# High-Throughput and Highly Selective Quantitative Lipidomics with the Stellar Mass Spectrometer – A Novel Hybrid Nominal Mass Instrument

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## Abstract

**Purpose:** This study aims to enhance lipidomics workflows by leveraging the Thermo Scientific™ Stellar™ mass spectrometer to improve specificity, sensitivity, and throughput in lipid quantitation, addressing limitations in existing clinical and translational lipidomics methods.

**Methods:** We evaluated the Stellar MS using a HILIC-MS/MS platform on the 1950 NIST plasma reference material, originally measuring over 1000 lipids in 12 minutes on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer. By integrating LipidCreator, we developed a targeted lipid panel with multiple transitions per species, enhancing annotation specificity and quantitative performance.

**Results:** The Stellar MS approach increased the diversity of screened lipid species by over 50% and improved peak definition and scan density by at least 20%, enabling more precise lipid identification. These advancements establish a high-throughput, selective, and sensitive method for lipidomics research and clinical applications.

## Introduction

The increasing prevalence of metabolic disorders underscores the need for robust, high-throughput clinical lipid panels and lipidomics methods to quantify lipid species comprehensively, with high precision and selectivity . Structurally related lipids, with varying fatty acid composition, were associated with distinct metabolic consequences and proven to have different biological roles. Thereby, measuring individual lipid species with highest selectivity possible is crucial for corroboration of clinical utility including the improved risk prediction and diagnostics.

We present an advanced methodology leveraging the unique capabilities of the new Stellar mass spectrometer. This nominal mass instrument addresses current limitations in specificity and throughput by enabling fast acquisition parallel reaction monitoring, and revolutionizing lipidomics workflows by delivering unprecedented specificity and quantitation. This system combines a quadrupole and a dual cell linear ion trap that significantly improves the ion accumulation efficiency and scan speed. This allows for the acquisition of full MS2 spectra including multiple transitions, thus improving the annotation specificity by resolving isomeric lipids and structurally similar species. This unique capacity of Stellar MS positions it as a practical solution for translational research, biomarker discovery and clinical lipidomics where rapid, sensitive and selective lipid quantitation is essential

## Materials and Methods

### Sample Preparation

Dried UltimateSPLASH ONE internal standard mixture (100 µL) was reconstituted in 25 µL of NIST human plasma SRM 1950 followed by the addition of 125 µL of isopropanol for lipid extraction. The supernatant was then transferred to LC vials for LC-MS analysis.

*Linearity assessment:* different volumes of Avanti Research™ UltimateSPLASH™ ONE internal standard mixture (0.5, 1, 5, 10, 50, 100, and 500 µL) were evaporated to dryness, followed by the reconstitution with 25 µL NIST Plasma and 125 µL of isopropanol for lipid extraction.

### Data Acquisition

Plasma lipid extracts were separated on a ACQUITY Premier BEH Amide column (1.7um, 100 mm x 2.1 mm I.D., Waters) and analyzed using a targeted LCMS lipidomics approach on Thermo Scientific™ Vanquish™ Duo UHPLC system coupled to a Stellar mass spectrometer (Figure 1)[1]. This method was built from a well-established selected reaction monitoring (SRM) method that we Converted to a parallel reaction monitoring (PRM) method by using only the unique precursors. As there are many isomeric lipids sharing the same mass, converting the method for the Stellar reduced 1,019 (579 positive mode; 440 negative mode) SRM precursors to 524 (233 positive mode; 291 negative mode) PRM precursors.

### Data Analysis

PRM data was processed using Skyline-Daily software[3]. Methods were optimized using PRM Conductor, a new Skyline-based external tool. Transition lists used for analysis were created using a third party, Skyline-based tool, LipidCreator[4]. Further analysis and figures were created using R studio.

## Results

**Figure 1. Stellar MS diagram[1]**

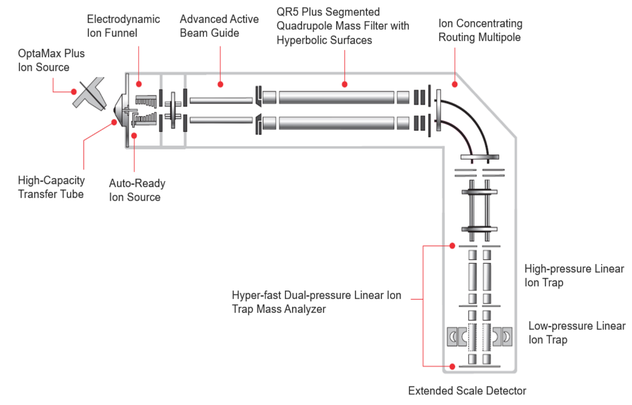
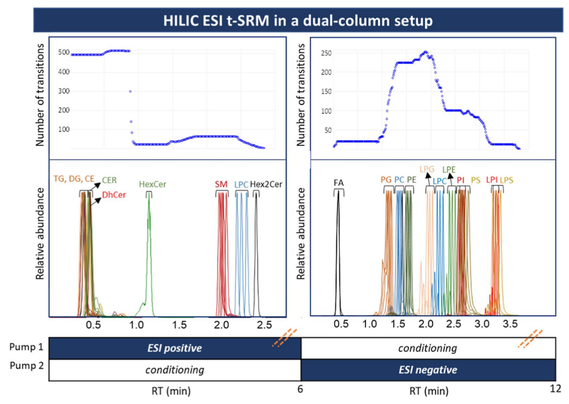


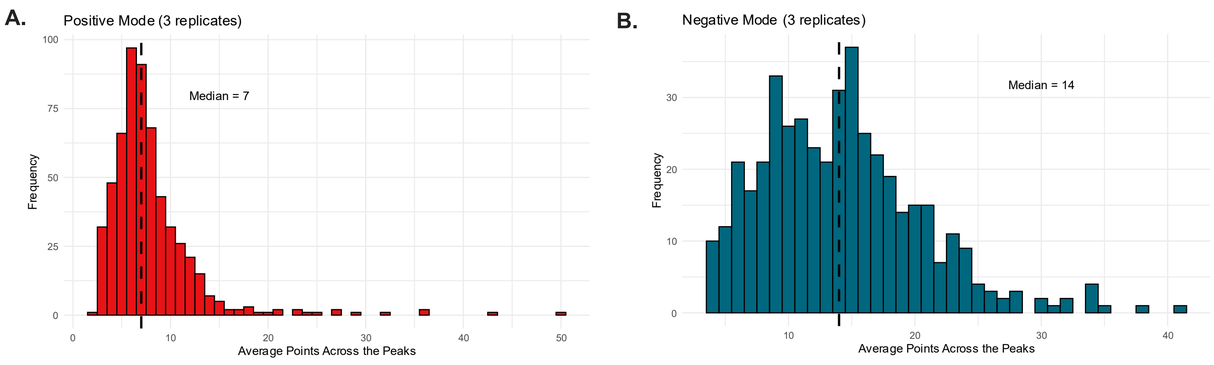

**Figure 2. Previously published SRM-based approach using HILIC chromatographic separation has become a well established lipidomics method[2]**




### Building on the High Throughput SRM-based Lipidomics Approach

A high coverage SRM-based targeted assay was previously reported by Medina et al.[2] for the semi-quantification of over 1000 lipid species. Lipids are quickly released from the column within the first 3.5 minutes leaving the rest of the time for the column to condition for the next run. Using the Vanquish Duo UHPLC system and two columns, both positive and negative modes can be acquired and conditioned in parallel all within 12 minutes.

**Figure 3. Comparing Converted SRM-based Method to PRM Stellar MS Method**

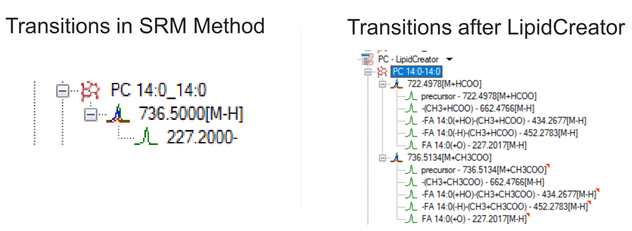



We initially validated the method on the Stellar MS using a selected reaction monitoring (SRM)-based approach, where each precursor ion was paired with a single product ion. All targeted precursor-product ion transitions were successfully detected, with an average of 7 data points per peak in positive mode and 14 data points per peak in negative mode, confirming the method's feasibility for implementation on Stellar MS (Figure 3).

### Reprocessing with Expanded Transitions to Evaluate Detection Efficiency

LipidCreator enabled automated and comprehensive access to all precursor-product ion transitions for lipid classes and species matching the original SRM method and exports directly into Skyline. In positive mode, the number of targeted molecules increased from 579 (with 579 transitions) to 2,713 molecules with 15,399 transitions. Similarly, in negative mode, coverage expanded from 440 molecules (with 440 transitions) to 2,611 molecules with 22,020 transitions, significantly enhancing lipid annotation and quantitation capabilities.(Figure 4).

**Figure 4. Expansion of Targeted Lipid Coverage Using LipidCreator for PRM-Based Analysis. Example PC 14:0-14:0 in negative mode**




Without acquiring new data, we utilized the expanded transition list from LipidCreator to reprocess existing data and assess transition detection. By applying the new list, we evaluated how many transitions were detected, refined by keeping those molecules with a ratio greater than 0.5 for a given precursor, demonstrating the improved coverage and sensitivity of the method.

Figure 5. illustrates a combined bar and line chart illustrating the number of detected lipid species by major lipid classes. The bars indicate total lipid counts, while the line represents average data points across peaks, with overall averages of 10 for positive mode and 15 for negative mode. This visualization highlights lipid abundance and sampling efficiency.

**Figure 5. Optimized Identification and Quantification of Sphingomyelins Using Stellar MS**

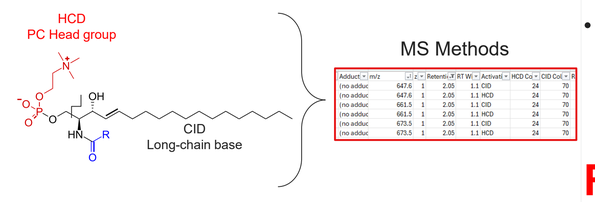



**Figure 5. Detection of Lipid Species and Peak Sampling Density Across Lipid Classes**

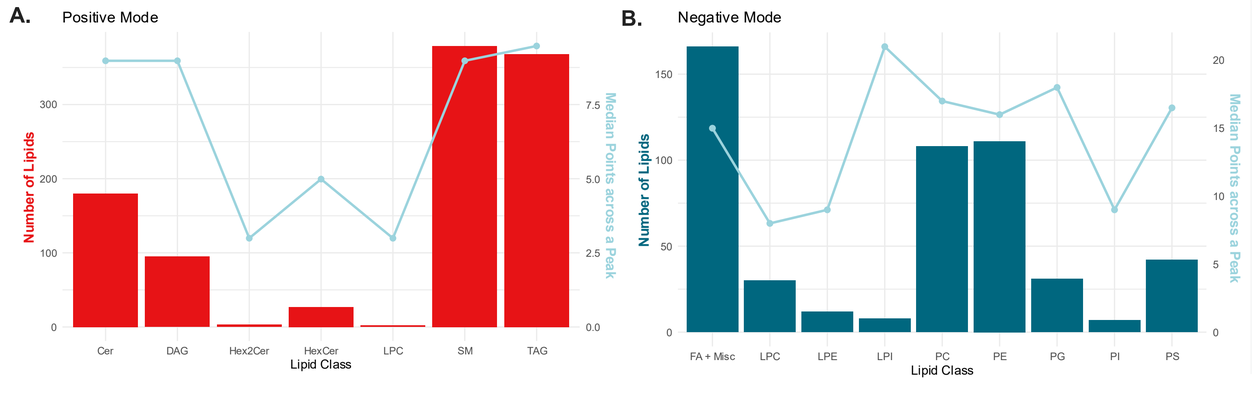



**Figure 7. Distribution of Transitions per Molecule Across Lipid Classes**


Unlike the original method, which utilized one transition per precursor, most lipids now feature over two transitions, with an average of over three transitions per molecule in both positive and negative modes (Figure 7). Further, we can see the variation in transition counts across different lipid species.

## Conclusions

- By integrating LipidCreator with Stellar MS, we expanded on an already thorough lipid workflow by increasing the number of targeted lipids (>50%) and transitions (>3/molecule) enabling higher confidence in lipid identification
- Multiple transitions per precursor, combined with fast scan speeds, enhanced both the specificity and accuracy of lipid quantification, particularly for isomeric species.
- Employing both HCD and CID simultaneously allowed for more robust characterization of sphingomyelins seen in the 2-3 transitions per species.
- This high throughput, targeted approach addresses key limitations and provides a efficient platform for biomarker discovery
- Also, look for our other poster showing a high-throughput targeted MS3 approach has been optimized on the Stellar MS, for the semi-quantification of TG species in human plasma.

## References

1. Remes et al. *BioRxiv* preprint, 2024. https://doi.org/10.1101/2024.05.31.596848
2. Medina et al. *Anal.Chem* 2023, PMID: 36716250
3. Adams et al. *J Proteome Res* 2020, PMID: 31984744
4. Peng et al. Nature Comm 2020, PMID:32345972

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