New LC Particle Technology Monodisperse Fully Porous Particles

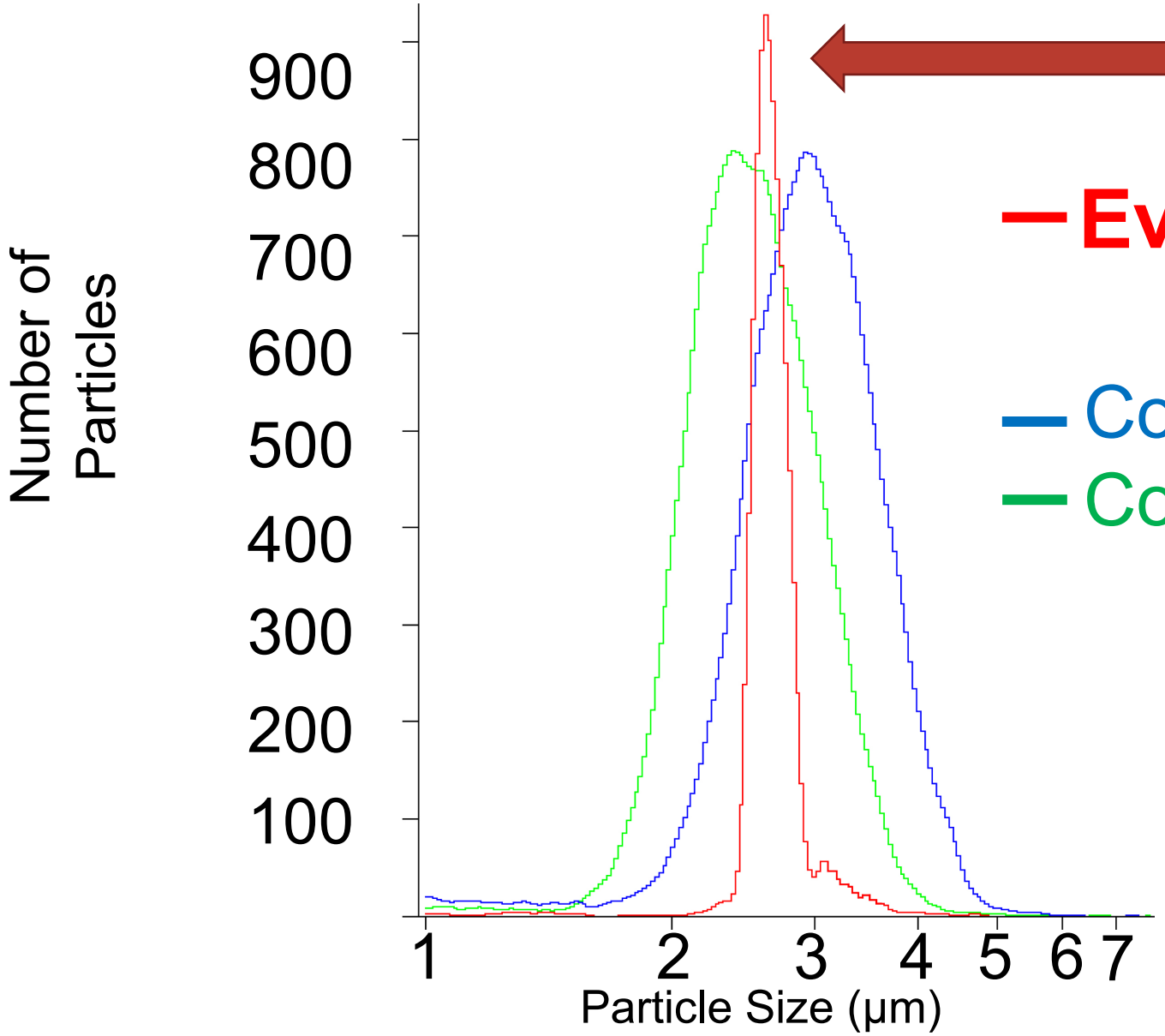
Presented by Geoff Faden – President

Evolution of U/HPLC Particles

- Morphology - Shape
- Size - Reduction
- Purity – Less Metals
- Size Distribution – Reduction in D90/D10

Ref: Historical Developments in HPLC and UHPLC Column Technology: The Past 25 Years, Dr. Ronald Majors

Particle Size Distribution Comparison



— Evosphere MFPP 3 µm

— Commercial FPP A 3 µm

— Commercial FPP B 3 µm

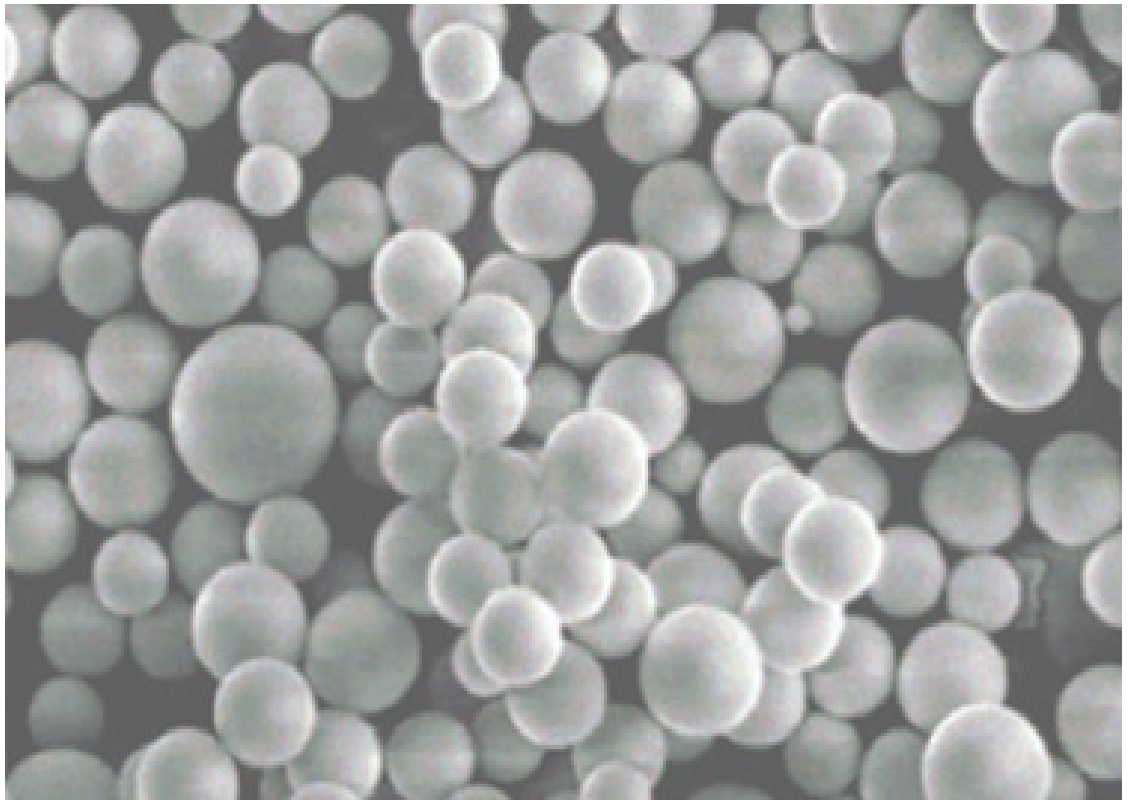
Particle Size Distribution Comparisons

	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

SEM Images of Particles Technologies



Polydisperse

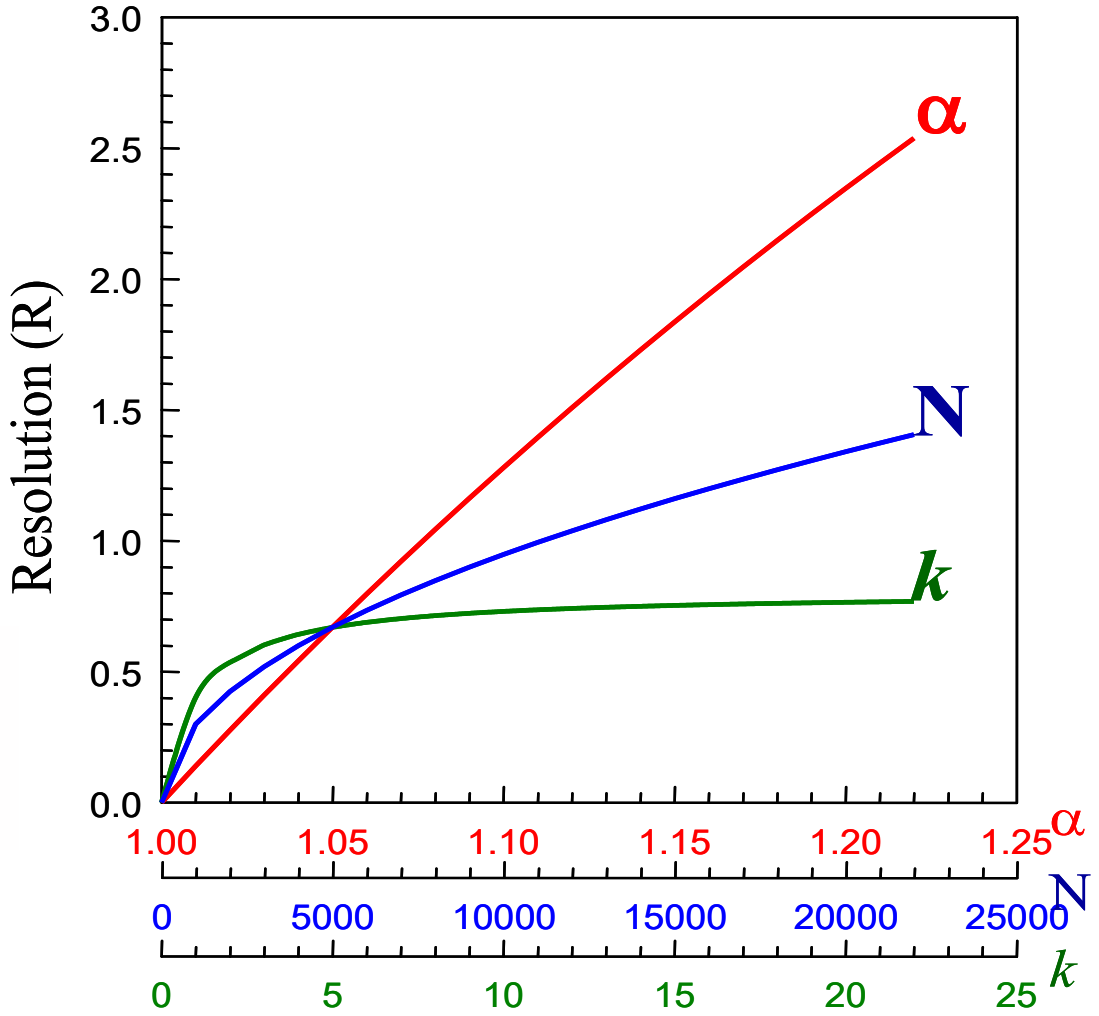
Monodisperse

Resolution Equation

Efficiency Retention Selectivity

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

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



Simplified Van Deemter Equation

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

H: Height Equivalent to a Theoretical Plate

A Term: Eddy Diffusion (Multipath Effect)

B Term: Longitudinal Diffusion (Molecular Diffusion)

C Term: Resistance to Mass Transfer (Mobile Phase to Stationary Phase Transition)

Expanded Van Deemter Equation

$$H = 2\lambda d_p + \frac{2\gamma D_m}{u} + \left(\frac{\omega d_p^2 u}{D_m} + \frac{R d_f^2 u}{D_s} \right)$$

- H = Plate Height
- λ is packing factor
- d_p is particle diameter
- γ, ω, and R are constants
- d_f is the film thickness (approaches 0 for LC)

- D_m is the diffusion coefficient of the mobile phase
- d_c is the capillary diameter
- D_s is the diffusion coefficient of the stationary phase.
- u is the linear velocity

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- D_s is the diffusion coefficient of the stationary phase.
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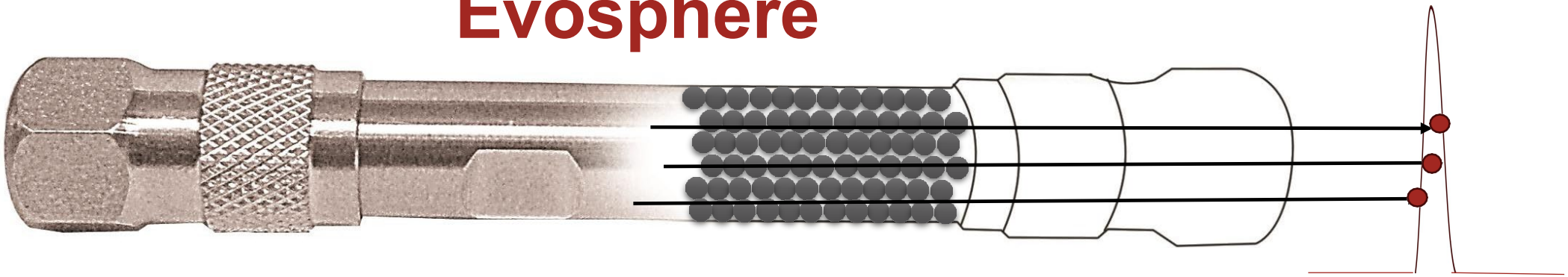
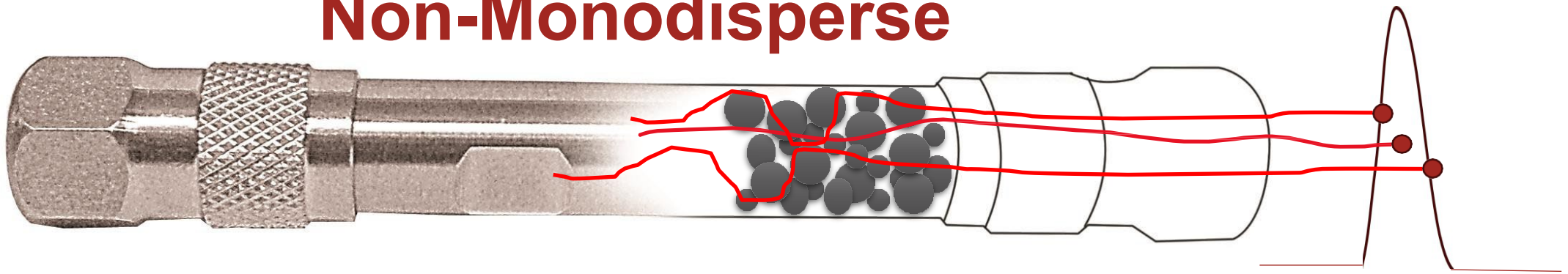
Visual Representation of Eddy Diffusion (“A Term”)

Evosphere

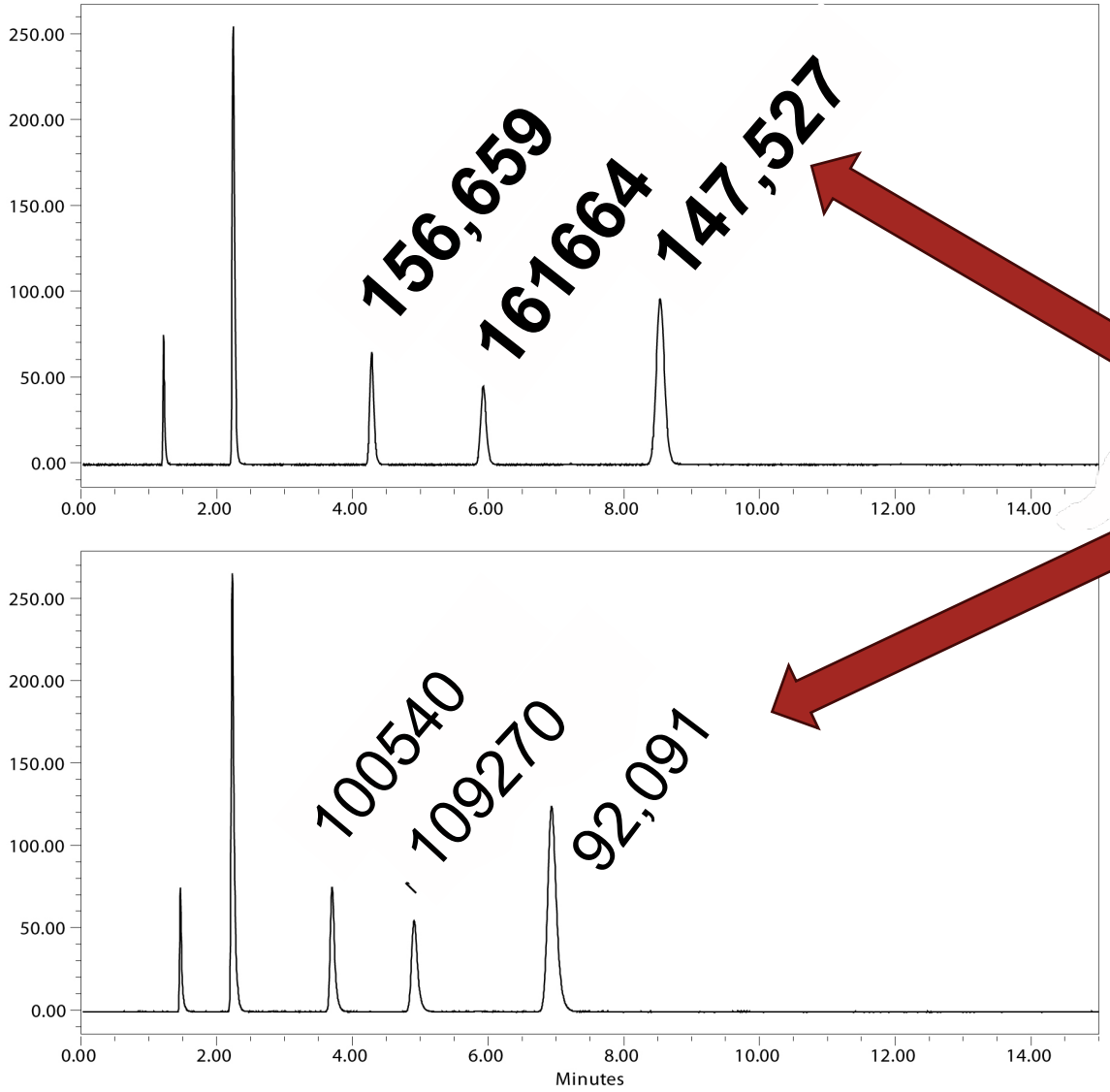


Non-Monodisperse



Flow through the column Evosphere vs. FPP

What does this look like chromatographically?



Evosphere C12

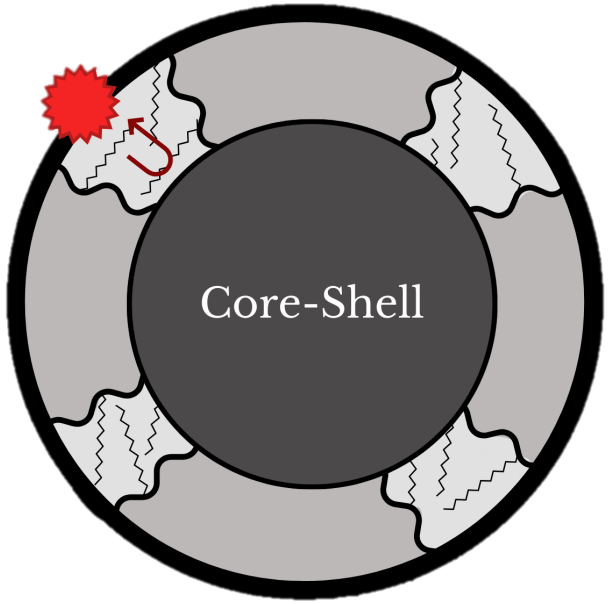
3µm, 4.6mm x 150 mm

60% Higher N

Popular Fully Porous C18
3µm, 4.6 x 150 mm

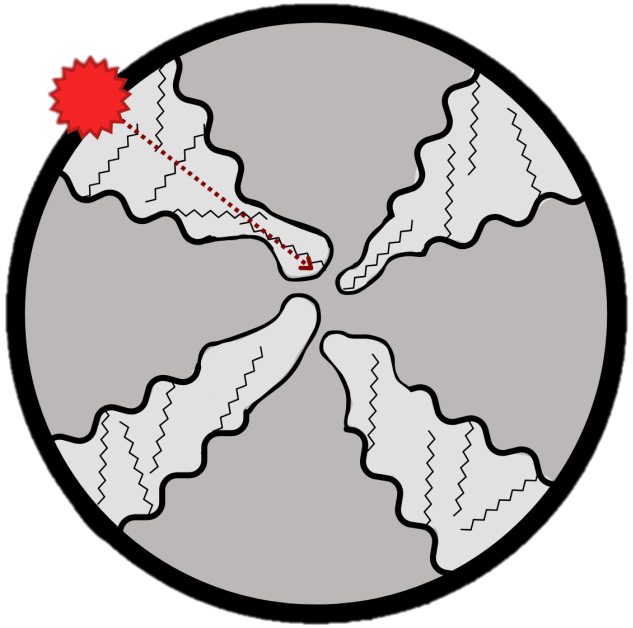
Core-Shell compared to Evosphere MFPP

Core-Shell



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

Evosphere

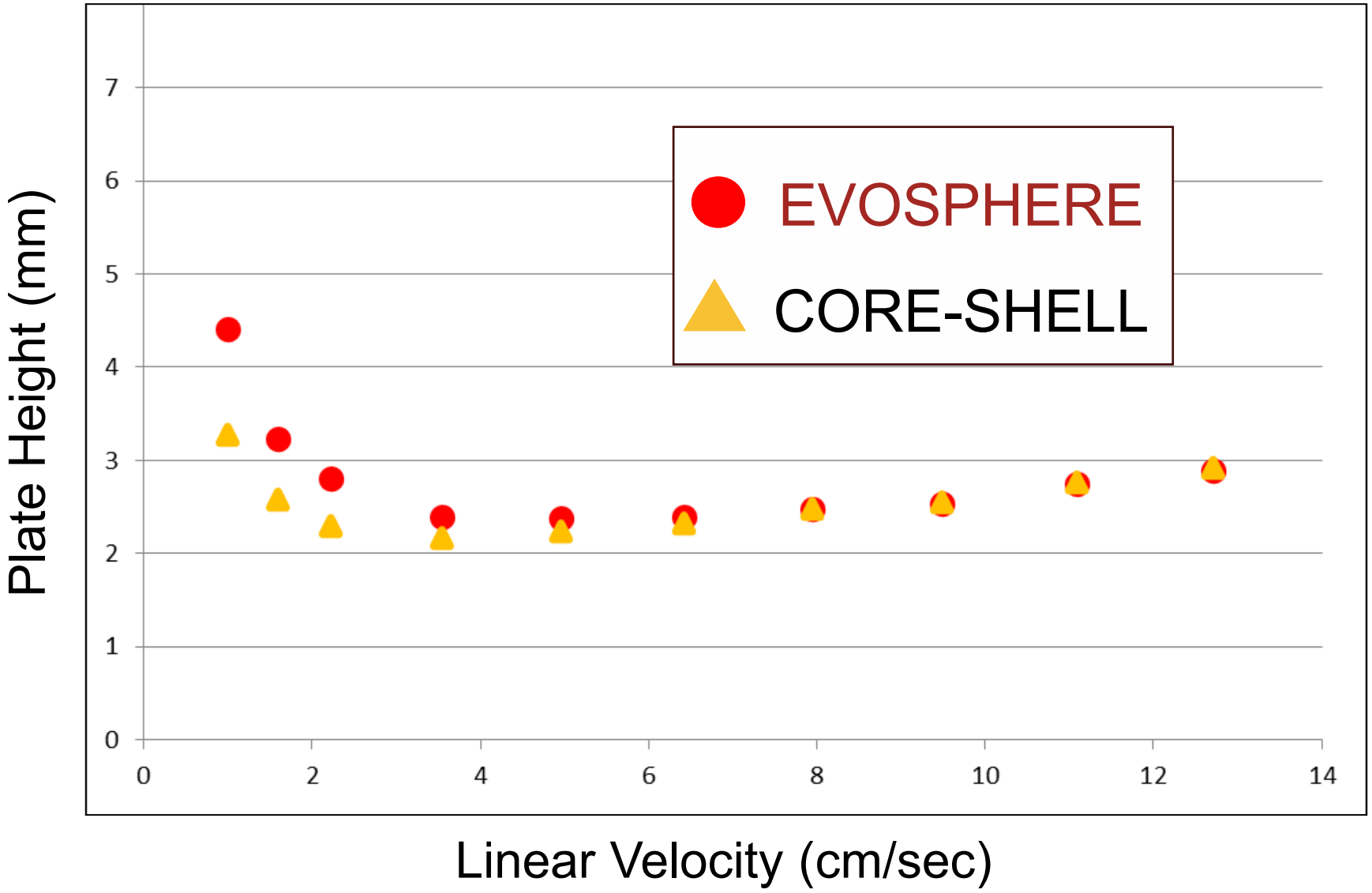


SA = ~130 m^2/g

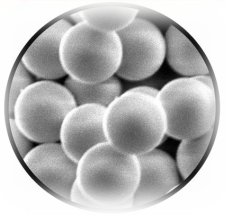
~3X Surface Area

SA = 350 m^2/g

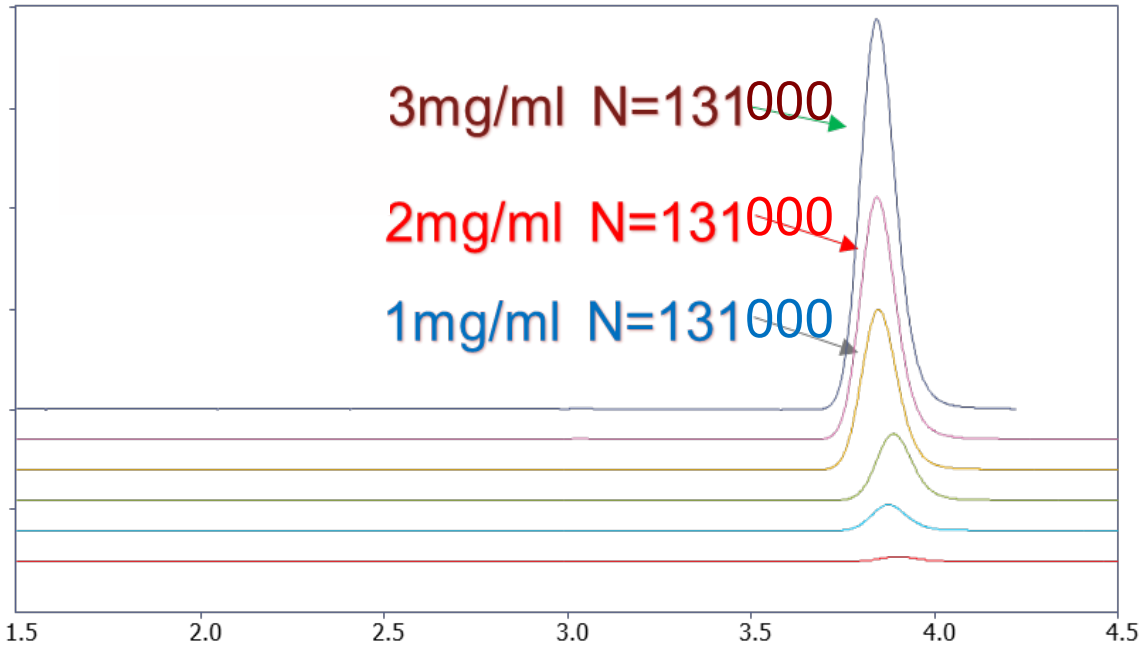
Van Deemter flattens at Elevated Linear Velocities



Fortis® Evosphere®
Improves Loading and Increases Retention

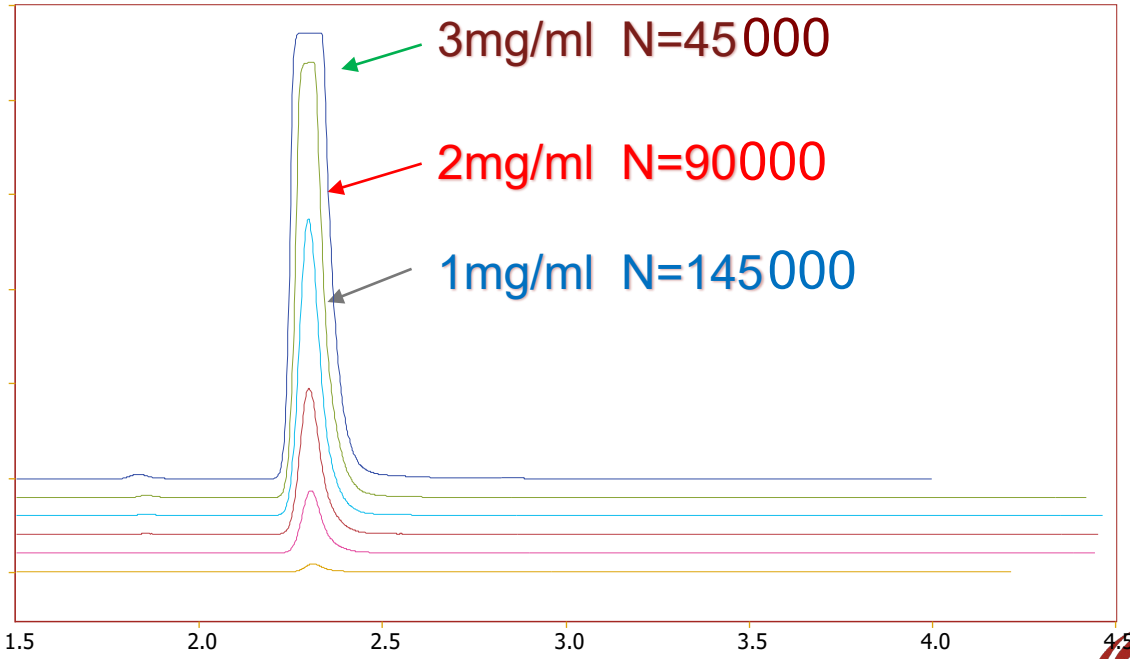


Evosphere L1 Surface Area = 350 m²/g



Rt = 3.8 min

Core Shell L1 Surface Area = 130 m²/g



Rt = 2.25 min

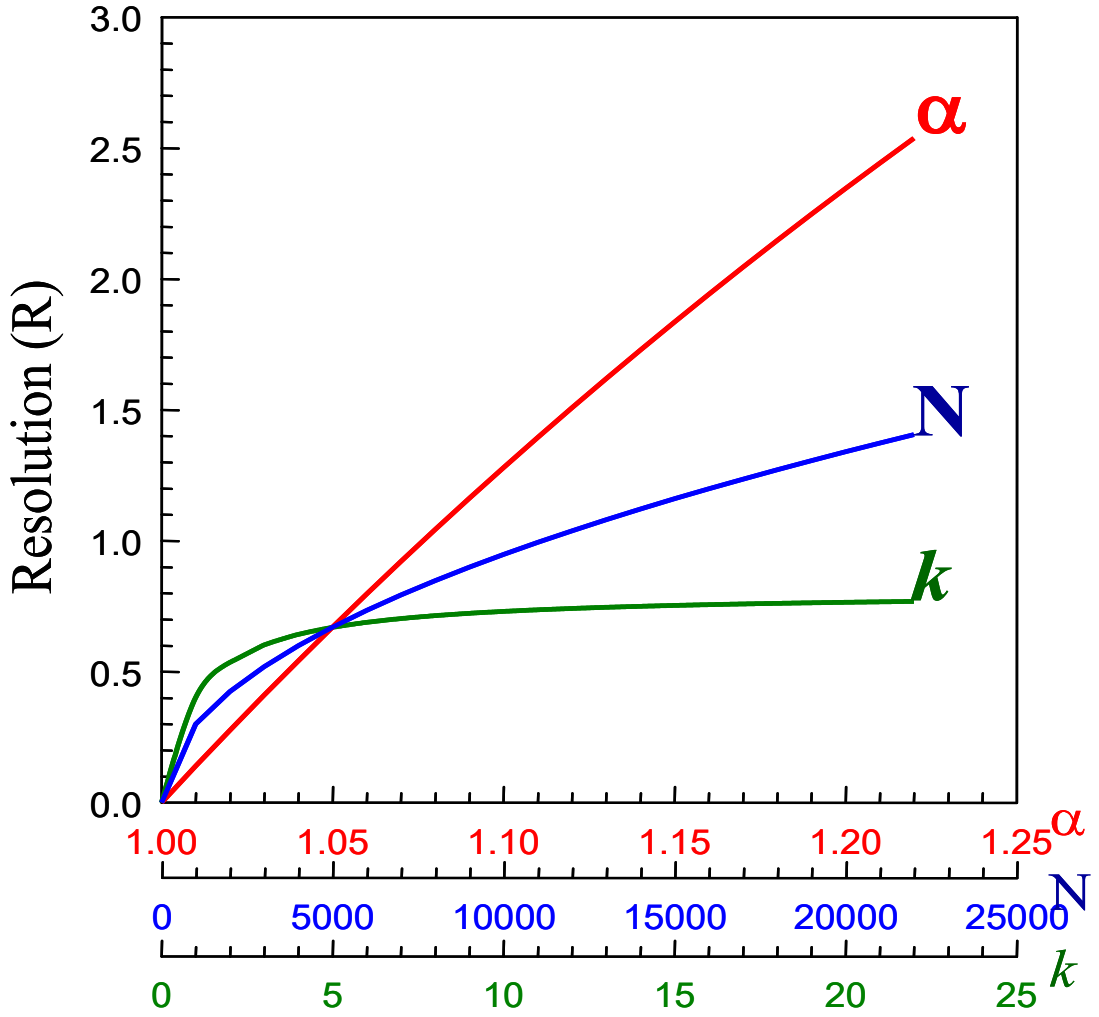
Resolution Equation

Efficiency Retention Selectivity

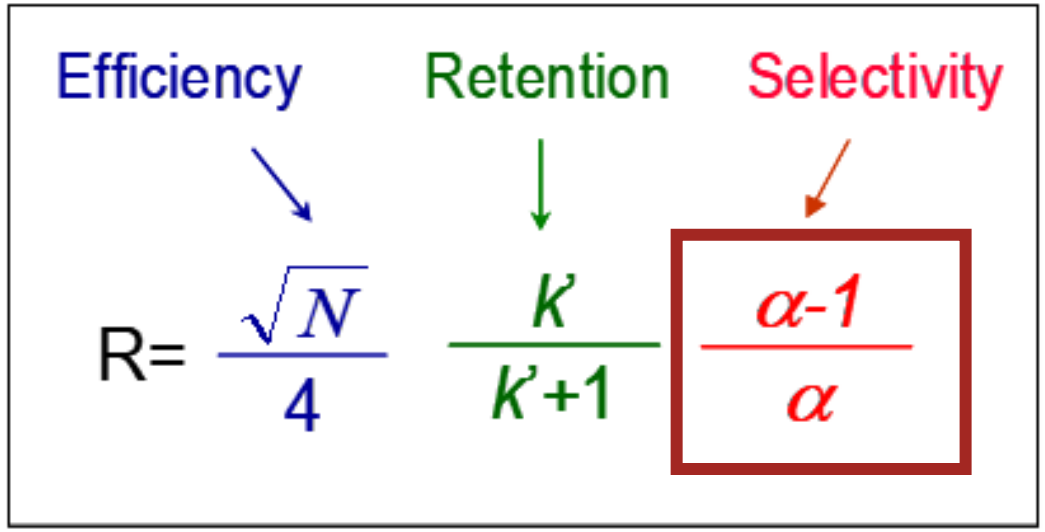
$$R = \frac{\sqrt{N}}{4} \quad \frac{k'}{k'+1} \quad \frac{\alpha-1}{\alpha}$$

$$\alpha = \frac{k_2}{k_1}$$

- Selectivity (α) has the greatest impact on improving resolution.



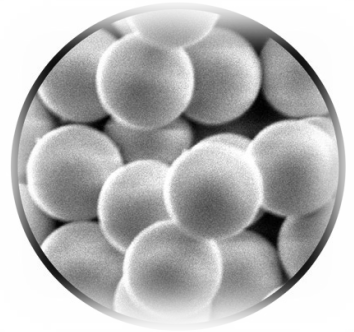
How does Evosphere Impact Selectivity?



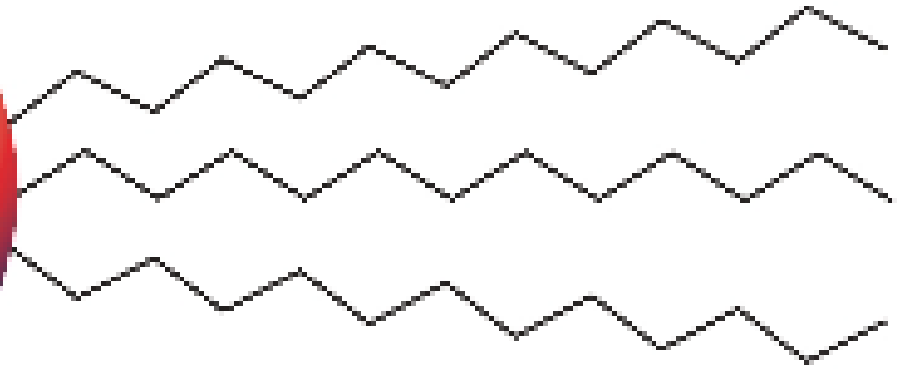
- **C12**
- **Diphenyl**
- **C18/PFP**
- **C18/AR**

- AQUA
- RP18-Amide
- Phenyl-Hexyl
- PFP

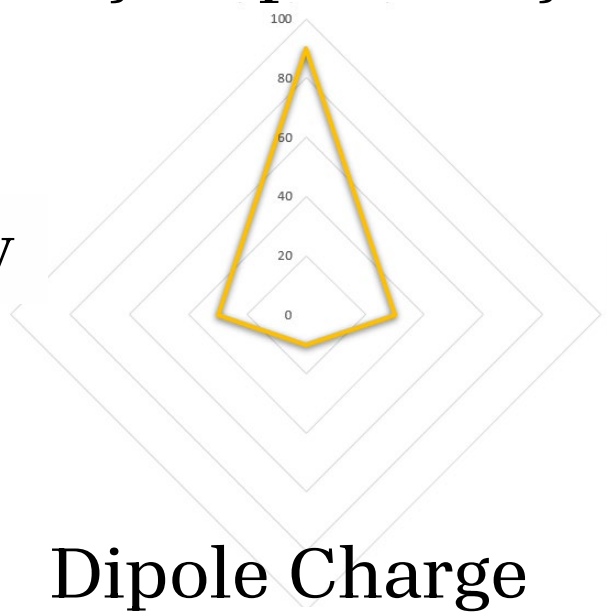
Evosphere C12



EVOSPHERE



Hydrophobicity

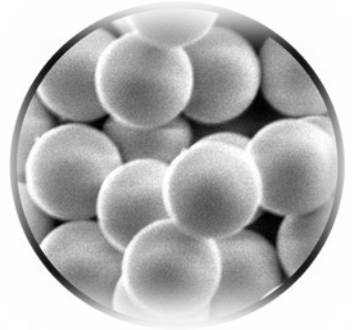


Steric Selectivity

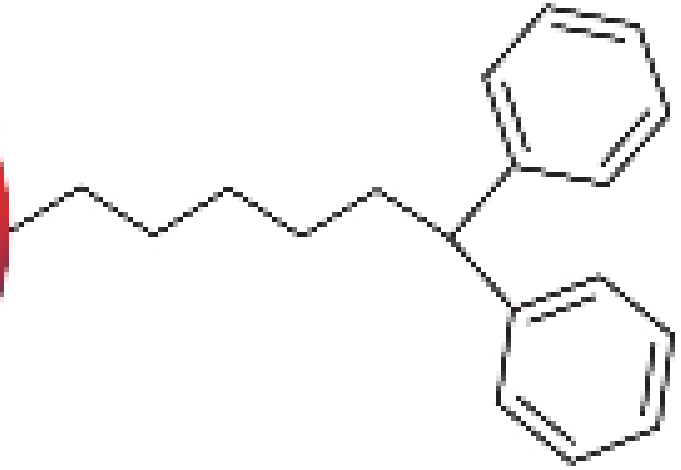
Polarity

Dipole Charge

Evosphere Diphenyl

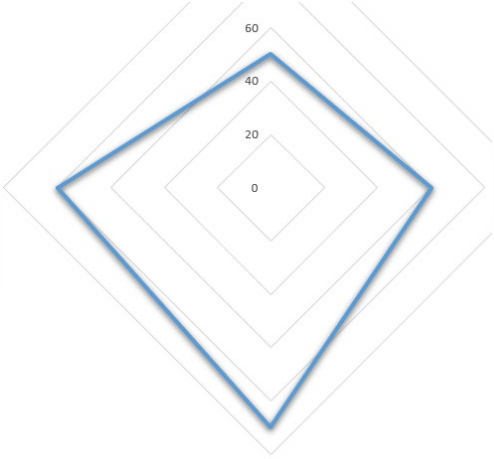


EVOSPHERE



Hydrophobicity

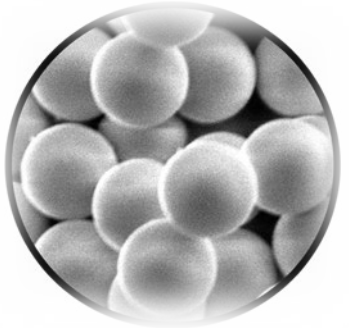
Steric Selectivity

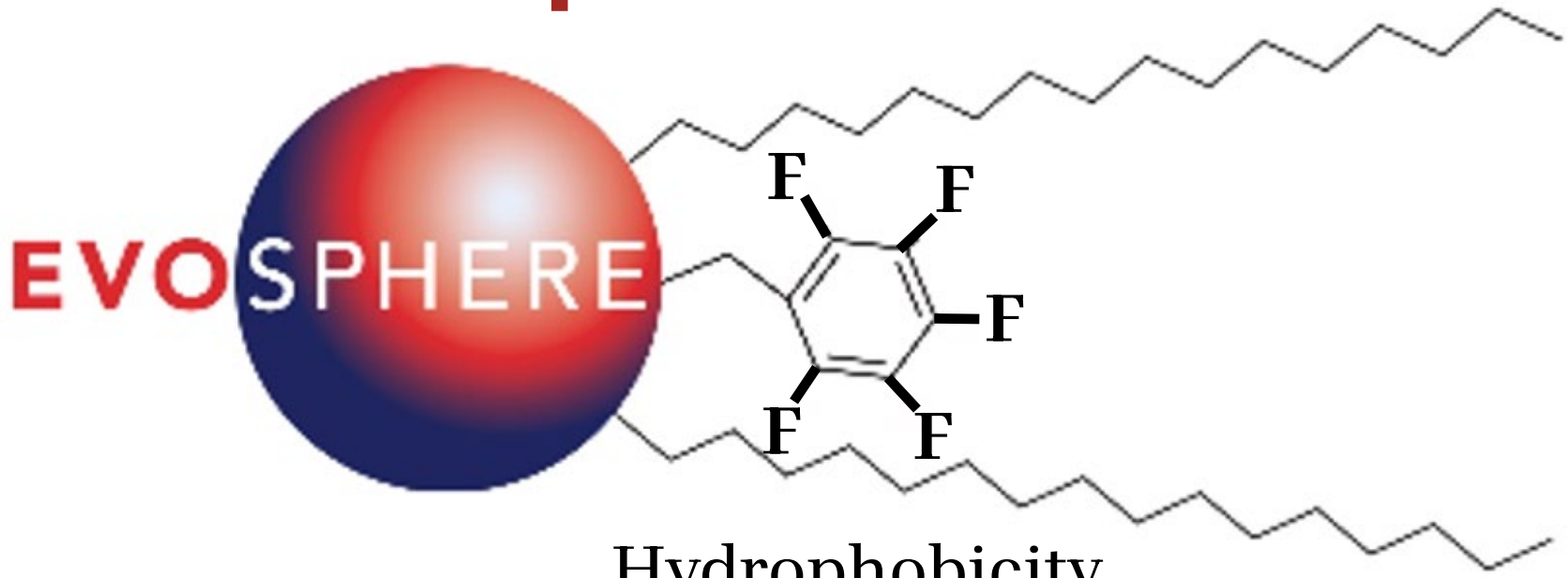


Polarity

Dipole Charge

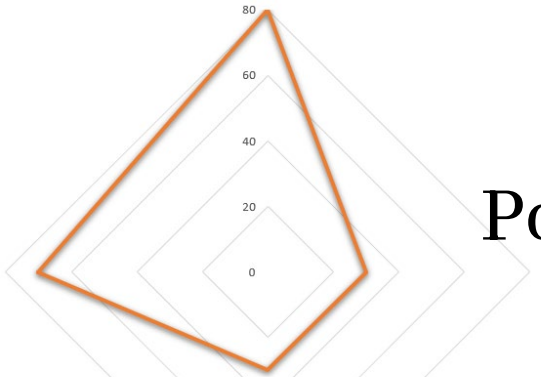
Evosphere C18/PFP





Hydrophobicity

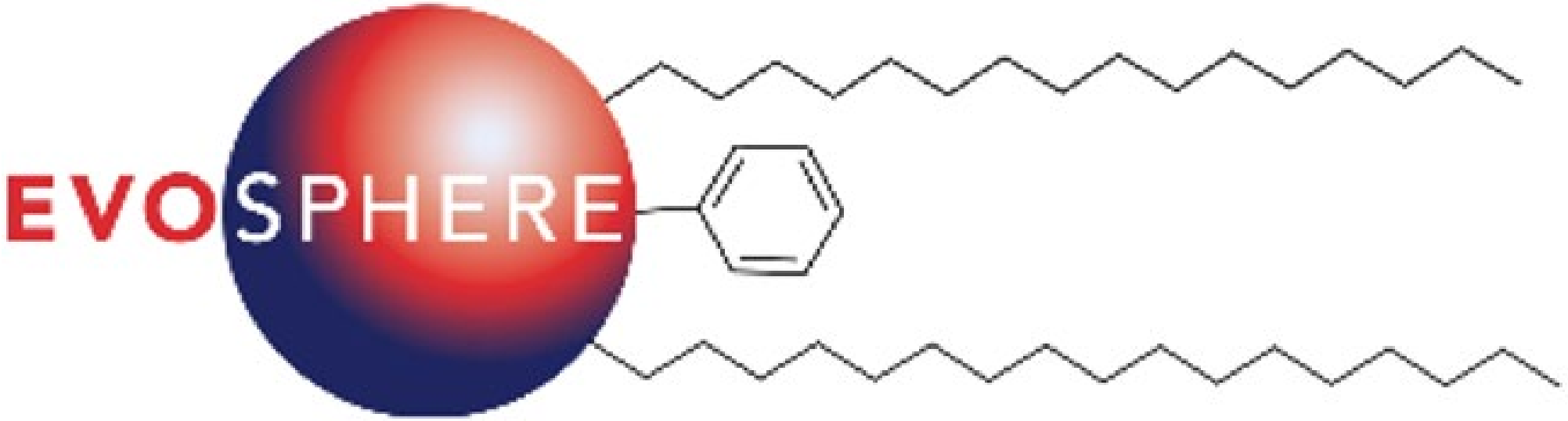
Steric Selectivity



Polarity

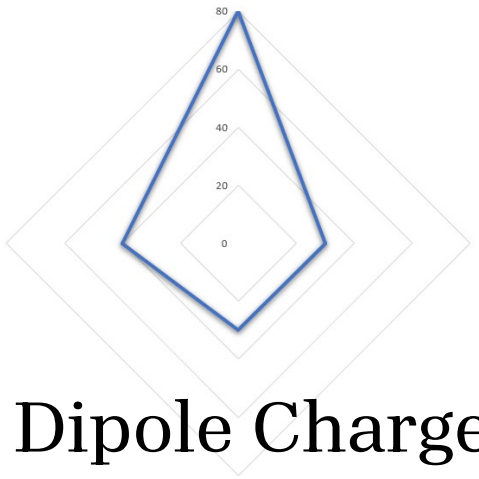
Dipole Charge

Evosphere C18/AR



Hydrophobicity

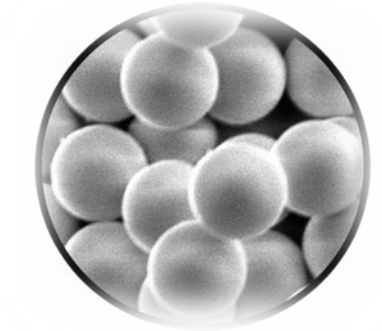
Steric Selectivity



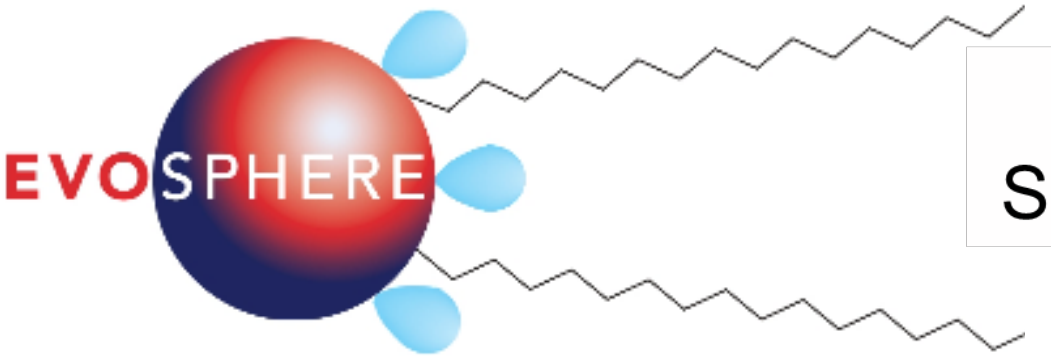
Polarity

Dipole Charge

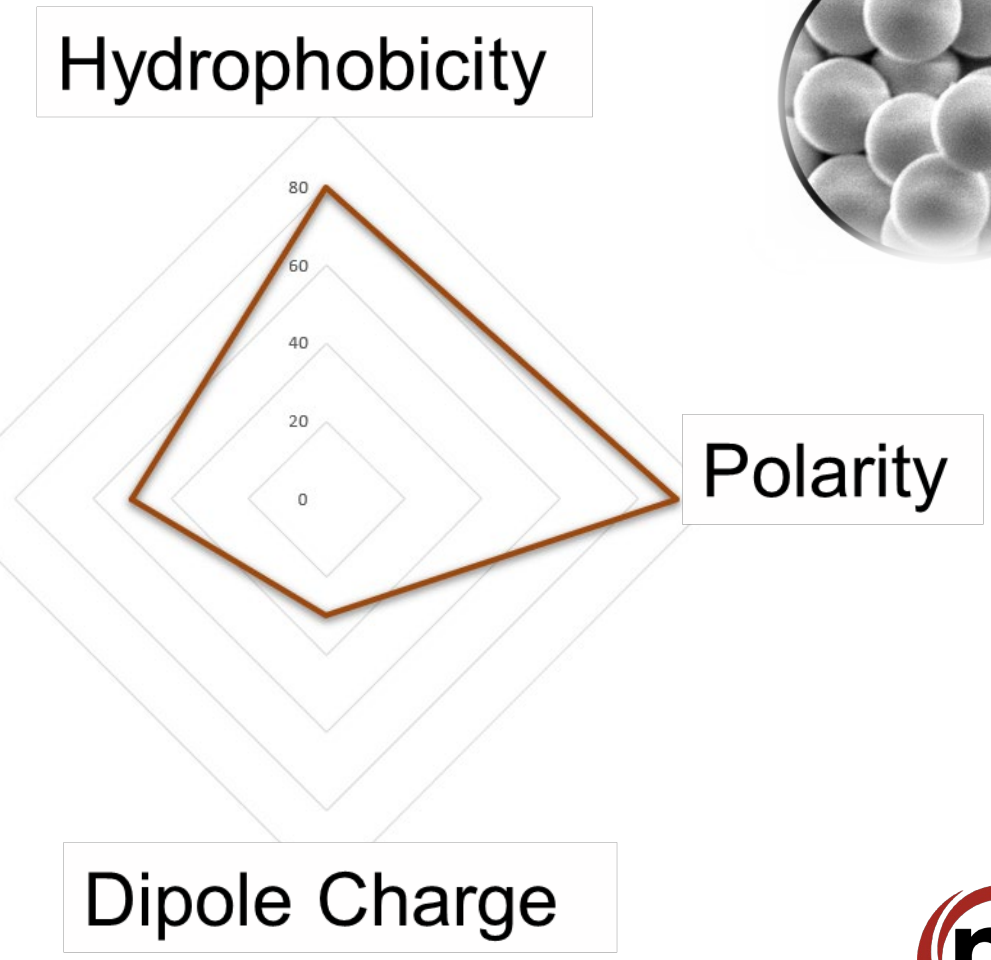
Evosphere AQUA



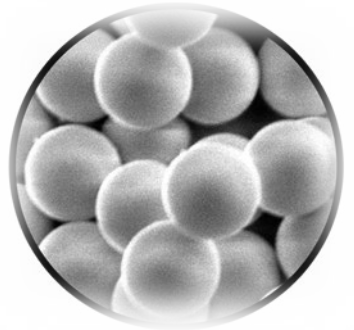
100% Aqueous Compatible!

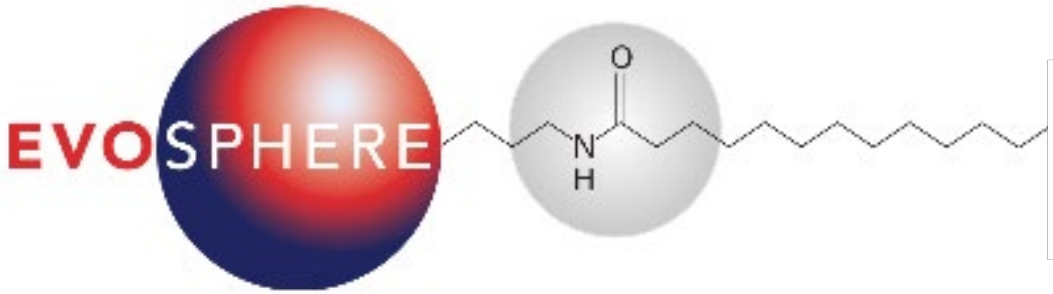


Steric Selectivity

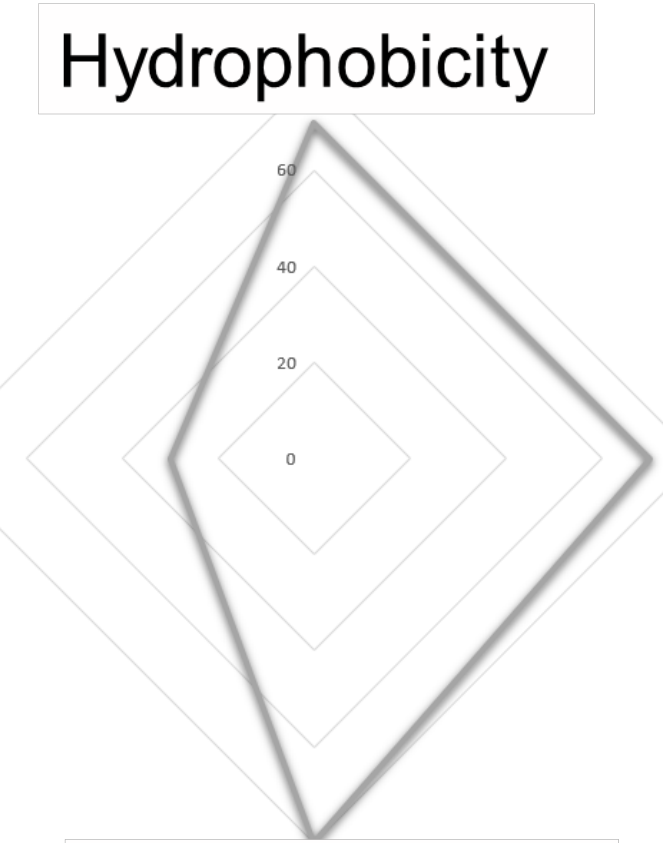


Evosphere RP18-Amide

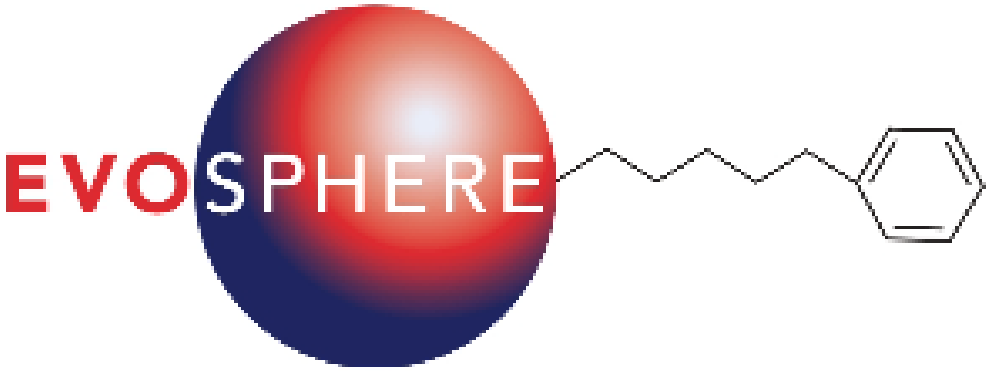




Steric Selectivity

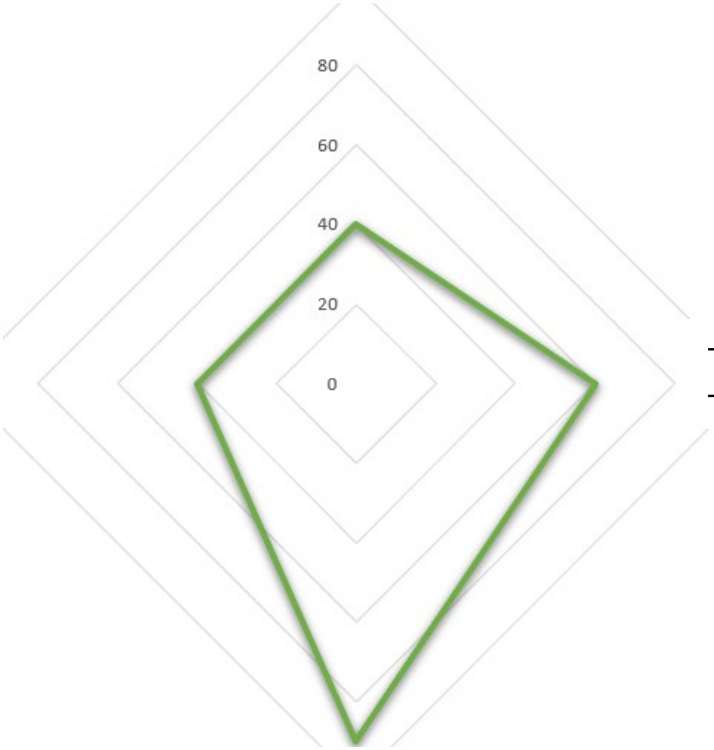


Evosphere Phenyl-Hexyl



Steric Selectivity

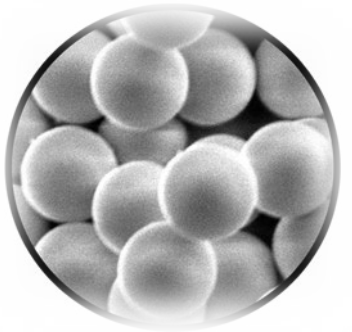
Hydrophobicity



Polarity

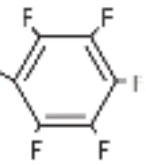
Dipole Charge

Evosphere PFP

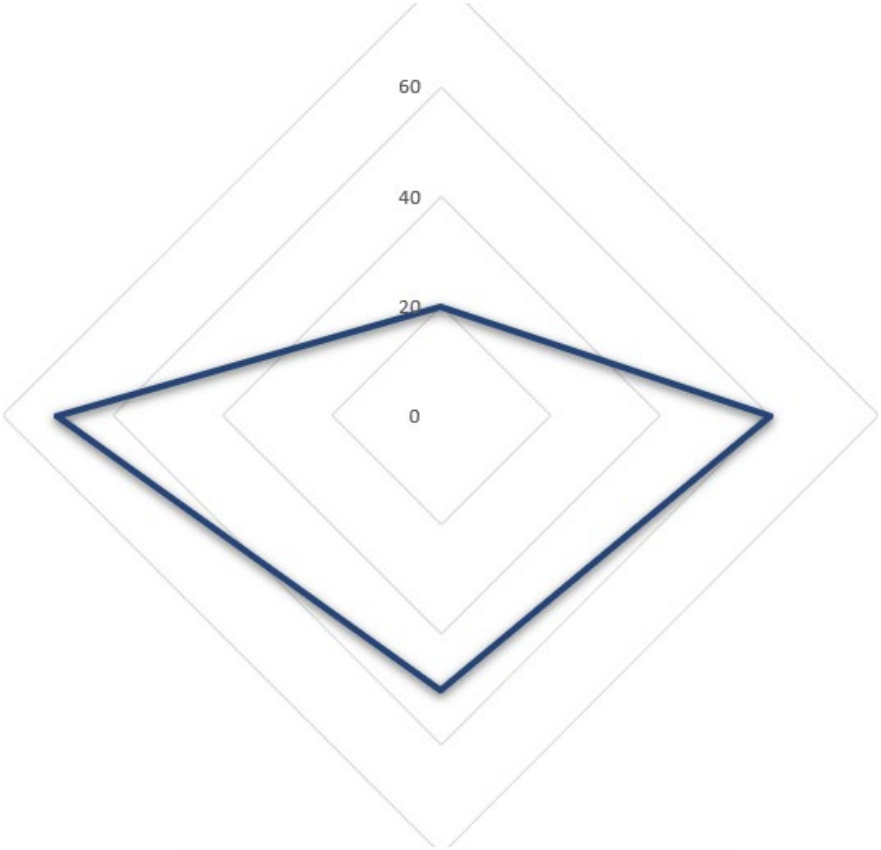


Hydrophobicity

EVOSPHERE



Steric
Selectivity

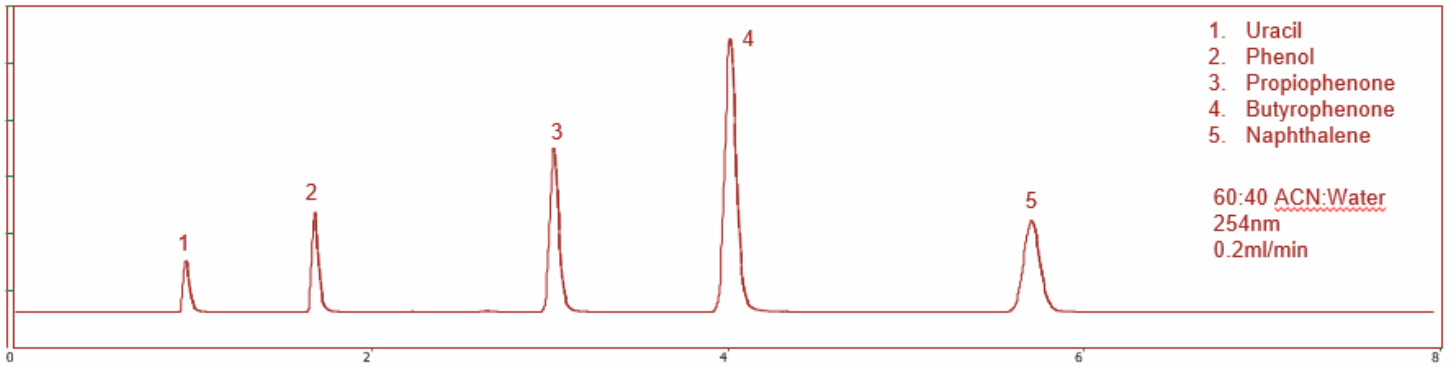


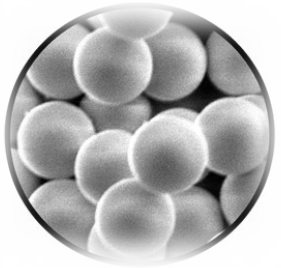
Polarity

Dipole Charge

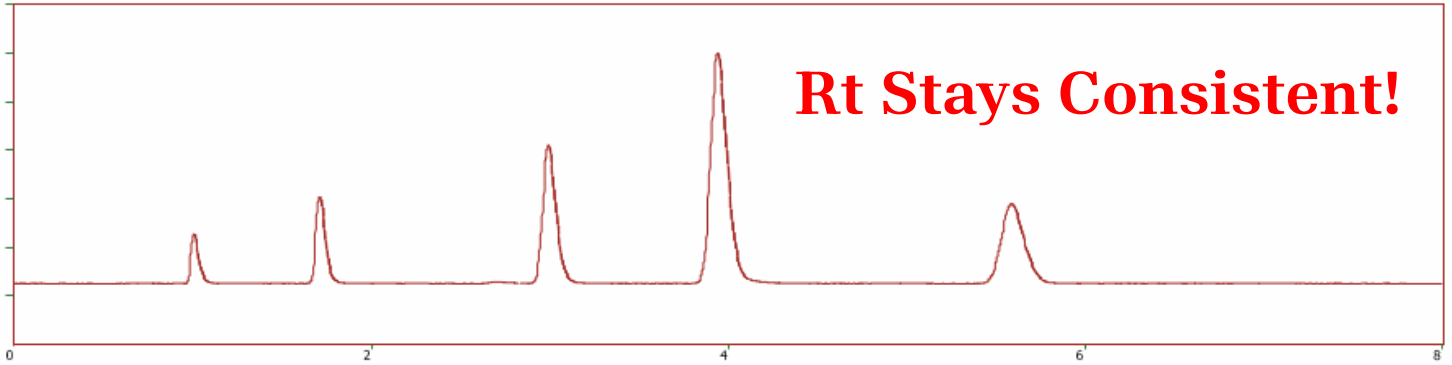
Translate the methods seamlessly for QC

C18/AR
1.7 µm
(2.1 mm x 100 mm)

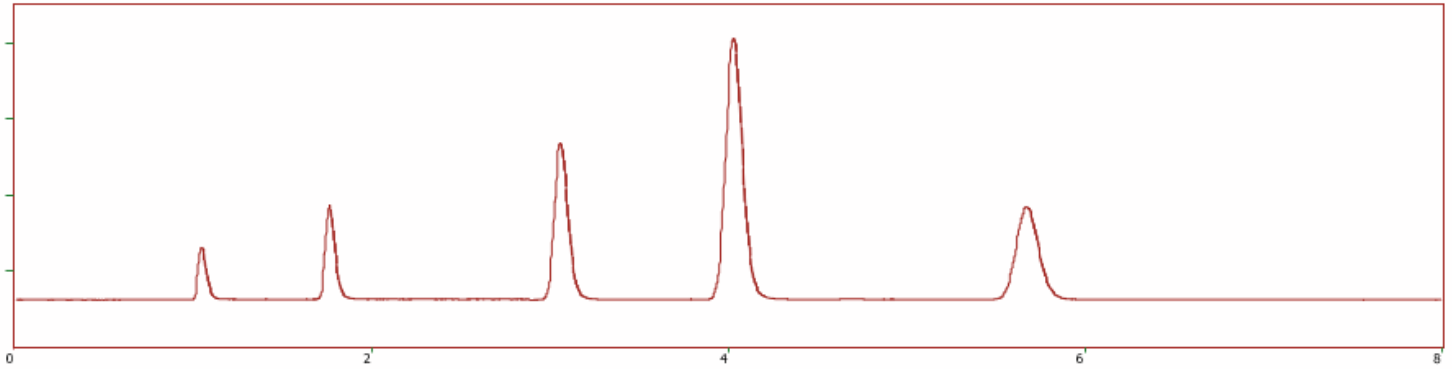




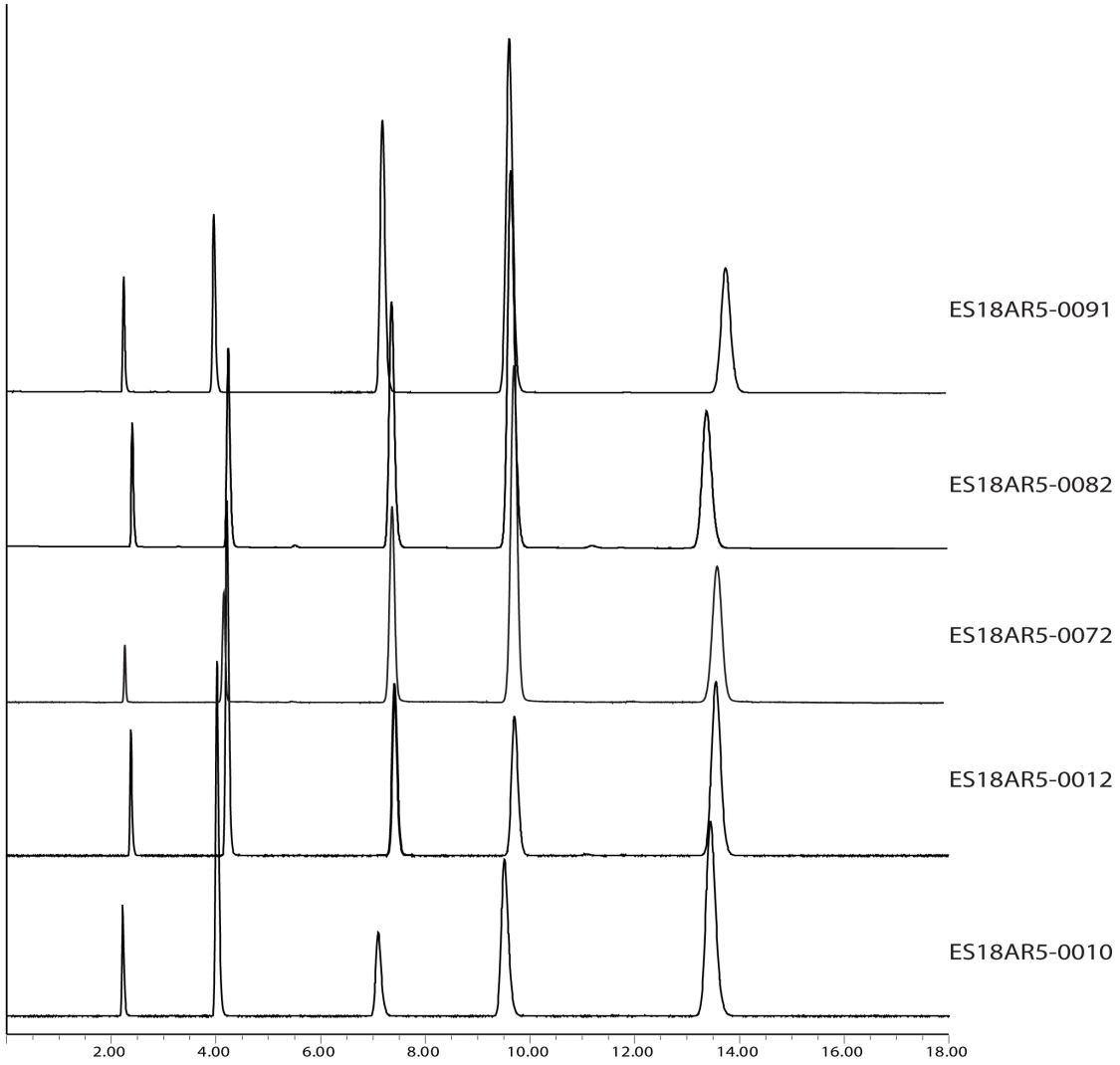
C18/AR
3 µm
(2.1 mm x 100 mm)



C18/AR
5 µm
(2.1 mm x 100 mm)



Batch to Batch Reproducibility Guaranteed



Current Production Batches of 5 C18/AR tested to ensure Batch to Batch Ruggedness and Reproducibility!


EVOSPHERE
Fortis
Technologies Ltd.
The Evolution of HPLC Columns

macmod

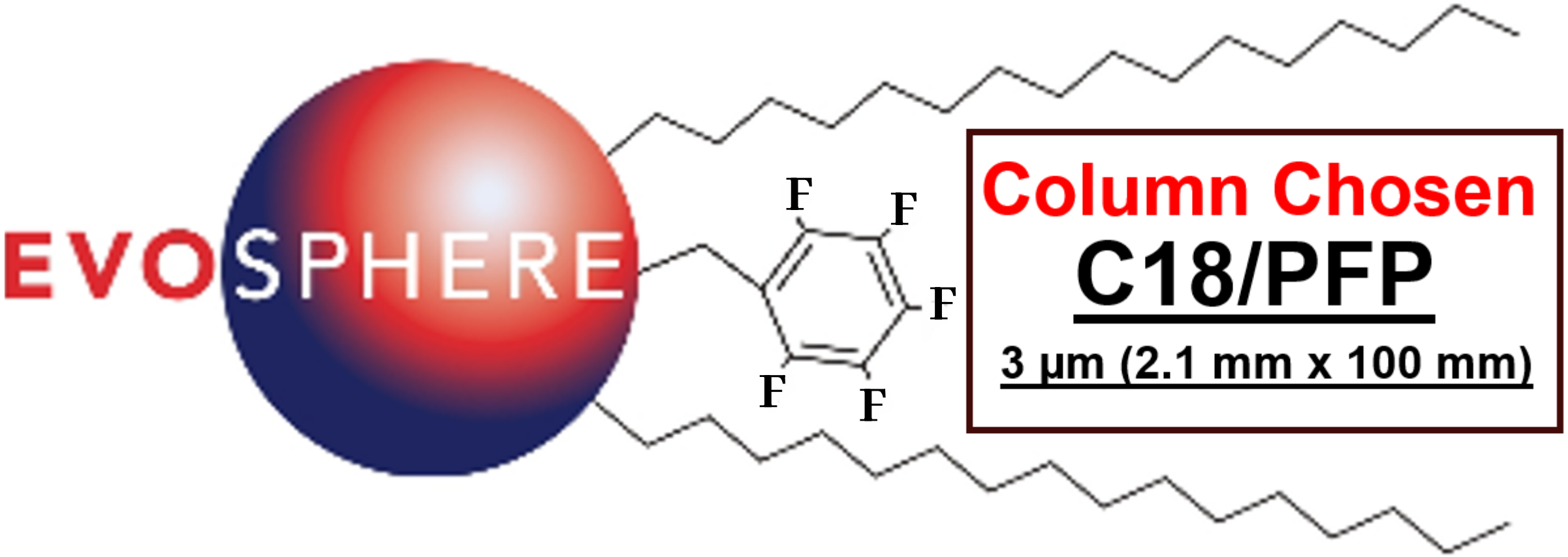
SMARTER CHROMATOGRAPHY

Untargeted Metabolomics Assay utilizing MFPP Technology

(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

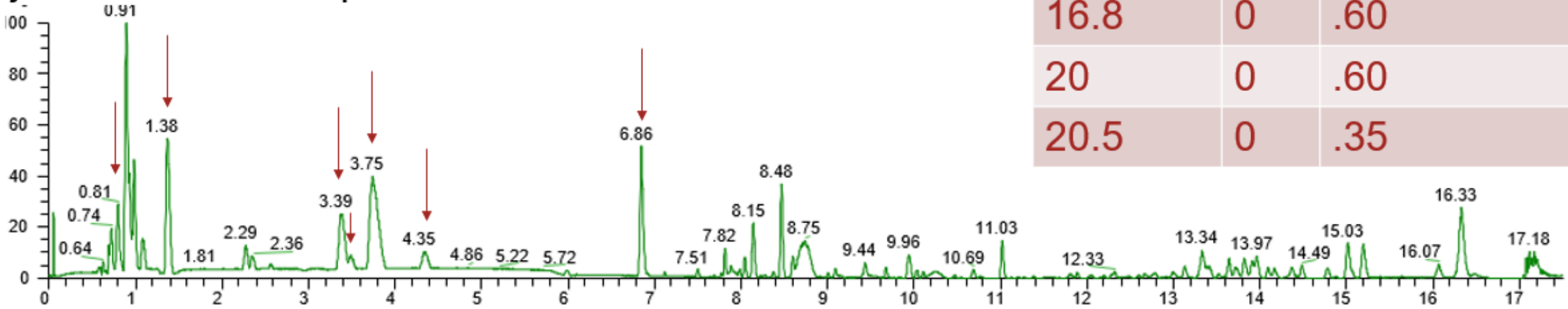
Mobile Phase A = 0.1% Formic Acid in H_2O

Mobile Phase B = Acetonitrile

Temperature = 25ºC

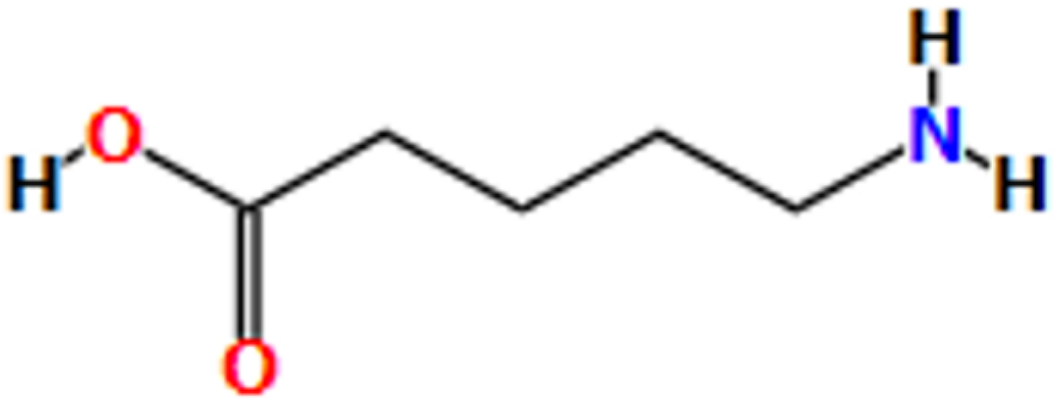
Injection volume = 2µL

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

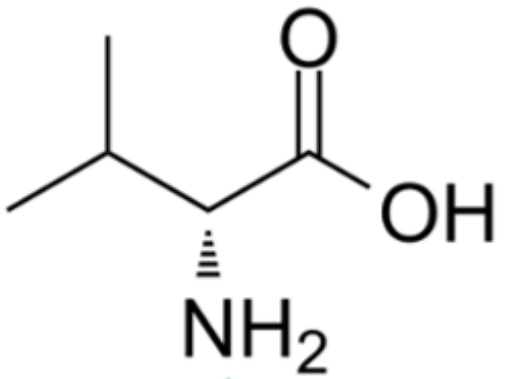


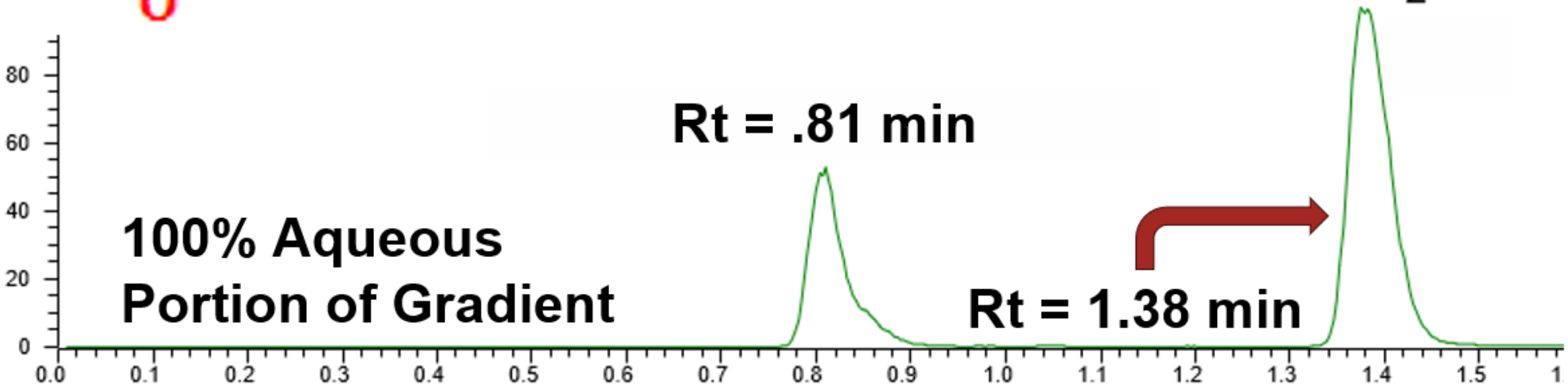
Polar Retention

5-Aminopentanoic Acid



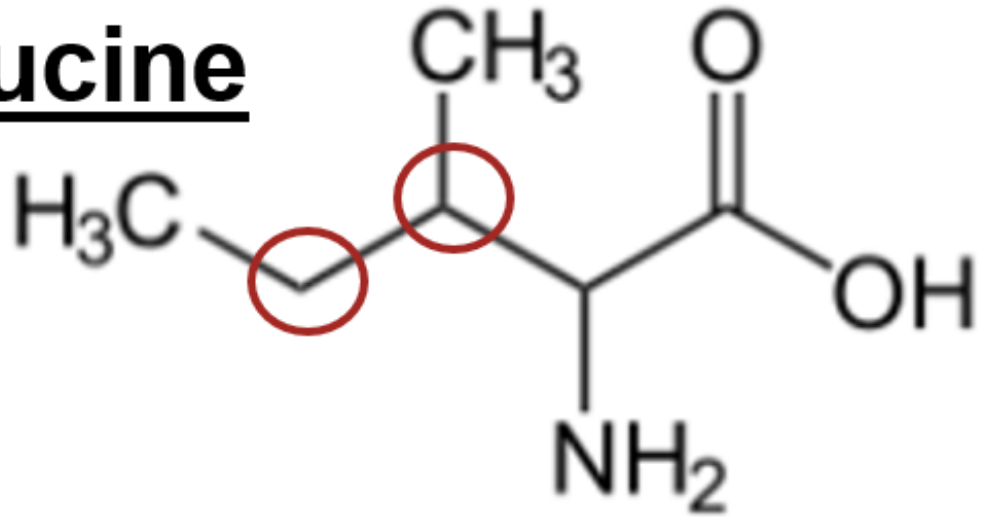
Valine



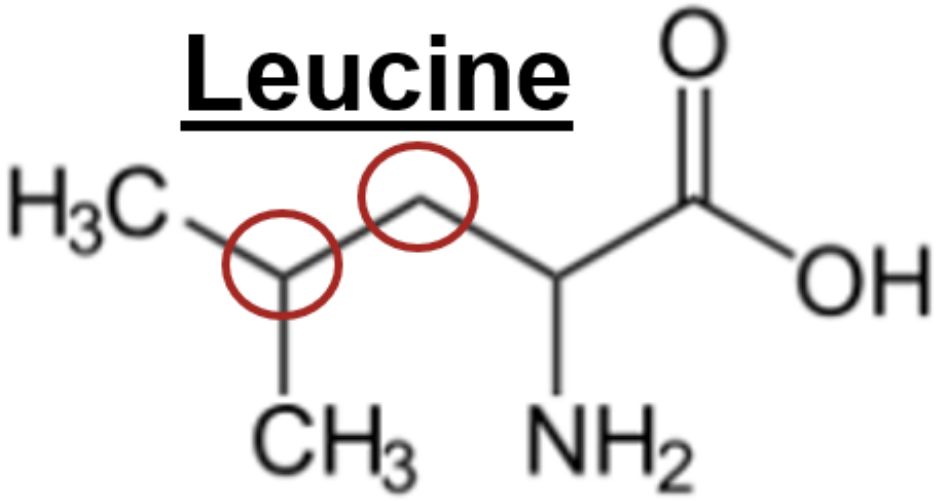


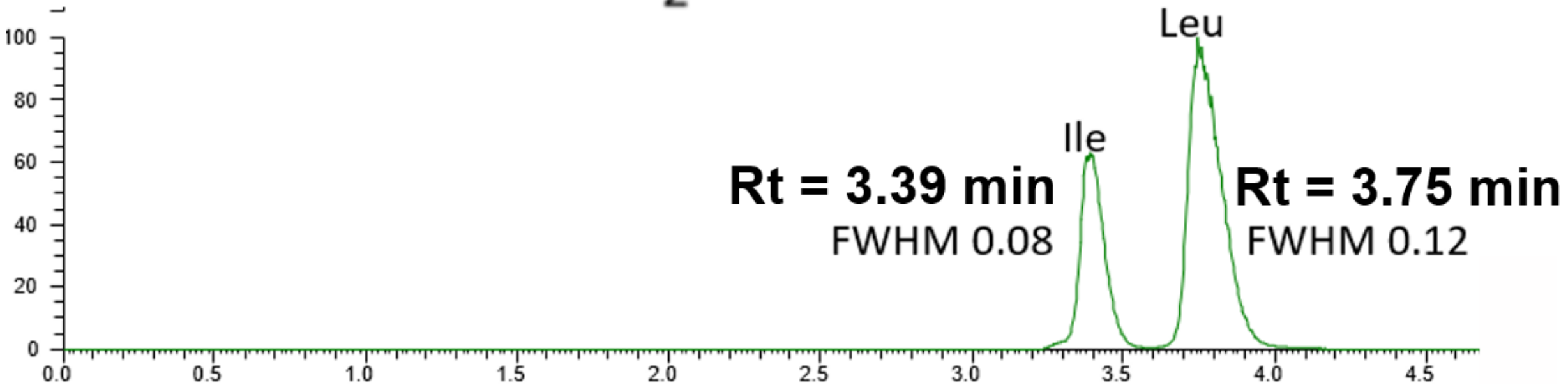
Methyl Position Switch

Isoleucine

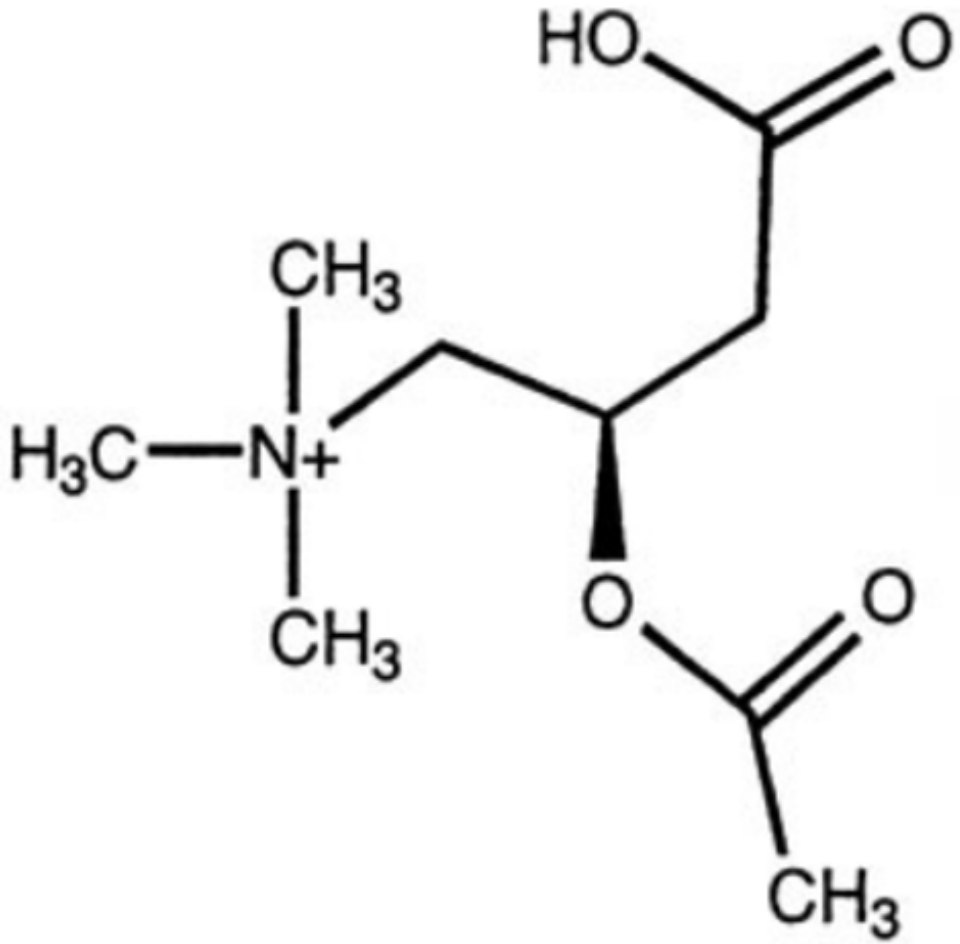


Leucine





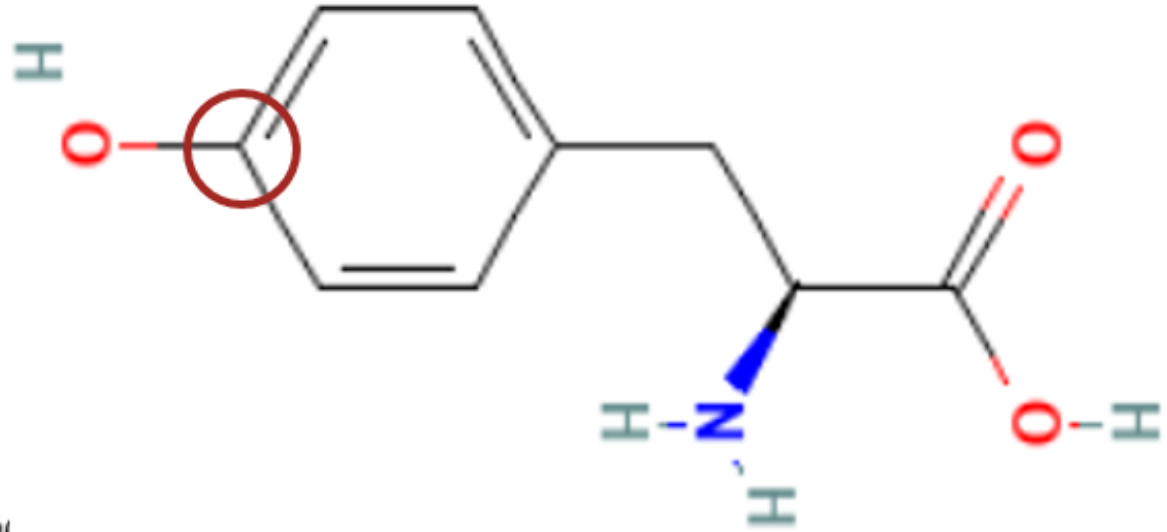
Optimal Peak Shape

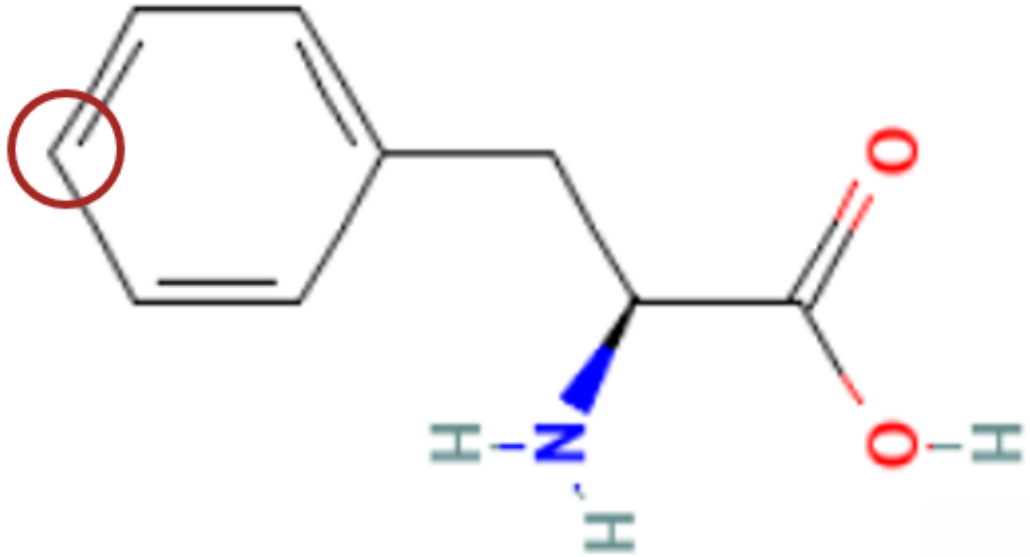


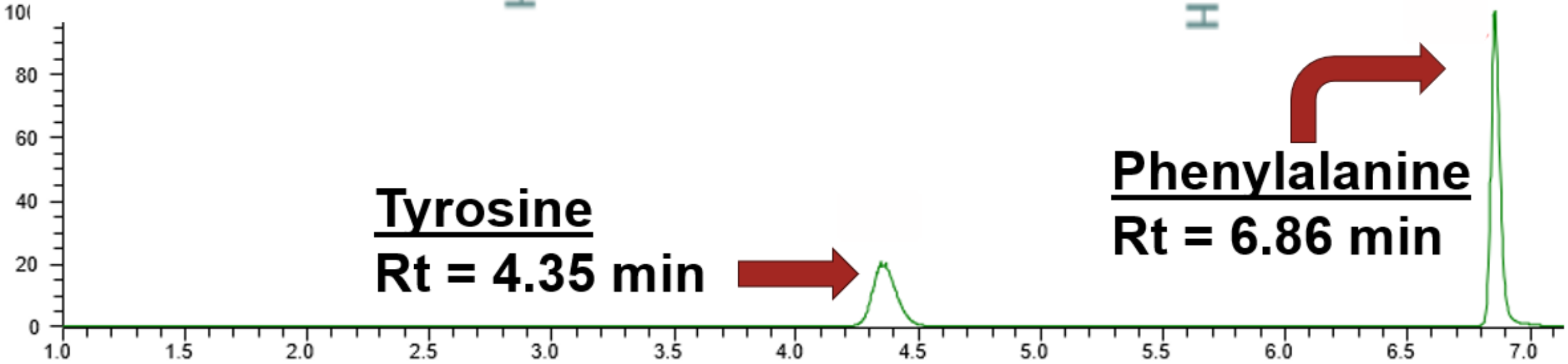
Acetyl-Carnitine

Rt = 3.51 min

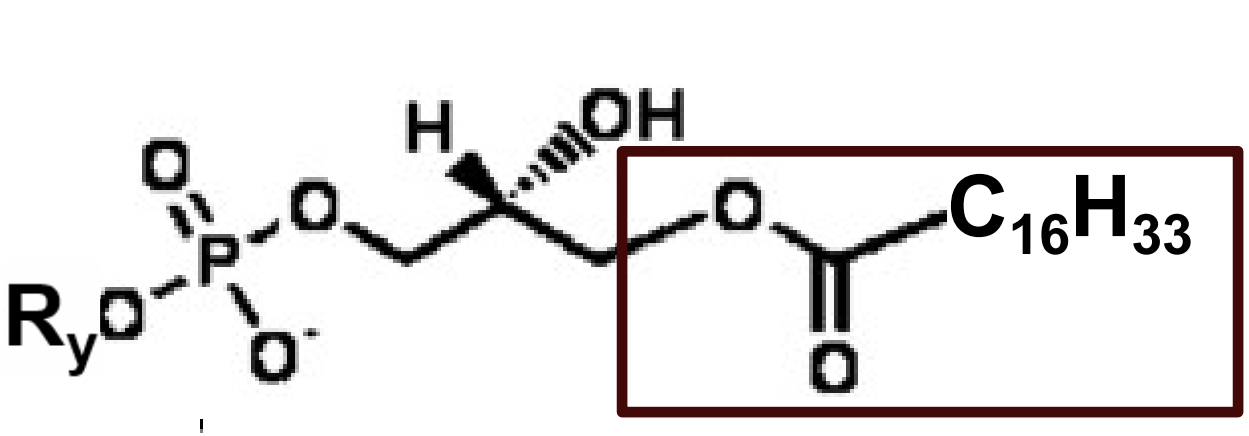
Strong Isomeric Selectivity

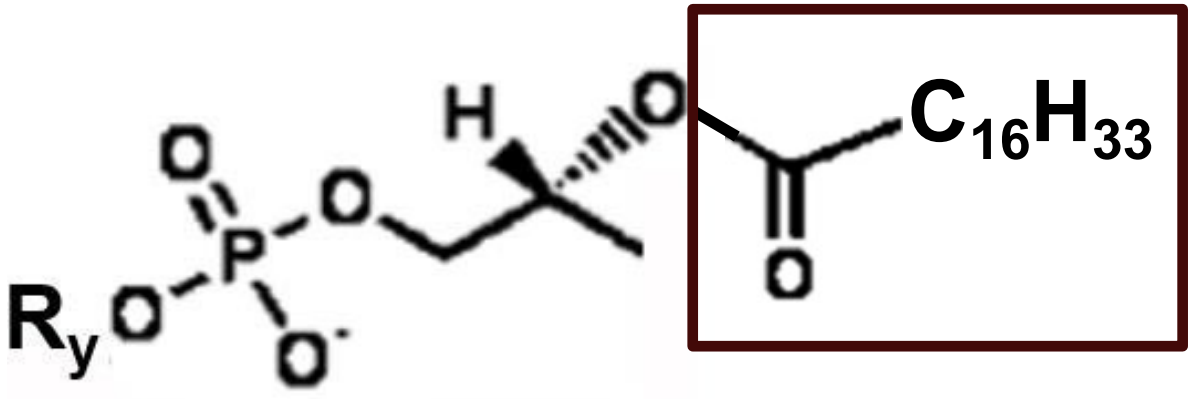




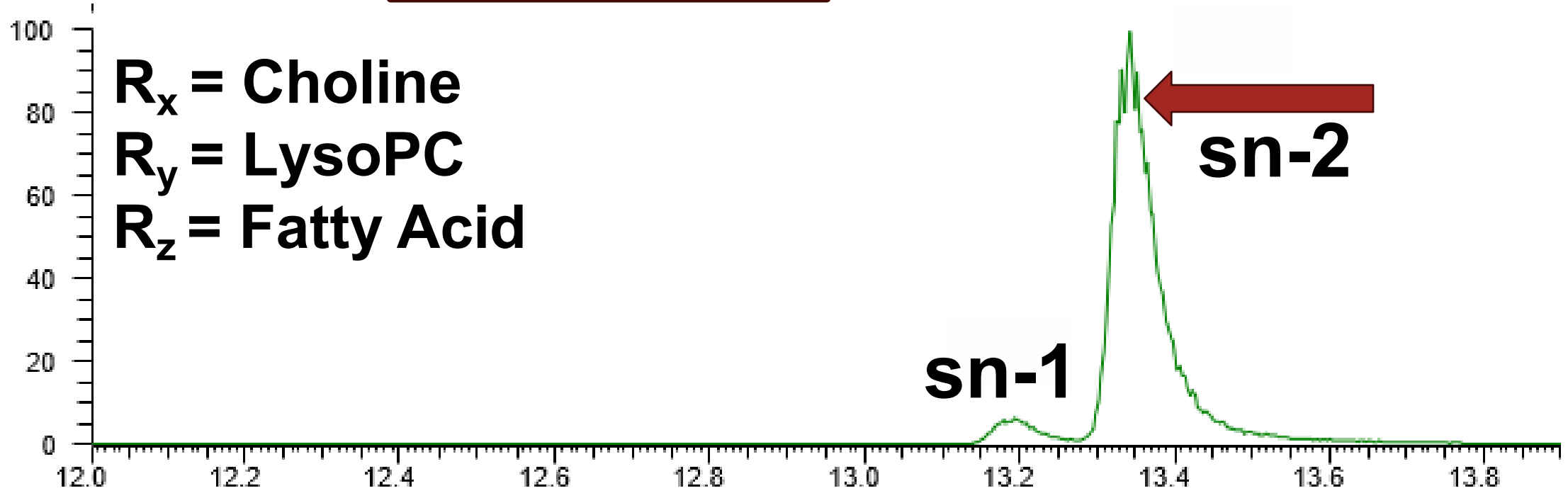


Lysophospholipid Isomers sn-1 vs sn-2 with C18/PFP





R_x = Choline
R_y = LysoPC
R_z = Fatty Acid



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Evosphere Product Line Extensions

Evosphere® BIO 300 Å Proteins and Peptides

- C12
- Diphenyl
- C4
- C18/AR

Evosphere® BIOMAX 100 Å Oligonucleotide separations – chemically inert hardware

- C12
- C18/AR

Evosphere® BIOMAX 300 Å Proteins and Peptides with chemically inert hardware

- C12
- Diphenyl
- C4
- C18/AR

Conclusion

Monodisperse fully porous particle technology eliminates the weaknesses and combines the strengths of traditional superficially porous and fully porous particles, giving you the best of both worlds. This new technology offers efficiencies similar to superficially porous particles, without loading limitations, and the ability to scale to preparative sizes, which provide significant method development advantages

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Thank You For Your Time