Cerno Application Note

Extending the Limits of Mass Spectrometry

GC/MSD Formula Determination for Forensic Chemistry

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Introduction

Single quadrupole GC/MS has become a workhorse instrument in many applications including forensic chemistry due to its reliability, cost advantage, ease-of-use, versatility in the types of compounds amenable for analysis, high sensitivity, and even portability or at least transportability. While considered sufficient for routine applications where a compound is known to belong to a given library (such as NIST MS library) these instruments are typically not used for unknown or new compound identification, which requires elemental composition determination, due to their nominally unit mass resolution and lack of tandem MS capabilities.

Elemental composition determination for the purpose of compound identification is a capability typically reserved for higher resolution systems such as qTOF or FTMS at a much higher ownership cost with larger instrument footprint. As has been shown elsewhere[1&2], even at unit mass resolution, a high degree of mass accuracy can be achieved, making elemental composition determination within reach of conventional users. In order to achieve the mass accuracy required a novel mass spectral calibration involving peak shape has been developed to be used beyond the conventional MS instrument calibration.[3] Fortunately for GC/MS users such a novel calibration is greatly facilitated by the readily available on-board tuning compound, perfluorotributylamine (PFTBA), which can be software-controlled through a valve on almost all commercially available GC/MS systems. Furthermore, with Electron Impact (EI) typically available on GC/MS systems, a molecular ion in many cases is fragmented into quite a few observable fragment ions which can also be measured with high mass accuracy through the same comprehensive MS calibration, providing additional structural information for unknown identification, all through a single MS experiment without a tandem MS.[4]

While this comprehensive MS calibration does allow for monoisotopic masses to be determined to within a few mDa typically, the mass accuracy, when reported in ppm mass error, is still too large for conventional formula determination. For example, at 7mDa standard mass error for an ion at 350Da, the mass accuracy would be 20ppm, resulting in too many possible formulas. Fortunately for quadrupole mass spectrometers very high Spectral Accuracy of 99% or better could be achieved[3] due to the wide linear dynamic range available on these systems. The available high Spectral Accuracy, combined with the moderately high mass accuracy, has been shown to enable formula determination

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on real chromatographic time scale on these otherwise conventional systems.

This short paper will demonstrate how high Spectral Accuracy combined with sufficient mass accuracy can be utilized to determine unknown elemental compositions for forensic chemistry using an Agilent GC/MSD system, a system found to be remarkably precise and accurate, based on extensive prior studies such as the one reported elsewhere.[3]

Experimental

Sample information: Calibration standard PFTBA available as tune compound from the MSD instrument and a prepared sample containing an unknown peak at RT=8.7min.

MS conditions: the PFTBA and standard were acquired in "raw scan" mode (non-peak detected) at a scan speed 2^2 (A/D samples = 4) over a mass range of 40-550 *m/z* with ion threshold set to zero so as to acquire full continuum raw mass spectral data. As is typical for MSD, the MS source is heated to 230ºC and the quadrupole is heated to 150ºC, conditions shown to provide high calibration stability lasting as long as a week without requiring recalibration, based on a previous study on the system.[3] A detailed data acquisition setup information can be found in a companion document.[5]

Unknown GC Separation and Internal PFTBA Calibration: After the 12min GC run, during which the unknown compound elutes at RT=8.7min, the EI Calibration Valve was programmed to be On at RT=12min and Off at RT=13.5min while the EMV Delta was set to -400V for the PFTBA data acquisition so as to avoid calibration ion signal saturation.

Data acquisition and analysis: The profile mode mass spectral scans were repeatedly collected for a total GC runtime of 12min. Similarly, the profile mode mass spectra of the PFTBA calibration standard were acquired immediately after the 12min GC runtime while the control valve was at ON position and GC oven was programmed to Hold. Figure 1 shows both the TIC (top) and the averaged PFTBA mass spectrum (bottom) for the given Rt window marked up in the TIC. A comprehensive mass spectral calibration can be created from the average of the PFTBA mass spectral scans within this time window using the MassWorks™ software from Cerno Bioscience and all available PFTBA (fragment) ions of sufficient abundances to cover the mass range of interest (40-550Da). Figure 2 shows the general flow of the data processing. The unique MassWorks calibration function calibrates both the mass (m/z) and the mass spectral peak shape function, the key for achieving high mass accuracy and, most importantly, high Spectral Accuracy. This calibration was then applied to each scans during the earlier GC part of the same run to transform each raw mass spectrum into its calibrated version with a mathematically defined symmetric peak shape located at accurate mass positions. Peak detection can then be applied to reliably and accurately calculate the monoisotopic mass locations for molecular ions/their fragment ions, from which a (typically long) list of candidate formulas is generated. From this list, the most probable one is sorted and ranked on the top according to Spectral Accuracy. Spectral Accuracy is a congruence measure between the calibrated and the theoretically calculated mass spectrum, all in vector form, for a given formula candidate. This refinement process based on Spectral Accuracy is called CLIPS (Calibrated Lineshape Isotope Profile Search).[6]

Results

Ten ions including the molecular ion of PFTBA are selected for the comprehensive MassWorks calibration. Their elemental compositions and theoretically calculated exact masses are listed in Figure 3 as part of the MassWorks calibration report. The average of scans 1372-1446 (RT=12.08-12.51min) is used to build the calibration, which transforms a raw mass spectrum into a fully calibration mass spectrum, both of which are shown in Figure 3 as overlays for one of the calibration ions, C5F10N+ (m/z 263.9871). Once a mass spectral scan has been fully calibrated to have a known peak shape, the mass spectral peaks can be accurately determined even at unit mass resolution. Figure 3 (bottom)

A Spectral Accuracy of better than 99.0% indicates that the signal-to-error ratio for all calibration ions is better than 100:1, i.e., there are no significant signal saturation or significant contaminants and the signal-to-noise ratio is higher than 100:1 for all calibration ions.

For the unknown peak eluted at RT = 8.7min, the averaged mass spectrum after applying the above calibration is shown in Figure 4 for its two largest ions/fragments. Since it's not known whether any of these two ions is the molecular ion, a universal CLIPS formula ID parameters with both even and odd electrons are used (Figure 5). A CLIPS formula determination on the 240Da ion resulted in 147 possible formulas, out of which C4H8N4O4S2+ is the top candidate with 98.7% Spectral Accuracy. This ion formula provides a nearly perfect match between the calibrated and the theoretical mass spectrum (Figure 4). If higher than 99% Spectral Accuracy is desired in order to better differentiate the correct formula candidate from the others on the list, a shorter mass spectral scan range could be used to increase the available signal to noise and therefore the Spectral Accuracy on this ion. At the conclusion of this blind test, it was confirmed that C4H8N4O4S2 is indeed the correct molecular formula of a highly toxic rodenticide, tetramethylenedisulfotetramine (TETS, DSTA, also called tetramine).

Tetramine is an odorless, tasteless, white, crystalline powder. This toxic material was reported to be responsible for many poisoning incidents in the past, in spite of the international ban on its use since 1984.[7] While GC/MS is an easy-to-use method for organic compound identification, it often requires comparing test samples with authentic standards from a MS library. Since the GC/MS spectrum of this compound is currently not in the NIST library, an independent identification is required and accomplished here through both mass accuracy and Spectral Accuracy on this GC/MSD system.

The same approach can be applied to the identification and elucidation of fragment ions. For example, the 211Da fragment ion for tetramine can be identified using the same CLIPS parameters shown in Figure 5, resulting in a list of 102 possible formulas, out of which C3H6N3O4S2+ is the top hit with a high Spectral Accuracy of 98.8% (Figure 6). It should be mentioned that although C3H8N4O3S2 and C2H8N6O2S2 also appear near the top of the list as #2 and #3 hits, they are not likely valid candidates for a sulfur containing molecule where each sulfur atom usually requires 2 oxygen atoms.

Conclusion

This application example demonstrates that the comprehensive mass spectral calibration involving both mass and peak shape enables formula determination on the GC/MSD system. The MassWorks calibration can be conveniently established using the on-board PFTBA tune standard through infusion measurement within the same GC/MS data acquisition. While the mass accuracy achievable on the system may not be the industry standard 5ppm for all ions at different m/z values, the combination of mass accuracy and Spectral Accuracy can indeed achieve formula determination on a real chromatographic time scale. Not only can this formula ID capability be applied to unknown identifications where there is no available library spectrum to compare with through conventional library search, it can also be utilized as an independent confirmation of library search results through formula ID of either the molecular ion or its fragment ions.

Acknowledgement

The blind test data used in this report was generated during a demonstration of this product at an accredited forensic laboratory. Prof. David Sparkman from the University of the Pacific and Dr. Ming Gu from Cerno Bioscience helped with the elucidation and confirmation of ion fragments

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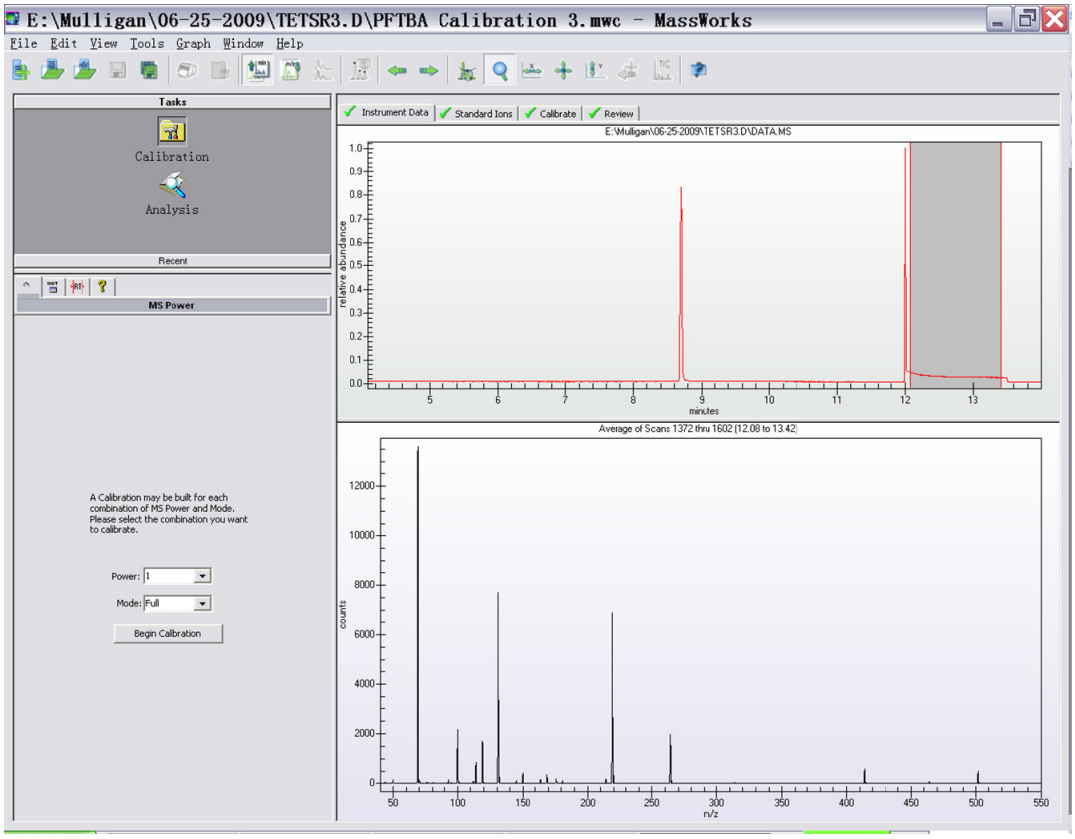

Figure 1. TIC (top) and the average PFTBA calibration spectrum from a single GC/MS acquisition (bottom).

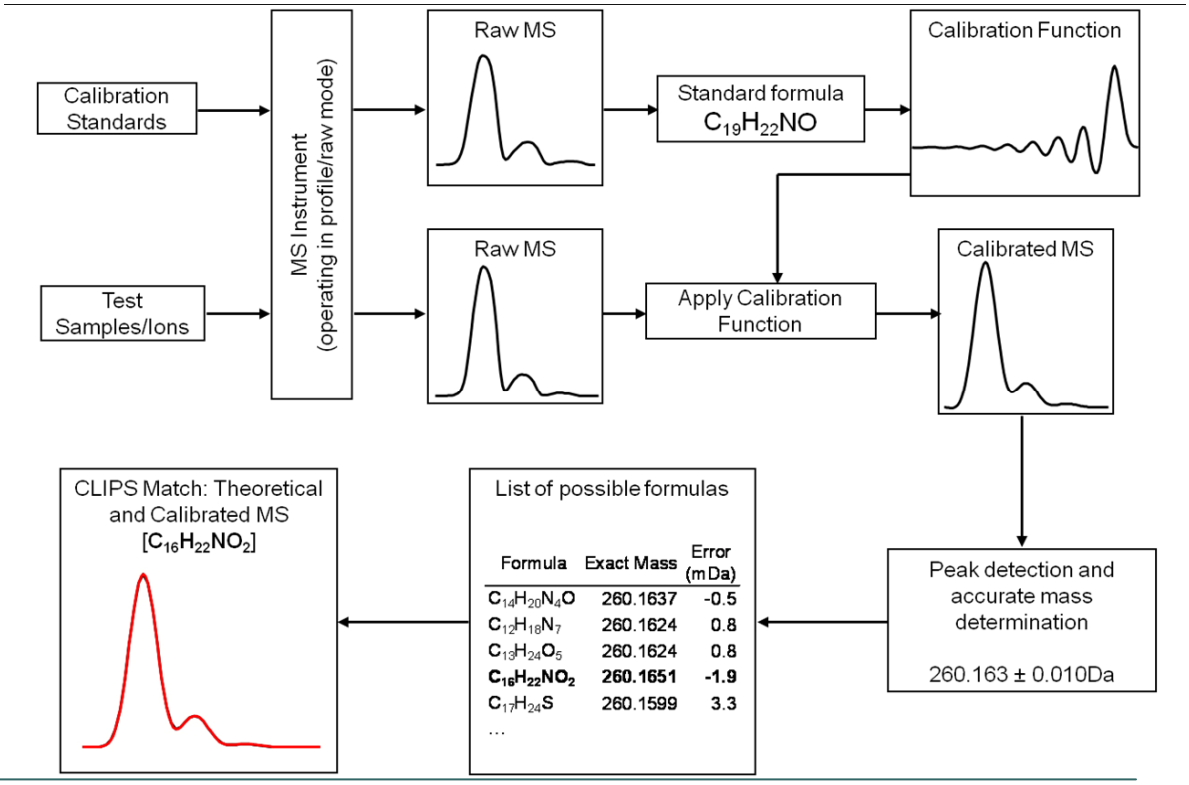

Figure 2. The general flow of MassWorks calibration and its elemental composition determination process.

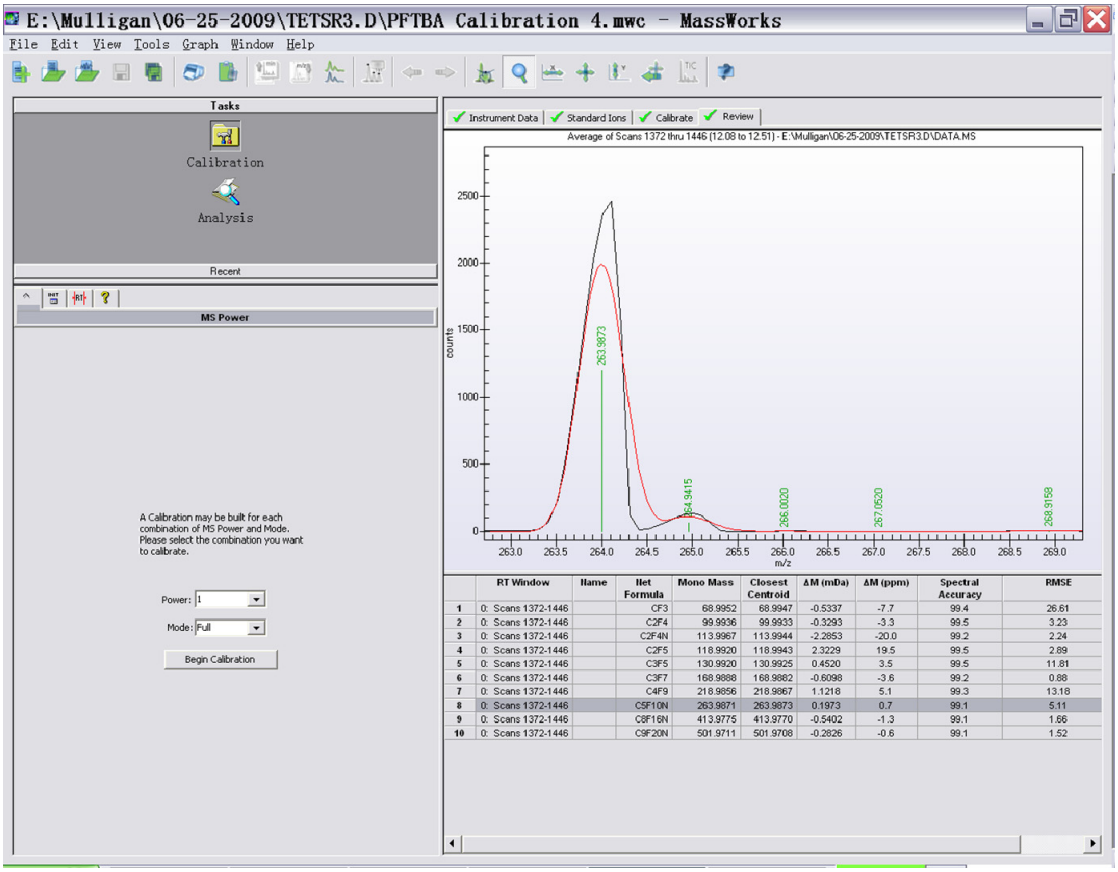

Figure 3. The raw (black) and the calibrated (red) mass spectrum for the PFTBA calibration fragment ion $C_5F_{10}N^+$ (exact mass 263.9871Da).

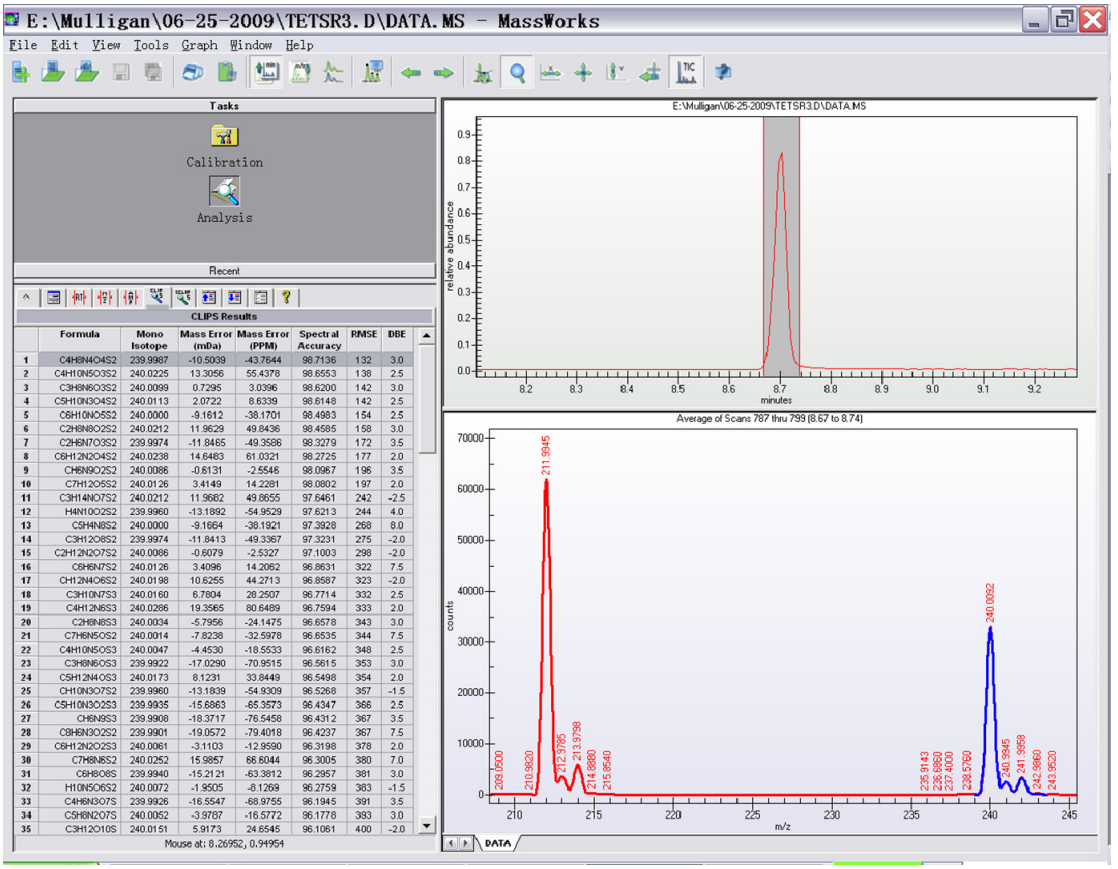

Figure 4. Averaged MS (Red) for the two large ions/ fragments from the unknown at RT=8.7min and the theoretical MS for C4H8N4O4S2 (Blue).

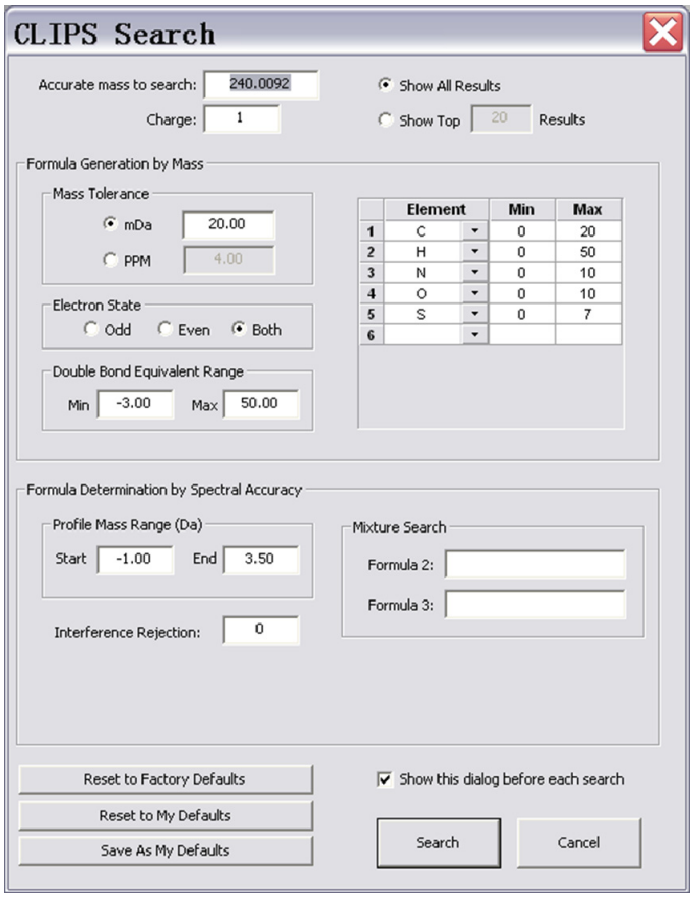

Figure 5. MassWorks CLIPS formula ID parameters.

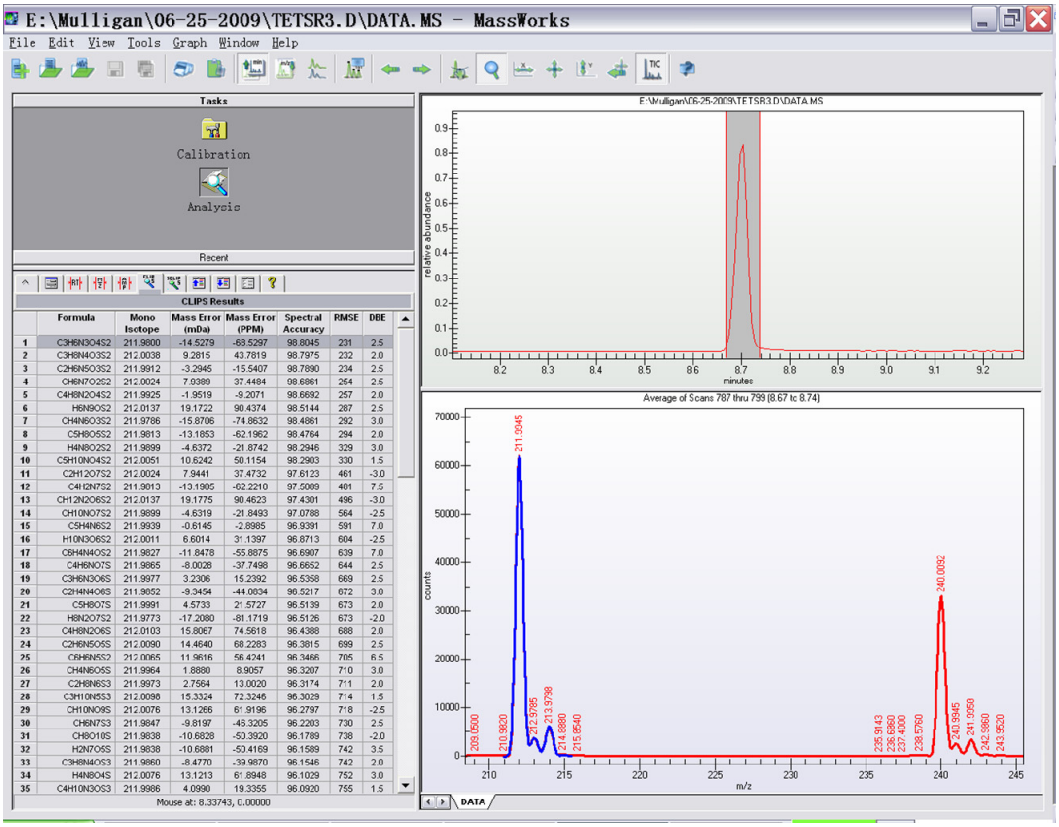

Figure 6. Averaged MS (Red) for the two large ions/ fragments from the unknown at RT=8.7min and the theoretical MS for C3H6N3O4S2 (Blue).