High-throughput lipidomic profiling using differential ion mobility separation

Infusion MS/MS^{ALL} workflow using the TripleTOF 6600+ system with a SelexION device

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Global lipid profiling has many biological implications in studying how drugs and disease alter biological lipid composition. A major consideration in any MS analysis of lipids is the issue of isobaric overlap, which arises because many lipids are extremely similar in molecular weight and differ only in the size of their individual head groups. Even though the Infusion MS/MS^{ALL} workflow can identify thousands of lipid species in a sample, isobaric overlap diminishes the capabilities of the mass spectrometer (MS) to appropriately separate all lipid classes.[1] Therefore, other modes of separation must be employed prior to MS analysis.

Traditionally, liquid chromatography separation has been employed to address the issue of isobaric overlap. However, the lengthy sample preparation, method development, and chromatographic run times necessary for adequate separation can diminish throughput. Differential ion mobility separation (DMS), on the other hand, uses a molecule's dipole moment rather than shape and size to separate it out from a matrix. A separation voltage is introduced to initially separate out the ions towards one of the electrodes depending on its dipole moment-induced mobility characteristics. A distinct compensation voltage value corrects the flight path of each ion as it travels to the MS.

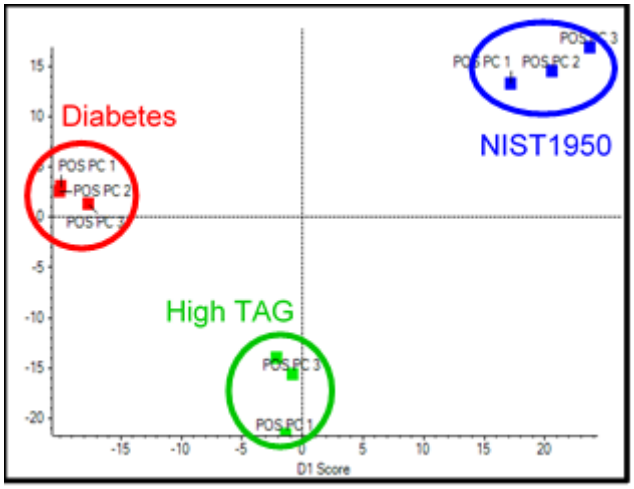

Figure 1. Method can differentiate phosphatidylcholine differences in reference plasma types. The described method effectively detects differences in lipid composition among different blood plasma types – blood plasma from diabetes patients, normal human blood plasma (NIST 1950), and blood plasma from patients with high triacylglycerol levels (High TAG).

Here, DMS is coupled with the Infusion MS/MS^{ALL} workflow to provide a fast, robust lipid profiling method for high-throughput lipid quantification.

Key feature of MS/MS^{ALL} with a SelexION device

- Differential mobility separation provides fast separation of isobaric species by resolving different lipid classes before MS analysis
  - Chemical modifiers can be used to assist in additional separation.[2]
  - SelexION device installs in front of the MS system, making it easy to install when needed
- Current method provides a comprehensive phospholipid profile in under 15 minutes, faster than typical LC methods
  - Can be applied to other lipid classes by adjusting the compensation voltage per species of interest
- Direct infusion technique does not require extensive sample preparation that an LC separation method would entail
- Provides reproducible, reliable, and robust data
- Seamless data analysis with LipidView and MarkerView software

Methods

Sample preparation: For workflow development and performance evaluation, 20 µL of the EquiSPLASH LIPIDOMIX Quantitative Mass Spec Internal Standard Kit (Avanti Polar Lipids) was diluted in 500 µL of 1:1 methanol/dichloromethane. For later experiments, 40 µL of bovine heart extract (BHE) from Avanti Polar Lipids was diluted in 1:1 methanol/dichloromethane with 20 mM ammonium acetate. Blood plasma samples were obtained from the National Institute of Standards and Technology (NIST). The three samples used were the Type 1 diabetes plasma, high triacylglyceride plasma, and normal human plasma. For blood plasma preparation, 25 µL of blood plasma was purified by liquid-liquid extraction and spiked with 40 µL of EquiSPLASH standard mix. The extracts were then resuspended in 1:1 methanol/dichloromethane with 10 mM ammonium acetate.

Mass spectrometry: The TripleTOF 6600+ system was equipped with a DuoSpray Turbo V ion source. Data was acquired using the Infusion MS/MS^{ALL} acquisition mode, consisting of a TOF MS scan and series of MS/MS scans.

Ion mobility: The SelexION device used 1-propanol as a modifier with the modifier composition set to low. The separation voltage (SV) was set to 3800 V, the DMS resolution enhancement set to off, and the DMS temperature was set to medium.

Data processing: LipidView software 1.3 was using to identify and quantify the detected lipids. Quantitative information on all lipids was then imported into MarkerView software for statistical analysis.

Table 1. Compensation voltages by class. The COV was ramped in both positive and negative ion mode, and the corresponding COV values were reported.

Lipid Class	Polarity	COV value
Phosphatidylcholine	*(+)*	*7*
Sphingomyelin	*(+)*	*12*
Phosphatidylcholine	*(-)*	*3*
Phosphatidylethanolamine	*(-)*	*5*
Phosphatidylserine	*(-)*	*12*

DMS separation of isomers

In the positive ion mode, EquiSPLASH standards were infused via syringe pump with the DMS cell on. The compensation voltage was ramped from -40 to +30 and a TOF scan was collected with the m/z range of 50-1000 Da. Phosphatidylcholine (PC) and sphingomyelin (SM) are two classes of compounds that in the positive ion mode share many M+1 isotopes and can lead to false positive identifications and possible overestimations. By finding a COV that is specific to each class, users can properly identify and quantify lipid species in each class (Figure 2). To demonstrate this separation and isobaric overlap removal, Figure 3 is a TOF spectrum that shows the potential for overlap. The top pane is a PC spectrum that was acquired with a COV value of 7 and the bottom pane was acquired with a COV value of 12. The green box highlights one example of overlap in the spectrum. At m/z 731.5 is sphingomyelin species SM(d18:1/18:1), however at 732.5 a PC(32:0) exists that would likely be lost in the isotopic spectrum of the more dominant SM lipid. By using the SelexION device, these classes are easily separated and this overlap minimized, allowing confident identification and quantification these lipids.

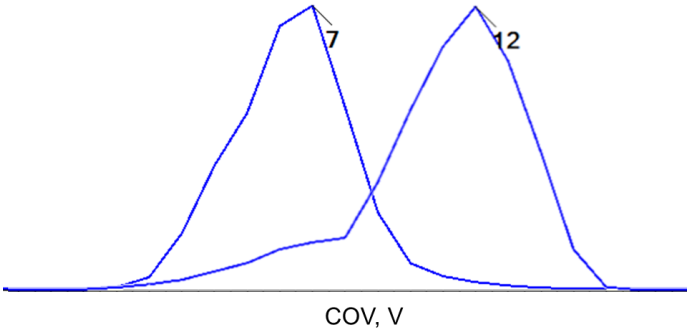

Figure 2. Detection of COV values for major lipid classes. Ramping the COV shows the two major lipid classes in positive ion mode can be separated by different COV value. For PC, that value is 7, and for SM, that value is 12. Similar analysis was conducted in negative ion mode and the appropriate COV values were determined, as listed in Table 1.

Improvements of identification

Using a direct infusion approach for high-throughput lipid analysis comes with the challenge of false positive identification in the negative ion mode due the isolation window of the MS/MS being 1 Da. In Figure 4 (left), a complex MS/MS spectrum was acquired at m/z 812.6 collected in bovine heart extract (BHE) and highlights the large degree of overlap for lipids. There are several possible lipid species present at that mass with the ability for multiple adducts to form. Often, these lipid species are all reported and could show significant changes, but are either not in the matrix or relative amounts can be over- or underestimated due to the isobaric overlap.

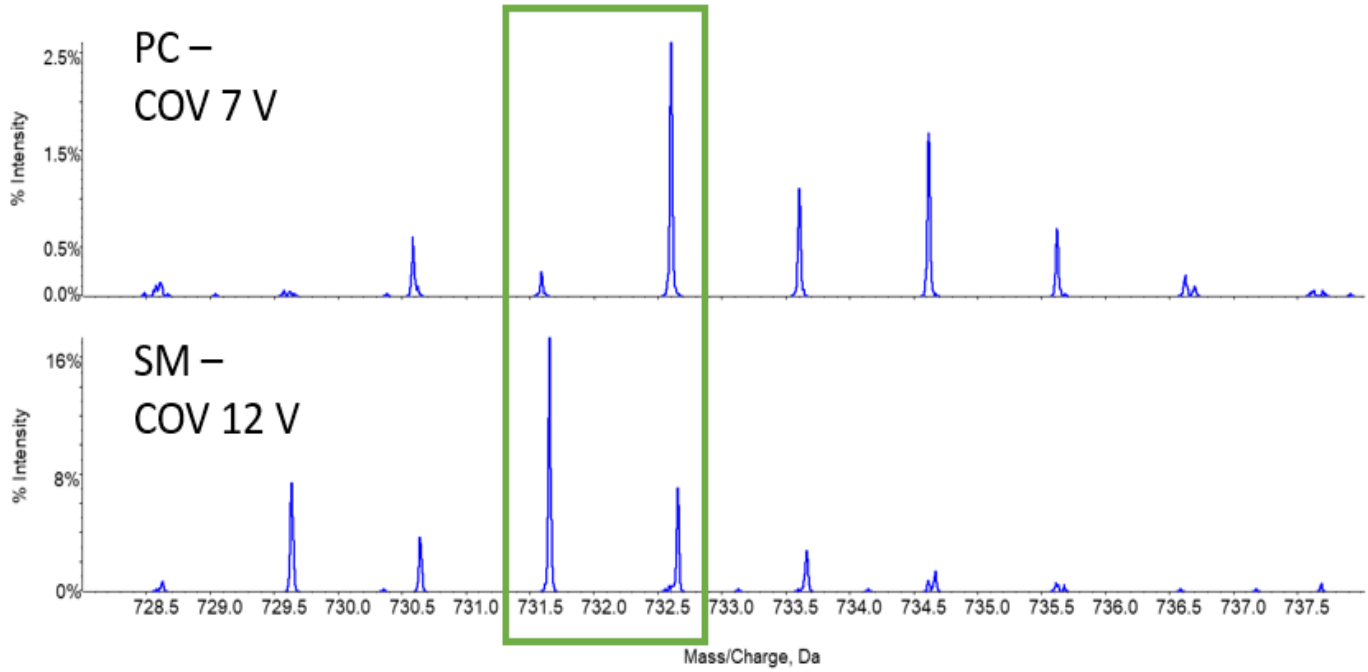

Figure 3. Separation of common isomers. A compensative voltage was selected for PC (7 V) and SM (12 V) and the TOF MS spectrum is displayed for each class. Outlined in the green box are common isobaric species that would be overlapping if no additional separation was applied. This is a core challenge of lipidomics and this overlap can lead to false positive identifications or an overestimation of one or more species from the contribution of isotopic overlap. Using the SelexION device, these overlapping classes can be easily separated and positively identified and quantified.

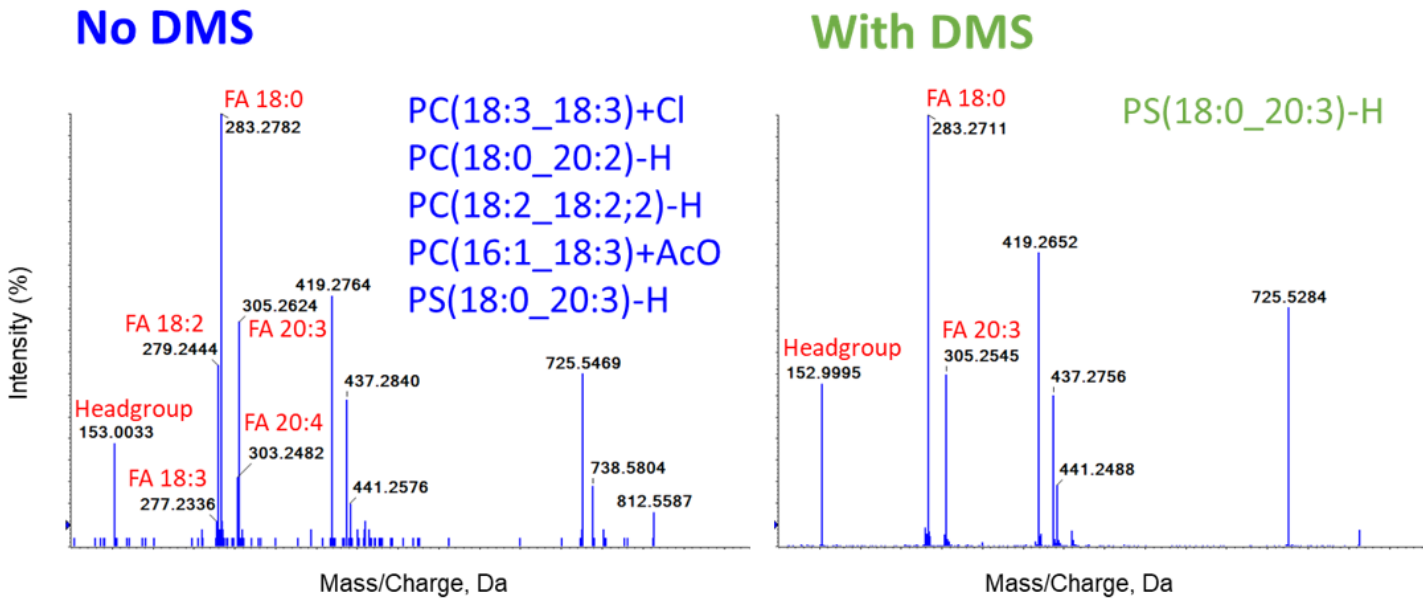

Figure 4. Confidence in identification. (Left) The MS/MS spectrum acquired at m/z 812.5 is annotated for lipid species and shows a number of different fatty acid species. There are several lipid species that are possible within this single mass unit that could belong to several classes with a multitude of adducts. (Right) By finding a COV that is exclusive to the PS class, a much simplified MS/MS spectrum is obtained and the identification can be more confidently confirmed to be PS(18:0_20:3).

Figure 4 (right) uses the DMS cell with a COV value of 12 to isolate phosphatidylserine (PS). The spectrum utilizing the DMS shows a single lipid species of PS(18:0_20:3) at the same m/z 812.6. This provides confidence in identification and the capability to accurately quantify with the fatty acid species.

Using this workflow, users have the ability to optimize the COV value for every lipid class of interest in both the positive and negative ion mode to lower the false discovery rate of lipid species annotation without sacrificing throughput. Each lipid species method requires an average of 1.6 minutes of acquisition. The six phospholipid subclasses in the negative ion mode would take less than 10 minutes of acquisition.

Biological samples and robustness

To demonstrate the effectiveness of this workflow, three types of NIST plasma were extracted and infused to evaluate changes in the 5 classes described above. Figure 1 shows the supervised PCA analysis, highlighting the PC1 vs. PC2 loadings plot from MarkerView software and illustrating both the reproducibility of the method and the ability to capture the differences between each plasma type. Table 2 provides the percent CV values for each of these groups.

Table 2. Percent CV by group. For the PC classes, intensities were summed and %CV are reported for each group of pooled plasma.

Group	%CV
Diabetes	*0.38*
High TAG	*3.52*
NIST Normal	*8.24*

To further investigate the changes in lipid species, data was exported from LipidView software to MarkerView software for statistical analysis. Figure 5 displays 3 different PC species that are significantly changing between the groups. Even though it is the brutto level of these lipid species that is under investigation, noticeable changes between the groups in overall carbon length and degree of desaturation can be observed. This high-throughput analysis allows quick investigation these changes to then guide biological research and gain further insight in the lipid composition of these possible markers.

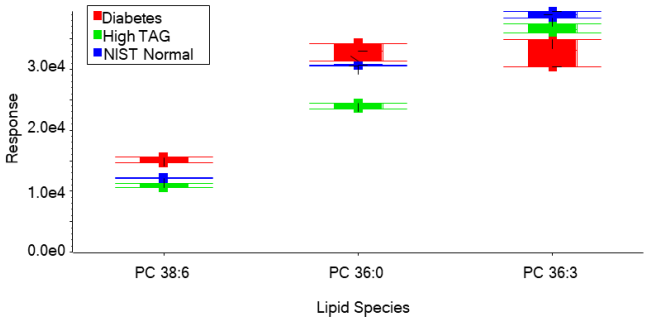

Figure 5. Differences in 3 PC species levels among blood plasma types. The lipid species PC(38:6), PC(36:0), and PC(36:3) are present in significantly higher quantities (p-value > 0.05) among the groups of plasma: Diabetes, High TAG, and NIST1950.

Conclusions

The SelexION device is a key technological advance in lipidomic research. Using DMS as an upfront orthogonal separation enables the fast gas phase separation of complex lipid mixtures into their individual lipid classes before MS analysis, effectively addressing the problem of isobaric interferences among different lipid classes and sub-classes.

Using the SelexION device coupled with the Infusion MS/MS^{ALL} workflow provides a seamless, high throughput strategy for lipidomics biomarker discovery and quantification delivering an automated, untargeted MS acquisition strategy. Here, the analysis was optimized to provide automated infusion with low carryover and excellent reproducibility to analyze user specified lipid classes in a fast, robust, selective method.

References

1. Molecular characterization and quantification of lipids with high resolution accurate mass tandem MS techniques. SCIEX technical note, RUO-MKT-02-13720-A.
2. Differential mobility separation for improving lipidomic analysis by mass spectrometry. SCIEX technical note, RUO-MKT-02-4802-A.

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