

# Direct Determination of Elemental Isotope Abundance Ratios Using Spectral Accuracy with Single Quadrupole MS Systems

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MP 459

## Introduction

Elemental isotope abundance ratio determination requires a very specialized type of MS instrumentation, Isotope Ratio MS (IRMS). IRMS typically comes with a (combustion) module to convert a given organic compound into simpler forms (CO<sub>2</sub>, SO<sub>2</sub>, H<sub>2</sub>, N<sub>2</sub>, etc.) before high resolution MS detection and analysis for that specific element contained in the compound. While the sensitivity and accuracy have made IRMS the gold standard for such analysis, it does suffer from the extra complexity, cost, and sample loss associated with the element-specific conversion process and corresponding hardware module. In this paper, we describe a direct approach to the determination of elemental isotope ratios without any conversion step or extra hardware module on an otherwise conventional system such as a unit mass resolution single quadrupole GC/MS.

## Method

For an ion of the elemental composition A<sub>a</sub>B<sub>b</sub>C<sub>c</sub>D<sub>d</sub>, the complete isotope distribution is given by<sup>1</sup>:

$$\left(\sum a_i A_i\right)^a \left(\sum b_i B_i\right)^b \left(\sum c_i C_i\right)^c \left(\sum d_i D_i\right)^d$$

where a, b, c, d are the number of atoms A, B, C, D, ..., respectively, and a<sub>i</sub>, b<sub>i</sub>, c<sub>i</sub>, d<sub>i</sub> are the natural abundances for isotopes A<sub>i</sub>, B<sub>i</sub>, C<sub>i</sub>, D<sub>i</sub>, respectively. This expression can be expanded and re-organized to give the mass locations and abundances of all expected isotope species. For example, for an EI fragment of PFTBA, C<sub>3</sub>F<sub>5</sub>, with the natural abundance for C and F given by

$$\begin{aligned} C^{12} &= 12.000000, & c_{12} & (0.9893, \text{ typically}^2) \\ C^{13} &= 13.003354, & c_{13} & (0.0107, \text{ typically}^2) \\ F^{19} &= 18.998403, & f_{19} & (1.0000, \text{ typically}^2) \end{aligned}$$

The isotope masses (m) and relative abundances (y) for this ion fragment can be calculated as

$$m = \begin{bmatrix} 3C^{12} + 5F^{19} \\ 2C^{12} + C^{13} + 5F^{19} \\ C^{12} + 2C^{13} + 5F^{19} \\ 3C^{13} + 5F^{19} \end{bmatrix} = \begin{bmatrix} 130.992015 \\ 131.995369 \\ 132.998723 \\ 134.002077 \end{bmatrix}$$

$$y = \begin{bmatrix} c_{12}^3 \\ 3c_{13}c_{12}^2 \\ 3c_{13}^2c_{12} \\ c_{13}^3 \end{bmatrix} = \begin{bmatrix} 9.6824 \times 10^{-1} \\ 3.1417 \times 10^{-2} \\ 3.3979 \times 10^{-4} \\ 1.2250 \times 10^{-6} \end{bmatrix}$$

Two notable observations from the above expansion:

(1) The ion abundance ratio of A+1 to A, in this case between [<sup>12</sup>C]<sub>2</sub><sup>12</sup>CF<sub>5</sub> and [<sup>12</sup>C]<sub>3</sub>F<sub>5</sub>, is given by

$$\frac{I_{A+1}}{I_A} = \frac{f_{c_{13}}c_{12}^2}{c_{12}^3} = \frac{f_{c_{13}}}{c_{12}} \quad \text{or} \quad c_{13} \text{ relative abundance} = \frac{c_{13}}{c_{13}+c_{12}} = \frac{I_{A+1}/I_A}{f + I_{A+1}/I_A}$$

In other words, the elemental [<sup>13</sup>C] to [<sup>12</sup>C] abundance ratio is equal to the intensity ratio between the ion species [<sup>12</sup>C]<sub>2</sub><sup>12</sup>CF<sub>5</sub> and [<sup>12</sup>C]<sub>3</sub>F<sub>5</sub> scaled down by a factor of f=3.

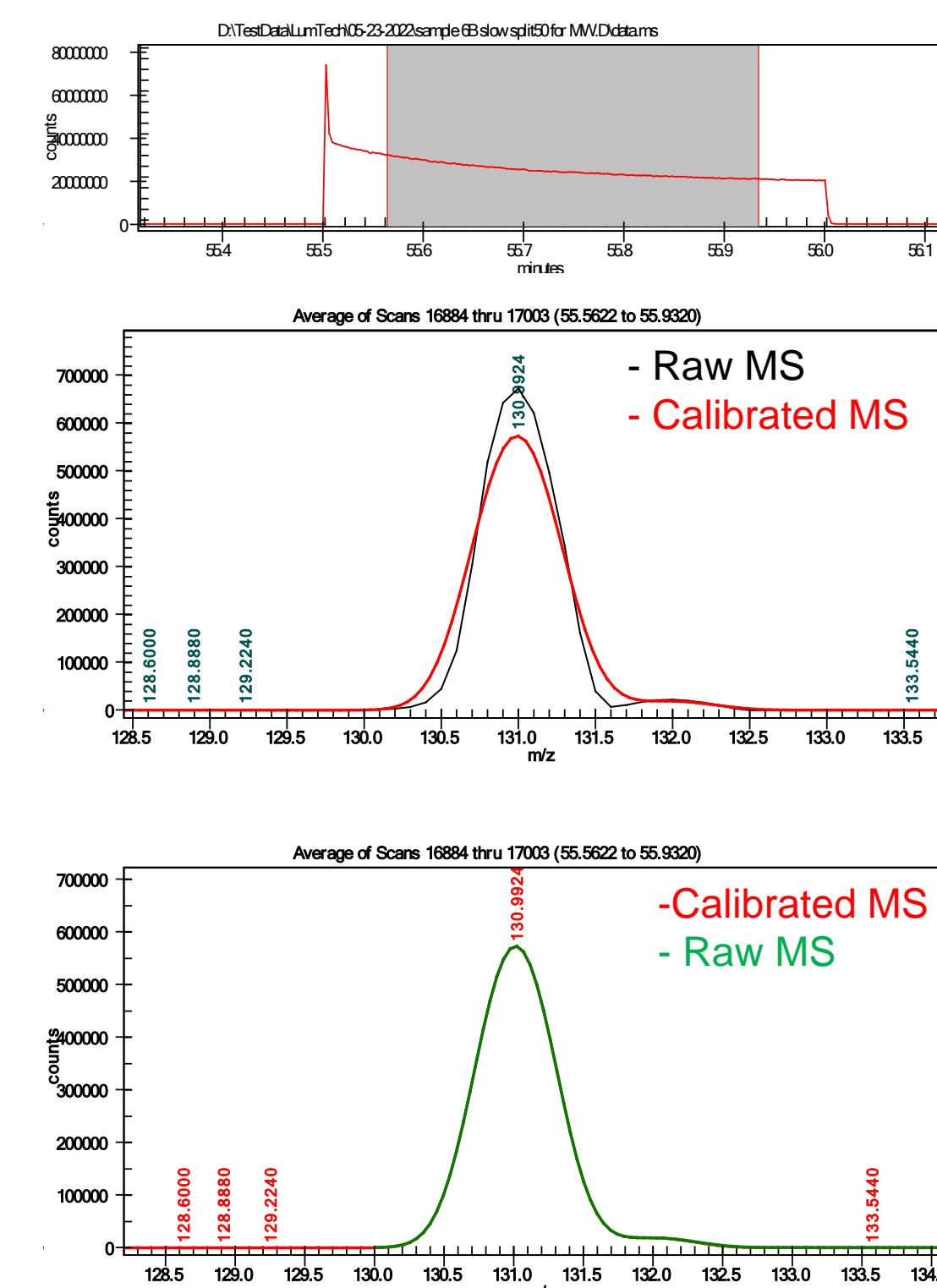
(2) The factor of f=3 comes from the below well-known binomial table with two stable isotopes, corresponding to 3 carbons contained in the EI fragment ion C<sub>3</sub>F<sub>5</sub>:

# Carbons	Factors									
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

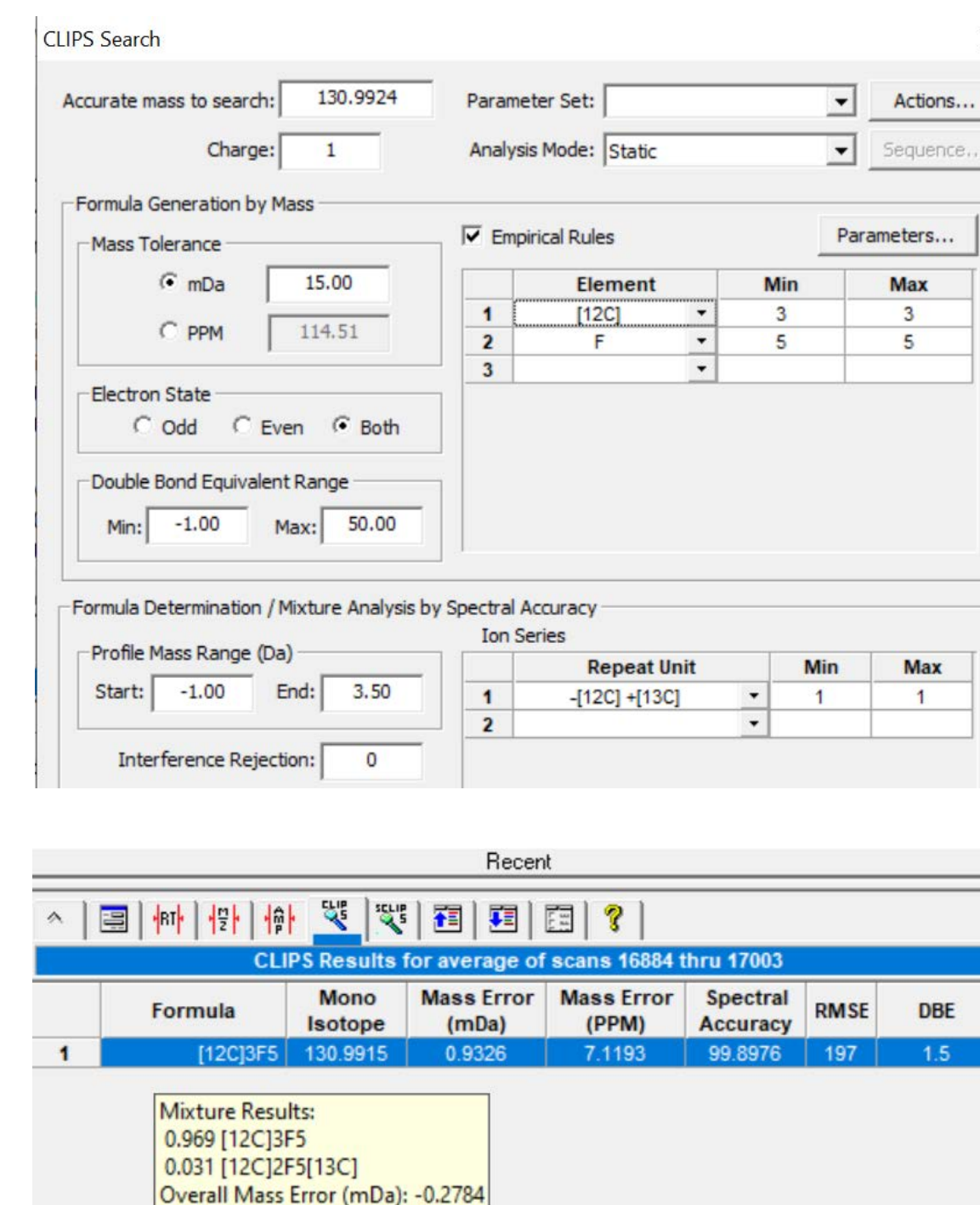
From the above analysis, it would seem to be quite straightforward to measure the A+1 abundance relative to A with the use of high resolution MS systems such as TOF or Orbitrap where there is baseline separation between A and A+1. Unfortunately, as published elsewhere<sup>3-4</sup>, high resolution MS systems may suffer from a systematic lack of spectral accuracy, sometimes depending on the operating conditions.<sup>5</sup> Our recently published work has shown that the relatively simple and lower cost single quadrupole MS system at unit mass resolution has high spectral accuracy for accurate relative quantitation of various differently labeled compounds, in spite of the mutual mass spectral overlaps among the ion species.<sup>6</sup> One obvious complication with isotope measurement at unit mass resolution is the spectral overlaps between (e.g., A and A+1) isotopes, making accurate measurement difficult or inaccurate, as the degree of overlap is dependent on the exact MS peak shape and the peak width, both of which are a function of the specific ion optics and the MS tune at the time of the MS measurement.

With the full spectral calibration process implemented in the MassWorks software<sup>3,7</sup>, not only the issue with uncertain or unknown MS peak shape is solved but the accurate quantitative analysis of ion mixtures with overlapping MS signals could also be achieved, applied previously to compounds with different isotope labels<sup>6</sup> and here where specific isotope species are of interest.

## Determination of <sup>13</sup>C% from Various EI Fragments of PFTBA Tune Gas



The PFTBA tune gas from a GC/MS system has many fragments with varying carbon numbers and the presence and absence of N atom, providing a great test case to evaluate this new approach. With accurate mass and spectral accuracy calibration and the ion series analysis available under the elemental composition search, it is possible to determine the key abundance ratios for the calculation of <sup>13</sup>C%.

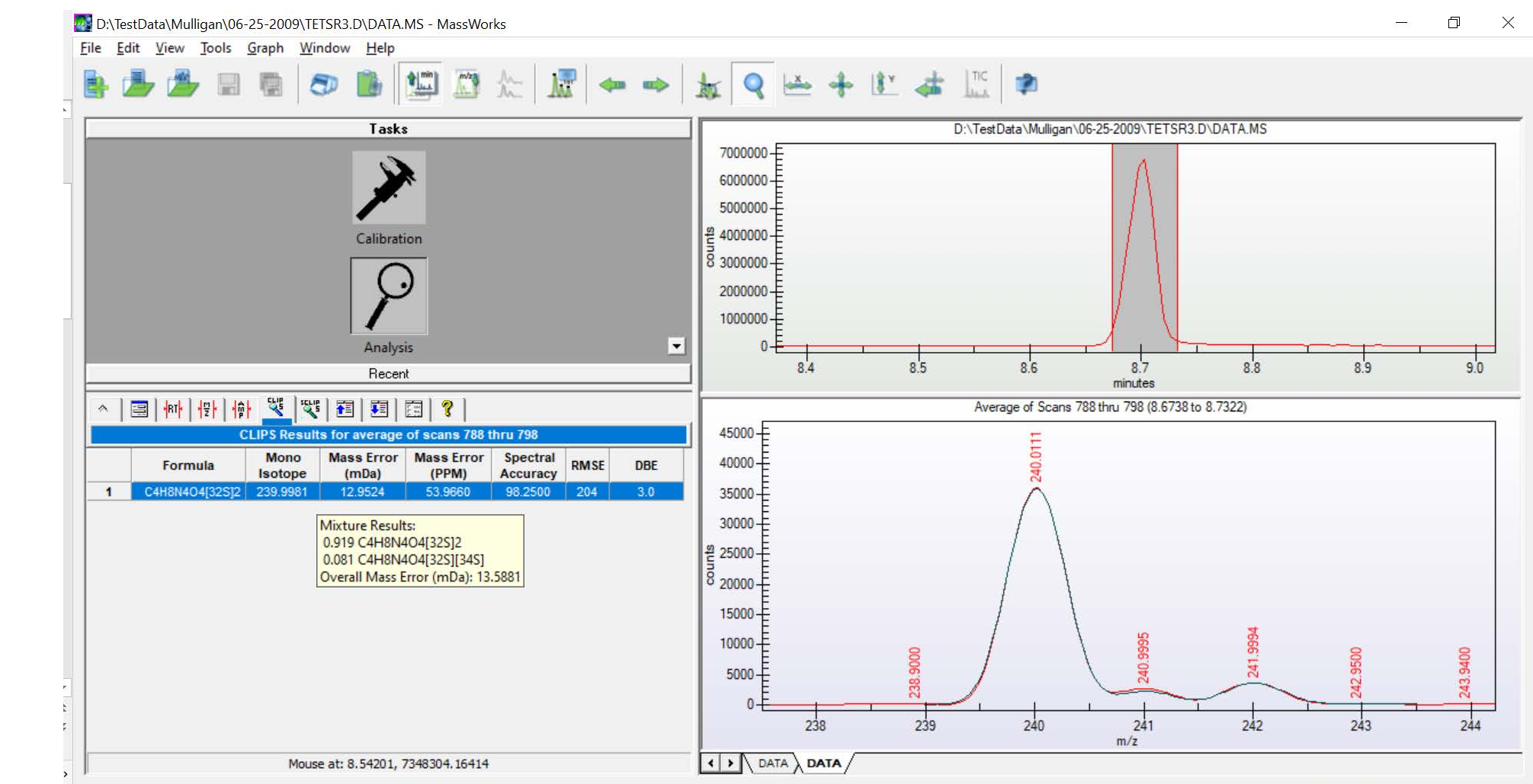


Promising results are obtained from a wide range of EI fragments even in the presence of <sup>15</sup>N isotope under A+1. The RSD% is within 4% when compared to the published value from NIST<sup>2</sup>.

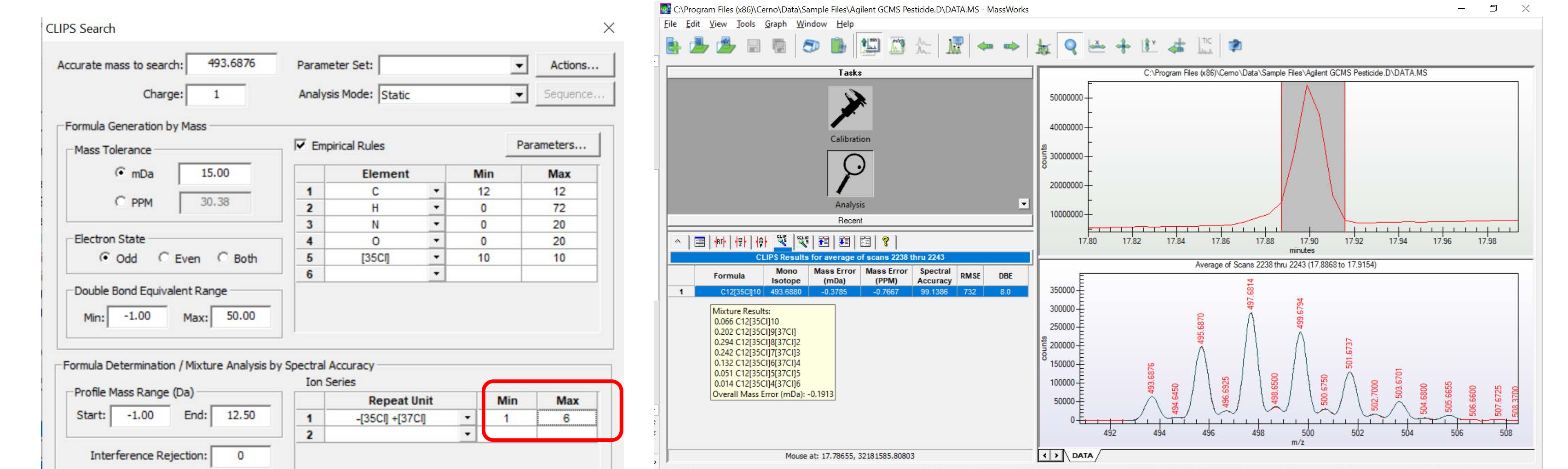
Formula	CF3+	C2F4+	C2F5+	C3F5+	C3F7+	C4F9+	C5F10+	C8F16N+	C9F18N+	C9F20N+
f factor	1	2	2	3	3	4	5	8	9	9
Exact Mass	68.9947	99.9931	118.9915	130.9915	168.9883	218.9851	263.9866	413.977	463.9738	501.9706
I <sub>A</sub>	0.989	0.979	0.981	0.969	0.97	0.959	0.951	0.923	0.919	0.914
I <sub>A+1</sub>	0.011	0.021	0.019	0.031	0.03	0.041	0.049	0.077	0.081	0.086
I <sub>A+1</sub> /I <sub>A</sub>	0.0111	0.0215	0.0194	0.0320	0.0309	0.0428	0.0515	0.0834	0.0881	0.0941
<sup>13</sup> C%	1.10	1.06	0.96	1.06	1.02	1.06	1.02	1.03	0.97	1.03
Average	1.03		NIST Value <sup>2</sup>	1.078						
Std Dev	0.04		RSD%	3.9						

## Determination of <sup>36</sup>S%, <sup>37</sup>Cl%, <sup>18</sup>O%, and <sup>15</sup>N%

For the same ion, it is possible to determine the isotope abundances for any one of the elements involved if all other elemental abundances are known (e.g., as NIST standard values).



The ion series analysis in MassWorks allows for multiple replacement of a given labeled isotope, providing good spectral accuracy fit across the whole m/z range and additional binomial or multinomial terms for analysis, beyond the 1<sup>st</sup> term used in this study.



From the summary results table below, it is seen that the higher number of a given element leads to higher measurement accuracy, indicating that there is more relevant isotope signal available for the determination of its relative abundance. Consistent with the study of <sup>13</sup>C measurement from the PFTBA EI fragments, the relative error of the measurement is less than a few percent, provided there is enough MS signal available and there are more than four atoms for a given element to work with, even for lower abundance isotopes such as <sup>15</sup>N and <sup>16</sup>O.

Formula	C33H41N2O9+	C33H41N2O9+	C33H41N2O9+	C12C10+	C12C10+	C4H8N4O4S2+	C4H8N4O4S2+	C4H8N4O4S2+
f factor	33	9	2	12	10	2	4	4
Isotope	<sup>13</sup> C	<sup>18</sup> O	<sup>15</sup> N	<sup>13</sup> C	<sup>37</sup> Cl	<sup>34</sup> S	<sup>15</sup> N	<sup>18</sup> O
I <sub>A</sub>	0.712	0.982	0.999	0.886	0.065	0.919	0.985	0.991
I <sub>A+1</sub>	0.248	0.018	0.001	0.114	0.206	0.081	0.015	0.009
I <sub>A+1</sub> /I <sub>A</sub>	0.348	0.0183	0.0010	0.129	3.17	0.0881	0.0152	0.0091
Measured%	1.04	0.20	0.05	1.06	24.07	4.22	0.38	0.23
NIST Values <sup>2</sup>	1.078	0.20514	0.36420	1.078	24.2410	4.2524	0.36420	0.20514
Error%	-3.1	-0.9	-86.3	-1.6	-0.7	-0.7	4.1	10.4

\* Purely for simplicity, less abundant <sup>33</sup>S and <sup>36</sup>S are ignored in this study

## Conclusions

- It is feasible to determine a given isotope's relative abundance knowing the elemental composition of the ion and the isotope abundances of other elements.
- If the relative abundance and the number of atoms for a given element could produce more than 1.0% in relative additional spectral response, the determination error can be controlled to within 4%.
- The full spectral calibration is the key to achieve the spectral accuracy required for accurate determination of specific isotope species required for relative abundance calculation.
- While the sensitivity and accuracy of this approach may be less than a dedicated IRMS system, the ease of use and the readily available MS instrumentation required make this an attractive solution.
- When there are multiple elements in a compound that need to be determined, one may use a nonlinear optimization algorithm to solve for more than one unknowns simultaneously (see reference<sup>8</sup>).

## References

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