Is High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Needed to Improve Metabolite Annotation?

Georgia Tech Chemistry and Biochemistry College of Sciences

Danning Huang[1], *Marcos Bouza*[1], *David A. Gaul*[1], *Arthur S. Edison*[2], *Facundo M. Fernández*[1]

[1]School of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 (USA)

[2]Department of Genetics and Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, University of Georgia, Athens GA 30602 (USA)

UNIVERSITY OF GEORGIA 1785

OVERVIEW

- One hundred and four metabolite standards were measured using high-resolution FT-ICR and Orbitrap MS platforms.
- Mass accuracy and elemental formulae assignment were evaluated using different combinations of automatic gain control (AGC: 5e4, 1e5, and 5e5) and resolution settings (R: 120K, 240K, and 500K) on an Orbitrap ID-X mass spectrometer.
- A data analysis pipeline was developed for preprocessing mass spectrometry data generated with the two instruments, using the same software package.
- The two leading analytical platforms were compared in their capabilities of assigning the correct elemental formulae for metabolite standards with high resolution accurate mass measurements and isotope patterns.

INTRODUCTION

Fourier transform ion cyclotron resonance (FT-ICR) and Orbitrap mass spectrometry (MS) are the highest-performing analytical MS platforms in metabolomics. Their high mass accuracy and resolution enables detailed investigation of biological metabolomes. Non-targeted MS experiments, however, yield extremely complex datasets that make metabolite identification very challenging, if not impossible. High resolution accurate mass measurements greatly facilitate this process by reducing mass errors and spectral overlaps. When applied together with isotope pattern matches, heuristic rules, and limits during searches, the number of candidate empirical formula(s) found can be significantly reduced. Here, we evaluate the quality of the mass measurements produced by FT-ICR and high field Orbitrap platforms, and how these affect the assignment of the correct elemental formulae in metabolite identification applications.

REFERENCES

1. Weber, R.J., Southam, A.D., Sommer, U., Viant, M.R., *Anal Chem*. **2011**; 83(10):3737-3743.
2. Barbier, S.H.P., Hohenester, U.M., Colsch, B., Tabet, J.C., Junot, C., Fenaille, F., *Anal Chem.* **2018**; 90(5): 3030-3035.4.
3. Pluskal, T., Uehara, T., Yanagida, M., *Anal Chem.* **2012**; 84(10): 4396-4403.

METHODS

Sample Preparation

- One hundred and four metabolite standard stock solutions were prepared at a concentration of 0.001 M in methanol. A pooled metabolite sample was prepared by mixing each stock solution with a final concentration of 5 µM.

Instruments and Experimental Conditions

- Column: Acquity UPLC BEH HILIC 1.7 µm, 2.1 mm*150 mm
- Mobile phase A: water + 10 mM ammonium formate + 0.1% formic acid.
- Mobile phase B: acetonitrile + 0.05% ammonium hydroxide.
- Instrument: Thermo Scientific Vanquish UPLC coupled to Thermo Scientific Orbitrap ID-X mass spectrometer. Automatic gain control (AGC: 5e4, 1e5, and 5e5) and resolution settings (R: 120K, 240K, and 500K)

- Bruker 12-T solariX FT-ICR mass spectrometer. Spectral Size: 8M; Avg. Scan: 200; Accum. Time: 0.05s

- Ionization mode: Positive (ESI+), Negative (ESI-).

Data Analysis

- MZmine 2.51 was used for data preprocessing of the pooled metabolite sample analyzed in the Orbitrap and FT-ICR mass spectrometers in both positive and negative ion modes.
- Empirical formula prediction of the extracted metabolite features was performed with a < 1 ppm (or 0.001 Da) mass error tolerance, $[C_{0\text{-}50}H_{0\text{-}100}N_{0\text{-}15}O_{0\text{-}20}P_{0\text{-}7}S_{0\text{-}8}+H]^+$ limits for positive mode or $[C_{0\text{-}50}H_{0\text{-}100}N_{0\text{-}15}O_{0\text{-}20}P_{0\text{-}7}S_{0\text{-}8}-H]^-$ for negative mode, heuristic rules, and isotope pattern matches applied through built-in functions in MZmine.

PRELIMINARY RESULTS

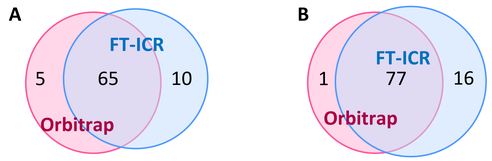


Fig. 1 (above) Venn diagrams showing the number of metabolites detected in **(A) positive** mode, and (**B**) **negative** mode. Metabolites detected with Orbitrap ID-X (R: **500K**, AGC: **1e5**) and FT-ICR (Size: **8M**) were represented in pink and blue colors, respectively. Determination of overlap in the Venn diagram was done by checking the proximity of peaks in m/z space.

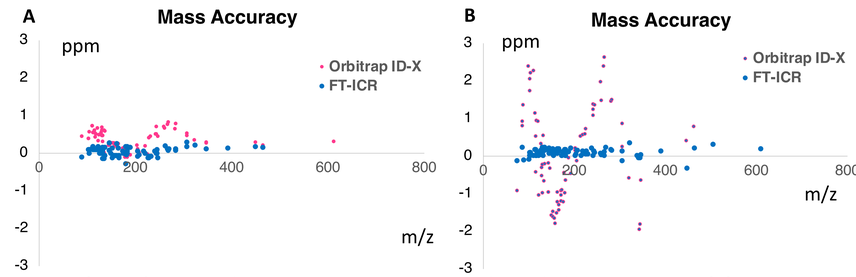
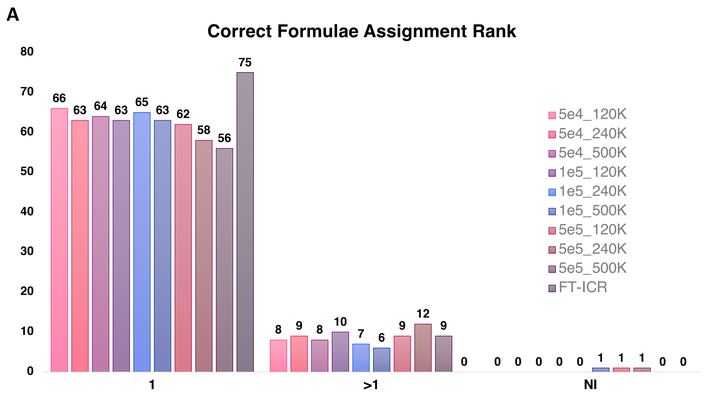


Fig. 2 (above) Scatter plots showing the mass error of metabolites detected in **(A) positive** mode, and **(B) negative** mode using Orbitrap ID-X (R: **500K**, AGC: **1e5**) and FT-ICR (Size: 8M). x-axis represents m/z, and y-axis represents mass error in ppm. Data were shown in same scale for comparison purposes.



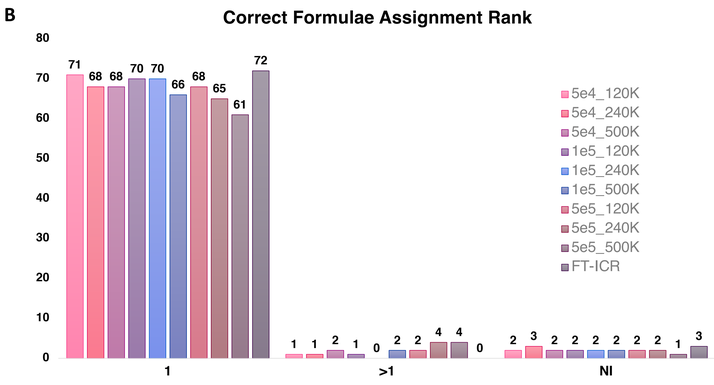


Fig. 3 (above, left and right) Clustered column plot showing the performance in assigning correct elemental formulae for detected metabolites using **(A) mass accuracy**, and **(B) mass accuracy and isotope pattern score** in **positive** mode. Number of metabolites detected using Orbitrap-IDX (with different combinations of R and AGC) and FT-ICR were represented in different colors, respectively. x-axis represents the ranking of the correct formulae assignments in top candidates. y-axis represents the number of metabolites detected in each category (1: correct assignment, >1: correct assignment not in the top 1 rank; NI: not identified or without correct assignment in top 10 identities. Formulae were assigned with a < 1 ppm (or 0.001 Da) mass error tolerance and 90% isotope pattern score.

Table 1 (below) Summary of mass accuracy of detected metabolites using Orbitrap-IDX (with different combinations of R and AGC) and FT-ICR in **positive** mode.

AGC/ R	5e4/ 120K	5e4/ 240K	5e4/ 500K	1e5/ 120K	1e5/ 240K	1e5/ 500K	5e5/ 120K	5e5/ 240K	5e5/ 500K	FT-ICR
Max	0.858	0.821	0.818	0.895	0.858	0.818	1.078	1.370	1.041	0.292
Standard Deviation	0.205	0.207	0.216	0.229	0.213	0.217	0.286	0.318	0.296	0.069
Mean	0.345	0.411	0.457	0.295	0.330	0.369	0.332	0.361	0.352	0.099

CONCLUSIONS

- FT-ICR and high field Orbitrap platforms were evaluated for the assignment of the correct elemental formulae in metabolite identification applications.
- At ~ 500K resolution, metabolites were detected with higher mass accuracy using FT-ICR compared with Orbitrap ID-X. For FT-ICR, elemental formulae were able to be correctly assigned using mass accuracy only. For Orbitrap ID-X, adding isotope pattern scores greatly improved the correct elemental formulae assignment.
- The advantage of FT-ICR high resolving power on the correct elemental formulae assignment was not indicated in the score, this is due to the algorithm that has higher weight on the major isotope peaks. Further investigation is needed in a more advanced algorithm that capturing minor isotope peaks.

Future Work

- Develop an advanced isotope score algorithm that capturing minor isotope peaks detected in high resolution settings.
- Analyze biological samples with spike-in metabolite standards for further evaluation of metabolite identification success using the FT-ICR and high field Orbitrap platforms with the developed methods and data analysis workflows.

ACKNOWLEDGEMENTS

FMF and ASE acknowledge support by NIH 1U2CES030167-01 and the CMaT NSF Research Center (EEC-1648035). FMF also acknowledges support by 1R01CA218664-01, and the NIH MoTrPAC Consortium (1U24DK112341-01).