE-153 were approximately equal. A similar change in PBDE-pattern has been observed in human samples from The Faroese Island and USA [36,37]. The reason for this is not fully understood yet, but possible explanations might be a higher persistence of BDE-153 compared to BDE-47, or a change in use of technical mixtures of PBDE.

The PCB levels were also investigated in the same serum pools. An opposite trend was observed compared to the PBDEs, with a decrease in the sum of five PCBs (CB-101, CB-118, CB-138, CB-153 and CB-180) from 1977 to 2003 (Fig. 3). The levels of PCB in human samples have been shown to be declining in several Nordic studies [15,16,38] which indicates that

Table 4
Mean accuracies (Acc.) and precision (RSD) of the different POPs found in spiked horse serum from the validation study[a]

Compounds	Level (pg/g)	*n*	Acc. (%)	RSD (%)	Compounds	Level (pg/g)	*n*	Acc. (%)	RSD (%)	Compounds	Level (pg/g)	*n*	Acc. (%)	RSD (%)
BDE-28	1.2	4	87	8.1	HCB[b]	3.0	4	78	15	CB-123	3.0	4	29	22
	2.4	4	102	8.0		6.0	4	62	14		6.0	4	69	12
	6.0	4	97	3.2		15	4	104	5.3		15	4	87	3.0
	18	1	99			60	1	97			60	1	106	
	120	4	82	8.8		300	4	97	1.9		300	4	102	1.4
BDE-37	1.2	4	97	11	OCS	3.0	4	89	6.2	CB-128	3.36	4	97	16
	2.4	4	98	1.5		6.0	4	100	10		6.72	4	94	10
	6.0	4	96	3.6		15	4	101	4.0		15.12	4	94	3.3
	18	1	90			60	1	98			60.48	1	96	
	120	4	83	8.1		300	4	102	3.2		302.4	4	97	1.7
BDE-47	1.2	4	64	4.4	CB-18[b]	3.36	4	n.a.[a]		CB-138	3.36	4	n.a.	
	2.4	4	94	17		6.72	4	n.a.	6.72		6.72	4	n.a.	
	6.0	4	99	6.5		15.12	4	n.a.	15.12		15.12	4	96	11
	18	1	89			60.48	1	65	60.48		60.48	1	94	
	120	4	71	7.9		302.4	4	88	11		302.4	4	96	1.4
BDE-85	1.2	4	101	3.0	CB-28[b]	3.36	4	n.a.		CB-153	3.36	4	n.a.	
	2.4	4	102	4.4		6.72	4	n.a.			6.72	4	n.a.	
	6.0	4	100	1.4		15.12	4	127	27		15.12	4	107	15
	18	1	99			60.48	1	97			60.48	1	104	
	120	4	92	2.7		302.4	4	97	3.7		302.4	4	103	0.6
BDE-99	1.2	4	109	25	CB-52[b]	3.36	4	n.a.		CB-156	3.0	4	108	6.1
	2.4	4	112	4.5		6.72	4	n.a.			6.0	4	109	1.8
	6.0	4	96	4.5		15.12	4	107	77		15	4	105	1.6
	18	1	90			60.48	1	98			60	1	102	
	120	4	84	2.2		302.4	4	88	8.6		300	4	101	0.7
BDE-100	1.2	4	100	3.4	CB-66[b]	3.36	4	105	15	CB-157	3.0	4	78	3.5
	2.4	4	101	3.3		6.72	4	76	20		6.0	4	83	10
	6.0	4	97	3.4		15.12	4	84	14		15	4	98	1.1
	18	1	88			60.48	1	101			60	1	102	
	120	4	81	1.5		302.4	4	106	4.3		300	4	101	1.1
BDE-119	1.2	4	95	3.4	CB-74	3.36	4	n.a.		CB-167	3.0	4	104	5.6
	2.4	4	91	3.5		6.72	4	n.a.			6.0	4	106	4.2
	6.0	4	94	3.5		15.12	4	94	5.1		15	4	101	2.2
	18	1	95			60.48	1	100			60	1	101	
	120	4	94	3.3		302.4	4	100	3.6		300	4	100	0.3
BDE-138	1.2	4	85	8.8	CB-81	3.36	4	88	11	CB-170	3.36	4	102	14
	2.4	4	97	2.5		6.72	4	103	10		6.72	4	105	5.0
	6.0	4	93	6.2		15.12	4	112	7.3		15.12	4	105	2.6
	18	1	97			60.48	1	113			60.48	1	103	
	120	4	87	1.2		302.4	4	105	1.7		302.4	4	104	0.6
BDE-153	1.2	4	96	4.4	CB-99	3.36	4	n.a.		CB-180	3.36	4	104	39
	2.4	4	99	2.8		6.72	4	n.a.			6.72	4	98	29

Table 4 (*Continued*)

Compounds	Level (pg/g)	*n*	Acc. (%)	RSD (%)	Compounds	Level (pg/g)	*n*	Acc. (%)	RSD (%)	Compounds	Level (pg/g)	*n*	Acc. (%)	RSD (%)
	6.0	4	98	2.2		15.12	4	97	11		15.12	4	103	4.4
	18	1	94			60.48	1	106			60.48	1	102	
	120	4	96	3.0		302.4	4	102	1.9		302.4	4	102	0.8
BDE-154	1.2	4	93	2.7	CB-101	3.36	4	n.a.		CB-183	3.36	4	106	16
	2.4	4	97	4.6		6.72	4	n.a.			6.72	4	109	7.1
	6.0	4	99	5.2		15.12	4	90	34		15.12	4	105	1.5
	18	1	95			60.48	1	114			60.48	1	93	
	120	4	90	0.6		302.4	4	102	1.9		302.4	4	91	2.3
BDE-183	1.2	4	101	2.0	CB-105	3.0	4	121	25	CB-187	3.36	4	107	37
	2.4	4	104	8.0		6.0	4	111	20		6.72	4	109	19
	6.0	4	98	7.4		15	4	107	1.5		15.12	4	106	7.5
	18	1	90			60	1	103			60.48	1	92	
	120	4	90	6.7		300	4	102	2.3		302.4	4	90	2.6
BDE-209	12	4	108	56	CB-110	3.36	4	n.a.		CB-189	3.0	4	79	3.8
	24	4	150	33		6.72	4	n.a.			6.0	4	96	3.5
	60	4	103	13		15.12	4	n.a.			15	4	100	0.4
	180	1	100			60.48	1	98			60	1	104	
	1200	4	95	1.3		302.4	4	112	4.5		300	4	102	0.5
PeBP	1.2	4	101	24	CB-114	3.0	4	99	2.6	CB-194	3.36	4	87	1.9
	2.4	4	119	14		6.0	4	102	4.3		6.72	4	97	2.4
	6.0	4	108	7.7		15	4	102	1.6		15.12	4	101	1.4
	18	1	99			60	1	102			60.48	1	102	
	120	4	104	5.9		300	4	101	1.1		302.4	4	102	0.5
TBBP-A	2.4	4	99	15	CB-118	3.0	4	n.a.		CB-209	3.36	4	90	3.8
	4.8	4	77	24		6.0	4	122	38		6.72	4	97	2.5
	12	4	123	21		15	4	100	14		15.12	4	100	1.4
	36	1	132			60	1	102			60.48	1	101	
	240	4	123	4.5		300	4	102	0.3		302.4	4	102	0.7

[a] Levels marked n.a. could not be accurately quantified because of high native concentrations of the compounds in the horse serum and/or procedural blanks.
[b] These compounds were determined in EI mode, the other PCBs and OCS were determined in CI mode.

Table 5
Serum concentrations of the BFRs and PCBs in ng/g lipids[a]. Compounds quantified below LOQ are presented as <LOQ

Serum pool	Lipids (%)	*n*	PBDE congeners									PCB congeners						TBBP-A[d]
			28	47	99	100	153	154	183	209	Sum 7[b]	101	118	138	153	180	Sum 5[c]	
1977	0.62	34	Nd	<0.60	<0.20	<0.1	<0.1	Nd	Nd	Nd	0.5	51	54	127	251	183	666	Nd
1982	0.72	18	Nd	0.84	0.20	0.13	0.18	Nd	<0.1	Nd	1.4	11	47	146	290	218	712	<0.1
1988	0.63	23	Nd	0.77	0.26	0.14	0.27	Nd	<0.1	<10	1.5	9.2	33	92	179	137	450	0.53
1991	0.72	19	0.16	2.8	0.65	0.32	0.92	<0.1	0.14	<10	5.1	8.5	20	60	122	98	308	1.0
1994	0.68	30	<0.1	1.8	0.48	0.31	0.65	Nd	<0.1	<10	3.4	5.6	15	52	108	96	277	0.66
1997	0.61	14	<0.1	2.4	0.56	0.36	1.1	<0.1	<0.1	<10	4.5	6.8	12	44	106	100	268	0.45
1998	0.66	20	<0.1	2.3	0.63	0.36	1.2	Nd	0.34	15	4.8	6.9	13	47	107	90	264	0.16
1999	0.72	20	0.18	1.7	0.53	0.27	1.2	<0.1	0.15	10	4.1	6.1	12	42	92	76	227	0.41
2000	0.60	20	<0.1	1.8	0.68	0.44	1.6	Nd	0.14	37	4.6	7.3	12	40	96	84	238	0.88
2001	0.62	20	0.25	1.5	0.35	0.27	1.5	Nd	0.13	<10	4.0	5.8	9.6	31	71	64	182	<0.1
2002	0.64	20	0.11	2.8	0.87	0.38	1.4	Nd	0.22	<10	5.8	6.2	8.6	26	61	53	155	<0.1
2003	0.73	20	<0.1	1.5	0.40	0.29	1.4	Nd	0.12	10	3.8	6.1	10	33	69	58	176	<0.1
0–4 years	0.51	29	<0.1	4.8	2.4	1.1	1.8	0.10	<0.1	<10	9.7	8.3	6.1	19	40	24	98	0.19
5–14 years, f	0.55	20	<0.1	2.6	1.2	0.67	1.3	<0.1	0.19	12	6.4	7.9	7.9	18	40	24	98	<0.1
5–14 years, m	0.54	20	<0.1	2.2	0.76	0.49	1.9	Nd	<0.1	10	5.4	9.1	5.2	14	31	18	78	<0.1
15–24 years, f	0.57	20	<0.1	1.3	0.39	0.28	0.95	Nd	0.11	21	3.1	6.8	<4.7	12	23	14	58	0.15
15–24 years, m	0.47	20	<0.1	1.3	0.41	0.21	1.5	Nd	0.12	<10	3.6	8.5	<4.7	14	34	26	84	0.44
25–59 years, f	0.67	20	<0.1	1.6	0.35	0.30	0.8	Nd	<0.1	<10	3.1	5.8	8.0	20	43	34	110	0.10
25–59 years, m	0.61	20	<0.1	1.3	0.34	0.20	1.3	Nd	0.18	<10	3.3	5.2	6.1	24	52	44	132	Nd
>60 years, f	0.75	20	0.10	1.3	0.38	0.28	0.91	Nd	<0.1	<10	2.9	4.7	25	60	123	95	308	2.0
>60 years, m	0.69	20	0.10	1.2	0.28	0.29	1.1	Nd	<0.1	<10	2.9	6.0	22	60	140	124	352	0.28

[a] The lipid content of the serum sample and number of individuals represented in each pool are given in column 2 and 3. Abbreviations f: females, m: males and Nd: not detected.
[b] Sum 7: the sum of BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183.
[c] Sum 5: The sum of CB-101, CB-118, CB-138, CB-153 and CB-180.
[d] The sum of TBBP-A and the native methylated TBBP-A.

these pooled serum sample are representative for the Norwegian population.

The serum levels of PBDEs and PCBs were also investigated in pooled samples from individuals with differing age and gender. As can be seen from Table 5, the PBDE concentrations in the serum samples from the youngest individuals were higher compared to the serum levels found in the other age groups, which is similar to what was found previously [18]. Food is regarded as an important source for human exposure to BFRs [39], and BFRs have been reported in breast milk [17,22,40] which is the main food source for infants. Prenatal exposure might also contribute to the elevated levels seen in infants, similar concentrations to that found in human milk have been reported in placenta [41]. However, air and dust may also be an important contribution source, as PBDEs have been reported in house dust both in the US and Europe [42,43]. Young children are particularly vulnerable to contaminants found in dust as they are in close contact with floors and dusty surfaces, and often put hands and objects in their mouths. The exposure of PBDEs through inhalation has been estimated to be 7% of total exposure (93% from diet) [9]. In contradiction, the sum of the five PCBs increased steadily from 98 ng/g lipid in the age group 0–4 years to 308 ng/g lipid and 352 ng/g lipid for women and men in the age group >60, respectively (Table 5). The difference between PBDEs and PCBs regarding age dependency might be explained by the fact that the PCBs have been present in the environment for a longer time, and thus a clearer accumulation with age is observed.

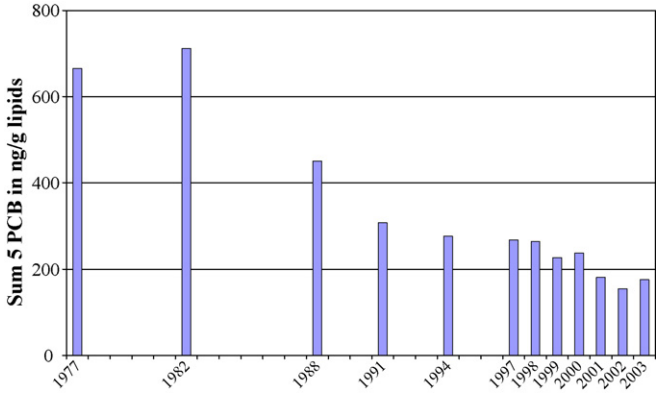

Fig. 3. The concentration of the sum of five PCBs (PCB 101, 118, 138, 153 and 180) in ng/g lipids in pooled serum samples from 40 to 50 year old Norwegian men sampled from 1977 to 2003.

TBBP-A was found in all serum pools from 1982 to 2003. Prior to derivatisation, the sample extracts were analysed for methylated TBBP-A, which was observed in all the samples. The presented values in Table 5 for TBBP-A are therefore the sum of TBBP-A and methylated TBBP-A. The concentration ranged from <LOQ (0.1 ng/g lipids) to 2.0 ng/g lipids, which is in the same order of magnitude as reported in the very limited number of studies available on TBBP-A in human samples [44,45].

BDE-209 was also detected in all serum pools, but the recovery was below 30% in the pools from 1977 and 1982 which have thus been excluded. These two sample pools were more viscous due to their long storage time and therefore difficult to work with.

The concentration of BDE-209 in the 21 serum pools ranged from <10 ng/g lipids to 37 ng/g lipids (Table 5), however, no similar trends as that seen for the other PBDEs was observed. The BDE-209 concentrations are comparable with results reported in serum samples from general populations in Europe [24,45] The concentration range BDE-209 in these studies however, is quite large and further investigations are needed to evaluate body burdens of BDE-209 in general populations.

4. Conclusion

The automated method developed for determination of POPs in human serum is fast compared to traditional manual SPE, simple and consumes low amounts of solvents compared to LLE. The automatic unit is capable of working around the clock, and with the SPE method outlined in this study up to 24 serum samples can be extracted within 24 h. For the additional clean-up step, which is less time consuming, up to 96 extracts can be cleaned-up within the same time period. The method has been validated, and the accuracy and precision was found satisfactory, except for BDE-209 and some of the PCBs and phenols due to their high background levels (Table 4). The method has been used to investigate the levels of PBDEs and PCBs in 21 pooled serum samples form the general Norwegian population and was found suitable for this application. This fast and simple multi-method enables us to perform cost-effective, large-scale analysis series, which are of utmost importance with respect to further investigations on POPs related to e.g. human exposure characterisations or possible health effects.

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