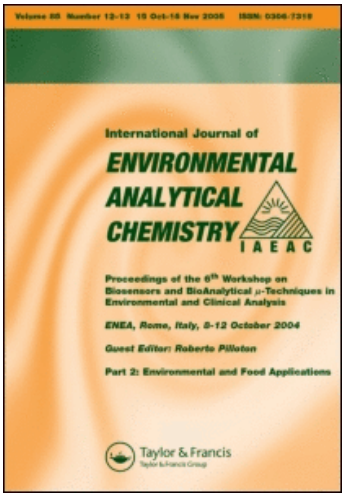

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Method development for simultaneous analysis of HBCD, TBBPA, and dimethyl-TBBPA in marine biota from Greenland and the Faroe Islands

Marie Frederiksen[a]; Katrin Vorkamp[a]; Rossana Bossi[b]; Frank Rigét[c]; Maria Dam[d]; Bo Svensmark[e]
[a] Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, University of Aarhus, 4000 Roskilde, Denmark [b] Department of Atmospheric Environment, University of Aarhus, Frederiksborgvej 399, 4000 Roskilde, Denmark [c] Department of Arctic Environment, University of Aarhus, Frederiksborgvej 399, 4000 Roskilde, Denmark [d] Food, Veterinary and Environmental Agency, Torshavn, Faroe Islands [e] Department of Chemistry, University of Copenhagen, Copenhagen Ø, Denmark

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Method development for simultaneous analysis of HBCD, TBBPA, and dimethyl-TBBPA in marine biota from Greenland and the Faroe Islands

MARIE FREDERIKSEN*†, KATRIN VORKAMP†, ROSSANA BOSSI‡, FRANK RIGÉT§, MARIA DAM¶ and BO SVENSMARK‖

†Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, University of Aarhus, Frederiksborgvej 399, 4000 Roskilde, Denmark
‡Department of Atmospheric Environment, University of Aarhus, Frederiksborgvej 399, 4000 Roskilde, Denmark
§Department of Arctic Environment, University of Aarhus, Frederiksborgvej 399, 4000 Roskilde, Denmark
¶Food, Veterinary and Environmental Agency, Falkavegur 6, FO-100, Torshavn, Faroe Islands
‖Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100, Copenhagen Ø, Denmark

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The brominated flame retardants hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) are high-production-volume chemicals. In recent years, their presence has been reported in sediment and biota from the marine environment. In this study, an analytical method was developed for the simultaneous determination of HBCD, TBBPA, and the possible metabolite dimethyl-TBBPA. The method was applied in a preliminary screening of egg, liver, and adipose tissue of marine biota from Greenland and the Faroe Islands. α-HBCD was detected in 35 of 36 analysed samples from the Arctic, indicating a ubiquitous presence of α-HBCD in the environment. β- and γ-HBCD were found in 10 and 14 samples, respectively. TBBPA and dimethyl-TBBPA were not detected in any of the samples indicating limited or no transport of these compounds to remote areas.

Keywords: Brominated flame retardants; GPC; GC-MS; LC-MS-MS; Arctic biota; PLE

1. Introduction

The positive effects of brominated flame retardants (BFRs) as lifesavers are indisputable; however, the drawback in their extensive use is their presence in the environment and the possible adverse effects on wildlife and human health. Tetrabromobisphenol A (TBBPA) is the most used BFR worldwide (145 113 t/yr, 2003), while hexabromocyclododecane (HBCD) is the third most used BFR (21 951 t/yr,

*Corresponding author. Fax: +45-46301114. Email: mafr@dmu.dk

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2003) [1]. Currently, the use of TBBPA and HBCD has not been regulated anywhere in the world, though they are included as 'priority' compounds in monitoring and assessment programmes in several countries [1].

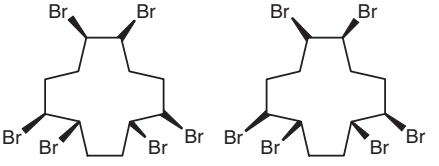
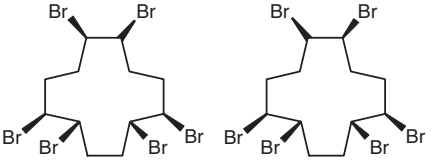
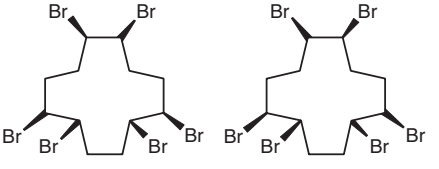
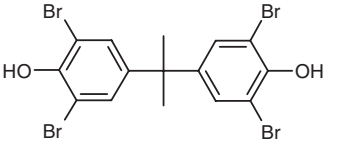
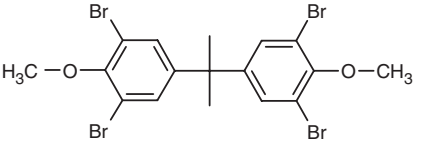
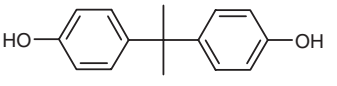
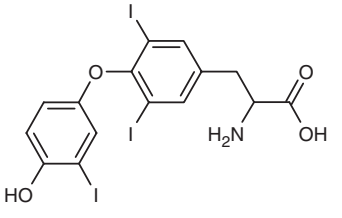
Technical HBCD primarily consists of three enantiomeric pairs, α, β, and γ (table 1), which make up approximately 12, 6, and 82%, respectively of the technical formulation [2] though the composition varies considerably. α-HBCD is the main isomer detected in biota, and several authors have concluded that α-HBCD biomagnifies to a higher extent than β- and γ-HBCD [3, 4]. α-HBCD seems to be more resistant to degradation than the β- and γ-isomers, which has been shown in biota and anaerobic sewage sludge [4–6]. Little information is available on the toxicity of HBCD, but one study has shown that exposure of HBCD to newborn mice results in alteration in the spontaneous behaviour as well as defects in learning and memory. Simultaneous exposure to PCBs has a synergistic effect and enhances the adverse effects of HBCD [7].

TBBPA is applied as a reactive flame retardant, which means that it is covalently bound in the epoxy laminate of circuit boards, for example, which reduces the risk of leakage while in use. The structure of TBBPA resembles that of bisphenol A, BPA, as well as the thyroid hormone (T_3/T_4) (table 1). The active form of thyroid hormones, triiodothyronine, T_3, is a very important hormone that influences many body functions, including growth. Additionally, T_3 is extremely important for normal development of the central nervous system during foetal life [8]. BPA is known to exhibit oestrogenic activity, while TBBPA has previously been reported to show thyroid hormonal activity [9]. TBBPA has been reported to be photolysed by UV-radiation [10], degraded to BPA under anaerobic conditions in sediment [11], and transformed *in vitro* by Gram-positive bacteria to the dimethyl ether (Me-TBBPA) (table 1) [12]. The transformation to the dimethyl ether reduces the polarity of TBBPA, and thus $\log K_{ow}$ is increased along with the risk of bioaccumulation ($\log K_{ow}$ TBBPA: 5.9; $\log K_{ow}$ Me-TBBPA: 6.4).

Previous studies have shown that HBCD is present in a wide variety of environmental matrices, e.g. sediment, fish, birds, and marine mammals [13–15]. Increasing concentrations between 35 and 170 ng g^{-1} lw HBCD have been reported in guillemot eggs (*Uria algae)* in the period 1969 to 2001 [16]. However, only a few researchers have investigated the presence of HBCD in the Arctic with reported levels of 32–59 ng g^{-1} lw in polar bear blubber from East Greenland [17]. The levels of HBCD in biota from the Faroe Islands have not been investigated yet. TBBPA has previously been reported in abiotic matrices such as sludge and sediment [18, 19], but reports of TBBPA in environmental biota samples are scarce [6, 20]. Levels of TBBPA in the range 0.1–418 ng g^{-1} lw have been measured in harbour porpoises (*Phocoena phocoena*), but no results have been reported from animals that live in remote areas of the Arctic. The possible metabolite, Me-TBBPA, has so far received little attention and has only been subject to a limited number of studies resulting in finding Me-TBBPA in both biotic and abiotic matrices [18, 19, 21].

Previously, the dominating analytical method for HBCD and TBBPA was GC-MS [17, 18]. When analysing TBBPA by GC-MS, derivatization is needed in order to obtain an adequate response and avoid peak tailing. No combined method for the determination of ΣHBCD, TBBPA (derivative), and Me-TBBPA has been reported so far. In the last few years, a shift towards LC-MS(-MS) analysis has taken place [22–24]. LC-MS allows separation of the HBCD-isomers and analysis of TBBPA without derivatization. The first combined analysis of HBCD-isomers and TBBPA

Table 1. Structure of α, β, and γ-HBCD, TBBPA, dimethyl-TBBPA, bisphenol A, and thyroid hormone, triiodothyronine (T_3).

Name	Structure
α-HBCD (α-hexabromocyclododecane)	
β-HBCD (β-hexabromocyclododecane)	
γ-HBCD (γ-hexabromocyclododecane)	
TBBPA (tetrabromobisphenol A)	
Me-TBBPA (dimethyl TBBPA)	
BPA (bisphenol A)	
T_3 (3,5,3′-triiodothyronine)	

using LC-MS was published in 2004 [20]. The aim of the current study was to combine the analysis of HBCD, TBBPA, and Me-TBBPA (in biota samples), and to apply the method to environmental biota samples.

2. Experimental

2.1 *Chemicals and reagents*

Standard solutions of α-, β-, and γ-HBCD were obtained from Wellington Lab. (Guelph, Ontario, Canada). $^{13}C_{12}$-TBBPA, Me-TBBPA, BDE-71 (2,3′,4,4′-tetrabromo diphenyl ether), and BDE-77 (3,3′,4,4′-tetrabromo diphenyl ether) (all purities >97%) were obtained from Cambridge Isotopes Laboratories (Andover, MA) along with the BDE congeners used for co-elution tests (same purity). TBBPA was a technical product of unknown purity from Promochem (Teddington, UK). Methanol and acetonitrile were from Merck (Darmstadt, Germany); cyclohexane and dichloromethane were from Rathburn (Walkerburn, UK) all of HPLC-grade; while acetone, *n*-hexane (Rathburn), diethyl ether (J.T. Baker, Phillipsburg, NJ), ethyl acetate, and isooctane (Merck, Darmstadt, Germany) were glass-distilled. The reagents ammonium acetate and concentrated sulfuric acid (Merck) were both pro-analysis. Water used for eluents was MilliQ grade (Super-Q, Millipore, Danvers, MA). Silica Gel 60, 0.063–0.2 mm (Merck, Germany) and Hydromatrix (Varian, San José, CA) were cleaned with dichloromethane (DCM) prior to use. Unfortunately, isotope labelled HBCD-isomer standards were not available within this project. All stock solutions prepared for LC-MS-MS analyses were in methanol and stored at −20°C. Stock solutions for GC-MS were prepared in isooctane and stored at 5°C.

2.2 *Method development*

The overall experimental outline and tested variables of the method development are shown in table 2. The tested extraction methods included Soxhlet and pressurized liquid extraction (PLE). In the Soxhlet experiment, two solvent mixtures were tested, hexane : acetone (3 : 1) and (4 : 1), while the other parametres were kept constant, i.e. 75°C for 7 h. In the PLE extraction experiment, both solvent and temperature were varied. First, three solvent mixtures were tested, and then the two best solvents were tested at four different temperatures (table 2).

The primary scope of the gel-permeation chromatography (GPC) cleanup was to remove the lipid fraction. Two types of solvent were tested, DCM, which was reported in the literature [20, 25], and the less toxic ethyl acetate : cyclohexane (1 : 1). Two separation columns with 100-mL and 300-mL internal volumes were tested with extracts of blue mussel. Specific programmes for different matrices (table 2) were developed by analysing small consecutive fractions of the eluate. The secondary clean-up step consisted of treatment of 2 mL of GPC-cleaned extract with 1 mL of concentrated sulfuric acid. This step was not optimized but applied as found in the literature [20]. The recovery of this cleanup step was estimated by treating 2 mL of standard solutions with 1 mL of sulfuric acid. The third clean-up step was carried out on a short silica column [20] preconditioned with 10 mL of isooctane and eluted with 12.5 mL of isooctane (discarded)

Table 2. Experimental outline.

Step	Type	Variable/experiment
Extraction	Soxhlet	Solvent: hexane : acetone (3 : 1) *vs.* (4 : 1)
	PLE	Solvent: hexane : acetone (3 : 1) *vs.* (4 : 1) *vs.* hexane : DCM (1 : 1)
		Temperature: 60, 100, 125, and 150°C
Clean-up	GPC	Solvent: DCM *vs.* ethyl acetate : cyclohexane (1 : 1)
		Column: PL Gel (600 × 25 mm) *vs.* Phenogel (300 × 21 mm)
	GPC	Fraction setting with different matrices (mussel homogenate, liver-, muscle-, and blubber tissue, and sand eel oil).
	$GPC + H_2SO_4$	Recovery of standard solutions[a] after H_2SO_4 treatment
	$GPC + H_2SO_4$ + silica column	Recovery of standard solutions[a] when eluted on a silica gel column
Analysis	GC-MS	Temperature programme incl. co-elution of PBDEs with or without derivatization
		MS-settings: ion source and quadrupole
	LC-MS-MS	Eluent: H_2O : methanol *vs.* H_2O : acetonitrile
		Eluent programme
		MS-MS settings and injection volume
		Column (5 *vs.* 15 cm C_{18})

[a]Standards used to assess to recovery of the single step as opposed to the entire clean-up. PLE: pressurized liquid extraction, GPC: gel-permeation chromatograph; DCM: dichloromethane.

and 40 mL of isooctane : diethyl ether (85 : 15). As in the case of the sulfuric acid treatment, this step was not further optimized. A recovery experiment of the silica column clean-up was conducted with standard solutions.

The GC temperature programme was optimized in order to separate not only HBCD, TBBPA, and Me-TBBPA but also the derivative of TBBPA (diacetyl-TBBPA) and, at the same time, to avoid co-elution of the analytes with other brominated compounds. Eleven BDE-congeners (BDE-17, 28, 49, 47, 71, 66, 100, 99, 153, 154, and 183) were analysed with the same instrumental method to investigate possible co-elution. In an attempt to increase the response, the same sample was analysed at different temperatures of the ion source (150, 200, and 250°C) and quadrupole (130, 180, and 196°C).

The LC-MS-MS method development was initiated by an infusion experiment in order to find and optimize parent and product ions for each compound. Two analytical columns were tested: a 5-cm Hypersil C_{18} (Thermo Scientific, Waltham, MA) and a 15-cm Zorbax C_{18} (Agilent Technologies, Santa Clara, CA). The columns were tested with water/methanol and water/acetonitrile as eluents, and the final HPLC gradient was developed. Finally, the injection volume and the gas pressure in the collision chamber were optimized.

2.3 *Instrumentation*

For the test of PLE as an alternative to Soxhlet, an ASE-200 system (Dionex, Sunnyvale, CA) was used. The GPC instrument consisted of a Gilson 322 pump, Gilson 233XL injector/fraction collector, Gilson 402 syringe pump, Gilson UV/VIS-155 detector, and a Gilson 506C interface (Gilson, Middleton, WI). The column used for the separation was a PL Gel (600 × 25 mm) packed with polystyrene/divinyl benzene, 10 µm particle, 50 Å pores (Polymer Laboratories, Church Stretton, UK).

The GC-MS instrument was an Agilent gas chromatograph (6890 series) coupled to a MSD 5973 mass spectrometer (Agilent Technologies, Santa Clara, CA). The injector

was operated in pulsed splitless mode with injection of 1 μL of sample; the temperature of the inlet was 220°C in an attempt to avoid degradation of the analytes. The analytes were separated on a DB-5 column (60 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA). The carrier gas was helium at a flow rate of 1.2 mL min^{-1}. The MS was operated in negative chemical ionization (NCI) mode using methane as ionization gas (1.9×10^{-4} Torr). The temperature of the ion source and quadrupole was 250 and 180°C, respectively. For the SIM mode (selected ion monitoring), the following m/z values were chosen: 78.9, 80.8, 160.0, 507.0, and 544.0 for the complete MS run.

The LC-MS-MS used in this study consisted of an Agilent HPLC (1100 series) and a Sciex API 2000 triple quadrupole (Sciex, Concorde, Ontario, Canada). A 15-μL sample was injected and separated on a Zorbax 15 cm C_{18}-column (Agilent Technologies, Santa Clara, CA) with a flow rate of 200 μL min^{-1}. Electrospray ionization (ESI) was used for ionization. The transition ions monitored were 641/79 and 641/81 (HBCDs); 543/79 and 543/81 (TBBPA); 555/79 and 555/81 ($^{13}C_{12}$-TBBPA).

2.4 *Sampling and sample preparation*

A screening study with the purpose of determining the presence of HBCD, TBBPA, and Me-TBBPA in various types of marine biota was performed. The samples analysed in the preliminary screening study included liver and adipose tissue from Faroese long-finned pilot whales (*Globicephala melas*) and northern fulmars (*Fulmarus glacialis*) as well as Greenland black guillemots (*Cepphus grylle*), shorthorn sculpins (*Myoxocephalus scorpius*), ringed seals (*Phoca hispida*), minke whales (*Balaenoptera acutorostrata*), and polar bears (*Ursus maritimus*). In addition, black guillemot eggs were also analysed. The sampling process has been described previously [26, 27], and included pooling of individual samples (typically pools of five subsamples based on equal amounts). Though pooling generally hides low or high values, the pooling was performed in order to obtain better estimates of levels in the general population with a limited number of analyses. Furthermore, the use of pools instead of individual samples allowed analyses of several species. The samples were stored at −20°C until analysis. For the Greenland samples, except minke whale, two pooled samples of each species from each location were analysed, and one pooled sample from each type of the sex and age groups of the Faroese species was analysed. Following homogenization, approximately 0.5 g of blubber, 4 g of liver, or 6 g of egg was mixed with at least an equal amount (weight) of diatomaceous earth for drying. A total of 36 samples were analysed with 12 samples in each batch, along with one blank, one duplicate and two samples of in-house reference material (sand eel oil) in each batch of samples. The samples were analysed with the analytical method that had been developed as part of this study (see section 3.5).

3. Results and discussion

3.1 *Extraction method development*

HBCD, TBBPA, and Me-TBBPA could be extracted with average recoveries between 91 and 98%, using Soxhlet extraction with 350 mL of hexane : acetone (3 : 1) for 7 h at 75°C (figure 1). However, the disadvantages are the large solvent consumption

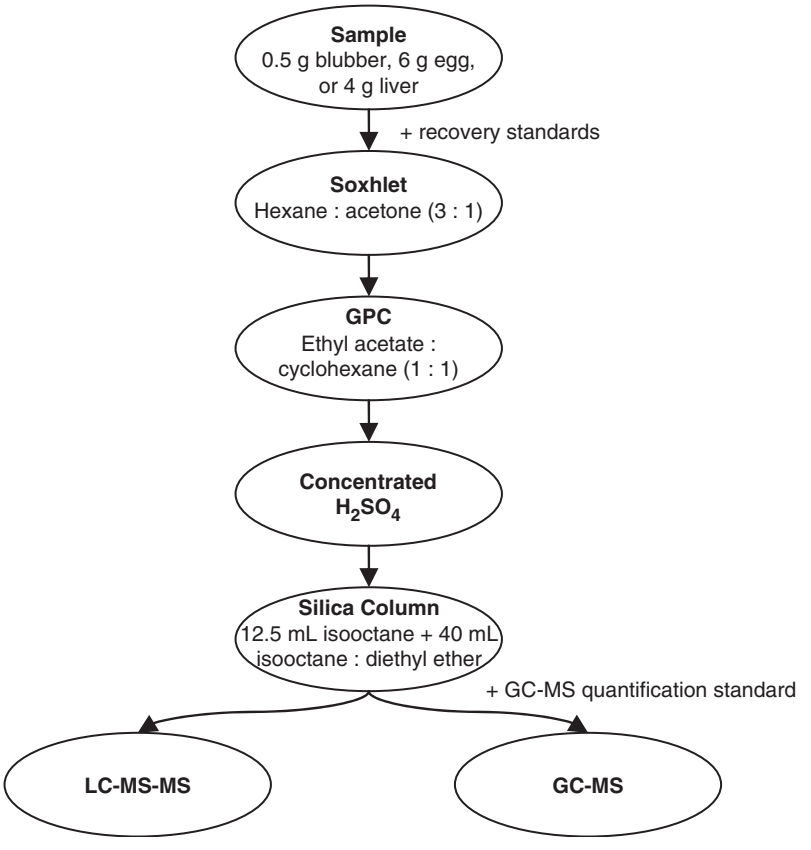


Figure 1. Flow chart of the final analytical method.

(350 mL per sample) and the long extraction time. Therefore, an attempt was made also to develop a PLE method. Despite many experimental varieties, regarding solvent combinations (acetone, hexane, and DCM), temperatures, and other instrumental parameters, it was not possible to extract TBBPA even from Hydromatrix spiked with a standard solution. The average recoveries of HBCD and Me-TBBPA were 99.7 and 99.1%, respectively, while the maximum recovery obtained for TBBPA was 14.7%, which was achieved by extracting with hexane : acetone (3 : 1) at 100°C for 5 min and two static cycles with a flush volume of 60%. As TBBPA has not previously been reported to be extracted by PLE either, it was concluded that TBBPA could not readily be extracted using PLE. Since the aim of the study was to develop a combined extraction procedure for HBCD, TBBPA, and Me-TBBPA, Soxhlet was preferred to PLE.

3.2 *Clean-up method development*

Column clean-up methods used for PBDE analysis did not give satisfactory results for HBCD and TBBPA (recovery: 120–150% and 0.15–0.17%, respectively) [26, 28].

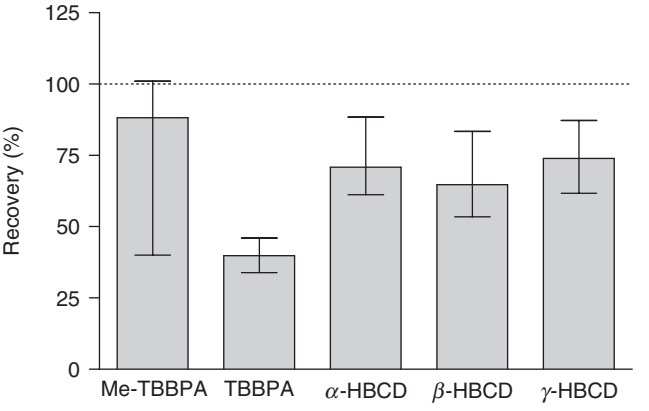


Figure 2. Recovery of analytes (in standard solutions) after silica column chromatography, showing the mean and range of seven replicates. Column clean-up is a critical step for the total recovery of TBBPA.

Therefore, GPC was tested for lipid removal. The experiments showed that a mixture of ethyl acetate and cyclohexane (1 : 1) was just as efficient for elution as DCM. It was noticed that the retention time of the analytes shifted with variations in the lipid content of the samples. Hence, different methods had to be developed for liver and adipose tissue, respectively. Consequently, the method was tested for different types of tissue by analysing small consecutive fractions of the eluting sample. For the analysed egg samples, the method for liver samples was applied, as egg and liver had a similar lipid content.

Analysis of the GPC-fractions revealed that GPC clean-up was not sufficient to remove lipids from biota samples. A treatment with sulfuric acid was applied, though there was some concern that it would also affect the recovery of the analytes. However, recoveries of TBBPA and the HBCD isomers were all close to 100% (93–97%) (both by LC-MS-MS), while the recovery of Me-TBBPA was only 63% (by GC-MS). The low recovery of Me-TBBPA was accepted, as the sulfuric acid clean-up was highly effective.

After purification with sulfuric acid, interfering peaks were still observed in the LC-MS-MS chromatogram of the internal reference material, sand eel oil (~100% lipid). These interferences were not observed in other types of matrices including liver and blubber. Thus, a third clean-up procedure on a short silica column was applied following GPC and sulfuric acid treatment [20]. A recovery experiment using standard solutions showed that this was a critical step in the clean-up procedure, particularly for TBBPA (figure 2). This is a step that should be considered for further optimization, e.g. by eluting with a larger volume, applying less column material, or replacement of silica with another type of sorbent.

3.3 *GC-MS method development*

A temperature programme for the separation of ΣHBCD, TBBPA, Me-TBBPA, and diacetyl-TBBPA was optimized and tested for co-elution with 10 BDE-congeners. However, analysis of native TBBPA revealed a poor response and peak tailing as expected (figure 3a), as well as a high sensitivity to impurities in the inlet.

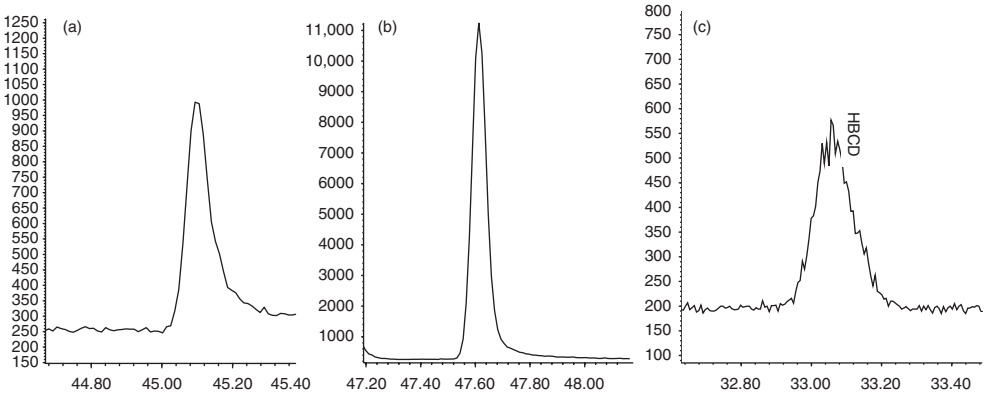


Figure 3. GC-MS chromatograms of (a) TBBPA (112 ng mL^{-1}) revealing peak tailing and poor response, (b) diacetyl-TBBPA (112 ng mL^{-1}) improved chromatography and higher response compared with TBBPA, and (c) HBCD resulting in a broad peak as a result of thermal breakdown on the column.

Hence, derivatization was considered necessary for the determination of TBBPA by GC-MS (figure 3b). The derivatization process is time-consuming and very sensitive to matrix effects. Thus, it was decided to analyse TBBPA by LC-MS-MS. Me-TBBPA showed sharp peaks and a good response when analysed by GC-MS.

When analysing HBCD by GC-MS, the response was low, and the peak was very broad [29] (figure 3c), which was probably caused by degradation on the column due to the high temperature and HBCD being thermally labile [30]. The 60-m DB-5 column helps separation of HBCD from other brominated compounds and thus reduces co-elution; however, the extended residence time of HBCD on the column probably increases the degradation on-column. Degradation in the inlet was also observed in terms of additional appearing peaks, but this was minimized by reducing the inlet temperature from 270 to 220°C. Analysis of ΣHBCD by GC-MS (NCI) excludes the use of isotope labelled standards, as they cannot be separated from native HBCDs by GC-MS in NCI mode.

MS parametres (temperature of ion source and quadrupole) were optimized to obtain the best possible response. It had previously been reported that the temperature of the quadrupole was not important [31]; however, an increased response could be obtained by increasing the temperature of the quadrupole along with the ion source. The final temperatures were 250°C for the ion source and 180°C for the quadrupole. Preliminary experiments indicated that the pressure of the ionization gas also influenced the response (data not shown), but this was not investigated further. It would probably also be possible to lower the detection limit by optimising the injection volume, as pulsed splitless allows larger injection volumes.

3.4 *LC-MS-MS method development*

A 15-cm C_{18}-column was selected after incomplete separation on a 5-cm C_{18}-column (figure 4a). The application of acetonitrile and water as eluent resulted in incomplete (~50%) separations of the α and β isomers (figure 4b). Application of methanol and water as eluents and the same gradient programme resulted in nearly complete

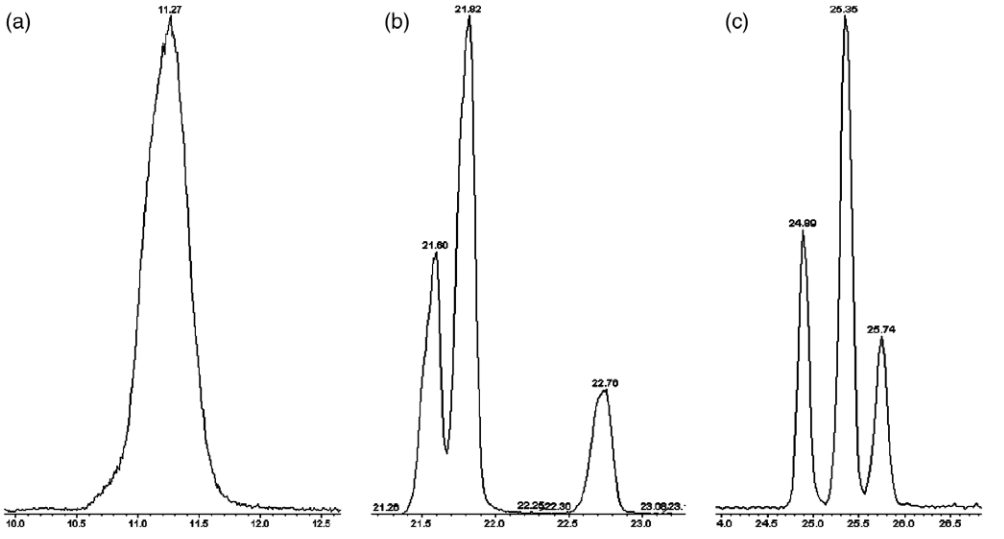


Figure 4. LC-MS-MS separation of the HBCD isomers ($1\,\mu g\,mL^{-1}$ of each) with different column and eluents, (a) 5 cm C_{18} w. water/methanol, (b) 15 cm C_{18} w. water/acetonitrile, and (c) 15 cm C_{18} w. water/methanol (final method).

separation of α and β; only a small overlap remained near baseline for β and γ (figure 4c). It is possible that the separation could still be improved by simultaneous application of both acetonitrile and methanol in combination with water; this combination has been applied previously [32, 33].

The injection volume (15 µL) was a compromise between the highest sensitivity obtained for HBCDs (optimal volume: 25 µL) and for good chromatography of TBBPA (optimal volume 15 µL).

3.5 *Summary of analytical method*

The outline of the method is shown in figure 1, and the details of the final method are as follows. The weighed samples were transferred to Soxhlet thimbles, recovery standards (BDE-77 and $^{13}C_{12}$-TBBPA) were added, and the samples were left for at least 8 h prior to Soxhlet extraction, which was carried out over 7 h at 75°C with *n*-hexane : acetone (3 : 1). The extracts were reduced to 1.5 mL by rotary evaporation and transferred to a GPC vial. The GPC eluent was ethyl acetate : cyclohexane (1 : 1) at a flow rate of $10\,mL\,min^{-1}$ (separation column: PL Gel 60 10 µm 50 Å). The analytes were collected between 15 and 23 min. The GPC-cleaned extracts were reduced to 2 mL by rotary evaporation, and 1 mL of concentrated sulfuric acid was added. After mixing, the samples were left at 5°C for 4–18 h to obtain a good separation of the aqueous and organic phases. The organic phase was transferred to a glass column (d: 10 mm) packed with 1.8 g of silicagel, to which one drop of water had been added. The column had been conditioned with 10 mL of isooctane and was eluted with 12.5 mL of isooctane (fraction discarded) and then 40 mL of isooctane : diethyl ether (85 : 15). The purified extracts were reduced to less than 1 mL, the GC-quantification standard (BDE-71) was added, and the volume was adjusted to 1 mL. Two hundred microlitres was taken

Table 3. Limits of detection on the instruments and in different types of tissue samples.

Compound	Instrument	LOD ($ng\,mL^{-1}$)	LOD_{liver} ($ng\,g^{-1}$ ww)	$LOD_{blubber}$ ($ng\,g^{-1}$ ww)	Average recovery (%)[a]	LOD_{liver}[b] ($ng\,g^{-1}$ ww)	$LOD_{blubber}$[b] ($ng\,g^{-1}$ ww)
Me-TBBPA	GC-MS	1.28	0.37	2.9	68	0.55	4.2
ΣHBCD	GC-MS	5.92	1.66	12.8	56	2.96	22.8
TBBPA	LC-MS-MS	1.53	0.11	0.79	28	0.38	2.83
$^{13}C_{12}$-TBBPA	LC-MS-MS	1.61	0.12	0.93	35	0.35	2.65
α-HBCD	LC-MS-MS	1.02	0.08	0.62	63	0.13	0.98
β-HBCD	LC-MS-MS	0.46	0.16	0.27	20	0.17	1.33
γ-HBCD	LC-MS-MS	1.59	0.13	0.98	43	0.30	2.29

[a]In piked sand eel oil ($n = 4$).
[b]Corrected for approximate recovery, weight, and dilution of sample.

for GC-MS analysis of Me-TBBPA and ΣHBCD. The temperature programme was 90°C (2 min), 15°C min^{-1} to 220°C, 3°C min^{-1} to 300°C, and 300°C held for 13 min, which resulted in a total run-time of 50 min. The remaining 800 μL was evaporated to dryness in a silanized vial and reconstituted in 200 μL methanol. The methanol extracts were analysed by LC-MS-MS for the HBCD-isomers and TBBPA. The analytes were eluted with a gradient of water (10 mM ammonium acetate) and methanol starting at 80/20 (1 min), ramp to 5/95 over 15 min, 5/95 held for 10 min, ramp to 80/20 in 4 min, and held for 10 min.

3.6 *Limit of detection*

The instrumental limit of detection (LOD) and quantification (LOQ) were determined as a signal-to-noise-ratio (S/N) of 3 and 5, respectively. A low-level sample was used to determine the noise level. This LOD can be used to calculate the detection limit in actual biota analyses with varying sample amounts. Since the recovery varied between compounds, the detection limit in the tissue was also corrected for the estimated recovery (table 3).

3.7 *Preliminary screening*

The newly developed method was applied in a preliminary screening of biota samples from Greenland and the Faroe Islands. Detailed information on the analysed samples has been published elsewhere [26]. The results of the in-house reference material were used to establish a control chart. The relative standard deviation between the six reference materials in the three batches was between 15 and 21% for Me-TBBPA, TBBPA, and α-HBCD. The recovery observed in the reference material was very similar to those reported in table 3 with measured concentrations around 40 $ng\,g^{-1}$ lw. The variation of α-HBCD concentrations in the duplicates was between 5 and 25% (not corrected for recovery). These variations are likely to decrease significantly when specific recovery standards are available. The target compounds were not found in the blank samples.

Neither TBBPA nor Me-TBBPA was detected in any of the samples. α-HBCD was detected in 35 of the 36 samples, while β and γ-HBCD were detected in 10 and 14 samples, respectively, though generally in much lower concentrations than the

Table 4. Levels of HBCD isomers in marine Arctic biota.[a]

Species and tissue	Lipid content (%)	α-HBCD (ng g^{-1} lw)	β-HBCD (ng g^{-1} lw)	γ-HBCD (ng g^{-1} lw)	ΣHBCD (LC) (ng g^{-1} lw)	ΣHBCD (GC) (ng g^{-1} lw)
Polar bear, adipose (E.Gr.)	89.48	5.38	n.d.	n.d.	5.38	8.92
	89.40	13.21	<0.54	<1.81	13.21	18.27
Polar bear, liver (E.Gr.)	4.85	2.85	n.d.	n.d.	2.85	5.40
	7.42	3.87	n.d.	n.d.	3.87	6.89
Ringed seal, blubber (E.Gr.)	96.70	2.50	<0.49	<1.66	2.50	4.69
	96.28	2.40	n.d.	n.d.	2.40	6.92
Ringed seal, liver (E.Gr.)	1.79	16.71	n.d.	n.d.	16.71	n.d.
	1.65	<8.61	<3.95	<13.36	<25.9	13.08
Ringed seal, blubber (W.Gr.)	97.45	<1.04	n.d.	n.d.	<1.04	1.85
	97.07	<1.01	<0.47	<1.57	<3.05	1.54
Ringed seal, liver (W.Gr.)	1.75	<7.94	n.d.	n.d.	<7.94	22.56
	2.33	<5.45	n.d.	<8.46	<13.9	n.d.
Minke whale, blubber. (W.Gr.)	83.78	3.04	n.d.	<1.90	3.04	4.45
Minke whale, liver (W.Gr.)	3.38	<3.81	n.d.	n.d.	<3.81	4.67
Shorthorn sculpin, liver	15.47	1.82	n.d.	n.d.	1.82	3.79
	12.53	1.78	n.d.	n.d.	1.78	4.92
Shorthorn sculpin, liver (W.Gr.)	12.27	<1.12	n.d.	n.d.	<1.12	n.d.
	10.43	<1.24	n.d.	<1.92	<3.15	1.21
Black guillemot, liver (E.Gr.)	5.43	11.20	n.d.	<10.20	11.20	22.54
	2.89	8.39	n.d.	n.d.	8.39	24.00
Black guillemot, egg (E.Gr.)	9.58	4.10	n.d.	n.d.	4.10	5.44
	10.36	1.92	n.d.	n.d.	1.92	3.86
Black guillemot, liver (W.Gr.)	4.65	<4.60	n.d.	n.d.	<4.60	4.02
	3.01	n.d.[b]	n.d.	n.d.	n.d.	8.63
Black guillemot, egg (W.Gr.)	10.67	<0.86	<0.39	n.d.	<1.25	1.01
	10.00	<0.91	n.d.	<1.41	<2.32	1.25
Lf. Pilot whale, blubber, juveniles (F.I.)	91.27	80.04	39.77	n.d.	119.81	217.55
Lf. Pilot whale, blubber, females (F.I.)	93.47	26.20	n.d.	<1.59	26.20	70.31
Lf. Pilot whale, blubber, males (F.I.)	90.33	90.92	<0.50	<1.70	90.92	137.71
Lf. Pilot whale, liver, juveniles (F.I.)	2.28	10.56	n.d.	n.d.	10.56	92.14
Lf. Pilot whale, liver, females (F.I.)	2.91	15.00	n.d.	n.d.	15.00	37.29
Lf. Pilot whale, liver, males (F.I.)	2.41	22.54	n.d.	n.d.	22.54	114.44
Fulmar, subcutaneous fat, females (F.I.)	56.90	19.20	<0.79	6.64	25.84	61.55
Fulmar, subcutaneous fat, males (F.I.)	69.31	41.94	<0.68	2.39	44.33	28.75
Fulmar, liver, females (F.I.)	3.65	10.28	<1.72	<5.81	10.28	12.10
Fulmar, liver, males (F.I.)	2.93	23.06	n.d.	n.d.	23.06	34.66

[a]Neither TBBPA nor Me-TBBPA was found in any of the analysed samples. <0.54 ng g^{-1} lw indicates that a peak was detected but below LOQ. n.d.: not detected; Gr.: Greenland; F.I.: Faroe Islands; E.: east; W.: west; Lf: Long finned.
[b]Only 1.66 g was available for analysis; usually 4 g is used; this has affected the detection limit (−60%). ΣHBCD (LC) is calculated as the sum of α, β, and γ-HBCD determined by LC-MS-MS. α, β, and γ-HBCD were not corrected for recovery and should be taken as minimum values. ΣHBCD (GC) is the direct determination of ΣHBCD by GC-MS.

α-isomer (table 4). The one sample in which none of the HBCD-isomers were found was only available in limited amounts, which affected the detection limit (60% reduction). Since the results are not corrected for recovery, the reported concentrations should be regarded as minimum values. The results reported in table 4 are normalized to lipid content, as it is a common procedure when reporting lipophilic compounds like BFRs. However, when the lipid content is extremely small (as in the case of the ringed seal livers), even small uncertainties in the lipid determination greatly influence the reported results in ng g^{-1} lw.

ΣHBCD concentrations determined by GC-MS are generally higher than the sum of the individual isomers determined by LC-MS-MS. However, the overall recoveries

of ΣHBCD (GC) and α-HBCD (LC) are very similar (table 3). The difference is likely to occur from the large uncertainty in the quantification of ΣHBCD by GC-MS caused by the low response and broad peak. However, ion suppression on LC-MS-MS might underestimate results if not corrected. LC-MS-MS was chosen for analyses of HBCDs and TBBPA over GC-MS because of its ability to separate the HBCD-isomers as well as better chromatography of both HBCD and TBBPA without thermal degradation. Hence, in the current study, the LC-MS-MS results are believed to be more reliable and are the only results considered in the following discussion.

The levels of HBCD measured in this study are comparable though slightly lower than previously reported concentrations of HBCD in the Arctic environment, e.g. polar bear adipose tissue from East Greenland (32.4–58.6 ng g^{-1} lw compared with 5.4–13.2 ng g^{-1} lw in the current study) and seal blubber from Svalbard (15–35 ng g^{-1} lw compared with 2.4–2.5 ng g^{-1} lw found in seal blubber from East Greenland in the current study) [17, 34]. The previously analysed Artic samples have all been analysed using GC-MS, which might result in differences from results obtained by LC-MS-MS. It should be kept in mind that the results in this study are to be considered as minimum values.

The concentration levels of the HBCDs in animals from East Greenland are generally higher than in the same species and tissues from West Greenland. The same effect has previously been described for other halogenated compounds such as PBDEs, PCBs, and DDT [26, 35]. Based on the blubber samples alone, there are indications of biomagnification of HBCD through the food web (ringed seal < polar bear). However, the liver samples from the same animals do not confirm this result. Hence, further investigations on the biomagnification of HBCD in various tissues of individual animals should be conducted.

The samples from the Faroe Islands generally contained higher concentrations of HBCD than the Greenland samples. The highest level of all analysed samples was observed in blubber of juvenile long-finned pilot whales (119.8 ng g^{-1} lw). Though the current dataset is very limited, there were indications of maternal transfer of HBCD from mother to offspring for long-finned pilot whales; however, this should be examined in more detail.

The fact that TBBPA was not detected in any of the analysed samples could be a result of concentrations below LOD (liver: 0.38 ng g^{-1} lw, blubber: 2.8 ng g^{-1} lw). TBBPA is mainly used as a reactive flame retardant, which might have markedly reduced the emission to the environment. However, TBBPA has previously been reported in marine biota closer to sources [6, 20]. It could also indicate that TBBPA is not persistent enough for the long-range transport to the Arctic, as it has been reported to be photodegradable [10]. Me-TBBPA possesses the physical properties to accumulate; nevertheless, it was not detected in the Greenland and Faroese samples. This could be explained by Me-TBBPA being present below the limit of detection or the absence of the parent compound in the remote Arctic.

4. Conclusion

An analytical method for the simultaneous determination of HBCD-isomers, TBBPA, and Me-TBBPA was developed using both GC-MS and LC-MS-MS. The advantage

of the method was that the individual HBCD-isomers could be determined, that TBBPA did not need derivatization, and that Me-TBBPA was determined with the same extraction as TBBPA. However, the low recoveries for some compounds show that further method optimization is advisable. It also became clear that individual mass-labelled HBCD-isomers would be an advantage for accurate determinations as the three isomers had different recoveries. The presence of HBCD in Arctic marine biota indicates both persistence and long-range transport of this compound, and along with the previous findings in the literature, it strongly indicates that HBCD is a ubiquitous chemical. HBCD possesses several of the same properties as the chemicals on the United Nations POP list [36]. Further studies on the presence, and not least the toxicology of the individual isomers, will be advisable. TBBPA has not been detected in the remote Arctic with the analytical method available at present. Despite the extremely large consumption of TBBPA, only relatively low levels have been reported in biota (though more frequently observed in abiotic samples).

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