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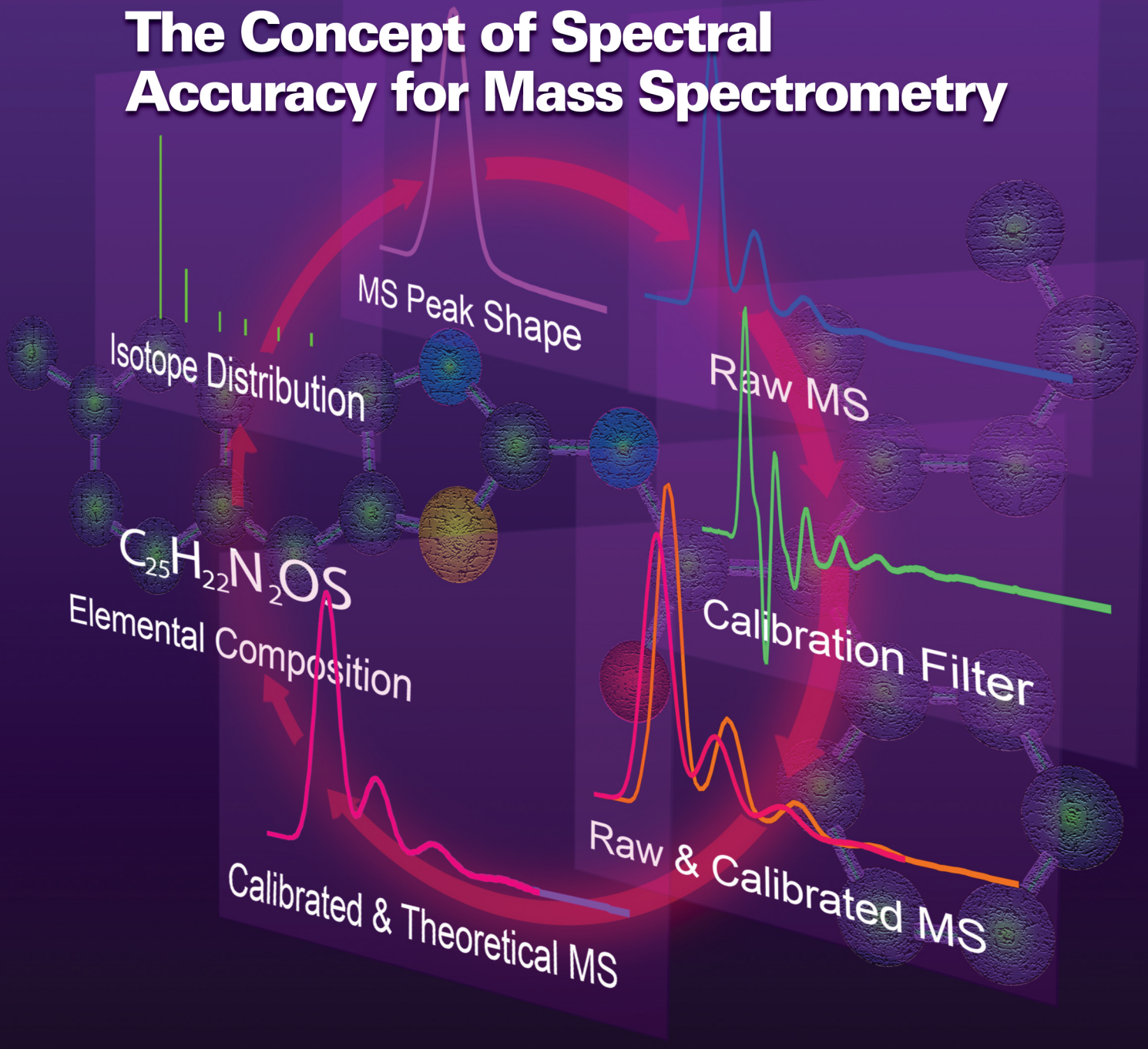

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The Concept of Spectral Accuracy for MS

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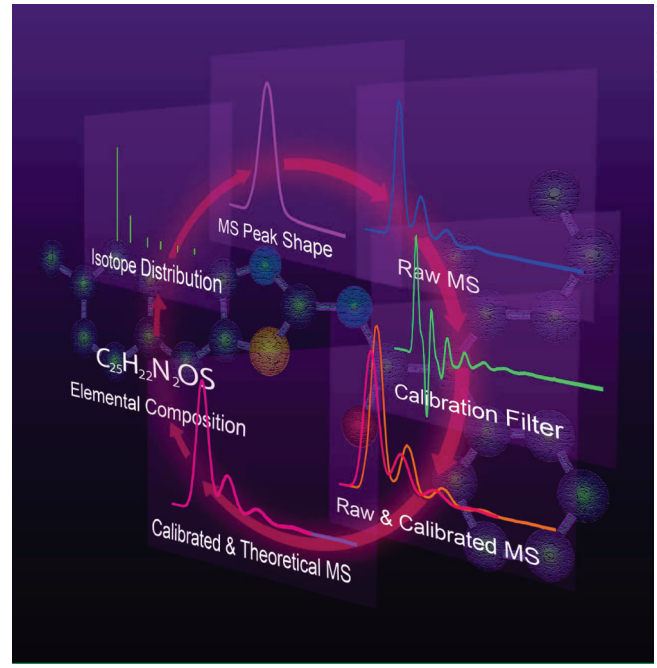
Though MS has always appeared to be quite different from spectroscopy to analytical chemists who are familiar with both, a careful examination from historical and theoretical perspectives reveals a striking similarity between the two. With the introduction of spectral accuracy, a companion concept to the better known mass accuracy, new capabilities previously thought unfeasible can now be enabled for MS. (To listen to a podcast about this article, please go to the *Analytical Chemistry* multimedia page at pubs.acs.org/page/ancham/audio/index.html.)



ROBERT GATES

MS is typically considered quite unique when compared to other analytical measurements such as UV/vis or FTIR spectroscopy. Besides using a different set of terminologies,[1] interesting ion chemistries,[2] and various ion experiments readily available inside an MS instrument,[3−6] at least three attributes distinguish an MS measurement:

First, for any given analyte ion, the theoretical MS response can be accurately calculated from its elemental composition and charge, *z*, using the relative isotope abundances of all elements involved. The actual algorithm for the calculation ranges from the more straightforward multinomial expansions[7] to a computationally more efficient approach involving FT.[8,9] At this point, MS is perhaps the only analytical measurement for which the theoretical response of an analyte can be so accurately calculated based solely on first principles. This theoretical response, called theoretical isotope distribution, is in the form of relative ion abundances and is a discrete function of the mass-to-charge (*m*/*z*) values (measured in the unit of Dalton, or Da)—that is, the non-zero ion abundances occur only at limited *m*/*z* values corresponding to specific combinations of elemental isotopes. Figure 1A shows the theoretical isotope distribution for $C_{25}H_{23}N_2OS^+$.

Second, even the most routine mass spectrometer, such as a single quadrupole with unit mass resolution (Full Width at Half Maximum, or FWHM, of 0.5−0.7 Da, typically) is able to resolve major isotope clusters, such as those dominated by ^{12}C and ^{13}C from a carbon-based organic compound, which are called A and A+1[1] and shown in Figure 1B for $C_{25}H_{23}N_2OS^+$. Observing such isotopic details with FTIR would require special experimental conditions such as a low pressure gas phase experiment and a higher-than-normal (∼1−4 cm^{-1}) interferometer and optical resolution for very simple compounds such as CH_4.[10]

Finally, in the early days of MS instrument development, the amount of data and the data collection rate from a mass spectrometer easily overwhelmed the state-of-art data system and data storage capability at the time. Consequently, early mass spectrometers were designed to reduce the acquired raw MS data (Figure 1B) into stick spectrum, or centroid, data (Figure 1C) in a process known as centroiding. This practice is carried over to modern mass spectrometers, even though data communication rate and storage capacity are no longer barriers on most systems. Furthermore, centroiding typically occurs on the fly as raw data are acquired and discarded subsequent to centroiding, leaving only the centroid data for most applications.

These factors lead most MS practitioners to think of mass spectral data as a collection of discrete sticks with non-zero intensities at limited *m*/*z* locations and zeros everywhere else, as

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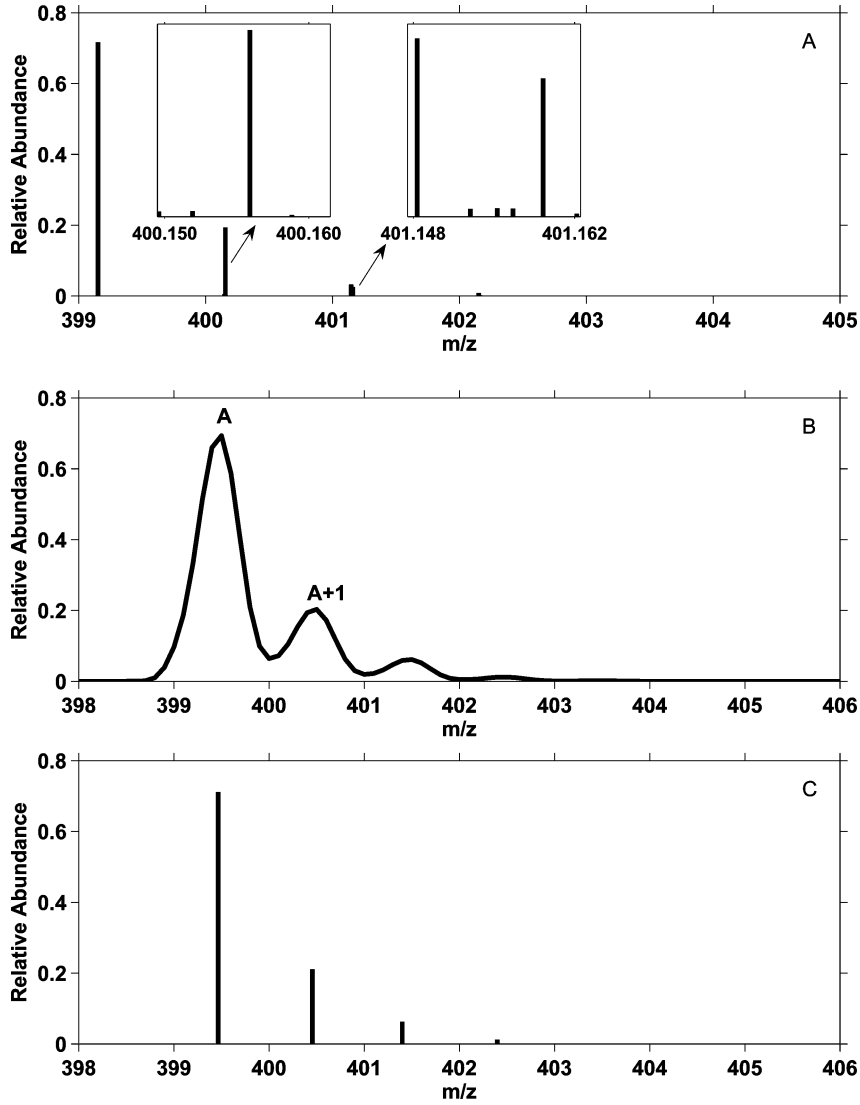

Figure 1. (A) Calculated theoretical isotope distribution, (B) measured profile mode MS response at unit mass resolution, and (C) measured MS data after centroiding for $C_{25}H_{23}N_2OS^+$.

if theoretical isotope distributions were being measured. Therefore MS data is typically presented as stick spectrum in a publication including MS (e.g., see ref. 11). Though having the clear advantage of significantly smaller (10−100×) data file sizes, centroid MS data are obtained at the expense of significant information loss, including noise characteristics, linearity of the ion signal, mass spectrally interfering ions, and isotope fine features (comparing Figure 1C to 1A, for example). Because of the discrete nature of centroid data, the associated information loss and nonlinearity, and the mass positioning error, MS centroid data are not easily amenable to a host of chemometrics methods commonly used in molecular spectroscopy, such as differentiation, derivative analysis, or multivariate regression[12] for either qualitative identification or quantitative analysis.[13]

MASS ACCURACY AND ELEMENTAL COMPOSITION DETERMINATION

In data for a typical small molecule organic compound such as that shown in Figure 1B, the first MS peak is called the monoisotopic peak because it comes from a straight combination of the lightest isotopes of all elements and is a mathematically pure peak. It originates from a single stick in the theoretical isotope distribution, e.g., the first stick in Figure 1A. Unlike the monoisotopic peak, the A+1 cluster can come from more than one combination of elemental isotopes. In the example given in Figure 1B, the A+1 cluster includes, in addition to other possible combinations, one ^{13}C and the lightest isotopes for all other elements and one ^{15}N and the lightest isotopes for the rest of the elements. For this reason, the information loss and error associated with MS data centroiding have the least impact on the monoisotopic peak of a given ion, especially for higher resolution mass spectrometers in which there is baseline separation between A and the A+1 cluster. Centroiding this type of data does not lead to typical complications arising from the mutual interferences between A and the A+1 clusters (Figure 1B).

The accurate determination of monoisotopic peak m/z has long been a goal of mass spectrometer design and development and remains as one of the key specifications for a commercial mass spectrometer. A unit mass resolution MS is conventionally

Table 1. Candidate Formulas for True Unknown Identification within 100 ppb Mass Tolerance*

Ion Formula	Exact Monoisotope Mass (Da)	Mass Error (mDa) **	Mass Error (ppb) **
$C_{25}H_{23}N_2OS^+$	399.152561	0.000	0
$C_4H_{19}N_{16}OF_2PNa^+$	399.152563	0.002	5
$C_{13}H_{26}N_6O_2SNa_3^+$	399.152554	–0.007	–18
$C_{20}H_{27}N_2FP_2Na^+$	399.152571	0.010	26
$C_{11}H_{27}N_2O_8F_2Na_2^+$	399.152538	–0.023	–58
$C_9H_{33}N_6O_3S_2P_2^+$	399.152530	–0.031	–78
$C_{10}H_{27}N_{10}OS_3^+$	399.152594	0.033	83

* Two formulas in the shaded rows contain more than one Na and may be eliminated. ** All mass errors are calculated relative to the first ion formula, $C_{25}H_{23}N_2OS^+$.

specified to have ±~0.2−0.5 Da mass error due to the complications and errors associated with the centroiding process under limited mass spectral resolution. For higher resolution systems such as TOFMS and FTMS, there has been a very strong (if not exclusive) focus on improving the mass accuracy, expressed in the form of parts per million (ppm)[14] mass measurement error e_m on the monoisotopic peak, given by:[15,16]

$$e_m = \frac{m_a - m_t}{m_t} \times 10^6 \propto \frac{1}{R\sqrt{S}}$$

where m_a is a measured accurate mass and m_t is the exact or theoretical mass (all m/z values in Da), R is the mass spectral resolving power defined as the ratio between a given m/z value and the corresponding MS peak FWHM, and S is the ion signal level (assuming only ion counting noise). From the above equation, it is easy to see why higher resolving power R has been pursued to obtain proportionally higher mass accuracy while maintaining the same ion signal level S. This is a classical challenge for the design of analytical instruments, including MS. Through multiple replicate measurements or other statistical estimation, a standard error s for the mass error e_m can be established and used as a better measurement of mass accuracy.

As one may expect, high mass accuracy measurement has important practical applications for qualitative analysis, including, for example, organic synthesis confirmation, pharmaceutical impurity identification, drug metabolism research, natural product chemistry research, food safety, and contaminant analysis. Assuming normal distribution for the mass measurement error e_m, with a given accurate mass measurement m_a and mass standard error s, a limited list of possible elemental compositions (formulas) could be found to satisfy the following equation:

$$\left| m_a - \left(\frac{1}{z} \sum_{i=1}^{k} n_i m_i \pm m_e \right) \right| \leq \varepsilon$$

where n_i is the number of elements for the i-th chemical element whose lightest isotope mass is m_i, k is the number of element types to be considered, m_e (0.000549 Da) is the static mass of an electron (with + for negative ion and − for positive ion) and ε is the mass tolerance. Other chemistry constraints such as the nitrogen rule and ring/double bond equivalence[17−20] may also be applied to further limit the possible formulas. Note that the mass tolerance ε, mass measurement error e_m, and the standard error s are different both conceptually and numerically, even though all three numbers are typically reported and measured in ppm. Depending on the exact statistical distribution of mass error e_m, the mass tolerance ε typically needs to be set at a few (e.g., three) times the standard error s to correspond to a certain statistical confidence interval.[21]

It was reported in the early days of accurate mass measurement that a mass accuracy corresponding to 5 ppm mass error would be accurate enough for high confidence formula confirmation/determination. Consequently, the *Journal of Organic Chemistry* requires this same level of accurate mass measurement for submitted manuscripts reporting newly synthesized organic compounds,[22] but not clear is whether 5 ppm mass accuracy refers to e_m, s, or ε. This level of mass accuracy for formula determination or compound confirmation, nonetheless, became widely accepted in academia[23,24] and turned into an industry standard for the developers of high resolution MS[25,26] as well as end users from pharmaceutical and other industries.[27] However, different opinions have also been reported.[28]

Fortunately for MS, the resolving power has been constantly improving, up to 1,000,000:1 on a few commercially available FT ion cyclotron resonance (ICR) mass spectrometers[29] and even higher within academic labs[30] for which the FWHM approaches zero. An instrument properly calibrated with sufficient care and maintenance could achieve a superb mass accuracy of better than ε = 100 parts per billion (ppb). For a monoisotopic peak at 400 Da, this means that the mass tolerance ε would be 0.04 mDa, in absolute terms, with standard mass measurement error s at a fraction (e.g., 1/3) of the mass tolerance (33 ppb or 0.013 mDa, respectively). For an example of a truly unknown ion observed under ESI (Table 1), for which all typical organic elements, including C (0-33), H (0-396), N (0-28), O (0-24), S (0-12), P (0-12), F (0-21), and Na (0−17), need to be considered, a total of

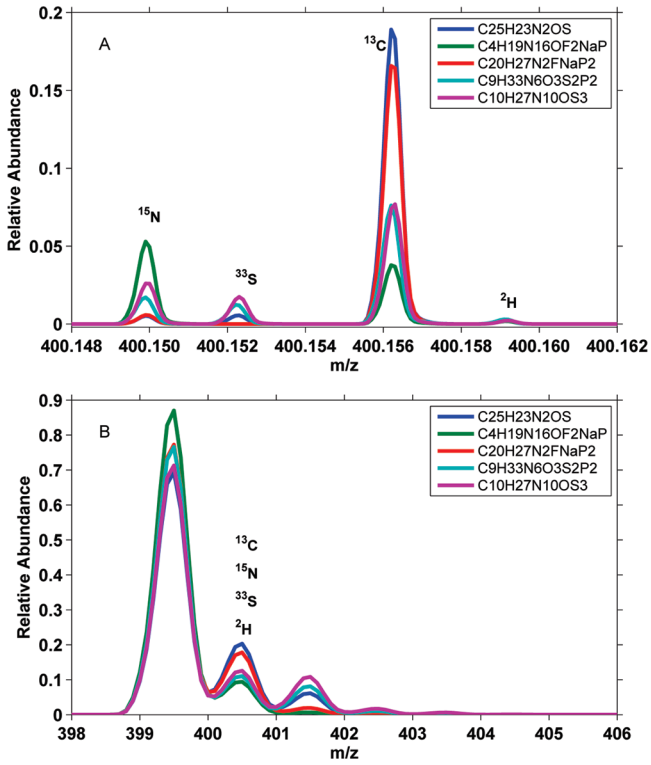

Figure 2. Computer simulated profile mode MS data for five candidate formulas: A+1 isotope cluster from (A) FTMS (R = 800,000) and (B) the complete MS response from a quadrupole system (R = 800).

seven possible formulas are found, of which two formulas contain more than one Na and can be eliminated considering the unknown ion's single charge state (though there is a risk of eliminating the correct formula). The challenging task at this point is to determine the correct formula out of the remaining five possible formula candidates.

The high mass positioning accuracy from high and ultrahigh resolution mass spectrometers is typically relevant only to the monoisotope peak of an unknown ion but does provide an efficient first order filtering to reduce the number of possible formula candidates. Other isotope clusters from the same ion, though more complex in structure and much more susceptible to complications and errors during centroiding, actually contain more relevant information about the types of elements involved in the formation of the particular ion and its elemental compositions that can help determine the correct formula from the list of candidates. In order to maintain the mass spectral integrity, however, these higher isotope clusters will have to be analyzed in the raw scan mode without data centroiding. Figure 2A shows the computer simulated A+1 isotope clusters of those five formula candidates listed in Table 1, and immediately clear is that there is more than enough spectral information from just the A+1 cluster to differentiate these five formula candidates. At such high resolving power, it is even possible to spectrally interpret each MS peak within the A+1 cluster and count the number of elements based on peak intensities. For isotope clusters higher than A+1, there would, in general, be more and more mutually overlapping isotope fine features, regardless of how high the MS resolving power, necessitating again the use of raw scan MS data for the analysis of these isotope clusters.

Because there are large spectral differences among these otherwise hard-to-differentiate formulas, an attempt at simulating their MS spectra at unit mass resolution was made, resulting in five low resolution mass spectra with all relevant isotope clusters (Figure 2B). The much reduced resolving power and the severe overlaps among the various isotopes within the same isotope cluster and between consecutive isotope clusters make the mass spectra far less interpretable than those in Figure 2A. Surprisingly, significant enough overall mass spectral differences remain for these formulas to be differentiated from each other, even at unit mass resolution! In order to analyze these low resolution MS data, however, it becomes even more important that the raw scan MS data are used for the analysis. Centroiding these raw scan data shown in Figure 2B would have created large enough errors to obliterate the critically important mass spectral differences among these formulas.

To take full advantage of the subtle mass spectral differences among the formula candidates, a measured mass spectrum must faithfully and accurately reflect all isotopes related to the ion of interest—i.e., the MS measurement has to be spectrally accurate. A recent publication[31] reviewed various available approaches to use the isotope information to help determine formula and carefully examined one popular FTMS instrument, the LTQ Orbitrap from Thermo Fisher Scientific, for its spectral accuracy at various resolving powers.

SPECTRAL ACCURACY

Contrary to the stick spectrum that typifies the majority of MS data, mass spectral signal at the time of detection is almost always a continuous signal sampled at some interval of time or other variable, such as displacement or distance, prior to its conversion into m/z. This continuous response is variably called raw scan data, profile mode data, or continuum data, depending on the particular MS instrumentation. What one actually obtains from any physical mass spectrometer with its finite resolving power, mass accuracy, and linear dynamic range is always a continuous response curve similar to the one shown in Figure 1B, which is a mathematical convolution between the discrete theoretical isotope distribution in Figure 1A and a continuous function called peak (or line) shape function.[13] The complete profile of a peak shape function provides a numerical representation of the ion dispersion or aberration in the mass spectrometer, including spatial and velocity dispersion inside the ion source.[32−34] This is not unlike the line broadening observed in optical systems after laser light enters the entrance slit of a spectrophotometer.[35]

On a TOFMS instrument, the peak shape function—sometimes with a sharp rise followed by a slower decline—is simply the arrival time distribution of a population of isotopically pure ions. This reflects the statistical distribution introduced by the TOF analyzer, which includes the energy and velocity spread inside the ion source and the collisions with the residual gas throughout the flight path. The peak shape function is a signature of the mass spectrometer and is specific to the instrument under a given set of conditions, called MS tune on commercial mass spectrometers, which includes mass analyzer geometries and field strengths such as voltages on various ion lenses. The FWHM defines the mass spectral resolution or resolving power, whereas the amount of

shift in the position of the peak shape function defines mass accuracy. Vestal and Juhasz provided a detailed mathematical treatment on resolution and mass accuracy for MALDI TOF.[36]

Because the profile mode mass spectrum has the convoluted contribution from the MS peak shape function, which is typically unknown, undefined, or unavailable to an end user and variable as a function of the MS tune and m/z, the measured profile mode mass spectrum is also undefined and variable to some degree, even with measurements from the same instrument. This uncertainty and variability make it difficult, if not impossible, to judge how spectrally accurate a mass spectral measurement is, let alone quantitatively measure spectral accuracy in a scientifically defendable way.

This uncertainty from the MS peak shape can, however, be calibrated out and removed through a novel and comprehensive calibration process that involves not just m/z, as is the case for all conventional MS calibration, but more importantly the peak shape. Because this process has been published and detailed elsewhere,[13,16,31] only a brief description will be given here. For any measured mass spectrum acquired in profile mode, $\mathbf{y}$, a vector of a certain dimension representing the number of data points sufficiently sampled across all significant isotope clusters of a given ion, can be expressed as a convolution operation (denoted as $\otimes$) between its theoretical isotope distribution $\mathbf{y}_0$ and the peak shape function $\mathbf{p}$ (also a vector of similar but generally different dimension):

$$\mathbf{y} = \mathbf{y}_0 \otimes \mathbf{p}$$

This actual peak shape function $\mathbf{p}$ can be converted into a mathematically definable function $\mathbf{d}$ (a perfect Gaussian, for example) through another convolution with a filter function $\mathbf{f}$ (which is capable of correcting for both m/z shift and peak shape distortion):

$$\mathbf{d} = \mathbf{p} \otimes \mathbf{f}$$

Combining the two equations and rearranging, we have:

$$\mathbf{y}_0 \otimes \mathbf{d} = \mathbf{y} \otimes \mathbf{f}$$

For a standard (calibration) ion with its actually measured $\mathbf{y}$, the theoretical isotope distribution $\mathbf{y}_0$ (calculated from its known elemental composition), and the mathematically specified peak shape function $\mathbf{d}$, the filter function $\mathbf{f}$ can be solved from the above equation to serve as a convolution filter to calibrate any applicable actual measurement $\mathbf{y}$ into a calibrated version $\mathbf{r}$, with its m/z calibrated and peak shape function fully defined by $\mathbf{d}$:

$$\mathbf{y} \otimes \mathbf{f} = \mathbf{r}$$

On a unit mass resolution system, this calibration is accomplished through the measurement of internal or external calibration standards whose elemental compositions are known.[13,16] On a GC/MS system, this type of calibration can be easily accomplished by using the computer controllable onboard tune gas perfluorotributylamine as the standard, as shown in a pharmaceutical drug impurity application.[37] On a higher resolution mass spectrometer for which the monoisotopic peak A is baseline-resolved from the A+1 isotope cluster, the monoisotopic peak measured of an unknown ion could be used as the actual peak shape in a self calibration process involving just the peak shape so as to keep the reasonably accurate mass-only calibration intact.[31] Figure 3 shows the key components of this comprehensive mass spectral calibration, including the calibration filter at the core of this calibration process. This calibration filter must be constructed as a linear operator so that all relative isotope information, either in the form of peak height or peak area or a continuously sampled mass spectrum with or without spectrally resolved isotope fine features, is preserved throughout the calibration process.[13]

Once a mass spectrum has been calibrated to have an accurately known and definable peak shape function, it is now possible to convolute a theoretically calculated isotope distribution for a given formula with this very same peak shape function to generate a theoretical mass spectrum (in continuum mode). Because no assumption or oftentimes arbitrary decision is made about the peak shape function to be used for either the calibrated or theoretical mass spectrum, this theoretical mass spectral vector $\mathbf{t}$ could then be accurately compared to the calibrated mass spectral vector $\mathbf{r}$ (also in the continuum mode) to measure the spectral accuracy. This is accomplished through a spectral error calculation, much like how mass accuracy is measured through calculated mass error. There are however two significant differences between mass accuracy and spectral accuracy.

First, unlike mass error, the spectral error could not be simply calculated as the difference between the calibrated and the theoretical mass spectrum because the scales for these two spectra may be quite different. While the scale for a theoretical mass spectrum can always be normalized to a consistent unit, the scale for the calibrated mass spectrum depends on quite a few experimental, hardware, and even software factors, including the concentration of the ion in the sample, the flow rate, ionization efficiency, ion transmission efficiency, electronic or software gain factors, and the number of mass spectra acquired and/or averaged. All these scaling factors, however, can be combined into one single scalar, c, through a fitting process such as least squares regression with the following linear equation:[13,21,31]

$$\mathbf{r} = c\mathbf{t} + \mathbf{e}_s$$

to arrive at the spectral error $\mathbf{e}_s$, a vector (always expressed in bold face) with the same dimension as $\mathbf{r}$ and $\mathbf{t}$. For an ideal mass spectrometer in the absence of either mass spectral interference or random noise, the theoretical mass spectrum $\mathbf{t}$ and calibrated mass spectrum $\mathbf{r}$ should be exactly the same after adjusting for the scaling factor c; i.e., the spectral error $\mathbf{e}_s$ should be identically zero. In any actual MS measurement, the spectral error $\mathbf{e}_s$ should be composed of mostly random noise arising from ion measurement statistics, i.e., the spectral error $\mathbf{e}_s$ should be at a level comparable to the ion shot noise (see for example, ref. 13). Any systematic deviation in $\mathbf{e}_s$ from this ion measurement shot noise serves as a strong indication for either hardware limitations such as limited linear dynamic range including detector saturation or space charge effect,[38] excess electronic noise, on-board or post processing errors

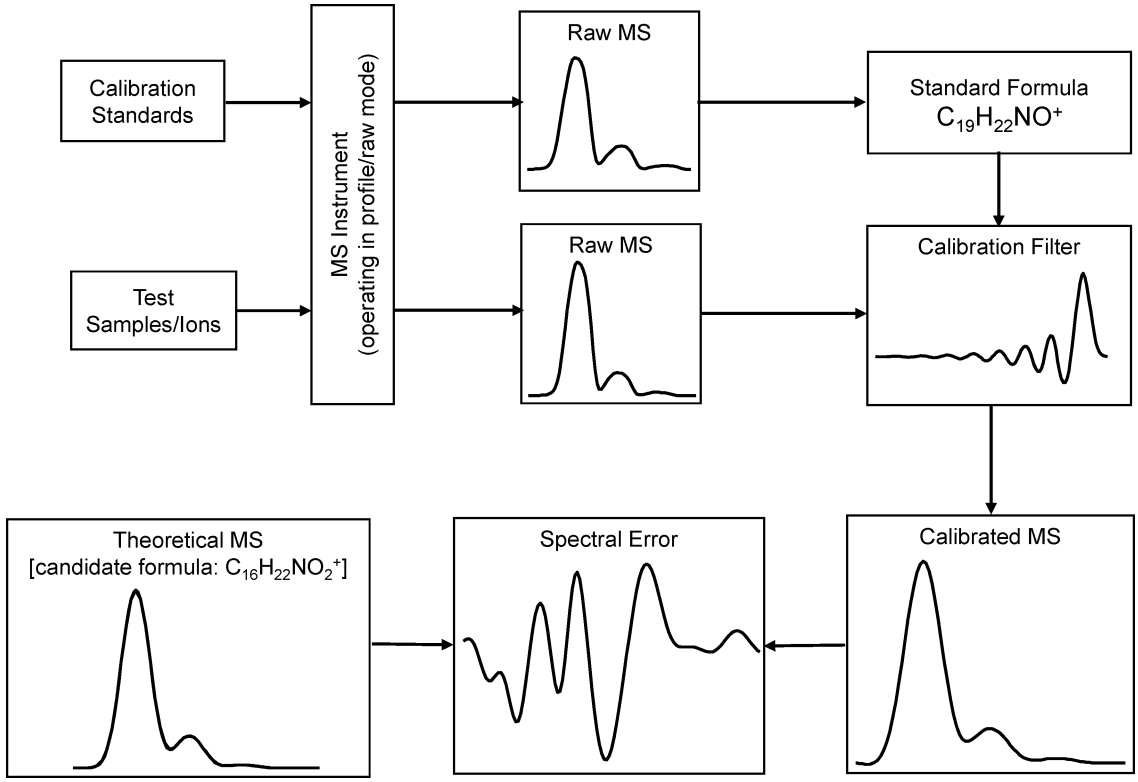

Figure 3. The comprehensive mass spectral calibration and the spectral accuracy calculation at unit mass resolution.

such as thresholding, or experimental issues such as the presence of a mass spectral interference.[31]

Second, whereas mass accuracy reflects the measurement error at a single point of a monoisotopic peak position, the spectral accuracy reflects the correctness of the complete mass spectral response of an ion in the form of continuously sampled spectral error as a function of all relevant m/z values (Figure 3). This goes far beyond the simplistic isotope ratio or peak area measurement typically used because it does not require relevant isotopes be mass spectrally resolved and individually measured. Though less informative, a single scalar based on root mean squared error can be estimated using standard statistics[21] to reflect an overall measurement of the spectral error at all m/z values:

$$e_s = \frac{||\mathbf{e}_s||_2}{||\mathbf{r}||_2}$$

where $||.||_2$ represents the square root of the sums of squares of all elements in a vector and e_s is the relative spectral error,[31] approximating the inverse of signal-to-noise ratio of an MS measurement in the absence of systematic errors such as mass spectral interferences.[13]

APPLICATION OF SPECTRAL ACCURACY

With mass spectral data calibrated for spectral accuracy according to Figure 3, the mass accuracy from a quadrupole mass spectrometer can be significantly improved by the now known peak shape function and the gain from noise filtering through the calibration filter, which can greatly simplify the centroiding process and reduce the mass determination error. A standard mass error on the order of a few millidaltons (±0.00x Da, versus the conventional ±0.2−0.5 Da) has been reported for both LC/MS[16] and GC/MS[39] applications on a real chromatographic time scale.

When tested with the 400 Da ion from Figure 2 as an example, a mass error of 6.8 mDa (17 ppm) was observed from the calibrated mass spectral data shown in Figure 4A, which now has a fully defined peak shape function, a Gaussian with FWHM = 0.600 Da. Such mass accuracy is typically considered insufficient for formula determination because there are a total of 4110 possible formulas within the mass tolerance window $\varepsilon = 20$ mDa (51 ppm) under the same most general search conditions required of a true unknown. The theoretical mass spectrum conforming to the same Gaussian peak shape function of FWHM = 0.600 Da for each of these possible formula candidates could be calculated and accurately compared to the calibrated mass spectrum of the unknown (also shown in Figure 4A) to arrive at a corresponding spectral error e_s for each. When sorted by spectral accuracy (from the lowest to highest spectral error), the correct formula $C_{25}H_{23}N_2OS^+$ is the 2nd hit out of a total 4110 formulas with a spectral error of 1.0%, reflected in the nearly perfect spectral overlay shown in Figure 4A. Worth noting is that there would have been 411 possible formula candidates within the industry standard mass tolerance of 5 ppm (2 mDa). This not only demonstrates the feasibility for unknown formula determination on a unit mass resolution quadrupole mass spectrometer—even though the fine isotope features are almost never resolved on these instruments—but also clearly shows that spectral accuracy is more important than mass accuracy at differentiating a large number of formula candidates, regardless of the mass spectral resolving power used. A recent systematic study has shown that >99% of the incorrect formula candidates could be eliminated

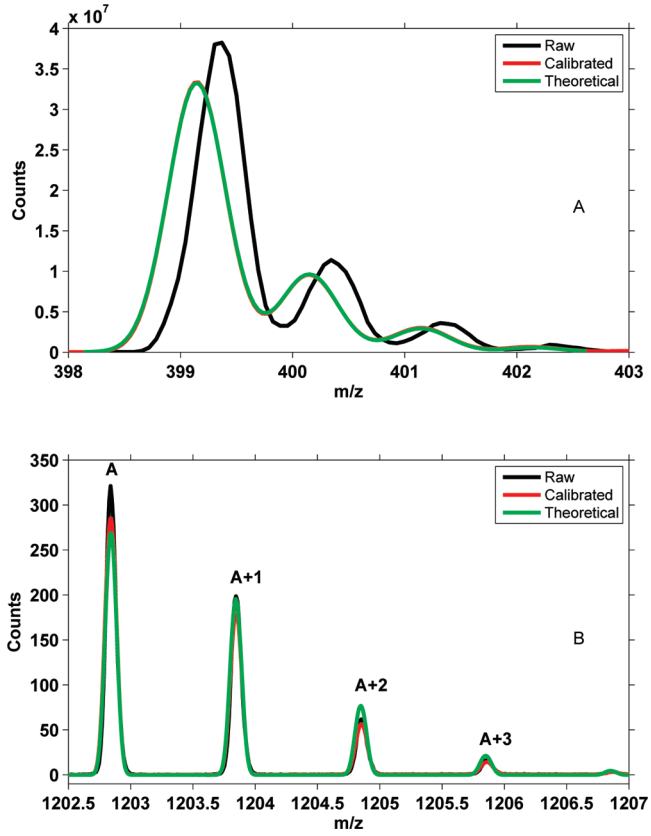

Figure 4. The raw (black), calibrated (red), and theoretical (green) mass spectrum from (A) a quadrupole mass spectrometer for an unknown ion ($C_{25}H_{23}N_2OS^+$) with 1.0% spectral error and (B) a higher resolution TOF system for a standard ion ($C_{62}H_{111}N_{11}O_{12}^+$) with 9.4% spectral error.

based on spectral accuracy from the highly mass-accurate Orbitrap FTMS system when operating at the moderate 15,000 resolving power, where high spectral accuracy can be attained.[31]

Some higher resolution TOFMS systems are sensitive to time-related variations such as ambient temperature fluctuations, which necessitates alternating or simultaneous introduction of internal standards to ensure mass accuracy (see, e.g., ref. 40). However, a single quadrupole mass spectrometer has been reported to have remarkable calibration stability both in terms of its achievable mass accuracy and more importantly, its high spectral accuracy, through an external calibration lasting one week or more at a time.[37,41] This has allowed for interesting applications on such a conventional mass spectrometer: e.g., the correct identification of intermediate ion fragments inside an electron impact ionization (EI) source.[42]

A lack of spectral accuracy in an MS experiment may be caused by the presence of a mass spectral interference, which could be taken into account during the spectral accuracy calculation to accommodate additional ions and accomplish exact analysis of ion mixtures, a capability uniquely enabled by the comprehensive mass spectral calibration process. Previously reported examples of exact mixture analysis include the evaluation of mass spectral overlap between the EI fragment of nicotine $(M-H)^+$ and the molecular ion M^+,[37] a radiocarbon-labeled drug metabolism study in which the native and ^{14}C-labeled version of the parent drug mass spectrally overlap,[43] investigation of co-existing oxidation products due to the loss of H_2,[31] and analysis of more complex mixtures such as those encountered in petroleum applications.[44] In all these examples, relative concentrations of components can be simultaneously obtained as part of the spectral accuracy calculation to accomplish both qualitative and quantitative analysis.

MS detector saturation can have significant impact on the mass accuracy of a TOF instrument,[45] which will certainly manifest through lack of spectral accuracy. Similarly, other instrument- or hardware-related issues could be diagnosed through spectral accuracy. For example, the TOF mass spectrum of a standard (Cyclosporin A) measured with zero ion threshold and with the manufacturer's suggested saturation limit in mind resulted in a poor spectral accuracy (spectral error at 9.4%), indicating a systematic error. The spectral overlay in Figure 4B shows that the A+1 isotope cluster has lower-than-expected abundance, and this intensity deficiency becomes relatively more and more pronounced for the weaker A+2 and A+3 clusters, which is the opposite of what is expected of TOF detector saturation. This provides an MS user with an important piece of instrument diagnostic information to further improve the TOF operating conditions. This could also serve as constructive feedback for the development of future generations of TOF instruments.

Another example of a spectral accuracy application has to do with chemical samples composed of elements whose isotope abundances are different from those derived from Earth's petrochemical sources. In this case, the lack of spectral accuracy for a given elemental composition reflects changes in isotope abundances for one or more elements involved, which provides another approach for isotope ratio measurement without the use of highly specialized MS hardware such as isotope ratio MS or accelerator MS and/or a combustion process.[46]

An interesting analogy could be drawn here between molecular spectroscopy and MS. For quite some time in the development of IR spectroscopy and its applications, the mid-IR region (like high resolution MS) has been considered uniquely important for reliable organic compound identification because of the spectral interpretability of the various vibrational bands in this fingerprint region of the spectrum.[47] However, NIR (like unit mass resolution MS), with its many overlapping 2nd and 3rd overtones and the associated lower optical resolving power, was considered not suitable for qualitative analysis until more advanced chemometrics approaches made it a routine practice starting 20 years ago.[48−50] Just like in NIR spectroscopy, unit mass resolution mass spectrometers, though unable to spectrally resolve fine spectral features, may be more stable with easily achievable wider linear dynamic range in the form of high spectral accuracy to compensate for their lack of resolving power and to help solve even the most demanding analytical problems.

CONCLUSION

Though MS appears unique, it shows surprising similarities to other analytical techniques if the profile mode MS data are used and a more comprehensive MS calibration carried out to achieve high spectral accuracy. This process allows it to benefit greatly from the many well researched and proven approaches from other analytical fields. Not only could this enable reasonably accurate mass measurement and formula determination on an otherwise conventional quadrupole mass spectrometer, the concept of spectral accuracy could also be used as an important instrument

diagnostic tool for mass spectrometers and to further enhance the formula determination on high resolution instruments. Other more challenging applications such as the qualitative and quantitative analysis of complex mixtures from petroleum research to proteomics may also benefit from the use of spectral accuracy. Finally, it is intellectually gratifying to learn that there is unity among the diverse analytical measurement techniques after all.

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Yongdong Wang is founder and president of Cerno Bioscience, a startup software company dedicated to the practical applications of modern mathematical techniques and chemometrics to MS for both qualitative and quantitative analysis of small and large molecules. Presently, Cerno Bioscience has developed a software product (MassWorks) incorporating both mass accuracy and spectral accuracy for the determination of elemental compositions and exact mixture analysis with either unit mass or higher resolution mass spectrometers (e.g., quadrupole GC/MS or FTICR MS). Ming Gu is vice president of research at Cerno Bioscience and is responsible for MS research and application development. The idea for Cerno Bioscience came from the cross-disciplinary interactions between Yongdong, primarily interested in chemometrics and its applications to optical spectroscopy, and Ming, primarily interested in MS instrumentation. Address correspondence to Yongdong at Cerno Bioscience, 14 Commerce Drive, Danbury, CT 06810 (yongdong.wang@cernobioscience.com). (To watch a flash demo about this topic, please go to www.cernobioscience.com/CernoDemo.html).

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on July 20, 2010, with an error in the x-axis label for the Figure 1A inset. The corrected version was reposted on August 5, 2010.

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