

Chlorine Isotope Analysis of Organic Contaminants Using GC-qMS: Method Optimization and Comparison of Different Evaluation Schemes

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S1. Details on Chlorine Isotope Ratio Computations

Deriving Relative Isotopologue Abundances. Expressing the relative isotopologue abundances of molecular chlorinated compounds and corresponding fragments is the first step to compute the chlorine isotope ratio. Taking PCE as an example and light ^{35}Cl (L %) and heavy ^{37}Cl (H %) chlorine atoms; the probability of encountering a PCE molecule containing two ^{35}Cl and two ^{37}Cl is $6 \cdot H \cdot H \cdot L \cdot L$. The number, “6”, indicates six possible patterns of heavy and light isotope compositions (i.e. HHLL, HLHL, HLLH, LHHL, LHLH and LLHH). The computed probability stands for the relative abundance of $\text{C}_2^{35}\text{Cl}_2^{37}\text{Cl}_2$. The probability that an event will occur “ k ” times out of “ n ” trials can be computed by the binomial formula (1):

$$P = \binom{n}{k} p^k (1-p)^{n-k} \quad (\text{S1})$$

$$\binom{n}{k} = \frac{(n-k+1) \cdot (n-k+2) \cdots (n-1) \cdot (n)}{k!} \quad (\text{S2})$$

where n is the number of trials, k is the specific number that an event will occur, and p is the probability that the event is to occur on each particular trial. Eqn. S2 expresses the binomial coefficient, which represents the number of possible patterns (1).

For the general case of chlorinated compounds, this equation can be used to compute the relative abundance of the chlorinated isotopologue containing “ k ” ^{37}Cl atoms out of a total of “ n ” chlorine atoms. This corresponds to:

$$A = \binom{n}{k} H^k L^{n-k} \quad (\text{S3})$$

where A is the relative abundance of a certain isotopologue of a chlorinated compound, H (%) is the abundance of ^{37}Cl , L (%) is the abundance of ^{35}Cl , n is the total number of chlorine atoms in a certain chlorinated compound, k is the number of ^{37}Cl atoms in a specified

isotopologue. Here, the binomial coefficient is defined as the number of possible positions of chlorine atoms on a certain molecule.

Due to the excessive electron impact (EI) energy in the ion source of mass spectrometry, not only charged molecular ions but also fragment ions exist in the mass spectrum. Isotopologues with different chlorine isotope compositions are identified by the corresponding mass over charge ratios (m/z). Based on the statistical method explained above, all the relative ion abundances of molecular chlorinated ethenes and their corresponding fragments are mathematically expressed and summarized in Figure S1.

PCE	Molecular group	Mass	Relative abundance	Chlorine isotope ratio	
	$^{12}\text{C}_2^{35}\text{Cl}_4$	164*	L ⁴ (1)	$R_M \rightarrow$	
	$^{12}\text{C}_2^{35}\text{Cl}_3^{37}\text{Cl}$	166*	4L ³ H (2)		$1/4 \cdot (2)/(1)$
	$^{12}\text{C}_2^{35}\text{Cl}_2^{37}\text{Cl}_2$	168	6L ² H ² (3)		$2/3 \cdot (3)/(2)$
	$^{12}\text{C}_2^{35}\text{Cl}^{37}\text{Cl}_3$	170	4LH ³ (4)		$3/2 \cdot (4)/(3)$
	$^{12}\text{C}_2^{37}\text{Cl}_4$	172	H ⁴ (5)		$4 \cdot (5)/(4)$
	Fragment group 1				
	$^{12}\text{C}_2^{35}\text{Cl}_3$	129*	L ³ (6)	$R_{F1} \rightarrow$	
	$^{12}\text{C}_2^{35}\text{Cl}_2^{37}\text{Cl}$	131*	3L ² H (7)		$1/3 \cdot (7)/(6)$
	$^{12}\text{C}_2^{35}\text{Cl}^{37}\text{Cl}_2$	133	3LH ² (8)		$(8)/(7)$
	$^{12}\text{C}_2^{37}\text{Cl}_3$	135	H ³ (9)		$3 \cdot (9)/(8)$
	Fragment group 2				
	$^{12}\text{C}_2^{35}\text{Cl}_2$	94*	L ² (10)	$R_{F2} \rightarrow$	
	$^{12}\text{C}_2^{35}\text{Cl}^{37}\text{Cl}$	96*	2LH (11)		$1/2 \cdot (11)/(10)$
	$^{12}\text{C}_2^{37}\text{Cl}_2$	98	H ² (12)		$2 \cdot (12)/(11)$
	Fragment group 3				
	$^{12}\text{C}_2^{35}\text{Cl}$	59*	L (13)	$R_{F3} \rightarrow$	
	$^{12}\text{C}_2^{37}\text{Cl}$	61*	H (14)		$(14)/(13)$
TCE	Molecular group				
	$^{12}\text{C}_2\text{H}^{35}\text{Cl}_3$	130*	L ³ (15)	$R_M \rightarrow$	
	$^{12}\text{C}_2\text{H}^{35}\text{Cl}_2^{37}\text{Cl}$	132*	3L ² H (16)		$1/3 \cdot (16)/(15)$
	$^{12}\text{C}_2\text{H}^{35}\text{Cl}^{37}\text{Cl}_2$	134	3LH ² (17)		$(17)/(16)$
	$^{12}\text{C}_2\text{H}^{37}\text{Cl}_3$	136	H ³ (18)		$3 \cdot (18)/(17)$
	Fragment group 1				
	$^{12}\text{C}_2^{35}\text{Cl}_2\text{H}$	95*	L ² (19)	$R_{F1} \rightarrow$	
	$^{12}\text{C}_2^{35}\text{Cl}^{37}\text{ClH}$	97*	2LH (20)		$1/2 \cdot (20)/(19)$
	$^{12}\text{C}_2^{37}\text{Cl}_2\text{H}$	99	H ² (21)		$2 \cdot (21)/(20)$
	Fragment group 2				
	$^{12}\text{C}_2\text{H}^{35}\text{Cl}$	60*	L (22)	$R_{F2} \rightarrow$	
	$^{12}\text{C}_2\text{H}^{37}\text{Cl}$	62*	H (23)		$(23)/(22)$
DCE	Molecular group				
	$^{12}\text{C}_2\text{H}_2^{35}\text{Cl}_2$	96*	L ² (24)	$R_M \rightarrow$	
	$^{12}\text{C}_2\text{H}_2^{35}\text{Cl}^{37}\text{Cl}$	98*	2LH (25)		$1/2 \cdot (25)/(24)$
	$^{12}\text{C}_2\text{H}_2^{37}\text{Cl}_2$	100	H ² (26)		$2 \cdot (26)/(25)$
	Fragment group 1				
	$^{12}\text{C}_2\text{H}_2^{35}\text{Cl}$	61*	L (27)	$R_{F1} \rightarrow$	
	$^{12}\text{C}_2\text{H}_2^{37}\text{Cl}$	63*	H (28)		$(28)/(27)$
VC	Molecular group				
	$^{12}\text{C}_2\text{H}_3^{35}\text{Cl}$	62*	L (29)	$R_M \rightarrow$	
	$^{12}\text{C}_2\text{H}_3^{37}\text{Cl}$	64*	H (30)		$(30)/(29)$

FIGURE S1. Evaluation scheme for chlorine isotope ratio determination of chlorinated ethenes based on relative abundances of different isotopologues. The masses selected in the multiple ion method are labelled (*). Arrows indicate the expression for chlorine isotope ratio determination. R_M and R_F are the partial chlorine isotope ratios from the molecular and fragment ion groups, respectively.

Conversion of Mass Spectra into Partial Chlorine Isotope Ratios. The partial chlorine isotope ratio can be determined indirectly using the relative abundances of neighboring isotopologues with a difference of two-mass units. For instance, the partial chlorine isotope ratios of PCE can be determined as follows:

$$R_{M(PCE)} = \frac{1}{4} \cdot \frac{I_{166}}{I_{164}} \quad (\text{S4})$$

$$R_{F1(PCE)} = \frac{1}{3} \cdot \frac{I_{131}}{I_{129}} \quad (\text{S5})$$

$$R_{F2(PCE)} = \frac{1}{2} \cdot \frac{I_{96}}{I_{94}} \quad (\text{S6})$$

$$R_{F3(PCE)} = \frac{I_{61}}{I_{59}} \quad (\text{S7})$$

For TCE similar equations can be derived as follow:

$$R_{M(TCE)} = \frac{1}{3} \cdot \frac{I_{132}}{I_{130}} \quad (\text{S8})$$

$$R_{F1(TCE)} = \frac{1}{2} \cdot \frac{I_{97}}{I_{95}} \quad (\text{S9})$$

$$R_{F2(TCE)} = \frac{I_{62}}{I_{60}} \quad (\text{S10})$$

Calculation of Overall Chlorine Isotope Ratios. In order to calculate the overall chlorine isotope ratio that represents the isotopic composition of a specific compound, the weighted average of partial $^{37}\text{Cl}/^{35}\text{Cl}$ ratios from different ion groups needs to be taken into account. In comparison to the previous work of Sakaguchi-Soeder et al. (2), here, the weight factors are

defined as the relative abundance of the two most abundant ions in each ion group, instead of only the major ion. Hence, both ions contributing to the partial chlorine isotope ratio computation are taken into account. The overall isotope ratio can then be determined as follows:

$$R_{PCE} = a \times R_M + b \times R_{F1} + c \times R_{F2} + d \times R_{F3} \quad (S11)$$

$$a = \frac{I_{166} + I_{164}}{(I_{166} + I_{164}) + (I_{131} + I_{129}) + (I_{96} + I_{94}) + (I_{61} + I_{59})}$$

$$b = \frac{I_{131} + I_{129}}{(I_{166} + I_{164}) + (I_{131} + I_{129}) + (I_{96} + I_{94}) + (I_{61} + I_{59})}$$

$$c = \frac{I_{96} + I_{94}}{(I_{166} + I_{164}) + (I_{131} + I_{129}) + (I_{96} + I_{94}) + (I_{61} + I_{59})}$$

$$d = \frac{I_{61} + I_{59}}{(I_{166} + I_{164}) + (I_{131} + I_{129}) + (I_{96} + I_{94}) + (I_{61} + I_{59})} \quad (S12)$$

where I is the corresponding molecular/fragment ion abundance at different m/z values. The weight factors a , b , c and d are defined as the relative ion intensities among two major ions from each group. R_M , R_{F1} , R_{F2} and R_{F3} are the partial chlorine isotope ratios determined from two major ions of each group. Similarly, for TCE, DCE and VC (vinyl chloride) the equations are:

$$R_{TCE} = a \times R_M + b \times R_{F1} + c \times R_{F2} \quad (S13)$$

$$a = \frac{I_{130} + I_{132}}{(I_{132} + I_{130}) + (I_{97} + I_{95}) + (I_{62} + I_{60})}$$

$$b = \frac{I_{97} + I_{95}}{(I_{132} + I_{130}) + (I_{97} + I_{95}) + (I_{62} + I_{60})}$$

$$c = \frac{I_{62} + I_{60}}{(I_{132} + I_{130}) + (I_{97} + I_{95}) + (I_{62} + I_{60})} \quad (S14)$$

$$R_{DCE} = a \times R_M + b \times R_{F1} \quad (S15)$$

$$a = \frac{I_{96} + I_{98}}{(I_{96} + I_{98}) + (I_{61} + I_{63})} \quad b = \frac{I_{61} + I_{63}}{(I_{96} + I_{98}) + (I_{61} + I_{63})} \quad (S16)$$

$$R_{VC} = R_M \quad (S17)$$

Differently from the multiple ion method, the complete ion method uses complete mass spectral data to compute the total abundances of ^{35}Cl and ^{37}Cl atoms in each ion group. The partial $^{35}\text{Cl}/^{37}\text{Cl}$ ratios of PCE molecule/fragment then can be determined by taking ratios of heavy over light chlorine atom abundance. For PCE the equations correspond to:

$$R_M = \frac{I_{166} + 2I_{168} + 3I_{170} + 4I_{172}}{4I_{164} + 3I_{166} + 2I_{168} + I_{170}} \quad (S18)$$

$$R_{F1} = \frac{I_{131} + 2I_{133} + 3I_{135}}{3I_{129} + 2I_{131} + I_{133}} \quad (S19)$$

$$R_{F2} = \frac{I_{96} + 2I_{98}}{2I_{94} + I_{96}} \quad (S20)$$

$$R_{F3} = \frac{I_{61}}{I_{59}} \quad (S21)$$

Then, the overall $^{35}\text{Cl}/^{37}\text{Cl}$ ratio for PCE can be determined by taking weighted mean

$$R_{PCE} = a \cdot R_M + b \cdot R_{F1} + c \cdot R_{F2} + d \cdot R_{F3} \quad (S22)$$

$$a = \frac{\sum_{j=1}^{n_M} I_j}{\sum_{k=1}^{n_T} I_k} \quad b = \frac{\sum_{j=1}^{n_{F1}} I_j}{\sum_{k=1}^{n_T} I_k}$$

$$c = \frac{\sum_{j=1}^{n_{F2}} I_j}{\sum_{k=1}^{n_T} I_k} \quad d = \frac{\sum_{j=1}^{n_{F3}} I_j}{\sum_{k=1}^{n_T} I_k} \quad (\text{S23})$$

where the n_M , n_{F1} , n_{F2} , and n_{F3} are the number of ions in each ion group, respectively, n_T is the total number of ion in the mass spectra, a , b , c and d are weight factors.

Similarly, the corresponding equations for TCE are:

$$R_M = \frac{I_{132} + 2I_{134} + 3I_{136}}{3I_{130} + 2I_{132} + I_{134}} \quad (\text{S24})$$

$$R_{F1} = \frac{I_{97} + 2I_{99}}{2I_{95} + I_{97}} \quad (\text{S25})$$

$$R_{F2} = \frac{I_{62}}{I_{60}} \quad (\text{S26})$$

$$R_{TCE} = a \cdot R_M + b \cdot R_{F1} + c \cdot R_{F2} \quad (\text{S27})$$

a , b and c are the weight factors which can be computed by Eqn. S23.

S2. List of Instrumental Parameters

The principle GC-qMS settings used in this study are the following:

Injection parameters

Injection mode: split

Split ratio: 10:1

Injected volume: 500 μL

Column and oven parameters

GC column: 60 m \times 250 μm , 1.4 μm film thickness; Restek, USA

Carrier gas: Helium at 1ml/min

Oven program: 40 $^{\circ}\text{C}$ (2 min) \rightarrow 110 $^{\circ}\text{C}$ @ 25 $^{\circ}\text{C}/\text{min}$ \rightarrow 200 $^{\circ}\text{C}$ @ 15 $^{\circ}\text{C}/\text{min}$ (5min)

Mass spectrometer parameters

Type of mode: selected ion monitoring (SIM)

Ion Source Temperature: 230 °C

SIM list: See selected mass/ion (see Fig. 2) or complete mass/ion for cis-DCE

EI voltage: 70 eV

S3. Experimental dataset

In the following we report in a tabular format the experimental data shown in Figure 3, and the comparison of the selected evaluation methods for TCE and PCE.

Table S1. Evaluation of chlorine isotope ratios of PCE and TCE using the conventional and modified multiple ion methods.

Compound	n ^b	Aqueous Conc. [µg/L]	Conventional multiple ion method (2)				Modified multiple ion method		
			Mass on Column [pmol]	³⁷ Cl/ ³⁵ Cl Ratio	STDV (1σ)	RSD [%]	³⁷ Cl/ ³⁵ Cl Ratio	STDV (1σ)	RSD [%]
TCE	5	20	3.2	0.3237	0.00076	2.4	0.32344	0.00066	2.0
	5	50	8	0.3230	0.00062	1.9	0.32266	0.00061	1.9
	5	100	16	0.3224	0.00038	1.2	0.32208	0.00039	1.2
	5	200	32	0.3227	0.00032	1.0	0.32240	0.00032	1.0
	5	300	48	0.3231	0.00026	0.8	0.32277	0.00022	0.7
	5	400	64	0.3229	0.00024	0.7	0.32263	0.00025	0.8
	5	500	80	0.3230	0.00023	0.7	0.32273	0.00023	0.7
PCE	5	20	4.6	0.32159	0.00053	1.7	0.32144	0.00048	1.5
	5	50	11.5	0.32228	0.00030	0.9	0.32216	0.00036	1.1
	5	100	23	0.32210	0.00026	0.8	0.32195	0.00024	0.8
	5	200	46	0.32214	0.00038	1.2	0.32199	0.00038	1.2
	5	300	69	0.32233	0.00028	0.9	0.32217	0.00028	0.9
	5	400	92	0.32233	0.00018	0.6	0.32217	0.00018	0.6
	5	500	115	0.32232	0.00012	0.4	0.32216	0.00013	0.4

Table S2. Evaluation of chlorine isotope ratios of PCE and TCE mixtures (at 200 µg/L) using the conventional and modified multiple ion methods, the molecular ion method and the complete ion method.

Evaluation schemes	TCE				PCE			
	n	³⁷ Cl/ ³⁵ Cl Ratio	STDV(1σ)	RSD [%]	n	³⁷ Cl/ ³⁵ Cl Ratio	STDV(1σ)	RSD [%]
Molecular ion method	5	0.32122	0.00039	1.2	5	0.32002	0.00062	1.9
Conventional multiple ion method	5	0.32270	0.00032	1.0	5	0.32214	0.00038	1.2
Modified multiple ion method	5	0.32240	0.00032	1.0	5	0.32199	0.00038	1.2
Complete ion method	5	0.32488	0.00055	1.7	5	0.33570	0.00083	2.5

As shown in table S2, the complete ion method resulted in the highest chlorine isotope ratios with the lowest precision (1.7‰ for TCE, 2.5‰ for PCE). The conventional and modified ion method showed the highest precision (1‰ for TCE, 1.2‰ for PCE). Note that here the results using the complete ion method are based on a full mass spectral data of PCE and TCE (see Figure S1). A larger number of ions (i.e. 14 ions for PCE, 9 for TCE) were added into the SIM mode of MS, therefore significantly lowering the scan rates.

S4. Long-Time Monitoring of the Chlorine Isotope Ratios

Chlorine isotope ratios for PCE and TCE measured over a period of approximately 6 months are reported in Table S3.

Table S3: Chlorine isotope ratios of PCE and TCE over time

Date	PCE		TCE	
	³⁷ Cl/ ³⁵ Cl Ratio	STDV (1σ)	³⁷ Cl/ ³⁵ Cl Ratio	STDV(1σ)
07-Jul-09	0.32107	0.00034	0.32219	0.00078
16-Jul-09	0.32163	0.00064	0.32215	0.00061
22-Jul-09	0.32159	0.00032	0.32270	0.00090
30-Jul-09	0.32222	0.00039	0.32299	0.00019
05-Jan-2010 ^a	0.32195	0.00026	0.32266	0.00061
06-Jan-10	0.32217	0.00028	0.32276	0.00020
13-Jan-10	0.32199	0.00038	0.32240	0.00032
	PCE		TCE	
	Average^b		0.322	Average 0.32255
	STDV (1σ)^c		4E-04	STDV (1σ) 0.00031

a: changing of GC column: before: DB-5 capillary column (30 m × 250 μm I.D., 0.25 μm film); after: Restek capillary column (60 m × 250 μm, 1.4 μm film thickness)

b: average of ³⁷Cl/³⁵Cl ratio over time;

c: standard deviation of ³⁷Cl/³⁵Cl ratio over time

S5. Evaluation of ¹³C Error

Differences of two mass units in the MS spectra are not only caused by ³⁵Cl and ³⁷Cl atoms, but can also be caused by two ¹³C atoms. Neglecting the influence of ¹³C atoms results in an error on ³⁷Cl/³⁵Cl ratios. This can be quantified using the equation reported by Aeppli et al. (3), which reads as:

$$Error = R_{chlorine} - R'_{chlorine} = \frac{1}{n_{Cl}} \times \frac{n_C(n_C - 1)}{2} \times R_C^2 \quad (S28)$$

where $R_{chlorine}$ is the apparent ³⁷Cl/³⁵Cl ratios (experimental values), $R'_{chlorine}$ is the theoretical ³⁷Cl/³⁵Cl ratios (corrected values), n_{Cl} is the number of chlorine atoms per molecule, n_C is the number of carbon atoms per molecule.

Table S4: Quantification of error caused by ^{13}C

N_{C}^{a}	N_{Cl}^{b}	$R_{\text{chlorine}} - R'_{\text{chlorine}}$	Overestimated Error [%] ^c
2	1	R_{carbon}^2	0.100
2	2	$\frac{1}{2} \cdot R_{\text{carbon}}^2$	0.050
2	3	$\frac{1}{3} \cdot R_{\text{carbon}}^2$	0.033
2	4	$\frac{1}{4} \cdot R_{\text{carbon}}^2$	0.025
6	1	$15 \cdot R_{\text{carbon}}^2$	1.500
6	2	$\frac{15}{2} \cdot R_{\text{carbon}}^2$	0.750
6	3	$5 \cdot R_{\text{carbon}}^2$	0.500
6	4	$\frac{15}{4} \cdot R_{\text{carbon}}^2$	0.375
6	5	$3 \cdot R_{\text{carbon}}^2$	0.300
6	6	$\frac{5}{2} \cdot R_{\text{carbon}}^2$	0.250

a: Number of carbon atoms in molecule or fragment

b: Number of chlorine atoms in molecule or fragment

c: Assuming $R_{\text{carbon}}=1\%$

In addition, the stable hydrogen isotopes (i.e. ^1H and ^2H) can also contribute to the potential error with the presence of two deuterium atoms in certain molecules and fragments. Similarly, the error can be also determined in a way that three elements, chlorine, carbon and hydrogen, are all involved into the probability computation. However, due to the extremely low natural abundance of deuterium (about 0.0001) (4), this error can be neglected unless the considered compounds has a very large number of hydrogen atoms.

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