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

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ABSTRACT

Purpose: Showcase 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\(^2\) 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 lower dynamic range spanning 6 orders of magnitude.

INTRODUCTION

Metabolomics is an exciting field that aims to identify and quantify as many metabolites as possible in a given sample. Experiments are broken down into targeted analysis, where preidentified 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 high quantitative data is collected in parallel by the ion trap.

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-Altech) was spiked with a series of dilutions of labeled amino acids and extracted with 60% methanol.

Instrumentation

A Thermo Scientific™ Hypersil GOLD™ column connected to a Thermo Scientific™ Vanquish™ FTU LC. Orbitrap X™ and Orbitrap Accela were used to separate metabolites and perform SQUAD analysis.

Data-dependent MS\(^2\) was collected in Orbitrap, while the Ion trap acquired targeted MS\(^2\) for low-intensity standards in positive ion mode. The same sample was run using Thermo Scientific™ AccurateQuand™ to automatically generate an exclusion list for background compounds and focus fragmentation on metabolite ions.

Data Analysis

Data were processed using Thermo Scientific™ Compound Discoverer™ 3.3 and Thermo Scientific™ TraceFinder™ 3.1 software.

RESULTS

Figure 1. Targeted Quantitation Analysis - Quantitation

Experiment is Discovery analysis and is performed in the Orbitrap detector. The method is set to perform a data dependent scan with Intensity and Dynamic Exclusion filters. Untargeted data in, Untargeted Metabolites, is used for the discovery of new metabolites with high confidence.

Figure 2. Method editor set up

Figure 3. Comparison of number of MS\(^2\) scans – In Orbitrap and Ion trap

In fig 3, ion trap sensitivity is high enough to identify and quantify the low concentration metabolites while targeted MS\(^2\) was able to quantify the high concentration metabolites.

Figure 4. MS\(^2\) scans NAE(16:0) lipid

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

Figure 5. Linear Dynamic Range Phenylalanine

60.08078 is observed in the Ion trap, while the Orbitrap detector was unable to observe it.

Figure 6. Comparison of Ion Trap – Orbitrap

In fig 5, Linear Dynamic Range Phenylalanine – 1.25 Stm & zoom in on low concentrations

In fig 6, the linear dynamic range for the quantified (phenyl alanine) is 6 orders of magnitude.

CONCLUSIONS

Data-dependent MS\(^2\) was obtained for labeled standards and NIST SRM plasma metabolites while targeted MS\(^2\) was obtained for the labeled standards. The targeted experiments using the Ion trap, it was found that MS\(^2\) yielded the diagnostic fragments at sufficient intensity and enough scan points across the peak for quantification. Preliminary data demonstrates LODD and LLOQ lower than 10 ng/mL for phenylalanine on the column for the targeted analyses utilizing the ion trap. High resolution MS\(^2\) were analyzed by Compound Discoverer to generate annotations from multiple sources including mzCloud. The full scan for identified compounds have enough scan points 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

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