A Novel Approach to Achieve High Mass Accuracy for LC/MS Metabolite Identification

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Introduction

Accurate mass (AM) measurements have been widely utilized for a variety of applications including metabolite identification, peptide database search, and confirmation of pharmaceuticals/impurities and degradation products. The attractiveness of the AM technique is its capability to determine the elemental composition of an unknown molecule with the mass accuracy at low or below ppm level. As a result, AM measurements alone can often provide the definite structure for the molecule, eliminating the need for tandem mass spectrometry (MS/MS). When applied to MS/MS, AM analysis of precursor and product ions can always give valuable confirmation for structural elucidation for the molecules of interest. While AM technique has generated very impressive results and is becoming increasingly popular, the conventional wisdom is that AM can only be achieved on higher resolution instruments such as Fourier Transform mass spectrometer (FTMS), double focus sector instrument, tandem time of flight (TOF/TOF), and quadrupole time of flight (qTOF). Cerno Bioscience has developed a set of novel mass spectrometry calibration and spectral peak processing technologies, MSIntegrity™, to enable AM analysis on conventional mass spectrometers while improving AM performance on high resolution mass spectrometers. Here we present some theoretical background of MS peak analysis in general and AM measurement in particular, computer simulation results, and experimental results from Buspirone metabolite studies on a conventional ion trap as well as a higher resolution qTOF instruments.

Theory

The dependence of mass accuracy (expressed as standard error σ in ppm) on signal strength (S) and mass spectral resolving power (R) has been reported in literature[4],

$$\sigma_{ppm} = \frac{10^6}{CRS^{1/2}}$$

where the constant C includes such factors as signal unit conversion to real ion counts, peak area, peak sampling interval, peak analysis and mass determination algorithms etc. It was reported in same literature[4] that, for MicroMass qTOF operating @ 5,000 resolving power using MassLynx™ software with reserpine as lockmass,
C = 2.9
or 9ppm standard error for signal strength of Imax = 100 ion counts.

It should be noted that the loss of mass accuracy due to a lower mass spectral resolving power can be compensated for by the increase in signal strength, suggesting the potential of achieving high enough mass accuracy on even conventional mass spectrometers.

The value of C is closely connected with the peak analysis algorithm used for mass determination. A well designed mass spectral peak analysis algorithm requires addressing at least the following issues: (1) noise filtering; (2) peak shapes and their variations; (3) short and long term instrument drift; (4) nonlinear mass calibration in full mass range; (5) peak picking and centroiding. MSIntegrity™ was designed with these requirements in mind.

Computer Simulation

MSIntegrity™ was first tested using computer simulation for three typical mass spectrometers, MS with unit mass resolution, qTOF, and FTMS. Figures 1-3 presents the results of these simulations with Imax = 100 after N=30 trials using Poisson distribution.

Figure 1. Unit Mass (R=1,000) Simulation with 100 detected ions
(σ = 12ppm, C = 20)

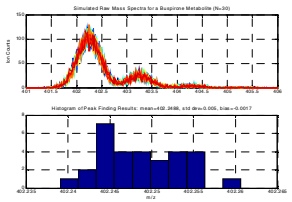


Figure 2. qTOF (R=5,000) Simulation with 100 detected ions
(σ = 3.3ppm, C = 8 vs reported 9ppm w/C=8/2.8)

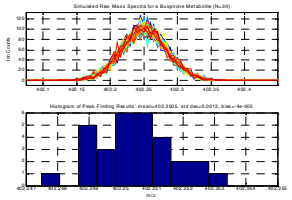
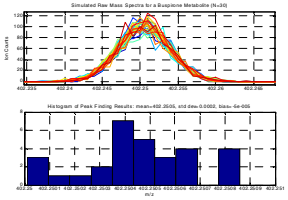


Figure 3. FTMS (R=50,000) Simulation with 100 detected ions
(σ = 0.5ppm, C = 3.6)



Experimental Results

Besides ion counting statistics and instrument drift, high mass accuracy depends heavily on the performance of mass spectral peak analysis algorithm. Figures 4a-b show the peak analysis results from MassLynx™ and MSIntegrity™.

Figure 4a. Peak Centroiding Results from MassLynx™
(I_{592}/I_{591} = 55% vs True 29.68%)

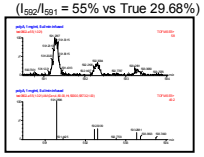
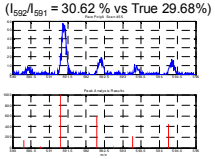


Figure 4b. Peak Centroiding Results from MSIntegrity™
(I_{592}/I_{591} = 30.62 % vs True 29.68%)



A poly-alanine solution was infused into an MicroMass qTOF II instrument to acquire several hundred continuous scans (see Figure 5 for one such scan). An MSIntegrity™ instrument calibration was first applied to all scans followed by MSIntegrity calibration update using a single internal standard, 8-A, before reporting the exact masses of all other known peaks in each scan. The ppm standard error for each peak is presented in Figure 6 along with results from exactly the same data using MassLynx™ software. An overall factor of 2 improvement can be achieved.

Figure 5. A typical MS scan from MicroMass qTOF II

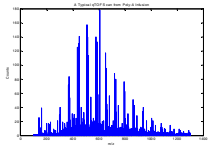
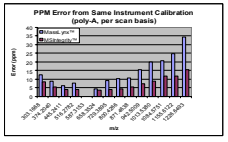


Figure 6. Standard Errors for Mass Determination



LC/MS analysis was conducted on Thermo LCQ classic and MicroMass qTOF II instruments which were respectively coupled with µLC and an Alliance separations module, both from Waters for separation. LCQ instrument calibration was performed with sodium trifluoroacetate prepared according the procedures[5]. LCQ internal calibration during LC/MS runs was done by infusing 5 mg/ml Loperamide water solution at 0.01 µl/min through a syringe pump. The instrument calibration for qTOF II employed 1 mg/ml poly-alanine in 0.01% trifluoroacetic acid in 1:1 ratio of water and ACN. Reserpine of 0.5 mg/ml was infused at 0.1 µl /min as a lock mass for LC/MS analysis. Z-spray ion capillary voltage was set to 2800V, while cone voltage was tuned to 25V. The source block and desolvation temperatures were at 90°C and 100°C, respectively.

All incubations were conducted in 0.1 M potassium phosphate buffer (pH 7.4) and consisted of Buspirone, human liver microsomes and an NADPH regenerating system. The samples were incubated for 1 hour at 37°C. The reactions were quenched with 1 ml of ice-chilled methanol. Microsomal proteins were pelleted by centrifugation at 14,000 rpm for 10min. The supernatants were transferred into clean tubes and evaporated to dryness. The residues were reconstituted with 250µl of water.

AM results from LCQ are shown in Figure 7 and those from qTOF II are shown in Figure 8.

Figure 7. Metabolite Identification on Finnigan LCQ LC/MS
(Mass Error @ 1ppm)

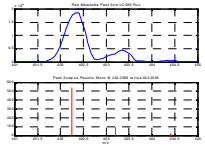
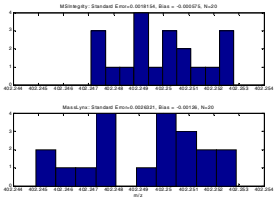


Figure 8. Metabolite Identification on Waters µLC-qTOF II
(MSIntegrity™: 4.5ppm/scan, 1.4ppm/peak; MassLynx™: 6.6ppm/scan, 3.1ppm/peak)



Conclusions

The loss in mass accuracy at unit mass resolution can be compensated for by higher signal strength, allowing for high mass accuracy good enough for metabolite identification and molecular formula search on a conventional LC/MS instrument, provided that great care is taken for both mass spectral calibration, calibration updating, and spectral peak analysis. MSIntegrity™ from Cerno Bioscience has achieved these objectives and enables highly accurate mass determination on conventional MS systems.

On MS instruments with higher resolution such as MicroMass qTOF II, even higher accuracy could be achieved with MSIntegrity™ with a gain factor of ~3x in mass accuracy.

Not only does MSIntegrity™ achieves high mass accuracy from low to higher resolution MS systems, it also gives much better quantitative results due to the intrinsic advantages built into the set of algorithms.

Cerno Bioscience is actively seeking academic and industrial partners to further develop and validate MSIntegrity™. Please contact us if you have demanding applications requiring high mass accuracy, accurate quantitation, and reliable peak picking/centroiding or if you have complex mass spectral data with many peaks and ions that need to be elucidated.

Notes

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