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Comparison of Orbitrap Mass Accuracy Using External and Internal Lock Mass Correction Methods

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ABSTRACT

Purpose: Compare the Orbitrap mass accuracy under a variety of acquisition conditions using two different lock mass techniques, internal and external.

Methods: Lock mass acquisitions are acquired on a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer configured with an Easy-ETD reagent ion source for generation of the lock mass species (202 *m/z*, from fluoranthene).

Results: Comparison of the mass accuracy obtained with the external versus internal lock mass corrections indicate that the external correction method yields results that are within approximately 2 ppm of the internal correction results, and that overall, less than 5 ppm RMS intra-scan mass accuracy is achieved with the external correction method over a wide range of acquisition conditions.

INTRODUCTION

Parts per million (ppm) mass accuracy and mass stability over time are two key performance metrics of the Orbitrap (OT) mass analyzer.[1] While the Orbitrap performs quite well on both counts using a standard external mass calibration, both metrics can be improved by use of an internal lock mass correction via injection of a species of known m/z into the mass analyzer.[2] This approach provides a real time (scan-to-scan) recalibration of mass error, but the additional time required to inject the internal lock mass species (approximately 15 msec including injection and overhead) can adversely affect the instrument scan rate and duty cycle, which has limited its adoption in many applications. To address this deficiency, we explored the possibility of operating an OT mass analyzer with lock mass correction factor that is acquired independent from the analytical scan, and at a much lower frequency.

MATERIALS AND METHODS

Sample Preparation

Thermo Scientific™ Pierce™ FlexMix calibration solution, used as delivered. Thermo Scientific™ Pierce™ HeLa protein standard, see below.

Test Method(s)

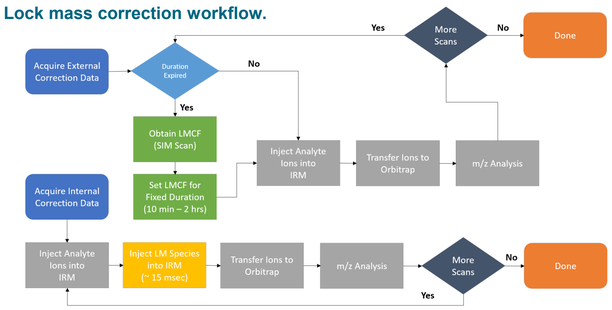
We characterized the performance of internal versus external lock mass correction of OT data under a variety of conditions known to impact the OT mass calibration (e.g., space charge and resolution) via infusion and LC-MS/MS based acquisitions. For the infusion experiments, mass accuracy was monitored from infusion of FlexMix at 4 µL/min using the ESI source, while LC-MS/MS runs were 1 µgram on-column injections of the HeLa standard using a Thermo Scientific™ Easy-nLC™ 1200, a 120-minute gradient, and 30,000 MS/MS resolving power.

Figure 1 depicts the workflow for both types of lock mass correction experiments. During external lock mass correction experiments, the lock mass correction factor (LMCF) is extracted from a SIM scan that solely consists of the lock mass ion. The external LMCF is then applied to subsequent analytical scans for a predetermined amount of time (approximately 10 minutes for the infusion data, and once per sample injection for the LC-MS/MS data). In contrast, internal lock mass scans combine the lock mass ion with the analytical ion population prior to m/z analysis in every scan.

Data Analysis

Mass accuracy from the infusion data was extracted from a custom instrument control script, while the LC-MS/MS runs were searched using Thermo Scientific™ Proteome Discoverer™ (PD) 2.4 software, against the UniProt human database, and peptide spectral matches (PSMs) were filtered to a 1% false discovery rate using Percolator.

Figure 1. Lock mass correction workflow.



RESULTS

Acquisition of external lock mass correction factor

The external correction factor is acquired from a SIM scan (5 Th isolation width) of the lock mass species at 120k Orbitrap resolution, and an automatic gain control (AGC) target of 1e4 charges. Figure 2 shows a representative scan of the lock mass species acquired in this manner.

Infusion Data

Results from the FlexMix infusion data are presented in Figure 3. We find that the external correction method can provide less than 5ppm RMS intra-scan mass accuracy regardless of the acquisition conditions, and while it generally gives higher residuals than the internal correction method, it is thought to be a reasonable trade off for the increase in scan duty cycle the approach affords. Figure 3d further demonstrates that the external lock mass correction technique provides similar RMS mass accuracy when the uncorrected mass accuracy is artificially forced out to +15 ppm, which may be beneficial for counteracting long term Orbitrap *m/z* drift.

Figure 2. External lock mass correction factor SIM scan. 1e4 AGC target, 120K OT resolution.

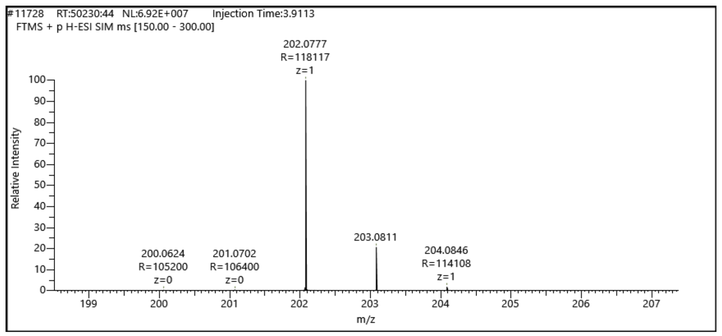
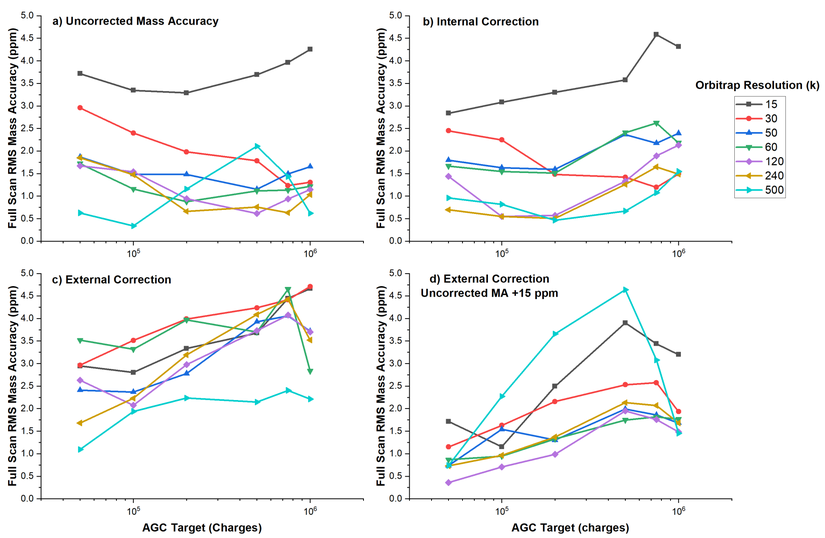


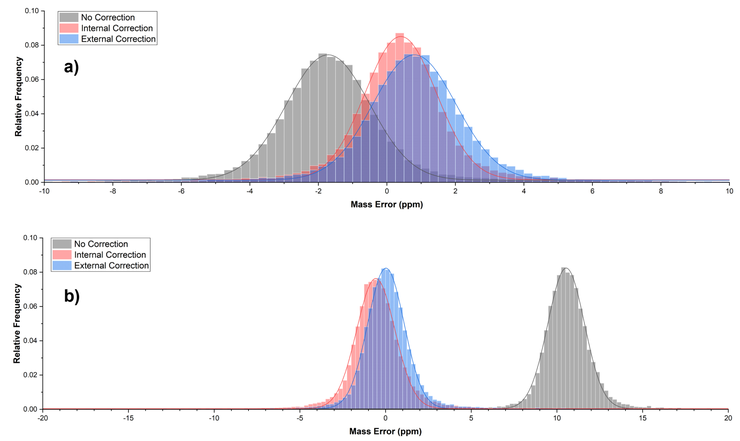
Figure 3. Full scan RMS mass accuracy from FlexMix peaks for uncorrected, internally corrected, and externally corrected data as a function of AGC target and Orbitrap resolution. a) Uncorrected, b) Internal correction, c) External correction, d) External correction when the mass accuracy is nominally +15 ppm before correction.



LC-MS/MS Data

LC-MS/MS acquisitions present a dynamic environment to test the external correction approach since the distribution and abundance of charge the instrument sees is constantly changing throughout the gradient. The histograms in Figure 4 show the performance of the external calibration approach in two situations. a) In comparison to internal correction when the uncorrected mass accuracy is good, and b) when the uncorrected mass accuracy has drifted by ~ 10 ppm. Both histograms are generated from the PSM's that met the filtering conditions as described in the Data Analysis section. The mass accuracy from external correction in Figure 4b fits to a 2σ standard deviation of ± 2.5 ppm.

Figure 4. Histogram of the mass accuracy of peptide spectral matches (PSMs) extracted from PD. Full scan Orbitrap resolution 60k. a) Comparison of all three data acquisition types, b) External correction when the nominal mass accuracy is ~ +10 ppm.



CONCLUSIONS

- External mass correction provides mass accuracy comparable to that of internal correction, with improved scan duty cycle. While the improvement in duty cycle is strongly method dependent, the time savings gained by removing the internal calibrant injection from MS2 scans during LC-MS/MS experiments similar to those conducted here, is on the order of fifteen minutes throughout the duration of the gradient, affording the acquisition on many thousands of additional MS/MS spectra.
- The difference in mass accuracy between the two methods, either infusion or LC-MS/MS based, is on the order of 1-2 ppm in favor of the internal correction and is largely attributed to the fact that the external lock mass species does not experience the space charge of the analytical scan.
- While higher in magnitude than the standard OT mass accuracy specification of 3 ppm RMS, the mass accuracy quoted for external correction (5 ppm RMS) spans a much larger range of acquisition conditions, with less stringent environmental requirements.
- Although not shown, the external lock mass correction method should alleviate one of the major shortcomings associated with the internal method, namely, incorrect or non-assignment of the lock mass correction factor due to interfering peaks during LC-MS acquisition.

REFERENCES

1. Makarov, A., Denisov, E., Lange, O., and Horning, S. *J Am Soc Mass Spec*, **2006**, 17, 977-982.
2. Olsen, J.V. et al. *MCP*, **2011**, 4(12), 2010-2021.

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