Automated Iterative LC-MS/MS Data Acquisition Improves Coverage of the Plasma Lipidome

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METABOLOMICS 2018 Conference

POSTER P-239

Introduction

A major challenge in mass spectrometry-based lipidomics is the comprehensive characterization of a large and diverse set of lipid species, spanning a wide concentration range within a biological sample. Coupling liquid chromatography (LC) to MS helps reduce the complexity of the ionized lipid population and can also help elucidate isomeric lipid species. Confident lipid annotation requires data acquisition at the MS/MS level to enable product-ion spectral matching against in-silico generated databases. However, comprehensive lipid annotation from data-dependent (Auto) LC-MS/MS data is generally limited by the number of precursors that can be selected for fragmentation during chromatographic elution. Due to concentration bias this strategy often misses important lipid species of low abundance. Here we introduce a new, fully-automated mode of Q-TOF data acquisition where precursors previously selected for MS/MS fragmentation are excluded on a rolling basis. It was previously demonstrated with iterative exclusion that by excluding both background ions and previously selected high abundance lipid ions for fragmentation, sequential injections provide fragmentation of lower abundance lipid species.[1] As a proof-of-principle, we applied the new Agilent Iterative MS/MS functionality to a complex plasma lipid extract to explore the potential benefits.

Experimental

Sample Preparation

Lipids from NIST SRM 1950 pooled human plasma were extracted with a modified Folch method. Following phase separation, the pooled lower organic layers were evaporated, and the dried lipid extracts were resuspended using a mixture of 9:1 methanol/chloroform.

Data Acquisition

Lipids extracts were analyzed by reverse-phase LC-MS using a 1.9µm, 2.1x150mm Agilent InfinityLab Poroshell 120 EC-C18 column with a 40-minute LC-MS/(MS) method.

Agilent MassHunter LC/MS Data Acquisition software Version B.09 was used that offers the new Iterative MS/MS functionality.

The Agilent 6545 LC/MS Q-TOF with 1290 Infinity II LC system

Agilent 6545 LC/MS Q-TOF Mass Spectrometer	
Polarity / Mode	Positive or Negative / EDR 2 GHz
MS Mass range	100-1200 *m/z*
MS Acquisition rate	3 spectra/s
MS/MS Mass range	80-1200 *m/z*
MS/MS Acq rate	4 spectra/s
Isolation width	Narrow (~1.3 *m/z*)
Precursors/cycle	6
Collision energy	Fixed: 20, 35 V
Threshold for MS/MS	500 counts and 0.01%
Active Exclusion	4 spectra, exclude for 0.1 minutes
Isotope Model	Common organic molecules
Iterative MS/MS mass tolerance	(+/-) 20 ppm
Iterative RT exclusion tolerance	(+/-) 0.1 min

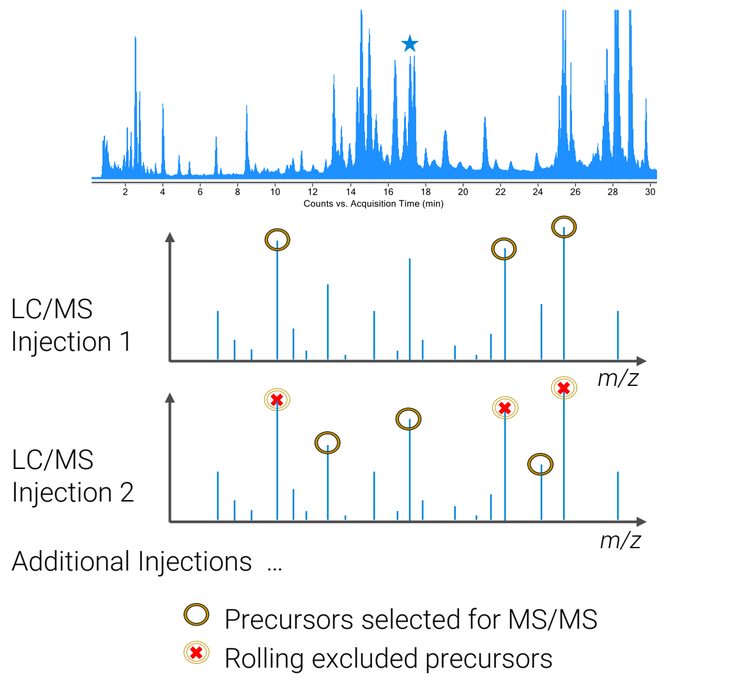
Data Analysis

LipidMatch software[2], an R-based tool that simulates over 300,000 lipid species spanning over 60 lipid types, was used for rule-based lipid identification. MS-only, conventional Auto-MS/MS, and Iterative MS/MS datafiles from plasma lipids and appropriate mock extraction controls were submitted in batch format to the LipidMatch GUI. Excel was used to compare/contrast the resulting lipid identifications.

Experimental

Principle of Iterative MS/MS

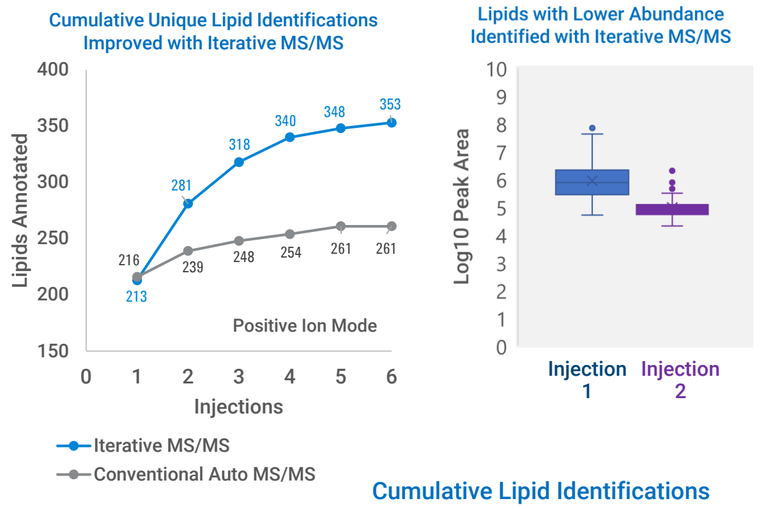
The strategy of iterative MS/MS acquisition is illustrated below



Results and Discussion

Iterative MS/MS Increases Lipid Identifications and Enriches Lipid Ions of Low Abundance

The number of cumulative unique lipid identifications across multiple Iterative MS/MS data acquisition files increased compared to conventional Auto MS/MS (Fig 1) files. Additionally, sequential injections with Iterative MS/MS selected lipid ion precursors of lower abundance.



Cumulative Lipid Identifications

	Positive	Negative	Combined
Iterative	353	166	439
Conventional	261	73	297

Figure 1. Plasma lipid extract results for positive ion mode. Cumulative unique lipid identifications from LipidMatch software across multiple data acquisition files (left panel). Boxplot of MS1 feature peak areas corresponding to all identified lipids from two sequential Iterative MS/MS injections (right panel). The table compares the cumulative number of lipid identifications from negative, positive, and combined polarities for six injections.

Results and Discussion

Iterative MS/MS Improved Coverage for Select Lipid Classes

Lipid classes such as acylcarnitines (AcCas), cholesterol esters (CEs), diacylglycerols (DGs), LPCs, LPEs and others were enriched with Iterative MS/MS (Fig 2).

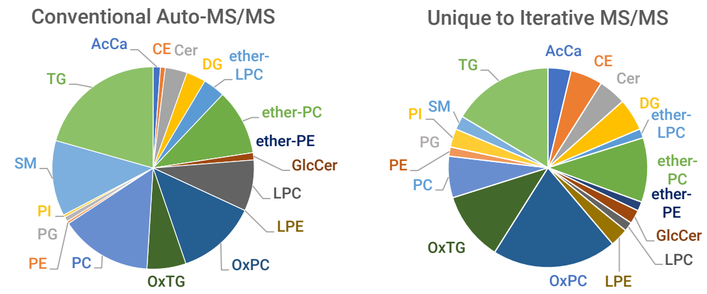


Figure 2. Distribution of lipid classes from cumulative identifications in positive-ion datafiles (n=6) acquired with conventional MS/MS versus additional, unique identifications in datafiles with Iterative MS/MS.

As an example of lipid class enrichment, Figure 3 demonstrates identification of 2 CE species from a 1st injection. An additional 4 low-abundant CE species were identified from the 2nd injection after exclusion of overlapping TGs and CEs of higher abundance.

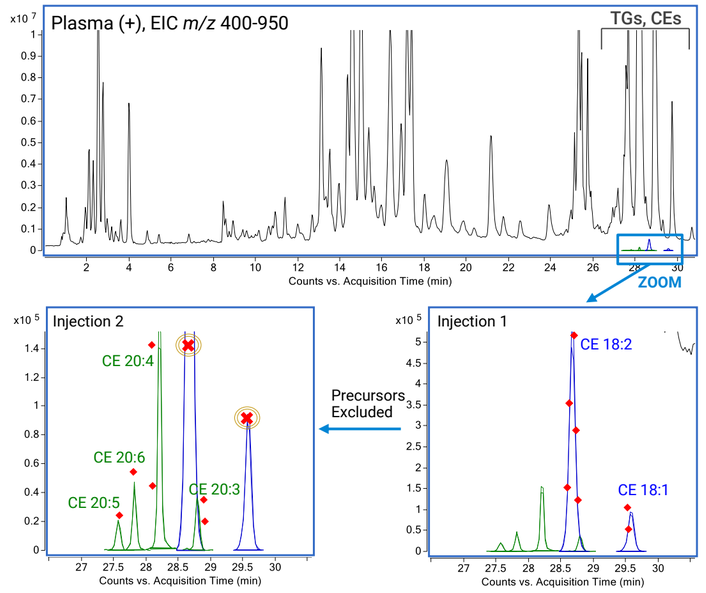


Figure 3. Extracted MS/MS chromatograms from a plasma lipid extract, showing Iterative selection of cholesterol ester ($M+NH_4$) ion precursors.

Conclusions

The newly developed automated Iterative MS/MS Acquisition functionality in MassHunter Acquisition software:

- Significantly increased lipid identifications in complex plasma extracts (>45% with combined polarities)
- Showed specific advantage for lipid species of low abundance and in spectra-dense regions of the chromatogram

Together these results demonstrate that lipidome coverage can be improved with Iterative MS/MS, a useful addition to the lipidomics toolbox.

References

1. Koelmel, J.P. et al. *Expanding Lipidome Coverage using LC-MS/MS Data-Dependent Acquisition with Automated Exclusion List Generation*. J. Am. Soc. Mass Spectrom. 2017 Mar; 28: 908-17
2. Koelmel, J.P. et al. *LipidMatch: an Automated Workflow for Rule-Based Identification using Untargeted High-Resolution Tandem Mass Spectrometry Data*. BMC Bioinformatics. 2017 18:331