1 **Determination of priority PBDEs by Isotope Dilution GC(EI)MS using**
2 **^{81}Br-labeled standards.**

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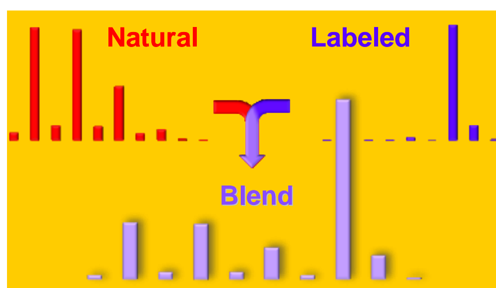
14 

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Abstract

A mixture of ^{81}Br-labeled polybrominated diphenyl ethers (PBDEs), previously synthesized in our laboratory, was separated by Liquid Chromatography for the individual isolation of different ^{81}Br-labeled PBDEs containing from 3 to 6 bromine atoms. The different fractions were collected and a mixed labeled standard was then prepared adequate for the determination of priority PBDEs (congeners 28, 47, 99, 100, 153 and 154) in environmental samples. The spike mixture was then characterized using GC(EI)MS both in isotope composition and concentration in combination with multiple least squares. Contamination from natural abundance BDEs 153 and 154 was detected in the spike mixture and a new isotope dilution equation developed to take into account the natural abundance contribution from the spike. The spike mixture was shown to be stable during at least four months and no isotope exchange between natural abundance and labeled PBDEs was detected during this period of time.

Finally, the ^{81}Br-labeled PBDEs standard was used for the determination of congeners 28 (+33), 47, 49, 99, 100, 153 and 154 in a standard reference material (Lake Michigan fish tissue SRM 1947) using three different sample to spike ratios. No methodological calibration needed to be prepared as no isotopic effects were detected using this labeling mode. Concentrations found were in agreement with the certified concentrations (recoveries between 89 and 116%) and reproducibility was always below 7% RSD. Kragten procedure was used to calculate expanded uncertainties. Very low limits of detection were obtained for all compounds (between 0.02 and 0.9 ng.g^{-1}) using the procedure developed here.

Keywords: PBDEs, Isotope Dilution Mass Spectrometry, GC(EI)MS, isotope pattern deconvolution.

Introduction

The general expression “brominated flame retardants” (BFRs) refers to a group of bromine containing organic compounds which are employed as additives in polymers to inhibit combustion processes.[1,2] From this group, polybrominated diphenyl ethers (PBDEs) are some of the most widely used BFRs. These flame retardants are not chemically bound to the polymers[3,4] and, therefore, PBDEs can be easily released into the environment. Several release routes have been described such as volatilization or dust formation from polymers and emissions during manufacture, waste disposal or during the recycling of PBDE-containing products.[5] Thus, PBDEs are nowadays widely spread in the environment. As a result, these compounds have been detected in air, sediments, sludge and soils as well as indoor air, house dust and even in foodstuffs.[6,7] They have also been found in living organisms such as birds, fish, terrestrial animals and in humans (adipose tissue, serum and breast milk).[5] This wide distribution of PBDEs in the environment has raised concerns about the potential risks of PBDEs exposure to human health. PBDEs show high lipophilicity,[4] they are resistant to chemical and biological degradation[8] and posses high bioaccumulation and biomagnification potential[9]. Some toxicological studies suggest that they are linked to adverse physiological effects.[10] Consequently, new regulations about the control of those compounds in environmental samples have been published.[11] For example, the European Water Framework Directive requires the determination of priority BDEs (congeners 28, 47, 99, 100, 153 and 154) in continental waters[11] at levels below 0.5 ng L^{-1}. It is clear that, for routine analysis of PBDEs, further improvements in the determination techniques are still needed if we are to meet the analytical requirements.[12]

Analytical methods employed for the determination of PBDEs in biological and environmental samples are very similar to those employed for PCBs.[13] These methodologies require a complex sample preparation procedure which usually involves several steps such as drying of solid samples followed by extraction of the analytes from the sample matrix with organic solvents and, finally, the clean up and

fractionation of the organic extracts.[12] The techniques most widely used for the determination of PBDEs are gas chromatography (GC) coupled to a halogen specific detector such as electron capture (ECD) or, more often, Mass Spectrometry (MS) either in negative chemical ionization (NCI) or in positive electron ionization (EI) modes.[14] Recently, the inductively coupled plasma (ICP) source has been proposed also for the determination of PBDEs with very low detection limits.[15] To correct for losses during the sample preparation steps, commercially available $^{13}C_{12}$-labeled analogues are usually selected as internal standards in combination with Isotope Dilution Mass Spectrometry (IDMS). Unfortunately, the use of these labeled standards limits the choice of the ion source. Only electron ionization can be employed[12] since the higher sensitive NCI[14] or ICP[15] sources typically produce monoatomic negative or positive Br ions (m/z = 79 and 81) which do not allow the discrimination between the analyte and the ^{13}C-labeled internal standards.[16]

Recently we have synthesized a series of ^{81}Br-labeled PBDEs which would allow the use of any of these three different ionization sources for their determination by IDMS. In that work,[17] the main congeners obtained in the crude product were BDEs 28, 47 and 99 and they were characterized in concentration and isotopic composition both by GC(EI)MS and GC(ICP)MS. Finally, a calibration-free IDMS procedure based on multiple linear regression[18] was developed and applied to the determination of congeners 28, 47 and 99 in spiked water samples at ng L^{-1} levels. The crude synthetic mixture used previously[17] showed also detectable amounts of other BDEs such as 49, 100, 153 and 154 but in a very low concentration level. It is clear that, for IDMS calculations, the concentration ratio between analyte and spike should be within certain limits to minimize error propagation.[19] That means that we need to prepare a new PBDEs mixture with similar concentration levels for all PBDEs for the simultaneous determination of all priority congeners[11] in environmental samples.

Consequently, this work focused on the isolation of the different ^{81}Br-labeled congeners from the crude synthetic mixture by Liquid Chromatography. Then, an appropriate mixture was prepared and characterized and, finally, an analytical IDMS procedure was developed and validated using a Lake Michigan fish tissue reference

1 material (SRM 1947). GC(EI)MS was used in this work as this source, unlike higher
2 sensitive NCI and ICP, provides information of molecular clusters, which allows the
3 study of isotope exchange reactions. This study must be carried out in order to
4 evaluate the suitability of the ^{81}Br-labeled standard for IDMS experiments using any
5 of the mentioned ionization sources. Particular attention was paid to the application
6 of IDMS procedures which do not require the construction of a methodological
7 calibration graph[18] and could be suitable for routine analysis of these priority
8 pollutants.
9

10 **Experimental**

11

12 **Reagents and materials**

13 Individual certified standards of 6 BDEs (congeners 28, 47, 99, 100, 153 and 154, 50
14 μg mL^{-1} in nonane) were obtained from Cambridge Isotope Laboratories Inc.
15 (Andover, MA, USA). The tetrabrominated $^{13}C_{12}$-BDE 47 (99% isotopic purity, 50 μg
16 mL^{-1} in nonane) was also obtained from Cambridge Isotope Laboratories. Reference
17 materials SRM 1947 (Lake Michigan Fish Tissue) and SRM 2257 (PBDE Congener
18 mixture in 2,2,4-Trimethylpentane) were both obtained from the National Institute of
19 Standards and Technology (NIST).
20

21 All solvents used in this work were of the highest purity. Acetone, methanol and
22 hexane were purchased from Fluka (Steinheim, Germany) and dichloromethane and
23 diethyl ether from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water was
24 obtained from a Milli-Q Gradient A10 water purification system (Millipore S.A.S,
25 Molsheim, France). Working standard solutions of labeled and unlabeled PBDEs and
26 SRM 2257 were prepared in isooctane (Sigma-Aldrich) and stored in the dark at 4 ºC
27 until use. All dilutions were performed on a weight basis.
28

29 All glassware used for the sample preparation was cleaned with detergent (Mucasol
30 from Brand GmbH + COKG, Wertheim, Germany), rinsed with Milli-Q water, dried in
31 an oven and brought to room temperature. Then it was rinsed twice with hexane and
32 acetone and allowed to dry at room temperature just before its use. Anhydrous

sodium sulfate (Merck, Darmstadt, Germany) was used to dry the samples and silica gel (0.063 -0.200 mm) for column chromatography (Merck) was used in the clean up and fractionation steps during sample preparation.

Instrumentation

A HPLC model 1100 Series (Agilent Technologies, Waldbronn, Germany) has been used in this work for the purification of PBDEs. The system consisted of a four-channel on-line degasser, a standard binary pump, a micro wellplate autosampler, a thermostated column compartment and a UV-VIS (190 - 700 nm) diode array detector (DAD). Solutions (20 μL) of the crude synthetic mixture were injected automatically in a Zorbax Eclipse XDB-C18 separation column (Agilent Technologies, Waldbronn, Germany).

A GC model 6890N (Agilent Technologies, Waldbronn, Germany) fitted with a split/splitless injector and equipped with a MSD model 5975B (Agilent Technologies, Tokyo, Japan) has been used for the analytical work. Solutions (2 μL) were injected automatically by an autosampler model 7683 (Agilent). The chromatographic separation was carried out using a low polarity capillary column DB-5MS Ultra Inert (J&W Scientific, Folsom, CA, USA; 30m x 0.25mm i.d., 0.25μm film thickness), as it has been one of the most used and tested for PBDEs.[12] 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).

Procedures

PBDEs purification

For the separation of each PBDE present in the synthesis crude product,[17] the original solvent (dichoromethane) was evaporated to dryness and the residue redissolved in acetone. Finally, 20 μL of the mixture dissolved in acetone were injected into the HPLC system. The mobile phase consisted in 92% methanol 8%

water in isocratic mode at a flow rate of 1 mL min^{-1}. Six fractions were manually collected in different amber glass vials. Then, each fraction was evaporated to dryness under a gentle stream of nitrogen and redissolved in isooctane. The separation procedure was repeated as many times as necessary to get a sufficient amount of each BDE congener for future work. Finally, the solutions containing individual ^{81}Br-labeled congeners were preconcentrated under nitrogen to a final volume of ca.1 mL, and 2 μL of each solution were injected in the GC(EI)MS to check the purity of each fraction.

Characterization of ^{81}Br-labeled PBDEs

The isotopic composition of bromine in the ^{81}Br-labeled PBDEs synthetic mixture had been previously determined by GC(ICP)MS monitoring both m/z 79 and 81 and using a mixture of natural abundance PBDEs for mass bias correction.[17] The isotopic composition of the purified congeners was now evaluated again by GC(EI)MS using a multiple least square procedure.

The concentrations of the congeners 28, 47, 49, 99, 100, 153 and 154 in the new ^{81}Br-labeled mixture were determined by reverse isotope dilution analysis using the certified reference material SRM 2257 (certified PBDEs mixture) as standard. To do that, a mixture of the SRM 2257 and the labeled standard was prepared and injected in the GC(EI)MS system and the concentrations were calculated by the isotope pattern deconvolution procedure described previously.[17]

Determination of PBDEs in fish tissue SRM 1947

Samples of Lake Michigan Fish Tissue (SRM 1947) were prepared following a previously described sample preparation procedure [20] with some modifications. Homogenized fish tissue was ground in a mortar with anhydrous sodium sulfate and allowed to dry for three hours. Then, the samples were spiked with an appropriated amount of the ^{81}Br-labeled PBDEs standard. After that, the PBDEs were extracted using a Soxhlet system 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 second fractionation silica gel column (2% H_2O) with hexane, hexane/diethyl ether (85:15, v/v) and diethyl ether. Samples were then evaporated under nitrogen to a few microliters and injected in the GC(EI)MS system.

IDMS procedure

The concentrations of the different BDE congeners were calculated by the isotope pattern deconvolution procedure described previously.[17] In brief, the peak areas corresponding to n=5 selected masses for each compound were measured in SIM mode. Then, the isotope abundances for each mass in the mixture, A^i_{mix} were calculated by dividing each peak area by the sum of all peak areas for each compound. The molar fractions of natural and labeled BDEs, X_{nat} and X_{lab}, were calculated by multiple least squares from the equation:

$$\begin{bmatrix} A^1_{mix} \\ A^2_{mix} \\ \ldots \\ A^n_{mix} \end{bmatrix} = \begin{bmatrix} A^1_{nat} & A^1_{lab} \\ A^2_{nat} & A^2_{lab} \\ \ldots & \ldots \\ A^n_{nat} & A^n_{lab} \end{bmatrix} \cdot \begin{bmatrix} X_{nat} \\ X_{lab} \end{bmatrix} + \begin{bmatrix} e^1 \\ e^2 \\ \ldots \\ e^n \end{bmatrix}$$

Where the isotope composition of the natural and labeled PBDEs, A^i_{nat} and A^i_{lab}, are known. Finally, the number of mols of natural abundance BDEs can be calculated using:

$$\frac{N_{nat}}{N_{lab}} = \frac{X_{nat}}{X_{lab}} \qquad (1)$$

Please note that this final equation (1) provides directly the concentration of the analyte without requiring the construction of a methodological calibration graph as no isotopic effects are expected from the changes in the isotope composition of bromine.[21]

Results and discussion

1 *Isolation of individual BDE congeners*

2 In order to obtain individual labeled standards from the synthesis crude product their 3 chromatographic separation with UV detection was carried out. A reverse phase C18 4 column was used in this work as it had been previously tested for the separation of 5 brominated flame retardants showing good resolution in the separation of PBDE 6 congeners present in commercial penta mixtures (congeners 28, 47, 99, 100, 153 7 and 154)[16,22] which are the same compounds found in the synthetic mixture. The 8 mobile phase consisted of methanol:water (92:8, v/v) at 1 mL min^{-1} in isocratic mode 9 following a previously described procedure for the separation of these BDE 10 congeners.[16] Acetone turned out to be the most appropriate solvent for these PBDEs, 11 allowing good resolution for the separation of the six congeners when injecting 20 µL 12 of a natural abundance mixture (5 ppm of each congener) and with relatively short 13 chromatographic run times. UV spectra were recorded over the range of 200 - 280 14 nm showing an optimal absorption wavelength between 200 - 210 nm, which is in 15 agreement with the values found in literature.[22,23] Therefore, detection at 206 nm was 16 selected in this work as it allowed the detection of all the congeners of interest at the 17 selected chromatographic separation conditions.

18

19 Once optimized the separation conditions, individual natural standards of the six 20 BDEs of interest were injected in the HPLC system in order to identify each congener 21 in the mixture by their retention time. Then the labeled standard mixture (in acetone) 22 was injected into the same chromatographic system for the fraction collection. The 23 initial and final collection time for each fraction were set daily by comparison of the 24 retention times with a natural abundance standard mixture. Figure 1 shows overlaid 25 chromatograms for the natural abundance and labeled mixtures of PBDEs. Collected 26 fractions from F1 to F6 are also indicated in Figure 1. As can be seen on the natural 27 standard chromatogram the optimized conditions seamed to allow a complete 28 separation of each congener of interest. In the chromatogram obtained for the ^{81}Br- 29 labeled mixture there are some congeners that cannot be observed by UV absorption 30 (100, 153 and 154). However, previous injections of the crude product in our 31 GC(EI)MS system confirmed their presence in the synthetic mixture. Therefore, these 32 fractions were collected and treated in the same way as the others even though their

corresponding congeners were present at such a low concentration in the labeled mixture that could not be detected.

Each fraction was evaporated to dryness under nitrogen to remove the mobile phase and was redissolved in isooctane. Then, equivalent fractions obtained from successive injections were collected together and preconcentrated under nitrogen to a final volume of ca. 1 mL. Finally, the six extracts were injected in the GC(EI)MS to check the purity of each fraction and get a preliminary estimate of their concentration. Figure 2 shows the chromatograms obtained for fractions F1 to F6 and for a natural standard mixture. As can be seen each fraction contained only one congener except for F2 which showed another tetrabrominated congener that eluted earlier than BDE-47. This compound was identified by its retention time (in comparison with SRM 2257) and its mass spectrum and turned out to be BDE-49.

Preparation of the ^{81}Br-labeled PBDEs standard

After the separation of the different BDEs a mixed spike mixture was prepared taking into account the most common congener profiles found in environmental and biological samples. Environmental samples usually show congener patterns similar to the composition of the commercial penta-mix formula (Bromkal 70-5DE),[24] whereas in biological samples BDE 47 is usually the major congener, representing sometimes up to 60-70% of the total BDEs content, followed in most cases by BDE 99 and BDE 100 or BDE 153.[25] Therefore, a BDEs mixture with a congener profile that would allow the simultaneous determination of all priority BDEs both in environmental and biological samples was prepared. As BDE 49 was present in fraction F2 together with BDE 47 and it has also been found in real samples, although at lower concentration levels, it was decided to include and certify this congener also in the final spike mixture.

Determination of the isotope composition of the ^{81}Br-labeled compounds

The isotopic composition of bromine in the original synthesized mixture had been previously determined by GC(ICP)MS for congeners 28, 47 and 99 showing isotopic abundances (atom %) of 99.53 for isotope 81 and 0.47 for isotope 79.[17] So, these

values were initially given to all congeners isolated since no changes in the isotopic composition were expected during the HPLC separation process. However, it was observed that, for some labeled congeners, e.g., BDE-153 and BDE-154, the experimental isotopic profiles of the cluster corresponding to the molecular ion (M^+) did not match the profiles calculated using the theoretical abundance of 99.53% for ^{81}Br.[26] This could be due to two main reasons. First, the isotopic composition of bromine could be different from the observed value for the main synthetic products (99.53% ^{81}Br) and, second, there could have been contamination with the congeners of natural abundance during the synthesis, chromatographic separation or preconcentration processes. The discrimination between isotope enrichment and natural contamination was carried out by studying the molecular cluster M^+ for each congener using a GC(EI)MS. This study can not be performed when ICP or NCI sources are employed instead of EI.

In order to figure out which of these two reasons was responsible for the modified isotopic profiles observed in the hexabrominated BDEs 153 and 154 a multiple linear regression procedure was employed to fit the experimental isotope pattern to some theoretical isotope patterns. In this procedure, the experimentally observed isotope patterns of the molecular cluster M^+ for ten consecutive masses were compared to two sets of theoretically generated isotope patterns which took into account, in the first set, possible changes in the isotope enrichment of ^{81}Br (with no natural contamination) and, in the second set, the contamination from natural abundance BDEs (at the nominal enrichment of 99.53% ^{81}Br). Theoretical patterns for ten consecutive masses were generated[26] using a linear mixing model:

$$\begin{bmatrix} A^1_{theo} \\ A^2_{theo} \\ \ldots \\ A^9_{theo} \\ A^{10}_{theo} \end{bmatrix} = x^{sp}_{lab} \begin{bmatrix} A^1_{lab} \\ A^2_{lab} \\ \ldots \\ A^9_{lab} \\ A^{10}_{lab} \end{bmatrix} + x^{sp}_{nat} \begin{bmatrix} A^1_{nat} \\ A^2_{nat} \\ \ldots \\ A^9_{nat} \\ A^{10}_{nat} \end{bmatrix}$$

Where a given molar fraction of the labeled compound (X^{sp}_{lab}) at a given isotope enrichment of bromine 81, A^i_{lab} was mixed with a given molar fraction of natural

abundance compound (X^{sp}_{nat}) of isotope composition A^{i}_{nat}. The theoretical abundances for the different A^{i}_{theo} were computed then for different molar fractions of natural and labeled compound.

Finally, the theoretical abundances were subtracted from the experimental abundances and the residual sum of squares (RSS) calculated for different combinations of isotope enrichment and natural contamination. Figure 3 shows the results obtained for BDE-153. If we assumed the natural contamination negligible the best isotope enrichment of ^{81}Br resulted to be 98% (black dots). However, a much better fit was obtained when the isotope composition of bromine was assumed to be the nominal isotope enrichment of 99.53% and we considered a contribution of 41.7% of natural abundance BDE-153 in the spike (white dots). The comparison between the theoretical abundances calculated for both situations and the experimental abundances measured are shown in the supplementary information (Figure S1).

The same procedure was applied to the other 6 congeners. In all cases the best fit resulted in an isotope enrichment of 99.53% for ^{81}Br with natural contamination for some congeners. Fortunately, only BDE-154 showed a noticeable contribution from natural contamination with a 24.4% of natural abundance compound in the isolated fraction. For comparison, Figure S2 in the supplementary information shows the RSS plot for BDE-47 where the minimum found at the isotope composition of 99.53% is clearly observed with no significative natural contribution. Until now the source of the natural contamination for the hexabrominated BDEs 153 and 154 could not be found.

For isotope dilution analysis of congeners 153 and 154 in real samples the natural contribution must be taken into account in the calculations because the addition of the spike carries also some natural abundance compound. We have modified the isotope dilution equation (1) to take into account the natural abundance contribution in the spike. So, for congeners 153 and 154 the equation used was:

1 $$\frac{N_{nat}}{N_{lab}} = \frac{X_{nat}}{X_{lab}} \cdot X^{sp}_{lab} - X^{sp}_{nat} \quad (2)$$

2

3 In equation (2), the determined natural contribution to the spike, X^{sp}_{nat}, corresponding
4 to 0.417 for BDE-153 and 0.244 for BDE-154, was used as a correction factor.

5

6 *Characterization and stability of the ^{81}Br-labeled PBDEs standard*

7

8 The concentrations of the different BDE congeners in the labeled mixture were
9 determined by reverse isotope dilution analysis using a certified reference material
10 (SRM 2257) as natural abundance reference. For this purpose, an appropriate
11 mixture between the reference material and the ^{81}Br-labeled PBDEs standard was
12 injected in the GC(EI)MS system. In all cases 10 consecutive masses were selected
13 for the isotope pattern deconvolution calculations. The results obtained for 5
14 independent determinations are shown in Table 2. As can be observed, BDE-47 is
15 the main component of the spike mixture. Please note that the concentrations given
16 correspond to the total concentration including the natural contribution when
17 applicable (congeners 153 and 154). Total combined uncertainties were calculated
18 using Kragten procedure and the contribution of the different uncertainty sources are
19 included in Table 2. In all cases, two uncertainty sources were dominant: the
20 uncertainty in the concentration of the natural reference standard SRM 2257, and the
21 uncertainty in the experimental measurement of the blend ratio (equations 1 and 2).
22 For BDEs 49, 153 and 154 the main uncertainty source was the analytical
23 measurement while for BDEs 28, 47, 99 and 100 the main source was the
24 uncertainty in the reference standard.

25

26 The spike stability was evaluated in two different forms. First, possible isotope
27 exchange between bromine atoms from the natural abundance compounds and the
28 spike was evaluated. An aliquot of the spike was mixed with a natural abundance
29 standard containing congeners 28, 47, 99, 100, 153 and 154 and this mixture was
30 measured on different days during a period of four months. The results obtained are
31 shown in the supplementary information Figure S3. No changes in the ratio of molar

fractions X_{nat}/X_{lab} was observed for any of the compounds measured during this period indicating that no isotope exchange between bromine atoms from the natural abundance and labeled compounds took place. This is an important fact as it demonstrates the validity of this mode of labeling as alternative to the standard ^{13}C labeling.

Second, the spike stability was evaluated in terms of concentration as a function of time for a period of four months also. Concentrations of each labeled BDE congener were obtained on different days by reverse isotope dilution analysis using mixtures between the natural and labeled standards which were prepared on the same day they were measured. The results obtained are also included in the supplementary information Figure S4. The measured concentrations remained constant throughout the studied period. So, we can conclude that the spike is reasonably stable and does not suffer for isotope exchange when mixed with the natural abundance compound.

Determination of PBDEs in a Lake Michigan fish tissue SRM 1947

Once the ^{81}Br-labeled spike was demonstrated to be suitable for its use in isotope dilution analysis, the previously developed methodology, based on isotope pattern deconvolution,[17] was applied to the determination of PBDEs in a Lake Michigan fish tissue reference material. The SRM 1947 samples were treated as described in the procedures section. Two independent experiments and a blank were performed at three increasing spike levels (indicated as blend 1, blend 2 and blend 3). The different spike levels were selected in order to detect possible spectral interferences during the quantitation procedure. Also, the three spike levels were selected in order to study error propagation in isotope pattern deconvolution. In principle, all spike levels would provide molar fraction ratios (X_{nat}/X_{lab}) for all compounds in the range 0.1 - 10 since better precision in the measurements are expected under these conditions.

Quantitation of PBDEs in SRM 1947 samples was carried out by the isotope pattern deconvolution procedure described previously[17] selecting here five masses for each compound (except BDE-28 where only for masses were employed). Three of these

masses corresponded to the most abundant masses of natural abundance BDEs and the other two masses corresponded to the most abundant masses in the labeled compound (for details, see Table S1 in the supplementary information). The obtained results are shown in Table 3. As can be observed, the concentrations are in good agreement with the certified values, except for BDE-28+33, for the three studied spike levels. Recoveries were between 89% and 116% in all cases, which can be considered acceptable in ultratrace analysis. With regards to BDE-28, it is worth stressing that the indicative value is given for the mixture of BDEs 28 and 33 which can not be resolved in our chromatograph. Furthermore, it was observed that, for spiked SRM 1947 samples, the experimental isotopic profile did not match the typical isotopic profile for mixtures between labeled and unlabeled tribrominated congeners, showing unexpected interferences at masses 407.8 and 410.8. This fact could be observed by checking the residuals of the multiple linear regression. The residuals at mass 410.8 were very large so this mass was excluded from the calculations. Anyway, the final concentrations found for BDE-28+33 after excluding mass 410.8, given in Table 3, do not agree with the certified values indicating the presence of spectral interferences for this congener also for other measured masses.

The experimental reproducibilities in the measured concentrations between samples spiked at the same level were calculated as RSD (%). The values obtained were always below 7% although, in most cases, reproducibilities below 3% were found. 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 0.9 $ng \cdot g^{-1}$, expressed as three times the standard deviation of the measured blanks, were obtained.

Uncertainties and error propagation studies

The concentrations of the natural abundance compounds in this mode of IDMS are calculated from the ratio of molar fractions, $R = X_{nat}/X_{lab}$, using equation (1) without requiring the construction of a methodological calibration graph as no isotopic effects were expected.[18] The molar fractions X_{nat} and X_{lab} are calculated from a multiple linear regression procedure which allows the estimation of the uncertainties in both

parameters, s_{Xnat} and s_{Xlab}, from the regression results for each injection. Also, X_{nat} and X_{lab} are correlated variables of constant sum ($X_{nat} + X_{lab} = 1$) so the correlation factor between these variables, r = -1, need to be taken into account for error propagation studies.[26] In summary, we have developed an equation for the calculation of the relative uncertainty in the ratio R from the measured uncertainties in X_{nat} and X_{lab} taking into account the correlation between both variables. Equation (3) is the final equation obtained:

$$\frac{s_R}{R} = \sqrt{\left(\frac{s_{X_{nat}}}{X_{nat}}\right)^2 + \left(\frac{s_{X_{lab}}}{X_{lab}}\right)^2 + 2\frac{s_{X_{nat}} s_{X_{lab}}}{X_{nat} X_{lab}}} \qquad (3)$$

For the three blends prepared in the determination of PBDEs in SRM 1947 we performed a duplicate sample preparation and each sample was injected 5 times in the GC(EI)MS system. For each single injection we can calculate the relative uncertainty in the ratio of molar fractions and plot this relative uncertainty as a function of the measured ratio as it is usual in IDMS calculations for the optimization of the spike addition.[19] The results obtained for the three blends and the different congeners measured are shown in Figure 4. For each congener and blend we have ten data points in the graph. Almost all values of R lie between 0.1 and 10. As can be observed in the graph values of R close to R = 1 provide minimum error propagation values.

Figure 4 also contains two theoretical error propagation curves (red lines) calculated from equation (3) and assuming two extreme values for the uncertainties in the X_{nat} and X_{lab} molar fractions. In the best case, s_X=0.001, relative errors in R will be lower than 2% for R values between 0.1 and 10. In the worst case, s_X = 0.01, the relative error in R will be lower than 12% for the same range of R values. Please note that, in both cases, an optimum is found for R=1. The experimentally obtained s_X values were in all cases between these two extreme values (typically the average s_X was 0.003) and so almost all data points are in between the two red curves.

Therefore, in view of the obtained results, any of the three studied spike levels can be considered acceptable for the determination of PBDEs in the certified reference material. However, blend 2 provided overall the lowest propagated uncertainties (Figure 4).

Expanded uncertainties.

For the calculation of expanded uncertainties in the measured blends all uncertainty sources need to be taken into account including the experimental measurement uncertainty (Figure 4) and the uncertainties of other parameters such as the concentration of the spike, the deviation between duplicate samples, and the uncertainties in the sample weights taken. We have included the expanded uncertainties (k=2) in Table 3. As can be observed, very similar expanded uncertainties are obtained for the different blends prepared indicating that, within the given limits of R, the overall experimental uncertainty is similar for all blends. It is remarkable the high relative uncertainties calculated for BDE-28+33 which can be adscribed mainly to high uncertainty in the blend ratio R caused by spectral interferences. In most cases, the larger contribution to the expanded uncertainty in the uncertainty in the concentration of the spike (see table 2) and not so much the experimental measurement of the blend ratio R (Figure 4). For example, for BDE-47 the contribution of the uncertainty in the concentration of the spike is ca. 80% of the total uncertainty. Values between 60 and 80% were obtained for the contribution of the uncertainty in the spike concentration for the other PBDE congeners.

Use of a calibration curve

For a comparison purpose, PBDEs were also determined in the certified reference material by means of a methodological calibration graph prepared from SRM 2257. Two independent samples and a blank were treated following the same sample preparation procedure but in this case $^{13}C_{12}$-BDE-47 was added as internal standard for all studied congeners. Quantitation was carried out by monitoring the most abundant mass for each congener as well as for the internal standard. The concentrations obtained are summarized in the supplementary information Table S2. Except for BDE-28+33, the recoveries ranged from 89 (BDE-47) to 151% (BDE-153)

depending on the compound with relatively large expanded uncertainties. For BDE-47 the recovery using the same compound as ^{13}C-labeled internal standard was 89% which can be considered satisfactory at this concentration level. For the hexabrominated congeners BDE-153 and BDE-154 the recoveries obtained were not satisfactory. This could be due to the fact that $^{13}C_{12}$ BDE-47 is not the best internal standard for these compounds as indicated in EPA method 1614.

In summary, the overall recoveries obtained using IDMS with the ^{81}Br-labeled compounds are a bit better than those obtained using the classical calibration graph with internal standard. However, the expanded uncertainties are sometimes better when using the calibration graph (e.g. for BDE-47) as the uncertainties in the concentrations of the labeled standards do not need to be taken into account.

Conclusions

We have demonstrated that the ^{81}Br-labeled PBDEs standard prepared in our laboratory can be extremely useful for the routine determination of priority PBDEs by GC(EI)MS in solid environmental samples. The labeled standard proved to be stable without noticeable isotopic exchange between bromine atoms. The suitability of the ^{81}Br-labeled standard for its use in IDMS experiments (particularly the lack of isotope exchange reactions) was demonstrated using a GC(EI)MS. The proposed IDMS method was validated by the analysis of SRM 1947 with good accuracy (recoveries between 89 and 116% except for BDE-28) and reproducibility (below 7%). The method does not require the construction of a methodological calibration graph as no isotopic effects were detected and each injection can provide a concentration result with an uncertainty value associated. This alternative labeled standard could be also useful in the analysis of these compounds by IDMS using other more sensitive ion sources such as the negative chemical ionization source which cannot be used for the determination of these six priority pollutants using ^{13}C-labeled standards. The increased propagated uncertainty observed due to the uncertainty in the concentration of the spike could be minimized by using a natural abundance certified standard with lower concentration uncertainties. Anyway, the procedure could be

useful for fast and accurate routine analysis of PBDEs in environmental samples as the construction of a calibration graph is not required. We expect that these labeled standards will be commercially available in the near future.

Acknowledgements

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3 **Table 1.** GC(EI)MS operating conditions
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GC and interface parameters	
Column	DB-5MS (30 m × 0.25 mm ×0.25 μm)
Injection mode	Pulsed splitless
Splitless time	2 min
Pulse	30 psi, 1 min
Injection volume	2 μL
Carrier gas / Flow	He / constant flow 2 mL·min^{-1}
Injection temperature	290 ºC
Oven programme	90 ºC (2 min) to 200 ºC at 30 ºC min^{-1} to 255 ºC at 1.5 ºC min^{-1} and to 300 ºC (10 min) at 30 ºC
Interface temperature	280 ºC
EI ion source and MS parameters	
Source temperature	230 ºC
Analizer temperature	150 ºC
Adquisition mode	SIM
Dwel time	20 ms
Solvent delay	3.5 min

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2 **Table 2.** Concentrations of the labeled PBDEs in the spike mixture using SRM 2257 as
3 reference. Uncertainties expressed as total combined uncertainty from n=5 independent
4 measurements. The two main sources of uncertainty are indicated.

Congener	Concentration (ng g^{-1})	Uncertainty sources (%)	
		Concentration of natural reference standard	Measurement of blend ratio
BDE-28	378 ± 15	90	4
BDE-47	1810 ± 75	75	19
BDE-49	31 ± 4	3	96
BDE-99	313 ± 8	56	28
BDE-100	169 ± 4	50	27
BDE-153	372 ± 16	13	81
BDE-154	28 ± 2	6	93

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2 **Table 3.** Concentration of priority PBDEs in SRM 1947 determined by Isotope Dilution Mass
3 Spectrometry. Mean values correspond to two separate extractions measured n=5 times
4 each. Uncertainties correspond to expanded uncertainty (k=2).

Congener	Concentration (ng g^{-1})			Certified concentration (ng g^{-1})
	Blend 1	Blend 2	Blend 3	
BDE-28+33	6.3 ± 4.1	6.7 ± 3.8	7.5 ± 3.8	2.26 ± 0.46*
BDE-47	79.4 ± 7.4	74.2 ± 7.9	77.2 ± 7.2	73.3 ± 2.9
BDE-49	4.2 ± 1.1	4.0 ± 1.0	4.2 ± 1.0	4.01 ± 0.1
BDE-99	21.4 ± 1.6	20.1 ± 1.7	21.1 ± 1.4	19.2 ± 0.8
BDE-100	19.9 ± 1.3	18.4 ± 1.5	19.1 ± 1.3	17.1 ± 0.6
BDE-153	4.6 ± 0.7	3.9 ± 0.6	3.4 ± 0.8	3.83 ± 0.04
BDE-154	8.0 ± 1.9	7.2 ± 1.5	7.8 ± 1.6	6.88 ± 0.52

5 * Not certified. Indicative value only.

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1 **Figure 1.** HPLC chromatograms for a natural abundance PBDE standard (containing BDEs
2 28, 47, 99, 100, 153, 154 and 183) and the ^{81}Br-labeled mixture showing the six fractions
3 collected.

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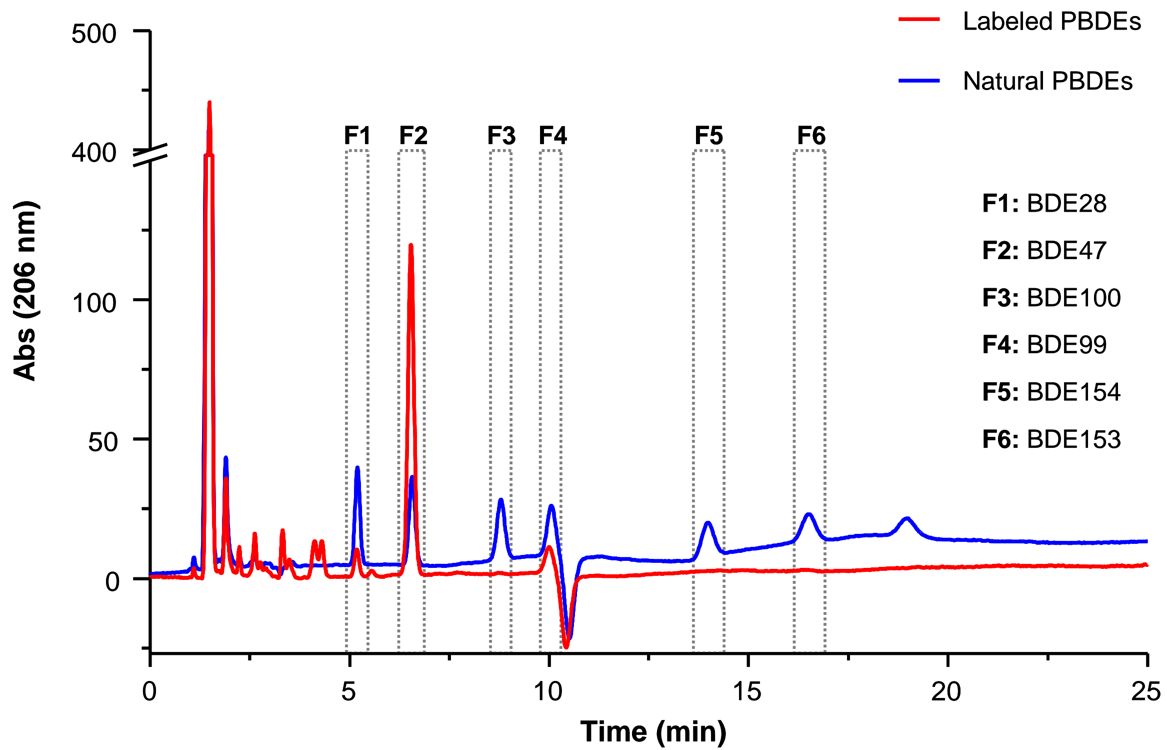

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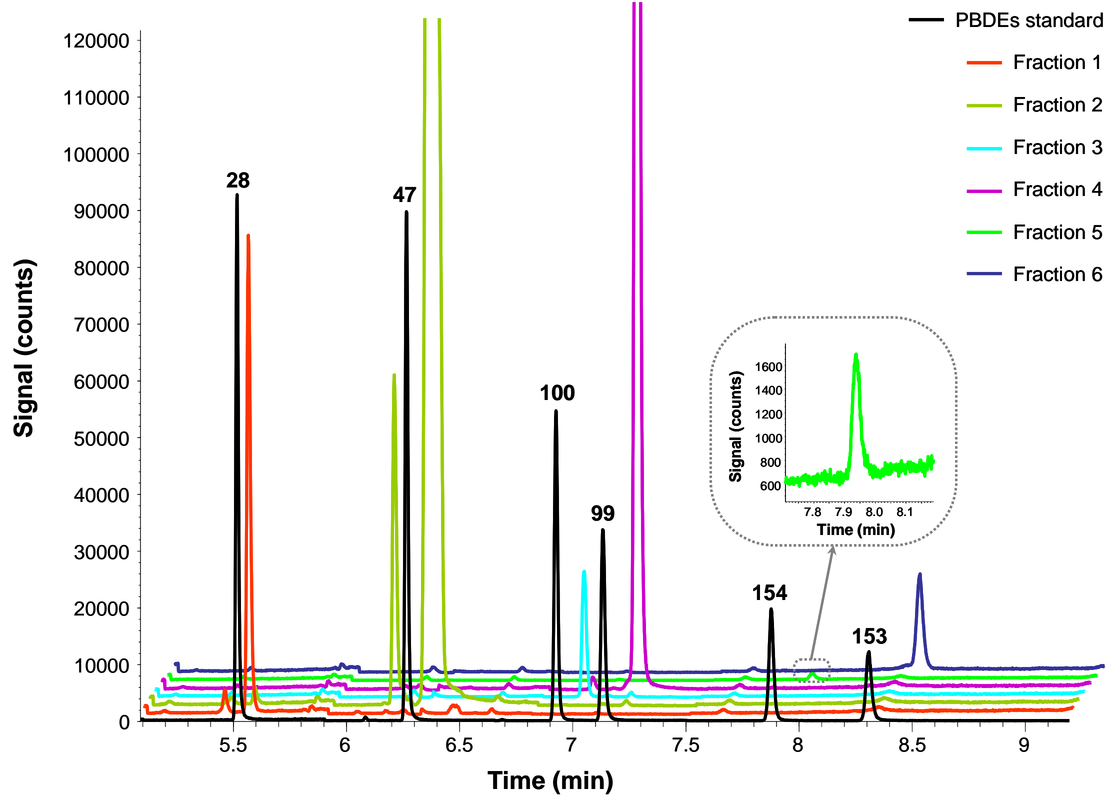
1 **Figure 2.** GC(EI)MS chromatogram for the six fractions collected and for a natural standard
2 mixture of BDEs 28, 47, 99, 100, 153 and 154.

3



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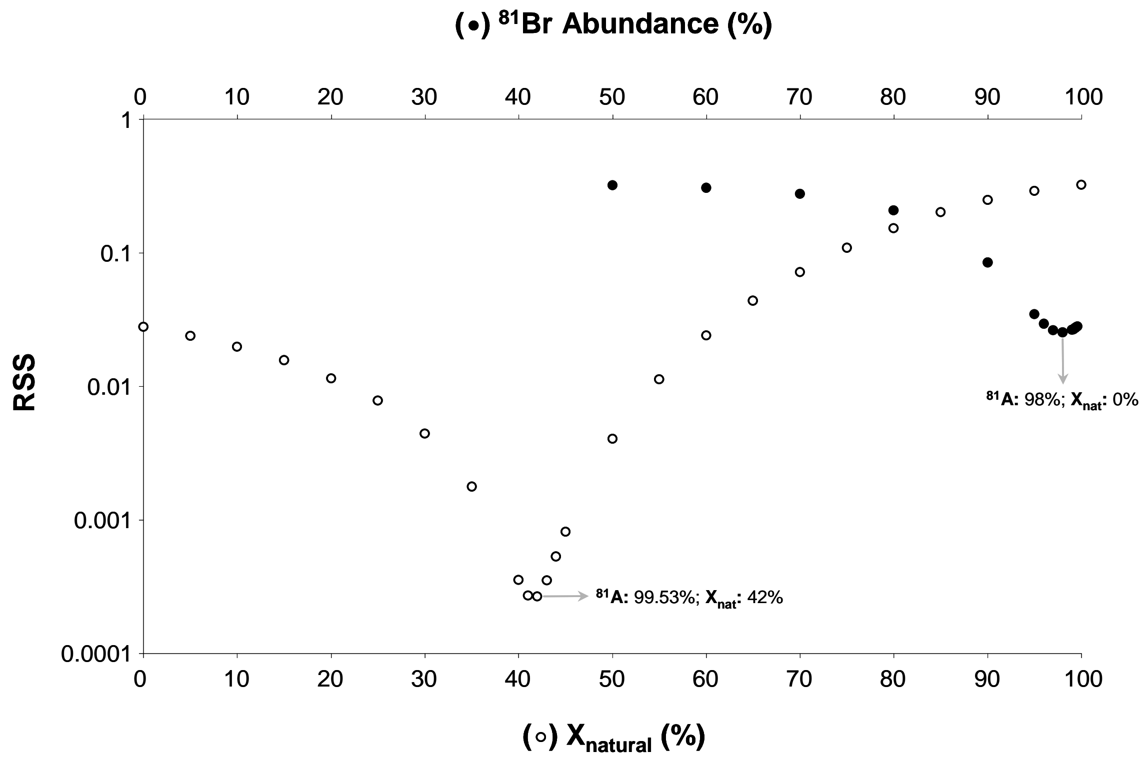
1 **Figure 3.-** Residual sum of squares (RSS) for the multiple linear regression for BDE-153
2 calculated for different ^{81}Br enrichments (black points) and different impurity levels of the
3 natural abundance congener in the ^{81}Br-labeled standard (white points).

4



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1 **Figure 4.** Error propagation studies in IDMS using molar fraction ratios.

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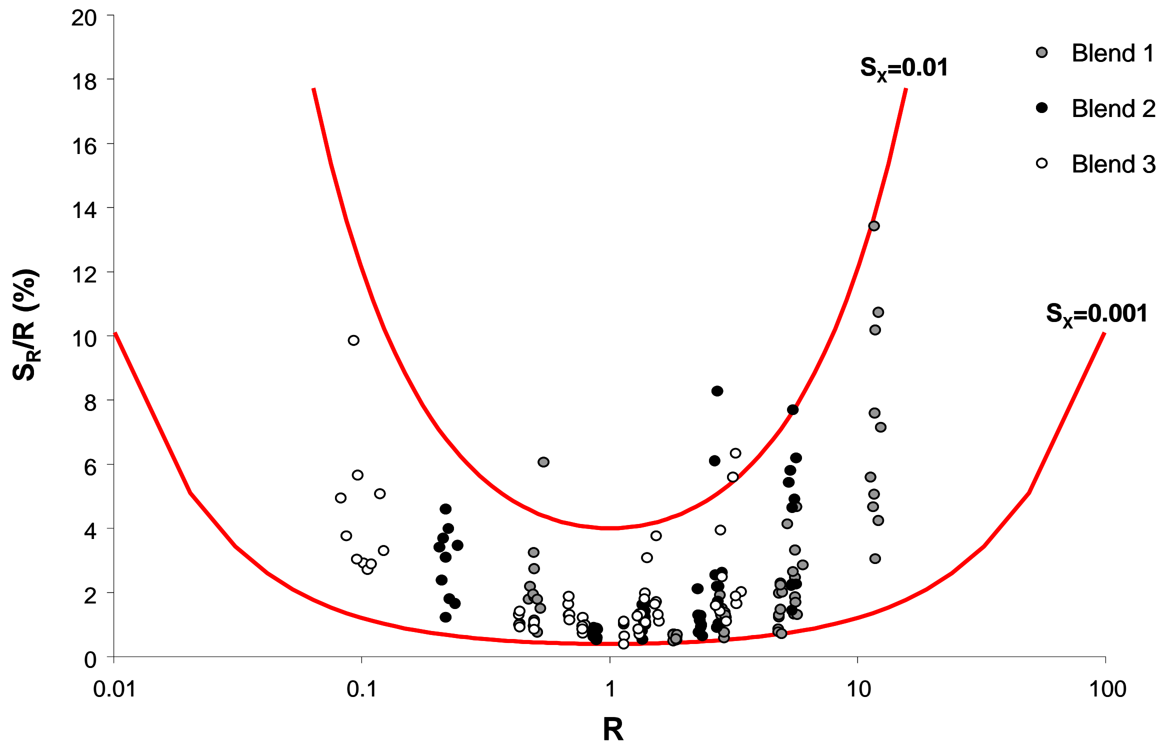

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