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Potential reference measurement procedures for PBDE in surface water at levels required by the EU Water Frame Directive



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ABSTRACT

Polybrominated diphenylethers (PBDE), used as flame retardants, are named as priority substances in the Directive 2000/60/EC of the European parliament and of the council establishing a framework for Community action in the field of water policy. An annual average environmental quality standard (EQS) for inland surface waters of 0.0005 µg/L (0.0002 µg/L for other surface waters) for PBDE congeners involved in the technical penta-PBDE mixtures containing PBDE with five bromine atoms has been established. The directives focus especially on the congeners PBDE 28, 47, 99, 100, 153 and 154 contained in the penta-PBDE mixture. Up to now, no reference measurement procedures have been established reaching the limits of quantification (LOQs) and the associated uncertainties as defined in the directives with results traceable to the SI. Within a recent European project on metrology, different approaches for the traceable quantification of PBDE, based on liquid/liquid or solid phase extraction followed by the detection with gas chromatography coupled to either inductively coupled plasma mass spectrometry or triple quadrupole mass spectrometry, were investigated and the related LOQs and expanded uncertainties of the results were compared. A complete uncertainty budget for each method was estimated according to the Guide to the Expression of Uncertainty in Measurement (GUM). All presented analytical procedures can serve as reference measurement procedures regarding the LOQs and their associated expanded uncertainties for monitoring the six priority PBDEs named above. LOQs as low as 0.026 ng/kg with an associated expanded uncertainty of 0.002 ng/kg could be achieved

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1. Introduction

Polybrominated diphenylethers (PBDE) are widely used flame retardants. The production of penta-PBDE was phased out in the EU in 1997. The annual world-wide production of PBDE at that time was estimated at 40,000 t/a thereof about 10% were penta-PBDE [1]. These additive PBDEs were mainly used in polyurethane foams included in car headrests, domestic furniture such as mattresses and foam-based packaging [2]. To a small extent they were also used in textiles. As most items containing penta-PBDE have a long life-time, they can still mimic as a source for the release of

penta-PBDE into the environment. The commercially sold mixture contained penta-PBDE at around 0.50–0.62 g/g, tetra-PBDE at around 0.24–0.38 g/g and the rest consisting of tri-PBDE and hexa-PBDE [1]. PBDE are included in the list of priority substances of Directive 2008/105/EC [3] which amends Directive 2000/60/EC [4] (in the following called European Union Water Framework Directive (EU-WFD)), establishing a framework for Community action in the field of water policy, due to their persistence and ubiquity. A risk assessment published in 2001 by the European Community proposes the classification of PBDEs as very toxic to aquatic organisms, because of a “lack of biodegradation seen in standard tests and the high bioconcentration factors”. Although the water solubility of PBDEs is low (2–10 µg/L) [5], the bioaccumulation and the persistence in the environment as well as the hints that they might act as thyroid hormones [6] make them potentially dangerous also to public health. The EU Member States, therefore,

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expressed concerns about “the biopersistent nature of penta-PBDE, its systemic toxicity following oral and dermal exposure, and observations of penta-PBDE in human breast milk” [7]. Hence, environmental quality standards (EQS) of 0.5 ng/L and 0.2 ng/L were specified for each congener named in the EU-WFD for inland and other surface waters, respectively, in the Directive 2008/105/EC [3]. New toxicological assessments gave rise to change these values in the amendment Directive 2013/39/EU [8] As the EQS values discussed for the amendment were too low to be measured directly in water, EQS values in biota were chosen instead. For water, maximum allowable concentrations (MAC) are given of 0.14 µg/L for inland and 0.014 µg/L for other surface waters.

The low concentrations allowed in surface water for the congeners contained in commercial penta-PBDE mixtures require very sensitive detection methods as well as the enrichment and matrix separation of the PBDE of concern from the analysed water samples. As such methods were not available at the time when the EQS values were established in 2008, several efforts were undertaken with respect to the development of suitable methods. The Mandate 424 [9] is concerned with the development of measurement procedures suitable for measuring PBDE concentrations in water samples by routine laboratories, which are responsible for monitoring the water bodies in the EU. In order to develop a method which is capable to measure PBDEs in water at low concentration level and to ensure the traceability of the results to the SI, the project ENV08 (Traceable measurements for monitoring critical pollutants under the European Water Framework Directive (WFD 2000/60/EC)) was launched in the framework of the European Metrology Research Programme (EMRP). The results of this project will be presented here. When the project started in 2011, the former version of the EU-WFD from 2008 was in force and, therefore, the EQS values given at that time were relevant for the project design and, thus, for the method development. Even though the matrix in which the EQS is determined in the recent version of the EU-WFD has changed, the determination of PBDEs directly in water is still relevant due to several reasons: to be able to draw conclusions from the PBDE concentrations in fish in comparison to their concentrations/partitioning in water also data, reflecting the water concentration are needed. Indeed, to allow comparisons between different water bodies in Europe, ideally the same fish species at the same age with the same body weight has to be chosen. Furthermore, fish is mobile. So the results obtained from measuring PBDE in fish are representative of an average of the PBDE taken up and excreted during its life time. Living most of the time in clean water and a short time in highly contaminated water may result in the same concentration in fish tissue as living its whole life in medium contaminated water. PBDE contamination effects on the water body and its inhabitants, however, might be rather different.

After a close survey of the existing literature, the most promising analytical methods were chosen for further investigations. Various extraction procedures and detection methods were published hitherto, mainly for measuring PBDE concentrations in plastics at relatively high concentration levels. For the measurement in water, gas chromatography (GC) coupled to various mass spectrometric techniques such as single quadrupole mass spectrometry (MS) [10,11], triple quadrupole MS (MS/MS) [12,13] or inductively coupled plasma mass spectrometry (ICP-MS) [14,15] represents the method of choice, since liquid chromatography (LC) results in an unacceptable dilution of the analytes [16,17], which hampers the detection of the low concentrations usually found in water. For the extraction of PBDE from water, liquid/liquid extraction (LLE) [18] as well as solid phase extraction (SPE) using SPE cartridges [19] or SPE discs [20,21] have been applied. A more detailed review about the extraction and measurement of PBDE concentrations is given by Fulara and Czaplicka (2012) [22]. As

Directive 2008/105/EC requires the extraction of PBDE from whole water without prior filtration, thus containing also solid phase particulate matter (SPM), all methods face the challenge of extracting PBDE bound to humic acid as well as to other types of particles, which can be found in natural waters. Often the SPM phase is separated from the water and extracted separately using LLE or Soxhlet [23]. Recently, Novak et al. [24] published a method for the quantification of PBDE in water samples with LOQs required by Directive 2008/105/EC. However, the suitability of the proposed method remains questionable, in particular when looking at the peak areas and the signal/noise ratio shown for the method optimisation at 400 ng/L PBDE. Some questions arise when comparing this data with the results presented for mass concentrations of 0.025 ng/L for the various congeners in real water samples. Furthermore, they found that the mass concentration of PBDE 47 is the lowest of all PBDE found in the water samples, which is highly unusual as PBDE 47 and 99 were the most concentrated ones in the commercial available penta-PBDE mixes. This seems to be preserved in environmental samples and many authors, therefore, use PBDE 47 and 99 as indicators for the presence of contamination with penta-PBDE [25–27].

The optimisation of a GC-ICP-MS method for the separation and detection of PBDE at concentration levels of about the EQS value named in Directive 2008/105/EC was described by González-Gago et al. [28] in detail. This work was also performed within the EMRP project ENV08. The method described in the publication has been adapted and validated for coastal waters and it is included here to be compared with other methods and to discuss the advantages and disadvantages of the various methods.

In this work, various approaches such as LLE and SPE using discs or cartridges were tested and compared. The goal was to extract and analyse PBDE in whole water without separation of SPM from water. The PBDE congeners 28, 47, 99, 100, 153 and 154 were quantified using GC coupled to ICP-MS and EI-MS, respectively, and the results were compared. Additionally, real water samples were analysed to show the applicability of the developed methods for whole water analysis as required by the Directive 2008/105/EC.

2. Experimental

All solutions were prepared gravimetrically and stored in the dark at 4 °C.

2.1. Preparation of PBDE standard solutions

The pure PBDE compounds (Chiron, Trondheim, Norway), PBDE 28, 47, 99, 100, 153 and 154, were accurately weighed into brown glass bottles at PTB and dissolved with 2,2,4-trimethylpentane (isooctane) (SupraSolv[®], Merck, Darmstadt, Germany), which results in individual congener solutions with an approximate mass fraction of 80 µg/g. The purity of the compounds in solution was measured both with GC-MS and GC with flame ionization detection (GC-FID). The purity between 0.984 g/g (PBDE 100) and 0.999 g/g was found for all congeners. These solutions were then sent to all partners involved in this research for further characterisation. Diluted working solutions have been prepared with a solvent soluble in water such as 1-propanol or methanol, to allow a good mixing of the targeted PBDE congeners and the water phase. All dilutions were performed gravimetrically using an analytical balance.

2.2. Preparation of labelled PBDE spike solutions

For measuring PBDE concentrations with ICP-MS using isotope

Table 1

Mass concentration \pm standard measurement uncertainty and isotopic abundances as amount-of-substance fractions for the various PBDE used as species specific spikes in ID-ICP-MS as given by the provider.

Congener	Mass concentration \pm standard measurement uncertainty ($\mu\text{g/mL}$)	Amount-of-substance fraction $^{79}\text{Br}/$ (mol/mol)	Amount-of-substance fraction $^{81}\text{Br}/$ (mol/mol)
$^{81}\text{PBDE}$ 28	1.948 ± 0.033	0.0047	0.9953
$^{81}\text{PBDE}$ 47	2.881 ± 0.052	0.0047	0.9953
$^{81}\text{PBDE}$ 99	2.144 ± 0.042	0.0047	0.9953
$^{81}\text{PBDE}$ 100	2.65 ± 0.04	0.315	0.685
$^{81}\text{PBDE}$ 153	2.055 ± 0.035	0.0047	0.9953
$^{81}\text{PBDE}$ 154	2.35 ± 0.03	0.247	0.763

dilution mass spectrometry (IDMS), stock solutions of the various PBDE isotopically labelled with ^{81}Br were purchased from ISC Science (Oviedo, Spain). The mass concentration and isotope abundances are given in Table 1.

For measuring PBDE concentrations with MS, stock solutions of ^{13}C -labelled PBDE (PBDE 3, 15, 28, 47, 99, 100, 153, 154, 183, and 209) in *n*-nonane were used to implement IDMS.

2.3. Preparation of samples mimicking surface water

A variety of cleaning procedures for the glassware was applied by the different partners involved in the project ENV08 with respect to reduce the blank mass fractions. Usually, a multistage process involving the rinsing with solvents of different polarity with or without a thermal processing was used by most partners.

For the preparation of the PBDE containing water samples, brown glass bottles were used to prevent the degradation of PBDE by light during storage and transport [29]. Mineral water was spiked with humic acid provided by the Joint Research Center-Institute for Reference Materials and Measurements (JRC-IRMM) (Geel, Belgium) resulting in a dissolved organic carbon content (DOC) of about 5 mg/L and 15 mg/L, respectively. These matrices were used as a kind of model surface water. The two different concentration levels for the humic acid were chosen to mimic water with a low DOC such as groundwater and most surface waters (usually below 5 mg/L) [30] and water with a high content of DOC such as some lakes and rivers in wetlands with DOC > 10 mg/L [31]. As natural water often contains SPM, an additional model water sample was prepared containing 5 mg/L humic acid and SPM. To add the SPM to water, slurry was prepared using sediment with a known amount of the various congeners. The SPM was further milled to an average particle size of about 10 μm which was then suspended in water. This slurry was pipetted into the humic acid containing mineral water, resulting in a mass concentration of the various PBDE congeners in the model water of around 1 ng/L. The procedure is described in more detail in [32].

2.4. Real water samples

To test the developed methods for use in analysis of real water samples, freshwater samples were collected from two rivers in London area (UK), in the following referred to as River 1 and River 2, as well as water from the Elbe river estuary and the North Sea near the German coast. The samples were stored in dark at around 4 °C until analysis. For recovery studies, the water samples were spiked with a solution containing the native PBDEs under investigation. Unspiked water samples were also analysed to measure the natural PBDE background level.

2.5. Sample preparation

Different extraction and pre-concentration procedures were developed by the project partners of ENV08, all based on the two different principles LLE and SPE using SPE discs. The details can be found in the [Electronic Supplementary Information \(ESI\)](#).

Briefly: LLE was performed using organic solvents such as *iso*-octane, hexane, dichloromethane or mixtures of these solvents. Due to the effects caused by a complex matrix in some samples, the crude extract had to be treated further. In case the water samples contained humic acid, an emulsion which formed between water and the extraction solvent, had to be broken by adding sulphuric acid. For real surface water samples such as river waters it was necessary to clean the extracts more elaborately using an additional solid phase clean-up step to remove other interfering bromine containing compounds. The coastal and estuary samples also required a further cleanup of the crude extracts to remove interfering substances.

The SPE approach used C18 discs for the extraction of the PBDE from water. After retaining the congeners on the disks they were eluted using either a mixture of ethyl acetate and dichloromethane or acetone and hexane. The extracts were also purified further using acidic or basic silica columns.

In all cases, the extracts were finally evaporated using a nitrogen stream reducing the sample volumes to about 100–200 μL . Enrichment factors of at least 5000 for the targeted PBDE could be achieved by all the proposed sample preparation methods in the analysed water samples.

2.6. Measurement procedure

All partners used GC for the separation of the various PBDE congeners and ICP-MS or organic MS for their detection and quantification. Details about the applied analytical methods can be found in the ESI.

The Agilent GC 7890A or 6890 (Agilent Technologies, Santa Clara, USA) equipped with either a CTC CombiPAL (CTC Analytics AG, Zwingen, Switzerland) or an Agilent 7683 autosampler was coupled to either an Agilent ICP-MS 7500 or 7700. In cases where GC-MS/MS was used either a Trace 1310 GC coupled to TSQ Quantum XLS (both Thermo Scientific, Waltham, USA) or a GC 7890 (Agilent Technologies) coupled to a Waters Quattro Micro (Waters, Milford, USA) have been utilised for the measurements. For the injection of the extracts into the GC either split-splitless, programmed temperature vaporiser (PTV) or cool-on-column systems were used.

For the separation, most partners used a DB-5MS column with either, 30 m length, 0.32 mm diameter and 0.25 μm film thickness or 15 m length, 0.25 mm diameter and 0.1 μm film thickness (Agilent Technologies). Besides, also ZB-5HT Inferno (Phenomenex, Torrance, USA) and Optima 5 (Macherey-Nagel, Düren, Germany), both with 15 m length, 0.25 mm diameter and 0.1 μm film thickness, were used. All columns have a similar stationary phase for the separation consisting of 5% phenyl and 95% dimethylpolysiloxane. The carrier gas flow rate and the oven temperature program were optimised to achieve a good separation of the priority PBDE from each other as well as from the interfering substances of the matrix. For more information about the measurement conditions see the ESI.

3. Results and discussion

3.1. Blank mass fractions

As PBDE were also used in many equipments commonly found

in laboratories or production sites of chemicals, a control of lab and procedural blanks is important since they strongly influences the overall sensitivity of the proposed methods. All partners performed a thorough cleaning of all glassware before use. All solvents were tested regarding their blank contents for the PBDE investigated. A summary of the blank mass fractions of the various PBDE for the different methods found when extracting mineral water samples without adding any PBDE is given in Table 2. A GC-ICP-MS (LLE) method by PTB, LGC and HZG, GC-EI-MS/MS (LLE) method by LNE and UME and GC-EI-MS/MS (SPE) method by SYKE were applied. Presented data in Table 2 were determined by calculating mean of the results, which were reported by each institute, performing in triplicate.

3.2. Identification and selectivity

For the identification of the various congeners the retention times (RT) of the bromine (Br) containing analytes and the RT of the pure PBDE congeners were compared in case of GC-ICP-MS measurements. For GC-MS analyses, the identification was achieved by the RT and maximal two characteristic mass to charge ratios (m/z -ratios) or transitions when MS/MS was implemented. To ensure adequate selectivity, the partners used different multi-component solutions such as the reference material of the National Institute of Standards and Technology NIST SRM 2257 (NIST, Gaithersburg, USA) containing 38 different congeners applying the same conditions as for the analyses of the water samples (Fig. 1).

The extract clean-up procedures employed by some partners remove a significant part of the potentially co-eluting and Br-containing interfering compounds, especially when aggressive reagents like concentrated acids and bases are used in addition to a solid-phase clean-up. Therefore, the method selectivity is further increased by the use of chemical clean-up procedures during the sample preparation procedure.

3.3. Repeatability, reproducibility and LOQ

The repeatability was evaluated by applying the developed methods on mineral water samples containing humic acid and 1 ng/L of each PBDE congener. Each method was applied at least in four replicates and the samples were injected onto the GC column at least three times. Relative standard deviation (S_{rep}) of the results were calculated and presented in Table 3.

In order to determine the reproducibility, also mineral water samples containing humic acid and 1 ng/L of each PBDE congener were analysed at least in triplicate on the same day and on three different days. All samples were measured three times by GC. Reproducibility was identified by calculating relative standard deviation (S_{rep}) of the results and presented in Table 4.

The samples with mass concentrations close to the WFD EQS of 0.5 ng/L were prepared and measured by each method in ten replicates and standard deviation of the results were multiplied by

Table 2
Calculated blank mass fractions for the investigated PBDE congeners applying the different methods.

Congener	GC-ICP-MS (LLE) /(ng/kg)	GC-EI-MS/MS (LLE) /(ng/kg)	GC-EI-MS/MS (SPE) /(ng/kg)
28	< 0.01	< 0.01	0.011
47	0.04	0.06	0.027
99	0.02	0.05	0.021
100	0.01	0.02	0.011
153	0.05	< 0.01	0.006
154	0.004	< 0.01	0.016

w: mass fraction.

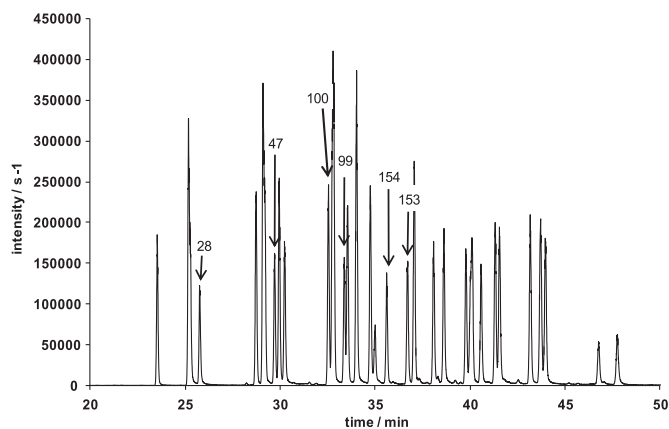


Fig. 1. Chromatogram of the PBDE reference solution NIST 2257 achieved using GC-ICP-MS. The congeners listed in Annex I of the amendment of the WFD are labelled.

Table 3
Repeatability in % achieved for the different measurement procedures.

Congener	GC-ICP-IDMS (LLE) $S_{rep}/\%$		GC-EI-MS/MS (LLE) $S_{rep}/\%$	GC-EI-MS/MS (SPE) $S_{rep}/\%$
	Without clean-up	With clean-up		
28	2.2	3.5	2.7	1.7
47	2.8	5.8	4.6	0.65
99	4.6	7.9	5.6	2.8
100	3.0	5.0	5.4	11
153	2.5	14	5.2	10
154	5.3	11	6.3	11

S_{rep} : relative standard deviation.

9 to determine LOQ values. Then, they were classified according to mass fraction of humic acid in water and presented in Table 5. As it is mentioned above GC-ICP-MS (LLE) method by PTB, LGC and HZG, GC-EI-MS/MS (LLE) method by LNE and UME and GC-EI-MS/MS (SPE) method by SYKE were applied. While LNE, SYKE and PTB were studied the water sample containing 5 mg/kg of humic acid, LGC and UME applied their own method to the water sample containing 15 mg/kg of humic acid. SYKE determined their LOQ values only with pure water samples without humic acid, which were indicated "without matrix" in Table 5.

3.4. Estimation of measurement uncertainty according to Guide to the Expression of Uncertainty in Measurement (GUM)

The water samples containing humic acid with 5 mg/kg or 15 mg/kg mass fractions were prepared with mass concentrations of about 1 ng/L for each PBDE congener and studied in triplicate. The samples were injected into the GC for three times. Preparation of the water samples, extraction and quantification procedure were repeated on two different days. The mass concentration was chosen as it can be measured easily with all the developed methods and provides, therefore, a good basis for comparing with all the methods. Based on this experimental set up, a complete uncertainty budgets was estimated by each institute for the individual methods and which are presented in Table 6. Matching of institute and method was presented in the previous section. The uncertainty values were calculated according to GUM [33] using either GUM Workbench Pro Version 2.4.1.392 (Metrodata GmbH, Weil am Rhein, Germany) or Wincert software Version 3.1 (IM-PLEX, Lyon, France). The equations used for the estimation of the expanded uncertainties associated to the results obtained with various methods are given in the ESI. As the congeners behave differently during sample preparation and measurement, a

Table 4
Reproducibility in % achieved for the different measurement procedures.

Congener	GC-ICP-IDMS $s_{repr}/\%$		GC-EI-MS/MS (LLE)/ $s_{repr}/\%$	GC-EI-MS/MS (SPE)/ $s_{repr}/\%$
	Without clean-up	With clean-up		
28	5.3	9.2	2.4	6.4
47	4.8	10	6.3	21
99	6.3	13	6.8	14
100	2.2	10	5.1	42
153	6.4	14	7.3	27
154	7.5	12	4.6	23

Table 5
LOQs achieved for the different measurement procedures (a) LOQs achieved for samples with a humic acid mass fraction of about 5 mg/kg in mineral water. (b) LOQs achieved for samples with a humic acid mass fraction of about 15 mg/kg in mineral water applying additional clean-up.

(a) Humic acid mass fraction of 5 mg/kg

Congener	GC-ICP-IDMS LOQ/(ng/kg)		GC-EI-MS/MS (SPE) ^a LOQ/(ng/kg)	GC-EI-MS/MS (LLE) LOQ/(ng/kg)
	Without clean-up	With clean-up		
28	0.05	0.015	0.0016	0.058
47	0.14	0.056	0.0060	0.058
99	0.10	0.074	0.0074	0.055
100	0.31	0.047	0.0065	0.056
153	0.065	0.024	0.0022	0.053
154	0.41	0.031	0.098	0.053

(b) Humic acid mass fraction of 15 mg/kg

Congener	GC-ICP-IDMS w_{LOQ} /(ng/kg)	GC-EI-MS/MS (LLE) w_{LOQ} /(ng/kg)
28	0.13	0.023
47	0.11	0.021
99	0.13	0.044
100	0.12	0.044
153	0.13	0.081
154	0.12	0.16

w: mass fraction.

^a These values were measured without matrix.

complete uncertainty budget was compiled for each congener separately.

3.5. Samples mimicking real water and real water samples

As most real water samples contain SPM besides humic acid, artificially water sample mimicking real water containing 5 mg/L humic acid, SPM and PBDE with a mass concentration resulting in 1 ng/L for each PBDE congener was produced and the performance of one method for both SPM containing and non-SPM containing water samples was compared.

The LOQs and associated uncertainties at a mass concentration level of 1 ng/L for each PBDE congener achieved in both cases are summarised in Table 7.

In addition to these artificially produced water samples mimicking real water, two different river water samples from the London area (UK) were analysed regarding their inherent PBDE mass fractions. Two PBDE congeners could be detected in the River 1 water sample (PBDE 47 and 99) but their mass fractions were below the respective LOQs. In both river water samples other bromine containing compounds, eluted before the lowest retained PBDE congener (PBDE 28), were detected. Therefore, an elaborated clean-up of the crude extract was necessary to successfully remove the interference. The river water samples were spiked with PBDE at mass fractions close to the EQS and used to test the developed

Table 6
Relative expanded uncertainty (U_{rel}) using a coverage factor of $k=2$ for the different measurement procedures at a mass concentration of 1 ng/L for each congener in water. (a) U_{rel} achieved for samples with a humic acid mass fraction in water of about 5 mg/kg. (b) U_{rel} achieved for samples with a humic acid mass fraction in water of about 15 mg/kg applying additional clean-up.

(a) Humic acid concentration of 5 mg/kg

Congener	GC-ICP-IDMS $U_{rel}/\%$		GC-EI-MS/MS (SPE) $U_{rel}/\%$	GC-EI-MS/MS (LLE) $U_{rel}/\%$
	Without clean-up	With clean-up		
28	5.6	1.7	3	5.9
47	8.8	4.2	7	21.8
99	8.0	2.8	5	17.1
100	18	4.8	16	10.2
153	20	3.8	9	6
154	13	7.3	8	4.3

(b) Humic acid concentration of 15 mg/kg

Congener	GC-ICP-IDMS $U_{rel}/\%$	GC-EI-MS/MS (LLE) $U_{rel}/\%$
28	13.0	5.5
47	8.0	5.3
99	15	7.0
100	6.1	8.7
153	21	15
154	6.9	28

Table 7

(a) LOQs and (b) U_{rel} ($k=2$) achieved for the measurement of 1 ng/L for each PBDE congener in water containing humic acid with and without additional SPM using SPE disc extraction followed by GC-MS.

(a) LOQs

Congener	Without SPM $w/$ (ng/kg)	With SPM $w/$ (ng/kg)
28	0.0016	0.012
47	0.0060	0.1
99	0.0074	0.2
100	0.0065	0.04
153	0.0022	0.02
154	0.098	0.02

(b) Expanded uncertainty at 1 ng/L

Congener	Without SPM $U_{rel}/\%$	With SPM $U_{rel}/\%$
28	3	1.2
47	7	10
99	5	20
100	16	4.0
153	9	2.0
154	8	2.0

GC-ICP-MS method with LLE regarding recovery and expanded measurement uncertainty (Table 8). The river water 1 was spiked with about 0.1 ng/kg for each congener, while the river water 2 was spiked with about 1 ng/kg for each congener. The relative

Table 8
Recovery and relative measurement uncertainty ($U_{rel}/\%$) ($k=2$) of PBDE in water samples of two rivers from the London area (UK).

Congener	River 1 water spiked with 0.1 ng/kg		River 2 water spiked with 1 ng/kg	
	Recovery/%	$U_{rel}/\%$	Recovery/%	$U_{rel}/\%$
28	105–120	31	99–101	13
47	109–121	41	95–106	6
99	101–129	39	99–106	15
100	97–105	22	98–102	6
153	94–112	32	94–107	21
154	91–107	26	99–101	7

expanded uncertainty (U_{rel}) ($k=2$) corresponds to the highest obtained expanded measurement uncertainty. Five replicates were measured. The results in Table 8 were obtained by LGC.

Besides the river water samples, estuary and sea water samples were analysed as this matrix poses a special challenge due to the high salt content. The two types of water samples showed PBDE mass fraction levels below the LOQs for the six priority congeners. These samples were also analysed directly and after spiking them with native PBDE for recovery studies. The results are summarised in Table 9. Elbe river water was spiked with about 0.25 ng/kg while the tested North Sea water was spiked with about 0.35 ng/kg for each PBDE congener. The relative expanded uncertainty (U_{rel}) (with $k=2$) corresponds to the highest obtained expanded measurement uncertainties. Five replicates were measured. The results in Table 9 were obtained by HZG.

3.6. Discussion

The measurement of the PBDE concentrations in unfiltered natural water samples at the levels as required by EU-WFD of 2008 is challenging due to a number of factors. The environmental water samples are likely to contain a complex mix of anthropogenic and naturally occurring compounds and particles. Additionally, natural waters can also contain an ecosystem of microorganisms along with inorganic and organic SPM. PBDE might be dispersed among these different compartments: incorporated into microorganisms and adsorbed onto inorganic and organic SPM, as well as being present in the so called “dissolved fraction”. After sampling, changes due to alteration of aerobic and anaerobic conditions can occur. These changes are reflected in micro-flora growth with the possibility of aggregation leading to the sedimentation of SPM. PBDE may adhere to such particles or to the walls of the sampling container. Therefore, a thorough investigation of the various extraction procedures and a strategy for the correction of possible losses had to be performed by the project partners. Considering that PBDE are widely spread, special attention has also to be paid to the contamination control, especially

Table 9
Recovery and relative measurement uncertainty ($U_{rel}/\%$) ($k=2$) of PBDE in water samples from the Elbe river estuary (Germany) and North Sea.

Congener	Elbe river estuary spiked with 0.25 ng/kg		North Sea spiked with 0.35 ng/kg	
	Recovery/%	$U_{rel}/\%$	Recovery/%	$U_{rel}/\%$
28	92–98	5.8	92–97	5.1
47	120–128	7.1	119–124	6.0
99	102–107	5.0	99–103	4.0
100	87–100	14	92–105	12
153	89–93	4.4	89–92	3.2
154	85–93	8.8	85–92	6.6

when high volume sample pre-concentration is employed.

PBDE 47 and PBDE 99 were most often encountered in the blanks as they are the most abundant congeners in the technical penta-PBDE mixtures. Therefore, the water used to produce model water has to be chosen carefully. In our case, mineral water from Evian was finally chosen as it shows the lowest blank mass fractions. All the developed methods show comparable blank mass fractions. Considering the very low concentrations of PBDE in the samples both reproducibility and repeatability of all developed methods are satisfactory.

The recovery of spiked PBDE congeners from the model water samples was found to be close to 100% for all methods indicating that the equilibration of spike and sample could be achieved. The recovery of the PBDE in artificial samples is similar to that in real water samples as shown in Tables 8 and 9.

All partners used GC for the separation of the various PBDE congeners and ICP-MS or organic MS for their detection and quantification, since HPLC coupled to ICP-MS or MS is not suited to meet the required LOQs. For all developed measurement procedures, the EQS level defined in the EU-WFD from 2008 [3] could be reached. For some compounds, however, the required LOQ of 30% of the EQS is still challenging. Difficulties were encountered in case of LLE when analysing samples with humic acid. The shaking during extraction caused severe foaming and resulted in incomplete recovery of the organic extraction solvent after phase separation. Even though the loss of analyte does not influence the results when applying IDMS, it can happen that the already low mass fractions of PBDE decrease under the LOQ of the detection method. Samples with a humic acid content of about 5 mg/L DOC allowed the extraction of the PBDE despite the foaming. However, samples with a higher DOC could be difficult to analyse. The methods in Table 5a which include a clean-up step show, in general, better LOQs than the method without clean-up. For the samples containing only 5 mg/L humic acid this clean-up included at least passing the extract through a column containing sodium sulphate before reducing the extract to the final volume.

In case of real water samples, the measurements using GC-ICP-MS encountered a problem with Br containing interferences such as co-eluting compounds which required a more elaborate sample clean-up. The developed sample preparation involved acidic and basic clean-up steps followed by a SPE clean-up using an AgNO₃ impregnated silica gel with a small plug of anhydrous Na₂SO₄ (for more detailed information see the ESI). When analysing water samples with a high humic acid content, this elaborate clean-up led to a higher LOQ achieved with the whole measurement procedure (Table 5b). Such an extended clean-up was not necessary when using GC-MS/MS as in this case the identification of the analyte was achieved using specific molecular ion transitions during MRM which was not affected by the Br containing compound, interfered the ICP-MS measurements. Therefore, the LOQ for most analytes is a factor of 2–4 better using GC-MS/MS instead of GC-ICP-MS.

Another approach to avoid problems with foaming is the use of SPE disc systems with C18 SPE discs. This approach is also recommended in a draft standard for measuring PBDE concentrations in water in routine laboratories by the Mandate 424 participants [34]. Combining this procedure with the quantification of the various congeners using IDMS achieved the lowest LOQs and associated expanded uncertainty (Tables 5 and 6).

The use of isotopically labelled spikes of the various PBDE congeners enabled the monitoring of possible degradation of higher brominated PBDE to lower brominated species during sample preparation and measurement. However, no such degradation could be observed for the investigated PBDE during this project. For IDMS two different approaches were used: double IDMS and single IDMS. Double IDMS provided advantages over

single IDMS for traceable and accurate quantification since the enriched spike concentration is not required to be traceably characterized and accurately known as it is not included in analyte mass fraction calculations. Additionally, with double IDMS calibration, the mass-bias affected the result of the ICP-MS measurement to much lesser extent compared to single IDMS. However, it was found that samples containing humic acid or SPM behaved quite different to reference solutions prepared with water. As it is difficult to prepare a reference samples exactly matching the matrix of surface water, single IDMS was investigated in this project in addition to double IDMS. In GC-MS/MS measurements IDMS also had the advantage that spike and analyte experience the same matrix suppression in the ionisation source.

The uncertainty of the isotope ratio measurements in sample and sample-spike blend accounts for the greatest part of the total uncertainty in GC-ICP-IDMS measurements using single IDMS, while the main source of uncertainty in case of double IDMS derives from the observed isotope ratios in sample-spike and calibration-spike blends. If it is possible to reduce the uncertainty in the isotope ratio measurements, the total uncertainty of the measurement of PBDE concentrations in water can be further reduced. The use of high resolution ICP-MS such as sector field instruments would enable a more accurate measurement of the isotope ratio. However, due to the interferences of $^{40}\text{Ar}_2^1\text{H}$ and $^{40}\text{Ar}^{38}\text{Ar}^1\text{H}$ the use of a resolution of at least 5000 is required. This reduces the sensitivity of the sector field ICP-MS to an extent that the LOQs required in the Directive 2008/105/EC cannot be achieved anymore. Therefore, the higher uncertainty was accepted to ensure sufficiently low LOQs for the investigated PBDE. Furthermore, a high enrichment in ^{81}Br in the PBDE used as spike material is needed for a reliable quantification using IDMS. For PBDE 100 and 154, there are currently only solutions commercially available with a very low isotope enrichment in ^{81}Br (Table 1) which leads to a large expanded uncertainty for these compounds when using GC-ICP-IDMS. The high uncertainty observed for PBDE 100 and PBDE 154 is mainly caused by the low isotope enrichment of the spike. To achieve a reasonable isotope ratio in the blend a high amount of spike has to be added. Especially for PBDE 100 a ratio of 2:1 ($^{81}\text{PBDE}:^{79}\text{PBDE}$) can hardly be reached as the spike itself has only a ratio of 2.3:1. Furthermore, it can be shown that for such low isotope enrichment small variations in the isotope ratio of the spike lead to great changes in the measured concentration of the PBDE in the sample. Therefore, the isotope ratio in the spike has to be measured very accurately, which is difficult using a quadrupole ICP-MS. So a spike material with a higher enrichment in ^{81}Br is urgently needed. For PBDE 153 the higher uncertainty is due to a higher uncertainty in the reference solution caused by difficulties in dissolving the solid compound in *iso*-octane.

In case of GC-MS/MS the main contributions to the uncertainty turned out to be the repeatability of the measurement and the sample preparation. The use of triple quadrupole analyzer in SRM mode is one of the most selective and sensitive approaches for trace analysis in environmental pollutants. However, the optimization of the MS/MS parameters had to be done carefully to raise the maximum signal for each PBDE. In particular higher brominated compounds (with more than six bromine atoms in their molecule) typically requires high collision energies. The dwell time parameter had to be optimised to obtain at least 15 data points per peak, to provide a good peak shape, and, at the same time, maintain sufficient sensitivity. As a result, depending on the number of MS/MS transitions in each particular time segment, large values had to be chosen. Moreover, to intensify the signal of higher brominated PBDEs that provided less intensive signal compared to lower brominated PBDEs, a higher electron multiplier gain had to be set.

After the development of the measurement procedures, samples closer to real water and water samples were measured. As the EU-WFD requires the measurement of PBDE concentrations in water samples without prior filtration, SPM was added to the water samples containing 5 mg/L humic acid. It can be seen in Table 7 that both the LOQ and the expanded uncertainty increases significantly when SPM is present in the samples. Water samples from two rivers in the London area (UK) spiked with PBDE were used to demonstrate the applicability of the GC-ICP-MS methods to real water samples. River 2 water spiked with around 1 ng/kg of each congener and River 1 water spiked with around 0.1 ng/kg of each congener were analysed and the recovery and expanded uncertainty was calculated (Table 8). It can be observed that the recovery variations are more important at the lower mass fraction and that the measurement uncertainty increases but remains in all cases below the required level of 50%. Furthermore, water samples with a higher salt content were investigated originating from the Elbe river estuary and the North Sea (Table 9). The salt content seems to affect neither the extraction efficiency nor the measurement uncertainty.

4. Conclusion

All analytical procedures presented can serve as reference measurement procedures with respect to the requirements of the EU-WFD from 2008 regarding the LOQs and their associated expanded uncertainties for the six PBDE listed as priority substances in the EU-WFD and associated directives. The traceability to the SI was achieved by either tracing the isotopically labelled PBDE congeners back to the reference material SRM 2257 or to in-house standards prepared from the solid congeners after verification of the purity of the material.

LLE without additional clean-up of the extracts is the easiest way to extract PBDE from unfiltered natural water samples as long as the humic acid concentration is low. In all other cases, an elaborate clean-up has to be applied. However, this requires skilled personnel and is time consuming. LOQs and expanded uncertainties of the results obtained with the method using SPE discs combined with GC-MS/MS recommended by Mandate 424 as draft standard [34] are in good agreement with LOQs and expanded uncertainties of results for the PBDE congeners obtained with the other methods investigated in this work. Combined with IDMS it can serve as a reference measurement procedure. GC-ICP-IDMS is also a suitable separation and detection method. However, as it requires $^{81}\text{PBDE}$ labelled congeners which are currently only available for PBDE 28, 47, 99 and 153 with enrichment in ^{81}Br that is acceptable for IDMS; therefore this method cannot provide reliable measurement results for all PBDE congeners defined in the EU-WFD. Especially the low enrichment of $^{81}\text{PBDE}$ 100 caused difficulties when measuring PBDE 100 in water samples using ICP-IDMS.

However, as none of the applied methods is interference free and considering the number of organic molecules – artificial as well as natural – that might interfere with the various PBDE congeners in real water samples, more than one method is necessary to achieve highly accurate results. As GC-MS/MS and GC-ICP-MS are based on different principles-the first detects molecules while the latter detects only Br ions – they can complement each other for the identification of possible systematic errors and interferences.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2016.01.066>.

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