Received: 17 October 2019 | Revised: 14 February 2020 | Accepted: 16 February 2020

DOI: 10.1002/rcm.8758

RESEARCH ARTICLE

Rapid Communications in Mass Spectrometry WILEY

Compound-specific carbon isotope analysis for mechanistic characterization of debromination of decabrominated diphenyl ether

Xifen Zhu[1,2] | Yin Zhong[1] | Heli Wang[1,2] | Dan Li[1,2,3] | Yirong Deng[1,2,4] | Shutao Gao[1] | Ping'an Peng[1]

[1]Guangdong Provincial Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry, Guangzhou, 510640, China

[2]University of Chinese Academy of Sciences, Beijing, 100049, China

[3]School of Environment and Civil Engineering, Dongguan University of Technology, Dongguan, 523808, China

[4]Guangdong Provincial Academy of Environmental Science, Guangdong Key Laboratory of Contaminated Sites Environmental Management and Remediation, Guangzhou, 510045, China

Correspondence
Y. Zhong, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Wushan, Guangzhou 510640, China.
Email: zhongyin@gig.ac.cn

Funding information
Guangdong Foundation for Program of Science and Technology Research, Grant/Award Number: 2017B030314057; National Natural Science Foundation of China, Grant/Award Number: 41773132;41473100; State Key Laboratory of Organic Geochemistry, GIGCAS, Grant/Award Numbers: SKLOGA2016A04, SKLOG-201910

Rationale: Decabrominated diphenyl ether (BDE-209) is a notorious persistent organic pollutant widely found in the environment. Developing a compound-specific isotope analysis (CSIA) method is much needed in order to trace its transport and degradation processes and to evaluate the effectiveness of the remediation of BDE-209 in the environment. However, the conventional CSIA method, i.e. gas chromatography (GC) combustion isotope ratio mass spectrometry, is not appropriate for BDE-209 because of its high thermal instability and incomplete combustion.

Methods: We developed a high-performance liquid chromatography (HPLC) method for the separation and purification of BDE-209 that prevents its thermal reactivity as occurred in prior GC-based methods. The $\delta^{13}C$ value of the purified BDE-209 was determined using offline elemental analyzer isotope ratio mass spectrometry (EA/IRMS). This two-step method was applied to determine the $\delta^{13}C$ values of BDE-209 in two commercial samples and to characterize carbon isotope fractionation associated with the debromination of BDE-209 via nanoscale zero-valent iron.

Results: The mean values of daily $\delta^{13}C$ analyses of six replicates of a BDE-209 standard varied from −27.66‰ to −27.92‰, with a standard deviation ranging from 0.07‰ to 0.16‰, indicating a good reproducibility of EA/IRMS. The EA/IRMS analysis of the purified BDE-209 standard indicated no obvious isotope fractionation during the sample purification. The impurity content in commercial BDE-209 samples may contribute additional variation of the $\delta^{13}C$ values of BDE-209. The $\delta^{13}C$ values of BDE-209 gradually changed from −27.47 ± 0.37‰ to −24.59 ± 0.19‰ when 74% of the BDE-209 standard was degraded within 36 h. The estimated carbon isotope enrichment factor was −1.72 ± 0.18‰.

Conclusions: The two-step method based on HPLC and EA/IRMS avoids the thermal instability of BDE-209 in the traditional CSIA method. It offers a novel approach for elucidating the degradation mechanisms of BDE-209 in the environment and for source identification in contaminated sites.

1 | INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are the most widely used brominated flame retardant additives in such commercial products as plastics, vehicles, textiles, buildings and electronic equipment.[1] Decabrominated diphenyl ether (BDE-209) is the dominant PBDE homologue, constituting approximately 75% of the worldwide use of PBDEs.[2] Because of its persistent, bioaccumulative and toxic effects,[3] BDE-209 has been phased out in Europe and was added to Annex A under the Stockholm Convention on Persistent Organic Pollutants.[4] However, it is continues to be emitted from existing PBDE-containing products, leading to its wide occurrence in the environment. Surface soil samples collected from sites of plastic/PBDE manufacturers in China were shown to have PBDE concentrations ranging from 73 216 to 226 906 ng g^{-1} dry weight, of which BDE-209 accounted for 68.8–100%.[5] Once in the environment, BDE-209 may undergo biodegradation and abiotic degradation (i.e. photolysis, oxidation and reductive degradation) to form less-brominated PBDE congeners, some of which are more toxic and bioavailable than the parent BDE-209.[6,7] Characterizing the degradation of BDE-209 for environmental samples is key to assessing environmental risk and to developing effective treatment methods for remediation of BDE-209-contaminated sites.

Compound-specific isotope analysis (CSIA) is a powerful tool for source identification and process characterization involving such persistent organic pollutants as hexachlorocyclohexanes, polychlorinated biphenyls and PBDEs.[8-10] Prior studies showed that organic compounds having lighter carbon isotopes (i.e. ^{12}C) are preferentially transformed in abiotic and biotic degradation processes, resulting in the enrichment of heavier carbon isotopes (i.e. ^{13}C) in the remaining organic reactant. Determining the carbon isotope composition for a target organic pollutant requires sophisticated separation and purification procedures since background organic carbon and organic compounds with similar structures overshadow the carbon isotope signature of the target organic pollutant. The traditional CSIA method uses gas chromatography–combustion isotope ratio mass spectrometry (GC/C-IRMS) and has been successfully applied in characterizing bioaccumulation processes and abiotic and biotic degradation of a wide range of persistent organic pollutants in the environment.[11,12] Such a GC/C-IRMS method was also used by Mai and colleagues for the measurement of the carbon isotope compositions of tri- to hexa-BDEs in sediments and biological samples.[12-15] However, GC/C-IRMS is not suitable for highly brominated PBDEs, especially for BDE-209, because BDE-209 is not thermally stable and tends to be incompletely combusted due to the inhibitory effect of Br^-.[13] Thermal instability during the GC separation can lead to the fractionation of stable carbon isotopes among the parent compound and thermal degradation products, whereas incomplete combustion in the combustion oven after GC separation and before IRMS analysis can cause large inaccuracy in isotope measurement.

The goal of the study reported here was to develop an alternative CSIA method that can avoid the effects of both thermal instability and incomplete combustion of BDE-209. Chen et al[16] used elemental analyzer–isotope ratio mass spectrometry (EA/IRMS) for determining the $\delta^{13}C$ values of commercial BDE-209 samples. They found that BDE-209 could be completely combusted at 950°C in the presence of O_2 and sufficient catalysts. It should be noted, however, that the reported EA/IRMS method used solid samples that may contain impurities and the accuracy of its isotope analysis could not be assessed. Thus, a pretreatment procedure for the separation and purification of the target BDE-209 is needed before EA/IRMS can be applied to determine $\delta^{13}C$ values for the single compound. In the study reported here, we used a high-purity BDE-209 standard to develop a high-performance liquid chromatography (HPLC) method as the first step for purification and verified an offline EA/IRMS method as the second step for precisely determining the carbon isotopic composition of the purified BDE-209. The two-step method was further applied to determine the $\delta^{13}C$ values of BDE-209 in two commercial samples and to characterize carbon isotope fractionation associated with the debromination of BDE-209 by nanoscale zero-valent iron (nZVI).

2 | EXPERIMENTAL

2.1 | Chemicals and materials

The BDE-209 standard was purchased from Alfa Aesar (Ward Hill, MA, USA; >99.0% pure, powder). Two commercial BDE-209 samples were purchased from two different suppliers, namely BDE-209I (>96.4% pure, Dr. Ehrenstorfer GmbH, Augsburg, Germany) and BDE-209II (>95.0% pure, powder, TCI Tokyo Chemical Industry Company, Tokyo, Japan). Methanol (Merck, Darmstadt, Germany) and tetrahydrofuran (THF; HiPure Chem, Elmsford, NY, USA) of HPLC grade were used as received. HPLC-grade dichloromethane (DCM; HiPure Chem) was distilled before use. Tin capsules (4 mm × 4 mm × 11 mm; Aladdin, i-Quip, Shanghai, China) were ultrasonically cleaned in organic solvent and used as containers for loading samples to be combusted for $\delta^{13}C$ measurement by EA/IRMS. Before analysis, samples were weighed, transferred into the tin capsules and subsequently sealed and folded. Deionized water (18.2 MΩ cm) produced by Unique (Research Water Purification Technology Co. Ltd, Xiamen, China) was used in all experiments. Concentrated hydrochloric acid (HCl, 37% w/w), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, AR, 99%) and sodium borohydride ($NaBH_4$, 98%) of guaranteed reagent grade were purchased from Guangzhou Chemical Reagents Factory (Guangzhou, China). nZVI was synthesized following the method described by Li et al.[17]

Reference materials RM 8540 (IAEA-CH-7, polyethylene foil) and RM 8542 (IAEA-CH-6, sucrose) were obtained from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). RM USGS40 (L-glutamic acid) was prepared by Reston Stable Isotope Laboratory of the United States Geological Survey (Reston, VA, USA). The protein (casein) standard OAS (B2155, certificate no. 114859) was purchased from Elemental Microanalysis (Okehampton, UK). The

information for the certified $\delta^{13}C$ values of these reference materials is presented in Table S1 (supporting information).

2.2 | EA/IRMS method

The EA/IRMS method used in Chen et al[16] was verified in this study using a Flash 2000 elemental analyzer coupled to a Delta V Advantage isotope ratio mass spectrometer via a ConFlo IV interface (all from Thermo Fisher Scientific, Waltham, MA, USA). A fixed amount of a solid sample of the high-purity BDE-209 standard was loaded into a tin capsule, which was placed in the oven of the elemental analyzer and combusted in a combustion CuO tube at 950°C. The generated CO_2 was carried by helium gas to the isotope ratio mass spectrometer for carbon isotope analysis. The stable carbon isotope ratios were expressed in the delta (δ) notation relative to the international standard Vienna Peedee Belemnite (VPDB) according to Equation (1)[18]:

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \quad (1)$$

where R_{sample} represents the $^{13}C/^{12}C$ ratio measured for the sample and $R_{standard}$ is that of the VPDB standard. The raw data (i.e. the $\delta^{13}C$ values value produced by the instrumental software) were relative to a laboratory standard CO_2 gas that was introduced at the beginning of each run. The laboratory standard gas was calibrated to VPDB by reference CO_2 standards (RM 8540, RM USGS40 and RM 8542). The protein (casein) standard OAS was analyzed after every nine samples to verify the instrumental reliability and stability. As presented in Table S1 (supporting information), the certified $\delta^{13}C$ value of the protein (casein) standard OAS was −26.98 ± 0.13‰ (mean ± expanded uncertainty). Data were processed using Isodat NT software (version 3.0, Thermo Fisher Scientific).

A series of sample weights ranging from 100 to 400 μg was analyzed to determine the optimal mass of the compound required for carbon isotope analysis of the three international reference materials. The results showed that the optimal mass was 200 μg. To assess the reproducibility of the method, six replicates of the BDE-209 standard (200 μg) were analyzed on six consecutive days.

2.3 | Blank correction

The presence of trace levels of organic impurities introduced during the separation, purification and combustion procedures may affect the accuracy of the carbon isotope analysis method developed in this study. To evaluate such effects, a two-step procedure (a HPLC method combined with an offline EA/IRMS method) described below was run for three BDE-209-free blanks. The results showed that the average mass of blank carbon was 2.94 ± 0.32 μg and the $\delta^{13}C_{Blank}$ value was −26.74 ± 0.15‰. The blank correction can be made using a two-component mixing model (sample carbon and blank carbon) according to Equations (2) and (3)[19]:

$$A_{Sample} = A_{Total} - A_{Blank} \quad (2)$$

$$\delta^{13}C_{Sample} = \frac{\delta^{13}C_{Total} \times A_{Total} - \delta^{13}C_{Blank} \times A_{Blank}}{A_{Sample}} \quad (3)$$

where A is the peak area of the m/z 44 peak, in coulombs, representing the measured sample weight; A_{Total}, A_{Blank} and A_{Sample} represent the peak area of the total carbon, blank carbon and sample carbon, respectively; and $\delta^{13}C_{Total}$, $\delta^{13}C_{Blank}$ and $\delta^{13}C_{Sample}$ represent the stable carbon isotope ratio of the total sample, blanks and target sample, respectively.

2.4 | Purification of BDE-209

The purification procedure was developed using the high-purity BDE-209 standard (Figure S1, supporting information) and verified using an impure commercial BDE-209 sample. For method development, 8 mL of BDE-209 solution in a THF–water (60:40 v/v) mixture (50 μg mL^{-1}) was extracted three times, each with an equal volume of DCM to remove inorganic impurities. The extracts were combined, concentrated with a rotary evaporator and transferred to a 2-mL glass vial. The solvent was dried by volatilization. The dried residue was solubilized in 0.5 mL of THF and further purified using HPLC performed with an LC-10A system (Shimadzu, Kyoto, Japan) equipped with a UV detector (SPD-10AV) and an autosampler (SIL-20A), and coupled to an automated fraction collector (FRC-20A; Shimadzu). A Zorbax Eclipse Plus-C18 reversed-phase column (250 mm × 4.6 mm i.d., 5 μm film thickness; Agilent, Santa Clara, CA, USA) was used. The mobile phase consisted of 96% methanol and 4% deionized water and was delivered at 1.2 mL min^{-1}. The wavelength was set at 240 nm and the injection volume was 10 μL. The HPLC chromatogram of the commercial BDE-209I sample is shown in Figure 1. Complete baseline separation was achieved for BDE-209. The BDE-209 fraction was collected over the retention time range from 19.0 to 21.0 min for the measurement of stable carbon isotope ratio. This relatively wide fraction window was chosen to avoid any potential error caused by retention time variability.

For each sample, the BDE-209 fractions from approximately 40 injections were combined and mixed with deionized water and DCM for liquid–liquid extraction which was repeated twice. The three batches of the DCM phase were combined, rotary-evaporated and dried via volatilization. The residue was re-dissolved in 0.5 mL of DCM and the effectiveness of the above BDE-209 purification procedure evaluated using gas chromatography–mass spectrometry (GC/MS) as described below. After purity evaluation, the DCM solution was transferred to a tin capsule and the solvent was evaporated before the sample was used in EA/IRMS analysis.

The GC/MS purity evaluation was performed using a GCMS-QP2010 Plus single-quadrupole mass spectrometer (Shimadzu) equipped with a DB5-MS capillary column (15 m × 0.25 mm i.d.,

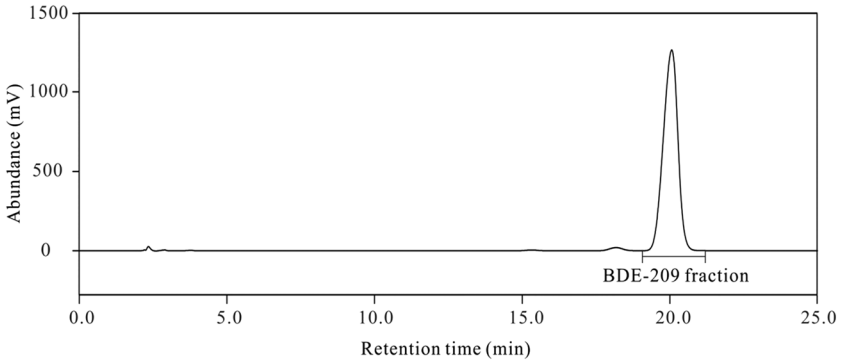

FIGURE 1 HPLC chromatogram of commercial BDE-209I

0.10 μm film thickness; Shimadzu). The column temperature was initiated at 110°C (held for 5 min) and increased to 200°C at 20°C min^{-1} (held for 4.5 min), and to a final temperature of 310°C at 10°C min^{-1} (held for 30 min). The temperature of the injection port was 250°C. Methane (99.999%; Messer Griesheim, Foshan, Guangdong, China) was used as the chemical ionization gas and helium (99.999%; Messer Griesheim, Duisburg, Germany) was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The ion source and interface temperatures were set to 250°C. The mass spectrometer was operated in negative chemical ionization mode. Acquisition in full-scan mode was performed with scan speed at 3333 *m/z* units per second and a scan range from *m/z* 50 to 1000.

2.5 | Debromination

Duplicate batch experiments were conducted in an anaerobic glovebox using 800-mL screw-capped glass bottles containing 500 mL of a THF–water (60:40 v/v) suspension of 1.0 g L^{-1} nZVI and 50 μg mL^{-1} BDE-209. The BDE-209 stock solution was prepared in THF. The reaction was initiated by adding an appropriate amount of BDE-209 stock solution into the suspensions. The bottles were wrapped with aluminum foil to exclude photochemical reactions and placed on a magnetic stirrer at 300 rpm at room temperature (28 ± 2°C). At a given sampling time, an aliquot of 1 mL of suspension was sampled for the analysis of the residual concentration of BDE-209, and larger volume of BDE-209 (>8 mL) was taken out for stable isotope analysis. An amount of 40 μL of HCl (5 M) was added to each vial for dissolving nZVI solids to release BDE-209 into the solution phase. The concentrations of BDE-209 were determined with HPLC-UV.

3 | RESULTS AND DISCUSSION

3.1 | Verification of EA/IRMS method

3.1.1 | Accuracy and precision

The protein (casein) standard OAS was used for the estimation of accuracy and precision of the EA/IRMS method. Two replicates of casein were analyzed on each of five consecutive days. The measured $\delta^{13}C$ value of ten replicates of casein was −27.03 ± 0.17‰, compared with the certified $\delta^{13}C$ value of −26.98 ± 0.13‰ (mean ± expanded uncertainty). The absolute difference between the measured and certified $\delta^{13}C$ values was 0.05‰, suggesting that the accuracy of this method is good. The precision (i.e. standard deviation) of casein of this method is 0.17‰.

3.1.2 | Reproducibility

To obtain more accurate results with good reproducibility, it is important to determine the optimal mass of BDE-209 required for carbon isotope analysis by EA/IRMS. We thus analyzed a series of sample weights ranging from 100 to 400 μg following the same combustion procedure. The mean $\delta^{13}C$ values of the BDE-209 standard slightly decreased from −27.73 ± 0.11‰ to −28.05 ± 0.06‰ when the sample weights increased from 100 to 400 μg (Table 1). The precisions were within the analytical precision (± 0.17‰) of casein determined by EA/IRMS. A small dependence of the mean $\delta^{13}C$ values on the sample weight was observed (Figure 2), although the mean $\delta^{13}C$ value (−27.97 ± 0.11‰) of 250 μg of BDE-209 was similar to that (−27.97 ± 0.06‰) of 300 μg of BDE-209. As the variation of the $\delta^{13}C$ values of the BDE-209 standard over sample weights in the range 100–200 μg was not significant at the 0.05 level of significance (Table 1 and Figure 2) according to the one-way analysis of variance (ANOVA) test, these sample weights were chosen for subsequent analyses.

The day-to-day and within-day reproducibility of the $\delta^{13}C$ measurement of a BDE-209 standard by EA/IRMS was verified by analyzing six replicate samples (200 μg) on each of six consecutive days. As presented in Table 2, the mean values of daily $\delta^{13}C$ analyses varied from −27.66‰ to −27.92‰ over the six days. The precision of the $\delta^{13}C$ value of BDE-209 ranged from 0.07‰ to 0.16‰, less than the EA/IRMS analytical precision (±0.17‰) of casein. A one-way ANOVA test (Table S2, supporting information) showed that the day-to-day variation was not significant at the 0.05 level ($P = 0.09$). The day-to-day variation accounted for 26% of the total measurement variability, much smaller than the contribution of the within-day variation (74%). These results demonstrated good reproducibility of the stable carbon isotope ratios of BDE-209 measured by EA/IRMS.

TABLE 1 Carbon isotope ratios ($\delta^{13}C$ values, ‰) of BDE-209 standard at different sample weights determined by EA/IRMS

Sample weight (μg)	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	SD
100	−27.80	−27.76	−27.74	−27.50	−27.78	−27.79	−27.73	0.11
150	−27.67	−27.76	−27.80	−27.78	−27.79	−27.79	−27.77	0.05
200	−27.80	−27.86	−27.64	−27.86	−27.89	−27.87	−27.82	0.09
250	−27.88	−27.94	−27.84	−28.01	−28.11	−28.07	−27.97	0.11
300	−27.98	−27.92	−27.90	−27.95	−28.03	−28.03	−27.97	0.05
350	−27.97	−28.10	−28.01	−27.96	−28.05	−27.97	−28.01	0.06
400	−27.96	−28.03	−28.13	−28.10	−28.09	−28.02	−28.05	0.06

3.1.3 | Uncertainty estimation

In order to comprehensively estimate the measurement uncertainty, the uncertainty u of this method derived from reference materials, repeatability and blank correction was evaluated according to Eurachem CITAC Guide.[20] The standard uncertainty $u(x)$ was calculated according to Equation (4)[21]:

$$u(x) = \mathrm{SD}/\sqrt{n} \quad (4)$$

where SD is the standard deviation of the replicated samples and n is the number of replicates. The standard uncertainties derived from RM 8542, RM USGS40 and RM 8540, repeatability and blank correction, named as u_{ref1}, u_{ref2}, u_{ref3}, u_{rep} and u_{cor}, were determined to be 0.0173, 0.0115, 0.0173, 0.0173 and 0.0866, respectively. The combined standard uncertainty u_c was calculated according to Equation (5)[20]:

$$u_c = \sqrt{\sum\left((u_{ref1})^2 + (u_{ref2})^2 + (u_{ref3})^2 + (u_{rep})^2 + (u_{cor})^2\right)} \quad (5)$$

The combined standard uncertainty of measurement was determined as ±0.09‰. The expanded uncertainty u was calculated from the combined standard uncertainty u_c and the coverage factor k using Equation (6)[20]:

$$u = ku_c \quad (6)$$

The expanded uncertainty of the measurement was determined as ±0.39‰ (k = 4.3, 95% confidence level). The percentage contribution of uncertainty sources to the combined uncertainty was calculated. As presented in Table S3 (supporting information), the contribution of the blank correction to the measurement uncertainty was estimated to be 87.9%, suggesting that blank correction was the major uncertainty source.

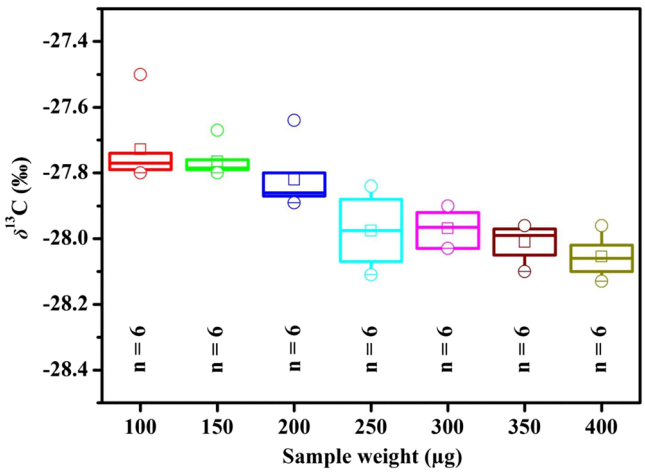

FIGURE 2 Box plot of carbon isotopic compositions of BDE-209 with respect to sample weight. The box stretches from the 25th percentile to the 75th percentile. The solid line within each box represents the median. The square represents the mean value. The whiskers indicate the minimum and maximum observed value or 1.5 times the interquartile range. Circles outside each box represent outliers lower or higher than 1.5 times the interquartile range [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Purification of BDE-209

In order to investigate the isotope fractionation during the sample purification process, the $\delta^{13}C$ values of the BDE-209 standard before and after sample purification were measured. As presented in Table 3, the mean $\delta^{13}C$ values of the BDE-209 standard before and after purification were −27.87 ± 0.03‰ and – 27.68 ± 0.07‰, respectively. The difference in the mean $\delta^{13}C$ values of the BDE-209 standard before and after sample purification process was 0.19‰, which was less than the expanded uncertainty (±0.39‰) of the EA/IRMS analysis, demonstrating a stable isotopic ratio conservation throughout the entire sample preparation procedure.

This sample preparation procedure was applied in the determination of the $\delta^{13}C$ value of BDE-209 in two commercial samples (BDE-209I and BDE-209II). These commercial samples contained some impurities identified as nonabrominated diphenyl ethers (nona-BDEs) by GC/MS (Figure 3A). After purification, the nona-BDE peaks disappeared and only the BDE-209 peak was present in the GC/MS chromatogram (Figure 3B), indicating that the nona-BDE congeners were successfully removed from the samples and the purified BDE-209 could be analyzed by EA/IRMS. As presented in Table 3, the average $\delta^{13}C$ values (± standard deviation)

TABLE 2 Carbon isotope ratios ($\delta^{13}C$ values, ‰) of 200 μg of BDE-209 standard in day-to-day and within-day analyses

Sample	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
No. 1	−27.80	−27.90	−27.73	−27.68	−27.81	−27.91
No. 2	−27.86	−28.06	−27.77	−27.73	−27.52	−27.79
No. 3	−27.64	−27.89	−27.75	−27.87	−27.85	−27.97
No. 4	−27.86	−27.88	−28.05	−27.46	−27.88	−27.50
No. 5	−27.89	−27.94	−27.51	−27.67	−27.87	−27.74
No. 6	−27.87	−27.84	−27.88	−27.55	−27.96	−27.66
Average	−27.82	−27.92	−27.78	−27.66	−27.82	−27.76
SD	0.09	0.07	0.16	0.13	0.14	0.16

TABLE 3 Mean $\delta^{13}C$ values of BDE-209 in one BDE-209 standard and two commercial BDE-209 samples. $\delta^{13}C_0$ is the $\delta^{13}C$ value of BDE-209 before purification and $\delta^{13}C_{purified}$ is its $\delta^{13}C$ value after purification. Values are expressed as means and standard deviations (SD, n = 3)

Sample	Sample purity (%)	$\delta^{13}C_0$ ± SD (‰)	$\delta^{13}C_{purified}$ ± SD (‰)[a]	$\triangle\delta^{13}C$ (‰)[b]
BDE-209 standard	>99.0	−27.87 ± 0.03	−27.68 ± 0.07	0.19
Commercial BDE-209I	96.4	−28.03 ± 0.06	−27.73 ± 0.17	0.30
Commercial BDE-209II	95.0	−27.86 ± 0.15	−28.29 ± 0.24	−0.43

[a] $\delta^{13}C_{purified}$ values were corrected for the contribution of blank carbon.
[b] $\triangle\delta^{13}C$ (‰) = $\delta^{13}C_{purified}$ (‰) − $\delta^{13}C_0$ (‰).

of commercial BDE-209I and BDE-209II determined using EA/IRMS were −28.03 ± 0.06‰ and −27.86 ± 0.15‰, respectively. After purification, the values changed to −27.73 ± 0.17‰ and −28.29 ± 0.24‰, respectively. The absolute difference in $\delta^{13}C$ values before and after purification was 0.43‰ for BDE-209II (>95% purity), which was higher than the expanded uncertainty (0.39‰) of EA/IRMS, suggesting that the impurity content in commercial BDE-209 samples may contribute additional variation to the $\delta^{13}C$ values. Thus, it is very important and necessary to purify commercial BDE-209 samples before stable carbon isotope analysis.

3.3 | Application of the method

ZVI has been considered as a promising treatment technology for halogenated compounds (i.e. trichloroethene, tetrachloroethene,

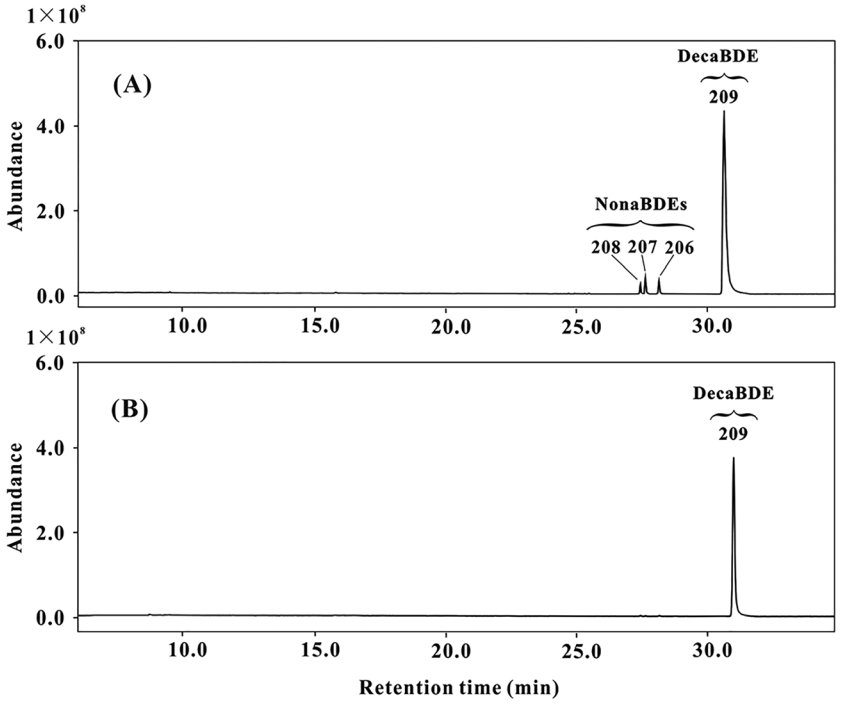

FIGURE 3 Full-scan GC/MS chromatograms of unpurified (A) and purified (B) commercial BDE-209I sample

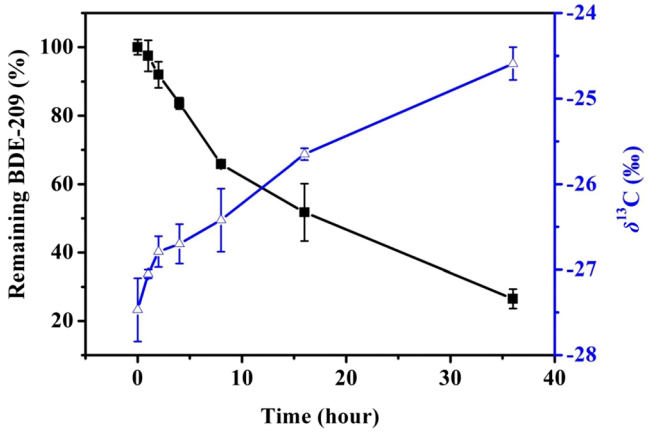

FIGURE 4 Remaining percentage and $\delta^{13}C$ values of BDE-209 during the degradation process of BDE-209 via nZVI. The mean value and standard deviation of three replicates are shown [Color figure can be viewed at wileyonlinelibrary.com]

polychlorinated biphenyls, PBDEs). It has been widely applied to the remediation of underground water and soils contaminated by halogenated compounds.[22-24] Fu et al[25] reported that BDE-209 could be removed from contaminated soils by solubilizer-enhanced electrokinetics coupled with a ZVI-permeable reactive barrier. Recently, nZVI has been attracting much more attention due to its higher reactivity and larger reactive surface area. Keum and Li[24] reported that nZVI exhibited approximately sevenfold greater reactivity with BDE-209 than microscale ZVI with a smaller surface area. nZVI can effectively and reductively debrominate BDE-209 into di- to nona-BDEs.[26,27]

In this study, a two-step method based on HPLC and EA/IRMS was applied to investigate the carbon isotope fractionation during the degradation of BDE-209 by nZVI. As presented in Figure 4, approximately 74% of the BDE-209 could be debrominated by nZVI within 36 h. The debrominated products formed were successfully separated from BDE-209 by HPLC (Figure 5) and the $\delta^{13}C$ values of the purified BDE-209 were then determined by

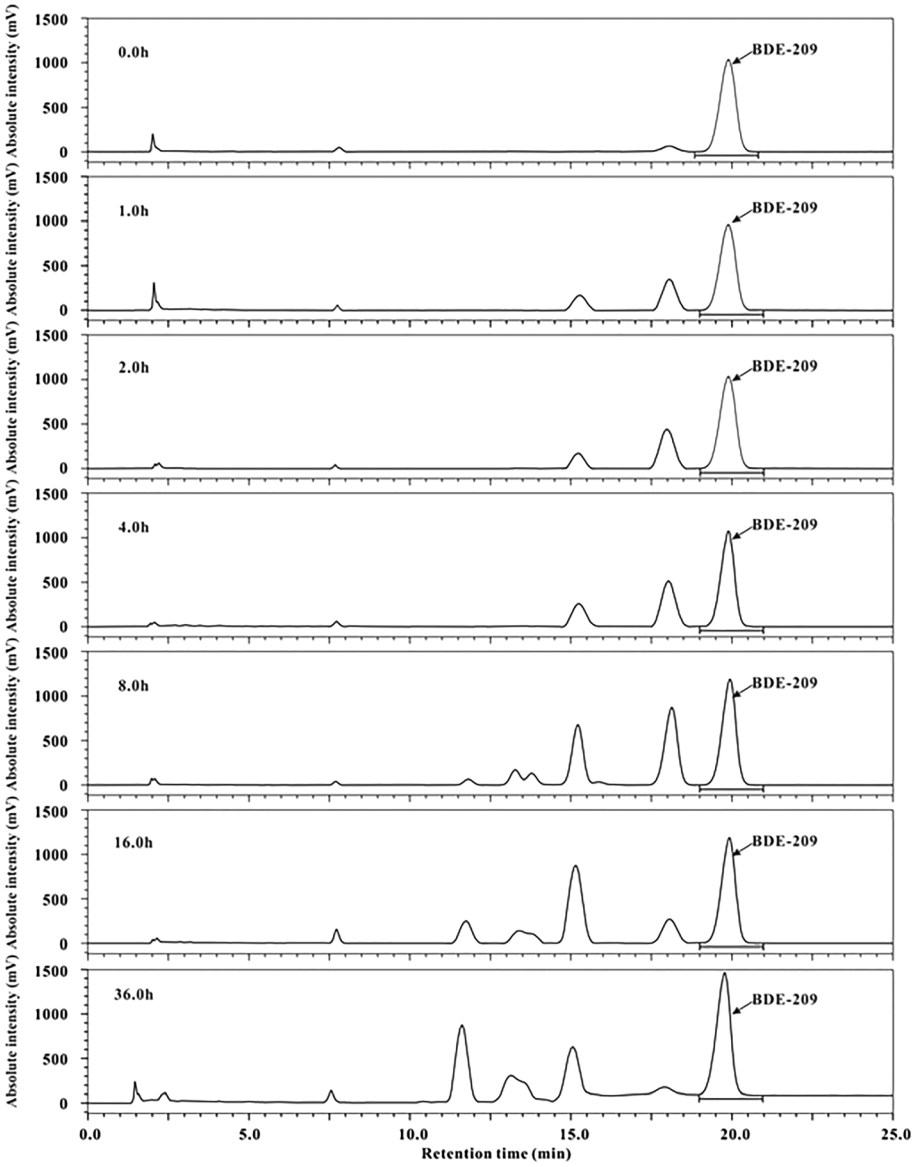

FIGURE 5 HPLC isolation of BDE-209 fraction from debrominated products generated from BDE-209 degradation via nZVI

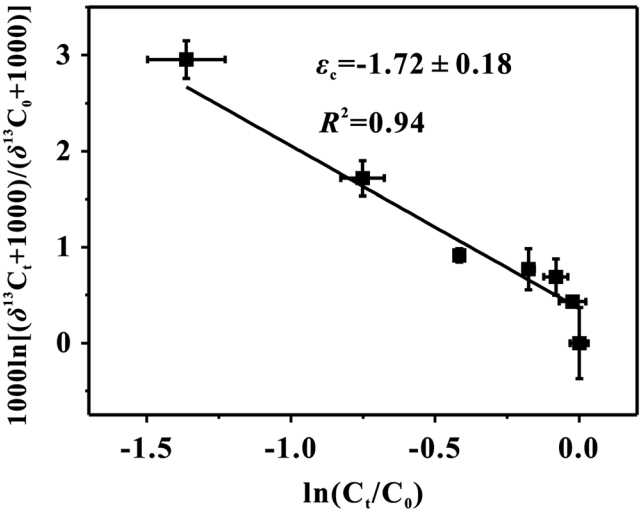

FIGURE 6 Rayleigh plot of carbon stable isotope fractionation for BDE-209 degradation via nZVI. Line represents linear regression of the datasets. The carbon isotope enrichment factor (ε_c) was derived from the slope of the linear regression lines. The correlation coefficient (R^2) of the linear regression is presented. Data shown are based on measurements from three replicates (mean ± SD, n = 3)

EA/IRMS. It was observed that the average δ^{13}C values of BDE-209 increased gradually from −27.47 ± 0.37‰ to −24.59 ± 0.19‰, suggesting that significant carbon isotope fractionation occurred in the reductive debromination of BDE-209 (Figure 4).

The relationship between isotopic fractionation and extent of debromination of BDE-209 by nZVI was modeled by a simple isotopic model, known as the Rayleigh model (Figure 6). The Rayleigh model Equation (7) describes the evolution of the isotope ratio (R) of BDE-209 during debromination (from t = 0 to t = max) as a function of the remaining fraction f of BDE-209 and the fractionation factor α for the debromination of BDE-209 to product:

$$\frac{R_t}{R_0} = f^{(\alpha-1)} \quad (7)$$

The carbon isotope enrichment factor, ε_c, is given by $\varepsilon_c = 1000(\alpha - 1)$. This was derived from the slope of the linear regression line, which can express the isotopic preference of a reaction. In this study, ε_c for the debromination of BDE-209 was −1.72 ± 0.18‰, which was much smaller than those (from −7.6 ± 0.7‰ to −29.4 ± 2.1‰) for dehalogenation processes of other halogenated pollutants by nZVI, including tribromoneopentyl alcohol, trichloroethylene and chlorinated methanes.[28-30] The lower carbon isotope enrichment factor for BDE-209 debromination may be attributed to the large number of nonreactive carbon atoms in the molecule.[31]

4 | CONCLUSIONS

A two-step method based on HPLC and EA/IRMS has been developed to determine the δ^{13}C value of BDE-209. In this method, the use of HPLC for separation and purification of BDE-209 prevents its thermal reactivity as occurs in the traditional GC-based method. The δ^{13}C value of the purified BDE-209 was determined by offline EA/IRMS. The EA/IRMS analyses showed good reproducibility of the stable carbon isotope ratios of BDE-209. This method was applied to the stable carbon composition analysis of two commercial BDE-209 samples. The results showed that the impurity content in commercial BDE-209 samples may contribute additional variation of the δ^{13}C values of BDE-209.

This method was then applied to quantify the carbon isotope fractionation in the reductive debromination of BDE-209 by nZVI. The δ^{13}C values of BDE-209 gradually increased from −27.47 ± 0.37‰ to −24.59 ± 0.19‰ with ε_c estimated at −1.72 ± 0.18‰. These results indicated that the carbon isotope composition of BDE-209 was a promising probe for evaluating the debromination process of BDE-209 by nZVI. Future application of this method in the carbon isotope analysis of BDE-209 might provide insightful information for evaluating the effectiveness of remediation processes using nZVI-based materials as reactive agents.

ACKNOWLEDGEMENTS

This study was supported financially by the National Natural Science Foundation of China (grant nos. 41773132 and 41473100), Guangdong Foundation for Program of Science and Technology Research (grant no. 2017B030314057) and the State Key Laboratory of Organic Geochemistry, GIGCAS (grant nos. SKLOGA2016A04 and SKLOG-201910). This is contribution no. IS-2817 from GIGCAS.

ORCID

Yin Zhong https://orcid.org/0000-0002-0968-124X

REFERENCES

1. Alaee M, Arias P, Sjödin A, Bergman Å. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ Int*. 2003;29(6):683-689.
2. Shi JQ, Qu RJ, Feng MB, et al. Oxidative degradation of decabromodiphenyl ether (BDE209) by potassium permanganate: Reaction pathways, kinetics, and mechanisms assisted by density functional theory calculations. *Environ Sci Technol*. 2015;49(7):4209-4217.
3. Wang ST, Wu C, Liu ZS, You H. Studies on the interaction of BDE-47 and BDE-209 with acetylcholinesterase (AChE) based on the neurotoxicity through fluorescence, UV-vis spectra, and molecular docking. *Toxicol Lett*. 2018;287:42-48.
4. UNEP. The 16 New POPs under the Stockholm Convention. 2017. http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx.
5. Deng C, Chen Y, Li JH, Li Y, Li HF. Environmental pollution of polybrominated diphenyl ethers from industrial plants in China: A preliminary investigation. *Environ Sci Pollut Res*. 2016;23(7):7012-7021.
6. Pan YH, Tsang DCW, Wang YY, Li Y, Yang X. The photodegradation of polybrominated diphenyl ethers (PBDEs) in various environmental matrices: Kinetics and mechanisms. *Chem Eng J*. 2016;297:74-96.
7. Zhao CH, Yan M, Zhong H, et al. Biodegradation of polybrominated diphenyl ethers and strategies for acceleration: A review. *Int Biodeter Biodegr*. 2018;129:23-32.

8. Luo XJ, Zeng YH, Chen HS, Wu JP, Chen SJ, Mai BX. Application of compound-specific stable carbon isotope analysis for the biotransformation and trophic dynamics of PBDEs in a feeding study with fish. *Environ Pollut*. 2013;176:36-41.
9. Wu LP, Liu YQ, Liu X, et al. Isotope fractionation approach to characterize the reactive transport processes governing the fate of hexachlorocyclohexanes at a contaminated site in India. *Environ Int*. 2019;132:105036.
10. Tang B, Luo XJ, Huang CC, Ren ZH, Zeng YH, Mai BX. Characterizing the influence of metabolism on the halogenated organic contaminant biomagnification in two artificial food chains using compound- and enantiomer-specific stable carbon isotope analysis. *Environ Sci Technol*. 2018;52(18):10359-10368.
11. Vetter W, Gaul S, Armbruster W. Stable carbon isotope ratios of POPS – A tracer that can lead to the origins of pollution. *Environ Int*. 2008;34(3):357-362.
12. Zeng YH, Luo XJ, Chen HS, Wu JP, Chen SJ, Mai BX. Separation of polybrominated diphenyl ethers in fish for compound-specific stable carbon isotope analysis. *Sci Total Environ*. 2012;425:208-213.
13. Zeng YH, Luo XJ, Chen HS, Chen SJ, Wu JP, Mai BX. Method for the purification of polybrominated diphenyl ethers in sediment for compound-specific isotope analysis. *Talanta*. 2013;111:93-97.
14. Huang CC, Zeng YH, Luo XJ, et al. In situ microbial degradation of PBDEs in sediments from an e-waste site as revealed by positive matrix factorization and compound-specific stable carbon isotope analysis. *Environ Sci Technol*. 2019;53(4):1928-1936.
15. Tang B, Luo XJ, Zeng YH, Mai BX. Tracing the biotransformation of PCBs and PBDEs in common carp (*Cyprinus carpio*) using compound-specific and enantiomer-specific stable carbon isotope analysis. *Environ Sci Technol*. 2017;51(5):2705-2713.
16. Chen LZ, Shouakar-Stash O, Ma T, Wang CL, Liu L. Significance of stable carbon and bromine isotopes in the source identification of PBDEs. *Chemosphere*. 2017;186:160-166.
17. Li D, Mao Z, Zhong Y, Huang WL, Peng PA. Reductive transformation of tetrabromobisphenol A by sulfidated nano zerovalent iron. *Water Res*. 2016;103:1-9.
18. Coplen TB. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun Mass Spectrom*. 2011;25(17):2538-2560.
19. Werner RA, Bruch BA, Brand WA. ConFlo III – An interface for high precision δ^{13}C and δ^{15}N analysis with an extended dynamic range. *Rapid Commun Mass Spectrom*. 1999;13(13):1237-1241.
20. Eurachem/CITAC Guide (2012). Quantifying Uncertainty in Analytical Measurement. 3rd ed.
21. ISO Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM 1995 with minor corrections), JCGM 100:2008, 1st ed. Geneva, Switzerland: BIPM; 2008.
22. Dries J, Bastiaens L, Springael D, Agathos SN, Diels L. Competition for sorption and degradation of chlorinated ethenes in batch zero-valent iron systems. *Environ Sci Technol*. 2004;38(10):2879-2884.
23. Zhuang Y, Ahn S, Seyfferth AL, Masue-Slowey Y, Fendorf S, Luthy RG. Dehalogenation of polybrominated diphenyl ethers and polychlorinated biphenyl by bimetallic, impregnated, and nanoscale zerovalent iron. *Environ Sci Technol*. 2011;45(11):4896-4903.
24. Keum YS, Li QX. Reductive debromination of polybrominated diphenyl ethers by zerovalent iron. *Environ Sci Technol*. 2005;39(7):2280-2286.
25. Fu RB, Wen DD, Chen X, Gu YY, Xu Z, Zhang W. Treatment of decabromodiphenyl ether (BDE209) contaminated soil by solubilizer-enhanced electrokinetics coupled with ZVI-PRB. *Environ Sci Pollut Res*. 2017;24(15):13509-13518.
26. Li A, Tai C, Zhao ZS, et al. Debromination of decabrominated diphenyl ether by resin-bound iron nanoparticles. *Environ Sci Technol*. 2007;41(19):6841-6846.
27. Yu K, Gu C, Boyd SA, et al. Rapid and extensive debromination of decabromodiphenyl ether by smectite clay-templated subnanoscale zero-valent iron. *Environ Sci Technol*. 2012;46(16):8969-8975.
28. Kozell A, Yecheskel Y, Balaban N, et al. Application of dual carbon-bromine isotope analysis for investigating abiotic transformations of tribromoneopentyl alcohol (TBNPA). *Environ Sci Technol*. 2015;49(7):4433-4440.
29. Han YL, Yan WL. Reductive dechlorination of trichloroethene by zero-valent iron nanoparticles: Reactivity enhancement through sulfidation treatment. *Environ Sci Technol*. 2016;50(23):12992-13001.
30. Lee M, Wells E, Wong YK, et al. Relative contributions of *Dehalobacter* and zerovalent iron in the degradation of chlorinated methanes. *Environ Sci Technol*. 2016;49(7):4481-4489.
31. Elsner M, Zwank L, Hunkeler D, Schwarzenbach RP. A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. *Environ Sci Technol*. 2005;39(18):6896-6916.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhu X, Zhong Y, Wang H, et al. Compound-specific carbon isotope analysis for mechanistic characterization of debromination of decabrominated diphenyl ether. *Rapid Commun Mass Spectrom*. 2020;34:e8758. https://doi.org/10.1002/rcm.8758