



Determination of halogenated flame retardants by GC-API-MS/MS and GC-EI-MS: a multi-compound multi-matrix method

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Received: 29 September 2017 / Revised: 16 November 2017 / Accepted: 21 November 2017 / Published online: 13 December 2017
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Abstract

The extensive application of halogenated flame retardants has led to their widespread distribution in the environment. Recently, concerns emerged regarding their potential persistence, (bio)accumulation, and/or toxicity. Particularly halogenated flame retardants based on norbornene structures, like Dechlorane Plus as well as other brominated PBDE replacements, generically called *emerging*, *novel*, or *alternative* flame retardants, are in the focus of interest. A comprehensive analytical method for the determination of 21 halogenated flame retardants (HFRs) of different substance classes (dechloranes, brominated aromates, brominated ethers, cyclic BFR) in a broad variety of matrices (tree leaves, fish fillet, birds eggs, suspended particles) was developed in order to assess their environmental levels as well as temporal trends, especially for the use within environmental specimen banks (ESBs). In addition to the *alternative* HFRs, a set of 24 PBDEs were measured in the same samples, however using GC-EI-MS for detection. Samples were extracted using accelerated solvent extraction (ASE) with dichloromethane:hexane (exception: soxhlet extraction for suspended particles) followed by a multi column clean-up. Quantification was performed by API-GC-MS/MS as a modern, gentle, and sensitive technique for simultaneous detection of compounds throughout a wide range of masses and fragmentation characteristics (exception: PBDE detection using GC-EI-MS). With the exception of BDE 209, instrumental precisions of target compounds ranged from 1% to 16 % (at levels of 2 pg injection⁻¹ for HFR, 20 pg injection⁻¹ for DBDPE, 7–36 pg injection⁻¹ for PBDEs). Interday precisions of the entire analytical method including extraction and clean-up were mostly below 25% for all validation matrices at spiked levels of 100 pg sample⁻¹ for HFR (DBDPE: 1000 pg sample⁻¹) and 1200–6000 pg sample⁻¹ for PBDEs. The majority of analytes were investigated with expanded measurement uncertainties of less than 50%.

Keywords Dechlorane Plus · Brominated flame retardants · nBFR · German environment specimen bank · Atmospheric pressure ionization

Introduction

Over the last decades, the need for fire prevention has led to an extensive use of halogenated flame retardants (HFR)

especially in furniture, building materials, isolation materials, and household articles. HFRs react with radicals formed during initial stages of the combustion process, help to exclude oxygen, and also enhance charring, all of which inhibit the propagation of fire (cited in [1]). HFRs can also significantly reduce the amount of smoke produced during combustion, enhancing vision for escape from the fire zone [1]. More than 175 different flame retardant substances are known to have been manufactured, among which the brominated flame retardants (BFRs) are the largest group in terms of production volume and market demand [2]. In the past, polybrominated diphenyl ethers (PBDEs) have dominated the market for BFRs. In 2009 certain congeners contained in commercial pentabromodiphenyl ethers (PentaBDE) and octabromodiphenyl ethers (OctaBDE) were added to Annex A of the Stockholm Convention. In 2017, DecaBDE was added to Annex A of the Stockholm Convention with several exceptions [3]. However, several so called *emerging*, *novel*, or

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00216-017-0784-x>) contains supplementary material, which is available to authorized users.

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alternative flame retardants meet the market demand with an estimated amount of 100,000–180,000 tons annually [4], suggesting more frequent occurrence in environmental matrices in the future as a result of increasing usage as substitutes. Besides a variety of alternative BFRs, chlorinated flame retardants such as Dechlorane Plus (DP) are increasingly applied. Many of these flame retardants have similar properties as persistent organic pollutants (POPs), e.g., a high degree of halogenation or high lipophilicity, and therefore POPs-like environmental behavior and adverse environmental effects can be anticipated. Thus, a growing number of scientific studies investigate the environmental fate and effects of these substances. Therefore, there is an ongoing need for multi-compound methods that are suited to be applicable to a wide range of environmental matrices.

HFR were usually extracted by soxhlet extraction or accelerated solvent extraction (ASE). Solvents used comprise hexane, dichloromethane (DCM), hexane:acetone, or hexane:DCM in various mixtures but usually 1:1 (v:v). Clean-up procedures were usually performed with (multilayer)silica, alumina, and/or florisil columns of differing degree of deactivation or acidification, also depending on the acid stability of the target analytes. In general, biota samples were additionally cleaned up using gel permeation chromatography (GPC; usually using Bio-Beads SX3) or sulfuric acid. In the past, instrumental analysis of HFR mostly relied on GC-MS or GC-MS/MS with negative chemical ionization (NCI) as instrumental techniques [5–10]. Less frequently, GC-high resolution MS (HRMS) was applied [11–13]. The analysis of HFR was reviewed, e.g., by [14–16]. Though with HRMS, sensitive instrumentation is available, certain drawbacks such as high price, difficult handling, or a limited mass range as used in voltage scan mode make it interesting to look towards other techniques. Over the last years more sensitive techniques such as, e.g., atmospheric pressure ionization GC-API-MS/MS offered possibilities to quantify amounts at the low to mid femtogram on-column range. General suitability of that technique could recently be demonstrated for some BFRs [9, 17–20]. As noted by Papachlimitzou et al. [14], there are a number of studies on *novel* flame retardants that have reported extensive information on quality assurance/quality control (QA/QC) and method validation, although for many others such important information was lacking and data have been published without sufficient evidence that the method used was fit for purpose.

The objective of this study was to develop a sensitive, robust, and selective method for a variety of chlorinated and brominated flame retardants – with a focus on Dechlorane Plus and other dechloranes – that can be applied later on to a wide range of samples of the German environment specimen bank (ESB) such as tree leave samples, fish and marine biota samples, bird egg samples, or riverine suspended particulate matter. Due to the matrix diversity, most developmental efforts

were put into sample preparation, i.e., different clean-up procedures, as different matrix constituents such as lipids or polar substances may lead to interferences hampering a decent analytical performance. The present study is structured in such a way that the final method is described in detail in the ‘Materials and method’ section, whereas all validation parameters as well as challenges of the method and challenges during the method development are discussed in the “Results and discussion” section.

Materials and method

Standards and reagents

All chemicals and standards used are shown in the Electronic Supplementary Material (ESM) (Tables S1, S2; Fig. S1). The following target compounds were analyzed: TBA, ATE, BATE, β -/ γ -TBECH, PBT, PBEB, HBBz, DPTE, BEHTBP, EHTeBB, BTBPE, Dec602, Dec603, Dec604, DPMA, Cl₁₀-antiDP, Cl₁₁-antiDP, synDP, antiDP, DBDPE, and a series of important PBDEs (BDE 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209).

Sample material

As the method should be capable of analyzing a broad range of halogenated flame retardants in several specimen types of the German ESB, final method validation was performed on pooled surplus material from the following ESB sample types: spruce shoots for complex plant materials, bream fillet for marine and fresh water biota, herring gull eggs content for birds egg samples, and riverine suspended particulate matter for solid environmental materials with complex organic constituents.

Sample preparation and extraction

Samples were freeze-dried (–76 °C, overnight) and homogenized with a stainless steel blender. The amount of samples used were 5 g dry weight (dw) for spruce shoots, 2.5 g dw for fish, 2 g dw for egg, and 2 g dw for suspended particles. Samples were directly spiked with a mixture of mass-labeled (isotopically-labeled) internal standards (¹³C-HBBz, ¹³C-BTBPE, ¹³C²H-EHTeBB, ¹³C²H-BEHTBP, ¹³C-Dec602, ¹³C-SynDP, ¹³C-AntiDP, ¹³C-DBDPE, ¹³C-TriBDE 28, ¹³C-TetraBDE 47, ¹³C-PentaBDE 99, ¹³C-HexaBDE 153, ¹³C-HeptaBDE 183, ¹³C-OctaBDE 197, ¹³C-NonaBDE 207, ¹³C-DecaBDE 209, ESM Table S1) in nonane at levels of 125 to 5000 pg sample^{–1}. For determination of method performance characteristics, target compounds were spiked to original sample material at a level of 100 pg sample^{–1} for

HFR (DBDPE: 1000 pg sample⁻¹) and 1200–6000 pg sample⁻¹ for PBDEs. Standard addition levels are given in Table S3.

All samples were extracted after a settling time of at least 1 h after spiking. For biota matrices, sample extraction was performed using accelerated solvent extraction (ASE) with dichloromethane (DCM):n-hexane 1:1 (v:v) [21] over three static cycles, 3 min extraction time at 125 °C each. For extraction, samples were thoroughly mixed with diatomaceous earth in order to prevent clogging and to reduce the dead volume of the extraction cell. For riverine suspended matter, soxhlet extraction over 8 h was used. For details see ESM Table S5. The combined extracts were evaporated using a rotary evaporator (Büchi, Switzerland) to near dryness (0.2 mL), filled up to 2 mL n-hexane, and transferred to the clean-up procedure. For suspended matter, 1.5 g copper was activated with hydrochloric acid and added before clean-up in order to remove sulphur [6].

Sample clean-up

The final clean-up method was chosen on the basis of preceding experiments (see ESM). It was designed in a way that of each matrix type the lipid content of the analyte-containing fractions was minimized without losing significant amounts of the target compounds. Thus, the final clean-up was a multi-column clean-up, which is schematically presented in Fig. S2 (see ESM). The first clean-up step was a 15 mm inner diameter (i.d.) column filled with 2 g silica. The column was prewashed with 20 mL n-hexane, then the sample solution was applied and the column was eluted with 20 mL of DCM. The eluate was evaporated nearly to dryness using 50 µL n-nonane as a keeper and filled up to 1 mL with ethylacetate:cyclohexane 1:1 (v:v) (called GPC eluent in the following). The second clean-up step consisted of gel permeation chromatography on a reusable 20 mm i.d. GPC column filled with 33 g Bio-Beads S-X3 (on the basis of [22]), conditioned with the GPC eluent. Sample eluates from clean-up step 1 of 1 mL were applied to the GPC column and the flask rinsed twice with 0.5 mL GPC eluent. The column was eluted with 1st 70 mL GPC eluent (this fraction was discarded), 2nd with 85 mL GPC eluent (fraction containing the target compounds), and 3rd with 20 mL GPC eluent for preparation of the column for reuse. The analyte fraction was evaporated again to near dryness by rotary evaporation. After volume setting to 1 mL, as third and final step, a clean-up using a florisil column was performed. A 12 mm i.d. column was filled with 2.5 g of 5% water deactivated florisil. The analytes were eluted in two fractions. The first fraction consisted of 10 mL of n-hexane and contained the major part of the analytes, i.e., TBA, ATE, BATE, β-/γ-TBECH, PBT, PBEB, HBBz, DPTE, Dec602, Dec603, Dec604, DPMA, Cl₁₀-antiDP, Cl₁₁-antiDP, synDP, antiDP, DBDPE as well as PBDEs. Three substances (BEHTBP, EHTeBB, and BTBPE and their respective mass-

labeled standards) were eluted in a second fraction, which consisted of 15 mL toluene/n-hexane 1:1 (v:v) and 5 mL n-hexane. For suspended particle samples, the third clean-up step proved not to be necessary, resulting in one single fraction for all analytes.

Fifty µL of n-nonane as well as 50 µL of ¹³C₁₂-HexaBDE138 recovery standard (syringe standard) solution at 250 pg sample⁻¹ were added to each fraction. Afterwards, both fractions were evaporated separately to approximately 50 µL final volume each, for instrumental analysis. For covering the entire set of analytes, the instrumental analyses were split into three separate runs per sample. Finally, 45 HFR were integrated into the method.

Instrumental separation and detection

Over the last decade, dechloranes and other halogenated flame retardants were analyzed using different mass spectrometric techniques, mainly by GC-NCI-MS but also by GC-HRMS, GC-MS/MS, and recently GC-API-MS/MS [9, 20]. Prior to establishing the analytical method, an evaluation of alternative instrumentation was performed (see ESM). As result, GC-API-MS/MS (XEVO TQ-S, Waters, Eschborn, Germany) equipped with a 7890 GC oven and a 7693A automated liquid sampler (both Agilent, Waldbronn, Germany) were found to be the most suitable instrumentation for dechloranes, and *alternative* BFRs. PBDEs were not analyzed by GC-API-MS/MS because stability of the analytical performance (particularly instrumental sensitivity and peak shape) was significantly better using GC-EI-MS.

The final GC-API-MS/MS method was as follows: injections took place using a splitless injection with a 1 µL injection volume in pulsed splitless mode at 280 °C. For maximum inertness, a Siltek-deactivated gooseneck-liner (Restek, Bad Homburg, Germany) was used. The GC column was a fused silica capillary column (Agilent DB5ht; 15 m; 0.25 µm dF; 0.25 mm i.d.) run with helium as a carrier gas. The flow program started at 4 mL min⁻¹ constant flow for 10 min and was then ramped up to 12 mL min⁻¹ with a rate of 1 mL min⁻². The analytical GC column was amended by a 0.3 m uncoated, deactivated fused silica pre-column (retention gap, Siltek guard column, Restek, Bad Homburg, Germany) and a 0.5 m post-column (transfer line). The temperature program was 120 °C for 2 min.; 10 °C min⁻¹ to 200 °C; 20 °C min⁻¹ to 300 °C; 40 °C min⁻¹ to 340 °C for 5 min. The transfer line was maintained at 340 °C. The detector was used in API ionization mode under dry conditions and multi reaction mode (MRM) using 2 ions per compound with individually adapted ionization parameters. Detailed information about the GC conditions as well as MS source and acquisition parameters are presented in Tables S6–S8 in the ESM. Information on the evaluation of instrumentation is presented in the ESM.

The GC-EI-MS conditions for the analysis of PBDEs were as follows: PBDE were analyzed on an Agilent 6890 GC coupled to an Agilent 5973 single quad mass spectrometer. Injections took place with 3 μL injection volume using a PTV injector (Gerstel KAS) at 150 $^{\circ}\text{C}$ for 15 s hold, then ramping at 300 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$. Purge flow was 100 mL min^{-1} over 2 min. For maximum inertness, a Siltek-deactivated baffled 1.5 mm liner (Restek) was used. The GC column used was a fused silica capillary column (Restek RTX1614, 15 m, 0.1 μm dF, 0.25 mm i.d.) run with helium 5.2 as a carrier gas at 1.5 ml min^{-1} at constant flow. The temperature programme was 130 $^{\circ}\text{C}$ for 1 min.; 12 $^{\circ}\text{C min}^{-1}$ to 275 $^{\circ}\text{C}$; 12.5 $^{\circ}\text{C min}^{-1}$ to 330 $^{\circ}\text{C}$ for 5 min. The detector was used in EI ionization mode at 150 $^{\circ}\text{C}$ source temperature. Mass calibration was performed using FC43 (heptacosane). For data registration, 2 ions per compound were registered in 0.7 u mass resolution mode. Detailed information about the GC conditions as well as MS source and acquisition parameters are presented in ESM Table S8.

QA/QC and quality aspects

General QA/QC measures

The laboratory equipment used was thoroughly pre-cleaned by a standard laboratory dishwasher, a drying oven for glassware operated at 300 $^{\circ}\text{C}$, and a final rinsing step with acetone. In order to shield the partially well brominated and UV sensitive analytes from UV radiation, light exclusion (e.g., by using alumina foil, closing shutters, keeping off unnecessary halogen lights, using brown glassware as far as possible) was performed. Sixteen mass-labeled internal standards (eight PBDEs, eight other HFRs; ESM Table S1) were used to correct for analyte losses or irregularities during the analytical process.

Instrumental QA/QC measures

For control of GC performance, it was observed that peak separation was not the critical factor for chromatography quality but rather peak form. Therefore, the tailing factor for hexabromobenzene (HBBz) has been used as a quality criterion as particularly HBBz was critical regarding tailing (ESM Figs. S3, S4). The Tailing factor TF was calculated as $\text{TF} = 2b/ab$, with a = peak width at 10% peak height towards peak start and b = peak width at 10% peak height towards peak end and an acceptance criterion of $\text{TF} \leq 2.5$. Daily GC/MS-system performance in terms of sensitivity was assessed by monitoring an indicator. The intensity of ^{13}C -Dec602 has been chosen with a demanded minimum of signal to noise (S/N) ratio of 500:1 for 2 $\text{pg } ^{13}\text{C}$ -Dec602 on column as Dec602 elutes near the center of the chromatogram and is a non-sensitive analyte with respect to GC behavior. General information on sensitivity and robustness of instrumental performance were obtained

by running multi-point calibrations over the development process. Instrument linearity was determined using a 14 point calibration in the range of 0.01–200 $\text{pg } \mu\text{L}^{-1}$ (DBDPE 0.1–2000 $\text{pg } \mu\text{L}^{-1}$). For quantification, the isotope dilution method or, in case of substances with no compound-specific mass-labeled equivalent, the internal standard method was applied.

Method validation

The method was validated for each of the validation matrices (spruce shoots, bream fillet, herring gull egg, riverine suspended particulate matter) regarding instrumental detection limits (IDL) and method quantification limits (MQL), working range, laboratory performance (recovery rates of mass-labeled standards), instrumental measurement precision, repeatability (intraday precision), intermediate precision (interday precision) trueness, and estimation of measurement uncertainty with one validation level.

MQLs were defined using a worst-case approach on the basis of instrument sensitivity (expressed as IDL), the method working range (calibration), and standard deviation of background blanks (expressed as $\text{MQL} = 10 \cdot s \cdot \sqrt{2}$ with s = standard deviation of background blanks based on [23]). Information on blanks is given in Table S4 (see ESM). With the exception of BDE 47, BDE 99, BDE 100, BDE 183, and BDE209, PBDEs were detected in less than 20% of the blank samples. In contrast, most alternative HFRs investigated in the present study were frequently detected in blank samples. This also reflects the lower IQLs for substances determined by the GC-API-MS/MS.

A working range was established for each individual substance. Criterion was the stability of the relative response factor (RRF) of the analyte's response to its respective quantification standard with an allowed maximum deviation of $\pm 20\%$ of the average RRF for isotope dilution quantification via average RRF [24].

Recovery rates for the mass-labeled internal standards were calculated as a measure of general laboratory performance and monitored against the recovery standard $^{13}\text{C}_{12}$ -HexaBDE 138 for every analysis. Instrumental precision was determined by running a standard solution at a level of 2 pg for HFRs (DBDPE: 20 pg) and 7–36 pg for PBDEs per injection for six times. Measurement precision was expressed as standard deviation of the peak areas of the target analytes normalized by the internal standard.

For the determination of intraday and interday precision, standard solutions containing native analytes at a general spiking level of 100 pg sample^{-1} for HFRs, 1000 pg sample^{-1} for DBDPE, and 1200–6000 pg sample^{-1} for PBDEs were added to matrix samples used for validation (see section samples) and analyzed with the final procedure. The results of in total 15 analyses per matrix were used for the determination of intraday precision ($n = 6$) and interday precision ($n = 4 \cdot 3$).

In addition, analyses of the same samples, but unspiked, were performed ($n \geq 3$) in order to determine the intrinsic amount of target compounds in the samples. The total nominal spiking concentration of the analytes could thus be calculated and served as a basis for the assessment of trueness [expressed as recovery rate of native analytes (actual concentration observed in the spiked sample divided by the nominal concentration in the same sample)].

An estimation of the measurement uncertainty was performed applying a scheme by considering random errors from the precision data as well as method bias [25]. The latter was estimated due to lack of certified reference materials and laboratory round robin tests by use of the data generated for analyte recovery/trueness.

Results and discussion

Chromatographic separation

General chromatograms of the final instrumental methods are presented in Fig. 1. Overall, analyte separation was good. In the rare case of co-elution, correct quantification was achieved due to sufficient selectivity of mass spectrometric detection. However, performance of β - and γ -TBECH proved to be critical due to instabilities of their relative ratio under different clean-up conditions as well as in the GC system. Owing to its close elution to β -TBECH, the chromatographic peak of α -TBECH was integrated together with the β -TBECH peak.

DBDPE was found to be prone to degradation at higher temperatures and long residence times, e.g., in the GC-System. Therefore, instrumental analysis was sped up as far as possible in order to reduce residence time of DBDPE in the system. This was achieved by using a high temperature (280 °C) in the injection liner, raising transfer line temperature to a minimum of 340 °C and applying ramped flow during the analysis. Application of ramped flow improved the DBDPE performance significantly without impacting the separation of the other analytes investigated. Furthermore, a short (15 m) column with a surrounding as inert as possible (deactivated injection liners, a pre-column, and a separate transfer line to the detector run at high temperature) was used.

During optimization of the instrumental method, it could be observed that generally, the chromatographic behavior of brominated compounds differed from that of chlorinated compounds, resulting in a usually higher sensibility of brominated species towards GC system inertness, which was observed from faster appearance of tailing effects (ESM Figs. S3, S4). As a consequence, it proved to be necessary to frequently and meticulously maintain the system, including complete and regular pre-column replacement instead of column cutting or even frequent column changes. Thus, cleanliness of the

extracts proved to be crucial in the analysis of brominated flame retardants.

IDL, MQL, working range

IDLs and MQLs are presented in Table 1. For dechloranes, IDLs (GC-API-MS/MS) and MQLs were in the range of 0.01 to 0.02 $\text{pg } \mu\text{L}^{-1}$ and 1.2 to 27 pg sample^{-1} , respectively. For *alternative* BFRs investigated (GC-API-MS/MS), they ranged from 0.01 $\text{pg } \mu\text{L}^{-1}$ to 0.5 $\text{pg } \mu\text{L}^{-1}$ and 2.5 to 900 pg sample^{-1} , respectively. IDLs and MQLs for PBDEs (GC-EI-MS) were between 0.24 and 1.2 $\text{pg } \mu\text{L}^{-1}$ as well as 10 and 710 pg sample^{-1} . As the typical sample amount used for the analysis differed between the matrices, MQLs were best for spruce shoots and worst for herring gull eggs. As shown in Table 1, the lower limits of the working ranges were up to a factor of 5 (DBDPE 20) higher than the IDLs, mainly because of deviating response factors or mass-labeled signals from internal standards interfering on quantification mass traces of the target compounds. Additionally, blank levels impacted the lower limits of the working ranges for some compounds, i.e., the instrument's sensitivity cannot be used completely for quantification due to such hampering factors. On the upper end of the working range high concentrations of native analytes affected the mass traces of corresponding internal standards. Also, even though mass-labeled standards are considered as the *gold standard* of ultra-trace analysis, some limitations of the working range were identified as mass fragmentation of native and mass-labeled compounds may result in signals affecting the mass traces of the respective other compounds, i.e., in samples with very low analyte concentrations such as, e.g., blank samples, there might be signals on the target analyte's mass traces that arose not from the target analyte itself but from its corresponding internal standard, which can easily be mistaken as background blank signals. This is due to typical fragmentation pathways as discussed by Guo et al. [7] and leads – depending on the extent of this effect – to a limitation of constancy of the relative response factors and therefore narrows down the method working range on both ends of the intended range as well as the method MQL. It has to be noted that the GC-API-MS/MS instrument itself gives linear response down to 0.01 pg per injection for each compound at $r^2 \geq 0.995$.

This study's IDLs were lower than those of a multi-compound method recently published by Gustavsson et al. [26] using GC-EI-MS, GC-NCI-MS, and GC-EI-MS/MS for detection (about 1–400 $\text{pg } \mu\text{L}^{-1}$ for same analytes). IDLs for dechloranes detected by GC-NCI-MS/MS were reported to be 0.02 to 0.08 pg injected by Baron et al. [27] and were slightly higher than those of the present study. The same authors published IDLs of 0.1 to 16 pg injected for 19 BFRs (PBDE, HBBz, PBEB, DPDPE) detected by GC MS/MS, which were higher than IDLs of this study. In agreement with the present

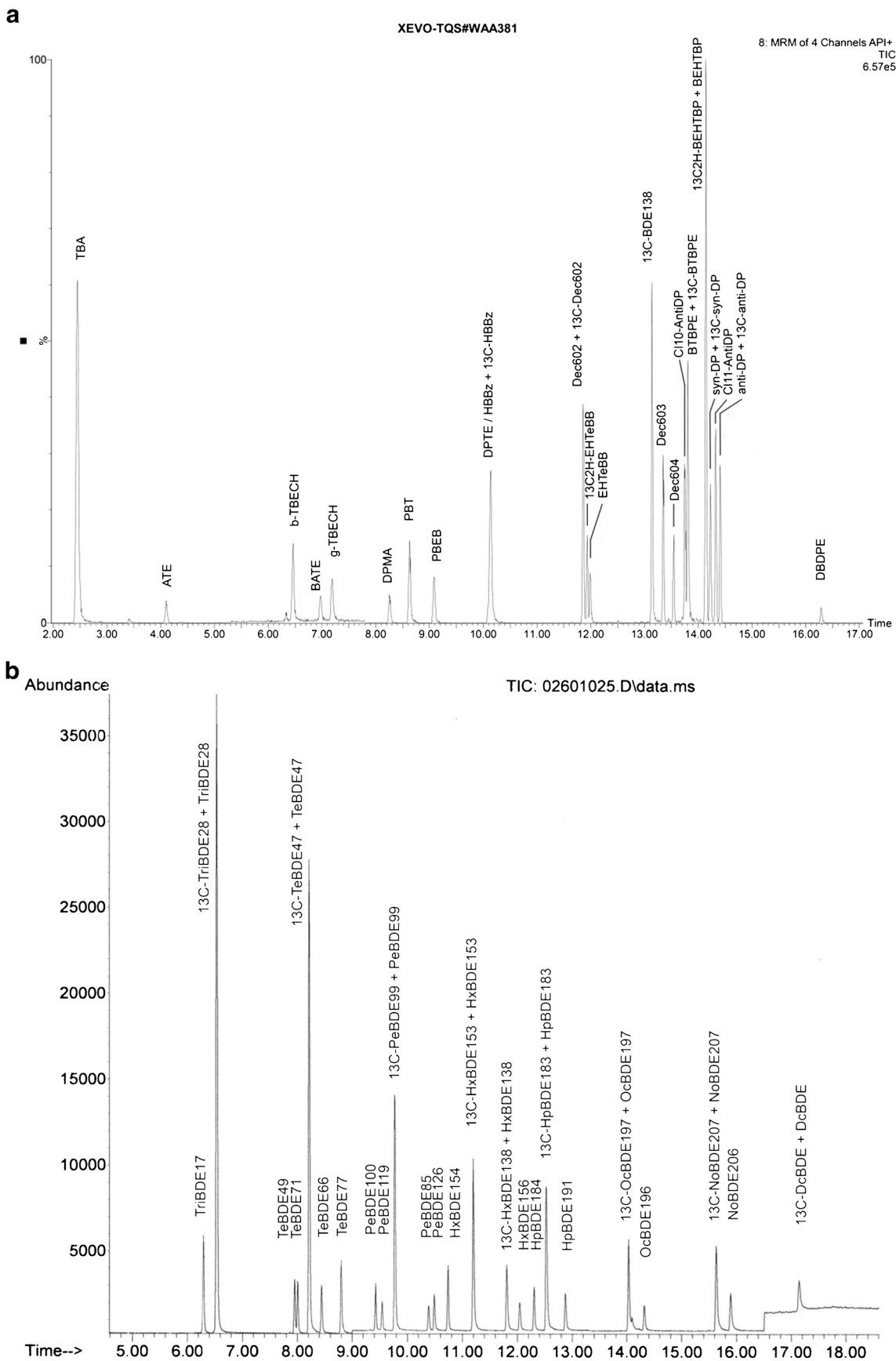


Fig. 1 Total ion chromatogram of (a) HFRs on the API-GC-MS/MS and (b) PBDEs on the GC-MS

Table 1 Instrumental limits of detection (IDL), working range, and method quantification limits (MQL)

Acronym	IDL pg μL^{-1}	working range pg μL^{-1}	MQL pg	MQL spruce needles pg g^{-1} dw	MQL bream fillet pg g^{-1} dw	MQL SP pg g^{-1} dw	MQL herring gull eggs pg g^{-1} dw
syn-DP	0.01	0.02 - 40	24	4.8	9.6	9.6	48
anti-DP	0.01	0.02 - 40	27	5.5	10.9	10.9	54.6
Cl ₁₀ -AntiDP	0.01	0.02 - 40	1.5	0.3	0.6	0.6	3.1
Cl ₁₁ -AntiDP	0.01	0.02 - 40	1.3	0.3	0.5	0.5	2.7
Dec602	0.01	0.05 - 40	6.9	1.4	2.7	2.7	13.7
Dec603	0.01	0.05 - 100	2.5	0.5	1	1	5
Dec604	0.02	0.05 - 100	5.5	1.1	2.2	2.2	10.9
DPMA	0.02	0.02 - 40	1.0	0.2	0.4	0.4	2
TBA	0.01	0.02 - 20	170	34	68	68	340
ATE	0.02	0.05 - 40	52	10.3	20.6	20.6	103.2
BATE	0.1	0.1 - 40	17	3.3	6.6	6.6	33.2
DPTE	0.05	0.5 - 40	320	64	128	128	640
BTBPE	0.2	0.2 - 200	99	19.7	39.4	39.4	197.2
PBT	0.01	0.05 - 40	43	8.6	17.2	17.2	86.1
PBEB	0.01	0.05 - 40	2.5	0.5	1	1	5
HBBz	0.01	0.05 - 40	10	2	4.1	4.1	20.5
β -TBECH	0.01	0.05 - 20	11	2.3	4.5	4.5	22.5
γ -TBECH	0.02	0.02 - 20	14	2.9	5.8	5.8	28.9
EHTeBB	0.05	0.2 - 200	130	26	52	52	260
BEHTBP	0.05	0.2 - 200	460	92	184	184	920
DBDPE	0.5	10 - 1100	900	180	360	360	1800
BDE 17	0.24	0.6 - 600	10	2	4	4	20
BDE 28	0.24	0.6 - 600	13	3	5	5	26
BDE 49	0.24	0.6 - 600	10	2	4	4	20
BDE 71	0.24	0.6 - 600	10	2	4	4	20
BDE 47	0.24	0.6 - 600	220	44	88	88	440
BDE 66	0.24	0.6 - 600	10	2	4	4	20
BDE 77	0.24	0.6 - 600	10	2	4	4	20
BDE 100	0.24	0.6 - 600	30	6	12	12	60
BDE 119	0.24	0.6 - 600	10	2	4	4	20
BDE 99	0.24	0.6 - 600	120	24	48	48	240
BDE 85	0.24	0.6 - 600	10	2	4	4	20
BDE 126	0.24	0.6 - 600	10	2	4	4	20
BDE 154	0.48	1.2 - 480	20	4	8	8	40
BDE 153	0.48	1.2 - 1200	20	4	8	8	40
BDE 138	0.48	1.2 - 1200	20	4	8	8	40
BDE 156	0.48	1.2 - 1200	20	4	8	8	40
BDE 184	0.48	1.2 - 1200	20	4	8	8	40
BDE 183	0.48	1.2 - 1200	50	10	20	20	100
BDE 191	0.48	1.2 - 1200	20	4	8	8	40
BDE 197	0.48	1.2 - 1200	200	40	80	80	400
BDE 196	0.48	1.2 - 1200	20	4	8	8	40
BDE 207	1.2	3 - 3000	50	10	20	20	100
BDE 206	1.2	3 - 3000	50	10	20	20	100
BDE 209	1.2	3 - 3000	710	140	280	280	1420

dw: dry weight; SP: riverine suspended particles

study, IDLs were better for lower brominated BDEs. IDL for DBDPE were up to factor of 50 higher than those of other *alternative* BFRs [27]. IDLs for *alternative* BFRs reported for GC-APCI-MS/MS by Lv et al. [17] and Geng et al. [19] were in slightly higher ($<0.05 \text{ pg } \mu\text{L}^{-1}$; $0.075\text{--}0.1 \text{ pg } \mu\text{L}^{-1}$, respectively). Lower IDLs for PBDEs, DPDPE, BTBPE were determined by GC-API-MS/MS of 0.001 to 0.025 pg by Portoles et al. [9]. Sales et al. [20] published DP IDLs below 1 f. μL^{-1} for a GC-ECNI-MS/MS method. However, IDLs for Dec602/603 or other *alternative* BFRs were factor 2 to 10 higher than those of the present method. IDLs of 3 to 300 pg were reviewed by Papachlimitzou et al. [14] for most *alternative* BFRs. DBDPE IDLs were usually about 10 times higher than those of other BFRs (40 pg and 900 pg) [14].

Papachlimitzou et al. [14] reviewed MDLs and MQLs of *alternative* BFRs in biota samples in the sub ng g^{-1} range with different normalization depending on the sample matrix, e.g., MQLs of several *alternative* BFRs in fish using LC-APPI-MS or GC methods were between 4.5 and a few hundred pg g^{-1} ww, which were evaluated as being equivalent to those obtained by other GC methods. MQLs for *alternative* BFRs in gull eggs were between 50 and 100 pg g^{-1} wet weight (ww), excluding DBDPE for which it was about 300 pg g^{-1} ww [14]. MQLs of *alternative* BFRs in food samples analyzed by GC-APCI-MS/MS were about 2 pg g^{-1} ww [17]. For the analysis of soil and sediment, MDLs and MQLs were summarized as being in the 100s of pg g^{-1} range [14]. Reported MDLs for dechloranes were, e.g., 0.1 to 1.3 pg g^{-1} dry weight (dw) for sediment, 2.3 to 21.1 pg g^{-1} lipid weight (lw) for fish ([27]; GC-NCI-MS/MS), 9 to 15 pg g^{-1} lw for fish ([28]; GC-ECNCI-MS), 1 to 4 pg g^{-1} dw for fish and sediment ([29]; GC-MS), and 5 to 8 pg g^{-1} ww or 20 to 55 pg g^{-1} lw for silver eel ([30]; GC-NCI-MS). In their multi-compound method on 54 HFRs in benthic fish and sediment, Sühling et al. [29] published MDLs from 0.75 pg g^{-1} dw for TBCT to 4.8 ng g^{-1} for BEHTBP. For 19 BFRs (PBDEs, HBBz, PBEB, DPDPE), Baron et al. [31] published MDLs of 0.01 to 1.65 ng g^{-1} dw for sediment, 0.04 to 10.6 ng g^{-1} lw for fish, 0.03 and 3.50 ng g^{-1} lw for bird eggs.

Recovery rates of mass-labeled standards

Average recovery rates of mass-labeled internal standards were generally between 50% and 140% and thus well within the range required by EPA 1614A [24] for the determination of PBDEs in water, soil, sediment, and tissue (25%–150%, BDE 209 up to 200%; Fig. 2) and were in a similar range as values of 10 mass-labeled BFRs in fish and sediment reported for another multi-compound method (42% to 154 % [29]). Few exceptions were ^{13}C -EHTeBB, ^{13}C -BTBPE, and ^{13}C -DPDPE in herring gull egg as well as ^{13}C -BTBPE and ^{13}C -BDE 209 in suspended particulate matter. However, native compounds' levels are corrected by the internal standards resulting – except for BTBPE in spruce needles – in a good performance of the method with respect to trueness (see below).

Precision

Instrumental measurement precisions of target compounds detected by GC-API-MS/MS were between 4% (DPMA) and 16% (DBDPE). For PBDEs (detected by GC-EI-MS), instrumental precisions were between 1% (BDE 153) and 38% (BDE 209) with increasing values with increasing degree of bromination (Fig. 3). Instrumental precision was better for PBDEs compared with the *novel* HFR investigated, probably because validation levels were higher and the substance group less diverse. With the exception of DPDPE, instrumental precision of the present method was similar to values reported by Baron et al. [31] for the analysis of 19 BFRs (PBDEs, HBBz, PBEB, DPDPE; 3%–19%) using GC MS/MS and Baron et al. [27] for the analysis of several dechloranes (3%–8.5%) using GC-NCI MS/MS.

Interday precision of the entire method was generally between 5% (HBBz, suspended particles) and 37% (DBDPE, spruce) for investigated *novel* HFRs, and between 3% (BDE 47, bream fillet) and 36% (BDE 85, spruce shoots) for PBDEs. In bream, it was not possible to compile reasonable TBECHE interday precision. Similarly, β -TBECHE precision in

Fig. 2 Recovery rates of mass-labelled internal standards during the validation process. Red lines: acceptable limit according to EPA 1614A [20] (Deca BDE up to 200%)

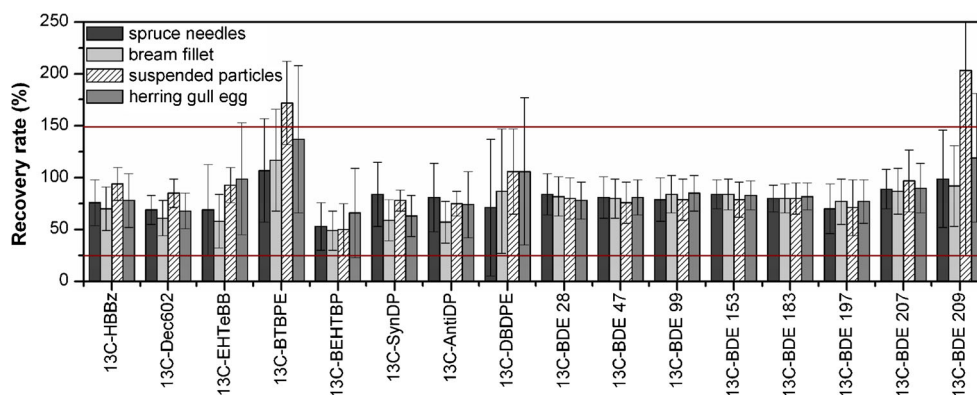
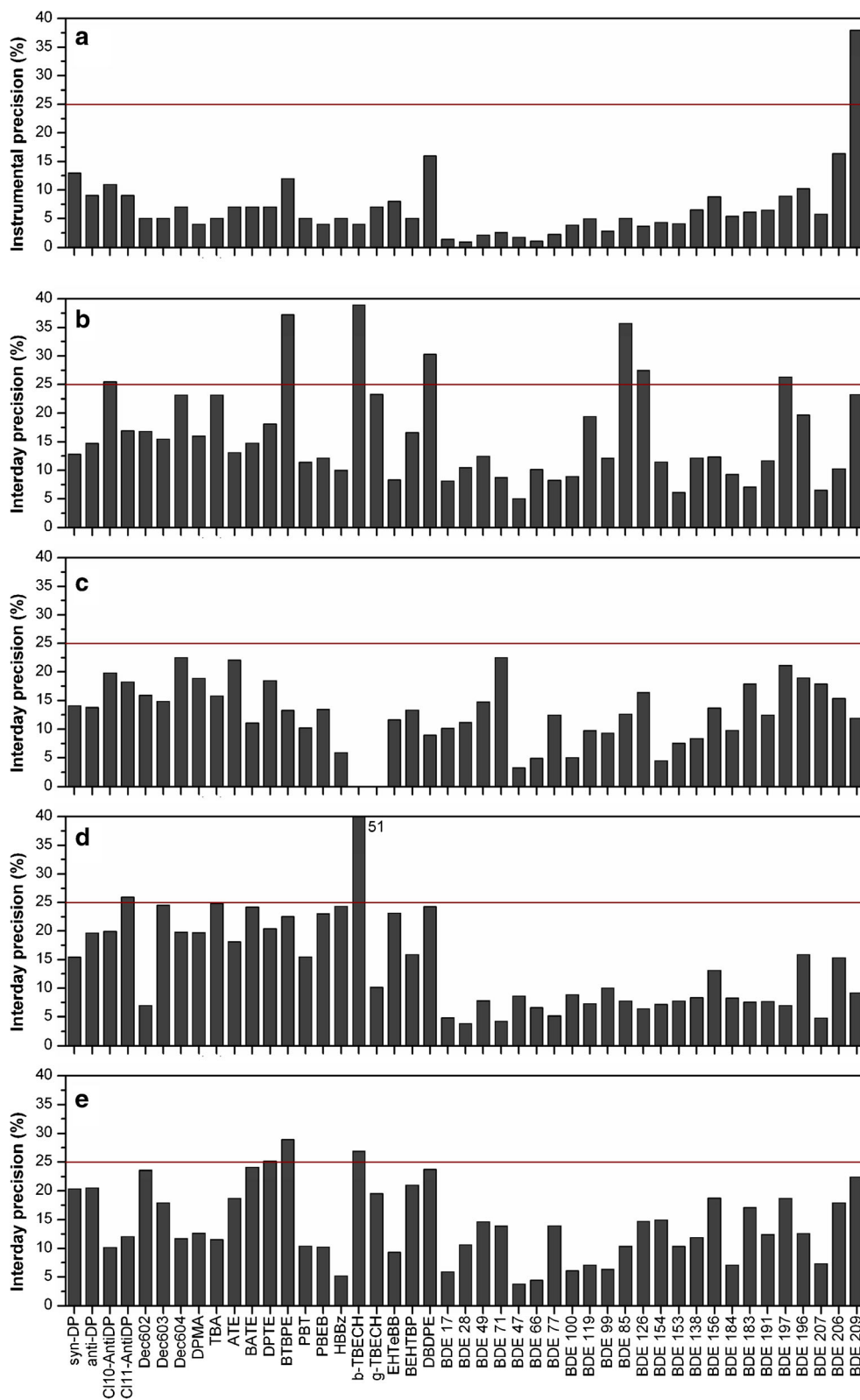


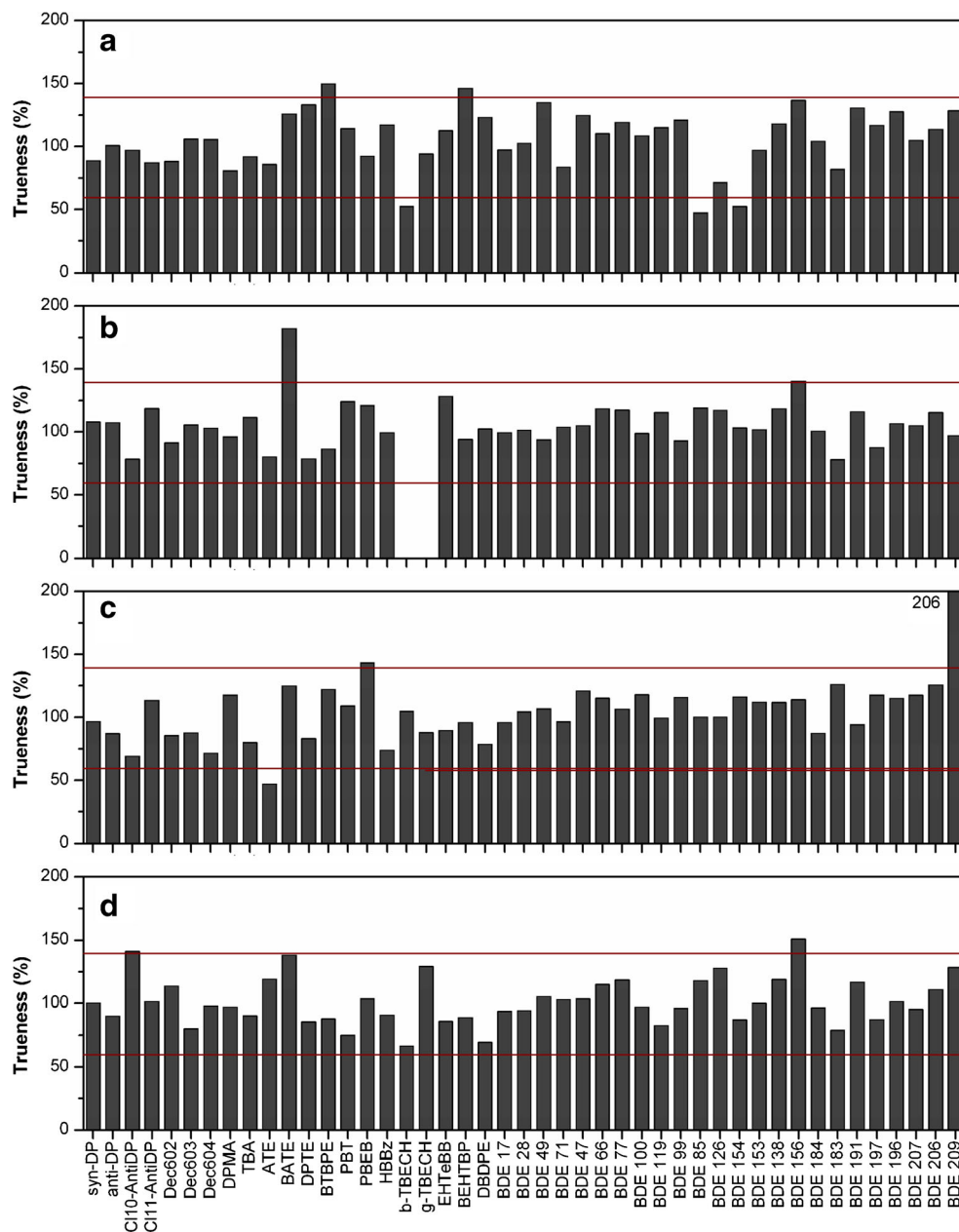
Fig. 3 Instrumental (a) GC-API-MS/MS for alternative HFRs, GC-MS for PBDEs) and interday precision (b) spruce needles, (c) bream fillet, (d) herring gull egg, (e) riverine suspended particles. Red line: acceptable limit for routine trace analysis [28]. TBECH in bream could not be analyzed



spruce and herring gull egg was at such a high level (up to 53%) that TBECH values for that matrix are rather of qualitative nature. Overall, precision was better for PBDEs than for

the *alternative* HFRs investigated. For most target analytes, values were below 25% and thus in a normal range for routine trace analytical methods [32]. Moreover, with these values,

Fig. 4 Trueness expressed as recovery rate of native analytes. **(a)** spruce needles, **(b)** bream fillet, **(c)** herring gull egg, **(d)** riverine suspended particles. Red line: acceptable limit for routine trace analysis [20]. TBECH in bream could not be analyzed



the method meets the performance criterion of 40% mentioned by US EPA 1614A [24] for the analysis of PBDEs. Elevated values for interday precision as well as the inability to reliably analyse TBECH in bream fillet and herring gull egg resulted from chromatographic interferences of matrix constituents.

Except for DBDPE in herring gull egg samples, interday precisions of the present method were in a similar range as those reported by Baron et al. [31] for 19 BFRs (PBDEs, HBBz, PBEB, DPDPE) in similar matrices (sediment 2%–17%; fish 3%–19%; bird egg 2%–20%). Values were slightly higher than those published for a method investigating dechloranes only in fish

samples (<7%) [28]. Portoles et al. [9] reported intraday precision of <20 % for PBDEs, DPDPE, BTBPE determined by GC-API-MS/MS. Compared with the instrumental precision and depending on the matrix, interday precision of the method varied from ‘in the same range as’ (e.g., for DP) to exceptionally ‘up to a factor of 10 higher’ (e.g., BDE 66 in spruce or BDE 17 in bream) indicating the impact of interfering matrix constituents. Interestingly, for BDE 209 in bream fillet, interday precision was lower than the instrumental precision, probably as result of matrix-induced improved chromatographic performance or reduced degradation in the presence of matrix [26].

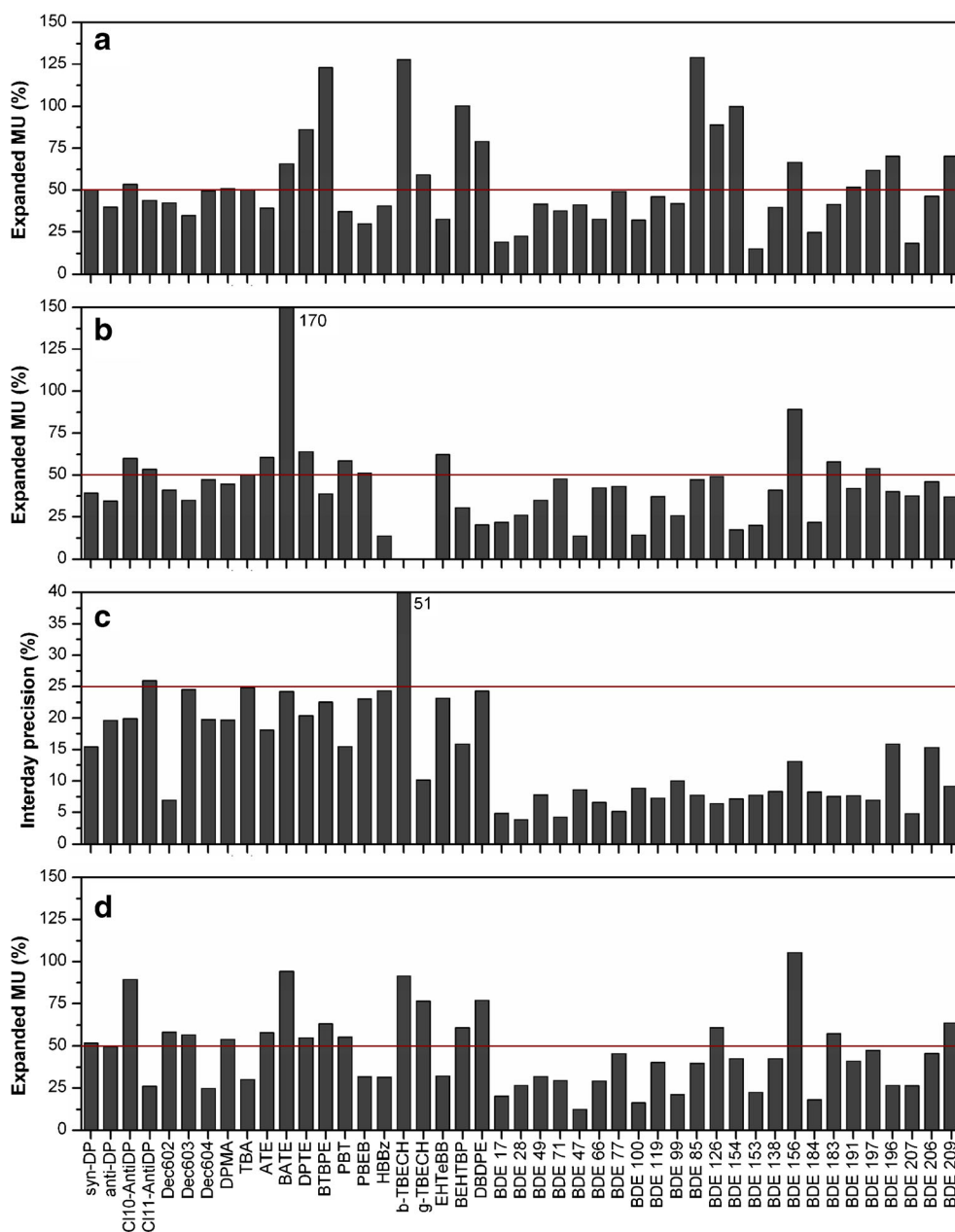
Trueness

The method trueness expressed as recovery rate of native analytes was mostly in the acceptable range of 60% to 140% (50%–200% for BDE 209, [24]; Fig. 4). Values outside of this range or close to its limits were partially observed and rather scattered in all matrices, and, with few exceptions, concerned substances where there was no compound-specific mass-labeled internal standard available (e.g., PBEB, ATE, or BATE) and/or the intrinsic contamination of the validation matrix was relatively high with respect to the amount of spiked standard (e.g., DPTE, BEHTBP, DBDPE; ESM Table S3) showing the limits of using real samples for

validation purposes. Matrix-specific influences can also be anticipated due to the observed scattering. Similar to precision, trueness was better for PBDEs than for the investigated *alternative* HFRs, which might be explained by the higher spiking levels applied and appeared to be better for bream fillet and suspended particles than for spruce shoots and herring gull egg.

Similar to the present study, Baron et al. [27] reported recovery rates of native dechloranes between 65% and 114% in sediment and fish. Recoveries of native dechloranes determined by GC-ECNCl-MS were between 78% and 95% [28]. For native PBDEs they ranged from 75% to 96%, from 57% to 77%, and from 53% to 82% in sediment, fish, bird egg,

Fig. 5 Expanded measurement uncertainty (MU); (a) spruce needles, (b) bream fillet, (c) herring gull egg, (d) riverine suspended particles. Red line: limit according to the water frame work directive at EQS level [29]; TBECHE in bream could not be analyzed



respectively [31]. For HBB, PBEB, and DBDPE, 103% to 105% in sediment, 68% to 80% in fish, and 70% to 78% in bird eggs were reported [31].

Measurement uncertainty

Overall, the approach of validating using real samples and multiple matrix types bring along the need to characterize the background contamination patterns and concentrations, which in the end increases measurement uncertainty (as expressed via an additional calculation term for the measurement uncertainty (MU) of the unspiked reference matrix). Nevertheless, for the majority of the target compounds, the expanded measurement uncertainty was below 60% (Fig. 5) and about 2/3 meet the water frame work directive's criteria for priority substances of 50% expanded MU at the environmental quality standard (EQS) level [33]. Partially, elevated expanded measurement uncertainties were observed for analytes that occurred at elevated intrinsic concentrations in the validation matrices, e.g., in herring gull egg; yet not all analytes at such high intrinsic concentrations (ESM Table S3) resulted in high values for the expanded measurement uncertainties. Besides TBECH, compounds of elevated measurement uncertainties were, e.g., brominated aromatic BFR with free double bonds (ATE, BATE), possibly making these analytes prone to degradation or reactions.

Conclusion

A method for the determination of 45 halogenated flame retardants (21 *alternative* HFRs and 24 PBDEs) in different environmental matrices (spruce shoots as representatives for plant materials, bream fillet as representative for animal tissue, herring gull eggs as representatives for bird eggs, and riverine suspended particulate matter as representatives for organic matter rich solids) using GC-API-MS/MS and GC-EI-MS was developed and validated. Of course, a method on 45 HFRs of different physical-chemical properties at trace concentrations in different environmental matrices is always a compromise. This method also has its limitations observable as elevated uncertainties, e.g., for compounds with a high degree of bromination such as DBDPE or BDE 209, for volatile compounds, where there is no compound or compound group-specific mass-labeled internal standard (such as TBA, ATE, TBECH), or for samples rich in extractable lipophilic matrix constituents. Overall, the encountered constraints can be attributed to a couple of basic facts, being mainly:

- (a) Varying individual matrix constituents in different environmental samples (composition and amount) interfering with the target's detectability or impairing the method's performance regarding validation parameters,

particularly against the background of non-destructive clean-up methods and low spiking levels.

- (b) Validation on real samples as validation matrices, i.e., the absence of analyte-free matrices for validation.
- (c) Absence of mass-labeled internal standards for quantification and monitoring of method performance for all compound groups. In this study, method performance was generally poorer for compounds where a corresponding internal standard was not available.
- (d) Gas chromatographic performance that may quickly decline within only few analytical runs of even thoroughly cleaned sample extracts; mainly affecting (highly) brominated compounds.

Thus it is not (only) the most sensitive instrument deciding on the suitability and applicability of a method on the analysis of HFRs at trace levels but its robustness towards such constraints. Considering these effects, the overall results of the validation demonstrate the method's suitability for the investigation of HFRs in a broad range of environmental matrices, especially for the dechlorane-type flame retardants being primary objective of the development. However, reasonable care has to be taken if additional substances or matrices are included in the method and adjustments might become necessary, e.g., reduction of the sample amount analyzed in order to overcome the heavy matrix interferences. Future studies on the analysis of HFRs should also apply additional mass-labeled standards, especially for the most volatile HFRs (e.g. TBA, ATE, TBECH) as soon as these are available. Furthermore, we propose using multiple syringe standards with respect to a better formal control of the laboratory performance.

Published as well as acknowledged standard methods were often validated on relatively high concentration levels at single analytical series and/or simple substitute matrices (e.g., plant oil) only. However, such type of validation merely demonstrates analytical sensitivity and performance of instrumentation, analytes, or internal standards and often underestimates real uncertainties. Thus validation on multiple matrices might be a valuable tool in order to evaluate capabilities and limitations of analytical methods at trace concentrations.

Acknowledgments The authors thank their laboratory staff for the dedicated work on the project, particularly Kay Kelterer, Judith Söhler, and Steffi Rolle. The authors are grateful to Heinz Rüdél (Fraunhofer IME) as well as Caren Rauert and Peter Lepom (German Environment Agency) for valuable discussions. The German Environment Agency is acknowledged for funding (AZ 93 04/25).

Compliance with ethical standards Herring gull egg sampling was performed as part of the routine operation of the German Federal Environmental Specimen Bank. It was approved by the state authorities and followed the respective guidelines.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kolic TM, Shen L, MacPherson K, Favez L, Gobran T, Helm PA, et al. The analysis of halogenated flame retardants by GC-HRMS in environmental samples. *J Chromatogr Sci*. 2009;47:83–91.
2. Chen D, Letcher RJ, Burgess NM, Champoux L, Elliott JE, Hebert CE, et al. Flame retardants in eggs of four gull species (Laridae) from breeding sites spanning Atlantic to Pacific Canada. *Environ Pollut*. 2012;168:1–9.
3. Stockholm Convention. <http://chm.pops.int>. 15.09.2017
4. Law RJ, Losada S, Barber JL, Bersuder P, Deaville R, Brownlow A, et al. Alternative flame retardants, Dechlorane Plus and BDEs in the blubber of harbour porpoises (*Phocoena phocoena*) stranded or caught in the UK during 2008. *Environ Int*. 2013;60:81–8.
5. Guerra P, Fernie K, Jimenez B, Pacepavicius G, Shen L, Reiner E, et al. Dechlorane Plus and related compounds in Peregrine falcon (*Falco peregrinus*) eggs from Canada and Spain. *Environ Sci Technol*. 2011;45:1284–90.
6. Guo J, Li Z, Sandy AL, Li A. Method development for simultaneous analyses of multiple legacy and emerging organic chemicals in sediments. *J Chromatogr A*. 2014;1370:1–8.
7. Guo T, LaBelle B, Petreas M, Park J-S. Mass spectrometric characterization of halogenated flame retardants. *Rapid Commun Mass Spectrom*. 2013;27:1437–49.
8. Liu L-Y, Salamova A, Heb K, Hites RA. Analysis of polybrominated diphenyl ethers and emerging halogenated and organophosphate flame retardants in human hair and nails. *J Chromatogr A*. 2015;1406:251–7.
9. Portolés T, Sales C, Gómara B, Sancho JV, Beltrán J, Herrero L, et al. Novel analytical approach for brominated flame retardants based on the use of gas chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry with emphasis in highly brominated congeners. *Anal Chem*. 2015;87:9892–9.
10. Suehring R, Barber JL, Wolschke H, Koetke D. Fingerprint analysis of brominated flame retardants and dechloranes in North Sea sediments. *Environ Res*. 2015;140:569–78.
11. Schlabach M, Remberger M, Brorström-Lundén E, Norström K, Kaj L, Andersson H, Herzke D, Borgen A, Harju M. Brominated flame retardants (BFR) in the Nordic environment. *TemaNord*. 2011;528. <http://www.diva-portal.org/smash/get/diva2:701100/FULLTEXT01.pdf>.
12. De la Torre A, Alonso MB, Martínez MA, Sanz P, Shen L, Reiner EJ, et al. Dechlorane-related compounds in Franciscana dolphin (*Pontoporia blainvillei*) from southeastern and southern coast of Brazil. *Environ Sci Technol*. 2012;46:12364–72.
13. Muñoz-Arnanz J, Roscales JL, Vicente A, Aguirre JI, Jimenez B. Dechlorane Plus in eggs of two gull species (*Larus michahellis* and *Larus audouinii*) from the southwestern Mediterranean Sea. *Anal Bioanal Chem*. 2012;404:2765–73.
14. Papachlimitzou A, Barber J, Losada S, Bersuder P, Law R. A review of the analysis of novel brominated flame retardants. *J Chromatogr A*. 2012;1219:15–28.
15. Wang P, Zhang Q, Zhang H, Wang T, Sun H, Zheng S, et al. Sources and environmental behaviors of Dechlorane Plus and related compounds – a review. *Environ Int*. 2016;88:206–20.
16. Covaci A, Harrad S, Abdallah MA-E, Ali N, Law RJ, Herzke D, et al. Novel brominated flame retardants: a review of their analysis, environmental fate, and behavior. *Environ Int*. 2011;37:532–56.
17. Lv S, Niu Y, Zhang J, Shao B, Du Z. Atmospheric pressure chemical ionization gas chromatography mass spectrometry for the analysis of selected emerging brominated flame retardants in foods. *Sci Rep*. 2017;7:1–8.
18. Ballesteros-Gómez A, de Boer J, Leonards PEG. Novel analytical methods for flame retardants and plasticizers based on gas chromatography, comprehensive two-dimensional gas chromatography, and direct probe coupled to atmospheric pressure chemical ionization-high resolution time-of-flight-mass spectrometry. *Anal Chem*. 2013;85:9572–80.
19. Geng D, Kukucka P, Jogsten IE. Analysis of brominated flame retardants and their derivatives by atmospheric pressure chemical ionization using gas chromatography coupled to tandem quadrupole mass spectrometry. *Talanta*. 2017;162:618–24.
20. Sales C, Poma G, Malarvannan G, Portolés T, Beltrán J, Covaci A. Simultaneous determination of dechloranes, polybrominated diphenyl ethers and novel brominated flame retardants in food and serum. *Anal Bioanal Chem*. 2017;409:4507–15.
21. Na G, Wei W, Zhou S, Gao H, Ma X, Ge L, et al. Distribution characteristics and indicator significance of dechloranes in multi-matrices at Ny-Ålesund in the Arctic. *J Environ Sci (China)*. 2015;28:8–13.
22. Suehring R, Byer J, Freese M, Pohlmann J-D, Wolschke H, Moeller A, et al. Brominated flame retardants and dechloranes in European and American eels from glass to silver life stages. *Chemosphere*. 2014;116:104–11.
23. Ellison SLR, Williams A (eds) Eurachem/CITAC guide: quantifying uncertainty in analytical measurement. 3rd edn. 2012. Available from www.eurachem.org.
24. US EPA. Method 1614A - Brominated diphenyl ethers in water, soil, sediment, and tissue by HRGC/HRMS. United States Environmental Protection Agency. 2010. https://www.epa.gov/sites/production/files/2015-08/documents/method_1614a_2010.pdf.
25. Magnusson B, Näykki T, Hovind H, Krysell M. Handbook for calculation of measurement uncertainty in environmental laboratories. Nordtest Report TR 537. 2003. www.nordtest.org
26. Gustavsson J, Ahrens L, Nguyena MA, Josefsson S, Wiberg K. Development and comparison of gas chromatography-mass spectrometry techniques for analysis of flame retardants. *J Chromatogr A*. 2017;1481:116–26.
27. Baron E, Eljarrat E, Barcelo D. Analytical method for the determination of halogenated norbornene flame retardants in environmental and biota matrices by gas chromatography coupled to tandem mass spectrometry. *J Chromatogr A*. 2012;1248:154–60.
28. Chen C-L, Tsai D-Y, Ding W-H. Optimization of matrix solid-phase dispersion for the determination of dechlorane compounds in marketed fish. *Food Chem*. 2014;164:286–92.
29. Suehring R, Busch F, Fricke N, Kötke D, Wolschke H, Ebinghaus R. Distribution of brominated flame retardants and dechloranes between sediments and benthic fish – a comparison of a freshwater and marine habitat. *Sci Total Environ*. 2016;542:578–85.
30. Suehring R, Moeller A, Freese M, Pohlmann J-D, Wolschke H, Sturm R, et al. Brominated flame retardants and dechloranes in eels from German rivers. *Chemosphere*. 2013;90:118–24.
31. Baron E, Eljarrat E, Barcelo D. Gas chromatography/tandem mass spectrometry method for the simultaneous analysis of 19 brominated compounds in environmental and biological samples. *Anal Bioanal Chem*. 2014;406:7667–76.
32. USP NF 39. USP NF 39 <736> mass spectrometry p. 5, table 1: Analytical Measurement Requirements (Category II)
33. Philipp R, Gonzales A, Cea AR. Traceable measurements for monitoring critical pollutants under the European Water Framework Directive (WFD-2000/60/EC). Joint Research Project ENV08 V6.0. 2015