

Simultaneous Quantitation and Discovery (SQUAD) metabolomics: an intelligent combination of targeted and untargeted workflows using the novel Orbitrap Astral mass spectrometer

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Abstract

A single injection, high-throughput, and accurate metabolomics approach that enhances productivity by utilizing the novel Thermo Scientific™ Orbitrap™ Astral™ instrument for parallel quantitation of predefined compounds as well as unknown identifications of potential biological significant features was developed.

LC-MS quantitation of isotopically labeled amino acids spiked in NIST SRM 1950 plasma reference standard and untargeted characterization of the NIST plasma metabolites was performed on Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer. Full-scan Orbitrap HRAM MS¹ was acquired in parallel to fast and sensitive DDA MS² on the Astral™ mass analyzer.

Data showed an increased percentage (i.e., 90%) of fragmented compounds using the novel Astral mass analyzer resulting in improved annotation capability for deeper discovery analysis, and faster high-quality MS¹ scans on the Orbitrap that enable an accurate and sensitive quantitative analysis (i.e., LOQ down to 10 femtomoles with 5 orders of magnitude linearity range) within the single injection approach.

Introduction

Here we introduce a single injection **simultaneous quantitation and discovery (SQUAD)** metabolomics method that combines targeted and untargeted workflows on the Orbitrap Astral instrument, Figure 1. SQUAD is used for the confident identification and accurate quantitation of targeted metabolites. It also allows the untargeted discovery analysis to look for global metabolic changes that were not part of the original focus. This offers a way to strike the balance between targeted and untargeted approaches in one single experiment¹.

Single Injection SQUAD analysis!!

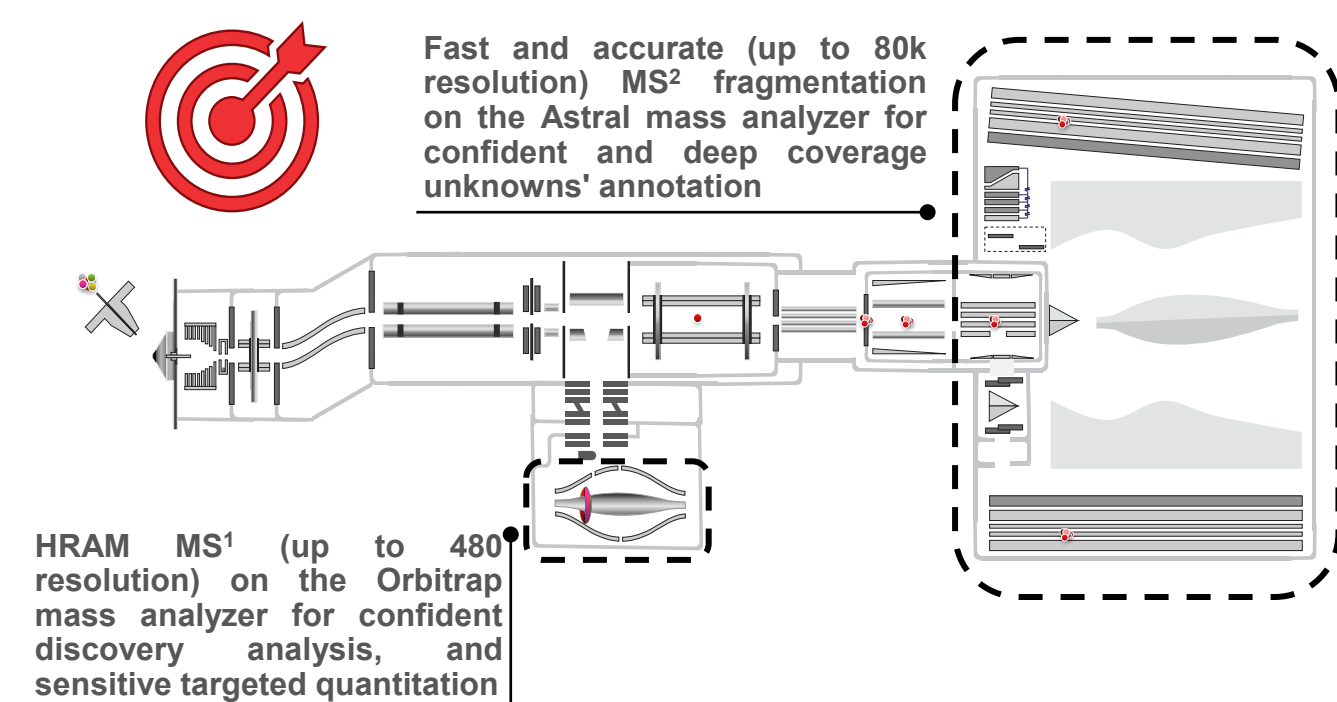


Figure 1. Single injection simultaneous quantitation and discovery (SQUAD) metabolomics analysis on Orbitrap Astral mass spectrometer.

In parallel, the Thermo Scientific™ Astral system can run a full-scan Orbitrap HRAM MS¹ analysis, and a fast and sensitive DDA MS² on the Astral mass analyzer. This allows a confident untargeted and accurate targeted analysis utilizing the same injection, thus, eliminating the variability of using multiple instruments and the need to re-inject limited biological samples.

Materials and methods

Metabolite Reference Standard NIST SRM 1950 plasma sample and isotope-labeled amino acids were purchased from Sigma and CL, respectively. The plasma was spiked with a dilution series (1 nM – 2.5 mM) of isotope-labeled amino acids before extraction with 80% methanol, Figure 2.

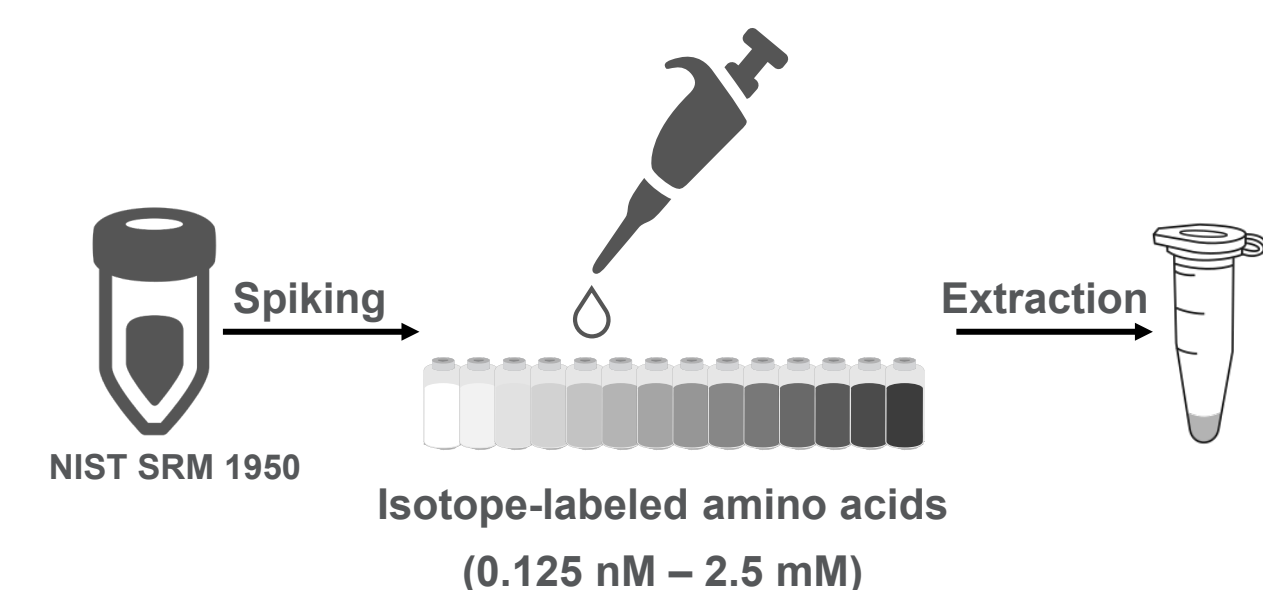


Figure 2. Single injection simultaneous quantitation and discovery (SQUAD) metabolomics analysis on Orbitrap Astral mass spectrometer.

Reversed-phase chromatography was applied as the technique of choice for the pre-separation of the metabolites using Thermo Scientific™ Hypersil GOLD™ column attached to Thermo Scientific™ Vanquish™ Horizon UHPLC as a pre-separation technique. Data were acquired in full-scan Orbitrap MS¹ in parallel to DDA MS² (top 30) on a Thermo Scientific Orbitrap Astral mass spectrometer. Thermo Scientific™ TraceFinder™ 5.1 and Compound Discoverer™ 3.3 software were used for data processing, analytes quantitation, and unknown annotation, Figure 3.

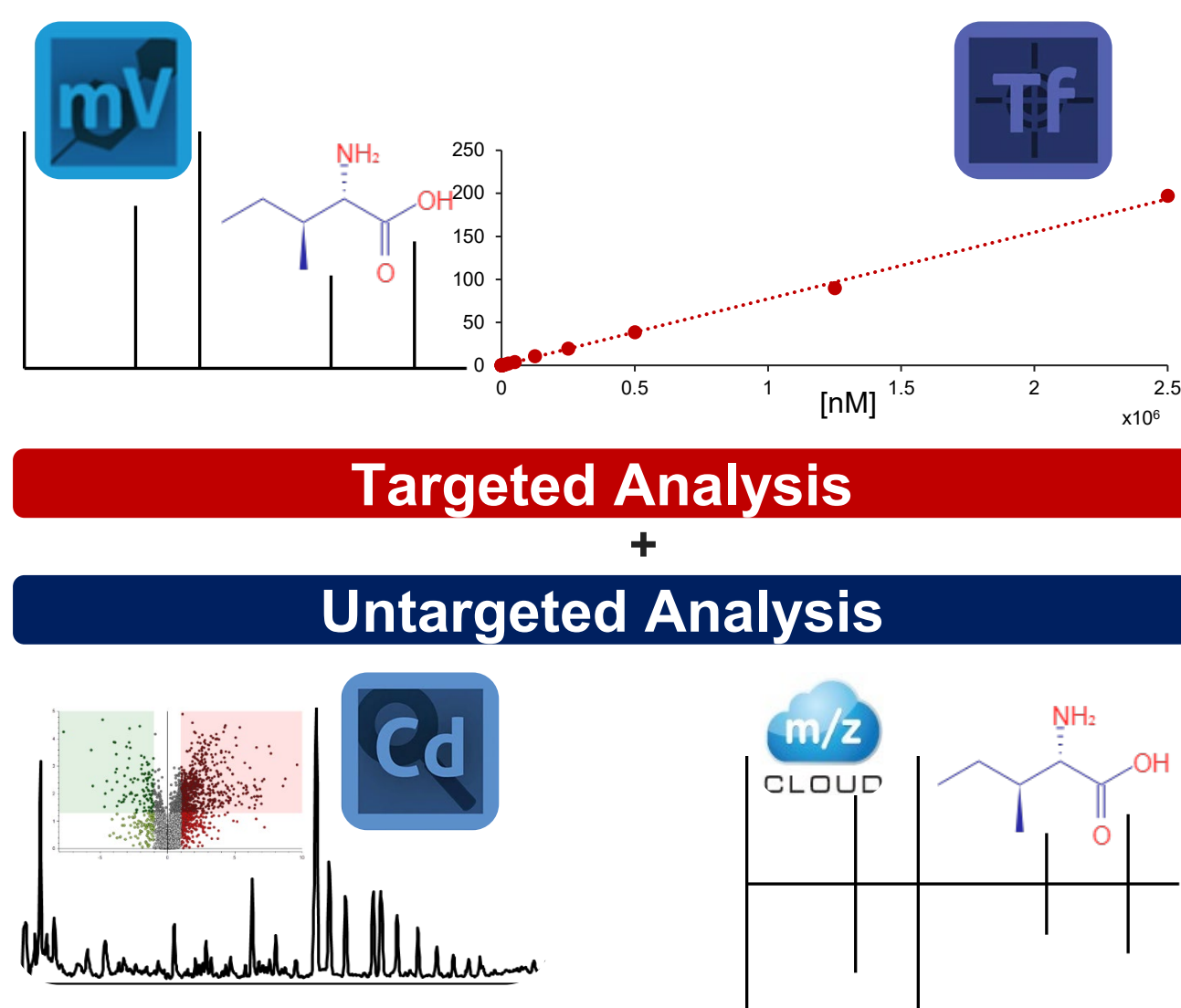


Figure 3. SQUAD workflow provides both targeted quantitation and untargeted discovery data analysis.

Results

The parallel data acquisition utilizing the two mass analyzers (i.e., Orbitrap and Astral) results in faster Orbitrap HRAM scans (sub-ppm mass accuracy), this empowers the Orbitrap to measure sufficient and high-quality scans per peak at low concentrations (Figure 4) that are required for accurate and sensitive MS¹-based quantitation.

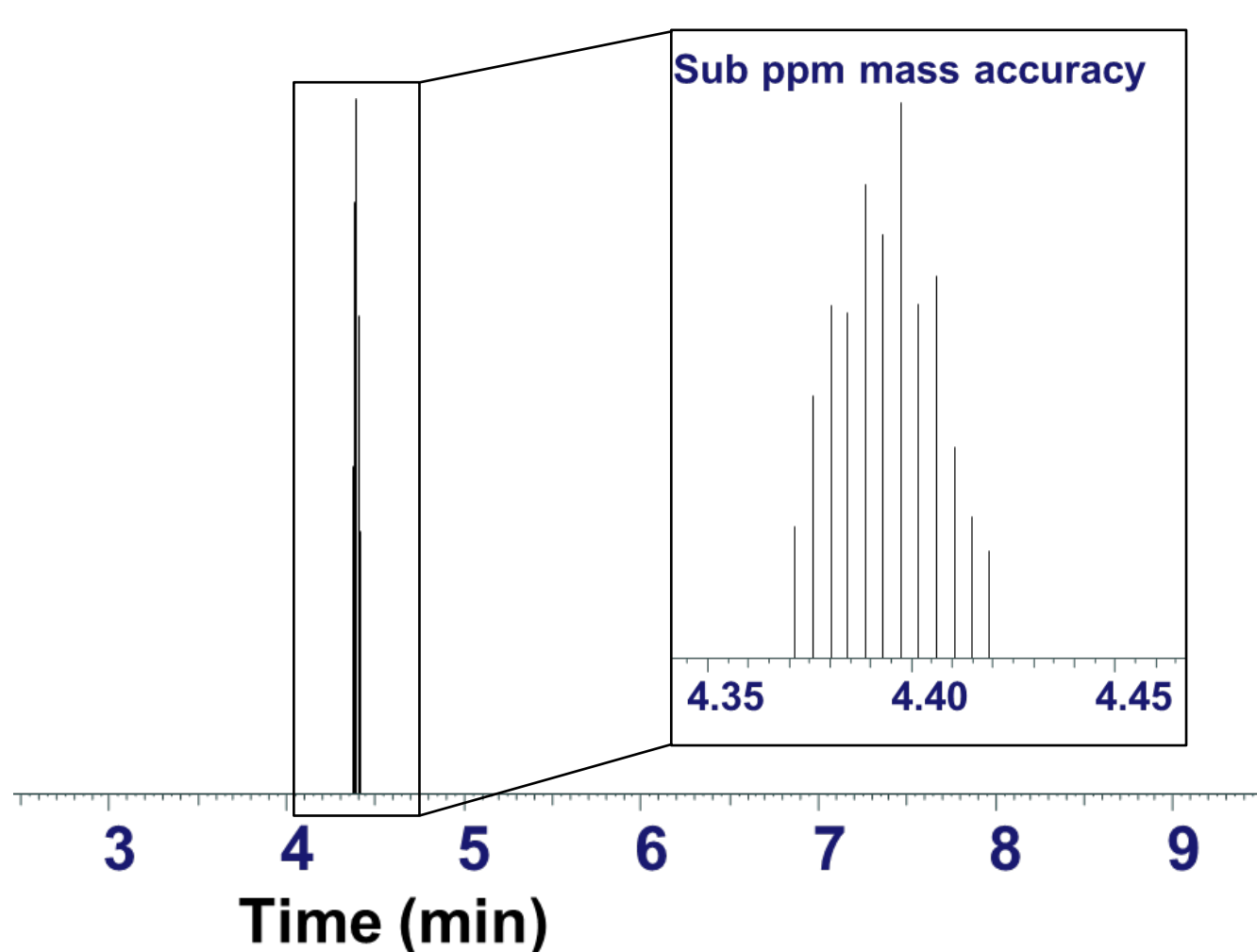


Figure 4. Orbitrap mass analyzer for accurate and sensitive targeted analysis. A sufficient number of high-quality MS¹ scans of the spiked isotope-labeled phenylalanine in NIST SRM 1950 plasma were observed; 12.5 nM.

As a result, the Orbitrap Astral mass spectrometer demonstrated high sensitivity and extended linear dynamic range MS¹-based quantitative analysis, Figure 5 and Table 1.

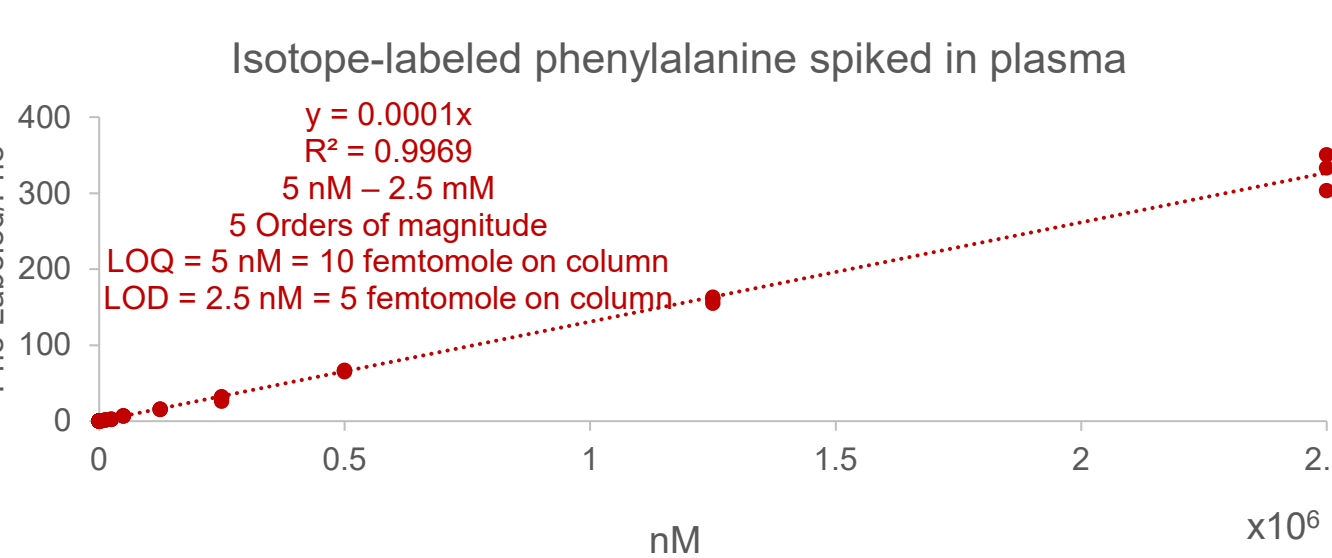


Figure 5. Absolute quantitation results for isotope-labeled phenylalanine spiked in NIST SRM 1950 plasma reference standard.

Table 1. Absolute quantitation results (i.e., linear dynamic range, LOQ, and LOD) for isotope-labeled amino acids spiked in NIST SRM 1950 plasma reference standard.

Analyte	Calibration linear dynamic range (5 orders of magnitude)	LOQ (fmole on column)	LOD (fmole on column)
Phenylalanine	5 nM – 2.5 mM	10	5
Isoleucine	12.5 nM – 2.5 mM	25	10
Leucine	12.5 nM – 2.5 mM	25	10
Tyrosine	25 nM – 2.5 mM	50	25
Tryptophan	25 nM – 2.5 mM	50	25

Alongside, the parallel fast analysis secures a higher number of high-quality (up to 80k resolution) MS² scans compared to the number of fragments measured by Orbitrap-Orbitrap mode, Figure 6. This is crucial for deep coverage and confident untargeted discovery analysis.

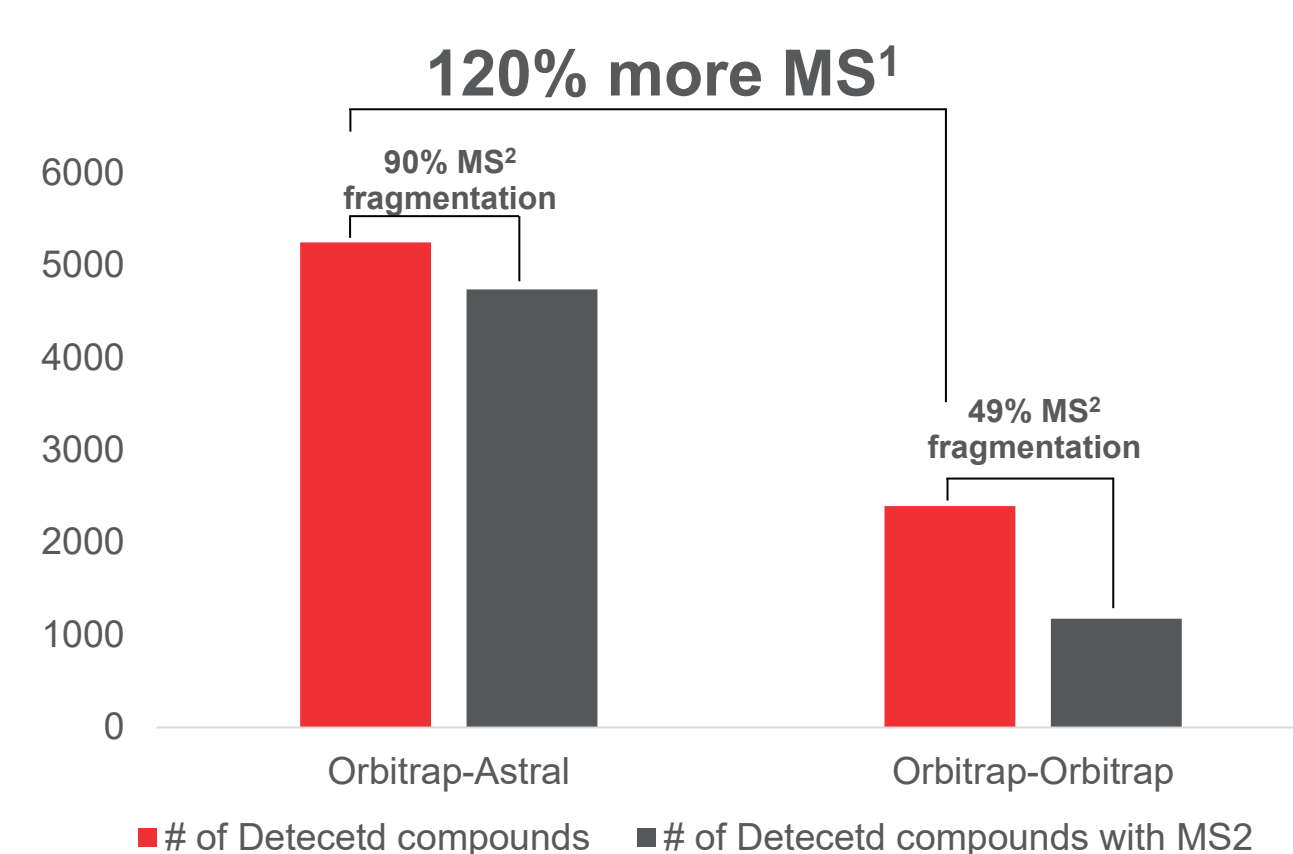


Figure 6. The total number of detected compounds in NIST plasma and percentage of spectra fragmentation using parallel Orbitrap MS¹ and Astral DDA MS² (Orbitrap-Astral) vs. Orbitrap MS¹ and Orbitrap DDA MS² (Orbitrap-Orbitrap). Data collected from Compound Discoverer analysis using ≥ 5 spectra/peak threshold.

In addition, the Orbitrap Astral platform allows for reducing the LC gradient (i.e., 3 times shorter) without compromising the number of MS¹ scans and signal-to-noise (Figure 7), nor the spectra MS² fragmentation ratios (Figure 8). Thus, providing a golden opportunity for the development of high-throughput SQUAD analysis on the Orbitrap Astral mass spectrometer.

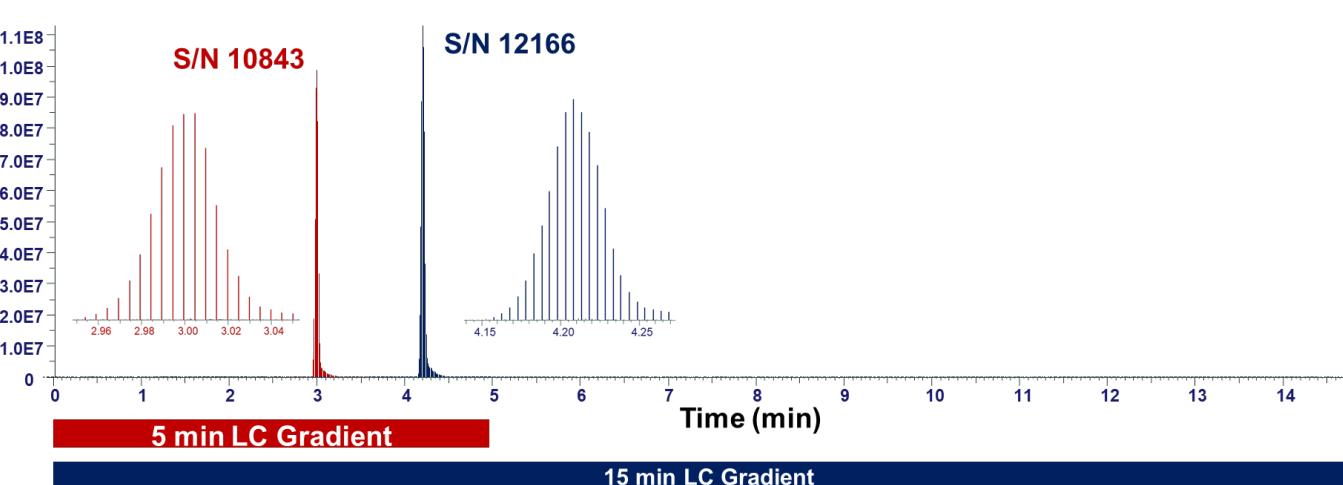


Figure 7. Signal-to-noise and the number of scans per peak for phenylalanine in NIST plasma using two different LC gradients and Orbitrap-Astral SQUAD analysis.

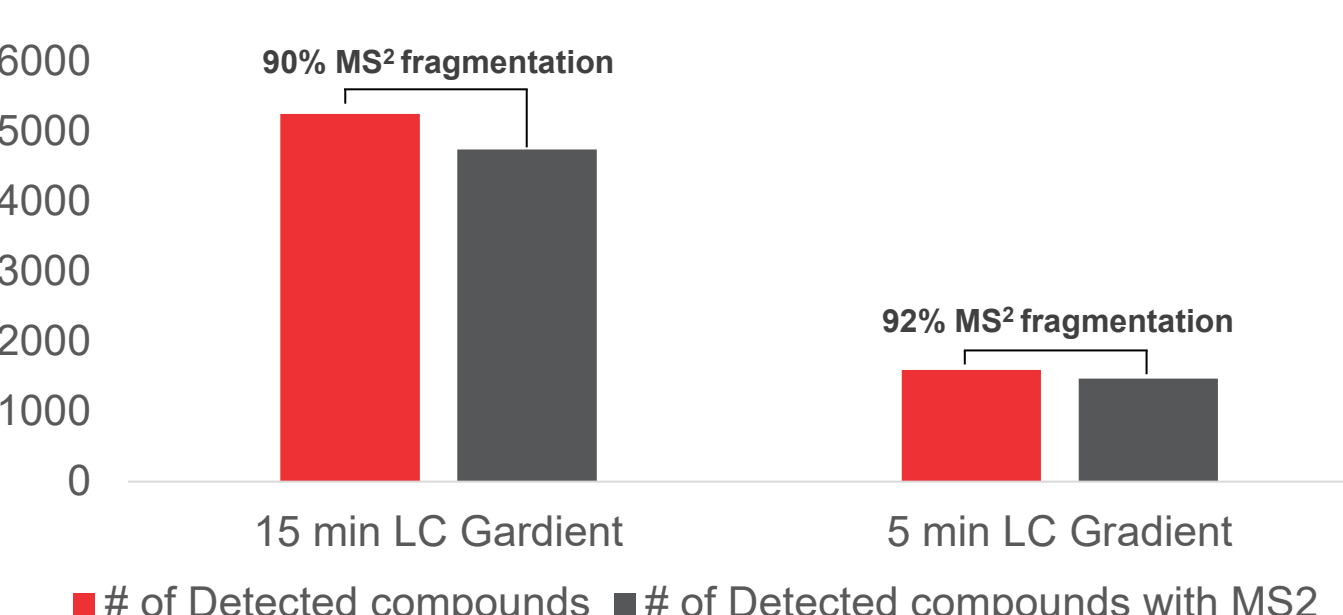


Figure 8. The total number of detected compounds in NIST plasma and percentage of spectra fragmentation using parallel Orbitrap MS¹ and Astral DDA top 30 MS² with 15- and 5-min LC gradients. Data collected from Compound Discoverer analysis using ≥ 5 spectra/peak threshold.

Moreover, the number of detected compounds with MS² in the Orbitrap-Astral 5 min LC gradient experiment was 25% higher than in the Orbitrap-Orbitrap 15 min LC gradient experiment, Figure 9.

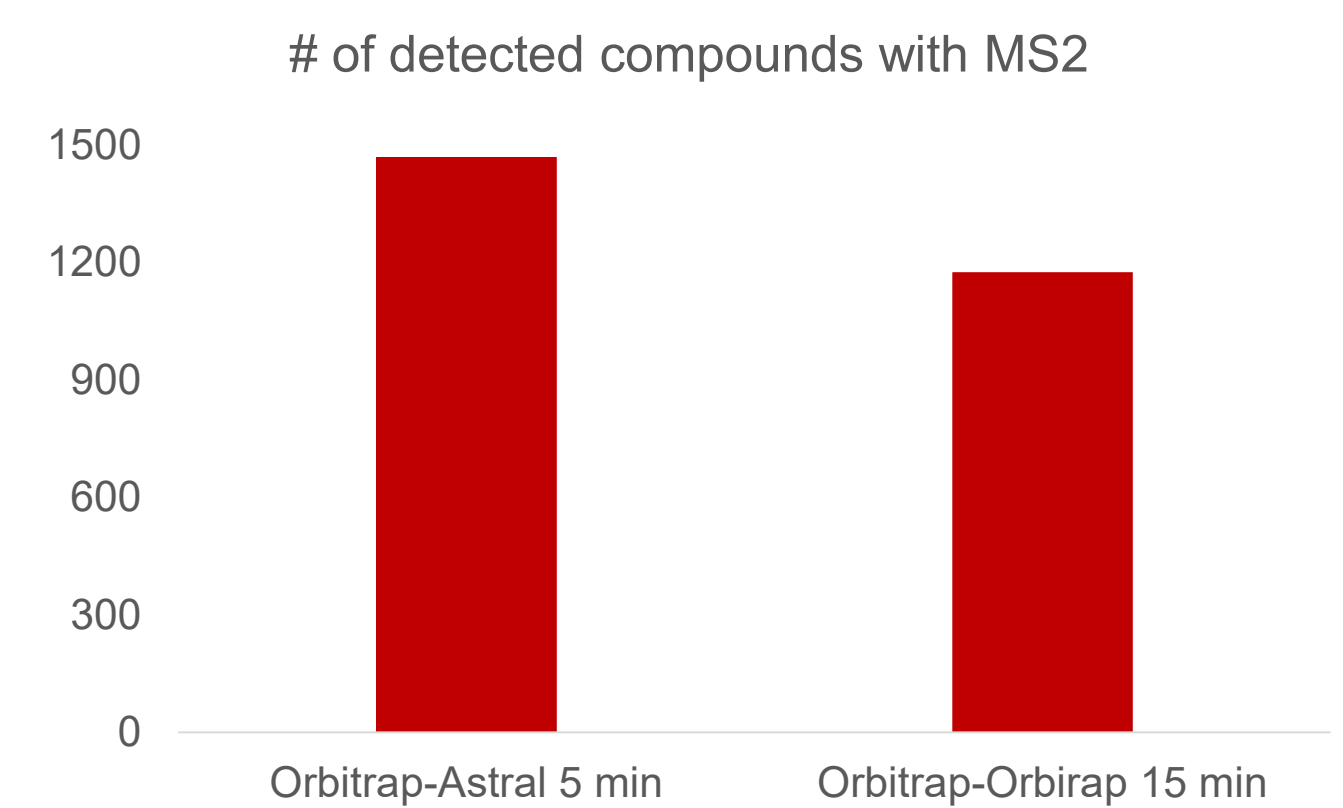


Figure 9. The total number of detected compounds in NIST plasma with MS² fragmentation using parallel Orbitrap MS¹ and Astral DDA top 30 MS² with 5-min LC gradient, and Orbitrap MS¹ and Orbitrap MS² with 15-min LC gradient. Data collected from Compound Discoverer analysis using ≥ 5 spectra/peak threshold.

This increases the number of annotated unknowns when using Orbitrap-Astral mode, Figure 10 A, due to higher scan speed and enhanced peak quality, Figure 10 B.

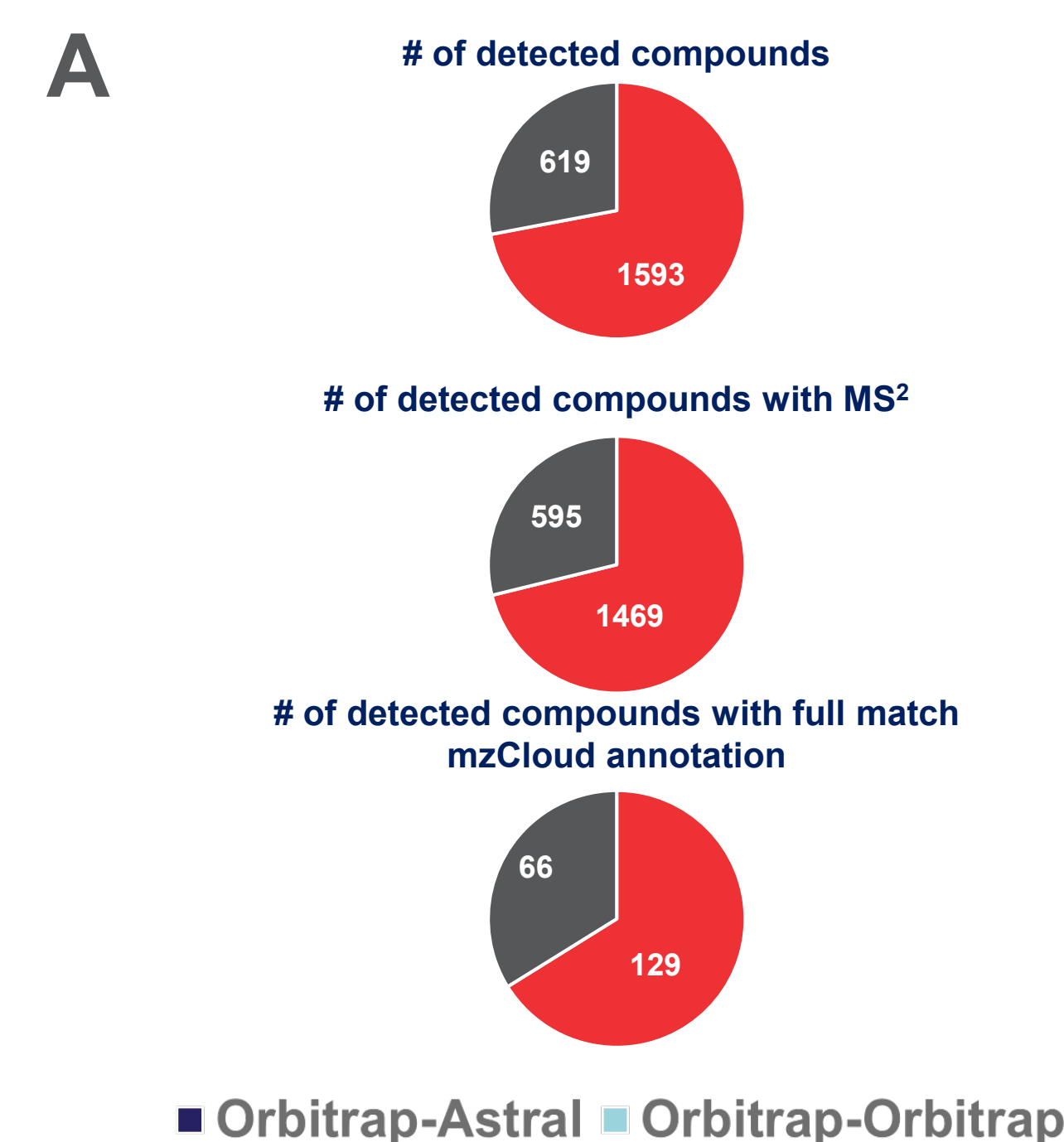


Figure 10. A) The total number of detected compounds in NIST plasma and percentage of spectra fragmentation, and B) the number of MS¹ scans per peak using parallel Orbitrap MS¹ and Astral DDA MS² (Orbitrap-Astral) vs. Orbitrap MS¹ and Orbitrap DDA MS² (Orbitrap-Orbitrap). Data collected from Compound Discoverer analysis using ≥ 5 spectra/peak threshold.

Conclusions

The novel Orbitrap Astral mass spectrometer provides a unique opportunity to perform a high-throughput SQUAD analysis without compromising the sensitivity and coverage utilizing the parallel operation of Orbitrap and Astral analyzers.

References

1. Amer, B., Deshpande, R., and Bird, S., Simultaneous Quantitation and Discovery (SQUAD) Analysis: Combining the Best of Targeted and Untargeted Mass. *Metabolites*, 2023, 13, 648.

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