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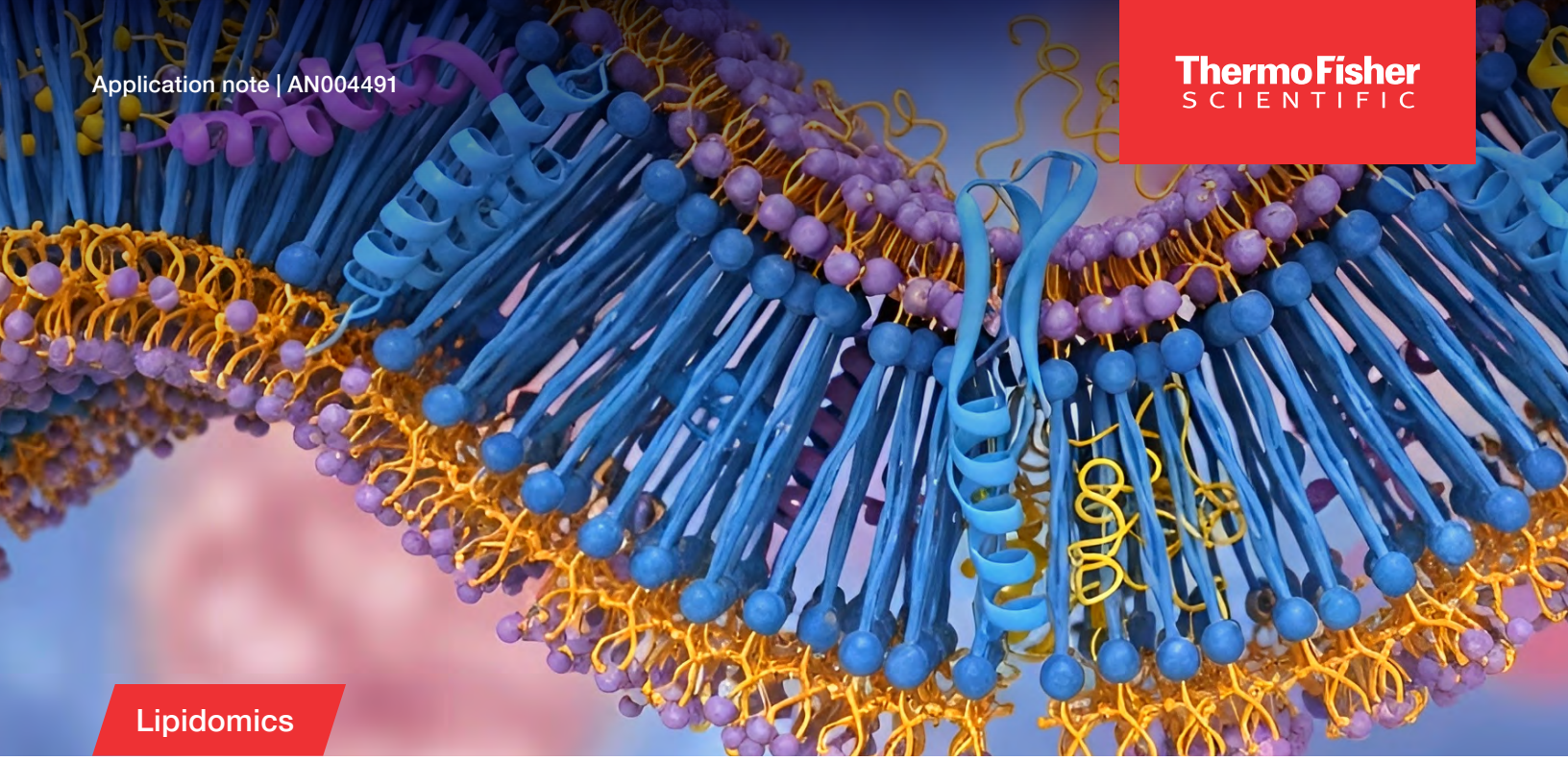

Lipidomics

Go beyond standard targeted lipidomics: Elevating SRM methods with PRM on the Stellar mass spectrometer

Seamlessly transition from SRM to PRM for faster, broader, and more confident lipid quantification

Authors

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Keywords

Parallel reaction monitoring (PRM), linear ion trap, selective reaction monitoring (SRM), multiple reaction monitoring (MRM), targeted lipidomics, Stellar MS, SRM-to-PRM method transfer, HILIC LC–MS/MS, full-scan MS^2 acquisition, expanded product-ion coverage, quantitative lipid analysis, isomeric lipid identification, LipidCreator tool, Skyline software, high-throughput lipid profiling

Goal

Demonstrate that existing selective reaction monitoring (SRM) based targeted lipidomics methods can be seamlessly transferred to full-scan parallel reaction monitoring (PRM) acquisition on the Thermo Scientific™ Stellar™ Mass Spectrometer, delivering broader lipid coverage, improved structural insight, and enhanced quantitative performance without requiring extensive method redevelopment.

Introduction

Global lipid profiling is transforming how researchers map metabolic pathways, uncover disease mechanisms, and discover biomarkers. However, the same SRM workflows that provide quantitative precision can also limit qualitative selectivity by capturing only the predefined product ions selected during method development. Many laboratories have invested significant time developing targeted SRM assays on triple-quadrupole mass spectrometers (QQQ MS), carefully optimizing transitions, validating retention times, and building extensive method libraries. These methods have long represented the gold standard for targeted lipidomics analyses, but recent advances in mass spectrometry make many of the inherent limitations of SRM unnecessary.

To address these limitations while maintaining the strengths of established workflows, the Stellar mass spectrometer allows researchers to preserve the value of their existing SRM methods while expanding analytical performance through PRM. The Stellar MS is designed to select precursors using a quadrupole mass filter, dissociate in a second quadrupole, scan in a linear ion trap, and then detect in a photomultiplier. Using the same precursor mass-to-charge information, retention times, and normalized collision energies, existing SRM assays can be transferred directly to a Stellar MS PRM workflow with minimal adjustment. This transition is straightforward yet transformative.

A key improvement lies in how the Stellar MS handles data acquisition. Because the instrument acquires full-scan MS^n spectra, only unique precursor ions need to be targeted. Removing redundant transitions frees acquisition time that can be used to collect more data points across each chromatographic peak, adding the ability to improve quantitative precision or to monitor a greater number of precursor ions in a single run. When combined with hydrophilic-interaction liquid chromatography (HILIC) for class-based lipid separation, the PRM workflow provides broad lipid coverage, consistent retention behavior, and reproducible performance across complex biological samples.

Building on this streamlined acquisition, the LipidCreator™ tool[1] within Skyline™ software[2] simplifies both method setup and data analysis. It automatically generates processing files that include all product ions for each lipid species rather than only the few predefined transitions used in SRM. This comprehensive approach provides a complete view of each lipid's fragmentation pattern, greatly enhancing structural interpretation and confidence in annotation, particularly for distinguishing isomeric and isobaric species.

Together, these capabilities form the foundation for the work described here. This application note demonstrates how the previously published HILIC global lipid SRM method by Medina et al.[3] can be easily transferred and enhanced on the Stellar MS. The workflow delivers faster setup, broader coverage, and higher-quality quantitative data while revealing deeper structural insights. These advances enable laboratories to extend the value of their SRM expertise and accelerate the next generation of targeted lipidomics with minimal disruption and maximum analytical benefit.

Methods

Sample preparation

All analyses were performed using the NIST SRM 1950 human plasma reference material[4] and the Avanti Research™ UltimateSPLASH™ ONE Internal Standard Kit[5]. Following the original protocols from Medina et al., 100 µL of the internal-standard mix was evaporated to dryness, after which 25 µL of plasma and 125 µL of isopropanol were added for lipid extraction. Three replicate samples were analyzed for each ESI polarity to evaluate reproducibility, while a six-point calibration curve was prepared to assess quantitative sensitivity and linearity.

LC–PRM analysis

Chromatographic separation followed the methods originally described by Medina et al. and was performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC System. HILIC chromatography was used to separate lipids by head-group polarity, producing class-based separation and a predictable elution order that supports high reproducibility and broad lipid coverage. The method used a 4.5-minute effective gradient and a 12-minute sample-to-sample injection cycle, with two injections per sample to capture both positive and negative polarity data.

The published method was originally implemented on a Thermo Scientific™ Vanquish™ Duo UHPLC System, which uses two independent pumps and column compartments to enable continuous operation. In that configuration, one column re-equilibrates while the other performs the sample analysis, allowing throughput of up to 120 samples per day. The same chromatographic parameters were applied here on a single-channel Vanquish Horizon system, demonstrating that the workflow remains compatible and robust even without the dual-channel setup.

All mass-spectrometric data was acquired on the Stellar MS operating in PRM mode. Precursor *m/z* values, retention times, and absolute collision energies (CE) based on the original SRM method were imported directly as starting parameters. Table 1 lists representative values for one lipid species per subclass with the corresponding SRM method settings that were directly transferred from the published method on the Thermo Scientific™ TSQ Altis™ Plus Mass Spectrometer to the Stellar MS. All targeted lipid species within a subclass were analyzed using the same parameters and assumptions except for the product ion *m/z* values and minimum dwell time settings. Because the Stellar MS collects full-scan MS^2 spectra, only unique precursor ions needed to be targeted, eliminating redundant scan events. The acquisition time gained from removing duplicate scan events was distributed to the reduced scan events within a cycle, improving the number of data points acquired per LC peak. The PRM method used a 1 Th Q1 isolation width, an AGC target of 10,000 ions, and a minimum fill time of 5 milliseconds. With a scan speed of 125 kDa/s and parallel ion-packet management, up to 140 PRM scan events were achieved per second with consistent sensitivity across lipid classes. The set of PRM scan events were acquired in similar scheduled retention time windows in both ESI polarities as originally defined in the SRM method, further simplifying method transfer process.

Table 1. List of PRM acquisition parameters used on the Stellar MS. One representative lipid species is listed per subclass and read directly from the original TSQ Altis Plus MS analysis using SRM acquisition.

Lipid species summary composition	Molecular ion	Retention time (min)	RT window (min)	Precursor *m/z*	Collision energy (V)	Min dwell time (ms)	RF lens (V)
CE 18:1	$[M+NH4]^+$	0.4	0.8	668.6	15	0.866	66
Cer d16:1_22:0	$[M+H]^+$	0.4	0.8	594.6	24	0.866	54
Cer 44:1;O2	$[M+H]^+$	0.4	0.8	678.6	24	0.866	54
CL 72:5	$[M-2H]^{2-}$	1.75	0.8	726.5	30	1.342	73
DG 42:6	$[M+NH_4]^+$	0.4	0.8	714.6	20	1.18	76
Cer 42:1;O2	$[M+H]^+$	0.4	0.8	650.9	34	0.866	93
FA 10:0	$[M-H]^-$	0.52	0.8	201.1	5	26.486	73
HexCer 40:0;O2	$[M+H]^+$	1.05	1	768.9	30	1.12	125
Hex2Cer 40:0;O2	$[M+H]^+$	2.2	1	930.6	38	5.38	177
HexCer 34:1;O2	$[M+H]^+$	1.05	1	682.6	30	1.12	125
LPC 14:0	$[M+AcO]^-$	2.3	0.8	526.3	30	0.813	73
LPC O-16:0	$[M+H]^+$	2.33	1	482.4	24	5.38	86
LPC P-16:0	$[M+H]^+$	2.33	1	480.3	24	5.38	86
LPE 14:0	$[M-H]^-$	2.5	0.8	424.2	25	1.929	124
LPG 16:0	$[M-H]^-$	2.1	0.8	483.3	26	0.813	101
LPI 16:0	$[M-H]^-$	3.26	0.8	571.3	35	1.944	130
PC 30:0	$[M+AcO]^-$	1.63	0.8	764.5	35	0.813	140
PC O-32:0	$[M+AcO]^-$	1.63	0.8	778.6	35	0.813	140
PC P-32:0	$[M+AcO]^-$	1.63	0.8	776.6	35	0.813	140
PE 34:4	$[M-H]^-$	1.75	0.8	710.5	30	1.342	124
PE O-40:5	$[M-H]^-$	1.75	0.8	778.6	30	1.342	124
PG 34:1	$[M-H]^-$	1.37	0.8	747.5	37	1.736	131
PI 32:0	$[M-H]^-$	2.6	0.8	809.5	47	2.362	140
PS 35:1	$[M-H]^-$	2.6	0.8	774.5	38	1.929	116
SM 31:1;O2	$[M+H]^+$	2.05	1	661.5	24	5.38	86
TG 42:1	$[M+NH_4]^+$	0.4	0.8	738.7	24	1.18	98

Data processing

Targeted lipid species lists were generated using the LipidCreator tool within Skyline software. Using the same precursor list from the original SRM method, LipidCreator automatically created in silico product-ion libraries that included all potential fragments rather than only those predefined in an SRM transition list. The resulting PRM datasets were processed with Skyline software, providing a more complete MS^2 profile for each lipid species. Accumulating and measuring all product ions increased structural information and improved confidence in annotation, particularly for isomeric and isobaric species. By expanding the processing list without altering the original precursor set, approximately 25% more well-defined lipid species were identified with multiple confirming product ions. Final identifications were curated in Skyline software using refinement tools with confidence metrics based on 75% product-ion ratio agreement and %CV values across replicates, yielding a comprehensive, high-confidence profile of the plasma lipidome.

Results and discussion

PRM simplifies targeted method creation and increases acquisition efficiency

In this study, a high-throughput assay for a broad range of lipid species was converted from the previously published SRM method[3] performed on a QQQ MS to the Stellar MS PRM workflow. Transferring the established global lipid SRM method to the Stellar MS PRM workflow required no new LC development or transition redesign. The original SRM method targeted 1,019 lipid species, while the Stellar MS PRM method expanded coverage to approximately 1,290 species across 24 lipid classes, maintaining a 12-minute total sample-to-sample cycle time using a 4.5-minute HILIC gradient. The QQQ MS method relied on one transition per lipid species and implemented multiple SRM transitions based on the same precursor to expand isomeric lipid coverage, whereas PRM acquisition using the Stellar MS captured all product ions per species as shown in Figure 1. The

Stellar MS leverages the instrument configuration to perform synchronous ion packet management, meaning that while the initial set of MS^n product ions is being scanned out of the low-pressure linear ion trap for detection, a second scan event can be initiated using Q1 to filter a different precursor to generate the next ion packet formed and accumulated in the ICRM. Simultaneous ion packet management reduces the elapsed scan time required per PRM event, increasing detection efficiency.

Because HILIC chromatography separates lipids by head group, co-elution of lipid classes occurs. Thus, one PRM-based tMS2 scan event can form, accumulate, and measure a range of product ions from multiple co-eluting lipid species. This enables comprehensive post-acquisition data extraction and comparative expression-level analysis of isomeric and isobaric lipids without requiring high-resolution or accurate-mass analysis. Furthermore, this allows for more cycle time to be spent on the unique precursors rather than having multiple events used on the same precursor. An example of the improved data acquisition efficiency is presented for the isomeric contribution for TG 50:4 (Table 2). Each SRM transition listed measures one fatty acid chain loss requiring seven different scan events to evaluate the resulting biology, significantly increasing scan event concurrency. Extending this approach to many more TGs and other lipids with multiple acyl chains limits the dwell time to <2 ms due to the increased scan event concurrency. Using the short dwell times results in a duty cycle around 42% (i.e. the instrument is acquiring data only 42% of the cycle time and either switching electronics or re-establishing precursor-product ion beam) that can compromise quantitative sensitivity, accuracy, and precision.

Table 2. List of TG 50:4 lipid species that are differentiated based on one fatty acid chain loss following beam-type dissociation (HCD). The lipid species color-coded in bold font were originally evaluated using SRM transitions sequentially measured in the original study.

Lipid species	RT (min)	Precursor *m/z*	Product *m/z*
50:4 – 14:0	**0.4**	**844.6**	**599.3**
50:4 – 14:1	0.4	844.6	601.3
50:4 – 16:0	0.4	844.6	571.3
50:4 – 16:1	**0.4**	**844.6**	**573.3**
50:4 – 16:2	0.4	844.6	575.3
50:4 – 16:3	0.4	844.6	577.3
50:4 – 18:0	0.4	844.6	543.3
50:4 – 18:1	**0.4**	**844.6**	**545.3**
50:4 – 18:2	**0.4**	**844.6**	**547.3**
50:4 – 18:3	**0.4**	**844.6**	**549.3**
50:4 – 20:3	**0.4**	**844.6**	**521.3**
50:4 – 20:4	**0.4**	**844.6**	**523.3**

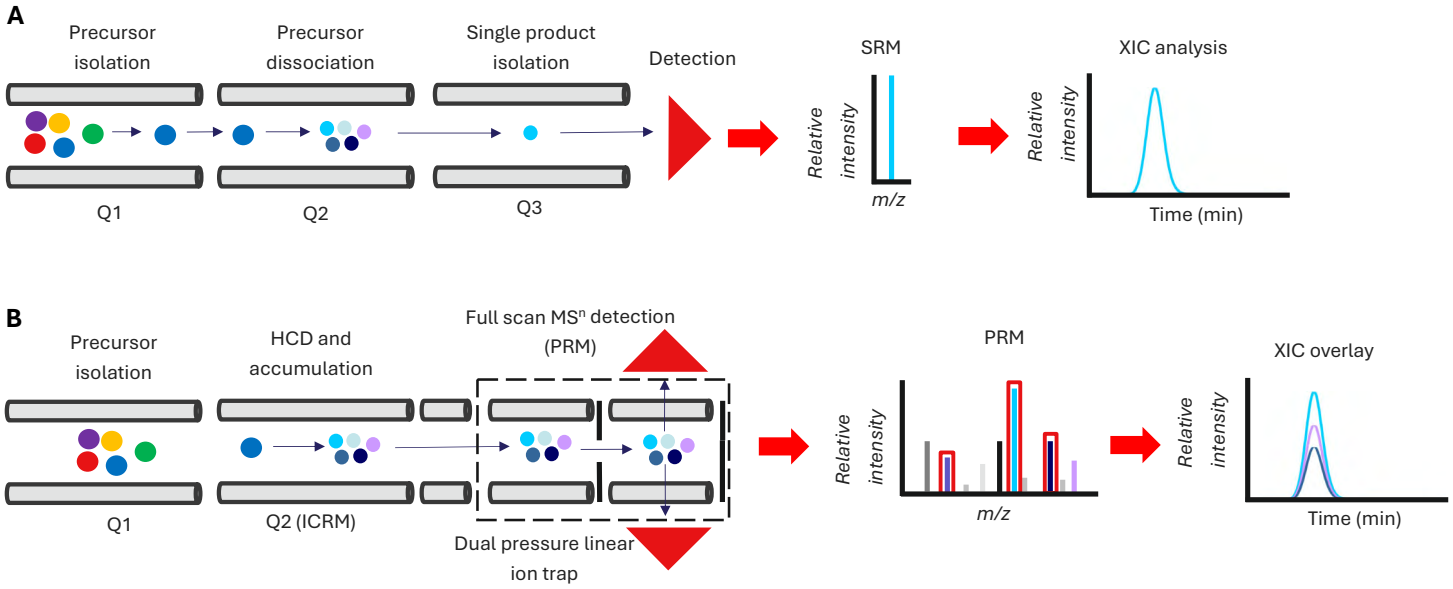

Figure 1. Schematic representation of targeted data acquisition schemes based on (A) SRM acquisition on a QQQ MS and (B) PRM acquisition using the Stellar MS. The acquisition scheme shows the ion flight path components performing tandem MS steps (i.e., precursor isolation, dissociation, and detection) as well as the data stream and processing steps to perform relative or absolute quantitation.

PRM analysis of the TG 50:4 lipid provides more information in one scan event than the collective SRM data set. Figure 2 shows the PRM spectrum for the 50:4 precursor with the highlighted set of product ions measured in the seven successive SRM scan events using QQQ MS. The resulting PRM spectrum shows not only presence/absence of targeted lipid species but also the relative abundance of each product ion based on the fatty acid chain loss. The PRM spectrum was acquired in nine milliseconds of instrument time with only six milliseconds of product ion accumulation time for 67% duty cycle.

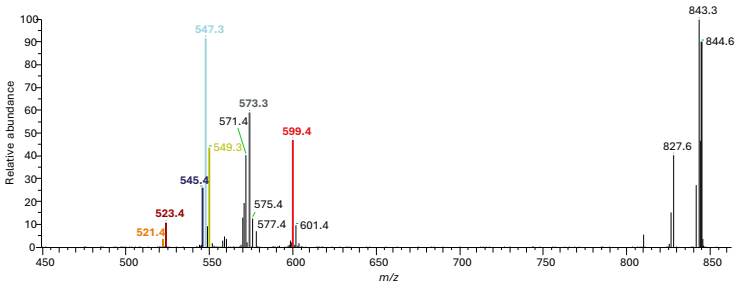

Figure 2. PRM spectrum at 0.4 minutes for the entire set of TG 50:4 lipid species. The narrowed product ion mass range shows the unreacted precursor *m/z* 844 ion (black) and set of color-coded product ions listed in Table 2 representing the neutral loss of one fatty acid chain (-FA).

In addition to the seven product ions targeted in the original SRM experiment, the resulting PRM spectrum measured an additional six product ions associated with the 50:4 isomeric mixture. These additional product ions represent the entire 18:X series covering FA chains with 0 to 4 double bonds, 16:X series covering 0 to 3 double bonds, 14:X for 0 and 1 double bonds and the originally defined loss of the FA chains 20:3 and 20:4 that collectively cover thirteen different FA chains in nine milliseconds of instrument time. This is almost equivalent to the estimated 8.5 milliseconds of combined instrument time for the seven SRM scan events measuring seven neutral loss FA chains originally implemented in the QQQ MS research method.

PRM also enables post-acquisition data processing to determine which specific sets of product ions are used for qualitative and quantitative analysis. By measuring all product ions per data point, researchers can specify any product ion *m/z* value to perform post-acquisition XIC analysis from any PRM scan event, determining the area under the curve (AUC), signal-to-noise ratio (S/N), and LC peak symmetry if assigning more than one product ion to a specific lipid species. Figure 3 shows a subset of quantitative product ions originally listed in Table 2, demonstrating the advantage of PRM acquisition for comparative analysis for the TG 50:4 isomeric contributions. XIC analysis shows an asymmetrical peak that is about 6 seconds wide for each of the five product ions. At least six data points were acquired across the peak width despite over 400 TG species targeted in the scheduled RT window and a 40X AUC difference between the base peak at *m/z* 547, representing the 18:2 FA loss, and the lowest intensity product ion at *m/z* 521, representing the 20:3 FA loss.

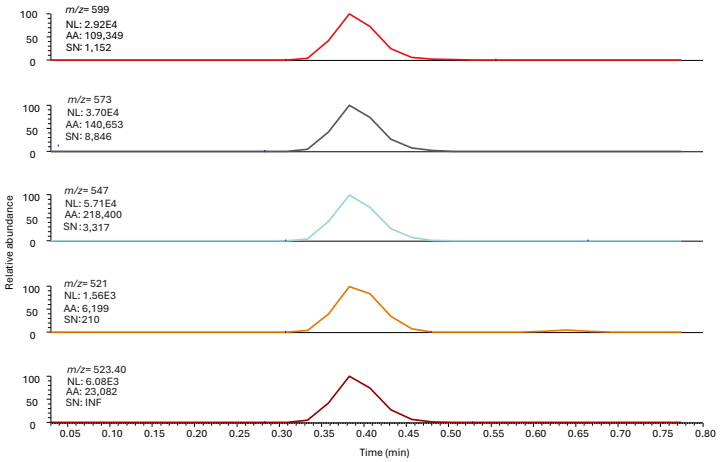

Figure 3. Comparative XIC analysis for the subset of quantitative PRM product ions representing neutral FA loss following beam-type dissociation. Each XIC trace is color-coded with the specific product ion listed in Table 2. The measured integrated peak information is provided for each XIC, where NL is the normalized apex intensity, AA is the measured area value, and SN is signal-to-noise, with INF indicating there is no noise measured at the time interval used to determine the signal assigned to the noise.

As described above, PRM analysis in combination with HILIC separation extends sample coverage with excellent quantitative accuracy and precision while ensuring massive sample throughput. Targeted acquisition methods focused on unique precursors significantly reduce scan event concurrency to drive higher duty cycles per lipid, further simplifying method development. To demonstrate the improvement, two conditions were evaluated on the required cycle time needed to ensure high data quality, focused on the number of lipids versus method set up to measure unique precursor *m/z* values within a scheduled RT window. Figure 4 shows the calculated cycle times needed to measure the number of scan events per lipid species based on the two approaches. Due to HILIC separations, there are “hot spots” in the resulting high-throughput methods where the majority of the targeted lipid species elute. Our PRM method focused on unique precursors, substantially reducing scan event concurrency throughput the method. Thus, during the first two minutes of +ESI scheduling, the estimated cycle time is 3X less using our method (Figure 4B) than that estimated using the SRM acquisition needed to acquire six to seven data points 3X in +ESI and almost 2X in -ESI.

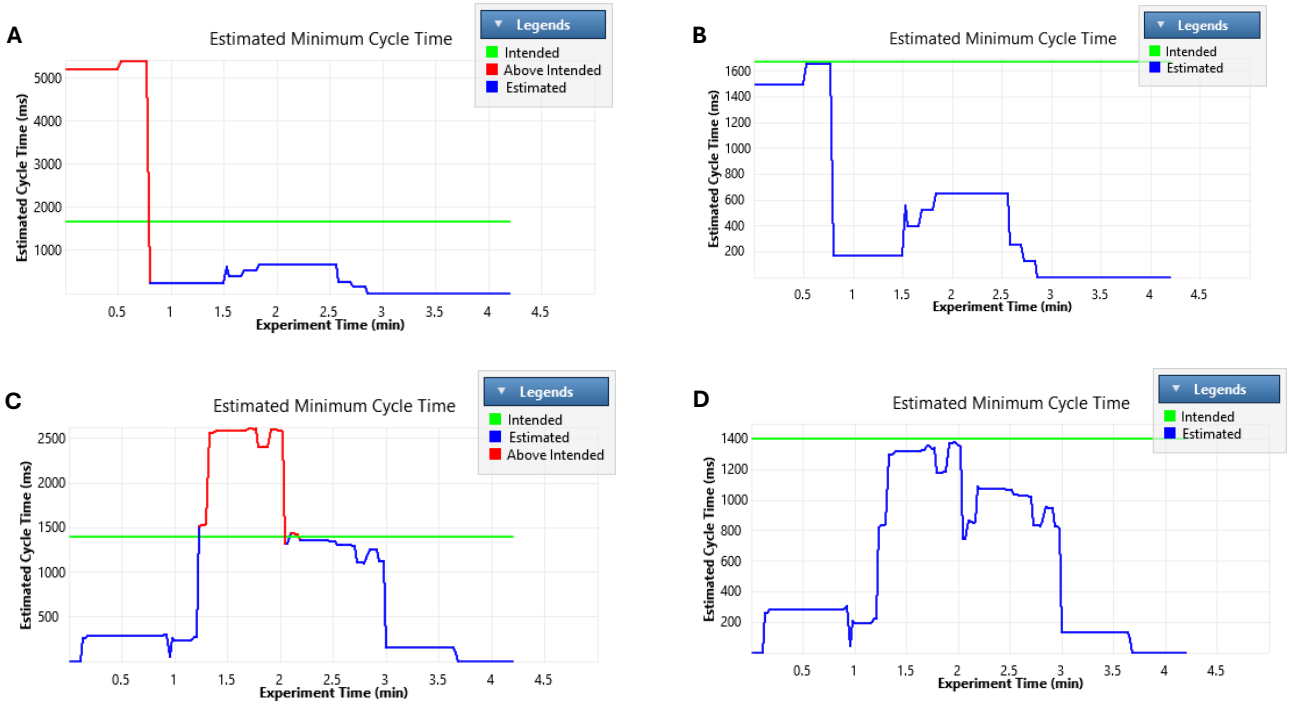

Figure 4. Visualization within the Stellar MS instrument control software helps to determine impact of scan event currency on estimated cycle times. The intended cycle time is shown in green based on the estimated HILIC peak width of 6 seconds and the desired number of data points (cycles) acquired across the peak. Superimposed on the plot is the estimated cycle time needed to address the scan event concurrency for (A) SRM acquisition covering 579 lipid species and (B) PRM with 233 targeted precursors in +ESI mode. The red portion of the second line shows where the estimated cycle time needed to support the scan event concurrency exceeds the intended cycle time, while the blue trace fits within the user-defined goals. Similar analysis was performed in -ESI mode considering (C) SRM acquisition covering 440 lipid species while (D) represents our PRM method targeting 291 precursors.

Expanded product-ion coverage and transition analysis

The full-scan acquisition capability of the Stellar MS PRM workflow greatly increased the amount of structural information obtained per lipid species. As demonstrated above for the analysis of the TG 50:4 isomers, collecting all product ions enables comprehensive analyses. On average, PRM scan events per lipid precursor generated approximately three product ions in +ESI mode, although a larger percentage of HexCer were measured with four product ions (Figure 5). The results for -ESI showed that most lipid subclasses had four to seven product ions per PRM. This expanded product-ion coverage enabled confident differentiation of isomeric variants and improved the accuracy of molecular annotation as demonstrated above.

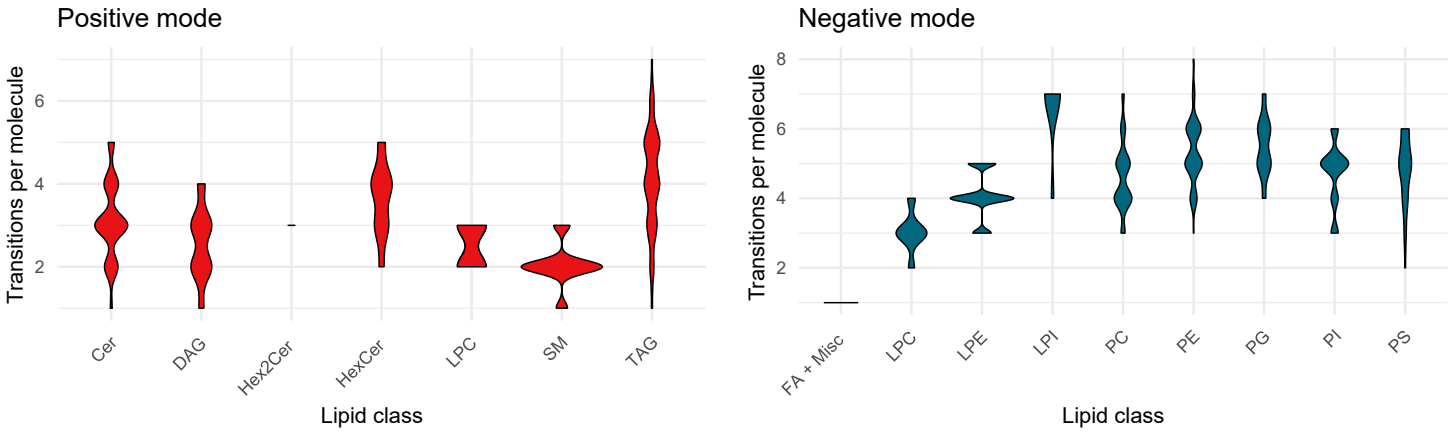

Figure 5. Expanded product-ion coverage across lipid classes using Stellar MS PRM. Violin plots show the number of product ions detected per lipid species across major lipid classes.

The richness of the resulting PRM spectra enabled exhaustive post-acquisition data processing on an extended set of lipid species, facilitated by the LipidCreator tool in Skyline software. The LipidCreator tool generates precursor and product ion *m/z* values using in silico modeling based on user-defined lipid subclasses, the range of acyl chain lengths, and degree of saturation. The LipidCreator tool can export the resulting table of predicted precursor/product ions per molecular lipid species into the Skyline workbook for reprocessing with minimal effort. As demonstrated with the TG 50:4 lipid species, the number of candidates screened and identified was significantly increased without changing the instrument method or re-acquiring PRM data. The stepwise consideration applied for the NIST SRM 1950 sample analysis using the PRM-based tMS2 acquisition strategy is summarized in Table 3.

Table 3. List of steps to convert an original SRM transition method to a PRM acquisition method and manage data acquisition and all subsequent data processing events in Skyline software using the LipidCreator tool.

Step #	Description	ESI polarity	Analytes	Scan events	Transitions
1	Extract information from the original SRM method	+ESI	579	579	579
		-ESI	440	440	440
2	Convert it to a PRM acquisition using the Stellar MS	+ESI	579	233	
		-ESI	440	291	
3	Acquire the PRM data and import all raw files into the Skyline workbook				
4	**Perform *in silico* molecular lipid species creation based on the set of original targeted lipid species**	**+ESI**	**2,715**	**233**	**15,399**
		-ESI	**2,611**	**291**	**22,020**
5	Process the resulting data in Skyline software, which takes minutes to test each XIC trace associated with the set of lipid species				
6	Apply scoring filters with the settings as listed and remove those entries not meeting both requirements using one mouse click	• Product ion ratio calculations >70% • Transition ion CV ≤15%			
7	**Determine final candidates for report generation**	**+ESI**	**775**	**233**	**2,587**
		-ESI	**515**	**291**	**1,855**

The results listed in Table 3 show that performing PRM acquisition on the Stellar MS increased the number of molecular lipid species quantified by 34% in +ESI mode and 18% in -ESI mode despite substantially fewer scan events. Additionally, a total of 5,326 different molecular lipid species were processed using 37,419 product ions without changing the acquisition method, reacquiring data, or manually creating search parameters. The results of the new LipidCreator/Skyline post-acquisition processing routine identified 1,290 total lipid species qualified and quantified using 4,442 transitions, demonstrating the enormous experimental benefits the Stellar MS provides for targeted lipid analysis researchers.

The set of 1,290 lipid species that satisfied the acceptance criteria listed above were distributed between the subclasses, demonstrating the increased sample coverage delivered using the Stellar MS (Figure 6). While the set of TGs quantified between the original QQQ MS method and that described here are similar,

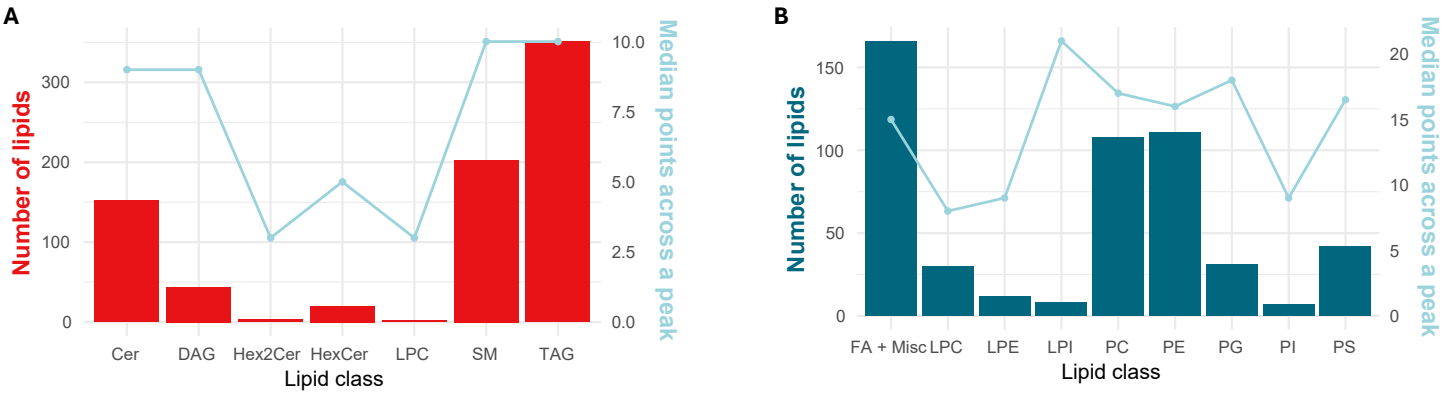

Figure 6. Increased lipid identifications and improved data-point density using Stellar PRM. Total number of confidently identified lipid species per lipid class and median number of data points collected across the chromatographic peak in (A) positive mode and (B) negative mode.

other subclasses such as ceramides, sphingomyelins, and all subclasses targeted in -ESI mode resulted in 2X to 10X more molecular lipid species than that using static SRM data.

Quantitative performance

By eliminating redundant transitions and targeting only unique precursor ions, Stellar MS PRM provided greater quantitative performance as compared to the published SRM results. Two critical factors influencing quantitative performance are analysis time and number of data points measured to define the LC elution peak. For the Stellar MS, parallel ion packet management results in the ion fill time as the rate determining step, defining the analysis time. The reduced cycle burden allowed longer ion fill times, which is critical to improve quantitative performance for low-abundance species as well as increase PRM data quality at the leading and tailing edges of the LC peak.

To demonstrate the performance benefits using PRM acquisition on the Stellar MS, three replicate injections per polarity were performed on the NIST SRM 1950 plasma sample to evaluate reproducibility and quantitative precision. Quantitative confidence for the1,290 lipid species was assessed using the coefficient of variation (%CV) of integrated peak areas and the average number of data points collected across each chromatographic peak, which were generally less than six seconds wide at 20% peak height.

As a result, the average data points across each chromatographic peak increased from 5–7 in SRM to approximately 7–8 in +ESI with a median variation calculated to be 14.5%. (Figures 7A and 7B, respectively) For -ESI, an average of 14 data points were acquired per LC peak width and the median variance was 7.4% (Figures 7C and 7D).

Overall, the Stellar PRM workflow maintained the robust quantitative precision of traditional SRM methods while offering substantially higher structural detail, broader lipid coverage, and improved acquisition efficiency. These results confirm that laboratories can transition from SRM to PRM without sacrificing data quality, gaining both richer information content and greater confidence in quantitative outcomes. Further, this is seen with a tighter R-square distribution in comparison to the SRM method (Figure 7).

Two additional metrics were assessed to determine the quantitative performance using PRM acquisition relative to the SRM acquisition. The median LOQ and standard deviation measurements become essential to determine the effectiveness of the two competing methods. It should be noted that the original experimental results using SRM acquisition on the TSQ Altis Plus mass spectrometer established new performance metrics for the scale of targeted lipid species measured at a 120 samples per day (SPD) rate using HILIC separation. As such, the dwell

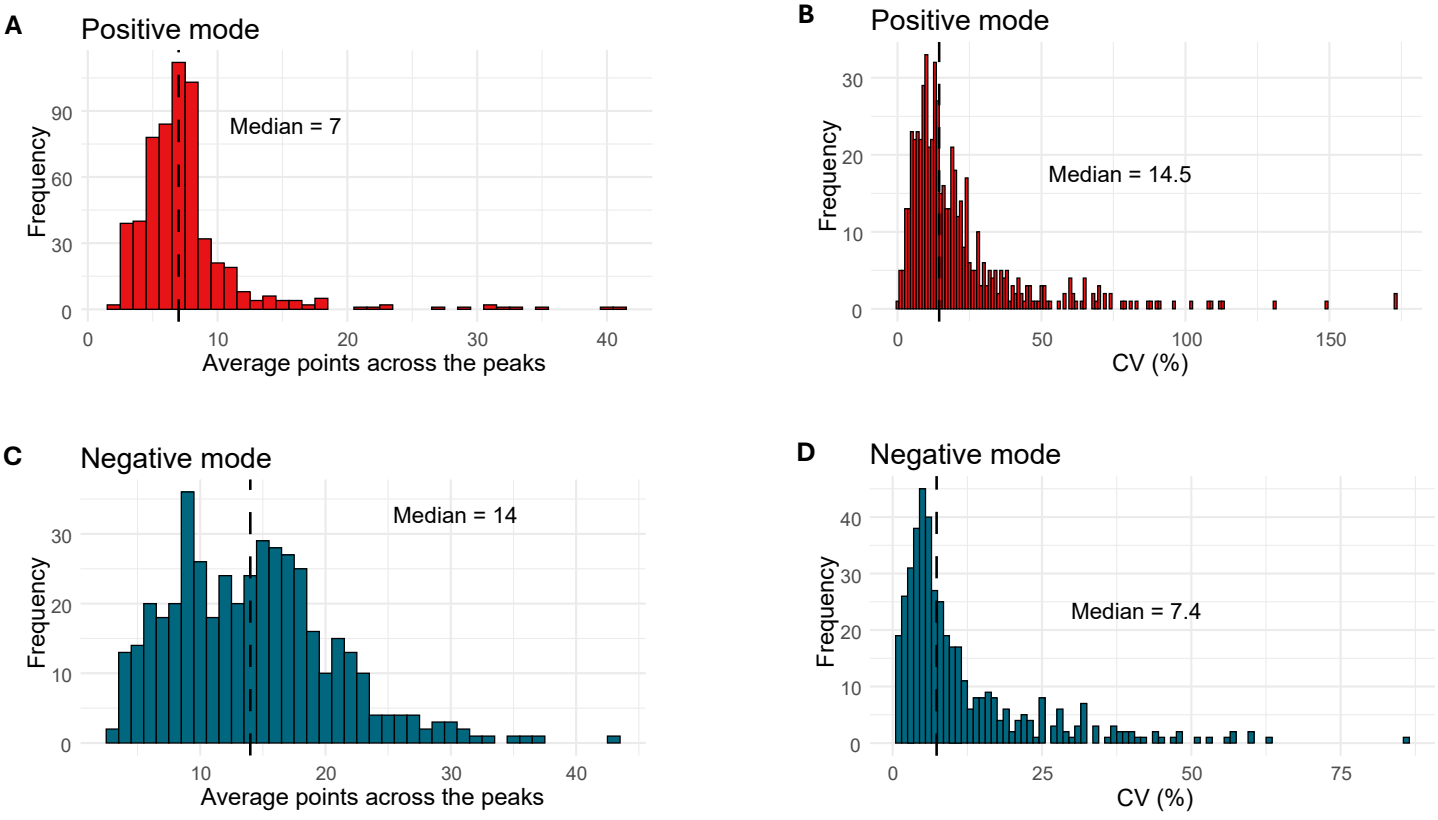

Figure 7. Quantitative performance of Stellar PRM using predefined SRM product ions. (A–B) Positive mode: median seven points across the peak and median CV of 14.5%. (C–D) Negative mode: median fourteen points across the peak and median CV of 7.5%.

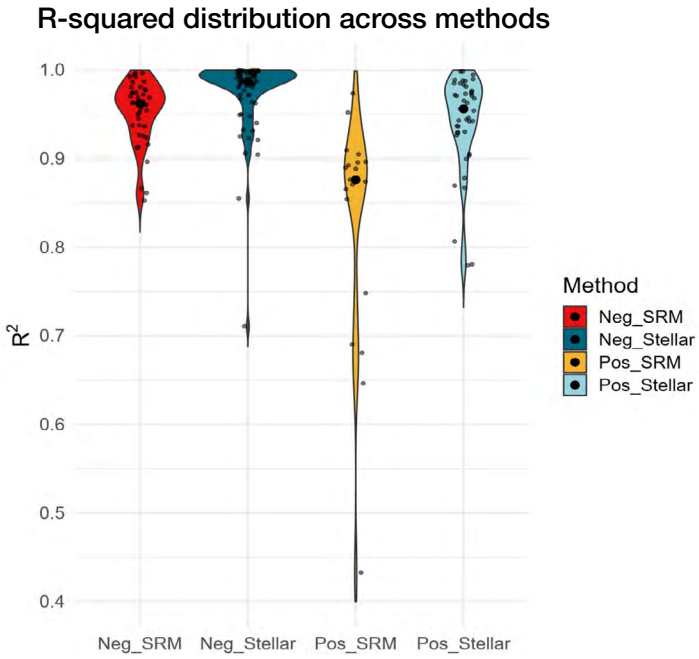

Figure 8. R^2 distributions for SRM and PRM calibration curves in positive and negative ionization modes. PRM on the Stellar MS shows more consistent R^2 values compared with the SRM method, indicating improved linearity.

times needed to achieve this metric were often <1 ms, which can become challenging on all QQQ MS. Table 4, however, shows the Stellar MS acquisition strategy using PRM improves median LOQ determination by 1.5X in -ESI mode as compared to the published SRM results, while significantly improving median standard deviation at the measured LOQ over 30X. For +ESI mode, the Stellar MS results improved the quantitative sensitivity 2.5X while improving the reproducibility at the stated LOQ levels 1.5X.

Table 4. The median LOQ for the lipid standards and corresponding standard deviation across the three replicate measurements at the determined LOQ concentration. Comparative values were measured using PRM on the Stellar MS and QQQ MS-based SRM from previously published results.

Polarity	Method	Median LOQ (nM)	Median STDEV at LOQ
-ESI	SRM	0.055	19.9%
	PRM	0.035	0.6%
+ESI	SRM	0.602	38.5%
	PRM	0.233	25.9%

Conclusion

This work demonstrates how laboratories can expand the power of their existing SRM-based lipidomics workflows by transitioning to PRM acquisition on the Stellar mass spectrometer. Using identical (optimized) precursor information, retention times, and collision energies, the previously published global lipid SRM method (Medina et al.) was transferred without extensive redevelopment and produced immediate gains in both coverage and data quality.

By acquiring full-scan MS^2 spectra for every precursor, Stellar MS PRM captured a comprehensive set of fragment ions, providing threefold greater product-ion coverage across lipid classes and more than five fragments for many phospholipids. These richer spectra improved isomeric and structural resolution and increased the total number of confidently identified lipid species by approximately 25%. The reduction of redundant transitions further optimized cycle times and data density, enabling 9 to 14 points across each chromatographic peak and enhancing quantitative precision.

Quantitatively, Stellar MS delivered equal or better reproducibility compared with SRM, achieving median coefficients of variation around 20% and limits of quantitation that improved up to 2.5-fold. Together, these results demonstrate that Stellar PRM provides deeper structural insight, broader lipid coverage, and improved quantitation—all without sacrificing throughput or requiring extensive method redevelopment.

In summary, the Stellar mass spectrometer combines the familiarity of SRM with the flexibility of PRM to create a streamlined, future-ready approach for global lipidomics. The ability to reuse existing SRM parameters while gaining full MS^2 spectral data allows researchers to extract more information from each analysis, enabling faster, more confident, and more comprehensive characterization of the lipidome.

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