<u>**SUPPORTING INFORMATION**</u>

SPECIES-SPECIFIC ISOTOPE DILUTION ANALYSIS AND ISOTOPE PATTERN DECONVOLUTION FOR BUTYLTIN COMPOUNDS METABOLISM INVESTIGATIONS

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Abstract

This section details the mathematical development followed in this manuscript for the isotope deconvolution of the species present in the different samples under investigation. The isotope abundances of the species determined in the samples are expressed as a linear combination of the isotope abundances of the initially administered isotopically enriched species and the natural abundances of the corresponding element.

Isotope pattern reconstruction using least-squares.

Although this methodology can be applied to a wide variety of elements, the development of the mathematical approach will be explained for the particular case of the three different species of tin studied (MBT, DBT and TBT) each enriched in a different Sn isotope. Accordingly to the definitions explained in the manuscript equation [1] can be obtained for the contribution of enriched MBT, DBT and TBT and natural Sn for any given compound measured (MBT, DBT or TBT) using ***n*** isotopes:

$$\begin{pmatrix} A_{m,1} \\ A_{m,2} \\ \cdot \\ A_{m,i} \\ \cdot \\ A_{m,n} \end{pmatrix} = \begin{pmatrix} \alpha \\ \beta \\ \gamma \\ 1-(\alpha+\beta+\gamma) \end{pmatrix} \cdot \begin{pmatrix} A_{MBT,1} & A_{DBT,1} & A_{TBT,1} & A_{nat,1} \\ A_{MBT,2} & A_{DBT,2} & A_{TBT,2} & A_{nat,2} \\ \cdot & \cdot & \cdot & \cdot \\ A_{MBT,i} & A_{DBT,i} & A_{TBT,i} & A_{nat,i} \\ \cdot & \cdot & \cdot & \cdot \\ A_{MBT,n} & A_{DBT,n} & A_{TBT,n} & A_{nat,n} \end{pmatrix} \qquad [1]$$

In this way, the following expression is obtained for isotope *i*:

$$A_{m,i} = \alpha A_{MBT,i} + \beta A_{DBT,i} + \gamma A_{TBT,i} + [1-(\alpha+\beta+\gamma)]A_{nat,i} \qquad [2]$$

As we have more parameters than unknowns, least square fitting can be applied to calculate the values for these unknowns. The uncertainty function to be minimised is:

$$U = \sum_{i=1}^{n} \left(A_{m,i} - \hat{A}_{m,i}\right)^2 \qquad [3]$$

where $A_{m,i}$ is the experimental value of the abundance for the isotope *i* in the considered species present in a sample *m* and $\hat{A}_{m,i}$ the calculated value. For the case of equation [2] we obtain:

$$U = \sum_{i=1}^{n} \left(A_{m,i} - \alpha A_{MBT,i} - \beta A_{DBT,i} - \gamma A_{TBT,i} - A_{nat,i} + \alpha A_{nat,i} + \beta A_{nat,i} + \gamma A_{nat,i}\right)^2 \qquad [4]$$

and rearranging we obtain:

$$U = \sum_{i=1}^{n} \left[A_{m,i} - A_{nat,i} + \alpha \left(A_{nat,i} - A_{MBT,i} \right) + \beta \left(A_{nat,i} - A_{DBT,i} \right) + \gamma \left(A_{nat,i} - A_{TBT,i} \right) \right]^{2} \qquad [5]$$

As the condition for U to be a minimum is:

$$\frac{\partial U}{\partial \alpha} = 0 \qquad [6]$$

$$\frac{\partial U}{\partial \beta} = 0 \qquad [7]$$

$$\frac{\partial U}{\partial \gamma} = 0 \qquad [8]$$

the following set of equations is obtained by taking the parcial derivatives in equation [5]:

$$\frac{\partial U}{\partial \alpha} = 2 \sum_{i=1}^{n} \left[\left(A_{nat,i} - A_{MBT,i} \right) \cdot \left[A_{m,i} - A_{nat,i} + \alpha \left(A_{nat,i} - A_{MBT,i} \right) + \beta \left(A_{nat,i} - A_{DBT,i} \right) + \gamma \left(A_{nat,i} - A_{TBT,i} \right) \right] \right] = 0 \qquad [9]$$

$$\frac{\partial U}{\partial \beta} = 2 \sum_{i=1}^{n} \left[\left(A_{nat,i} - A_{DBT,i} \right) \cdot \left[A_{m,i} - A_{nat,i} + \alpha \left(A_{nat,i} - A_{MBT,i} \right) + \beta \left(A_{nat,i} - A_{DBT,i} \right) + \gamma \left(A_{nat,i} - A_{TBT,i} \right) \right] \right] = 0 \qquad [10]$$

$$\frac{\partial U}{\partial \gamma} = 2 \sum_{i=1}^{n} \left[\left(A_{nat,i} - A_{TBT,i} \right) \cdot \left[A_{m,i} - A_{nat,i} + \alpha \left(A_{nat,i} - A_{MBT,i} \right) + \beta \left(A_{nat,i} - A_{DBT,i} \right) + \gamma \left(A_{nat,i} - A_{TBT,i} \right) \right] \right] = 0$$

[11]

Rearranging equation [9]:

$$\sum_{i=1}^{n}\left[\begin{array}{l}\left(A_{nat,i}\cdot A_{m,i}-A_{MBT,i}\cdot A_{m,i}\right)+\alpha\cdot\left(A_{nat,i}-A_{MBT,i}\right)^{2}+\beta\cdot\left(A_{nat,i}-A_{DBT,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)\\+\gamma\cdot\left(A_{nat,i}-A_{TBT,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)-A_{nat,i}^{2}+A_{nat,i}\cdot A_{MBT,i}\end{array}\right]=0$$

[12]

$$\begin{array}{l}\alpha\cdot\sum_{i=1}^{n}\left(A_{nat,i}-A_{MBT,i}\right)^{2}+\beta\cdot\sum_{i=1}^{n}\left(A_{nat,i}-A_{DBT,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)+\gamma\cdot\sum_{i=1}^{n}\left(A_{nat,i}-A_{TBT,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)\\+\sum_{i=1}^{n}A_{nat,i}\cdot A_{m,i}+\sum_{i=1}^{n}A_{nat,i}\cdot A_{MBT,i}-\sum_{i=1}^{n}A_{nat,i}^{2}-\sum_{i=1}^{n}A_{MBT,i}\cdot A_{m,i}=0\end{array}$$

[13]

$$\begin{array}{l}\alpha\cdot\sum_{i=1}^{n}\left(A_{nat,i}-A_{MBT,i}\right)^{2}+\beta\cdot\sum_{i=1}^{n}\left(A_{nat,i}-A_{DBT,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)+\gamma\cdot\sum_{i=1}^{n}\left(A_{nat,i}-A_{TBT,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)\\+\sum_{i=1}^{n}\left(A_{m,i}-A_{nat,i}\right)\cdot\left(A_{nat,i}-A_{MBT,i}\right)=0\end{array}$$

[14]

Rearranging equation [10]:

$$\sum_{i=1}^{n}\left[\begin{array}{l}\left(A_{nat,i}\cdot A_{m,i}-A_{DBT,i}\cdot A_{m,i}\right)+\alpha\cdot\left(A_{nat,i}-A_{MBT,i}\right)\left(A_{nat,i}-A_{DBT,i}\right)+\beta\cdot\left(A_{nat,i}-A_{DBT,i}\right)^{2}\\+\gamma\cdot\left(A_{nat,i}-A_{TBT,i}\right)\cdot\left(A_{nat,i}-A_{DBT,i}\right)-A_{nat,i}^{2}+A_{nat,i}\cdot A_{DBT,i}\end{array}\right]=0$$

[15]

$$\alpha \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right) + \beta \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{DBT,i}\right)^2 + \gamma \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{TBT,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right)$$
$$+ \sum_{i=1}^{n} \left(A_{m,i} - A_{nat,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right) = 0$$

[16]

Rearranging equation [11]:

$$\sum_{i=1}^{n} \begin{bmatrix} \left(A_{nat,i} \cdot A_{m,i} - A_{TBT,i} \cdot A_{m,i}\right) + \alpha \cdot \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) + \beta \cdot \left(A_{nat,i} - A_{DBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) \\ + \gamma \cdot \left(A_{nat,i} - A_{TBT,i}\right)^2 - A_{nat,i}^2 + A_{nat,i} \cdot A_{TBT,i} \end{bmatrix} = 0$$

[17]

$$\alpha \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) + \beta \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{DBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) + \gamma \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{TBT,i}\right)^2 \cdot$$
$$+ \sum_{i=1}^{n} \left(A_{m,i} - A_{nat,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) = 0$$

[18]

Finally, from equation [14], [16] and [18] the following equations are obtained:

$$\alpha \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{MBT,i}\right)^2 + \beta \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{DBT,i}\right) \cdot \left(A_{nat,i} - A_{MBT,i}\right) + \gamma \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{TBT,i}\right) \cdot \left(A_{nat,i} - A_{MBT,i}\right)$$
$$= \sum_{i=1}^{n} \left(A_{nat,i} - A_{m,i}\right) \cdot \left(A_{nat,i} - A_{MBT,i}\right)$$

[19]

$$\alpha \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right) + \beta \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{DBT,i}\right)^2 + \gamma \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{TBT,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right)$$
$$= \sum_{i=1}^{n} \left(A_{nat,i} - A_{m,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right)$$

[20]

$$\alpha \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) + \beta \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{DBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) + \gamma \cdot \sum_{i=1}^{n} \left(A_{nat,i} - A_{TBT,i}\right)^2 \cdot$$
$$= \sum_{i=1}^{n} \left(A_{nat,i} - A_{m,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right)$$

[21]

which can be expressed as matrix notation with equation [22]:

$$\begin{bmatrix} \alpha \\ \beta \\ \gamma \end{bmatrix} \cdot \begin{bmatrix} \sum_i \left(A_{nat,i} - A_{MBT,i}\right)^2 & \sum_i \left(A_{nat,i} - A_{DBT,i}\right) \cdot \left(A_{nat,i} - A_{MBT,i}\right) & \sum_i \left(A_{nat,i} - A_{TBT,i}\right) \cdot \left(A_{nat,i} - A_{MBT,i}\right) \\ \sum_i \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right) & \sum_i \left(A_{nat,i} - A_{DBT,i}\right)^2 & \sum_i \left(A_{nat,i} - A_{TBT,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right) \\ \sum_i \left(A_{nat,i} - A_{MBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) & \sum_i \left(A_{nat,i} - A_{DBT,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) & \sum_i \left(A_{nat,i} - A_{TBT,i}\right)^2 \end{bmatrix} =$$
$$\begin{bmatrix} \sum_i \left(A_{nat,i} - A_{m,i}\right) \cdot \left(A_{nat,i} - A_{MBT,i}\right) \\ \sum_i \left(A_{nat,i} - A_{m,i}\right) \cdot \left(A_{nat,i} - A_{DBT,i}\right) \\ \sum_i \left(A_{nat,i} - A_{m,i}\right) \cdot \left(A_{nat,i} - A_{TBT,i}\right) \end{bmatrix}$$

Once the isotopic composition of each compound in the sample *m* is measured, it is clear that, we end up with three equations and three unknowns, which can be easily resolved using Kramer´s rule, or by inverting the matrix of the factors, computing therefore the values of α, β, γ and δ.