Quantification of Lambda (Λ) in multi-elemental compound-specific isotope analysis

Patrick Höhener, Gwenael Imfeld

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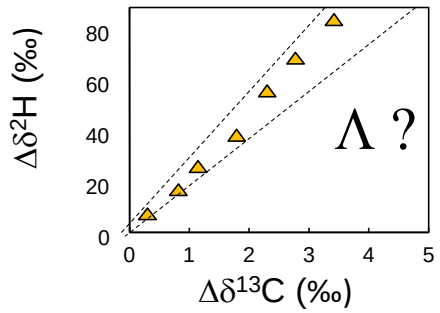

Highlights

The parameter Λ represents dual element stable isotope data

Two conventions for quantifying Λ give different Λ values

Linear regressions of delta values in a dual element plot overestimate Λ

We show that only the ln-transformed isotope ratios should be fitted

ABSTRACT

In multi-elemental compound-specific isotope analysis the lambda (Λ) value expresses the isotope shift of one element versus the isotope shift of a second element. In dual-isotope plots, the slope of the regression lines typical reveals the footprint of the underlying isotope effects allowing to distinguish degradation pathways of an organic contaminant molecule in the environment. While different conventions and fitting procedures are used in the literature to determine Λ, it remains unclear how they affect the magnitude of Λ. Here we generate synthetic data for benzene δ^2H and δ^{13}C with two enrichment factors ε_H and ε_C using the Rayleigh equation to examine how different conventions and linear fitting procedures yield distinct Λ. Fitting an error-free data set in a graph plotting the δ^2H versus δ^{13}C overestimates Λ by $0.225\% \cdot \varepsilon_H/\varepsilon_C$, meaning that if $\varepsilon_H/\varepsilon_C$ is larger than 22, Λ is overestimated by more than 5%. The correct fitting of Λ requires a natural logarithmic transformation of δ^2H versus δ^{13}C data. Using this transformation, the ordinary linear regression (OLR), the reduced major-axis (RMA) and the York methods find the correct Λ, even for large $\varepsilon_H/\varepsilon_C$. Fitting a dataset with synthetic data with typical random errors let to the same conclusion and positioned the suitability of each regression method. We conclude that fitting of non-transformed δ values should be discontinued. The validity of most previous Λ values is not compromised, although previously obtained Λ values for large $\varepsilon_H/\varepsilon_C$ could be corrected using our error estimation to improve comparison.

Key Words

Stable isotopes, pollution, assessment, bioremediation

1. Introduction

Multi-elemental Compound-Specific Isotope Analysis (ME-CSIA) is increasingly used to assess the fate of pollutants such as hydrocarbons (Vogt et al., 2016), chlorinated solvents solvents (Palau et al., 2014, Audi-Miro et al., 2015, Palau et al., 2016), nitrates (Xue et al., 2009), perchlorates (Sturchio et al., 2012) and pesticides (Ponsin et al., 2019, Melsbach et al., 2020) in the environment. The slope of the dual-isotope plot (Lambda, Λ) reflects changes of the isotope ratios of each element, which can be specific to a reaction mechanism, and thus inform about transformation processes in the laboratory or in the field. (Vogt et al., 2016, Elsner, 2010) Several studies (Masbou et al., 2018, Huntscha et al., 2014, Lian et al., 2019, Bouchard et al., 2018, Vogt et al., 2016, Elsner, 2010, Ojeda et al., 2019) refer to Λ using the simple definition in eq. 1, which is written here as an example for hydrogen vs carbon δ values (eq. 1).

$$\Lambda = \frac{\Delta\delta\,^{2}H}{\Delta\delta^{13}C} \approx \frac{\varepsilon_H}{\varepsilon_C} \qquad \text{eq. 1}$$

where Δδ is the change of isotope ratios from initial values, and ε are the enrichment factors for hydrogen and carbon. The Lambda (Λ) is an important parameter in ME-CSIA. It is a practical and unitless number which characterizes a specific process. It can be determined either by simply using the two enrichment factors and the right-hand side of equation 1 on one hand, or from regression analysis in a dual-isotope plots with isotope data of one element versus data of another element in the same compound (Figure 1). Lambda values were obtained in many studies (Ojeda et al., 2019, Palau et al., 2017, Rosell et al., 2007, Rodriguez-Fernandez et al., 2018, Rodriguez-Fernandez et al., 2018, Dogan-Subasi et al., 2017, Cretnik et al., 2013, Audi-Miro et al., 2013, Palau et al., 2014, Lian et al., 2019, Badin et al., 2016, Mogusu et al., 2015, Ponsin et

al., 2019, McKelvie et al., 2009, Pati et al., 2012) from the regression analyses in dual-isotope plots (i.e., ratios of one isotope as a function of another isotope as delta values; Figure 1A).

Another mathematical notation for Λ has been described in detail in (Wijker et al., 2013) (eq. 2), noted here for hydrogen and carbon isotopes:

$$\Lambda = \frac{ln\left[\left(\delta\ ^{2}H/1000+1\right)/\left(\delta\ ^{2}H_0/1000+1\right)\right]}{ln\left[\left(\delta^{13}C/1000+1\right)/\left(\delta^{13}C_0/1000+1\right)\right]} \approx \frac{\varepsilon_H}{\varepsilon_C} \qquad \text{eq. 2}$$

Figure 1B shows an example of a dual-isotope plot to determine Λ using eq. 2, named below the ln-transformed δ data. This way of obtaining Λ was used e.g. in (Schilling et al., 2019 a+b).

Apart from those two different conventions for plotting isotope data, different methods of linear regression were proposed to obtain Λ. These include the ordinary linear regression (OLR), the reduced major axis regression (RMA), and the York linear regression, which have been compared recently (Ojeda et al., 2019).

The objective of this short comment is to compare the two conventions (i.e., A, with eq. 1 and B, with 2) to determine Λ values and the associated uncertainty from a dual-isotope plot. Two synthetic datasets were generated, one without random error, and a second one with random errors mimicking measurement uncertainties. Each dataset was fitted with the ordinary linear regression (OLR), the reduced major-axis (RMA) and the York regression methods and results were compared.

2. Methods

The Rayleigh equation (eq. 3) (Aelion et al., 2010) was used to generate 10 synthetic exact data points for each element (i.e., C and H). We used isotope enrichment factors for carbon and

hydrogen corresponding to methanogenic degradation of benzene: ε_C = -2.0 and ε_H = -59.5 ‰. (Mancini et al., 2003) The remaining fraction (f) of benzene was varied from 1 to 0.1 in steps of 0.1 (see data set in the supplementary data).

$$\frac{R}{R_0} = f^{(\alpha-1)} \qquad \text{eq. 3}$$

Where R is the isotope ratio, R_0 is the initial isotope ratio (chosen as the R of international standard R_{std}), f is the fraction of compound remaining (C/C_0), and α is the isotope fractionation factor (equal to $\varepsilon/1000 +1$). The resulting isotope ratios were expressed as δ values [$\delta = (R/R_{std} - 1)*1000$; $R_{std,H}$=1.5575E-4; $R_{std,C}$=0.011237] and plotted in Figure 1A ($\Delta\delta^2H$ vs $\Delta\delta^{13}C$, eq .1) and 1B (ln-transformed data, eq. 2). The resulting slopes should reflect the ratio of original isotopic enrichment values, -59.50/-2.00, thus Λ=29.75.

A second dataset was generated using the same enrichment factors but introducing random errors in the calculated δ values (see Table S1 in supplementary data). The δ values of this set had a random error of up to ± 0.5 ‰ for carbon and up to ± 5.0 ‰ for hydrogen, which corresponds to the typical total analytical uncertainties.

Finally, 25 more datasets (data not shown) were generated in the same manner as dataset 1 without random error, keeping ε_C = -2.0 ‰ and varying ε_H over $\varepsilon_H / \varepsilon_C$ ratios from 2 to 50. Each of these data sets was fitted with OLR, and the overestimation of fit A over fit B was quantified and plotted in Figure 2 as a function of $\varepsilon_H/\varepsilon_C$.

The datasets were generated with Excel (Microsoft), Vs. 2011), and linear regressions (OLR, RMA and York) were calculated with a script adapted from Ojeda et al. (2019) and were not forced through the origin.

3. Results

The dataset 1 with the raw $\Delta\delta$ values (eq. 1) does not plot on a perfect straight line (Figure 1A). The slope becomes steeper with increasing δ values (smaller f). An OLR gives a mean Λ of 31.70 ± 0.21 (R^2 >0.99), which overestimates the true Λ of 29.75 by 6.6 %. In contrast, the dataset 1 with ln-transformed δ values (eq. 2) plots perfectly on a straight line with a slope of 29.74 ± 0.02 with an R^2 of 1.0000 (Figure 1B), which matches the true Λ.

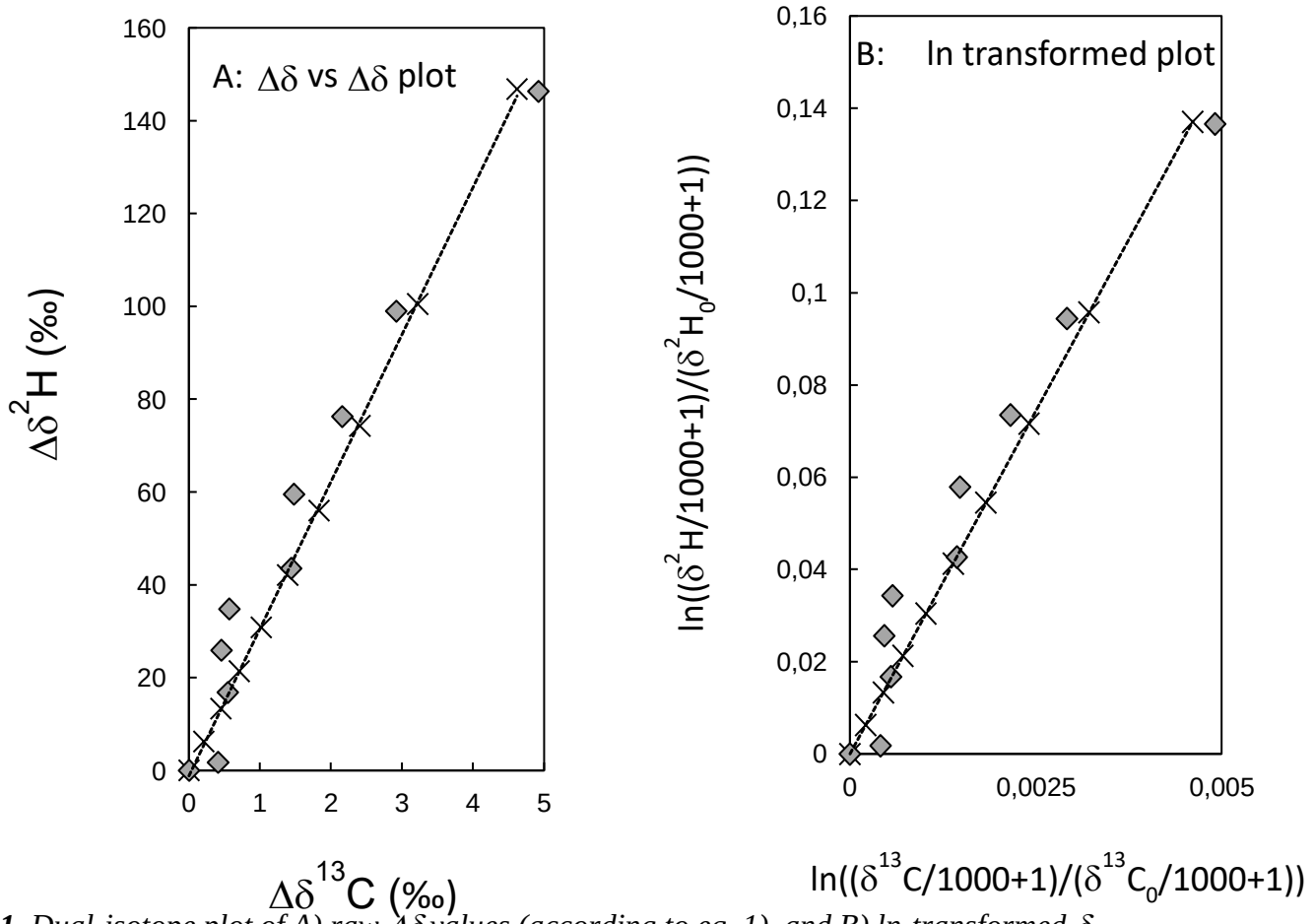

Fig. 1. *Dual-isotope plot of A) raw $\Delta\delta$ values (according to eq. 1), and B) ln-transformed δ values (according to eq. 2). Crosses correspond to exact datapoints (dataset 1) and grey diamonds are datapoints with random error (dataset 2).*

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119 ***Table 1:*** *Comparison of Λ calculated with the raw $\Delta\delta$ values (convention A, eq. 1) and the ln-transformed δ values (convention B, eq.*

120 *2) using the OLR, RMA and York methods, for the exact data points (dataset 1) and data generated with a random error (dataset 2).*

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	Exact data points (dataset 1)						***Random error (dataset 2)***					
	$\Delta\delta$ vs $\Delta\delta$			***ln-transformed***			***$\Delta\delta$ vs $\Delta\delta$***			***ln-transformed***		
	Λ	***SE***	***R^2***	***Λ***	***SE***	***R^2***	***Λ***	***SE***	***R^2***	***Λ***	***SE***	***R^2***
OLR	*31.70*	*0.21*	*>0.99*	*29.74*	*0.02*	*1.00*	*30.08*	*2.13*	*0.96*	*28.15*	*2.16*	*0.95*
RMA	*31.71*	*0.19*	*>0.99*	*29.74*	*0.02*	*1.00*	*30.67*	*1.90*	*0.98*	*28.80*	*1.93*	*0.98*
York	*31.71*	*3.77*	*>0.99*	*29.74*	*3.56*	*1.00*	*31.17*	*3.68*	*0.96*	*29.33*	*3.50*	*0.95*

122 *SE: Standard error of Λ* 128

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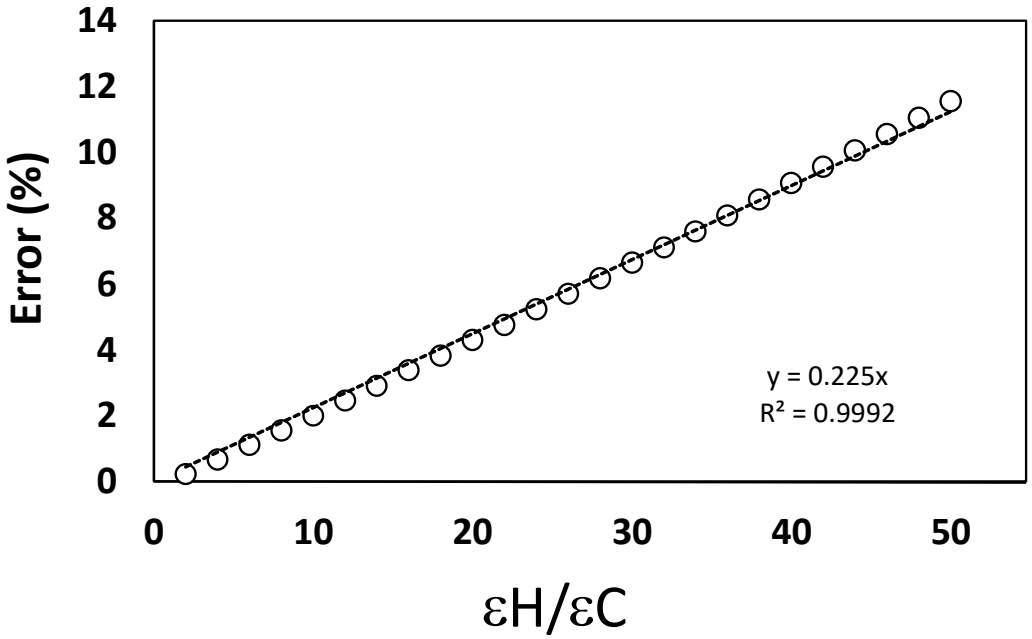
129 The overestimation of Λ calculated with convention A compared to convention B was quantified
130 as a function of $\varepsilon_H/\varepsilon_C$ ranging from 2 to 50 (Fig. 2; OLR method)).



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132 ***Fig. 2*** *Overestimation of Λ (%) as a function of* $\varepsilon_H/\varepsilon_C$ *(symbols) when convention A (eq. 1) is*
133 *used. The straight dotted line is the mean error increase of* 0.225% per $\varepsilon_H/\varepsilon_C$.

134 Figure 2 shows that the error in a graph plotting the δ values like in Fig. 1.A overestimates Λ by
135 11.5 % when $\varepsilon_H / \varepsilon_C$ reaches 50. The increase of the error is almost linear with a slope of 0.225%
136 per $\varepsilon_H / \varepsilon_C$.

137 **4. Discussion**

138 The use of the exact (error-free) synthetic dataset to compare conventions A (eq. 1) and B (eq. 2)
139 emphasized that Λ calculated with convention A is linearly overestimated (eq.1). The difference

of Λ obtained with convention A and B has a pure mathematical cause (Wijker et al., 2013): equation 1 is derived from eq. 2 by a Taylor series expansion which is only approximate.

Höhener and Atteia (Höhener and Atteia, 2014) derived mathematically the dependence of the slope Λ on the remaining, non-degraded fraction *f* in a dual-isotope plot (eq. 4) based on the theory of Rayleigh distillation.

$$\Lambda = \frac{\Delta\delta\,^{2}H}{\Delta\delta^{13}C} = \frac{f^{\frac{\varepsilon_H}{1000}-1}}{f^{\frac{\varepsilon_C}{1000}-1}} \qquad \text{eq. 4}$$

Equation 4 (eq. 16 in (Höhener and Atteia, 2014)) shows that Λ is increasing with decreasing *f* , as observed in Figure 1A. Thus, for *f* close to one, Λ is 29.75, while for *f* = 0.1, Λ is 31.80.

All three regression methods tested for convention A with dataset 1 gave a similar Λ of 31.7, although their standard errors (SE) differed (Table 1). OLR and RMA methods gave a narrow SE (0.21 and 0.19, respectively), leading us to the wrong conclusion that Λ is > 31. Regression with the York method gave a larger SE (Λ = 31.71 ± 3.68, Tab. 1), which represents a correct but inaccurate description of the true Λ of 29.75. For convention B and dataset 1, all three regression methods find the true Λ, although only the OLR and RMA method yielded accurate Λ within narrow error limits.

Measured isotope ratios are always affected by random errors from measurements, which were accounted for in dataset 2 to calculate Λ (Table 1). All three methods predicted Λ > 31 using convention A, and Λ was associated with large SE, ranging from 1.90 to 3.68. Using convention B, Λ ranged from 28.15 to 29.33, with SE ranging from 1.9 (RMA) to 3.5 (York). For dataset 2, RMA was the best fitting method, yielding the narrower SE, while both OLR and York gave

accurate predictions also with higher error. All regressions match thus the true value of 29.75 within their error limits.

To sum up, the error-free data in a dual-isotope plot with $\Delta\delta$ *vs* $\Delta\delta$ values do not lie on a straight line and thus should not be fitted with any linear regression. The slope in a $\Delta\delta$ *vs* $\Delta\delta$ plot is per definition a function of the progress of reaction *f* (eq. 4). A non-linear curve is obtained, especially when the orders of magnitude of the enrichment factors differ. Linear regressions in such plots yield Λ that overestimate the true Λ and should be discontinued. The correct convention to linearize data is provided in eq. 2 and should be applied as in Figure 1B to obtain accurate Λ. OLR and RMA regression methods yield narrower error estimates, whereas the York method finds the true Λ within a larger error margin. The validity of most previously obtained Λ values with convention A might not be compromised given the total uncertainty of the experimental and analytical methods. However, in a few cases with large $\varepsilon_H/\varepsilon_C$ ratios, corrections might be applied in order to compare optimally all Λ values. The simple procedure to follow consists in using Fig. 2 of our manuscript, selecting the appropriate ratio of epsilons, reporting the corresponding error percentage (which is the percentage of overestimation) to lower Λ by this percentage. Worthy of note, if experimental data still plotting nonlinearly on a ln-transformed plot with eq. 2, as e.g. in (Dorer et al., 2014), another process may be involved, including a very strong hydrogen fractionation (tunneling), concentration-dependent fractionation and/or instrumental non-linearity. In these specific cases, Λ cannot be expressed as a constant number.

Acknowledgments

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Supplementary data

Table of synthetic datasets used in this work.

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SUPPLEMENTARY DATA

Quantification of Lambda (Λ) in multi-elemental compound-specific isotope analysis

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Contents:

Table S1: Datasets used in this work

Table S1: *Synthetic data shown in Figure 1 and used for fitting.*

Remaining fraction f	Exact data points (dataset 1) $\delta^{13}C$ ‰	$\delta^{2}H$ ‰	Random error (dataset 2) $\delta^{13}C$ ‰	$\delta^{2}H$ ‰
1	0	0	0	0
0.9	0.21	6.29	0.41	1.79
0.8	0.45	13.37	0.55	16.87
0.7	0.71	21.45	0.46	25.95
0.6	1.02	30.86	0.57	34.86
0.5	1.39	42.10	1.44	43.6
0.4	1.83	56.03	1.48	59.53
0.3	2.41	74.26	2.16	76.26
0.2	3.22	100.50	2.92	99.0
0.1	4.62	146.83	4.92	146.33

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