APPLICATION OF TARGETED LIPIDOMICS TO DETERMINE CHANGES IN THE PLASMA LIPIDOME OF MALE RATS FOLLOWING REPEAT ORAL ADMINISTRATION OF METHAPYRILENE

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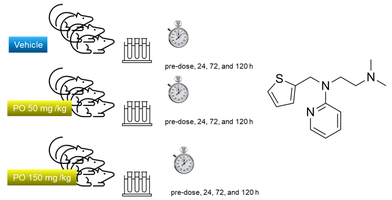
INTRODUCTION

Omics-based biomarker technologies including metabolic profiling and lipidomics are making a significant impact on disease understanding, drug development, and translational research. A wide range of pathophysiological processes involve lipids and monitoring changes in lipid concentration can give valuable insights into drug toxicity and off target pharmacology. Methapyrilene, an antihistamine and anticholinergic, has been shown to cause cancer following chronic administration [1]. Here we report changes detected by targeted HILIC-MS/MS in the plasma lipidome of male Wistar rats following the oral administration of methapyrilene over 5 days at 0, 50 and 150 mg/kg/day.



Blood was collected via vena cava 24, 72 and 120 h post dose (D1, D3, D5). A study QC was constructed by pooling 10 µL of plasma from each sample. Plasma samples and QCs (25 µL) were protein precipitated with 125 µL IPA/ACN (1:2, v/v) containing Avanti EQUISPLASH™ lipid stable label isotope mix diluted 1:500, then vortex mixed and incubated at 2ºC 2 h (shaken every 30 min) then centrifuged for 10 min. The supernatant was transferred to sample vials (Waters Total Recovery) for analysis.

A panel of 435 unique lipids were measured using an 8 min HILIC UPLC method (Waters ACQUITY Premier UPLC system™ with Premier BEH Amide Column, 1.7 µm, 2.1 mm X 100 mm) coupled to a tandem quadrupole MS (Waters Xevo™ TQ-Absolute) operating in successive positive, then negative ion MRM mode [2]. The MRM provide lipid IDs. The LC peak areas were determined using Skyline (MacCoss lab [3]) and exported to MetaboAnalyst 6.0 [4] for statistical analysis.

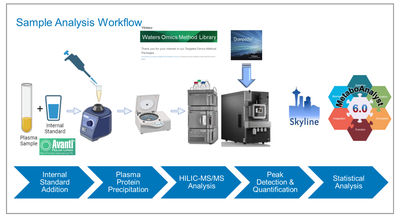


Figure 1. LipidQuan™ complete targeted lipidomics workflow used for preparing and analyzing the plasma extracts [2]

TARGETED UPLC-MS/MS

The rat plasma extracts (n = 2) from the three dose groups over the 5 day period were analyzed with HILIC chromatography that separates the lipids classes by head group [2]. MS/MS detection in +ve and -ve ESI mode using 450 MRM transitions (see ref [2]) quantified 430 lipid species using single-point calibration to the corresponding deuterated lipid internal standard in the same class. Batch QC samples were evenly distributed throughout the analysis, and each sample was analyzed in duplicate. Representative extracted ion chromatograms from the pool QC are shown in **Figures 2A** and **2B,** respectively.

The rapid MRM data acquisition capability of the TQ-Absolute MS gave accurate, reproducible acquisition of the 430 MRM channels (+ve/-ve) ESI modes, consistent assay performance. The method showed excellent stability and reproducibility over the course of the batch, with %CV for the EQUISPLASH mix in the batch QC ranging from 1.5 to 12%, for the 16:1 d7 LPC and 15:0_18:1 d7 PS respectively in **Figure 3.**

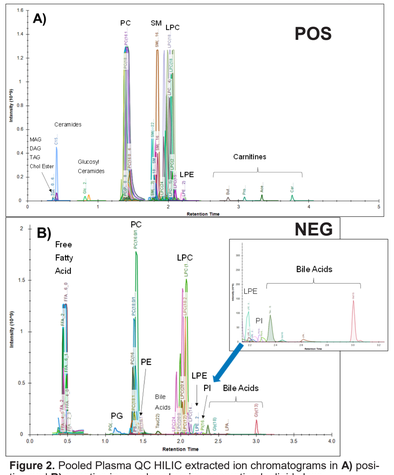


Figure 2. Pooled Plasma QC HILIC extracted ion chromatograms in **A)** positive and **B)** negative ion modes showing separation by lipid class.

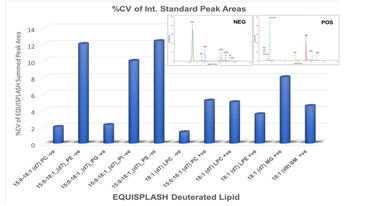


Figure 4. % Coefficient of Variation (%CV) of EQUISPLASH internal standard total peak areas in batch pooled QCs

STATISTICAL ANALYSIS OF TARGETED LIPIDOMICS DATA

Principle Component Analysis (PCA) of the chromatographic peak areas from the 3 groups showed no observable difference in the vehicle only rat on all three sampling days (D1, 3 & 5; 24h post dose). The D1 24h samples from both the 50 and 150 mg/kg methapyrilene dosed group clustered with the vehicle only samples as did the D3 50 mg/kg dosed samples. However, the D3 150 mg/kg samples were separated from the vehicle samples. The D5 50 and 150 mg/kg dose group samples were both separated from the vehicle samples and from the D3 50 mg/kg rats. These two D5 groups did not cluster together either, suggesting differences in the lipid profiles of the 50 and 150 mg/kg dose group samples on D5 as seen in **Figures 5A** and **5B**.

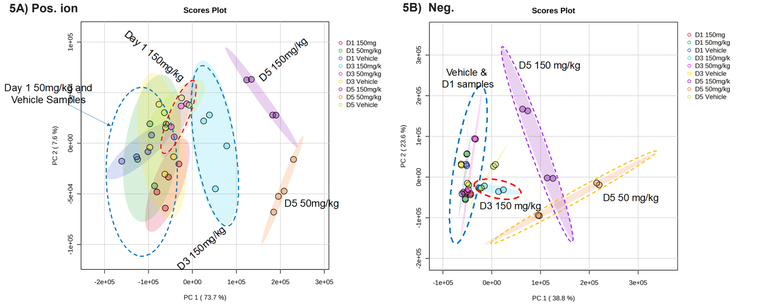


Figure 5: PCA scores plots from the statistical analysis of the **(A)** +ve mode; and **(B)** -ve mode ESI HILIC-MS/MS analysis of rat plasma for vehicle (control), 50 mg/kg dose, and 150 mg/kg dose taken on Day 1 (D1), Day 3 (D3), and Day 5 (D5)

VARIABLE IMPORTANCE PLOT OF LIPID MARKERS

Variable Important Plots (VIP) provide heat maps of highest varying specific lipid chromatograph peak areas amongst the three conditions (vehicle, 50 mg/kg dose, and 150 mg/kg dose. In +ve ESI mode, ceramides (Cer), glucosyl ceramides (GlcCer). and carnitines contributed most significantly to the observed variance in the data. While in -ve ESI mode, free fatty acids (FFA), bile acids, PI, PE, and LPEs all showed some degree of dysregulation, contributing to the observed variation in the statistical analysis in **Figures 6 A** and **B**, most significantly for 50 mg/kg and 150 mg/kg methapyrilene doses on day 5 (D5).

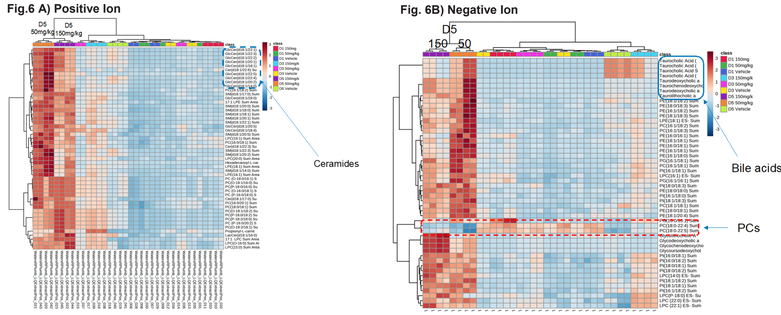


Figure 6: Variable Importance Plot (VIP) obtained from the statistical analysis of the **(A)** +ve ESI mode; and **(B)** -ve ESI mode HILIC-MS/MS analysis of rat plasma over the time course of methapyrilene administration

DISCUSSION

Changes the in the abundance of Glucosyl ceramides (GlcCer) and Carnitines were compared between the dose groups by sample time as shown in **Fig. 6A.** The data in **Figure 7** illustrate the increase in GlcCer (d18:1/22:1) and GlcCer(d18:1/16:0) relative to the other GlcCer for the 150 mg/kg dose group.

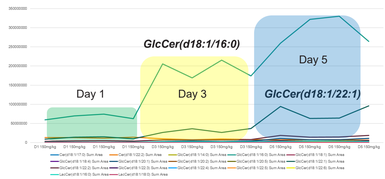


Figure 7: Variation in Ceramide and GlcCer abundance following dosing with methapyrilene at 150 mg/kg sampled over five days (pos. ion).

Similarly, the relative abundances of Hexadecanoyl-L-carnitine (C16:0) and Oleoyl-L-carnitine (C18:1) significantly increased on day 5 at the 150 m/g kg dose relative to D1 and D3, and relative to the other carnitines as in **Figure 8.**

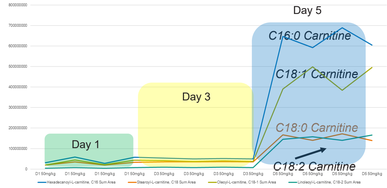


Figure 8: Variation in Carnitine abundance following dosing with methapyrilene at 150 mg/kg over 5 days (pos. ion).

Changes in lipid abundance were also observed in the -ve ESI data. Free fatty acid (FFA) (C20:0) decreased over the entire five day dosing period at 150 mg/kg dose vs. other FFAs, such as C22:3. The downward trend was consistent over all 150 mg/kg dosed rats, illustrated in **Figure 9.**

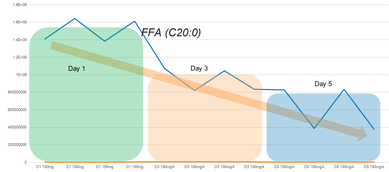


Figure 9: Variation in free fatty acid (FFA) abundance following dosing with methapyrilene at 150 mg/kg (neg. ion).

Glycochendexoycholic acid (GCDCA), glycoursodeoxycholic acid (GUDCA), and glycodeoxycholic acid were most upregulated bile acids All three significantly increased on D5 in both the 50 mg/kg and 150 mg/kg samples vs. the vehicle. The 150 mg/kg dosed samples also showed greater increase in these bile acids relative to the 50 mg/kg samples on day 5, seen below in **Figure 10**.

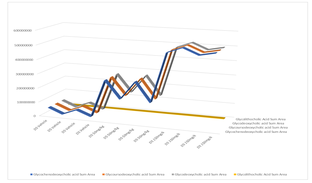


Figure 10: Variation in Bile Acid abundance following dosing with methapyrilene at 50 mg/kg and 150 mg/kg seen on day 5 (D5)

CONCLUSIONS

- **The dysregulation of the plasma lipidome following oral administration of methapyrilene in male Wistar rats was studied using a rapid (8 min.) UPLC HILIC-MS/MS assay on a tandem quad MS with MRM for lipid identification.**
- **A broad range of lipid classes, including mono-, di-, triglycerides, FFA's, and cholesterol esters ceramides, hexosyl ceramides, PG, PC, SM, LPC, LPE, PS, PA, PI, LPA, and LPI were quantified using stable labelled isotopes.**
- **The HILIC-MRM method displayed excellent reproducibility and accuracy through the course of the analytical batch with CV ranging from 1.5 – 12% for internal standards.**
- **Selected bile acids, carnitines, LPEs and GlcCer lipids increased following dosing with methapyrilene, while selected FFA and PE lipids decreased post dose, with biggest changes seen at Day 5 (D5) using statistical analyses of LC-MSMS data.**

References

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The authors declare no competing financial interest.