

# Identification of Co-eluted Components by High Mass Accuracy and Spectral Accuracy with Quadrupole GC-MS Systems

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## Overview

>The novel peak shape calibration technology and its application for unit mass resolution GC/MS systems to achieve high mass accuracy and high spectral accuracy are described.

>With improved mass accuracy, high spectral accuracy is demonstrated to play an important role on identification of co-eluted compounds which were not successfully identified by NIST library search.

## Introduction

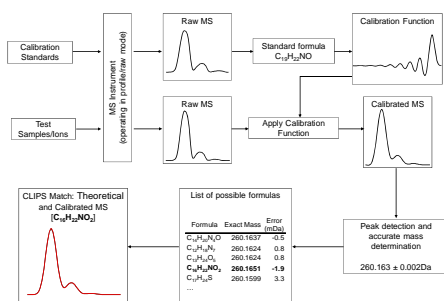
Identification of unknown compounds by unit mass resolution GC-MS systems essentially relies on searching an existing database/library (such as NIST) for spectral match. One critical requirement to achieve successful compound identification is to have pure mass spectra available, which can be obtained by either rigorous GC separation or spectral deconvolution of co-eluted components. When library search could not deliver high-confidence compound identification results, high mass accuracy through high resolution MS systems can provide elemental composition information to facilitate unknown identification. Here we report an effective alternative approach to performing elemental composition determination on a unit mass resolution GC/MS system through innovative instrument line shape calibration technology. Briefly, this comprehensive mass spectral technology calibrates not only m/z values, but more importantly, the mass spectral peak shape. Consequently, symmetrical and mathematically well-defined mass spectral peak shape is obtained to allow accurate mass measurement on unit mass resolution mass spectra even when the monoisotope peak (A) is overlapped with the corresponding A+1 peak. In addition to the high enough mass accuracy thus achieved, the high spectral accuracy available from a quadrupole system allows for exact isotope modeling to achieve elemental composition determination, much like one would do with a higher resolution MS system such as TOF or qTOF.

## Methods

The sample used in the experiments was a dry powder that is used in the food industry to make au jus sauce and it contains many different flavor compounds, which lead to a complex chromatogram. The sampling technique used was Headspace-SPME with a polyacrylate fiber, injected onto a Thermo Scientific TRACE 1310 GC with TG-WaxMS column 60 m x 0.25 mm ID x 0.5 µm dF. The mass spectrometer used was a Thermo TSQ8000 GC-MS/MS operated in full scan mode (mass range 10 – 450 u). Data was acquired in continuum (profile) mode at a rate of 6,197 u/s (0.071 dwell time, 0.075s cycle time) and the acquisition threshold was set to zero. Mass spectra of the sample were calibrated by MassWorks™ 4.0 (Cerno Bioscience, Norwalk, CT) employing perfluorotributylamine (PFTBA) as internal calibration standards acquired in the same data file of the sample, as discussed by Thermo Application Note xxxx concurrently released. Since no MS/MS scan was used, exactly the same experimental setup and analysis would apply to the single quad GC/MS system with the same results.

All profile data were calibrated by instrument line shape calibration as illustrated in Fig.1. This unique calibration will not only correct for mass errors but also transform asymmetric peak shape from raw spectra to mathematically well-defined symmetric peak shape to achieve high spectral accuracy. The determination of elemental composition in unknown samples will automatically result from spectral accuracy calculation on calibrated spectra of the unknowns against possible theoretically calculated spectra.

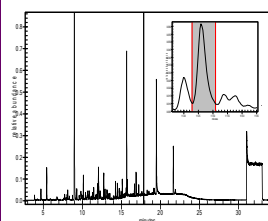
**Fig 1. MassWorks Calibration+CLIPS Formula ID**



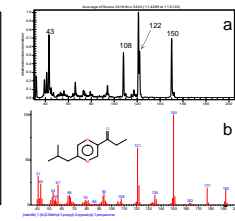
## NIST Library Search

The dry powder sample containing multiple flavor compounds was separated with a 25-minute gradient followed by data collection on PFTBA as shown in Figure 2. The two co-eluted compounds were found at RT = 11.5 min (insert of Figure 2) with major ions observed at m/z 150, 122, and 108 (Figure 3a). Through NIST library search, the acquired spectra were found to have the most likely match against the library spectrum of 1-(5-(2-Methyl-1-propyl)-2-pyrazinyl)-1-propanone (Figure 3b, C11H16N2O, m/z 192). However, the poor search results with forward match factor of 656, reverse match factor of 613, and probability of 2.48% strongly suggest positive identification on the unknown peak was not achieved.

**Fig 2. A TIC of the sample and PFTBA with the co-eluted peak (insert).**



**Fig 3. NIST library search results for mass spectrum of co-eluted components.**



## Results and Discussion

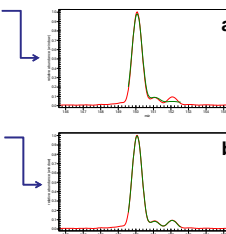
### Elemental Composition Determination by MassWorks

Elemental composition determination with unit mass resolution data by MassWorks relies on improved mass accuracy and more importantly spectral accuracy, which, by definition, is a measure of the spectral congruency between the calibrated mass spectrum and the theoretically calculated version using the same peak shape function. Since MassWorks peak shape calibration transforms ill-defined mass spectral peak shape into mathematically well-defined and symmetric peak shape, the calibrated spectra contain only chemical composition information (plus random noise) from measured compounds to allow spectral accuracy to be a highly selective metric to rank and interactively evaluate various possible elemental compositions based on mass accuracy and mass tolerance.

**Table 1. MassWorks search results**

Rank	Formula	Mass (amu)	Mass Error (amu)	Elemental Accuracy (%)	Match (%)	Score
1	C <sub>6</sub> H <sub>14</sub> S <sub>2</sub>	260.163	0.002	98.3	98.3	17.660
2	C <sub>7</sub> H <sub>10</sub> N <sub>2</sub>	260.163	0.002	98.3	98.3	17.471
3	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	260.163	0.002	98.3	98.2	17.612
4	C <sub>10</sub> H <sub>16</sub> O	260.163	0.002	98.3	98.2	17.593
5	C <sub>10</sub> H <sub>14</sub> O	260.163	0.002	98.3	98.2	17.522
6	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub>	260.163	0.002	98.3	98.2	17.511
7	C <sub>10</sub> H <sub>12</sub> O	260.163	0.002	98.3	98.1	17.511
8	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub>	260.163	0.002	98.3	98.1	17.500
9	C <sub>10</sub> H <sub>10</sub> O	260.163	0.002	98.3	98.0	17.499
10	C <sub>10</sub> H <sub>10</sub> N <sub>2</sub>	260.163	0.002	98.3	98.0	17.498
11	C <sub>10</sub> H <sub>8</sub> O	260.163	0.002	98.3	97.9	17.497
12	C <sub>10</sub> H <sub>8</sub> N <sub>2</sub>	260.163	0.002	98.3	97.9	17.496
13	C <sub>10</sub> H <sub>6</sub> O	260.163	0.002	98.3	97.8	17.495
14	C <sub>10</sub> H <sub>6</sub> N <sub>2</sub>	260.163	0.002	98.3	97.8	17.494
15	C <sub>10</sub> H <sub>4</sub> O	260.163	0.002	98.3	97.7	17.493
16	C <sub>10</sub> H <sub>4</sub> N <sub>2</sub>	260.163	0.002	98.3	97.7	17.492
17	C <sub>10</sub> H <sub>2</sub> O	260.163	0.002	98.3	97.6	17.491
18	C <sub>10</sub> H <sub>2</sub> N <sub>2</sub>	260.163	0.002	98.3	97.6	17.490
19	C <sub>10</sub> H <sub>2</sub> S <sub>2</sub>	260.163	0.002	98.3	97.6	17.489
20	C <sub>10</sub> H <sub>2</sub> O <sub>2</sub>	260.163	0.002	98.3	97.6	17.488
21	C <sub>10</sub> H <sub>2</sub> N <sub>2</sub> O	260.163	0.002	98.3	97.6	17.487

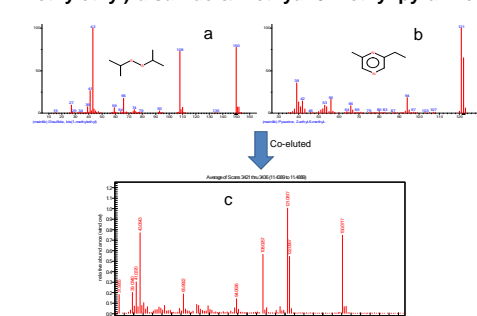
**Fig 4. Spectral overlay of calibrated and calculated**



As shown in Table 1a, initial elemental composition determination of the m/z 150 ion considering only C/H/N/O resulted in a total of 21 possible candidates. The elemental composition C<sub>6</sub>H<sub>14</sub>O<sub>2</sub> was ranked as the best match, but achieved spectral accuracy of only 94.3%, significantly below commonly expected value of 98% for a confident determination. The poor spectral accuracy is illustrated by the spectral overlay between the calibrated spectra (red, Figure 4a) and theoretically calculated spectra of C<sub>6</sub>H<sub>14</sub>O<sub>2</sub> (green, Figure 4a), suggesting the elemental composition of the m/z 150 may contain elements other than just C/H/N/O.

Indeed, when including an additional element sulfur for the search, we found a very different elemental composition, C<sub>6</sub>H<sub>14</sub>S<sub>2</sub>, with the best spectral accuracy of 98.3% among 31 possible candidates. These candidates include three kinds of compounds that contain two, one, and zero sulfur atoms (Table 1b). Since one naturally occurring sulfur atom has 100% of <sup>32</sup>S (A peak) and about 4% <sup>34</sup>S (A+2 peak), compounds with different number of sulfur atoms should exhibit distinct isotope distribution. Taking advantages of this information, the spectral accuracy calculation clearly points out that the most likely elemental composition of the m/z 150 is C<sub>6</sub>H<sub>14</sub>S<sub>2</sub>. The nearly perfect match between the calibrated spectrum of the m/z 150 (red, Figure 4b) and its theoretically calculated version (green, Figure 4b) is illustrated through spectral overlay.

**Fig 5. Identification of Co-eluted components: bis (1-methylethyl) disulfide & 2-ethyl-6-methyl pyrazine**



Further elemental composition determination of other major ions at the m/z 108 and 122 from the co-eluted components was found to have elemental compositions of C<sub>3</sub>H<sub>6</sub>S<sub>2</sub> and C<sub>7</sub>H<sub>10</sub>N<sub>2</sub> (both top ranking) with spectral accuracy of 98.1%. Once the elemental compositions of the major ions are proposed, identification of the co-eluted compounds becomes straightforward. Searching NIST library with elemental composition C<sub>3</sub>H<sub>6</sub>S<sub>2</sub> as input, a total of 13 isomers were found. Ten compounds can be ruled out immediately as they do not have fragment ions at m/z 108. Among the remaining three possibilities, the spectrum from the compound, bis (1-methylethyl) disulfide, has three dominant ions at the m/z 150, 108, and 43 with similar intensities, mostly resembling partial spectrum of the co-eluted components. With the same approach, C<sub>7</sub>H<sub>10</sub>N<sub>2</sub> was further identified as 2-ethyl-6-methyl pyrazine. By examining all the spectra in Figure 5, it is obvious to see that the spectrum from co-eluted components (Figure 5c) are a composite spectrum arising from both bis (1-methylethyl) disulfide (Figure 5a) and 2-ethyl-6-methyl pyrazine (Figure 5b).

## Conclusions

With the novel peak shape calibration technology embedded in MassWorks software, the most accessible unit-mass resolution quadrupole mass spectrometers can achieve sufficient high mass accuracy and spectral accuracy to enable elemental composition determination of even total unknowns. While the improved mass accuracy does provide a list of possible elemental compositions (up to a few dozen candidates) for consideration, the achievable high spectral accuracy is capable of further narrowing down the correct answers to be among the top ranking of spectral accuracy. Taking full advantage of molecular fingerprint information, this approach demonstrated that elemental composition determinations of completely co-eluting ions could be achieved, providing a complementary solution to the identification of unknown chemicals through NIST library search.