Simultaneous Quantitation and Discovery (SQUAD) metabolomics workflow implementing parallel analysis on Thermo Ascend Tribrid instrument

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ABSTRACT

Purpose: Develop a single injection metabolomics approach that enhances productivity by utilizing the Ascend Tribrid system for both unknown identifications of potential biological significant features as well as accurate quantitation of already known selected compounds.

Methods: LC-MS quantitation of isotopically labeled internal standards spiked in NIST SRM 1950 plasma reference standard was performed on a Thermo Scientific™ Orbitrap Ascend™ mass detector using Thermo Scientific™ Hypersil GOLD™ HPLC as a pre-separation technique. Data were acquired in full-scan orbitrap MS^1 discovery in parallel to tMS^2 linear ion trap quantitation of targeted analytes.

Results: Ion trap quantitative results showed excellent sensitivity (e.g., down to 5 femto mole) and a great dynamic range (6 orders of magnitude) for the selected compounds. Data also showed 55% more MS^1 ions and 25% more MS^2 ions are measured with Ascend compared to other Tribrid platforms, which improved the number of annotated unknown compounds (i.e., 15% more annotated compounds).

INTRODUCTION

Here we introduce a single injection simultaneous quantitation and discovery (SQUAD) metabolomics that combines targeted and untargeted workflows on the Thermo Scientific™ Ascend Tribrid, Figure 1. SQUAD is used for the confident identification and accurate quantitation of a targeted set of metabolites. It also allows data retro-mining 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 experiment and address the two's limitations.

Figure 1. Single injection simultaneous quantitation and discovery (SQUAD) metabolomics.

In parallel, the Thermo Scientific™ Ascend Tribrid can run a quantitative assay using the sensitive linear ion trap without sacrificing the untargeted assay performed on the high-resolution accurate mass orbitrap analyzer. This fast alternating eliminates 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 and organic acids were purchased from Sigma and CIL, respectively. Plasma was spiked with a dilution series (1 nM – 2.5 mM) of the labeled compounds quantified against the corresponding endogenous compounds in NIST SRM 1950 plasma, Figure 2. Extraction was performed with 80% methanol. Reversed-phase chromatography was applied as the technique of choice for the pre-separation of the metabolites.

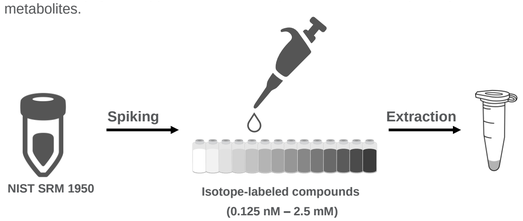

Figure 2. Spiking plasma with dilution series (1 nM – 2.5 mM) of isotopically labeled standards.

Data acquisition was performed on a Thermo Scientific™ Orbitrap Ascend™ system, a Tribrid-based mass spectrometer equipped with a high-resolution orbitrap and sensitive linear ion trap mass analyzers. Ascend facilitates a sensitive and high dynamic range tMS^2 quantitation utilizing the linear ion trap. It also allows MS^1 scanning on the orbitrap for higher annotation rates. AcquireX workflow was used to maximize the number of relevant compounds interrogated by MS/MS, resulting in higher confidence annotation. Thermo Scientific™ TraceFinder™ 5.1 and Thermo Scientific™ Compound Discoverer™ 3.3 software were used for data processing, analytes quantitation, and unknown annotation, Figure 3.

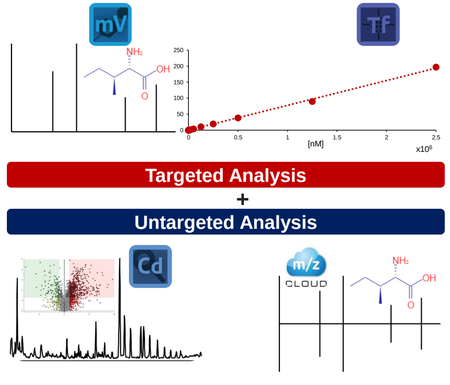

Figure 3. SQUAD workflow provides both targeted quantitation and untargeted discovery data analysis.

Results

The linear ion trap of the Tribrid system showed a higher sensitivity compared to the orbitrap analyzer. This was demonstrated by its higher ability to detect and quantify selected metabolites at low levels compared to the orbitrap, Figure 4.

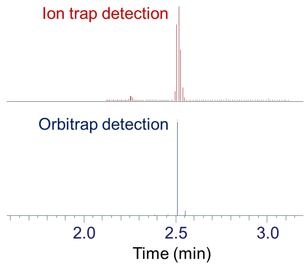

Figure 4. Linear ion trap mass analyzer for accurate and sensitive targeted analysis.

Using the SQUAD workflow on Ascend mass spectrometer enables sensitive tMS^2-based quantitation of selected metabolites with an extended linear dynamic range utilizing the linear ion trap, Figure 5 and Table 1.

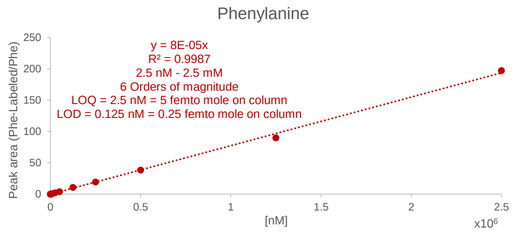

Figure 5. Absolute quantitation results for isotope-labeled phenylalanine spiked in NIST SRM 1950 plasma reference standard.

The Ascend linear ion trap operates at a range of user-specified scan rates to secure sufficient scans per peak for accurate quantitation at low concentrations, Figure 6.

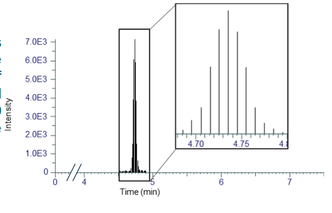

Figure 6. Linear ion trap mass analyzer for accurate and sensitive targeted analysis. The number of scans of the spiked isotope-labeled phenylalanine in NIST SRM 1950 plasma; 5 femto mole on the column.

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

Analyte	Calibration linear dynamic range	LOQ (femto mole on column)	LOD (femto mole on column)
Phenylalanine	2.5 nM – 2.5 mM (6 orders of magnitude)	5	0.25
Isoleucine	2.5 nM – 2.5 mM (6 orders of magnitude)	5	0.5
Leucine	2.5 nM – 2.5 mM (6 orders of magnitude)	5	0.25
Tyrosine	2.5 nM – 2.5 mM (6 orders of magnitude)	5	0.5
Tryptophan	2.5 nM – 2.5 mM (6 orders of magnitude)	5	0.5

The HRAM Orbitrap data and the increased percentage of fragmented compounds using the advanced deep scan AcquireX workflow resulted in improved annotation capability compared to traditional DDA on a wider dynamic range of plasma compounds. In addition, data acquisition on Ascend resulted in 55% more MS^1 ions and 25% more MS^2 ions compared to other Tribrid platforms, which improved the number of annotated unknown compounds (i.e., 15% more annotated compounds), Figure 7.

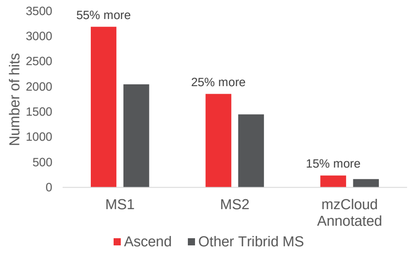

Figure 7. Number of ions with MS1, MS2, and mzCloud annotation using Ascend vs. other Tribrid MS.

CONCLUSIONS

SQUAD metabolomics technique is a promising alternative, offering researchers a new way to merge untargeted and targeted approaches in one single experiment. The recent advancement in the Ascend Orbitrap Tribrid mass spectrometer is a golden opportunity to perform a SQUAD analysis utilizing the sensitive linear ion trap for the quantitation of metabolites without sacrificing the discovery portion of the untargeted assay performed on the high-resolution accurate mass orbitrap analyzer. This fast alternating eliminates the variability of using multiple instruments and the need to re-inject limited biological samples.

TRADEMARKS/LICENSING

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