U/HPLC Monodisperse Particle Column Technology and its Applied Use in Metabolomics and Lipidomics

Webinar: 2 – Chromatography in 20

Presented by Geoffrey Faden

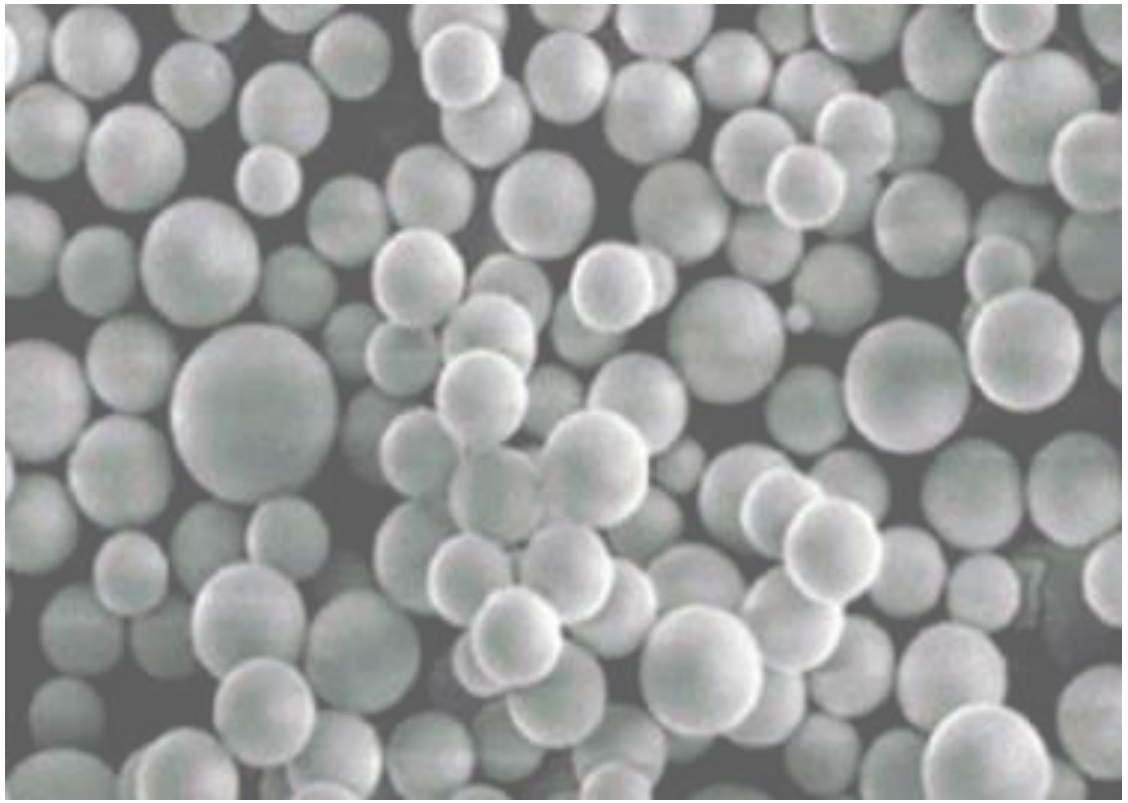
Executive Summary

- Monodisperse U/HPLC Particle Technology - Review
- Monodisperse UHPLC Column Technology – Untargeted Metabolomics Study
- Monodisperse UHPLC Column Technology – Lipidomics Comparison Study

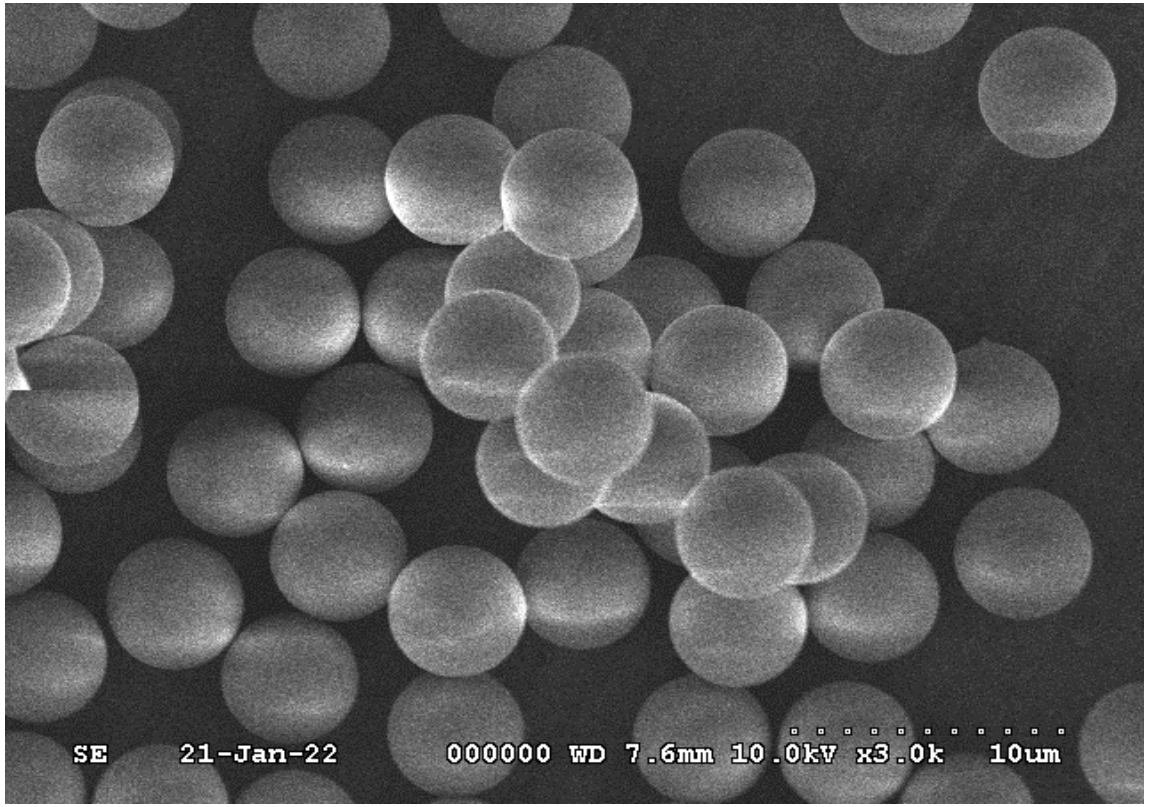
Evosphere®

Monodisperse U/HPLC Particle Technology

SEM Images of Particles Technologies

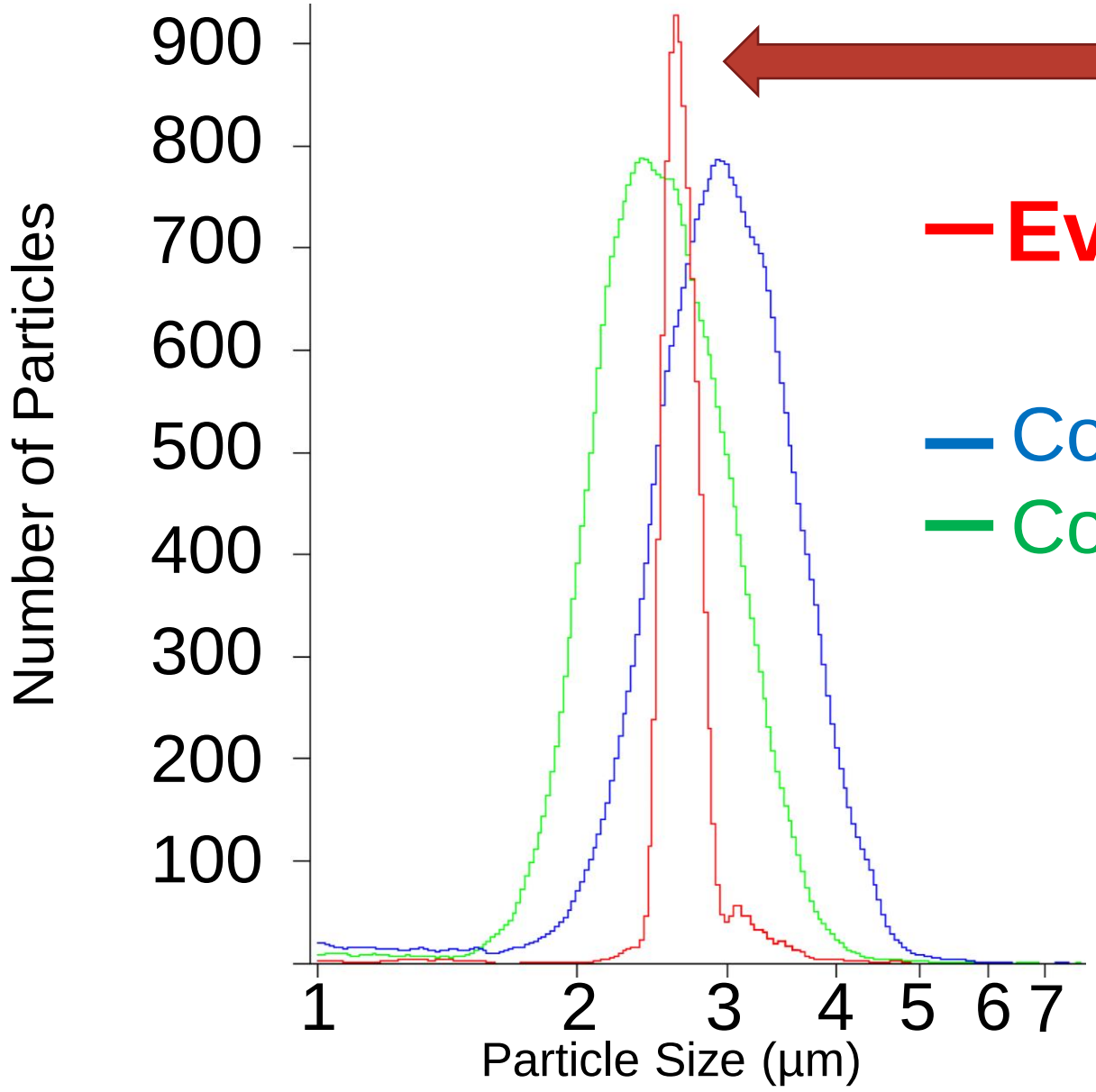


Polydisperse



Monodisperse

Particle Size Distribution Comparison



Evosphere MFPP 3 µm

Commercial FPP A 3 µm

Commercial FPP B 3 µm

Particle Size Distribution Comparisons

	Monodisperse silica	Commercial 3u silica - A	Commercial 3u Silica-B
Mean particle size (d50) *	2.66µm*	2.48µm	2.97µm
SEM particle diameter	3.0µm	2.8µm	3.3µm
D90/10	1.12	1.58	1.61
Pore volume	0.89	0.88	0.89

40% Reduction in D90/10

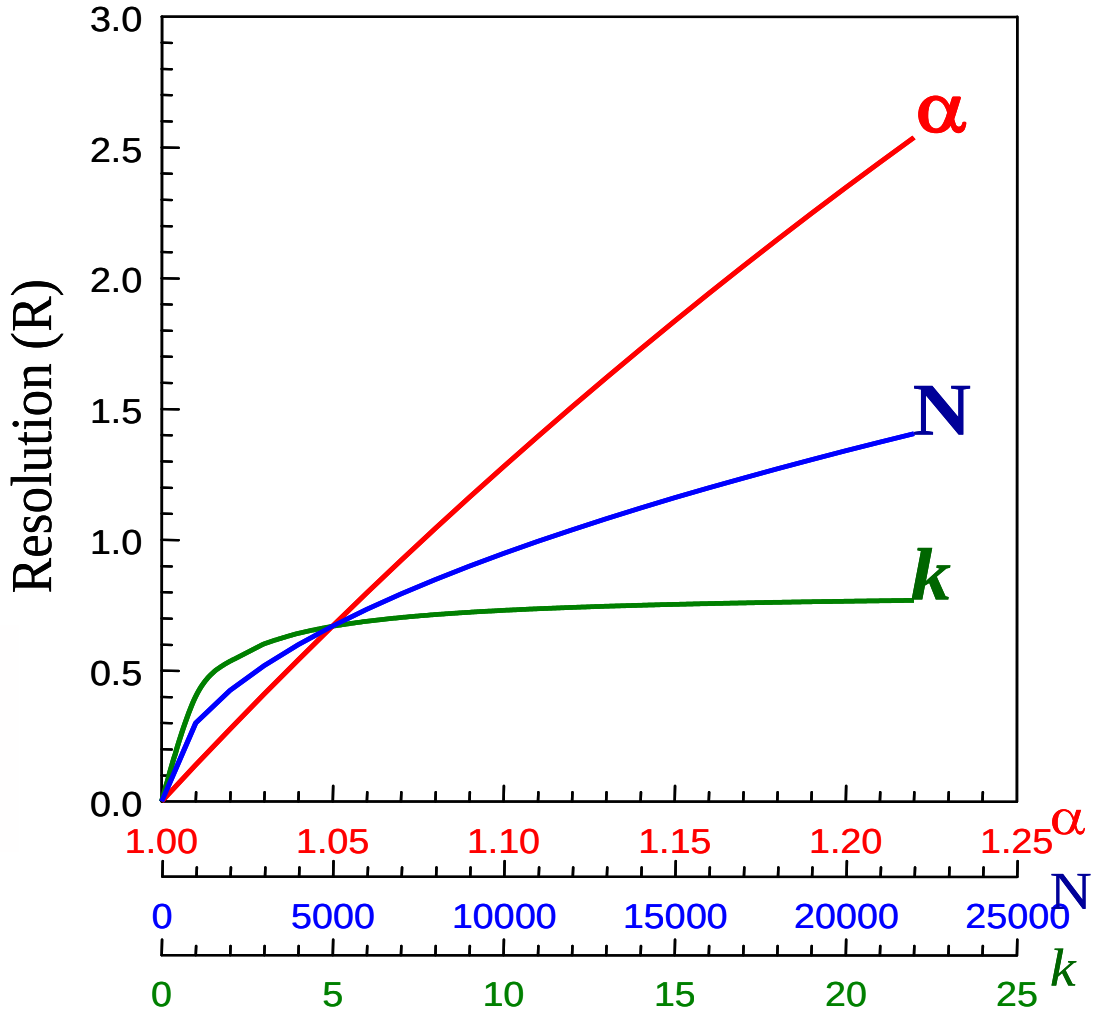
*Measured by Coulter Counter

Resolution Equation

Efficiency Retention Selectivity

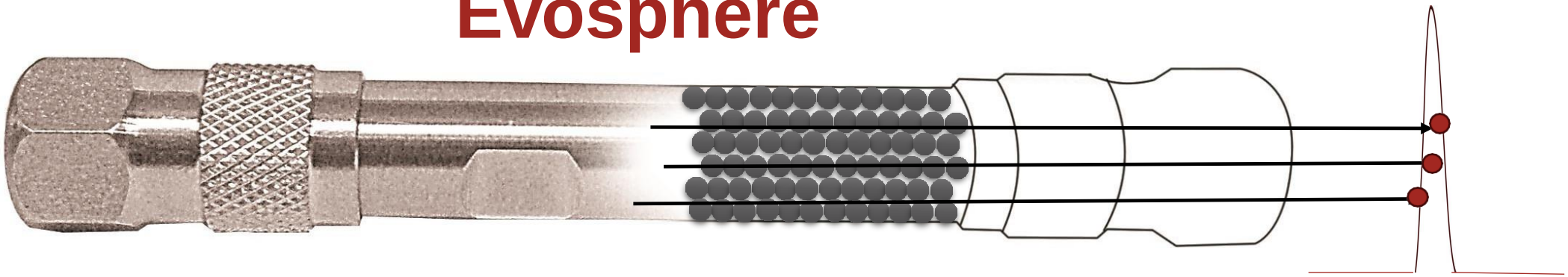
$$R = \frac{\sqrt{N}}{4} \quad \frac{k'}{k'+1} \quad \frac{\alpha-1}{\alpha}$$

$$N = \frac{Length\ of\ Column}{HETP}$$

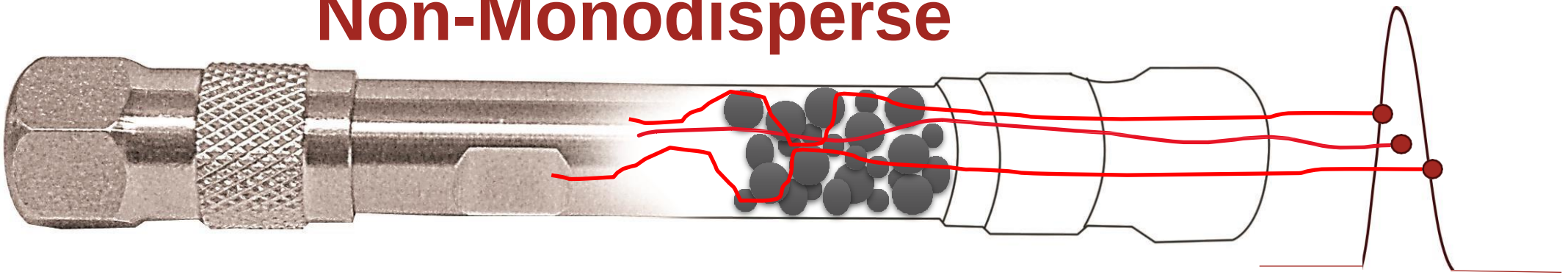


Visual Representation of Eddy Diffusion (“A Term”)

Evosphere

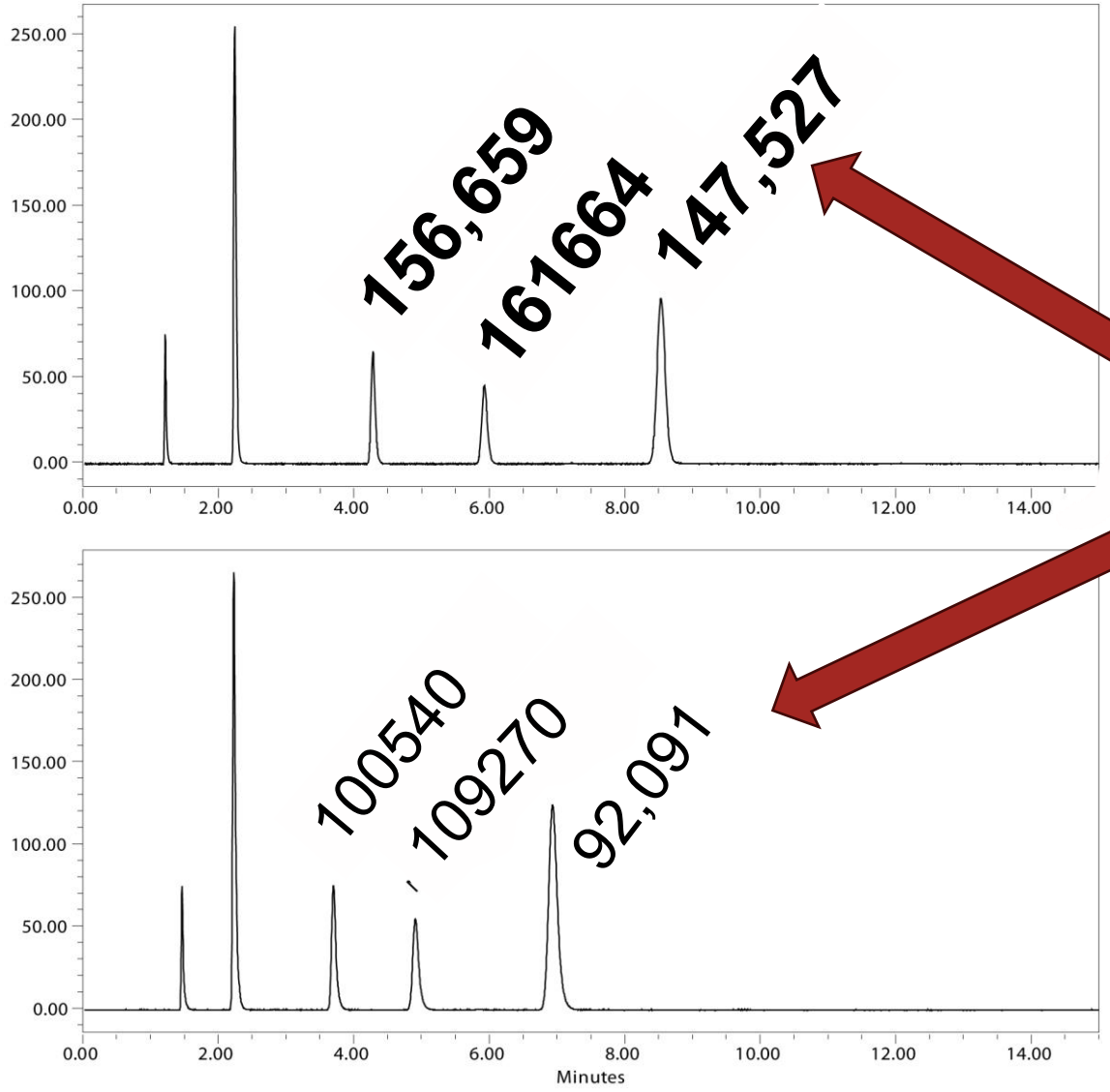


Non-Monodisperse



Flow through the column Evosphere vs. FPP

What does this look like chromatographically?



Evosphere C12

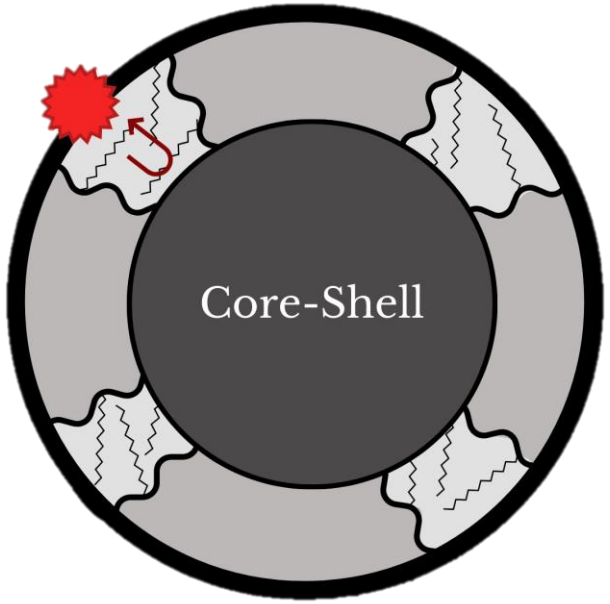
3 µm, 4.6 x 150 mm

60% Higher N

Popular Fully Porous C18
3 µm, 4.6 x 150 mm

Evosphere MFPP compared to Core-shell Materials

Core-Shell

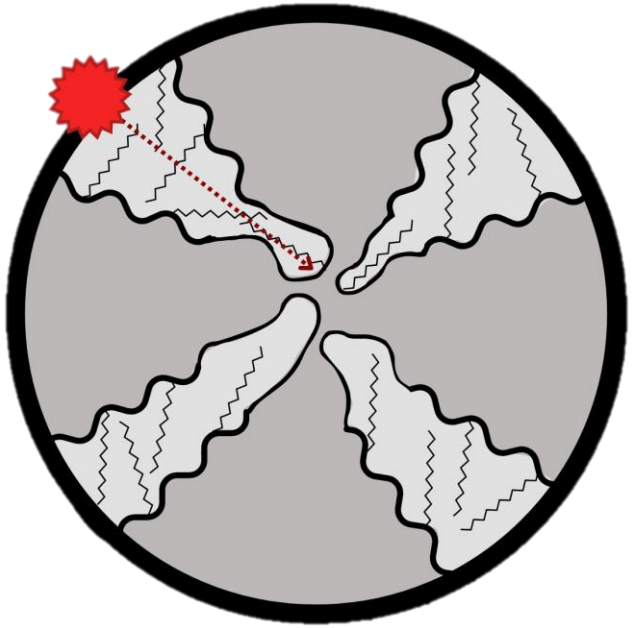


SA = ~130 m²/g

- Similar Efficiencies
- Greater Loading Capacity
- Scalability to Prep
- Increased Retention

~3X Surface Area

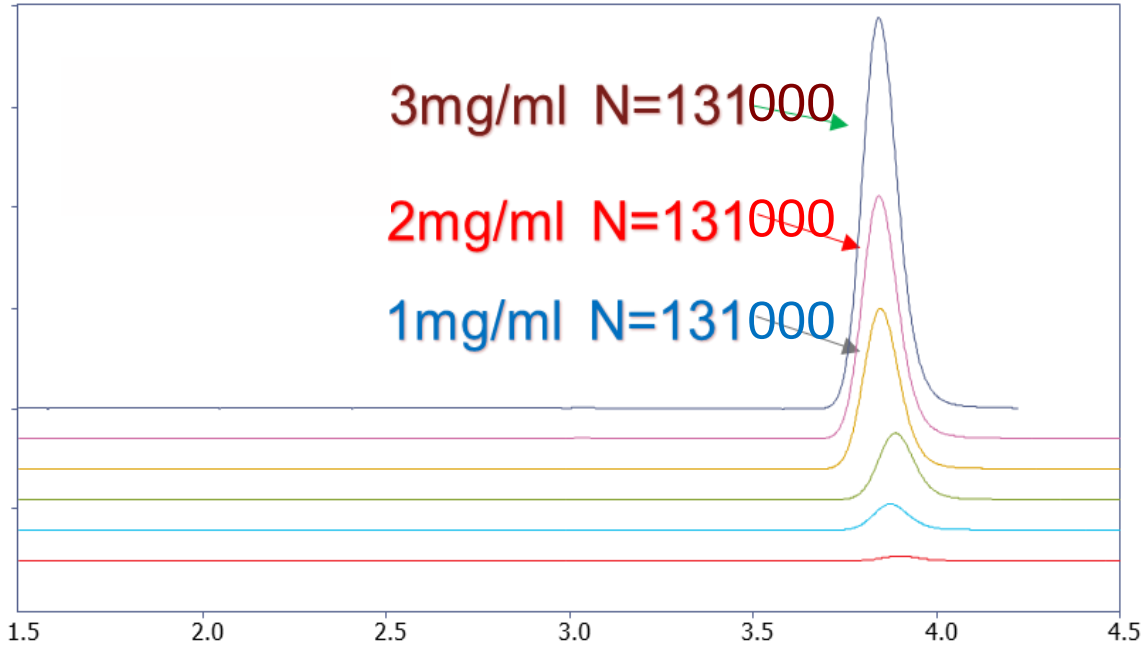
Evosphere



SA = 350 m²/g

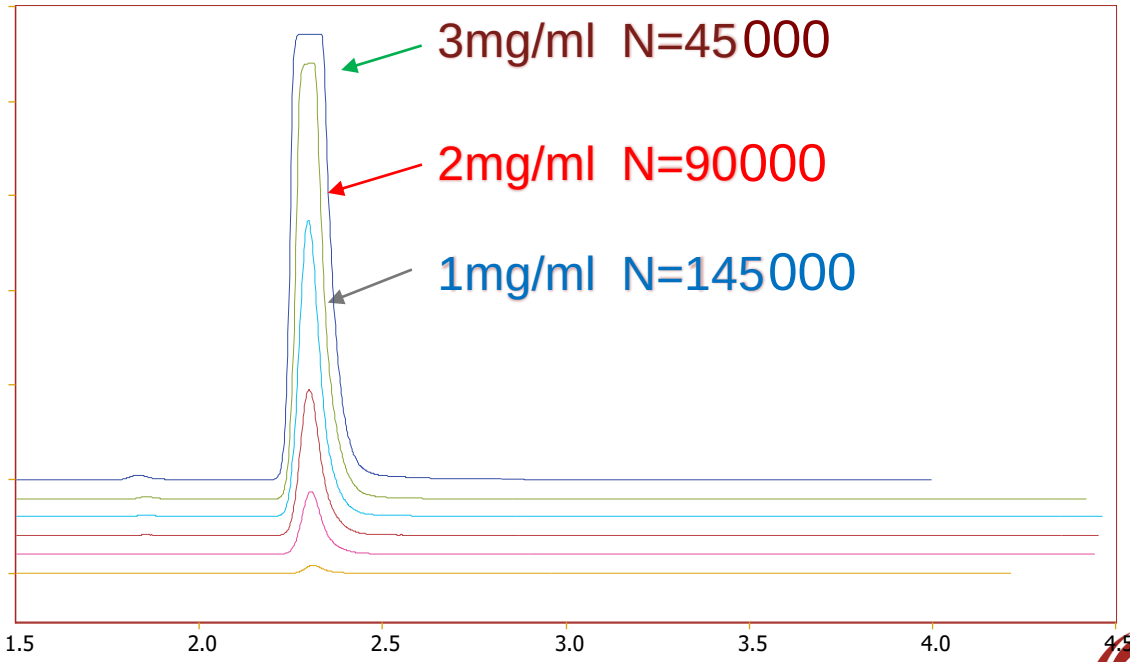
Fortis® Evosphere®
Improves Loading and Increases Retention

Evosphere L1 Surface Area = 350 m^2/g



Rt = 3.8 min

Core Shell L1 Surface Area = 130 m^2/g



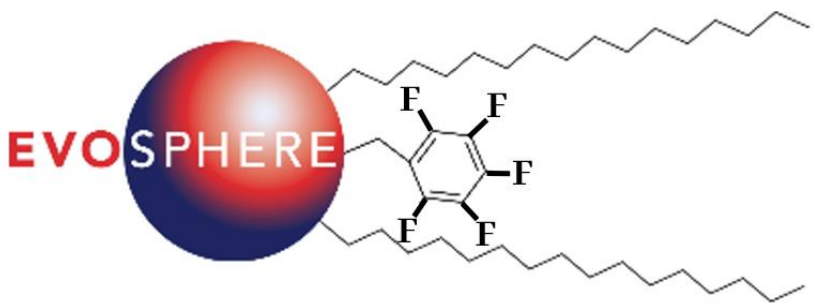
Rt = 2.25 min

Unique Selectivities for Omics Work

Efficiency Retention Selectivity

$$R = \frac{\sqrt{N}}{4} \quad \frac{k'}{k'+1} \quad \boxed{\frac{\alpha-1}{\alpha}}$$

Metabolomics: C18/PFP



Lipidomics: C12

macmod

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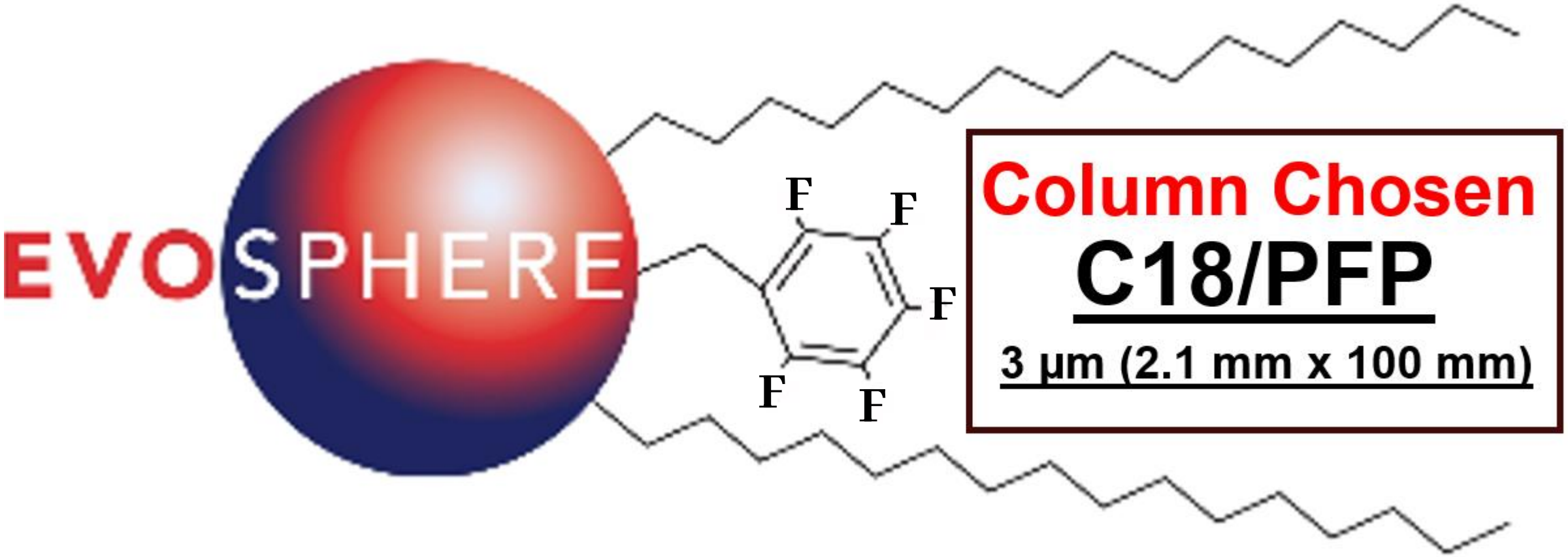
Monodisperse Particle Column Technology Applied to Metabolomics

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

Untargeted Metabolomics on Plasma Extract

Work done by collaborator Dr. Timothy Garrett at the University of Florida



Total Ion Chromatogram

Column Phase – Evosphere C18/PFP

Dimensions - 3 µm (2.1 mm x 100 mm)

Instrument - Thermo Q-Exactive with Dionex UHPLC

Sample – Plasma Extract

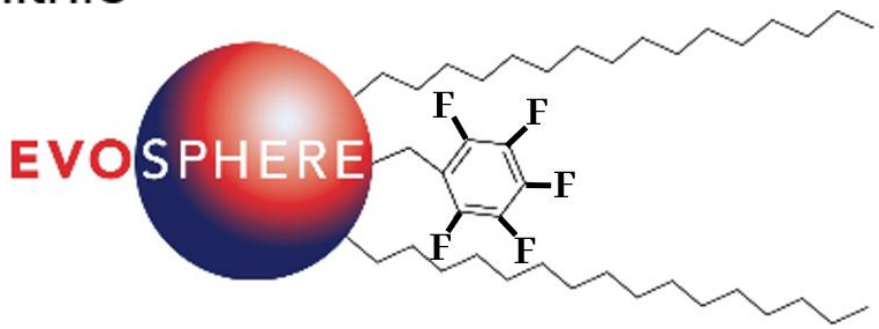
Mobile Phase A = 0.1% Formic Acid in H_2O

Mobile Phase B = Acetonitrile

Temperature = 25ºC

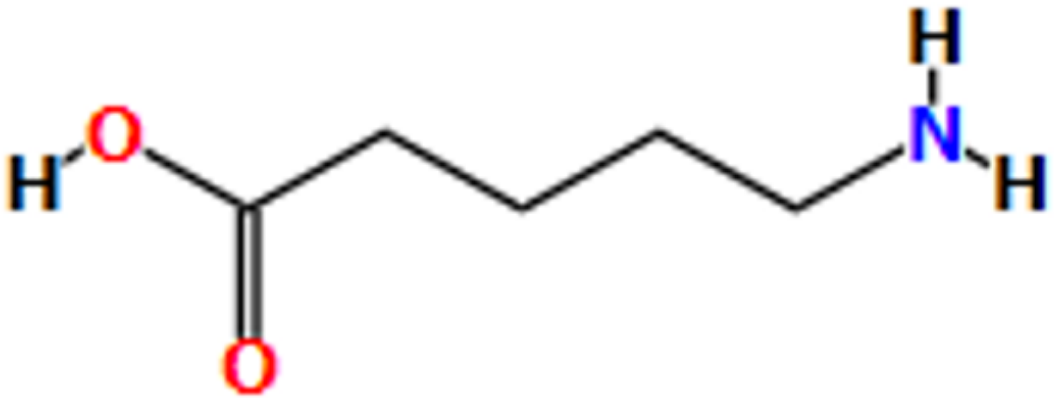
Injection volume = 2µL

Time	% B	Flow Rate (mL/min)
3 min	0	.35
13 min	80	.35
16 min	80	.35
16.5	0	.35
16.8	0	.60
20	0	.60
20.5	0	.35

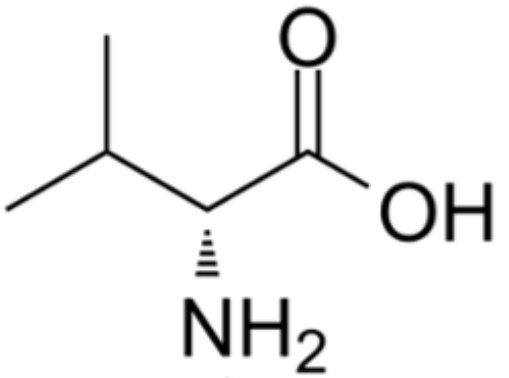


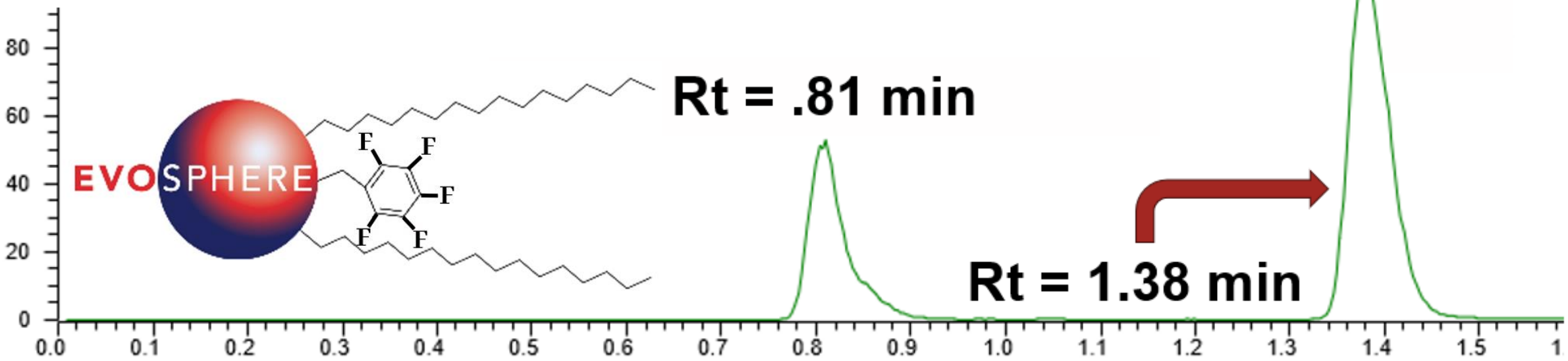
Polar Retention

5-Aminopentanoic Acid



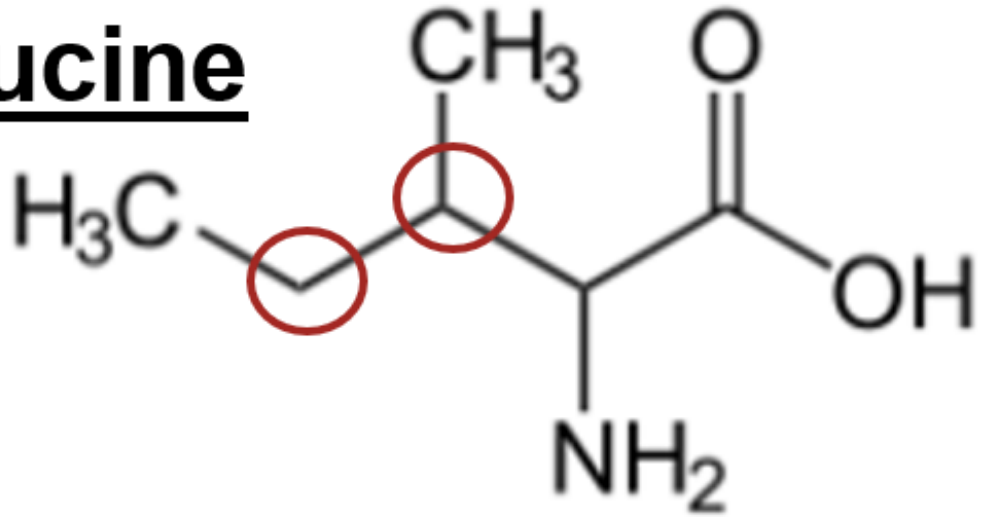
Valine



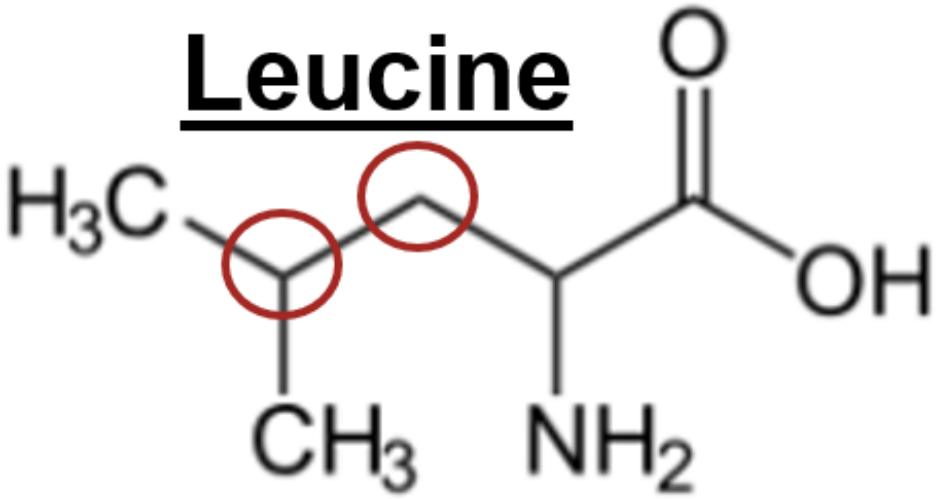


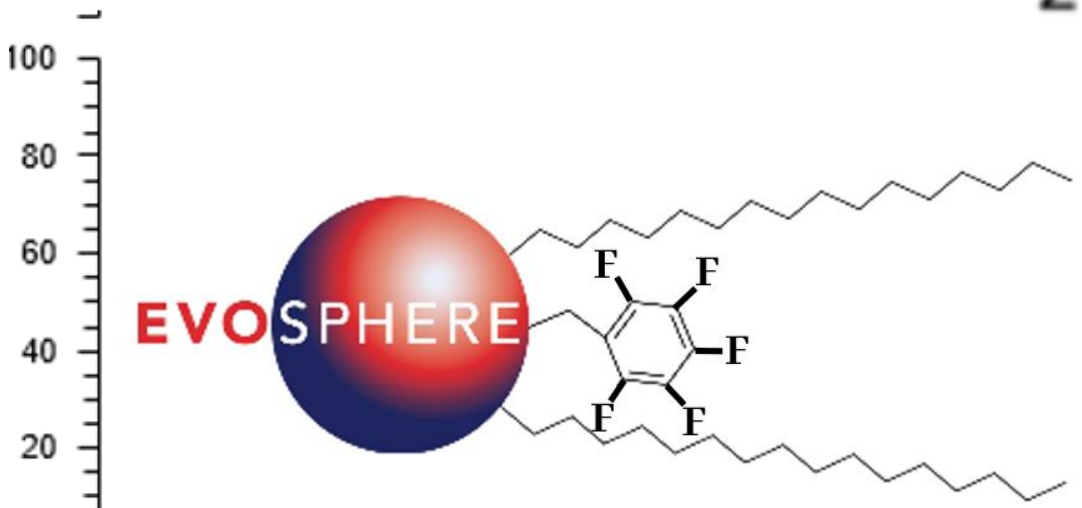
Methyl Position Switch

Isoleucine

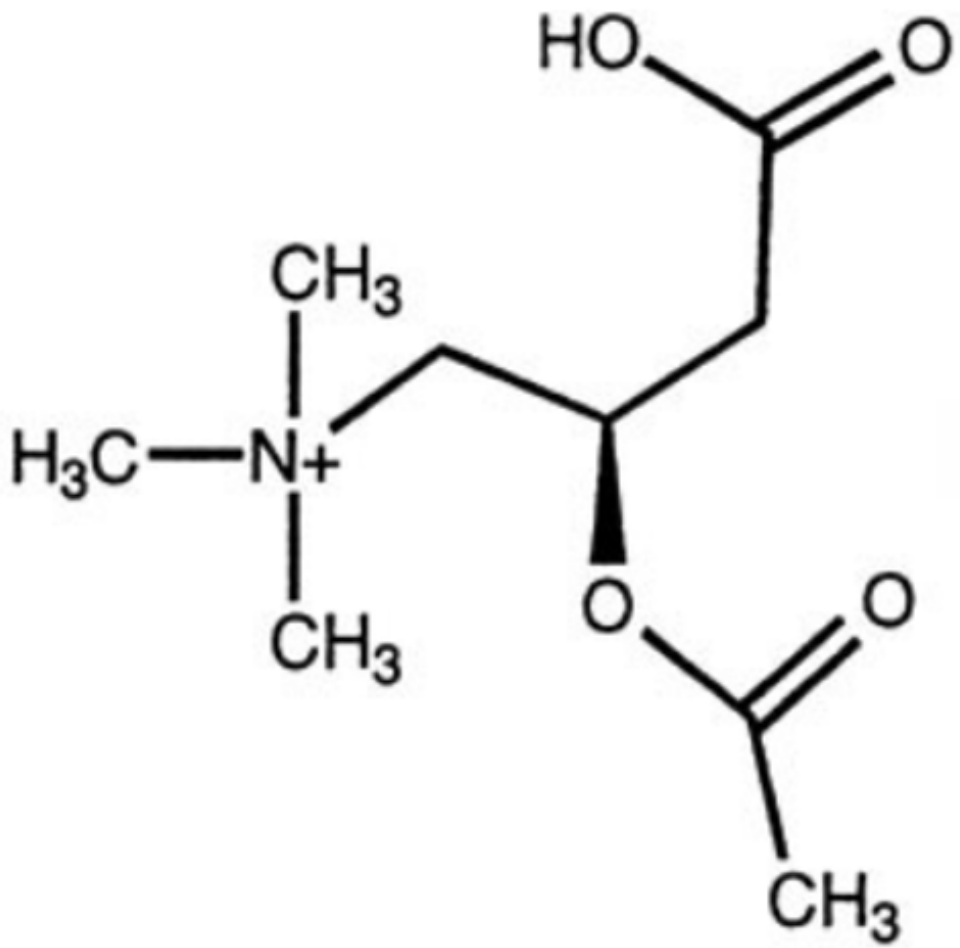


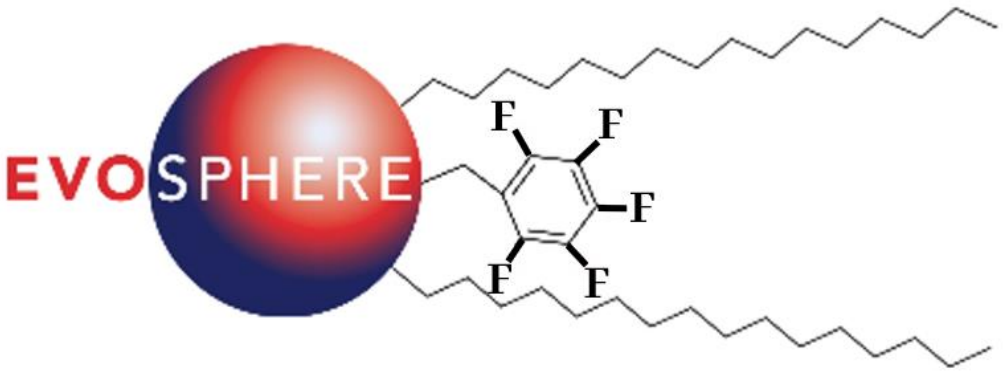
Leucine



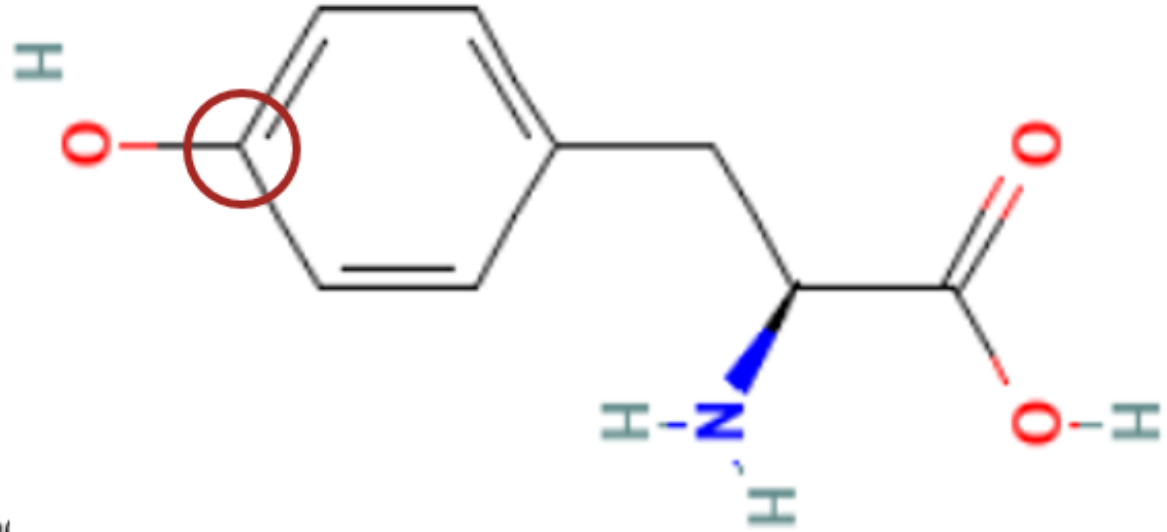


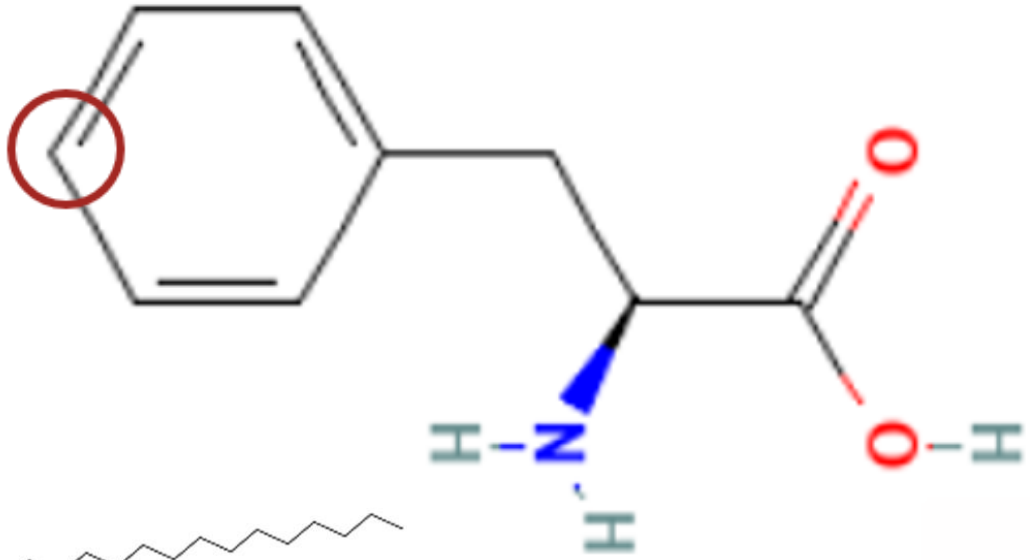
Optimal Peak Shape

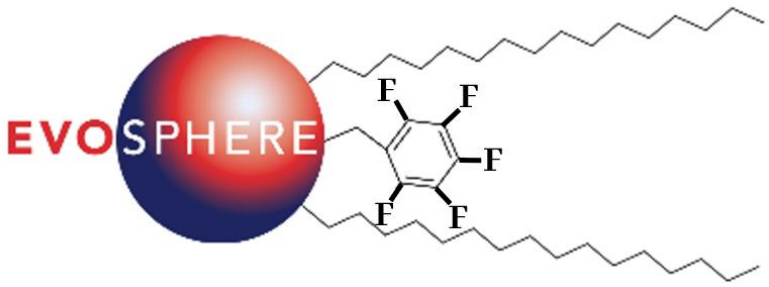




Strong Isomeric Selectivity







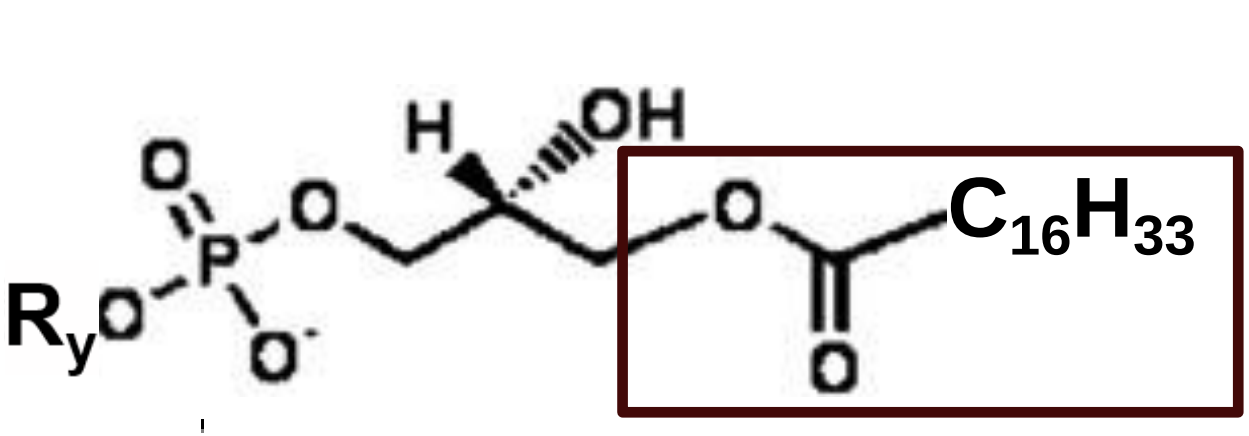
Tyrosine

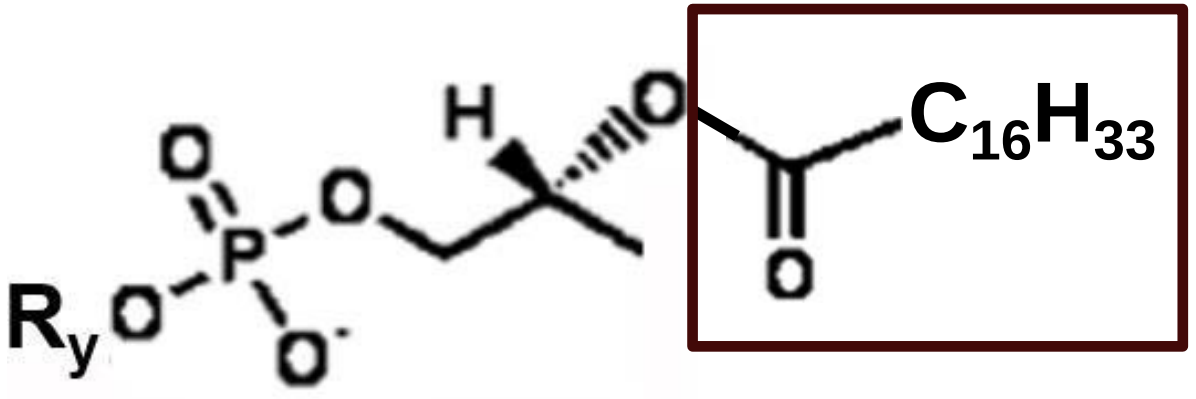
Rt = 4.35 min

Phenylalanine

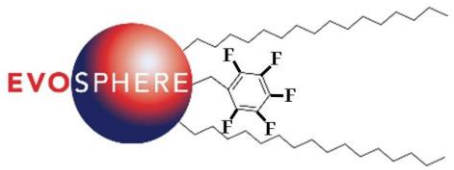
Rt = 6.86 min

Lysophospholipid Isomers sn-1 vs sn-2 with C18/PFP

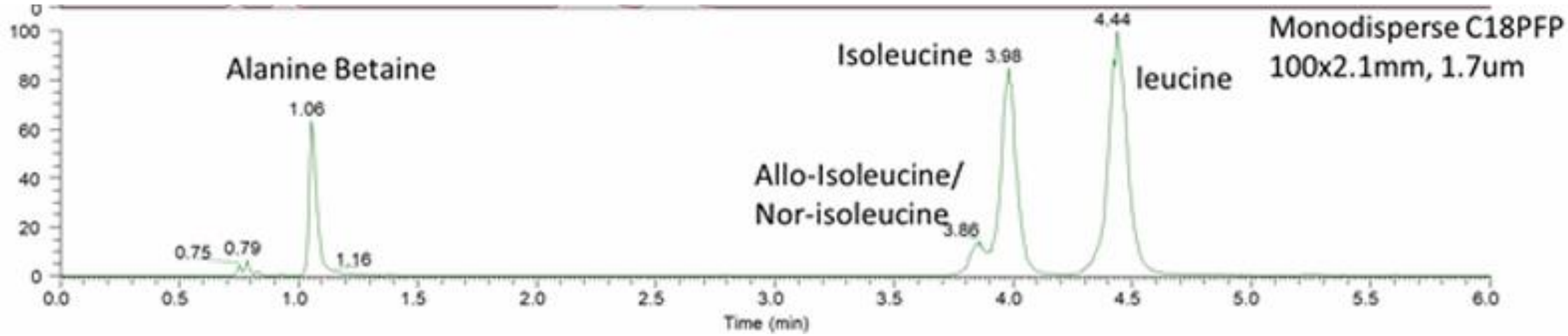




R_x = Choline
R_y = LysoPC
R_z = Fatty Acid



Power of 1.7 µm Monodisperse Particle Columns for Metabolomics



macmod

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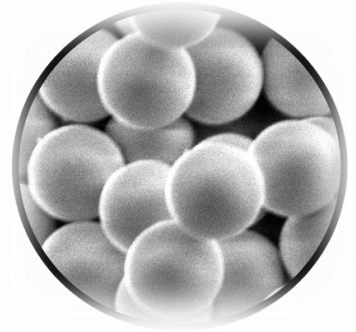
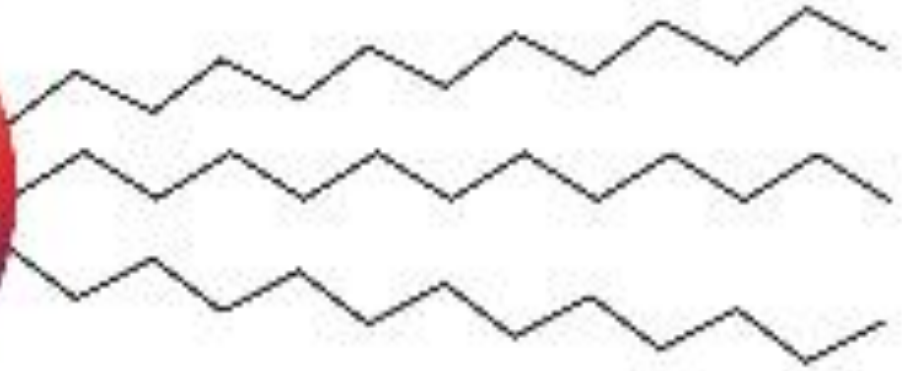
Column Comparison for Lipidomics

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

Evosphere C12

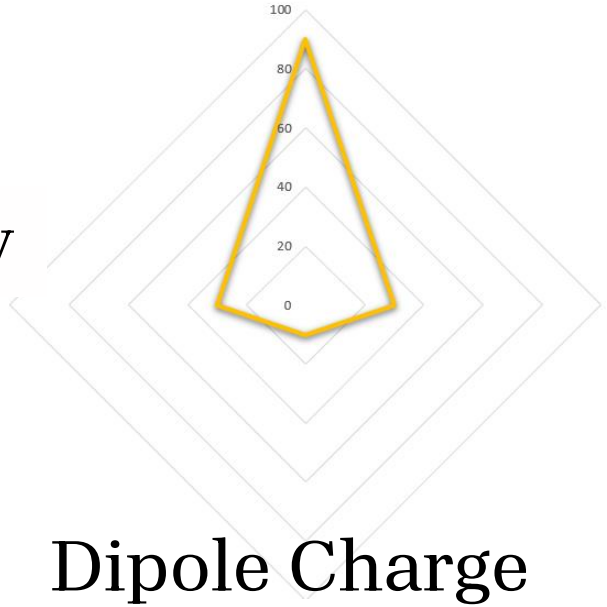


EVOSPHERE



Hydrophobicity

Steric Selectivity



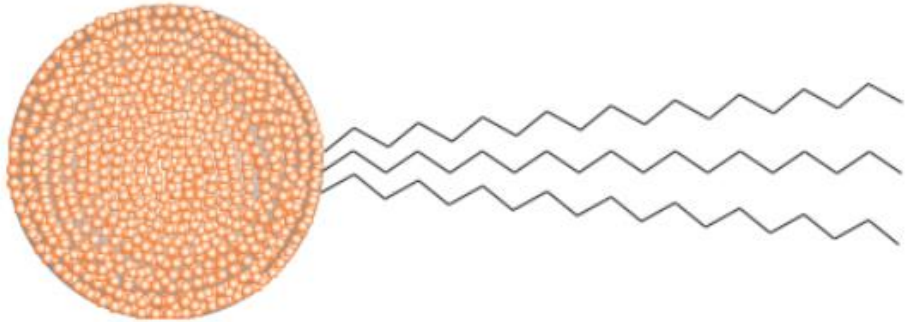
Polarity

Dipole Charge

HPLC/UHPLC Columns Compared

- Competitor C18 (2.1 x 50 mm) **1.7 µm**
- **Evosphere C12 (2.1 x 50 mm)** **3.0 µm**

C12 3 µm vs **C18** 1.7 µm



Goals of Experimental Comparison

- Explore whether new Monodisperse Fully Porous Particle improves resolution or chromatographic factors as compared to industry standard competitor columns.
- Investigate optimal bonded phase (C18 vs. C12) ligand length for analyte set.
- Utilize established lipidomic method analysis parameters to gauge the above experiments.

Experimental Conditions

Mobile Phase A = 60/40 ACN/H_2O with 0.1% FA and 10 mM NH_4HCO_2

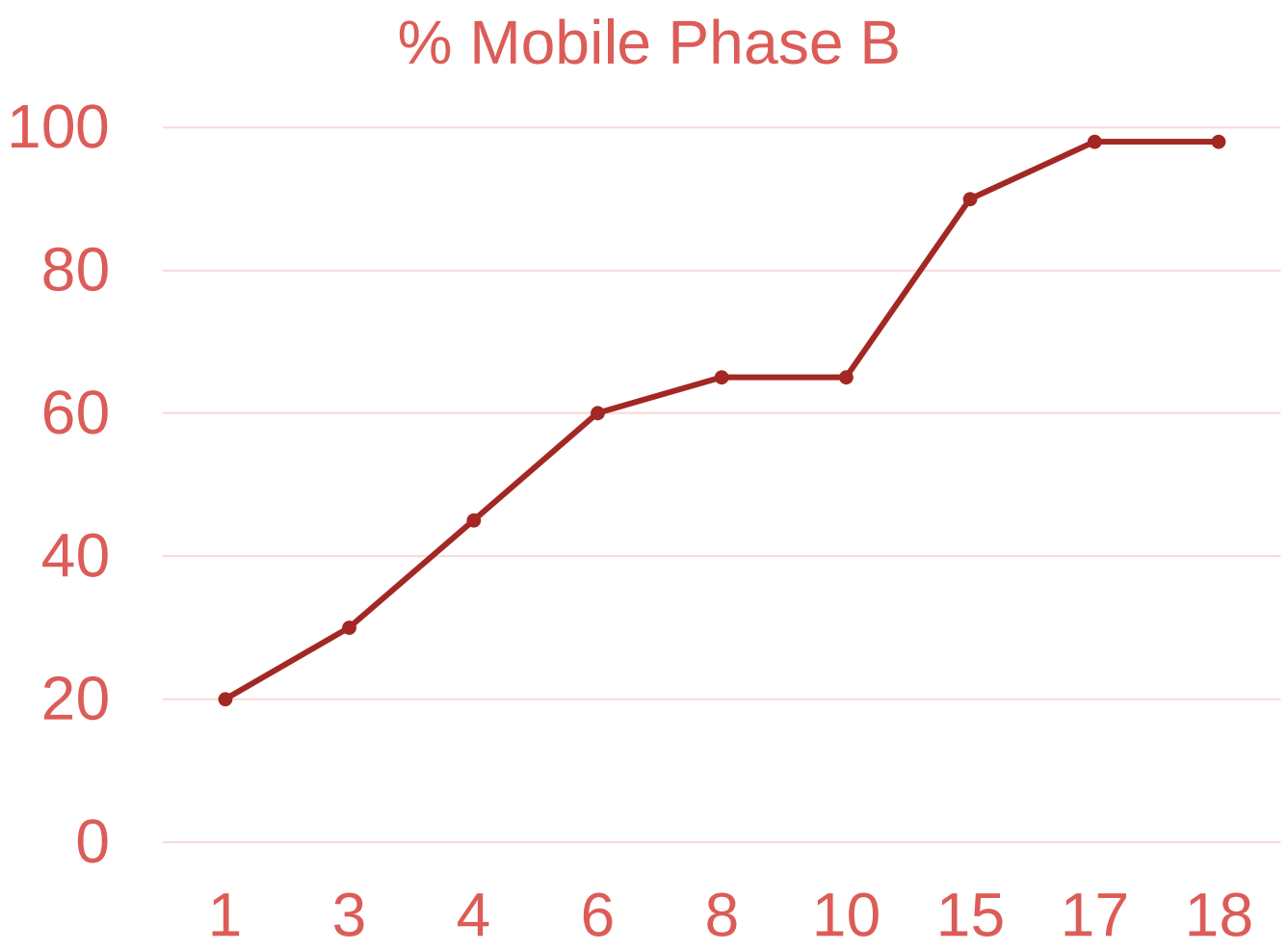
Mobile Phase B= 90/8/2 IPA/ACN/ H_2O with 0.1% FA and 10mM NH_4HCO_2

Flow Rate = 500 µL/min,

Temperature = 50°C

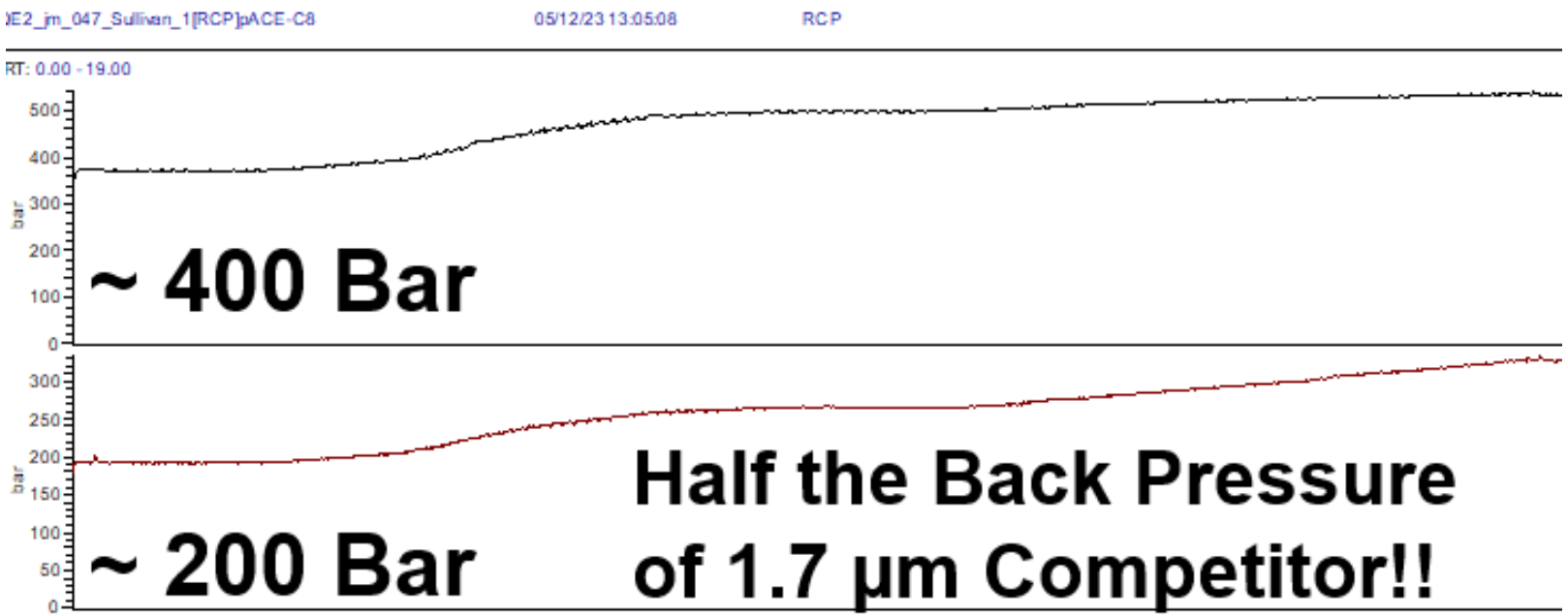
Multi-step gradient starting at 80/20 and ending at 2/98

Gradient Charts



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

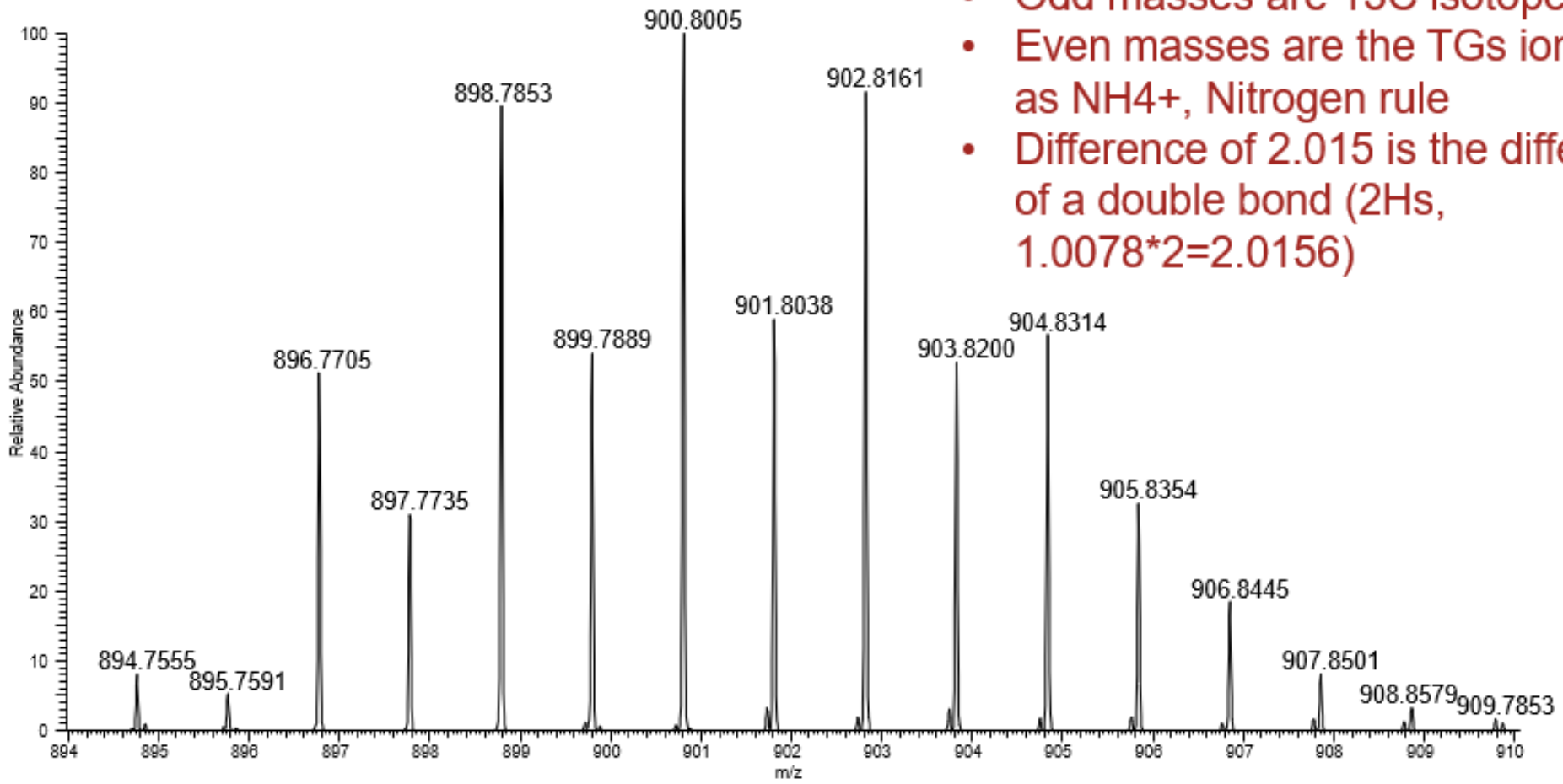
Pressure Difference



Competitor C18
(1.7 µm Particle Size)

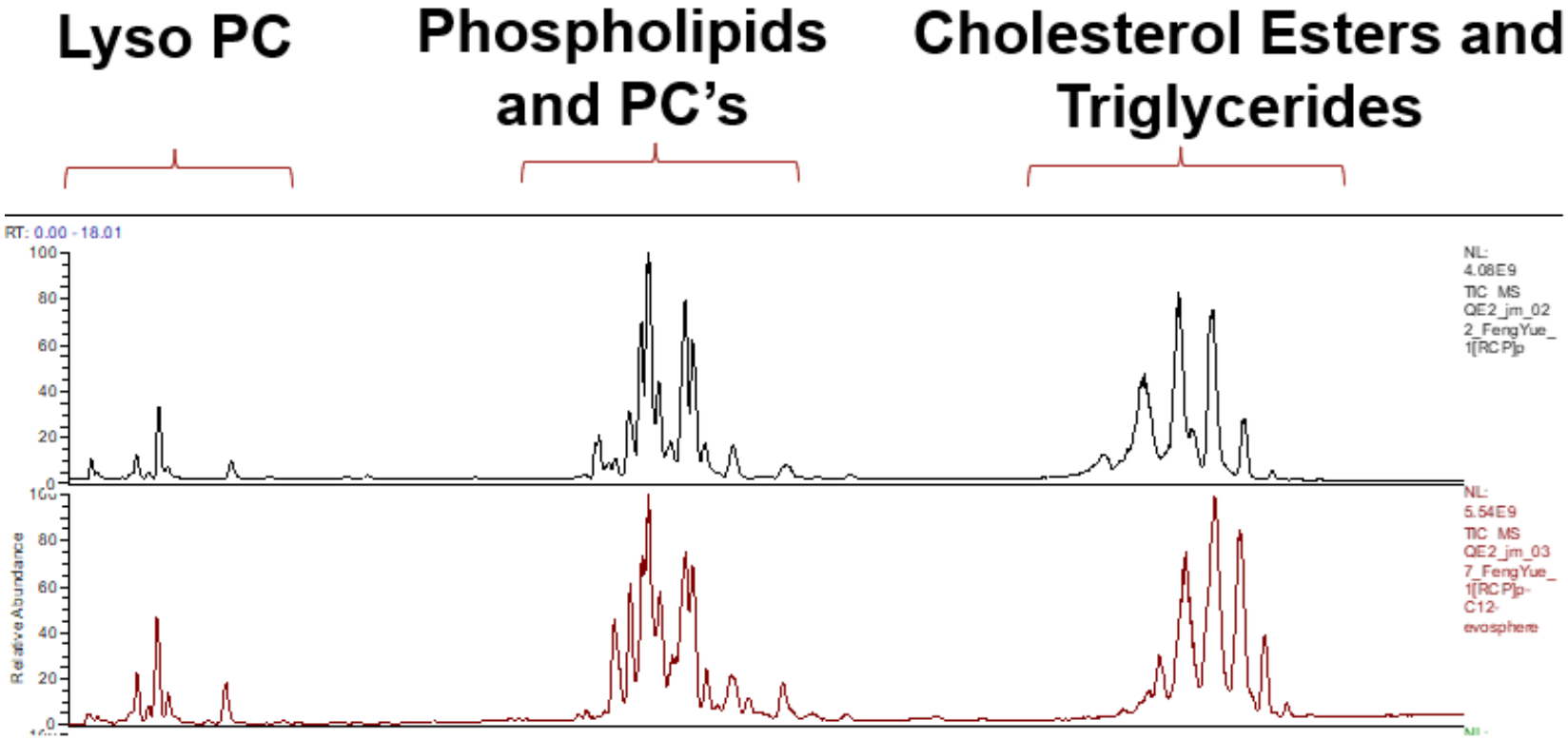
Evosphere C12
(3.0 µm Particle Size)

TG envelope around m/z 900



- Odd masses are 13C isotopes
- Even masses are the TGs ionized as NH4+, Nitrogen rule
- Difference of 2.015 is the difference of a double bond (2Hs, 1.0078*2=2.0156)

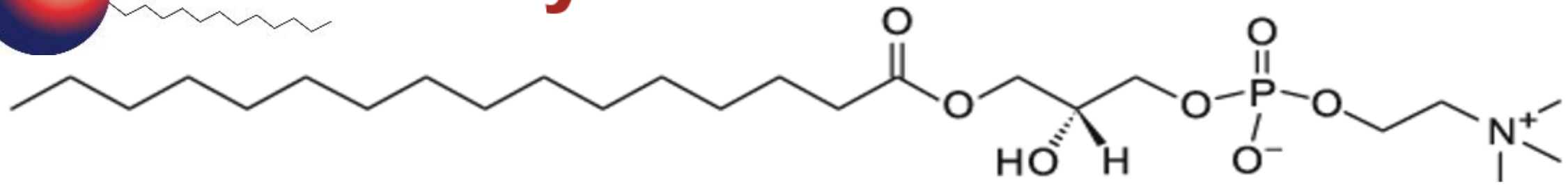
Total Ion Trace

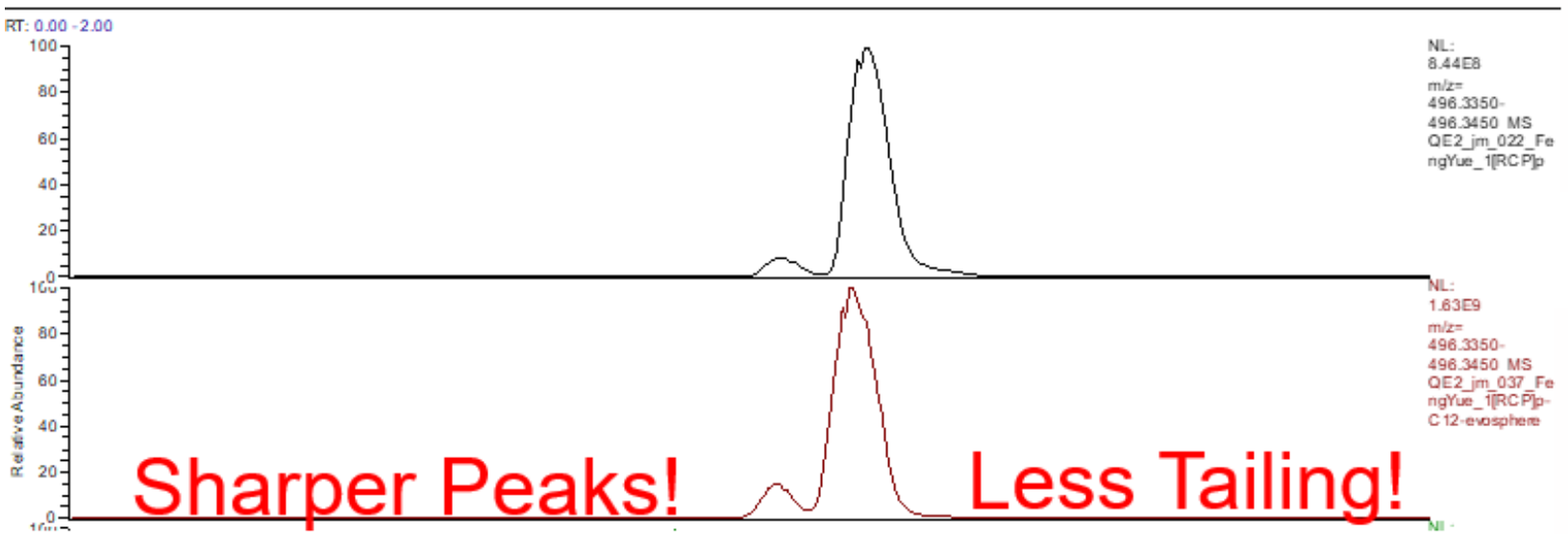


Competitor C18
(1.7 µm Particle Size)

Evosphere C12
(3.0 µm Particle Size)

Lyso PC 16:0

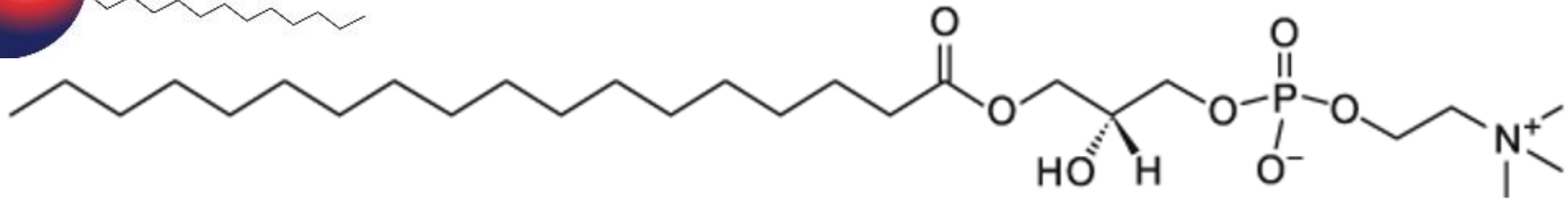


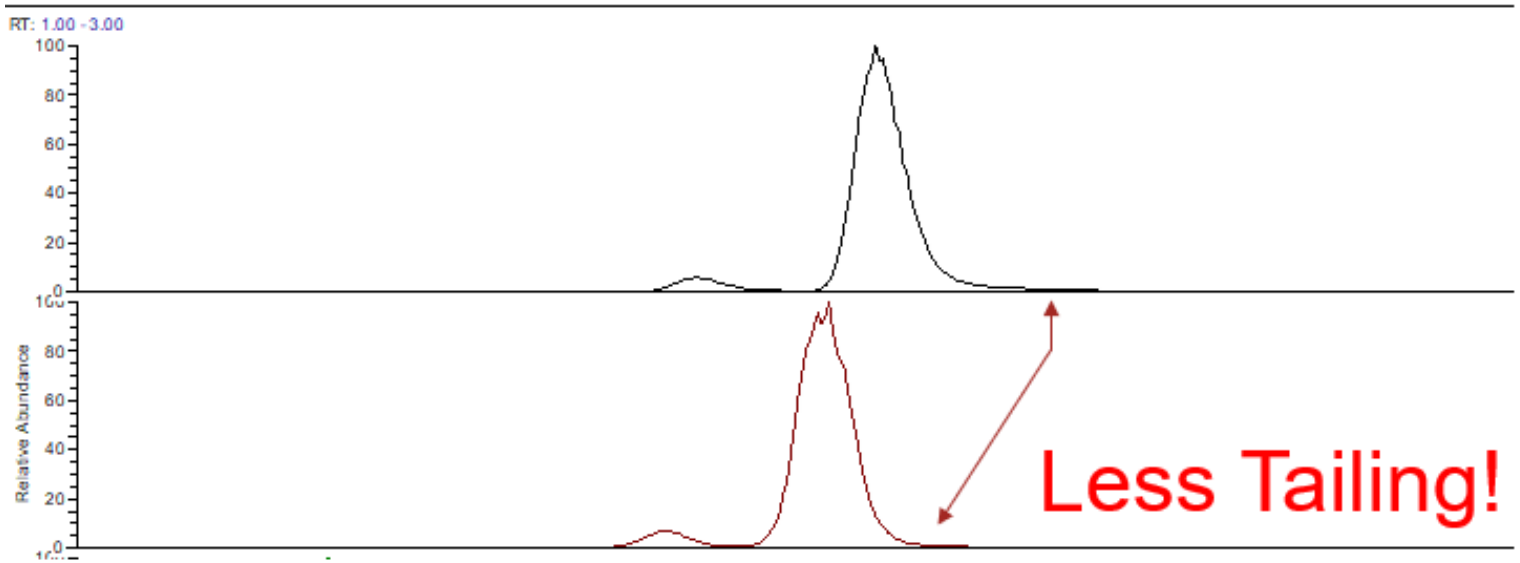


Competitor C18
(1.7 µm Particle Size)

Evosphere C12
(3.0 µm Particle Size)

LysoPC 18:0

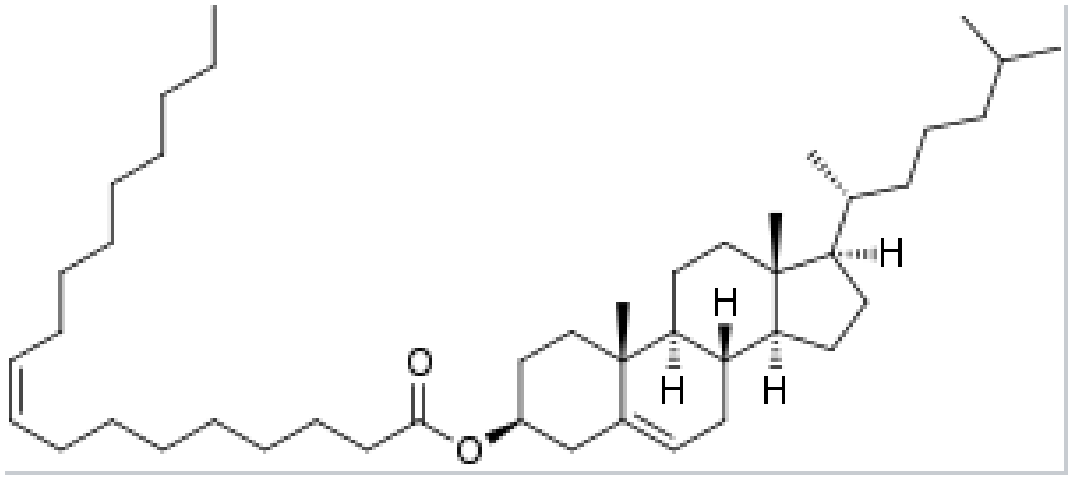


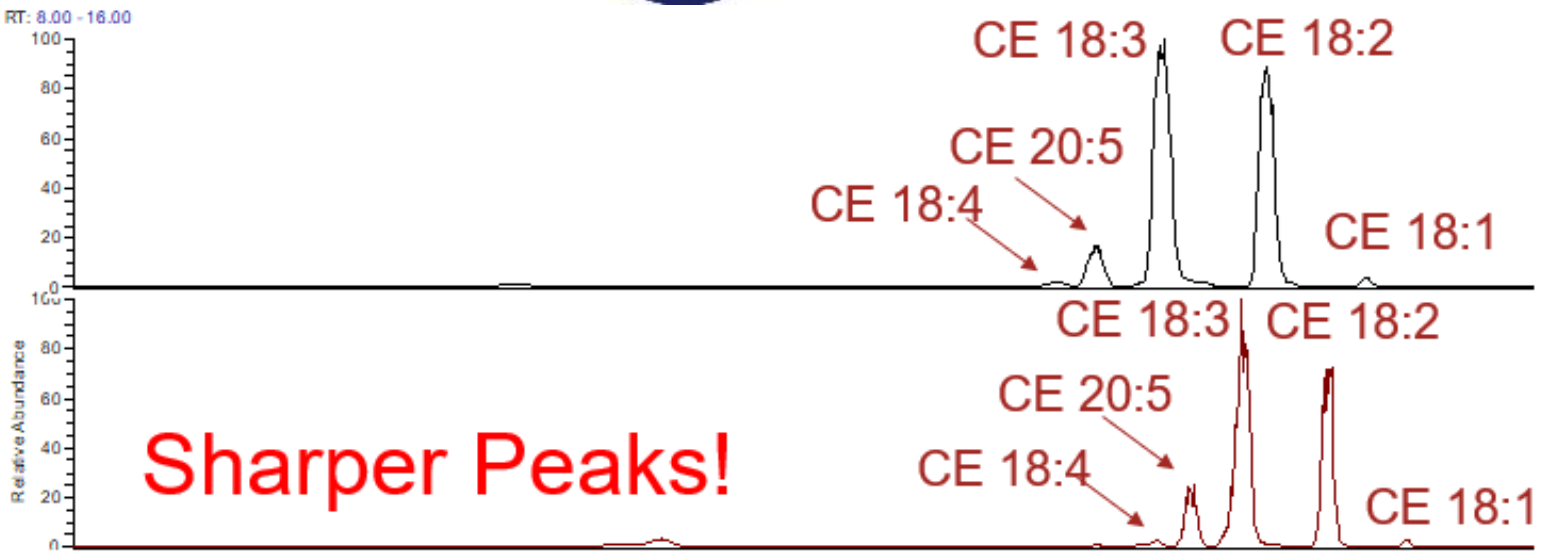


Competitor C18
(1.7 µm Particle Size)

Evosphere C12
(3.0 µm Particle Size)

Cholesterol Esters



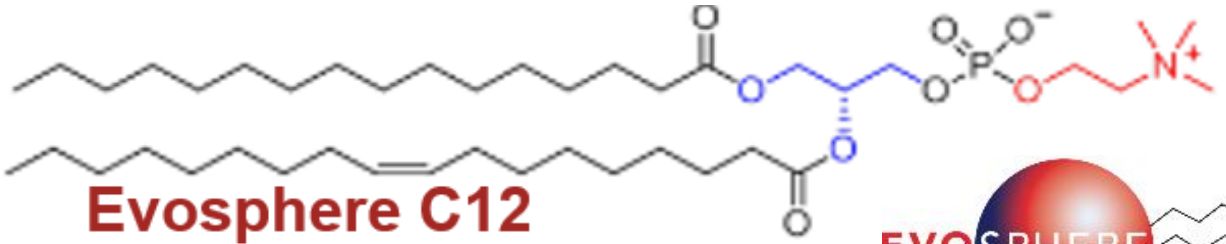


Competitor C18
(1.7 µm Particle Size)

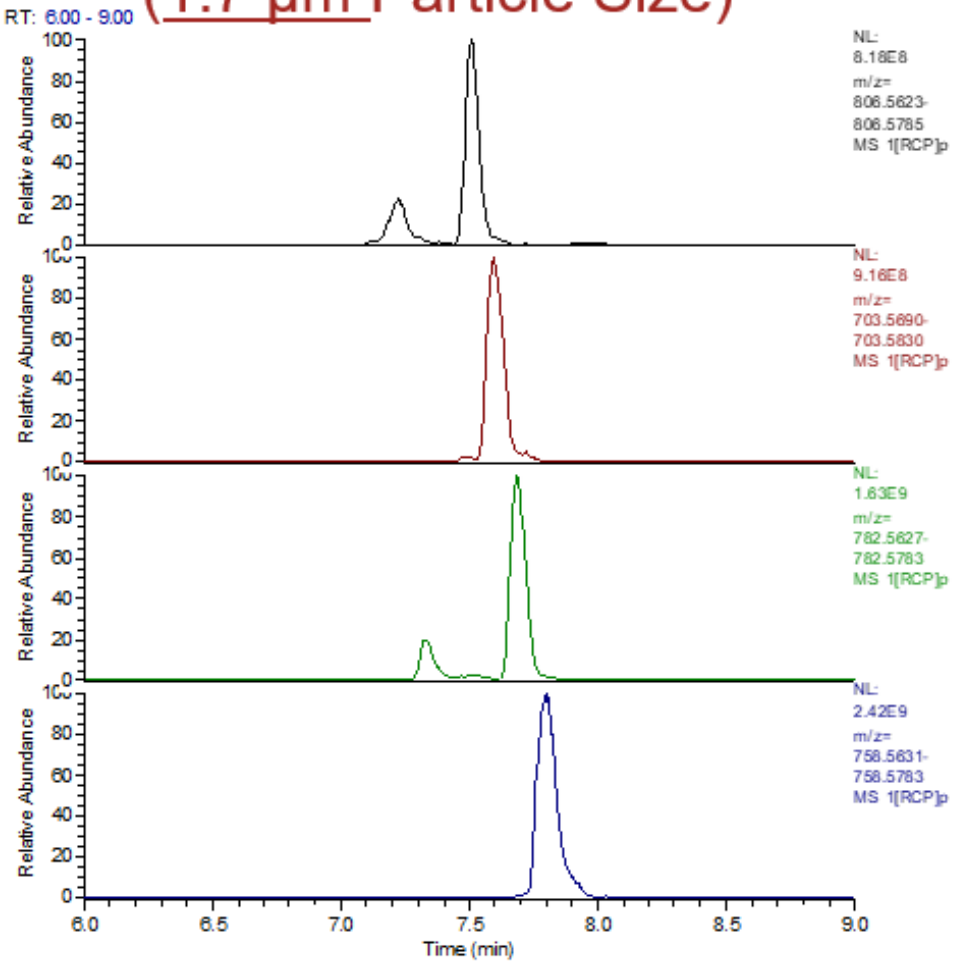
Sharper Peaks!

Evosphere C12
(3.0 µm Particle Size)

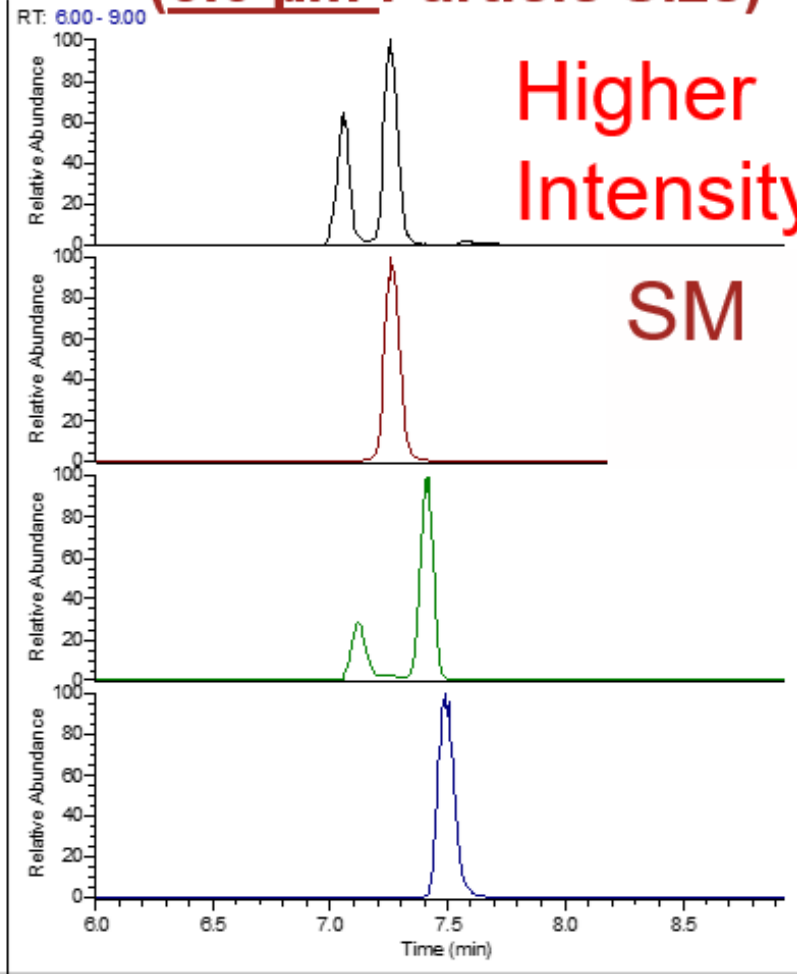
Phospholipids



Competitor C18 (1.7 µm Particle Size)



Evosphere C12 (3.0 µm Particle Size)



Higher Intensity!

PC 38:6

SM (d18:1/16:0)

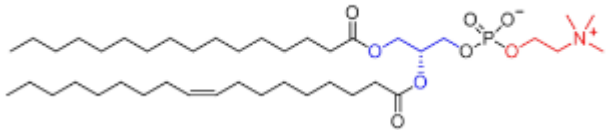
PC 36:4

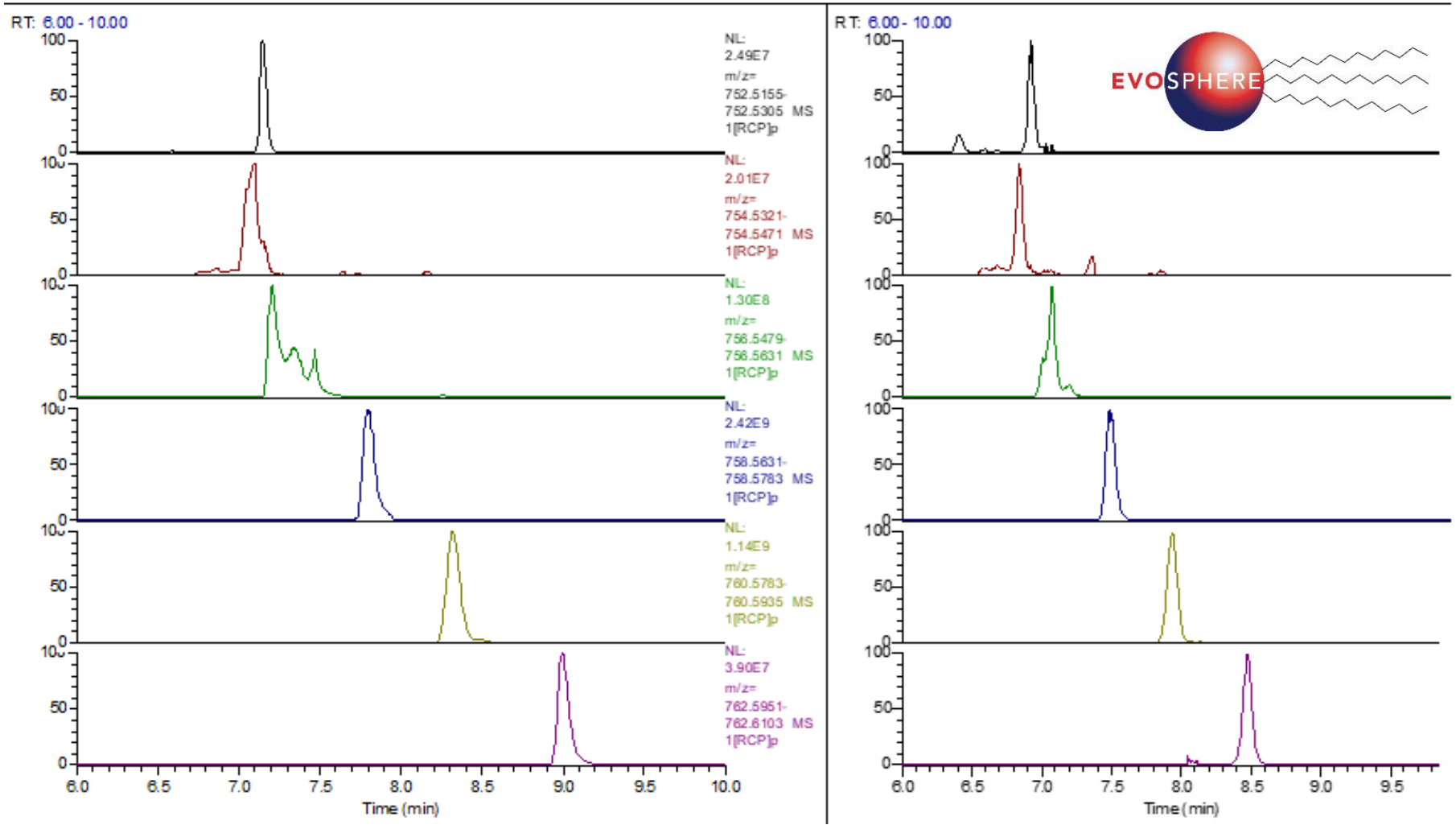
PC 34:2

PCs with different degrees of unsaturation

Competitor C18
(1.7 µm Particle Size)

Evosphere C12
(3.0 µm Particle Size)





PC 34:5 (Mass = 752)

PC 34:4 (Mass = 754)

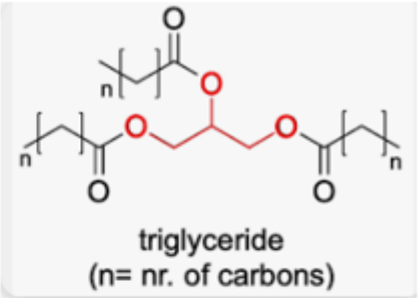
PC 34:3 (Mass = 756)

PC 34:2 (Mass = 758)

PC 34:1 (Mass = 760)

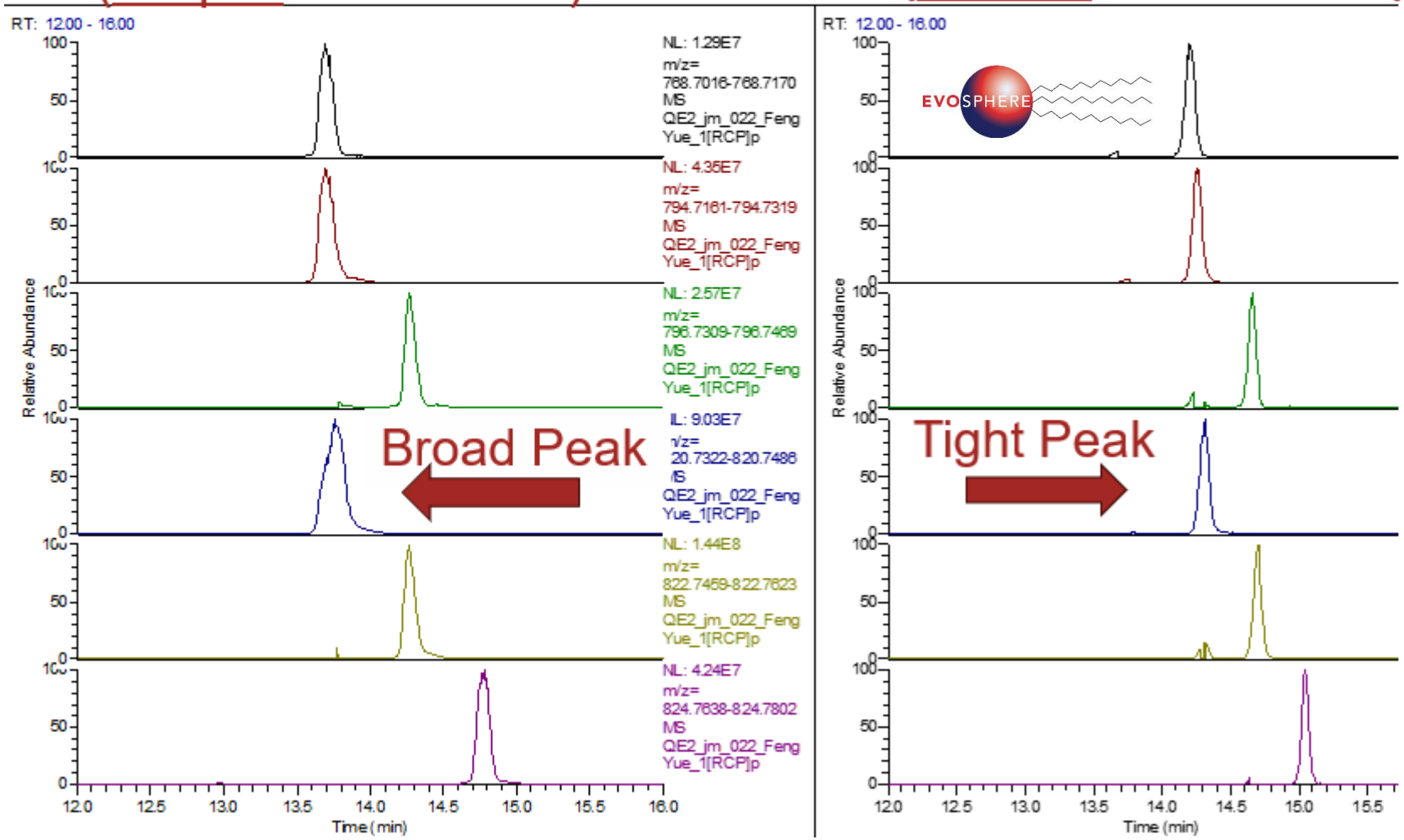
PC 34:0 (Mass = 762)

Triglycerides 44-48 total carbons



Competitor C18 (1.7 µm Particle Size)

Evosphere C12 (3.0 µm Particle Size)



TG 44:0 (Mass = 768)

TG 46:1 (Mass = 794)

TG 46:0 (Mass = 796)

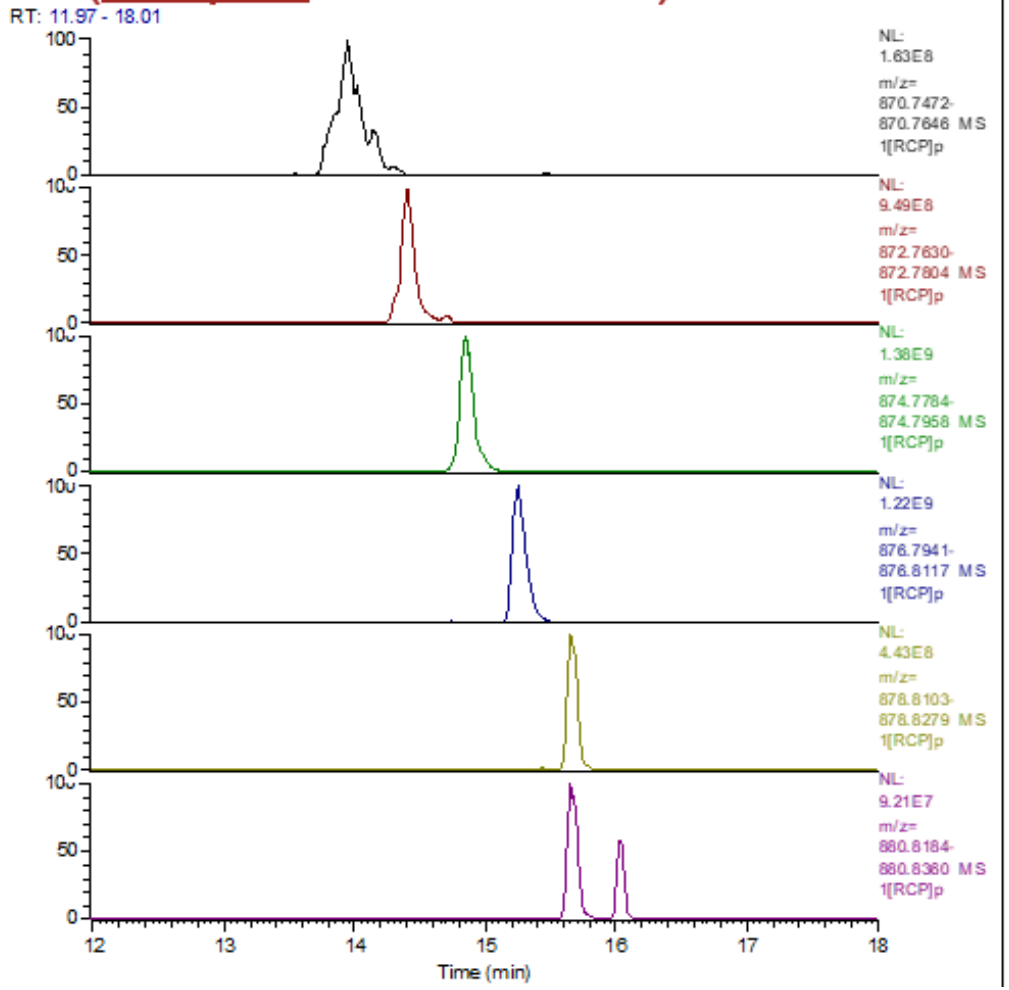
TG 48:2 (Mass = 820)

TG 48:1 (Mass = 822)

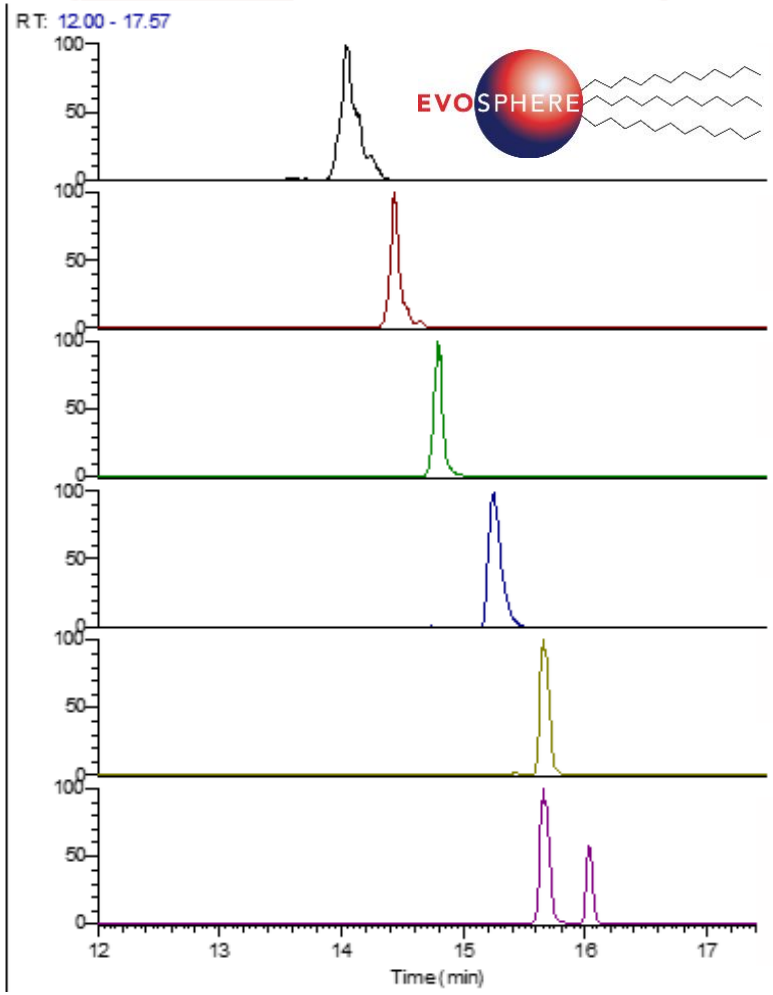
TG 48:0 (Mass = 824)

Triglycerides with 52 total carbons

Competitor C18 (1.7 µm Particle Size)



Evosphere C12 (3.0 µm Particle Size)



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

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Thank You For Your Time