ELSEVIER

Journal of Chromatography B, 750 (2001) 1–11

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of phenolic flame-retardants in human plasma using solid-phase extraction and gas chromatography–electron-capture mass spectrometry

C. Thomsen[a,*], K. Janák[a], E. Lundanes[b], G. Becher[c]

[a]*National Institute of Public Health, P.O. Box 4404 Torshov, N-0403 Oslo, Norway*
[b]*Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, N-0315 Oslo, Norway*
[c]*Norwegian Institute for Water Research, P.O. Box 173 Kjelsaas, N-0411 Oslo, Norway*

Received 21 March 2000; received in revised form 13 June 2000; accepted 13 June 2000

Abstract

A method for determination of phenolic flame-retardants in human plasma utilizing solid-phase extraction (SPE) and gas chromatography with electron-capture mass spectrometric detection (GC–ECMS), has been developed. The plasma lipids were decomposed by application of concentrated sulphuric acid directly on the polystyrene–divinylbenzene SPE column. The method has been validated for 2,4,6-tribromophenol (TriBP), pentabromophenol (PeBP), tetrachlorobisphenol-A (TCBP-A) and tetrabromobisphenol-A (TBBP-A) in the concentration range 1.2–25, 0.4–40, 4–200 and 4–200 pg g^{-1} plasma, respectively. The average absolute recovery of the analytes ranged from 51 to 85%. Tetrabromo-*o*-cresol and chlorotribromobisphenol-A were found suitable as internal standards, and the average recovery of the analytes relative to the internal standards was in the range 93–107%. The repeatability of the method was in the range 4–30% relative standard deviation. The estimated detection limits of TriBP, PeBP, TCBP-A and TBBP-A were 0.3, 0.4, 3.0 and 0.8 pg g^{-1} plasma, respectively. The method has been used for analysis of plasma samples from potentially occupationally exposed human individuals.

Keywords: Phenolic flame-retardants

1. Introduction

Humans are exposed via food, water and air to a large number of environmental contaminants that are taken up and transported by the blood. Recently, much research interest has been focused on anthropogenic compounds and their metabolites mimicking the effect of hormones. Exposure to such endocrine-disrupting chemicals has been associated with various adverse effects on the endocrine and reproductive functions in animals [1,2], and has also been linked to negative health outcomes in humans [2–4]. Due to structural similarities with hormones, such as 17β-estradiol and thyroxine, phenolic compounds with one or two hydroxyl groups may play a key role as competitors of the natural hormones [5–7].

Halogenated phenolic compounds are widely used as flame-retardants [8]. Being both lipophilic and persistent, they accumulate in the food chain and finally in humans, and are thus a potential environ-

*Corresponding author. Tel.: +47-22-042-341; fax: +47-22-042-686.

E-mail address: cathrine.thomsen@folkehelsa.no (C. Thomsen).

0378-4347/01/$ – see front matter
PII: S0378-4347(00)00357-1

mental health problem. In this study we have focused upon 2,4,6-tribromophenol (TriBP), pentabromophenol (PeBP), tetrachlorobisphenol A (TCBP-A) and tetrabromobisphenol-A (TBBP-A) (Fig. 1).

TBBP-A is the most commonly used flame retardant worldwide; over 60 000 tons per year are used, which comprises about 30% of the total amount of brominated flame-retardants [8,9]. It is mainly incorporated as a reactive flame retardant in epoxy resins used in printed circuit boards, but also as an additive in ABS (acrylonitrile–butadiene–styrene) systems [8]. TriBP and PeBP are widely used as well, while TCBP-A is used to somewhat lesser extent [8].

Leakage of the brominated flame-retardants into the environment might occur by evaporation from electronic equipment, as discharges from industry or from wastes at dismantling plants. TBBP-A has been found in sediments [10,11], in sewage sludge [11,12] and in indoor air [13]. In environmental samples trace amounts of TBBP-A have been determined by GC with electron-capture detection (ECD) [10,13,14], GC–MS with electron ionization [10] or GC–ECMS [11,12,15]. Recently, an overview on the occurrence of brominated flame-retardants in the environment was published [16].

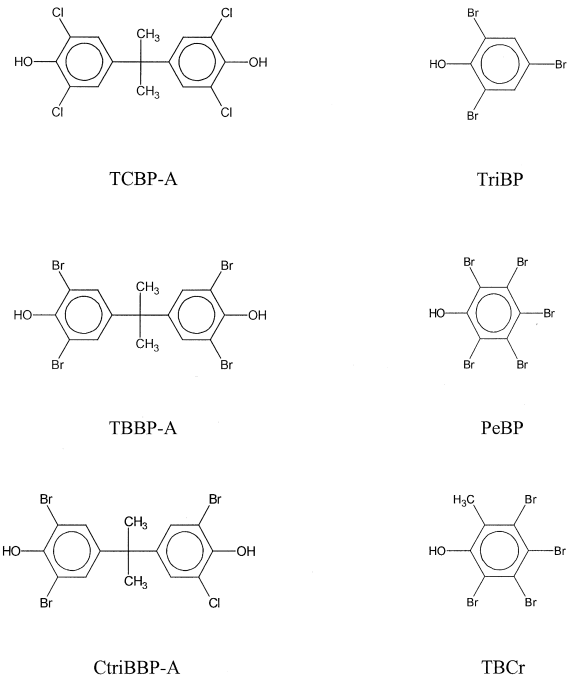

Fig. 1. Chemical structures of the investigated phenols and the internal standards.

Limited knowledge concerning human exposure to phenolic flame-retardants exists. To our knowledge, only one method, which utilized liquid–liquid extractions (LLE) for sample preparation, has been published on determination TBBP-A in human plasma [15].

The objective of this study was to develop a fast and simple method for determination of some phenolic flame-retardants in human plasma, namely TriBP, PeBP, TCBP-A and TBBP-A. Trace analyses of plasma are usually based on repeated liquid–liquid extractions, often in combination with time- and resource-consuming clean-up steps. Solid-phase extraction (SPE) offers fast and efficient methods with lower solvent consumption, less risk of contamination, and often higher selectivity. In the present work a method for sample preparation using SPE with lipid decomposition by concentrated sulphuric acid directly on the solid-phase column, is presented. As the concentrations of phenolic flame-retardants in human plasma are expected to be low, GC–ECMS was chosen for their determination, due to its high selectivity and sensitivity towards halogenated compounds.

2. Experimental

2.1. Materials and reagents

TCBP-A, TBBP-A and chlorotribromobisphenol-A (CtriBBP-A) were kindly supplied by the Wallenberg Laboratory (University of Stockholm, Sweden). TriBP, tetrabromo-*o*-cresol (TBCr) and *N*-methyl-*N*-nitrosoamine (diazald) were purchased from Aldrich (Milwaukee, WI, USA), PeBP from Acros (Geel, Belgium), 1,3,5-tribromobenzene (TriBB) from Fluka (Buchs, Switzerland) and 3,3′,4,4′-tetrabromobiphenyl (TBB) from AccuStandard (New Haven, CT, USA).

All solvents were pesticide grade from Labscan (Dublin, Ireland) and used as supplied. Formic acid, sulphuric acid and sodium acetate–trihydrate were of analytical grade (Merck, Darmstadt, Germany). Water was purified using Elga Option 4 Water

Purifier device (Elga, Bucks, UK). Nitrogen (99.999%), helium (99.998%) and methane (99.95%) were obtained from Aga (Oslo, Norway).

The Isolute ENV+ SPE columns (200 mg, 6 ml) were purchased from International Sorbent Technology (Mid Glamorgan, UK).

All glassware, except volumetric equipment, was washed in 2.5% RBS 25 foaming cleaner (Chemical Products, Brussels, Belgium), rinsed with distilled water and then heated at 450°C for 4 h.

2.2. Plasma samples

Plasma samples stabilized with citrate solution (CPD) were obtained using plasmapheresis at the Blood Center at Ullevål Hospital (Oslo, Norway). The lipid content of the validation plasma was determined at The National Hospital of Norway (Oslo, Norway) according to a method described by Grimvall et al. [17], and was 0.55% (w/w). The plasma was stored at −18°C until analysis.

2.3. Analytical procedures

2.3.1. Preparation of standard and calibration solutions

Stock solutions of 2 mg ml^{-1} TriBP, PeBP, TCBP-A, TBBP-A, TriBB and TBCr were prepared separately by dissolving an accurate amount in 10.0 ml ethyl acetate. TBB was obtained as 35.00 μg ml^{-1} in isooctane (2,2,4-trimethylpentane) and CtriBBP-A as 2.19 mg ml^{-1} in toluene. All further dilutions were done in ethyl acetate using volumetric equipment. The four analytes were combined in the next dilution step and the amount of each was adjusted to give as equal response as possible. Solutions of internal standards were combined and diluted similarly. In the final dilution step the internal standards were added to the analyte solutions. This resulted in standard solutions covering the concentration range 0.0207–4.16 pg TriBP, 0.0311–6.24 pg PeBP and 0.331–66.6 pg TCBP-A and TBBP-A/μl ethyl acetate. The concentration of the internal standards were 0.622 and 1.88 pg μl^{-1} for TBCr and CtriBBP-A, respectively. A separate solution containing only the internal standards at the same concentration was also prepared. A GC–MS quantification standard solution was made by diluting the TriBB and TBB solutions to a final concentration of 1.78 and 5.33 pg μl^{-1}, respectively.

GC–MS calibration solutions were prepared by adding 30 μl of standard solution to concentrated extracts of 5.0 g (non-spiked) plasma, and derivatization was proceeded as described below. The final concentration range of the GC–MS calibration solutions were 0.0124–1.25 pg TriBP, 0.0186–1.87 pg PeBP and 0.199–9.98 pg TCBP-A and TBBP-A/μl plasma extract. The concentrations of the internal standards were 0.187 pg TBCr and 0.563 pg CtriBBP-A/μl plasma extract.

All solutions were stored in amber glass containers at −18°C.

2.3.2. Validation samples

Frozen plasma was thawed overnight in a refrigerator (4°C) and brought to room temperature, before 30 μl standard solution containing analytes and internal standards were added to each plasma aliquot of 5.0 g. In addition, four samples added internal standard solution only, were prepared. All samples were kept overnight at 4°C before further preparation. The concentrations of the different spiking levels are given in Section 2.3.6.

2.3.3. Sample preparation

A method developed for determination of polychlorinated biphenyls in human blood [18] was modified for extraction of phenolic compounds from plasma. Five ml formic acid–2-propanol (4:1, v/v) were added to the plasma sample (5.0 g), and the sample was sonicated in an ultrasonic bath (Transsonic 460, Elma, Singen, Germany) for 5 min. After 60 min, the sample was diluted with 5.0 ml water–propanol (19:1, v/v), sonicated for 5 min and subjected to SPE. The SPE columns were mounted in a 12-port Visiprep SPE Vacuum Manifold (Supelco, Bellefonte, PA, USA) and the procedure was performed as described in Table 1. The first drying step was performed to expel most of the non-adsorbed water and the second to dry the column completely before elution. Two droplets of methanol–water (9:1, v/v) were added 3 times during the drying period to facilitate this process.

Table 1
SPE procedure for extraction of phenolic flame-retardants from human plasma

Step	Process	Reagent	Flow rate (ml min^{-1})
1	Conditioning	3 ml methanol	2
2	Conditioning	3 ml dichloromethane	2
3	Conditioning	6 ml dichloromethane–methanol (1:1, v/v)	2
4	Conditioning	5 ml methanol	2
5	Conditioning	5 ml water	2
6	Loading	15 ml diluted plasma	1
7	Washing	7 ml water–propanol (19:1, v/v)	2
8	Drying	0.5 bar N_2 for 2 min	
9	Lipid decomposition	6 ml concentrated H_2SO_4	0.5
10	Washing	14 ml water	2
11	Washing	7 ml 0.1 *M* Na acetate buffer	2
12	Washing	14 ml water	2
13	Washing	7 ml water–methanol (9:1, v/v)	2
14	Washing	7 ml water–methanol (3:2, v/v)	2
15	Drying	0.5 bar N_2 until dryness (~20 min)	
16	Elution	6 ml dichloromethane–methanol (1:1, v/v)	1

2.3.4. Derivatization

Diazomethane was produced in a base-catalyzed decomposition of *N*-methyl-*N*-nitrosoamine [19]. Before derivatization the sample extracts were concentrated under a gentle stream of nitrogen at 50°C to about 30 μl, and transferred to a microvial. When GC–MS calibration solutions were prepared, 30 μl standard solution were added at this point and further concentrated to 30 μl. Subsequently 50 μl diazomethane solution were added, the vial capped, vortex mixed and kept dark for 30 min. Excess derivatization reagent was evaporated by heating at 50°C for 15 min. Finally 15 μl of GC–MS quantification standard solution of TriBB and TBB were added and the samples stored at −18°C until analysis.

2.3.5. Instrumentation

A HP (Avondale, PA, USA) 6890 gas chromatograph equipped with a HP 7683 automatic liquid sampler was operated by ChemStation B 02.05. A CP-Sil 8 CB fused-silica capillary column (30 m× 0.25 mm I.D., 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands) was connected to the injector via a deactivated retention gap of 1.5 m× 0.32 mm I.D. (J&W Scientific, Folsom, CA, USA). The injector temperature was 250°C and samples of 1 μl were injected in pulsed splitless mode with a pulse pressure of 1.72 bar for 1.5 min. Helium was used as carrier gas and separation was performed at a constant flow of 1.2 ml min^{-1}. The column temperature was initially 70°C for 1 min, then raised by 23°C min^{-1} to 270°C, 4°C min^{-1} to 280°C and 23°C min^{-1} to 325°C, which was held for 2 min. For determination of TCBP-A the temperature program had to be slightly adjusted: 70°C for 1 min, then raised by 23°C min^{-1} to 325°C, which was held for 2 min.

The mass spectrometer, a HP 5973 MSD with chemical ionization (CI) option, was operated in the electron capture mode with methane as buffer gas. TCBP-A was monitored at m/z 35/37 and all other compounds at m/z 79/81. The compounds were confirmed by controlling the isotope abundancy ratio. The temperature was 106, 250 and 300°C for the quadrupole, the ion source and the interface, respectively, and an electron energy of 86.6 eV was used.

2.3.6. Quantification and validation

The method was validated by spiking plasma samples at six levels covering the concentration range 0.249–25.0 pg TriBP and 0.373–37.5 pg PeBP/g plasma, and at five levels covering 3.98–200 pg TCBP-A and TBBP-A/g plasma. Four plasma samples were added internal standards only, for investigation of halogenated flame-retardants initially present. The validation samples were randomly prepared during 3 days.

Peak heights were used for quantification of TCBP-A, whereas peak areas were used for all other compounds. All peaks were manually integrated. The validation results were based on two GC–MS replicates, except TCBP-A, for which only one injection was performed.

The absolute recovery was determined using TriBB or TBB as quantification standard.

3. Results and discussion

3.1. Sample preparation

3.1.1. Solid-phase extraction

The selection of sorbent for SPE was restricted to materials which could withstand treatment with concentrated sulphuric acid, and Isolute ENV+, a sorbent of a styrene–divinylbenzene copolymer, proved to work well [18]. This polystyrene material also offers high sample capacity and wetting of the sorbent is less critical, compared to silica-based materials. The critical parameters regarding the extraction method have been thoroughly discussed elsewhere [18].

The halogenated phenolic compounds were strongly retained by the solid phase, and none of them were detected in the eluates from the washing steps. Different combinations of ethyl acetate, dichloromethane and methanol were tested for elution, and dichloromethane–methanol (1:1, v/v) provided the highest recovery. A completely dry sorbent was required to obtain efficient elution from the solid phase. This was achieved by drying with suction under a nitrogen atmosphere, and by application of two droplets of methanol–water (9:1, v/v) 3 times during the drying period. The methanol facilitates water extrusion from the micropores of the sorbent material.

3.1.2. Derivatization

Improved detector response was sought by addition of more halogen by derivatization using fluorinated acylating reagents. However, the yield of acylphenolates was unacceptably low, probably because of sterical hindrance by the bulky halogen substituents. On the other hand, derivatization with diazomethane turned out to be fast, easy and efficient. Excess diazomethane had to be removed before chromatography, both with respect to its health hazard and to an interfering background. Being highly volatile, unreacted diazomethane was completely evaporated by heating at 50°C for 15 min, without any loss of the methylated analytes.

The SPE procedure followed by derivatization with diazomethane resulted in an extract, which needed no further clean-up before GC–EC–MS analysis.

A variety of brominated phenols and methoxylated phenols have been identified in marine organisms [20]. In order to investigate the presence of native methoxylated phenols in human samples, analysis both prior and after derivatization is recommended.

3.2. Choice of internal and recovery standards

Preliminary experiments showed that internal and quantification standards should be detected as ions produced by the same mechanism as the analytes. This because the GC–MS response seemed to change differently for compounds detected as molecular ions compared to compounds detected as fragmented ions, as the ion source was getting dirty. Regarding quantification, isotopically labeled internal standards are recommended in mass spectrometric analysis of biological samples, but such are of no value when using halogen ion detection in ECMS. Therefore, internal standards which are ionized in MS by the same EC mechanism, and which behave similarly to the analytes during sample preparation, were sought. TBCr was found suitable as internal standard for TriBP, PeBP and TCBP-A and CtriBBP-A for TBBP-A (Fig. 1). TriBB was used as GC–MS quantification standard for determination of absolute recovery for TriBP and PeBP, and TBB was used for TCBP-A and TBBP-A.

3.3. Chromatography

The chromatograms in Fig. 2 shows that the halogenated flame-retardants, the internal standards (I.S.) and the quantification standards (QS) are baseline separated within 15 min. All compounds were detected as fragmented ions Br^- or Cl^-. An ion source temperature of 100°C was recommended by the manufacturer, but was adjusted to 250°C because

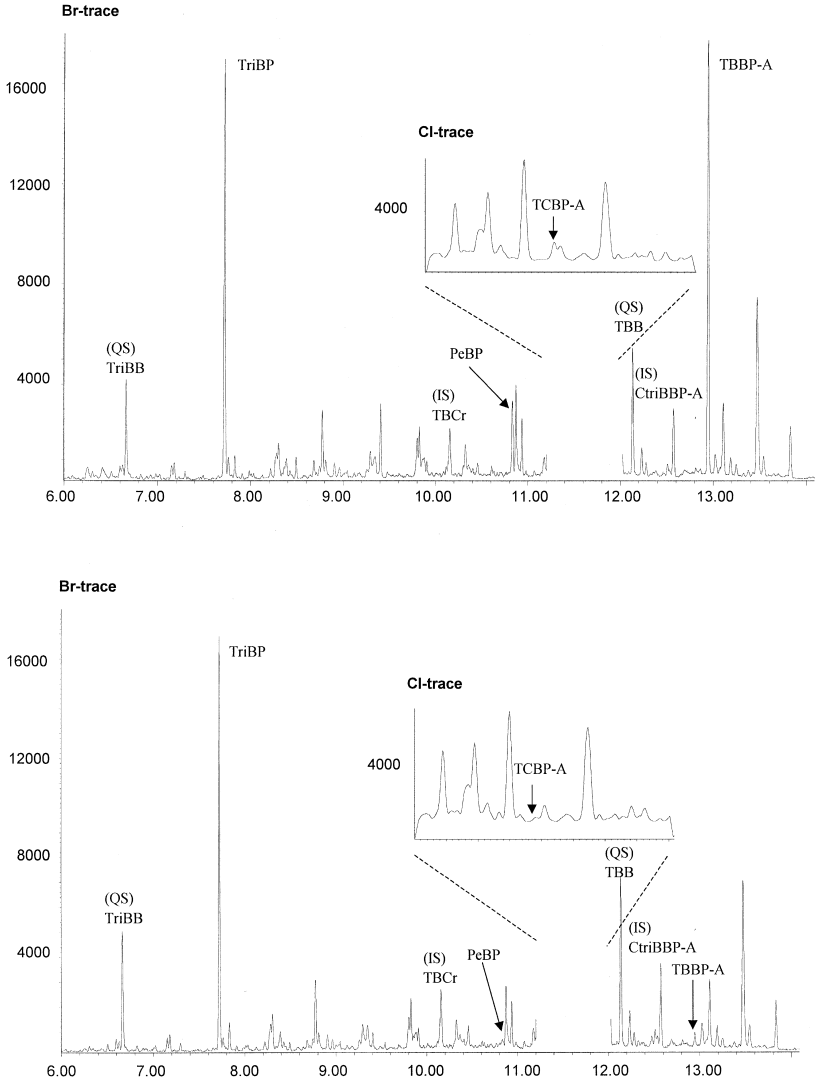

Fig. 2. Chromatogram of a spiked plasma sample (5 g) (upper) and a non-spiked plasma sample (5 g) (lower) after SPE extraction and derivatization. The plasma sample was spiked with 3.8 pg TriBP, 5.6 pg PeBP, 60 pg TCBP-A and 60 pg TBBP-A/g plasma. The halogenated flame-retardants, the internal standards (I.S.) and the quantification standards (QS) were detected as m/z 35/37 (Cl-trace) or m/z 79/81 (Br-trace).

the halogenide response was more stable at this temperature. Six consecutive injections of a spiked plasma sample resulted in a precision of $<0.01\%$ relative standard deviation (RSD) and $<5\%$ RSD for retention time and area ratio, respectively. Further analyses were thus performed with duplicate injections.

In plasma samples an interfering compound was eluting close to TCBP-A. Although we tried to optimize the temperature program, the two components were still not baseline separated (Fig. 2), and peak heights were used for quantification of TCBP-A. The identity of the interfering compound is not known. A solution containing 31 polychlorinated biphenyls (PCB) normally present in human plasma [18] was injected and detected by selected ion monitoring at m/z 35/37. All PCB were well separated from the halogenated flame-retardants and

the selectivity was thus considered appropriate. Brominated compounds were unfortunately not available.

3.4. GC–MS calibration

When analyzing plasma extracts a matrix effect was observed, which is well known in analysis of biological samples [21–24]. When higher response for the same amount of added analyte is found when residues from the sample is present, this can be explained as an effect of the ability of the sample matrix to protect the analyte from adsorption in the injector, which leads to an increased transfer from the injector to the GC column [21]. Different attempts, such as altering the injection temperature and pressure, were made to enhance the transfer from neat solutions and some improvements were observed. However, the effect was still severe for the latest eluting compounds, and GC–MS calibration standards were therefore prepared in plasma extracts. Solution to this inconvenience is under further investigation.

3.5. Method validation

The concentrations of the halogenated flame-retardants in human plasma were expected to be low, and thus the method was validated by spiking plasma from the detection limits and upward. However, the presence of TriBP in the plasma batch used for validation, made spiking of TriBP at the lowest level impossible. The standard deviation for determination of the amount initially present exceeded the added amount. Remarkably, so far no plasma samples were found that did not contain TriBP. As a consequence, the method for determination of TriBP was validated in a smaller concentration range and the lowest level had a relatively high uncertainty.

The procedural blank (no plasma) showed a low contamination of TriBP and TBBP-A. The concentration levels were quite stable and thus made no impact on the validation results. The contamination is believed to arise from the laboratory air, and will be subjected to further investigation.

3.5.1. Linearity

The GC–MS calibration standards, injected in triplicate, were used to investigate the linearity of the GC–MS method. Peak area (or height) ratios with respect to internal standard were plotted against the corresponding concentration ratio. The response was found to be linear in the validated range, with correlation coefficients (r) better than 0.999 for all compounds except TriBP, for which r was 0.994.

The validation samples were used for estimation of the linearity of the whole method (Table 2). The correlation coefficients were somewhat lower compared to the GC–MS method, but nevertheless acceptable.

3.5.2. Limit of quantification/detection

Signal-to-noise (S/N) ratios for the lowest concentration level of the validation samples are given in Table 3, and were used for a rough estimation, by extrapolation, of the detection and quantification limits, $S/N=3$ and $S/N=10$, respectively. Preliminary analysis using GC–ECD for determination of the same compounds resulted in detection limits at an at least 100 times higher concentration level. Due to the loss during sample preparation and higher background noise for plasma samples, the S/N-ratios are somewhat lower for plasma extracts compared to standards in ethyl acetate, despite the matrix effect.

Table 2
Regression data for extracted spiked plasma[a]

	TriBB	PeBP	TCBP-A	TBBP-A
Slope (b)	1.494	0.7354	0.2715	1.149
SD of slope (S_b)	0.059	0.020	0.0071	0.013
Intercept (a)	−0.1375	0.07339	−0.1578	−0.1094
SD of intercept (S_a)	0.17	0.070	0.18	0.12
Degrees of freedom (df)	16	30	12	28
Correlation coefficient (r)	0.988	0.990	0.996	0.998

[a] The response was corrected for the blank content and the point 0.0 equally weighted as the other points in the regression line.

Table 3
Signal-to-noise ratios and estimated limits of detection (LOD) ($S/N=3$) and limits of quantification (LOQ) ($S/N=10$).

Compound	Validation plasma samples		Standard solutions		Estimations	
	Spike level (pg μl^{-1})	S/N	Concentration (pg μl^{-1})	S/N	LOD (pg g^{-1} plasma)	LOQ (pg g^{-1} plasma)
TriBP	–	–	0.006	4	0.3[a]	1.0[a]
PeBP	0.019	3	0.019	4	0.4	1.3
TCBP-A	0.20	4	0.10	8	3.0	10
TBBP-A	0.20	15	0.10	10	0.8	2.7

[a] Estimations are based on the calculated amount TriBP in blank plasma and the corresponding S/N ratio.

As can be seen from Table 3 the lowest spike level is quite close to the detection limits for PeBP and TCBP-A, which explains the relatively poor precision revealed below.

3.5.3. Recovery

The absolute recovery of the method is shown in Table 4. Average absolute recoveries of 85, 79, 64 and 51% were found for TriBP, PeBP, TCBP-A and

Table 4
Mean recoveries of halogenated flame-retardants and internal standards in spiked plasma samples using TriBB or TBB as GC–MS quantification standard[a]

Compound	GC–MS quantification standard	Amount added (pg g^{-1} plasma)	n	Amount found (pg g^{-1} plasma)[b]	Mean recovery (%)	RSD (%)
TriBP	TriBB	1.24	3	1.14±0.74	92	65
		3.76	1	3.52	94	
		12.5	4	8.59±1.9	69	22
		25.0	1	20.9	84	
PeBP	TriBB	0.373	4	0.345±0.16	93	48
		0.746	1	0.473	63	
		1.86	4	1.58±0.23	85	14
		5.63	1	4.50	80	
		18.7	4	15.1±2.0	81	13
		37.4	1	27.2	72	
TCBP-A	TBB	3.98	4	2.41±1.7	61	70
		7.96	1	5.81	73	
		19.9	4	12.1±2.5	61	21
		60.1	1	38.5	64	
		200	4	124±21	62	17
TBBP-A	TBB	3.98	4	1.85±0.18	46	10
		7.96	1	3.59	45	
		19.9	4	10.9±1.4	55	13
		60.1	1	33.0	55	
		200	4	112±20	56	18
TBCr	TriBB	3.73	15	2.55±0.21	68	8.4
CtriBBP-A	TBB	11.3	15	6.04±0.69	54	11

[a] The point 0.blank response was included in the calibration curve used for determination of the recovery.
[b] Mean±one standard deviation (SD).

TBBP-A, respectively. The lower recovery of the bisphenols may be partly due to a reduced persistence to concentrated sulphuric acid. On the other hand, the lower recovery can also be explained by poorer desorption from the solid-phase column, which might be due to the compounds having two aromatic rings and four halogen atoms, which give rise to strong $\pi-\pi$ interactions with the sorbent. This has also been observed by others [18].

3.5.4. Repeatability and accuracy

The repeatability of the method, established at the concentration levels 1, 3 and 5 shown in Table 5, was fairly good, except for the lowest level of TriBP, PeBP and TCBP-A. Due to the lack of a reference material or another validated method, the accuracy of the method was determined as recovery relative to the internal standard (Table 5). The average accuracies were in the range 93–107%. The between-months variation was established by spiking four plasma samples, at level 3, at 1 and 2 months after the main validation experiment. The results are based on one randomly selected sample from each month (Table 6). The between-months variation of TBBP-A was satisfactory, while the between-months variation of PeBP and TCBP-A was about 25%, which is considered acceptable. Due to the high native plasma content, the RSD for determination of TriBP was high and the results were thus left out.

3.5.5. Stability and robustness

The GC–MS calibration standards and the standard solutions, kept in a freezer at -18°C, were stable during the time course of this study. The volumes of solvents used during the sample preparation procedure in Table 1 were not volumetrically added and the flow-rates were only manually controlled. In combination with the results of analyst B in Table 6, this is an indication of the method being robust.

Table 5
Repeatability and accuracy[a]

Compound	Internal standard	Amount added (pg g^{-1} plasma)	*n*	Amount found (pg g^{-1} plasma)[b]	Mean accuracy (%)	RSD (%)
TriBP	TBCr	1.24	3	1.26±1.1	101	88
		3.76	1	4.63	123	
		12.5	4	11.5±2.4	92	21
		25.0	1	27.5	110	
PeBP	TBCr	0.373	4	0.418±0.21	112	51
		0.746	1	0.602	81	
		1.86	4	2.10±0.21	113	10
		5.63	1	6.00	107	
		18.7	4	19.8±2.4	106	12
		37.4	1	34.1	91	
TCBP-A	TBCr	3.98	4	3.10±2.8	78	90
		7.96	1	7.04	88	
		19.9	4	18.6±5.4	93	29
		60.1	1	59.0	98	
		200	4	211±33	106	16
TBBP-A	CtriBBP-A	3.98	4	4.02±0.17	101	4.3
		7.96	1	7.39	93	
		19.9	4	18.6±0.78	93	4.2
		60.1	1	54.0	90	
		200	4	192±9.5	96	4.9

[a] The response was corrected for the blank content and the point 0.0 included in the regression line.
[b] Mean±SD.

Table 6
Between-months variation[a]

Compound	Internal standard	Month	Analyst	Amount added (pg g^{-1} plasma)	Amount found (pg g^{-1} plasma)	Mean amount found (pg g^{-1}plasma)[b]	RSD (%)
PeBP	TBCr	1	A	1.86	1.89		
		2	A	1.86	1.23	1.46±0.37	25
		3	B	1.86	1.27		
TCBP-A	TBCr	1	A	19.9	13.6		
		2	A	19.9	8.44	11.5±2.7	24
		3	B	19.9	12.6		
TBBP-A	CtriBBP-A	1	A	19.9	18.0		
		2	A	19.9	17.5	17.7±0.23	1.3
		3	B	19.9	17.6		

[a] $n=3$.
[b] Mean±SD.

3.6. Application

We are presently utilizing this method for investigation of the body burden of phenolic halogenated flame-retardants in potentially occupationally exposed persons and in control persons. The chromatogram from the analysis of one of the plasma samples is shown in Fig. 3. The results will be presented in a forthcoming publication.

4. Conclusion

To our knowledge this is the first method published utilizing SPE and GC–ECMS for determination of phenolic flame-retardants in human plasma. The method is faster, simpler, consumes lower amount of solvents, demands less equipment and presents a lower risk of contamination compared to traditional methods based on LLE. Up to 12 samples

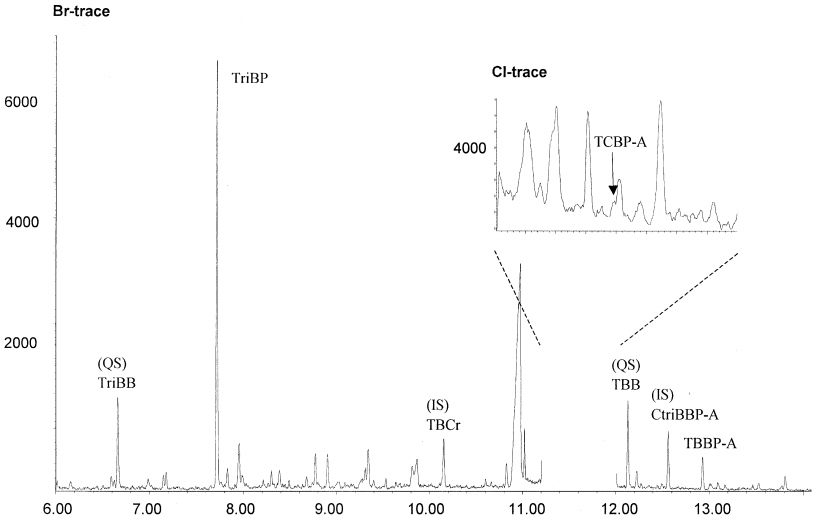

Fig. 3. Chromatogram of a 5-g plasma sample from a possibly occupationally exposed person.

can be prepared simultaneously within a day. The method shows sufficient sensitivity and is reproducible in a relatively wide concentration range, which is 1.2–25 pg TriBP, 0.4–40 pg PeBP and 4–200 pg TCBP-A and TBBP-A/g plasma. The possibility to extend the method to comprise other brominated flame-retardants and other sample types will be pursued.

Acknowledgements

The Research Council of Norway financially supported this project.

References

[1] T. Colborn, C. Clement, in: Chemically Induced Alterations in Sexual and Functional Development: the Wildlife/human Connection, Princeton Scientific Publications, Princeton, NJ, 1992.
[2] A. Brouwer, D.C. Morse, M.C. Lans, A.G. Schuur, A.J. Murk, E. Klasson-Wehler, Å. Bergman, T.J. Visser, J. Toxicol. Indust. Health 14 (1998) 59.
[3] R.M. Sharp, N.E. Skakkebæk, Lancet 341 (1993) 1392.
[4] T. Colborn, F.S. vom Saal, A.M. Soto, Environ. Health Perspect. 101 (1993) 378.
[5] J.A. Dodge, Pure Appl. Chem. 70 (1998) 1725.
[6] K. Korach, P. Sarver, K. Chae, J. McLachlan, J.D. McKinney, Mol. Pharmacol. 33 (1988) 120.
[7] V.J. Kramer, W.G. Helferich, Å. Bergman, E. Klasson-Wehler, J.P. Giesy, Toxicol. Appl. Pharmacol. 144 (1997) 363.
[8] WHO, Environmental Health Criteria 172, Tetrabromobisphenol A and derivatives, World Health Organization, Geneva, Switzerland, 1995
[9] WHO, Environmental Health Criteria 192, Flame-retardants: a general introduction, World Health Organization, Geneva, Switzerland, 1997
[10] I. Watanabe, T. Kashimoto, R. Tatsukawa, Bull. Environ. Contam. Toxicol. 31 (1983) 48.
[11] U. Sellström, B. Jansson, Chemosphere 31 (1995) 3085.
[12] U. Sellström, A. Kierkegaard, T. Alsberg, C. Wahlberg, C. de Wit, Organohalogen Compounds 40 (1999) 383.
[13] Å. Bergman, C. Östman, R. Nybom, A. Sjödin, H. Carlsson, U. Nilsson, C.A. Wachtmeister, Organohalogen Compounds 33 (1997) 414.
[14] L. Hovander, M. Athanasiadou, L. Asplund, E. Klasson-Wehler, Organohalogen Compounds 35 (1998) 115.
[15] E. Klasson-Wehler, L. Hovander, Å. Bergman, Organohalogen Compounds 33 (1997) 420.
[16] C. de Wit, Organohalogen Compounds 40 (1999) 329.
[17] E. Grimvall, L. Rylander, P. Nilsson-Ehle, U. Nilsson, U. Strömberg, L. Hagmar, C. Östman, Arch. Environ. Contam. Toxicol. 32 (1996) 329.
[18] K. Janák, E. Jensen, G. Becher, J. Chromatogr. B 734 (1999) 219.
[19] T. Howard Black, Aldrichim. Acta 16 (1983) 3.
[20] G. Gribble, Organohalogen Compounds 41 (1999) 23.
[21] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole, J. Chromatogr. 638 (1993) 57.
[22] M. Vincenti, C. Minero, M. Sega, C. Rovida, J. High Resolut. Chromatogr. 18 (1995) 490.
[23] U. Sellström, A. Kierkegaard, C. de Wit, B. Jansson, Environ. Toxicol. Chem. 17 (1998) 1065.
[24] M. Godula, J. Hajšlová, K. Alterová, J. High Resolut. Chromatogr. 22 (1999) 395.