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ORIGINAL PAPER

Determination of ultra-trace levels of priority PBDEs in water samples by isotope dilution GC(ECNI)MS using ^{81}Br-labelled standards

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Abstract A gas chromatography electron capture negative ionization mass spectrometry (GC(ECNI)MS) procedure for the determination of priority polybrominated diphenyl ethers (PBDEs; congeners 28, 47, 99, 100, 153 and 154) in water samples at regulatory EU levels has been developed. The method is based on the use of ^{81}Br-labelled PBDEs for isotope dilution analysis and the measurement of ^{79}Br/^{81}Br isotope ratios in gas chromatography peaks with the electron capture negative ionization technique. The suitability of this ion source for the precise and accurate measurement of bromine isotope ratios has been demonstrated. The general ECNI-IDMS procedure was evaluated by the analysis of NIST SRM 1947 (Lake Michigan fish tissue) with satisfactory results. For the analysis of water samples, 500 mL of the samples were spiked with the labelled PBDEs and extracted with 10 mL isooctane for 30 min. The extract was evaporated down to ca. 100 μL and injected in the GC(ECNI)MS. Detection limits ranged from 0.014 $^{-1}$ to 0.089 pg mL^{-1} depending on the congener. Recoveries from real water samples, spiked at a level of 0.5 pg mL^{-1}, ranged from 77% to 102%.

Keywords Isotope dilution mass spectrometry · Polybrominated diphenyl ethers · ^{81}Br-labelled standards · Electron capture negative ionization

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Introduction

Polybrominated diphenyl ethers (PBDEs) are a family of 209 chemical substances (congeners) which are added as flame retardants to a wide range of polymeric materials [1, 2]. These compounds are not chemically bound to the polymers so they can be easily released into the environment [3]. Consequently, PBDEs have been distributed worldwide and are nowadays being found in nearly all environmental compartments [3, 4]. Special attention has been paid by environmental scientists to the constituents of the pentaBDE technical product [5], as these compounds are volatile enough to permit their long-range transport [6]. Furthermore, it has been found that congeners with less than seven bromine atoms are more bioaccumulative than heavier PBDEs [7], exhibiting large biomagnification factors through the food chain [8]. As a result, the congeners present in the pentaBDE technical product, have been detected at increasing levels not only in environmental samples (sediments, sludge, house dust and indoor and outdoor air) but also in biological samples (human adipose tissues, serum, breast milk, fish, birds and marine mammals) and foodstuffs [2, 9]. Besides, observed adverse effects are generally more pronounced for congeners constituting the pentaBDE technical product, which seem to cause toxic effects at lower doses than the higher brominated BDEs [10]. Consequently, the production and use of pentaBDE mixtures has been banned in Europe and in several states in the USA since 2004 [11]. The European Union has issued a Directive [12] in which congeners number 28, 47, 99, 100, 153 and 154 will need to be measured in European freshwaters at levels below 0.5 ng·L^{-1}. The US EPA has included congeners 47, 99 and 100 in the unregulated contaminants list to be measured in freshwaters and congeners 28, 47, 99, 100, 153, 154, 183

and 209 have also been proposed to be included in a European monitoring programme for feed and food [13].

Due to these analytical challenges, new methods for the determination of these compounds have been developed but these still need further improvement in terms of sensitivity, precision and accuracy [14]. These methodologies require a sample preparation stage which usually involves several steps such as drying of solid samples followed by extraction of the analyte from the sample matrix with organic solvents and finally clean up and fractionation of the organic extracts [14, 15]. The techniques most widely used for the determination of PBDEs are gas chromatography (GC) coupled to electron capture or mass spectrometry (MS) detectors [9]. Low resolution (LR) mass spectrometers are more frequently used in routine analysis since high resolution (HR) instruments are more expensive and require experienced users. HR-MS is almost exclusively operated in electron impact (EI) mode, but LR-MS has been widely employed working either in electron capture negative ionization (ECNI) or in EI modes [14].

Several analytical methodologies have been developed for the determination of PBDEs in biological and environmental samples, although procedures based in isotope dilution mass spectrometry (IDMS) are usually preferred since they provide better results in terms of precision and accuracy [16]. However, to date, only ^{13}C-labelled standards are commercially available, so IDMS is only suitable when using ion sources which generate carbon-containing ions as both compounds coelute in the gas chromatograph [17]. Therefore, the EI source in positive mode has been often selected for the determination of PBDEs by IDMS using both HR [16, 18] and LR [19] analysers. Unfortunately, under standard conditions, the ECNI source provides negative ion mass spectra dominated by non specific bromide ions, so most PBDEs (except BDE-209) cannot be quantified by ECNI in combination with IDMS using the commercially available ^{13}C-labelled standards [20, 21]. However, by modifying the source conditions Ackerman et al. [17] could detect carbon-containing negative ions suitable for ^{13}C IDMS for high-level samples. On the other hand, the ECNI source is often selected for ultra-trace analysis of PBDEs as it provides lower detection limits than the EI source using the same mass analyzers.

We have prepared and characterised a series of ^{81}Br-labelled PBDE standards which would allow the combination of the high sensitivity ECNI source with the accuracy and precision provided by IDMS. The synthesis of the ^{81}Br-labelled analogues has been reported [22] and their purification and characterisation in both concentration and isotopic abundances has been given [23]. Using GC(EI)MS, it was observed that no isotopic exchange took place between bromine ions from the analyte and the labelled standards demonstrating the suitability of these compounds for IDMS [23].

In this work, the ^{81}Br-labelled standards have been applied for the determination of the six European priority BDEs (28, 47, 99, 100, 153 and 154) in river waters at ng·L^{-1} levels. The determination of priority PBDEs was carried out by IDMS using a quadrupole MS fitted with an ECNI source as GC detector. First, the capabilities of the GC(ECNI)MS system for elemental bromine isotope ratio measurements were evaluated in two different instruments. Then, the proposed methodology was evaluated by analyzing a certified reference material SRM 1947 (Lake Michigan fish tissue) and applied for the analysis of river waters at regulatory EU levels.

Experimental

Reagents and materials

Individual standards of 6 PBDE congeners (28, 47, 99, 100, 153 and 154, 50 μg·mL^{-1} in nonane) were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). SRM 1947 (Lake Michigan fish tissue) and SRM 2257 (PBDE Congeners in 2,2,4-trimethylpentane) were both obtained from the National Institute of Standards and Technology (NIST), Gaithersburg, USA.

All solvents used in this work were of the highest purity. Acetone and hexane were obtained from Fluka (Steinheim, Germany) and dichloromethane and diethyl ether from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water was obtained from a Milli-Q Gradient A10 water purification system (Millipore S.A.S, Molsheim, France). Working standard solutions of labelled and unlabelled PBDEs were prepared by weight in methanol or isooctane, both from Sigma-Aldrich, and stored in the dark at 4 °C until use.

Sampling bottles (500 mL, amber glass) and all glassware used for sample preparation were cleaned with detergent (Mucasol from Brand GmbH, Wertheim, Germany), rinsed with Milli-Q water, dried in an oven, brought to room temperature and stored. Just before use, all glassware was rinsed twice with hexane and acetone and allowed to dry at room temperature. Anhydrous sodium sulphate (Merck, Darmstadt, Germany) was used to dry the SRM 1947 samples and silica gel (0.063–0.200 mm) for column chromatography (Merck) was used in the clean up and fractionation steps for the SRM 1947 sample preparation.

Instrumentation

Two GC(ECNI)MS instruments were compared for bromine isotope ratio measurements in PBDEs. The GC model 6890N (Agilent Technologies, Waldbronn, Germany) was

located at the VU University in Amsterdam, The Netherlands and was equipped with a split/splitless injector and a MSD model 5975B (Agilent Technologies, Tokyo, Japan). Injections (1 μL) were carried out automatically by an autosampler model 7683 (Agilent). The chromatographic separation was carried out using a low-polarity capillary column SGE BPX5 (SGE Analytical Science, Bester, Amstelveen, The Netherlands; 50 m×0.25 mm i.d., 0.25 μm film thickness).

The second instrument was a GC(ECNI)MS model QP2010 Plus (Shimadzu, Kyoto, Japan) and was installed at the University of Oviedo, Spain. This instrument was fitted with a split/splitless injector. Sample, spikes and standard solutions (2 μL) were injected in each case automatically by an autosampler model AOC-5000 (Shimadzu). The chromatographic separation was carried out using a low-polarity capillary column DB-5MS Ultra Inert (J&W Scientific, Folsom, CA, USA; 30 m×0.25 mm i.d., 0.25 μm film thickness), as it has been one of the most used and tested for PBDEs [14]. All analytical determinations in SRM 1947 and river water samples were performed in this second instrument. Operating conditions are summarized in Table 1.

All standard solutions and mixtures were prepared gravimetrically using an analytical balance model AB204-S (Mettler-Toledo GmbH, Greifensee, Switzerland). A mechanical shaker (Heidolph REAX 2, Kelheim, Germany) was used for the liquid–liquid extraction of PBDEs from water samples.

Table 1 GC(ECNI)MS operating conditions for the Shimadzu instrument

GC and interface parameters	
Column	DB-5MS UI (30 m×0.25 mm×0.25 μm)
Injection mode	Pulsed splitless
Splitless time	2 min
Pulse	200 kPa, 1.5 min
Injection volume	2 μL
Carrier gas / Flow	He / 2 mL·min^{-1}
Injection temperature	300 °C
Oven programme	120 °C to 300 °C (10 min) at 30 °C·min^{-1}
Interface temperature	280 °C
ECNI ion source and MS parameters	
Source temperature	230 °C
Source voltage	70 V
Emission current	150 μA
Reagent gas	CH_4
Acquisition mode	SIM
Selected *m*/*z*	79 and 81
Dwell time	50 ms
Solvent delay	3.5 min

Procedures

Determination of PBDEs in SRM 1947

Samples of Lake Michigan fish tissue (SRM 1947) were prepared following a previously described sample preparation procedure [24] with some modifications. In brief, homogenized fish tissue was ground in a mortar with anhydrous sodium sulphate and allowed to dry for 3 h. Then, the samples (ca. 1 g) were spiked with the ^{81}Br-BDEs standard mixture at three spike levels (blends 1, 2 and 3). For blend 2 the spiked concentrations were 16.7 (BDE 28), 1.40 (BDE 49), 81.2 (BDE 47), 14.2 (BDE 99), 7.68 (BDE 100), 15.8 (BDE 153) and 1.09 (BDE 154) ng·g^{-1}. For blend 1, the spike concentrations were half and for blend 3 double of those indicated for blend 2. Spiked samples were Soxhlet extracted for 12 h with hexane/acetone (3:1, *v*/*v*). The extract was concentrated, cleaned up on acidic silica gel columns (40% H_2SO_4) and eluted with dichloromethane/hexane (3:7, *v*/*v*). The collected fraction was concentrated under nitrogen and eluted over a fractionation silica gel column (2% H_2O) with hexane, hexane/diethyl ether (85:15, *v*/*v*) and diethyl ether. The two first fractions, containing the PBDEs, were mixed and then evaporated under nitrogen down to a few microlitres and stored in the dark at 4 °C until analysis. Finally, 2 μL of the organic extract were injected in the GC(ECNI)MS system. Concentrations were calculated for each congener by the elemental isotope dilution equation [25].

River water collection

The study area is located in the northwest of Spain along the River Nalón. Water samples (500 mL) were collected in pre-weighed graduated amber glass bottles fitted with PTFE coated PBT screw caps during July 2010 at the five sampling sites shown in Fig. 1 and in duplicate. The first sampling site was the closest to the river source and it was selected before the confluence of River Nalón with any of its main tributaries. The other four sampling sites were located downstream just beyond its confluence with each of its main four tributaries (rivers Caudal, Trubia, Nora and Narcea). No filtration of the samples was performed according to EU regulations as, for organic compounds, the suspended particulate phase should be included in the analyses [12].

Determination of PBDEs in river water samples

Sample preparation was performed in the same glass bottles used for sampling. All samples collected were weighed in the lab and one of the two samples collected at the same sampling site were spiked (by weight) with a mixture of

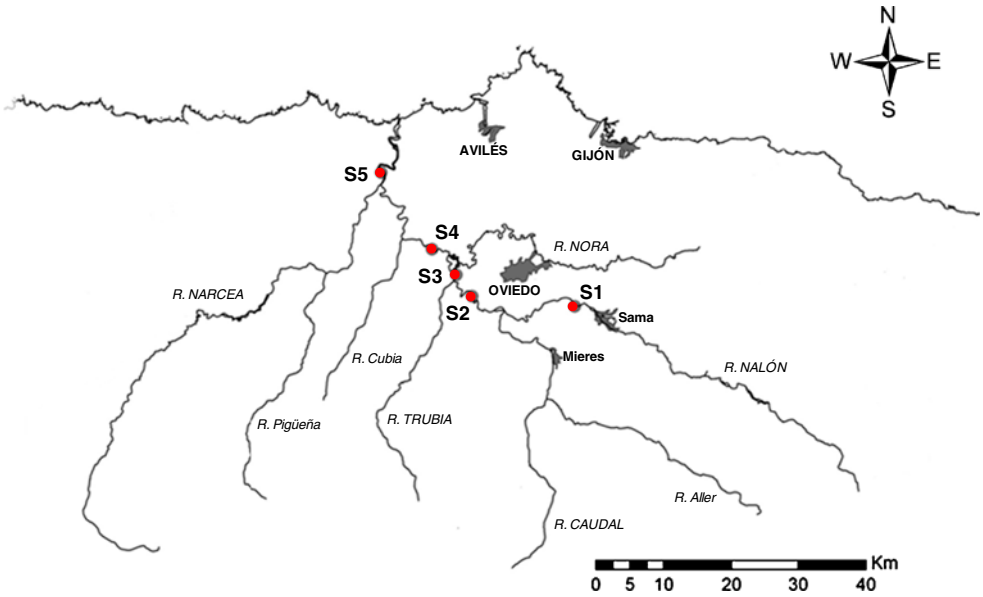

Fig. 1 Sampling locations in the River Nalón

natural abundance certified PBDEs in methanol at 0.5 pg g^{-1} for recovery studies. Then, a known amount of the ^{81}Br-labelled standard in methanol was added to all samples to get a final spike concentration of 1.36 (BDE 28), 6.53 (BDE 47), 1.14 (BDE 99), 0.62 (BDE 100), 1.19 (BDE 153) and 0.08 (BDE 154) pg g^{-1} respectively. Finally, approximately 10 mL of isooctane were transferred to each river water bottle. The samples were mechanically shaken for 30 min and then the organic extracts separated and centrifuged to break up the formed emulsions. Next, the organic extracts were removed and pre-concentrated under nitrogen to a final volume of ca. 100 μL and stored in the dark at 4 °C until analysis. Finally, 2 μL of each organic extract were injected in the GC(ECNI)MS system, the isotope ratios ^{79}Br/^{81}Br measured as peak area ratios and the concentrations calculated for each priority congener by the elemental isotope dilution equation [25].

Results and discussion

Evaluation of the ECNI source for bromine isotope ratio measurements

The ECNI source has been widely used for the determination of PBDEs in different samples, especially when very low concentrations need to be determined, since it provides, in general, lower detection limits than the EI source when working at LR [14]. One of the characteristics of the ECNI source in the analysis of PBDEs is that, under standard conditions, it produces almost exclusively bromide ions ($^{79}Br^-$ and $^{81}Br^-$) showing barely molecular ions and/or fragments containing C atoms. So, our ^{81}Br-labelled standard would allow the determination of PBDEs in different samples by IDMS using an ECNI source. In principle, concentrations could be obtained using the elemental isotope dilution equation in a way similar to other determinations carried out by GC(ICP)MS [26–28].

To our knowledge, the ECNI source has not been employed previously in IDMS experiments in combination with ^{81}Br or any other heteroatom labelling. So, the GC (ECNI)MS system needs to be evaluated for elemental isotope ratio measurements in terms of precision and accuracy. Mass bias, spectral interferences and detector linearity are traditional factors influencing the accuracy of isotope ratio measurements in elemental ion sources such as the ICP [25]. On the other hand, isotope ratio precision is usually limited by counting statistics and ion source fluctuations. However, none of those factors have previously been described for instruments containing the ECNI source. In this paper, two instruments from two different manufacturers are evaluated.

Spectral interferences (the presence of contributions other than the analyte at the *m*/*z* of interest) can affect the accuracy in isotope ratio measurements. In fact, elemental IDMS can only be carried out if two isotopes of the element to be determined are free of spectral interferences [25]. The ECNI source in combination with MS is a very selective technique towards aromatic brominated compounds as only electrophilic molecules can be ionized. This fact makes the ECNI source very suitable for the determination of brominated aromatic compounds by IDMS as spectral interferences are seldom expected [29]. Mass bias, the differential transport of ions through the mass spectrometer, is another parameter that can affect the accuracy in isotope ratio measurements in transient signals. To date, this parameter has never been evaluated for the ECNI source as quantitation has always been carried out by means of a

calibration graph. However, mass discrimination effects must be taken into account for quantitation by elemental IDMS. Finally, the linearity of the detector can affect the accuracy of the IDMS results for isotope ratios far from 1 as one ion can be out of the linearity range while the other ion still within this range.

In order to evaluate all these parameters in a single experiment, standard mixtures of natural abundance PBDEs (from tri- to hexa-brominated congeners) at different concentration levels were injected in the two commercial instruments (single injections). The fact that both instruments employed different analytical columns was not considered relevant as the chromatographic conditions will not influence the measurement of bromine isotope ratios. The isotope ratio results from both instruments (measured as $^{79}Br/^{81}Br$ peak area ratios) were plotted versus the measured ^{79}Br peak area. The results obtained for all congeners in both instruments are shown in Fig. 2. First, no influence of the nature of the congener on the measured isotope ratio was evident, so this information is not included in Fig. 2. Second, calibration graphs obtained by plotting the measured peak areas versus the concentration of each congener were linear both at *m*/*z* 79 and 81 for the studied concentration ranges (from ca. 0.5 $ng \cdot g^{-1}$ to 2,000 $ng \cdot g^{-1}$ in both instruments, data not shown), why problems with a non-linear detector response are not expected.

As can be seen in Fig. 2 at high concentrations, where isotope ratios are more precise, the measured isotope ratios were slightly biased towards the heavier isotope in the Agilent instrument whereas the Shimadzu instrument showed a somewhat larger bias towards the lighter isotope. The different mass discrimination effects observed for each instrument can be due to the specific system configuration and might occur during the extraction, mass analysis and detection of the negative ions [30]. No experiments were performed to study the influence of the ion lens settings on mass bias as both instruments were only optimised for maximum signal. Therefore, in further IDMS determinations, the mass bias factor (ratio of measured to theoretical isotope ratio) must be measured using a natural isotope abundance PBDE standard as optimum instrumental settings can change on a daily basis in the GC(ECNI)MS system. The measured isotope ratios do not seem to vary with the measured peak areas at mass 79 at increasing concentrations for both instruments (Fig. 2), which means that detector non-linearity effects (e.g. detector dead time) are negligible for this concentration range.

The statistics of ion counting and ion source fluctuations are the main parameters affecting the precision in isotope ratio measurements when working in ion counting mode [25]. In single collector (ICP)MS work, the isotope ratio precision follows Poisson statistics for low counting rates, improves for high ion counting rates and is finally limited by plasma fluctuations [25]. The precision in the experimental isotope ratios, expressed as RSD (%) for $n=3$ injections of spiked samples and standards, is shown in Fig. 3 as a function of the measured area at $m/z=79$ for both instruments. The grey points correspond to the theoretical RSD(%) based on the Poisson distribution for each data point calculated as:

$$\frac{s_R}{R}(\%) = 100 \times \sqrt{\frac{1}{I_{79}} + \frac{1}{I_{81}}}$$

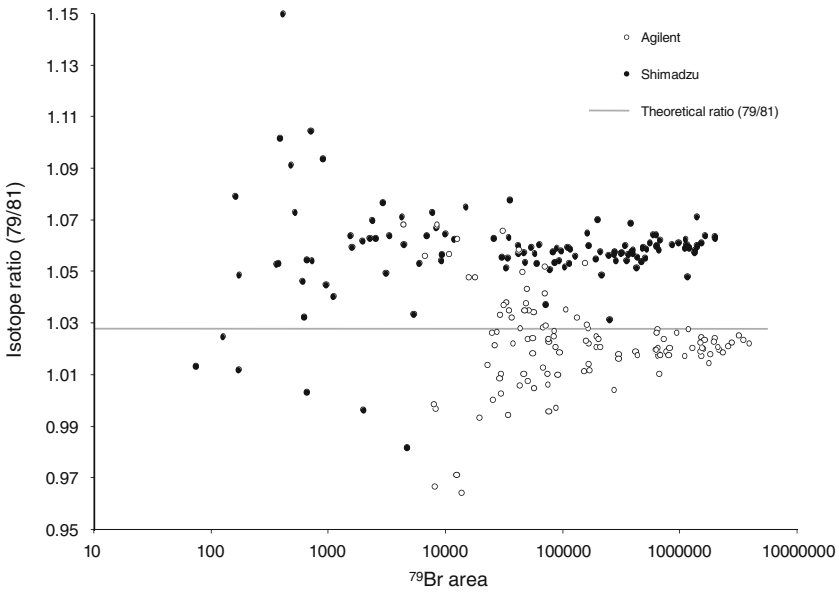

Fig. 2 Isotope ratios $^{79}Br/^{81}Br$ measured in the Agilent and Shimadzu instruments as peak area ratios at different PBDE concentration levels. Single injections at each concentration level

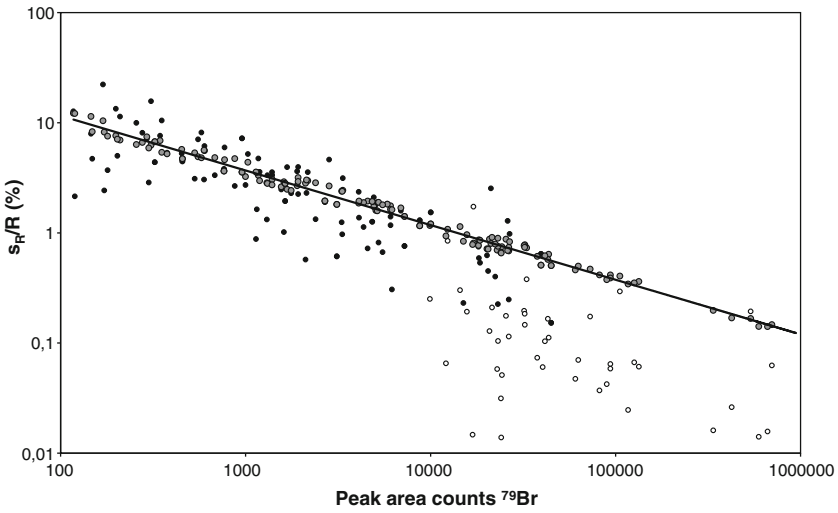

Fig. 3 Variation of the relative standard deviation (%) on the measured isotope ratios in spiked samples (n=3 injections) with the peak areas measured for ^{79}Br in both analytical instruments. Data and legends as in Fig. 2. The *grey points* correspond to the theoretical values for a Poisson distribution and the *solid line* is the best fit for the theoretical values

The best fit of the theoretical Poisson distribution is shown as the solid line. As can be observed, the precision follows approximately the theoretical curve for both instruments showing RSD values below 1% for ^{79}Br areas higher than ca. 10,000 counts and below 0.1% when areas increase above 1,000,000 counts as predicted by Poisson statistics. In all cases, the areas for ^{81}Br were higher than those for ^{79}Br (spiked samples). So, ion source fluctuations show a negligible contribution to the observed reproducibility in contrast to other elemental ion sources (such as the ICP) where isotope ratio precisions in GC(ICP)MS mode rarely go below 0.5% regardless of the measured ion counts [25].

Characterisation of the ^{81}Br-labelled PBDEs spike

The concentration, isotope composition and stability of the labelled spike were described previously [22, 23]. In brief, the crude synthetic mixture, containing large amounts of BDE-47 with respect to the other congeners, was characterised in isotope composition by GC(ICP)MS showing an isotope enrichment of 99.53% for ^{81}Br in BDE-47 [22]. After preparative reverse-phase HPLC separation and preconcentration, a second mixture was prepared containing similar amounts of BDEs 28, 47, 99, 100, 153 and 154. This second mixture was characterised both in isotope composition and concentration by GC(EI)MS [23]. It was observed that BDEs 28, 47, 99 and 100 showed an isotope enrichment similar to that measured previously for BDE-47 by GC(ICP)MS. However, both BDE 153 and 154 showed a mass isotopomer profile that was consistent with the isotopic enrichment of the other congeners but contaminated with the presence of 41.8% BDE-153 and 24.4% BDE-154 of natural isotopic composition. In order to confirm these results, the ^{81}Br-labelled PBDE standard was injected in the Shimadzu GC(ECNI)MS instrument. The obtained results, corrected for mass bias using a natural abundance PBDE standard, are summarized in Table 2 in comparison with those measured using other ion sources. As can be observed, the measured isotope composition for BDE-47 matches that determined by GC(ICP)MS and that calculated by GC(EI)MS. For the other PBDEs, similar results were obtained by GC(EI)MS and GC(ECNI)MS. Please note that

Table 2 Bromine isotope enrichment for the labelled PBDEs measured with different ion sources

Congener	Isotope abundance ^{81}Br (%)			Concentration (ng/g) GC(EI)MS[23]
	GC(ICP)MS	GC(EI)MS[a]	GC(ECNI)MS	
BDE-28 (2,4,4′ - tri BDE)	–	99.0±0.3	99.36±0.01	378±15
BDE-47 (2,2′,4,4′ - tetra BDE)	99.53±0.02	99.3±0.3	99.53±0.01	1,810±75
BDE-99 (2,2′,4,4′,5 - penta BDE)	–	99.0±0.5	99.28±0.02	313±8
BDE-100 (2,2′,4,4′,6 - penta BDE)	–	98.2±0.5	99.38±0.03	169±4
BDE-153 (2,2′,4,4′,5, 5′ - hexa BDE)	–	78.6±0.9	80.5±0.2	372±16
BDE-154 (2,2′,4,4′,5,6′ - hexa BDE)	–	87.3±5.9	89.0±0.3	28±2

Their concentrations were determined by reverse IDMS[23]

[a] Calculated by least squares optimisation by comparison of experimental and calculated profiles

with GC(EI)MS, the isotopic compositions are not measured directly but calculated by least squares optimisation in the measured isotope profile of the molecular ion in comparison with theoretically calculated isotope patterns. The concentrations of the PBDEs in the spike were determined by reverse isotope dilution analysis, using SRM 2257 as reference, by GC(EI)MS [23] and are also included in Table 2. This spike was shown to be stable for over 4 months and no isotope exchange between bromine atoms from the natural abundance PBDEs and the labelled spike was detected [23].

Optimisation of the IDMS procedure

The optimum sample to spike ratio needs to be selected when studying any IDMS procedure. In this regard, the random error propagation theory must be taken into account in order to achieve good precision in the results obtained by IDMS [25]. In the absence of other relevant uncertainty sources, the relative uncertainties associated to the concentrations depend mainly on the relative uncertainties of the measured isotope ratios in the mixture, R_m, following the expression [31]:

$$\left[\frac{s(C_s)}{C_s}\right] = \left[\frac{R_m(1 - R_{sp}R_s)}{(R_m - R_{sp})(1 - R_mR_s)}\right]\left[\frac{s(R_m)}{R_m}\right]$$

Where $s(C_s)/C_s$ is the relative error in the determined concentration, $s(R_m)/R_m$ the relative error in the measured isotope ratio in the mixture of natural and labelled compounds. The other term in the equation is the so-called error magnification factor, which depends on R_m (isotope ratio in the mixture), R_s (isotope ratio in the sample of natural abundance), and R_{sp} (isotope ratio in the spike). As R_s and R_{sp} are constant for a particular element and labelled standard, only R_m can be optimised to minimize the error magnification factor. That means that the amount of spike added to the sample must be optimised in order to obtain adequate isotope ratios in the mixture. Therefore, a previous approximate knowledge of the concentration of the element in the sample is required to select the optimum amount of spike to be added.

Using the data shown in Table 2 for GC(ECNI)MS, three different isotope ratios in the spike were obtained. A R_{sp} value of ca. 0.006 for BDEs 28, 47, 99 and 100 and values of 0.242 and 0.124 for BDEs 153 and 154, respectively because of the natural contamination. As bromine natural abundances are well known [32], the error magnification factor can be minimized as a function of R_m. The error magnification curves obtained for our labelled standard are shown in Fig. 4. As can be seen, for BDEs 28, 47, 99 and 100, R_m values between 0.02 and 0.50 provide error magnification factors below 2, which can be considered

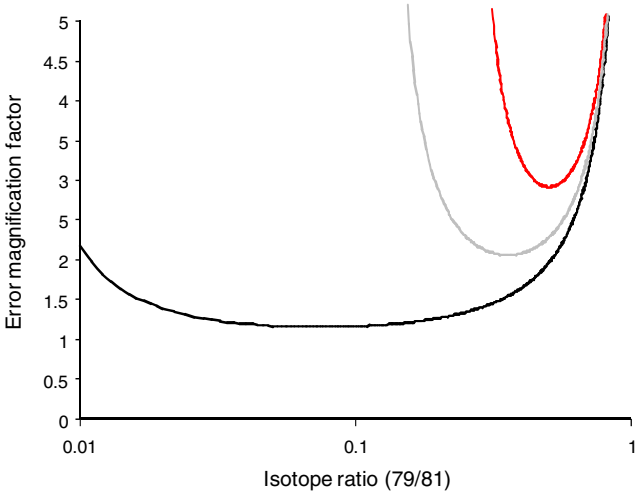

Fig. 4 Variation of the error magnification factor with R_m for BDEs 28, 47, 99 and 100 (*solid black line*), 154 (*grey line*) and 153 (*red line*)

acceptable, showing a minimum error magnification factor of 1.15 around R_m=0.08. These R_m values correspond to natural to labelled concentration ratios in the range of 1:35–1:0.5, respectively with the minimum error magnification factor for a ratio of ca. 1:6. Unfortunately, for BDEs 153 and 154 the optimum range in the error magnification curves is much narrower with higher propagated error as can be observed in the figure.

Evaluation of the ID-GC(ECNI)MS procedure for the determination of PBDEs

Once the GC(ECNI)MS system was evaluated and working conditions were optimised for its use in isotope ratio measurements, the proposed methodology consisting of the determination of the six priority PBDEs in environmental and biological samples, was validated by using a standard reference material (Lake Michigan fish tissue SRM 1947). BDE 49 was also included in the validation stage as it was present in the labelled standard and it was one of the certified congeners in the reference material. Samples were treated as described at the procedures section. Two independent samples and a blank were analysed at three different and increasing spike levels (indicated as blend 1, blend 2 and blend 3) in order to detect possible systematic errors in the procedure.

The concentrations of the BDE congeners in the SRM 1947 samples were obtained by using the elemental isotope dilution equation [25]. The obtained concentrations were in agreement with the certified values [33] for the seven congeners at the three studied spike levels (Table 3) with the exception of BDE-154 in blends 1 and 3. Recoveries were between 90% and 105% for congeners 47, 49, 99,100 and 153. BDE-28+33 showed recoveries of about 80% for the three studied spiked samples. However, it must be taken

Table 3 Concentrations of PBDEs in SRM 1947 determined by ID-GC(ECNI) MS

Congener	Concentration ($ng \cdot g^{-1}$)			Certified concentration ($ng \cdot g^{-1}$)
	Blend 1	Blend 2	Blend 3	
BDE-28+33	1.9±0.2	1.8±0.2	1.8±0.2	2.26±0.46[a]
BDE-47	72.3±5.5	70.0±5.3	71.7±6.1	73.3±2.9
BDE-49	3.7±0.8	3.6±0.8	3.6±0.8	4.01±0.1
BDE-99	19.1±1.0	18.3±1.0	18.8±1.2	19.2±0.8
BDE-100	17.7±1.0	17.0±1.0	17.4±1.2	17.1±0.6
BDE-153	4.0±0.3	3.7±0.9	4.2±0.5	3.83±0.04
BDE-154	8.5±1.4	8.2±1.3	8.5±1.2	6.88±0.52

Mean values correspond to two separate extractions measured $n=5$ times each. Uncertainties correspond to expanded uncertainties ($k=2$)

[a]Not certified

into account that the reference value for BDE 28 was, due to coelution, actually given for the sum of BDEs 28 and 33. BDE-154 showed high recoveries in the range of 120–125% for the three studied spiked samples. This could be due to coelution with an unknown brominated compound (e.g. BB-153). The variability in the results, expressed in terms of expanded uncertainties, were calculated following the Kragten spreadsheet method [34] and taking into account all parameters involved in the calculation of the final concentrations and their respective uncertainties. The same results for the three different blends and duplicate samples are shown in Fig. 5 as a function of the obtained peak area for ^{79}Br. As can be observed, the results follow a similar trend that the isotope ratios given in Fig. 2: increasing variability at lower counts with recoveries in the range 80–120%.

The experimental reproducibilities of the measured concentrations between different samples of each blend were calculated as RSD (%). All the values found were always below 4%, although RSDs lower than 2% were obtained for the lower brominated congeners (tri-, tetra- and penta-) in the three blends. Detection limits were calculated from the variation in the three blank measurements performed during the analysis of the reference material. Detection limits between 0.02 and 0.4 $ng \cdot g^{-1}$, expressed as three times the standard deviation of the three measured blanks ($n=5$), were obtained.

Determination of PBDEs in river water samples

Finally, the validated methodology was applied to the determination of the six priority PBDEs (28, 47, 99, 100, 153 and 154) [12] in river water samples. The samples were collected in July 2010 along the River Nalón, which was selected for this work as it is the longest and most important river in the Autonomous Community of Asturias (4,830 km^2 of basin surface covering 46% of the Asturian territory). Different sampling points were selected along the river, beyond its confluence with each of its main four tributaries (Rivers Caudal, Trubia, Nora and Narcea) in order to better estimate the possible location area of the BDEs contamination source. Two separate samples were collected at each sampling site and one sample from each site was spiked by weight with a mixture of the six priority PBDEs in methanol for recovery purposes, at the concentration set by the Water Framework Directive (WFD) as Environmental Quality Standard (EQS) for inland surface waters (0.5 pg g^{-1}) [12].

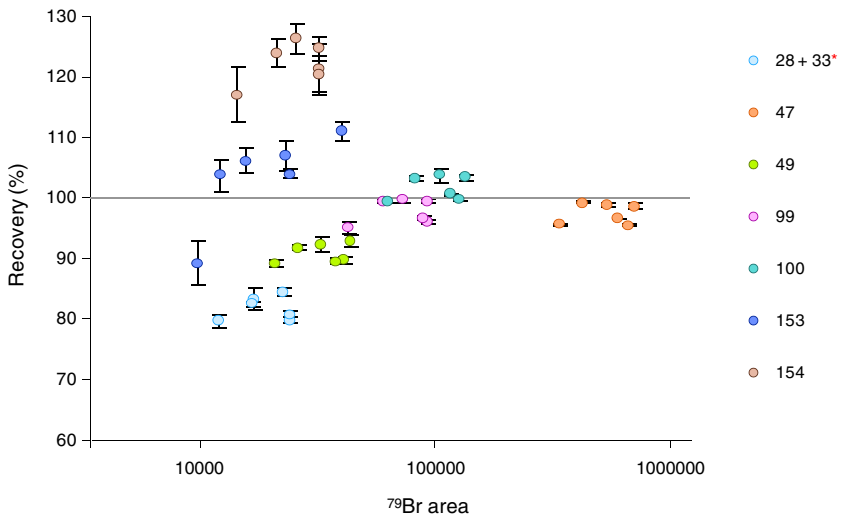

Fig. 5 Recovery of PBDEs from NIST SRM 1947 as a function of the area counts observed for ^{79}Br

Table 4 PBDE recoveries in milli-Q water samples spiked at different concentration levels

Congener	Recovery (%)				
	0.2 pg·g^{-1}	0.5 pg·g^{-1}	1 pg·g^{-1}	5 pg·g^{-1}	10 pg·g^{-1}
BDE-28	84.7±2.4	89.3±1.6	91.6±0.8	95.8±1.6	92.7±1.6
BDE-47	77.9±1.4	84.4±1.3	87.4±0.8	97.5±0.8	95.2±0.6
BDE-99	83.6±1.8	90.7±1.2	93.5±0.7	98.9±1.6	98.2±1.7
BDE-100	83.7±2.9	93.1±0.7	95.0±1.2	97.8±3.2	99.1±5.0
BDE-153	62±11	78.2±1.9	84.8±4.9	96.2±4.6	95.8±3.9
BDE-154	84±12	82.8±2.7	82.9±5.7	92±32	94±37

The uncertainty is indicated as standard deviation for n=5 independent samples

In order to evaluate the analytical characteristics of the proposed methodology milli-Q water samples were also fortified by weight with a mixture of natural abundance of the six priority PBDEs in methanol at different concentration levels (from 0.2 to 10 pg g^{-1}). All samples (river and milli-Q water) were treated as indicated at the procedure section and spiked with the same amount of the ^{81}Br-labelled standard. Concentrations were obtained by using the elemental isotope dilution equation [25] and mass bias correction was carried out by using a natural abundance standard mixture containing the six PBDEs of primary interest and the average natural isotopic abundances of bromine published by the IUPAC [32] as reference.

Table 4 shows the recoveries obtained for milli-Q water samples spiked at different concentration levels. Recoveries at 0.2 pg g^{-1} could be considered acceptable for such concentration level. For samples spiked at the EQS level (0.5 pg g^{-1}) recoveries were in the range of 78–93%. For samples spiked at the higher levels of 1, 5 and 10 pg g^{-1} recoveries were between 83% and 99%. Table 5 shows the limits of quantification, expressed as ten times the standard deviation of ten individual blanks and the precision, in terms of repeatability and reproducibility, expressed as RSD (%). The limits of quantification obtained are below the EQS required by the WFD. Good precisions (below 4% RSD) were obtained for all congeners except for BDE-154 at the two studied concentration levels. The higher RSDs found for this congener at 5 pg g^{-1} can be due to its low concentration in the ^{81}Br-labelled standard (10–100 times lower than the other congeners). As the same amount of ^{81}Br-labelled standard was added to all the samples for an expected concentration of around 0.5–1 pg·g^{-1} for each congener, measured isotope ratios for BDE-154 in this blend were around 1, very close to the natural abundance isotope ratio and, therefore, completely out of the range which minimizes error propagation (see Fig. 4).

Finally, the six priority congeners of interest were determined in the river water samples. Obtained concentrations for both, fortified and non fortified river water samples are summarized in Table 6. Concentration values were reported when the corresponding congener was present at a concentration that was above the limit of detection even if that concentration was below the LOQ because those data can provide an estimate of the LOQs in real samples confirming the LOQs obtained with blank experiments. The variability in the results is expressed in terms of expanded uncertainties, following the Kragten method [34]. As can be seen, concentrations found for non fortified samples are below the limits of detection (n.d.) for most congeners in several samples. In general, when detected, concentrations were of the order or lower than the LOQs (Table 5) and so, their values were lower than their expanded uncertainties. The obtained concentrations were always below the annual average Environmental

Table 5 PBDE limits of quantification (LOQ) and precision at 0.5 and 5 pg g^{-1} in milli-Q water samples

Congener	LOQ (pg·g^{-1})	Precision at 0.5 pg·g^{-1} (%RSD)		Precision at 5 pg·g^{-1} (%RSD)	
		Repeatability[a]	Reproducibility[b]	Repeatability[a]	Reproducibility[b]
BDE-28	0.28	0.8	1.8	1.3	1.3
BDE-47	0.30	1.4	1.5	0.5	0.4
BDE-99	0.05	0.6	1.4	0.8	1.0
BDE-100	0.05	1.6	0.8	2.6	2.7
BDE-153	0.28	2.0	2.5	2.5	3.9
BDE-154	0.06	6.3	3.3	26	17

[a] m=5 injections of the same sample

[b] n=3 individual samples

Table 6 Determination of BDEs 28, 47, 99, 100, 153 and 154 in five river water samples from River Nalón

Congener	S1	S2	S3	S4	S5
			River water samples		
BDE-28	n. d.	n. d.	n. d.	n. d.	n. d.
BDE-47	n. d.	n. d.	n. d.	0.1±0.4	n. d.
BDE-99	0.03±0.07	0.06±0.08	0.04±0.05	0.1±0.2	n. d.
BDE-100	0.02±0.06	0.02±0.07	0.02±0.04	0.1±0.2	n. d.
BDE-153	n. d.	n. d.	n. d.	0.1±0.2	n. d.
BDE-154	0.02±0.04	0.04±0.05	n. d.	0.1±0.2	n. d.
			Fortified river water samples		
BDE-28	0.52±0.02 (93.3)	0.5±0.1 (95.1)	0.51±0.08 (91.6)	0.50±0.05 (91.7)	0.50±0.01 (90.8)
BDE-47	0.49±0.02 (85.2)	0.54±0.07 (94.2)	0.51±0.06 (88.7)	0.49±0.05 (86.7)	0.54±0.03 (94.7)
BDE-99	0.50±0.02 (90.4)	0.57±0.09 (101.7)	0.54±0.05 (96.9)	0.51±0.03 (92.9)	0.49±0.03 (88.1)
BDE-100	0.48±0.02 (88.6)	0.51±0.06 (91.4)	0.47±0.05 (85.2)	0.46±0.02 (85.2)	0.47±0.02 (85.8)
BDE-153	0.5±0.2 (91.2)	0.46±0.08 (81.3)	0.44±0.04 (78.5)	0.5±0.2 (89.0)	0.5±0.1 (80.7)
BDE-154	0.4±0.1 (76.9)	0.4±0.2 (86.1)	0.4±0.2 (82.2)	0.4±0.1 (83.9)	0.6±0.2 (83.4)

Uncertainties correspond to expanded uncertainties (k=2). Bottom: concentrations and recoveries (%) from fortified samples at the EQS level

Quality Standard set by the WFD [12]. The most contaminated sample was sample S4 taken just downstream from the merging of the Nora tributary. As exemplified by the fortified (0.5 pg g^{-1}) sample S5, very clean chromatograms can be obtained with ECNI (Fig. 6). Concentrations together with their respective expanded uncertainties are also shown in Table 6 for the fortified river water samples. Satisfactory recoveries (in brackets), in the range 77–102%, were found for the six priority congeners. For future studies other extraction solvents will need to be evaluated to check the extraction of particle-bound PBDEs.

Conclusions

A procedure for the determination of the six priority PBDE congeners in water samples at regulatory EU levels has been developed. In comparison with the GC(EI)MS procedure previously described [23] the method does not permit compound identification by fragment ions but provides much improved sensitivity suitable for the determination of very low levels of PBDEs in water samples. Detection limits and recoveries at the EQS level are within those required by the legislation. The method is

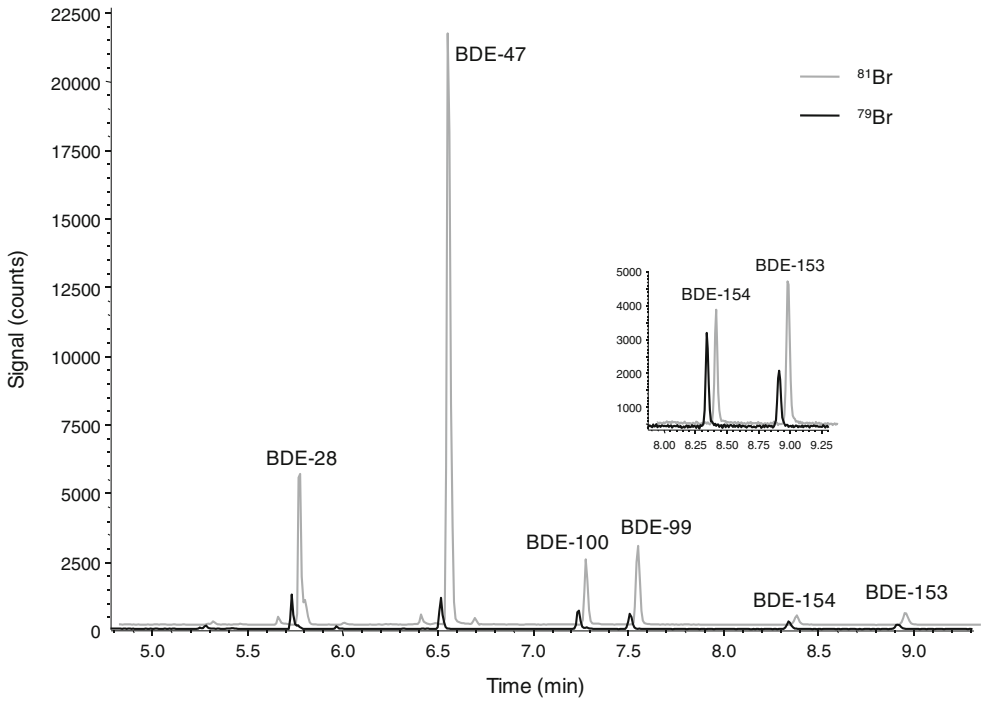

Fig. 6 Chromatogram of a fortified (at the EQS level) real sample of river water (S5) after spiking with the ^{81}Br-labelled spike

fast and simple to perform and does not require the construction of a methodological calibration graph as it uses the classical elemental isotope dilution equation. During method development, the performance of the ECNI source for bromine isotope ratio measurements was evaluated with satisfactory results in terms of isotope ratio precision and accuracy. The ^{81}Br-labelled compounds will be available commercially in the near future.

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