Supercritical fluid chromatography-mass spectrometry methods for lipidomics profiling

Anne E. Blackwell, Joseph L. Hedrick

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Introduction

Long chromatography methods are often used for metabolomics and lipidomics profiling experiments due to the highly complex nature of biological samples and matrices, often resulting in extremely low throughput. When combined with the large sample numbers needed to ensure good statistics, this work is time consuming and expensive. The use of supercriticial fluid chromatography (SFC) instead of liquid chromatography offers significantly shorter runs while preserving or even improving chromatographic separation for certain classes of lipids. Little work exists with annotated features, as such there is not widespread understanding of lipid and lipid class elution patterns by SFC. With better understanding of lipid class behavior in different SFC separations, scientists can better select the best chromatography for their needs.

SFC-MS was found to be an excellent platform for lipidomics profiling experiments. The chromatographic reproducibility was more than sufficient, and the approach had several unique strengths, the most obvious of which was speed. Different stationary phases were investigated to uncover different separation profiles to assist future method development for specific lipidomics applications. Triglycerides (TG) are a lipid class where SFC is particularly advantageous, where equal carbon number TGs can be readily separated by degrees of saturation.

Experimental

An Agilent 1260 Infinity Analytical SFC was coupled to a 6500 series Agilent QTOF mass spectrometer, where the full flow from the SFC was sent to the Dual Agilent Jet Stream ESI source. Bovine liver (Avanti Polar Lipids), and human liver were analyzed using C18, cyano (CN), and silica columns. Cooking oils were also analyzed, using C18 and CN columns. Several lipid standards representing a variety of lipid classes were also analyzed in order to better characterize elution profiles of lipid classes in these methods. For the human liver samples comparisons were made between LC and SFC approaches. All SFC methods and the LC method used are described in the tables that follow. For all SFC methods the back pressure regulator and temperature were 130 bar and 60 °C, respectively.

SFC Conditions – C18 Methods				
Method	General C18		Shallow C18	
Column	Agilent Zorbax SB-C18, 3 x 150 mm,3.5 µm			
Column Temp	40 °C			
Needle Wash	20 sec in wash port (50:50 MeOH/IPA)			
Modifier & Make Up	MeOH with 1 mM NH_4OAc			
Flow Rate	2.0 mL/min			
Gradient Program	t (min)	% Modifier	t (min)	% Modifier
	0	2	0	5
	1	2	4	5
	11	60	11	10
	12	60	12.5	10
	13	2	14	60
Make Up Flow	0.3 mL/min		0.6 mL/min	
Stop/Post Time	13 min/2 min		14 min/3 min	

SFC Conditions – CN & SIL Methods				
Method	Cyano		Silica	
Column	Agilent Zorbax CN, 4.6 x 150 mm, 5 µm		Agilent Zorbax RX-SIL, 3.0 x 100 mm, 1.8 µm	
Column Temp	40 °C			
Needle Wash	20 sec in wash port (50:50 MeOH/IPA)			
Modifier & Make Up	MeOH with 5 mM NH_4OAc			
Flow Rate	2.0 mL/min			
Gradient Program	t (min)	% Modifier	t (min)	% Modifier
	0	5	0	2
	4	5	1	2
	11	10	13	60
	12.5	10	15	60
	14	60	15.1	2
Make Up Flow	0.6 mL/min			
Stop/Post Time	14 min/3 min		15.1 min/5 min	

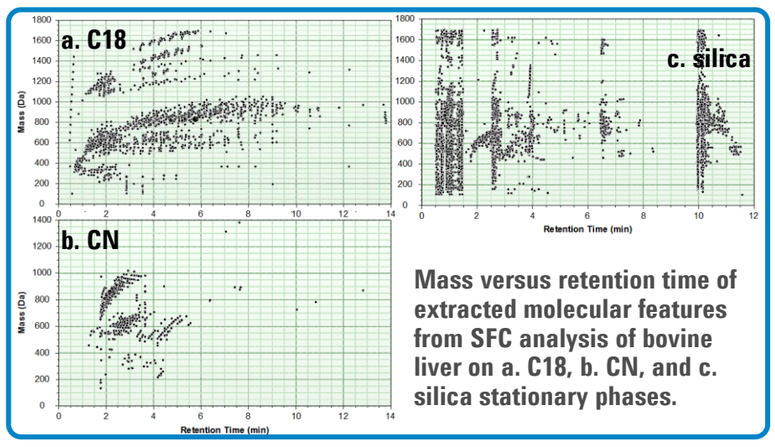
Experimental

LC Conditions		
Column	Agilent Zorbax EclipsePlus RRHD C18, 2.1 x 100 mm, 1.8 µm	
Column Temp	50 °C	
Needle Wash	20 sec in wash port (50:50 MeOH/IPA)	
Mobile Phase	A = 50:10:40 IPA/MeOH/H_2O with 5 mM NH_4OAc and 0.1% CH_3COOH B = 99:1 IPA/H_2O with 5 mM NH_4OAc and 0.1% CH_3COOH	
Flow Rate	0.350 mL/min	
Gradient Program	Time (min)	% B
	0	0
	3	0
	5	20
	25	30
	35	95
	36	95
	38	0
Stop/Post Time	38 min/10 min	

Results and Discussion

SFC Elution Profiles

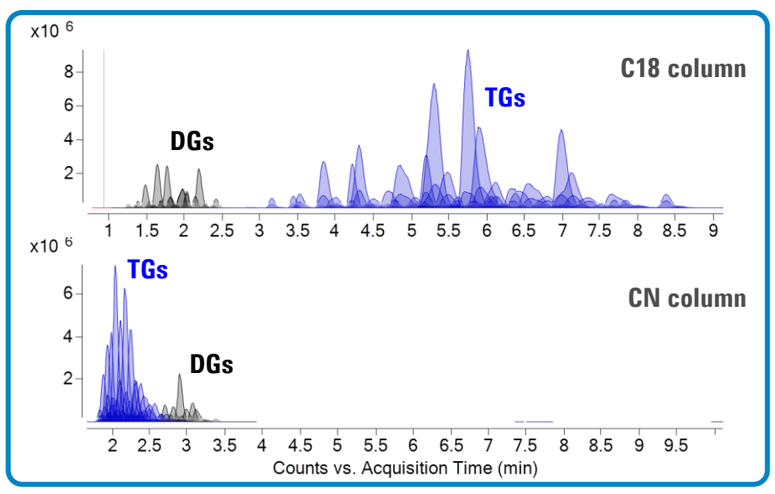
As seen below, plotting mass versus retention time for each chromatographic feature quickly reveals distinct differences between the stationary phases. For both C18 and CN, there is a general trend of retention time increasing with mass, while there is no such relationship for the silica elution profile. Despite a shallow gradient, there is relatively little retention using the CN column.



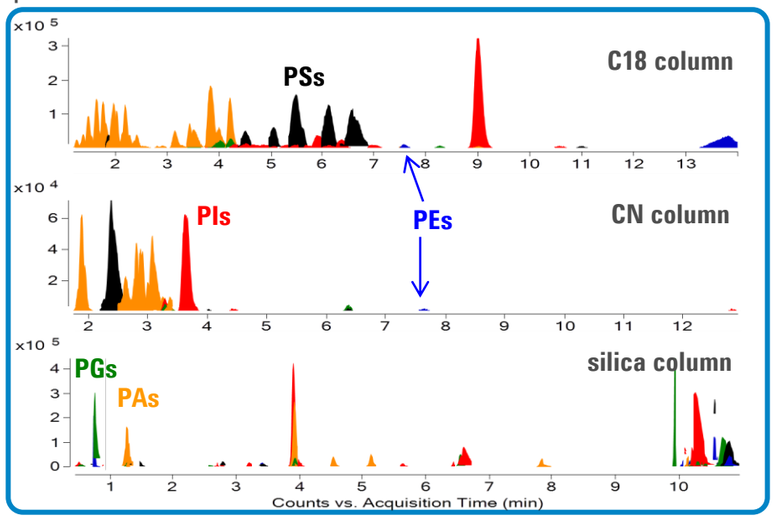
Mass versus retention time of extracted molecular features from SFC analysis of bovine liver on a. C18, b. CN, and c. silica stationary phases.

Lipid Class Patterns in Bovine Liver

Glycerolipids elute in the opposite order from CN and C18 columns, while few di- and triglycerides were detected and annotated using the silica column.



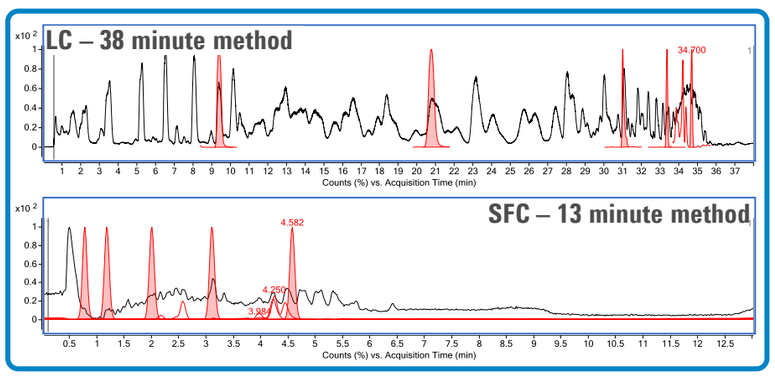
Several phospholipid classes were detected, with some class-based separation on both C18 and CN columns, though this trend is not as clear as can be seen by normal-phase LC methods.



Results and Discussion

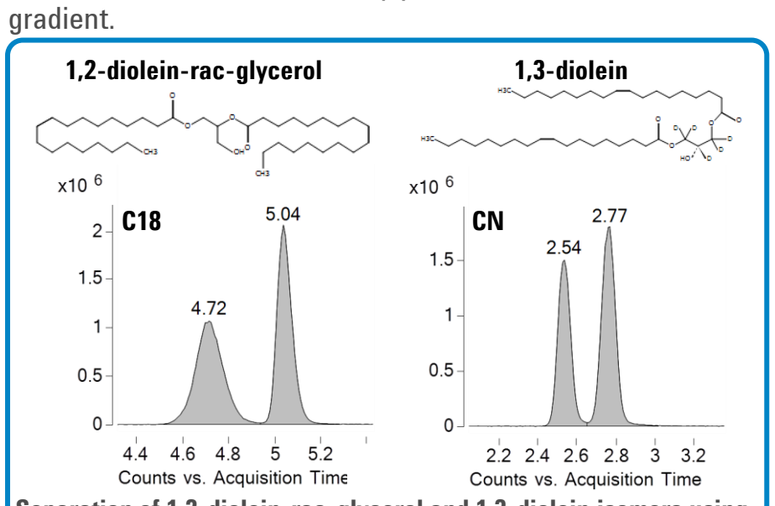
Comparison of SFC and LC for Triglycerides (TG)

The biggest advantage of SFC over LC is the ability to obtain equal or better separation in a fraction of the time. Human liver samples were analyzed by both LC and the general C18 SFC method described earlier. The sample had 5 TG standards spiked in – C:8 – C:16. Using a 38 minute H2O-IPA LC gradient, the five TG standards elute over a 35 minute period. However with a 13 minute SFC method, the same five TG standards elute completely resolved within 5 minutes.



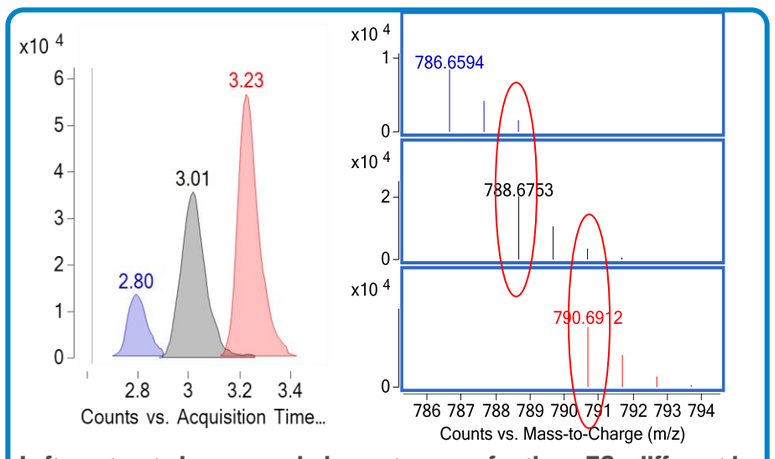
SFC is Especially Powerful for Glycerolipids

SFC separations using a C18 column appear to be particularly useful when targeting triglycerides, resulting in good separation based on both carbon length and degree of saturation. Additionally for diglycerides, it is possible to distinguish between the 1,2 and 1,3 isomers. Separation of 1,2-dioleate and 1,3-dioleate standards is shown below on both the CN and C18 stationary phases with a shallow gradient.



Separation of 1,2-diolein-rac-glycerol and 1,3-diolein isomers using C18 (left) and CN (right) stationary phase

Close study of the extracted triglycerides reveals multiple examples of baseline separation of TGs that differ by a single degree of saturation. This separation is especially valuable for obtaining pure precursor isolation for MS/MS experiments. An example of baseline separated TGs is shown below.



Left - extracted compound chromatograms for three TGs different by 1 double bond. Right - corresponding precursor isotope clusters that would overlap without chromatographic separation

Conclusions

- C18, CN, and silica columns produce very different elution profiles. CN has relatively little retention.
- C18 separation appears driven by lipid class and chain length, while separation is more class-driven for the CN column.
- SFC has clear speed advantages for labs looking for higher throughput and solvent savings

Thank you to Dr. Xiang Zhang and his group at the University of Louisville for generously sharing the human liver samples.