Large Scale Lipid Profiling of a Human Serum Lipidome Using a High Resolution Accurate Mass LC/MS/MS Approach

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Introduction

Lipids play a key role in cell, tissue and organ physiology with diseases such as cancer and diabetes which involve disruption of their metabolic enzymes and pathways. Identification of unique lipid biomarkers to distinguish healthy humans compared to those with a disease can have an impact on the early detection of diseases and personalized medicine.

Because of the complexity of lipidome, which includes 8 major categories of lipids, over 80 major classes, 300 sub-classes and thousands of lipid species[1], HPLC MS/MS methods are often used to separate many overlapping isomeric or isobaric molecular ions. It is critical that the adapted LC/MS platform offers the capability to separate and identify the isobars and isomers from the biological lipid extracts. C30 HPLC columns uniquely offer high shape selectivity for separation of structurally related isomers and provide improved lipid isomer separation efficiency compared to C18 columns. Here we report that more than nine hundreds of lipid molecules from human serum sample were simultaneously identified and quantified using a new small particle (1.9µm) Acclaim C30 prototype column and a quadrupole Orbitrap HRAM MS platform.

Methods

LC-MS Sample Preparation. Three Human serum and one human plasma samples were provided by NIST. Table 1 shows the detail description about these four samples. The lipids were extracted from the serum samples using the solvents of Chloroform, Methanol, and water (Table 1).

HPLC Method. A Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system performed HPLC separations using the gradient conditions as shown in Table 2. Mobile phase A was 60:40 Acetonitrile / Water and mobile phase B was 90:10 IPA / Acetonitrile; both A and B contained 10mM ammonium formate and 0.1% formic acid. The column was an C30 prototype column (2.1x250mm, 1.9µm) operated at 45°C, flow rate of 200 µL/min and the injection volume was 2 µL.

MS Conditions. A Thermo Scientific™ Q Exactive™ HF mass spectrometer was employed. The instrument method and operating conditions are shown in Table 3.

Data Analysis Software. Thermo Scientific™ LipidSearch™ Software was used for lipid identification and quantification.

TABLE 1. Human Serum and Plasma Sample Description and Lipid Extraction Procedure.

NIST ID	Sample Type	Sample Description
SRM 1950	Human plasma	Equal number of men and women
2378-1	Human serum	Donors took fish oil supplements
2378-2	Human serum	Donors took flaxseed oil supplements
2378-3	Human serum	Donors did not take fish or flaxseed oil

Lipid Extraction Procedure

1. Pipette 80 µL sample aliquots into 4-mL glass tubes
2. Add multiple internal standards in Chloroform
3. Add 600 µL of Methanol, vortex
4. Add 1000 µL Chloroform, vortex
5. Add 500 µL of Water, vortex
6. Centrifuge (3000 rpm,10 min)
7. Collect the lower (Chloroform) phase
8. Add additional 600 µL of Chloroform, repeat steps 6 to 7
9. Evaporate the combined organic to dryness in a vacuum centrifuge
10. Reconstitute extracted lipids in 100 µL of IPA/Methanol (50:50) for storage

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TABLE 2. HPLC Gradient Conditions.

Time, min	% A	% B
0	60	40
7	45	55
8	35	65
12	35	65
30	30	70
31	12	88
51	5	95
53	0	100
63	0	100
63.1	60	40
75	60	40

TABLE 3. MS Operating Conditions.

HESI Source	Q Exactive HF
Sheath gas 40	Pos. Ion (250-1200 amu) Neg. Ion (200-1200 amu)
Aux gas 8	MS Resolution, R = 120K FWHM at m/z 200
Spray Voltage 3.5 kV	Top15 dd-MS^2, R = 30K FWHM at m/z 200
S-Lens 50	MS^2 Isolation Width 1 Da
Cap. Temp. 320°C	Stepped NCE Pos. Ion: 25, 30 Neg. Ion: 20, 24, 28
Heater Temp. 300°C	AGC target 1E+6 MS, 50 msec max. 1E+5 MS^2, 80 msec max.

Results

Improved lipid isomers separation efficiency using the Acclaim C30 column.

The Acclaim C30 column is designed to provide high shape selectivity for separation of hydrophobic structurally related isomers. Combining with further improved efficiency using small particle size (1.9µm) and longer column length (25 cm), the new C30 column detected more lipid isomers compared to regular 15 cm C18 columns. Figure 1 shows one example of TG (55:3) isomer separation. Fourteen TG (55:3) isomer peaks were detected using the C30 column, while only five TG (55:3) isomer peaks were detected using a 15 cm C18 column. Significant more lipid species including all isomers from a bovine heart lipid extract were identified using the C30 column compared with those using conventional C18 columns (Figure 2).

FIGURE 1. TG (55:3) Isomer Peak Detection from a Serum Sample with C30 Column and C18 Column.

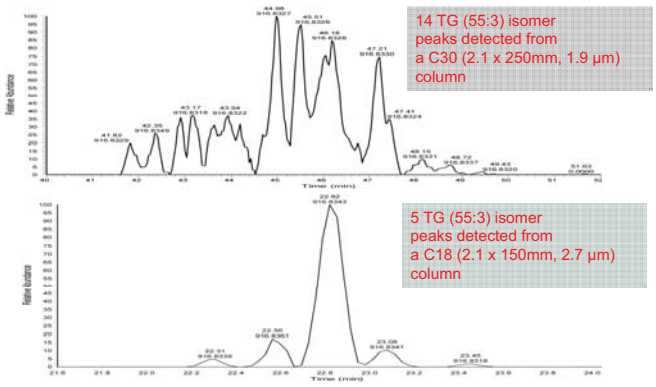


FIGURE 2. Comparison of Lipid IDs (including all isomers) across a C30 column and Three type of C18 Columns.

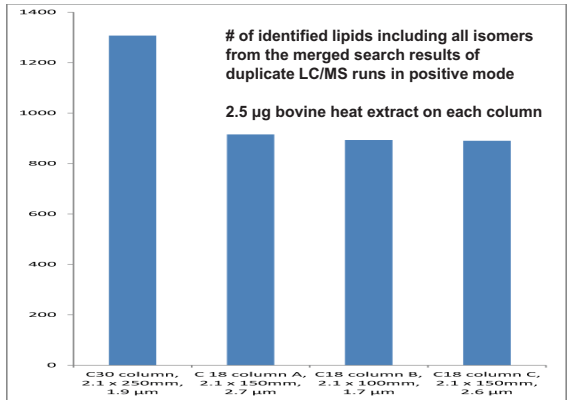
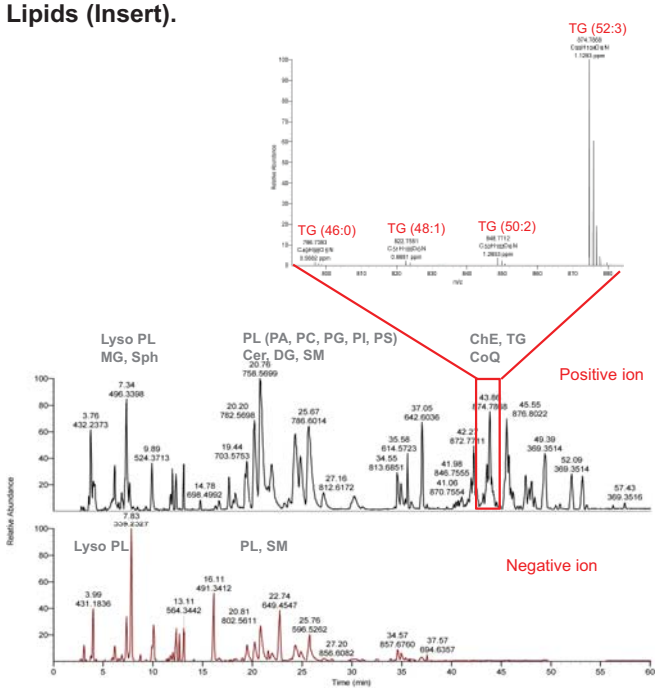


FIGURE 3. Base Peak Chromatograms of a Serum Sample and one Example of MS Spectrum of TG Lipids (Insert).



Simultaneous lipid IDs and quantitation of the human serum/plasma samples.

The lipid extracts from the three human serum and one plasma samples were analyzed using the LCMS conditions described above with positive ion and negative ion mode, respectively. Each sample was run triplicate in both positive and negative mode, yielding 24 raw files on the total. Lipid Search 4.1 software was used to identify the lipid species and align each raw file's search results together for lipid profiling (Figure 4).

After filtering and manual validation[2], 981 lipid species were confidently identified and quantified from the human serum/plasma samples (Figure 5 and Figure 6).

FIGURE 4. Lipid IDs and Relative Quantification Using the Lipid Search Software.

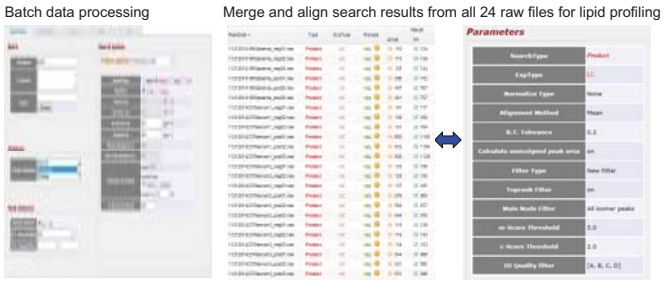


FIGURE 5. Lipid Class File of the Human Serum and Plasma Samples.

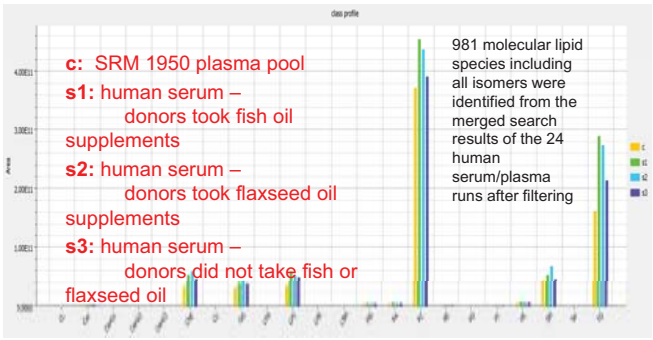
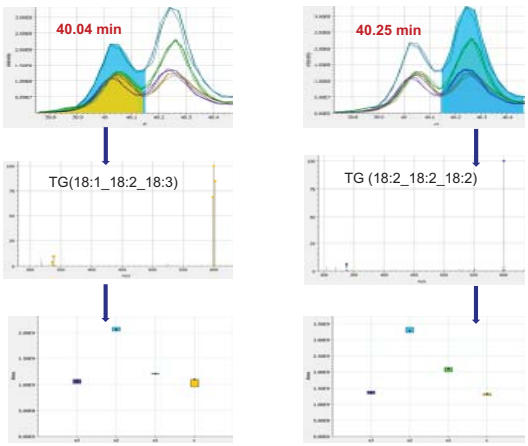


FIGURE 6. ID and Quan Results for Two TG (54:6) Isomers.



Conclusion

- Developed an optimum LC/MS workflow for lipid profiling by using a long small particle size C30 column for efficient lipid isomers separation, a new generation Thermo Scientific™ Orbitrap™ detector for increased lipid identification coverage and quantitative accuracy and LipidSearch software for high throughput lipid identification and quantification.
- The optimized LC/MS workflow was successfully used to carry out the human serum lipidome profiling experiments.
- Approximately 1000 molecular lipid species (including all isomers) were identified and quantified from the four serum/plasma samples with excellent analytical precision. The coefficient of variation of most quantified lipids were less than15%.

References

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2. Processing of a Complex Lipid Dataset for the NIST Inter-laboratory Comparison Exercise for Lipidomics Measurements in Human Serum and Plasma; David A. Peake et. al., Lipid Maps 2015 Poster #30.

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