New LC Particle Technology Monodisperse Fully Porous Particles – Part 1

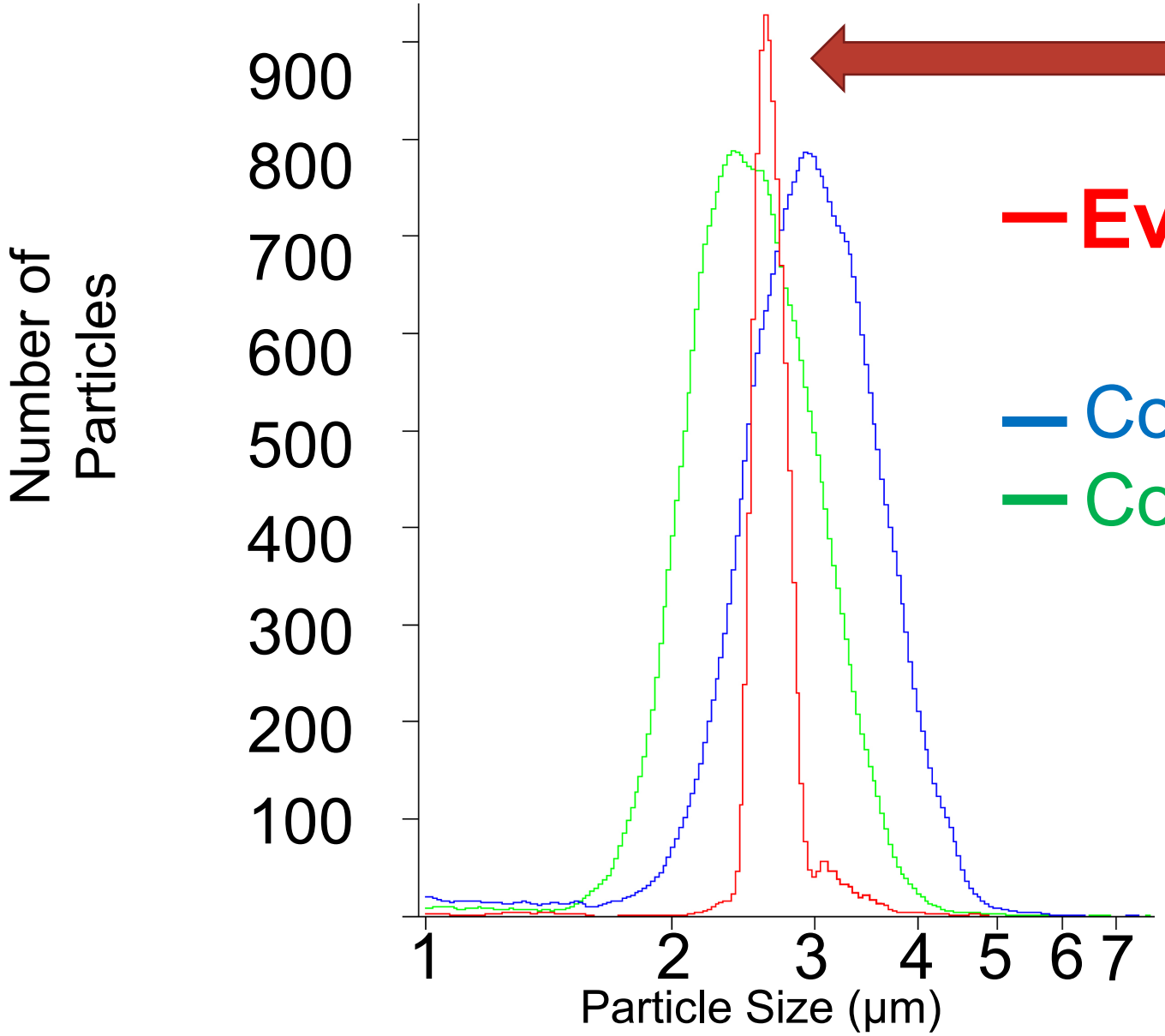
Presented by Geoff Faden – President

Evolution of UHPLC and HPLC Particles

- Morphology: Irregular►Pellicular ►Spherical Porous
- Size: 100 µm ►50 µm ►10 µm ►5 µm ►3 µm ► 1.7 µm
- Purity: [ ] Metals – Type A ►Type B
- Particle Size Distribution – ▼ 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

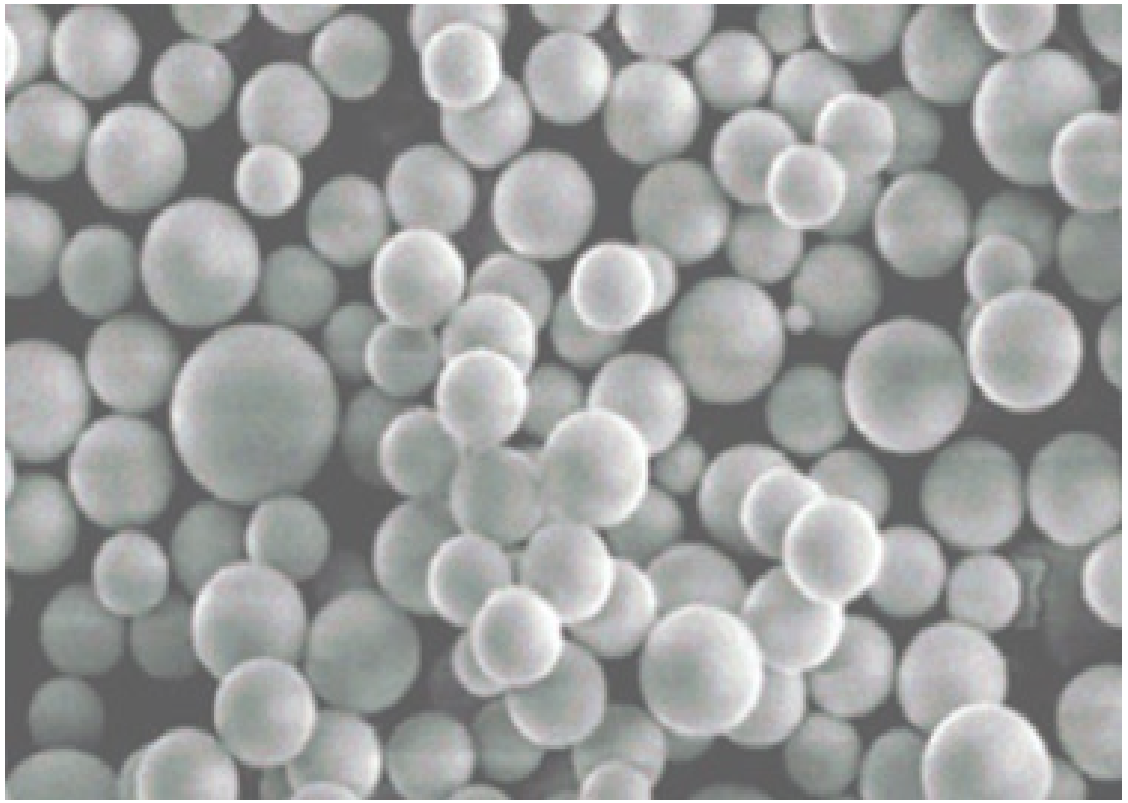
Particle Size Distribution Comparisons

	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

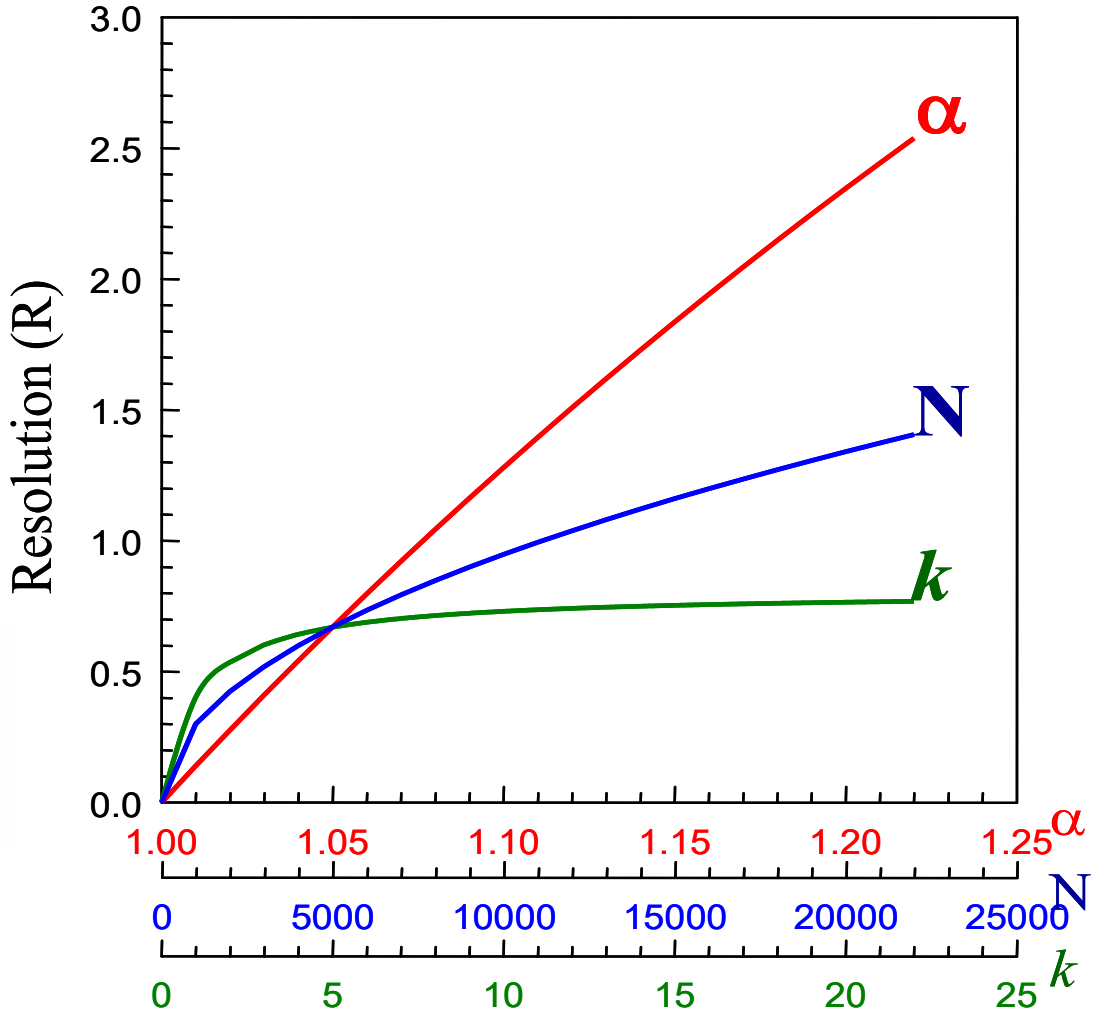
Monodisperse

Impact of N (Efficiency) on Resolution

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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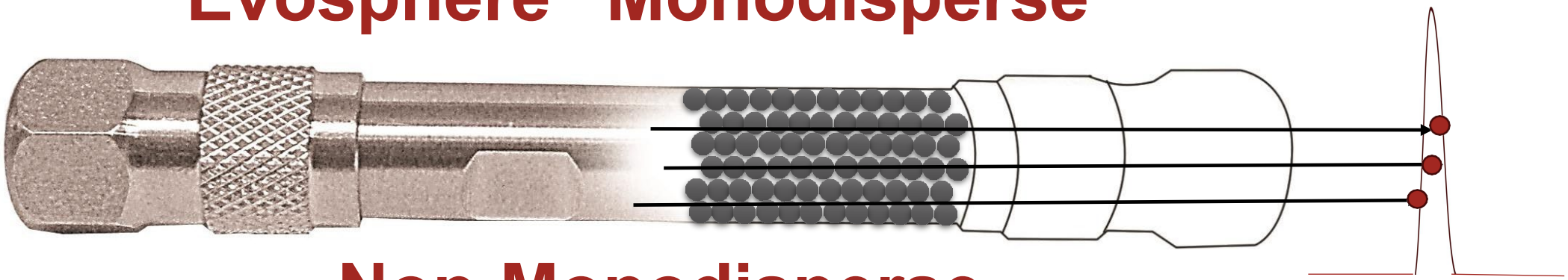
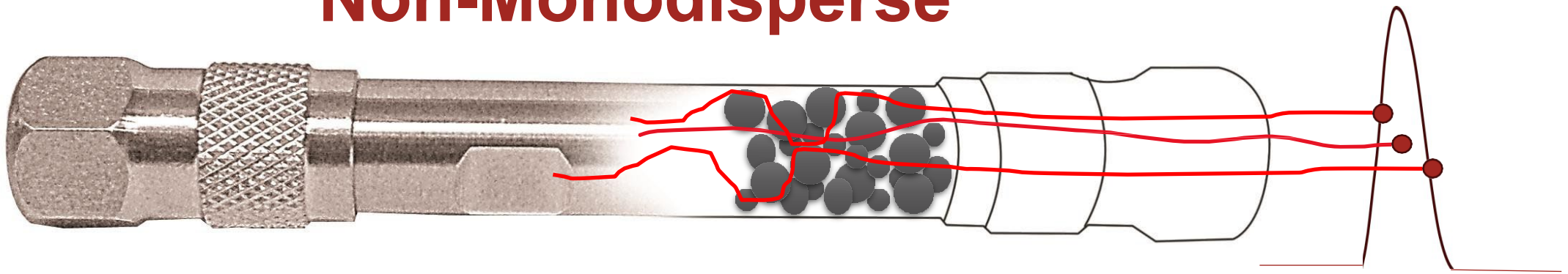
- D_s is the diffusion coefficient of the stationary phase.
- u is the linear velocity

Visual Representation of Minimizing Eddy Diffusion (“A Term”)

Evosphere® Monodisperse

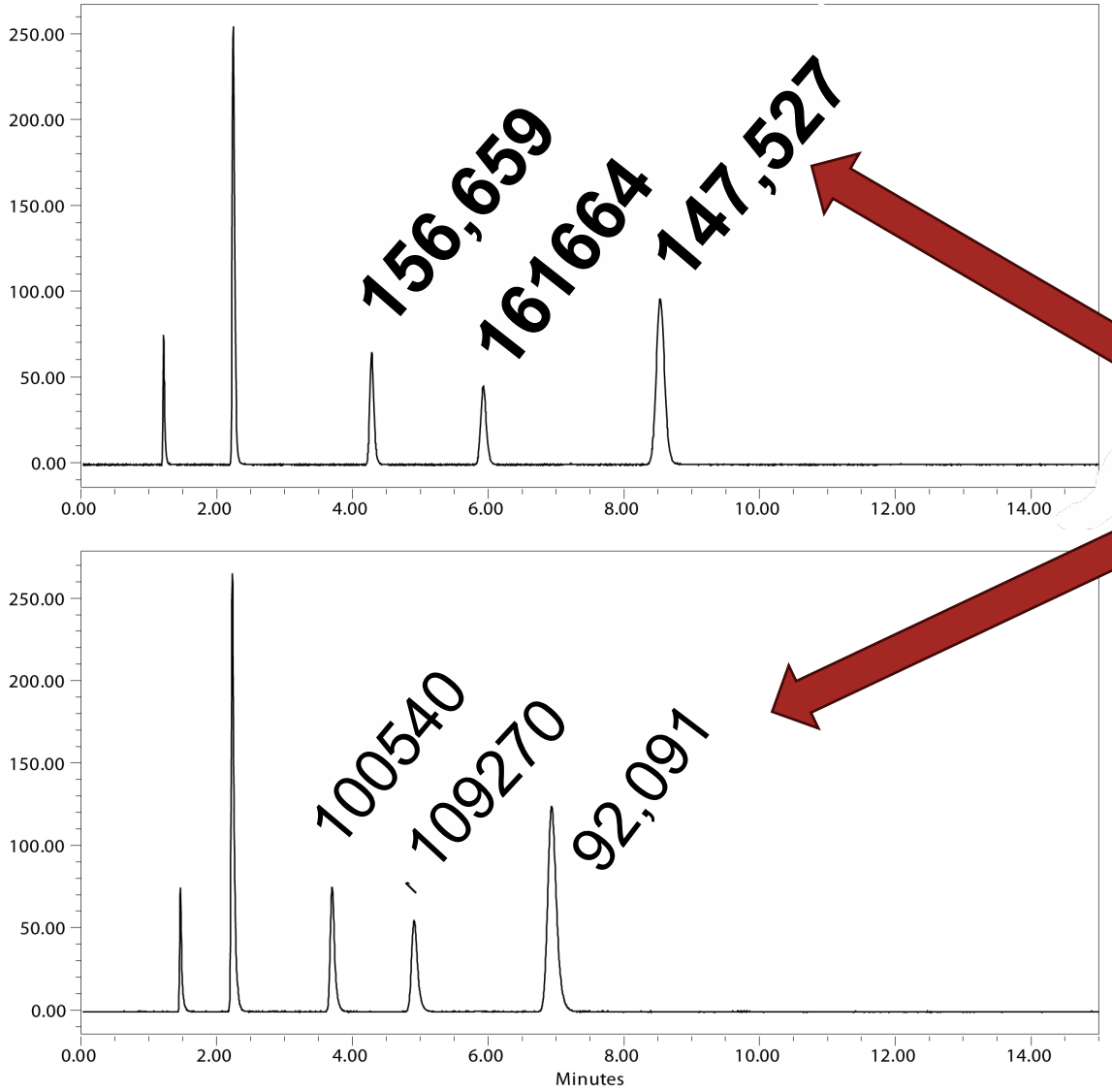


Non-Monodisperse



Flow through the column Evosphere Monodisperse vs. Non-Monodisperse Packed Column

What does this look like chromatographically?



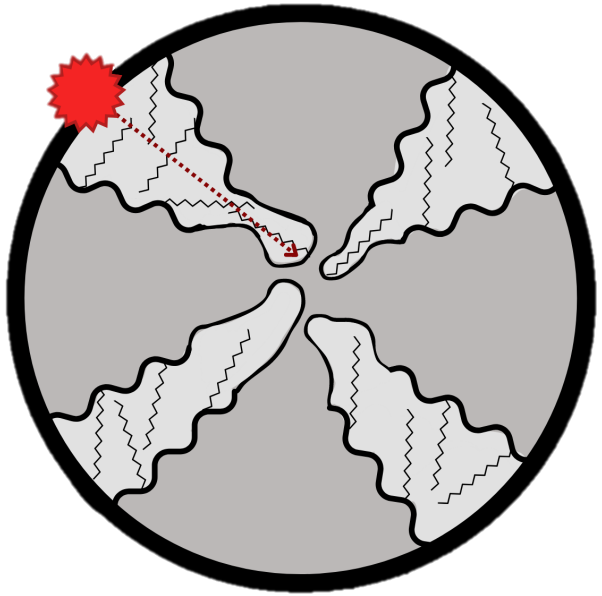
Evosphere C12
3 µm, 4.6 x 150 mm

60% Higher N

Fully Porous C18
3µm, 4.6 x 150 mm

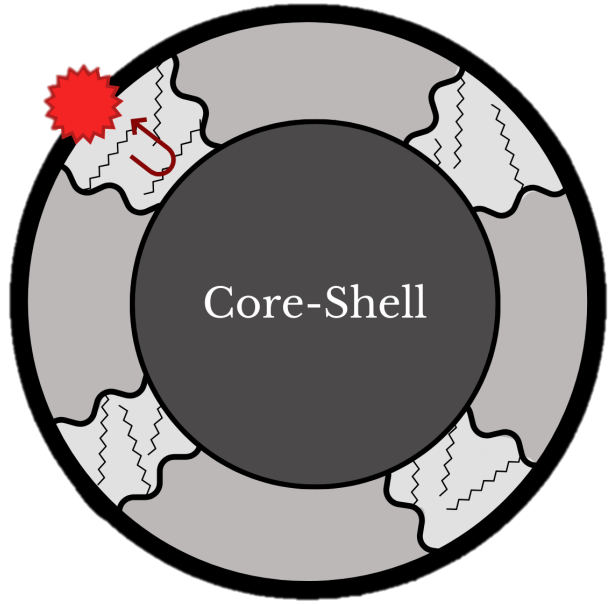
Evosphere Monodisperse compared to Core-Shell Technology

Evosphere®



1. Similar Plates (N)
2. ▲ Loading Capacity
3. Unlock Scalability to Prep and Semi-Prep
4. Increased Retention due to Surface Area

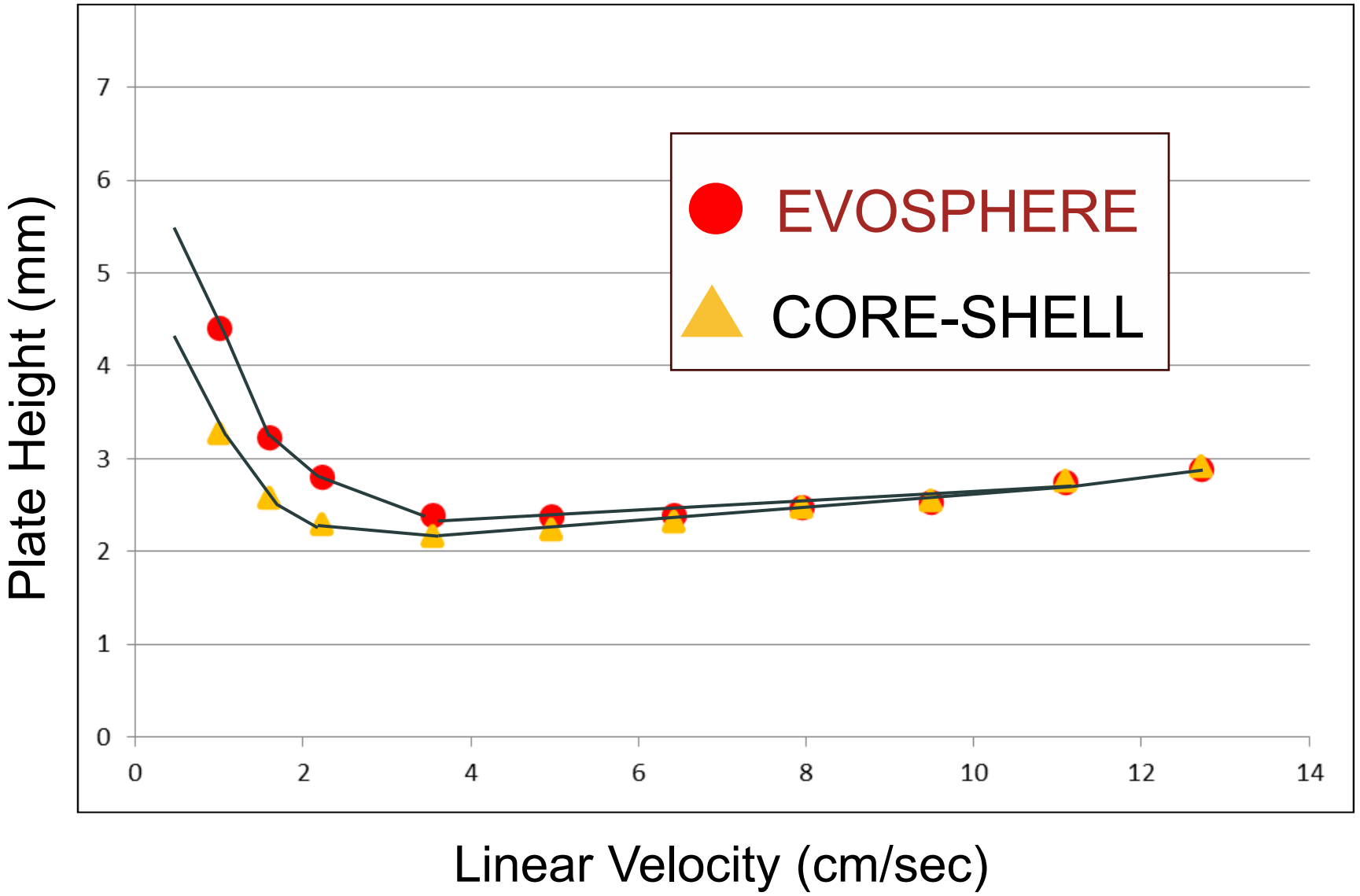
Core-Shell



Surface Area = 350 m^2/g > **Surface Area ~130 m^2/g**

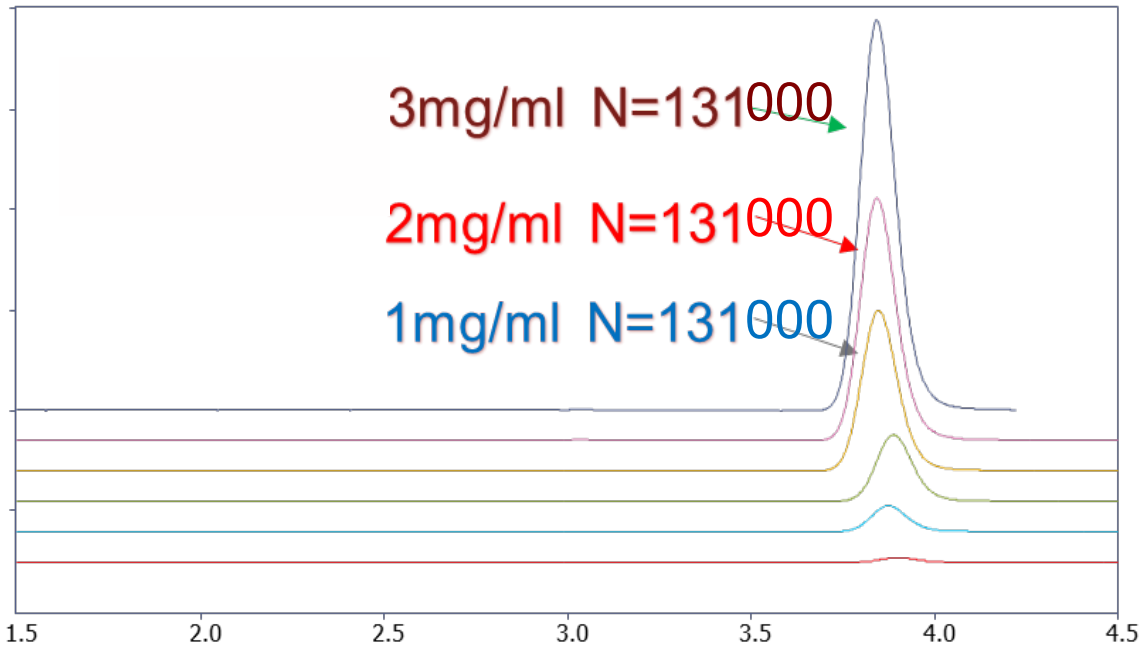
~3x Surface Area

Van Deemter flattens at Elevated Linear Velocities



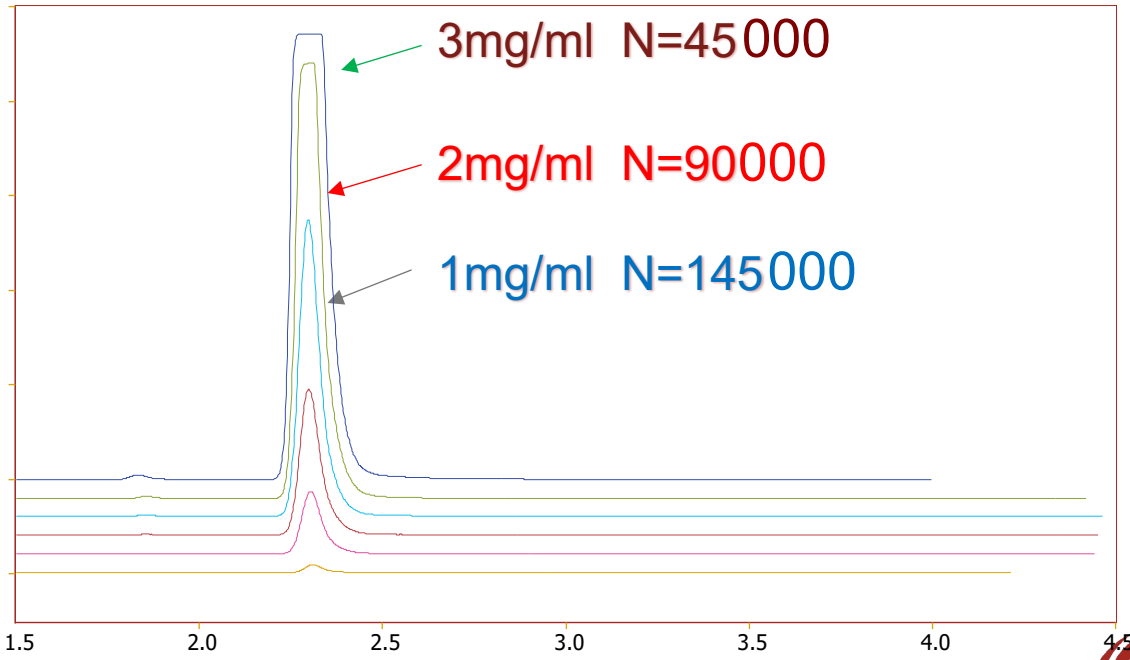
Evosphere®
Improves Loading and Increases Retention

Evosphere
USP L1 Surface Area = 350 m^2/g



Rt = 3.8 min

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



Rt = 2.25 min

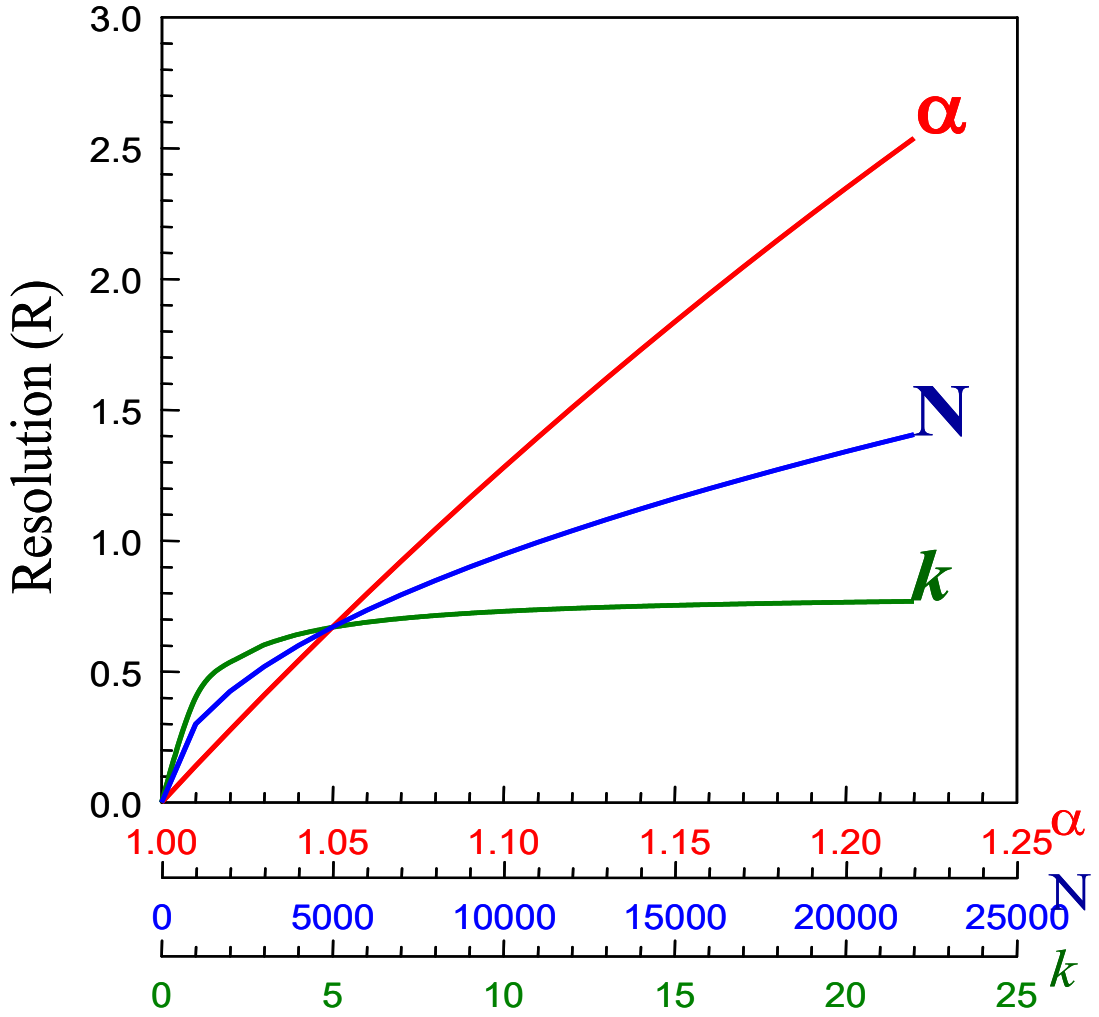
Resolution Equation

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.

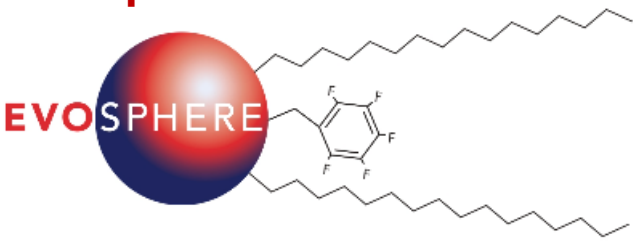


How does Evosphere Impact Selectivity?

Evosphere C12

Evosphere Diphenyl

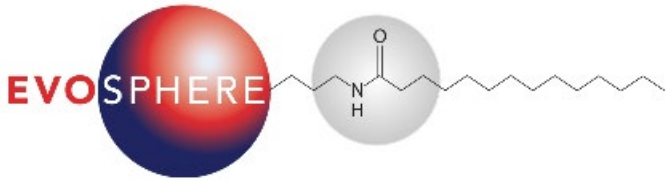
Evosphere C18/PFP



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

Efficiency Retention Selectivity

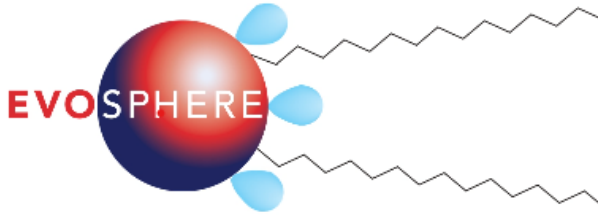
Evosphere RP18-Amide



Evosphere C18/AR

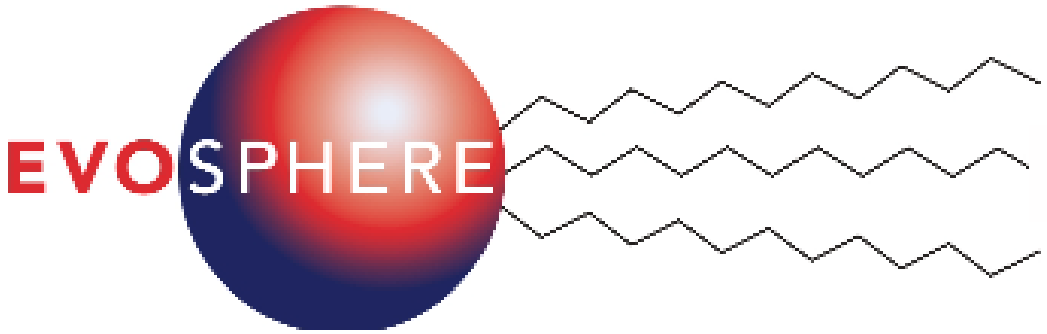
Evosphere Phenyl-Hexyl

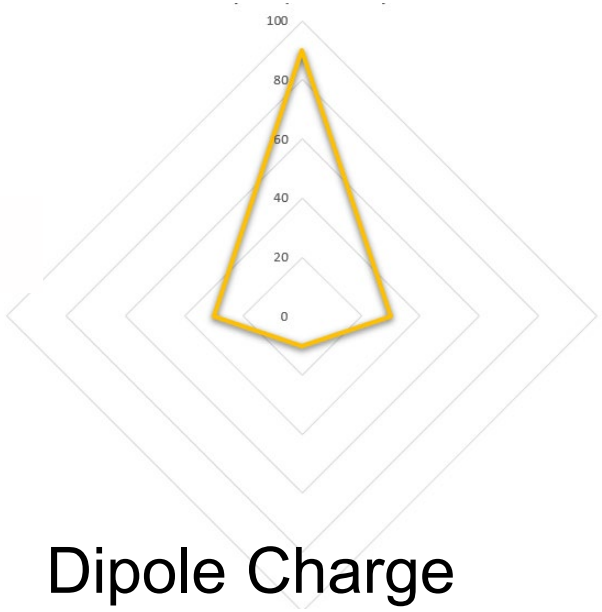
Evosphere AQUA



Evosphere PFP

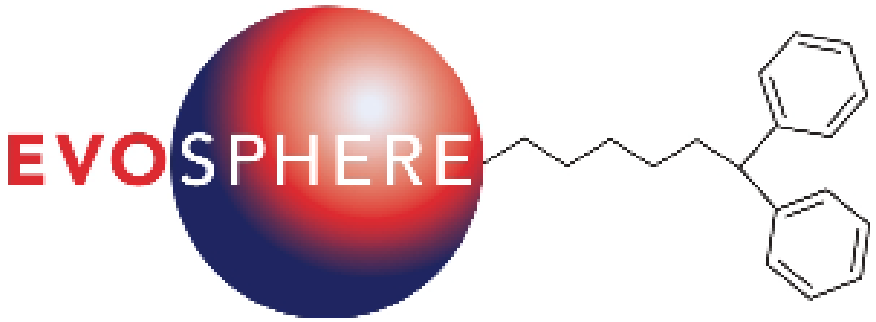
Evosphere C12

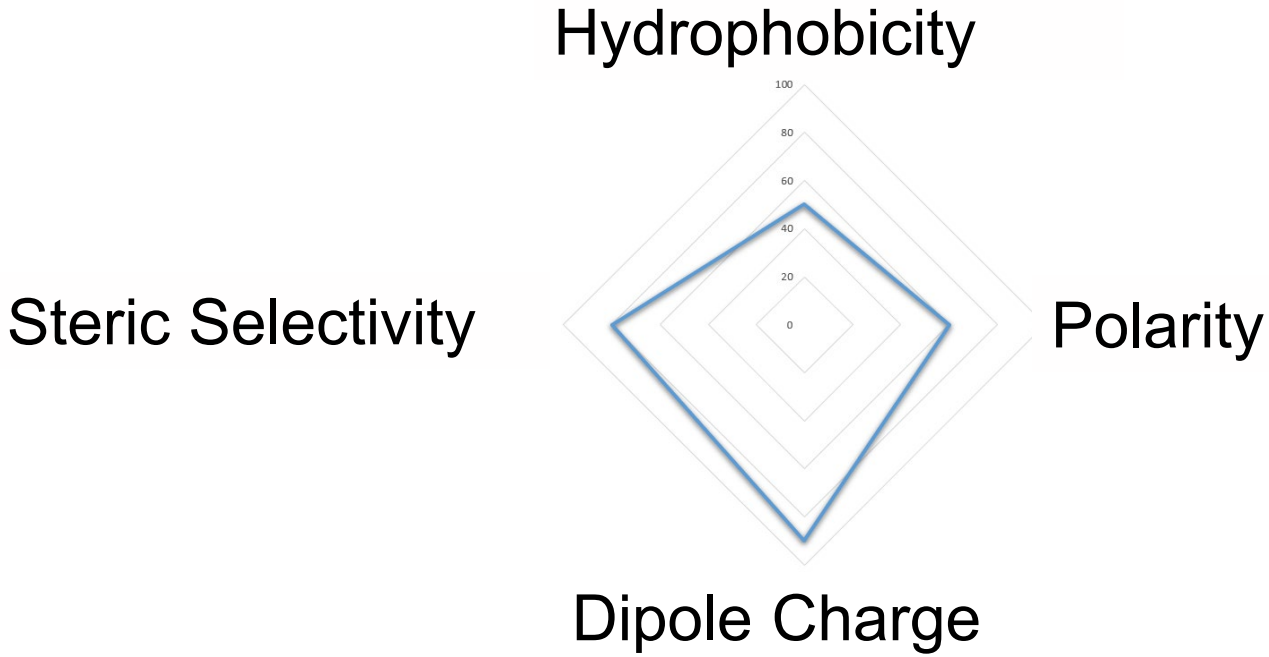




- Predominantly Hydrophobic
- High surface area protection
  - pH limit 2 – 11
- Ideal for mixtures of neutrals, acids and bases
- Excellent for peptide mixtures with FA or TFA

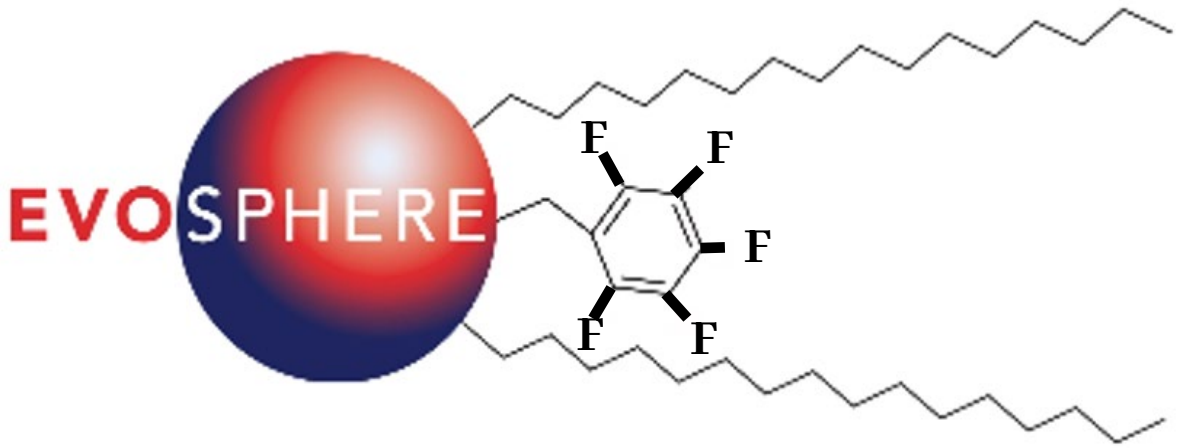
Evosphere Diphenyl

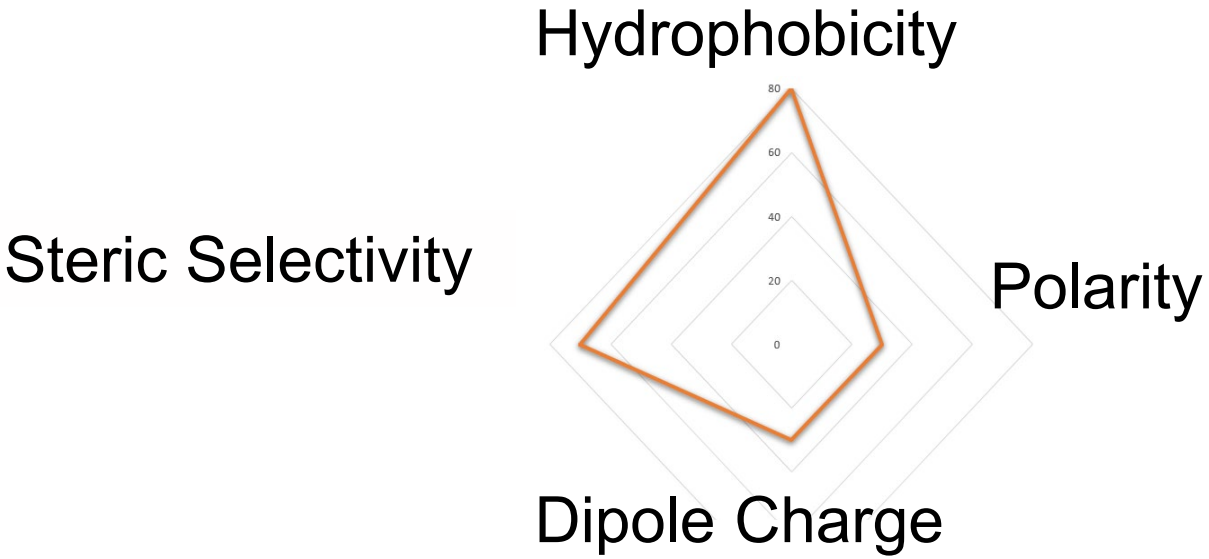




- Predominantly Dipole, moderately hydrophobic due to hexyl ligand
- Excellent positional isomer separations
- No bleed – excellent MS choice
- Alternate selectivity to C18 and Biphenyl

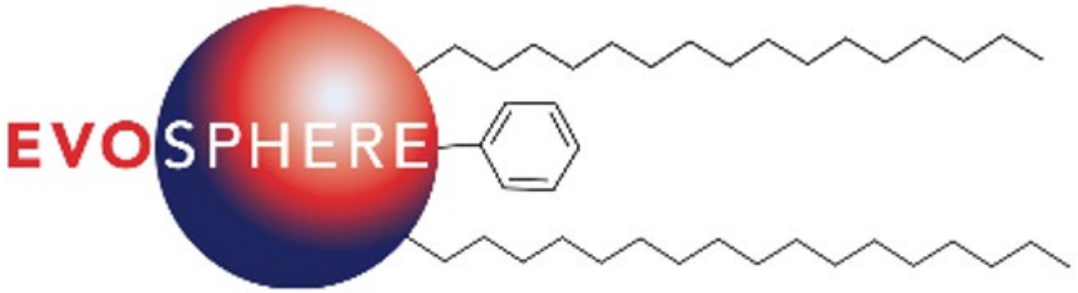
Evosphere C18/PFP

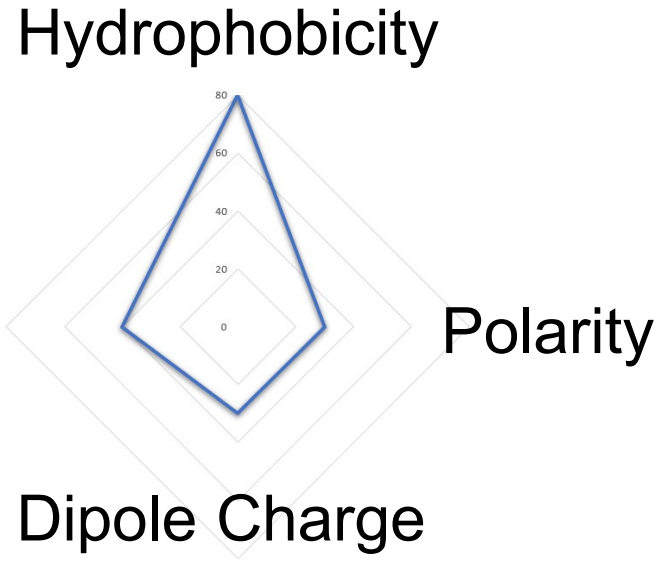




- Hydrophobic, Steric and Dipole Selectivity
- Excellent for regioisomer separations
- Alternative selectivity for PFP – more hydrophobic

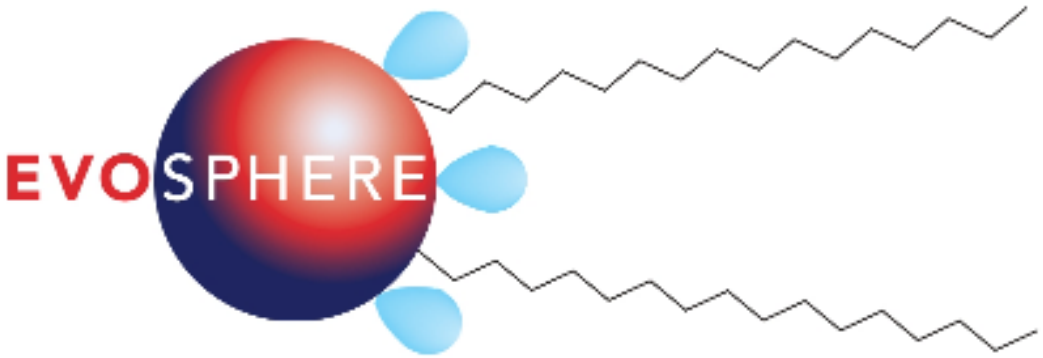
Evosphere C18/AR

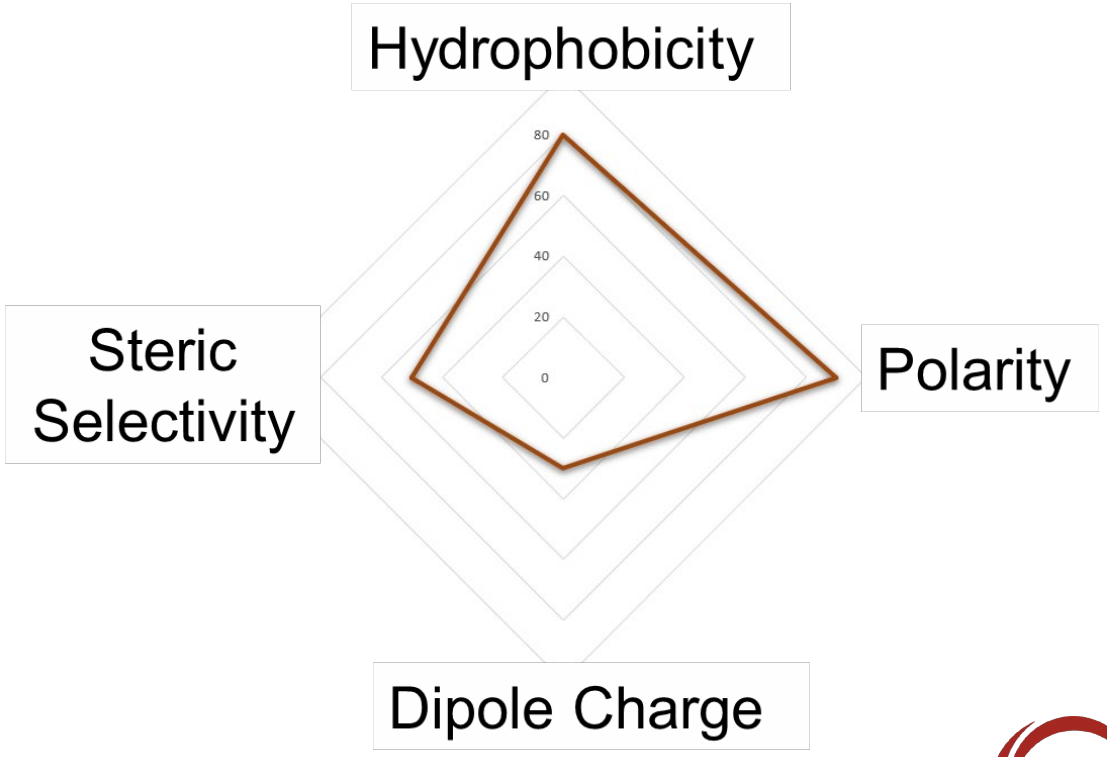




- Hydrophobic, and Steric selectivity
- Excellent for peptide separations
- Alternative selectivity for Phenyl – more hydrophobic

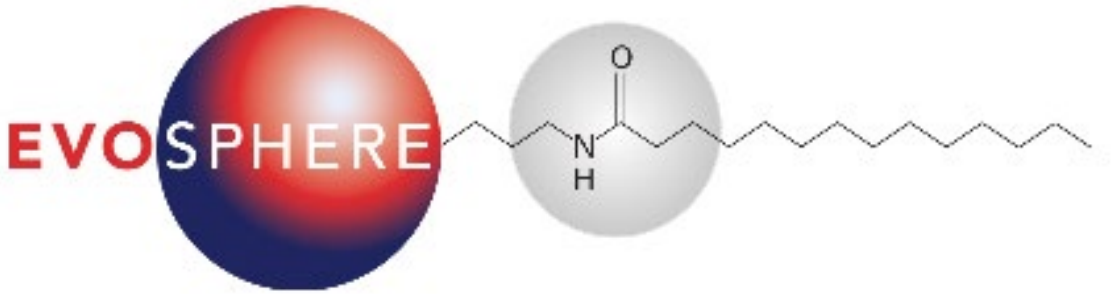
Evosphere AQUA



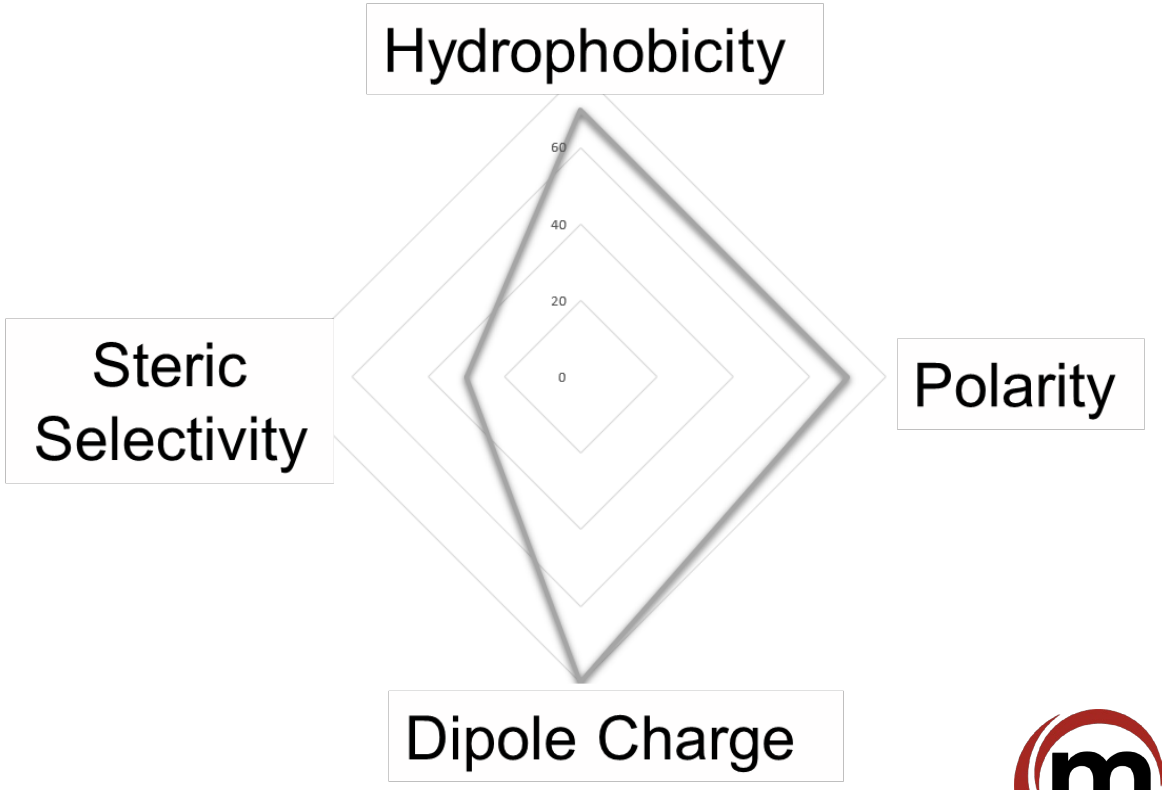


- 100% Aqueous Compatible
- Excellent for Nitrosamines via LC-UV
- Excellent polar retention for (Nitro) containing compounds
- Excellent Stability and robustness

Evosphere RP18-Amide

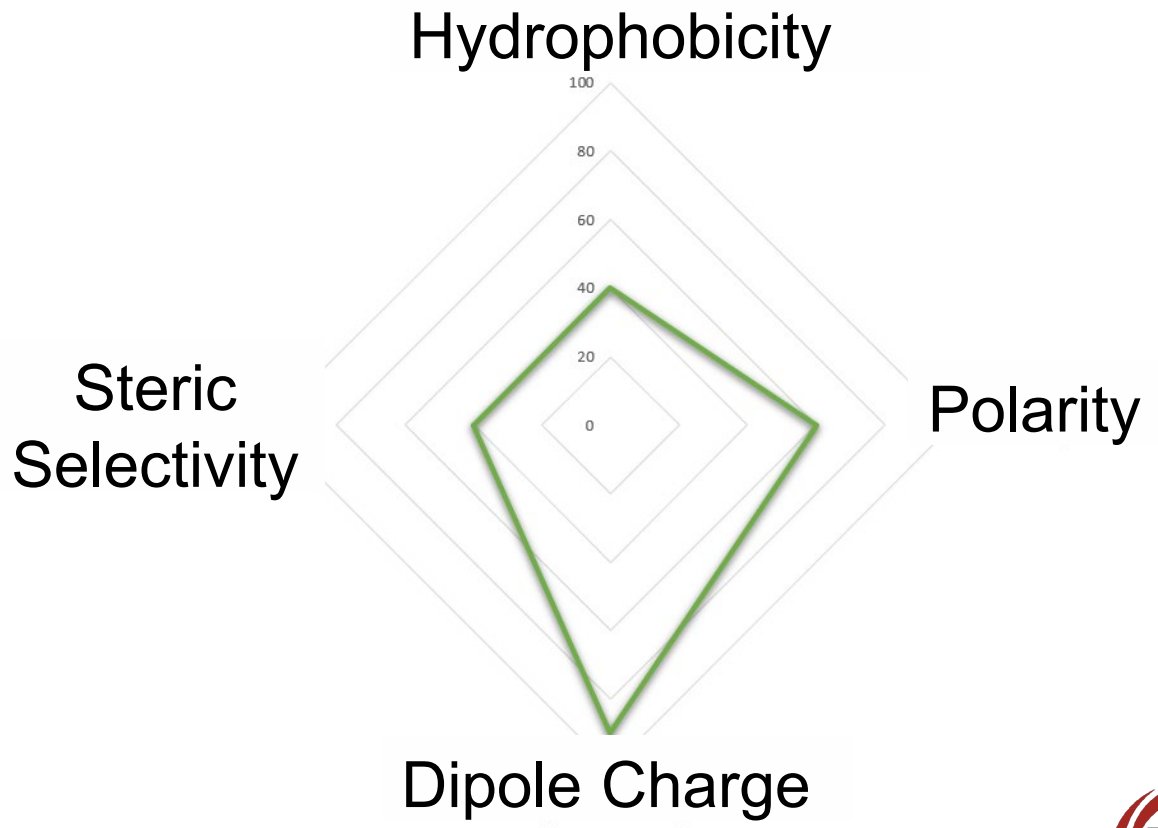


- Great for peak shape for bases at low pH
- Added dipole for charged species
- Hydrophobic and Hydrophylic interactions

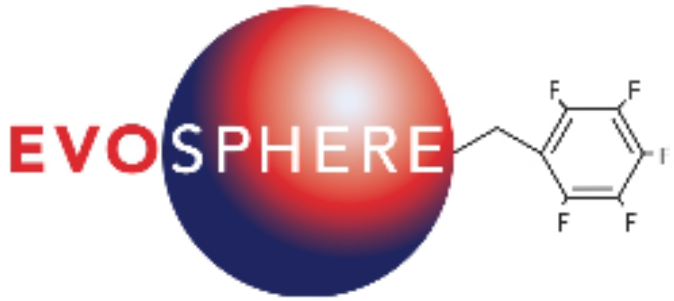


Evosphere Phenyl-Hexyl

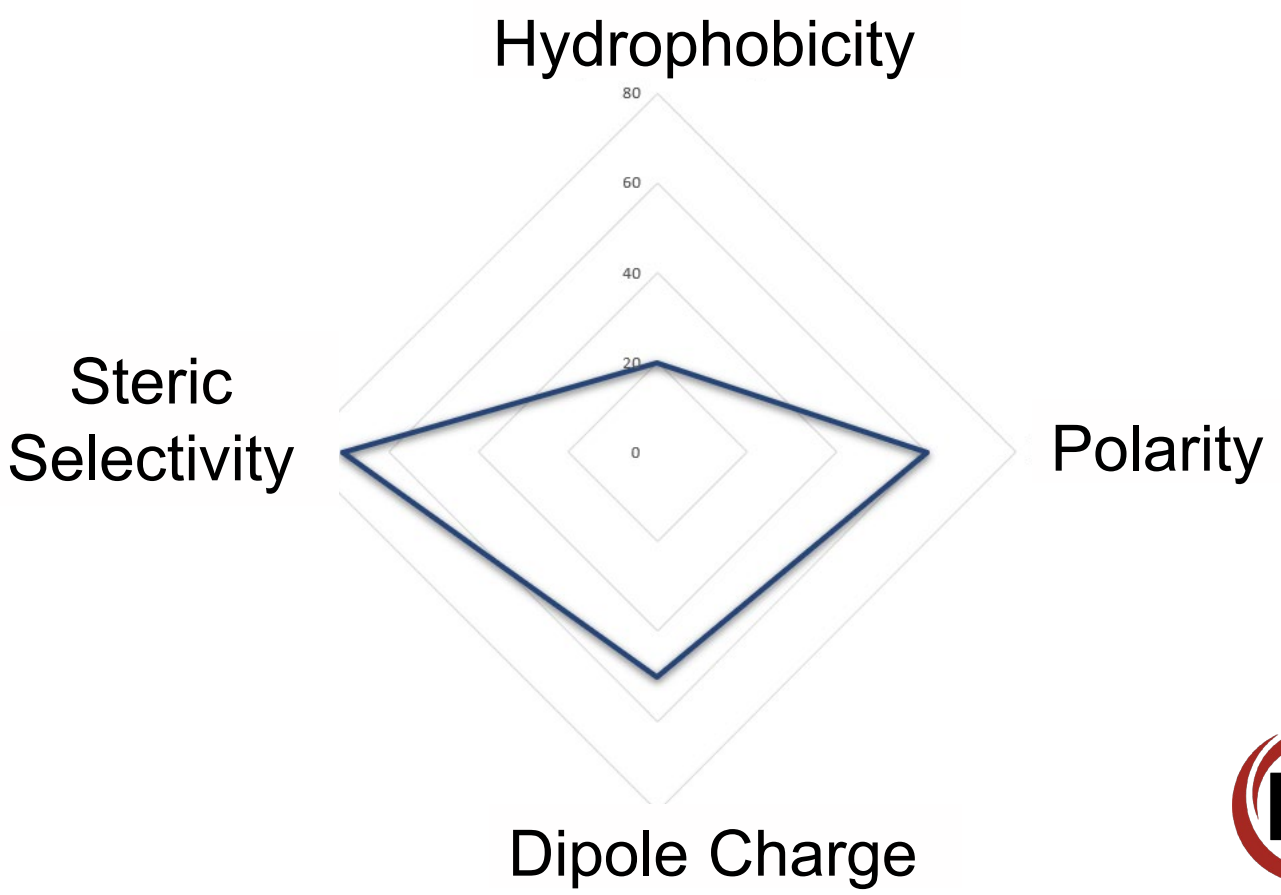
- Alternate selectivity to short-chain phenyl, and C12
- Excellent resolution



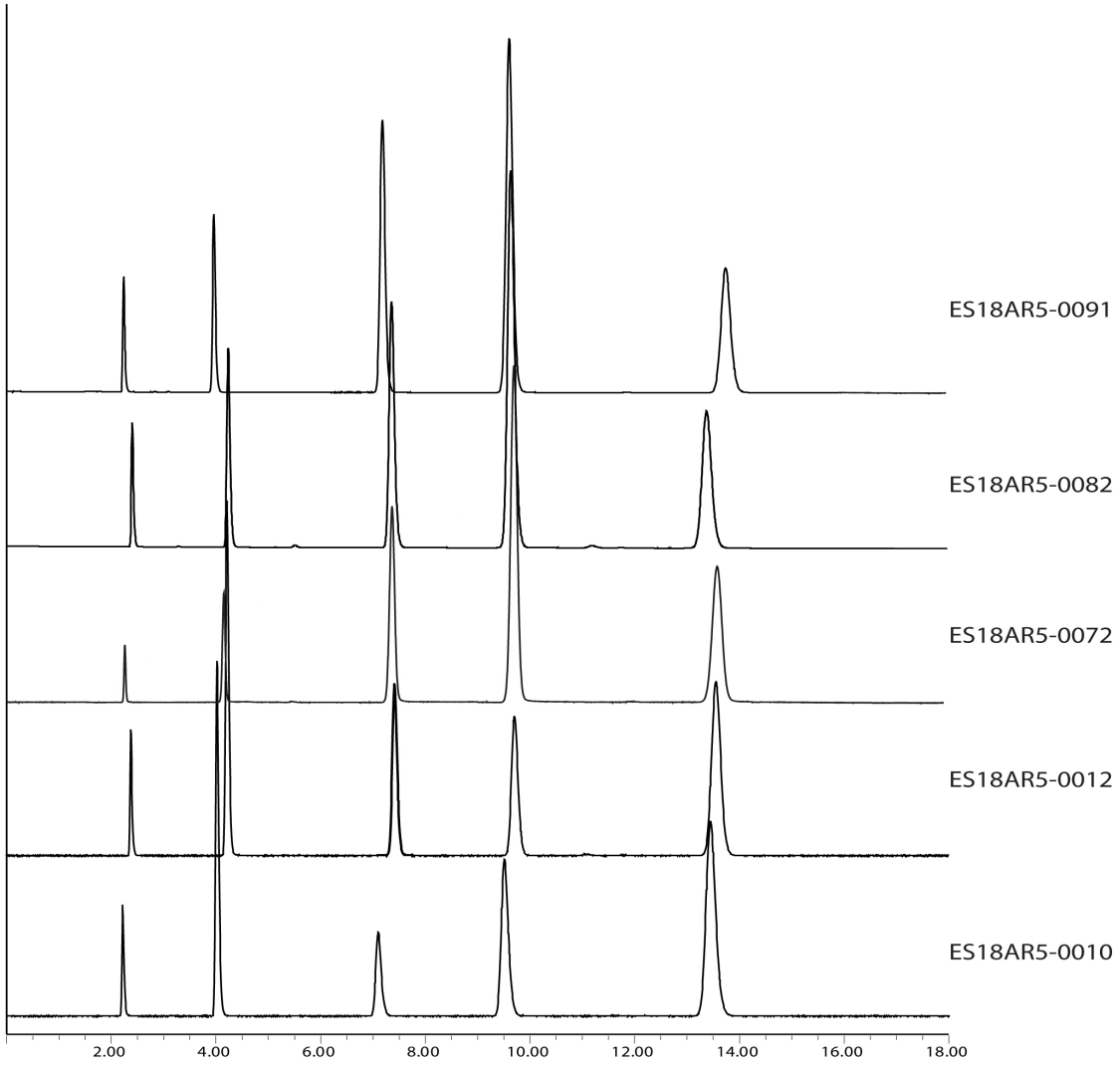
Evosphere PFP



- Excellent shape selectivity
- Alternate selectivity C18/PFP
- HILIC compatible



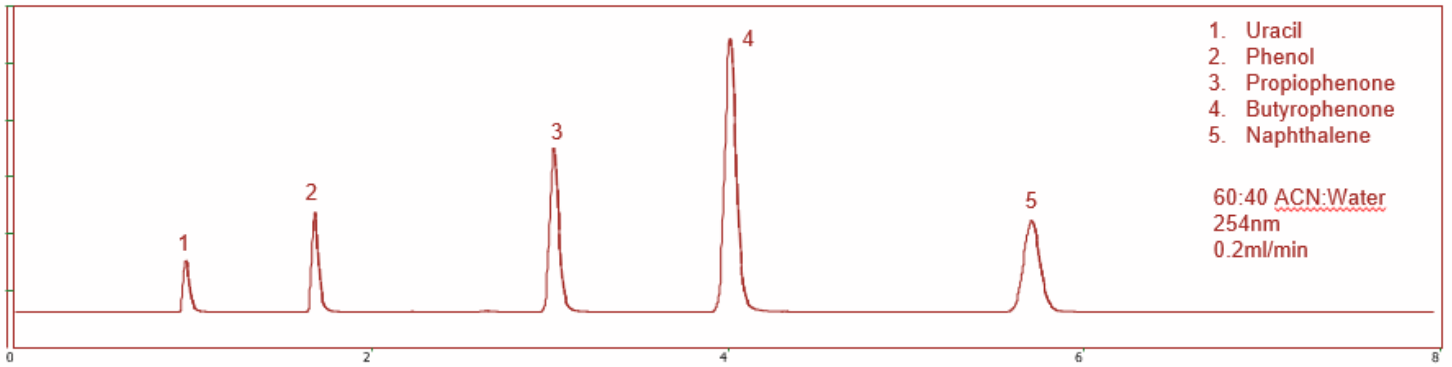
Batch to Batch Reproducibility Guaranteed

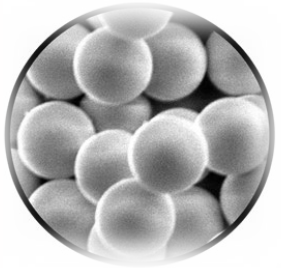


Current Production Batches of 5 C18/AR tested to ensure Batch to Batch Ruggedness and Reproducibility!

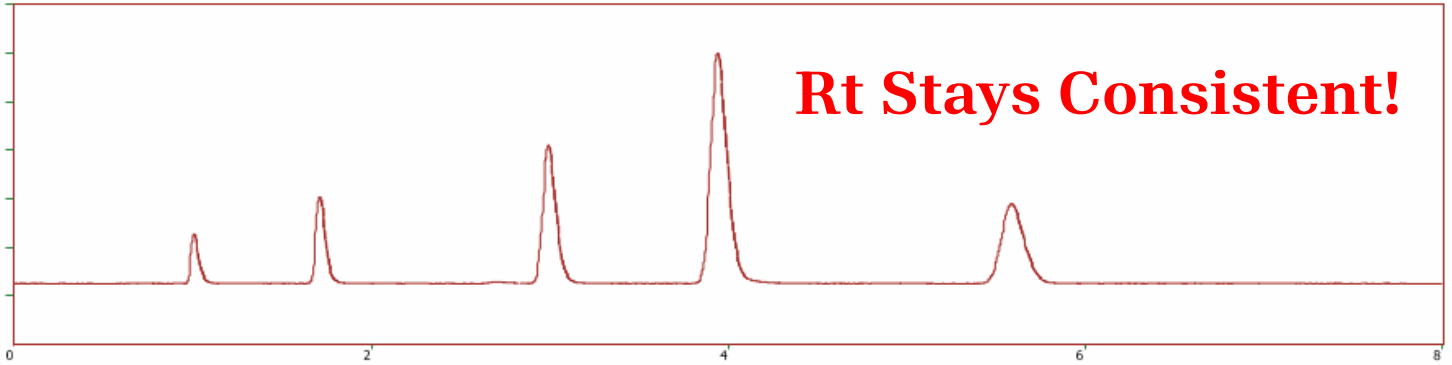
Translate the methods seamlessly for QC

C18/AR
1.7 µm
(2.1 mm x 100 mm)

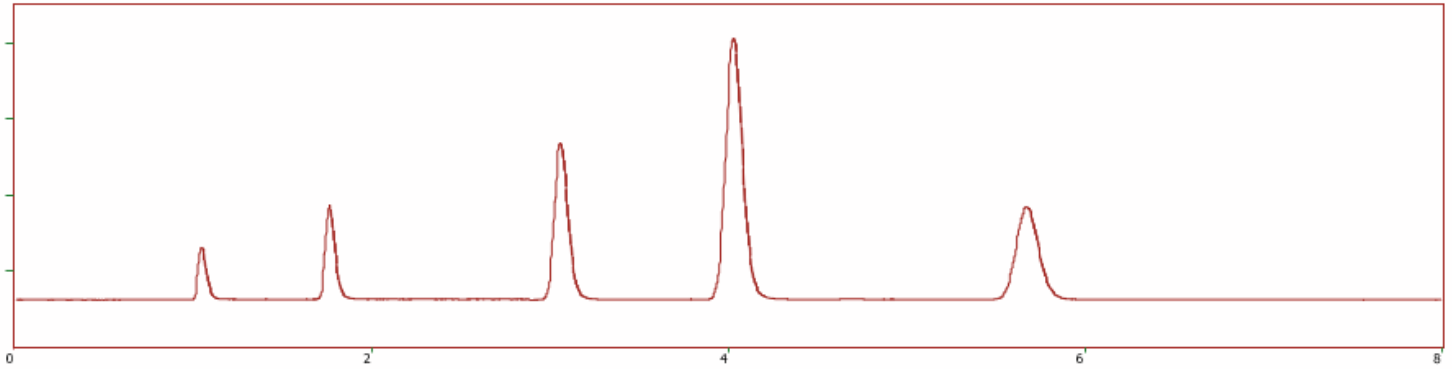




C18/AR
3 µm
(2.1 mm x 100 mm)



C18/AR
5 µm
(2.1 mm x 100 mm)



macmod

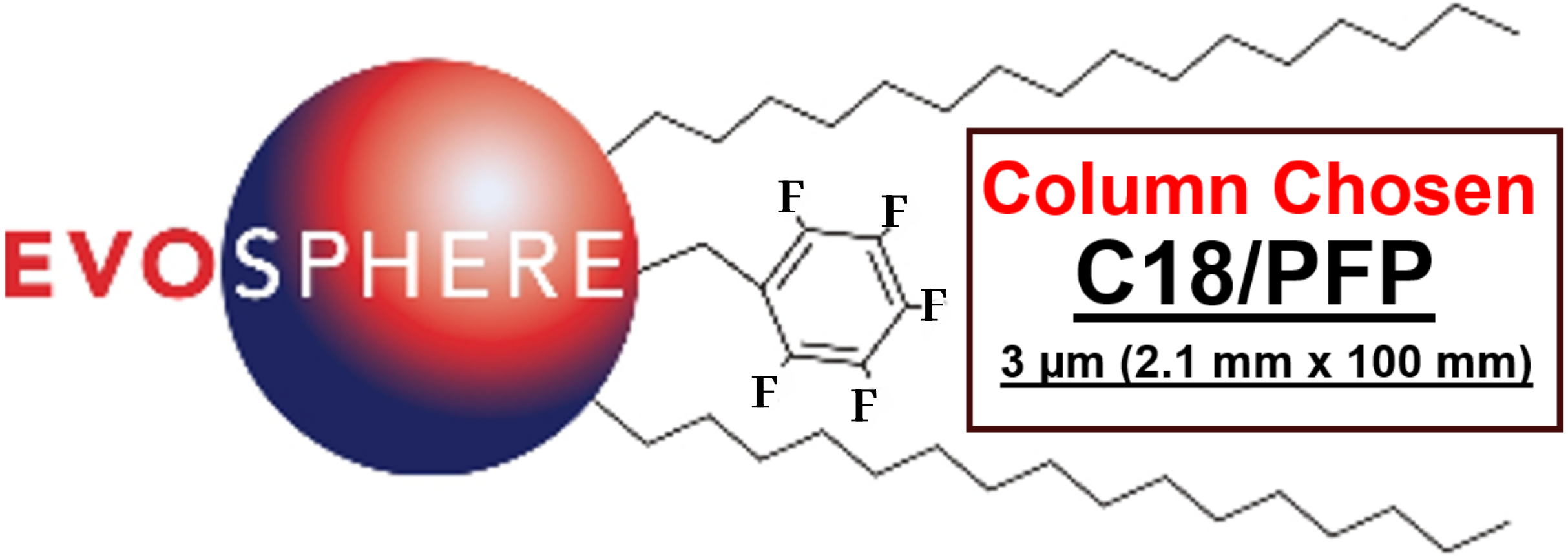
SMARTER CHROMATOGRAPHY

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

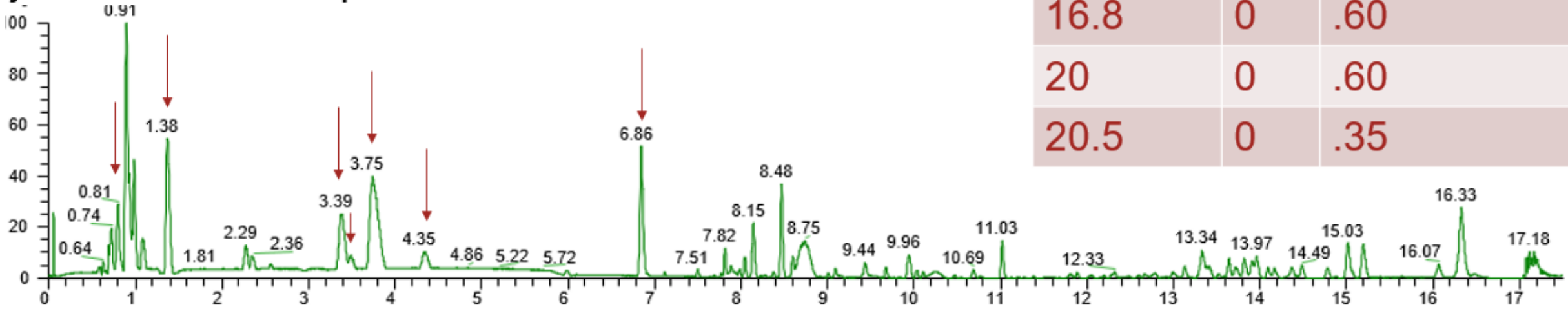
Mobile Phase A = 0.1% Formic Acid in H_2O

Mobile Phase B = Acetonitrile

Temperature = 25ºC

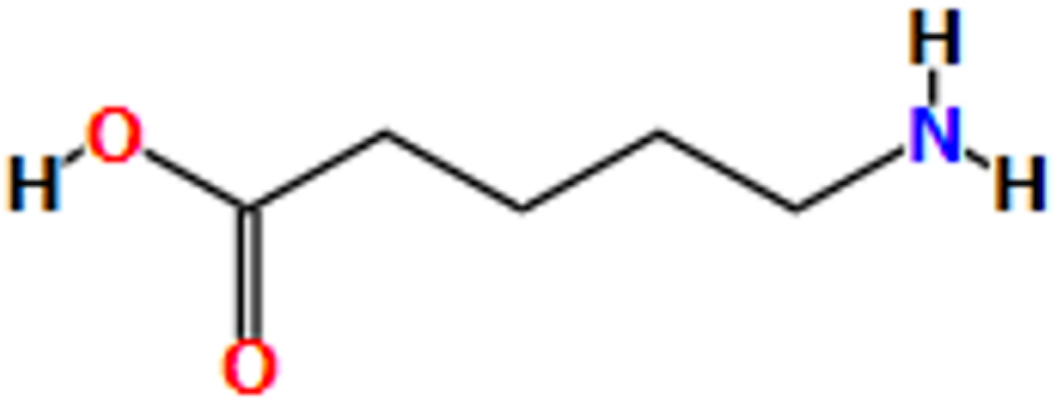
Injection volume = 2µL

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

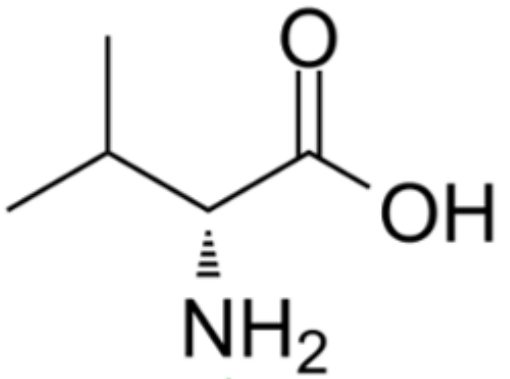


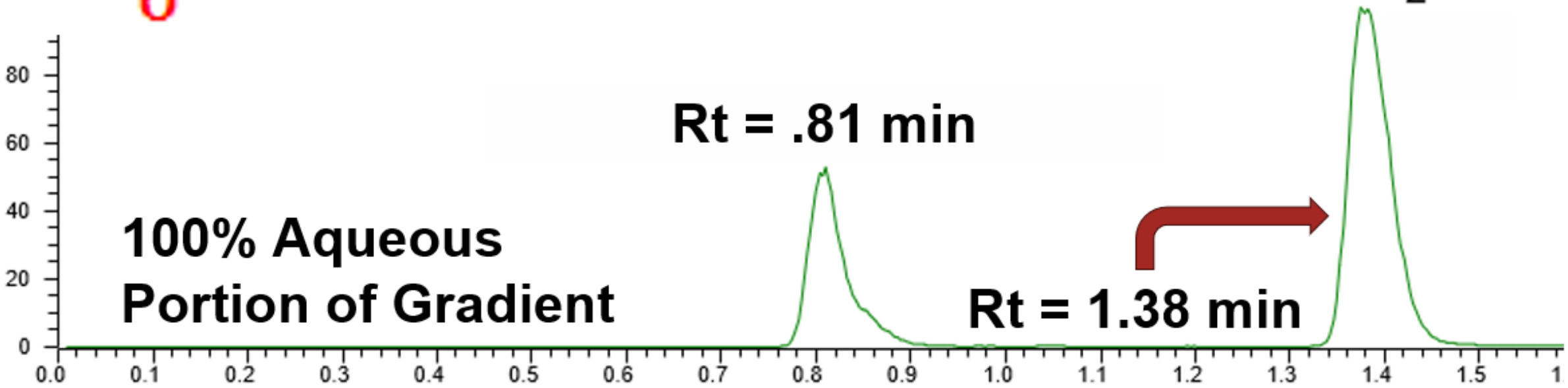
Polar Retention

5-Aminopentanoic Acid



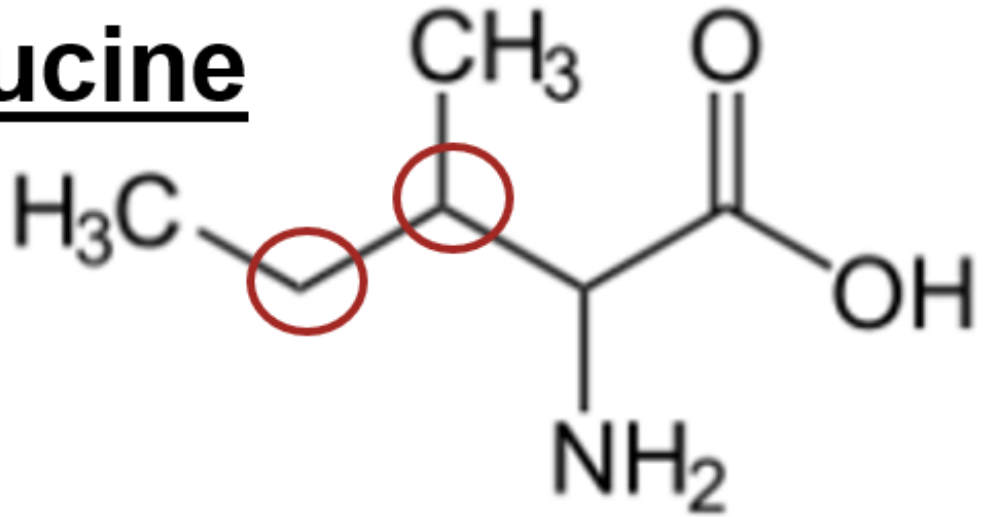
Valine



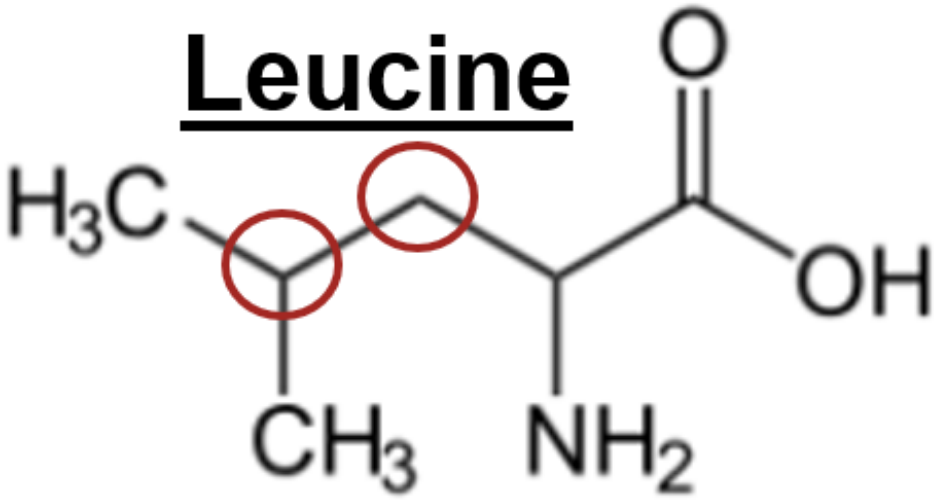


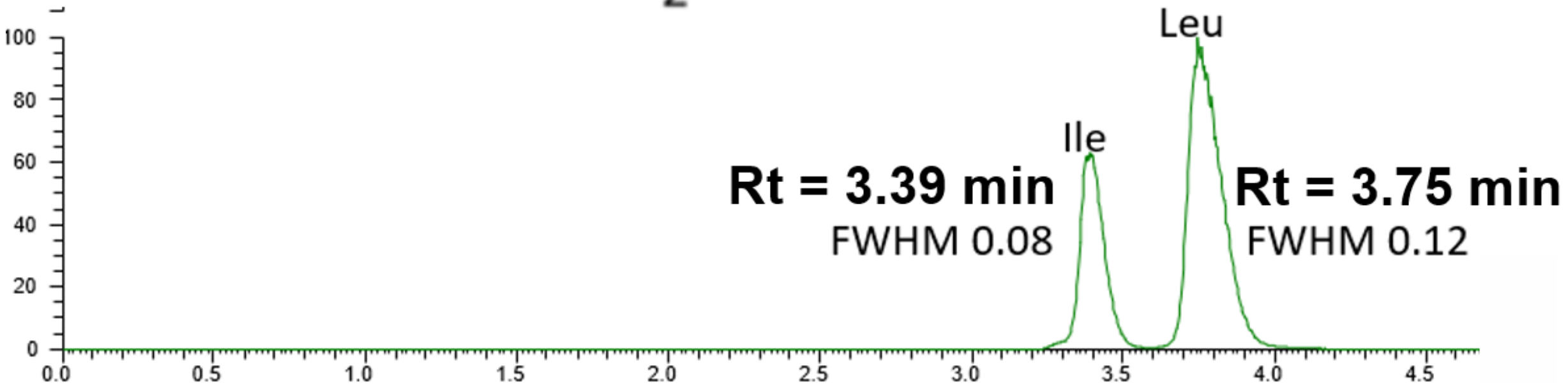
Methyl Position Switch

Isoleucine

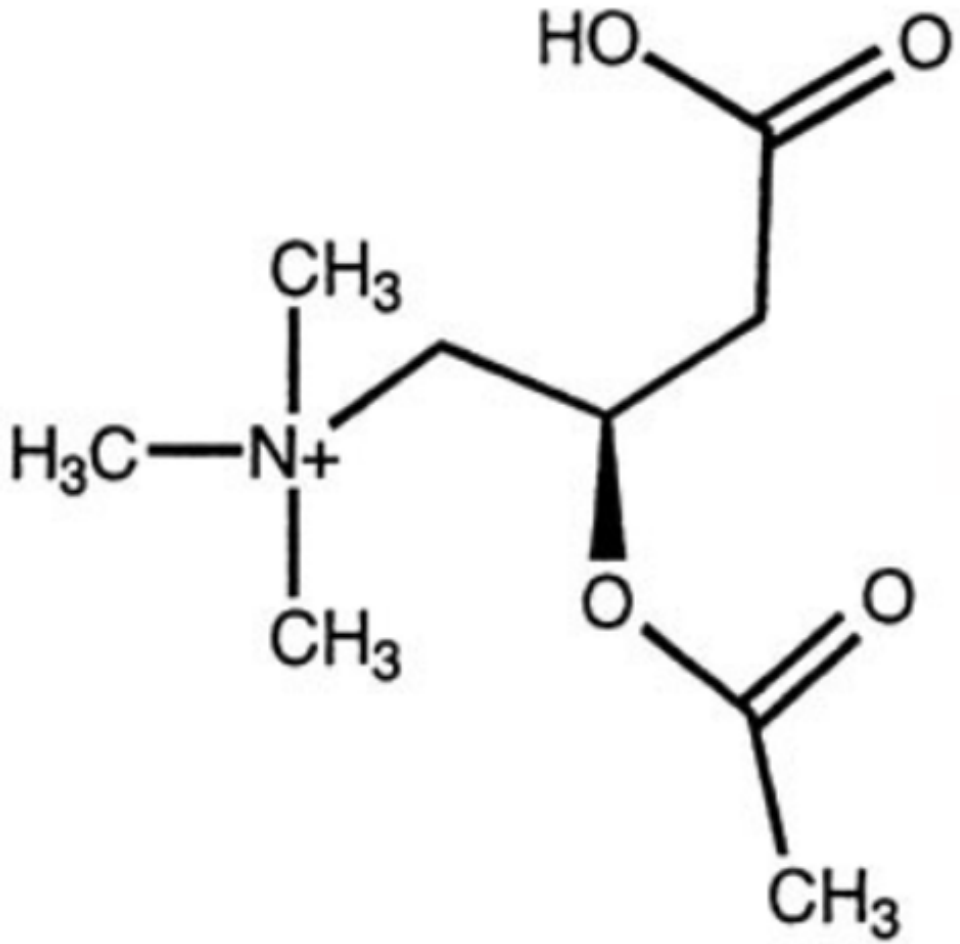


Leucine





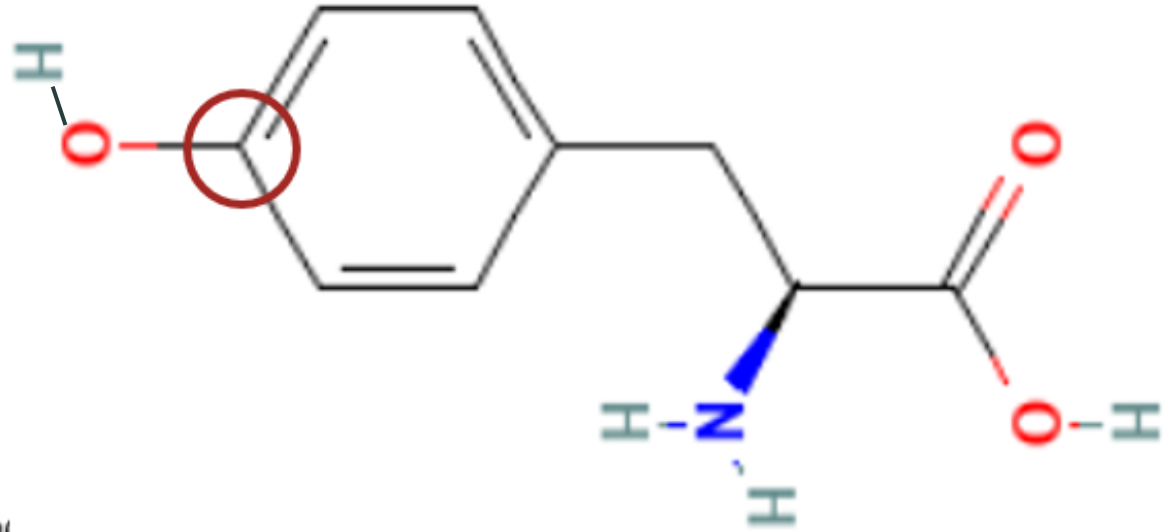
Optimal Peak Shape

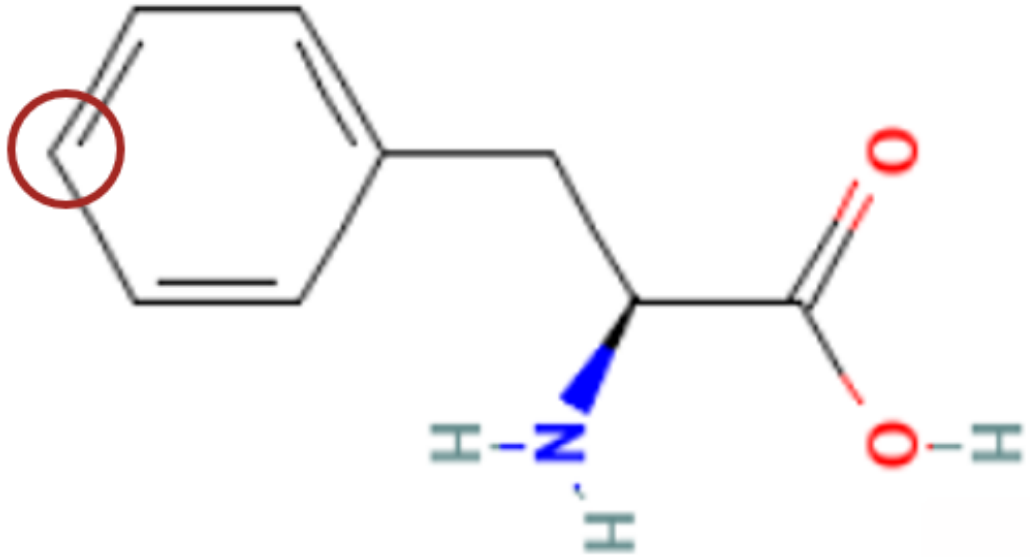


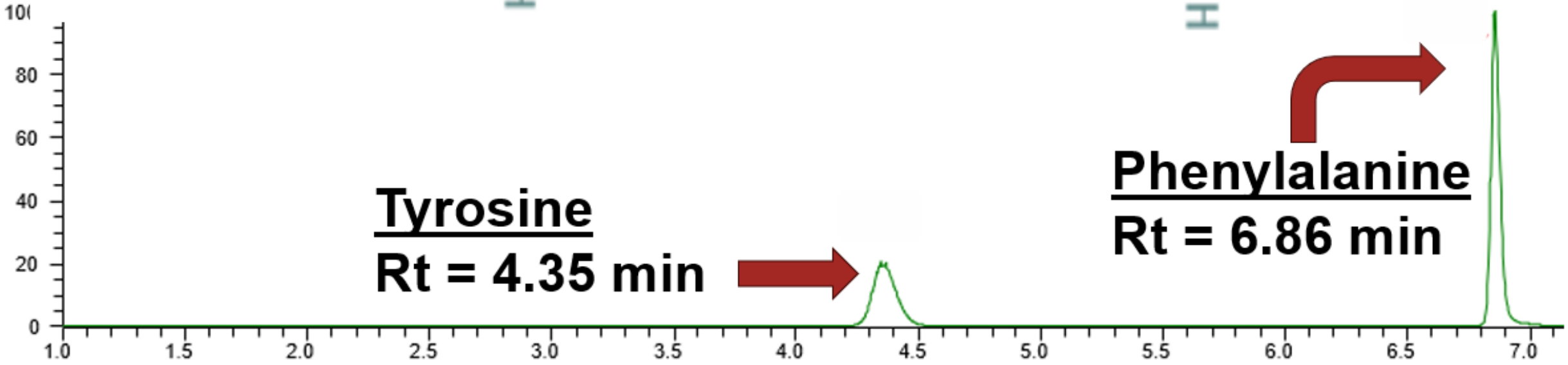
Acetyl-Carnitine

Rt = 3.51 min

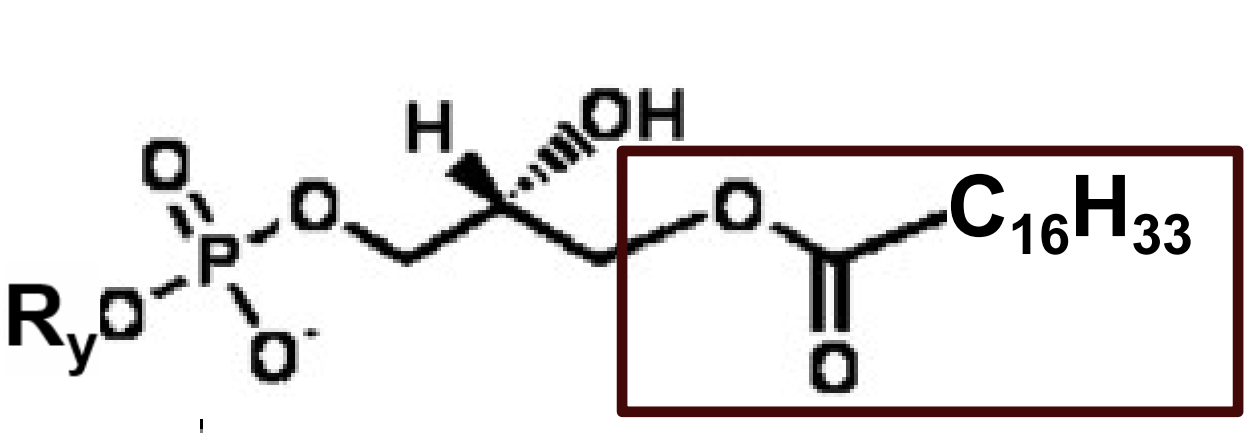
Strong Isomeric Selectivity

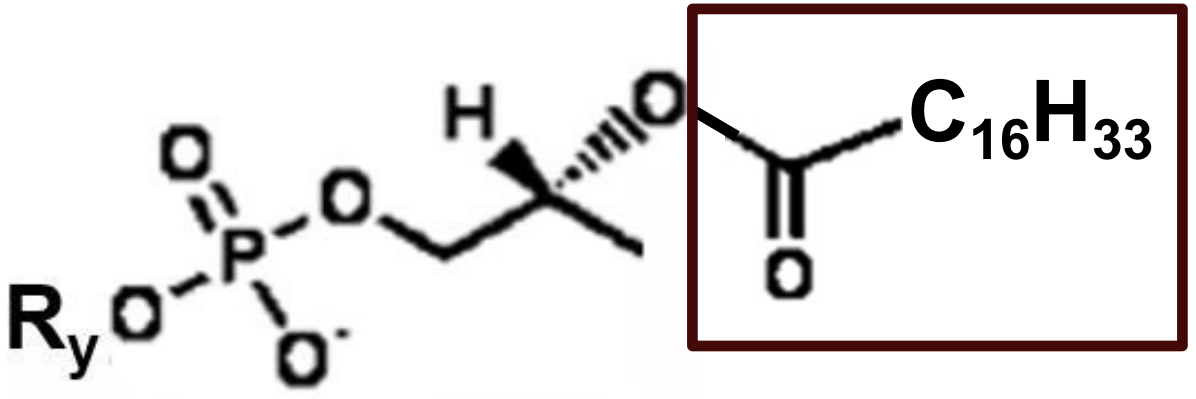




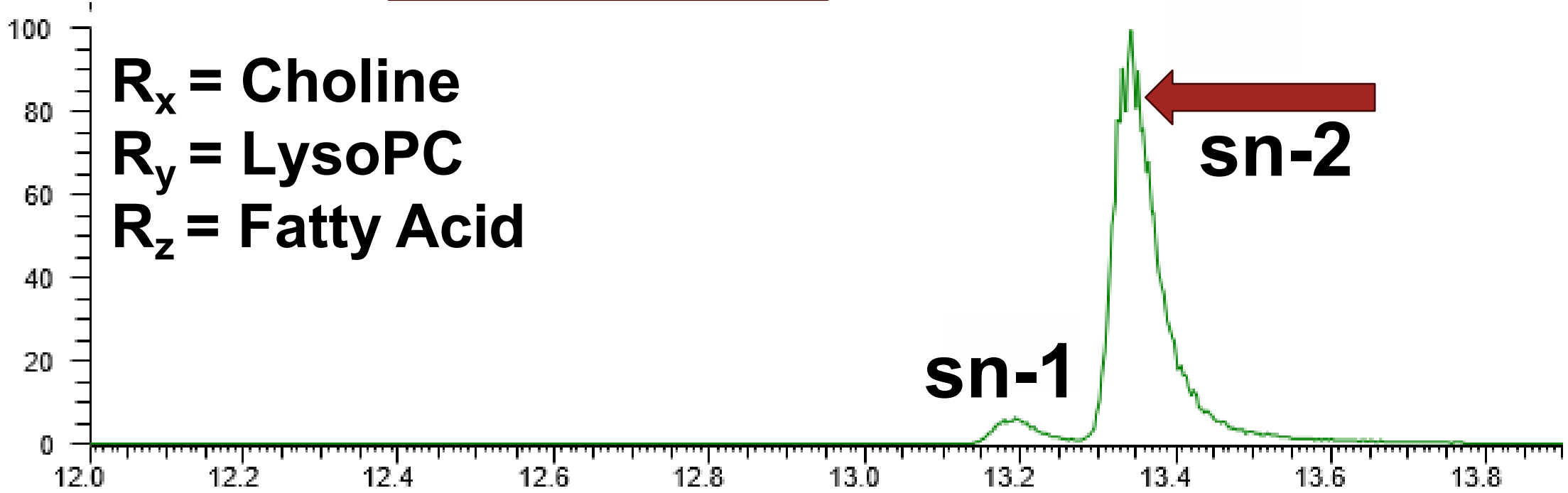


Lysophospholipid Isomers sn-1 vs sn-2 with C18/PFP





R_x = Choline
R_y = LysoPC
R_z = Fatty Acid



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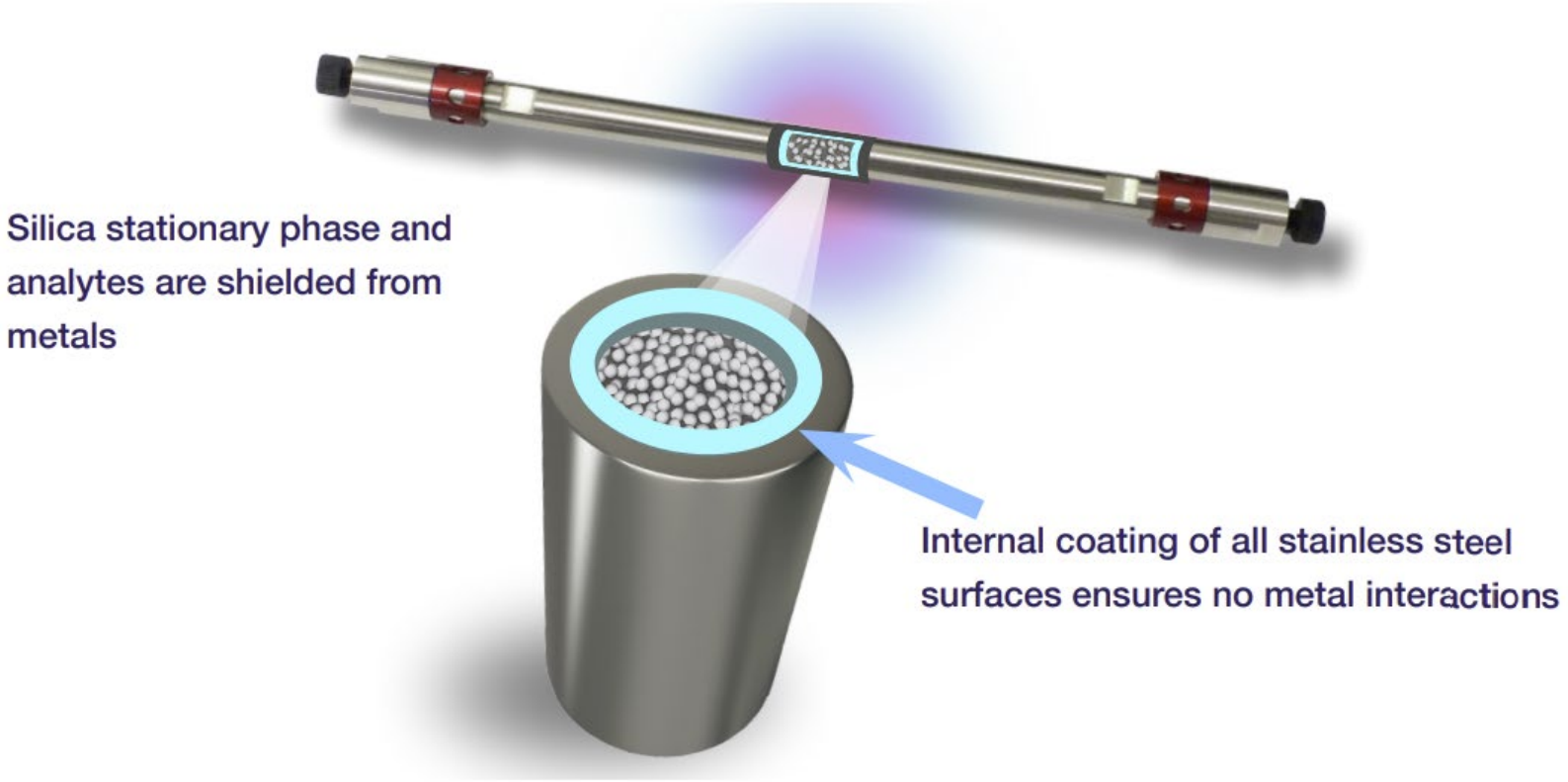
New Monodisperse Fully Porous Particle Products

Introducing Evosphere® MAX™ 100 Å UHPLC and HPLC Columns



- All bonded phases available in inert-coated MAX hardware
- Hardware available in 2.1, 3.0, 4.6, 10.0, 21.2 and 30.0 mm Column Internal Diameters
- 1.7, 3.0 and 5.0 µm particle sizes available
- Inert-Coated Hardware – Applications for small/single-stranded oligonucleotides and chelators among other small sticky molecules

Introducing NEW Evosphere BIO 300 Å UHPLC and HPLC Columns

Reversed-Phase Peptide and Protein Separations

Evosphere BIO 300 Å C12

Evosphere BIO 300 Å C4

Evosphere BIO 300 Å Diphenyl

Evosphere BIO 300 Å C18/AR

3 µm and 5 µm particle sizes

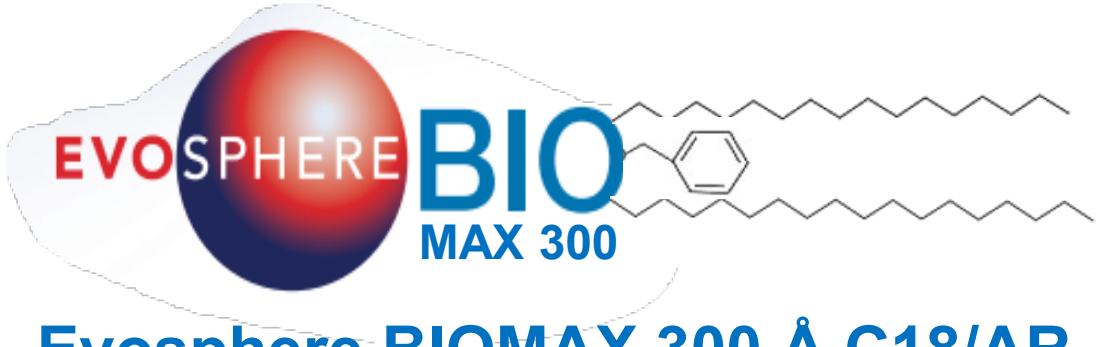
Introducing NEW Evosphere BIOMAX 300 Å UHPLC and HPLC Columns

Reversed-Phase Peptide and Protein Separations

Evosphere BIOMAX 300 Å C12

Evosphere BIOMAX 300 Å C4

Evosphere BIOMAX 300 Å Diphenyl



Evosphere BIOMAX 300 Å C18/AR

3 µm and 5 µm particle sizes

Conclusion

Monodisperse fully porous particle technology eliminates the weaknesses and combines the strengths of traditional superficially porous and fully porous particles.

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.

The introduction of MFPP in wide pore as well as MAX coated hardware further extends the unlimited application breadth of the new particle platform.

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Stay Tuned for Part II of the webinar series in partnership with CACA!
Leveraging this MFPP platform for Non-Ion Pair Oligonucleotide separations on February 5th

We will also give a sneak peak on further MFPP BIO additions