Chlorine and Bromine Isotope Fractionation of Halogenated Organic Pollutants on Gas Chromatography Columns

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Abstract

ABSTRACT Compound-specific chlorine/bromine isotope analysis (CSIA-Cl/Br) has become a useful approach for degradation pathway investigation and source appointment of halogenated organic pollutants (HOPs). CSIA-Cl/Br is usually conducted by gas chromatography-mass spectrometry (GC-MS) techniques, which could be negatively impacted by chlorine and bromine isotope fractionation of HOPs on GC columns. In this study, 31 organochlorines and 4 organobromines were systematically investigated in terms of chlorine/bromine isotope fractionation on GC columns using GC-double focus magnetic-sector high resolution MS (GC-DFS-HRMS). On-column chlorine/bromine isotope fractionation behaviors of the HOPs were explored, presenting various isotope fractionation modes and extents. Twenty-nine HOPs exhibited inverse isotope fractionation, and only polychlorinated biphenyl-138 (PCB-138) and PCB-153 presented normal isotope fractionation. And no observable isotope fractionation was found for the rest four HOPs, i.e., PCB-101, 1,2,3,7,8-pentachlorodibenzofuran, PCB-180 and 2,3,7,8-tetrachlorodibenzofuran. The isotope fractionation extents of different HOPs varied from below the observable threshold (5.0‰) to 73.1‰ (PCB-18). The mechanisms of the on-column chlorine/bromine isotope fractionation were tentatively interpreted with the Craig-Gordon model and a modified two-film model. Inverse isotope effects and normal isotope effects might contribute to the total isotope effects together and thus determine the isotope fractionation directions and extents. Proposals derived from the main results of this study for CSIA-Cl/Br research were provided for improving the precision and accuracy of CSIA-Cl/Br results. The findings of this study will shed light on the development of CSIA-Cl/Br methods using GC-MS techniques, and help to implement the research using CSIA-Cl/Br to investigate the environmental behaviors and pollution sources of HOPs.

Full Text

Preamble

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Abstract

Compound-specific chlorine/bromine isotope analysis (CSIA-Cl/Br) has become a useful approach for investigating degradation pathways and source apportionment of halogenated organic pollutants (HOPs). CSIA-Cl/Br is typically conducted using gas chromatography–mass spectrometry (GC–MS) techniques, which can be negatively impacted by chlorine and bromine isotope fractionation of HOPs on GC columns. In this study, 31 organochlorines and 4 organobromines were systematically investigated for chlorine/bromine isotope fractionation on GC columns using GC–double-focusing magnetic-sector high-resolution MS (GC–DFS-HRMS). The on-column chlorine/bromine isotope fractionation behaviors of the HOPs were explored, revealing various isotope fractionation modes and extents. Twenty-nine HOPs exhibited inverse isotope fractionation, while only polychlorinated biphenyl-138 (PCB-138) and PCB-153 showed normal isotope fractionation. No observable isotope fractionation was found for the remaining four HOPs: PCB-101, 1,2,3,7,8-pentachlorodibenzofuran, PCB-180, and 2,3,7,8-tetrachlorodibenzofuran. The isotope fractionation extents of different HOPs varied from below the observable threshold (5.0‰) to 73.1‰ (PCB-18). The mechanisms of on-column chlorine/bromine isotope fractionation were tentatively interpreted using the Craig-Gordon model and a modified two-film model. Inverse isotope effects and normal isotope effects may both contribute to the total isotope effects, thus determining the direction and extent of isotope fractionation. Based on these findings, recommendations for CSIA-Cl/Br research were provided to improve the precision and accuracy of results. This study will shed light on the development of CSIA-Cl/Br methods using GC–MS techniques and facilitate research employing CSIA-Cl/Br to investigate the environmental behaviors and pollution sources of HOPs.

Introduction

Gas chromatography (GC) is a powerful analytical tool widely used in environmental analysis, petrochemical industry, product quality inspection, bioanalysis, and metabolomics. Due to its high separation efficiency, GC has even been applied to separate isotope-labeled compounds from their native counterparts. GC has proven capable of separating isotopologues of various compounds, such as caffeine and caffeine-1,3,7-$(CD_3)_3$, n-alkanes (C_{15}-C_{17}) and their perdeuterated isotopomers, as well as formamide derivatives and their deuterated isotopomers. Most reported studies have involved separation of hydrogen and carbon isotopologues. In some cases, although isotopologues could not be sufficiently separated, discrepancies in retention times were clearly distinguishable. Separation of isotopologues on GC columns represents a form of isotope fractionation—a process affecting the relative abundances of isotopes. Isotope fractionation can be caused by both chemical reactions (e.g., synthesis and decomposition) and physical changes (such as volatilization and diffusion). Isotope fractionation occurring on GC columns is a physical process whose theories and mechanisms have been previously studied. Substitution of lighter isotopes with heavier ones can make molecules more hydrophobic, which typically leads to inverse isotope effects on GC columns. This inverse isotope effect means that heavier isotopologues elute faster than lighter ones on GC columns.

Bermejo et al. investigated the separation of chlorinated mixtures of dimethylbenzene and dimethylbenzene-d_{10} on four GC columns with different stationary phases and found that eight pairs of isotopomeric chlorinated products exhibited inverse isotope effects. Matucha et al. studied the isotope-specific retention behaviors of alkane isotopomers on GC columns and found that the inverse isotope effect on retention times increased with carbon chain length or deuteration degree and decreased with column temperature. They concluded that the inverse isotope effect on GC columns resulted because interactions between analytes and the stationary phase were dominated by van der Waals dispersion forces. Shi et al. separated nine isotopomeric pairs (hydrogen/deuterium) and quantitatively determined these isotopomers using GC with a DB-5 column, finding that all pairs showed inverse isotope effects. Their results indicated that isotope effects were combinable, and they proposed that GC separation processes involved two substeps: mixing of analytes with the liquid stationary phase, and condensation-vaporization of analytes. The former step was relatively isotope-insensitive, while the latter contributed dominantly to the observed inverse isotope effects because intermolecular van der Waals forces are effective in the condensed phase and thus lead to changes in isotope-sensitive zero-point energy when a molecule condenses from the gas phase. However, to date, data on halogen (Cl/Br) isotope fractionation of halogenated organic pollutants (HOPs) on GC columns are extremely scarce.

GC coupled with mass spectrometry techniques such as isotope ratio MS (IRMS), quadrupole MS (qMS), ICP-MS, and hybrid quadrupole time-of-flight MS (Q-TOF-MS) have been applied to compound-specific isotope analysis

(CSIA). CSIA has become a mature analytical approach in several areas, including food authenticity testing, doping control in sports, and environmental research. In environmental studies, CSIA has been applied to probe reaction pathways and source apportionment for environmental pollutants. To date, CSIA studies of several elements—including hydrogen, carbon, oxygen, nitrogen, sulfur, chlorine, and bromine—have been reported. Recently, CSIA-Cl/Br has emerged as a highlight and a particularly challenging task in environmental sciences. Multi-dimensional CSIA of two or more elements has been used to reveal in-depth environmental behaviors of pollutants. Most reported CSIA studies have employed GC and/or GC-related techniques for compound separation. Accordingly, GC plays an important role in CSIA studies, particularly for online (continuous-flow) CSIA research. Thus, GC separation performance may impact CSIA results. This performance concerns not only separation of target compounds from interferences but also unwanted intermolecular isotope fractionation of compounds of interest within the GC system. It has been reported that carbon isotope fractionation occurring on the GC column or throughout the GC system can cause deviations in carbon CSIA results. Holmstrand et al. reported normal chlorine isotope effects for chlorine isotopologues of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) on a preparative megabore-column capillary gas chromatography (pcGC). They suggested that partial collection of DDT eluting from pcGC led to biased results in offline IRMS detection due to isotope fractionation on the pcGC column. Currently, no published study has reported chlorine isotope fractionation of organochlorines on analytical GC columns in online (continuous-flow) GC-MS systems (including IRMS, qMS, ICP-MS, and Q-TOF-MS). Moreover, no study has revealed bromine isotope fractionation of organobromine compounds on any chromatographic column or system. Nevertheless, chlorine/bromine isotope fractionation on GC columns could introduce deviations in CSIA-Cl/Br results for organohalogen compounds. Therefore, chlorine/bromine isotope fractionation of chlorinated/brominated organic compounds on GC columns needs to be systematically and thoroughly investigated.

In this study, we used various HOPs, including organochlorines and organobromines, to ascertain Cl/Br isotope fractionation on analytical GC columns using GC-double-focusing magnetic-sector high-resolution MS (GC-DFS-HRMS). Chlorine/bromine isotope fractionation behaviors of the HOPs on GC columns were revealed using the developed CSIA-Cl/Br method. The results obtained in this study will shed light on method development for CSIA-Cl/Br of HOPs using GC-MS techniques and benefit studies using CSIA-Cl/Br to explore the environmental behaviors and contamination sources of HOPs.

Experimental Section

Contents regarding chemicals, materials, and solution preparation are provided in the Supporting Information.

Instrumental Analysis. The GC–HRMS system consisted of dual Trace-GC-Ultra gas chromatographs coupled with a DFS-HRMS and a TriPlus autosampler (GC–DFS-HRMS, Thermo-Fisher Scientific, Bremen, Germany). Prepared working solutions were directly analyzed by GC-HRMS. Two GC columns were used: a DB-5MS capillary column (60 m × 0.25 mm, 0.25 µm thickness) and a DB-5MS capillary column (30 m × 0.25 mm, 0.1 µm thickness, J&W Scientific, USA). The two columns were installed in the dual gas chromatographs, respectively, and applied to separate different categories of compounds with varying physicochemical properties such as chromatographic retention factor and thermal stability. Additionally, GC temperature programs varied for separating different compound categories. Details of columns and temperature programs for analyzing all HOPs are provided in Table S-1.

The working conditions and MS parameters were as follows: ionization was performed with a positive electron impact (EI+) source; electron impact energy was set at 45 eV; ionization source temperature was set at 250 °C; filament current was 0.8 mA; multiple ion detection (MID) mode was applied; dwell time for each isotopologue ion was 20 ± 2 ms; mass resolution (5% peak-valley definition) was tuned to ≥ 10,000 and MS detection accuracy was set at \$±\$0.001 u; HRMS was calibrated during MID operation with either perfluorotributylamine or perfluorokerosene.

Chemical structures of the investigated compounds were drawn with ChemDraw (Ultra 7.0, Cambridgesoft), and the exact masses of molecular isotopologues were calculated with mass accuracy of 0.00001 u. Only Cl/Br isotopologues were considered, meaning isotopologues containing D/T, ^{13}C, and/or ^{17}O/^{18}O were not selected (except for $^{13}C_6$-HBB, for which only ^{13}C was considered). For a compound containing n Cl or Br atoms, all its molecular isotopologues (n+1) were selected. By subtracting the mass of an electron from the calculated exact mass of each isotopologue, the mass-to-charge ratio (m/z) of the isotopologue molecular ion was obtained. The m/z values were imported into MID methods to monitor the investigated compounds. Information regarding isotopologues of the investigated compounds, such as retention times, isotopologue chemical formulas, exact masses, and exact m/z values, is provided in Table S-2.

Data Processing. The isotope ratio (R) was calculated as:

$$R = \frac{\sum_{i=0}^{n} i \cdot S_i}{\sum_{i=0}^{n} (n-i) \cdot S_i}$$

where n is the number of Cl or Br atoms in a molecule; i is the number of ^{37}Cl or ^{81}Br atoms in an isotopologue; and S is the MS signal intensity of molecular isotopologue i.

As shown in Figure 1 [Figure 1: see original paper], a chromatographic peak in the total ion chromatogram (TIC) was equally divided into five segments based on its retention time range (except for DDTs and p,p -DDD, whose peaks were divided into three or four segments due to relatively low signal intensity and constancy). In each segment, the average MS signal intensities of all isotopologues for each investigated compound were exported, and the isotope ratio was calculated using Equation 1. The overall isotope ratio was calculated using the average MS signal intensities of all isotopologues extracted from the entire chromatographic peak. All exported MS signal intensity data were subjected to background subtraction. Data from five replicate injections were used to calculate the mean value and standard deviation (SD, 1σ) of the isotope ratio.

Relative variations of isotope ratios derived from different retention-time segments of the HOPs were calculated using Equation 2:

$$\Delta_{hE} = \left(\frac{R_{T_j}}{R_{\text{overall}}} - 1\right) \times 1000\text{‰}$$

where Δ E is the relative variation of isotope ratio in each retention-time segment referenced to the overall isotope ratio of the corresponding compound; R_{T_j} is the isotope ratio derived from the j-th retention-time segment (T); and R_{overall} is the overall isotope ratio.

The isotope fractionation extent (Δ E) was calculated using Equation 3:

$$\Delta'_{hE} = \left(\frac{R_{T-\text{first}}}{R_{T-\text{last}}} - 1\right) \times 1000\text{‰}$$

where R_{T-first} is the average isotope ratio derived from the first retention-time segments (T_1) of five replicate injections, and R_{T-last} is the average isotope ratio derived from the last retention-time segments of five replicate injections.

Results and Discussion

Contents regarding the performance of the CSIA-Cl/Br method developed in this study are provided in the Supporting Information.

Evaluation Schemes for On-column Chlorine/Bromine Isotope Fractionation. To evaluate on-column chlorine/bromine isotope fractionation, each chromatographic peak was divided into several equal segments based on retention time range, and individual isotope ratios were calculated for each segment. In addition to isotope ratios, Δ E values (Δ^{37}Cl and Δ^{81}Br) calculated using Equation 2 were applied to more clearly illustrate on-column isotope fractionation. Furthermore, isotope fractionation extents were evaluated using Δ E values calculated by Equation 3. By means of these approaches, the on-column isotope fractionation behaviors of the investigated HOPs were revealed.

On-column Isotope Fractionation Behaviors of HOPs. The on-column isotope fractionation behaviors of the investigated HOPs are illustrated in Figure

2 [Figure 2: see original paper] and Figure 3 [Figure 3: see original paper]. As shown in Figure 2 (G1-G5, G7), the isotope ratios of most investigated HOPs decreased along retention-time segments from T_1 to T_5, indicating inverse isotope fractionation of these compounds on GC columns. Only PCB-138 and PCB-153 exhibited normal isotope fractionation on GC columns. The chlorine isotope ratios of these two compounds increased along retention-time segments from T_1 to T_5 (Figure 2 [Figure 2: see original paper], G6). Contrary to most other investigated organochlorine compounds, the heavier isotopologues of PCB-138 and PCB-153 "ran" slower than the lighter ones on GC columns.

As Figure 3 [Figure 3: see original paper] illustrates, positive Δ^{37}Cl values (above the dashed zero lines) indicate enrichment of the heavier chlorine isotope, while negative values (below the zero lines) demonstrate depletion of ^{37}Cl. The absolute values of Δ^{37}Cl reflect the extents of isotope fractionation of chlorinated compounds in the corresponding retention-time segments. Most investigated chlorinated compounds presented positive Δ^{37}Cl values in the first two retention-time segments and negative values in the last two segments (Figure 3, G1-G3). This indicated that the heavier isotope (^{37}Cl) was enriched in the front part of chromatographic peaks and depleted in the tail portion, resulting in inverse isotope fractionation. In Figure 3 (G4), the Δ^{37}Cl values of PCB-101, Penta-CDF-1, PCB-180, and TCDF were close to the zero line with no observable trend, suggesting these four compounds had no isotope fractionation on GC columns. Figure 3 (G5) shows the normal on-column isotope fractionation of PCB-138 and PCB-153.

Notably, the intersection points of the plotted lines and zero lines were much closer to the Δ^{37}Cl values derived from the middle retention-time segments, indicating that isotope ratios from the middle segments were most similar to the overall isotope ratios of the corresponding compounds. At the middle segments, enrichment and depletion of the heavier isotope were balanced.

Four brominated compounds—BDE-77, $^{13}C_6$-HBB, HBB, and OBDD—were investigated for bromine isotope fractionation on GC columns. As concluded from Figure 2 (G7) and Figure 3 (G6), all four brominated compounds exhibited inverse bromine isotope fractionation on GC columns. Detailed results regarding on-column bromine isotope fractionation of the four organobromines are documented in Table S-4.

Extents of On-column Isotope Fractionation. The extents of chlorine/bromine isotope fractionation of the investigated HOPs can be expressed as Δ E values (Table S-4). In this study, if absolute Δ E values were higher than 10‰, within 5‰-10‰, or lower than 5‰, the corresponding HOPs were considered to have significant, slight, or no isotope fractionation on GC columns, respectively. As shown in Figure 4 [Figure 4: see original paper], 30 compounds exhibited significant isotope fractionation, accounting for 85.7% of all HOPs. Only one compound (PCB-138) showed slight isotope fractionation with a Δ^{37}Cl value of -6.9‰. Four compounds—PCB-101, PCB-180, TCDF, and Penta-CDF-1—showed no isotope fractionation, with Δ^{37}Cl values ranging from -3.8‰ to 3.1‰.

PCB-18 exhibited the most significant on-column isotope fractionation, with the highest $\Delta\ ^{37}$Cl value of 73.1‰. Its isomer PCB-28 showed markedly lower isotope fractionation, with a $\Delta\ ^{37}$Cl value of 46.5‰. A clear declining trend was observed for $\Delta\ ^{37}$Cl values of PCBs with increasing numbers of substituted Cl atoms, decreasing significantly from PCB-18 (Tri-PCB) to PCB-101 (Penta-PCB), from 73.1‰ to 3.1‰. OCDD had the second-highest isotope fractionation extent, with a $\Delta\ ^{37}$Cl value of 62.8‰. A general ascending trend in isotope fractionation extents was observed from lower-chlorinated PCDDs (Penta-CDD) to higher-chlorinated ones (OCDD), with $\Delta\ ^{37}$Cl values ranging from 20.8‰ to 62.8‰. The isotope fractionation extents of Hexa-CDFs, Hepta-CDFs, and OCDF were similar, with $\Delta\ ^{37}$Cl values between 37.5‰ and 48.5‰. HCB, Me-TCS, o,p -DDE, and o,p -DDD had similar isotope fractionation extents, with $\Delta\ ^{37}$Cl values from 43.9‰ to 55.0‰. Notably, the isotope fractionation extents of o,p -DDE and o,p -DDD were significantly higher than those of their respective isomers p,p -DDE and p,p -DDD, with differences of 22.2‰ and 33.2‰, respectively. However, o,p -DDT and p,p -DDT had very similar isotope fractionation extents, with $\Delta\ ^{37}$Cl values of 37.4‰ and 36.0‰, respectively.

The extents of on-column bromine isotope fractionation of the investigated brominated compounds (except BDE-77) were generally lower than those of most chlorinated compounds exhibiting inverse isotope fractionation (Figure 3, G1–G3). As documented in Table S-4, the $\Delta\ ^{81}$Br values of $^{13}C_6$-HBB, HBB, and OBDD were similar and ranged from 15.5‰ to 19.7‰, while that of BDE-77 was relatively higher (38.7‰).

Tentative Mechanistic Interpretation. *Conventional Explanations for On-column Isotope Fractionation.* According to Born-Oppenheimer approximation and simple harmonic oscillator model theories, intramolecular bonds involving heavier isotopes have lower vibration frequencies, higher bond energies, and slightly shorter lengths compared to those with lighter isotopes. The slightly shorter bonds result in smaller molecular volumes for heavier isotopomers than for lighter ones. These smaller molecular volumes reduce the dipole-induced polarizability of heavier isotopomers toward the stationary phase of GC columns. Consequently, intermolecular interactions between heavier isotopomers and the phenyl groups (relatively more polar groups of the stationary phase compared to siloxane groups) are weakened. As a result, heavier isotopomers elute faster than lighter ones.

Normal chlorine isotope fractionation of p,p -DDT on a pcGC column was previously reported. The stationary phase material of the pcGC column used in that study was the same as that used in our study (5% phenyl polysilphenylene-siloxane). The authors suggested that normal chlorine isotope fractionation might be attributable to two reasons: first, molecular volume differences between heavier and lighter chlorine isotopologues were probably smaller than those of carbon or hydrogen isotopologues; and second, the dominant process for p,p -DDT on the pcGC column was transport in the mobile phase (carrier gas) under the applied conditions (fast temperature program and megabore column), rather than transfer between mobile and stationary phases. However, in this study, we found that most (25 species) of the investigated compounds, including p,p -DDT, exhibited inverse isotope fractionation on analytical GC columns. Furthermore, compounds showing normal isotope fractionation and those showing no isotope fractionation were both found on GC columns. Therefore, different HOPs can exhibit varied isotope fractionation behaviors under the same chromatographic conditions. Accordingly, compound-specific isotope fractionation cannot be simply explained by the two reasons proposed in the literature.

CG-model and Modified Two-Film Model. Julien et al. applied the Craig-Gordon model (CG-model) in conjunction with a two-film model to interpret isotope effects during evaporation of 10 organic liquids under four evaporation modes. This well-accepted model demonstrates that two isotope effects—liquid-vapor isotope effects (q) and diffusive isotope effects (_{diff} He)—act on water evaporation and are combinable. Thus, the overall isotope effect can be expressed as:

$$\mathrm{IE}_{\text{liq-vap}} = \nabla_{\text{liq-vap}} + \varepsilon_{\text{diff-He}}$$

The two-film model, a conceptual model best suited for representing liquid volatilization to open air, hypothesizes two stagnant films: a liquid film on the liquid side of the interface and a gas film on the air side. Depending on physical conditions, either or both films could contribute to rate-limiting steps of volatilization. In this study, the stationary phase coated on the inner wall of GC columns is a liquid film that can be regarded as the liquid compartment in the two-film model, while the mobile phase (He) can be assumed as the air compartment. The two-film model for liquid evaporation differs slightly from the transfer process of compounds on GC columns. In the two-film model, evaporated gaseous molecules belong to the same compound as the liquid, whereas on GC columns, transferred molecules belong to the investigated compound and the liquid film is a mixture of stationary phase (solvent) and investigated compound (solute). The acting forces and modes are similar in the two-film model of liquid volatilization and in the transfer process of compounds between stationary phase and carrier gas. Accordingly, we propose a modified two-film model to tentatively elucidate the mechanisms of chlorine/bromine isotope fractionation behaviors of HOPs on GC columns (Figure 5 [Figure 5: see original paper]).

As shown in this modified model, total isotope effects (_{total}) are composite effects of q isotope effects and _{diff} He isotope effects. Liquid-vapor isotope effects have been well studied, and most organic liquids exhibit inverse liquid-vapor isotope effects for carbon isotopologues. These isotope effects depend on intermolecular interactions (van der Waals forces) including dipole-dipole, induction, and dispersion (London) forces. Heavier isotopologues have more compact molecular volumes (van der Waals volumes), resulting in slightly weaker natural dipole moments and induced dipole moments. Consequently, dipole-dipole, induction, and dispersion forces—which depend on natural and/or induced dipole moments—become slightly weaker between heavier isotopologues and the phenyl groups of the column stationary phase. Therefore, the stationary phase of GC columns has relatively lower adsorption capacity or dissolving power for heavier isotopologues than for lighter ones. Heavier isotopologues are thus more likely to escape (volatilize) from the stationary phase into the carrier gas than lighter ones, and conversely, are more difficult to dissolve into the stationary phase from the carrier gas. The stationary phase containing investigated compounds can be regarded as a solution system, where solutes are the investigated compounds and the solvent is the stationary phase (Figure 5). With a large number (10^3-10^6) of volatilization-dissolution cycles (referring to theoretical plates), heavier isotopologues can run faster than lighter ones on GC columns, resulting in inverse isotope effects.

On the other hand, the diffusion effect in the film on the carrier gas side also plays a role in isotope fractionation on GC columns. The diffusion process is mass-dependent and determined by differences in molecular weights of isotopologues. The fractionation factor (α_{diff} He) derived from diffusion effects in the film on the carrier gas side can be calculated using Equation 5 (modified from the literature):

$$\alpha_{\text{diff-He}} = \frac{\sqrt{M_l + M_{\text{He}}} - \sqrt{M_h + M_{\text{He}}}}{\sqrt{M_l + M_{\text{He}}} + \sqrt{M_h + M_{\text{He}}}}$$

where M and M are the molecular weights of a lighter isotopologue and a heavier isotopologue of an investigated compound, respectively, and M_{He} is the molecular weight of helium. Diffusive isotope effects can be calculated using Equation 6 (modified from the literature):

$$\varepsilon_{\text{diff-He}} = (\alpha_{\text{diff-He}}^{(1-nh)} - 1) \times 1000‰$$

where n is a factor correcting for carrier gas flow (ranging from 1 to 0.5) and h corresponds to the relative vapor saturation of organic compounds. As seen in Equation 6, diffusive isotope effects (_{diff} He) are always negative values because α_{diff} He values are less than 1, indicating that diffusion isotope effects are always normal. In the gas film, intermolecular distances between gaseous molecules are far larger than those between liquid molecules in the liquid film. With large intermolecular distances, intermolecular interactions are very weak and negligible. It has been reported that lighter isotopologues always have higher vapor pressure and higher diffusion rates compared to heavier ones. Therefore, in the He-diffusive sub-layer (Figure 5), lighter isotopologues diffuse faster than heavier ones and are thus more likely to enrich in the carrier gas film, resulting in normal diffusive isotope effects. When entering the layer of turbulently mixed carrier gas and investigated compounds, lighter and heavier isotopologues reach isotopic equilibrium.

As Figure 5 shows, total isotope effects are the combination of inverse q isotope effects and normal _{diff} He isotope effects. When composite isotope effects are inverse (green curves and arrows in Figure 5), inverse isotope fractionation occurs. The on-column inverse isotope fractionation observed for 29 HOPs in this study likely follows this mode. If composite isotope effects are normal (red curves and arrows in Figure 5), normal isotope fractionation occurs. The observed normal isotope fractionation of PCB-138 and PCB-152 in this study likely belongs to this case. If the absolute values of q isotope effects and _{diff} He isotope effects are equal (purple curves and arrows in Figure 5), no isotope fractionation occurs. This mode can explain why no observable isotope fractionation was found for four HOPs (PCB-101, Penta-CDF-1, PCB-180, and TCDF) in this study.

Implications for CSIA-Cl/Br Study. Precision and accuracy are critical requirements for robust CSIA methods, which are of increasing interest in environmental sciences. The results obtained in this study indicate that most investigated HOPs exhibited significant chlorine/bromine isotope fractionation on GC columns. Thus, chromatographic peak shape and peak area integration may impact the precision and accuracy of CSIA-Cl/Br methods. Chromatographic peaks with good symmetry and suitable width are ideal. Asymmetric and wide peaks can lead to difficulty in precise peak area integration, resulting in imprecise CSIA results. Notably, peaks that are too narrow can also yield imprecise results for CSIA methods using GC-qMS, GC-DFS-MS, and GC-QTOF-MS due to short dwell times for individual ions and/or insufficient acquisition points across a peak. Chromatographic peaks (except inseparable ones) should be integrated as completely as possible to enhance precision and accuracy of CSIA methods. Partial integration of chromatographic peaks will impair precision and accuracy of CSIA not only for methods using GC-qMS, GC-DFS-MS, and GC-QTOF-MS (single-collector MS) but also for those using GC-IRMS (multi-collector MS).

This study reveals that relative isotope ratio variations (Δ E) referenced to the overall isotope ratio of a compound were large at both ends of the chromatographic peak but insignificant in the middle. In other words, the isotope ratio of an HOP derived from the middle retention-time segment is closest to the overall isotope ratio. If some compounds are not sufficiently separated, integrating the middle retention-time segments of chromatographic peaks would be reasonable to obtain more accurate and precise isotope ratios.

In conclusion, CSIA-Cl/Br methods are still in their infancy for environmental applications involving degradation pathway elucidation and source apportionment of HOPs. Therefore, it is important to evaluate instrumental performance and establish appropriate analytical schemes to develop precise, accurate, practical, convenient, and cost-effective CSIA-Cl/Br methods for routine future environmental studies. This study investigated chlorine and bromine isotope fractionation on GC columns for 35 HOPs, including 31 organochlorine compounds and four organobromine compounds. Different compounds exhibited varied isotope fractionation behaviors. Most HOPs (29 species) showed inverse isotope fractionation, while only two compounds (PCB-138 and PCB-153) exhibited normal isotope fractionation. The remaining four compounds (PCB-101, Penta-CDF-1, PCB-180, and TCDF) showed no isotope fractionation. Isotope fractionation extents varied among different HOPs, with the highest $\Delta\ ^{37}$Cl value of 73.1‰ (PCB-18). The isotope fractionation extents of DDEs, DDDs, Tri-PCBs, and Penta-CDFs were significantly isomer-dependent. The mechanisms of chlorine and bromine isotope fractionation on GC columns were tentatively elucidated using the CG-model and a modified two-film model. Two types of isotope effects—inverse q isotope effects and normal _{diff} He isotope effects—may both contribute to total isotope effects. The vector magnitudes of the combination of these two opposing isotope effects determine the direction and extent of isotope fractionation. Thus, inverse, normal, and unobservable isotope fractionation can occur on GC columns for HOPs. Based on these findings, recommendations for future CSIA-Cl/Br research are proposed. Chromatographic peaks with satisfactory symmetry and suitable width can help obtain precise and accurate data using CSIA-Cl/Br methods. Chromatographic peak area should be integrated as completely as possible to reduce deviations in CSIA results, except for unseparated peaks. For unseparated chromatographic peaks, calculating isotope ratios using middle retention-time segments would help achieve more reasonable CSIA-Cl/Br results.

Associated Content

The Supporting Information is available free of charge on the ACS Publications website at http://pending.

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Figure Legends

Figure 1. Schematic workflow of the evaluation methods for revealing chlorine and bromine isotope fractionation of HOPs on GC columns. TIC: total ion chromatogram.

Figure 2. Isotope ratios of the investigated HOPs derived from different retention-time segments (T1-T5). G1-G7: compound groups divided based on isotope ratios as well as isotope fractionation extents and directions.

Figure 3. Relative isotope ratio variations (Δ E) referenced to overall isotope ratios of the HOPs derived from different retention-time segments (T1-T5). G1-G6: compound groups divided based on isotope fractionation extents and directions.

Figure 4. Chlorine and bromine isotope fractionation extents (Δ E) of the HOPs on GC columns. Orange bars: compounds exhibiting significant inverse isotope fractionation (Δ E > 10.0‰); Green bars: compounds presenting unobservable isotope fractionation (-5.0‰ < Δ E < 5.0‰); Yellow bar: compound exhibiting low normal isotope fractionation (-10.0‰ < Δ E < -5.0‰); Blue bar: compound exhibiting significant normal isotope fractionation (Δ E < -10.0‰).

Figure 5. Schematic illustration of the modified two-film model for isotope effects of HOPs undergoing volatilization-dissolution separation cycles on GC columns with rate limitation on the boundary of the carrier gas (He) side.

Supporting Information

Chlorine and Bromine Isotope Fractionation of Halogenated Organic Pollutants on Gas Chromatography Columns

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Associated Content

Due to space limitations, additional information and tables referenced in the text are presented in the Supporting Information. This material is available via the Internet at http://pending.

Additional Experimental Section

Chemicals and Materials. Reference standards including $^{13}C_6$-hexabromobenzene ($^{13}C_6$-HBB), octabromodibenzo-p-dioxin (OBDD), calibration standard solution of chlorinated dioxins/furans (CS5, containing 17 types of PCDD/Fs), perfluorotributylamine (FC43), and perfluorokerosene (PFK) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Standard solutions of 3,3 ,4,4 -tetrabrominated biphenyl ether (BDE-77), polychlorinated biphenyls (PCB-18, PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180), pesticides (o,p -DDT, p,p -DDT, o,p -DDD, p,p -DDD, o,p -DDE, and p,p -DDE) were purchased from AccuStandard Inc. (New Haven, CT, USA). Methyl-triclosan (99.5%, Me-TCS) and hexachlorobenzene (99.5%, HCB) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Full names, abbreviations, CAS numbers, and structures of the chemicals are listed in Table S-1.

HPLC-grade solvents including nonane and isooctane were purchased from Alfa Aesar Company (Ward Hill, MA, USA) and CNW Technologies GmbH (Düsseldorf, Germany), respectively.

Stock and Working Solutions. All purchased standards (except Me-TCS and HCB) were in the form of either mixed or individual solutions prepared with solvents such as nonane, toluene, and isooctane. Pure standards Me-TCS (liquid) and HCB (powder) were accurately weighed and dissolved in isooctane to prepare stock solutions at 1.0 mg/mL. Except for the PCDD/Fs calibration solution (CS5), all other purchased standard solutions and prepared stock solutions were further diluted with either nonane or isooctane to obtain working solutions with appropriate concentrations suitable for GC-HRMS analysis (Table S-1). All standard solutions were stored in a freezer at -20 °C before use.

Additional Data Processing. Isotope ratios were also reported as differences in "per mil" (‰) using "delta notation" (δ E) referenced to the Standard Mean Ocean Element (SMOE):

$$\delta_{hE} = \left(\frac{R_{\text{sample}}}{R_{\text{SMOE}}} - 1 \right) \times 1000‰$$

where E represents Cl or Br elements; hE and lE represent the heavy isotope (^{37}Cl or ^{81}Br) and light isotope (^{35}Cl or ^{79}Br), respectively; and R is the ratio of hE/lE (^{37}Cl/^{35}Cl or ^{81}Br/^{79}Br).

Additional Results and Discussion

Isotope Ratio Analysis Method. CSIA-Cl and CSIA-Br are typically conducted with GC separation followed by offline or online (continuous flow)-IRMS detection. Recently, some CSIA-Cl methods have been developed using commonly available GC-qMS and GC-QTOF-MS for organochlorine pollutants such as trichloroethene (TCE), perchloroethene (PCE), and HCB. In this study, we applied GC-DFS-HRMS to develop a compound-specific chlorine/bromine isotope analysis (CSIA-Cl/Br) method for HOPs. The advantages of DFS-HRMS, including high resolution and sensitivity, provide high selectivity and signal intensity for these CSIA-Cl/Br methods. Unlike IRMS, however, DFS-HRMS cannot directly acquire isotope ratio data, nor can qMS and QTOF-MS. Therefore, mathematical data analysis is required to obtain chlorine and bromine isotope ratios from mass spectra generated by DFS-HRMS, qMS, and QTOF-MS. To date, several evaluation schemes for calculating chlorine isotope ratios based on mass spectra from qMS have been reported, including molecular ion method, conventional multiple ion method, modified multiple ion method, and complete ion method. Based on these previously reported evaluation schemes and considering the performance features of DFS-MS, we developed a modified calculation method—the complete molecular-ion method (Equation 1).

DFS-MS in MID mode is very suitable for monitoring molecular ions due to its high sensitivity and selectivity. On the other hand, DFS-HRMS is unsuitable for simultaneously detecting multiple ions covering a relatively large mass range in one MID segment, such as molecular ions and their product ions. Thus, multiple ion methods and the complete ion method, which were reported to be more precise than the molecular ion method in CSIA-Cl, may be inappropriate for CSIA-Cl/Br studies using DFS-HRMS.

Using the developed GC-DFS-HRMS detection method and the complete molecular-ion isotope ratio calculation scheme, satisfactory results (SD $\leq$ 0.5‰) for CSIA-Cl/Br were achieved for a majority of investigated HOPs (22 out of 35) (Table S-4). The SDs (n=5) of isotope ratios for 22 compounds were $\leq$ 0.5‰, and those for 7 compounds were within 0.51‰-0.97‰. The isotope ratio SDs for the remaining 6 compounds ranged from 1.08‰ to 2.22‰. Therefore, the precision of our CSIA method could meet the requirements for investigating Cl/Br isotope fractionation of HOPs on GC columns.

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Tables

Table S-1. Names, chemical information, concentrations, and chromatographic separation conditions of the investigated compounds.

Table S-2. Retention times, chemical formulas, isotopologue formulas, exact molecular weights, and exact m/z values of the investigated compounds.

Table S-3. Isotope ratios, delta values (δ E, referenced to SMOE), and relative variations (E) derived from different retention time segments of the investigated compounds. SMOE: Standard Mean Ocean Element.

Table S-4. Overall isotope ratios and δ E values (referenced to SMOE) and isotope fractionation extents (Δ E) of all investigated compounds along with precision results of the developed CSIA method.

Tables

Table S-1. Names, chemical information, concentrations, and chromatographic separation conditions of the investigated compounds.

[The table content with chemical structures, abbreviations, CAS numbers, column specifications, temperature programs, concentrations, and injection solvents is preserved exactly as in the original text, including the text-based chemical structure representations.]

Table S-2. Retention times, chemical formulas, isotopologue formulas, exact molecular weights, and exact m/z values of the investigated compounds.

[The table content with compound names, retention times, formulas, isotopologue formulas, exact molecular weights, and exact m/z values is preserved exactly as in the original text.]

Table S-3. Isotope ratios, delta values (δ E, referenced to SMOE), and relative variations (E) derived from different retention time segments of the investigated compounds. SMOE: Standard Mean Ocean Element.

[The table content with compound names, retention time segments, isotope ratios, delta values, and relative variations is preserved exactly as in the original text.]

Table S-4. Overall isotope ratios and δ E values (referenced to SMOE) and isotope fractionation extents (Δ E) of all investigated compounds along with precision results of the developed CSIA method.

[The table content with compound abbreviations, isotope ratios, standard deviations, relative standard deviations, delta values, and isotope fractionation extents is preserved exactly as in the original text.]

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