Supporting Information

Compound-specific chlorine isotope analysis of the herbicides atrazine, acetochlor and metolachlor

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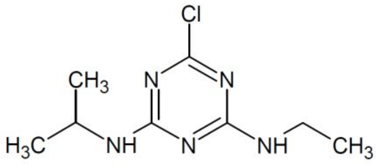
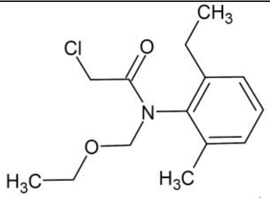
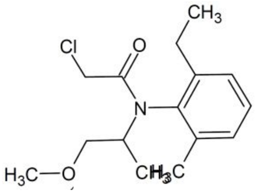
I. Target analytes

Table S1. Relevant properties of atrazine (ATR), acetochlor (ACETO) and metolachlor (METO).

	Atrazine (ATR)	**Acetochlor (ACETO)**	**Metolachlor (METO)**
Chemical structure			
Empirical formula	$C_8H_{14}ClN_5$	$C_{14}H_{20}ClNO_2$	$C_{15}H_{22}ClNO_2$
Molecular weight (g/mol)	215.7	269.8	283.8
Melting point (°C)	175.8	10.2	-62.1
Boiling point (°C)	200	172	n.a.
Solubility in ethyl acetate (g/L)	24	500	n.a.

n.a. not available

II. Methods

II.1. qMS method

Figure S1. Chromatogram of ATR, ACETO and METO standards (Single Ion Monitoring) corresponding to 10 ng on column for ATR (0.046 nmol Cl) and METO (0.035 nmol Cl) and 15 ng on column for ACETO (0.056 nmol Cl).

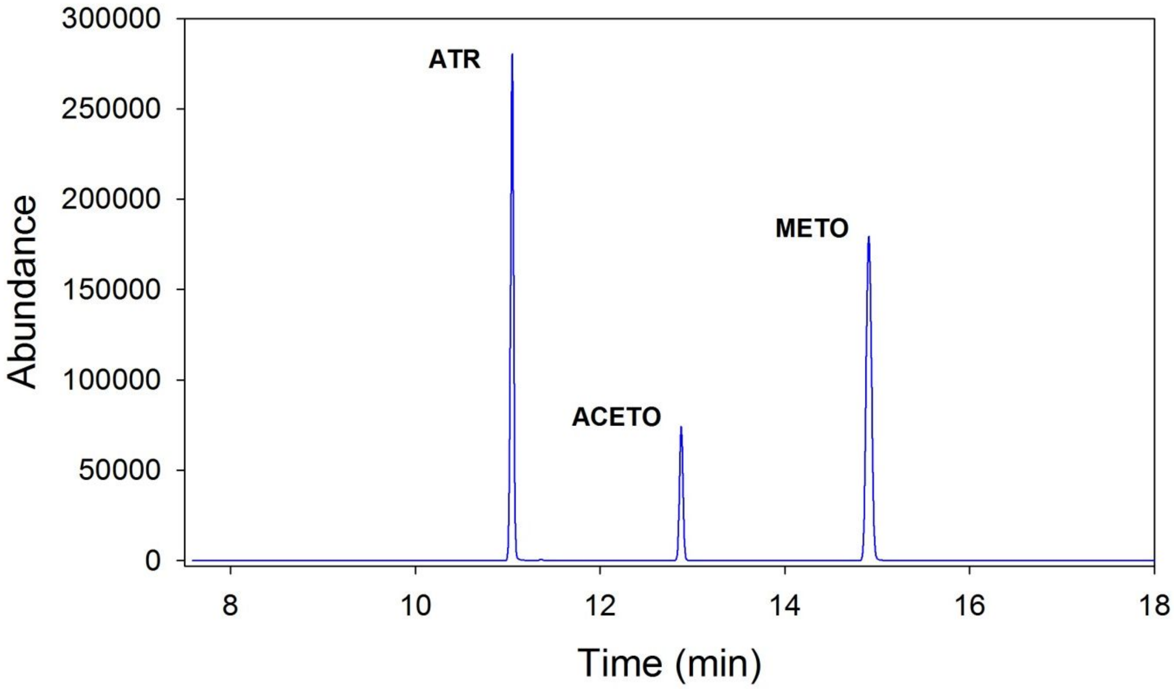
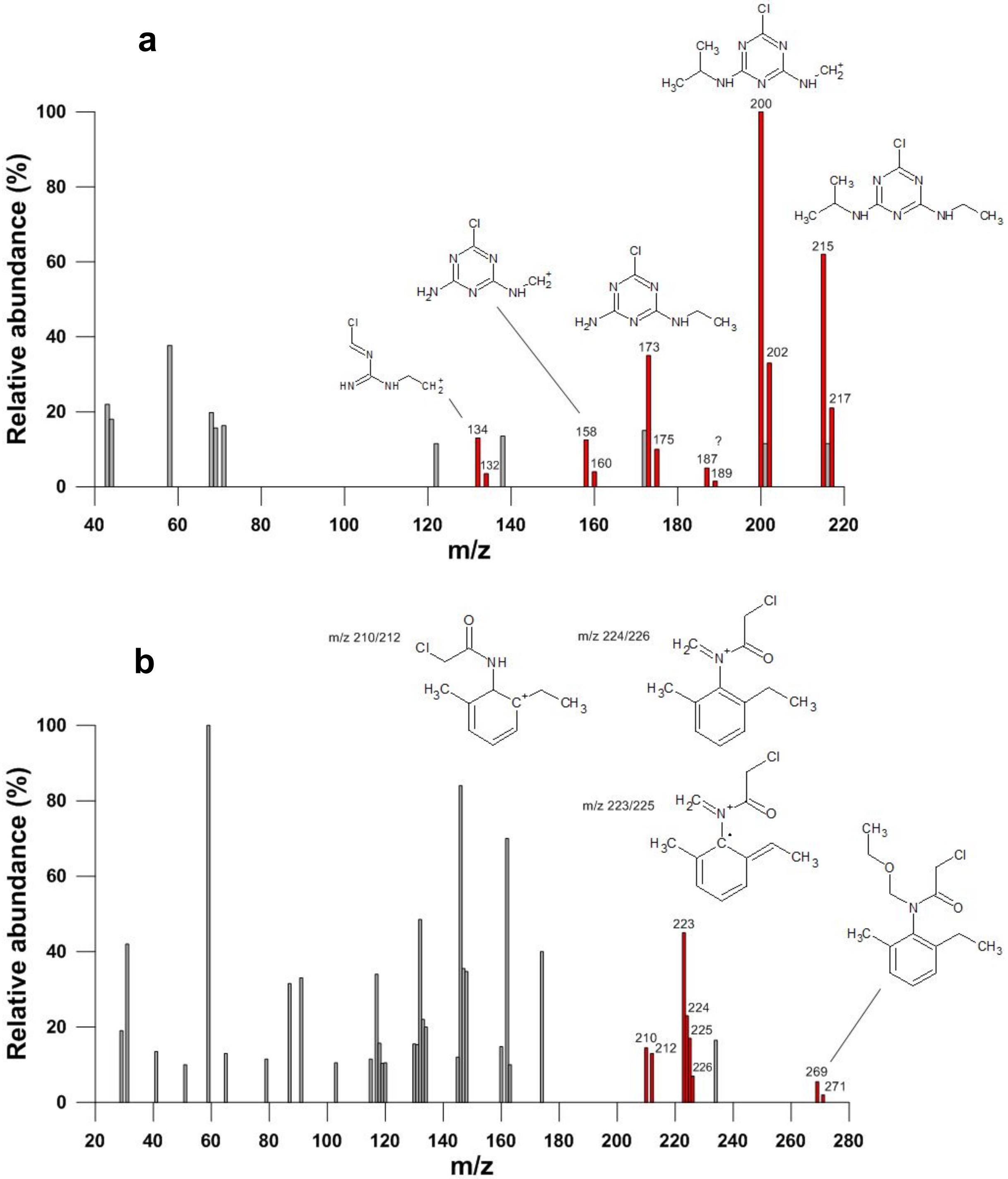
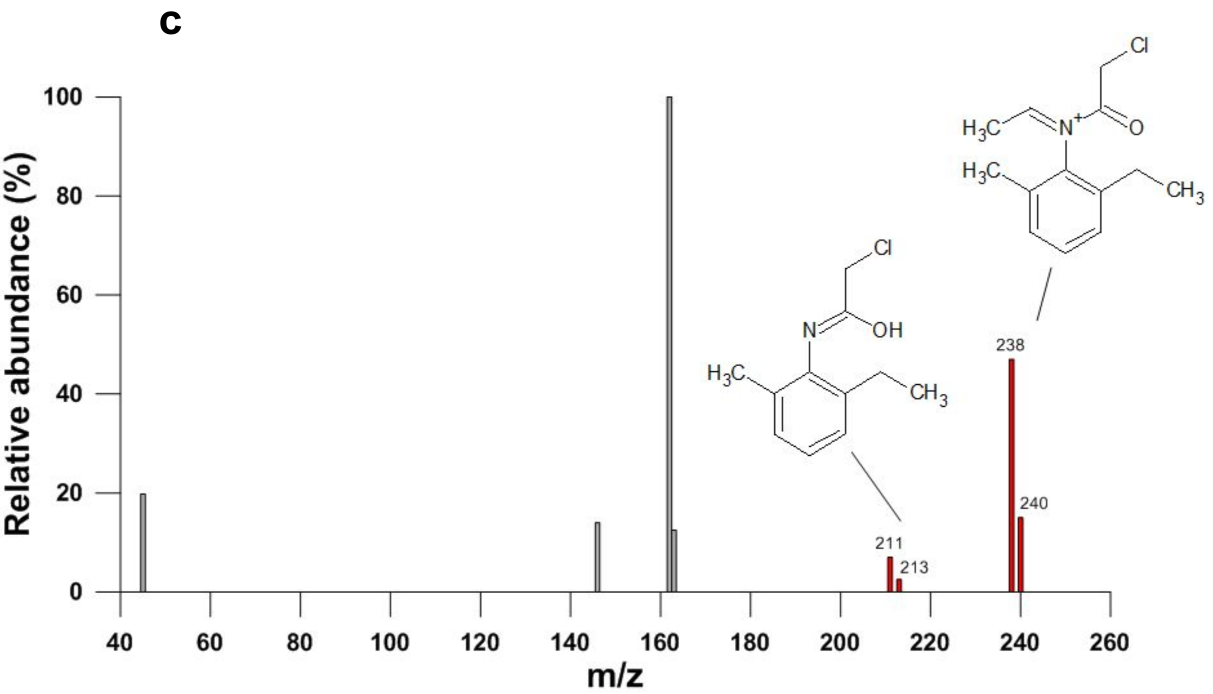


Figure S2. Spectra obtained in electron ionization mode for a) ATR, b) ACETO and c) METO. Only fragments with relative abundance > 10% are shown (fragments with a chlorine atom are all displayed, even those with a relative abundance < 10%). Fragments containing a chlorine atom are represented in red.




c
Relative abundance (%)
100
80
60
40
20
0
40
60
80
100
120
140
160
180
200
220
240
260
m/z
211
213
238
240
Cl
N
OH
H_3C
CH_3
Cl
H_3C
N^+
O
H_3C
CH_3

II.2. Calculations of chlorine isotope ratios

When two ions were used, the isotope ratio (*R*) was obtained from the ratio of the corresponding isotopologues according to Eq. S1 following the *most abundant ions* method[1]:

$$R = \frac{^{37}Cl}{^{35}Cl} = \frac{^{37}\mathrm{p}}{^{35}\mathrm{p}} = \frac{k}{(n-k+1)} \cdot \frac{^{37}Cl_{(k)}{}^{35}Cl_{(n-k)}}{^{37}Cl_{(k-1)}{}^{35}Cl_{(n-k+1)}} \qquad \text{Eq. (S1)}$$

where ^{37}p and ^{35}p are the probabilities of encountering ^{37}Cl and ^{35}Cl, *n* is the number of Cl atoms in the fragment, *k* is the number of ^{37}Cl isotopes in the "heavy" isotopologue, and ^{37}Cl(k)^{35}Cl(n-k) and ^{37}Cl(k-1)^{35}Cl(n−k+1) represent the isotopologues containing *k* and (*k*-1) heavy isotopes, respectively. For ATR, the isotopologues $[^{37}Cl^{12}C_7{}^1H_{11}{}^{14}N_5]^+$ (m/z 202), $[^{35}Cl^{12}C_7{}^1H_{11}{}^{14}N_5]^+$ (m/z 200), $[^{37}Cl^{12}C_8{}^1H_{14}{}^{14}N_5]^+$ (m/z 217), and $[^{35}Cl^{12}C_8{}^1H_{14}{}^{14}N_5]^+$ (m/z 215) were used. Similarly, the following isotopologues were used for ACETO: $[^{37}Cl^{12}C_{12}{}^1H_{14}{}^{14}N^{16}O]^+$ (m/z 225), $[^{35}Cl^{12}C_{12}{}^1H_{14}{}^{14}N^{16}O]^+$ (m/z 223), $[^{37}Cl^{12}C_{12}{}^1H_{15}{}^{14}N^{16}O]^+$ (m/z 226) and $[^{35}Cl^{12}C_{12}{}^1H_{15}{}^{14}N^{16}O]^+$ (m/z 224). For METO, the isotopologues $[^{37}Cl^{12}C_{13}{}^1H_{17}{}^{14}N^{16}O]^+$ (m/z 240) and $[^{35}Cl^{12}C_{13}{}^1H_{17}{}^{14}N^{16}O]^+$ (m/z 238) were monitored. Therefore, the following equations apply for the target compounds (*n*=*k*=1):

$$R\,(ATR) = \frac{^{202}I}{^{200}I};\ R\,(ACETO) = \frac{^{225}I}{^{223}I};\ R\,(METO) = \frac{^{240}I}{^{238}I} \qquad \text{Eq. (S2)}$$

where *I* indicates the ion peak intensities. For ATR and ACETO, the *modified multiple ion* method[2] was also tested, monitoring four ions thus obtaining the isotope ratio from the ratio of the corresponding isotopologues according to Eq. S3:

$$R = a \cdot R_{F1} + b \cdot R_{F2} \qquad \text{Eq. (S3)}$$

where R_{F1} is the isotope ratio of the fragments 217/215 for ATR and 226/224 for ACETO and R_{F2} is the isotope ratio of the fragments 202/200 for ATR and 225/223 for ACETO. The weight factors *a* and *b* are determined as the relative ion pairs intensities:

$$a\,(ATR) = \frac{I_{217} + I_{215}}{(I_{217} + I_{215}) + (I_{202} + I_{200})};\ b\,(ATR) = \frac{I_{202} + I_{200}}{(I_{217} + I_{215}) + (I_{202} + I_{200})} \qquad \text{Eq. (S4)}$$

$$a\ (ACETO) = \frac{I_{226} + I_{224}}{(I_{226} + I_{224}) + (I_{225} + I_{223})};\ b\ (ACETO) = \frac{I_{225} + I_{223}}{(I_{226} + I_{224}) + (I_{225} + I_{223})} \qquad \text{Eq. (S5)}$$

II.3. Relative abundance of fragments interfering with the measure of ^{37}Cl-containing fragment

Probability of occurrence for the different fragments studied was calculated with the binomial law (see Equation S1 in Section II.2).

Due to the very low natural abundance of ^{2}H (0.000115)[3], its influence was not taken into account as the occurrence of fragments containing two ^{2}H but no ^{37}Cl is expected to be negligible compared to other interfering fragments. Note that occurrences have been estimated based on natural abundances and will evolve if abundances of the different isotopes change.

Table S2. Occurrence of targeted and non-targeted fragments when monitoring m/z containing ^{37}Cl for ATR, ACETO and METO.

Atrazine (ATR) m/z monitored: 202			
		Composition of the fragment	**Occurrence m/z = 202^{a} (%)**
Target fragment	$[^{37}Cl^{12}C_7{}^{1}H_{11}{}^{14}N_5]^+$	one ^{37}Cl	98.77
Non-target fragments	$[^{35}Cl^{12}C_5{}^{13}C_2{}^{1}H_{11}{}^{14}N_5]^+$	two ^{13}C	0.76
	$[^{35}Cl^{12}C_6{}^{13}C^{1}H_{11}{}^{14}N_4{}^{15}N]^+$	one ^{13}C and one ^{15}N	0.43
	$[^{35}Cl^{12}C_7{}^{1}H_{11}{}^{14}N_3{}^{15}N_2]^+$	two ^{15}N	0.04
Acetochlor (ACETO) m/z monitored: 225			
		Composition of the fragment	**Occurrence m/z = 225^{a} (%)**
Target fragment	$[^{37}Cl^{12}C_{12}{}^{1}H_{14}{}^{14}N^{16}O]^+$	one ^{37}Cl	96.89
Non-target fragments	$[^{35}Cl^{12}C_{10}{}^{13}C_2{}^{1}H_{14}{}^{14}N^{16}O]^+$	two ^{13}C	2.34
	$[^{35}Cl^{12}C_{11}{}^{13}C^{11}H_{14}{}^{15}N^{16}O]^+$	one ^{13}C and one ^{15}N	0.14
	$[^{35}Cl^{12}C_{12}{}^{1}H_{14}{}^{14}N^{18}O]^+$	one ^{18}O	0.62
Metolachlor (METO) m/z monitored: 240			
		Composition of the fragment	**Occurrence m/z = 240^{a} (%)**

Target fragment	$[^{37}\mathrm{Cl}^{12}\mathrm{C}_{13}{}^{1}\mathrm{H}_{17}{}^{14}\mathrm{N}^{16}\mathrm{O}]^{+}$	one ^{37}Cl	96.47
Non-target fragments	$[^{35}\mathrm{Cl}^{12}\mathrm{C}_{11}{}^{13}\mathrm{C}_{2}{}^{1}\mathrm{H}_{17}{}^{14}\mathrm{N}^{16}\mathrm{O}]^{+}$	two ^{13}C	2.75
	$[^{35}\mathrm{Cl}^{12}\mathrm{C}_{12}{}^{13}\mathrm{C}_{1}{}^{1}\mathrm{H}_{17}{}^{15}\mathrm{N}^{16}\mathrm{O}]^{+}$	one ^{13}C and one ^{15}N	0.16
	$[^{35}\mathrm{Cl}^{12}\mathrm{C}_{13}{}^{1}\mathrm{H}_{17}{}^{14}\mathrm{N}^{18}\mathrm{O}]^{+}$	one ^{18}O	0.62

[a] based on natural abundance for each stable isotope considered (^{12}C: 0.9893; ^{13}C: 0.0107; ^{35}Cl: 0.7578; ^{37}Cl: 0.2422; ^{14}N: 0.99636; ^{15}N: 0.00364).[3]

II.4. Hydrolysis experiments

Before GC-qMS analyses, the 5 mL-aliquots were extracted by SPE, following a method modified from Torrentó et al.[4] Briefly, empty 6-mL SPE polyethylene cartridges were packed with 0.2 g of the Sepra ZT (Phenomenex) sorbent. They were first rinsed with 3 mL ethyl acetate, conditioned with two times 3 mL methanol and finally washed with two times 3 mL of ultrapure water. Samples were extracted at a flow rate of 5 mL/min. After the loading step, the sorbent was rinsed with 3 mL MilliQ water and dried under vacuum for 15 min to remove the excess of water. The eluates were eluted two times with 1.5 mL of ethyl acetate. The eluates were then evaporated until dryness followed by reconstitution with appropriate volume of ethyl acetate for GC-qMS injections.

The performance of the method was first evaluated in terms of extraction efficiency for ATR, ACETO and METO. Duplicated extraction tests were performed in 5 mL of distilled water spiked to 0.5 to 50 mg/L each analyte (Figure S3). Recoveries for ATR were satisfactory (higher than 85 %). Acceptable recoveries were also obtained for METO (higher than 60 % in most cases), whereas lower recoveries were achieved for ACETO.

For evaluating the effect of the SPE extraction procedure on δ^{37}Cl values, standards of ATR, ACETO and METO of known isotope ratios were spiked into 5 mL distilled water samples to give concentrations in the range of 0.5 to 50 mg/L (Figure S4). The SPE method induced negligible chlorine isotope fractionation, which was within the uncertainty of analysis (σ_m = ±1.0‰ for ATR and ±0.5‰ for ACETO and METO), except for the chloroacetanilides at high concentration. It is worth noting that precise δ^{37}Cl values for ACETO and METO were obtained despite relatively low extraction efficiencies.

Figure S3. Performance of the SPE method. Mean recoveries (%) and RSDs (error bars) obtained on loading 5 mL of distilled water spiked to 0.5 to 50 mg/L each analyte on cartridges containing 0.2 g of Sepra ZT.

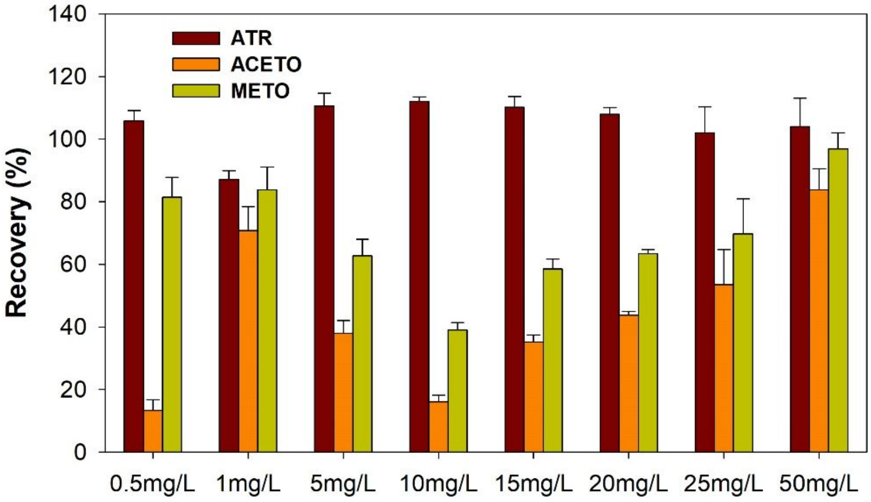
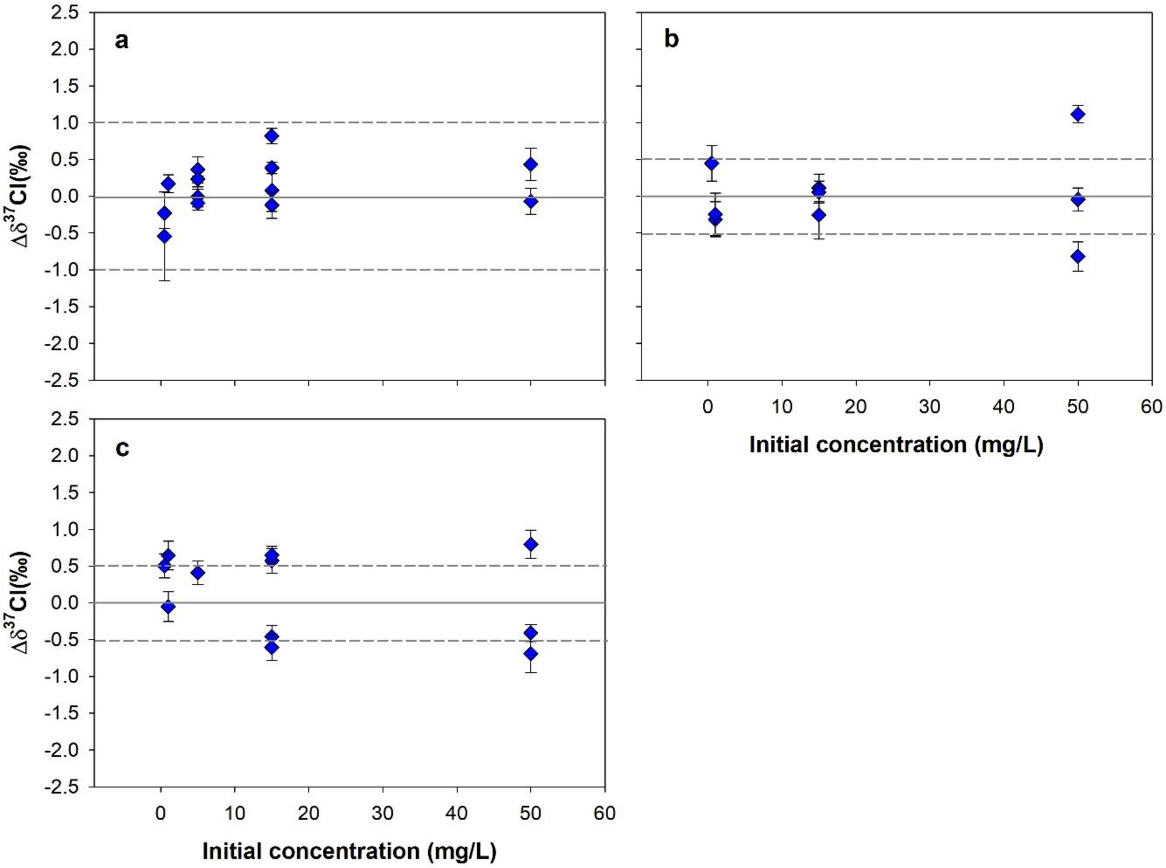


Figure S4. Validation of the SPE-CSIA procedure for the determination of chlorine isotope ratios of the target compounds in 5-mL water samples (blue diamonds) spiked with 0.5 to 50 mg/L ATR (a), ACETO (b) and METO (c). Triplicate tests were performed at each concentration level. The grey lines represent the reference isotope signatures determined by GC-MC-ICPMS ($\Delta\delta = 0$), with σ_m intervals (dashed lines) of ±1.0‰ for ATR and ±0.5‰ for ACETO and METO. Error bars show uncertainty (σ_m) for n = 20 injections.



II.5. Estimation of the uncertainty of δ^{37}Cl values for hydrolysis and SPE samples

To evaluate the total uncertainty associated with δ^{37}Cl measurements made with a GC-qMS, uncertainties associated with the measurement of the sample and the two standards have to be taken into account. We first determined a confidence interval for values predicted by the calibration curve with the equation proposed by Miska et al.[5]:

$$S_{x,sample} = \left(\frac{S_r}{|a|}\right)\sqrt{\frac{1}{n}+\frac{1}{N}+\frac{\left(y_{sample}-y_{avg}\right)^2}{a^2\sum_{i=1}^{N}\left(x_i-x_{avg}\right)^2}} \qquad \text{Eq. (S6)}$$

where S_r is the standard deviation of the calibration's regression, a is the slope of the calibration curve, n is the number of replicate measurements, N is the number of calibration pairs, y_{sample} is the average of the measured y value to be calculated, y_{avg} is the average y value of the calibration standards, x_i and their average x_{avg} are the x values (i.e. target values, the reference isotope signatures determined by GC-MC-ICPMS) of the calibrations standards.

We applied then error propagation still according to Miska et al.[5] as follow:

$$S_{final} = \sqrt{\left(S_{x,sample}\right)^2+\left(S_{x,STDA}\right)^2+\left(S_{x,STDB}\right)^2} \qquad \text{Eq. (S7)}$$

where $S_{x,STDA}$ and $S_{x,STDB}$ are the uncertainties associated with the two isotopic standards expressed as standard error of the mean (Eq. 5).

III. Results and discussion

III.1. Method performance

Figure S5. Amount-dependency of the precision and limit of precise δ^{37}Cl analysis for ATR (a), ACETO (b) and METO (c). The linear regression between amplitudes and injected concentrations is also shown (black lines). Vertical bars indicate the limit of precise isotope analysis (LPIA) determined according to the moving mean procedure[6] with σ_m intervals of ±1.0‰ (blue lines and blue bars) or ±0.5‰ (green lines and green bars). Moving means are indicated by dashed gray lines. Error bars show uncertainty (σ_m) for n = 20 to 60 injections.

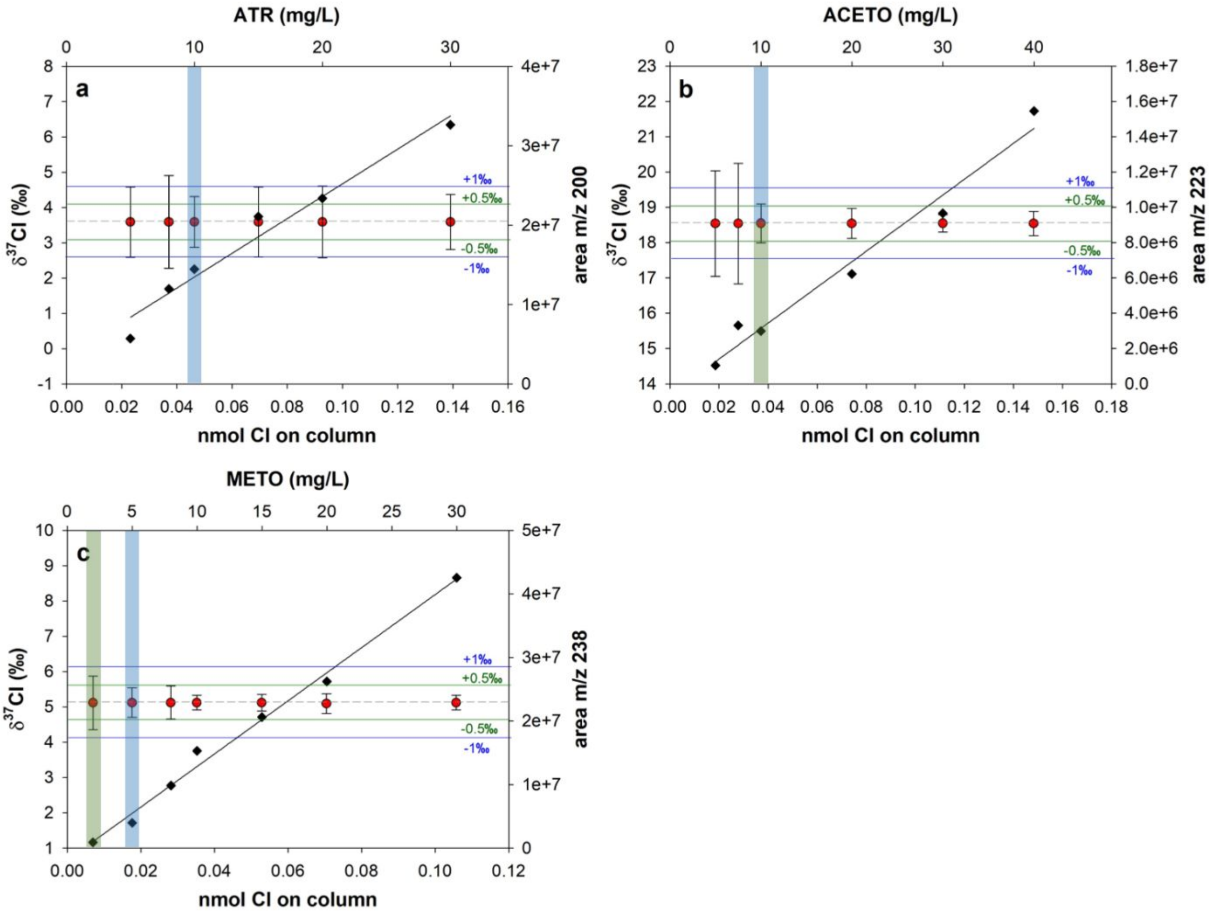
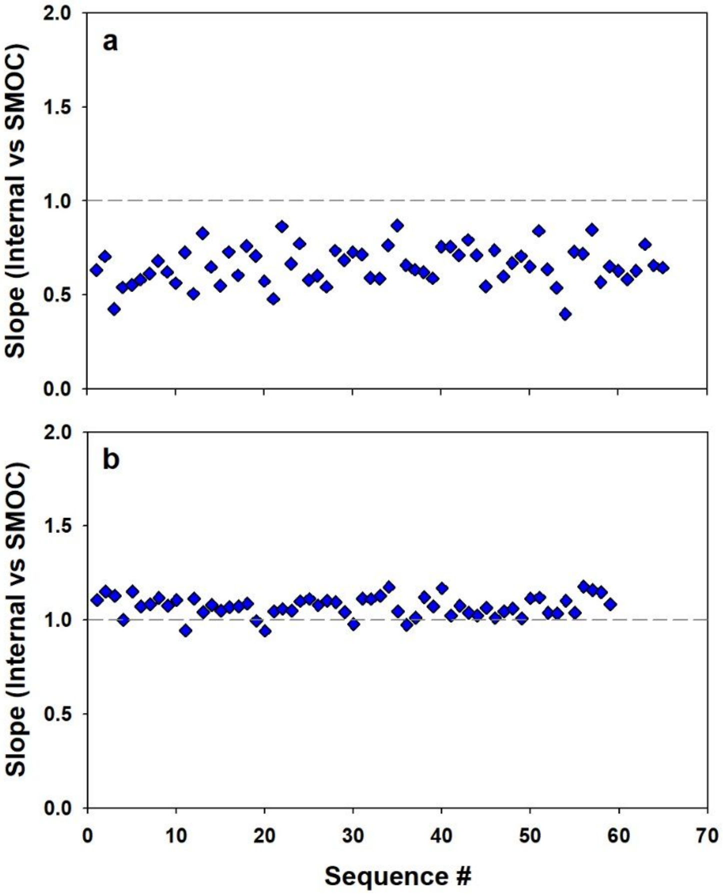


Figure S6. Comparison of the calibration slope obtained for METO over time using (a) standards with smaller differences in chlorine isotope values (group 2: METO_A and METO_B, $\Delta\delta^{37}$Cl = 2.74 ‰) and (b) standards with larger differences in chlorine isotope values (group 1: METO-I and METO-F, $\Delta\delta^{37}$Cl = 9.40 ‰). With the standards that are 2.74 ‰ apart on the SMOC scale (upper panel), the calibration slope ranged from 0.40 to 0.87 (average of 0.66±0.10) in 65 different measurement sequences within a 16-month period at injected concentration between 5 and 20 mg/L, whereas with the standards that are 9.40 ‰ apart (lower panel), the slope ranged from 0.94 to 1.18 (average of 1.07±0.05) in 59 different measurement sequences within a 13-month period at injected concentration between 5 and 45 mg/L.



III.2. Correction for two ^{13}C atoms

Table S3. Evaluation of chlorine isotope data of ATR, ACETO and METO with and without correction for two-^{13}C isotopologues, using two-point calibration with the pairs of standards selected in this work (group 1) and assuming a range of enrichment for δ^{13}C of a sample from -30‰ to -10‰ (maximum enrichment of +20‰). Corrections were calculated according to Aeppli et al.[7] following Eq. 6.

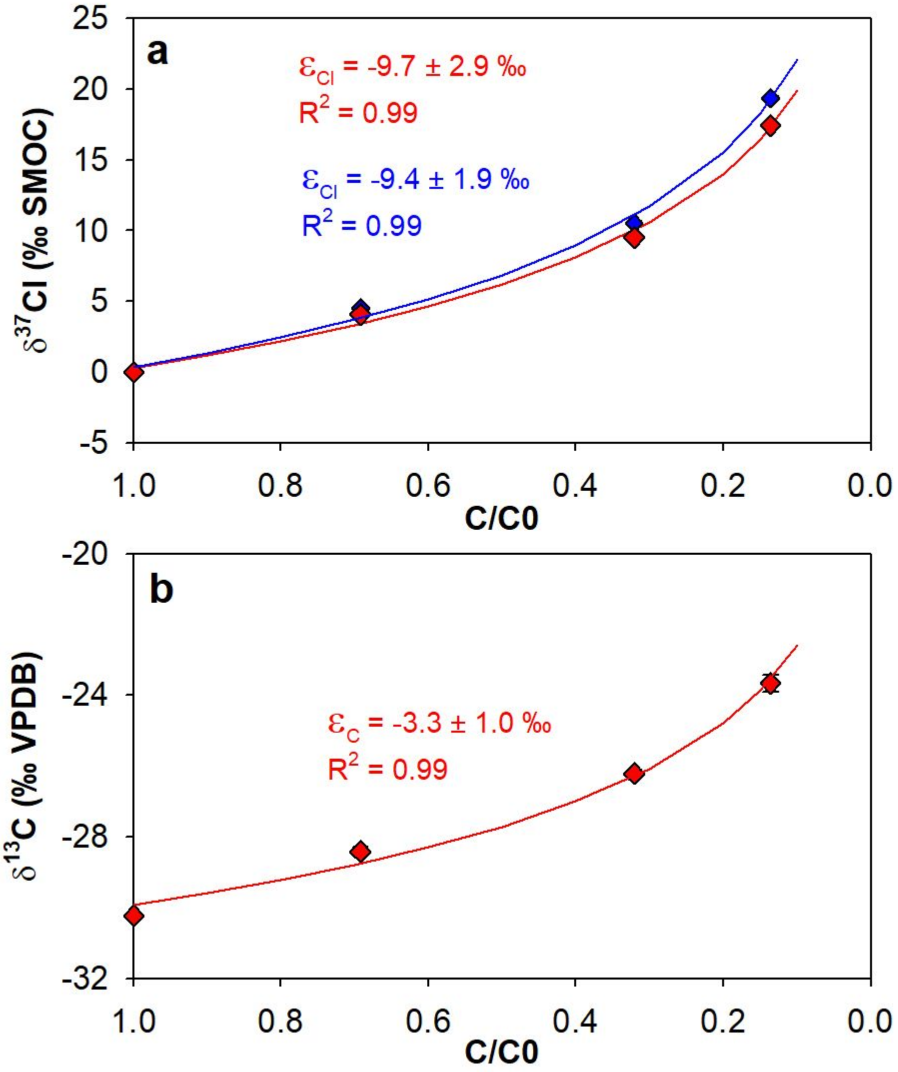
	$\delta^{13}C_{std}$ (1) [‰]	**$\delta^{13}C_{std}$ (2) [‰]**	**$\delta^{13}C$ $_{sample}$ [‰]**	**$R_{std}(1)$ [-]**	**$R_{std}(2)$ [-]**	**R_{sample} [-]**	**$corr_{std}(1)$ [-]**	**$corr_{std}(2)$ [-]**	**$corr_{sample}$ [-]**	**$\delta^{37}Cl$ [‰]**	**Offset [‰]**
ATR											
without correction	-26.4	-28.2	-30	0.3240	0.3256	0.3230	-	-	-	-3.58	
with correction	-26.4	-28.2	-30	0.3240	0.3256	0.3230	0.0025	0.0025	0.0025	-3.52	0.07
	-26.4	-28.2	-20	0.3240	0.3256	0.3230	0.0025	0.0025	0.0025	-3.66	-0.08
	-26.4	-28.2	-10	0.3240	0.3256	0.3230	0.0025	0.0025	0.0026	-3.80	-0.22
ACETO											
without correction	-27.8	-16.4	-30	0.3825	0.3893	0.3810	-	-	-	-3.87	
with correction	-27.8	-16.4	-30	0.3825	0.3893	0.3810	0.0079	0.0081	0.0078	-3.89	-0.02
	-27.8	-16.4	-20	0.3825	0.3893	0.3810	0.0079	0.0081	0.0080	-4.34	-0.47
	-27.8	-16.4	-10	0.3825	0.3893	0.3810	0.0079	0.0081	0.0082	-4.80	-0.93
METO											
without correction	-28.6	-22.5	-30	0.3349	0.3383	0.3350	-	-	-	-4.08	
with correction	-28.6	-22.5	-30	0.3349	0.3383	0.3350	0.0093	0.0094	0.0093	-3.99	0.09
	-28.6	-22.5	-20	0.3349	0.3383	0.3350	0.0093	0.0094	0.0095	-4.54	-0.46
	-28.6	-22.5	-10	0.3349	0.3383	0.3350	0.0093	0.0094	0.0097	-5.10	-1.02

III.3. Method application

Table S4. Trueness of chlorine isotope measurements after SPE-Cl-CSIA of drainage water samples spiked with sub-microgram per liter concentrations of ATR, ACETO and METO standards of known isotope signature. Recovery was estimated from GC-qMS responses.

	Recovery (%)	$\Delta\delta^{37}$Cl (‰)	σ_m
ATR			
5 µg/L, 10 L	66	0.0	0.6
1 µg/L, 10 L	56	0.1	0.5
0.5 µg/L, 2 × 10 L	14	-0.9	0.6
ACETO			
5 µg/L, 10 L	60	0.5	0.9
1 µg/L, 10 L	61	1.5	0.4
1 µg/L, 10 L	60	-0.5	0.4
1 µg/L, 10 L	69	-0.6	0.6
METO			
5 µg/L, 10 L	65	-0.5	0.4
1 µg/L, 10 L	67	-0.7	0.5
1 µg/L, 10 L	65	-0.6	0.7
1 µg/L, 10 L	63	-0.2	0.4
0.5 µg/L, 2 × 10 L	16	0.2	0.6

Figure S7. Method application: alkaline hydrolysis of METO with a) δ^{37}Cl as a function of the remaining fraction; blue diamonds represent chlorine isotope values uncorrected for fragments with two ^{13}C atoms while red diamonds represent chlorine isotope values corrected for fragments with two ^{13}C atoms, and b) δ^{13}C as a function of the remaining fraction. The solid lines stand for the logarithmic fitting curves for corrected (red) and uncorrected (blue) chlorine isotope values. Error bars may be smaller than symbols.



IV. References Supporting Information

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