thermoscientific

# Performance metrics and example applications: Orbitrap mass spectrometry at resolving power 1M

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## ABSTRACT

**Purpose:** This work surveys key performance problems and their solutions in Orbitrap mass spectrometry at high resolving powers and presents example applications benefiting from high-resolution Orbitrap analysis.

**Methods:** A Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer was modified by (1) ultra-precisely machined Orbitrap electrodes; and (2) carefully controlled injection conditions.

**Results:** Improvements in performance enabling routine analysis at resolving power 1,000,000 (at *m/z* 200), are presented, along with results from applications in metabolomics and lipid fluxomics.


## INTRODUCTION

Since its commercial introduction in 2005, the Orbitrap mass analyzer has been widely adopted by the mass spectrometry community. Historically, applications in which Orbitrap analysis has been particularly useful are those which require high resolving power. For example, in many proteomics studies, resolution of 60k or 120k (at *m/z* 200) is more than sufficient to accurately determine the monoisotopic mass of multiply charged peptides across the mass range of interest. However, some applications require higher resolutions and until now they remained served only by Fourier Transform Ion Cyclotron Resonance (FT-ICR) instruments. Here we describe an Orbitrap-based mass spectrometer capable of dependable and routine analysis at a resolving power of 1M. Performance criteria are discussed and applications examples are provided.

## MATERIALS AND METHODS

***Samples.*** For calibration and characterization, a standard calibration solution was used, which consisted of n-butylamine, caffeine, MRFA, and Ultramark 1621 dissolved in 50:49:1 acetonitrile, methanol, and formic acid. For the metabolite standards experiment, 24 common metabolites were mixed at a concentration of 100 pg/µL. 20 µL were then injected on to a Thermo Scientific™ Hypersil GOLD™ column (100x2.1 mm). Analytes were separated using a 20-min gradient starting at 5% buffer B (99.9:0.1 acetonitrile:formic acid) and increasing to 35% B (buffer A was 99.9:0.1 water:formic acid), at a flow rate of 300 µL/min. For the flavonoid and lipid fluxomics work, sample information can be found in the referenced presentations.

***Mass spectrometry.*** All mass spectrometry was performed on a Orbitrap Fusion Lumos Tribrid mass spectrometer equipped with the 1M Option (Fig. 1). The 1M Option consists of ultra-precisely machined high-field Orbitrap electrodes used in combination with a C-trap and injection optics with improved manufacturing tolerances. The UHV system was baked at a temperature of approximately 120°C until the base pressure was ≤ 5 × $10^{-11}$ torr. Applied voltages were calibrated according to an automated calibration routine as described below.

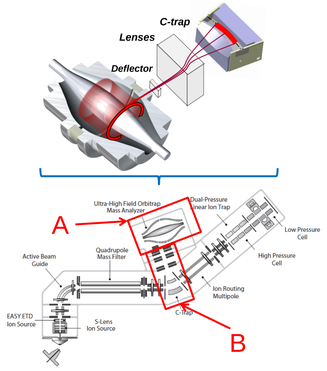



**FIGURE 1. Orbitrap Fusion Lumos mass spectrometer equipped with the 1M Option: (A) high-precision Orbitrap electrodes; (B) C-trap and injection optics with improved manufacturing tolerances.**

## RESULTS

**Performance considerations and validation**

In FTMS measurements, ion cloud coherence determines the length of time over which useful measurements can be made. Cloud coherence can be compromised especially for less dense ion clouds, and this can lead to intensity-dependent decay. This in turn can lead to inaccurate isotope abundances, as shown below.

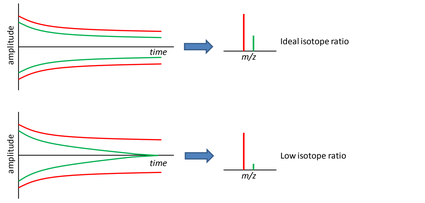



**FIGURE 2. Intensity-dependent signal decay can lead to inaccurate isotopic abundances for smaller ion clouds (green) relative to larger (red).**

The problem described above is worse for longer transients, i.e., high resolving powers. To ensure quantitative accuracy and adherence to all performance objectives, we employ an automated calibration routine. This builds upon the routine previously described [1], which employs a genetic algorithm and tailored scoring in order to navigate the multidimensional optimization surface that arises in C-trap/Orbitrap calibration.

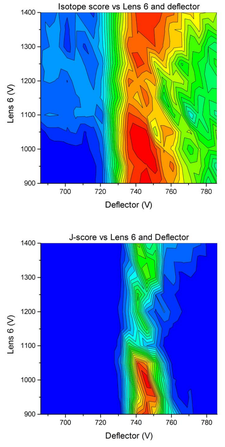
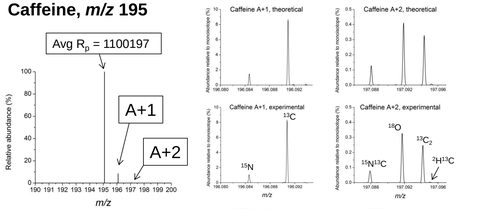



**FIGURE 3. Top left, complex surface representing the response of an isotope score (a composite of isotope fidelity measurements for caffeine, MRFA, and UM1522) to changes in two important optics elements, the deflector and an adjacent lens. While complex, this 2-D surface captures only a portion of the complexity of the entire multi-dimensional surface, which includes responses from 6 other optics elements.**

**Bottom left, this complexity can be significantly reduced by using a more selective score (see ref [1]), which makes the true optimum more clear.**

### Caffeine, *m/z* 195




### MRFA, *m/z* 524

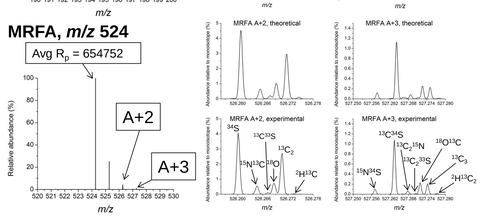
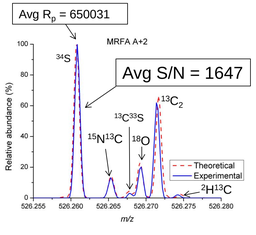



**FIGURE 4. With the improvements shown described in methods and with proper calibration, good results can be obtained as shown above. Each species is quad-isolated (20 *m/z* isolation width), AGC target 5e4 ions.**

**FIGURE 5. The above results can be improved slightly by exercising more control over the ion population. At right is the quad-isolated A+2 peak of MRFA, (isolation width 1 Da), AGC target 2000 ions. With internal calibration turned on, mass accuracy is << 1 ppm.**



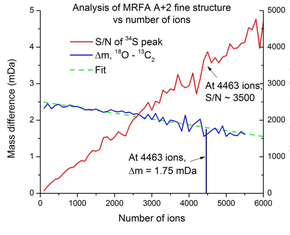
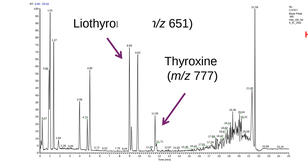



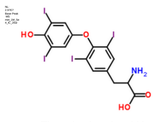

**FIGURE 6. We characterize the high-resolution behavior of the C-trap/Orbitrap combination by finding the signal-to-noise ratio at which the $^{18}O$ and $^{13}C_2$ peaks start to lose baseline separation. This starts when the mass difference (Δm) between the two reaches about 1.75 mDa (about 2.5 mDa theoretical), as the number of ions in the peak cluster increases.**

## Example applications

The figures below show fine isotopic structure revealed in a metabolites standard, on an LC timescale.

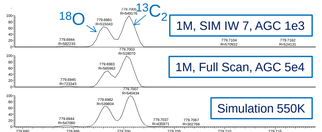
### *LC/MS of metabolite standards*

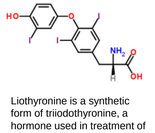


Thyroxine is a thyroid hormone responsible for regulation of metabolism.

Thyroxine, A+2



Liothyronine is a synthetic form of triiodothyronine, a hormone used in treatment of thyroid conditions.

Liothyronine, A+2

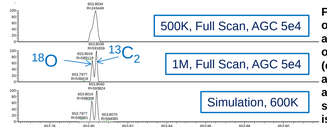
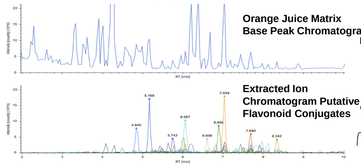



**FIGURE 7. Two examples of metabolite fine structure are shown at left, acquired on an LC timescale (chromatography trace above). Data were acquired with different strategies, showing that isolation and control of the ion population yields the best results.**

### *Flavonoids in orange juice*



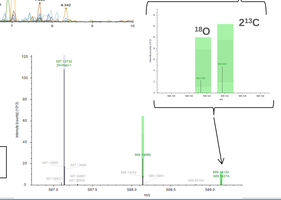




**FIGURE 8. Using fine isotope structure as a pattern filter allowed detection of 128 putative flavanoid conjugates in a mixture containing >4000 compounds.**

For more, see ASMS '17 Proceedings: WP457, T. Stratton et al.

### *Lipid Fluxomics*

In *fluxomics,* the objective is the quantitation of functional molecular turnover over many different molecular species of interest.

The figure below describes measurements of lipid biosynthesis. Normally such measurements are made using tritium labeling; however, very few laboratories are able to practice this method due to strict controls on radioactive materials and handling. Here deuterium labeling is used, and the labeled and unlabeled forms can be baseline separated using ultra-high resolution Orbitrap MS.

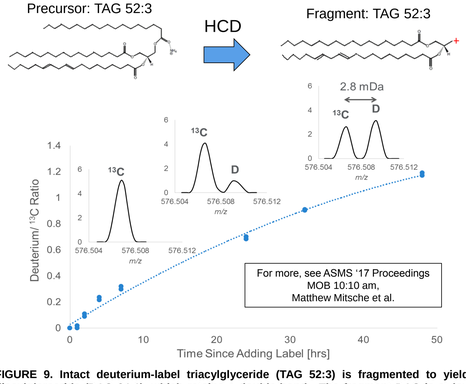



**FIGURE 9. Intact deuterium-label triacylglyceride (TAG 52:3) is fragmented to yield a diacylglerceride (DAG 34:1), which retains a double-bond. The fragment DAG is at lower *m/z* where higher resolving power allows the $^{13}C$ and D peaks to be easily separated.**

## CONCLUSIONS

- Using ultra-precisely machined Orbitrap electrodes and employing exact control over ion injection conditions, transients of 2 seconds yield good performance at a mass resolving power of 1,000,000 (at *m/z* 200).
- Optimized calibration and tuning provide accurate isotope fidelity and robust space charge performance
- Data obtained with the 1M Option provide important information about a variety of research topics.

## REFERENCES

1. J. Canterbury et al., Poster M309, ASMS 2013, "Automatic Isotope Ratio Optimization on High-field Orbitrap Mass Analyzers."

## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING



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