

# A Semi-Targeted Tribrid Method Template for Combining Ion Trap and Orbitrap Analysis for single run Qualitative and Quantitative Metabolomics Results

Sunandini Yedla<sup>1</sup>, Brandon Bills<sup>1</sup>, Bashar Amer<sup>1</sup>, Rahul Deshpande<sup>1</sup>, Elys. P. Rodriguez<sup>2</sup>, Uri Keshet<sup>2</sup>, Oliver Fiehn<sup>2</sup>, Susan Bird<sup>1</sup>, Vlad Zabrouskov<sup>1</sup>  
<sup>1</sup>Thermo Fisher Scientific, 355 River oaks Pkwy, San Jose, CA 95134, USA ; <sup>2</sup>UCDAVIS West Coast Metabolomics Centre, DAVIS, CA

## 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 MS<sup>2</sup> 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.

Orbitrap Mass Analyzer  
 Discovery Untargeted Analysis

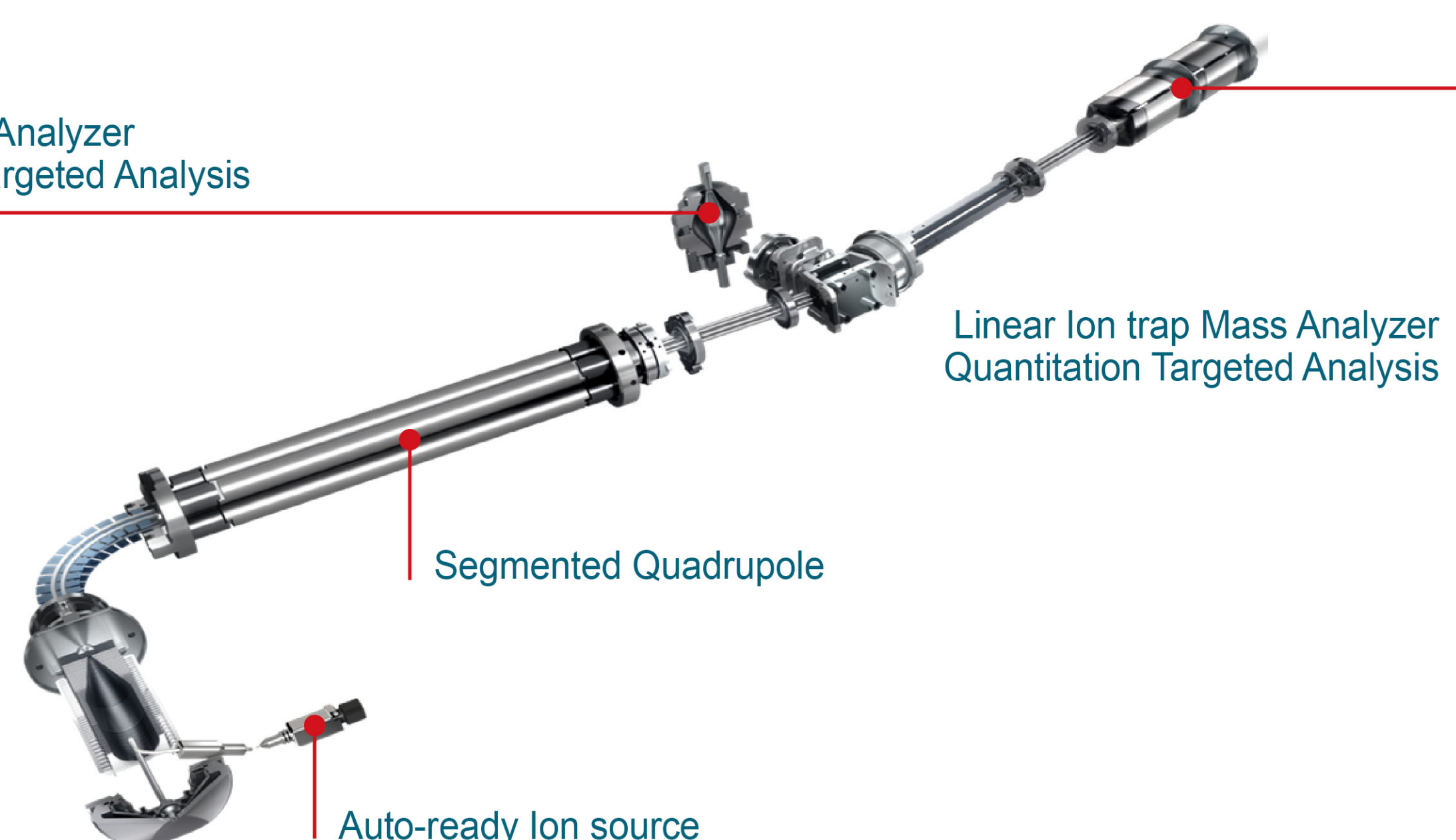


Figure 1 Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ mass spectrometer Instrument View

## 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

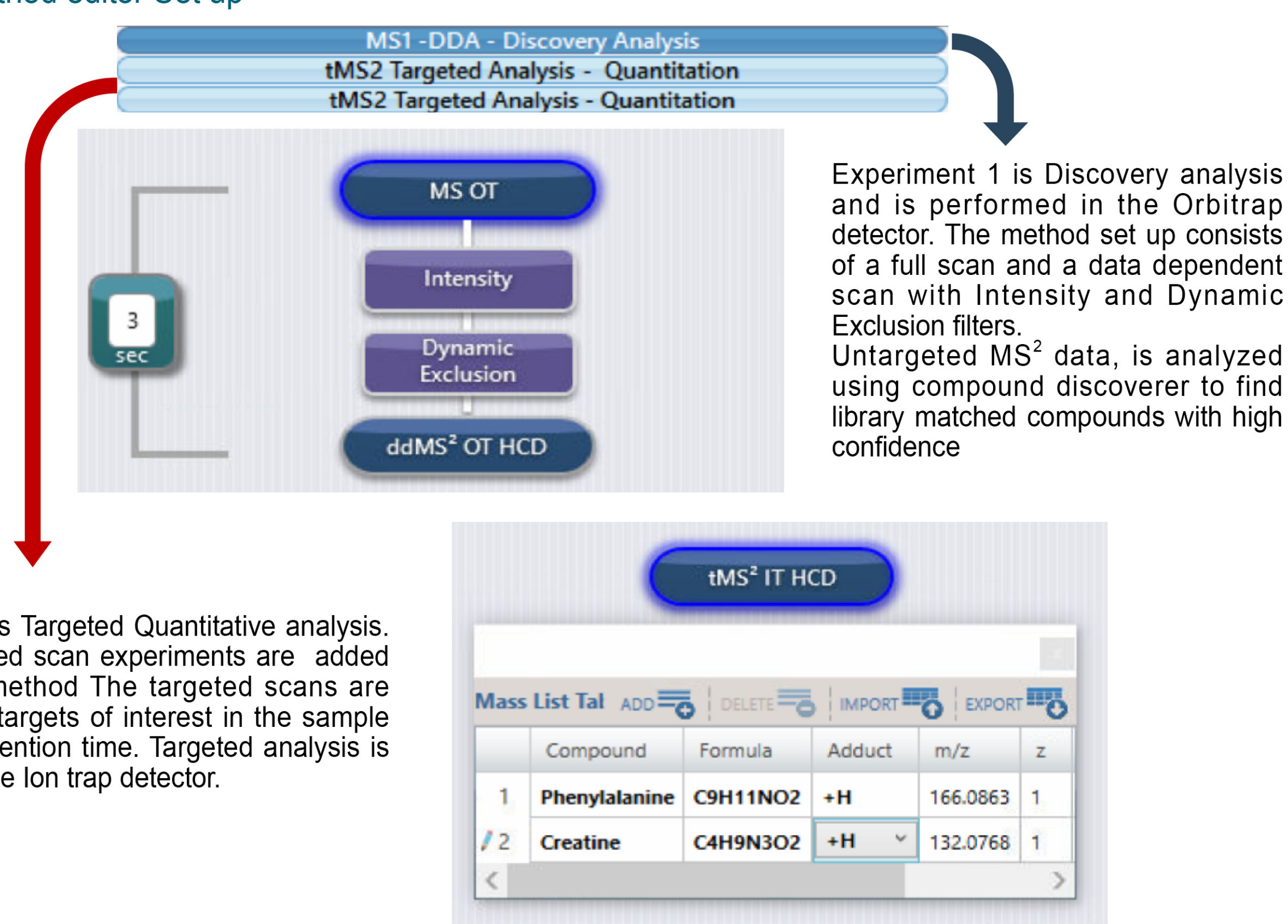
A Thermo Scientific™ Hypersil GOLD™ column connected to a Thermo Scientific™ Vanquish™ Horizon LC, Orbitrap IQ-X and Orbitrap Ascend were used to separate metabolites and perform SQUAD analysis.

Data-dependent MS<sup>2</sup> was collected in Orbitrap, while the Ion trap acquired targeted MS<sup>2</sup> 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

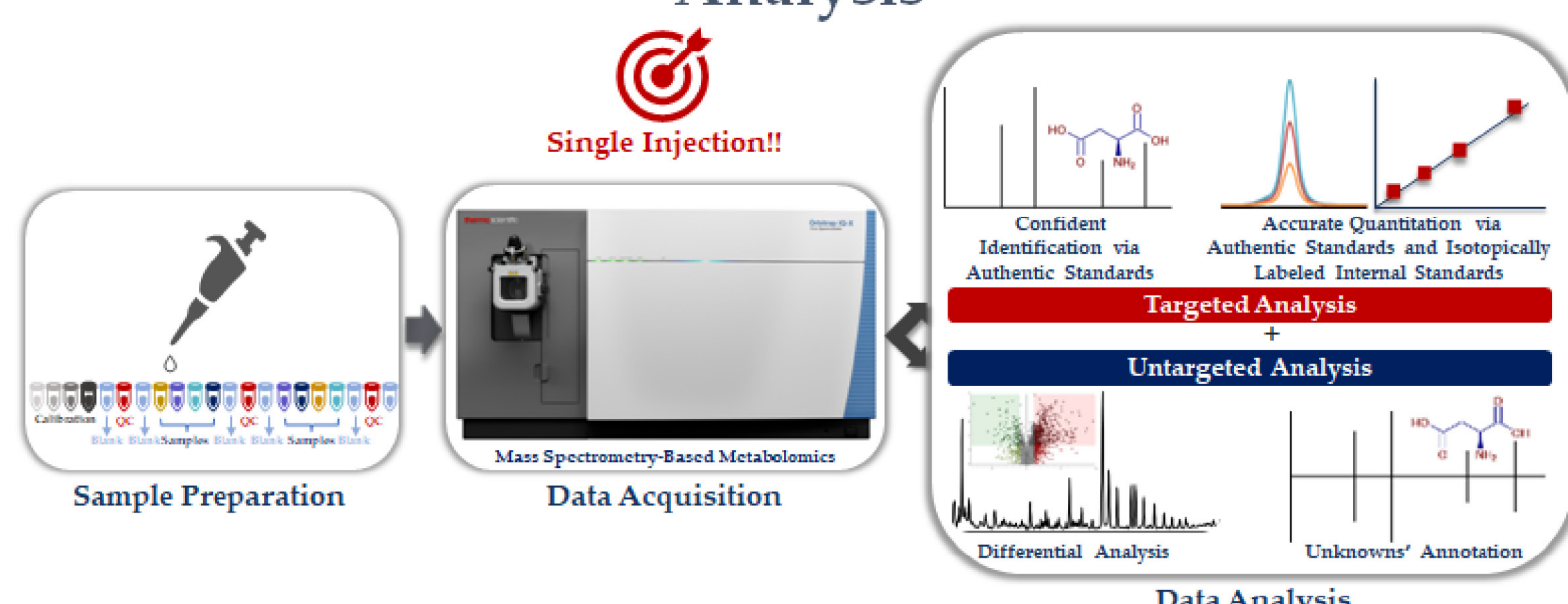
Figure 2 Method editor Set up



Experiment 2 is Targeted Quantitative analysis. Multiple targeted scan experiments are added in the same method. The targeted scans are aimed for the targets of interest in the sample with known retention time. Targeted analysis is performed in the ion trap detector.

Figure 3 Complete SQUAD workflow

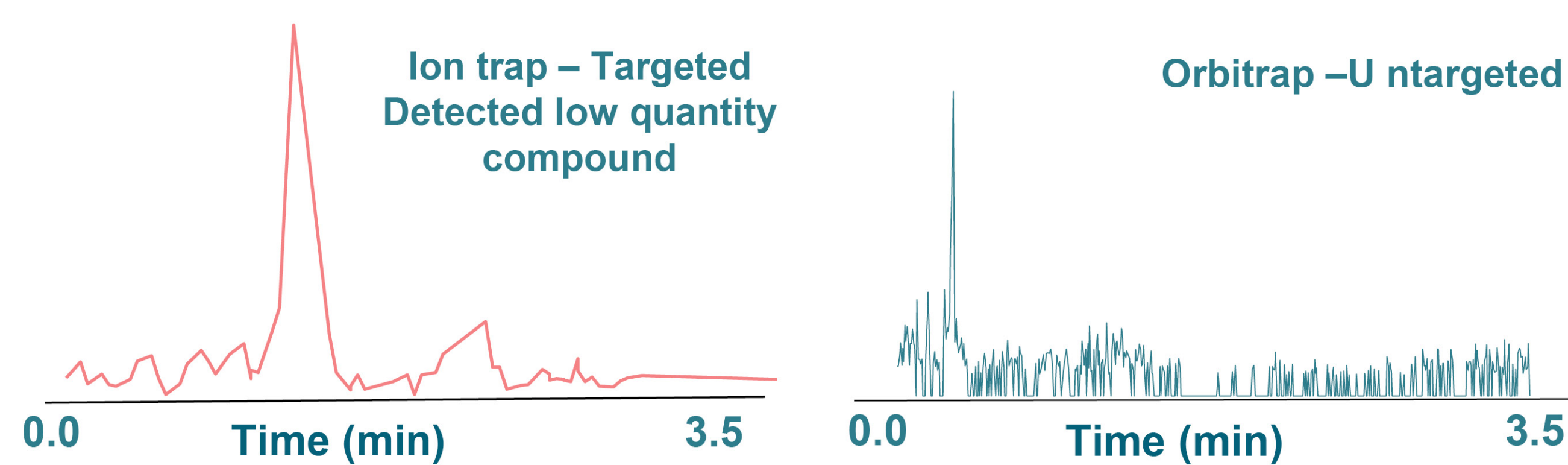
## Simultaneous Quantitation and Discovery (SQUAD) Analysis



SQUAD analysis: Combining both of Targeted and Untargeted MS based metabolomics. Bashar A, et al. (in preprint), 2023

## RESULTS

Figure 4 MS<sup>2</sup> 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 MS<sup>2</sup> scans – In Orbitrap and Ion trap SM (d18:1/16:0)

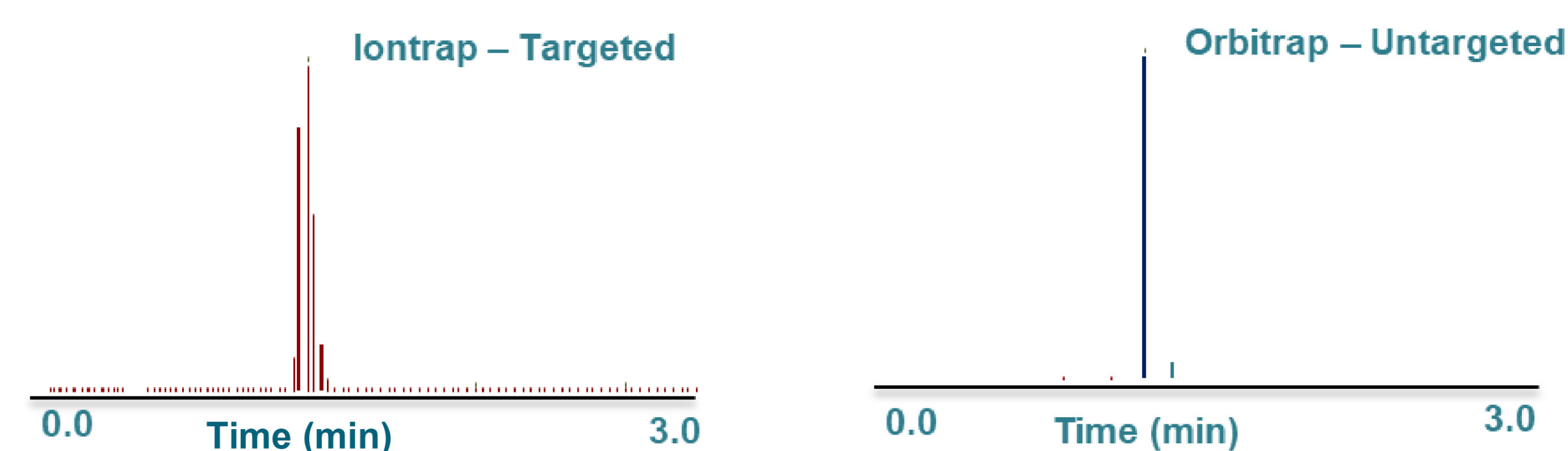
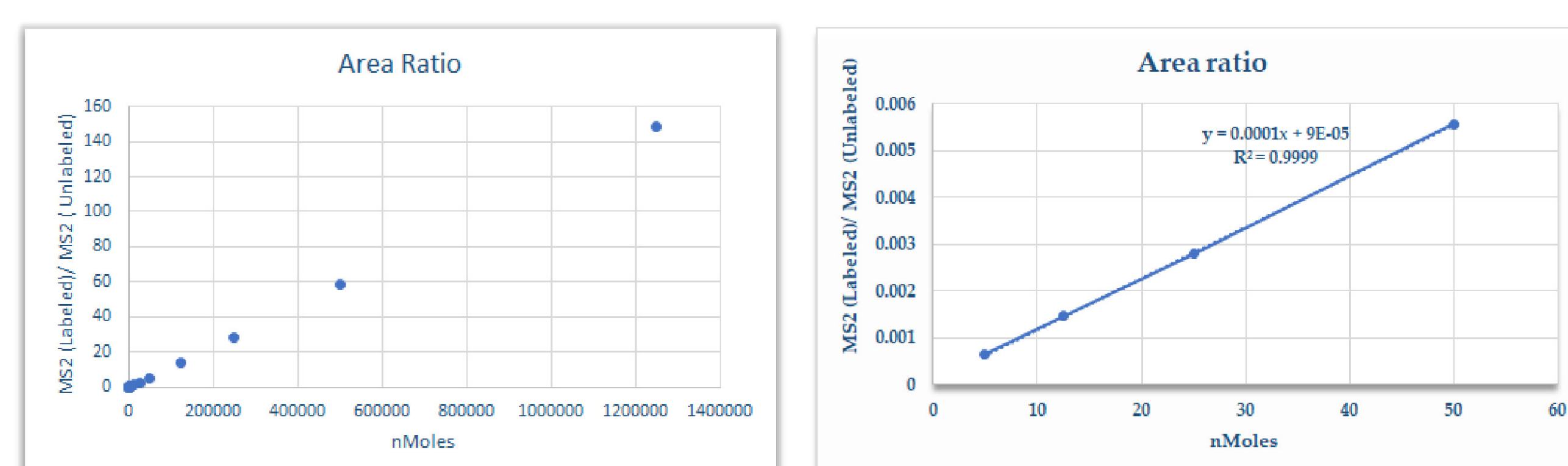
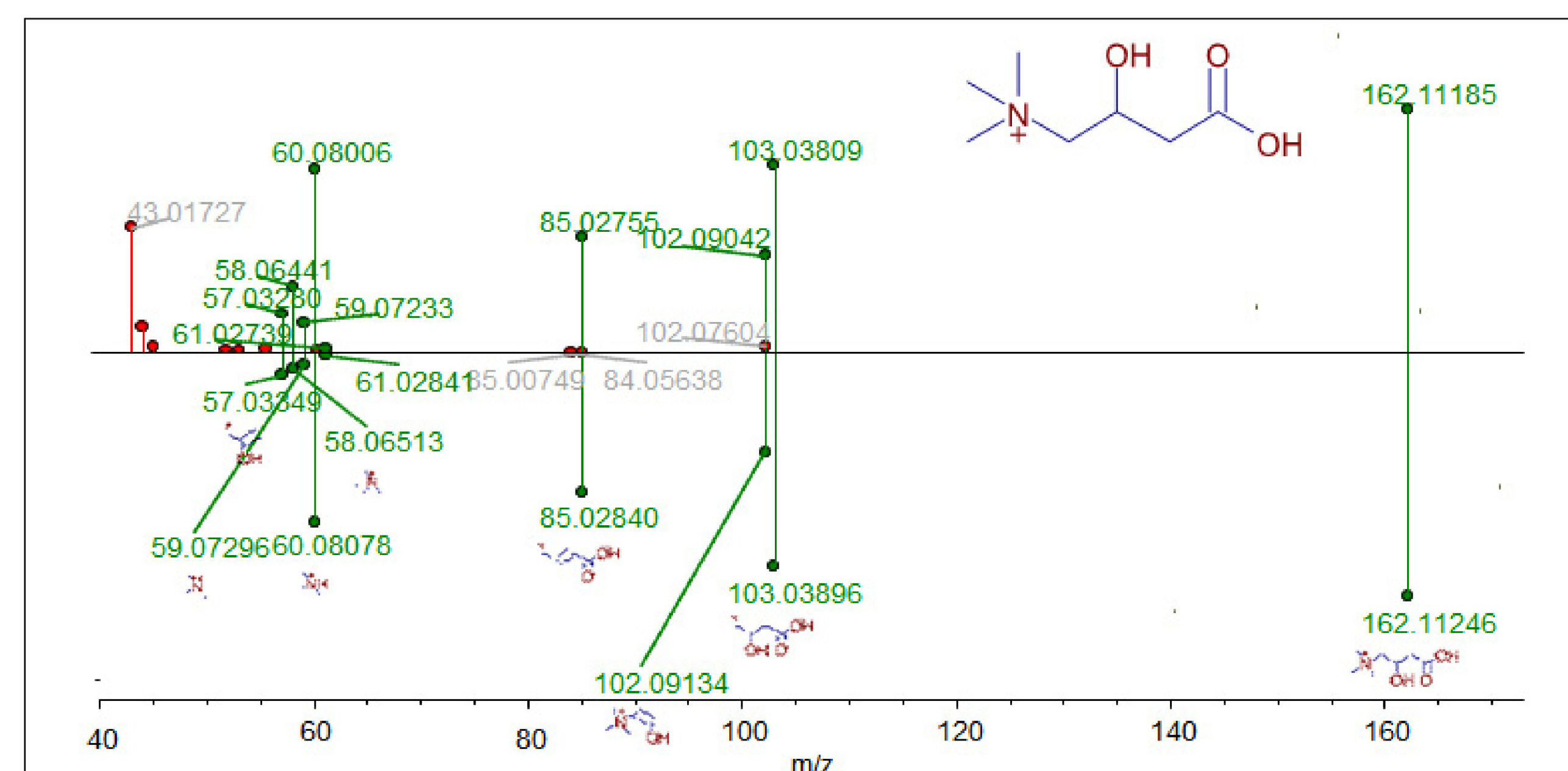


Figure 6 Ion Trap - Linear Dynamic Range Phenylalanine –1.25 nM & zoom in on low concentrations



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 MS<sup>2</sup> 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 MS<sup>2</sup> was obtained for labeled standards and NIST SRM plasma metabolites while targeted MS<sup>2</sup> was obtained for the labeled standards. The In targeted experiments using the ions trap, it was found that MS<sup>2</sup> 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 MS<sup>2</sup> 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) 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.

## ACKNOWLEDGEMENTS

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## TRADEMARKS/LICENSING

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