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Analysis of Intact Lipids from Biologics Matrices by UPLC/High Definition MS

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APPLICATION BENEFITS

The combination of the ACQUITY UPLC® System and the SYNAPT™ G2 High Definition Mass Spectrometry™ (HDMS™) System provides an ideal platform for the analysis of lipids. The method described in this work is simple, effective, and yields rapid results with better resolution and sensitivity than previously described HPLC methods.[1,2]

- UPLC/HDMS provides the ability to rapidly separate and identify different lipid classes.
- Analysts performing lipid analysis maintain the highest levels of data quality
- Laboratories operate in a more cost-effective manner

WATERS SOLUTIONS

ACQUITY UPLC
SYNAPT G2 HDMS

KEY WORDS

Separation of lipid classes, lipidomics, MS^E, ion mobility and time-of-flight mass spectrometry

INTRODUCTION

Lipids play an important role in energy storage, structure, and signaling in the human body. Information about lipids and their individual classes can aid in understanding the pathogenesis of many disease states. The task of separating lipid classes from complex biological matrices is a complicated procedure for many laboratories. Most mobile phase and column combinations do not give the desired separation of the majority of lipid classes simultaneously. This is often overcome by multiple separations, fraction collection, and re-separation of lipids.

Historically, lipids were separated by thin layer chromatography (TLC), which is very time consuming and labor intensive. Further analysis of fatty acid moieties required that they be scraped from the TLC plates and then reacted to form the methyl ester of the fatty acid. The methyl esters were then analyzed by GC/MS.

HPLC separations of lipids were developed using normal-phase chromatography, which was a dramatic improvement over TLC and allowed the analyst to develop LC/MS methods. However, these methods were typically long and yielded broad, poorly-resolved peaks. This application note describes a UPLC® method that separates lipid classes with outstanding resolution and is faster and more sensitive than previous HPLC methods.

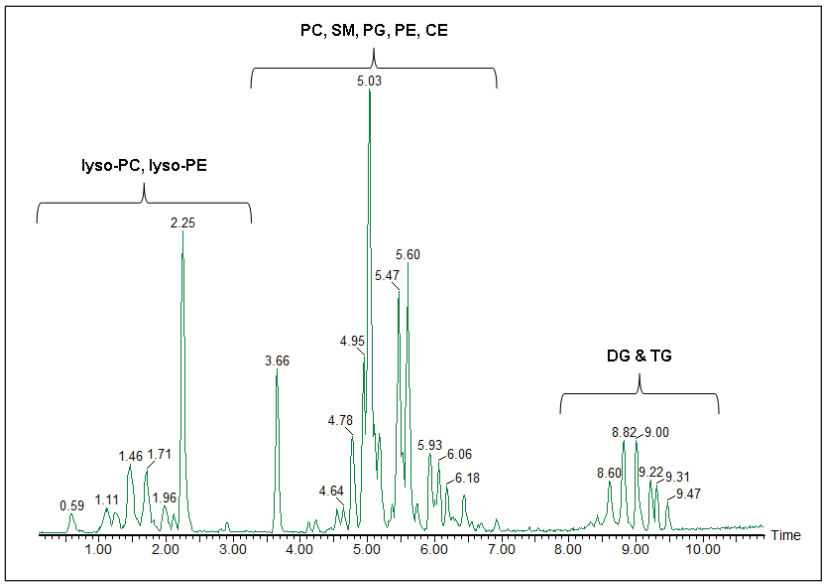

Figure 1. UPLC chromatogram. ES+, of lipid classes extracted from human plasma.

EXPERIMENTAL

Sample Preparation

Lipids were extracted from human plasma by adding 30 µL of plasma followed by 180 µL of methanol, and then 360 µL of dichloromethane. The sample was centrifuged at 13,000 RPM and the organic layer was extracted. This was diluted 5 times with mobile phase A. 10 µL was injected into the system.

LC conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC HSS T3 Column 2.1 x 100 mm, 1.8 µm
Column temp.:	65 °C
Flow rate:	500 µL/min
Mobile phase A:	Acetonitrile/Water (40:60) with 10 mM Ammonium Acetate, pH 5.0
Mobile phase B:	Acetonitrile/Isopropanol (10:90) with 10 mM Ammonium Acetate, pH 5.0
Gradient:	40 to 100% B/10 min

MS conditions

MS system:	Waters SYNAPT G2 HDMS System
Ionization mode:	ESI positive/negative
Capillary voltage:	3200 V
Cone voltage:	35 V
Desolvation temp.:	400 °C
Desolvation gas:	800 L/Hr
Source temp.:	120 °C
Acquisition range:	100 to 1000 m/z
Mobility carrier gas:	Nitrogen 32 mL/min

RESULTS AND DISCUSSION

The analysis of lipids in human plasma by UPLC with ion mobility/time-of-flight mass spectrometry (IMS-Tof MS) gave rise to good separation and resolution of major lipid classes. UPLC chromotagraphy coupled to ESI-positive data collection shows the major lipid classes present in plasma.

The expected lipid classes are shown such as lyso-phosphatidylcholines (lyso-PC), lyso-phosphatidylethanolamines (lyso-PE), phosphatidylcholines (PC), sphingomyelin (SM), phosphatidylglycerols (PG), phosphatidylethanolamines (PE), cholesterol esters (CE), diacylglycerols (DG), and triacylglycerols (TG) (Figure 1).

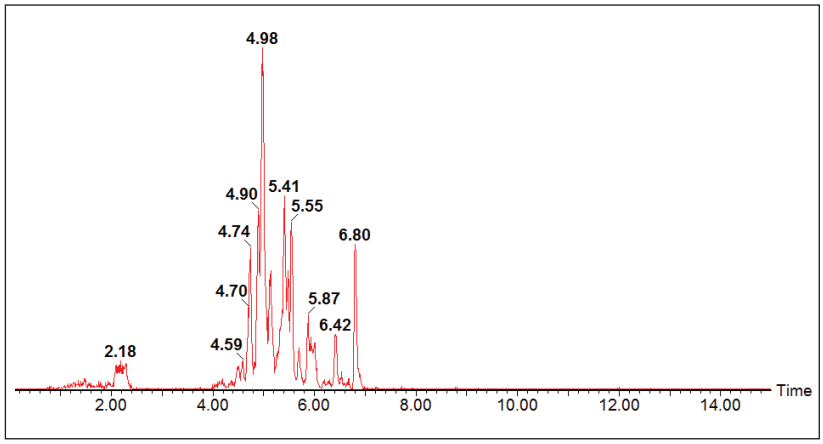

Figure 2. Extracted ion chromatogram for m/z 184.0760 from MS^E data with a 5 mDa window.

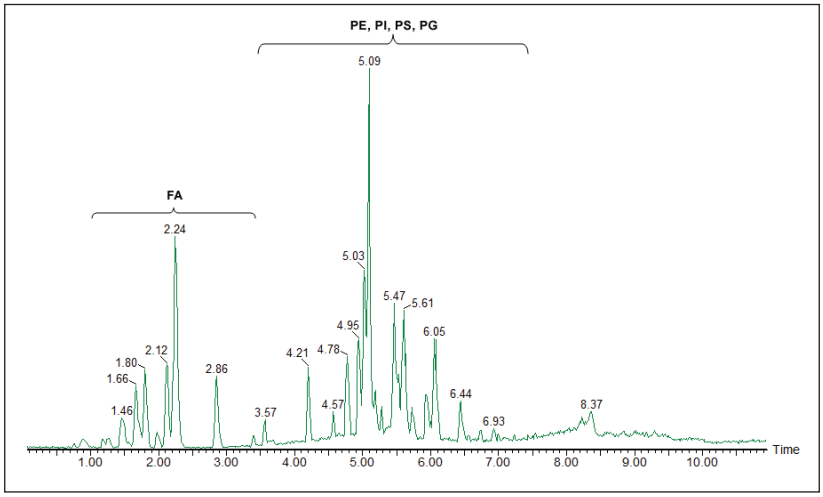

Figure 3. UPLC chromatogram, ES-, of lipid classes extracted from human plasma

The extracted ion chromatogram for the m/z 184.0760 from the MS^E data shows the separation of lipids containing the choline polar head group fragment typically found in sphingomyelins, lysophatidylcholines, and phosphatidylcholines (Figure 2). MS^E functionality was used where the first function obtained data at low collision energy while the second function simultaneously obtained data at high collision energy.

UPLC chromatography coupled to ESI-negative data collection shows the major lipid classes present in plasma. The expected lipid classes are shown such as fatty acids (FA), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and PG (Figure 3).

CONCLUSION

The ACQUITY UPLC System used in conjunction with the SYNAPT G2 HDMS System for IMS-Tof MS provides an ideal platform for the analysis of lipids. The UPLC method described is simple, effective, and yields rapid results with better resolution and sensitivity than previously described HPLC methods.[1,2] The UPLC/HDMS solution provides increased coverage and the ability to rapidly separate and identify different lipid classes. These improvements allow the analyst to operate in a more cost effective manner while maintaining the highest levels of data quality.

References

1. Christie W. Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light-scattering) detection. J. Lipid Res. 1985, 26; 507-512.
2. Sommer U, et. al. LC-MS-based method for the qualitative and quantitative analysis of complex lipid mixtures. J. Lipid Res. 2006, 47;804-814.

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