Chlorine and Bromine Isotope Analysis of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers Using Gas Chromatography-Quadrupole Mass Spectrometry

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ABSTRACT

A compound-specific chlorine/bromine isotope analysis (Cl-/Br-CSIA) method was developed using gas chromatography-quadrupole mass spectrometry for polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), which are toxic to human health and are frequently detected in various abiotic and biotic media. For PCB congeners, the molecular ion method for a concentration of 0.5–10.0 ppm, a dwell time of 20–100 ms, a relative EM voltage of 200 V, an electric current of 34 μA, and an ionization energy of 70 eV was determined as the most suitable scheme, which obtained standard deviations (SDs) of chlorine isotope ratios ranging from 0.00008 to 0.00068. As for the PBDE congeners, the lowest SDs, ranging from 0.00050 to 0.00172, were determined using the top four ion method with a concentration of 5–10 ppm and a dwell time of 20–50 ms. Both the chlorine and bromine isotope ratios showed strong concentration dependencies. Therefore, external standardization or detecting chlorine and bromine isotope ratios at a uniform concentration level is necessary to eliminate the concentration effect. In addition, ^{13}C-correction is critical to remove interference from carbon isotopes. This newly developed Cl-/Br-CSIA method successfully determined the chlorine/bromine isotope ratios of PCBs/PBDEs in technical mixtures and traced the chlorine/bromine isotope ratio variations of PCBs/PBDEs in photodegradation experiments, thereby suggesting that it is a promising tool for assessing the sources and transformation processes of PCBs and PDBEs in the environment.

1. Introduction

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are well-known man-made organohalogen compounds (OHCs) that have attracted increasing attention over the past few decades owing to their persistence, bioaccumulation, and toxicity [1-3]. From an environmental point of view, understanding the fate of these OHCs in the environment is essential for as-sessing the possible impacts of contaminations on humans and environmental systems. Conventional methods based on the concentrations of contaminants are susceptible to considerable misperception because multiple emission sources, complex environmental pathways, and a number of physical factors (e.g., diffusion, sorption, and volatilization) may alter their original composition, thereby preventing the understanding of the sources and transformation processes [4]. However, compound-specific stable isotope analysis (CSIA) has played an increasingly important role in source appointment and biotic (abiotic) degradation discrimination of organic contaminants in the field [5-7].

CSIA allows the evaluation of the source and transformation of organic contaminants by measuring the differences in the stable isotope composition of elements in a molecule over a location or time as a "fingerprint" of the sources as well as the transformation processes [8]. Routine methods are available for carbon sta-

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ble isotope analysis (C-CSIA) of environmental pollutants, including PCBs and PBDEs [9–11]. However, single C-CSIA is not always sufficient for identifying transformation in many cases, as factors such as mass transfer into cell membranes [12] and enzyme-substrate binding [13,14] can mask the kinetic isotope effect. In contrast, isotope analysis of two or more elements in the molecule is more robust because it allows further delineation of the involved degradation pathways [4].

As for the transformation of PCBs and PBDEs in the environment, many transformation pathways involve breaking of carbon-halogen bonds, which is similar to legacy organic halogen compounds [15-17]. Therefore, chlorine ($^{37}Cl/^{35}Cl$) and bromine ($^{81}Br/^{79}Br$) stable isotope analysis (Cl-CSIA and Br-CSIA, respectively) is a particularly promising approach to gain insights into the fate of PCBs and PBDEs, respectively. However, the application of Cl-CSIA or Br-CSIA is currently limited because of the numerous analytical challenges associated with Cl-CSIA and Br-CSIA. In the past, offline methods, including thermal ionization mass spectrometry (TIMS), dual-inlet isotope ratio mass spectrometry (DI-IRMS), and fast atom bombardment IRMS were proposed for the isotope analysis of OHCs [18,19]. However, these methods have limitations that require the isolation of large quantities of pure standard substances in advance, which is usually time-consuming and labor-intensive [18,19]. In the last few years, the online method of Cl-/Br-CSIA using gas chromatography interfaced with multiple-collector inductively coupled plasma mass spectrometry (GC-MC-ICPMS) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) were developed to measure the chlorine and bromine isotope signatures of halogenated organic contaminants in environmental samples [20-22]. Although these online methods are significantly more convenient and can obtain greater precision and accuracy of Cl-/Br-CSIA, their application is limited to very few laboratories in the world because of their high cost.

Recently, gas chromatography-quadrupole mass spectrometry (GC-qMS) was proven to carry out $^{37}Cl/^{35}Cl$ and $^{81}Br/^{79}Br$ isotope analysis in individual organic compounds with limits of detection in the pmol–nmol range [23-25]. Compared with previous methods, the GC-qMS technique avoids laborious offline separation and conversion to measurable species (such as methyl or CsCl) to achieve performances comparable to those of GC-IRMS while improving the sensitivity [26]. As a result, GC-qMS is a promising analytical method for $^{37}Cl/^{35}Cl$ and $^{81}Br/^{79}Br$ isotope measurements.

The GC-qMS technique is based on the use of multiple injections ($n = 8$–10) of the sample to measure the abundances of molecular ions or fragment ions of the target compounds. Sequential mass detection by a single detector in GC-qMS may be more prone to drift and discrimination compared with the simultaneous detection of several masses in IRMS technology; thus, the choice of computational schemes to derive chlorine and bromine isotope ratios from qMS spectra is crucial [25]. In addition, previous researches showed that the achievable precision using the GC-qMS method also depends on the compound analyzed and the instrumental setting, including the dwell time, split ratio, and collision energy [25].

Therefore, the goal of this study was to establish a strategy for $^{37}Cl/^{35}Cl$ and $^{81}Br/^{79}Br$ isotope analysis of PCBs and PBDEs. We applied and evaluated various computational schemes (i.e., molecular ion, conventional and modified multiple ion, and complete ion methods) and proposed alternative schemes (complete molecular ion method and top four and top two ion methods) to determine the chlorine and bromine isotope ratios of PCBs and PBDEs by GC-qMS. The appropriate concentration ranges of PCB/PBDE congeners and the instrument settings, including the dwell time, relative EM voltage, electric current, and ionization energy, were systematically optimized to improve the precision and reproducibility of the GC-qMS method. Furthermore, the optimized method was tested on samples from manufactured products and photodegradation experiments to verify its practicability in source apportionment and degradation discrimination of PCBs and PBDEs.

2. Experimental Section

2.1. Materials

Nine representative PCB congeners from Di- to Hepta-PCBs (PCB 4 (2-2), PCB 18 (25-2), PCB 77 (34-34), PCB 85 (234-24), PCB 132 (234-236), PCB 136 (236-236), PCB 138 (234-245), PCB 174 (2345-236), and PCB 183 (2346-245)) and eight dominated PBDE congeners in the environment (BDE 47 (24-24), BDE 77 (34-34), BDE 85 (234-24), BDE 99 (245-24), BDE 100 (246-24), BDE 153 (245-245), BDE 154 (245-246), and BDE 183 (2346-245)) were purchased from AccuStandard, Inc., USA. Isooctane (chromatographic grade) was purchased from CNW Technologies GmbH (Dusseldorf, Germany).

2.2. Chlorine or Bromine Isotope Analysis by Gas Chromatography-Quadrupole Mass Spectrometry

An Agilent 7890A GC-5975C qMS system with an electron impact (EI) ion source in selective ion monitoring mode was used to determine the chlorine and bromine isotope ratios of PCBs and PBDEs, respectively. The PCB and PBDE congeners were separated using a DB-5MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). A 1 μL portion of the solution was injected splitless in a split/splitless injector maintained at 290°C. High-purity helium was used as the carrier gas at a flow rate of 1.1 mL/min and 1.5 mL/min for PCBs and PDBEs, respectively. The temperatures of the interface, ion source, and MS quad were set at 285°C, 250°C, and 150°C, respectively. The oven temperature programs of PCBs and PBDEs are given in the Supporting Information (SI). The full scanning ion mass spectrograms of different analytes are shown in Figure S1. The optimization procedure of Cl-/Br-CSIA followed the order as shown in Figure S2, and the corresponding sequences (Seqs.) and detailed parameter settings were given in Table S1. Firstly, considering the key influence of computational schemes to Cl-/Br-CSIA, different selected ion groups were monitored for various computational schemes (Table S2) during oven temperature programs to determine the most suitable computational schemes (Seqs. 1-5 for PCBs and Seqs. 1-7 for PBDEs; seeing Table S1). Secondly, various concentration gradients, set according to environmental concentrations and instrument detectable concentrations, were tested with the optimal computational schemes (Seqs. 8-13; seeing Table S1). Finally, to improve the reproducibility and precision of the isotope ratios, the instrument settings, such as the dwell time, electric tension, electric current, and ionization energy, were systematically optimized with Seqs. 14-27 as listed in Table S1.

2.3. Computational Schemes

For a chlorinated or brominated organic contaminant, the relative abundance of an isotopologue containing k ^{37}Cl or ^{81}Br atoms out of n chlorine or bromine atoms ($I_{(n,k)}$) can be expressed as follows:

$$I_{(n,k)} = \binom{n}{k} H^k L^{n-k} \tag{1}$$

$$\binom{n}{k} = \frac{(n-k+1) \cdot (n-k+2) \cdots (n-1) \cdot n}{k!} \tag{2}$$

where H and L are the abundances of heavy isotopes (^{37}Cl, ^{81}Br) and light isotopes (^{35}Cl, ^{79}Br) in the studied chlorinated or brominated organic contaminants, respectively. Therefore, the isotope ra-

tios (R; $^{37}Cl/^{35}Cl$ or $^{81}Br/^{79}Br$) can be calculated from the ratio of any pair of molecules or fragment ions with two mass unit differences, as follows:

$$R = \frac{k}{n-k+1} \cdot \frac{I_{(n,k)}}{I_{(n,k-1)}} \quad (3)$$

Considering the bias or drift and the abundance difference of molecular or fragment ions, different computational schemes can obtain varying isotope ratios with different precision. In the current study, we first tested the published computational schemes [27], including 1) the molecular ion method considering only the two most abundant molecular ions of the analyte; 2) the conventional and modified multiple ion methods, which obtain the weighted average of all the isotope ratios calculated from the two most abundant ions of each ion group; and 3) the complete ion method considering all the ions of the analyte that contain chlorine atoms. In addition, we tested three new evaluation schemes, namely 1) the complete molecular ion method considering all the molecular ions of the analyte, which was the improvement on the complete ion method for the balance of measurement precision and elimination discrimination; 2) the top two ion method considering the two most abundant ions of the analyte; and 3) the top four ion method that determines the weighted average of the isotope ratios calculated from the two most abundant molecular ions and two most abundant fragment ions. The top two and top four ion methods, as alternative options of molecular ion method, were only applied to PBDE congeners because the molecular ion group was not the most abundant ion cluster in the PBDE full scan mass spectra, while it was the most dominant ion cluster for PCBs (Figure S1). A detailed introduction and computational formulas are given in the SI.

2.4. Correction for ^{13}C

Chlorine or bromine isotope ratios tend to be overestimated in the presence of an increasing number of ^{13}C and D atoms in the analyte [28]. Given the numerous carbon and hydrogen atoms of PCBs and PBDEs ($0 < H \leq 10$; $C = 12$) (Figure S3), correction of the chlorine or bromine isotope ratio was necessary in the present study. Considering the natural abundance of ^{13}C (1.11%) and D (0.16‰) [29], the interference from D was very small and commonly lower than the measurement accuracy of GC-qMS (Table S4). Therefore, we only corrected the bias originating from ^{13}C based on the following equation [28]:

$$R_{Cl/Br}^{corr} = R_{Cl/Br} - \frac{n_C \cdot (n_C - 1) \cdot R_C^2}{2 \cdot n_{Cl/Br}} \quad (4)$$

where $R_{Cl/Br}^{corr}$ and $R_{Cl/Br}$ are the ^{13}C-corrected and measured chlorine or bromine isotope ratios, respectively; R_C is the measured carbon isotope ratio; and n_C and $n_{Cl/Br}$ are the numbers of carbon and chlorine/bromine atoms per fragment, respectively. For computational schemes needing more than one ion group, such as top four ion method, the isotope ratios calculated from each ion group were ^{13}C-corrected firstly and then weighted average to gain the final isotope ratios.

2.5. Photodegradation Experiment

The photodegradation experiments were performed with a photochemical reactor (BL-GHX-V; Shanghai Bilon Instrument Co., Ltd. Shanghai, China). For PCBs, PCB 18 dissolved in 35 mL of n-hexane (Hex) was irradiated using a 100 W mercury lamp. The initial concentrations were set at 3 mg/L, and the sampling points were set at 0, 10, 20, 40, 70, and 120 min. The detailed information about photodegradation experiments has been published in previous literature [30]. For PBDEs, BDE 99 dissolved in 30 mL of Hex was

irradiated using simulated sunlight. The sampling points were set at 0, 3, 6, 9, 12, and 15 h. The PBDEs roughly had the same experiment process as PCBs except that the light source was replaced by a 1000 W xenon lamp and eight quartz filters were used around the lamp, $\lambda > 280$ nm, purchased from Shanghai Depai Biotech. Co. Ltd., China. At each sampling time, 5 mL of the solution was collected to determine the concentration and carbon and halogen (Cl/Br) isotope compositions. Detailed instrument analyses of the concentration and carbon isotope composition of PCBs and PBDEs have been published in previous studies [9,31].

2.6. Quality Assurance and Quality Control

All the pure solid standards of PCBs and PBDEs were dissolved in isooctane at initial concentrations of 1 mg/mL. The purity of all the dissolved PCB and PBDE congeners was checked first using GC-MS with an EI ion source in full scan mode to test whether interfering substances were introduced to the PCB and PBDE standards during the dissolution process (Figure S1). These dissolved standards were then diluted stepwise to 1 μg/mL to verify the dissolved concentrations using calibration curves constructed from eight concentrations of internal standard solutions. Six concentrations of PCB or PBDE mix standards, including 10.00 ppm, 5.00 ppm, 1.00 ppm, 0.50 ppm, 0.10 ppm, and 0.05 ppm, were obtained from these dissolved standards. For each evaluation scheme, concentration gradient, and instrument setting, 10 injections were performed. During sample testing, regular injection of 1.00 ppm mix standards was performed daily in the same instrument setting to ensure the stability of instrument response.

2.7. Statistical Analysis

SPSS 21.0 and Origin 8.0 software were used for statistical analysis and graph drawing. If there was no special illustration, then the isotope ratios were shown in the form of average ± standard deviation (AV ± SD) of 10 replicates. To compare the difference among the SDs with different evaluation parameters, homogeneity tests of 10 repetitive chlorine/bromine isotope ratios with different schemes were performed. The differences in the chlorine/bromine isotope ratios for each PCB and PBDE congener among different instrument settings were evaluated by one-way analysis of variance. The level of significance was set at $p < 0.05$ throughout the study.

3. Results and Discussion

3.1. Validation and Optimization of the Method

The computational scheme, concentration gradient, dwell time, electric voltage, electric current, and ionization energy were systematically optimized to improve the precision and reproducibility of Cl-/Br-CSIA using the GC-qMS method as much as possible. The effects of the standardization procedure, temporal drift, and ^{13}C-correction on the Cl-/Br-CSIA were then evaluated.

3.1.1. Comparison of Different Computational Schemes
Various computational schemes, including the molecular ion method, conventional and modified multiple ion method, and complete ion method, have been used to determine the chlorine or bromine isotope ratios of halogenated organic compounds [23-25]. In addition to these published schemes, we also tested three new computational schemes, including the complete molecular ion method, top two ion method, and top four ion method, under the same GC-qMS instrument settings to establish robust evaluation schemes for PCB and PBDE congeners. On the one hand, complete ion and multiple ion methods possibly need high concentration to determine the response of each ion accurately. On the
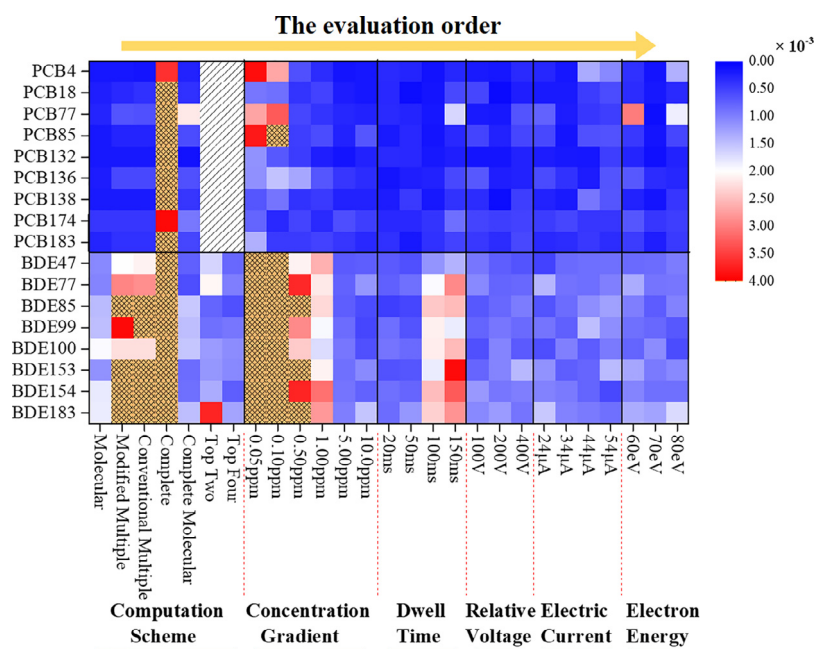
Fig. 1. Standard deviation of 10 replicates for different evaluation parameters (the symbol ⧄ indicates that the corresponding parameters were not monitored, and the symbol ▦ indicates that the corresponding SDs were larger than 0.00400; the detailed parameter settings for each evaluation scheme were given in Table S1).

other hand, the detectable concentration of Cl-/Br-CSIA should be as low as possible to expand this method's usefulness due to the trace levels of PCBs and PBDEs in the field. Balance these two aspects, concentration of 5.00 ppm was selected to evaluate various computational schemes.

With regard to PCBs, the complete ion method had the worst reproducibility with the largest SD values (SD > 0.00400) among those of the five applied schemes, thereby indicating that this method is not suitable for determining the chlorine isotope ratio of PCBs (Fig. 1). However, Jin et al. found that the complete ion method had higher precision at all concentrations for *cis*-1,2-dichloroethene relative to the molecular ion method and multiple ion methods [25]. This discrepancy might be caused by the difference of molecular structure and ionization properties on EI source between PCBs and chloroethylenes. Except for that of the complete molecular ion method for PCB 77, the reproducibilities of the molecular ion, conventional and modified multiple ion, and complete molecular ion methods for each PCB congener were similar, with SD values less than 0.00100 (Fig. 1 and Table S5). To further identify the most suitable evaluation method, 10 replicates of chlorine isotope ratios were used to evaluate the reproducibility difference among these four evaluation methods for each PCB congener with a homogeneity test. The results revealed that, except for PCBs 77, 85, and 174, there were no significant differences in reproducibility for PCB congeners among the four evaluation methods (Table 1). The molecular ion method had the smallest SD values for PCBs 77 (0.00029), 85 (0.00019), and 174 (0.00043) (Table S5). In addition, significant differences in the chlorine isotope ratios were found for each PCB congener among the four evaluation schemes (Table 1). Tang et al. reported that chlorine isotope fractionation can occur during fragmentation by EI-MS, and the molecular ion method can obtain the most precise and accurate isotope ratios [32]. Therefore, the molecular ion method was selected as the most suitable method to measure the chlorine isotope ratio of PCBs.

As for PBDEs, conventional and modified multiple ion and complete ion methods clearly did not apply for the measurement of bromine isotope ratios owing to their low precision (SD > 0.00400)

(Fig. 1 and Table S5). Significant differences ($p < 0.05$) in precision were observed among the other four evaluation methods for BDEs 47, 77, 154, and 183 with the smallest SD obtained from the complete molecular ion and top four ion methods and for BDE 85 with the smallest SD calculated from the top two ion and top four ion methods (Tables 1 and S5). Finally, the top four ion method was selected to further optimize the Br-CSIA using GC-qMS.

3.1.2. Optimization of Analytical Concentration

To evaluate the influence of the concentration on the reproducibility and precision of Cl-CSIA and Br-CSIA using GC-qMS, a series of concentrations ranging from 0.05 ppm to 10.00 ppm were continuously measured 10 times using the most suitable computational schemes for PCBs and PBDEs, respectively. The results suggested that the reproducibility of Cl-CSIA at 0.05–0.10 ppm was clearly worse than that at 0.50–10.00 ppm, whereas the precision of Br-CSIA at 0.05–1.00 ppm was clearly lower than that at 5.00–10.00 ppm (Fig. 1). Except for PCB 136, there were no significant differences in precision for Cl-CSIA and Br-CSIA at 0.50–10.00 ppm and 5.00–10.00 ppm, respectively (Table 1). These results indicated that the concentrations ranging from 0.50 ppm to 10.00 ppm for PCBs and 5.00 ppm to 10.00 ppm for PBDEs showed similarly good reproducibilities of Cl-CSIA and Br-CSIA using GC-qMS. It's worth noting that the halogen (Cl/Br) isotope ratios of most PCB and PBDE congeners showed significant differences ($p < 0.05$) among the various concentration levels, thereby suggesting concentration dependencies for PCBs and PBDEs (Table 1). Significant correlations ($p < 0.05$) between the concentrations and chlorine isotope ratios were found for PCBs 77, 85, 132, 136, 138, 174, and 183 (Fig. 2). Concentration dependence has been reported in Cl-CSIA of herbicides (atrazine, acetochlor, and metolachlor) and Br-CSIA of bromoform, 3-bromophenol, and 4-bromotoluene using GC-qMS [23,24]. This phenomenon highlights the necessity of comparing the chlorine and bromine isotope ratios of halogenated organic contaminants at the same concentration level when using GC-qMS for Cl-CSIA and Br-CSIA.

Table 1
The significant difference analysis of standard deviation (p_s) and average of chlorine or bromine isotope ratios for each analyte (p_i) among various setting for each evaluation parameter.

	Computation Schemes	Concentration gradients			Dwell Time	Relative EM Voltage	Electric Current	Ionization Energy
	p_s/p_i [a]	p_s/p_i [b1]	p_s/p_i [b2]	p_s/p_i [b3]	p_s/p_i [c]	p_s/p_i [d]	p_s/p_i [e]	p_s/p_i [f]
PCB4	0.21/0.00	0.06/0.88	0.07/0.24	0.00/1.00	0.07/0.00	0.31/0.35	0.01/0.00	0.15/0.00
PCB18	0.54/0.00	0.05/0.04	0.40/0.81	0.55/1.00	0.00/0.00	0.00/0.87	0.01/0.00	0.40/0.00
PCB77	0.00/0.00	0.32/0.00	0.21/0.00	0.28/1.00	0.00/0.01	0.00/0.37	0.47/0.00	0.00/0.73
PCB85	0.01/0.00	0.05/0.00	0.00/0.04	0.07/1.00	0.32/0.00	0.09/0.03	0.02/0.00	0.02/0.00
PCB132	0.32/0.00	0.05/0.14	0.00/0.07	0.29/1.00	0.48/0.00	0.56/0.00	0.00/0.00	0.11/0.00
PCB136	0.27/0.00	0.04/0.00	0.00/0.08	0.00/1.00	0.01/0.00	0.04/0.00	0.10/0.00	0.00/0.00
PCB138	0.27/0.00	0.07/0.00	0.00/0.03	0.04/1.00	0.01/0.00	0.00/0.00	0.03/0.00	0.09/0.00
PCB174	0.03/0.00	0.28/0.01	0.29/0.01	0.03/1.00	0.00/0.07	0.82/0.60	0.65/0.00	0.23/0.00
PCB183	0.41/0.00	0.81/0.05	0.06/0.08	0.36/1.00	0.23/0.00	0.66/0.59	0.68/0.00	0.05/0.00
BDE47	0.04/0.00	0.79/0.09	0.51/0.07	0.78/1.00	0.06/0.00	0.49/0.00	0.22/0.00	0.61/0.00
BDE77	0.00/0.00	0.37/0.01	0.00/0.00	0.35/1.00	0.00/0.04	0.41/0.00	0.60/0.00	0.67/0.00
BDE85	0.04/0.00	0.42/0.01	0.10/0.24	0.32/1.00	0.00/0.00	0.86/0.00	0.69/0.00	0.20/0.00
BDE99	0.15/0.00	0.13/0.33	0.25/0.05	0.13/1.00	0.04/0.02	0.86/0.00	0.65/0.08	0.98/0.00
BDE100	0.59/0.00	0.31/0.01	0.23/0.38	0.65/1.00	0.05/0.00	0.52/0.00	0.11/0.00	0.69/0.00
BDE153	0.50/0.00	0.96/0.46	0.36/0.01	0.48/1.00	0.00/0.15	0.66/0.00	0.37/0.00	0.26/0.00
BDE154	0.00/0.00	0.76/0.14	0.65/0.01	0.98/1.00	0.00/0.05	0.61/0.00	0.17/0.00	0.91/0.00
BDE183	0.01/0.00	0.52/0.01	0.81/0.00	0.25/1.00	0.04/0.13	0.26/0.00	0.38/0.00	0.45/0.00

[a] including molecular ion, conventional and modified multiple ion, and complete molecular ion methods for PCB congeners, and molecular ion, complete molecular ion, top two ion, and top four ion methods for PBDEs;

[b1, b2, b3] including concentration gradients of 0.50, 1.00, 5.00, and 10.00 ppm for PCBs and 5.00 and 10.00 ppm for PBDEs with un-correction, internal standard correction, and external standard correction, respectively;

[c] including dwell time of 20, 50, 100, and 150 ms for PCBs and PBDEs;

[d] including relative EM voltage of 100, 200, and 400 V for PCBs and PBDEs;

[e] including electric current of 24, 34, 44, and 54 μA for PCBs and PBDEs;

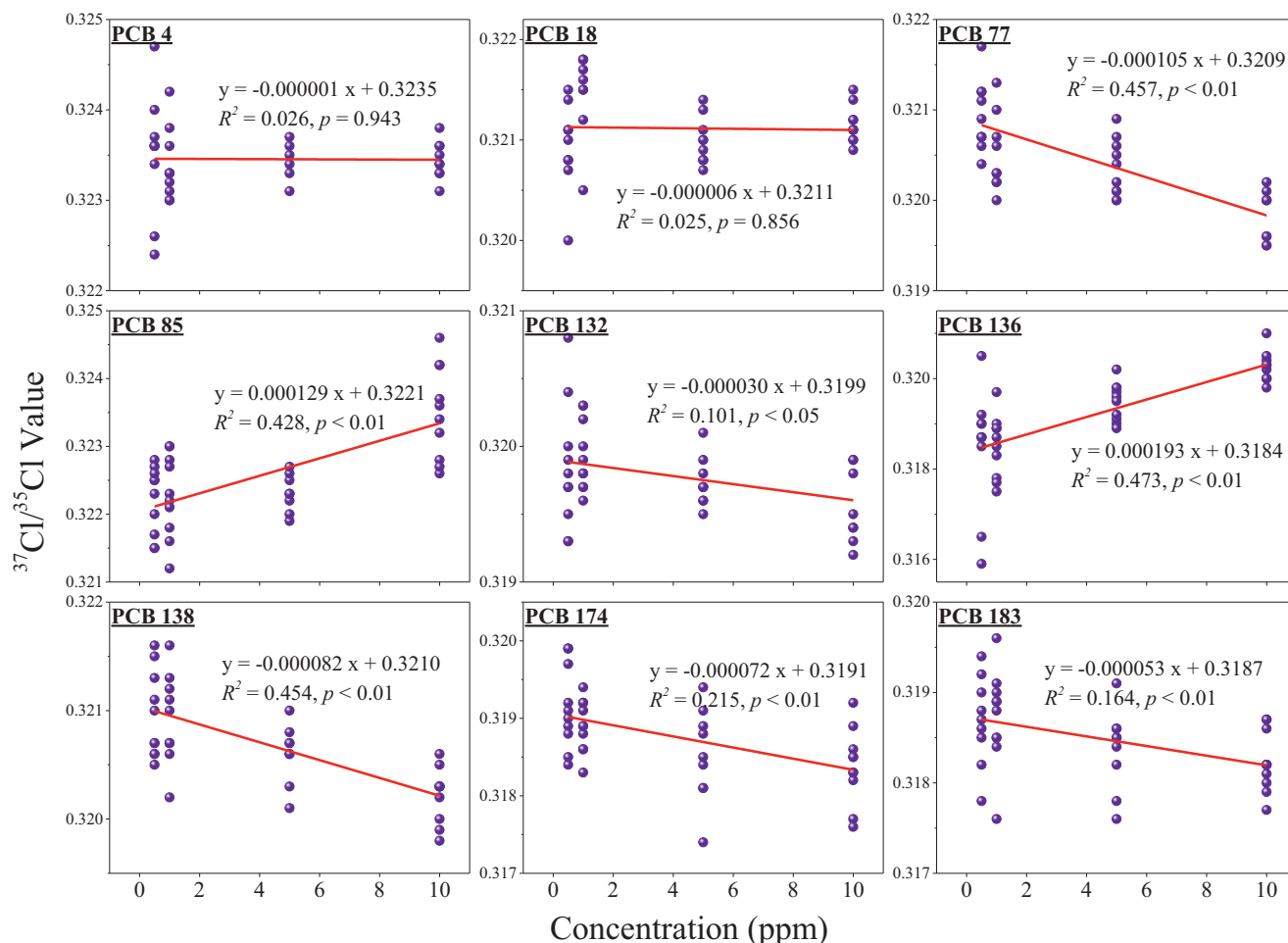[f] including ionization energy of 60, 70, and 80 eV for PCBs and PBDEs.

Fig. 2. Correlations between concentrations (0.50-10.00 ppm) and chlorine isotope ratios of PCB congeners.

3.1.3. Assessment of Instrumental Parameters

The instrumental parameters, including the dwell time of the monitoring ion, electric EM voltage, electric current, and ionization energy, could alter the stability of ionization and response of fragment ions, which would affect the accuracy and precision of Cl-CSIA and Br-CSIA using GC-qMS [23,25,32]. These factors were tested for Cl-CSIA and Br-CSIA of PCBs and PBDEs at 5.00 ppm with 10 replicates at each parameter setting. As shown in Fig. 1 and Table 1, the dwell times had a greater influence on the Br-CSIA of PBDEs than the Cl-CSIA of PCBs, whereas the relative EM voltage, electric current, and ionization energy only significantly affected some of the PCB congeners' chlorine isotope ratios. Generally, dwell times of 20–100 ms and 20–50 ms could obtain higher precision for PCBs and PBDEs, respectively (Fig. 1 and Table S5). Previous studies reported that the most suitable dwell times are 20 ms for bromoform, 3-bromophenol, and 4-bromotoluene, 30 ms for chlorinated herbicides, and 10–100 ms for chloroethylenes, varying with different GC-qMS [23-25, 33,34]. These results revealed that the optimized dwell time was compound-specific and instrument-specific. As for the relative EM voltage, electric current, and ionization energy, they only had a significant influence on the precision of the chlorine isotope ratios in PCBs 18, 77, and 138, in PCBs 4, 18, 132, and 138, and in PCBs 77 and 136, respectively (Table 1). Taking all the PCB congeners into consideration, Cl-CSIA obtained the best reproducibility when the relative EM voltage, electric current, and ionization energy were set at 200 V, 34 μA, and 70 eV, respectively (Fig. 1 and Table S5). In addition, the halogen (Cl/Br) isotope ratios of most of the PCB and PBDE congeners were significantly affected by the dwell time, relative EM voltage, electric current, and ionization energy (Table 1), and there were no external isotope standards of these analytes to calibrate the measured ratios to the standard mean ocean chlorine/bromine scale. Therefore, it is important to ensure that the Cl-/Br-CSIA is performed in the same instrument setting for the same set of experiments.

3.1.4. Effect of Standardization Procedure

Internal and external standard corrections are two important methods for reducing the measuring error. However, they are limited by the different ionization properties of the analyte *vs.* internal standard and the instrument performance fluctuation among different injections, respectively. To test the performances of these two correction strategies, internal and external standardizations were performed on the sequences of different concentration gradients of PCBs and PBDEs (Seqs. 8–13; seeing Table S1). For internal standardization, PCBs 24, 82, and 198 were selected as the internal standard of PCBs 4/18/77, PCBs 85/132/136/138, and PCBs 174/183, respectively; and PBDE 77 was selected as the internal standard for the remaining PBDE analytes due to no extra internal standard added in Br-CSIA. As for external standardization, because of no alternative PCB and PBDE standards, we used the former isotope ratio to correct the current isotope ratio of the same analytes in each sequence, e.g., using the isotope ratio of the first injection as the external standard of the isotope ratio of the second injection. As shown in Table 1, external standardization could completely remove the concentration dependencies for Cl-/Br-CSIA of PCBs and PBDEs, while internal standardization was useless for this. These findings were in accordance with a previous report on Cl-CSIA of herbicides [24].

3.1.5. Temporal Drift Monitoring and Correction for ^{13}C Atoms

The six sequences (Seqs. 1/12/16/19/22/26 for PCBs and Seqs. 7/12/16/19/22/26 for PBDEs; seeing Table S1) with the same detection conditions were selected to evaluate the temporal drift of the Cl-/Br-CSIA over a long period. As depicted in Figure S4, there was clearly no temporal drift for each PCB and PBDE congener. To test the stability of the variations between different isotope ratios of the same congeners, the variations of chlorine isotope ratios for PCB 18/77 and bromine isotope ratios for BDE 99 before and after photodegradation were determined in triplicate over 15 days. Each duplicate was calculated from 10 injections. The results showed that the variations were nearly constant with the range of 0.00365-0.00380, 0.00702-0.00722, and 0.00405-0.00423 for PCB 18, PCB 77, and BDE 99, respectively (Table S6). Moreover, the effects of the ^{13}C atom on the chlorine and bromine isotope ratios were evaluated using seq. 26 as an example. As shown in Figure S5, the carbon isotopic composition strongly affected the chlorine and bromine isotope ratios ($p < 0.05$) (Table S7). The error of the Cl-/Br-CSIA caused by carbon isotopes increased as the chlorine or bromine atoms in the analytes decreased (Figure S6). Similar patterns were found in Cl-CSIA of chlorinated ethenes and chlorinated benzenes using GC-qMS [25].

3.2. Method Application

The practicability of the newly developed Cl-/Br-CSIA using GC-qMS was tested by determining the chlorine/bromine isotope ratios of PCBs/PBDEs in technical mixtures (Fig. 3) and tracing the chlorine/bromine isotope ratio variations of PCBs/PBDEs in photodegradation experiments (Fig. 4).

3.2.1. Technical Mixtures of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers

The chlorine/bromine isotope ratios of PCBs/PBDEs in technical mixtures (PCB: Aroclor 1260; PBDE: DE-71 and DE-79) were determined (Table S8). As shown in Fig. 3, both the chlorine and bromine isotope ratios of PCBs and PBDEs decreased as the number of chlorine and bromine atoms in each commercial product increased. These findings are in accordance with the Cl-CSIA using TIMS, which reported that the δ^{37}Cl of PCB congeners decreased by -0.26‰ for each additional chlorine atom in the Aroclor and Clophen technical mixtures [35]. In addition, the same PBDE congeners commonly have different bromine isotope ratios (AV $\pm$ SD) in various commercial products, e.g., 0.94087 $\pm$ 0.00339 and 0.98287 $\pm$ 0.00223 of BDE 153 in Penta-BDE and Octa-BDE, respectively. (Table S8). These inherent properties are similar to the carbon isotope compositions of PCBs and PBDEs in different commercial products, which are important to determine the contaminant-sources in the complex environment [9,36].

3.2.2. Photodegradation of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers

The chlorine and bromine isotope ratios of PCB 18 and BDE 99 shifted by 0.00382 (PCB 18) and 0.00393 (BDE 99) over the course of irradiation, respectively, which were larger than their corresponding analytical errors with the largest SDs of 0.00051 (PCB 18) and 0.00129 (BDE 99) (Fig. 4). Moreover, the variations in the chlorine and bromine isotope ratios were significantly correlated with the irradiation time, Ln (C_t/C_0), and Ln (R_t/R_0)-C (Fig. 4). The calculated chlorine and bromine isotope enrichment factors (ε_{Cl} and ε_{Br}) were -6.0‰ and -3.0‰ for PCB 18 and BDE 99, respectively. Combined with the change in the carbon isotope ratio, the Λ values ($\varepsilon_{Cl}/\varepsilon_C$ and $\varepsilon_{Br}/\varepsilon_C$) were 2.25 and 0.56 for PCB 18 and BDE 99, respectively (Fig. 4). Although the ^{13}C-correction significantly changed the chlorine/bromine isotope ratios and altered the correlation coefficients between them and irradiation time, it did not cause changes in the ε_{Cl}, ε_{Br}, and Λ values ($\varepsilon_{Cl}/\varepsilon_C$ and $\varepsilon_{Br}/\varepsilon_C$) (Fig. 4). These results suggest that Cl-/Br-CSIA using GC-qMS is an effective tool for tracing the chlorine and bromine isotope ratio variations of PCBs and PBDEs in the process of degradation. However, there are
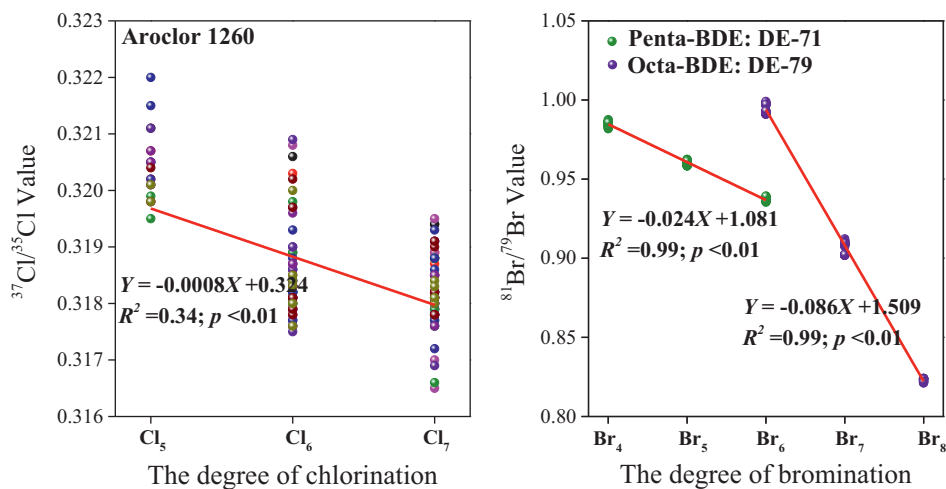
Fig. 3. Variation in the chlorine and bromine isotope ratios in polychlorinated biphenyl and polybrominated diphenyl ether commercial products.
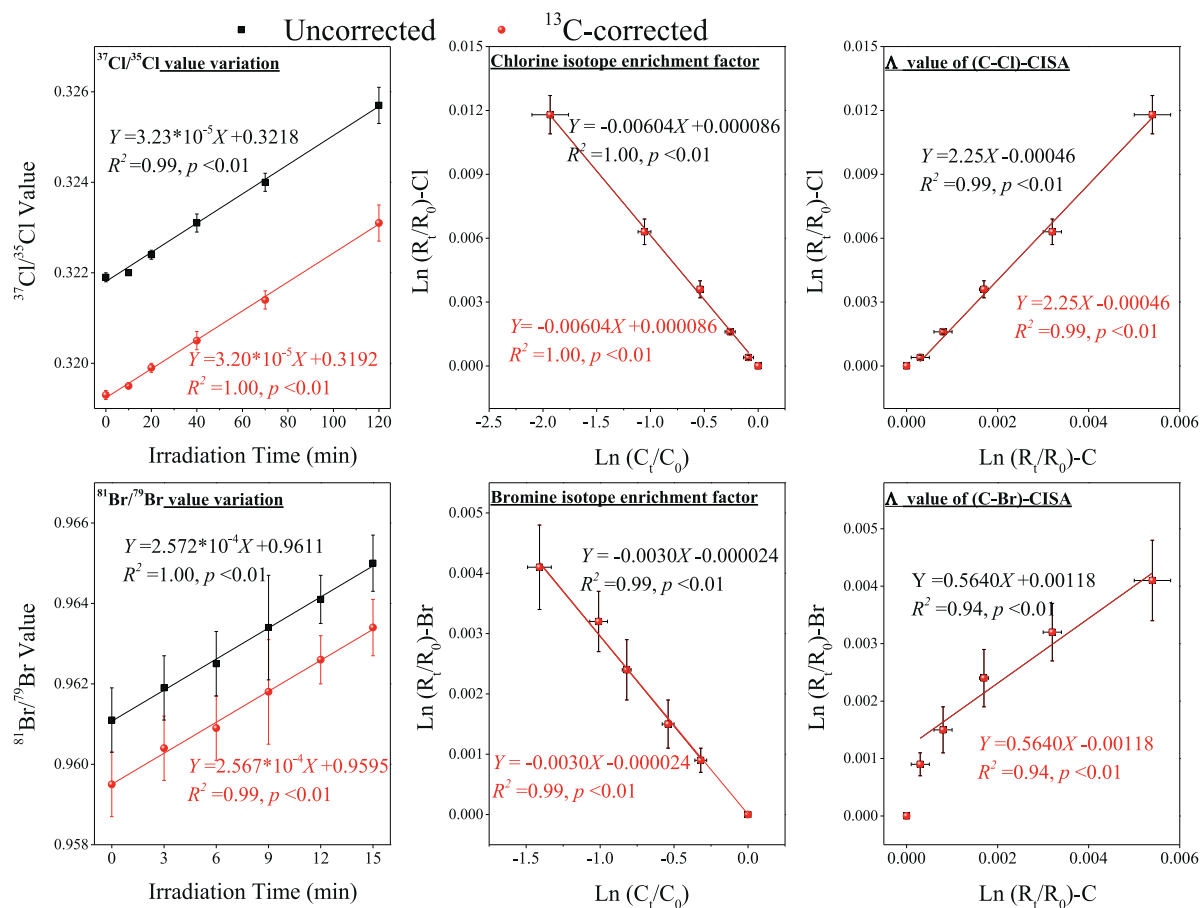

Fig. 4. Variation in the chlorine and bromine isotope ratios during the photodegradation of PCB 18 and PBDE 99.

no available external isotopic standards of the investigated compounds to calibrate these data to the standard mean ocean chlorine/bromine scale. Furthermore, previous research demonstrated that the chlorine/bromine isotope ratios of the same compounds detected by different GC-qMS systems had different correlations with their corresponding standard isotope ratios, which limits the application of Cl-/Br-CSIA using GC-qMS to some extent [22].

4. Conclusion and Implications

The current study developed a Cl-/Br-CSIA using the GC-qMS method for PCBs and PBDEs, which are toxic to human health and are frequently detected in various abiotic and biotic media [1,3,37]. The molecular ion and top four ion methods are the most suitable computational schemes for PCBs and PBDEs, respectively. Although concentration ranges of 0.50–10.00 ppm (PCBs) and 5.00–10.00

ppm (PBDEs) could obtain higher precision for Cl-/Br-CSIA, both the chlorine and bromine isotope ratios showed strong concentration dependencies. Therefore, external standardization or detection of chlorine and bromine isotope ratios at a uniform concentration level is necessary to eliminate the concentration effect. As for the instrument settings, the most precise chlorine isotope ratios were determined with a dwell time of 20–100 ms, relative EM voltage of 200 V, electric current of 34 μA, and ionization energy of 70 eV. The most precise bromine isotope ratios were obtained with a dwell time of 20–50 ms, and they were not significantly affected by the relative EM voltage, electric current, and ionization energy. The newly developed Cl-/Br-CSIA using GC-qMS successfully determined the chlorine/bromine isotope ratios of PCBs/PBDEs in technical mixtures and traced the chlorine/bromine isotope ratio variations of PCBs/PBDEs in photodegradation experiments, which are significant for evaluating the sources and degradation processes of PCBs and PBDEs in the field environment. Dual C–Cl or C–Br isotope analyses could provide more insights into the various degradation mechanisms of PCBs and PBDEs. ^{13}C-correction of Cl-/Br-CSIA is important for studying degradation, although it may not cause changes in the ε_{Cl}, ε_{Br}, and Λ values ($\varepsilon_{Cl}/\varepsilon_C$ and $\varepsilon_{Br}/\varepsilon_C$) owing to limited carbon isotope fractionation. However, we had no external isotopic (Cl/Br) standards of PCBs or PBDEs to calibrate the chlorine/bromine isotope data to international standards, which limited the comparison of different studies. Thus, future studies are warranted to further expand the practicability of Cl-/Br-CSIA by GC-qMS.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2020.461715.

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