A Platform to Identify Endogenous Metabolites Using a Novel High Performance Orbitrap and the mzCloud Library

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Overview

Purpose: To demonstrate the capability of Orbitrap Fusion Tribrid™ Mass Spectrometer in combination with MS^n library for metabolites structural identification.

Methods: UHPLC coupled to Orbitrap Fusion to simultaneously perform global profiling and structural elucidation with MS^n

Results: Within UHPLC timescale (2-3 second FWHM), >10 cycles can be finished with fast ion trap HCD MS^2 for top 11 precursors, each with ion trees of MS^2-MS^3(top2)-MS^4(top2).

Introduction

The structural identification of metabolites represents a significant challenge in metabolomics study. Multistage mass spectrometry (MS^n) is a powerful tool for compound identification and structural elucidation that goes beyond identifying the exact same compounds, to discovering additional compounds with structural homology to those in the library. Combining with high resolution accurate mass (HRAM) measurement, MS^n is highly effective in identifying the unknown but biologically relevant compounds in metabolomics studies[1]. However, the speed on current platforms is yet to be effectively compatible with UHPLC frontend, which limited such application in biological samples.

Presented here is a new platform to identify endogenous metabolites using a novel high performance Orbitrap hybrid instrument in conjunction with UHPLC and an MS^n library.

Methods

Sample Preparation

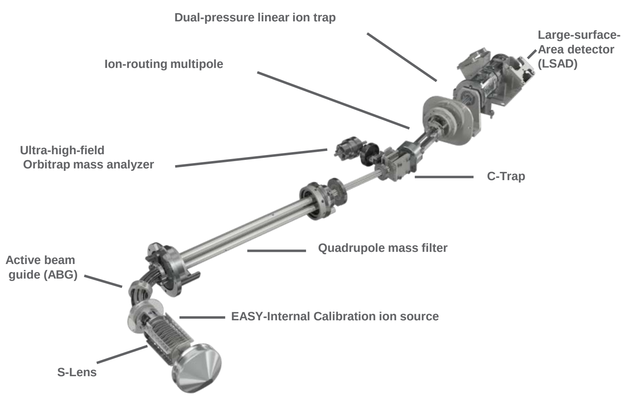
Urine samples from an adult male were analyzed. The urine samples were diluted 1 to 10 with water and processed with a 3kD MWCO filter.

For plasma samples, proteins were removed with cold methanol and centrifugation, followed by centrifugal evaporation at 35 ºC. The reconstituted residue in MeOH/Water, 1/9, and also processed with a 3kD MWCO filter.

Liquid Chromatography

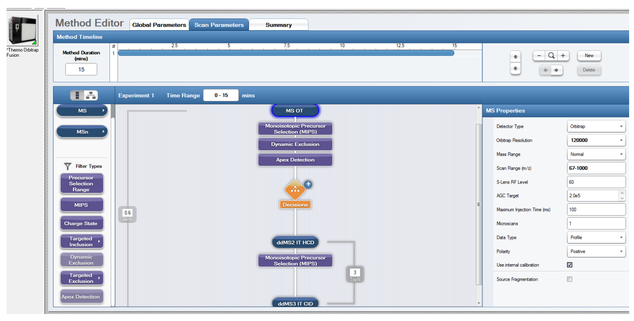
UHPLC separation was implemented on a Dionex Ultimate 3000 HPG (high-pressure gradient) pump using Hypersil GOLD RP C18 column at 450µL/min, column temperature at 55 ºC. LC solvents were 0.1% FA (A) and 0.1% FA in MeOH (B). Apply linear gradient from 0.5-50% B for 5.5min, followed by increasing to 98% at 6 min, hold 98% B for 6 min then decrease to 0.5% at 13min, then equilibrate for another 2 min.

FIGURE 1. Schematic of Orbitrap Fusion Tribrid™ Mass Spectrometer



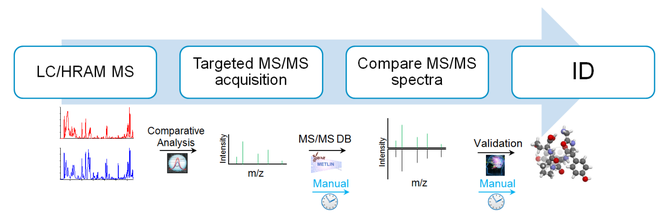
Results

FIGURE 2. Orbitrap Fusion and the Easy of Use Instrument Method Editor.

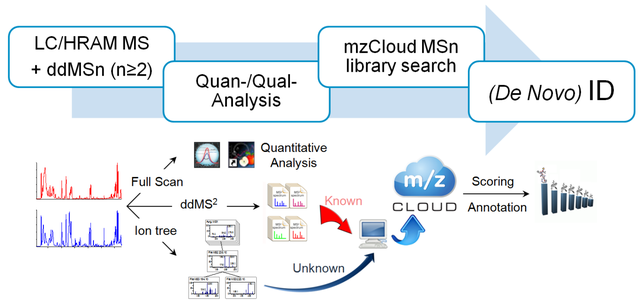


Improved Metabolomics Workflow

A traditional workflow for metabolite profiling and identification is based on full scan LC/MS experiment followed by targeted MS/MS confirmation.



Powered by the innovative mass spectrometer Orbitrap Fusion and the mzCloud library, an improved workflow is as below. It allows HCD MS/MS to be performed simultaneously with full scan MS on the UHPLC timescale; simultaneous ddMS^n ion tree with flexible combinations of CID and/or HCD fragmentation with ion trap and/or Orbitrap™ detection. *De novo* structural identification is made feasible by using this workflow, based on the spectral tree similarity, an algorithm called precursor ion fingerprinting (PIF) which enables the identification of compounds even if they are not in the library, and the fragmental peaks can be annotated [2].

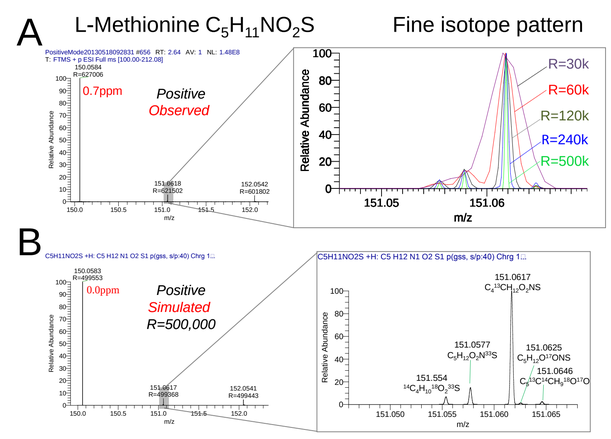


Metabolite Identification Requires High Resolution and Isotope Ratio Measurements

Structure elucidation of unknown small molecules by mass spectrometry is a great challenge. The first crucial step is to obtain correct elemental compositions.

Natural occurring elements can be monoisotopic (F, Na, P, I) or polyisotopic (H, C, N, O, S, Cl, Br) [1]. The molecule L-Methionine $C_5H_{11}NO_2S$ contains multiple polyisotopic elements, especially the S, which result in multiple isotopic peaks needing resolving power above 240,000 at m/z 200 (FWHM). Figure 3A shows the better isotope pattern of molecular ion $[M+1+H]^+$. With increasing resolution up to 450,000 (FWHM), all five isotope peaks are clearly resolved. Figure 3B shows the simulated isotope pattern.

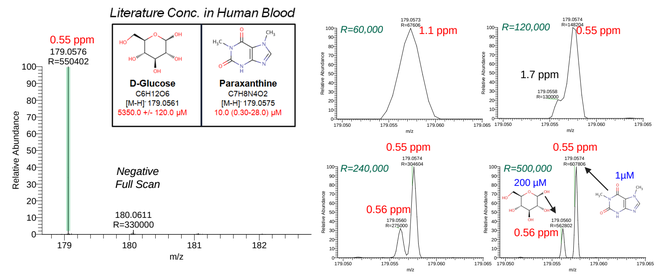
FIGURE 3. Ultrahigh resolution resolves the isotopes



Metabolite identification and quantitation in complex sample requires high resolution

D-Glucose $C_6H_{12}O_6$ and Paraxanthine $C_7H_8N_4O_2$ co-exist in human blood at distinct concentration levels: 5300 µM (D-Glucose) vs. 10 µM (Paraxanthine) [3]. Although D-Glucose is hundreds of fold more concentrated, it is less readily detectable because of its low ionization efficiency. Their masses are different by 0.0013 amu (179.0561 vs. 179.0574), making the detection of individual components very challenging. Figure 4 shows that, with >300,000 resolving power, the 200 µM (D-Glucose) and 1 µM (Paraxanthine) can be readily separated and quantified.

FIGURE 4. Ultrahigh resolution resolves isobaric metabolites



Metabolite Identification Requires High Accuracy of Mass Measurement in both MS Full Scan Level and MS/MS Level

For an unknown metabolite, the number of calculated chemically possible formula or structural isomers can be significantly reduced with high mass accuracy and MS^n techniques

The Orbitrap Fusion has a built-in internal calibration functionality (Easy IC). Figure 5 shows LC-MS/MS data of L-Tryptophan from an urine sample with and without internal calibration. Sub-ppm mass accuracy was observed for MS and MS^2 fragments with IC applied.

FIGURE 5. Easy Internal Calibration

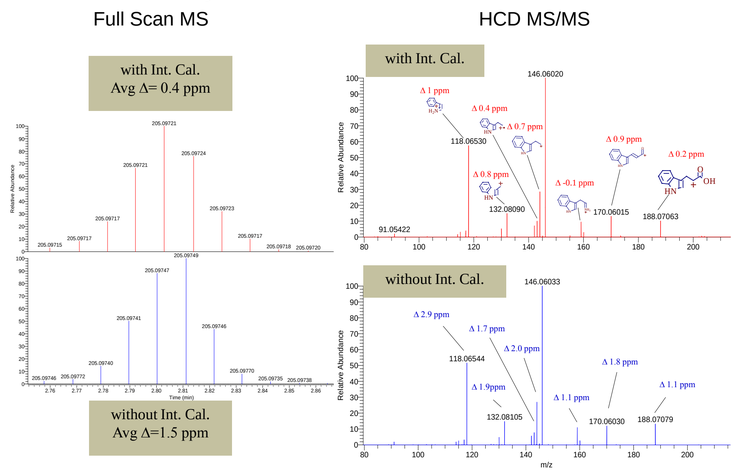
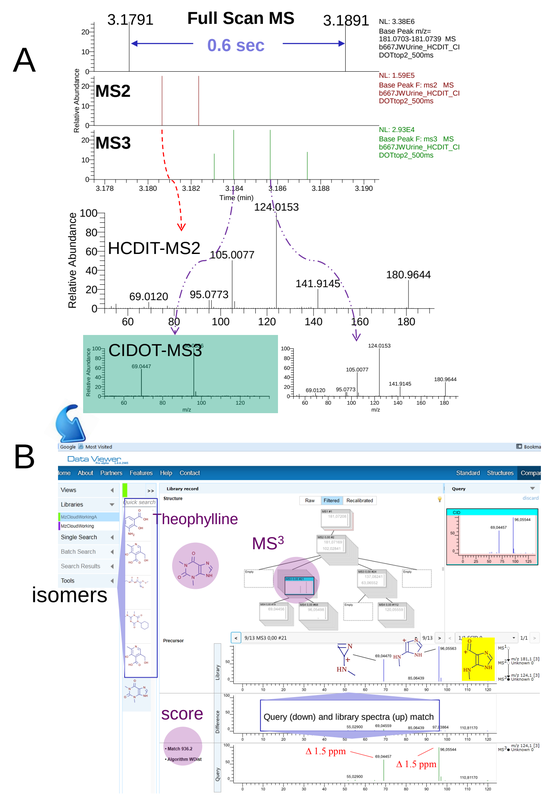


FIGURE 6. Rapid ion tree acquisition on UHPLC timescale from a urine sample (1 MS, 2 MS^2 and 4 MS^3 in 0.6sec/cycle).

(A) Ion trap HCD MS^2 followed by Orbitrap CID MS^3 were acquired for the precursor ion *m/z* 181.0721. (B) Spectral tree search in mzCloud matches with MS^3 from Theophylline with highest score, indicating the unknown must have the same substructure as Theophylline.



Conclusion

Orbitrap Fusion offers ultra high resolution and high fidelity in isotope ratio measurements of metabolites. This allows metabolites in complex mixture with a wide dynamic range be resolved and detected. The Easy-internal calibration allows compound assignment be readily done with confidence. OT Fusion provides ultimate flexibility that facilitates the examination of multiple structural pathways for more structural information at higher speed.

The mzCloud library allows metabolites identification with MS^2 and/or MS^n data in an automated fashion. The identification based on multistage MS spectral tree and PIF algorithm opens the possibility to identify unknown metabolites and isomers. An improved metabolomics workflow is enabled.

References

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2. Sheldon MT, Mistrik R, Croley TR, J. Am. Soc. Mass Spectrom. 2009, 20(3):370-376
3. http://www.hmdb.ca/metabolites/HMDB00122 & HMDB01860

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