Lipid profile of different biological matrices

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1 INTRODUCTION

Lipids play multiple roles in biological systems. They are the fundamental components of membranes; they serve as energy storage and also participate in signal transduction pathways, proliferation, apoptosis, and membrane trafficking in the cell. Alterations in their metabolism contribute to, or are the primary cause of many diseases. Thus, the study of the lipidome has become a promising area with a variety of applications, such as the discovery of prognostic and diagnostic biomarkers.

Since lipids are very diverse in their chemical structure it is very difficult to simultaneously determine the complete lipidome of a sample. Therefore, in OWL we have developed a platform in order to analyze some of the main lipid categories in different biological materials. This platform includes 612 compounds within the categories Glycerolipids, Sphingolipids, Glycerophospholipids, Sterol Lipids and Fatty Acyls, and employs specific sample preparation and analysis with Ultra Performance Liquid Chromatography (UPLC) tandem a Q-TOF mass spectrometer (MS).

2 METHODS

Figure 1. Sample preparation flow chart.

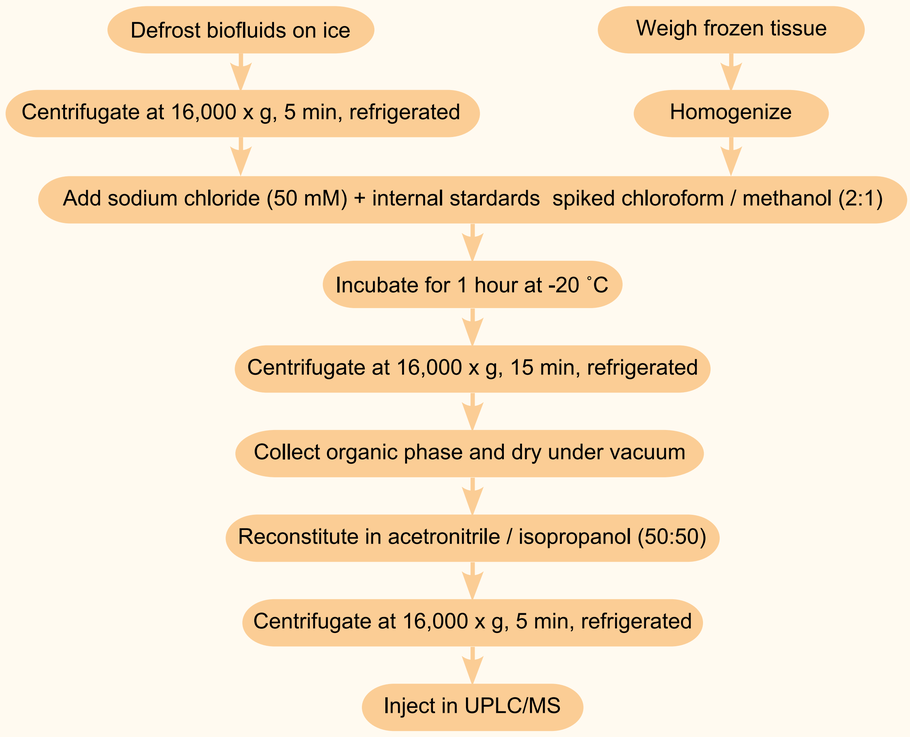


Table 1. Chomatographic separation (left) and mass spectrometric detection (right) conditions.

Instrument	Waters ACQUITY UPLC System
Column type	UPLC BEH C18, 2.1 x 100 mm, 1.7 µm
Flow rate	0.40 ml/min
Solvent A	H_2O + ACN + 10mM Ammonium Formate
Solvent B	ACN+ Isopropanol + 10mM Ammonium Formate
(%B), time	40%, 0 min
(%B), time	100%, 10 min
(%B), time	40%, 15 min
(%B), time	40%, 17 min

MS System	Waters XEVO G2 Q-TOF
Column temperature	60 °C
Injection volume	3 µl
Source temperature	120 °C
Nebulisation N_2 flow	1000 l / hour
Nebulisation N_2 temperature	500 °C
Cone N_2 flow	30 l / hour
Capillary voltage	3.2 kV
Cone voltage	30 V

3 RESULTS

1. COMPOUNDS

A total of 612 lipids have been identified in our platform, corresponding to the main lipid categories Glycerolipids, Sphingolipids, Glycerophospholipids, Sterol Lipids and Fatty Acyls.

In order to achieve the identification and subsequent incorporation of these compounds into the platform, previous MSMS experiments or internal standards injections were required. New identifications are constantly being carried out and thus the platform is enhanced.

Table 2. Lipid categories and number of compounds per class followed in the platform.

CATEGORY	CLASS	OWL CODE	NUMBER OF COMPOUNDS
Glycerolipids	Monoacylglycerides	MAG	19
	Diacylglycerides	DAG	70
	Triacylglycerides	TAG	184
Sphingolipids	Sphingomyelins	SM	32
	Ceramides	Cer	146
	Monohexosylceramides	CMH	4
Glycerophospholipids	Glycerophosphocholines	PC	73
	Glycerophosphoethanolamines	PE	44
	Glycerophosphoinositols	PI	9
Sterol Lipids	Cholesteryl Esters	ChoE	17
Fatty Acyls	Primary fatty amide	FAA	14

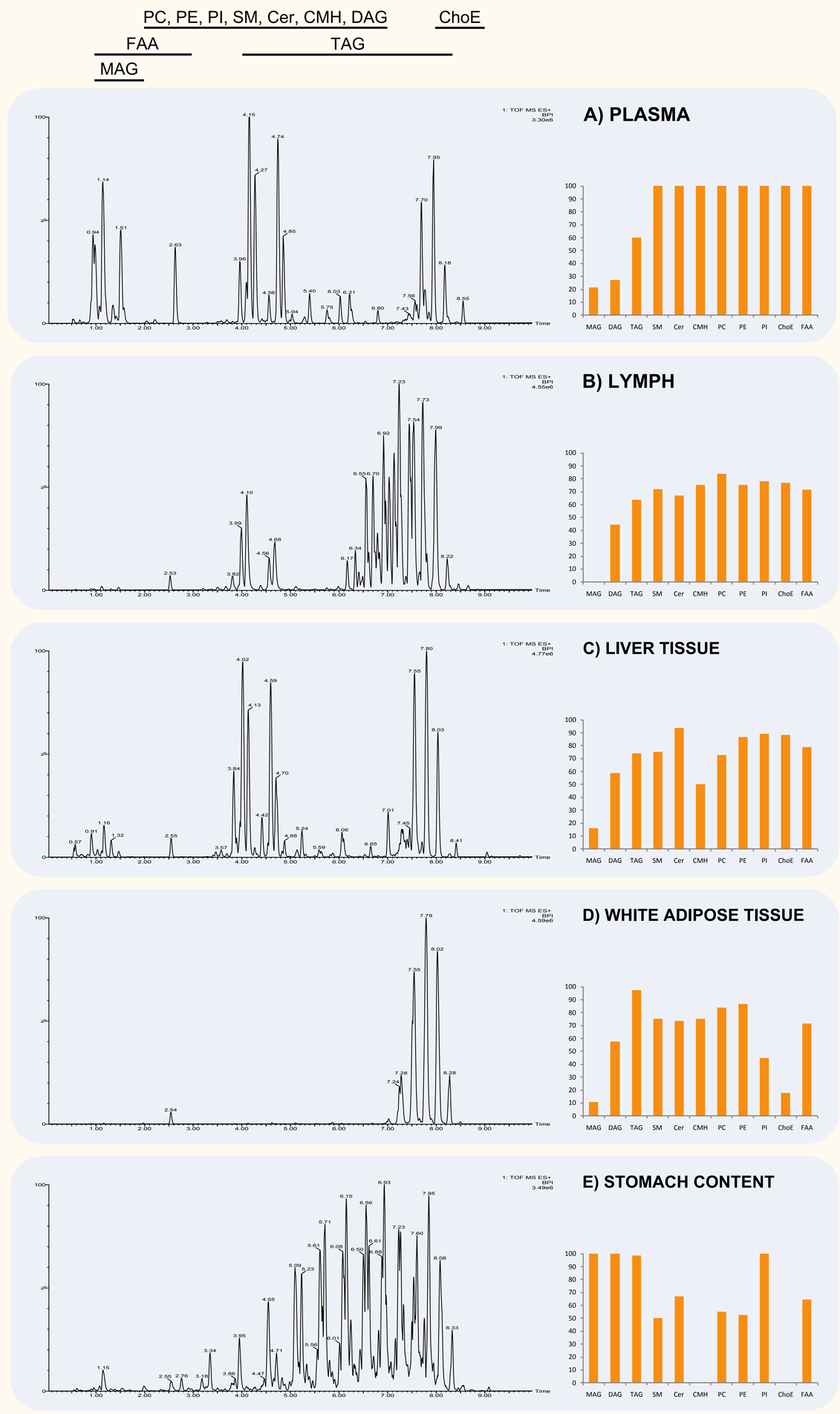
2. LIPID PROFILES

Chromatographic profiles obtained from five different rat matrices (plasma, lymph, liver tissue, white adipose tissue and stomach content) are displayed in figure 2. Graphs on the right hand side of the chromatograms show the percentage of the lipid groups which can be detected in each biological material.

In each chromatogram, the predominance of some classes above others can be observed. For instance, white adipose tissue (D) shows a prevalence of TAG, but the other classes also appear in this matrix. In contrast, stomach content chromatogram (e) displays peaks all over the run time, however some classes, such as CMH and ChoE are under the detection limit.

As expected, each matrix requires specific sample preparation, i.e. some need higher dilution than others to promote a proper peak detection. Therefore, profiles among different matrices cannot be compared *de visu*.

Figure 2. UPLC-TOF base peak ion intensity chromatograms for A) plasma, B) lymph, C) liver tissue, D) white adipose tissue and E) stomach content. Approximate retention time regions corresponding to identified metabolites are indicated at the top (see table 2 for abbreviations) on the left, and frequency barplots on the right.



4 CONCLUSIONS

The platform described allows the detection of over 600 compounds. These compounds belong to the lipid categories Glycerolipids, Sphingolipids, Glycerophospholipids, Sterol Lipids and Fatty Acyls.

The method has been optimized for the study of the lipidome in several biological materials, like plasma, lymph, liver tissue, white adipose tissue and stomach content, showing characteristic lipid profiles.