



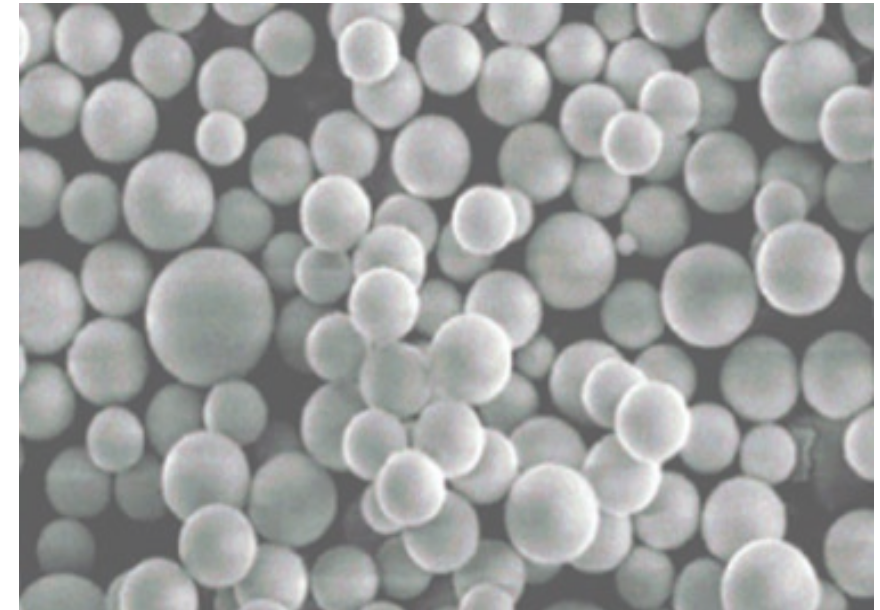
# **Advantages of Utilizing Monodisperse Fully Porous Particle Technology in the LC/MS study of Lipids**

**Presented by Edward Faden**

# What does Evosphere MDFPP particles look like compared to FPP?



Monodisperse Fully Porous Particle



Polydisperse Traditional Fully Porous Particle

Scanning Electron Microscope Images of Evosphere vs. Fully Porous Particles (FPP)



# How are Monodispersed Fully Porous Particles Made?

“The manufacture involves a modified Stober process (pioneered by Werner Stober in 1968) combined with surfactant porogens to generate monodisperse mesoporous silica spheres. By tightly controlling variations in these two processes we can control and manipulate the particle size, monodispersity and porosity of these silica particles.

“Therefore, no mechanical classification (i.e. removal of large/small particles) is required)”



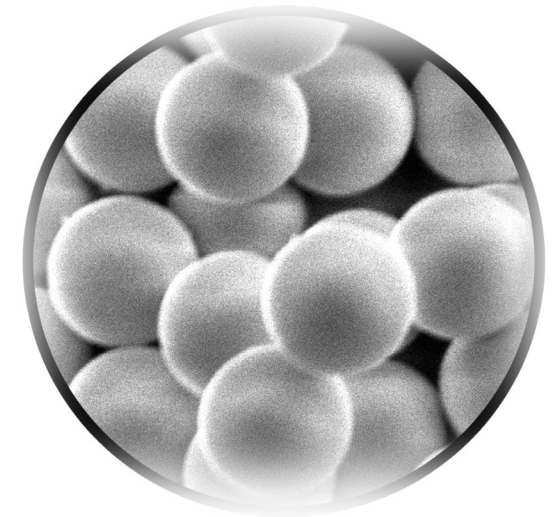
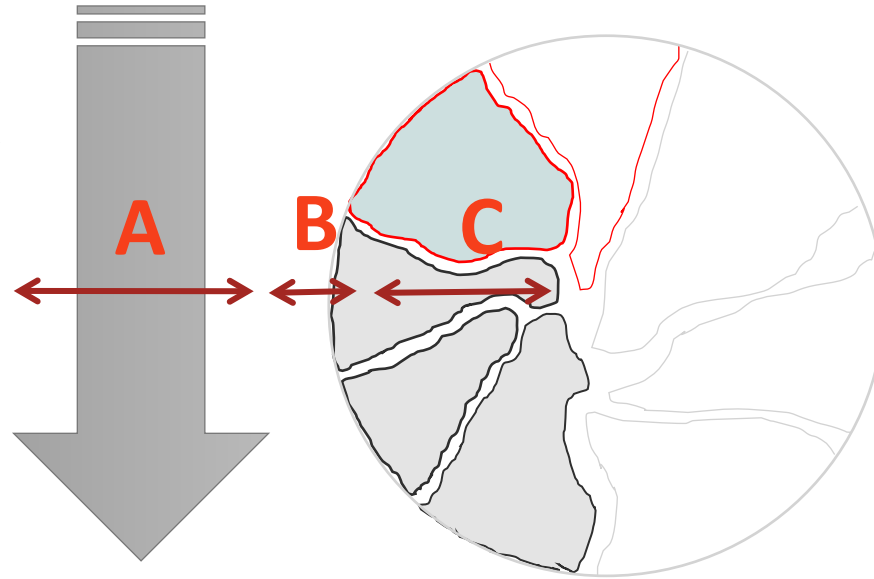
# How does Evosphere impact band broadening?

H: Height Equivalent to a theoretical plate

A: Eddy diffusion term

B: Longitudinal diffusion

C: Resistance to Mass Transfer

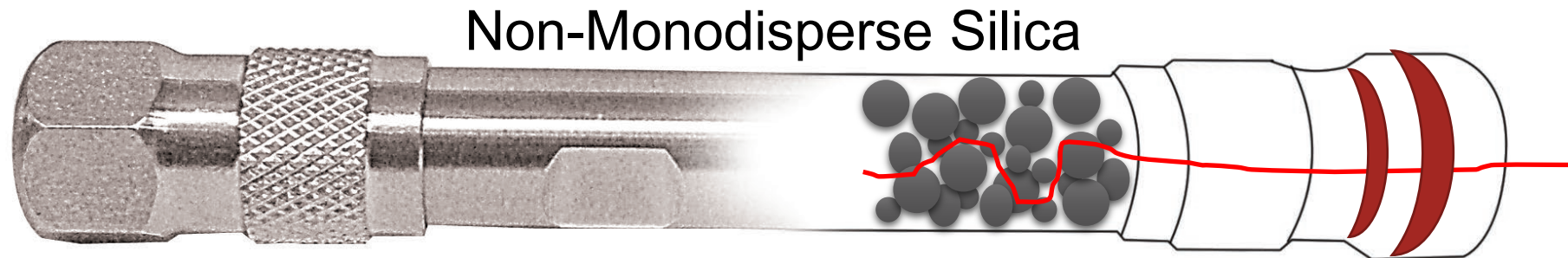
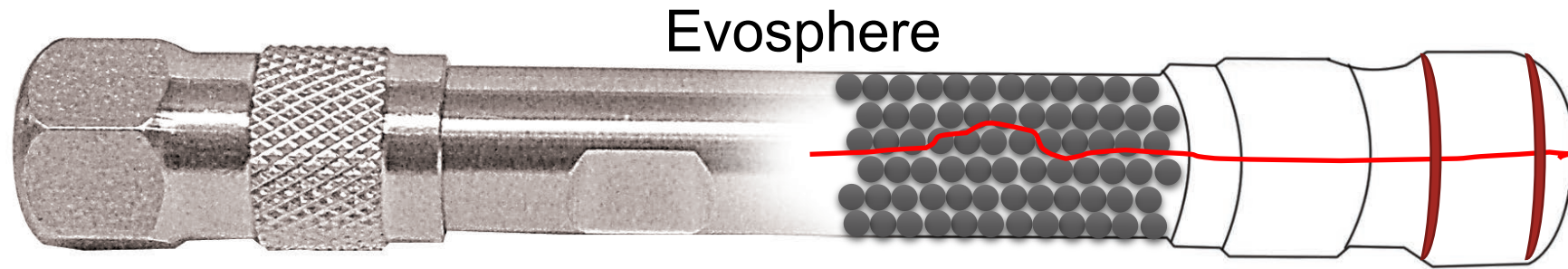


$$H = A + \frac{B}{u} + Cu$$

van Deemter Equation



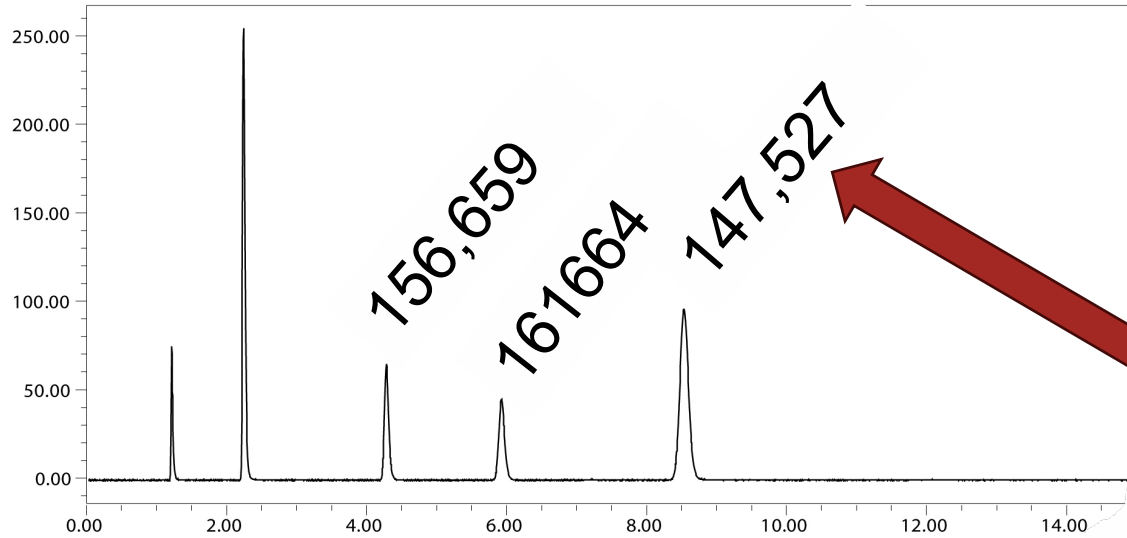
# What does this look like visually through the column?



Flow through the column Evosphere vs. FPP

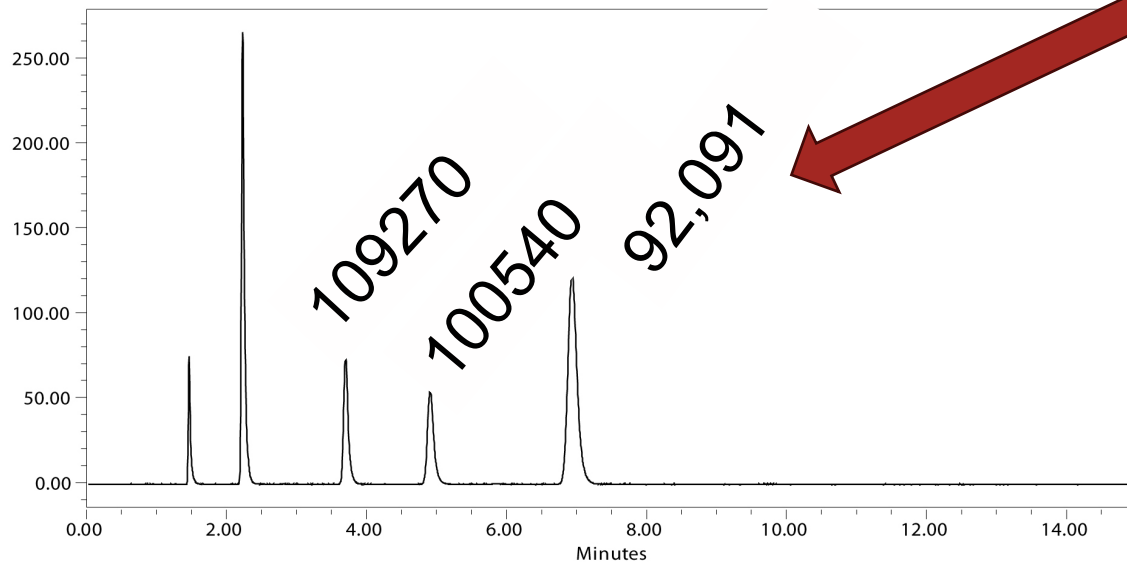


# What does this look like chromatographically?



**Evosphere C12**  
**3 $\mu$ m, 4.6mm x 150 mm**

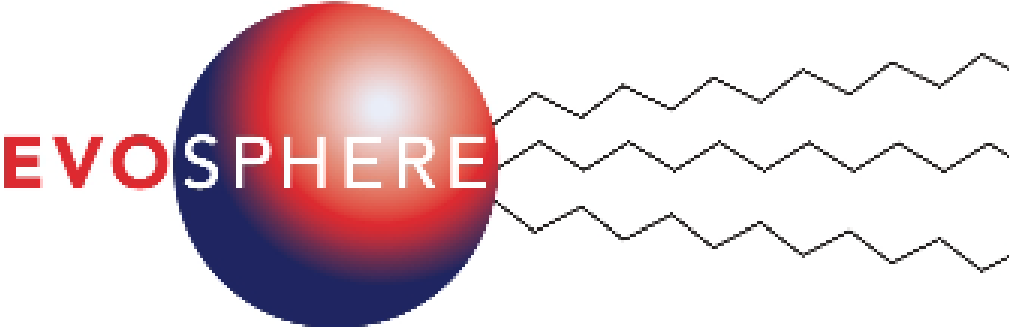
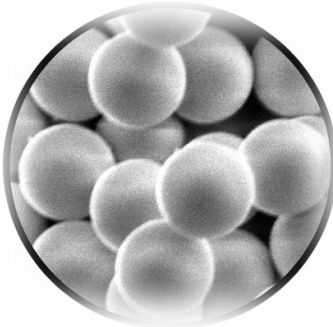
**60% Higher N**



**Popular Fully Porous C18**  
**3 $\mu$ m, 4.6 x 150 mm**

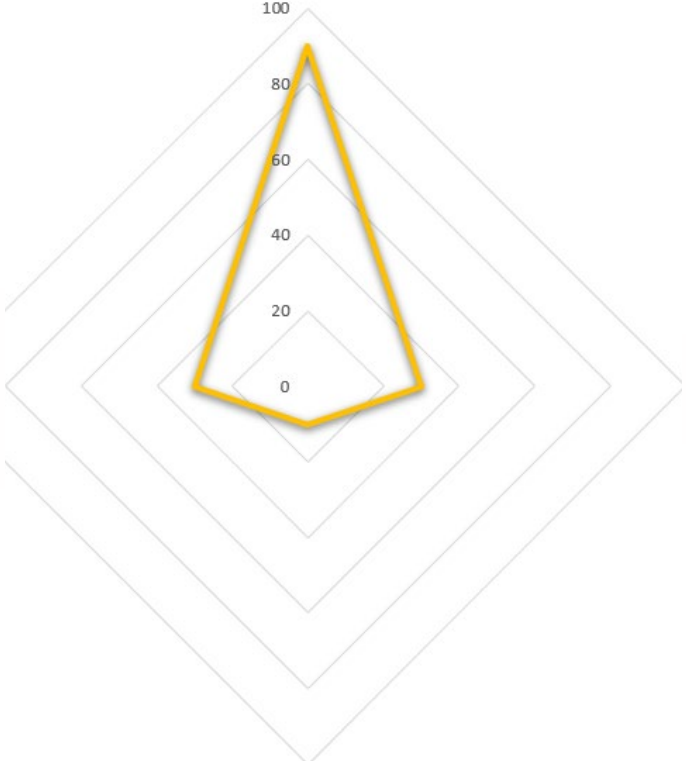


# Evosphere C12



Hydrophobicity

Steric  
Selectivity



Polarity

Dipole Charge





# Column Comparison for Lipid Panel Analysis

(Data Generated by Dr. Timothy Garrett  
at the University of Florida)



# HPLC/UHPLC Columns Compared

- Competitor C18 (50 mm x 2.1 mm) 1.7  $\mu\text{m}$
- **Evosphere C12 (50 mm x 2.1 mm) 3.0  $\mu\text{m}$**
- Competitor C8 (50 mm x 2.1 mm) 3.0  $\mu\text{m}$

**NOTE LARGER PARTICLE SIZE ON EVOSPHERE!!!**

Larger Particle Size  
SHOULD BE  
outperformed by 1.7  $\mu\text{m}$   
particle size column!!!



# Goals of Experimental Comparison

- Explore whether new Monodisperse Fully Porous Particle morphology is ideal for lipid panel analysis as compared to industry standard Polydisperse Fully Porous Particle morphologies.
- Investigate optimal bonded phase ligand length for lipid panel analysis.
- Utilize established lipidomic method analysis parameters to gauge the above experiments.



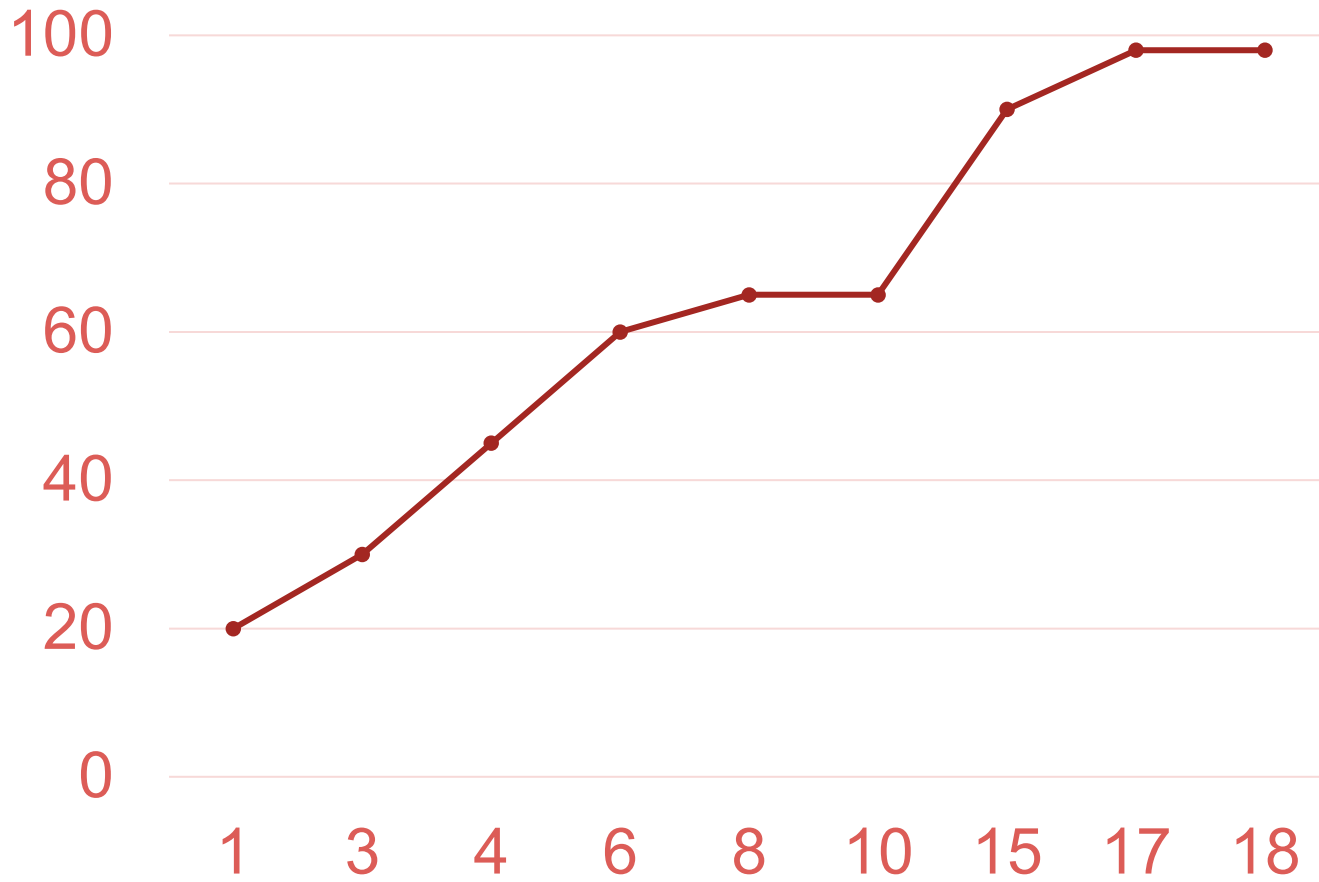
# Experimental Conditions

- Mobile Phase A = 60/40 ACN/H<sub>2</sub>O with 0.1% FA and 10 mM NH<sub>4</sub>HCO<sub>2</sub>
- Mobile Phase B= 90/8/2 IPA/ACN/ H<sub>2</sub>O with 0.1% FA and 10mM NH<sub>4</sub>HCO<sub>2</sub>
- Flow Rate = 500 μL/min,
- Temperature = 50°C
- Multi-step gradient starting at 80/20 and ending at 2/98



# Gradient Charts

% Mobile Phase B



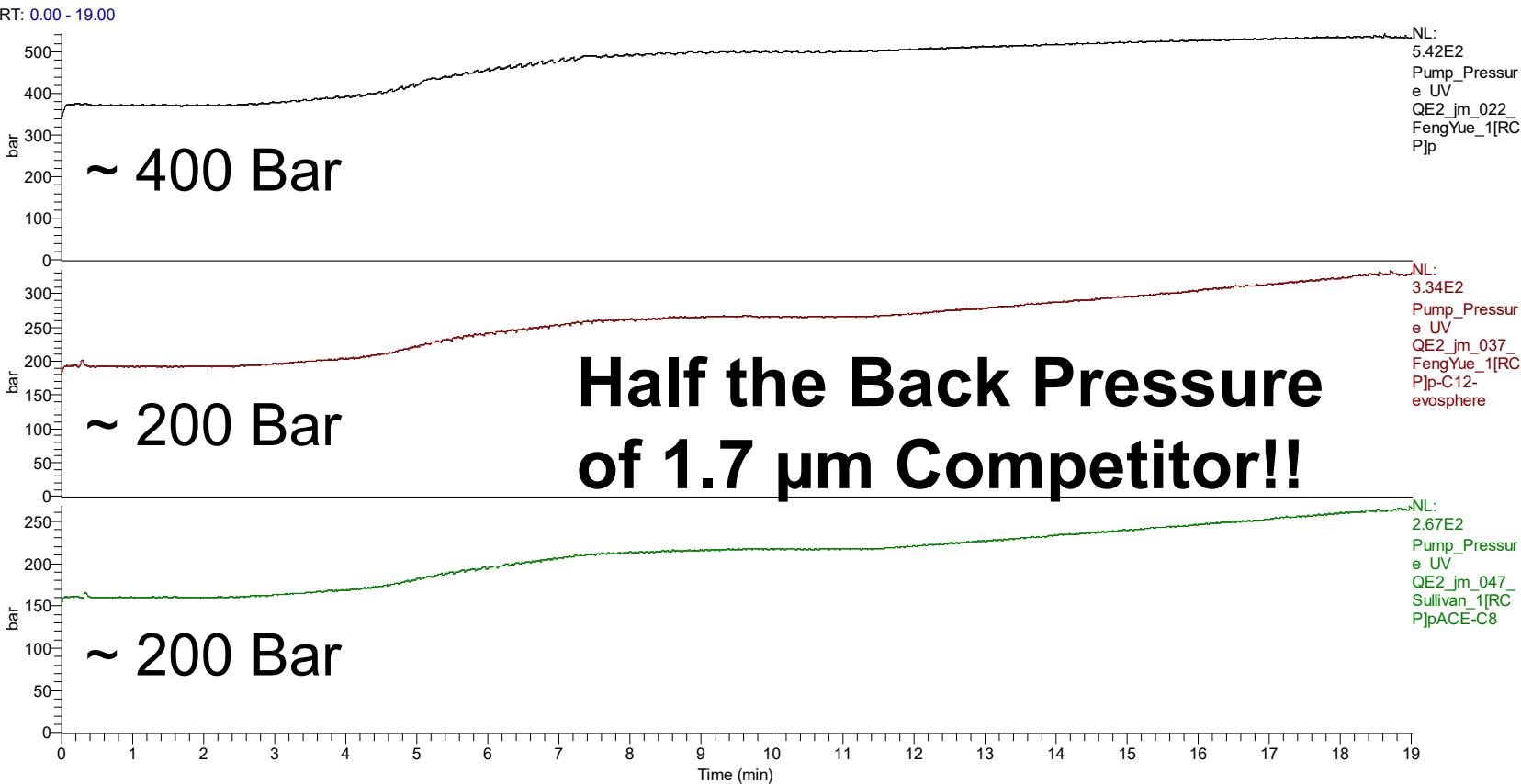
| Time   | %B |
|--------|----|
| 1 min  | 20 |
| 3 min  | 30 |
| 4 min  | 45 |
| 6 min  | 60 |
| 8 min  | 65 |
| 10 min | 65 |
| 15 min | 90 |
| 17 min | 98 |
| 18 min | 98 |

# Pressure difference

QE2\_jm\_047\_Sullivan\_1[RCP]pACE-C8

05/12/23 13:05:08

RCP



Competitor C18  
(1.7  $\mu\text{m}$  Particle Size)

Evosphere C12  
(3.0  $\mu\text{m}$  Particle Size)

Competitor C8  
(3.0  $\mu\text{m}$  Particle Size)

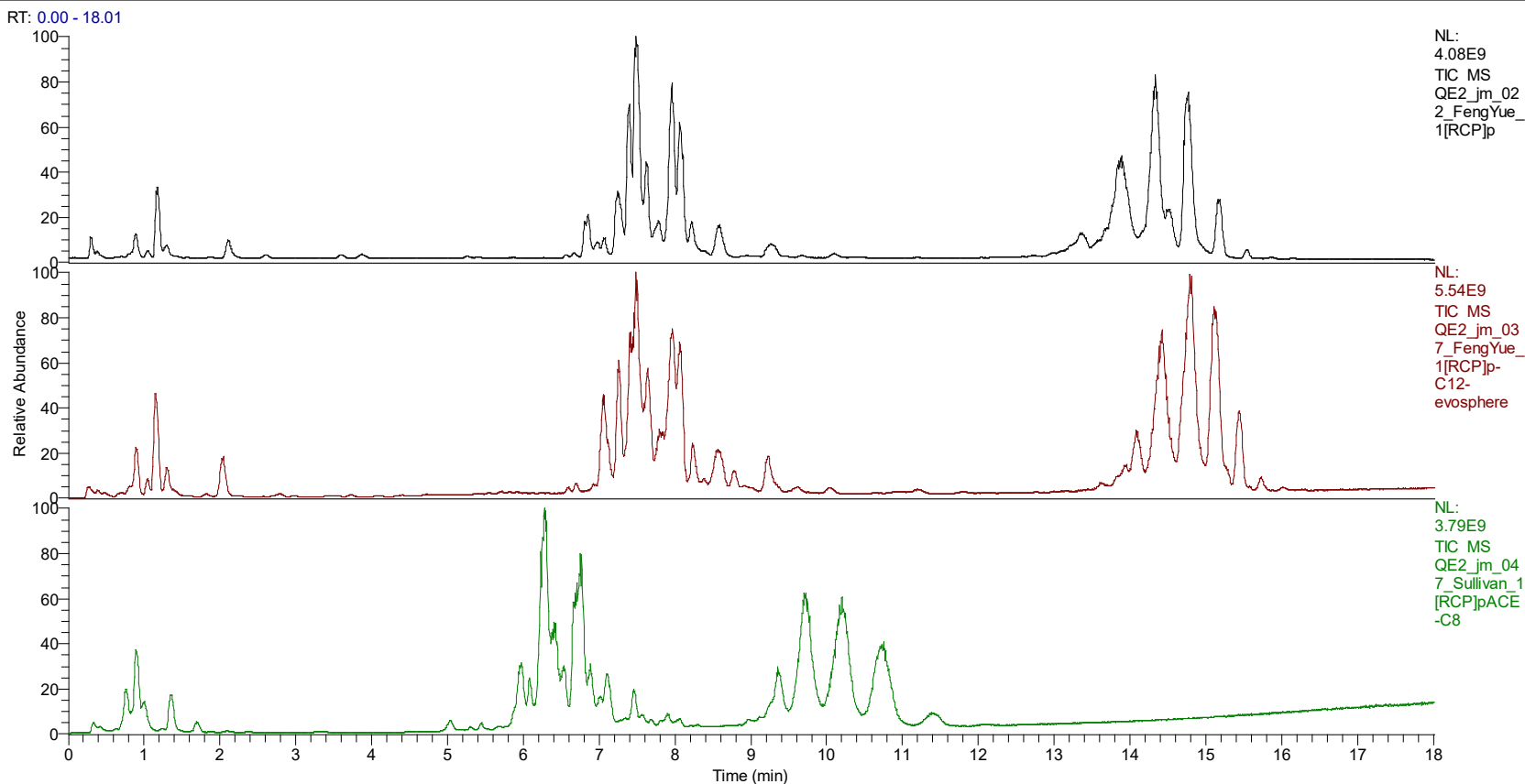


# Total Ion Trace

**Lyso PC**

**Phospholipids  
and PC's**

**Cholesterol Esters and  
Triglycerides**

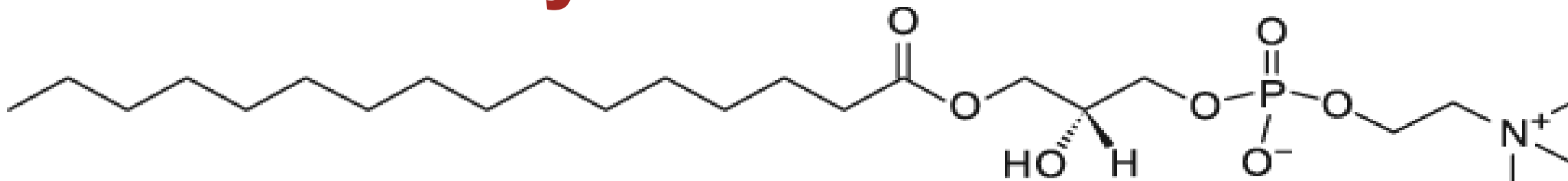


**Competitor C18**  
(1.7  $\mu\text{m}$  Particle Size)

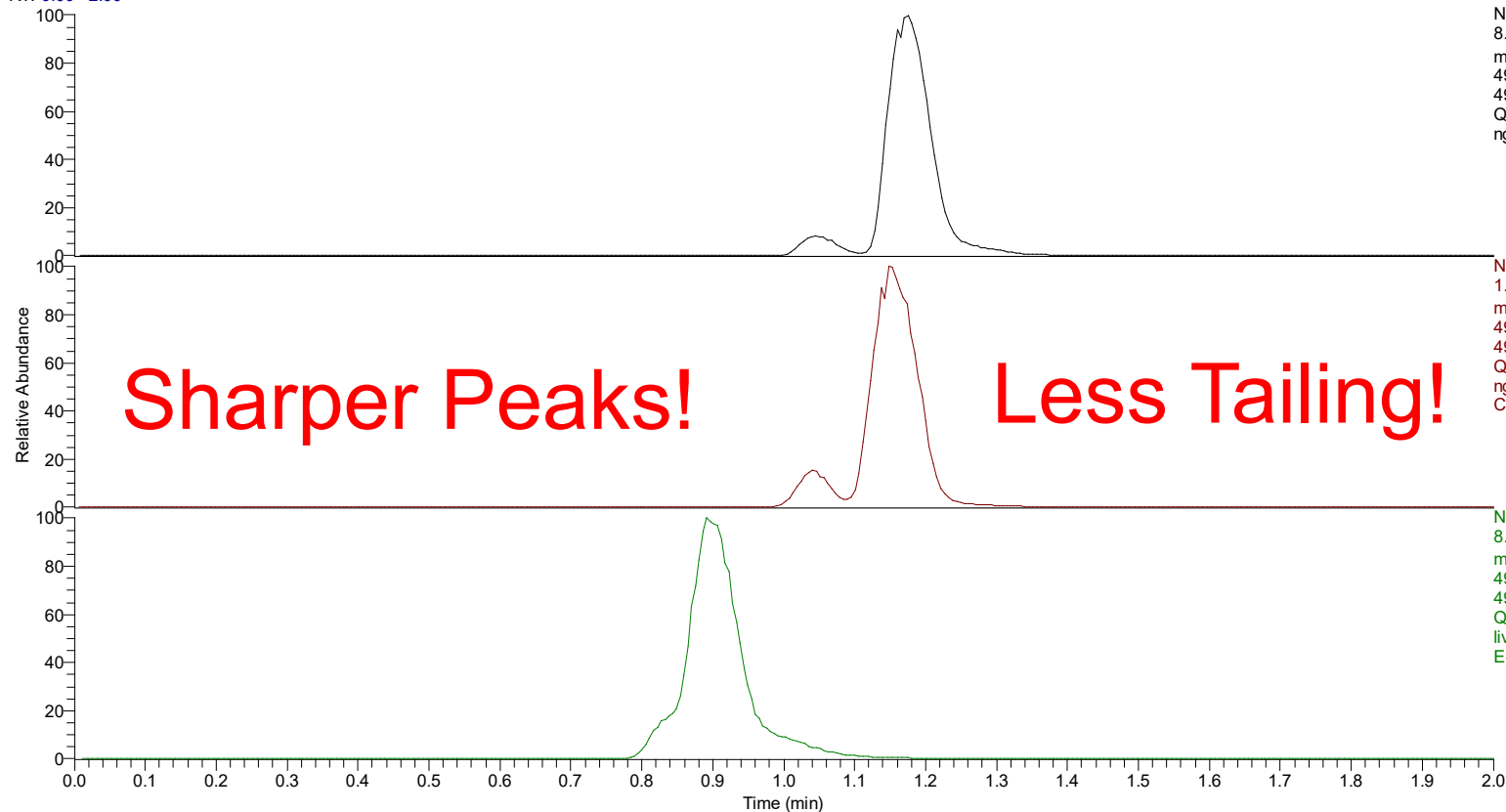
**Evosphere C12**  
(3.0  $\mu\text{m}$  Particle Size)

**Competitor C8**  
(3.0  $\mu\text{m}$  Particle Size)

# Lyso PC 16:0



RT: 0.00 - 2.00



NL:  
8.44E8  
m/z=  
496.3350-  
496.3450 I  
QE2\_jm\_0:  
ngYue\_1[R

**Competitor C18**  
**(1.7  $\mu\text{m}$  Particle Size)**

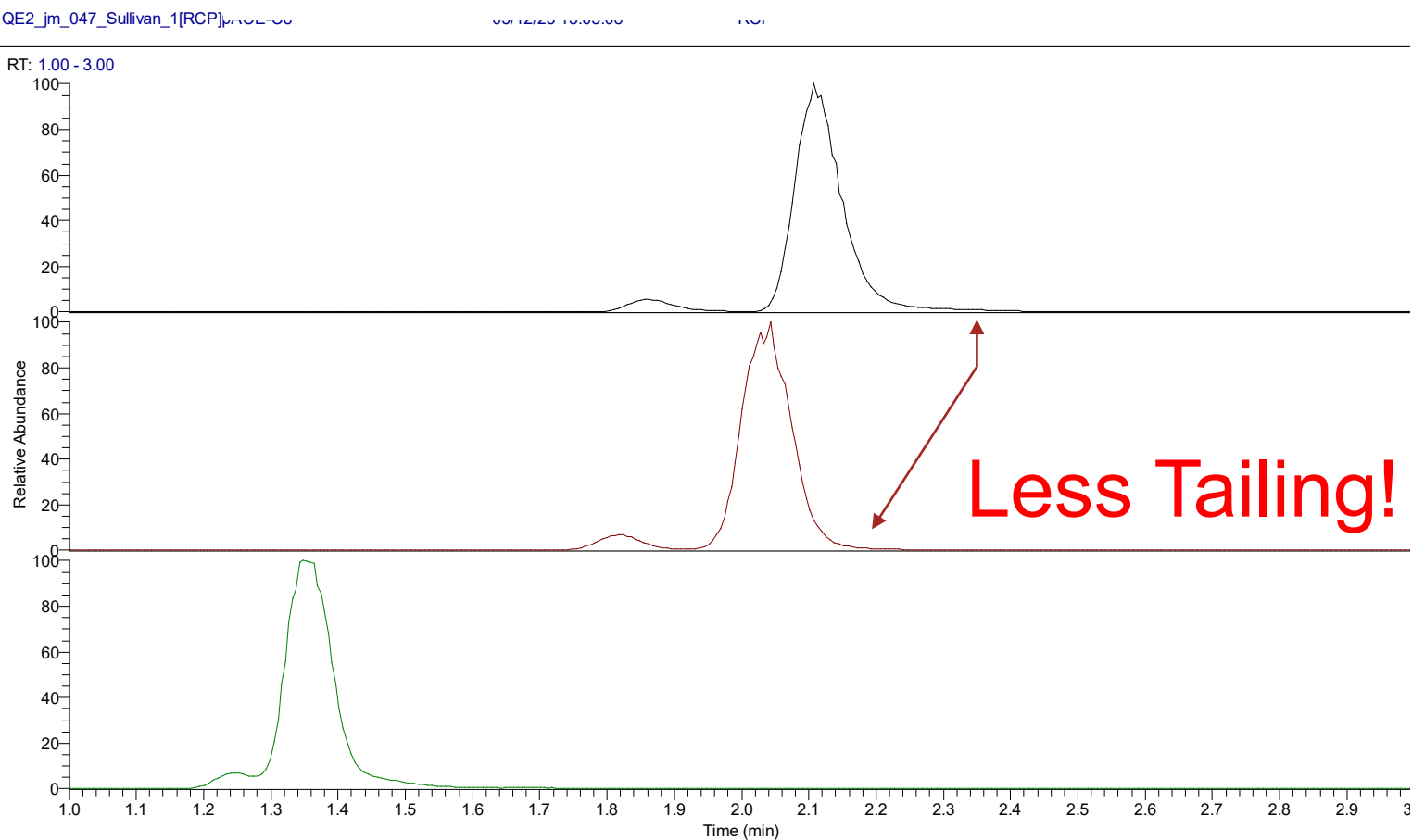
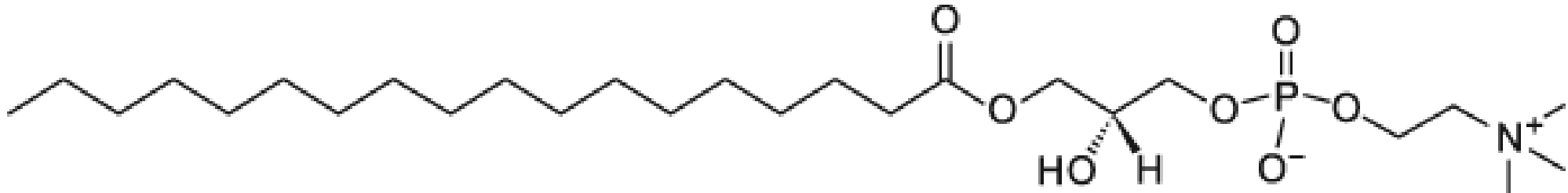
NL:  
1.63E9  
m/z=  
496.3350-  
496.3450 I  
QE2\_jm\_0:  
ngYue\_1[R  
C12-evosp

**Evosphere C12**  
**(3.0  $\mu\text{m}$  Particle Size)**

NL:  
8.90E8  
m/z=  
496.3350-  
496.3450 I  
QE2\_jm\_0:  
livan\_1[R  
E-C8

**Competitor C8**  
**(3.0  $\mu\text{m}$  Particle Size)**

# LysoPC 18:0



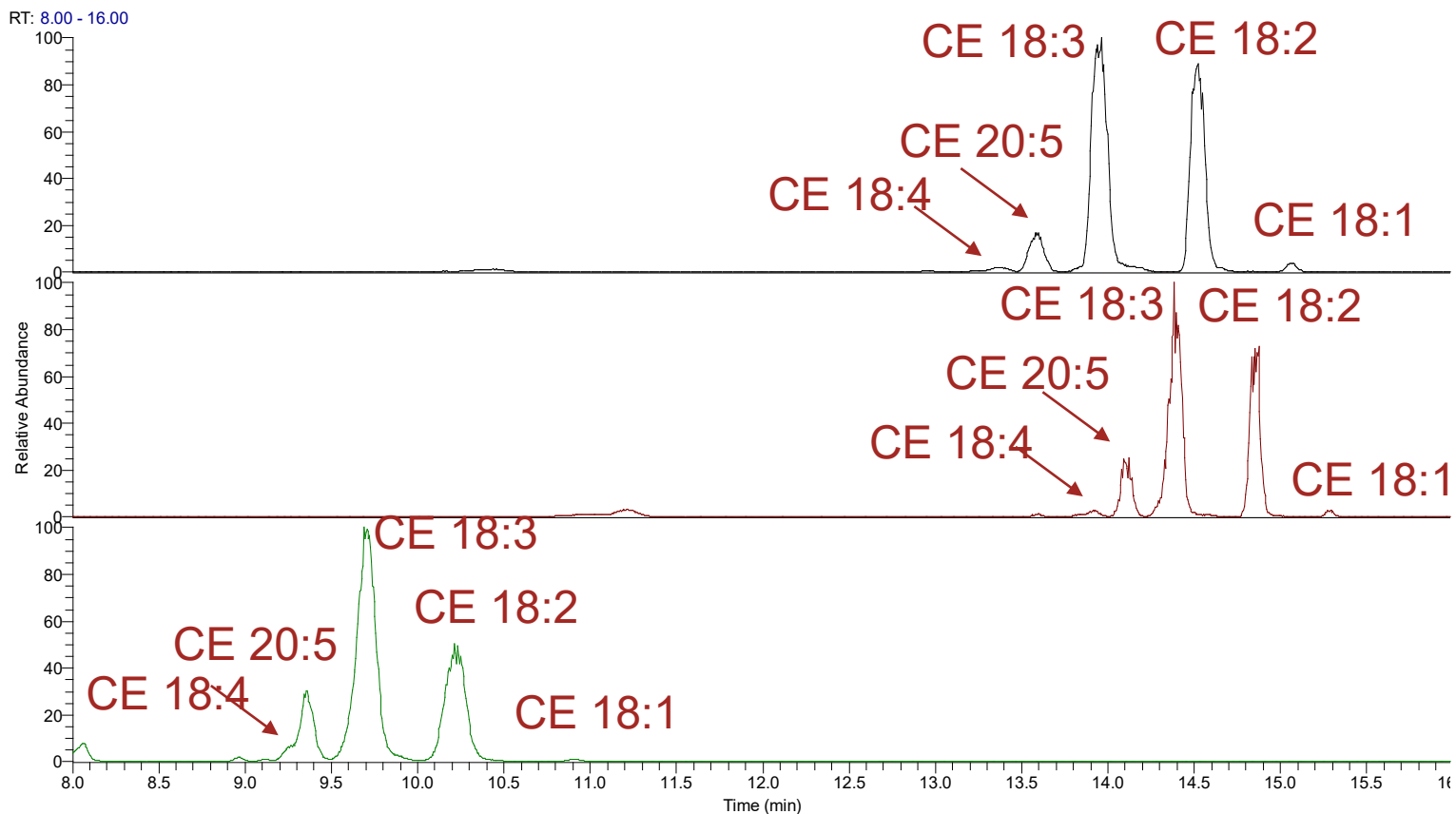
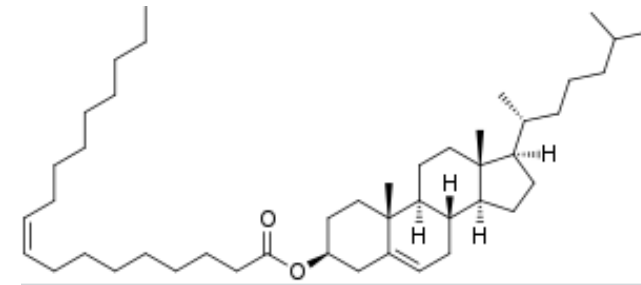
Competitor C18  
(1.7  $\mu\text{m}$  Particle Size)

**Evosphere C12**  
**(3.0  $\mu\text{m}$  Particle Size)**

Competitor C8  
(3.0  $\mu\text{m}$  Particle Size)



# Cholesterol Esters



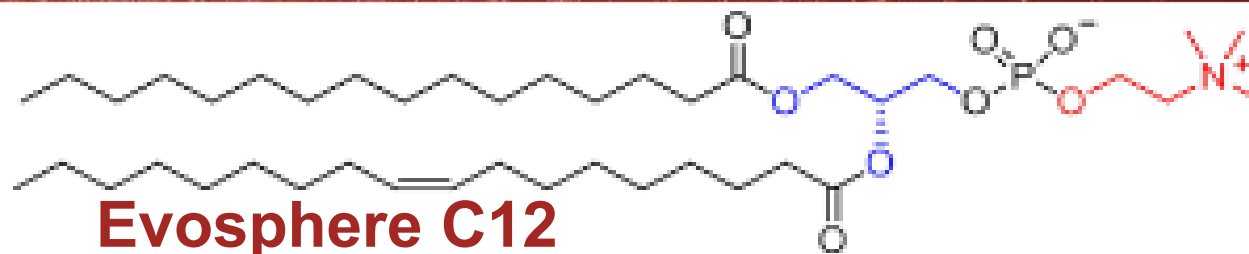
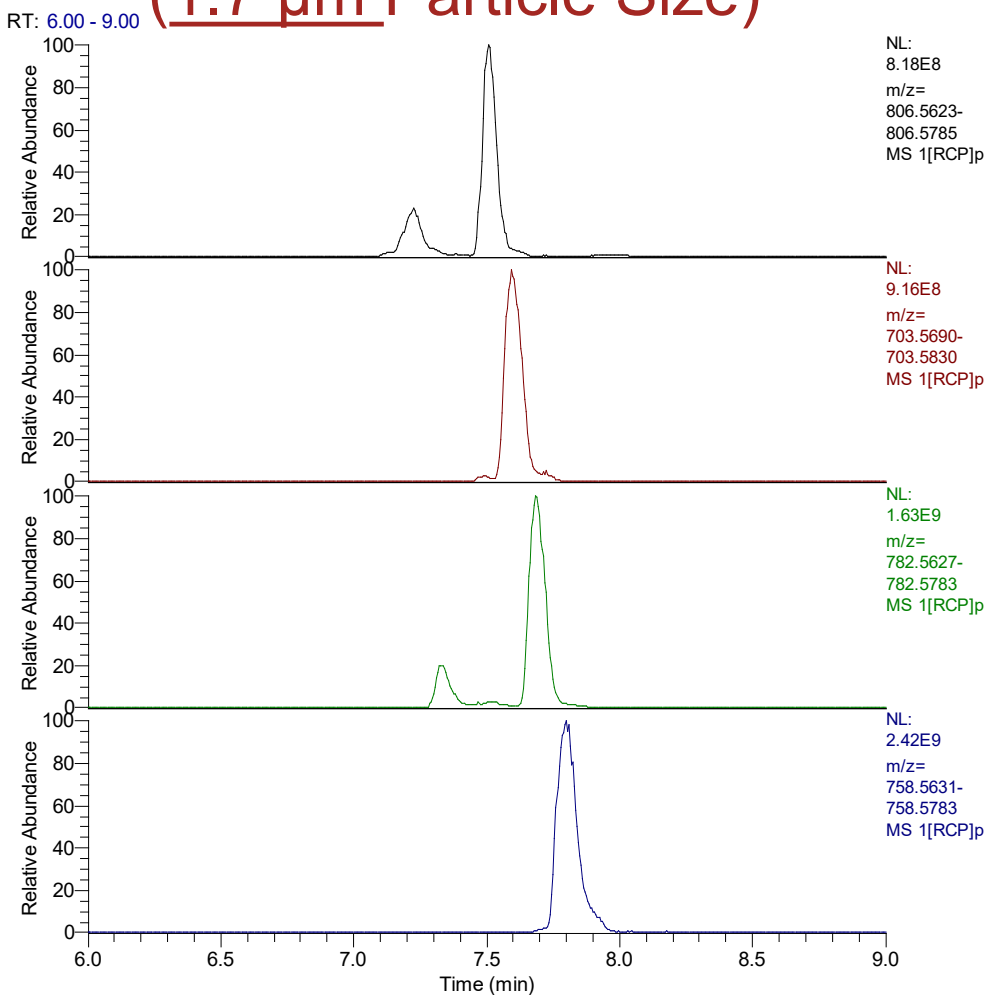
Competitor C18  
(1.7 μm Particle Size)

**Evosphere C12**  
**(3.0 μm Particle Size)**

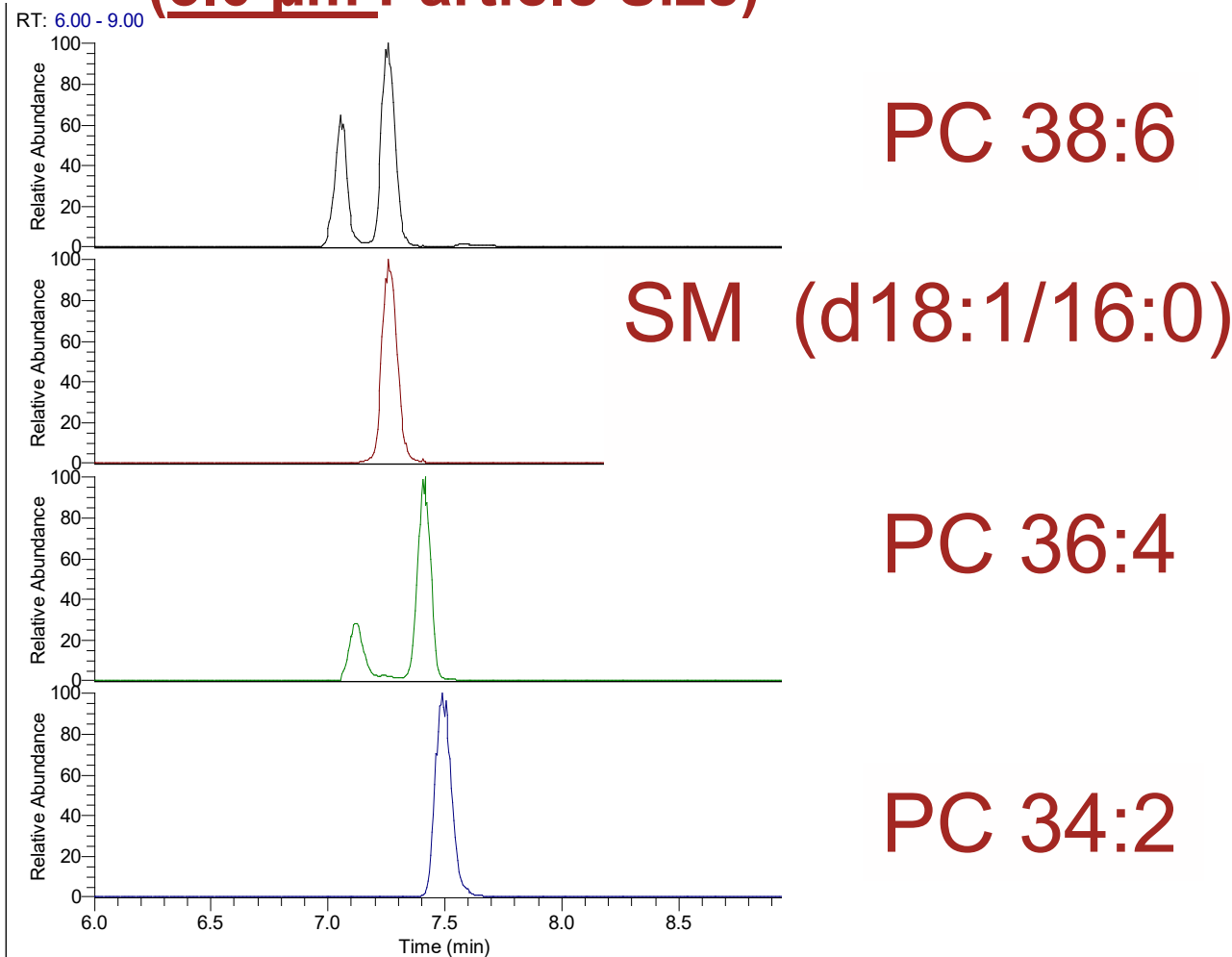
Competitor C8  
(3.0 μm Particle Size)

# Phospholipids

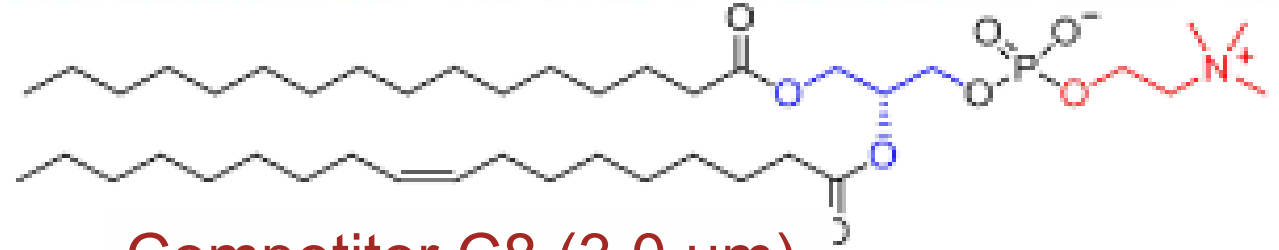
Competitor C18  
(1.7  $\mu\text{m}$  Particle Size)



Evosphere C12  
(3.0  $\mu\text{m}$  Particle Size)

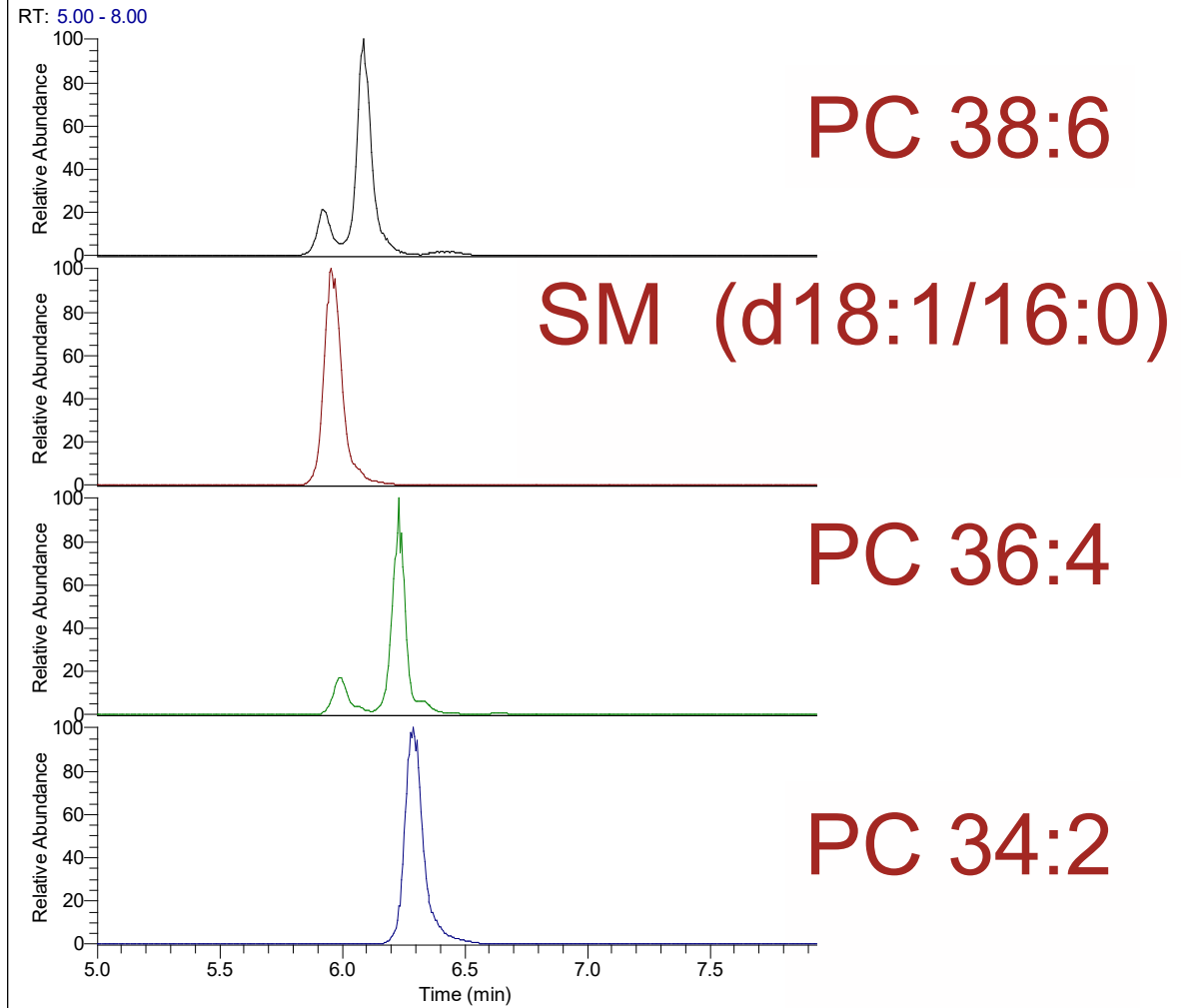
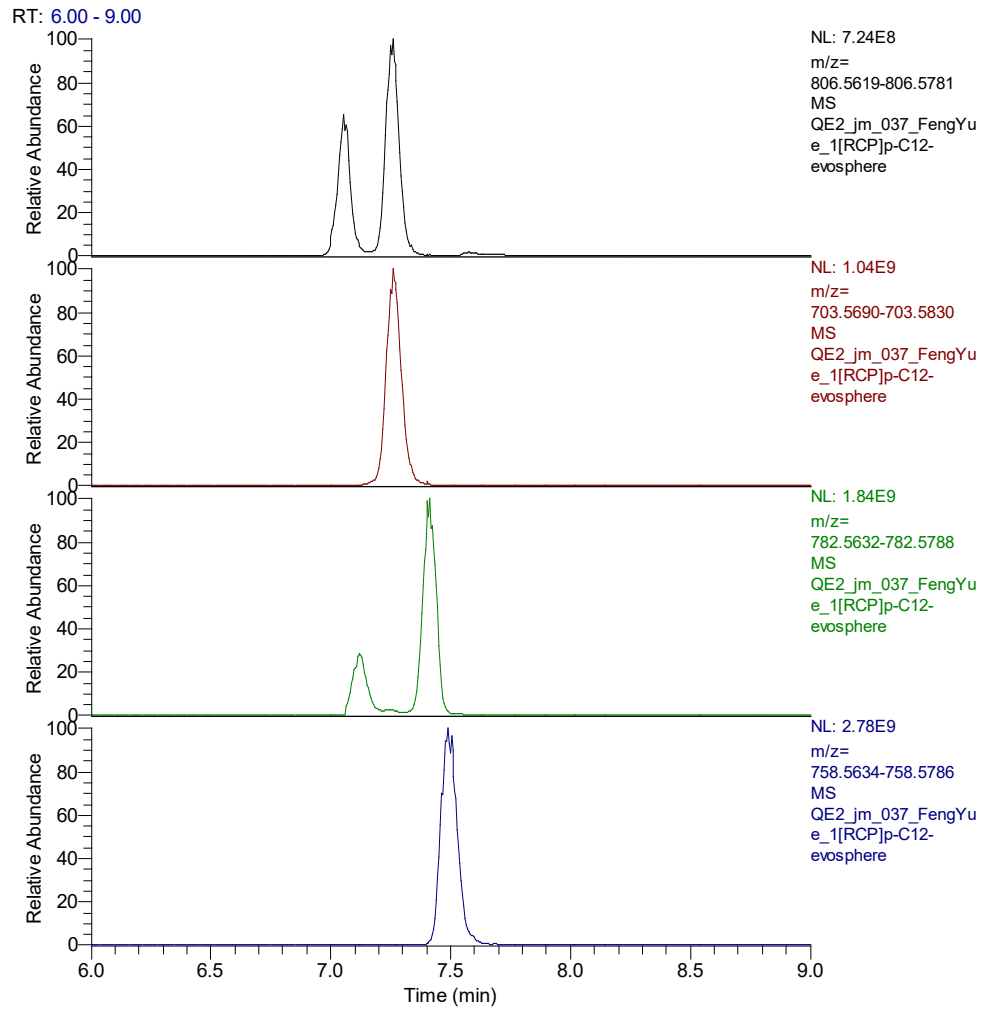


# Phospholipids



QE2\_jm\_ **Evosphere C12 (3.0  $\mu$ m)** RCP

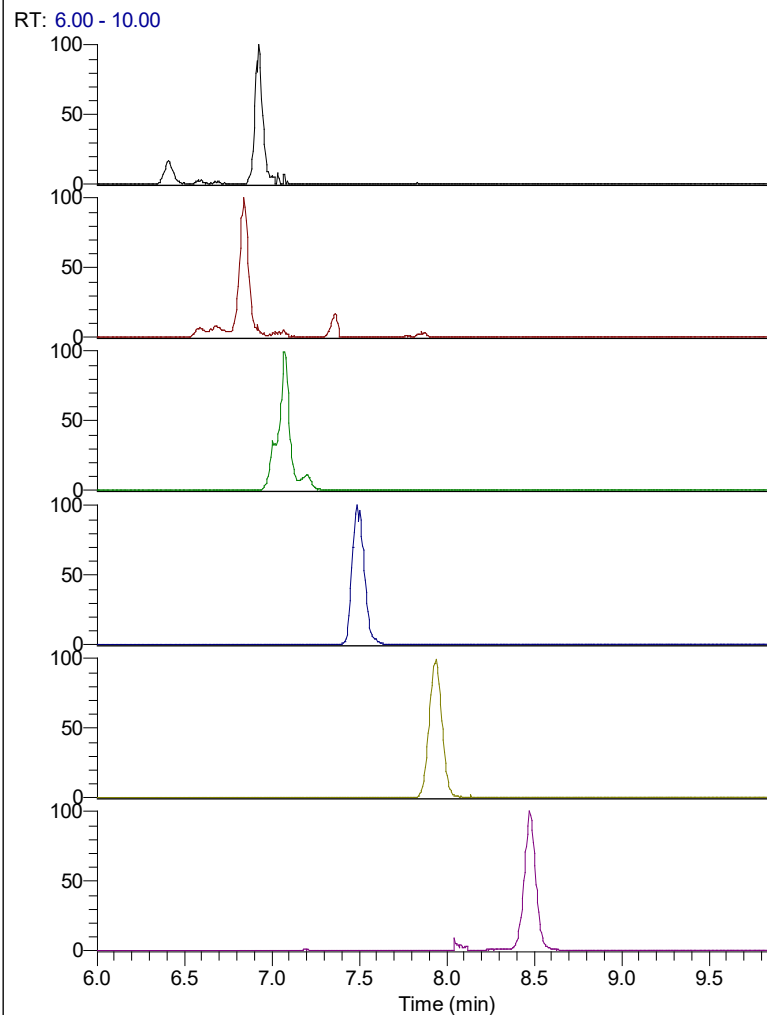
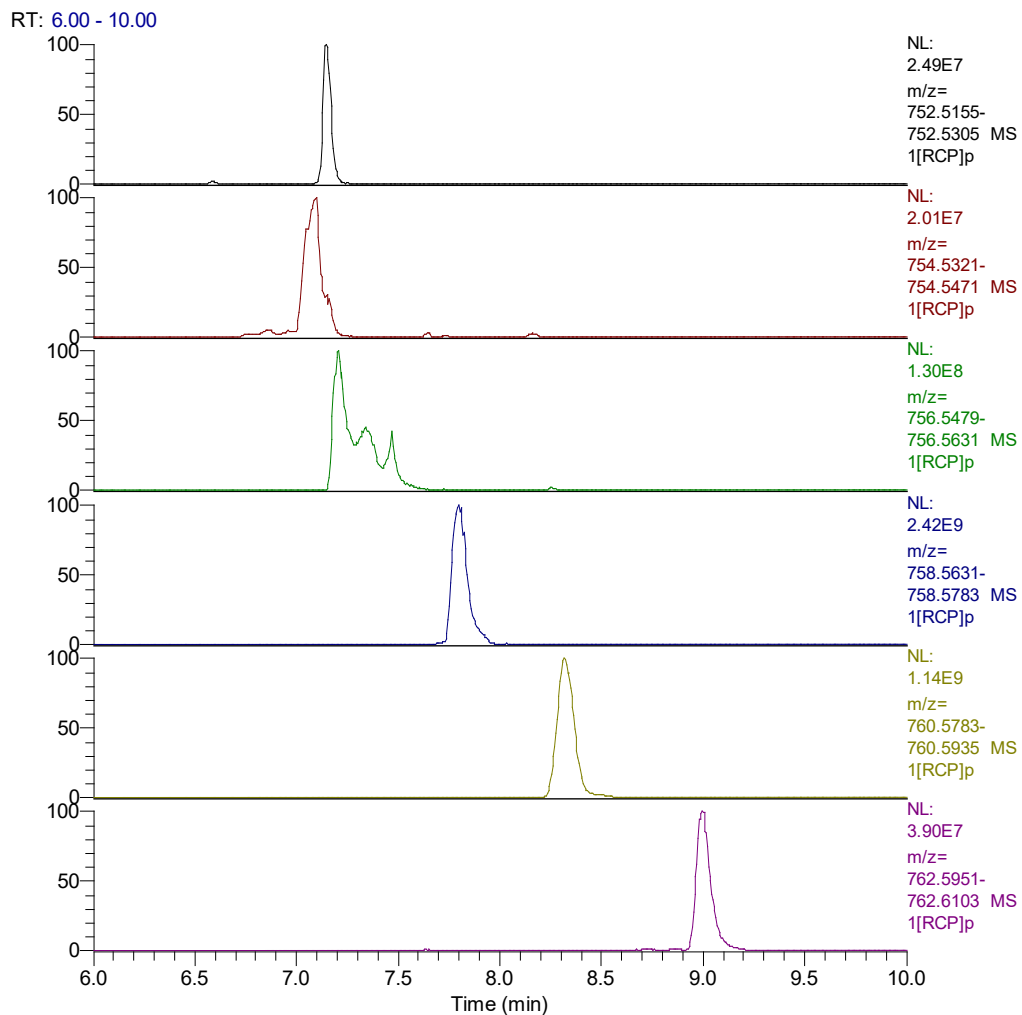
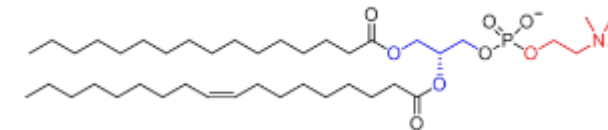
**Competitor C8 (3.0  $\mu$ m)**



# PC's with different degrees of unsaturation

Competitor C18  
(1.7  $\mu\text{m}$  Particle Size)

Evosphere C12  
(3.0  $\mu\text{m}$  Particle Size)



PC 34:5 (Mass = 752)

PC 34:4 (Mass = 754)

PC 34:3 (Mass = 756)

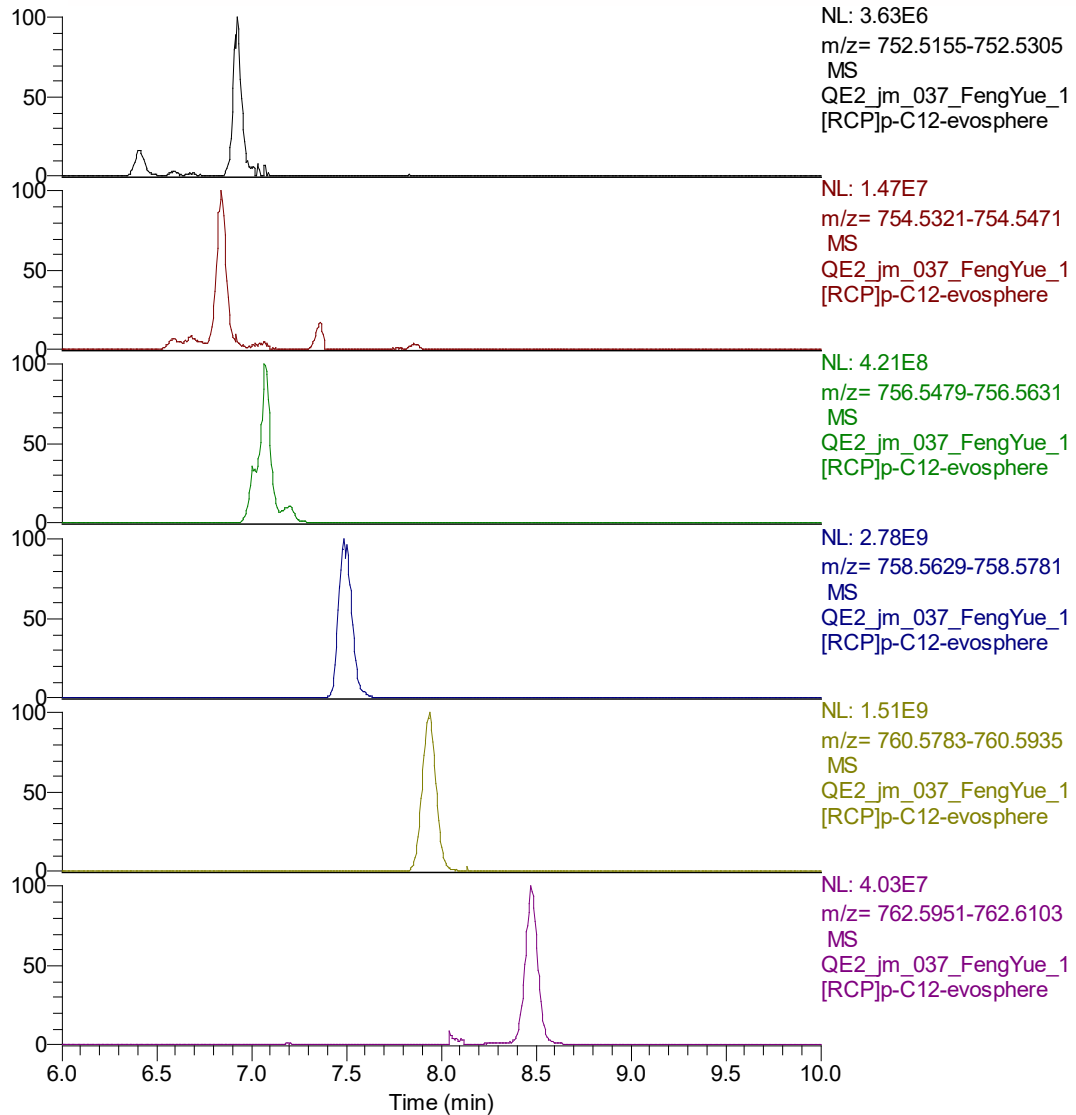
PC 34:2 (Mass = 758)

PC 34:1 (Mass = 760)

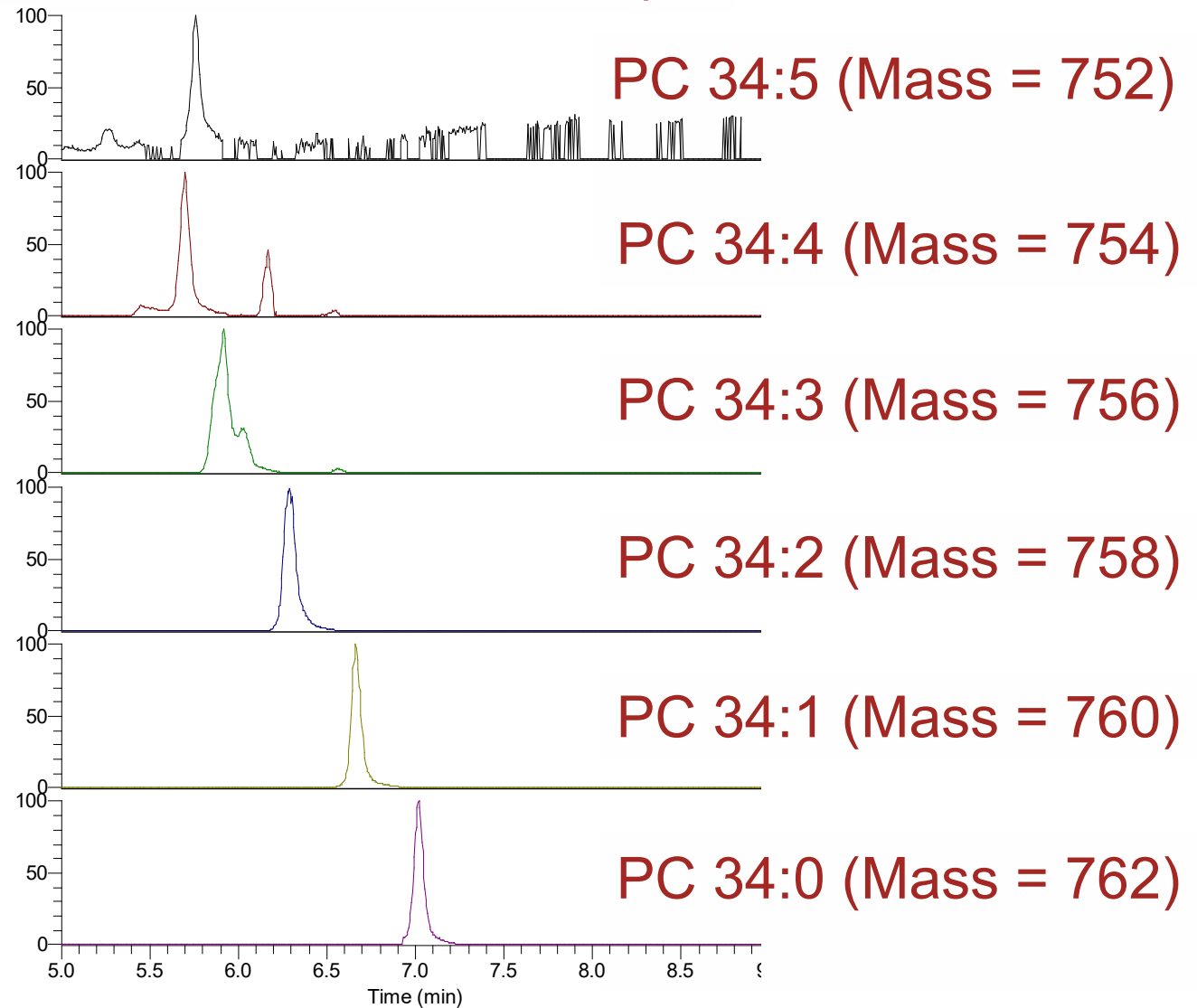
PC 34:0 (Mass = 762)

# PC's with Different Degrees of Unsaturation

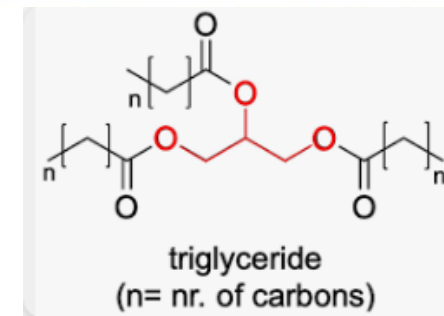
## Evosphere C12 (3.0 $\mu\text{m}$ )



## Competitor C8 (3.0 $\mu\text{m}$ )

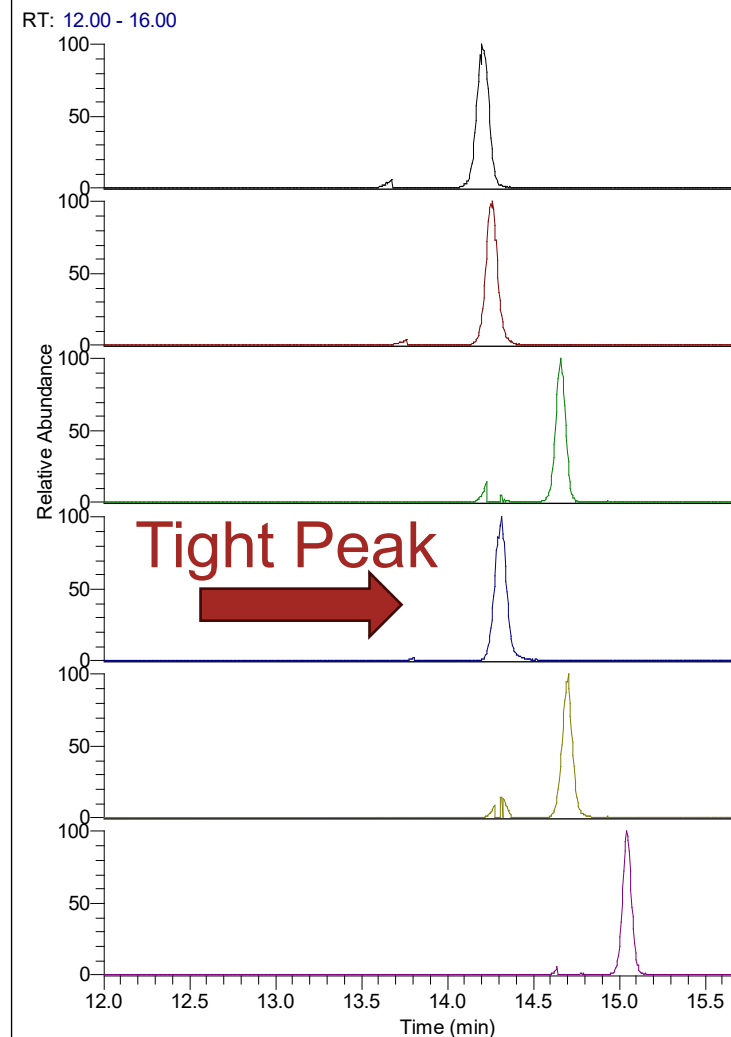
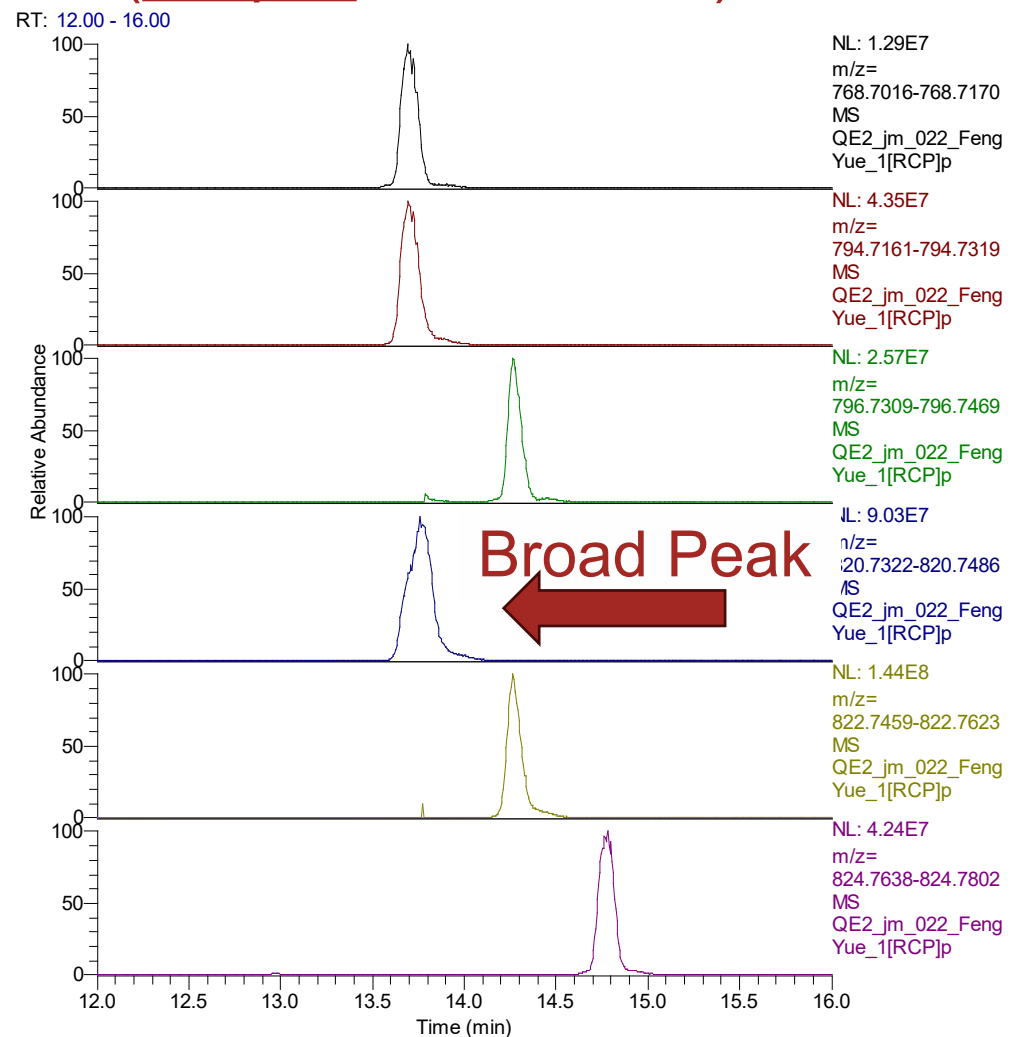


# Triglycerides 44-48 total carbons



Competitor C18  
(1.7  $\mu\text{m}$  Particle Size)

Evosphere C12  
(3.0  $\mu\text{m}$  Particle Size)



TG 44:0 (Mass = 768)

TG 46:1 (Mass = 794)

TG 46:0 (Mass = 796)

TG 48:2 (Mass = 820)

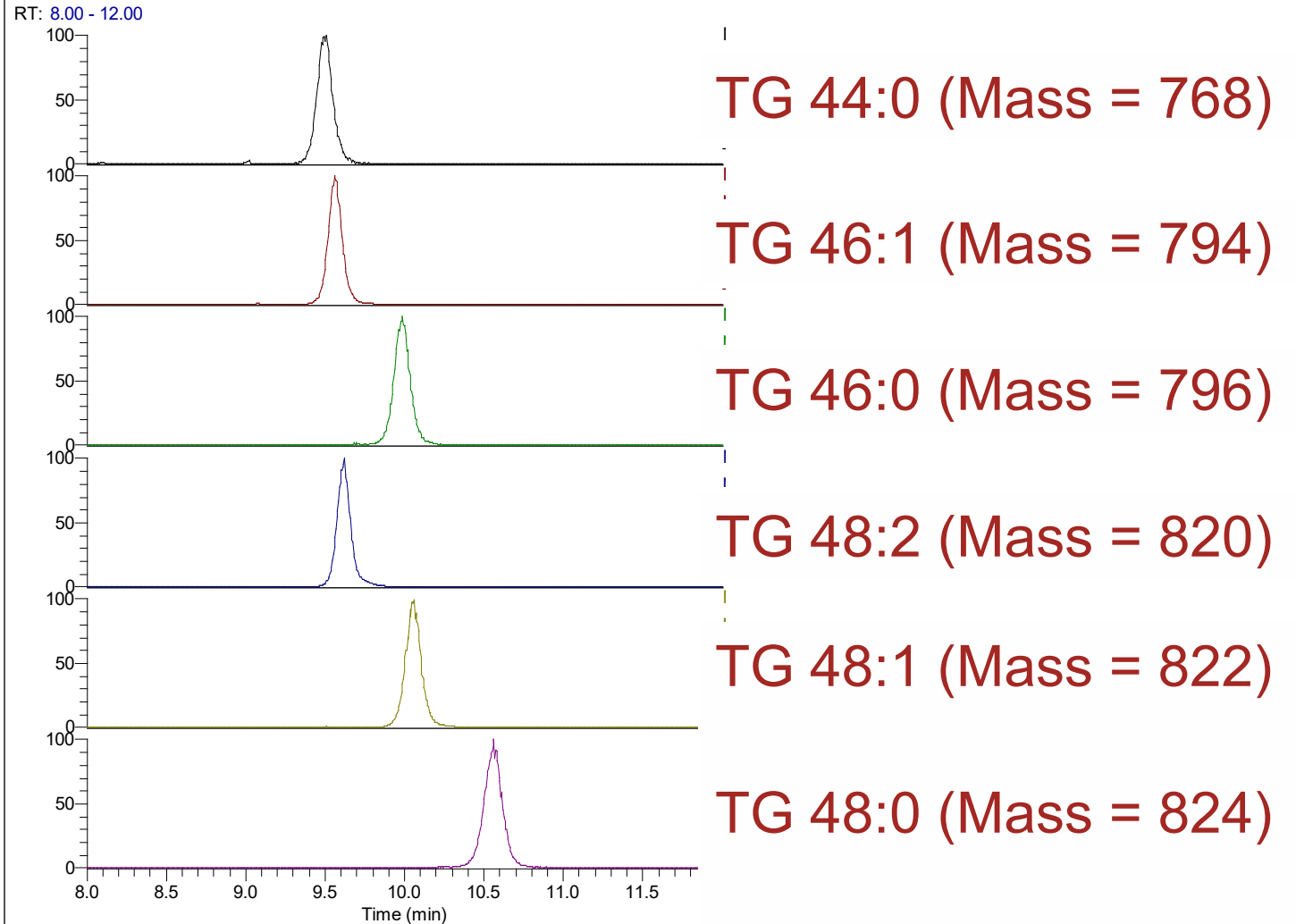
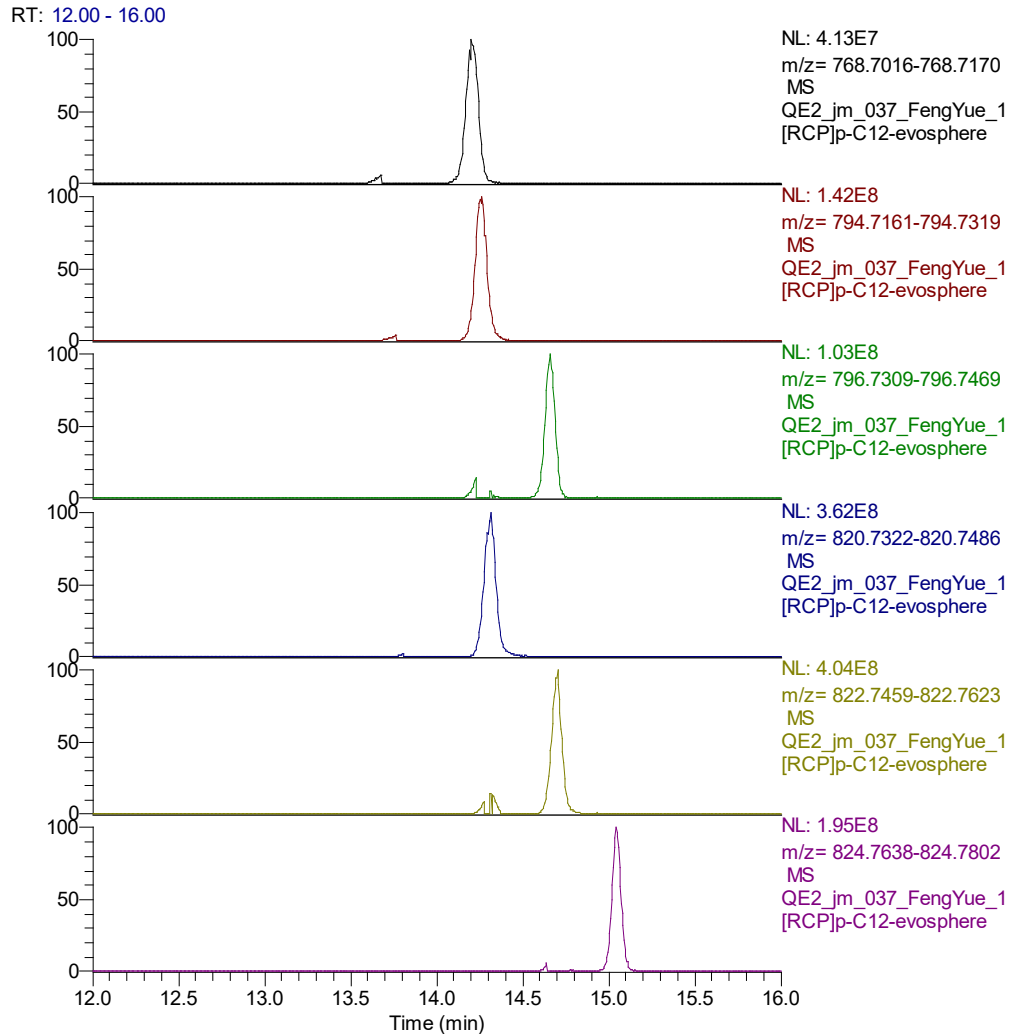
TG 48:1 (Mass = 822)

TG 48:0 (Mass = 824)

# Triglycerides 44-48 total carbons

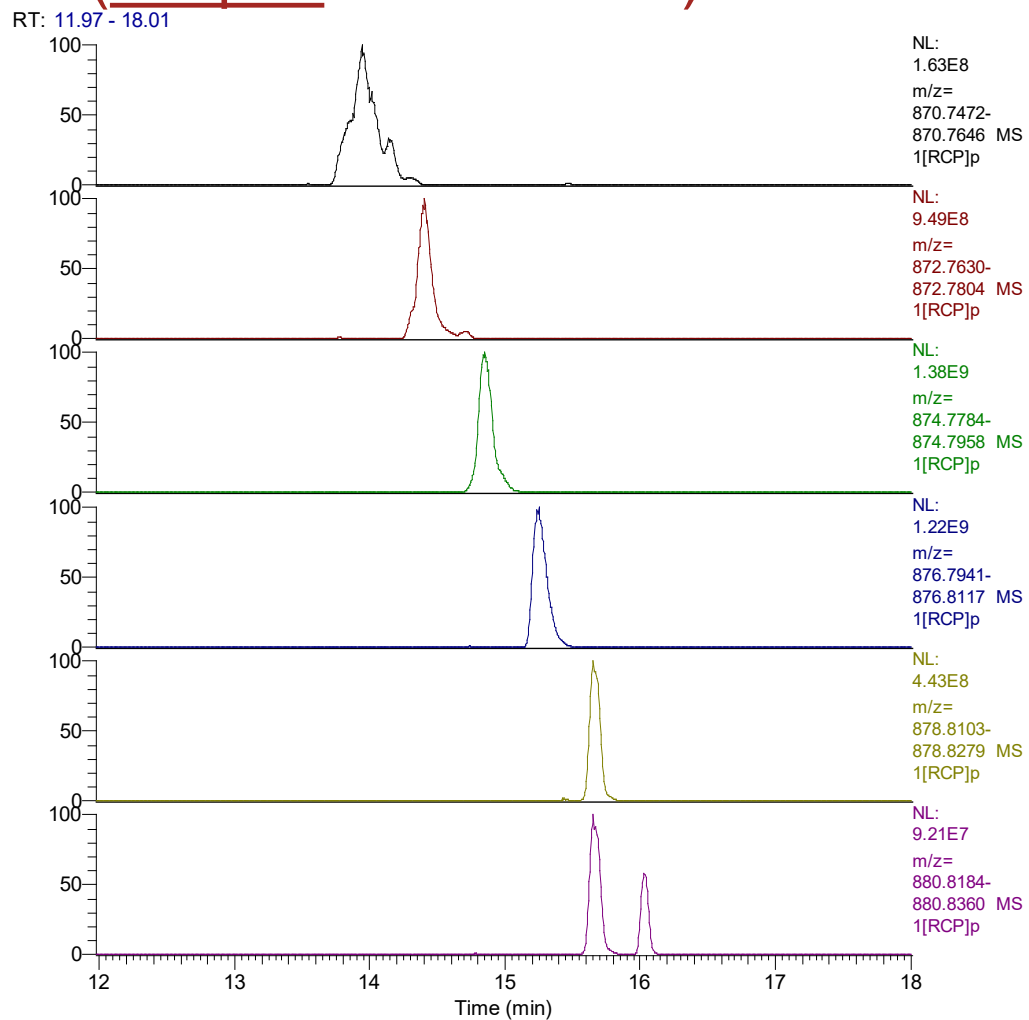
## Evosphere C12 (3.0 $\mu\text{m}$ )

## Competitor C8 (3.0 $\mu\text{m}$ )

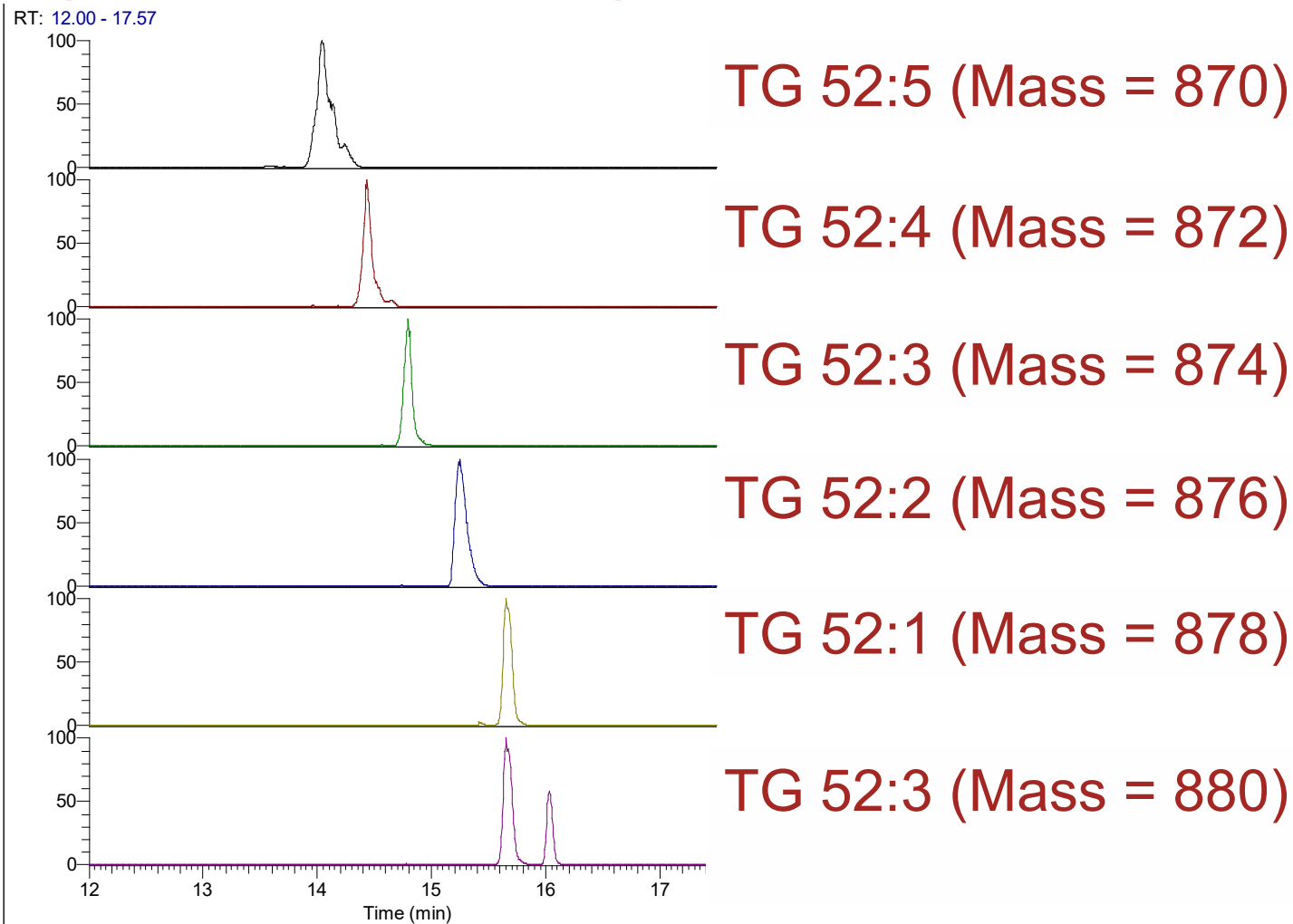


# Triglycerides with 52 total carbons

Competitor C18  
(1.7  $\mu\text{m}$  Particle Size)



Evosphere C12  
(3.0  $\mu\text{m}$  Particle Size)

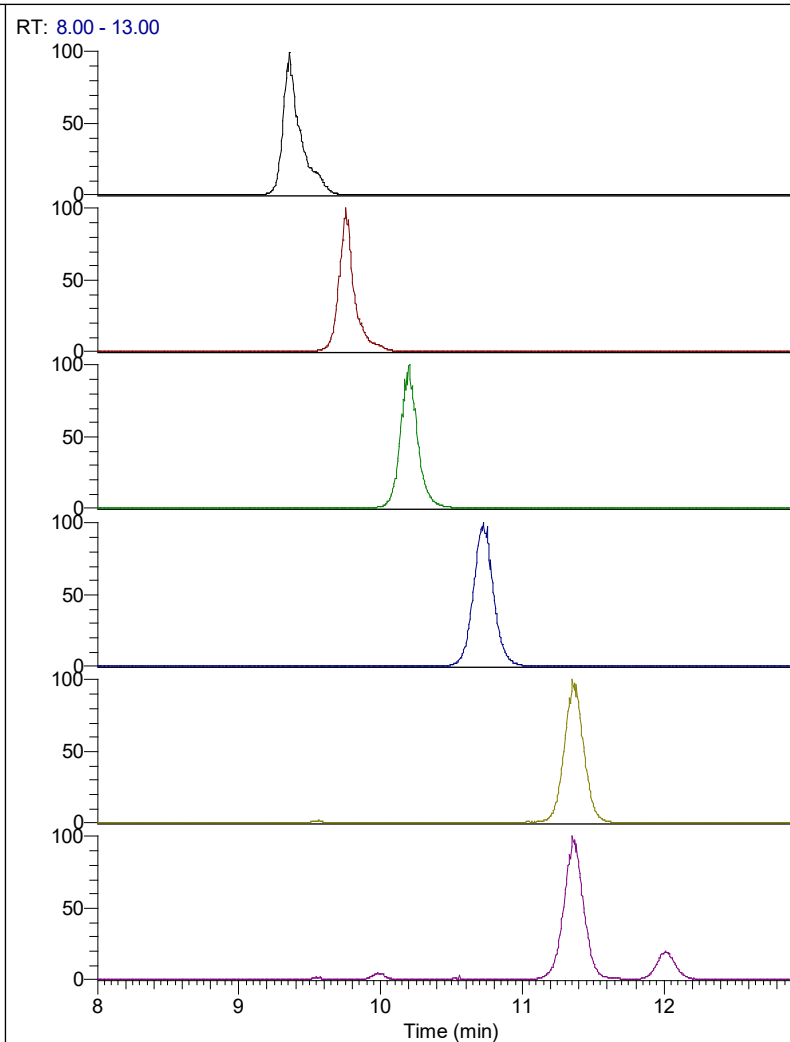
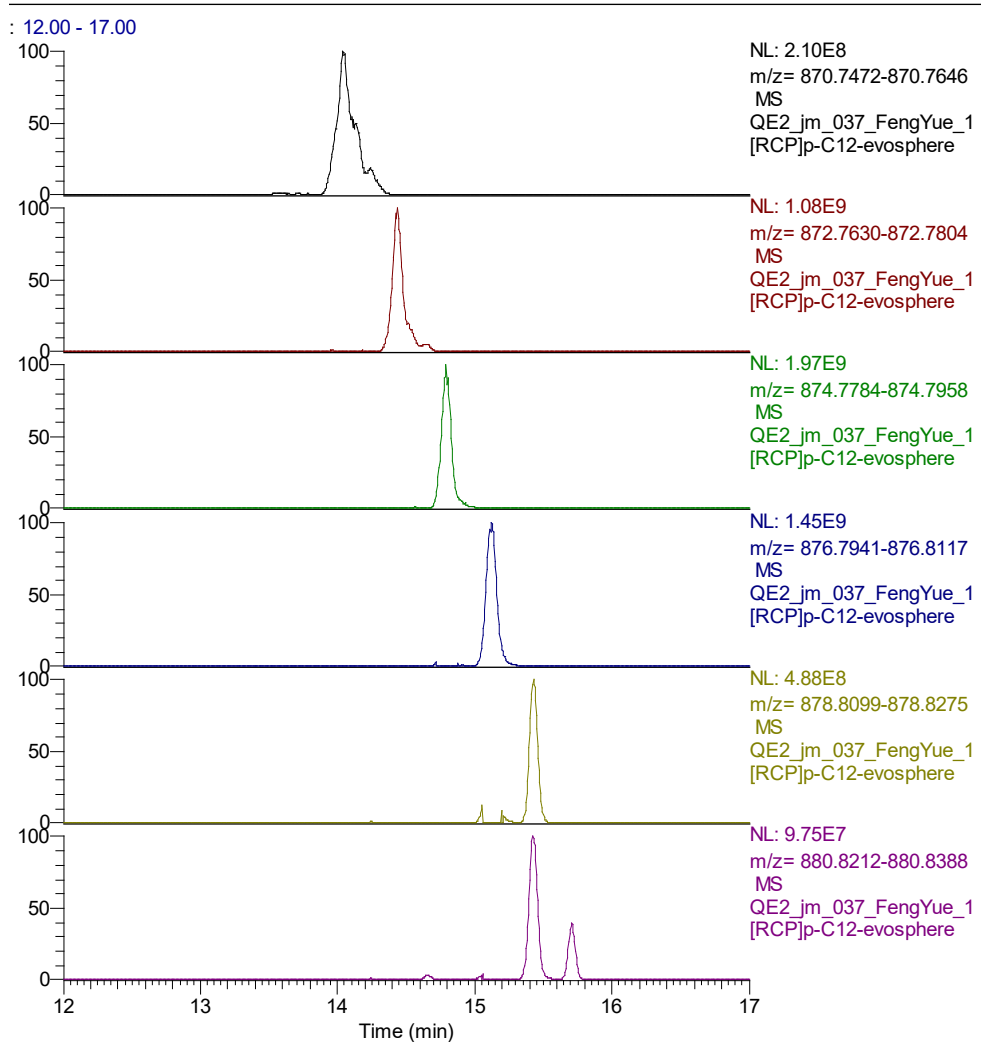




# Triglycerides with 52 total carbons

## Evosphere C12 (3.0 $\mu\text{m}$ )

## Competitor C8 (3.0 $\mu\text{m}$ )



TG 52:5 (Mass = 870)

TG 52:4 (Mass = 872)

TG 52:3 (Mass = 874)

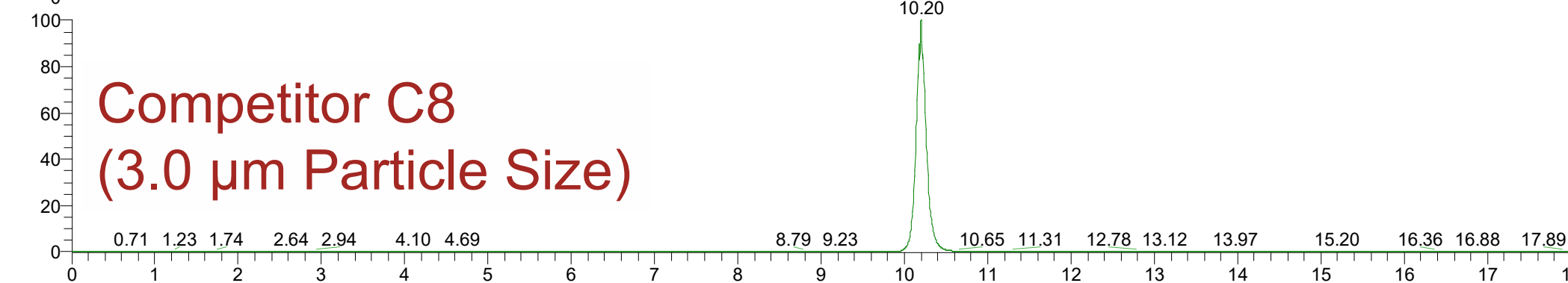
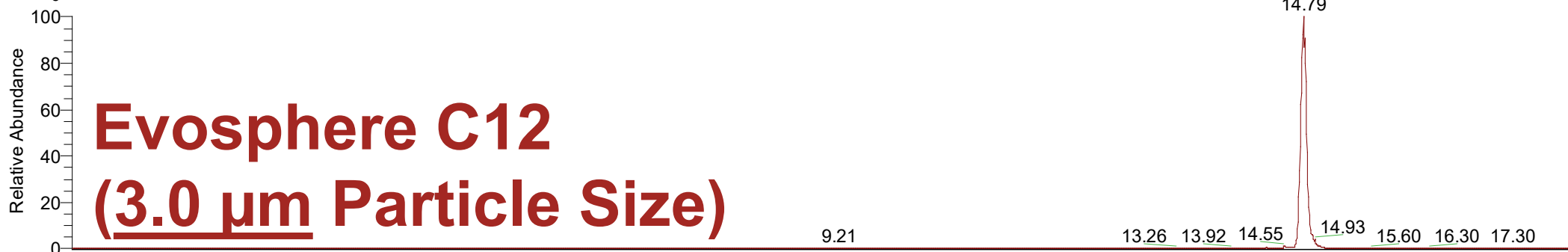
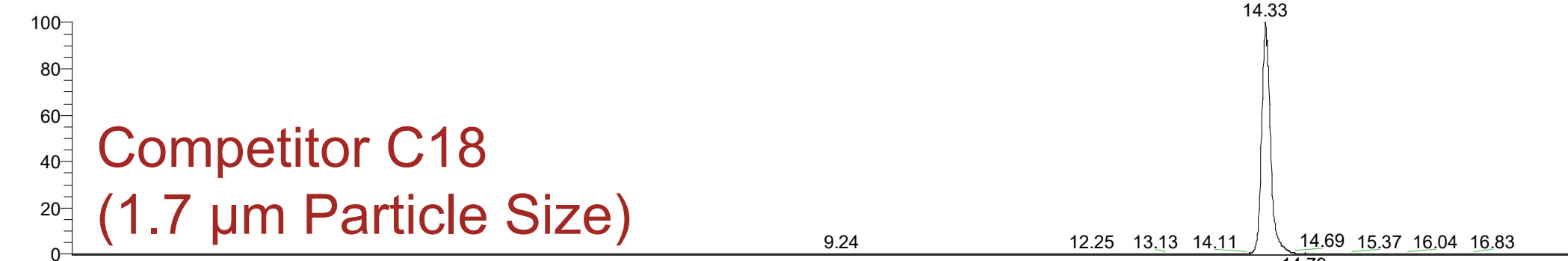
TG 52:2 (Mass = 876)

TG 52:1 (Mass = 878)

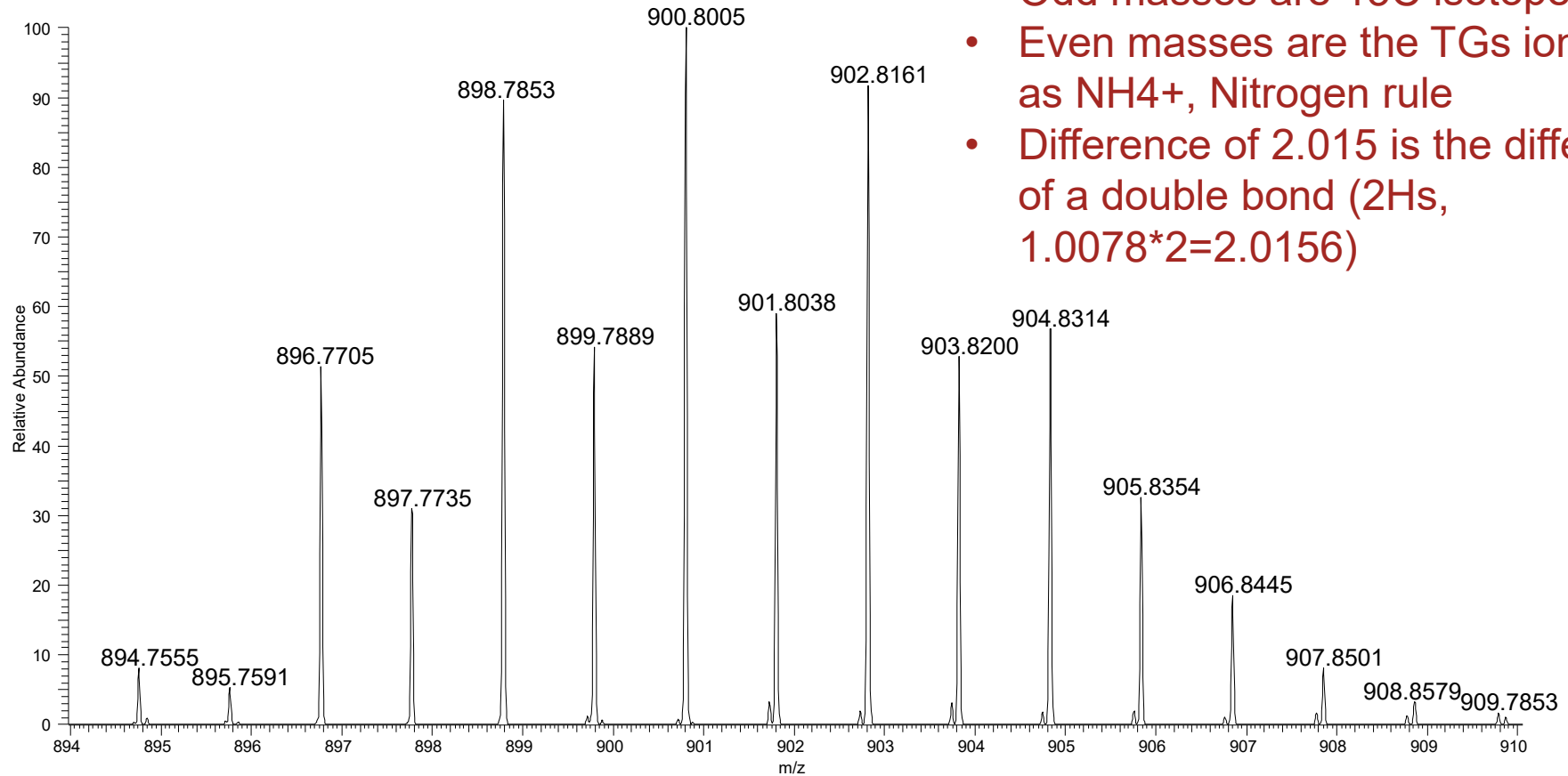
TG 52:3 (Mass = 880)

# Triglycerides 52:3

RT: 0.00 - 18.01



# TG envelope around m/z 900



- Odd masses are  $^{13}\text{C}$  isotopes
- Even masses are the TGs ionized as  $\text{NH}_4^+$ , Nitrogen rule
- Difference of 2.015 is the difference of a double bond ( $2\text{Hs}$ ,  $1.0078 \times 2 = 2.0156$ )





# Conclusion

- Fortis Evosphere is an innovative HPLC/UHPLC technology that provides significant method development advantages for lipid analysis.
- Fortis Evosphere C12 provided better performance than C8 or C18 for lipids panel!
- Fortis Evosphere in a 3  $\mu\text{m}$  particle size provided better performance than sub-2  $\mu\text{m}$  competition!!





**Thank You for Listening**