Cerno Application Note

Extending the Limits of Mass Spectrometry

Confident Compound Identification with High Mass Accuracy and Elemental Composition Specific NIST Library Search

Identification of unknown compounds by unit mass resolution GC-MS systems essentially relies on searching an existing database/library (such as NIST) by comparing spectra of an unknown compound with those in the library as molecular and fragment ions of a compound carry fingerprints of the molecule . 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 if two or more components co-elute. Otherwise, confidence in the conventional library search will be compromised, as the library search for the complex spectra often results in poor matching values. Such a challenging issue can usually be addressed by obtaining high mass accuracy and performing elemental composition determination on individual ions with high resolution mass spectrometers. However, the high cost of high resolution mass spectrometers remains a major obstacle for the majority of GC/MS users to take advantage of this approach. Here we report an effective and economic alternative to perform NIST library search with high mass accuracy and specified elemental composition utilizing unit mass resolution single quadrupole GC/MS systems. This is implemented through software interaction between MassWorks (Cerno Bioscience, Norwalk, CT) and NIST MS Search (NIST, Gaithersburg, MD) and illustrated with below examples.

Data acquisition of two samples was done on different GC/MS systems. One sample of food industry powder was introduced through Headspace-SPME with a polyacrylate fiber onto a Thermo Scientific TRACE 1310 GC coupled to TSQ8000 GC-MS/MS operated in full scan mode. Data was acquired in continuum (profile) mode at a rate of 6,197 u/s (0.071 dwell time, 0.075 s cycle time) and an acquisition threshold of zero. Another forensic sample was acquired in Agilent GC/MSD under following conditions: A raw scan mode was selected with a scan speed 2^2 (A/D samples = 4) over a mass range of 40-550 m/z with ion threshold set to zero. All data processing was conducted with MassWorks and NIST MS Search.

As shown in Figure 1, an unknown peak eluted at RT=11.5 min and has major ions observed at m/z 150, 121, 108, and 43. The largest ions at m/z 150 in the spectra, most likely molecular ions, were first analyzed by MassWorks to achieve high mass accuracy of 1.9 mD and high spectral accuracy of 99.2% as shown in Figure 2. The elemental composition for the m/z 150 was determined to be C8H14S2 with high confidence, because there is huge spectral accuracy differentiation about 2% between the first best and the second best candidates. In addition, very high spectral accuracy was found for the determination as illustrated by an almost perfect match between theoretical calculated and calibrated spectra (Figure 1, spectral overlay in insert). However, in order to identify the unknown compound(s), it is necessary to perform NIST library search to obtain structures of the molecules. With the newly released MassWorks 5.0, not only is conventional NIST library search available, but also restricted NIST library search with high mass accuracy or elemental composition as a constraint can be implemented to enhance the search performance.

Similar to all other third party search software, MassWorks can launch a conventional NIST library search without any specific constraints. It is noteworthy that under the MS Search menu item of "library search option/presearch", three choices, i.e., default, fast, and off, can be selected. When the choice "off" is selected, a more thorough search will be performed than with the other two selections,

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but that takes a little longer time. This more thorough search is recommended for a true unknown identification or a challenging case as shown below. With this conventional search, the most likely compound for the unknown peak was found to be 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-, but the search has very poor match values (reverse/forward match/probability = 667/705/10.6%). The mismatch is obvious as the base peak in the measured spectra is m/z 121 and there are abundant ions observed at m/z 43. In the library spectra of 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-, on the other hand, m/z 122 is the base peak instead of the m/z 121. More importantly, there are no ions at m/z 43 observed in the library spectra. Therefore, the unknown compound is not 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-.

Following the first unsatisfactory search, further investigation employed the second search routine with a constraint of high mass accuracy. In this example, a mass tolerance of 5 mD was used. This means the search would find all the compounds (in the NIST library) whose m/z values of molecular ions are within 5 mD mass error with respect to the accurate mass at m/z of 150.0550. This search has resulted in a total of 85 different compounds (Figure 3) compared with a total of 400 compounds listed in the results from the previous search (Figure 2). The significant reduction on possible candidates is evidently attributed to high mass accuracy used in the search. A close look at all the compounds from the second search list shows they have only five different elemental compositions including C6H6N4O, C6H14S2, C9H10S, C5H10O5, and C5H14OSSi. All of them have multiple isomers except for C5H14OSSi.

Using the elemental composition C6H14S2 determined by MassWorks as another constraint, the third search routine further reduced the number of candidates to 27 (Figure 4). By definition, these 27 compounds are isomers and have the same elemental composition of C6H14S2. Indeed, the correct compound is bis(1-methylethyl) disulfide, number 3 on the list, as highlighted in blue. The reason it has a poor match value of 591 and low probability of 23.8% is that the experimentally observed spectra are complex spectra that resulted from two co-eluted compounds. In other words, the bis(1-methylethyl) disulfide co-eluted with another compound, 2-ethyal-6-methyl Pyrazine, as reported previously (1).

In addition to the NIST library search, MassWorks can also directly link its determined elemental composition to the ChemSpider database for compound identification. This capability will provide extra confirmation when a compound is positively identified by any one of the three kinds of NIST library searches. It is particularly useful when the compound of interest does not have its GC/MS spectra included in the NIST library. For the example of forensic analysis, the molecular ions of an unknown were determined to have elemental composition of C4H8N4O4S2 with high spectral accuracy of 98.9% (Figure 5). However, no compound was found when searching the NIST library with the elemental composition C4H8N4O4S2 as a search constraint. By selecting the pop-up menu item of “Launch ChemSpider for C4H8N4O4S2”, the ChemSpider database search for the elemental composition was performed. As a result, the correct compound was identified as tetramethylenedisulfotetramine, the first on the search list (Figure 6).

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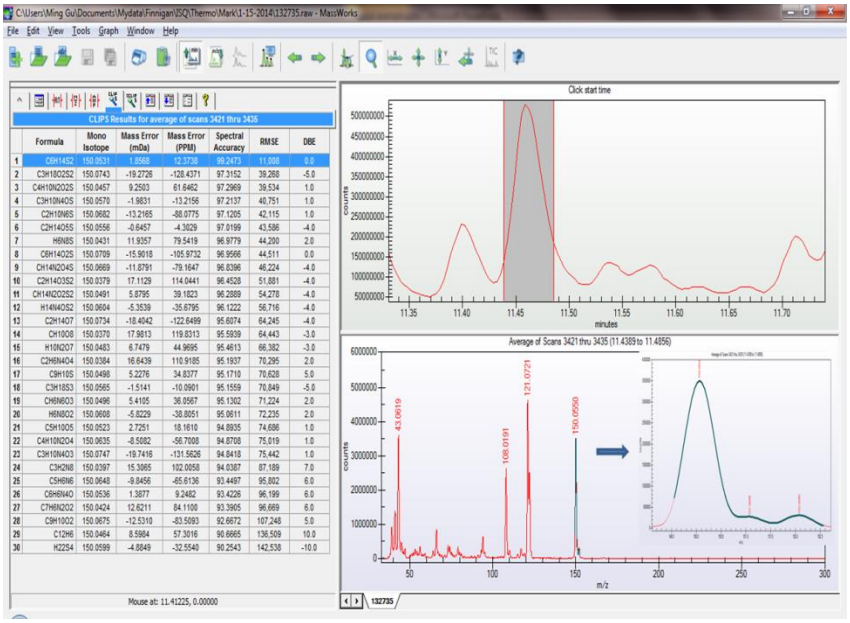

Figure 1. An unknown peak eluted at RT=11.5 and accurate mass measurements and elemental composition determination of m/z 150.

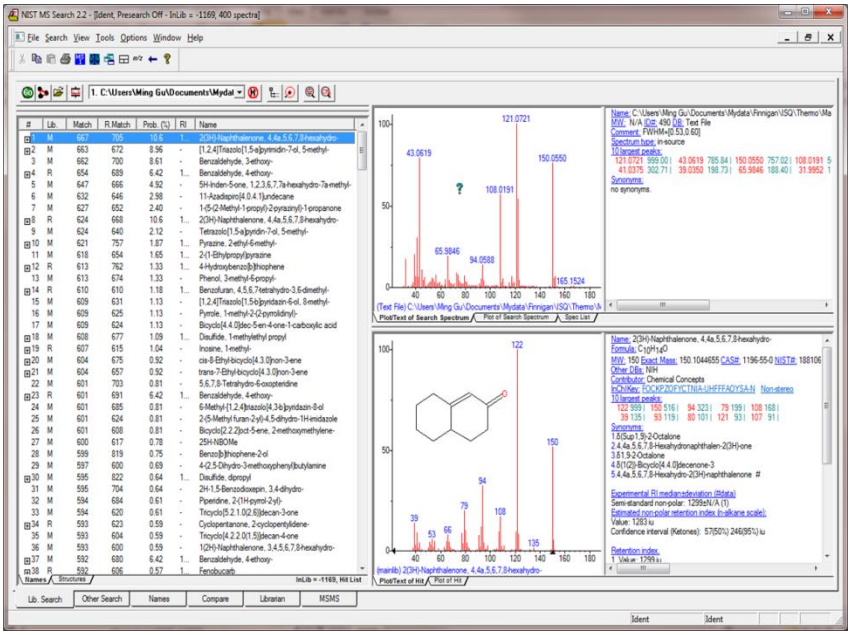

Figure 2. Conventional NIST library search for mass spectra of the unknown peak.

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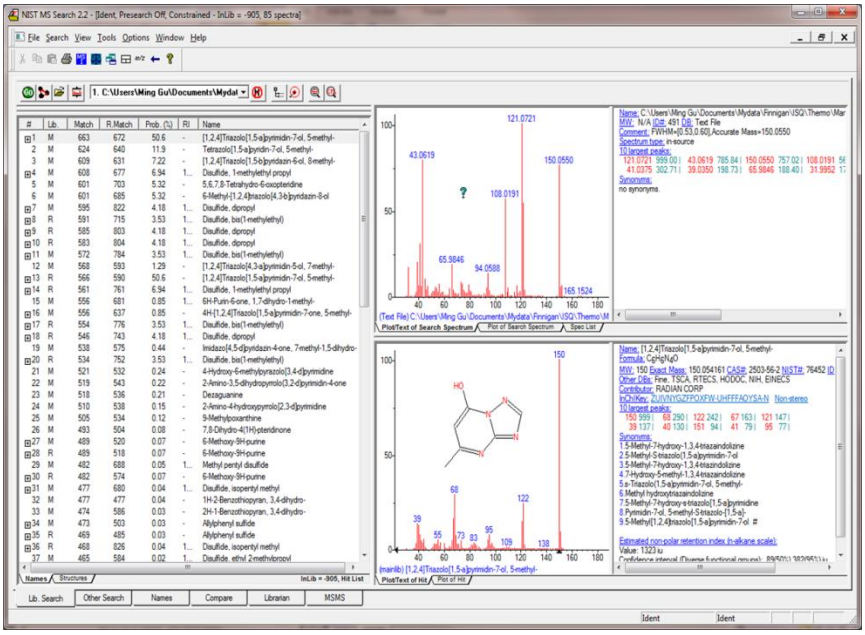

Figure 3. Accurate mass NIST library search for the unknown peak.

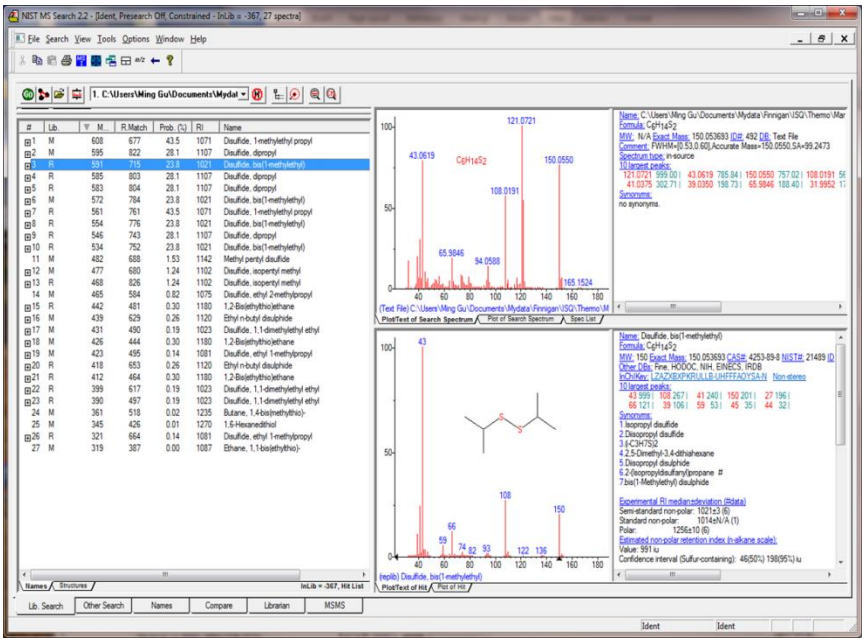

Figure 4. Elemental composition specific NIST library search for the unknown peak.

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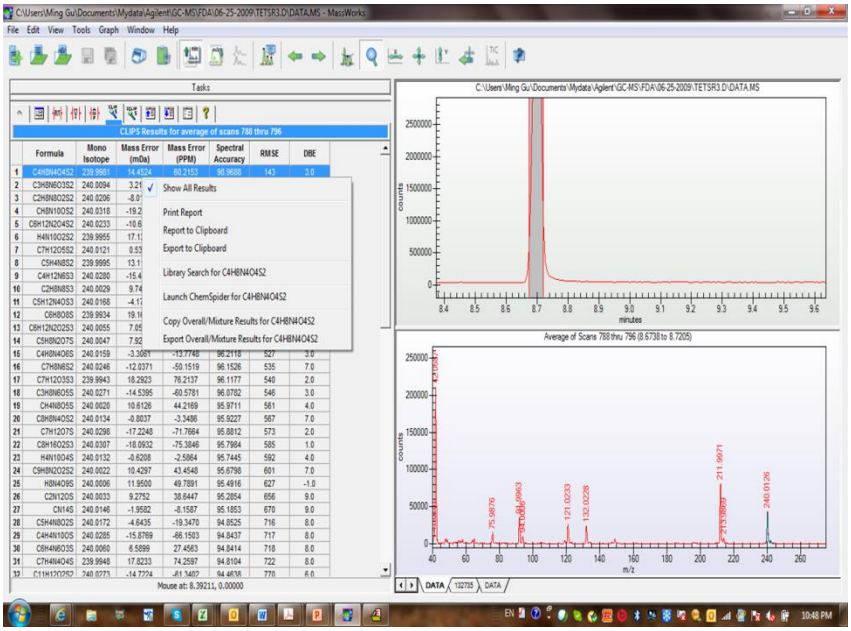

Figure 5. Launch ChemSpider search from MassWorks.

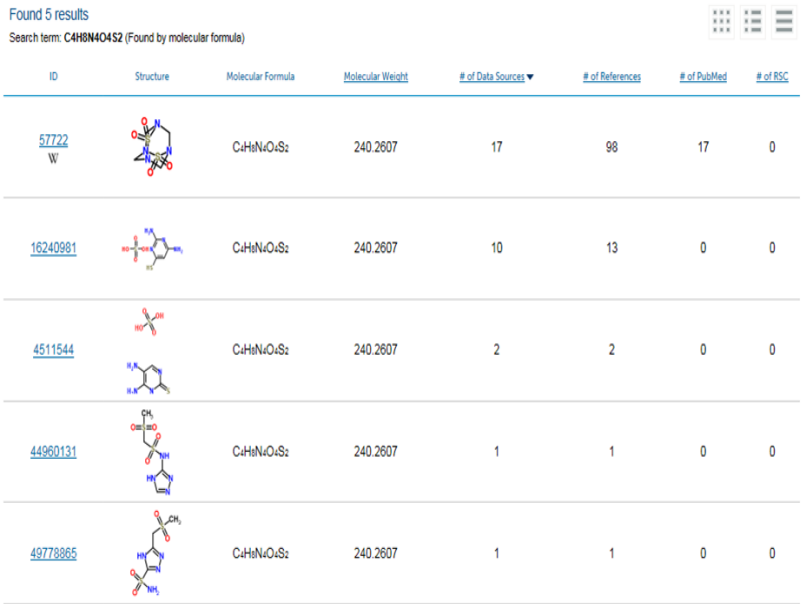

Figure 6. ChemSpider search results for C4H8N4O4S2

Reference

(1) Gu M, Xu HL, Wang YD. High Mass Accuracy Measurements and Elemental Composition Determination of Molecular Ions of Pesticides with a Single Quadrupole GC/MS System Proc. 53rd ASMS Conf. Mass Spectrometry and Allied Topics, St. Louis, MO, May 31 - June 4, 2015

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