Cerno Application Note

Extending the Limits of Mass Spectrometry

Elemental Composition Determination on a Single Quadrupole LC/MS System

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Single Quad instruments are the workhorses of the lab but fall short for applications that require compound ID. It is shown here that with proper calibration techniques, LC/MS Single Quad instruments can become powerful tools for unknown compound identification by using high mass accuracy in combination with a uniquely accurate approach to matching the compound isotope profile.

Introduction

With its reliability, cost advantage, ease-of-use, versatility in terms of the types of compounds, high sensitivity, and even portability or at least transportability, single quadrupole mass spectrometers have found wide applications from pharmaceutical research to industrial applications. In qualitative MS applications where the objective is to perform compound identification or mass confirmation, being able to measure mass (or m/z) with a high degree of mass accuracy is highly desirable, as it allows for nearly unique determination of elemental composition[1,2,3]. The need for high mass accuracy has led to the recent development and success of several new generations of MS instrumentation including TOF, qTOF, FTMS, and Orbi-Trap, all through the design of higher resolution MS hardware.

On a single quadrupole MS system typically operating at unit mass resolution, the conventional wisdom is that only 0.1-0.5Da mass accuracy can be achieved, relegating it for a rough and quick check of nominal m/z values, falling far short of the elemental composition determination required of key identifications and journal publications.

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As has been shown elsewhere[4,5] , even at unit mass resolution, a high degree of mass accuracy can be achieved, making it possible for elemental composition determination. Elemental composition analysis for the purpose of compound identification is a capability typically reserved for higher resolution systems such as qTOF or FTMS at a much higher cost with larger instrument footprint. In order to achieve the necessary high mass accuracy on a conventional unit mass resolution system, a very different and elaborate mass spectral calibration has to be performed outside the commercially available instrument systems.

In GC/MS applications with EI source, the requirement for high mass accuracy is alleviated due to the availability of multiple EI fragments and a library such as the one from NIST. Previous work has however shown that significant gains can be had as well with a much higher mass accuracy to allow for the identification of truly unknown compounds not included in the library or the elucidation of unknown ion fragments[6].

Using a real example from a single quadrupole LC/MS system, this application note will demonstrate that it is feasible to determine the elemental composition of an unknown compound without tandem MS ca-

pability based on a single observable ion (without the need for any other fragment ions) in the absence of a library, making it feasible now to identify truly unknown compounds on a routine basis on a conventional MS system.

Experimental

Sample information: Two commercially available compounds with nominal m/z at 260 and 280Da are obtained and dissolved into 1:1(v/v) water-methanol mixture at 2.5 uM. The 280Da ion with a known elemental composition of $C_{19}H_{22}NO^+$ will be used as the only internal calibration ion to determine the m/z of the 260Da ion accurately enough for elemental composition determination. This binary mixture is infused into an Agilent MSD LC/MS system for a short duration of ~1 min with a syringe pump at a flow rate of 1 ml/min.

MS conditions: Repeated MS scans were acquired in Scan mode over a mass range of 200-500 *m/z* with peak width setting at 0.05 min. The Agilent 1100 LC/MSD (G1946D) system was operated with an APCI source and ChemStation Rev. A 09.03 [1417] software. The ion counting threshold was set at zero with either 0.1 or 0.05Da as step size to allow for continuum profile mode data acquisition required of this analysis.

Data acquisition and analysis: Figure 1 shows the overall flow of data acquisition and analysis. The profile mode mass spectra of the binary mixture were acquired continuously for 1 min during the infusion process with a total of 62 scans. An elaborate mass spectral calibration can be created from the average of the mass spectral scans within a given time window between 0.2 and 0.9min. This calibration can be performed using the whole isotope envelope of the calibration ion $C_{19}H_{22}NO^+$ near 280Da with the calibration wizard in the MassWorks™ software from Cerno Bioscience. This unique calibration process calibrates both the mass and the mass spectral peak shape function, the key for achieving high mass accuracy. This calibration was then applied to each full MS scan to transform each raw mass spectrum into its calibrated version with a mathematically defined symmetric peak shape located at accurate mass values. Peak detection can then be applied to reliably and accurately calculate the m/z location of the 260Da ion for the purpose of elemental composition determination. The highly accurate m/z value thus obtained for the 260Da ion can now be used to find a limited number of formulas within a small mass tolerance window, e.g., ±5mDa. Not only does MassWorks report an accurate mass for the 260Da ion, it also provides a calibrated isotope profile or envelope with a known mathematical line-shape function as part of the comprehensive calibration performed. This list of possible formulas can be further refined and greatly shortened through the Calibrated Line-shape Isotope Profile Search or CLIPS™, also available in MassWorks[7], which utilizes whole isotope profile to determine an elemental composition, a highly selective capability made uniquely possible by the comprehensive MS calibration performed.

Results

For the calibration ion show in Figure 2, the raw mass spectral response (black) has a peak shape function of no particular given form and is typically nonsymmetrical, regardless of how careful the mass spectrometer has been tuned, making peak detection and accurate monoisotopic mass determination difficult, if not impossible. With MassWorks calibration function shown in the top right corner of Figure 1, this raw form of mass spectral data can be transformed into its calibrated version (red, in Figure 2), which now has symmetrical and mathematically definable peak shape, allowing for easy peak detection and accurate mass calculation. As a check on the calibration process itself, the mass error after calibration is at 0.2mDa or 0.6ppm. A true test of calibration mass accuracy would be to analyze the 260Da ion which is not used as one of the calibration ions.

In order to assess the suitability of this approach for applications at real chromatographic time scale, only 8 scans from scan #15 to 22 (lasting less than 7 seconds) are selected for accurate mass analysis, as shown in Figure 3. The same peak shape transformation and accurate mass determination have been performed, giving accurate mass readings of 260.1635Da for the M ion, 261.1657Da for the M+1 ion, and 262.1862Da for the M+2 ion.

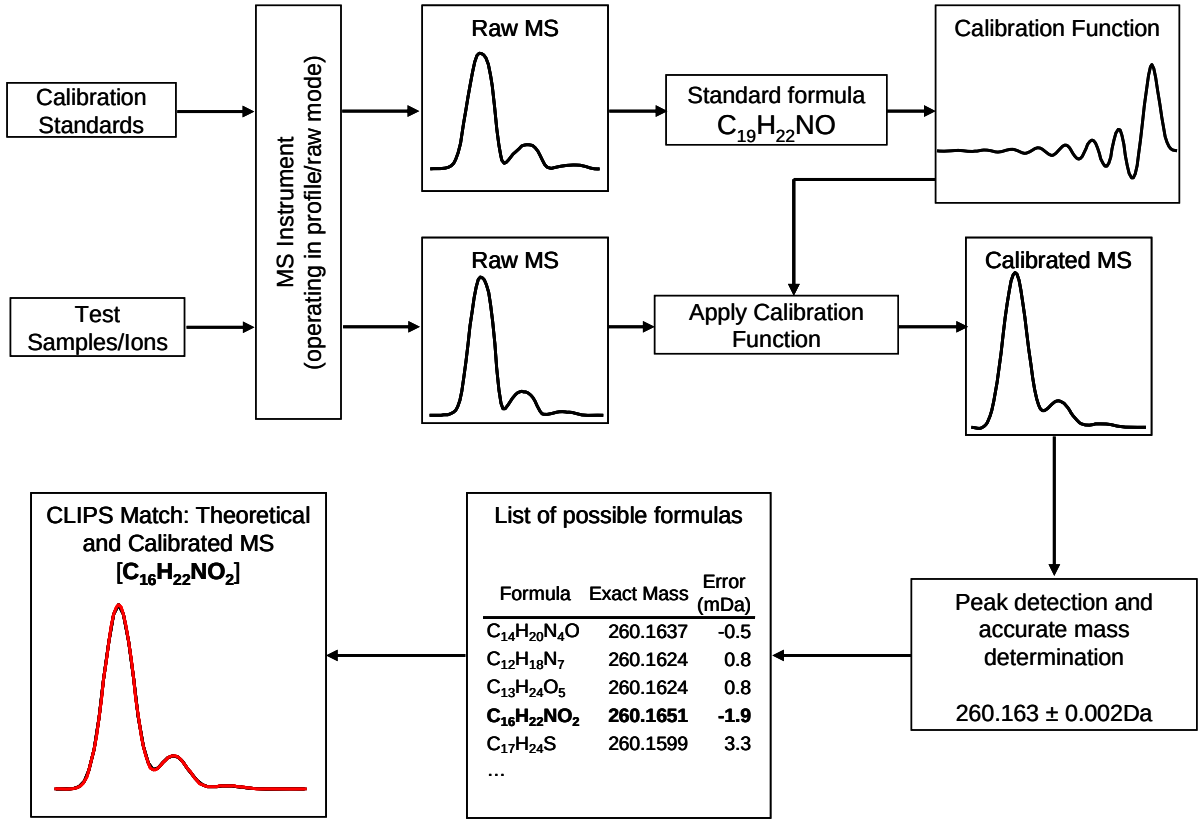

Figure 1. The general flow of MassWorks calibration and its elemental composition determination process.

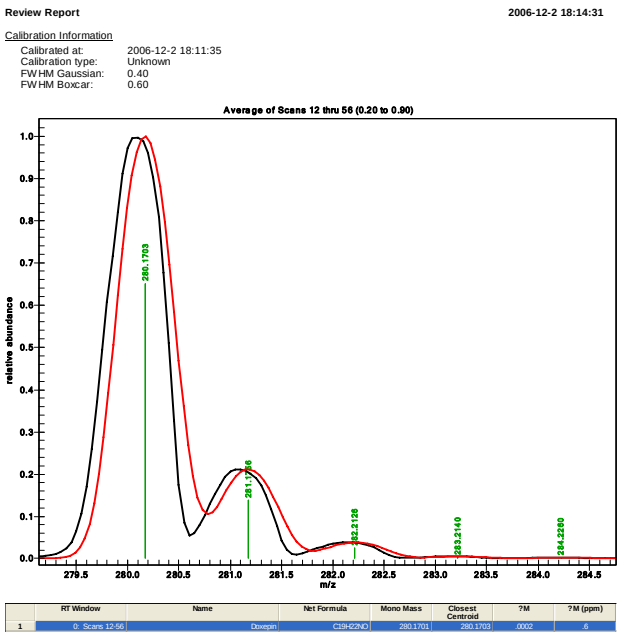

Figure 2. The raw (black) and the calibrated (red) mass spectrum for the calibration ion $C_{19}H_{22}NO^+$ (Accurate mass 280.1703 vs exact 280.1701Da).

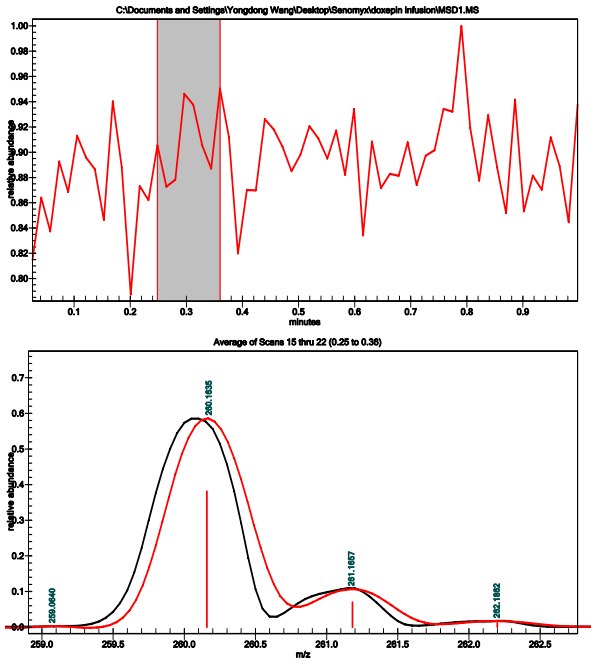

Figure 3. The raw and the calibrated mass spectrum for the unknown ion with M, M+1, and M+2 peaks detected and labeled.

With the mass accurately determined, an elemental composition search can be performed in the same fashion as has been done with accurate mass from high resolution systems such as TOF, qTOF, FTMS, or Orbitrap, including common elements C, H, N, O, and S as possible elements and generic upper bounds as shown in Table 1. With a tight mass tolerance of ±5mDa, five possible formulas are found as shown in Table 2. The correct formula ranks as the 4th hit on the list with -1.9mDa or -7.3ppm mass error. If the elemental composition is determined based on accurate mass measurement alone, the wrong formula $C_{14}H_{20}N_4O^+$ would have been proposed, which has the smallest mass error at -0.5mDa or -1.8ppm.

This ambiguity can be elegantly solved with the comprehensive calibration performed here. It is known that different elemental compositions not only generate different monoisotopic masses but also different isotope distributions. The difference in isotope distribution has been very hard to detect due to the typically unknown mass spectral peak shape functions, even for high resolution data where M, M+1, and M+2 etc are well separated. With the comprehensive calibration performed here that elaborately involves peak shape calibration as part of the calibration process, small differences in isotope distribution arising from different elemental compositions can now be assessed with a unique level of accuracy through CLIPS, even at unit mass resolution where M, M+1, and M+2 etc are partially overlapped. For each formula on the list in Table 2, a theoretically expected isotope profile or envelope can be calculated that conforms to the same peak shape function into which the raw mass spectrum in Figure 3 has been calibrated. A quantitative match can then be performed between the calibration raw mass spectrum and each of the calculated isotope envelopes, resulting in a residual measure reflecting the goodness of fit between the calibrated mass spectrum and the theoretically calculated isotope envelopes. Table 3 shows the same list of formulas after sorting by this residual measure. It is now clear that the correct formula has the smallest residual at 0.29% while all other formulas have residuals at least 1.7 times higher than that, demonstrating the high differentiating power of CLIPS match. Figure 4 shows the calibrated mass spectrum overlaid with the isotope envelope theoretically calculated for the correct formula $C_{16}H_{22}NO_2^+$.

Element	From	To
C	0	50
H	0	100
N	0	8
O	0	8
S	0	2

Table 1. Elemental composition search parameters.

Formula	Exact Mass (Da)	Mass Error (mDa)	Mass Error (ppm)
$C_{14}H_{20}N_4O$	260.1637	-0.5	-1.9
$C_{12}H_{18}N_7$	260.1624	0.8	3.1
$C_{13}H_{24}O_5$	260.1624	0.8	3.1
$C_{16}H_{22}NO_2$	**260.1651**	**-1.9**	**-7.3**
$C_{17}H_{24}S$	260.1599	3.3	12.7

Table 2. List of possible formulas with exact masses between 260.1585 and 260.1685Da.

Formula	Exact Mass (Da)	Mass Error (mDa)	Mass Error (ppm)	Residual (%)
$C_{16}H_{22}NO_2$	**260.1651**	**-1.9**	**-7.3**	**0.29**
$C_{14}H_{20}N_4O$	260.1637	-0.5	-1.9	0.52
$C_{12}H_{18}N_7$	260.1624	0.8	3.1	0.85
$C_{17}H_{24}S$	260.1599	3.3	12.7	1.01
$C_{13}H_{24}O_5$	260.1624	0.8	3.1	1.21

Table 3. List of formulas according to the CLIPS residual.

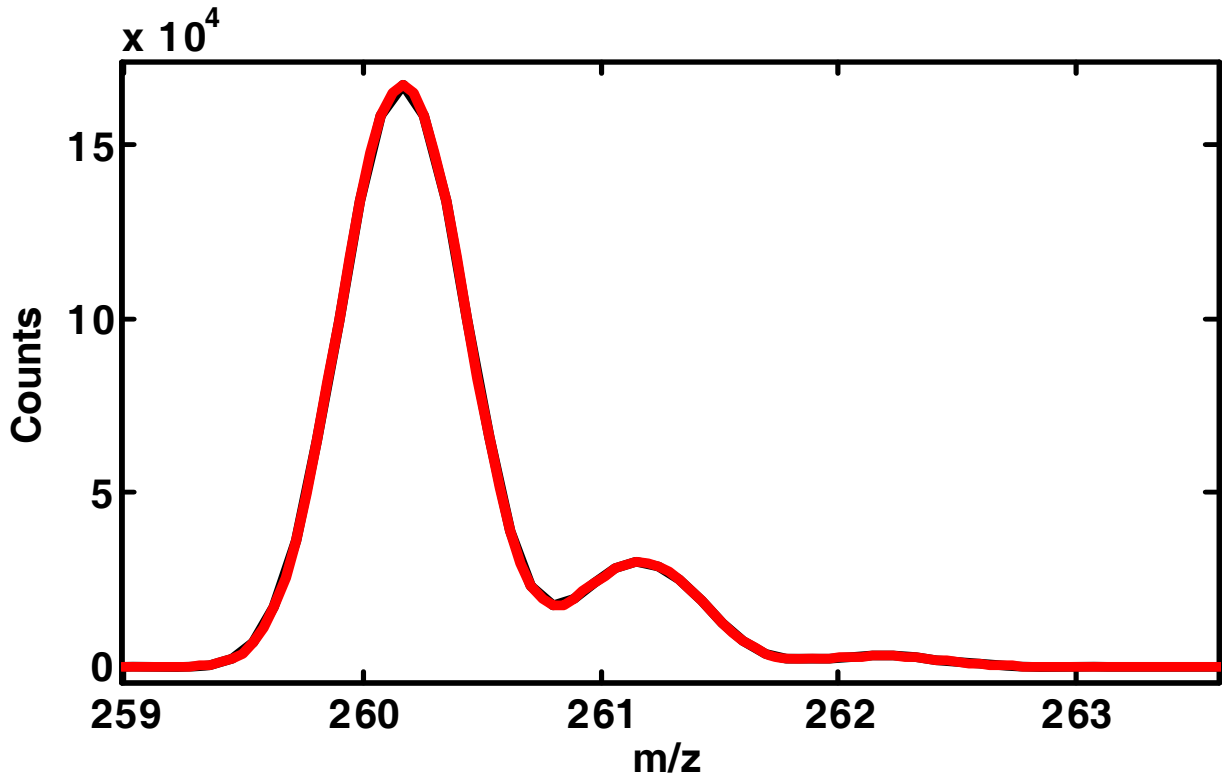

Figure 4. CLIPS Match between the calibrated (red) and theoretically calculated (black) isotope profile for $C_{16}H_{22}NO_2^+$.

Conclusion

On a single quadrupole LC/MS system, typically one and only one ion is observed for any given compound, it is therefore critical to achieve high mass accuracy to facilitate the compound identification. The simple experiment analyzed here demonstrated that it is feasible to achieve 100 times more mass accuracy through the use of an elaborate and comprehensive calibration approach involving both mass axis and peak shape. Even with mass accuracy approaching 5ppm on a real chromatographic time scale, mass accuracy alone could not uniquely determine the elemental composition of an unknown ion. With CLIPS taking advantage of both the mass accuracy and the calibrated peak shape function, however, unique elemental composition determination can be achieved on a unit mass resolution system with a single internal standard located 20Da away in mass. With its lower cost and ease of use, this new approach should open more doors for single quadrupole LC/MS systems, e.g., open access high mass accuracy measurement for organic synthesis support, among others.

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