

Omics

Changing the paradigm for global lipid analysis: Why PRM acquisition on the Stellar MS is superior to SRM/MRM

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San Jose, CA, USA**Keywords**Targeted lipidomics, lipid analysis, PRM, SRM, MRM, global lipidome analysis, Stellar Mass Spectrometer, MS³, CID, HCD**How can one mass spectrometer become the ideal solution for both global lipidome analysis and a focused lipid panel analysis when the requirements are so different?**

Lipids play a crucial role in human health, and therefore, are important biomolecules used in clinical health evaluations.^{1,2} The lipidome, however, is extremely complex, dividing lipids into multiple classes and subclasses, while each subclass can contain hundreds of molecular lipid species. Even at the lipid species level, there is further differentiation based on the sum composition (number of carbons and double bonds) and structural differentiation based on the acyl chain positions and double bonds. Extending confident quantitation across deeper lipid annotation levels enhances its biomarker potential for identifying health conditions and advance therapeutic targets.

Due to the complexity of the lipidome, researchers utilize liquid chromatography coupled to tandem mass spectrometry (LC-MSⁿ) to perform relative or absolute quantitation. To handle the lipidome complexity, timed or scheduled MSⁿ scan events (tMSⁿ) are implemented to facilitate more scan events within a method quantifying more lipids either at the head group and/or acyl chain level. Figure 1 shows a representative lipid structure for phosphatidylcholine (PC) with the sum composition of 36:1 and a molecular lipid structural level of 18:0_18:1. At least two product ions are required for qualitative and quantitative molecular lipid species analysis, including one product ion representing the head group to confirm it is a glycerolphospholipid and product ions containing either acyl chain from one of the two options shown in Figure 1.

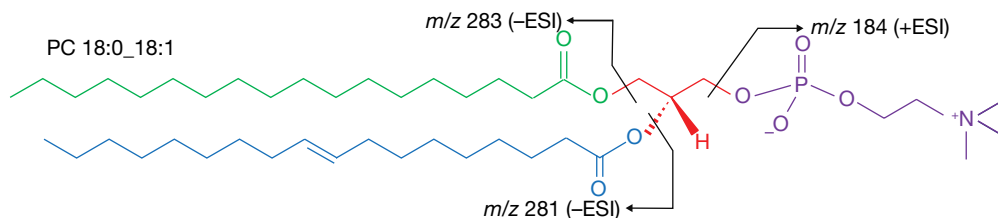


Figure 1. The challenges associated with quantification at the molecular lipid species level.

Representative glycerophospholipid with color-coded structure including the head group to define the subclass and acyl chain composition containing the double bond distribution. Using HCD-based MS² analysis, analysis in both positive and negative ESI polarity are needed to generate key product ions confirming the subclass based on the head group (purple) and acyl chains (green and blue). The latter provides critical information confirming double bond distribution between the acyl chains but does not localize site or orientation of the double bond.

Lipid research typically follows two complementary workflows to discover, validate, and apply putative lipid biomarkers: global lipidomics and targeted lipid subclass analysis. Global lipidomic analysis employs highly multiplexed methods that span more than 20 lipid subclasses and over 1,000 individual molecular lipid species. This comprehensive coverage enables unbiased profiling across complex biological samples. The resulting parallel reaction monitoring (PRM) methods are used to identify lipid subclasses and specific molecular species that are differentially expressed across health states or disease conditions, providing critical insight into biological mechanisms and potential biomarker candidates.

The second workflow, targeted subclass analysis, builds on these discoveries by applying PRM acquisition focused on a specific lipid subclass.^{3,4,5} By increasing molecular coverage within that

subclass, researchers can resolve structural characteristics—such as specific chain lengths and saturation per lipid species to directly compare isomeric contribution to the total response at the sum composition level.

The Thermo Scientific™ Stellar™ Mass Spectrometer uniquely enables and accelerates both workflows through key instrumental innovations. Hyper-fast PRM acquisition supports the scale and complexity of large lipid panels, while the extension of MS² acquisition to MS³ significantly improves analytical specificity. Combined with high sample throughput, these capabilities allow researchers to move seamlessly from broad discovery to deep structural characterization—making the Stellar MS a powerful platform for advancing lipid biomarker research with confidence and efficiency.

Key challenges in targeted lipidomics research

- **Fear of missing out (FOMO):** Researchers worry that limiting lipid targets may cause them to miss critical biomarkers. The Stellar MS addresses this through comprehensive PRM acquisition and retrospective data mining capabilities.
- **Method creation:** Developing highly multiplexed methods is traditionally time-consuming. The Stellar MS streamlines this process through software tools like Skyline Software with the LipidCreator™ tool, which automatically generate optimized methods from discovery data.
- **Achieving sufficient specificity:** Lipids present complex isobaric and isomeric challenges. MS³ acquisition combined with dual dissociation methods (HCD and CID) provides the specificity needed for confident identification and quantitation.
- **Sample throughput at scale:** Population studies require processing hundreds to thousands of samples. Thermo Scientific™ Vanquish™ Dual-Channel Systems and optimized acquisition speeds address throughput needs without compromising data quality.
- **Handling precious samples:** Single-cell studies, biopsies, and animal models provide limited material. Single-cell studies, biopsies, and animal models provide limited material. The Thermo Scientific™ Vanquish™ Neo UHPLC System extends sensitivity 100-fold compared to analytical flow rates, enabling analysis of low-input samples.
- **Workflow robustness:** Laboratories need reliable, reproducible performance over extended studies. The Stellar MS combines robust front-end design with comprehensive quality control features to ensure maximum uptime and data confidence.

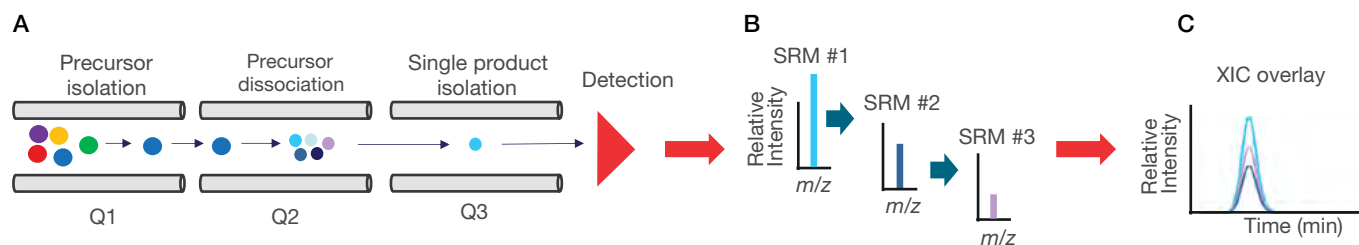


Figure 2. SRM data acquisition and processing schemes used in QQQ MS. Schematic representation of a QQQ MS performing an SRM scan event. Figure 2A shows the precursor-product ion beam formed with precursor m/z values selected in Q1, transmitted to Q2 for beam-type dissociation (termed HCD), and Q3 selects the user-defined product ion m/z transmitted to the on-axis detector to record counts per unit time. Following detection, the RF/DC offsets for Q1 and Q3 are changed to create a new SRM transition that is sequentially measured (Figure 2B) and post-acquisition XIC analysis can be performed to evaluate relative/absolute amounts.

How does PRM acquisition using the Stellar MS create a new paradigm for highly multiplexed lipid analysis?

Triple quadrupole mass spectrometers (QQQ MS) using selected reaction monitoring (SRM) have been the predominant instrument of choice for targeted lipid quantitation.^{1,2,3} SRM acquisition introduces two levels of selectivity filtering precursor (Q1) and product ions (Q3), has a detector scheme enabling single-ion sensitivity, and can be set up to perform SRM scan events in less than 2 milliseconds before switching to the next SRM scan event (Figure 2), enabling highly multiplexed lipid quantitation with increased quantitative sensitivity.² In addition, QQQ MS can perform fast polarity switching (around 5 milliseconds) to extend lipid structural coverage, acquiring SRM transitions in both positive and negative ESI polarities to cover both the head group and acyl chain product ions.⁶

The SRM bottleneck for comprehensive lipid analysis

Instrument methods performing global lipidome analysis covering close to one thousand lipid species generally acquire only one SRM transition per lipid species due to the scale of the experiment.^{1,6} This approach, however, severely limits specificity and data confidence. To gain adequate confidence in lipid species assignment, multiple experiments are performed to extensively characterize potential contaminants and co-elution prior to finalizing the SRM method and data processing routines. Multiple SRM transitions per precursor are needed, but this multiplies the number of scan events required.

Each SRM scan event also requires switching time between transitions, significantly reducing data acquisition efficiency. Each ion manipulation step performed for SRM acquisition is separated by space, thus managing only one ion beam within a scan event. When performing hundreds of SRM scan events within a scheduled retention time segment, the duty cycle (defined as the percentage of scan event time used for analysis) is about 66% as shown in Figure 3. The lower the duty cycle, the higher the risk of poor reproducibility as measured by the coefficient of variance. This risk is magnified when measuring target lipid species at lower concentration ranges or poorer

ionization efficiencies. Often, the scale of global lipidome analysis eliminates the opportunity to extend the number of SRM scan events improving sample coverage or introduces additional SRM transitions per lipid to advance quantitation to the molecular species level.

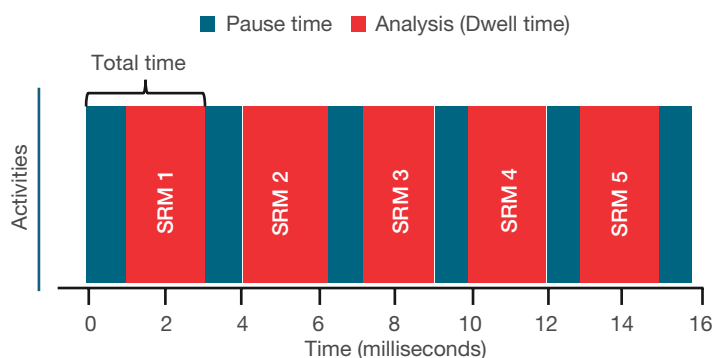


Figure 3. Time allotment strategy for highly multiplexed SRM acquisition on QQQ MS. Each SRM transition includes not only the dwell time for data acquisition, but the switching time needed to quench the ion beam from the previous SRM scan event, set the new RF/DC offset, and establish the next precursor-product ion beam.

PRM: A paradigm shift in acquisition efficiency using the Stellar MS

PRM takes a fundamentally different approach. PRM acquisition isolates one precursor m/z value (like SRM) but measures all resulting product ions from that single precursor m/z value in one full-scan MS² spectrum.⁷ Thus, PRM acquisition captures all co-eluting isomeric information and provides comprehensive product ion coverage without the need for multiple scan events. In addition, the Stellar MS extends Q2 capabilities to not only performing HCD but accumulating the resulting set of product ions as managed by Automatic Gain Control (AGC) to ensure high-quality, reproducible data.⁸ Finally, using a dual-pressure linear ion trap maintains ideal static gas pressures to ensure hyper-fast scan rates at 125 kDa/s, measuring wider product ion mass ranges within 5 milliseconds of scan time for a total product ion mass range of 625 Th while maintaining quantitative sensitivity or reproducibility.

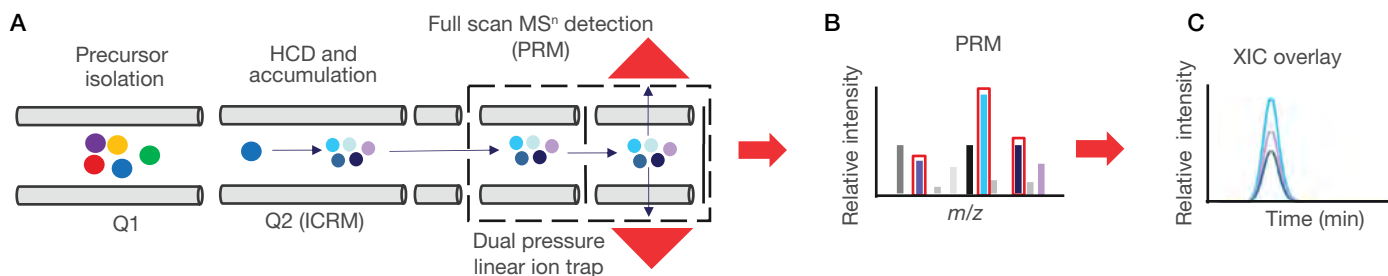


Figure 4. PRM data acquisition and processing schemes on the Stellar MS. Figure 4A shows the precursor-product ion beam formed with precursor m/z values selected in Q1, transmitted to Q2 for beam-type dissociation (termed HCD) and ion accumulation. Once the charge density of the resulting product ions reaches pre-defined AGC levels, all product ions are transferred to the dual-pressure linear ion trap for hyper-fast full scan detection. Post-acquisition processing is performed based on user-defined product ion m/z values and corresponding mass tolerance settings (Figure 4B) to generate the XIC analysis.

The Stellar MS also performs synchronized ion packet management to further extend differentiated method performance versus QQQ MS. As shown in Figure 4, the ability to accumulate ions in Q2 (outside of the linear ion trap) enables simultaneous ion packet management to improve duty cycle while acquiring PRM data to improve instrumental efficiency. Ion management activities as a function of time are shown in Figure 5 where the first set of HCD product ions are scanned out of the low-pressure linear ion trap while the next precursor-product ion beam has been formed between Q1 and Q2, dissociated with ion accumulation. The example shown below equates to a duty cycle of 75%, while all product ions are formed from a 1 Th precursor Q1 filter as opposed to one product ion.

What is the benefit of acquiring full scan tMSn as compared to SRM?

The benefits of PRM acquisition using the Stellar MS are evident as shown by the comparative analysis of TG 50:4. Figure 6 shows the resulting data acquired from a single SRM transition m/z 844 $\rightarrow$ 547 representing the neutral loss of one fatty acid chain with 18 carbons and 2 double bonds. Monitoring this single -FA MS² product ion provides no information on the other two acyl chains or potential co-eluting TG 50:4 isomers. To increase sample coverage and putative isomeric mapping, a series of SRM scan events must be set up and acquired within the scheduled RT window, which increases scale and the corresponding challenges outlined above. The previously published QQQ MS study used seven sequential SRM scan events as listed in the Figure 6 inset table to increase isomeric analysis of TG 50:4.¹

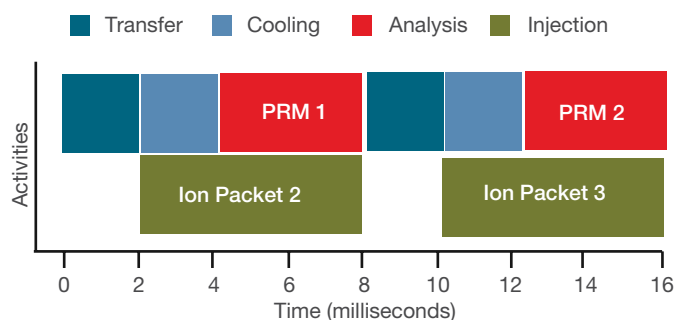


Figure 5. Time allotment strategy for highly multiplexed PRM acquisition on the Stellar MS. PRM acquisition sequence is divided into two steps—product ion formation and accumulation (labeled Injection) in Q2 and PRM analysis in the dual-pressure linear ion trap. Step 1 includes transferring and “cooling” or collisional dampening of motion, increasing transfer efficiency from Q2 to the high-pressure linear ion trap. There is a 2-millisecond delay between each PRM event.

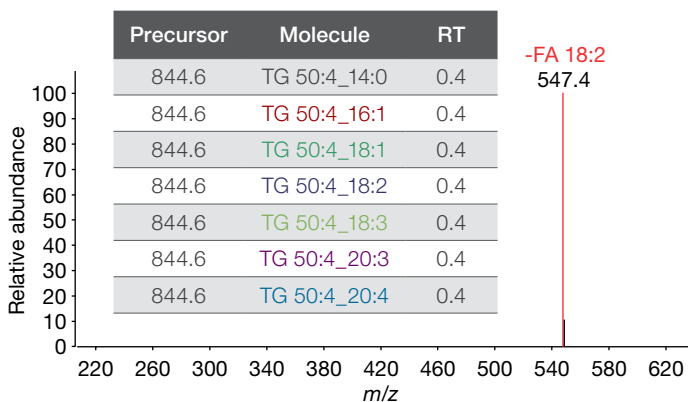


Figure 6. SRM analysis for TG 50:4 precursor at m/z 844.6 eluting at 0.4 minutes. The resulting data were generated from the SRM acquisition, which is limited by the user-defined Q3 mass transmission settings. To compensate for slow acquisition speeds, a series of SRM transitions is acquired sequentially as listed in the inset table to cover the TG 50:4 isomeric contribution.

PRM acquisition isolates the same precursor m/z value as published in the SRM method but forms, accumulates, and measures all resulting product ions in one hyper-fast scan event. Figure 7 shows the depth of product ion information measured in 9 milliseconds of instrument time. All product ions targeted using the published SRM method are simultaneously measured as well as signal attributed to an additional five product ions corresponding to other neutral FA chain losses contributing to the total TG 50:4 sample amount. Importantly, the lack of measured ion signal is also critical to extend isomeric characterization without having to acquire additional scan events. In summary, one PRM scan event confidently measured the originally published seven SRM product ions plus an additional five FA chains, as well as supporting the probability an additional six FA chains are either present at extremely low levels or not biologically formed in the plasma sample under study. For a QQQ MS to compete with the Stellar MS, 18 different scan events would be needed for one isomeric analysis. Extending this level of SRM coverage to the 400+ TG species targeted in the recent QQQ MS study becomes an insurmountable challenge.

What benefit does resonant RF dissociation provide relative to beam-type dissociation in a collision cell?

The Stellar MS offers two complementary dissociation methods—higher-energy collisional dissociation (HCD) and collision-induced dissociation (CID)—while triple quadrupole systems are limited to HCD alone. This dual-fragmentation capability significantly expands the structural information available for lipid characterization.

Beam-type dissociation: HCD

In HCD, the target precursor is isolated in Q1 and transmitted to Q2, where a voltage ramp accelerates ions, causing them to collide with nitrogen molecules. This beam-type dissociation fragments not only the target precursor but also all subsequently formed product ions, which can undergo further dissociation. The result is a product ion spectrum enriched in lower m/z fragments due to this cascading fragmentation process.

For many lipid classes analyzed in positive ion mode, HCD efficiently generates head group-specific product ions that confirm lipid class and subclass identity. However, HCD often provides limited information about individual acyl chain composition.

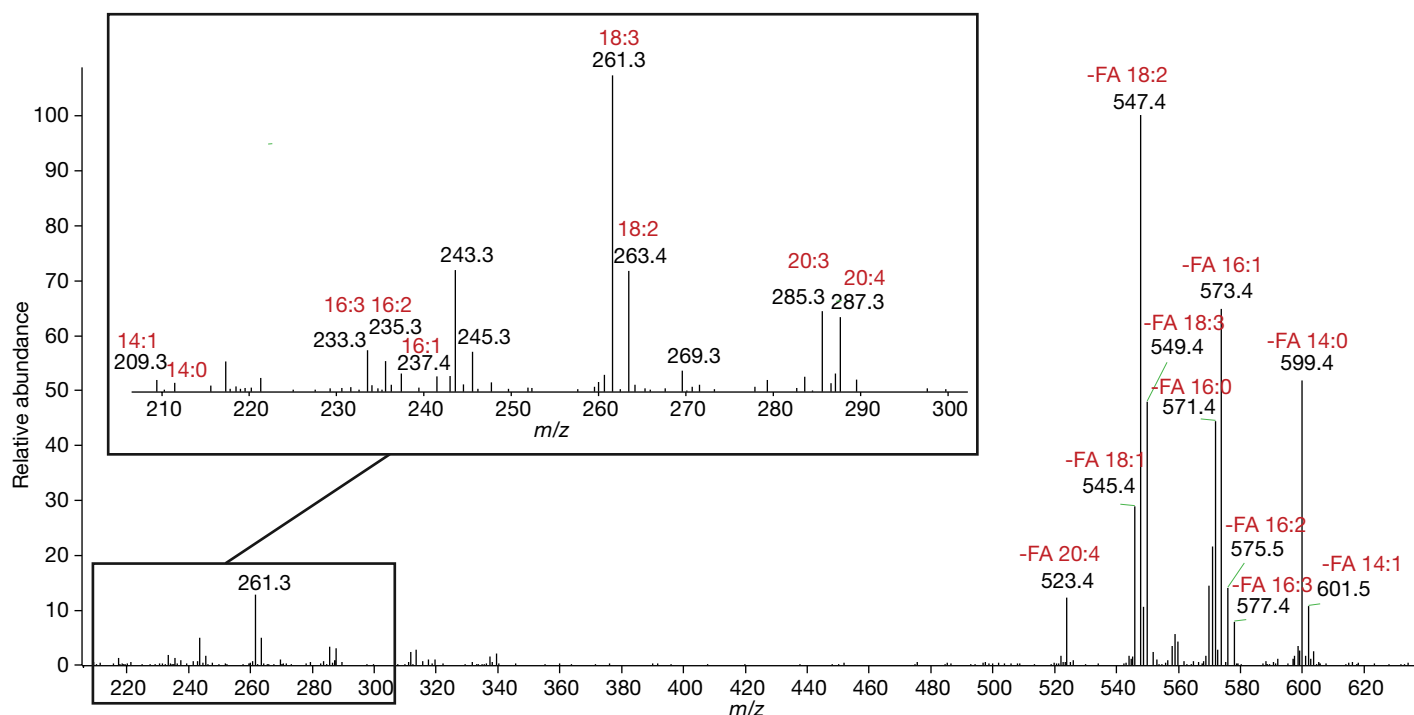


Figure 7. PRM analysis for TG 50:4 precursor at m/z 844.6 eluting at 0.4 minutes. The resulting full scan MS² spectrum shows two mass ranges of clustered product ions representing fatty acid chains either carrying the charge (low-mass range) or product ions with neutral FA loss in the high-mass range. Utilizing HCD on the Stellar MS overcomes the $\frac{1}{2}$ product ion mass range limitation normally associated with ion trap mass analyzers, enabling the measurement of the FA product ions for additional confirmation. Annotation of the resulting product ions was performed using the LipidCreator tool in Skyline software.

Resonant RF dissociation: CID

Ion trap CID takes a different approach. After precursor isolation in Q1 and accumulation in Q2, ions are transferred to a high-pressure linear ion trap where resonant radiofrequency (RF) is applied. This RF selectively accelerates only ions with m/z values matching the resonant frequency—in this case, the precursor ion.

The accelerated precursor undergoes thousands of low-energy collisions with helium (a much smaller neutral gas than nitrogen) over 2 to 5 milliseconds. Crucially, once product ions form, their m/z values no longer match the resonant frequency, so they are no longer accelerated nor fragmented. Instead, they are collisionally cooled and trapped. This “softer” fragmentation preserves higher m/z product ions that provide direct evidence of acyl chain composition.

Complementary information from HCD and CID

For sphingomyelin (SM) lipids in positive ion mode, HCD generates the characteristic m/z 184 product ion corresponding to the phosphocholine head group, confirming lipid class identity. However, HCD provides minimal information about the two acyl chains.

CID of the same precursor generates product ions corresponding to neutral losses of individual acyl chains, revealing not only their presence but also their carbon length and degree of unsaturation (Figure 8). This allows researchers to distinguish between molecular lipid species with the same total carbon count but different acyl chain distributions.

Why this matters: Traditional triple quadrupole systems require switching to negative ion mode and acquiring separate SRM transitions to obtain acyl chain information. Thus, to confirm both the head group and acyl chain composition, two SRM scan events are required as well as the accompanying switching time and polarity switching time. Increasing the number of SMs targeted in a method compromises scale. The Stellar MS, on the other hand, acquires both head group and acyl chain diagnostic product ions in a single CID-based PRM spectrum in positive mode, dramatically improving workflow efficiency.

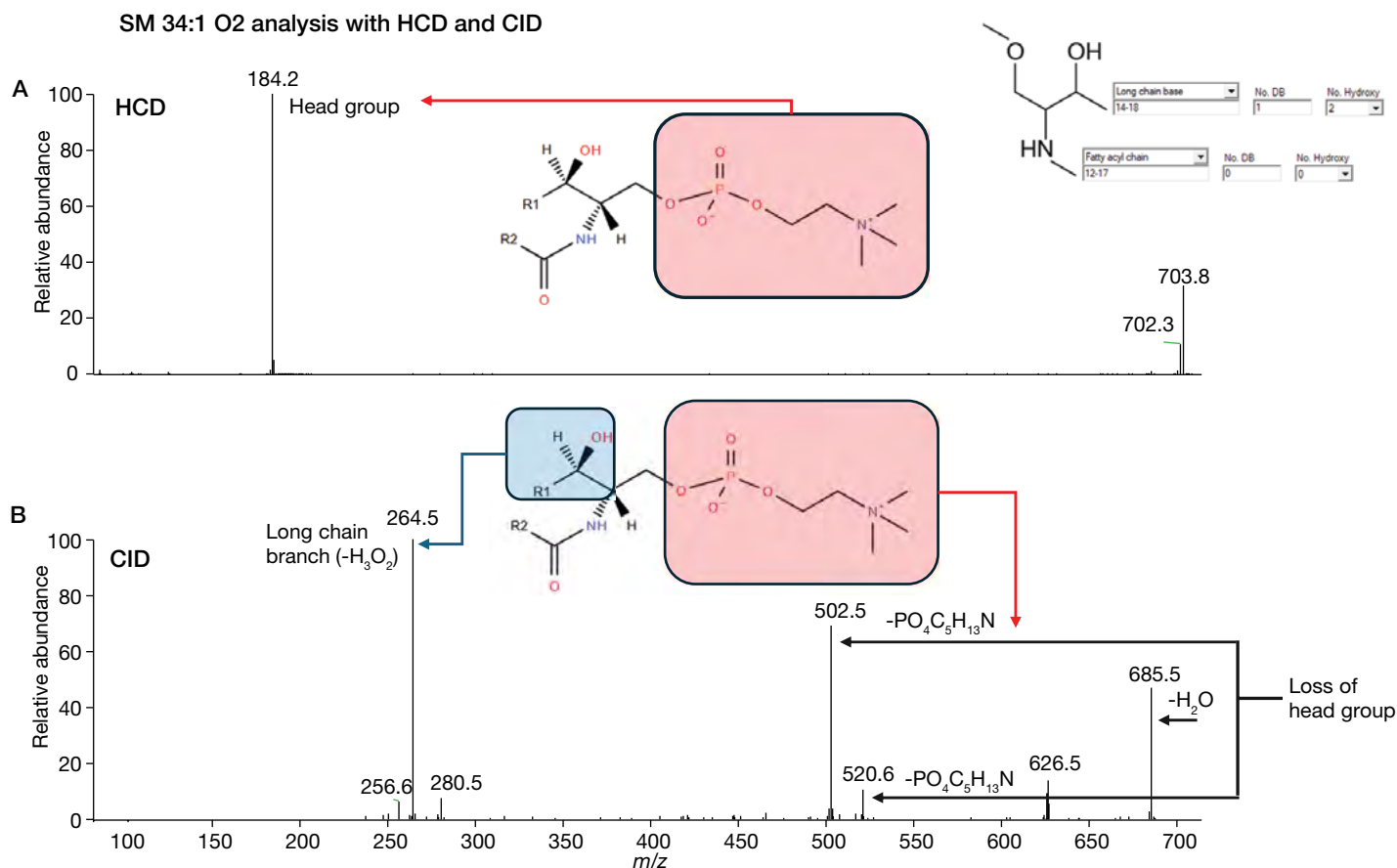


Figure 8. Comparing HCD and CID. An overview of SM34:1 O2 analysis with HCD (A) vs. CID (B).

How does PRM-based MS³ acquisition enhance specificity required for targeted lipid analysis at the molecular species level?

Even with comprehensive MS² product ion detection using PRM, resolving molecular lipid species containing multiple acyl chains remains challenging with HILIC chromatography. Figure 9 demonstrates the complexity of isomeric co-elution for the TG 50:4 species. Measuring either the neutral-loss product ion (-FA) or the charge-retaining FA at the MS² stage cannot determine localization of the three FA chains on a single isomer. The Stellar MS goes beyond MS² acquisition with fast tMS3 acquisition providing an additional dimension of specificity critical for targeted lipid panel validation through multiple levels of selectivity and specificity. This becomes a critical step transitioning from global lipid analysis to a targeted subclass interrogation to better understand biological impact.

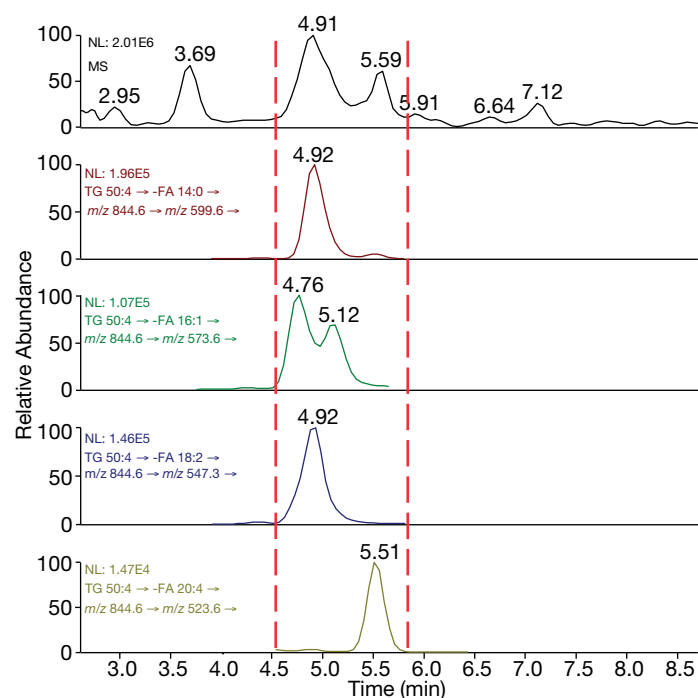


Figure 9. PRM-based tMS3 acquisition and processing strategy to extend relative quantitation to the molecular lipid species level for the set of TG 50:4 isomers eluting between 4.5 and 6 minutes. A set of XIC traces are presented, representing the MS signal for the precursor TG 50:4 species m/z 844.6 (black trace) and four of the seven specific tMS3 scan events. For each tMS3 scan event presented, the specific -FA loss and corresponding m/z value is presented for comparative analysis. The red dashed lines align the start and stop of the isomeric TG 50:4 species elution profiles to evaluate relative retention times.

The tMS3 acquisition steps

The Stellar MS is a hybrid quadrupole dual-pressure linear ion trap capable of fast and sensitive PRM-based MS³ acquisition at rates up to 40 scans per second. MS³ includes four stages of selectivity:

- 1. Scheduled MS³ acquisition at the expected chromatographic retention time:** Lipids are partially separated by LC for the first stage of chromatographic selectivity.
- 2. Precursor m/z isolation in Q1:** First stage of mass selectivity.
- 3. MS² product ion formation using CID:** Second stage of mass selectivity accompanied by isolation of the MS² product ion isolation common to multiple isomers.
- 4. CID-MS³ dissociation and detection:** Performing post-acquisition MS³ product ion XIC analysis from full-scan MS³ spectrum that are attributed to the isomer of interest.

To demonstrate the benefits of Stellar MS for the targeted subspecies analysis, a subset of TGs was identified and analyzed by PRM-based tMS3 acquisition. A total of 104 TGs were selected from the original SRM study¹ to define the target lipid species panel. For each targeted TG species, at least two MS² product ions were incorporated into 250 PRM-based tMS3 scan events. Selection of specific MS² product ions used to generate the resulting MS³ spectra shared a common neutral FA chain loss between two or more plausible isomers. A two-minute scheduled RT window was used for acquisition, resulting in a 3-second cycle-time ensuring 6 to 8 data points per targeted TG species. As described above in Figure 7, parallel ion packet management of the Stellar MS enabled longer ion fill times of 35 milliseconds to improve sensitivity and data quality but only took about 37 milliseconds of elapsed scan time, enabling over 80 tMS3 scan events acquired during any 3-second cycle.

Implementing tMS3 acquisition increased selectivity with the addition tandem MS level and increased specificity relative to MS² product ion analysis. Figure 9 shows the comparative XIC analysis for four different tMS3 scan events based on different neutral FA chain loss. Separate scan events enabled MS³ product ion determination to be associated with a specific species. Comparing the XIC traces based on the neutral FA chain loss shows multiple peaks within the 90-second peak width that is associated with isomeric contributions even at the MS² product-ion level.

Post-acquisition data mining

Because the Stellar MS acquires full-scan MS³ spectra, researchers can perform post-acquisition data mining to identify and quantify isomeric species that were not originally targeted. This is impossible with SRM-based methods, which only measure predefined transitions. The most complex XIC trace shown in Figure 9 is attributed to TG 50:4 species containing a 16:1 FA chain with two elution profiles having apexes at 4.76 and 5.12 minutes.

Figure 10 shows a pictorial representation of data processing strategy improving specificity to determine which isomeric species are present and absent. Left to right, the first two stages of selectivity listed above are leveraged for precursor isolation for the TG 50:4 isomers at the known retention time profile. The third step of selectivity is realized following CID-based MS² dissociation and intelligent isolation of the common MS² product ion between multiple isomers (Figure 10 middle). Following MS³ acquisition, one can perform XIC analysis based on predicted product ions per isomer to determine presence/absence

and relative/absolute contribution to the sum composition.

Increased data confidence is achieved based on the empirical measurements for three acyl chains in each isomer.

Performing the specific MS³ XIC analysis enables direct comparison of isobaric contribution of all product ions associated with each isomer. PRM-based MS³ acquisition enables co-addition of all product ions associated with individual FA chains shown above. Figure 11 shows the XIC integration strategy for two sets of MS³ product ions measured following the strategy outlined above to further separate the measured signal between two different isomers. There are two product ions associated with each FA chain that contain the TG backbone and the FA chain. The dashed line and the summed XIC trace to the right of each MS³ spectrum show that the summed product ion yields relatively abundant response (e5 signal) for the two isomers. In addition, the comparative XIC traces allow for peak symmetry determination that may be attributed to SN positions. For example, the XIC trace for the 16:1_16:1_18:2 has an extended tailing peak shape and almost a 12-second longer elution profile as compared to the 16:0_16:1_18:3 set of XIC traces.

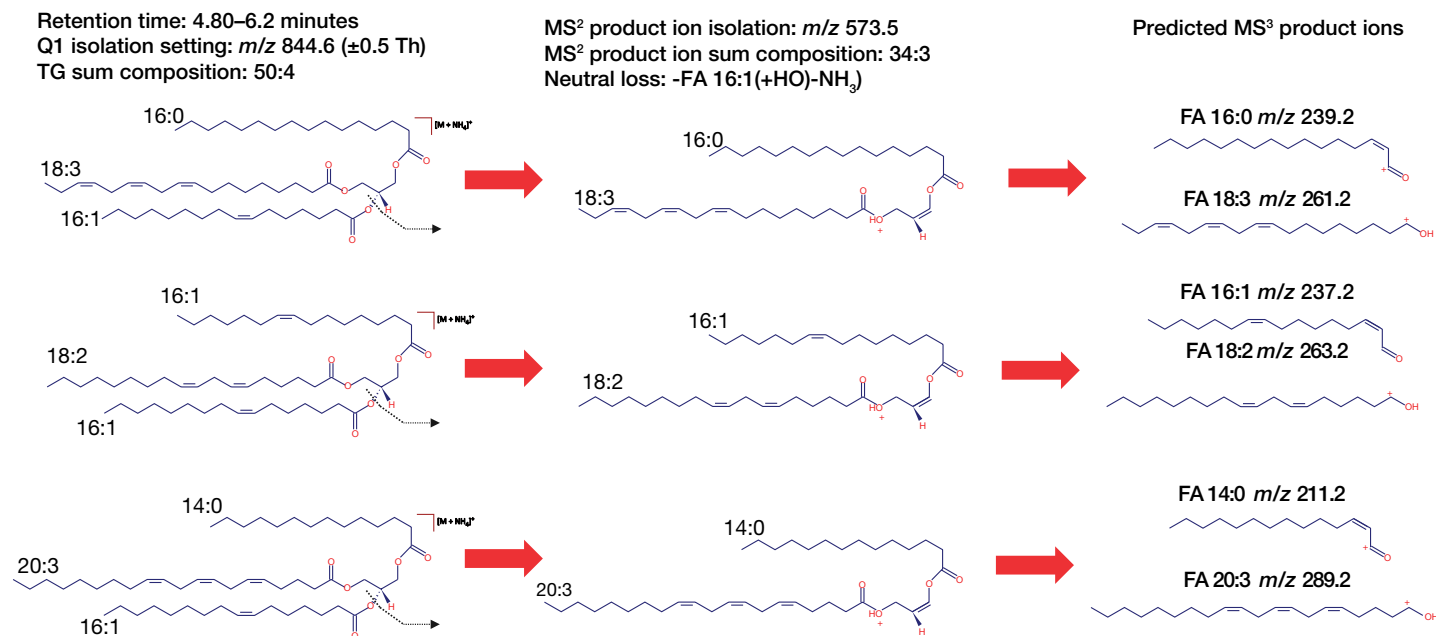


Figure 10. Targeted TG structural analysis and isomeric quantitation method using tMS3 acquisition. The set of plausible precursor structures under MS¹ are based on detected product ions from the single PRM-based MS² analysis satisfying the 50:4 sum composition as shown in Figure 7. The isolated MS² product ion results from the neutral loss of the 16:1 FA chain, leaving an MS² product ion with a sum composition of 34:3. Thus, the resulting MS³ spectra must contain the set of product ions listed on the right-side of the figure.

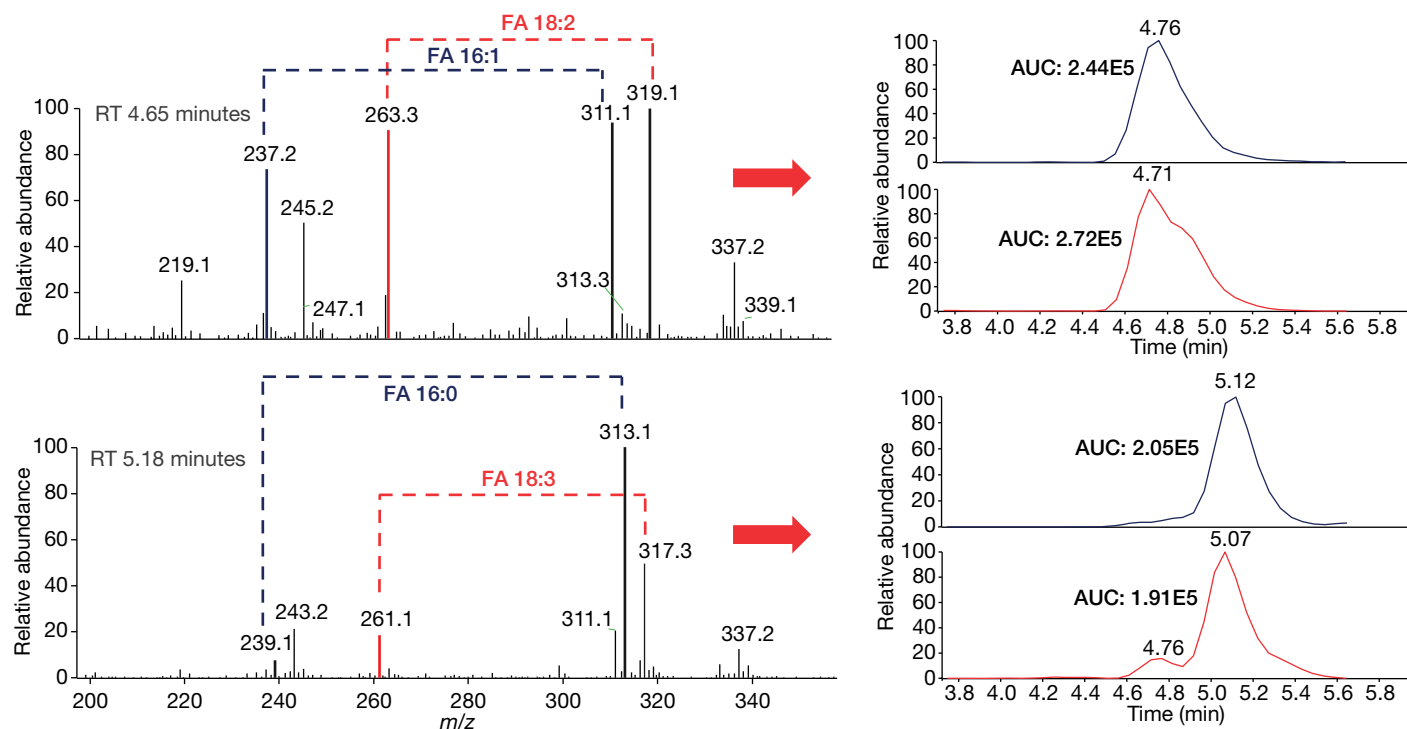


Figure 11. Comparative analysis of the tMS3 XIC elution profiles for the neutral loss of FA 16:1 MS² product ion from the precursor TG 50:4. Two MS³ spectra are presented covering at (top) 4.6 minutes with a proposed composition of TG 16:1_16:1_18:2 and 16:0_16:1_18:3 (bottom) eluting at 5.2 minutes. XIC analysis using the summed intensity response for the key product ions are presented to the right of each MS³ spectrum with measure AUC values.

A similar XIC strategy was carried out for each of the TG 50:4 tMS3 scan events to determine first if there is a measurable response and then the XIC profile was measured per MS² precursor and MS³ product ion sets. Table 1 lists the measured results for the seven different PRM-based tMS3 scan events where the AUC values were listed per LC peak. The set of product ions considered for extraction were dictated by the set of TG 50:4 MS² product ions measured and annotated in Figure 7. The results show those 50:4 molecular lipid species containing at least one 18:2 FA chain result in the greatest AUC values eluting in the first retention time window. Additionally, the set of tMS3 scan events allows for cross-referencing between different MS² product ions for confirmation. For example, the tMS3 scan event isolating the -FA 14:0 neutral loss shows the most intense response was attributed to XIC analysis of the proposed 18:2_18:2 MS³ product ion, which corresponds to resulting MS³ XIC trace associated with the 14:0_18:2 set of product ions resulting from the -FA 18:2 neutral loss MS² product ion.

Table 1 shows the differentiation of Stellar MS-based PRM acquisition to expand not only lipid sum composition coverage but also molecular lipid species coverage with incredible tMS3 efficiency relative to SRM acquisition using QQQ MS. The specified workflow extracted the original global lipidome

results to determine the set of TG species to target in the follow up experiment. User-defined selection of critical MS² product ions was used to create tMS3 scan events, and, finally, post-acquisition XIC analysis enabled comparative analysis for specific molecular lipid species with the prospects of normalizing against a heavy-labeled internal standard. Thus, the expansive nature of the experiment can be summarized as the following:

1. The sum composition for 104 TG lipid species was originally targeted from the global lipidome analysis.
2. Across a six-minute retention time window, 250 (or about 3 per sum composition) tMS3 scan events were acquired.
3. Method development used a single NCE-based CID method with no further optimization.
4. Over 1,000 plausible molecular lipid species were evaluated for presence/absence, relative or absolute quantitation.

It would take 10X longer to develop an SRM acquisition method on a QQQ MS as well as longer gradients to facilitate over 1,000 SRM transitions. Each SRM transition would need to be set up, and most likely, a single CE setting would be used to expedite method development. The results of which would reduce sample throughput decreasing laboratory productivity.

Table 1. List of TG 50:4 molecular lipid species evaluated at the MS³ level based on the isolated MS² product ion. The set of MS³ product ions are based on the specific neutral loss fatty acid chain product ion isolated at the MS² level and further dissociated. The resulting XIC profiles typically show one or two LC peaks as shown in Figure 11. The measured AUC value per LC peak is listed for each proposed molecular lipid species.

MS ² precursor	MS ³ product ions	LC peak width #1 start and stop (min)	LC peak width #2 start and stop (min)	AUC (1)	AUC (2)
-FA 14:0	FA 16:1_20:3	4.75–5.08	N/A	14,485	0
	FA 16:0_20:4	4.77–5.15	5.35–5.70	8,655	9,472
	FA 18:1_18:3	4.75–5.25	N/A	72,102	0
	FA 18:2_18:2	4.77–5.09	N/A	162,450	0
-FA 16:0	FA 14:0_20:4	4.97–5.23	5.32–5.70	3,699	65,688
	FA 14:1_20:3	4.93–5.38	5.49–5.65	8,921	1,000
	FA 16:0_18:4	4.97–5.25	5.43–5.81	7,121	7,272
	FA 16:1_18:3	4.95–5.30	N/A	8,086	0
	FA 16:2_18:2	4.95–5.33	N/A	19,888	0
	FA 16:3_18:1	4.94–5.28	5.28–5.65	6,470	3,973
-FA 16:1	FA 14:0_20:3	4.63–5.21	N/A	9,353	0
	FA 16:0_18:3	4.63–4.86	4.95–5.33	4,675	69,708
	FA 16:1_18:2	4.61–5.10	N/A	255,260	0
	FA 16:2_18:1	4.68–5.00	5.05–5.33	21,879	5,021
-FA 18:1	FA 14:0_18:3	4.82–5.23	N/A	56,722	0
	FA 14:1_18:2	4.63–5.11	N/A	43,273	0
	FA 14:2_18:1	4.66–5.30	N/A	7,988	0
	FA 16:0_16:3	4.78–5.18	N/A	4,340	0
	FA 16:1_16:2	4.63–5.22	N/A	14,220	0
-FA 18:2	FA 14:0_18:2	4.78–5.14	N/A	399,166	0
	FA 14:1_18:1	4.62–5.15	N/A	76,559	0
	FA 16:0_16:2	4.62–5.35	N/A	27,430	0
	FA 16:1_16:1	4.60–5.05	N/A	186,041	0
-FA 18:3	FA 14:0_18:1	4.80–5.30	N/A	170,333	0
	FA 16:0_16:1	4.95–5.40	N/A	278,234	0
-FA 20:4	FA 14:0_16:0	N/A	5.35–5.70	0	124,667

Why is the Stellar MS the ideal instrument for targeted lipid analysis?

As explained above, the Stellar mass spectrometer addresses both experimental scopes for translational lipid research through its unique design and capabilities:

- Overcoming SRM limitations for global analysis:** While SRM acquisition using triple quadrupole (QQQ) mass spectrometers is fast and selective, deciphering lipid complexity requires multiple product ions per precursor to reduce false positive rates. This creates overwhelming scan event concurrency on QQQ MS systems, significantly reducing the duty cycle and hindering effectiveness for global lipid analysis. The Stellar MS utilizes hyper-fast acquisition that increases data acquisition efficiency to capture all product ions per precursor in a single parallel reaction monitoring (PRM) scan event.
- Extended fragmentation capabilities:** QQQ MS systems are limited to MS² using only beam-type fragmentation, often requiring acquisition of the same precursor in negative electrospray ionization (ESI) mode to confirm the head group and characterize acyl chains. The Stellar MS can acquire collision-induced dissociation (CID) in one ESI polarity, generating diagnostic product ions that confirm both head group and acyl chain specificity in a single PRM spectrum.
- MS³ capabilities for isomer resolution:** The Stellar MS goes beyond MS² to perform fast, sensitive MS³ acquisition (up to 40 Hz), enabling the specificity required to quantify molecular lipid species contributions that would otherwise require extensive chromatographic optimization or remain unresolved.

Key advantages of PRM over SRM

- **Improved data acquisition efficiency:** 2–6X faster depending on lipid species, as only one scan event is needed per precursor rather than 2–4 separate SRM transitions.
- **Enhanced lipid species assignment:** Measuring multiple product ions per MS² spectrum provides greater confidence in structural identification.
- **Post-acquisition flexibility:** Researchers can retrospectively mine data to select different product ions for quantitation without reanalysis.
- **Reduced method development time:** No need to optimize individual transitions; one method covers all product ions.

Conclusion

This smart note presents significant advantages for translational lipidomics by combining Vanquish UHPLC Systems with the Stellar Mass Spectrometer, offering capabilities beyond traditional LC-QQQ MS systems performing SRM.

Key workflow advantages

- **Hyper-fast PRM acquisition:** The hybrid quadrupole dual-pressure linear ion trap architecture enables PRM acquisition rates up to 140 Hz for MS² and 40 Hz for MS³, extending both lipid subclass and lipid species coverage while maintaining excellent sensitivity.
- **Retrospective data mining:** PRM acquisition captures all product ions in every spectrum, enabling post-acquisition analysis to identify and quantify isomeric lipid species without reanalysis. This is fundamentally impossible with predefined SRM transitions.
- **Seamless workflow integration:** The ability to perform fast MS³-based PRM acquisition enables researchers to use the same LC-MS hardware throughout the translational workflow—from global lipidome analysis to targeted lipid panel validation—eliminating the need for separate discovery and validation platforms.
- **Comprehensive UHPLC solutions:** The Vanquish UHPLC platform addresses diverse experimental requirements including reversed-phase and HILIC separations, massive sample throughput with dual-channel configurations, and direct method transfer from analytical to microflow gradients for improved sensitivity with precious samples.
- **Dual dissociation methods:** HCD and CID fragmentation in a single ESI polarity provide complementary structural information, confirming both head group identity and acyl chain composition without requiring polarity switching or multiple acquisitions.

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