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Protocol

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A guide to precise measurements of isotope abundance by ESI-Orbitrap MS

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Supplementary Information for "A Guide to Precise Measurements of Isotope Abundance by ESI-Orbitrap MS"

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- Supplementary Description – A description of two alternative ways of implementing the one-point calibration of δ values.
- Supplementary Figure 1 – Flowchart of the functions provided within the isoorbi R package.

Supplementary Description

Different ways to calculate the one-point calibration for Orbitrap IRMS

All formulas are based on the example of the ratio of the ^{15}N-substituted nitrate ions over the unsubstituted nitrate ions $R\left(\frac{^{15}\mathrm{N}}{\mathrm{M0}}\right)$ in a measurement of a sample versus a standard. The final goal is to calculate δ values versus the stable-isotope primary reference material Air-N_2.

For simplicity, the ratio $R\left(\frac{^{15}\mathrm{N}}{\mathrm{M0}}\right)$ will be denoted simply as "R" in this document.

The standard is in this case the reference material USGS35, shortened as "35".

- **1st way to perform the one-point calibration**

The equation used by Hilkert et al. 2021 (https://doi.org/10.1021/acs.analchem.1c00944) to convert measured δ values of a sample ("sam") versus a standard to δ values versus a primary reference material (Air-N_2) is:

$$\delta_{\mathrm{sam/Air\text{-}N_2}} = \delta_{\mathrm{sam/35}} + \delta_{\mathrm{35/Air\text{-}N_2}} + \delta_{\mathrm{sam/35}} \times \delta_{\mathrm{35/Air\text{-}N_2}} \tag{1}$$

- **Details on the underlying derivations**

To understand where the two δ values $\delta_{\mathrm{sam/35}}$ and $\delta_{\mathrm{35/Air\text{-}N_2}}$ that are used as input in Eq. (1) come from, we have to differentiate between two different R_{35} terms. One is the ratio that is determined in the Orbitrap IRMS measurement, referred to as $R_{\mathrm{35,meas}}$. The second one is the USGS35 ratio reported by the USGS (United States Geological Survey), referred to as $R_{\mathrm{35,rep}}$. This value is what we consider the true value for the nitrate USGS35 material, and it gives the true $\delta_{\mathrm{35/Air\text{-}N_2}}$.

We can express these two δ values in the following way:

$$\delta_{\mathrm{sam/35}} = \frac{R_{\mathrm{sam}} - R_{\mathrm{35,meas}}}{R_{\mathrm{35,meas}}} \tag{2}$$

$$\delta_{\mathrm{35/Air\text{-}N_2}} = \frac{R_{\mathrm{35,rep}} - R_{\mathrm{Air\text{-}N_2}}}{R_{\mathrm{Air\text{-}N_2}}} \tag{3}$$

Next, substitute Eqs. (2) and (3) into Eq. (1):

$$\delta_{\text{sam/Air-N}_2} = \frac{R_{\text{sam}} - R_{35,\text{meas}}}{R_{35,\text{meas}}} + \frac{R_{35,\text{rep}} - R_{\text{Air-N}_2}}{R_{\text{Air-N}_2}} + \frac{R_{\text{sam}} - R_{35,\text{meas}}}{R_{35,\text{meas}}} \times \frac{R_{35,\text{rep}} - R_{\text{Air-N}_2}}{R_{\text{Air-N}_2}} \tag{4}$$

Expand the fractions so they have the same denominator:

$$\delta_{\text{sam/Air-N}_2} = \frac{(R_{\text{sam}} - R_{35,\text{meas}}) \times R_{\text{Air-N}_2} + (R_{35,\text{rep}} - R_{\text{Air-N}_2}) \times R_{35,\text{meas}} + (R_{\text{sam}} - R_{35,\text{meas}}) \times (R_{35,\text{rep}} - R_{\text{Air-N}_2})}{R_{35,\text{meas}} \times R_{\text{Air-N}_2}} \tag{5}$$

Multiply all the brackets; colored terms cancel each other out:

$$\delta_{\text{sam/Air-N}_2} = \frac{R_{\text{sam}} \times R_{\text{Air-N}_2} - R_{35,\text{meas}} \times R_{\text{Air-N}_2} + R_{35,\text{rep}} \times R_{35,\text{meas}} - R_{\text{Air-N}_2} \times R_{35,\text{meas}} + R_{\text{sam}} \times R_{35,\text{rep}} - R_{\text{sam}} \times R_{\text{Air-N}_2} - R_{35,\text{meas}} \times R_{35,\text{rep}} + R_{35,\text{meas}} \times R_{\text{Air-N}_2}}{R_{35,\text{meas}} \times R_{\text{Air-N}_2}} \tag{6}$$

Equation (6) results in:

$$\delta_{\text{sam/Air-N}_2} = \frac{-R_{\text{Air-N}_2} \times R_{35,\text{meas}} + R_{\text{sam}} \times R_{35,\text{rep}}}{R_{35,\text{meas}} \times R_{\text{Air-N}_2}} \tag{7}$$

Dividing by $R_{35,\text{meas}}$ gives:

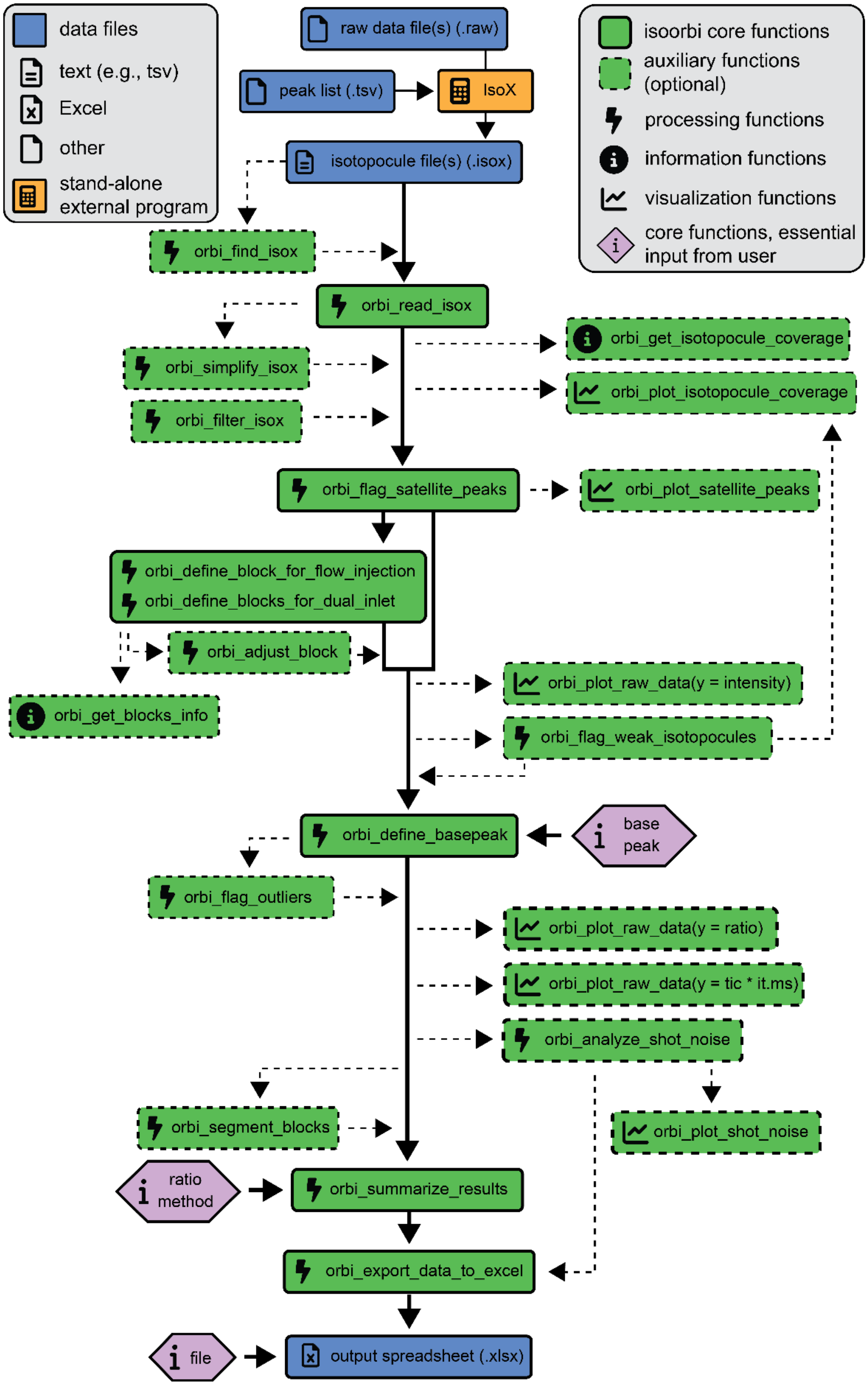
$$\delta_{\text{sam/Air-N}_2} = \frac{\left(\frac{R_{35,\text{rep}}}{R_{35,\text{meas}}}\right) \times R_{\text{sam}} - R_{\text{Air-N}_2}}{R_{\text{Air-N}_2}} \tag{8}$$

The term $\frac{R_{35,\text{rep}}}{R_{35,\text{meas}}}$ in Eq. (8) acts as a correction factor for the measured ratio in the sample based on the difference of the reported and measured ratios of the standard used.

- **2nd way to perform the one-point calibration**

A different way to calculate internationally referenced δ values: instead of calculating δ values versus a standard and then applying the conversion using Eq. (1), we do the calculation the other way around: first, correct the ratio via sample/standard comparison and then calculate δ values using only Eq. (8).

The ratio R_{sam} can be corrected for every sample injection by using the averaged correction factor of the preceding and following injections of the standard. That way, we get a corrected sample ratio as the result. Optionally, we can still calculate δ values using Eq. (1) for check which will be identical to those calculated using Eq. (8), as Eq. (8) is derived from Eq. (1). This has been demonstrated in this document and in the example dataset (Spreadsheet_Calculations.xlsx). Correcting the measured ratio R_{sam} instead of the δ value enables us to report the quality of our data to a wider variety of people since anyone can transfer ratios into any scale or unit required (ppm, %CV, δ, SEM…).



Supplementary Figure 1 | Flowchart of the functions provided within the isoorbi R package.