Comprehensive data acquisition workflow on Orbitrap Astral Zoom mass spectrometer to achieve deep lipidome coverage

Rahul Ravi Deshpande[1], Giorgis Isaac[1], Alan Baik[2], **Judith Ngere[3]**, Bashar Amer[1], Isha Jain[2] and Susan S. Bird[1],
[1]Thermo Fisher Scientific, San Jose, California, United States; [2]Gladstone Institute, San Francisco, California, United States; [3]Thermo Fisher Scientific, Bremen, Germany

Abstract

Purpose: We evaluated the modified Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer for deeper lipidome coverage and confident identification from control and high fat diet (HFD) 73 mice plasma lipid extracts.

Methods: Mice plasma lipid samples were extracted and separated using a Thermo Scientific™ Vanquish™ Neo UHPLC systems. High-resolution accurate mass (HRAM) Orbitrap MS1 full scans were collected in parallel with fast, sensitive HRAM Astral MS2 scans acquired through data-dependent acquisition (DDA) for untargeted lipidomics.

Results: The data showed a significant increase in the number of detected lipid species across the different lipid classes. The percentage of fragmented lipids were >90% using the novel Orbitrap Astral Zoom mass spectrometer greatly enhancing the lipid identification with a 15% increase with high quality grade A and B lipid annotation.

Introduction

The increasing prevalence of metabolic disorders underscores the need for robust, high-throughput clinical lipid panels and lipidomics methods to quantify lipid species comprehensively, with high precision and selectivity . Structurally related lipids, with varying fatty acid composition, were associated with distinct metabolic consequences and proven to have different biological roles. Thereby, measuring individual lipid species with highest selectivity possible is crucial for corroboration of clinical utility including the improved risk prediction and diagnostics.

Lipid identification is often considered the most challenging and bottleneck of the lipidomics workflow. This is due to the vast diversity of lipid structures, isomers and varying concentrations. Fragmentation of lipids generate characteristic fragments that help in lipid annotation, structural elucidation, isomer differentiation, and improved confidence in lipid identification. Having fragmentation for most of the lipids detected in a sample hence is highly desirable. This requires fast and sensitive instrumentation. Here we evaluated the new Orbitrap Astral Zoom mass spectrometer (Figure 1) for deeper lipidome coverage and confident lipid identification from 73 mice plasma extracts.

Materials and Methods

Sample Preparation

Lipids were extracted from plasma samples obtained from 73 mice fed with a high fat diet and a normal diet. Lipid standards and bovine liver lipid extracts were purchased from Avanti Research™.

LCMS Method

Plasma lipid extract was separated using Thermo Scientific™ nanoLC PepMap™ (C18, 75µmx150mm, 2µm) column connected to a Vanquish Neo UHPLC system.

Data acquisition was carried out on the Orbitrap Astral Zoom mass spectrometer using full-scan Orbitrap MS1 in parallel with data-dependent Astral MS2.

Data Analysis

Data processing was performed using Thermo Scientific™ Compound Discoverer™ 3.4 software. Compound Discoverer 3.4 software includes new features such Thermo Scientific™ LipidSearch™ software node for simplified lipid identification, QC for small molecule experiments using internal standards, automatically detects and groups MS1 fragments and other performance improvements.

Results

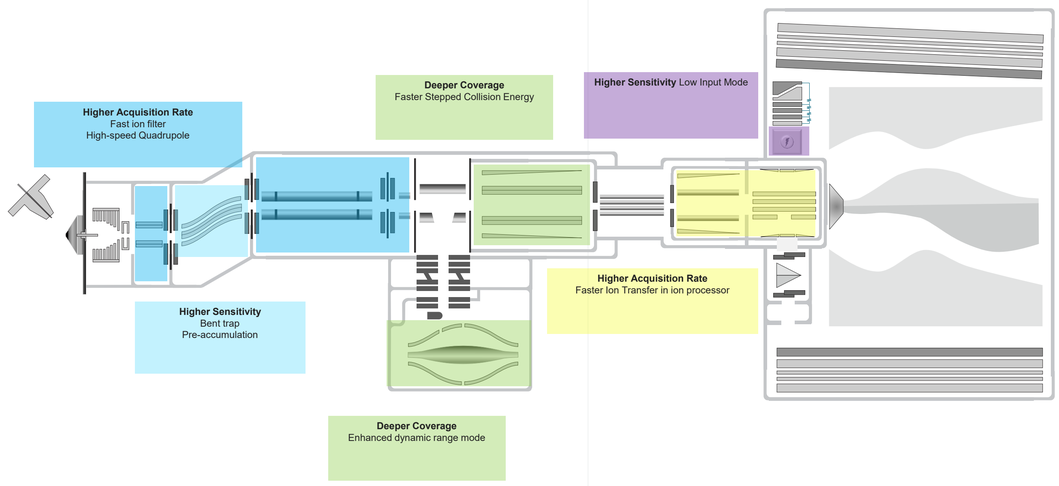


Figure 1. Instrument diagram of the Orbitrap Astral Zoom mass spectrometer.

Unprecedented speed and depth: dual analyzer power of Orbitrap Astral Zoom MS

The parallel data acquisition on Orbitrap Astral Zoom MS using both mass analyzers, the Orbitrap and Astral, enables a higher number of Orbitrap HRAM MS1 full scans with sub-ppm mass accuracy. Simultaneously, the fast Thermo Scientific™ Astral™ analyzer delivers a significantly greater number of high-quality MS2 scans at 80k resolution (77% more) compared to Orbitrap-Orbitrap configurations, which is essential for deep coverage and confident lipid identification (Figure 2).

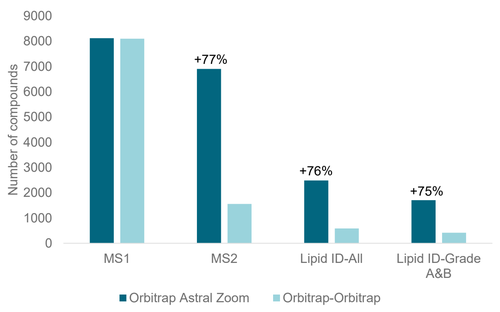


Figure 2. Orbitrap Astral Zoom MS1 and MS2 scans compared to Orbitrap-Orbitrap configurations. This dual-analyzer approach supports deep coverage and confident lipid identification.

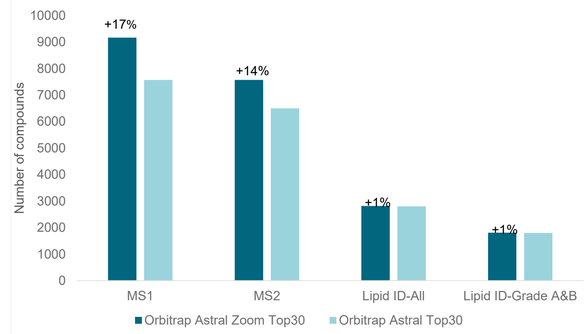


Figure 3. Orbitrap Astral Zoom MS provides more MS2 coverage and lipid identification.

This increased MS2 scan speed enables the detection of a broader range of lipid classes, facilitating deeper and more comprehensive untargeted lipidomics analysis (Figure 3). In the mice plasma extract, it delivers a 14% increase in MS2 coverage compared to the already comprehensive coverage of the Orbitrap Astral mass spectrometer.

Accelerated MS2 acquisition with stepped NCE for confident lipid annotation

The improved MS2 speed in Orbitrap Astral Zoom MS mode enables efficient data acquisition with Stepped NCE, providing comprehensive lipidome coverage without the delays associated with slower data collection with a 15% increase with grad A and B lipid annotation (Figure 4). This enhancement also positively impacts the annotation of unknown lipid species, delivering higher confidence in their identification thanks to the precision of the stepped collision energy (Figure 5).

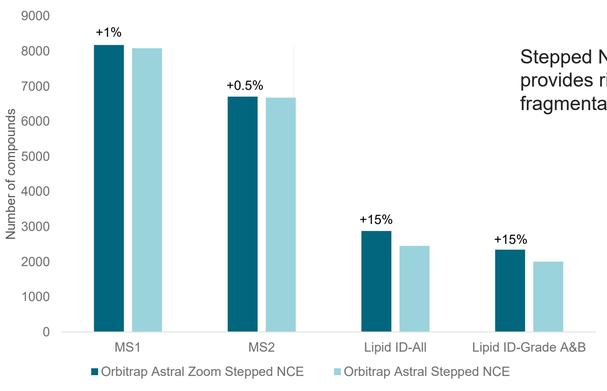


Figure 4. The enhanced MS2 speed in Orbitrap Astral Zoom MS mode, coupled with Stepped NCE, enables efficient data acquisition and comprehensive lipidome coverage.

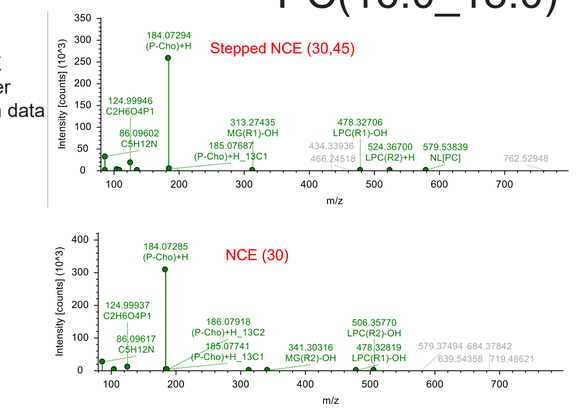
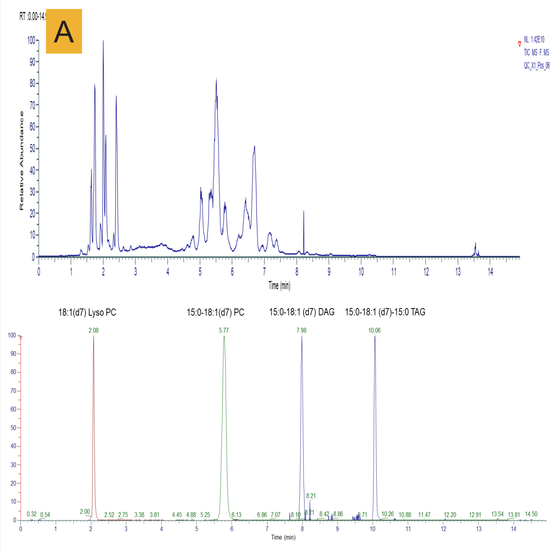


Figure 5. Besides high coverage, the Orbitrap Astral Zoom MS ensures increased confidence in lipid annotation with faster Stepped NCE.



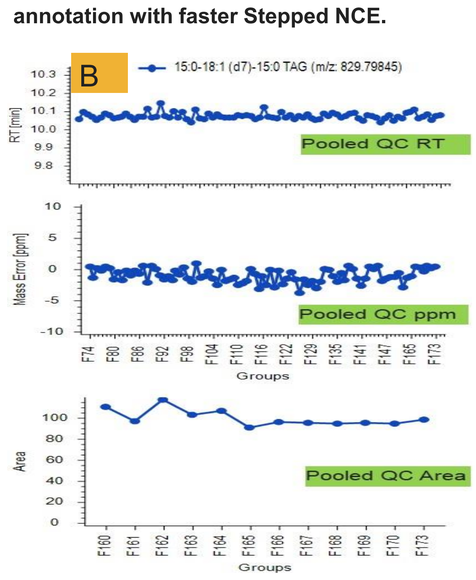
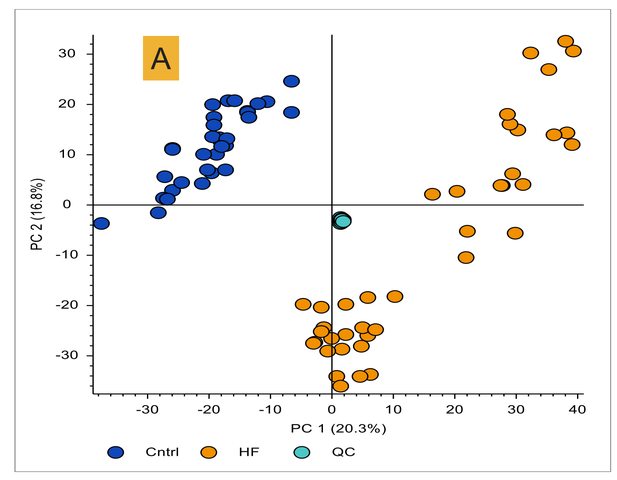


Figure 6. TIC using nano-flow with separation of various lipid class standards (A). Retention time, ppm error and peak area of the spiked internal standard 15:0-18:1(d7)-15:0 TAG using the QC internal standard workflow node (B).

Data quality was investigated using the spiked Avanti Research™ SPLASH™ LIPIDMIX™ for each lipid class. The combination of nano-flow and Orbitrap Astral Zoom MS provided robust and reliable data in retention time, ppm error and peak area for 15:0-18:1(d7)-15:0 TAG as shown in Figure 6B.

Based on the PCA plot, a significant difference was observed between control and high fat diet (Figure 7A). The pooled QC samples are tightly grouped at the center of the PCA plot which indicates the robustness of the Orbitrap Astral Zoom MS. The volcano plot shows 99 upregulated and 43 downregulate lipids in high fat diet. Initial investigation of the upregulated lipids show that most of these lipids are neutral lipids mainly triacylglycerides and cholesterol esters.



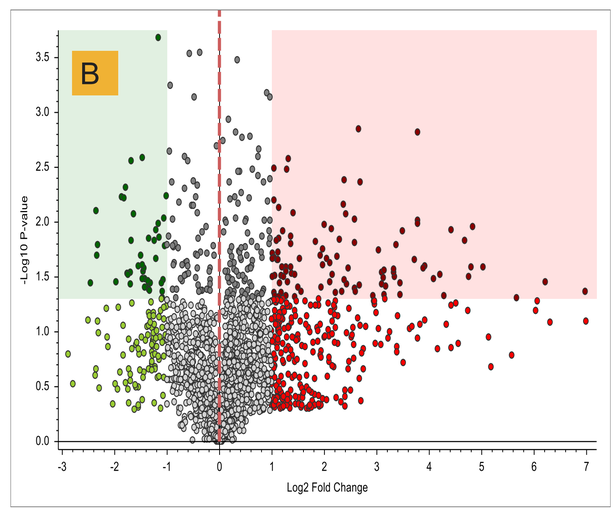


Figure 7. PCA plot control vs high fat diet (A). Volcano plot with 99 lipids upregulated (red) and 43 lipids downregulated (green) in the high fat diet.

Conclusions

- The enhanced MS2 speed in Orbitrap Astral Zoom MS mode, coupled with Stepped NCE, enables efficient data acquisition and comprehensive lipidome coverage with a 15% increase with grade A and B lipid annotation.
- The use of nano-flow increased the sensitivity and the number of detected lipids while achieving a significant reduction in the use of sample and solvent.
- A significant difference was observed between control and high fat diet mice samples with potential biomarker upregulated neutral lipids (triglycerides and cholesterol esters) in the high fat diet.

Trademarks/licensing

Learn more at thermofisher.com/orbitrapastralzoom