Accelerating Lipid Profiling Acquisition Strategies with Differential Mobility Spectrometry and SWATH® Data Collection

Eva Duchoslav[1]; Brad Patterson[2]; J. Larry Campbell[1]; Yves LeBlanc[1]
[1]SCIEX, Concord, ON, L4K 4V8 Canada; [2]SCIEX, Melbourne , Australia

ABSTRACT

Differential mobility spectrometry (DMS) provides effective separation of lipid species in shotgun lipidomics workflows[1,2]. A potential coupling of this methodology with SWATH® acquisition was investigated in light of accelerating accurate mass lipid profiling strategies and development of dedicated data processing tools.

INTRODUCTION

Shotgun lipidomics workflows that employ accurate mass spectrometry have been well established. These approaches have helped to solve challenges involving confident quantitation and identification of many isomeric lipid species, as well as lipids that share common MS/MS fragments, by advances in hardware and dedicated data processing.

Another improvement to these shotgun workflows is the addition of DMS which offers enhanced selectivity via orthogonal separation of lipids based on the geometry around a lipid's charge site. However systematic collection of MS/MS data for any candidate lipid at any compensation voltage (CV) can be time intensive. Alternate acquisition strategies that optimize how CV is surveyed in conjunction with required MS/MS specificity may have potential to improve the depth and throughput of lipidome profiling.

MATERIALS AND METHODS

Sample Preparation:
Bovine heart extract (Avanti Polar Lipids, Inc.) was standardized for total amount of lipid of approximately 10µM in 45/45/10 DCM/MeOH/water.

Instrumental Analysis:
ESI/DMS/MS/MS data were collected with a hybrid TOF mass spectrometer equipped with a planar DMS system, in both positive and negative modes (DMS temperature 150ºC, SV = 4000V, DR off, 2-propanol).

Multiple ESI data collection strategies were used.

- TOF MS with SWATH® acquisition
  - SWATH® window range from 650 to 900 m/z units, windows of equal width of 13 m/z units and accumulation time of 70ms (total cycle time 1.5s)
- DMS/TOF MS with information dependent acquisition (IDA)
  - 10 dependent experiments TOF MS/MS accumulation time 1s
- DMS/TOF MS with SWATH® acquisition
  - using either narrow (6 m/z units) or typical (13 m/z units) SWATH® windows covering m/z range from 650 to 900, TOF MS/MS accumulation time of 96ms, cycle time 2.2s or 5.1s.

Compensation voltage ramps covered range from -50V to +15V with steps of 0.25V for both positive and negative acquisitions.

Data processing:
Data were processed with research grade tools, namely an enhanced LipidView™ Method Exporter and the DMS Inspector plug-in for PeakView® software to construct and compare lipid profiles obtained with different methods as well as to determine confidence in molecular species assignment in light of the time required for instrumental analysis. The data processing workflow is illustrated in Figure 1.

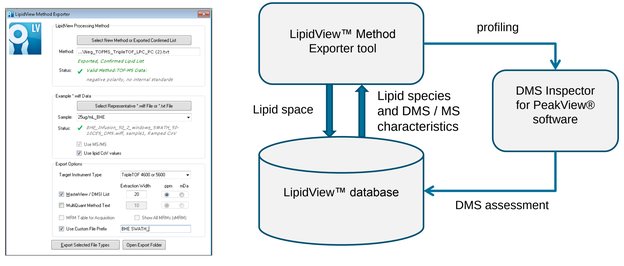


Figure 1. Data processing workflow utilized description of lipids and lipid MS and DMS behavior captured in LipidView™ database. LipidView Method Exporter tool collated required lipid species details to be used in the DMS Inspector (DMSI) Plug-in for PeakView® software. DMSI tool was used in 2 modes: 1/ DMS assessment mode to find differential mobility properties for lipid classes and update LipidView database, and 2/ profiling mode to search and profile lipids in biological samples.

RESULTS AND DISCUSSION

Similar to MasterView™ software DMS results are annotated in terms of their agreement with anticipated values for:

- Accurate mass
- Target RT (LC Data)
- Isotope pattern
- Target CV
- CV profile width

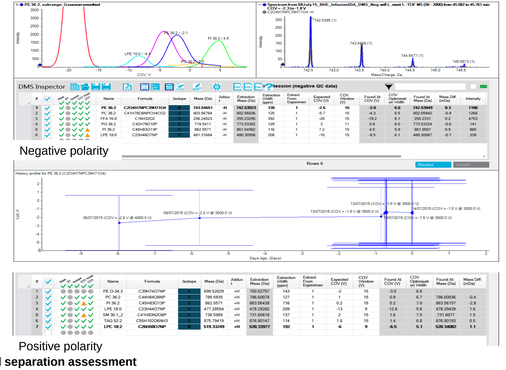


Figure 2. DMS lipid separation assessment
No internal standards were used in this study, therefore endogenous lipids were selected to confirm the dominant and complementary ion types as well as characteristic compensation voltage (CV) for lipid classes. DMSI supports archiving of historical CV values.

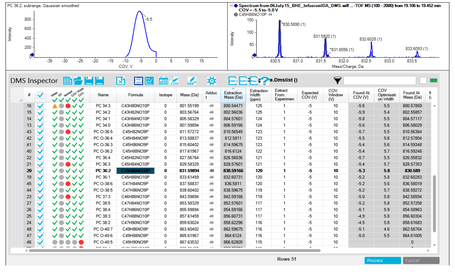


Figure 3: Understanding lipid profiles with the DMSI research tool.

51 PC lipids (by total composition) detected in negative ion mode as formate adducts. They were easily confirmed based on accurate mass, isotope pattern and CV profile. Entries with isotope pattern failure point to the necessity to remove isotope contribution from less saturated PC analogues.

When working with IDA data, TOF MS/MS were reviewed concurrently with TOF MS. In case of SWATH® data putative fragments were extracted to compose fatty acid profile for each class.

The typical lipid CV ionogram peak width at half height was 5V, suggesting that CV step size of 1V will yield sufficient number of points across each peak.

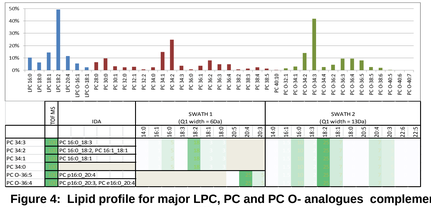


Figure 4: Lipid profile for major LPC, PC and PC O- analogues complemented with structure details available for lipids species with different acquisition strategies

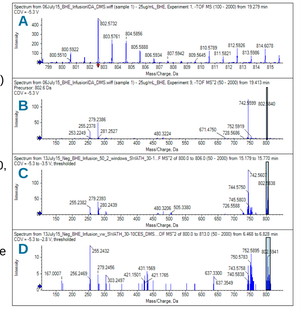
Figure 5. Example data from different acquisition strategies with varying Q1 isolation width

Panel A : part of TOF MS spectrum at CV for PC class.

Panel B: TOF MS/MS spectrum of m/z 802.6 (PC 16:0_18:2) and minor (PC 16:1_18:1)

Panel C: TOF MS/MS, SWATH® window width 6 m/z units, from m/z 800 to m/z 806 covering mono-isotopic masses of PC 34:n (n=1,2or 3) . m/z 303 fragment (arachidonic acid) is likely a product of isotope of PC 34:4.

Panel D: SWATH Spectrum, SWATH® window width of 13 m/z units, from m/z 800 to m/z 813 covering PC with 34:x carbons as well as PC P-36:y and PC O-36:z species (where x, y and z represent number of double bonds) since under the DMSi settings used these subclasses were not separated. Fragment chain distribution covers 16:0, 16:1, 18:0, 18:1:18:2 and 20:3, 20:4 and 20:5.



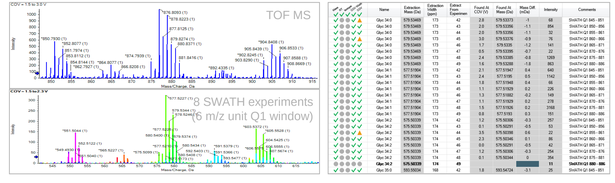


Figure 6: SWATH® profiles of TAG analogues. In positive ion mode, characteristic fragments are derived from the glycerol backbone with 2 acyl chains; the accurate neutral loss between TOF MS and TOF MS/MS points to a specific chain.

In summary, similar to previous studies[2], 450 lipids were identified in either positive or negative acquisition modes. With generic IDA triggering criteria, TOF MS/MS spectra were collected for 65% of major lipid species. SWATH® MS/MS data captured fragmentation for several lipid class analogues in parallel. The ease of deconvolution and confidence in species identification depended on the width of SWATH® Q1 window. With adjusting the CV range, CV step size of 1V and total cycle time of 12s the analysis time was less than 6 minutes per polarity making the DMS SWATH® data collection a powerful strategy for comprehensive lipid profiling.

CONCLUSIONS

Accurate mass measurements on a TripleTOF® platform in combination with DMS and data independent acquisition, provide an effective setup for lipid profiling in whole lipid extracts. Once the optimum DMS settings are established CV steps can be adjusted to minimize the acquisition time.

While DMS/ESI/IDA provided the most specific data, the MS/MS spectra were collected just for a fraction of the lipidome. TOF MS with SWATH® acquisition of 6 m/z unit windows offered the most comprehensive results, since just a limited number of lipid analogues were fragmented in parallel. TOF MS with wider SWATH® windows reduced significantly the time needed for the analysis and gave accurate overall information on chain profile for a given lipid class.

Research tools, such as enhanced LipidView™ Method Exporter and DMS Inspector were instrumental in data interrogation in support of this workflow.

REFERENCES

1 Lintonen TP, Baker PR, Suoniemi M, Ubhi BK, Koistinen KM, Duchoslav E, Campbell JL, Ekroos K: Differential mobility spectrometry-driven shotgun lipidomics, Analytical Chemistry 2014, 86 (19), 9662-9
2 Duchoslav E, Campbell JL, Baker PRS, Patterson BT ASMS conference 2013, ThP 28-586
3 Maccarone AT, Duldig J, Mitchell TW, Blanksby SJ, Duchoslav E, Campbell JL: Characterization of acyl chain position in unsaturated phosphatidylcholines using differential mobility-mass spectrometry, The Journal of Lipid Research 55, 2014, 1668-1677

TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

Document number: RUO-MKT-10-4077-A