Supplementary Material. Correction of Natural Isotopes of Elements in Mass Isotopomer Fragments

The natural isotopes of elements other than metabolic carbon can interfere in mass isotopomer abundance measurements and need correction before their use in estimating fluxes. The elements hydrogen, oxygen, sulfur and silicon are present in commonly measured metabolites (e.g. amino acids), and in commonly used derivatizing reagents (e.g. N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide [MTBSTFA]).

Previously, Fernandez and co-workers (1) reported a matrix-based correction method, which was improved by Wittmann and Heinzle (2) and then by van Winden and co-workers (3). However, the generation of the correction matrices in these papers is nontrivial, and may not be accessible to the general user. Here we present a novel algorithm to generate these correction matrices, based on the representation of mass isotopomers in an appropriate numeration system. We also demonstrate the application of this method to a mixture of known amounts of labeled and unlabeled amino acids.

Correction of Mass Isotopomer Abundances

The correction of a measured mass isotopomer abundance vector **m** to a corrected mass isotopomer abundance vector $\mathbf{m_c}$ involves premultiplication by a correction matrix **A** for each element other than metabolic carbon (3),

$$\mathbf{m}_c = \mathbf{A}^{-1} \cdot \mathbf{m} .$$

The correction matrix **A** has dimensions $n_0 \times n_I n_E$, where n_0 is the number of mass isotopomers before correction, n_I is the number of natural isotopes of the element being corrected for, and n_E is the number of atoms of this element in the compound of interest.

To demonstrate the generation of the correction matrix **A**, we have used the example of silicon isotopes in tBDMS-derivatized glycine. Glycine contains two metabolic carbon atoms ($n_0 = 2$), and tBDMS-derivatized glycine contains two silicon atoms ($n_E = 2$). Silicon has three natural isotopes, ^{28}Si [M+0], ^{29}Si [M+1], and ^{30}Si [M+2], with natural abundances p_0, p_1, and p_2 respectively. Since each silicon atom in tBDMS-derivatized glycine can be ^{28}Si, ^{29}Si, or ^{30}Si, $\left(n_I + 1\right)^{n_E} = 9$ isotopomers are possible (Table S-2.1).

We propose that the isotopomers be numbered in a numeration system of base n_I+1, with exactly n_E digits. The mass gain Δm (increase in the mass of the molecule) due to the isotopomer is then the sum of these digits, and the abundance of this isotopomer is $\prod_{i=1}^{n^E} p_i$. For silicon in tBDMS-derivatized glycine, $n_I + 1 = 3$. The isotopomer (^{28}Si–^{30}Si) or [M+0][M+2] is represented as 02, its mass gain Δm is 0+2 = 2 Da, and its abundance p is $p_0 p_2$.

The correction matrix A can now be updated for each isotopomer,

$$A_{j,\Delta m+j} = A_{j,\Delta m+j} + p .$$

We measured mass isotopomer abundances of three representative amino acids glycine, alanine, and glutamic acid from a mixture containing predetermined amounts of U-^{13}C and naturally abundant algal amino acids. The expected abundances of the mass isotopomers (from the weights of U-^{13}C and naturally abundant algal amino acids in the mixture) were corrected for the natural isotopes of hydrogen, oxygen, non-metabolic carbon, and silicon by generating correction matrices as described here. The

corrected mass isotopomer abundances were closer to the measured mass isotopomer abundances than the uncorrected mass isotopomer abundances (Figure S-2.1).

Table S1. Isotopomers of a fragment consisting of $n_E = 2$ silicon atoms. Silicon has $n_I = 3$ isotopes with masses [*M*+0], [*M*+0], and [*M*+2], and natural abundances p_0, p_1, and p_2 respectively. The total number of isotopomers is $(n_I+1)^{n_E} = 9$. When isotopomers are represented in base $n_I+1 = 3$, the mass gain due to each isotopomer is the sum of the digits, and the abundance of the isotopomer is the product of the probabilities corresponding to each isotope contained in the isotopomer.

Isotopomer	**Base (n_I+1) representation**	**Mass gain**	**Abundance**
[*M*+0][*M*+0]	00	0	p_0^2
[*M*+0][*M*+1]	01	1	p_0p_1
[*M*+0][*M*+2]	02	2	p_0p_2
[*M*+1][*M*+0]	10	1	p_1p_0
[*M*+1][*M*+1]	11	2	p_1^2
[*M*+1][*M*+2]	12	3	p_1p_2
[*M*+2][*M*+0]	20	2	p_2p_0
[*M*+2][*M*+1]	21	3	p_2p_1
[*M*+2][*M*+2]	22	4	p_2^2
		Total	$(p_0 + p_1 + p_2)^2 = 1$

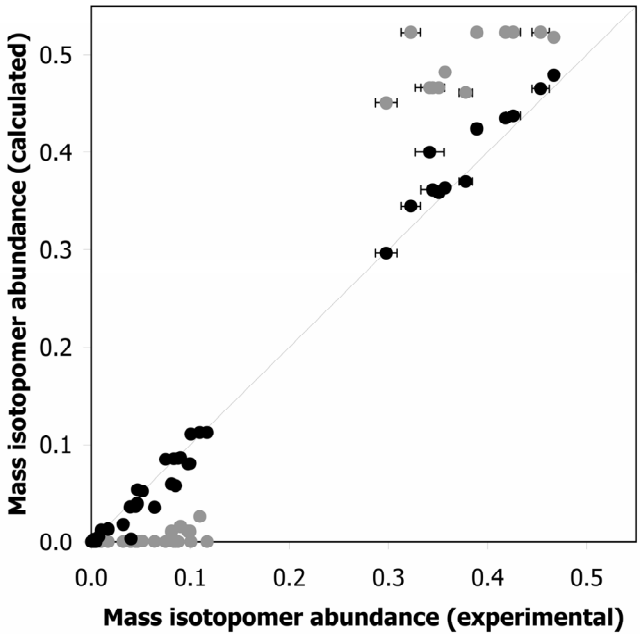

Figure S1. Measured and calculated isotopomer abundances of three representative amino acids glycine, alanine, and glutamic acid from a mixture of U-^{13}C and naturally abundant algal amino acids. Calculated abundances are either corrected for natural abundances of isotopes of elements other than the carbons in the amino acid and for [*M*-n]$^+$ peaks (black circles) or uncorrected (grey circles). Isotopomer abundances were measured by derivatizing a mixture of predetermined amounts of U-^{13}C and naturally abundant algal amino acids and analyzing on a GC-MS in EI mode. Correction of isotopomer abundances was performed as explained in text.

References

1. Fernandez, C. A., Des Rosiers, C., Previs, S. F., David, F., and Brunengraber, H. (1996) *J Mass Spectrom* **31**(3), 255-262
2. Wittmann, C., and Heinzle, E. (1999) *Biotechnol Bioeng* **62**(6), 739-750
3. van Winden, W. A., Wittmann, C., Heinzle, E., and Heijnen, J. J. (2002) *Biotechnol Bioeng* **80**(4), 477-479