Metabolomics

High throughput targeted metabolomics library generation on a novel mass spectrometer applied to microbiome analysis

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Abstract

Purpose: The main objective of this poster is to showcase the capabilities of the Thermo Scientific™ Stellar™ mass spectrometer in developing and implementing a comprehensive LC-MS library for high-throughput, sensitive, and selective quantitation of fecal metabolites.

Methods: Three LC columns were used to separate compounds before analysis on the Thermo Scientific Stellar instrument with the Thermo Scientific™ Vanquish™ Horizon UHPLC system: Hypersil GOLD™ C18 reversed-phase, Accucore™-150-Amide-HILIC, and Acclaim™ Trinity™ P2.

Results: This study demonstrated the successful development and implementation of a fecal metabolites LC-MS library using the Stellar mass spectrometer, highlighting the instrument's capability for high-throughput quantitation analysis without compromising data quality.

Introduction

Recent studies underscore the essential role of the human gut microbiome in maintaining gastrointestinal health and influencing disease processes. Concurrently, there is a growing interest in exploring the fecal metabolome due to the promise of metabolomic analysis for clinical diagnostics. This connection between the microbiome and health highlights the value of metabolomic insights for diagnostic applications.

Targeted mass spectrometry (MS)-metabolomics techniques identify and quantify various fecal metabolites, including amino acids, fatty acids, lipids, and bile acids. However, traditional MS approaches often face limitations in speed and selectivity, which can restrict the number of metabolites analyzed. In response to these challenges, we developed a comprehensive fecal metabolite library using an innovative mass spectrometer; Thermo Scientific™ Stellar™ mass spectrometer. This advanced instrument combines rapid scan speed, high selectivity, and enhanced detection sensitivity, facilitating the analysis of both polar and non-polar fecal metabolites.

The Thermo Scientific Stellar mass spectrometer, shown in Figure 1, revolutionizes biomarker verification with its exceptional sensitivity and comprehensive compound coverage, outperforming existing technologies by tenfold and fivefold, respectively. By merging the robust quantitative capabilities of triple quadrupole technology with the rapid full scan MS^n acquisition of dual-pressure linear ion trap technology, the Stellar mass spectrometer expands its analytical reach to include a broader spectrum of compounds. Enhanced single-ion detection allows for precise quantification even with minimal sample loads, significantly reducing the risk of data loss.

Figure 1. The Thermo Scientific™ Stellar™ mass spectrometer.

Additionally, advanced software tools streamline the development, implementation, and data acquisition of complex targeted quantitative methods, eliminating the need for time-consuming replicate injections. These innovations position the Stellar mass spectrometer as an indispensable tool for transitioning potential biomarker candidates from discovery to validation in translational metabolomics and lipidomics research.

Materials and methods

Sample Preparation

A Fecal Metabolites library, containing over 500 compounds sourced from MetaSci, was prepared using various solvent mixes according to vendor instructions. Pure methanol was used for non-polar compounds, while methanol-water mixtures were used for medium and high-polar compounds. Calibration curves were created by combining aliquots of each standard.

Fecal samples were collected from 8-week-old mice subjected to different diets with varying fat sources for 29 days. Metabolites were extracted using 80% methanol before analysis.

Test Methods

Three LC columns were used to separate compounds before analysis on the Thermo Stellar instrument using a Thermo Scientific™ Vanquish™ Horizon UHPLC system: a Thermo Scientific™ Hypersil GOLD™ C18 reversed-phase column, a HILIC LC column Thermo Scientific™ Accucore™-150-Amide-HILIC, and a Thermo Scientific™ mixed mode column Acclaim™ Trinity™ P2. The autosampler maintained a temperature of 5˚ C, and LC-MS grade solvents were used to prepare mobile phase solutions. Each method used specific mobile phases and gradients: 0.1% formic acid in water and methanol for the reversed-phase method, 10 mM ammonium formate + 0.1% FA in water and 0.1% FA in acetonitrile for the HILIC method, and 40 mM ammonium acetate in water and 0.1% acetic acid in acetonitrile for the mixed mode method. Both long and short gradients were run to assess Stellar's high-throughput quantitation capabilities without compromising data quality.

Data Analysis

All data were acquired using Thermo Scientific™ Xcalibur™ Software. Targeted compound standards were used to prepare calibration solutions. Quantitation data were processed in Thermo Scientific™ TraceFinder™ Software 5.1.

Results

Intuitive and efficient development of high-scale targeted methods

The innovative mass spectrometer boasts rapid scanning capabilities (up to 140 Hz MS2 and 40 Hz MS3), swift polarity switching (5 ms +/-), and high sensitivity across a broad linear dynamic range. It provides exceptional selectivity with MS^n fragmentation using both Higher Energy Collisional Dissociation (HCD) and Collision Induced Dissociation (CID) techniques. These features streamline method development, optimizing parameters efficiently for each compound. Users can perform comprehensive experiments within a single method injection, including full-scan MS^1 with polarity switching and tandem MS^n experiments alternating HCD and CID modes, enhancing analytical flexibility and efficiency (Figure 2).

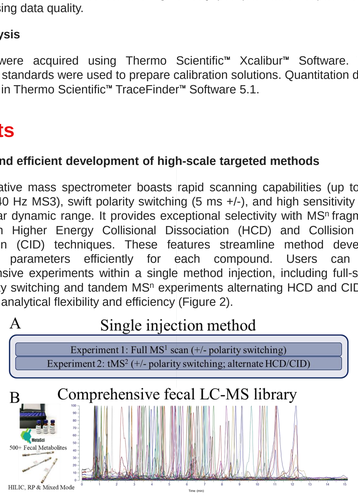

Figure 2. The innovative mass spectrometer's rapid scanning and high sensitivity enable intuitive method development and comprehensive analysis of fecal metabolites from the MetaSci library. These metabolites were separated using Thermo LC columns, including reversed-phase, HILIC, and mixed-mode columns.

High-throughput and sensitive targeted methods

The Stellar MS demonstrates rapid scanning capabilities, allowing the analysis of numerous analytes within a single injection method while maintaining accuracy and sensitivity by ensuring adequate scans per peak. Using gradients of less than 25 minutes, the methods showed heightened sensitivity and a broad linear dynamic range across five orders of magnitude, with tandem MS^2 quantitation preserving peak quality. In addition, the rapid scan speed and high selectivity of MS^n quantitation allowed for shorter LC gradients, highlighting the instrument's efficiency (Figure 3). These methods were used to measure metabolites in fecal extracts from mice subjected to different dietary interventions, with a heatmap (Figure 4) displaying quantified metabolites from the samples.

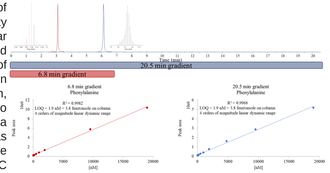

Figure 3. Illustration of the enhanced sensitivity and extended linear dynamic range achieved by employing gradients of less than 25 minutes on the mixed mode column, along with the ability to shorten LC gradients by a third. Phenylalanine was selected as an example from the mixed-mode LC gradient.

nM	SFA-rich diet	MUFA-rich diet	Standard diet
L-Tyrosine	93869	92406	79208
Citrulline	769	751	974
Adenine	2791	1745	2104
Methylguanidine	5492	1185	1286
Inosine	2889	873	1511
L-Phenylalanine	3331	5448	3486
Proline	11918	13195	6022
Thiamine	1784	2014	161
p-Hydroxymandelic acid	63	43	122
Carnitine	126	10	289
Choline	209	161	216
Atenolol	20	1	83
Kynurenic acid	127	9	302

Figure 4. Heatmap illustrating a subset of quantified metabolites from fecal samples collected from mice subjected to dietary interventions, using the established HILIC LC-MS method on the Stellar MS.

Methods robustness

The fragmentation mechanism of the Stellar was further assessed across diverse compounds, including numerous bile acids. This application note will demonstrate the fragmentation efficiency (HCD and CID) of a specific bile acid (i.e., deoxycholic acid – DCA) in comparison to counterparts found in Thermo Scientific™ mzCloud™ database. Figure 5 illustrates closely aligned CID fragmentation profiles between the Stellar and other platforms. However, notably enhanced HCD fragmentation was observed with the Stellar, characterized by the detection of fragment ions at lower collision energies compared to alternative platforms.

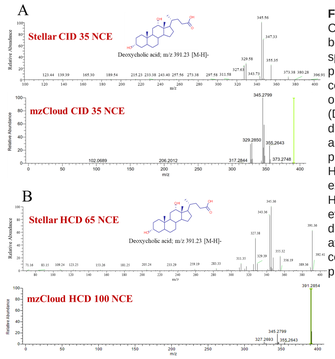

Figure 5. Comparison of CID fragmentation profiles between the Stellar mass spectrometer and other platforms for diverse compounds, with a focus on deoxycholic acid (DCA). The figure demonstrates closely aligned CID fragmentation profiles across platforms. However, the Stellar exhibits notably enhanced HCD fragmentation, as evidenced by the detection of fragment ions at lower collision energies compared to alternative platforms.

In a single method, both MS^1 full scan and PRM-based experiments were conducted to quantify bile acids in the mice's fecal samples, aiming for increased annotation rates and accurate quantitation. The RP-LC-based method exhibited high sensitivity (LOQ 12.7 femtomoles on the column for most analyzed bile acids) and a linear dynamic range spanning 5 orders of magnitude, Figure 6A. Isotope-labeled bile acids were utilized as internal standards (IS) to ensure precise quantitation and assess data quality, reliability, and measurement robustness, with metrics such as retention time, mass accuracy, and signal response evaluated. Minimal chromatographic shifts and consistent signal responses were observed, indicated by a low % coefficient of variation for sample replicates, while reproducible peak areas were consistently achieved for all internal standards throughout the acquisition period, Figure 6B.

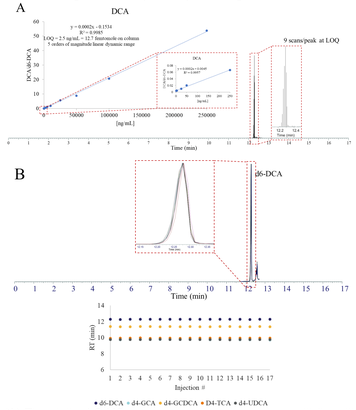

Figure 6. (A) The stellar LC-MS method demonstrates high sensitivity, with a limit of quantitation (LOQ) of 12.7 femtomoles on the column for most analyzed bile acids, and a linear dynamic range spanning 5 orders of magnitude. (B) Isotope-labeled bile acids served as internal standards (IS) to ensure precise quantitation and evaluate data quality, reliability, and measurement robustness. Deoxycholic acid (DCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA), and ursodeoxycholic acid (UDCA).

Methods selectivity

The proficient fragmentation capability of the Stellar mass spectrometer enables the detection of diagnostic MS^2 ions, facilitating the selective quantification of co-eluting isomers, particularly advantageous for high-throughput analyses employing short LC gradient-based methods. Figure 7 illustrates an exemplary application of utilizing MS^2 diagnostic ions for the selective quantification of co-eluting isomers.

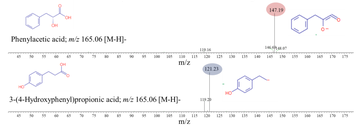

Figure 7. Demonstration of the utilization of diagnostic MS^2 ions for selective quantification of co-eluting isomers, enabled by the proficient fragmentation capability of the Stellar mass spectrometer.

The Stellar mass spectrometer further enhances selectivity by enabling rapid fragmentation and detection at both the MS^2 (up to 140 Hz) and MS^3 levels (up to 40 Hz). Initially, chromatographic separation of bile acid isomers GCDCA and GDCA required ion-pairing reagents, leading to lengthy methods and high solvent consumption (Figure 8A). However, efforts to reduce additives and flow rates resulted in chromatographic co-elution of the isomers, preventing gradient shortening (Figure 8B). MS^2 fragmentation produced similar patterns, hindering PRM quantitation (Figure 8C). Yet, the instrument's MS^n capability allowed the detection of diagnostic ions for each isomer, enabling accurate quantitation in high-throughput analyses without the need for ion-pairing reagents or high organic solvent flow rates (Figure 8D).

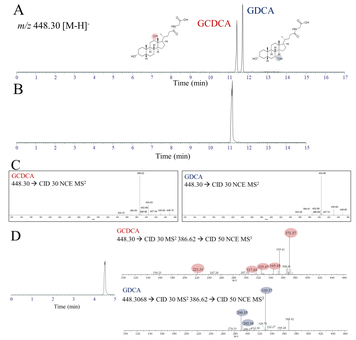

Figure 8. Illustration of the capabilities of the Stellar mass spectrometer, showcasing rapid MS^2 and MS^3 levels fragmentation to enhance the selectivity of co-eluting isomers. Initially, chromatographic separation of bile acid isomers GCDCA and GDCA necessitated ion-pairing reagents (A). However, reducing additives and flow rates led to co-elution of the isomers (B) precluding gradient shortening. MS^2 fragmentation generated similar patterns (C), impeding PRM quantitation. Yet, the instrument's MS^n capability facilitated the detection of diagnostic ions for each isomer, enabling accurate quantitation in high-throughput analyses without ion-pairing reagents or high solvent flow rates (D).

Conclusions

The Stellar MS's proficiency in handling complex sample matrices and co-eluting isomers through rapid MS^2 and efficient MS^3 level fragmentation further highlights its versatility and efficiency. Overall, the Thermo Scientific™ Stellar™ mass spectrometer emerges as an indispensable tool for high-throughput, sensitive, and selective biomarker verification, significantly advancing the field of metabolomics and lipidomics.

Acknowledgments

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