From Discovery to Targeted Analysis of Lipids

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Introduction

Targeted lipidomics is challenging because of the diversity of lipids both within a lipid class and at a global level. The amount of work needed to determine the targets and to ensure that the ID of each lipid target is validated is daunting. We have used a validated, well tested, rugged targeted lipidomics method that covers 35 lipid classes as a starting point to develop a workflow from discovery to targeted lipidomics[1]. We call the targeted lipidomics method the "Backbone" method. Our workflow allows the flexibility of adding panels of lipids to the original, validated "Backbone" method. We have expanded the backbone method to include a complete oxylipin panel.

We tested the backbone method and the complete workflow with two sets of experiments. The first experiment was by spiking known lipids into a yeast extract at two different concentrations and using the backbone method to make sure the retention times are stable with good recovery and a two-fold change of the spiked lipids. The second experiment is the discovery to targeted workflow. The starting point of this workflow is the discovery experiment using a QTOF. The study has been published.[2] Our focus from the discovery work is the expression of PG and BMP lipids. Here we show the methodology to bring in a panel of PG/BMP lipids from the lipid creator/skyline tools and add to the list of lipids and lipid classes covered by the backbone method.

Experimental

Sample Preparation

Yeast extract was purchased from Cambridge Isotope Lab and reconstituted in Methanol/IPA 50:50 to give a final concentration of 0.75 mg/mL or 1x108 Pichia pastoris cells. Table 1. shows the lipid classes and their concentrations that were spiked into the yeast extract. Mouse samples were extracted and prepared following the protocol in Reference 2..

LC Conditions

The LC column (Zorbax Eclipse Plus C18 (2.1x100mm 1.8µm,), Mobile solvent (Water/ACN/IPA with Ammonium Formate) and the gradient were kept the same as specified in Reference 1. No changes were made to the method in order to reproduce the retention times and retention time windows of the validated method .

6495C Ion Funnel QQQ Parameters

The Agilent Jet Stream source conditions and ion funnel were optimized for the best response over the broad range of lipid classes targeted in this method.

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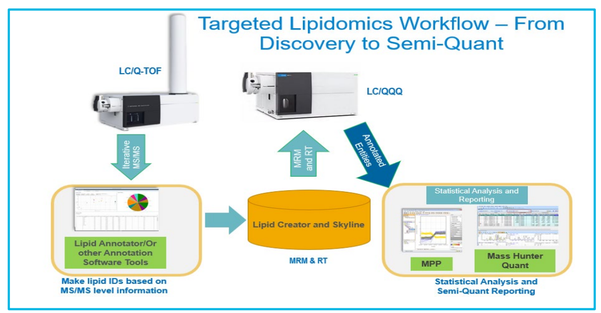
Experimental

Lipid Species	Concentration Range (pmole on Column)	Number of Compounds within a Class
PC	1.1-1.96	10
PE	1.4	1
LPC	1.89-1.96	2
LPE	2.05	2
PG	1.30-1.31	2
PS	1.32	1
SM	1.35-1.52	2
PI	1.33	1
Ceramide	1.81-1.86	2
Dihydroceramide	1.76	1
Sulfatides and Sulfatides-OH	1.1-1.2	10
Sphingosine	3.49	1
Chol-Ester	1.48	1
Free Cholesterol	2.43	1
MAG	2.62	1
DAG	1.65	1
TAG	1.2	1

Table 1: Shows the classes of lipids spiked into the yeast extract and their concentrations.

Figure 1 . Shows the LC/MS/MS instrumentation for discovery and targeted workflows consisting of an Infinity II LC stack which includes a multi sampler, column compartment with a switching valve, and high speed 1300 bar pump .

Figure 2 (Bottom). Shows the workflow from discovery to targeted analysis and getting a semi-quant report with the 6495C QQQ.



Results and Discussion

Results from the Spike Study

The chromatogram resulting from the spike study of the lipids in Table 1 and adding in the oxylipin panel is shown in Figure 3 below. The peaks are well separated with very good peak shape across the range of polarity.

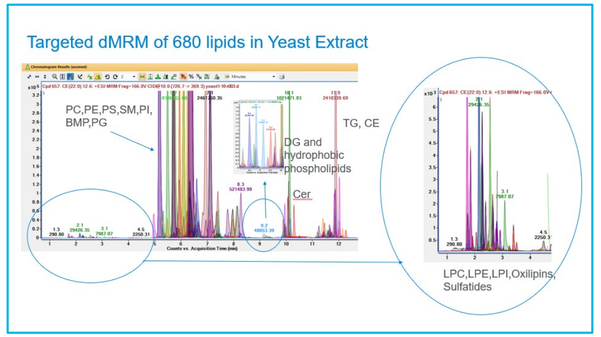


Figure 3: Chromatogram showing the separation of lipids

The fold change observed when comparing the spiked lipids at two different concentrations is shown below. The absolute value of the fold change in shown in the inset which is the experimental change in concentration between the spikes.

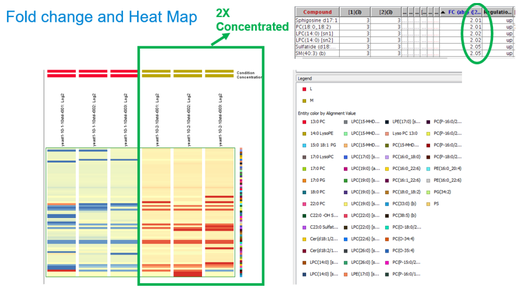


Figure 4. Fold change and heat map. Table on the top right of the Figure shows the absolute value of the fold change between the spikes.

From Discovery to the Targeted Analysis of Lipids

High resolution data from the QTOF is sent to Skyline, and with the help of the Lipid Creator and information from MS/MS spectra acquired on the QTOF, Specific MRMs are selected from the multiple theoretical possibilities in Lipid Creator, and the targeted list is sent to the QQQ. Figures 5 and 6 show the results from a targeted analysis of BMP and PG in the mouse model.[2]

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Results and Discussion

From Discovery with the QTOF to Semi-Quant on the QQQ

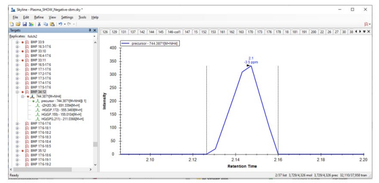

Figure 5. QTOF data in Skyline

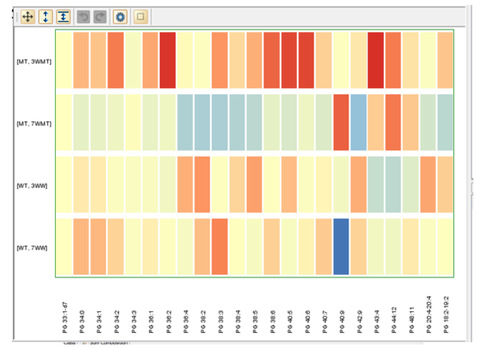

Figure 6. Heat map of changes in PG from targeted QQQ data

Conclusions

The workflow we present allows the flexibility of adding panels of lipids to a validated, tested, targeted dmrm method.

References

1. High-Throughput Plasma Lipidomics: Detailed Mapping of the Associations with Cardiometabolic Risk Factors, Kevin Huynh, Christopher K. Barlow, Kaushala S. Jayawardana, Jonathan E. Shaw, Brian G. Drew, Peter J. Meikle, Cell Chemical Biology, VOLUME 26, ISSUE 1 , P71-84.E4, JANUARY 17, 2019.

2. Lipidomic Analysis Reveals Altered Fatty Acid Metabolism in the Liver of the Symptomatic Niemann–Pick, Type C1 Mouse Model, Melissa R. Pergande, Fidel Serna-Perez, Sheher Banu Mohsin, Jonathon Hanek, Stephanie M. Cologna, Proteomics, 08 August 2019.