Synthesis of ^{81}Br-labeled polybrominated diphenyl ethers and their characterization using GC(EI)MS and GC(ICP)MS.

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Abstract

A mixture of different ^{81}Br-labeled polybrominated diphenyl ethers (PBDEs) was prepared and characterized for its future use as spike for the Isotope Dilution Analysis of PBDEs. The synthesis was carried out by direct bromination of diphenyl ether using ^{81}Br enriched Br_2 obtained after aqueous oxidation of bromide with potassium peroxymonosulfate and extraction into dichloromethane. The number of bromine atoms introduced in the diphenyl ether molecule depended on the molar ratio between bromine and diphenyl ether. The final mixture prepared contained a mixture of tri-, tetra-, penta- and hexabrominated PBDEs with a larger concentration of the tetrabrominated congener BDE 47. The isotopic composition of bromine in the resulting PBDEs mixture was determined by GC(ICP)MS and resulted in a 99.53% enrichment of the isotope 81 of bromine. The concentration of three of the PBDE congeners (28, 47 and 99) in the mixture was determined by reverse Isotope Dilution Analysis using a certified, natural abundance, PBDEs mixture and both GC(ICP)MS and GC(EI)MS. For this purpose, the fragmentation and isotope distribution patterns of the different PBDE cogeners in the positive electron ionization source were studied in detail both for natural abundance and labeled compounds. A procedure based on Isotope Pattern Deconvolution was developed which allowed the direct determination of the concentration of the labeled PBDEs in the spike mixture by GC(EI)MS. Finally, the GC(EI)MS Isotope Pattern Deconvolution procedure was applied for the determination of natural abundance congeners 28, 47 and 99 in spiked waters at ng L^{-1} levels. Detection limits below 0.5 ng L^{-1} could be obtained for all compounds using only 100 mL of sample and liquid-liquid extraction with isooctane.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardants which are used as additives in a wide range of household materials to prevent or reduce fire development by interfering with the combustion of polymeric materials.[1,2] However, these compounds can be easily released into the environment and accumulated by living organisms.[2] Their wide distribution in the environment together with their high lipophilicity, resistance to degradation and bioaccumulation has raised concerns about the potential risks of PBDEs exposure to human health and the environment. In fact, these compounds have been detected in many biological and environmental samples (human adipose tissues, serum and breast milk, fish, birds, marine mammals, sediments, sludge, house dust, indoor and outdoor air, and supermarket foods),[3] and toxicological studies suggest that they are linked to some adverse physiological effects.[4] Additionally, many national and international regulations now require the determination of different PBDE congeners in environmental samples. For example, the European Union has issued a recent Directive[5] in which congeners number 28, 47, 99, 100, 153 and 154 will need to be measured in European fresh waters at levels below 0.5 ng L^{-1}. Also, the US EPA include congeners 47, 99 and 100 in the unregulated contaminants list to be measured in fresh waters. Due to these analytical challenges, new methods for the determination of these compounds have been developed but they still need further improvement in terms of sensitivity, precision and accuracy.[6] Methods for the determination of PBDEs include GCECD (electron capture detector) and, more recently, GCMS. Different ionization sources have been used including Electron Ionization (EI),[7] Negative Chemical Ionization (NCI)[8] and Inductively Coupled Plasma (ICP).[9] In the last few years, GC(NCI)MS has become the technique of choice in many laboratories[10,11] because of its high sensitivity and the selective detection of bromine as negative ion at masses 79 and 81. The use of GC(ICP)MS is also increasing as the detection limits provided by this technique are, on average, ten times lower than those provided by GC(NCI)MS.[12]

The extensive sample preparation procedures required for the determination of PBDEs at ultratrace levels require the use of suitable internal standards for the correction of recoveries. In this regard, the use of labeled compounds as internal standards is a general procedure including fluorinated PBDEs and isotopically labeled compounds.[12] Many different laboratories have synthesized individual congeners both, labeled and unlabeled during the last ten years. Unlabeled PBDEs have been used as internal standards for the determination of PBDEs or for identification purposes. Labeled PBDEs have been employed mainly in toxicology studies (radioactive ^{14}C)[13] and for quantification purposes (^{13}C) either as internal standards[14] or using the isotope dilution method.[15-18] Isotope dilution procedures are ideal for the determination of PBDEs as no recovery corrections from sample preparation are required. So far, only fully labeled $^{13}C_{12}$ PBDEs can be obtained commercially for this purpose. Unfortunately, these compounds are only suitable for the determination of PBDEs when GC(EI)MS techniques are applied. When the more sensitive and selective GC(ICP)MS or GC(NCI)MS techniques are used for detection these ^{13}C-labeled compounds can not be used as the label is lost during the ionization process when using hard ionization sources such as ICP or the fragment containing the label coelute with the native compounds and cannot be then separated when using soft ionization sources such as NCI.

In the last few years the concept of heteroatom labeling has been developed in different laboratories around the world mainly in combination with ICPMS detection and for trace element speciation. The synthesis and application of isotopically labeled organometallic compounds of environmental interest such as butyltin compounds, methylmercury and organolead compounds has been described.[19] Using these labeled compounds, isotope dilution methodologies have been developed for both GC(ICP)MS and GC(EI)MS and some of those methodologies have been validated and accredited by testing laboratories.[20] The potential advantages of heteroatom labeling include the sensitive and selective detection of the label by ICPMS[12] and the possibility of avoiding the methodological calibration graph as both the labeled and unlabeled compounds behave exactly the same without any detectable isotopic effects.

The use of enriched isotopes of bromine in the labeling of organic molecules has not been described in the literature in spite of the fact that bromine can be detected selectively both by ICPMS and NCIMS with low detection limits.[12,18] So, the main objective of this work was the synthesis and characterization of some polybrominated diphenyl ethers labeled with ^{81}Br for its future use in the determination of PBDEs in environmental and biological samples by Isotope Dilution Analysis using both atomic and molecular ion sources. The synthesized compounds were characterized by GC(ICP)MS in isotopic composition and their fragmentation patterns were studied in detail using GC(EI)MS with a conventional electron ionization source in positive ionization mode. The determination of those compounds in the synthesized mixture was carried out by reverse Isotope Dilution Analysis using the Isotope Pattern Deconvolution algorithm recently applied in our laboratory for organic compounds and molecular ion sources.[21]

Experimental

Reagents and materials

Sodium bromide enriched in ^{81}Br (99.62 atom%) was purchased from Trace International Sciences Corp. (Richmond Hill, ON, Canada), natural abundance sodium bromide (99.995% purity) was obtained from Fluka (Buchs, Switzerland) and diphenyl ether (99.9%) was purchased from Fluka. Oxone® (potassium peroxymonosulfate) was obtained from Sigma-Aldrich (Steinheim, Germany), anhydrous aluminum chloride and dichloromethane were from Fluka and anhydrous sodium sulfate from Merck (Darmstadt, Germany). All glassware used for the synthesis of PBDEs was cleaned with detergent (Mucasol ® from Brand GMBH+COKG, Wertheim, Germany), rinsed with Milli-Q water and dichloromethane, dried in an oven and brought to room temperature before its use.

Individual certified standards of 7 PBDEs congeners (28, 47, 99, 100, 153, 154 and 183, 50 μg mL^{-1} in nonane) were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). The tetrabrominated $^{13}C_{12}$-BDE 47 (99% isotopic purity, 50 μg

mL^{-1} in nonane) was also obtained from Cambridge Isotope Laboratories. Working standard solutions of labeled and unlabeled PBDEs were prepared in isooctane (Sigma-Aldrich) by weight and stored in the dark at 4 ºC until use. Diluted solutions of BDEs 28, 47 and 99 were prepared in methanol (Fluka) for the water spiking experiments. Ultra-pure water was obtained from a Milli-Q Gradient A10 water purification system (Millipore S.A.S, Molsheim, France).

Instrumentation

A GC model 6890N (Agilent Technologies, Waldbrom, Germay) fitted with a split/splitless injector and equipped with a MSD model 5975B (Agilent Technilogies, Tokyo, Japan) has been used in this work. Two microlitre solutions were injected in each case automatically by an autosampler model 7683 (Agilent). The chromatographic separation was carried out using a low polarity capillary column HP-5MS (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.[6] Operating conditions are summarized in Table 1.

A GC model 6890 (Agilent Technologies, Palo Alto, CA, USA) fitted with a split/splitless injector was coupled to a quadrupole inductively coupled plasma mass spectrometer model 7500ce (Agilent Technologies, Tokyo, Japan) using the transfer line described in detail previously.[22] One microlitre solutions were injected manually in a low polarity capillary column TRB 5MS (Teknokroma, Barcelona, Spain; 30m x 0.25mm i.d., 0.25µm film thickness). Operating conditions for the GC(ICP)MS coupling are also 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.

Procedures

Synthesis of ^{81}Br-labeled PBDEs

Approximately 55 mg of ^{81}Br enriched NaBr, 1 g of Milli-Q water and 1.3 g of CH_2Cl_2 were introduced in a 7 mL amber vial. Then, 340 mg of oxone® were added carefully over the stirred mixture and it was allowed to react at room temperature. After 15 minutes the organic layer containing $^{81}Br_2$ was removed from the vial and then filtered and dried using a Na_2SO_4 (anhydrous) column on a glass Pasteur pipette. After that, ca. 50 mg of $AlCl_3$ (used as catalyst) and 0.7 g of diphenyl ether were added over the organic layer and the mixture was stirred again for 6 hours at 40 ºC. Once the reaction was finished and the mixture reached room temperature, the organic layer was washed 3 times with water to remove the catalyst and other inorganic impurities. Then, it was filtered, dried (anhydrous Na_2SO_4) and the organic solvent evaporated under reduced pressure. The resulting mixture of PBDEs was finally redissolved in approximately 6.5 g of CH_2Cl_2.

Characterization of ^{81}Br-labeled PBDEs

The isotopic composition of bromine in the PBDEs labeled mixture was determined by injecting 1 μL of a diluted labeled standard solution in the GC(ICP)MS system and both m/z 79 and 81 were measured. A natural abundance mixture of PBDEs was used for mass bias correction.

The concentrations of the congeners 28, 47 and 99 in the ^{81}Br-labeled standard were determined by reverse Isotope Dilution Analysis using natural abundance certified standards. Different mixtures of the natural and labeled standards were injected both in the GC(EI)MS and the GC(ICP)MS systems. The concentrations were calculated by Isotope Pattern Deconvolution[21] for GC(EI)MS and by the inorganic isotope dilution equation[19] for GC(ICP)MS.

Determination of congeners 28, 47 and 99 in water samples

Samples of 100 mL of Milli-Q water were placed in glass volumetric flasks with glass stoppers and spiked (by weight) with a mixture of natural abundance certified PBDEs (in methanol) to obtain three different concentration levels in water (ca. ~~0,~~ 1, 10 and 100 ng L^{-1}). Then, a known amount of the synthesized ^{81}Br-labeled standard and

approximately 4 mL of isooctane as extractant were added to each water sample. The mixture was shaken mechanically for 30 minutes and then most of the organic extract was removed, evaporated to a final volume of ca. 100 µL in an amber glass vial and stored in the dark at 4 ºC until analysis. Finally, 2 µL of the organic extract were injected into the GC(EI)MS. Detection of 5 masses in SIM mode was performed for each compound. The concentration of congeners 28, 47 and 99 was calculated using the Isotope Pattern Deconvolution procedure.

Results and discussion

Optimization of synthesis conditions

Optimization of the synthesis reaction was carried out using natural abundance sodium bromide. In this regard, different reaction methods were tried for the synthesis of PBDEs starting from diphenyl ether and sodium bromide. A priori, oxybromination of substituted aromatic compounds seemed to be the easiest and simplest alternative. This reaction consists of the electrophilic substitution of bromine generated *in situ* from bromide using an oxidizing agent.[23,24] Different chemical reaction parameters, such as temperature, amount and type of oxidizing agent, catalyst, solvent and time of reaction were evaluated. However, only low brominated congeners (tri- and tetrabrominated PBDEs) were obtained by this method at the optimal working conditions, so the reaction was not considered suitable since most of the PBDEs of primary interest[4] were not present in the mixture. Because of this, a two-step reaction was tried next. The first stage of this reaction consisted on the oxidation of bromide to bromine by an oxidizing agent, followed by a second stage of electrophilic adition of bromine to diphenyl ether. This reaction was carried out at the previously optimized working conditions (oxybromination) so only the molar ratio between bromine and diphenyl ether was evaluated in this case.

Experimental results showed that a molar ratio of approximately 5:1 (bromine to diphenyl ether) allowed obtaining a mixture of PBDEs containing mainly the congeners found at higher levels in different biological and environmental samples which are the tetra and pentabrominated congeners.[4] However, there are also other

congeners recommended by international organisms to be monitored in the environment, mainly congeners 100, 153 and 154. These congeners could also be obtained from the same reaction but at much lower concentrations, as can be seen in Figure 1. Once the working conditions were optimized the synthesis reaction was performed using ^{81}Br enriched sodium bromide as described in the procedures. Figure 1a shows the chromatogram obtained by injecting 2 μL of the synthesized ^{81}Br-labeled PBDE mixture in the GC(EI)MS instrument and detection in full scan mode while Figure 1b shows the chromatogram for the standard mixture of congeners 28, 47, 99, 100, 153, 154 and 183 at 1 μg g^{-1} for comparison purposes. As can be observed, the main compound in the mixture is congener 47 (tetrabrominated PBDE) with smaller amounts of congeners 28 (tribrominated PBDE) and 99 (pentabrominated PBDE). Also, congeners number 100 and 153 could be detected at much lower concentration levels (see Figure 1a, insert).

Characterization of ^{81}Br-labeled PBDEs by GC(ICP)MS.

The isotopic composition of bromine in the enriched mixture of PBDEs was determined by GC(ICP)MS. Figure 2 shows one of the chromatograms obtained for a ca. 80 fold dilution of the enriched mixture. As can be observed, the peak for congener 47 is the main peak in the chromatogram. The signal at mass 81 is much higher than that at mass 79 which is shown expanded in the insert of Figure 2. Mass bias correction was carried out by using a natural abundance standard containing the mixture of the PBDEs of primary interest (congeners 28, 47, 99, 100, 153, 154 and 183) and the average natural isotopic abundances of bromine published by IUPAC[25] as reference. The resulting isotopic abundances (atom%) for the labeled PBDE congeners 28, 47 and 99 were of 99.53 ± 0.02 for isotope 81 and 0.47 ± 0.02 for isotope 79 (close to the nominal enrichment of 99.62% for the $Na^{81}Br$ as supplied by Trace International Sciences Corp.). The bromine isotope enrichment for congeners 100 and 153 could not be determined because of its low concentration in the synthesis mixture.

The concentrations of the different PBDE congeners in the labeled standard were determined by reverse Isotope Dilution Analysis using, initially, GC(ICP)MS and the

certified natural abundance standards of PBDEs as reference. Figure 3 shows the chromatogram obtained at masses 79 and 81 (shifted for clarity) for one of the mixtures of natural abundance and enriched PBDEs by GC(ICP)MS. As can be observed, congener 47 shows enriched abundance while other congeners present at much lower concentration levels in the enriched mixture (e.g. congeners 100 and 153) shown nearly natural isotope abundances. When comparing this chromatogram with those shown in Figure 1, it can be observed that the retention times are larger and that the peak profiles are wide and show pronounced tailing. This is due mainly to the fact that the Gas Chromatograph used for GC(ICP)MS could not work at constant flow (only constant pressure) and that affected the retention time and shape of high boiling point compounds. Also, the coupling interface used between the GC and the ICP-MS[22] may not be completely suitable for high boiling point compounds such as PBDEs. The concentrations of the different compounds could still be determined based on the peak area ratios[19] but with high experimental uncertainties. The GC(ICP)MS results will be discussed below in comparison with those obtained by GC(EI)MS.

Characterization of ^{81}Br-labeled PBDEs by GC(EI)MS.

We have recently demonstrated[21] that Isotope Dilution Analysis can be performed in molecular Mass Spectrometry using Isotope Pattern Deconvolution in combination with minimal labeling, i.e. the use of a single ^{13}C label in the molecule. For that purpose, the isotope pattern measured for the blend of natural abundance and labeled compound is separated by multiple linear regression into two contribution factors: the molar fraction of the natural abundance compound and the molar fraction of the labeled compound. It was demonstrated[21] that the ratio of molar fractions was equal to the ratio of molar concentrations introducing the concept of calibration-free Isotope Dilution Analysis in organic Mass Spectrometry. In order to apply this procedure for the determination of PBDEs labeled with ^{81}Br (heteroatom labeling) we need first to study the fragmentation and isotope distribution patterns of the different PBDEs in the electron ionization source. Then, we need to select the best molecular cluster for the measurements taking into account the purity of the cluster and, finally, we need to perform the isotope dilution experiments.

Calculation of theoretical PBDEs isotope distribution patterns

The first requirement for the application of Isotope Pattern Deconvolution in organic IDMS is that the isotope distribution patterns, or "mass isotopomer" distribution patterns, of both the natural abundance compound and that of the labeled compound are known.[21] In our case, theoretical isotope patterns for the natural abundance and ^{81}Br-labeled PBDEs were calculated both for the molecular ion (M^+) and for the main fragmentation product where two bromine atoms were lost from the molecule ($M\text{-}2Br^+$) during fragmentation in the ion source.

For this purpose, a Visual Basic program was written as a macro for Excel using the calculation algorithm described by Kubinyi.[26] Data on the isotopic composition of the elements[25], both natural and enriched, and the exact mass of the isotopes were introduced in the Excel spreadsheet and were read from the Visual Basic program. Finally, the resulting isotopic composition was returned to the Excel spreadsheet as output. The program was tested by calculating the isotopic composition of bovine insulin ($C_{254}H_{377}N_{65}O_{75}S_6$) with satisfactory results in comparison to those published previously.[26]

The theoretical mass isotopomer distributions of tri-, tetra-, penta-, hexa- and hepta-PBDEs were computed using natural abundance C, H, O and Br and also using enriched ^{81}Br (99.53% enrichment as determined by GC(ICP)MS) both for the molecular ion and for the loss of two bromine atoms from the molecule. For example, the calculated mass isotopomer distributions for the natural and labeled tetrabrominated diphenyl ether (BDE 47) are shown in Table 2 for eight consecutive masses of the ion cluster both at the molecular ion M^+ and for the loss of two bromine atoms, $M\text{-}2Br^+$. As can be observed, the main peak in the M^+ cluster corresponds to exact mass 485.7 for the natural abundance compound while the main peak for the labeled compound is four masses higher at exact mass 489.7 with an isotope enrichment of ca. 86%. For the loss of two bromine atoms the mass difference was only two mass units with a higher isotope overlap particularly at the main mass of the labeled compound. Similarly, theoretical values for the isotope distributions of all

PBDEs, both natural abundance and labeled, were obtained. From these calculations it was concluded that the molecular ion, M^+, was the most suitable for the isotope dilution calculations as the mass overlap between natural abundance and labeled compounds was lower than for the loss of two bromine atoms in the molecule. Additionally, the molecular ion M^+ showed better selectivity (spectral interferences) than the $M\text{-}2Br^+$ cluster because of the higher mass and the larger mass defect due to the presence of bromine atoms in the fragment.

The developed mathematical procedure allowed also the computing of the standard uncertainties for the theoretical isotope distributions.[27] The uncertainties in the natural isotope abundances of carbon, oxygen, hydrogen and bromine were propagated to obtain the uncertainties in the isotope composition of the molecules.[27] These values will be discussed below in comparison with the experimental isotope distributions measured.

Measurement of the experimental PBDEs isotope distribution patterns

For the measurement of the experimental fragmentation patterns a mixture of natural PBDEs in hexane was injected in the GC(EI)MS in full scan mode. The mass spectrum showed two main ion clusters for all congeners corresponding to the molecular ion and for the fragment obtained by the loss of two bromine atoms. The relative intensity of these two clusters changed as the number of bromine atoms in the molecule increased so it was decided to measure both clusters for each PBDE congener. To study the fragmentation pattern, mass windows consisting of the ten most abundant consecutive exact masses for each cluster, M^+ and $M\text{-}2Br^+$, were selected. A mixture of natural abundance PBDEs of ca. 1 $\mu g\ g^{-1}$ of each congener in hexane and a diluted solution of the ^{81}Br labeled standard in hexane were injected in the GC(EI)MS in the SIM mode. Also, a solution of 1 $\mu g\ g^{-1}$ of the $^{13}C_{12}$ labeled standard (BDE 47) in hexane was also injected in order to compare the fragmentation pattern of this compound with the results obtained for the natural abundance and ^{81}Br-labeled BDE 47. The experimental mass isotopomer distribution was calculated by dividing the peak area measured for each mass by the sum of all peak areas measured for a given molecular cluster. The results obtained for

molecular ion M^+ of the three BDE 47 standards (natural abundance, ^{81}Br-labeled and $^{13}C_{12}$-labeled) are plotted in Figure 4 in comparison with the theoretical abundances calculated for these compounds. The experimental uncertainties correspond to the standard deviations from n=5 independent injections while the theoretical uncertainties were calculated using the error propagation theory as described before.[27]

As can be observed for all tetrabrominated compounds in Figures 4a, 4b and 4c, there is a good general agreement between the experimental and theoretical abundances. Similar results were obtained for all PBDEs considered. However, the experimental isotope abundances for low abundance masses are slightly higher than the theoretical values while the abundances of high abundance masses are slightly lower. This effect is particularly noticeable for the ^{81}Br-labeled compound in Figure 4b where the abundance of mass 488.7 is clearly higher than the theoretical value even when the uncertainty value is taken into account.

There are two possibilities in order to explain these results. First, it is possible that the mass peaks obtained in the quadrupole could spread to adjacent masses showing noticeable contribution to M-1 or M+1 ions or both. And, second, it is also possible that the measured clusters are not pure and are mixtures of, for example, M^+ and $M\text{-}H^+$ ions as has been observed before in the EI source.[28]

In order to understand this behavior, the main ion clusters for the mass calibration compound perfluorotributyl amine (PFTBA) were measured under the same experimental conditions as those employed for the PBDEs. The only difference was that the measurements were carried out on constant signals obtained with the calibration valve open during 1 minute for each considered cluster (ca. 180 consecutive measurements). This compound was selected as it does not contain hydrogen in the molecule so the formation of overlapping $M\text{-}H^+$ ions would be impossible. The results obtained for several clusters of this compound are shown in Table 3. As can be observed, there is a noticeable contribution at mass M-1, from 0.4 to 0.7%, which increased with the mass of the cluster considered. For the mass

range of the molecular ion of the PBDEs the contribution could be as high as 0.7%. This contribution is due to the tailing of the peak in the mass spectrum to the low mass side. On the high mass side, the contribution of natural abundance ^{13}C needs to be taken into account. For the clusters considered in Table 3 we have calculated the theoretical contribution at mass M+1 from the natural isotope abundance of ^{13}C as indicated by the IUPAC (1.07%).[25] As can be observed in the table, the measured M+1 contribution is in agreement with the theoretical contribution within its uncertainty range. This means two things: the tailing of the mass spectrum at the high mass side is negligible and no mass bias effects are present in the mass range considered.

From these results it seems that the differences found between the theoretical and experimental abundances for the PBDEs, as shown in Figure 4, could be explained, at least partially, by the tailing of the peak in the mass spectrum to the low mass side. However, the presence of $M\text{-}H^+$ ions in the spectra measured for the PBDEs needs to be evaluated.

Determination of the fragmentation patterns of PBDEs

A requirement for the application of Isotope Pattern Deconvolution in organic IDMS is that the fragmentation pattern of the target compound must be known in advance. Ideally, the ion cluster should be formed only by one ion type but this is not strictly required as long as the fragmentation pattern is constant. It is well known that many organic compounds form ion clusters where more than one ion types overlap. The loss of hydrogen atoms is usually observed and this fact has to be taken into account in the calculation of the isotope patterns to be used. To evaluate the possibility of isobaric overlaps, with the loss of one hydrogen atoms in the experimental isotope patterns, the procedure described by Meija *et al*[28] was followed. The theoretical isotopic composition of the different PBDE congeners was computed by the Visual Basic program described before assuming the possible loss of 0 or 1 hydrogen atom during fragmentation. Then, the theoretical patterns were compared to the experimentally observed fragmentation patterns and the contribution of the different isobaric fragment types evaluated by Isotope Pattern Deconvolution. It was observed

that the ion clusters for all PBDEs were consistent with a mixture of two components where the molecular ion was the main component and the loss of one hydrogen atom from the molecule was the minor component. The results obtained, as relative contribution to the ion cluster (in %) for all tested compounds are shown in Table 4 as the averages and standard deviations of the fragmentation patterns measured for a quintuplicate injection.

As can be observed for the molecular ions M^+, the fragmentation pattern is approximately constant and seems to be independent of the labeling of the molecule and the number of bromine atoms present. For the $M\text{-}2Br^+$ cluster, the contribution of the $M\text{-}2Br\text{-}H^+$ fragment is higher but still lower than 2%. A further consideration is the day-to-day variability of the fragmentation pattern and the possible modification of the fragmentation pattern with the ionization conditions in the ion source. Day-to-day variations in the fragmentation patterns were also evaluated by injecting the natural abundance standards in the GC(EI)MS on two additional days. The results obtained are shown in Table 5 for the fragmentation factor of the main clusters. As can be observed, very consistent fragmentation factors are obtained on a day-to-day basis. The effect of the ionization conditions in the ion source on the fragmentation of the PBDEs was studied at three different electron acceleration voltages: 35, 70 and 140 eV. The results obtained (not shown) indicated that no effects of the acceleration voltage were detected.

Based on the results shown in Tables 3, 4 and 5, the observed presence of the M-1 ion in the mass spectrum of the PBDEs is partially due to the tailing of the mass peak at the low mass side (ca. 0.6-0.7%) but the contribution of $M\text{-}H^+$ ions needs to be considered to explain the observed fragmentation factors. Both effects are computed simultaneously by Isotope Pattern Deconvolution. Finally, the selected measurement conditions are summarized in Table 6 together with the observed fragmentation factors. Finally, values of fragmentation factors of 0.987 for M^+ and 0.013 for M-1 (or $M\text{-}H^+$) were used for all compounds. For example, for each PBDE congener the isotope composition of the natural abundance compound at the M^+ ion cluster was calculated as:

$$\begin{bmatrix} A^{1}_{nat} \\ A^{2}_{nat} \\ A^{3}_{nat} \\ \dots \\ A^{n-1}_{nat} \\ A^{n}_{nat} \end{bmatrix} = 0.987 \cdot \begin{bmatrix} A^{1}_{M^+} \\ A^{2}_{M^+} \\ A^{3}_{M^+} \\ \dots \\ A^{n-1}_{M^+} \\ A^{n}_{M^+} \end{bmatrix} + 0.013 \cdot \begin{bmatrix} A^{1}_{M-H^+} \\ A^{2}_{M-H^+} \\ A^{3}_{M-H^+} \\ \dots \\ A^{n-1}_{M-H^+} \\ A^{n}_{M-H^+} \end{bmatrix} \qquad [1]$$

taking into account the theoretical composition for each of the pure clusters M^+ or $M\text{-}H^+$. These calculations were performed for all labeled and natural abundance compounds.

Determination of the concentrations of ^{81}Br-labeled PBDEs by GC(EI)MS

The fundamentals of Isotope Pattern Deconvolution as applied for organic Isotope Dilution Analysis were described elsewhere[21]. In brief, the isotope distribution in the measured mixture of natural abundance and labeled compound (A_{mix}) is decomposed into two known isotope patterns corresponding to the contribution of natural (A_{nat}) and labeled (A_{lab}) compound by multiple least squares. The basic Isotope Pattern Deconvolution equation is:

$$\begin{bmatrix} A^{1}_{mix} \\ A^{2}_{mix} \\ A^{3}_{mix} \\ \dots \\ A^{n-1}_{mix} \\ A^{n}_{mix} \end{bmatrix} = \begin{bmatrix} A^{1}_{nat} & A^{1}_{lab} \\ A^{2}_{nat} & A^{2}_{lab} \\ A^{3}_{nat} & A^{3}_{lab} \\ \dots & \dots \\ A^{n-1}_{nat} & A^{n-1}_{lab} \\ A^{n}_{nat} & A^{n}_{lab} \end{bmatrix} . \begin{bmatrix} x_{nat} \\ x_{lab} \end{bmatrix} + \begin{bmatrix} e^{1} \\ e^{2} \\ e^{3} \\ \dots \\ e^{n-1} \\ e^{n} \end{bmatrix} \qquad [2]$$

where n is the number of measured masses (here n=10 or n=5) and x_{nat} and x_{lab} are the unknown molar fractions of natural abundance and labeled compound respectively. As we have more equations than unknowns an error vector has to be included in the calculations. The values of the unknowns x_{nat} and x_{lab} are determined by multiple linear regression. The isotope dilution equation now reduces to:

$$\frac{N_{nat}}{N_{lab}} = \frac{x_{nat}}{x_{lab}} \qquad [3]$$

which indicates that the ratio of molar fractions is equal to the ratio of mols of natural and labeled compound in the spiked sample. As we know the mols of natural abundance compound added (in the case of a reverse ID experiment) the mols of the labeled compound can be determined directly using equation [3]. The main advantage of this mode of performing Isotope Dilution Analysis is that no calibration graph is required as the measurement of the molar fractions of natural and labeled analyte in the sample provide directly the concentration of the compound in the sample. For a direct isotope dilution experiment the mols of labeled compound added will be known and equation [3] will allow the determination of the mols of natural abundance compound in the sample.

Different mixtures of the natural abundance and the ^{81}Br-labeled compounds were prepared and then injected in the GC(EI)MS instrument in order to determine the concentrations of the synthesized congeners. For each congener three different blends were prepared by spiking the sample with each of the natural abundance standards sequentially (for BDEs 28, 47 and 99). Concentrations were calculated using equations [2] and [3] and taking into account the weights taken from sample and spike and the molecular weights of the compounds respectively. The results obtained are shown in Table 7 in comparison with those obtained previously by GC(ICP)MS using the inorganic isotope dilution equation[19] after mass bias correction. The mean and standard deviations from three independent blend measurements are given for each technique. As can be observed, better reproducibility was obtained by GC(EI)MS in comparison with GC(ICP)MS which could be due to the bad peak profiles obtained by the GC(ICP)MS coupling.

Evaluation of a simple methodology for the determination of PBDEs in water samples.

Current European legislation[5] require the routine determination of six PBDE congeners in continental waters at levels below 0.5 ng L^{-1}. In order to reach these low

detection limits several preconcentration procedures have been published in the literature including micellar cloud point extraction[29] and hollow fiber microporous membrane liquid-liquid extraction.[30] Using the ^{81}Br-labeled compounds we have evaluated a simple liquid-liquid extraction procedure in which 100 mL of the sample are measured in a glass volumetric flask and ca. 3-4 mL of isooctane are used for extraction. Because of the Isotope Dilution procedure, the extract can be evaporated to a small volume. In our case we have selected a final volume of ca. 100 µL resulting in a preconcentration factor of approximately 1000.

For the evaluation of the procedures samples of Milli-Q water were spiked with a mixture of the six priority congeners dissolved in methanol in order to reach water concentrations of 0 (blank), 1, 10 and 100 ng L^{-1} approximately. The amount of each congener added was controlled gravimetrically. Then, a mixture of the ^{81}Br-labeled PBDEs standard was spiked, a volume of ca. 3-4 mL of isooctane was added and, finally, the mixture shaken for 30 minutes in a rotary mechanical shaker.

After extraction most of the organic layer was removed and evaporated down to ca. 100 µL. From every sample five consecutive injections of 2 µL were performed in the GC(EI)MS instrument to evaluate the instrumental precision. For the Isotope Pattern Deconvolution procedure 5 masses were selected for each compound (n=5 in equation [2]) to improve counting statistics. Three of those masses corresponded to the most abundant masses of the natural abundance PBDE (for example, for BDE-47 these masses were 483.7, 485.7 and 487.7, see Table 2) and the other two masses corresponded to the most abundant masses in the labelled compound (for BDE-47 masses 489.7 and 490.7, see Table 2).

The final concentration results are given in Table 8 for the four samples prepared at different concentration levels. As can be observed, the recoveries can be considered quantitative for the concentrations of 1 and 10 ng L^{-1} spiked. However, at those concentrations experimental repeatability was variable and ranged between 3 and 30% RSD. For the 100 ng L^{-1} spiked sample the repeatability was better than 2% but the recoveries can not be considered quantitative. This fact could be due to lack of

isotope equilibration between natural and labeled analytes or because of the low solubility of these compounds in water. Obviously this point will need to be addressed in future studies.

An estimation of the method detection limit can be obtained from the standard deviation of the measured blank values. Based on those data detection limits for congeners 28, 47 and 99 can be estimated to be 0.1, 0.4 and 0.2 ng L^{-1} respectively based on three times the standard deviation of the blank.

It is clear that a proper methodological development will require much more studies and results. However, we have shown that the ^{81}Br-labeled compounds prepared may allow the development of routine analytical procedures capable of measuring PBDEs at the low concentration levels required by current international legislations.[5]

Conclusions

A mixture of different ^{81}Br-labeled polybrominated diphenyl ethers (PBDEs) was prepared by direct bromination of diphenyl ether using enriched ^{81}Br. The obtained mixture was characterized in isotope composition and concentration both by GC(ICP)MS and GC(EI)MS. A method for the determination of the concentration of three of the PBDEs congeners (28, 47 and 99) in the mixture by Isotope Pattern Deconvolution GC(EI)MS was developed. For this purpose, the capabilities of the GC(EI)MS coupling for the measurement of the isotope distribution patterns of the different PBDEs cogeners was studied in detail. The contribution of the M-1 ion observed was ascribed partially to the tailing of the mass peak to the low mass side but the presence of $M\text{-}H^+$ ions could not be ruled out. Finally, the prepared mixture was evaluated for the Isotope Dilution Analysis of PBDEs in spiked water samples with promising results.

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Table 1. Operating conditions

GC(EI)MS		GC(ICP)MS	
GC and interface parameters		GC and interface parameters	
Column	HP-5MS (30 m × 0.25 mm ×0.25 μm)	Column	TRB 5MS (30 m × 0.25 mm × 0.25 μm)
Injection mode	Pulsed splitless	Injection mode	Split/Splitless
Splitless time	1.5 min	Splitless time	1.5 min
Pulse	30 psi, 1 min		
Injection volume	2 μL	Injection volume	1 μL
Carrier gas / Flow	He / constant flow 2 $mL \cdot min^{-1}$	Carrier gas / Inlet pressure	He / constant pressure 25 psi
Injection temperature	300 ºC	Injection temperature	270 ºC
Oven programme	120 ºC to 300 ºC (11 min) at 30 ºC min^{-1}	Oven programme	120 ºC to 300 ºC (11 min) at 30 ºC min^{-1}
Interface temperature	255 ºC	Interface temperature	270 ºC
EI ion source and MS parameters		ICP ion source and MS parameters	
Source temperature	230 ºC	Rf power	1280 - 1300 W
Analizer temperature	150 ºC	Sampling depth	5.5 - 6.0 mm
Adquisition mode	SIM	Carrier gas flow rate	1.37 - 1.45 L min^{-1}
Selected ions	See Table 6	Intermediate gas flow rate	1 L min^{-1}
Dwel time	5 ms	Outer gas flow rate	15 L min^{-1}
Solvent delay	3.5 min	Selected ions	79, 81
		Integration time	0.05 s per m/z
		Ion lens setting	Daily optimization

Table 2. Theoretical fragmentation patterns for tetrabrominated PBDEs

Molecular Ion (M^+)			Loss of two bromine atoms ($M-2Br^+$)		
Exact mass of fragment	Natural abundance	^{81}Br-labelled	Exact mass of fragment	Natural abundance	^{81}Br-labelled
483.7	0.2248	0.0000	323.9	0.2243	0.0000
484.7	0.0302	0.0000	324.9	0.0302	0.0000
485.7	0.3295	0.0001	325.9	0.4387	0.0082
486.7	0.0442	0.0000	326.9	0.0589	0.0011
487.7	0.2156	0.0162	327.9	0.2167	0.8647
488.7	0.0288	0.0022	328.9	0.0289	0.1165
489.7	0.0538	0.8567	329.9	0.0022	0.0089
490.7	0.0071	0.1154	330.9	0.0001	0.0005

Table 3. Contribution at masses M-1 and M+1 (%) from the main M^+ peak of Perfluorotributyl amine (PFTBA).

Nominal mass (fragment)	M-1 contribution (%)	Std dev (%)	M+1 contribution (%)	Std dev (%)	Theoretical M+1 contribution (%)	Uncertainty of the theoretical value (%)
69 (CF_3^+)	0.389	0.010	1.108	0.004	1.08	0.04
131 ($C_3F_5^+$)	0.488	0.004	3.278	0.010	3.24	0.12
219 ($C_4F_9^+$)	0.404	0.004	4.343	0.011	4.32	0.16
414 ($C_8F_{16}N^+$)	0.652	0.013	9.091	0.055	9.01	0.30
502 ($C_9F_{20}N^+$)	0.673	0.014	10.219	0.063	10.10	0.33

Table 4. Experimental fragmentation pattern of PBDEs

Congener	Labeling	X_M	X_{M-H}	X_{M-2Br}	$X_{M-2Br-H}$
BDE-28	natural	98.8 ± 0.2	1.2 ± 0.2	93.6 ± 2.5	1.1 ± 2.4
	^{81}Br	97.7 ± 0.4	1.2 ± 0.3	-----	-----
BDE-47	natural	98.8 ± 0.2	1.0 ± 0.2	97.8 ± 0.5	1.9 ± 0.4
	^{81}Br	97.6 ± 0.7	1.6 ± 0.5	-----	-----
	^{13}C	98.5 ± 0.3	1.4 ± 0.3	98.0 ± 0.4	1.6 ± 0.4
BDE-99	natural	98.6 ± 0.3	1.3 ± 0.3	97.8 ± 0.5	1.7 ± 0.4
	^{81}Br	96.6 ± 0.6	1.7 ± 0.5	-----	-----
BDE-100	natural	98.9 ± 0.3	1.1 ± 0.2	97.4 ± 0.6	1.7 ± 0.5
BDE-153	natural	98.7 ± 0.5	1.3 ± 0.4	97.3 ± 0.9	1.6 ± 0.7
BDE-154	natural	98.8 ± 0.4	1.2 ± 0.4	97.8 ± 0.7	1.5 ± 0.6

Table 5. Stability of the fragmentation factors using natural abundance PBDEs

Cluster	Congener	Day 1	Day 2	Day 3
M^+	BDE-28	98.8 ± 0.2	98.7 ± 0.2	98.6 ± 0.4
	BDE-47	98.8 ± 0.2	98.9 ± 0.1	98.7 ± 0.4
	BDE-99	98.6 ± 0.3	98.7 ± 0.4	98.7 ± 0.5
	BDE-100	98.9 ± 0.3	98.7 ± 0.3	98.8 ± 0.5
	BDE-153	98.7 ± 0.5	98.3 ± 0.4	98.1 ± 0.5
	BDE-154	98.8 ± 0.4	98.7 ± 0.5	98.3 ± 0.6
$M-2Br^+$	BDE-28	93.6 ± 2.5	94.8 ± 1.2	94.8 ± 1.2
	BDE-47	97.8 ± 0.5	97.8 ± 0.4	97.4 ± 0.5
	BDE-99	97.8 ± 0.5	97.5 ± 0.5	97.2 ± 0.9
	BDE-100	97.4 ± 0.6	97.8 ± 0.7	97.1 ± 0.7
	BDE-153	97.3 ± 0.9	97.5 ± 0.5	97.0 ± 1.2
	BDE-154	97.8 ± 0.7	97.7 ± 0.4	97.5 ± 0.8

Table 6. Selected measurement conditions and obtained fragmentation factors by GC(EI)MS

Number of Br atoms	Ion cluster	Mass window	Main fragment (%)*	Loss of Hydrogen or M-1 tailing (%)
3	M^+	402.8-411.8	98.7	1.3
4	M^+	481.7-490.7	98.8	1.2
5	M^+	561.6-570.6	98.8	1.2
6	M^+	643.5-652.5	98.6	1.4

*Average from three measurement days

Table 7. Concentration of the ^{81}Br-labeled PBDEs obtained by GC(EI)MS and GC(ICP)MS

		GC(EI)MS	GC(ICP)MS
Congener	Cluster	Concentration (μg g^{-1})	Concentration (μg g^{-1})
BDE-28	M^+	93.2 ± 0.2	81 ± 7
BDE-47	M^+	1161 ± 2	1233 ± 48
BDE-99	M^+	185.3 ± 0.3	168 ± 10

Table 8. Concentration of PBDE congeners 28, 47 and 99 determined in spiked water.

	Blank	Level 1[a]		Level 2[a]		Level 3[a]	
Congener	Concentration[b] (ng L^{-1})	Concentration[b] (ng L^{-1})	Recovery[b,c] (%)	Concentration[b] (ng L^{-1})	Recovery[b,c] (%)	Concentration[b] (ng L^{-1})	Recovery[b,c] (%)
BDE-28	0.07 ± 0.04	1.8 ± 0.4	137 ± 29	13.5 ± 0.5	102 ± 3	100 ± 1	87 ± 1
BDE-47	0.3 ± 0.1	1.8 ± 0.2	128 ± 13	12 ± 2	91 ± 12	103 ± 2	89 ± 2
BDE-99	0.10 ± 0.08	1.7 ± 0.5	123 ± 38	12 ± 1	91 ± 10	98 ± 2	85 ± 1

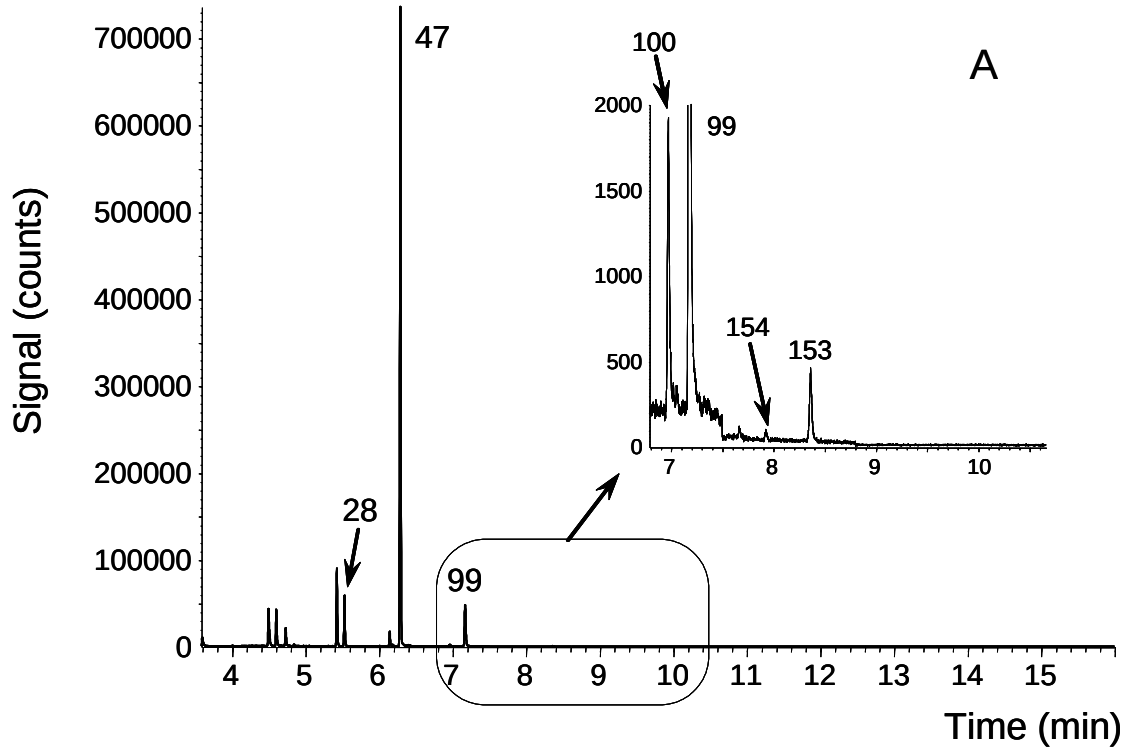
[a]Samples spiked at level 1, 2 and 3 with approximately 1, 10 and 100 ng L^{-1} of each congener respectively.

[b]The uncertainties correspond to the standard deviation from 5 injections of the same sample.

[c]The recovery was calculated by substracting the blank values and dividing by the theoretical concentration spiked.

Figure 1. GC(EI)MS Selected Ion Monitoring chromatogram for the synthesized spike[a] (A) and the natural abundance standard[b] (B)

[a] 2 µL of the synthesized ^{81}Br-labeled PBDEs



[b] 2 µL of 1µg g^{-1}

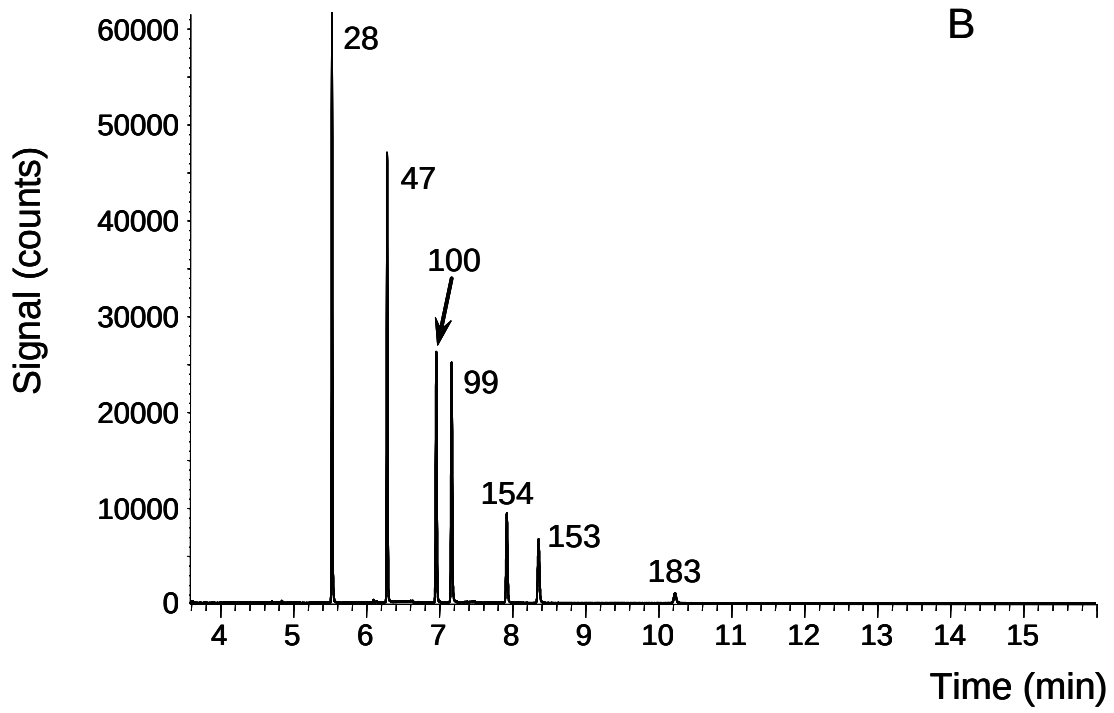


Figure 2. GC(ICP)MS chromatogram for the sinthesized PBDE mixture.

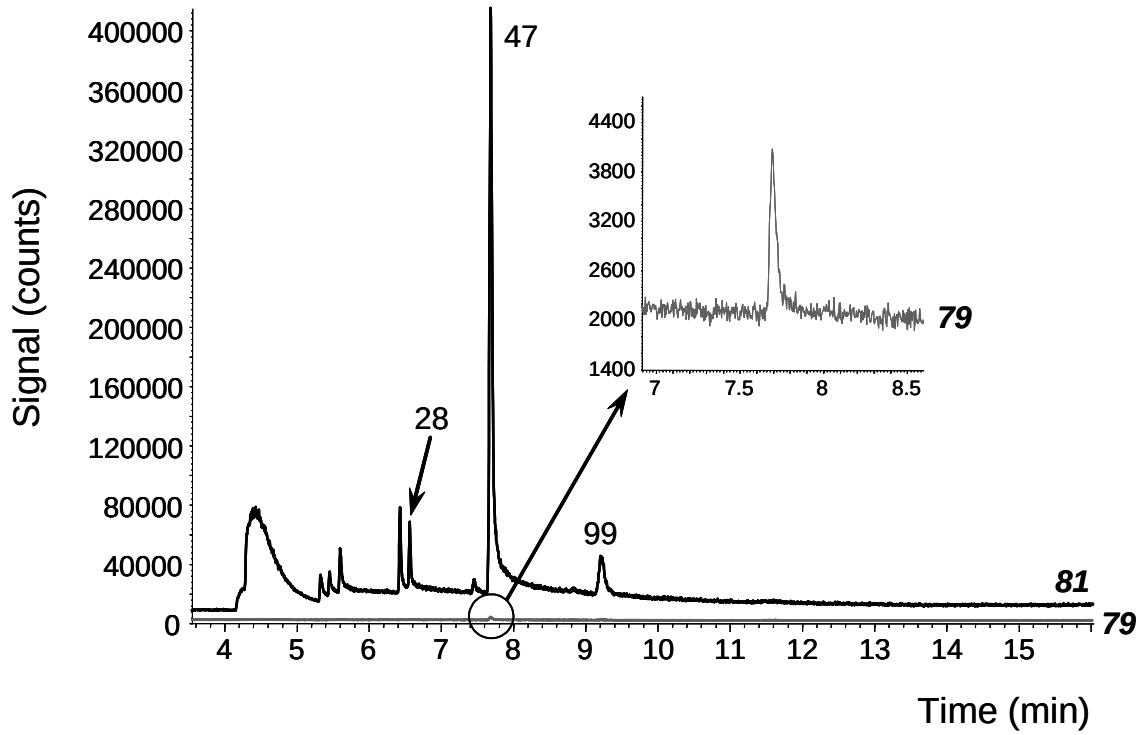


Figure 3. GC(ICP)MS chromatogram of a blend of natural and labeled PBDEs for reverse Isotope Dilution Analysis.

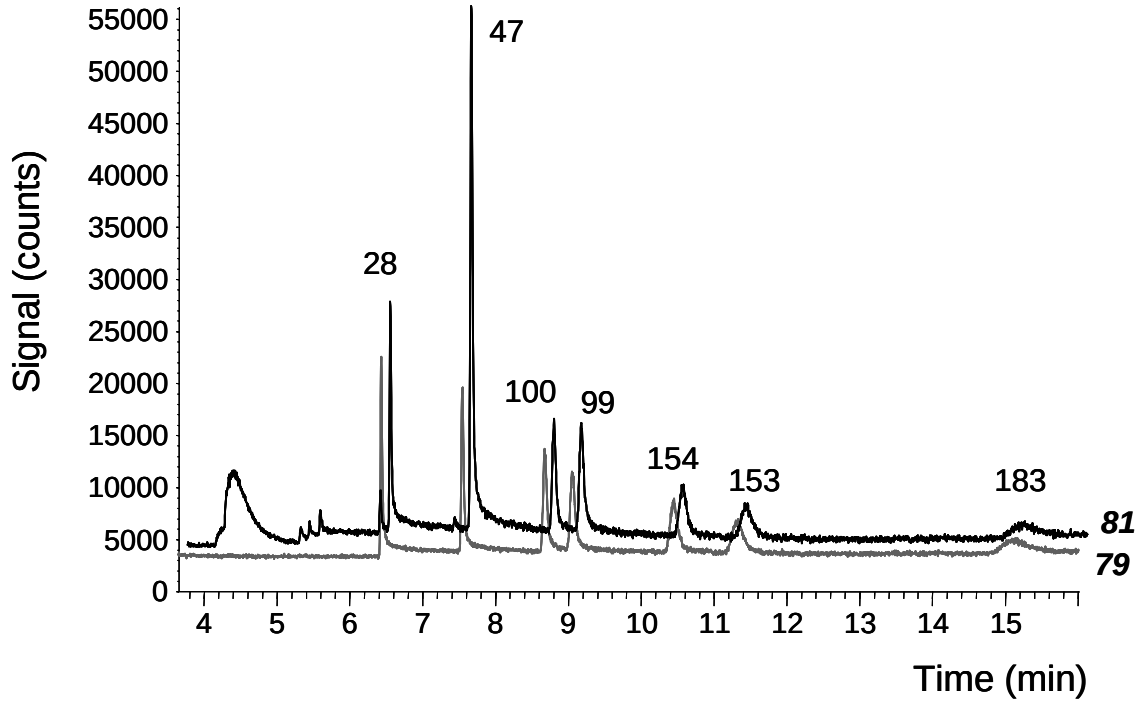
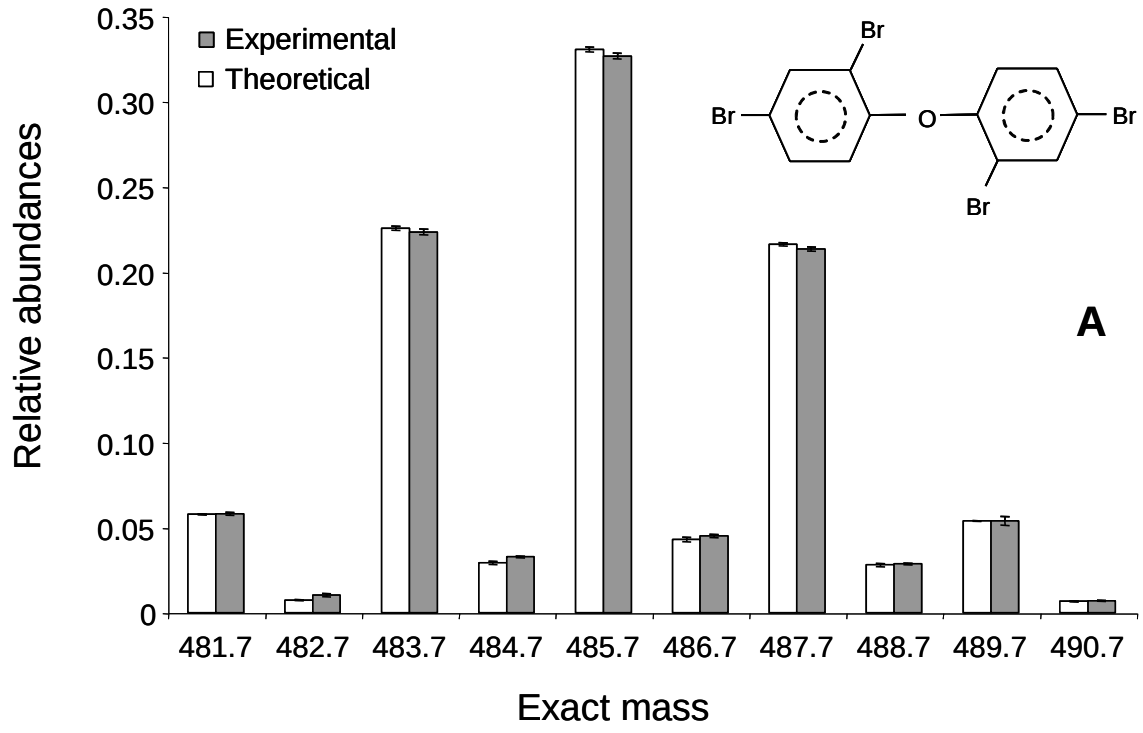
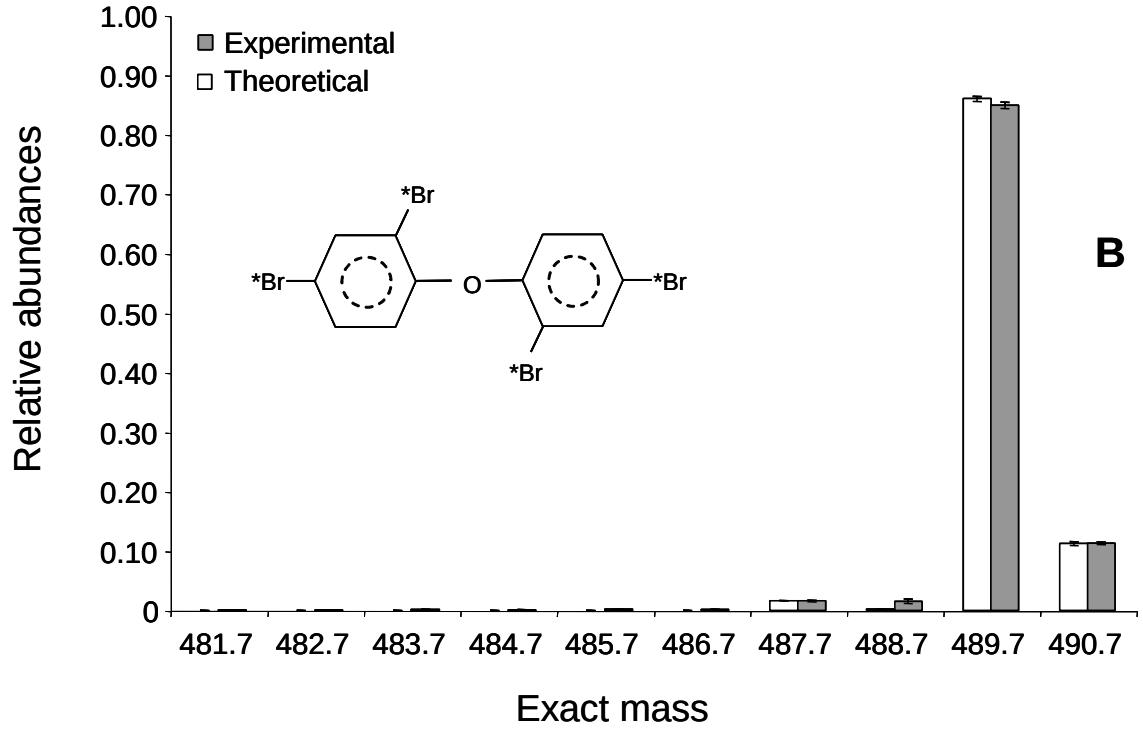
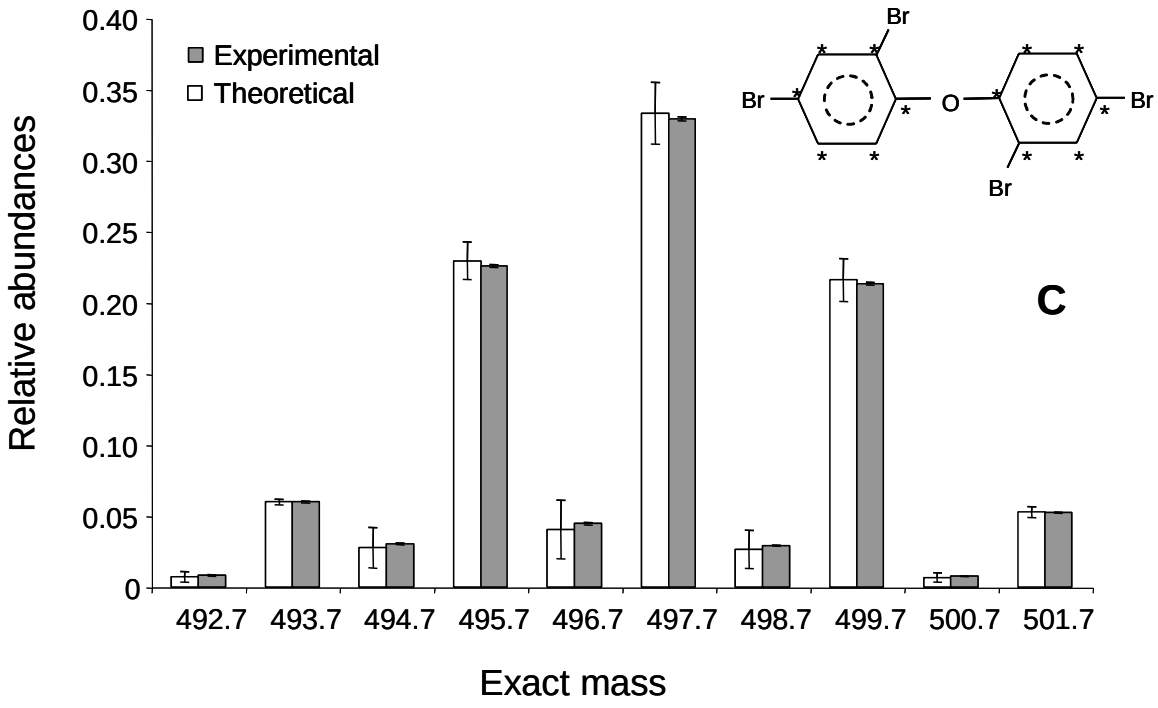


Figure 4. Comparison of the theoretical and experimental abundances for BDE-47 of (A) natural abundance, (B) ^{81}Br-labeled and (C) $^{13}C_{12}$-labeled.





1


Experimental
Theoretical
Relative abundances
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
492.7
493.7
494.7
495.7
496.7
497.7
498.7
499.7
500.7
501.7
Exact mass
Br
Br
O
Br
Br
C

2

3

1 References

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