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1 **Expanded uncertainty associated with determination of isotope enrichment**
2 **factors: comparison of two-points calculation and Rayleigh-plot**

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19 Running title: Determination of enrichment factor and associated expanded
20 uncertainty

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23

24 **ABSTRACT**

25 The Enrichment Factor (ϵ) is a common way to express Isotope Effects (IEs)
26 associated with a phenomenon. Many studies determine ϵ using a Rayleigh-plot,
27 which needs multiple data points. More recent articles describe an alternative method
28 using the Rayleigh equation that allows the determination of ϵ using only one
29 experimental point, but this method is often subject to controversy. However, a
30 calculation method using two points (one experimental point and one at t_0) should
31 lead to the same results because the calculation is derived from the Rayleigh
32 equation. But, it is frequently asked “what is the valid domain of use of this two-points
33 calculation?” The primary aim of the present work is a systematic comparison of
34 results obtained with these two methodologies and the determination of the
35 conditions required for the valid calculation of ϵ . In order to evaluate the efficiency of
36 the two approaches, the expanded uncertainty (U) associated with determining ϵ has
37 been calculated using experimental data from three published articles. The second
38 objective of the present work is to describe how to determine the expanded
39 uncertainty (U) associated with determining ϵ . Comparative methodologies using both
40 Rayleigh-plot and two-points calculation are detailed and it is clearly demonstrated
41 that calculation of ϵ using a single data point can give the same result as a Rayleigh-
42 plot provided one strict condition is respected: that the experimental value is
43 measured at a small fraction of unreacted substrate ($f < 30\%$). This study will help
44 stable isotope users to present their results in a more rigorous expression: $\epsilon \pm U$ and
45 therefore to define better the significance of an experimental results prior
46 interpretation.

47 Capsule: Enrichment factor can be determined through two different methods and the
48 calculation of associated expanded uncertainty allows checking its significance.

49

50 **Keywords**

51 Isotope Effects, isotope enrichment factor, expanded uncertainty, Rayleigh, two-
52 points

53

54 **INTRODUCTION**

55 Stable isotope analyses is widely used in different fields such as geochemistry [1],
56 biology [2] or environmental sciences [3]. The isotope ratio of light elements (C, H, O,
57 N, S or Cl) is known to change through processes such as (bio)chemical reactions or
58 physical processes due to preferential selection for the light or heavy isotope. This
59 physical phenomenon, called the Isotope Effect (IE), can occur at equilibrium where
60 heavy isotopes will be accumulated in one of the components of the system. Many
61 processes such as liquid-vapor transformation can be associated with an Equilibrium
62 Isotope Effect (EIE) [4]. IE can also be associated with a (bio)chemical reaction [5]
63 where the presence of heavy isotopes can influence the reaction rate constant.
64 Classically, this Kinetic Isotope Effect (KIE) is defined as the ratio of rate constants
65 between two isotopes of a given element $KIE = (k^{light}/k^{heavy})$ and depends on the
66 mechanism of the reaction/process and on environmental parameters such as
67 temperature or pH [6]. As a consequence, kinetic processes can be associated with
68 an enrichment ($KIE < 1$) or depletion ($KIE > 1$) of heavy isotope of the reaction
69 product(s) compared to the substrate(s). For a more detailed explanation, we
70 recommended to read the guide "Practice and Principles of Isotopic Measurements in
71 Organic Geochemistry" by J. M. Hayes where these concepts are explained in a very
72 pedagogical way [7].

73 IE is not directly observable but it induces an alteration of isotopic abundance in the
74 studied compounds: substrate(s) and/or product(s). This change in the isotope ratio,
75 called isotopic fractionation, can be determined using isotope analysis and is often
76 expressed as a fractionation factor (α) calculated from the equation described by
77 Bigeleisen and Wolfsberg [8]. In the present article, isotopic fractionation has been
78 expressed as an enrichment factor (ϵ) [9] instead of α (where $\epsilon = (\alpha - 1) * 1000$). This

79 notation has been chosen, because the sign of ε directly informs on the IE associated
80 with the studied process: when ε is negative, the IE is considered as normal (light
81 isotopes are preferentially used during the process) and when ε is positive, the IE is
82 inverse (transformation is faster using heavy isotopes). Enrichment factors can easily
83 be determined using a Rayleigh-plot where ε is the slope of the trend line [10, 11].
84 Therefore, knowing the sign and amplitude of the IE allows conclusions to be drawn
85 on the process and/or mechanism responsible for the production, degradation, or
86 transformation of a given molecule. ε (expressed in ‰) can be defined as follows:

$$87 \quad \varepsilon = \left(\frac{k^{\text{heavy}}}{k^{\text{light}}} - 1 \right) * 1000 \quad (1)$$

88 Note that in geo- and environmental sciences, in contrast to classical (bio)chemistry,
89 the ratio of the heavy to light isotope (and not *vice versa*) is commonly used.
90 However, what is usually measured during a given process is not directly the relative
91 rate of the reaction but rather the isotope ratio of the starting material and the product
92 at two or more points during the progress of the process/reaction. The link between
93 isotope ratios, extent of progress of the reaction f and ε for a kinetic process is given
94 by the Rayleigh equation [9, 12-14]:

$$95 \quad R \simeq R_0 \times f^{\varepsilon/1000} \quad (2)$$

96 where, R_0 is the isotope ratio at t_0 , R the isotope ratio at t_f and f is the unreacted
97 fraction of substrate at t_f . Equation 2 is a good approximation for all elements where
98 the heavy isotope is much rarer than the light isotope. In cases of near equal
99 abundance of isotopes, such as for B or when working with highly enriched
100 compounds, equation 2 must be replaced by that proposed by Hunkeler *et al.* in 2002
101 [14].

102 The most common method for determining ε is to use a Rayleigh-plot: the isotopic
103 composition of the remaining reactant is measured at different fractions of unreacted

104 substrate (f) and $\ln(R/R_0)$ is plotted as a function of $\ln(f)$ [10, 15]. The enrichment
105 factor ε can then be obtained from the slope of a linear regression fitted to all data
106 points. The variability of ε is then expressed from the standard deviation of the slope.
107 Most studies involving the determination of IEs have exploited this approach. In many
108 situations, such as field sampling, measurement conditions do not allow isotope
109 analysis at different times or different degrees of f to be determined [16].
110 Nonetheless, an enrichment factor can still be obtained by using equation 2 above
111 (Rayleigh equation). This calculation method only requires isotopic measurements on
112 the starting substrate (at $t = 0$, $f_0 = 1$) and on one data point (at $t = t_1$, $f_1 < 1$): this
113 calculation method is called two-points calculation in this article. The question is then:
114 how to express the variability of the results?

115 The goal of the present article is to compare these two approaches, namely
116 Rayleigh-plot and two-points calculation, both in terms of trueness and precision [17].
117 Note that these two ε determination approaches are derived from the Rayleigh
118 distillation equation, so they should lead to the same ε . We asked three fundamental
119 questions: “what is the domain of use of the Rayleigh equation?”, “how to express the
120 uncertainty of the results from the two-points calculation mode?” and “what is the
121 significance of the results based on the uncertainty determination in both ε
122 determination modes?”. Three articles have been selected, with the authors’ consent,
123 to compare these two methods for the determination of the enrichment factor. Using
124 data from these studies, we first compare ε values obtained with these alternative
125 approaches in order to evaluate under which conditions they give consistent results.
126 In a second part, we detail how to determine the limit of significance of the calculated
127 ε . Many studies only use the standard deviation (SD) of the isotopic measurement to
128 indicate the significant threshold of the determination of ε . But this method does not

129 include other sources of uncertainty such as the measurement of f , signal size or
130 linearity [18]. Calculation of the expanded uncertainty (named U) associated with the
131 determination of ε is explained in the section below and a more detailed U calculation
132 is described in the 'guide of expression of uncertainty in measurement' GUM [19].
133 Calculations of both ε and its associated U will help in determining IEs in a more
134 robust way and will provide a correct style to express the accuracy of the results.

135

136 **MATERIALS AND METHODS**

137 **Data collection**

138 Experimental data from three different articles have been collated, with the authors'
139 consent, to compare ε values obtained with Rayleigh-plot and the two-points
140 calculation and illustrate the advantage of performing the expanded uncertainty
141 calculation. These are:

142 Article A: Gray J. R. *et al.*, **2002**, *Environ. Sci. Technol.*, 36, 1931-1938 [20]

143 Article B: Cretnik S. *et al.*, **2014**, *Molecules*, 19, 6450-6473 [15]

144 Article C: Yamada K. *et al.*, **2014**, *Rapid Commun. Mass Spectrom.*, 28, 1821-1828
145 [22]

146 These articles have been chosen in order to explore diverse isotope fractionation
147 conditions (evaporation, biodegradation) and also to give examples with different
148 elements (^2H , ^{13}C , and ^{37}Cl). Moreover, this selection includes an article using
149 position-specific isotope analysis (PSIA) in order to confirm that these calculation
150 methods are applicable in such a case. In the present work, the articles are called
151 article A, B or C to make the text easier to read. Note that in all three articles a
152 Rayleigh-plot was used to calculate the isotope fractionation factor.

153 A part of article A from Gray *et al.* 2002 [20] deals with the determination of both
154 deuterium and carbon IEs associated with the aerobic biodegradation of methyl *tert*-
155 butyl ether (MTBE) by bacteria strains discovered in a military base located in
156 California. Bioremediation experiments have been performed *in vitro* using sediments
157 and groundwater extracted from the contaminated site. Stable isotope analysis was
158 performed by Gas Chromatography – Combustion – isotope ratio monitoring by Mass
159 Spectrometry (irm-GC/MS) [21] and the amount of unreacted substrate was
160 measured by gas chromatography for each experimental point.

161 In article B, Cretnik *et al.* 2014 [15] report a study of the *in vitro* anaerobic
162 biodegradation of tetrachloroethene (PCE) by *Desulfitobacterium sp.* strain Viet1.
163 Both carbon and chlorine isotope ratios were monitored throughout the
164 biotransformation using irm-GC/MS and f was determined by gas chromatography.

165 Article C, Yamada *et al.* 2014 [22], deals with position-specific isotope analysis of
166 acetic acid using on-line pyrolysis combined with GC-C-irm-MS (Py-irm-GC/MS) [23].
167 The authors determined position-specific isotope effects (PSIEs) associated with the
168 evaporation of acetic acid. For this experiment, 14 vials containing pure acetic acid
169 were placed in a hermetically-sealed chamber containing sodium hydroxide (NaOH)
170 to remove evaporated acetic acid. Vials are weighted at t_0 and at t_f in order to
171 determine f and PSIA were performed on each sample at variable f .

172 **Determination of the enrichment factor (ϵ)**

173 The two methods for ϵ determination are described in this section.

174 The most common method is the determination of ϵ using a Rayleigh-plot, in which
175 $\ln(f)$ is plotted on the x-axis and $\ln(R/R_0)$ on the y-axis [10, 11]. In this graph, the
176 enrichment factor (ϵ) corresponds to the slope of the trend line and the associated

177 root mean square (R^2) gives a first indication of the quality of the linearity. Note that
178 the regression is not forced through the origin following a recommendation by Scott
179 *et al.* in 2004 [24].

180 The second method is the determination of ε with the two-points calculation and has
181 been performed using the following relationship [25], in which the isotope ratios R (as
182 in equation 2) are expressed as isotopic composition δ :

$$183 \quad \varepsilon = \ln \left(\frac{\delta^A X_{tf} + 1000}{\delta^A X_{t0} + 1000} \right) \left(\frac{1000}{\ln f} \right) \quad (3)$$

184 where $\delta^A X_{t0}$ and $\delta^A X_{tf}$ are respectively the isotopic composition of the starting
185 reactant (initial state) and the remaining reactant at t_f . This relationship can be
186 approximated as described in the following equation [4]:

$$187 \quad \varepsilon = \frac{\Delta \delta^A X}{\ln f} \quad (4)$$

188 where $\Delta \delta^A X$ stands for the isotopic fractionation between the reactant at t_0 and at
189 time f ($\Delta \delta^A X = \delta^A X_{tf} - \delta^A X_{t0}$). These two equations give very similar results [9] and the
190 simplified equation 4 is employed here for the determination of ε with results from the
191 selected articles.

192 **Determination of expanded uncertainty (U)**

193 In order to determine the significance threshold of the enrichment factor, the
194 expanded uncertainty needs to be calculated. This article is the first which offers a
195 fully-described expanded uncertainty calculation method within this context.

196 The determination of the expanded uncertainty associated with the enrichment factor
197 determined using the Rayleigh-plot can be directly calculated using the function
198 "LINEST" in Microsoft ExcelTM, as described by Elsner *et al.* [10]. This function

199 calculates both the slope and the standard deviation of the trend line (STDV slope).

200 The expanded uncertainty can thus be calculated as follows:

$$201 \quad U = k \times \text{STDV slope} \quad (5)$$

202 where $k = 2$ for a coverage factor of 95% (t value in Student table).

203 In the case where ε is determined with a single data point, U can be calculated from
 204 the uncertainty estimation described in the 'guide of expression of uncertainty in
 205 measurement' GUM [19]. This evaluation criterion has been chosen because all
 206 parameters that can affect the final result are taken into account by U calculation,
 207 unlike in most articles in which authors only use the SD of the isotope analysis
 208 method as described by the instrument manufacturer. Parameters that need to be
 209 considered in the calculation of expanded uncertainty can be assembled in an
 210 Ishikawa diagram (also called fishbone diagram or cause-and-effect diagram, see
 211 Figure 1). Two contributions have to be taken into account, (i) the isotopic
 212 measurement of both the starting reactant (at t_0) and the remaining reactant (at t_f)
 213 along with the standard deviation of the measurements and (ii) the determination of f
 214 (and its associated variability) which can be performed by weighting, chromatography
 215 or spectroscopy (like NMR or Mass Spectrometry).

216 The expanded uncertainty can be calculated using the following equation:

$$217 \quad U = k \times \varepsilon \times \sqrt{\left(\frac{u(\delta^A X)}{\Delta \delta^A X}\right)^2 + \left(\frac{u(p)}{p}\right)^2} \quad (6)$$

218 where $k = 2$ for a coverage factor of 95% and p is the quantity measured during f
 219 determination (a mass, an area). Then, $u(\delta^A X)$ is the uncertainty associated with
 220 isotopic measurement at t_0 and t_f obtained by the propagation uncertainty law:

$$221 \quad u(\delta^A X) = \sqrt{SD^2 + SD^2} = \sqrt{2 \times SD^2} \quad (7)$$

222 where SD is the standard deviation of the isotopic measurement. The analyte at the
223 initial and the final states is measured using strictly the same protocol, i.e. there is no
224 linearity problem. The next step is the calculation of $u(p)$, namely the precision of the
225 analytical method used to determine f , which is expressed as an uncertainty using a
226 rectangular distribution (also called continuous uniform distribution) [19] and
227 calculated using the following equation:

$$228 \quad u(p) = \sqrt{\left(\frac{v}{\sqrt{3}}\right)^2} \times k \quad (8)$$

229 where v is the standard deviation of the analytical method used to determine f
230 (balance, spectrometric technique), p is the quantity measured (mass, area) and $k =$
231 2 for a coverage factor of 95%.

232 In the general equation for U calculation (equation 6), the term $\left(\frac{u(p)}{p}\right)^2$ is generally
233 negligible but needs to be taken into account in order to ensure that every step of the
234 uncertainty calculation is performed in a correct way. The precision of f determination
235 needs to be taken into account during ε determination, because both the
236 measurement method and the sampling can be associated with a systematic error
237 [26].

238 Once the enrichment factor and its associated expanded uncertainty are calculated,
239 the final result is expressed as $\varepsilon \pm U$. This expanded uncertainty value is slightly
240 overestimated but covers all sources of variability. In these conditions, the observed
241 isotope effect is considered significant when $|\varepsilon| > |U|$.

242

243 **RESULTS AND DISCUSSION**

244 **Comparison between the two approaches**

245 In this study, ϵ and the associated expanded uncertainty are calculated for each data
246 point of the selected articles and the values are compared with those obtained using
247 Rayleigh-plots. In this section, the studied element is specified in enrichment factor
248 expression in order to make the reading easier (i.e. ϵ_C in the case of ^{13}C enrichment
249 factor).

250 Figure 2 shows the Rayleigh-plots obtained for the datasets used in this study. In
251 most cases, the Rayleigh-plot trend-line fits very well with the experimental points,
252 with root mean square values (R^2) higher than 0.98, which clearly demonstrates the
253 linearity of the experimental data (Figure 2). The only exception is the trend line
254 obtained from the monitoring of $\delta^{13}\text{C}$ of the carboxyl group of acetic acid during
255 evaporation (Figure 2c) for which R^2 is about zero. This value is not surprising given
256 that the slope of the curve is 0 (implying that there is no significant isotope
257 fractionation associated with evaporation on this carbon-atom position).

258 For each dataset, ϵ was determined using the slope of the trend line. Values for ϵ
259 obtained from each data point using the two-points calculation (equation 3) are
260 presented in Table 1. In most cases, two-points calculation method gives very similar
261 results to those from Rayleigh-plots, but only for small values of f (which corresponds
262 to the fraction of unreacted substrate at t_i). As an example, results from article A
263 (Figure 2a) show a very high ϵ_H of -65.7‰ obtained from Rayleigh-plot and two-
264 points calculations give values comprised between -63.0 and -69.3‰ when f is lower
265 than 20%. The same phenomenon is observed in the case of ^{13}C with an ϵ_C of -1.5‰
266 according to Rayleigh-plot and very different values obtained with equation 3 when f
267 $> 24\%$. Rayleigh-plots drawn using experimental data from article B (Figure 2b) show
268 an ϵ_{Cl} value of -5.0‰ and an ϵ_C of -19.3‰. These data also demonstrate the trueness
269 of ϵ calculated using equation 3, with very similar values obtained when $f < 34.8\%$ in

270 the case of ^{37}Cl and $f < 31.1\%$ for ^{13}C . Figure 2c demonstrates that the two-points
271 calculation method is also efficient with PSIA data. Rayleigh-plot results from article
272 C indicate that acetic acid evaporation is associated with a $\epsilon_{\text{C-Bulk}}$ of -1.0‰ mostly
273 located on the methyl group ($\epsilon_{\text{C-Methyl}} = -2.0\text{‰}$) which is confirmed by the comparable
274 values obtained using the two-points calculation when $f < 39.0\%$. Calculated ϵ values
275 also confirm the absence of significant isotope effect located on the carboxyl
276 function.

277 According to data from Figure 2 and Table 1, the two methods seem to be exploitable
278 to determine ϵ from varied experimental data and elements. The limit of the two-
279 points calculation is the value of f , but this limit is not clearly established.
280 Determination of expanded uncertainty should help to fix the limit of the domain of
281 use of the Rayleigh equation.

282 **Expanded uncertainty**

283 Expanded uncertainties associated with ϵ determination using both Rayleigh-plot and
284 two-points calculation are detailed in Table 1. When ϵ is determined through
285 Rayleigh-plot using experimental data from Article A, the expanded uncertainty
286 associated with ^{13}C IE determination is very small ($U = 0.1\text{‰}$) compared to ϵ . That
287 result suggests that ϵ is significant. The U value is higher in the case of ^2H (3.7‰) but
288 this result is in accordance with the higher ϵ_{H} of -65.7‰ detected through deuterium
289 isotope analysis. These two examples demonstrate that an IE can be considered as
290 significant even if its associated uncertainty is high; when $|\epsilon| > |U|$ (or $|U/\epsilon| < 1$),
291 the result is significant. Carbon-13 bulk enrichment factors obtained in the other
292 articles are also significant with $\epsilon_{\text{C}} = -19.3 \pm 0.8\text{‰}$ in article B, $\epsilon_{\text{C-Bulk}} = -1.0 \pm 0.1\text{‰}$
293 and $\epsilon_{\text{C-Methyl}} = -2.0 \pm 0.1\text{‰}$ in article C (Table 1). The same conclusion can be made
294 with results from ^{37}Cl experimental data of article B with $\epsilon_{\text{Cl}} = -5.0 \pm 0.2\text{‰}$.

295 Enrichment factors calculated using the two-points approach are similar to those from
296 Rayleigh-plots, as previously described by Jeannotat and Hunkeler [25], but U
297 obtained with the two methods are very different and U seems to be increased with f
298 (Table 1), which is coherent with the observations made above. In each study, when
299 the process is not well advanced ($f > 30\%$), the equation gives different ε results than
300 those obtained with the Rayleigh-plot. When less than 70% of substrate has reacted,
301 the calculated ε is not significant, as demonstrated in Figure 3b with $|U/\varepsilon_{\text{Rayleigh}}| > 1$.
302 For instance, $\varepsilon_{\text{C-Bulk}}$ is $-1.0 \pm 0.1\text{‰}$ (article C) according to the Rayleigh-plot and the
303 same value is obtained from the two-points calculation with $|U/\varepsilon_{\text{Rayleigh}}|$ comprised
304 between 0.3 and 0.7‰ provided $f < 30\%$ (Figure 3b).

305 From all the data studied here, data points calculated at $f > 30\%$ give different values
306 from those determined using Rayleigh-plot and this difference is confirmed by high
307 expanded uncertainty values. The determination of $|U/\varepsilon|$ is an excellent tool to
308 ensure that the uncertainties associated with the determination of IE do not exceed
309 the measured ε [27].

310 **Significance of the results**

311 The results obtained from each article were used to draw three graphs (Figure 3) in
312 order to evaluate the trueness of the ε determined with the two-points calculation and
313 the minimum of substrate transformation that must be used to ensure that results are
314 significant.

315 The variation of the expanded uncertainty depending on the amount of remaining
316 substrate is described in Figure 3a. This result confirms the need to set a limit of f
317 value in order to use the two-points calculation in a correct way. The graph presented
318 in Figure 3b shows the correlation between the reaction yield ($1 - f$) and the degree
319 of significance of ε calculated using the two-points method (the result is considered

320 as significant when $|U/\varepsilon| < 1$). These data mean that results obtained with the two-
321 points calculation are significant on a large range of transformation yields, even if the
322 expanded uncertainty associated with the enrichment factor increases with the
323 amount of remaining substrate. This graph demonstrates that the results obtained
324 are significant when $1 - f > 0.7$ ($f < 30\%$). The relationship between the
325 transformation yield ($1 - f$) and the gap between ε values obtained with the two
326 methods $((\varepsilon - \varepsilon_{\text{Rayleigh}})/\varepsilon_{\text{Rayleigh}})$ clearly demonstrates the trueness of ε values
327 determined with the two-points calculation (see Figure 3c), considering that the
328 Rayleigh-plot allows the calculation of a true ε . Indeed, values obtained using the
329 two-points method have a deviation of 10% relative to the true value ($\varepsilon_{\text{Rayleigh}}$) when f
330 $< 20\%$ ($1 - f > 0.8$) and a deviation of 20% when $f < 30\%$ ($1 - f > 0.7$), which means
331 that the two-points method allows the calculation of true values of ε when the
332 selected data point has been measured at high transformation yield.
333 These results demonstrate that the two-points calculation method is efficient when
334 the amount of remaining substrate is below 30% (which corresponds to a reaction
335 yield of 70%).

336

337 CONCLUSIONS

338 Considering these observations, the determination of ε using a single experimental
339 point can be justified in conditions wherein the yield of the studied transformation is
340 above 70% ($f < 30\%$). Within this range, the ε values calculated are similar to those
341 obtained from Rayleigh-plots, and so is the associated uncertainty. Furthermore,
342 expanded uncertainty calculations are shown as an excellent and rigorous way to
343 check if the determined values of ε are significant and, in particular, are a good tool
344 to assess the validity of the two-points calculation.

345 Enrichment factors can therefore be directly determined from experimental data
346 using two different methods. The classical Rayleigh-plot approach is very efficient
347 and the resulting ε associated expanded uncertainty can be calculated using the
348 function "LINEST" of Microsoft ExcelTM. Equations that allow calculating enrichment
349 factor with only one experimental point can be very useful when it is difficult to collect
350 several samples during a transformation. When using this method, it is
351 recommended to work with transformations of more than 70%.

352 For both methods, determining the expanded uncertainty associated with a result is
353 highly recommended in order to draw any conclusion about the studied phenomenon.
354 This calculation requires the determination of precision for both the isotopic
355 measurement and the yield. In these conditions, the final result will take into account
356 all uncertainty sources associated with both the experiments and the measurements.
357 The final IE result can be expressed as $\varepsilon \pm U$ and considered as significant when
358 $|\varepsilon| > |U|$ (or $|U/\varepsilon| < 1$). In these conditions, the two-points calculation is an
359 excellent way to determine the IE associated with a transformation and the use of the
360 expanded uncertainty reinforces the validity of conclusions that can be made about
361 the studied mechanism.

362 Such calculations can be complex to set up; so this article provides a spreadsheet in
363 which both ε and U can be automatically calculated after appropriate input: the initial
364 isotopic composition ($t = 0$), the isotopic composition at $t = f$, the isotopic
365 measurement precision (SD), the transformation yield ($1 - f$), the quantification
366 measurement (mass or area) and the precision associated with f determination.

367

368 **Supporting information available**

369 EXCEL spreadsheet for calculation of enrichment factor and uncertainty by the two-
370 point method.

371

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378

379 **References**

- 380 [1] E.M. Galimov, Isotope organic geochemistry, *Org. Geochem.* 37 (2006) 1200-
381 1262.
- 382 [2] J.M. Hayes, Fractionation of Carbon and Hydrogen Isotopes in Biosynthetic
383 Processes, *Rev. Min. Geochem.* 43 (2001) 225-277.
- 384 [3] M. Elsner, M.A. Jochmann, T.B. Hofstetter, D. Hunkeler, A. Bernstein, T.C.
385 Schmidt, A. Schimmelmann, Current challenges in compound-specific stable isotope
386 analysis of environmental organic contaminants, *Anal. Bioanal. Chem.* 403 (2012)
387 2471-91.
- 388 [4] M. Julien, J. Parinet, P. Nun, K. Bayle, P. Höhener, R.J. Robins, G.S. Remaud,
389 Fractionation in position-specific isotope composition during vaporization of
390 environmental pollutants measured with isotope ratio monitoring by ¹³C nuclear
391 magnetic resonance spectrometry, *Environ. Pollut.* 205 (2015) 299-306.
- 392 [5] M.J. DeNiro, S. Epstein, Mechanism of carbon isotope fractionation associated
393 with lipid synthesis, *Science* 197 (1977) 261-263.
- 394 [6] M. Elsner, L. Zwank, D. Hunkeler, R.P. Schwarzenbach, A New Concept Linking
395 Observable Stable Isotope Fractionation to Transformation Pathways of Organic
396 Pollutants, *Environ. Sci. Technol.* 39 (2005) 6896-6916.
- 397 [7] J.M. Hayes, Practice and principles of isotopic measurements in organic
398 geochemistry, in: A. Sessions (Ed.),
399 2002, <http://web.gps.caltech.edu/~als/research_articles/other_stuff/hayespnp.pdf>.
- 400 [8] J. Bigeleisen, M. Wolfsberg, Theoretical and Experimental Aspects of Isotope
401 Effects in Chemical Kinetics, *Advances in Chemical Physics*, John Wiley & Sons,
402 Inc.2007, pp. 15-76.
- 403 [9] A. Mariotti, J.C. Germon, P. Hubert, P. Kaiser, R. Letolle, A. Tardieux, P.
404 Tardieux, Experimental determination of nitrogen kinetic isotope fractionation: Some
405 principles; illustration for the denitrification and nitrification processes, *Plant and Soil*
406 62 (1981) 413-430.

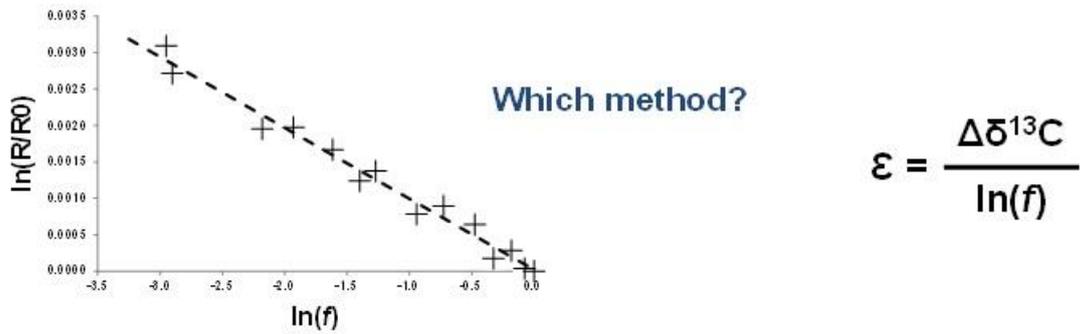
- 407 [10] M. Elsner, J. McKelvie, G. Lacrampe Couloume, B. Sherwood Lollar, Insight into
408 Methyl tert-Butyl Ether (MTBE) Stable Isotope Fractionation from Abiotic Reference
409 Experiments, *Environ. Sci. Technol.* 41 (2007) 5693-5700.
- 410 [11] P. Höhener, O. Atteia, Rayleigh equation for evolution of stable isotope ratios in
411 contaminant decay chains, *Geochim. Cosmochim. Acta* 126 (2014) 70-77.
- 412 [12] C. Kendall, J.J. Mc Donnell, *Isotope tracers in catchment hydrology*, Elsevier
413 Science B.V., Amsterdam 1998.
- 414 [13] M.C. Aelion, P. Höhener, D. Hunkeler, R. Aravena, *Environmental Isotopes in
415 Biodegradation and Bioremediation*, Taylor & Francis Group 2010.
- 416 [14] D. Hunkeler, Quantification of Isotope Fractionation in Experiments with
417 Deuterium-Labeled Substrate, *Appl. Environ. Microbiol.* 68 (2002) 5205-5207.
- 418 [15] S. Cretnik, A. Bernstein, O. Shouakar-Stash, F. Löffler, M. Elsner, Chlorine
419 Isotope Effects from Isotope Ratio Mass Spectrometry Suggest Intramolecular C-Cl
420 Bond Competition in Trichloroethene (TCE) Reductive Dehalogenation, *Molecules* 19
421 (2014) 6450.
- 422 [16] D.B. McNevin, M.R. Badger, H.J. Kane, G.D. Farquhar, Measurement of
423 (carbon) kinetic isotope effect by Rayleigh fractionation using membrane inlet mass
424 spectrometry for CO₂-consuming reactions, *Functional Plant Biology* 33 (2006) 1115-
425 1128.
- 426 [17] T.B. Coplen, Guidelines and recommended terms for expression of stable-
427 isotope-ratio and gas-ratio measurement results, *Rapid Commun. Mass Spectrom.*
428 25 (2011) 2538-2560.
- 429 [18] B. Sherwood Lollar, S.K. Hirschorn, M.M.G. Chartrand, G. Lacrampe-Couloume,
430 An Approach for Assessing Total Instrumental Uncertainty in Compound-Specific
431 Carbon Isotope Analysis: Implications for Environmental Remediation Studies, *Anal.
432 Chem.* 79 (2007) 3469-3475.
- 433 [19] JCGM/WG, Evaluation of measurement data- Guide to the expression of
434 uncertainty in measurement. Evaluation of measurement data- Guide to the
435 expression of uncertainty in measurement 100., 2008.
- 436 [20] J.R. Gray, G. Lacrampe-Couloume, D. Gandhi, K.M. Scow, R.D. Wilson, D.M.
437 Mackay, B. Sherwood Lollar, Carbon and Hydrogen Isotopic Fractionation during
438 Biodegradation of Methyl tert-Butyl Ether, *Environ. Sci. Technol.* 36 (2002) 1931-
439 1938.
- 440 [21] J.T. Brenna, T.N. Corso, H.J. Tobias, R.J. Caimi, High-precision continuous-flow
441 isotope ratio mass spectrometry, *Mass Spectrom. Rev.* 16 (1997) 227-258.
- 442 [22] K. Yamada, M. Kikuchi, A. Gilbert, N. Yoshida, N. Wasano, R. Hattori, S. Hirano,
443 Evaluation of commercially available reagents as a reference material for
444 intramolecular carbon isotopic measurements of acetic acid, *Rapid Commun. Mass
445 Spectrom.* 28 (2014) 1821-1828.
- 446 [23] T.N. Corso, J.T. Brenna, High-precision position-specific isotope analysis, *Proc.
447 Natl. Acad. Sci.* 94 (1997) 1049-1053.
- 448 [24] K.M. Scott, X. Lu, C.M. Cavanaugh, J.S. Liu, Optimal methods for estimating
449 kinetic isotope effects from different forms of the Rayleigh distillation equation,
450 *Geochim. Cosmochim. Acta* 68 (2004) 433-442.
- 451 [25] S. Jeannotat, D. Hunkeler, Chlorine and Carbon Isotopes Fractionation during
452 Volatilization and Diffusive Transport of Trichloroethene in the Unsaturated Zone,
453 *Environ. Sci. Technol.* 46 (2012) 3169-3176.
- 454 [26] D. Buchner, B. Jin, K. Ebert, M. Rolle, M. Elsner, S.B. Haderlein, Experimental
455 Determination of Isotope Enrichment Factors – Bias from Mass Removal by
456 Repetitive Sampling, *Environ. Sci. Technol.* 51 (2017) 1527-1536.

457 [27] D.A. Singleton, A.A. Thomas, High-Precision Simultaneous Determination of
458 Multiple Small Kinetic Isotope Effects at Natural Abundance, J. Am. Chem. Soc. 117
459 (1995) 9357-9358.

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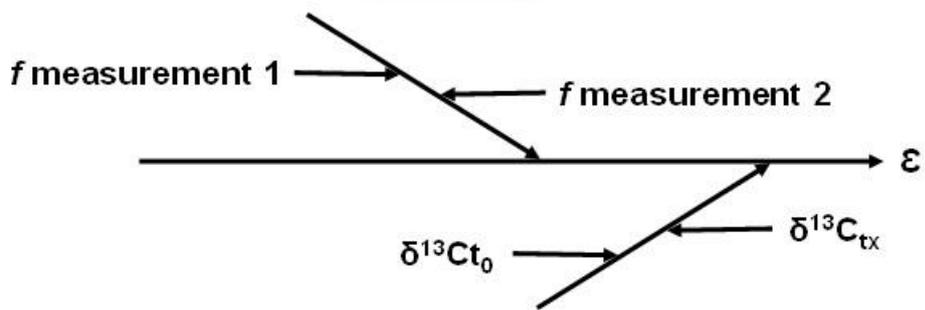
Graphical abstract



How to calculate?

$$\epsilon \pm U$$

Is it significant?



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Tables

475 **Table 1:** Values of ϵ calculated using each experimental point from (a) Gray *et al.*
 476 2002, (b) Cretnik *et al.* 2014 and (c) Yamada *et al.* 2014. Associated expanded
 477 uncertainties (U) are also listed and compared to ϵ values obtained from Rayleigh-
 478 plots.

¹³ C			² H			¹³ C			³⁷ Cl		
f	ϵ (‰)	U (‰)	f	ϵ (‰)	U (‰)	f	ϵ (‰)	U (‰)	f	ϵ (‰)	U (‰)
100.0%	-	-	100.0%	-	-	100.0%	-	-	100.0%	-	-
80.0%	0.0	6.3	97.7%	-42.8	484.2	92.3%	-15.5	17.5	92.3%	-5.7	7.0
48.7%	-1.0	2.0	80.4%	-45.9	51.9	83.7%	-13.4	8.0	83.7%	-6.0	3.2
42.6%	-0.9	1.7	64.3%	-29.4	25.6	76.6%	-6.8	5.3	76.6%	-2.3	2.1
33.9%	-1.2	1.3	19.3%	-68.7	6.9	71.7%	-13.1	4.2	71.7%	-4.2	1.7
31.3%	-1.1	1.2	18.8%	-62.9	6.8	70.4%	-11.7	4.0	70.4%	-3.8	1.6
26.1%	-1.2	1.1	12.9%	-69.3	5.5	68.5%	-14.4	3.7	68.5%	-4.6	1.5
23.5%	-1.4	1.0	9.2%	-64.1	4.8	61.0%	-17.7	2.9	61.0%	-4.8	1.1
19.1%	-1.3	0.9	6.4%	-67.4	4.2	56.1%	-14.0	2.4	56.1%	-4.1	1.0
15.7%	-1.4	0.8	3.2%	-67.5	3.8	49.0%	-15.6	2.0	49.0%	-4.5	0.8
8.7%	-1.4	0.6	1.6%	-63.0	4.4	34.8%	-16.7	1.3	34.8%	-4.6	0.5
8.7%	-1.6	0.6	Rayleigh plot	-65.7	3.7	31.1%	-16.9	1.2	31.1%	-4.8	0.5
5.2%	-1.4	0.5				14.6%	-18.6	0.8	14.6%	-5.3	0.3
4.3%	-1.5	0.5				7.8%	-18.9	0.7	7.8%	-4.9	0.2
3.5%	-1.4	0.4				Rayleigh plot	-19.3	0.8	Rayleigh plot	-5.0	0.2
2.6%	-1.4	0.4									
0.9%	-1.4	0.3									
0.3%	-1.4	0.4									
Rayleigh plot	-1.5	0.1									

¹³ C				
f	ϵ_{bulk} (‰)	ϵ_{methyl} (‰)	$\epsilon_{\text{carboxyl}}$ (‰)	U (‰)
100.0%	-	-	-	-
92.7%	-0.4	-0.6	-0.3	11.2
83.4%	-1.5	-3.4	0.4	4.7
72.2%	-0.5	-1.3	0.3	2.6
62.2%	-1.3	-1.7	-0.9	1.8
48.3%	-1.2	-1.3	-1.1	1.2
39.0%	-0.8	-1.8	0.2	0.9
28.0%	-1.0	-1.7	-0.4	0.7
24.7%	-0.9	-2.1	0.4	0.6
19.9%	-1.0	-1.8	-0.2	0.5
14.5%	-1.0	-1.9	-0.1	0.4
11.3%	-0.9	-1.9	0.2	0.4
5.5%	-0.9	-1.9	0.1	0.3
5.2%	-1.0	-1.8	-0.2	0.3
	-1.0	-	-	0.1
slope	-	-2.0	-	0.2
	-	-	0.0	0.1

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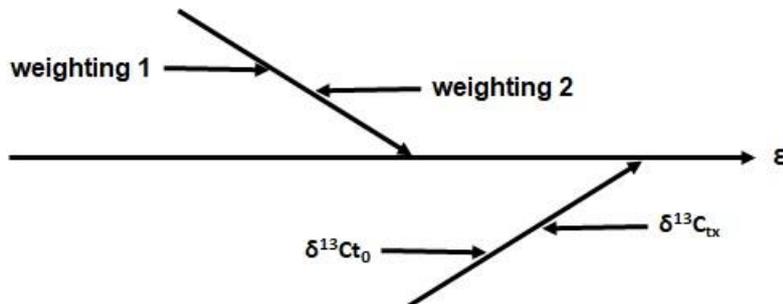
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Figure captions

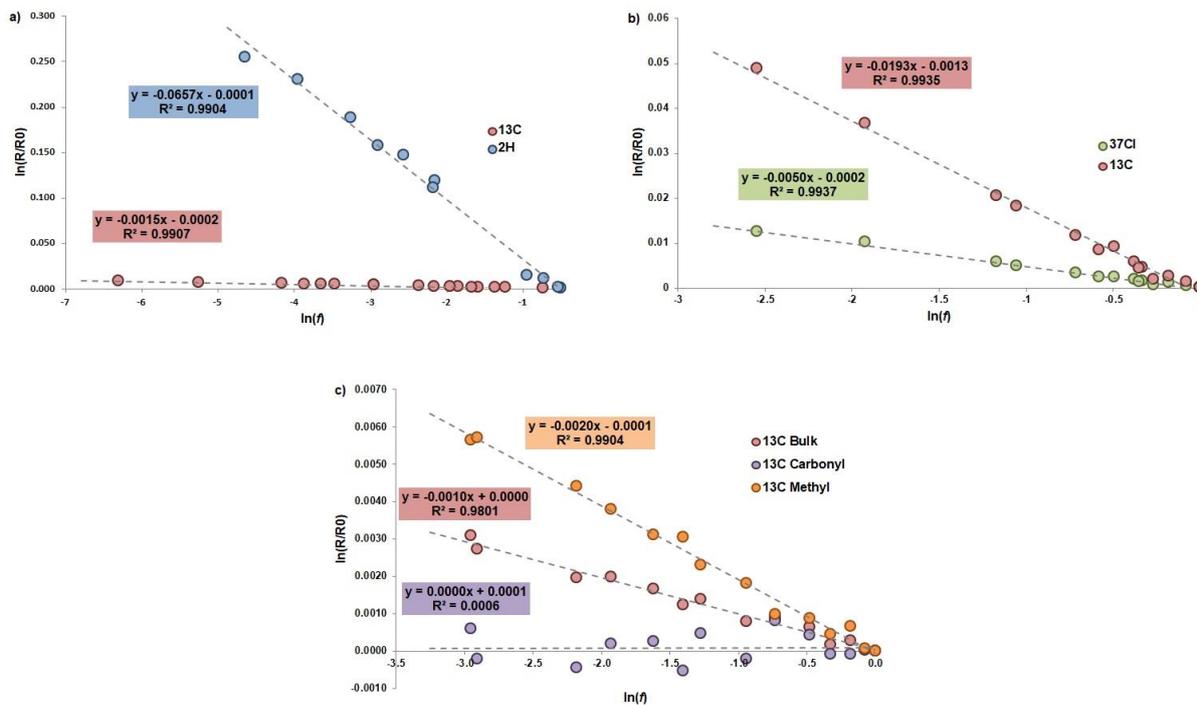
489 **Figure 1:** Ishikawa diagram describing all sources of uncertainty in the case of
 490 isotopic measurements of the studied compound at t_0 and t_x and two measurements
 491 required to determine f .



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494 **Figure 2:** Rayleigh-plots drawn from experimental data of (a) Gray *et al.* 2002, (b)
 495 Cretnik *et al.* 2014 and (c) Yamada *et al.* 2014.



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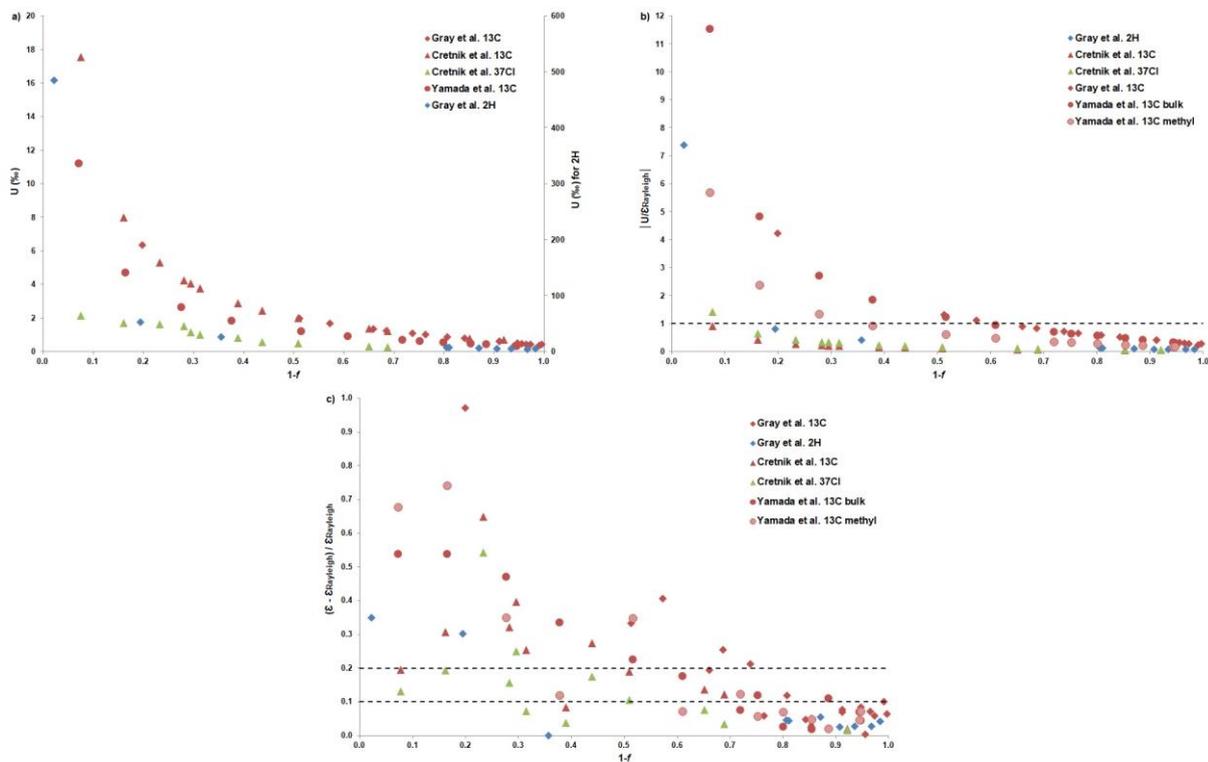
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503 **Figure 3:** Projection of the transformation yield (x-axis expressed as $1 - f$) and on the
 504 y-axis (a) U , (b) $|U/\epsilon_{\text{Rayleigh}}|$ and (c) $(\epsilon - \epsilon_{\text{Rayleigh}})/\epsilon_{\text{Rayleigh}}$. Dotted lines correspond to
 505 (b) the significance threshold ($|U/\epsilon_{\text{Rayleigh}}| = 1$) and (c) the gap between values
 506 obtained using the two-points calculation and the true value (fixed at 10 and 20%).

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