

Simultaneous Quantitation and Discovery (SQUAD) Tribrid Method Template Combining Ion Trap And Orbitrap Analysis

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ABSTRACT

Purpose: Showcasing a Tribrid method template utilizing both Ion Trap and Orbitrap for Simultaneous Quantitation and Discovery (SQUAD) analysis.

SQUAD analysis performs discovery in the Orbitrap, and in parallel, targeted MS² quantitation in ion trap. NIST SRM 1950 spiked with labeled amino acids was analyzed.

In a single run, both targeted ion trap data (for quantitation) and data dependent high resolution Orbitrap data (for unknown annotation) was collected. Data was analyzed using Compound Discoverer for annotating unknowns and TraceFinder for quantification.

The ion trap was capable of quantifying phenylalanine down to a LOD of 5 nM with a linear dynamic range spanning 6 orders of magnitude.

INTRODUCTION

Metabolomics is an evolving field that aims to identify and quantify as many metabolites as possible in a given sample. Experiments are broken down into targeted analysis, where predefined metabolites are quantified, and untargeted analysis for annotating unknown metabolites in a discovery experiment. A new method that includes both qualitative and quantitative analysis in one experiment is called Simultaneous Quantitation and Discovery (SQUAD).

The Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ Mass Spectrometer has a high-resolution Orbitrap and a highly sensitive Ion trap detector making it uniquely qualified for this type of experiment. An instrument control software method template was developed to acquire accurate mass data for untargeted analysis in Orbitrap, while highly quantitative data is collected in parallel by the ion trap.

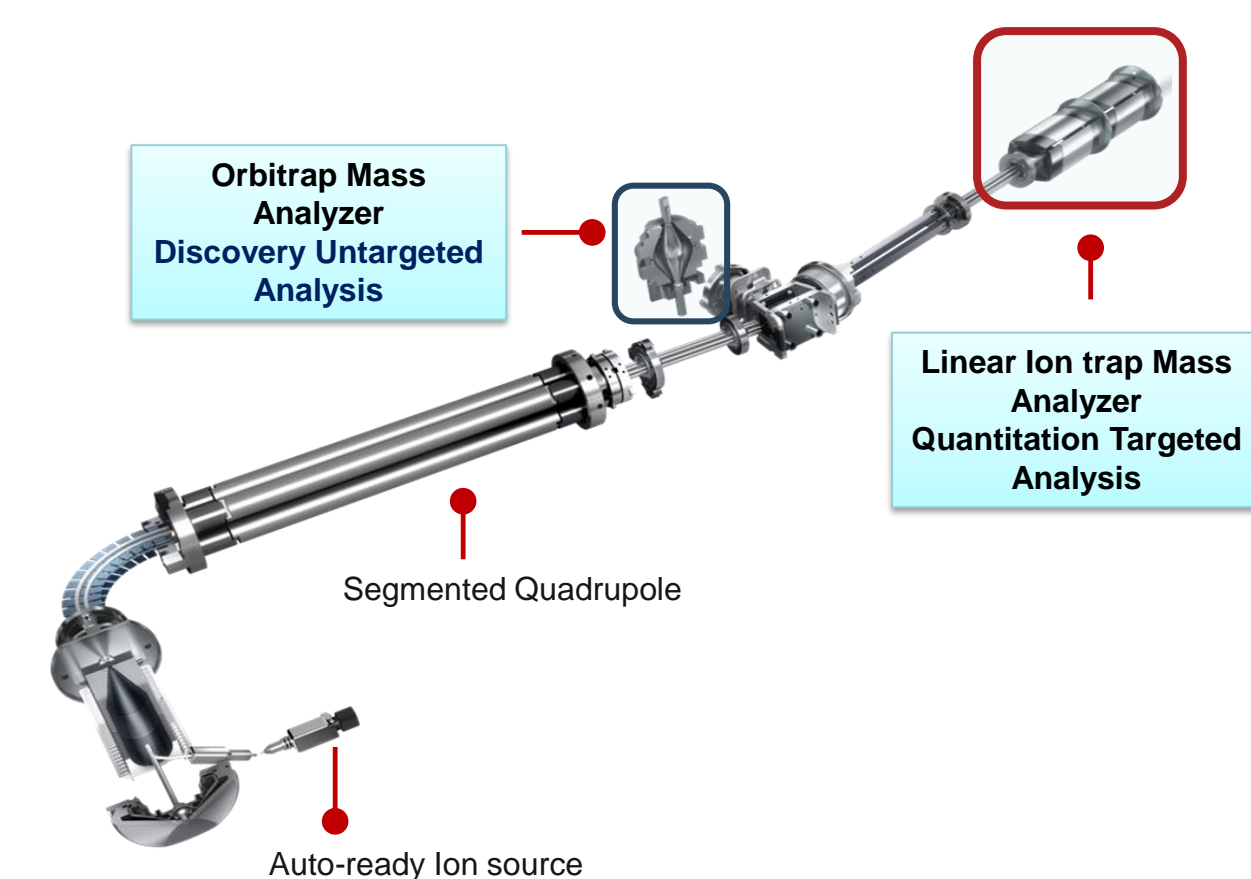


Figure 1. Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ mass spectrometer Instrument View

MATERIALS AND METHODS

Materials & Sample Preparation

LCMS grade Methanol, Water and formic acid were obtained from Fisher Scientific. Labeled amino acids were obtained from Cambridge Isotope Laboratories. NIST SRM 1950 (from Sigma-Aldrich) was spiked with a series of dilutions of labeled amino acids and extracted with 80% methanol.

Instrumentation

A Thermo Scientific™ Hypersil GOLD™ column connected to a Thermo Scientific™ Vanquish™ Horizon LC, Orbitrap IQ-X and Orbitrap Ascend were used to separate metabolites and perform SQUAD analysis.

Data-dependent MS² was collected in Orbitrap, while the Ion trap acquired targeted MS² for low-intensity standards in positive ion mode. The same sample was run using Thermo Scientific™ AcquireX™ to automatically generate an exclusion list for background compounds and focus fragmentation on metabolite ions.

Data Analysis:

Data were processed using Thermo Scientific™ Compounds Discoverer™ 3.3 and Thermo Scientific™ TraceFinder™ 5.1 software

Figure 2. Method editor Set up

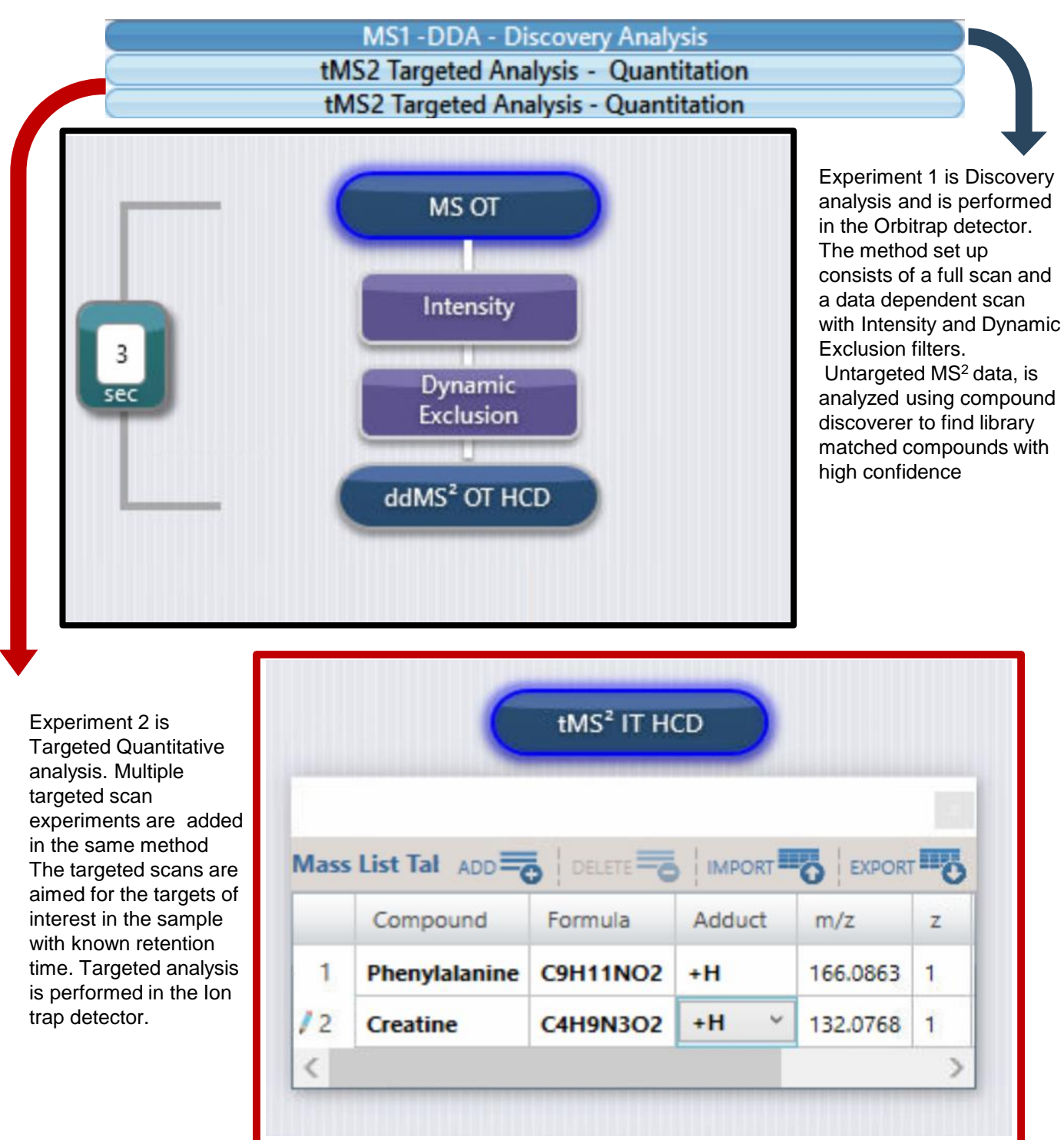
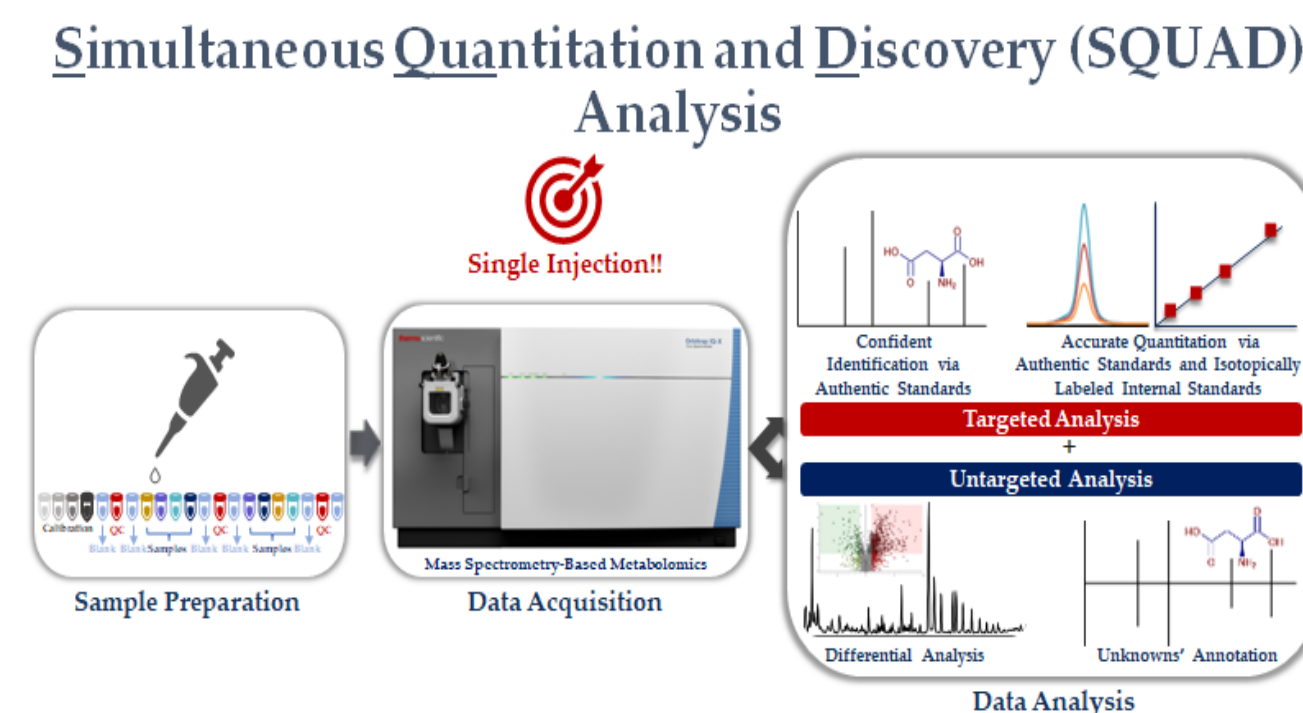


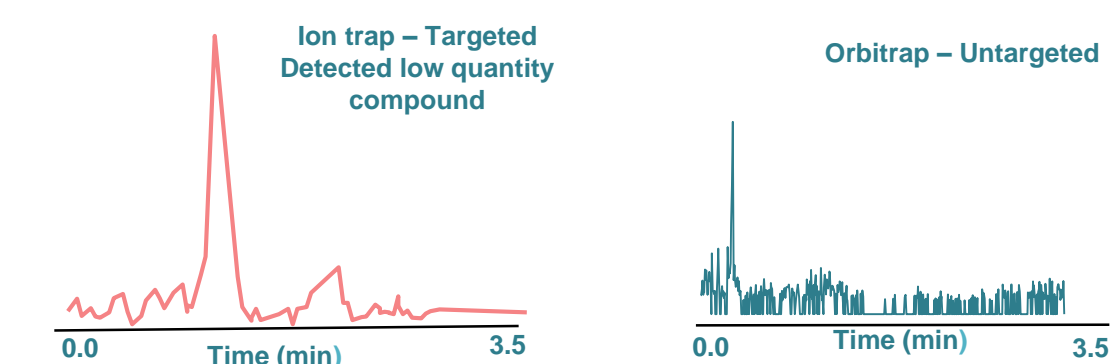
Figure 3. Complete SQUAD workflow



SQUAD analysis: Combining best of Targeted and Untargeted MS based metabolomics. Bashar A, et al. (in preprint), 2021

RESULTS

Figure 4. MS² scans NAE(16:0) lipid – In Orbitrap and Ion trap



In fig 4 Ion trap sensitivity is high enough to identify and quantify the low concentration metabolite

Figure 5. Comparison of number of MS² scans – In Orbitrap and Ion trap SM (d18:1/16:0)

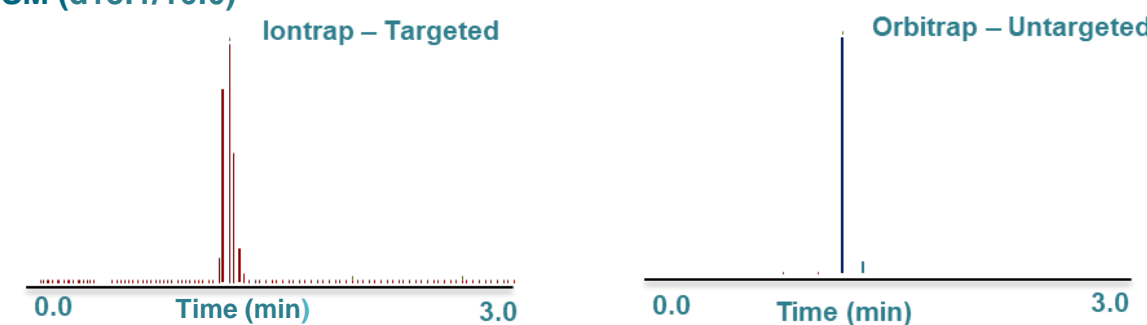
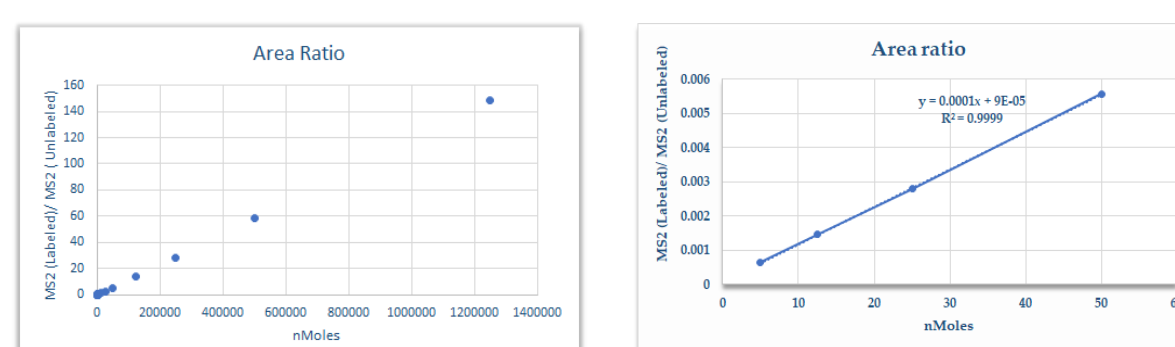
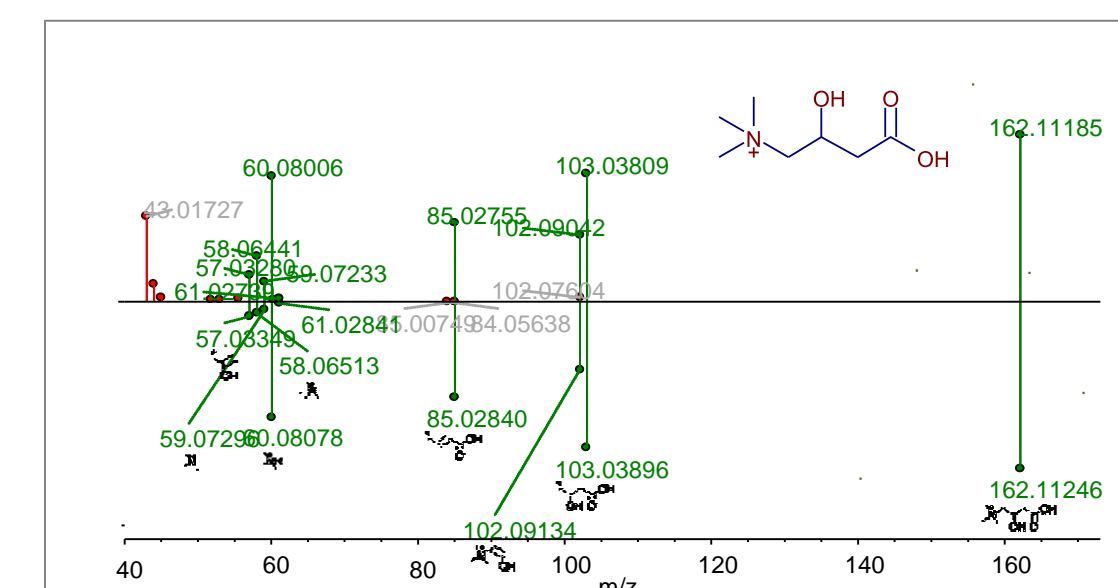


Figure 6. Ion Trap - Linear Dynamic Range Phenylalanine –1.25 5nM & zoom in on low concentrations



In fig 6 The Linear dynamic range for the quantified phenyl alanine is 6 orders of magnitude

Figure 7. Discovery analysis – Mirror plot – L-Carnitine – Identified and annotated in Compound discoverer



In fig 7 While tMS2 quantitative data was collected in the ion trap, untargeted discovery in the Orbitrap collected high resolution data to annotate unknown compounds such as L-Carnitine

CONCLUSIONS

Data-dependent MS² was obtained for labeled standards and NIST SRM plasma metabolites while targeted MS² was obtained for the labeled standards. The In targeted experiments using the ions trap, it was found that MS² yielded the diagnostic fragments at sufficient intensity and enough scan points across the peak for quantitation. Preliminary data demonstrates LLOD and LLOQ lower than 10 femtomoles for phenylalanine on the column for the targeted analytes utilizing the ion trap. High resolution MS² were analyzed by Compound Discoverer to generate annotations from multiple sources including mzCloud. The full scan for identified compounds have enough scans across peak for relative quantification. A method template that acquires high resolution Orbitrap data for confident identification of unknown compounds, that also employs Ion trap for identifying and quantifying low concentration targets serves as a unique method on all Orbitrap Tribrid mass spectrometers

REFERENCES

Abstract ID number: 315411
 Abstract title: Integrating quantification of low abundant targets into nontargeted LC-MS/MS screening using Simultaneous Quantitation and Discovery (SQUAD)
 Simultaneous Quantitation and Discovery (SQUAD) metabolomics: an intelligent combination of targeted and untargeted workflows using a novel mass spectrometer
 Simultaneous Quantitation and Discovery (SQUAD) metabolomics workflow implementing parallel analysis on Thermo Ascend Tribrid instrument.

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TRADEMARKS/LICENSING

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