SUPPORTING INFORMATION

Theoretical evaluation of inter-ion and intra-ion isotope effects in fragmentation: insights into chlorine and bromine isotope effects of halogenated organic compounds occurring in electron ionization mass spectrometry

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1 Theoretical derivation

1.1 Inter-ion isotope effects

For the compound RCl_4 (Figure 1), the probability of a product isotopologue possessing t ^{37}Cl atom(s) generated from the molecular ion by losing two Cl atoms is:

$$P(4,i,t,2)=\frac{C_{n-i}^{n-2-t}C_i^t}{C_4^2} \tag{2}$$

Then the probabilities of the product isotopologues produced from the molecular isotopologues can be obtained (Figure 1). We hypothesize the molar amounts of the molecular isotopologues are x_0, x_1, x_2, x_3 and x_4, and the mass spectrometry (MS) signal intensities of the product isotopologues are I_1, I_2 and I_3; We further define the proportionality constant of the molar amounts of the ions relative to their MS signal intensities as d, and then obtain the following equations:

$$1\cdot x_0+\tfrac{1}{2}\cdot x_1+\tfrac{1}{6}\cdot x_2+0\cdot x_3+0\cdot x_4=\tfrac{1}{d}I_1 \tag{S1}$$

$$0\cdot x_0+\tfrac{1}{2}\cdot x_1+\tfrac{2}{3}\cdot x_2+\tfrac{1}{2}\cdot x_3+0\cdot x_4=\tfrac{1}{d}I_2 \tag{S2}$$

$$0\cdot x_0+0\cdot x_1+\tfrac{1}{6}\cdot x_2+\tfrac{1}{2}\cdot x_3+1\cdot x_4=\tfrac{1}{d}I_3 \tag{S3}$$

Letting Eq (S1) × 2 + Eq (S2), we have

$$2\cdot x_0+\tfrac{3}{2}\cdot x_1+1\cdot x_2+\tfrac{1}{2}\cdot x_3+0\cdot x_4=\tfrac{2}{d}I_1+\tfrac{1}{d}I_2 \tag{S4}$$

And letting Eq (S3) × 2 + Eq (S2), we have

$$0\cdot x_0+\tfrac{1}{2}\cdot x_1+1\cdot x_2+\tfrac{3}{2}\cdot x_3+2\cdot x_4=\tfrac{2}{d}I_3+\tfrac{1}{d}I_2 \tag{S5}$$

Further letting Eq (S5) / Eq (S4), we have

$$\frac{0\cdot x_0+1\cdot x_1+2\cdot x_2+3\cdot x_3+4\cdot x_4}{4\cdot x_0+3\cdot x_1+2\cdot x_2+1\cdot x_3+0\cdot x_4}=\frac{2I_3+I_2}{2I_1+I_2} \tag{3}$$

Accordingly, by extrapolating Eq (3), we summarize a general equation as:

$$\frac{\sum_{i=0}^{n} i x_i}{\sum_{i=0}^{n} (n-i) x_i} = \frac{\sum_{i=0}^{n} \sum_{t=0}^{n-r} t C_{n-i}^{n-r-t} C_i^t x_i}{\sum_{i=0}^{n} \sum_{t=0}^{n-r} (n-r-t) C_{n-i}^{n-r-t} C_i^t x_i} \tag{4}$$

where n is the number of Cl atoms of a molecular ion; i is the number of ^{37}Cl atom(s) of an molecular isotopologue; r is the number of the lost Cl atom(s); t is the number of ^{37}Cl atom(s) of a product isotopologue derived from the molecular ion; x_i is the molar amount of molecular isotopologue i; C_{n-i}^{n-r-t} is a combination formula alternative to $\binom{n-i}{n-r-t}$, which is analogous to other relevant expressions in this text. Eq (4) can be mathematically proved as below. We define

$$R_{par} = \frac{\sum_{i=0}^{n} i x_i}{\sum_{i=0}^{n} (n-i) x_i} \tag{S6}$$

$$R_{pro} = \frac{\sum_{i=0}^{n} \sum_{t=0}^{n-r} t C_{n-i}^{n-r-t} C_i^t x_i}{\sum_{i=0}^{n} \sum_{t=0}^{n-r} (n-r-t) C_{n-i}^{n-r-t} C_i^t x_i} \tag{S7}$$

where R_{par} and R_{pro} are the isotope ratios of a parent ion and its product ion, respectively.

Proof:

$$R_{par} = R_{pro}$$

Proof process:

Functions $F(i, n)$ and $G(i, n)$ were defined as:

$$F(i,n) = \sum_{t=0}^{n-r} t C_{n-i}^{n-r-t} C_i^t \tag{S8}$$

$$G(i,n)=\sum_{t=0}^{n-r}(n-r-t)C_{n-i}^{n-r-t}C_i^t \tag{S9}$$

F (*i*, *n*) simplifies to

$$\begin{aligned}
F(i,n,r) &= \sum_{t=0}^{n-r} tC_{n-i}^{n-r-t}C_i^t\,(i-r\le t\le i, 0\le t\le n-r)\\
&=\sum_{t=0}^{r}(i-r+t)C_{n-i}^{t}C_i^{r-t}\\
&=\sum_{t=0}^{r}(i-r+t)C_{n-i}^{t}\,\frac{i!}{(r-t)!(i-r+t)!}\\
&=i\sum_{t=0}^{r}C_{n-i}^{t}\,\frac{(i-1)!}{(r-t)!(i-1-(r-t))!}\\
&=i\sum_{t=0}^{r}C_{n-i}^{t}C_{i-1}^{r-t}
\end{aligned} \tag{S10}$$

We can obtain the following equation according to combination principles:

$$\sum_{t=0}^{r}C_{n-i}^{t}C_{i-1}^{r-t}=C_{n-1}^{r} \tag{S11}$$

And *G* (*i*, *n*) simplifies to

$$\begin{aligned}
G(i,n) &= \sum_{t=0}^{n-r}(n-r-t)C_{n-i}^{n-r-t}C_i^t\,(i-r\le t\le i, 0\le t\le n-r)\\
&=\sum_{t=0}^{r}(n-i-t)C_{n-i}^{t}C_i^{r-t}\\
&=\sum_{t=0}^{r}(n-i-t)\frac{(n-i)!}{t!(n-i-t)!}C_i^{r-t}\\
&=(n-i)\sum_{t=0}^{r}\frac{(n-i-1)!}{t!(n-i-1-t)!}C_i^{r-t}\\
&=(n-i)\sum_{t=0}^{r}C_{n-i-1}^{t}C_i^{r-t}
\end{aligned} \tag{S12}$$

Similarly, we can obtain the following equation:

$$\sum_{t=0}^{r} C_{n-i-1}^{t} C_{i}^{r-t} = C_{n-1}^{r} \tag{S13}$$

Substituting Eqs (S10-S13) into Eq (S6), we have

$$\begin{aligned} R_{pro} &= \frac{\sum_{i=0}^{n}\sum_{t=0}^{n-r} t C_{n-i}^{n-r-t} C_{i}^{t} x_i}{\sum_{i=0}^{n}\sum_{t=0}^{n-r} (n-r-t) C_{n-i}^{n-r-t} C_{i}^{t} x_i} \\ &= \frac{\sum_{i=0}^{n} i C_{n-1}^{r} x_i}{\sum_{i=0}^{n} (n-i) C_{n-1}^{r} x_i} \\ &= \frac{\sum_{i=0}^{n} i x_i}{\sum_{i=0}^{n} (n-i) x_i} \\ &= R_{par} \end{aligned} \tag{S14}$$

Thus, $R_{par} = R_{pro}$ is proven.

1.2 Intra-ion isotope effects

The equilibrium constant between the cleavages of bond A_2 and bond A_3 can be expressed as:

$$K_a = \frac{\frac{b_2}{a_2 - b_2}}{\frac{b_3}{a_3 - b_3}} = \frac{b_2(a_3 - b_3)}{b_3(a_2 - b_2)} \tag{6}$$

And that between the cleavages of bond B_3 and bond B_2 can be expressed as:

$$K_b = \frac{\frac{a_3 - b_3}{b_3}}{\frac{a_2 - b_2}{b_2}} = \frac{b_2(a_3 - b_3)}{b_3(a_2 - b_2)} \tag{7}$$

Therefore, $K_a = K_b$.

Similarly, the equilibrium constants (K_2 and K_3) between the cleavages of bond A_2 and bond B_2, and bond A_3 and bond B_3 can be expressed as:

$$K_2 = \frac{\frac{b_2}{a_2 - b_2}}{\frac{a_2 - b_2}{b_2}} = \left(\frac{b_2}{(a_2 - b_2)}\right)^2 \tag{8}$$

$$K_3 = \frac{\frac{a_3 - b_3}{b_3}}{\frac{b_3}{a_3 - b_3}} = \left(\frac{a_3 - b_3}{b_3}\right)^2 \tag{9}$$

The isotope ratio of the parent ion derived from MI2 and MI3 ($R_{par(a2a3)}$) is

$$R_{par(a_2a_3)} = \frac{a_2 + a_3}{a_2 + a_3} = 1 \tag{10}$$

And the isotope ratio of the product ion generated by MI2 and MI3 ($R_{pro(a2a3)}$) is

$$R_{pro(a_2a_3)}=\frac{b_2+a_3-b_3}{b_3+a_2-b_2} \tag{11}$$

According to Eqs (6-9), we have

$$b_2=\frac{a_2}{1+\frac{1}{\sqrt{K_2}}} \tag{S15}$$

$$b_3=\frac{a_3}{1+\sqrt{K_3}} \tag{S16}$$

$$K_a=\sqrt{K_2K_3} \tag{S17}$$

Substituting Eq (S15) and Eq (S16) into Eq (11), we have

$$R_{pro(a_2a_3)}=\frac{\frac{a_2}{1+\frac{1}{\sqrt{K_2}}}+a_3-\frac{a_3}{1+\sqrt{K_3}}}{\frac{a_3}{1+\sqrt{K_3}}+a_2-\frac{a_2}{1+\frac{1}{\sqrt{K_2}}}} \tag{S18}$$

Substituting Eq (S17) into Eq (S18), gives

$$R_{pro(a_2a_3)}=\frac{\frac{K_a}{K_a+\sqrt{K_3}}+\frac{a_3}{a_2}\frac{\sqrt{K_3}}{1+\sqrt{K_3}}}{\frac{a_3}{a_2}\frac{1}{1+\sqrt{K_3}}+\frac{\sqrt{K_3}}{K_a+\sqrt{K_3}}} \tag{S19}$$

Letting $\sqrt{K_3}=x$ and $\frac{a_3}{a_2}=R^{'}_{a_2a_3}$, then we have

$$y=\frac{\frac{K_a}{K_a+x}+\frac{R'_{a_2a_3}x}{1+x}}{\frac{R'_{a_2a_3}}{1+x}+\frac{x}{K_a+x}} \tag{S20}$$

and further obtain

$$\frac{dy}{dx}=\frac{\left[\frac{-K_a}{(K_a+x)^2}+\frac{R'_{a_2a_3}}{(1+x)^2}\right]\left(\frac{R'_{a_2a_3}}{1+x}+\frac{x}{K_a+x}\right)-\left(\frac{K_a}{K_a+x}+\frac{R'_{a_2a_3}}{1+x}\right)\left[\frac{-R'_{a_2a_3}}{(1+x)^2}+\frac{K_a}{(K_a+x)^2}\right]}{\left(\frac{R'_{a_2a_3}}{1+x}+\frac{x}{K_a+x}\right)^2} \tag{S21}$$

which simplifies to

$$\frac{dy}{dx}=\frac{(R'_{a_2a_3}+1)\left[\frac{R'_{a_2a_3}}{(1+x)^2}-\frac{K_a}{(K_a+x)^2}\right]}{\left(\frac{R'_{a_2a_3}}{1+x}+\frac{x}{K_a+x}\right)^2} \tag{S22}$$

Because a$_2$ approximates to or equals a$_3$, thus letting $R'_{a_2a_3}=1$, then we have

$$y=\frac{\frac{K_a}{K_a+x}+\frac{x}{1+x}}{\frac{1}{1+x}+\frac{x}{K_a+x}} \tag{S23}$$

and

$$\frac{dy}{dx}=2\frac{(1-K_a)x^2+K_a{}^2-K_a}{\left(x^2+2x+K_a\right)^2} \tag{S24}$$

When $\frac{dy}{dx}=0$, we have $x=\pm\sqrt{K_a}$. Since $x\geq 0$, thus $x=\sqrt{K_a}$. Because $K_a>1$, then the function $f(x)=(1-K_a)x^2+K_a{}^2-K_a$ has a convex shape, and is larger than zero ($f(x)>0$) at $x\in[0,\sqrt{K_a})$, and lower than zero ($f(x)<0$) at $x\in(\sqrt{K_a},\infty)$. Therefore, the function $f(x)$ is monotonically increasing at $x\in[0,\sqrt{K_a})$, and monotonically decreasing at $x\in(\sqrt{K_a},\infty)$, and has a maximum at $x=\sqrt{K_a}$. When $K_3=0$, $IR_{pro(a_2a_3)}$ has the minimum:

$$R_{pro(a_2a_3)}=1=R_{par(a_2a_3)} \tag{12}$$

When $K_3=K_a$, $R_{pro(a_2a_3)}$ has the maximum:

$$R_{pro(a_2a_3)}=\frac{x^2+2K_ax+K_a}{x^2+2x+K_a}=\frac{K_a+2K_a\sqrt{K_a}+K_a}{K_a+2\sqrt{K_a}+K_a} \tag{S25}$$

$$R_{pro(a_2a_3)}=\sqrt{K_a} \tag{13}$$

When K_3 approaches infinity, $R_{pro(a2a3)}$ has the following limit:

$$\lim_{x\to\infty}f(x)=\lim_{x\to\infty}\frac{x^2+2K_ax+K_a}{x^2+2x+K_a}=1 \tag{S26}$$

Then for $K_3\in(0,\infty)$, we have

$$1<IR_{pro(a_2a_3)}\le\sqrt{K_a} \tag{14}$$

Hence, the isotope ratio of a product ion exceeds that of its parent ion when intra-ion isotope effects are present. The physicochemical meanings for the scenarios of $K_3=0$, $K_3\to\infty$ and $K_3=K_a$ are as follows. $K_3=0$: the bonds A_2 and A_3 are completely broken, while the bonds B_2 and B_3 are completely unbroken. In this scenario, the isotope ratio of the product ion is equal to that of its parent ion, indicating no intra-ion isotope effect taking place. This scenario is probably present for asymmetric molecules. If the difference in critical energies between the asymmetric C-Cl bonds are large enough, so that a bond can be cleaved and another completely non-cleaved or with a negligible fraction cleaved. $K_3 \to \infty$: this scenario is equivalent to that of $K_3 = 0$. $K_3 = K_a$: if the bonds A_2, B_2, A_3 and B_3 are structurally identical, then all these bonds are partially broken and more amounts of the C-^{35}Cl bonds are broken than the C-^{35}Cl bonds, indicating the occurrence of intra-ion isotope effects. For other scenarios, intra-ion isotope effects can occur, and the isotope ratio of the product ion is higher than 1 but lower than $\sqrt{K_a}$.

1.2.1 Impacts on apparent isotope ratio of a product ion

According to the reaction pathways illustrated in Figure 3 and Figure 4, the chlorine isotope ratio derived from all the isotopologues of the product ion (apparent isotope ratio, $R_{pro(app)}$) of the imaginary compound is

$$R_{pro(app)} = \frac{b_2 + a_3 - b_3 + a_4}{b_3 + a_2 - b_2 + a_1} \tag{15}$$

And the isotope ratio of the parent ion involving MI1, MI2, MI3 and MI4 (R_{par}) is

$$R_{par} = \frac{a_2 + a_3 + 2a_4}{a_2 + a_3 + 2a_1} \tag{16}$$

which transforms to

$$R_{par} = \frac{\frac{a_2 + a_3}{2} + a_4}{\frac{a_2 + a_3}{2} + a_1} \tag{S27}$$

If the bonds A_2, B_2, A_3 and B_3 are structurally identical, then we have $a_2 = a_3$, and Eq (S27) can thus simplify to

$$R_{par} = \frac{a_2 + a_4}{a_2 + a_1} \tag{17}$$

As $R_{pro(a_2a_3)} = \sqrt{K_a}$, we have

$$\frac{A_{^{37}Cl}}{A_{^{37}Cl+^{35}Cl}} = \frac{\sqrt{K_a}}{\sqrt{K_a}+1} \tag{S28}$$

$$\frac{A_{^{35}Cl}}{A_{^{37}Cl+^{35}Cl}} = \frac{1}{\sqrt{K_a}+1} \tag{S29}$$

where $A_{^{37}Cl}$ represents the abundance of ^{37}Cl; $A_{^{35}Cl}$ refers to the abundance of ^{35}Cl; $A_{^{37}Cl+^{35}Cl}$ denotes the total abundance of ^{37}Cl and ^{35}Cl. Substituting Eq (S28) and Eq (S29) into Eq (15), yields

$$R_{pro(app)} = \frac{2a_2\dfrac{\sqrt{K_a}}{\sqrt{K_a}+1}+a_4}{2a_2\dfrac{1}{\sqrt{K_a}+1}+a_1} \tag{S30}$$

Then, we compare $R_{pro(app)}$ with R_{par}. Letting $K_a = 1$, we obtain

$$R_{pro(app)} = \frac{2a_2\dfrac{1}{1+1}+a_4}{2a_2\dfrac{1}{1+1}+a_1} = \frac{a_2+a_4}{a_2+a_1} = R_{par} \tag{S31}$$

And letting $\sqrt{K_a} = x$, we have

$$y = \frac{2a_2\dfrac{x}{x+1}+a_4}{2a_2\dfrac{1}{x+1}+a_1} \tag{S32}$$

and further obtain

$$\frac{dy}{dx}=\frac{\frac{2a_2}{(x+1)^2}(a_1+2a_2+a_4)}{(2a_2\frac{1}{x+1}+a_1)^2} \tag{S33}$$

Therefore, y is a monotonically increasing function. Additionally, as $f(K_a)=\sqrt{K_a}=x$ is monotonically increasing, then the following function

$$y=g(x)=g[f(K_a)]=R_{pro(app)}=\frac{2a_2\frac{\sqrt{K_a}}{\sqrt{K_a}+1}+a_4}{2a_2\frac{1}{\sqrt{K_a}+1}+a_1} \tag{S34}$$

is monotonically increasing. When $K_a=1$, we have

$$R_{pro(app)}=R_{par} \tag{18}$$

Accordingly, when $K_a>1$, we obtain

$$R_{pro(app)}=\frac{2a_2\frac{\sqrt{K_a}}{\sqrt{K_a}+1}+a_4}{2a_2\frac{1}{\sqrt{K_a}+1}+a_1}>\frac{a_2+a_4}{a_2+a_1}=R_{par} \tag{19}$$

Thus, the apparent isotope ratio of the product ion is always higher than that of the parent ion, only if $K_a>1$.

For asymmetric molecules, letting $R_{pro(a_2a_3)}=K_u$, then we have $1\le K_u<\sqrt{K_a}$. Substituting $R_{pro(a_2a_3)}=K_u$ into Eq (15), leads to

$$R_{pro(app)} = \frac{(a_2 + a_3)\dfrac{K_u}{K_u + 1} + a_4}{(a_2 + a_3)\dfrac{1}{K_u + 1} + a_1} \tag{S35}$$

Defining function

$$R_{pro(app)} = y = f(K_u) = \frac{(a_2 + a_3)\dfrac{K_u}{K_u + 1} + a_4}{(a_2 + a_3)\dfrac{1}{K_u + 1} + a_1} \tag{S36}$$

we further obtain

$$\frac{dy}{dK_u} = \frac{(a_2 + a_3)(a_1 + a_2 + a_3 + a_4)}{(K_u + 1)^2[(a_2 + a_3)\dfrac{1}{K_u + 1} + a_1]^2} = \frac{(a_2 + a_3)(a_1 + a_2 + a_3 + a_4)}{[a_2 + a_3 + (K_u + 1)a_1]^2} \tag{S37}$$

Since $\frac{dy}{dK_u} > 0$, the function y is monotonically increasing. As

$$K_u = \frac{x^2 + 2K_a x + K_a}{x^2 + 2x + K_a} \qquad (x = \sqrt{K_3}) \tag{S38}$$

when $0 \le x \le \sqrt{K_a}$, the function $K_u(x)$ is monotonically increasing, thus the function $y = f(K_u) = f[K_u(x)]$ is monotonically increasing. When $x \ge \sqrt{K_a}$, the function $K_u(x)$ is monotonically decreasing, thus the function $y = f(K_u) = f[K_u(x)]$ is monotonically decreasing. When $x = \sqrt{K_a}$, the function y has the maximum:

$$y = \frac{(a_2 + a_3)\dfrac{\sqrt{K_a}}{\sqrt{K_a} + 1} + a_4}{(a_2 + a_3)\dfrac{1}{\sqrt{K_a} + 1} + a_1} \tag{S39}$$

When $x=0$, then $K_u = R_{pro(a_2a_3)} = 1 = R_{par(a_2a_3)}$, and the function has the minimum:

$$y = \frac{(a_2+a_3)\frac{1}{1+1}+a_4}{(a_2+a_3)\frac{1}{1+1}+a_1} = \frac{\frac{a_2+a_3}{2}+a_4}{\frac{a_2+a_3}{2}+a_1} = R_{par} \tag{S40}$$

namely, $R_{pro(app)} = R_{par}$. When $x \to \infty$, the limit of K_u can be given as

$$\lim_{x\to\infty} K_u = \lim_{x\to\infty} \frac{x^2+2K_a x+K_a}{x^2+2x+K_a} = 1 \tag{S41}$$

Hence, the limit of the function y is

$$\lim_{K_u\to 1} y = \lim_{K_u\to 1} \frac{(a_2+a_3)\frac{K_u}{K_u+1}+a_4}{(a_2+a_3)\frac{1}{K_u+1}+a_1} = \frac{(a_2+a_3)\frac{1}{1+1}+a_4}{(a_2+a_3)\frac{1}{1+1}+a_1} = \frac{\frac{a_2+a_3}{2}+a_4}{\frac{a_2+a_3}{2}+a_1} \tag{S42}$$

in other words, $R_{pro(app)} = R_{par}$. For general scenarios, as

$$K_u = R_{pro(a_2a_3)} = \frac{x^2+2K_a x+K_a}{x^2+2x+K_a} \tag{S43}$$

the $R_{pro(app)}$ can be expressed as

$$R_{pro(app)} = \frac{(a_2+a_3)\frac{K_u}{K_u+1}+a_4}{(a_2+a_3)\frac{1}{K_u+1}+a_1} \qquad \left(K_u = \frac{K_3+2K_a\sqrt{K_3}+K_a}{K_3+2\sqrt{K_3}+K_a} \right) \tag{20}$$

For $x \in (0,\infty)$, we have the following inequation:

$$R_{par} < R_{pro(app)} \leq \frac{(a_2 + a_3)\dfrac{\sqrt{K_a}}{\sqrt{K_a}+1} + a_4}{(a_2 + a_3)\dfrac{1}{\sqrt{K_a}+1} + a_1} \tag{21}$$

1.3 Impacts on isotopologue distribution and implications to isotope-ratio calculation schemes

1.3.1 Case of symmetric compounds

1.3.1.1 Perspective from the dechlorinated (unobserved) molecular ion

If the dechlorinated molecular isotopologues comply with binomial distribution, then we get

$$R' = \frac{i}{n-i+1} \cdot \frac{I'_i}{I'_{i-1}} \tag{S44}$$

where R’ is the chlorine isotope ratio of the dechlorinated molecular ion, and *I’* is the abundance of the dechlorinated molecular ion. The relative abundance of the dechlorinated molecular isotopologue to initial molecular isotopologue before dechlorination (c'_i) is expressed as:

$$c'_1 = \frac{I'_{1-1}}{I'_{(1-1)0}}, c'_2 = \frac{I'_{2-1}}{I'_{(2-1)0}} ... c'_i = \frac{I'_{i-1}}{I'_{(i-1)0}} ... c'_n = \frac{I'_{n-1}}{I'_{(n-1)0}}, c'_{n+1} = \frac{I'_n}{I'_{n0}} \tag{S45}$$

According to Eq (S44) and Eq (S45), the isotope ratio (R’) calculated with a random pair of neighboring isotopologues (i-1 and i) is

$$R' = \frac{c'_{i+1} I'_{i0}}{c'_i I'_{(i-1)0}} \cdot \frac{i}{n-i+1} \tag{S46}$$

which transforms to

$$\frac{I'_{i0}}{I'_{(i-1)0}} \cdot \frac{i}{n-i+1} = \frac{c'_i}{c'_{i+1}} R' = R_0 \tag{S47}$$

Similarly for the pair of isotopologues *i* and *i*+1, we obtain

$$\frac{I'_{(i+1)0}}{I'_{i0}} \cdot \frac{i+1}{n-i} = \frac{c'_{i+1}}{c'_{i+2}} R' = R_{all} \tag{S48}$$

If the isotopologues of the dechlorinated molecular ion comply with binomial distribution, then the isotope ratios calculated using random pairs of neighboring isotopologues are equal. Therefore, for any three adjacent isotopologues, we have

$$\frac{c'_i}{c'_{i+1}} \cdot R' = \frac{c'_{i+1}}{c'_{i+1+1}} R' \qquad \text{(S49)}$$

which simplifies to

$$\frac{c'_i}{c'_{i+1}} = \frac{c'_{i+1}}{c'_{i+1+1}} \qquad \text{(S50)}$$

Hence, the progression (c'_1, c'_2 … c'_i, c'_{i+1} … c'_n, c'_{n+1}) is geometric:

$$c'_i = c'_1 q'^{i-1} \qquad \text{(S51)}$$

of which the common ratio (q') is

$$q' = \frac{c'_2}{c'_1} = \frac{c'_{i+1}}{c'_i} \qquad \text{(S52)}$$

Due to inter-ion isotope effects, lighter molecular isotopologues are more liable to be dechlorinated compared with heavier ones, the common ratio is thus less than 1 ($q' < 1$). We hypothesize $n \to \infty$, then get the limit of c'_i:

$$\lim_{i \to n} c'_i = \lim_{i \to n} c'_1 q'^{i-1} = 0 \qquad \text{(S53)}$$

which contradicts the reality, because it is impossible that all the amounts of heavier isotopologues are completely non-dechlorinated. Therefore, in fact, the progression (c'_1, c'_2 … c'_i, c'_{i+1} … c'_n, c'_{n+1}) is non-geometric. We therefore conclude that the dechlorinated molecular isotopologues do not comply with binomial distribution neither.

1.3.2 Case of asymmetric compounds

We list three random adjacent pairs of mergeable similar terms of $f(n)$ as follows:

$$\beta p_{i-1} = \beta C_{n-1}^{i-1} \alpha_0{}^{n-1-(i-1)} \beta_0{}^{i-1} \tag{S54}$$

$$\alpha p_i = \alpha C_{n-1}^{i} \alpha_0{}^{n-1-i} \beta_0{}^{i} \tag{S55}$$

$$\beta p_i = \beta C_{n-1}^{i} \alpha_0{}^{n-1-i} \beta_0{}^{i} \tag{S56}$$

$$\alpha p_{i+1} = \alpha C_{n-1}^{i+1} \alpha_0{}^{n-1-(i+1)} \beta_0{}^{i+1} \tag{S57}$$

$$\beta p_{i+1} = \beta C_{n-1}^{i+1} \alpha_0{}^{n-1-(i+1)} \beta_0{}^{i+1} \tag{S58}$$

$$\alpha p_{i+2} = \alpha C_{n-1}^{i+2} \alpha_0{}^{n-1-(i+2)} \beta_0{}^{i+2} \tag{S59}$$

where p_i refers to $C_{n-1}^{i} \alpha_0{}^{n-1-i} \beta_0{}^{i}$. Then the isotope ratios (R_{i+1} and R_{i+2}) calculated with pairs of neighboring isotopologues are

$$R_{i+1} = \frac{\beta p_i + \alpha p_{i+1}}{\beta p_{i-1} + \alpha p_i} \cdot \frac{i+1}{n-i} = \frac{\beta C_{n-1}^{i} \alpha_0{}^{n-1-i} \beta_0{}^{i} + \alpha C_{n-1}^{i+1} \alpha_0{}^{n-1-(i+1)} \beta_0{}^{i+1}}{\beta C_{n-1}^{i-1} \alpha_0{}^{n-1-(i-1)} \beta_0{}^{i-1} + \alpha C_{n-1}^{i} \alpha_0{}^{n-1-i} \beta_0{}^{i}} \cdot \frac{i+1}{n-i} \tag{S60}$$

$$R_{i+2} = \frac{\beta p_{i+1} + \alpha p_{i+2}}{\beta p_i + \alpha p_{i+1}} \cdot \frac{i+2}{n-i-1} = \frac{\beta C_{n-1}^{i+1} \alpha_0{}^{n-1-(i+1)} \beta_0{}^{i+1} + \alpha C_{n-1}^{i+2} \alpha_0{}^{n-1-(i+2)} \beta_0{}^{i+2}}{\beta C_{n-1}^{i} \alpha_0{}^{n-1-i} \beta_0{}^{i} + \alpha C_{n-1}^{i+1} \alpha_0{}^{n-1-(i+1)} \beta_0{}^{i+1}} \cdot \frac{i+2}{n-i-1} \tag{S61}$$

which simplify and transform to

$$\frac{\beta p_i + \alpha p_{i+1}}{\beta p_{i-1} + \alpha p_i} \cdot \frac{i+1}{n-i} = \frac{\dfrac{\beta \alpha_0 \beta_0}{i(n-1-i)} + \dfrac{\alpha \beta_0{}^2}{i(i+1)}}{\dfrac{\beta \alpha_0{}^2}{(n-i)(n-i-1)} + \dfrac{\alpha \alpha_0 \beta_0}{i(n-1-i)}} \cdot \frac{i+1}{n-i} \tag{S62}$$

$$\frac{\beta p_{i+1}+\alpha p_{i+2}}{\beta p_i+\alpha p_{i+1}}\cdot\frac{i+2}{n-i-1}=\frac{\frac{\beta\alpha_0\beta_0}{(i+1)(n-1-i-1)}+\frac{\alpha\beta_0{}^2}{(i+2)(i+1)}}{\frac{\beta\alpha_0{}^2}{(n-i-1)(n-1-i-1)}+\frac{\alpha\alpha_0\beta_0}{(i+1)(n-1-i-1)}}\cdot\frac{i+2}{n-i-1} \tag{S63}$$

Then the proof of $R_{i+1}>R_{i+2}$ is equivalent to proving

$$\frac{\beta p_i+\alpha p_{i+1}}{\beta p_{i-1}+\alpha p_i}\cdot\frac{i+1}{n-i}>\frac{\beta p_{i+1}+\alpha p_{i+2}}{\beta p_i+\alpha p_{i+1}}\cdot\frac{i+2}{n-i-1} \tag{S64}$$

which is further equivalent to

$$\frac{\frac{\beta\alpha_0}{i(n-1-i)}+\frac{\alpha\beta_0}{i(i+1)}}{\frac{\beta\alpha_0}{(n-i)(n-i-1)}+\frac{\alpha\beta_0}{i(n-1-i)}}\cdot\frac{i+1}{n-i}>\frac{\frac{\beta\alpha_0}{(i+1)(n-1-i-1)}+\frac{\alpha\beta_0}{(i+2)(i+1)}}{\frac{\beta\alpha_0}{(n-i-1)(n-1-i-1)}+\frac{\alpha\beta_0}{(i+1)(n-1-i-1)}}\cdot\frac{i+2}{n-i-1} \tag{S65}$$

Letting $\frac{\beta\alpha_0}{\alpha\beta_0}=k$ and substituting it into Ineq (S65), yields

$$\frac{\frac{k}{i(n-1-i)}+\frac{1}{i(i+1)}}{\frac{k}{(n-i)(n-i-1)}+\frac{1}{i(n-1-i)}}\cdot\frac{i+1}{n-i}>\frac{\frac{k}{(i+1)(n-1-i-1)}+\frac{1}{(i+2)(i+1)}}{\frac{k}{(n-i-1)(n-1-i-1)}+\frac{1}{(i+1)(n-1-i-1)}}\cdot\frac{i+2}{n-i-1} \tag{S66}$$

which simplifies to

$$\frac{k(i+1)+(n-i-1)}{ki+(n-i)}>\frac{k(i+2)+(n-i-2)}{k(i+1)+(n-i-1)} \tag{S67}$$

This inequation can be transformed to

$$\frac{k(i+1)+(n-i-1)}{ki+(n-i)}>\frac{k[(i+1)+1]+[n-(i+1)-1]}{k(i+1)+[n-(i+1)]} \tag{S68}$$

We define a function H (i) as

$$H(i)=\frac{k(i+1)+(n-i-1)}{ki+(n-i)} \tag{S69}$$

and further obtain

$$\frac{dH(i)}{di}=\frac{(k-1)(ki-i+n)-(ki-i+k+n-1)(k-1)}{[ki+(n-i)]^2}=\frac{-(k-1)^2}{[ki+(n-i)]^2} \tag{S70}$$

When $k=1$, we obtain $\frac{dH(i)}{di}=0$ which indicates that H (i) always equals a constant ($H(i)=1$). Thus, we have

$$\frac{k(i+1)+(n-i-1)}{ki+(n-i)}=\frac{k(i+2)+(n-i-2)}{k(i+1)+(n-i-1)}=1 \tag{S71}$$

and

$$R_{i+1}=R_{i+2} \tag{S72}$$

Due to inter-ion isotope effects, the isotope ratio of the remaining Cl atoms on the specified position increases, and therefore we have

$$\frac{\beta}{\alpha}>\frac{\beta_0}{\alpha_0} \tag{S73}$$

which leads to

$$k=\frac{\beta\alpha_0}{\alpha\beta_0}>1 \tag{S74}$$

Hence, we have

$$\frac{d\mathrm{H}(i)}{di}<0 \tag{S75}$$

Accordingly, the function H (i) monotonically decreases in the definitional domain. Therefore, the following inequation $H(i) > H(i+1)$ is obtained, that is,

$$\frac{k(i+1)+(n-i-1)}{ki+(n-i)} > \frac{k[(i+1)+1]+[n-(i+1)-1]}{k(i+1)+[n-(i+1)]} \tag{S76}$$

which is equivalent to

$$R_{i+1} > R_{i+2} \quad (i = 0, 1, 2 . \tag{40}$$

2 Experimental section

2.1 Chemicals and materials

Reference standards of tetrachloroethylene (PCE, 99.0%) and trichloroethylene (TCE, 99.5%) of high performance liquid chromatography (HPLC) grade were bought from Dr. Ehrenstorfer (Augsburg, Germany, manufacturer-1), and the analytical-reagent grade PCE (99.0%) and TCE (99.0%) were obtained from Tianjin Fuyu Chemical Co. Ltd. (Tianjin, China, manufacturer-2). The standards were accurately weighed and dissolved in n-hexane to prepare stock solutions at 1.0 mg/mL. These stock solutions were further diluted with n-hexane to prepare cocktailed working solutions containing both PCE and TCE standards from individual manufacturers at 1.0 µg/mL. All the standard solutions were stored at −20 ºC condition prior to use. Hexane was of HPLC grade and bought from Merck Corp. (Darmstadt, Germany). Perfluorotributylamine (the reference standard for calibrating HRMS) was provided by Sigma-Aldrich LLC. (St. Louis, USA).

2.2 Instrumental measurement

The working solutions were directly analyzed by gas chromatography-high resolution mass spectrometry (GC-HRMS). The GC-HRMS system comprised dual gas chromatographers (Trace-GC-Ultra) coupled with a double focus magnetic-sector high resolution MS and a TriPlus auto-sampler (GC-DFS-HRMS, Thermo-Fisher Scientific, Bremen, Germany). The chromatographic separation was performed with a DB-5MS capillary column (60 m × 0.25 mm, 0.25 µm thickness, J&W Scientific, USA). Details of the temperature programs are tabulated in Table S-1.

The working conditions and parameters of the HRMS system are provided as follows: electron ionization source operated in positive mode (EI+) was used; EI energy was set at 45 eV; ionization source was maintained at 250 ºC; filament current of EI source was 0.8 mA; multiple ion detection (MID) mode was used for data acquisition; dwell time of each isotopologue ion was about 20 ms; mass resolution was ≥ 10000 (5% peak-valley definition) and the detection accuracy was ± 0.001 u. The HRMS system was calibrated in real time with perfluorotributylamine during MID operation.

Structures of PCE and TCE were sketched by ChemDraw (Ultra 7.0, Cambridgesoft), and the exact masses of the molecular isotopologues were calculated with mass accuracy of 0.00001 u. Only chlorine isotopologues were considered. For a compound containing *n* Cl atoms, the complete isotopologues (*n* + 1) were chosen. The mass-to-charge ratios (*m/z*) of ions were obtained by subtracting the mass of an electron from the exact mass of each isotopologue. The *m/z* values were imported into the MID module for data acquisition. The details of PCE and TCE in terms of retention times, isotopologue formulas, exact masses, exact *m/z* values, and isotopologue theoretical relative abundances are provided in Table S-2.

2.3 Data processing

Chlorine isotope ratio (R) was calculated with

$$R_{Comp\_Iso} = \frac{\sum_{i=0}^{n} iI_i}{\sum_{i=0}^{n} (n-i)I_i} \tag{23}$$

and

$$R_{IP\_Iso} = \frac{i}{n-i+1} \cdot \frac{I_i}{I_{i-1}} \tag{22}$$

where *n* is the number of Cl atoms of a molecule; *i* is the number of ^{37}Cl atoms of a molecular isotopologue; I_i is the MS signal intensity of the molecular isotopologue *i*; $\mathrm{R_{Comp\_Iso}}$ refers to the isotope ratio calculated with the complete molecular isotopologue scheme; $\mathrm{R_{IP\_Iso}}$: denotes the isotope ratio calculated with the molecular isotopologue-pair scheme. It is noteworthy that the complete molecular isotopologue scheme applied in our study is different from the previously reported “complete ion method” which involves both molecular and fragmental ions.[1]

All the measured chlorine isotope ratios in this study were raw values without being calibrated to the scale of the Standard Mean Ocean Chlorine (SMOC) due to unavailability of the external isotopic standards with known chlorine isotope compositions and identical structures as PCE and TCE. Since all the isotopologues of each compound were simultaneously monitored by HRMS, therefore the measured raw isotope ratios were accurate relative isotope ratios, viz. it was indeed real for the observed differences among the isotope ratios calculated with different pairs of neighboring isotopologues by the molecular isotopologue-pair scheme and between those calculated by different schemes (i.e., the molecular isotopologue-pair scheme and the complete molecular isotopologue scheme) for individual compounds. As a result, the lack of calibration of the measured isotope ratios to the SMOC scale cannot affect the conclusions of this study. The average MS signal intensity of each isotopologue within the whole chromatographic peak was extracted and used for isotope ratio calculation. Background subtraction was performed before exporting MS signal intensity by subtracting intensities of the baseline regions adjacent to the corresponding chromatographic peak. Data from five or six replicated injections were applied to calculation of each mean isotope ratio along with the corresponding standard deviation (1σ).

References

1 B. Jin, C. Laskov, M. Rolle, S. B. Haderlein, Chlorine isotope analysis of organic contaminants using GC-qMS: method optimization and comparison of different evaluation schemes. *Environ. Sci. Technol.,* 2011, 45, 5279-5286.

Tables

Table S-1. Names, structures, CAS number, concentrations and chromatographic separation conditions of tetrachloroethylene (PCE) and trichloroethylene (TCE).

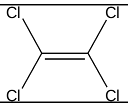
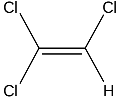
Compound	Abbreviation	Structure	CAS No.	Temperature program	Concentration (µg/mL)	Injection solvent
Tetrachloroethylene	PCE		127-18-4	Held at 40 ºC for 2 min, ramped to 65 ºC at 2 ºC/min, then ramped to 300 ºC at 40 ºC/min, held for 1 min (Inlet: 260 ºC; Transfer line: 280 ºC; flow rate: 1.0 mL/min)	1.0	Hexane
Trichloroethylene	TCE		79-01-6			

Table S-2. Retention times, chemical formulas, isotopologue formulas, *m/z* values, and isotopologue theoretical relative abundances of PCE and TCE.

Compound	Retention time (min)	Ion type	Formula	Isotopologue formula	*m/z* value (u)	Theoretical relative abundance
PCE	12.55	Molecular	C_2Cl_4	$C_2{}^{35}Cl_4$	163.87486	78.19
				$C_2{}^{35}Cl_3{}^{37}Cl$	165.87191	100.00
				$C_2{}^{35}Cl_2{}^{37}Cl_2$	167.86896	47.98
				$C_2{}^{35}Cl^{37}Cl_3$	169.86601	10.23
				$C_2{}^{37}Cl_4$	171.86306	0.82
TCE	8.38	Molecular	C_2HCl_3	$C_2H^{35}Cl_3$	129.91383	100.00
				$C_2H^{35}Cl_2{}^{37}Cl$	131.91088	95.90
				$C_2H^{35}Cl^{37}Cl_2$	133.90793	30.70
				$C_2H^{37}Cl_3$	135.90498	3.30