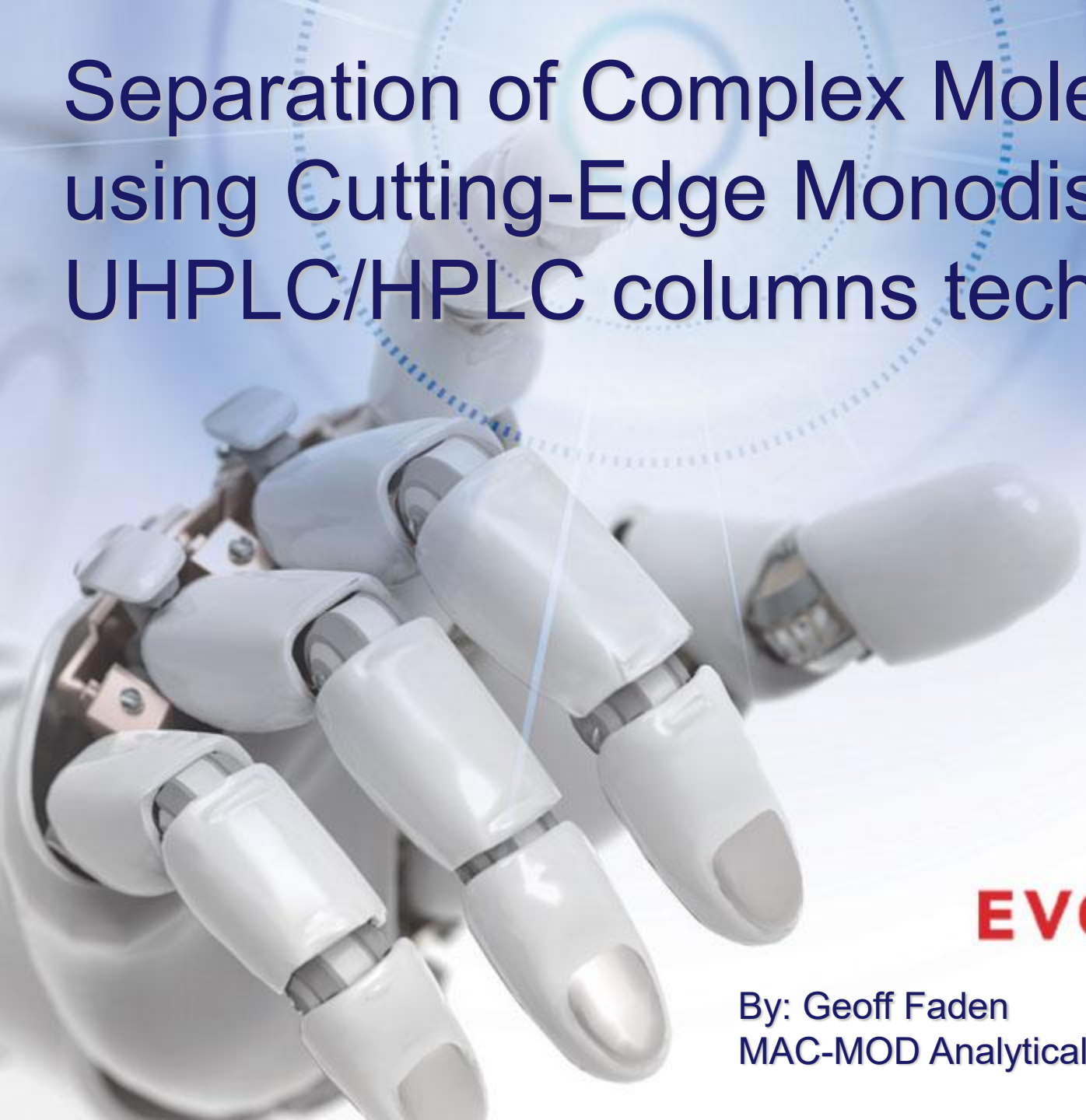
Separation of Complex Molecules using Cutting-Edge Monodisperse UHPLC/HPLC columns technology



EVOSPHERE

By: Geoff Faden
MAC-MOD Analytical INC.

Evolution of UHPLC & HPLC Particles

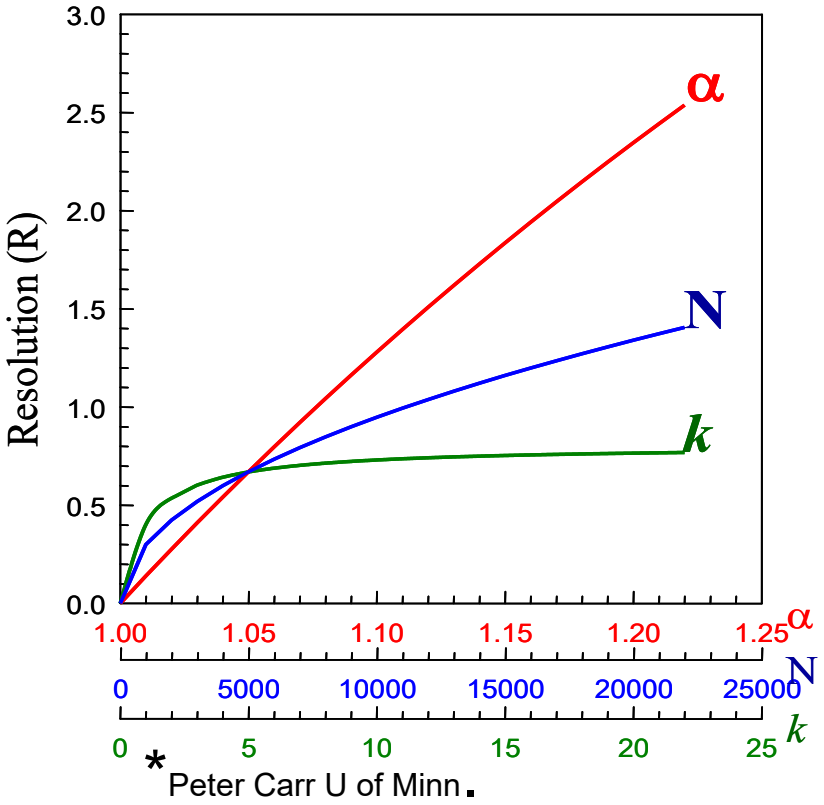
- Morphology – Shape
  - Irregular, spherical, core-shell, etc.
- Size – Reduction
  - 100 µm ⟶ 1.7 µm
- Silica Purity – Less Metals
- Size Distribution – Reduction in D90/D10
- Particle Hybridization –
  - Fully Silica, Coated Silica, Hybridized Silica

Resolution Equation

Efficiency Retention Selectivity

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

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



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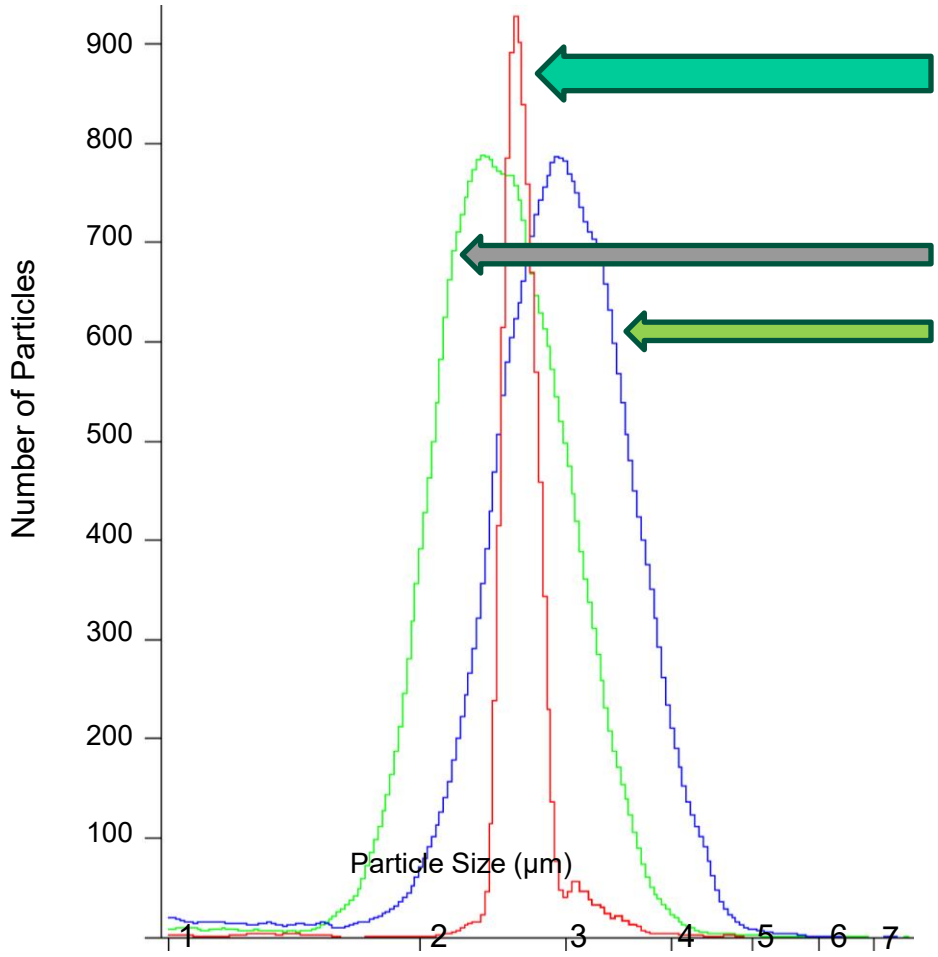
Monodisperse vs. Competitor Polydisperse Comparisons



Monodisperse FPP 3 µm

Commercial FPP A 3 µm

Commercial FPP B 3 µm

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Defining Monodispersity

	Monodisperse silica	Commercial 3u silica - A	Commercial 3u Silica-B
Mean particle size (d50) *	2.66µm*	2.49µm	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

*Measured by Coulter Counter

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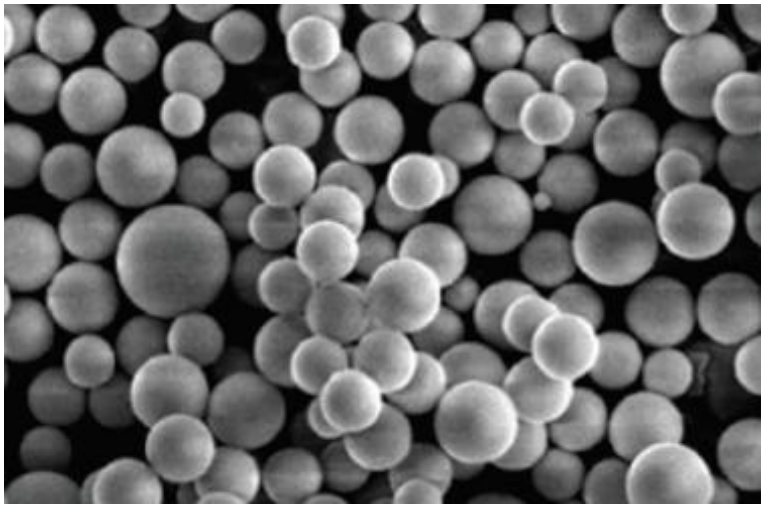
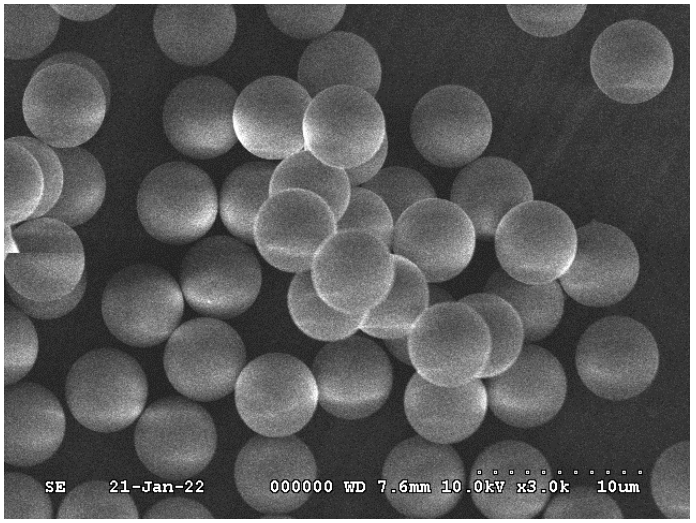
SEM Comparison of Particles



Traditional
porous particles



Monodisperse
Fully Porous Particles

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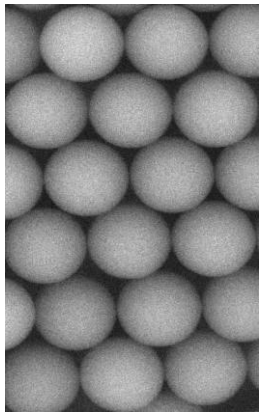
What is the Effect of Monodispersity

When particles have **very narrow size distribution**, they can arrange more regularly during column packing.



Effects include:
•More **uniform interstitial channels**
•Reduced **void heterogeneity**
•Less **local channeling**

This reduces the **variance of flow velocities** across the column.

However, **monodispersity alone does not directly shorten diffusion distances** inside a particle. Instead, it allows the bed to approach an **ordered packing structure** (often close to hexagonal or Face centred cubic (FCC) locally).

Effect of Improved Packed Bed Structure

The improved packing structure leads to:

- **Lower eddy dispersion (A-term)**
- **More uniform tortuosity**
- **Narrower flow path distribution**

In the **van Deemter framework**, this appears as a **smaller A-term**:

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

Monodisperse packings often achieve:

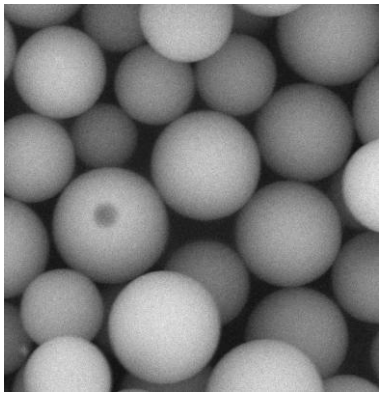
- **Reduced plate height** $h = H/d_p$ of **~1.5–2**
- Compared with **~2–3 for polydisperse packings**

This improvement arises from **more homogeneous interstitial flow paths**, not from a fundamentally different molecular diffusion mechanism.

Why Polydisperse Particles Give less Performance

If particle sizes vary:

- Smaller particles fill gaps between larger ones
- Packing becomes **structurally heterogeneous**
- Flow channels become **irregular**
- Some regions have **fast flow**, others **slow flow**



This increases **dispersion** by **eddy diffusion** because molecules experience different path lengths.

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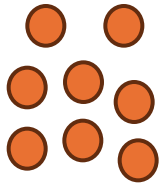
Key Findings of Monodispersity

The **reduced flow path dispersion** is:

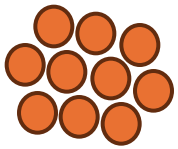
- **Not directly a property of monodispersity**

But……it Is a property of the improved bed geometry that monodispersity enables

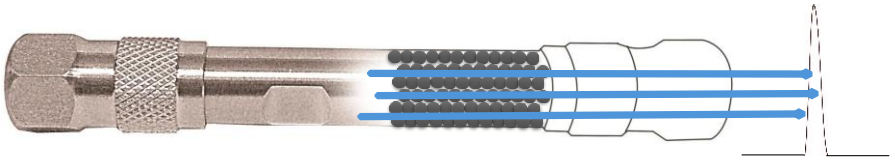
Monodisperse HPLC - Causal Effect



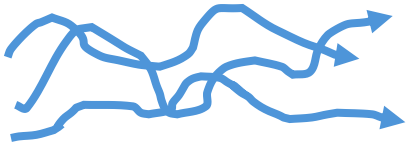
Monodisperse Particles



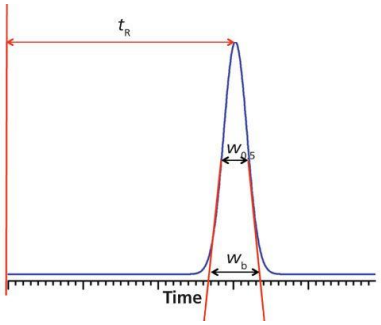
More Ordered Packing



More uniform flow channels



Reduced Eddy Dispersion



Lower plate height

Higher Efficiency

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Column Plate count Theory versus Reality

- **5 µm fully porous columns (150 mm)**
→ ~10,000–15,000 plates
→ **~70k–100k plates/m**

- **3 µm columns (100 mm)**
→ ~12,000–18,000 plates
→ **~100k–140k plates/m**

- **1.7 µm UHPLC columns (100 mm)**
→ ~20,000–35,000 plates
→ **~170k–250k plates/m**

- **2.6 µm core-shell columns (100 mm)**
→ ~18,000–25,000 plates
→ **~160k–240k plates/m**

What manufacturers typically report

Examples from common column types:

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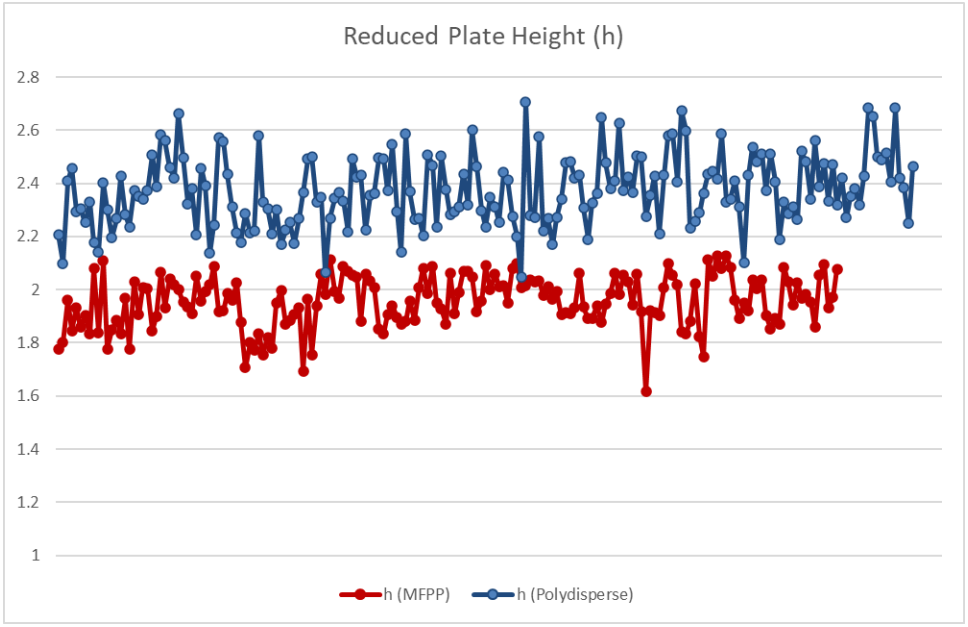
Monodisperse vs Polydisperse



	h		N/m
Max	2.817	=	71,042
Min	2.046	=	97,809
Ave	2.386	=	83,849

5um Polydisperse

Max	2.120	=	94,680
Min	1.615	=	123,842
Ave	1.950	=	102,585

5um Monodisperse

In other words:

- **Monodispersity → enables better packing**
- **Better packing → produces the shorter / more uniform flow paths**

The **reduced flow path in columns packed with monodisperse particles** is primarily a consequence of the **improved packed bed structure**, although **monodispersity is the enabling factor** that allows that packing structure to form.

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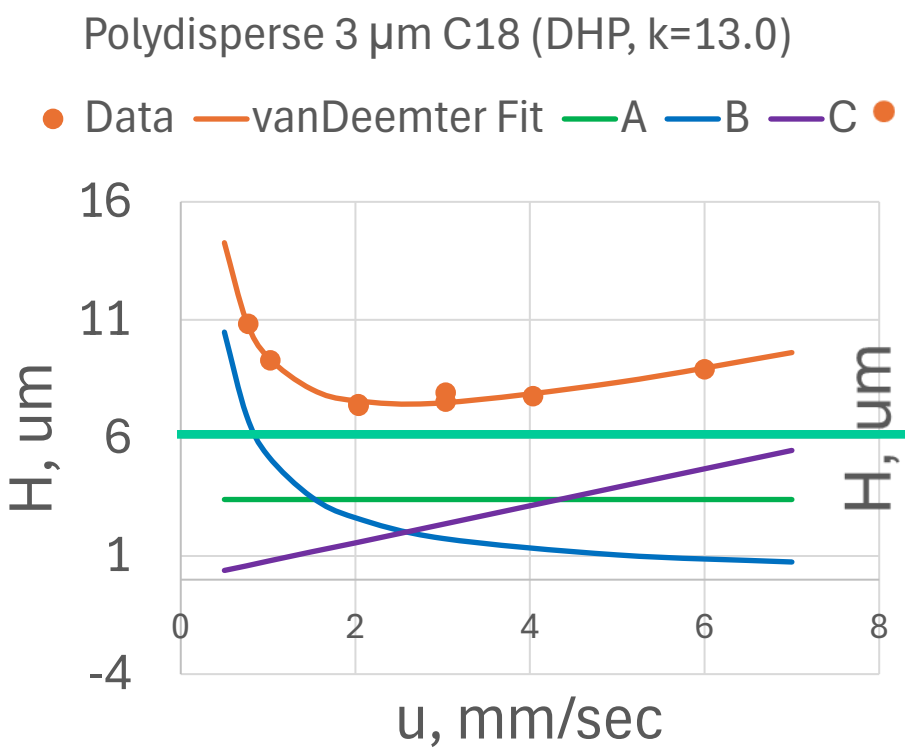
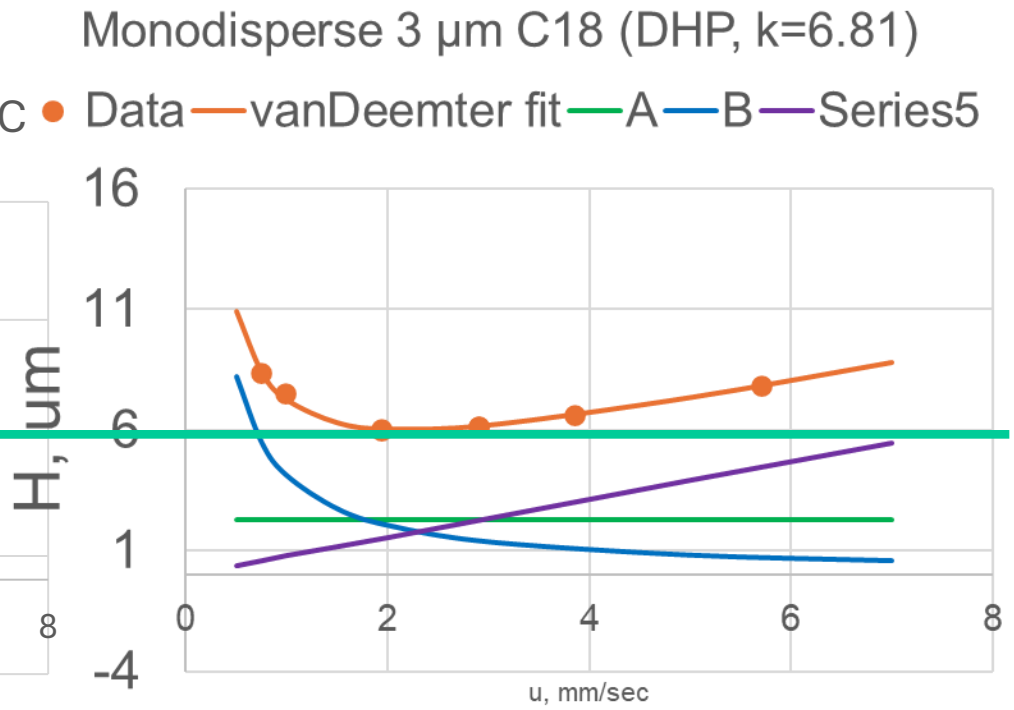
Experimental Van Deemter fits for Dihexylphthalate (DHP) of Polydisperse vs Monodisperse





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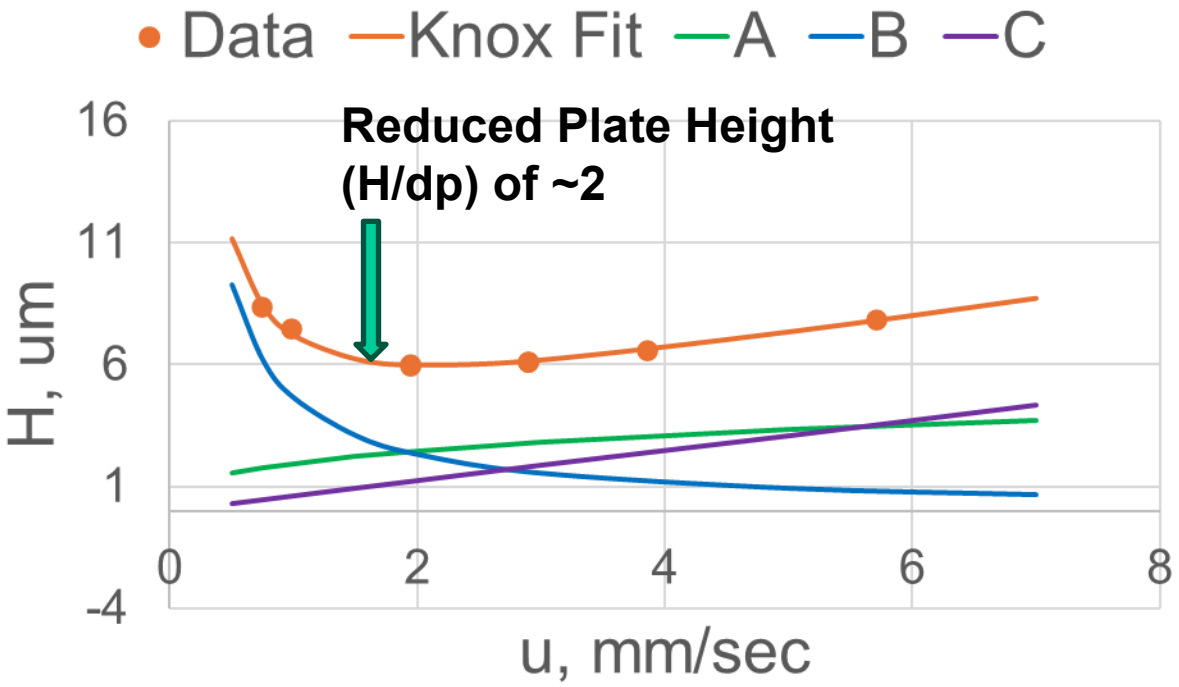
Monodisperse 3 µm (3.0 mm x 50 mm) Van Deemter Curve for Dihexylphthalate (DHP)



Data Generated by Merlin Bicking from ACCTA, Inc.

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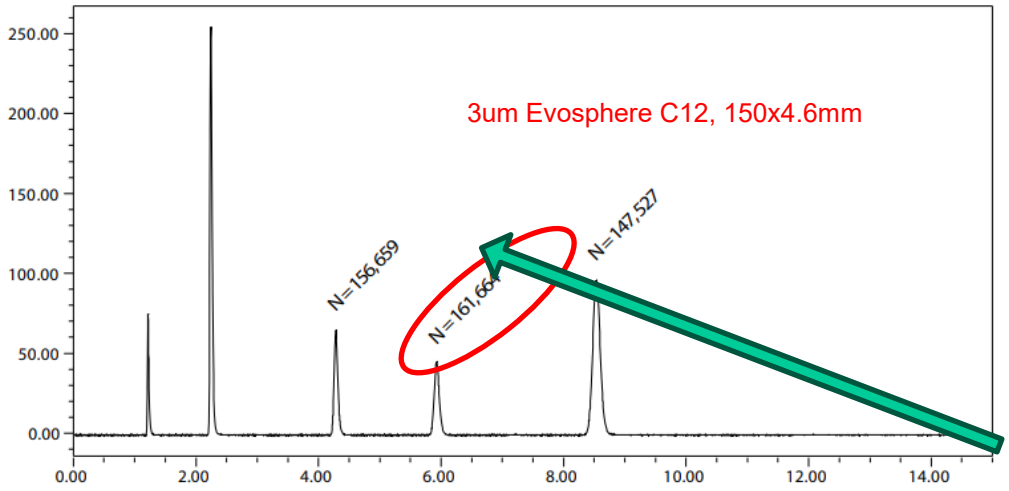
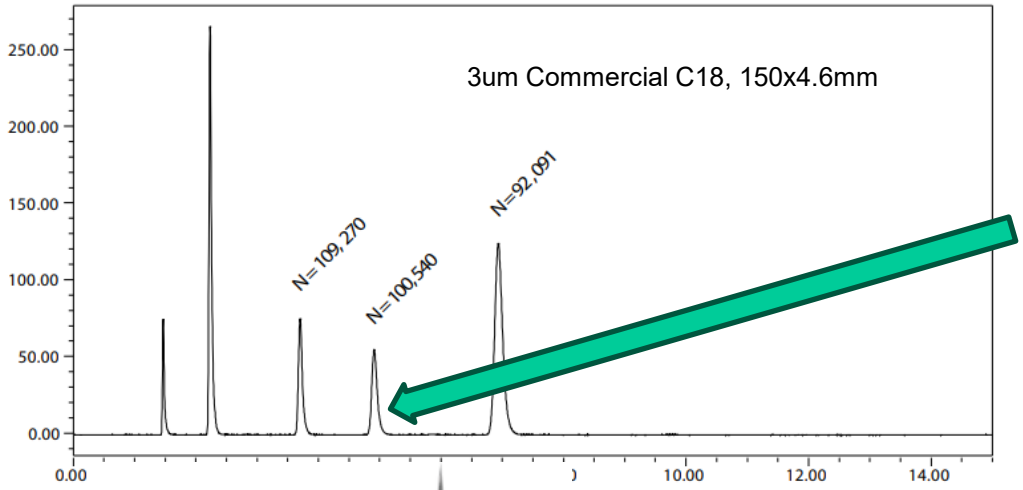
Unprecedented (N) Efficiency Gains



MONODISPERSE

60% Higher N



POLYDISPERSE

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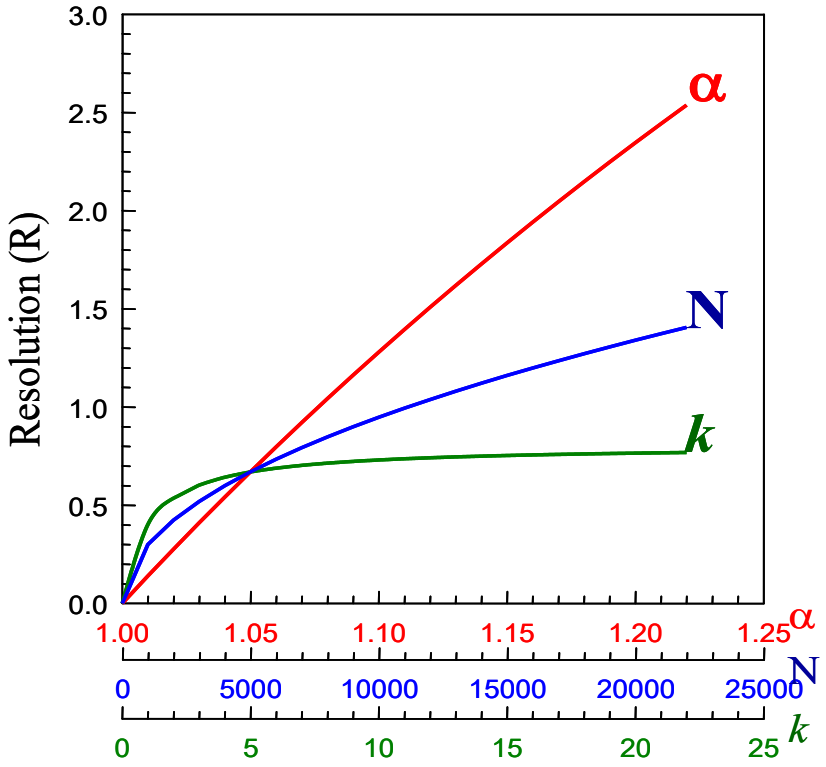
Resolution Equation Pt. 2

Efficiency Retention Selectivity

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



Selectivity is the Key to Resolution!

*P.W. Carr's Group U of M

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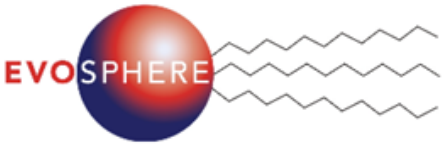
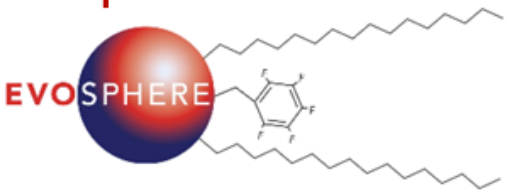
Powerful Selectivity Choices

Evosphere C12



Evosphere Diphenyl

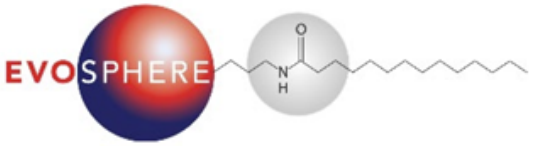
Evosphere C18/PFP



Efficiency Retention Selectivity

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

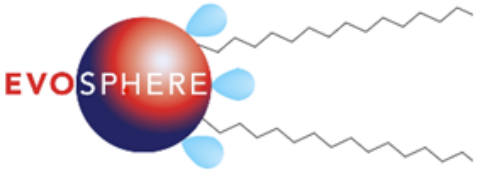
Evosphere RP18-Amide



Evosphere C18/AR

Evosphere Phenyl-Hexyl

Evosphere AQUA



Evosphere PFP

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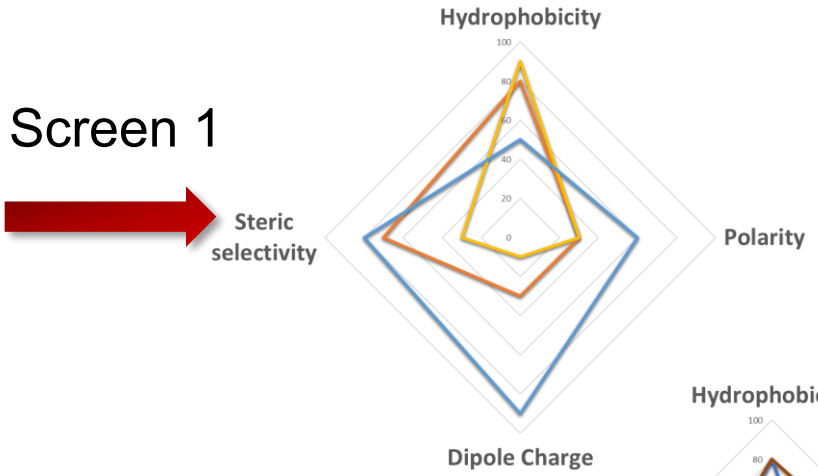
Orthoganol Selectivity

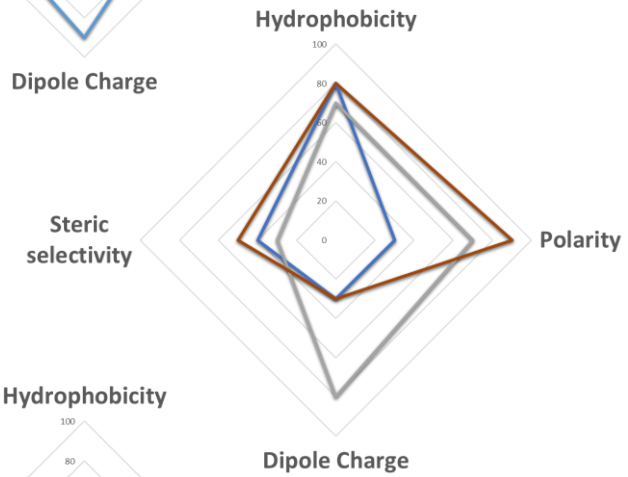
C12
Diphenyl
C18/PFP

Screen 1



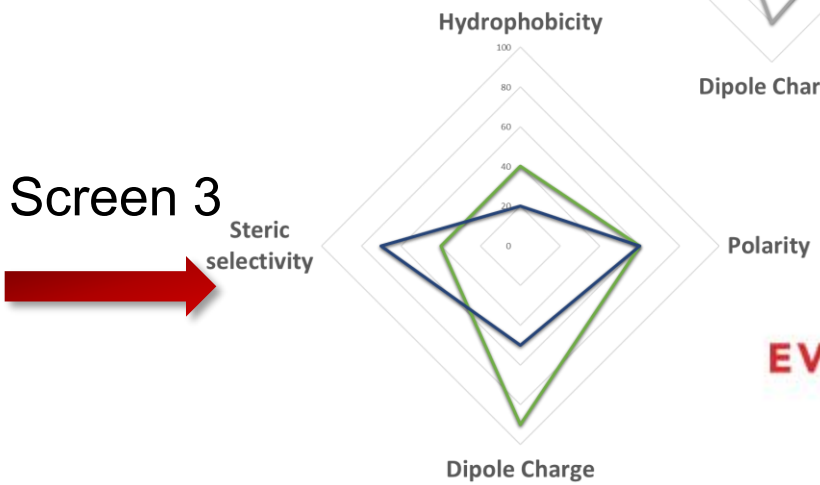
AQUA
C18/AR
RP18-Amide

Screen 2



PFP
Phenyl-Hexyl

Screen 3



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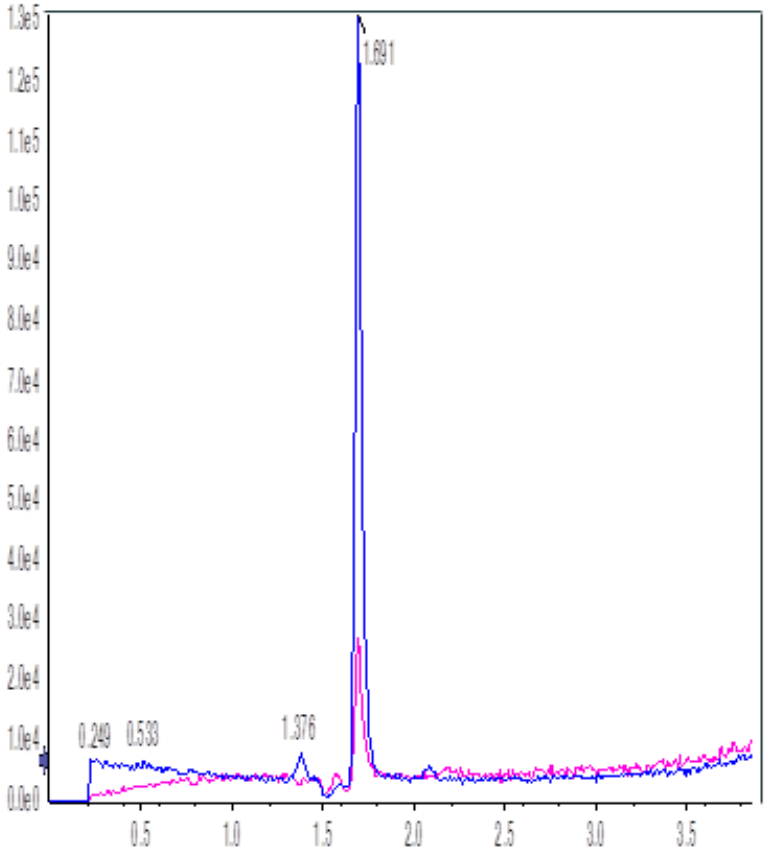
Challenges - a LC perspective

- Complex molecules require:
- Retention
  - *Polar compounds are difficult to retain in RP*
  - *Non-polar*
- Sensitivity
    - *Peak height (MS)*
    - *LOD*
- Resolution
    - *MS (limited MP choices) vs LC (expanded buffer ranges)*
    - *Reproducibility*
    - *Ruggedness*

Sugar Analysis in Reversed Phase
Polar Retention - Dextrose (D-Glucose) on Evosphere Diphenyl



Column: Evosphere Diphenyl
Column Format: 100 Å, 1.7 µm, 3.0 x 100 mm
Mobile Phase

- **A: 0.1% Formic Acid in Water**
- **B: 0.1% Formic Acid in 50% Methanol and 50% Acetonitrile**

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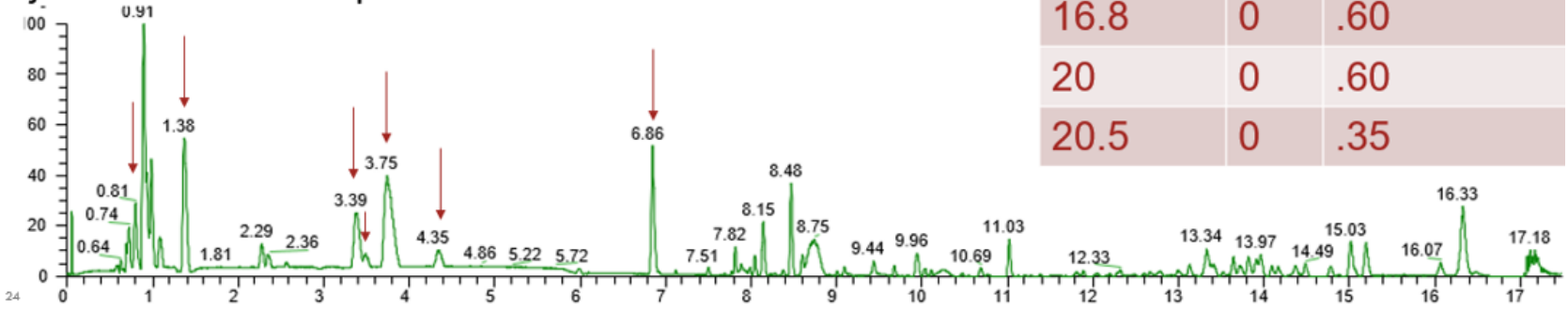
Untargeted Metabolomics Screen - TIC

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



Data Generated from Tim Garret PhD

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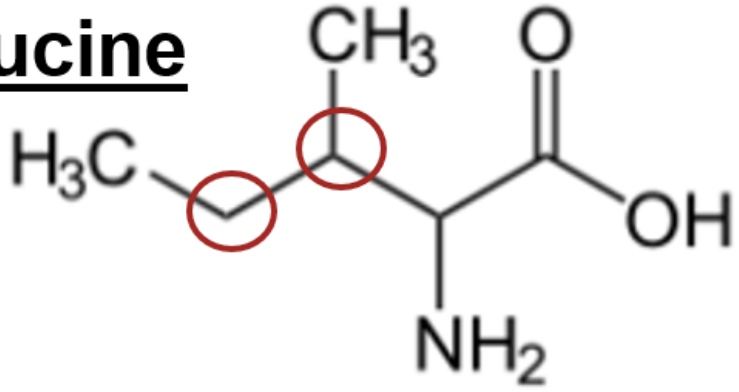
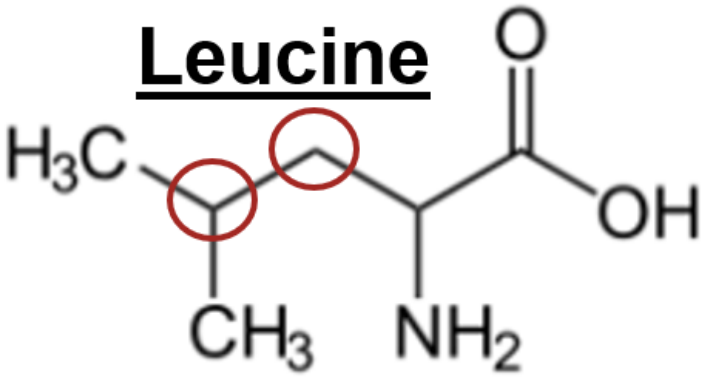
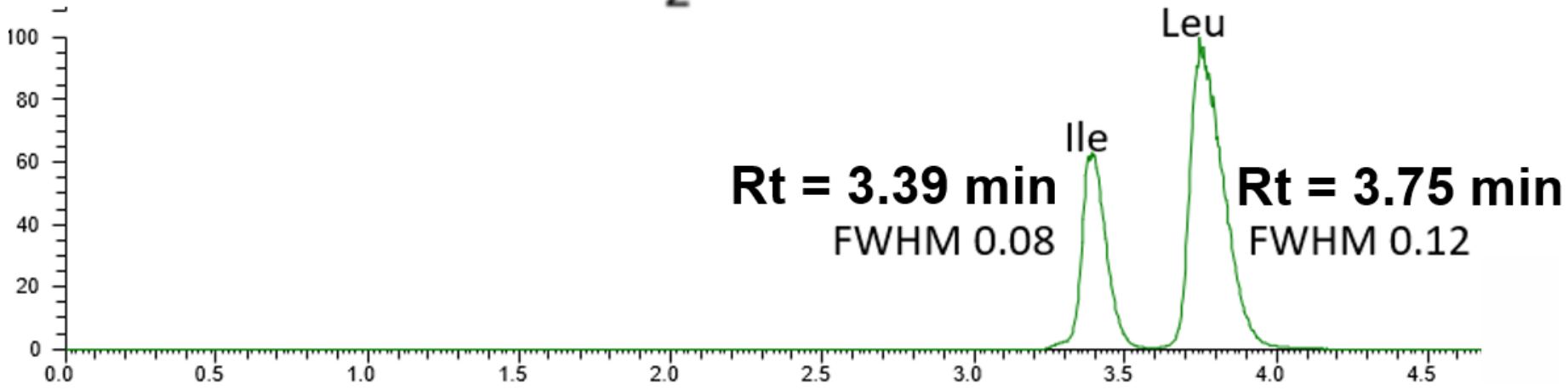
Methyl Position Switch

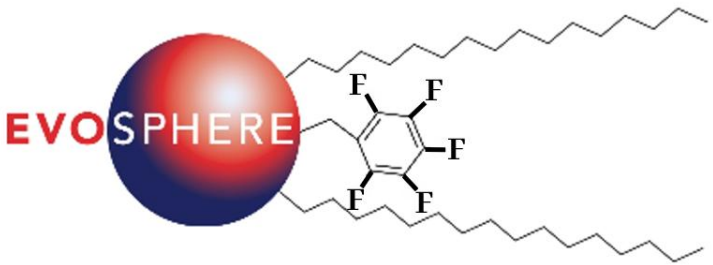
Isoleucine



Leucine







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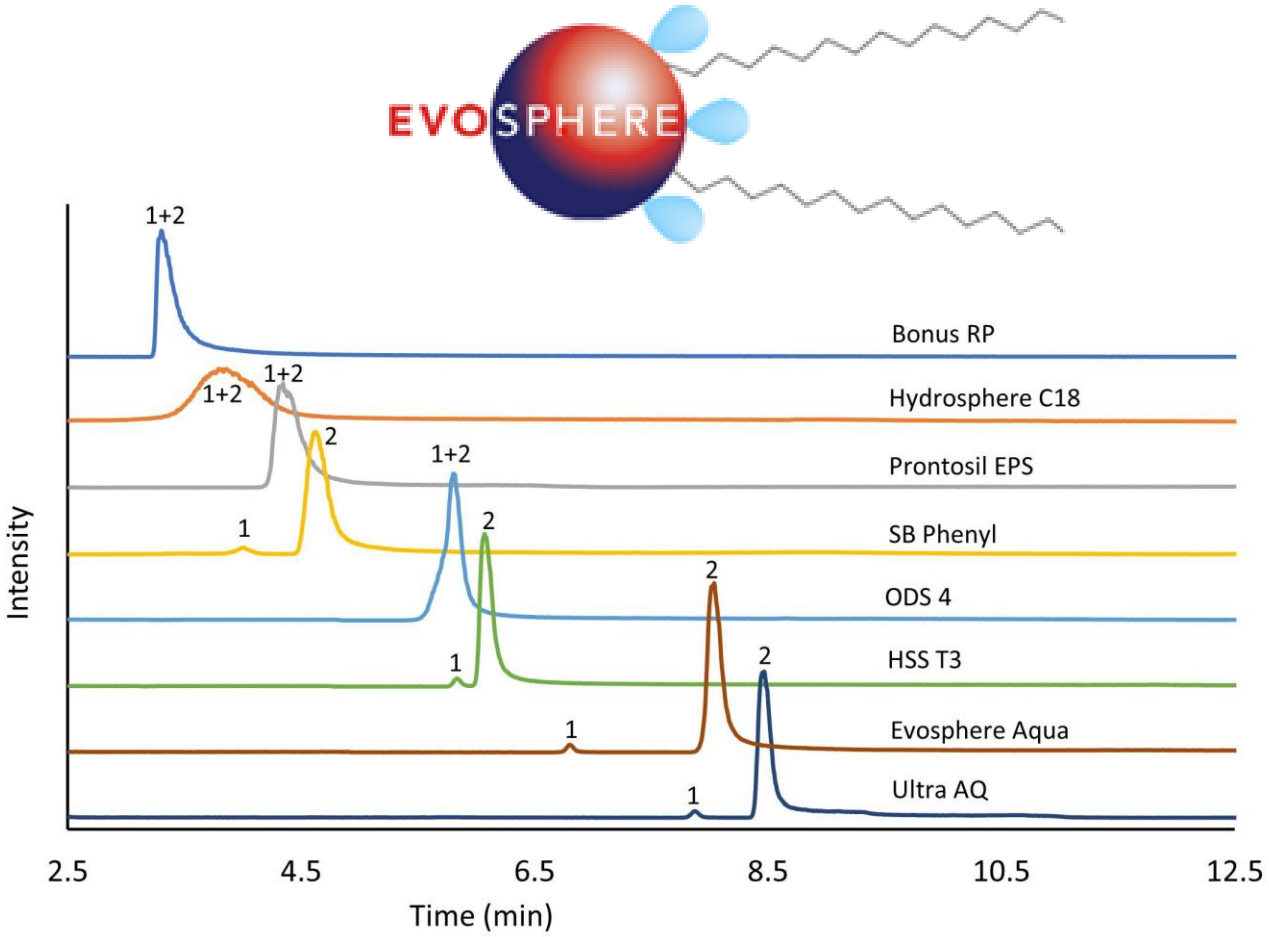
Initial Merck Screening wettable phase chemistries for Nitrosamine studies



The link to the online article - Analysis of N-Nitrosodimethylamine (Ndma) in Pharmaceutical Products in the Presence of High Concentration N,N-Dimethylformamide (Dmf) by Jinjian Zheng, Mikhaila D. Ritz, Ana I. Martinez, Timothy Yaroshuk, Xihui Liang, Xiaoyi Gong, Mark D. Mowery :: SSRN

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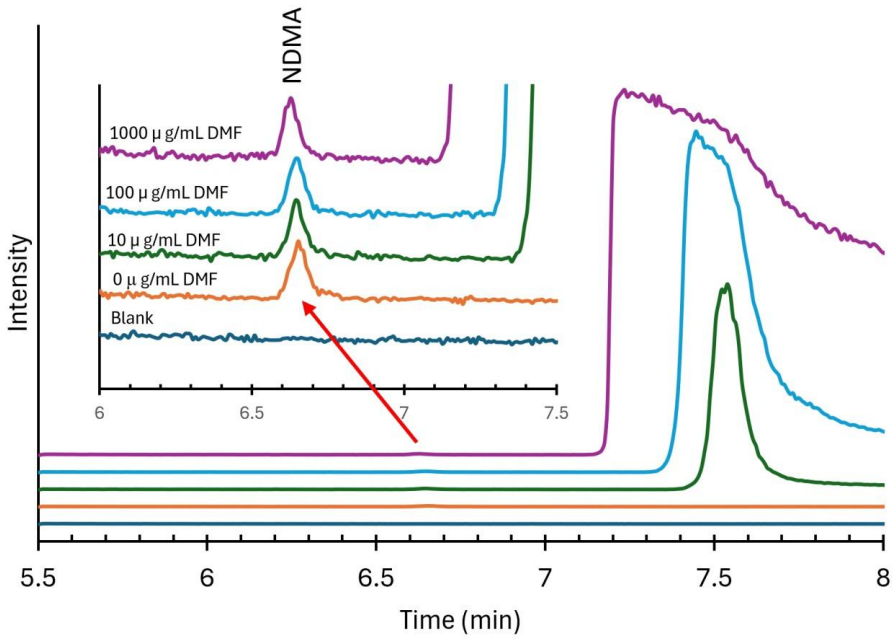
Why Evosphere Aqua was chosen:

7a.



- Significant resolution between DMF and NDMA
- Multiple lots were validated multiple sites
- Separation confirmation was done via single quad MS, but validated on LC-UV
- Enough Rs to overload $1x10^6$:1 DMF to NDMA

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Non-Ion-Pair Method for Oligos on Evosphere C18/AR INERT

Column: Evosphere C18/AR INERT 1.7 µm (2.1 mm x 100 mm)
Mobile Phase - (Unmodified Oligonucleotides) - 10-30% v/v MeOH in 10 minutes
Mobile Phase - (Modified Oligonucleotides) - 15-50% v/v MeOH in 10 minutes
Temperature - 30°C or 60°C
Flow Rate - 0.3 mL/min
Injection Volume - 0.5 µL
Instrument - Thermo Scientific™ Vanquish™ Horizon UHPLC system with DAD
Autosampler Temperature - 4°C
UV Wavelength – 260 nm

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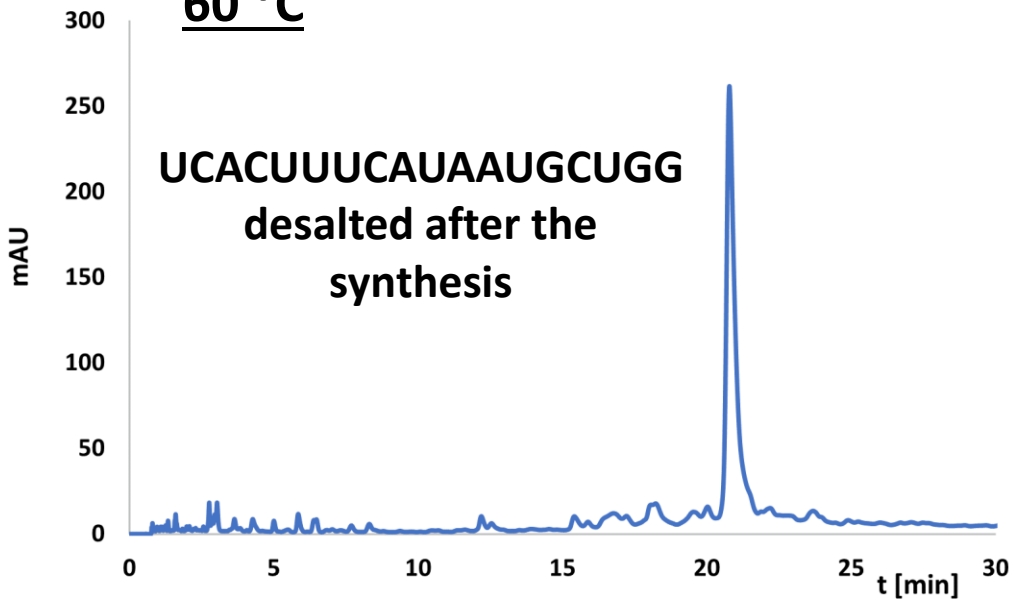
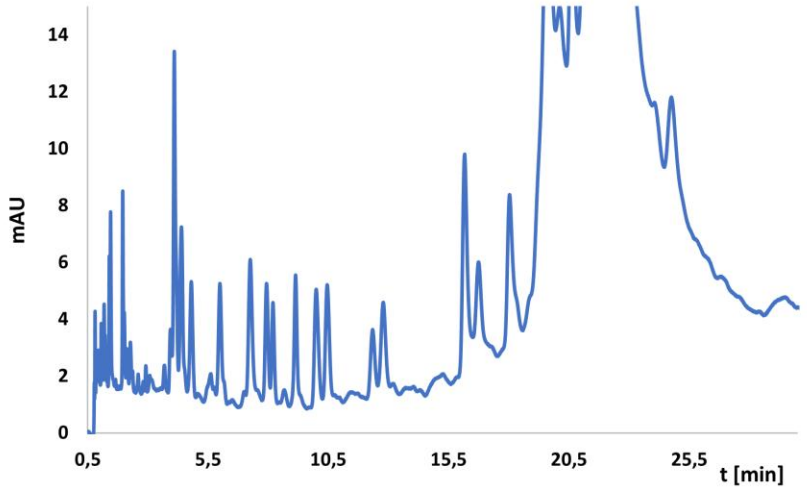
Unpurified Oligonucleotide Separation

25mM Amm. Ac. pH 6
2-8% MeOH 30min
60 °C



Magnified Chromatogram



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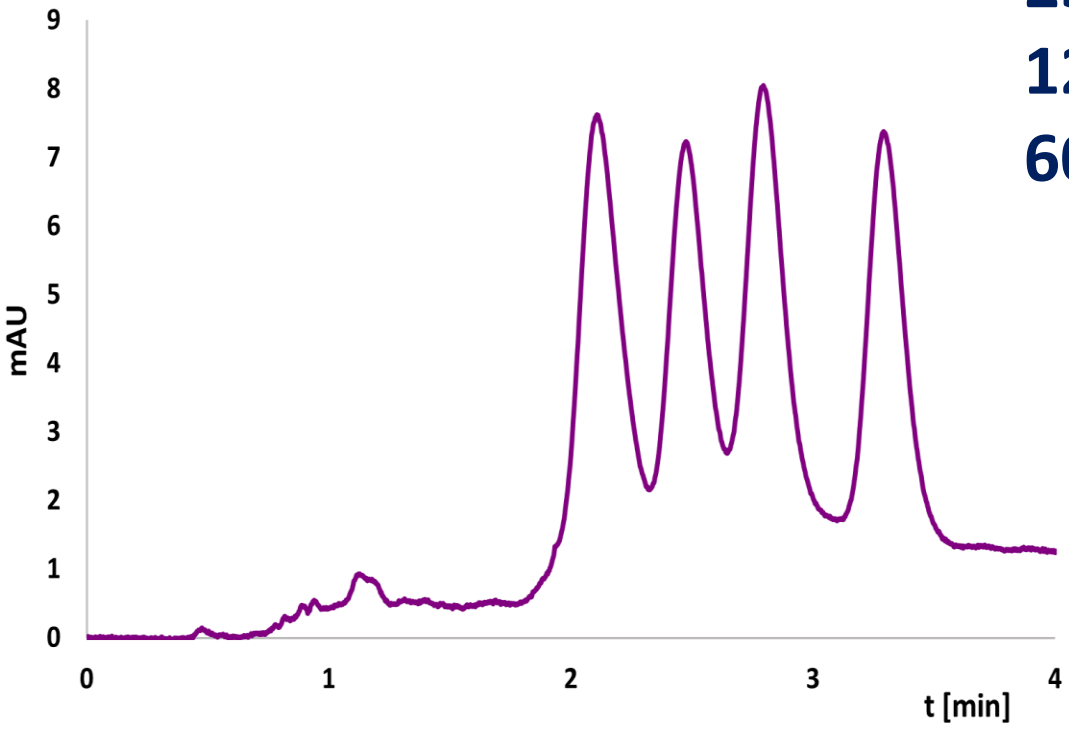
Separation of Unmodified Sequence Isomers



25mM AA pH=6

12-20 % MeOH in 10min

60 °C

1 - ATCGATCGATCGATCGATCG
2 - ATCGATCGATCGATCGATCT
3 - ATCGATCGATCGATCGATCC
4 - ATCGATCGATCGATCGATCA

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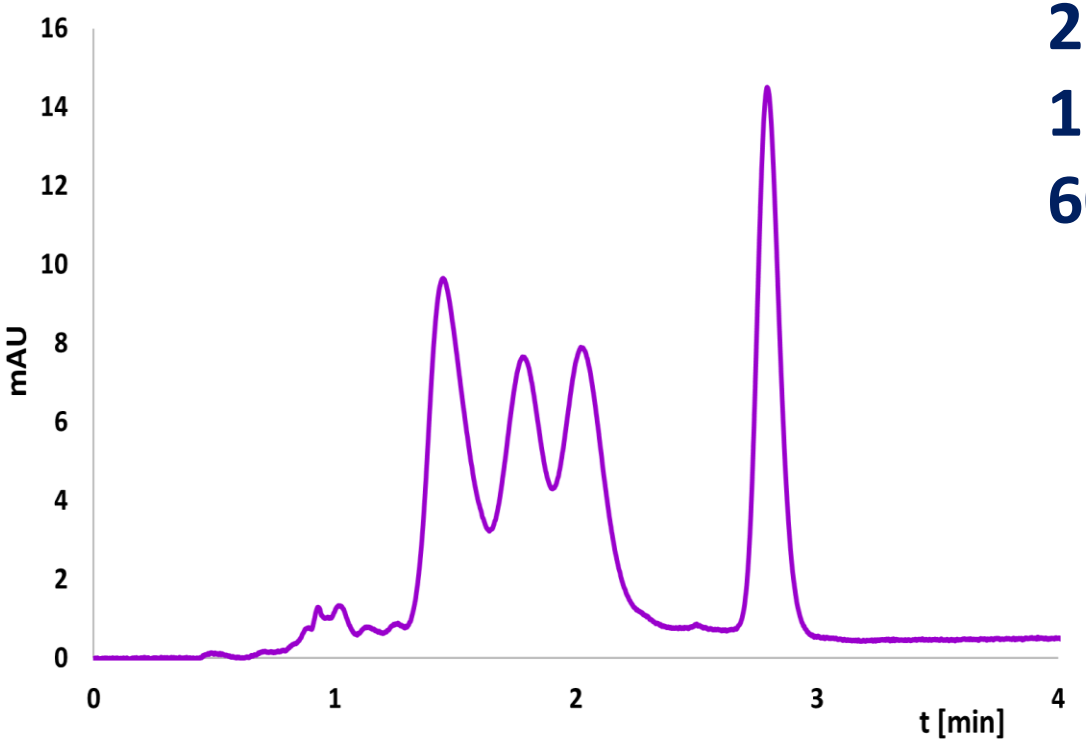
EVOSPHERE

Separation of Sequence Isomers



25mM AA pH=6
12-20 % MeOH in 10min
60 °C

1 - ATCGATCGAGCGATCGATCG
2 - ATCGATCGAACGATCGATCG
3 - ATCGATCGATCGATCGATCG
4 - ATCGATCGACCGATCGATCG

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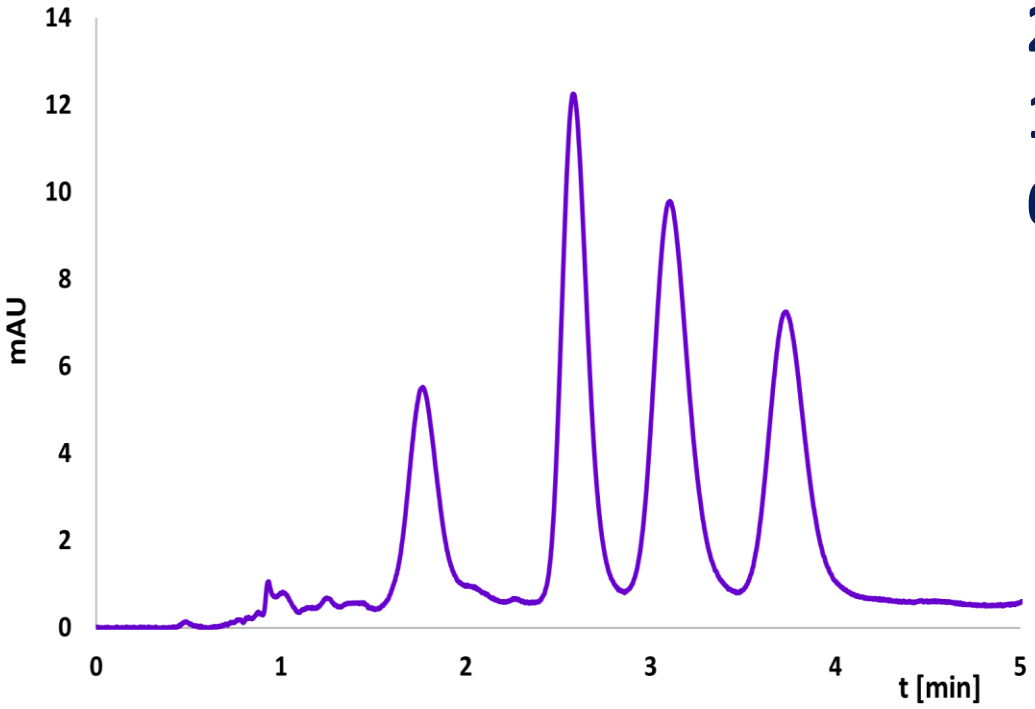
EVOSPHERE

Separation of Sequence Isomers



25mM AA pH=6

12-20 % MeOH in 10min

60 °C

1 - ATCGATCGAACGATCGATCG

2 - ATCGATCGATAGATCGATCG

3 - ATCGATCGATCGATCGAACG

4 - ATCGATCGATCGATCGATCA

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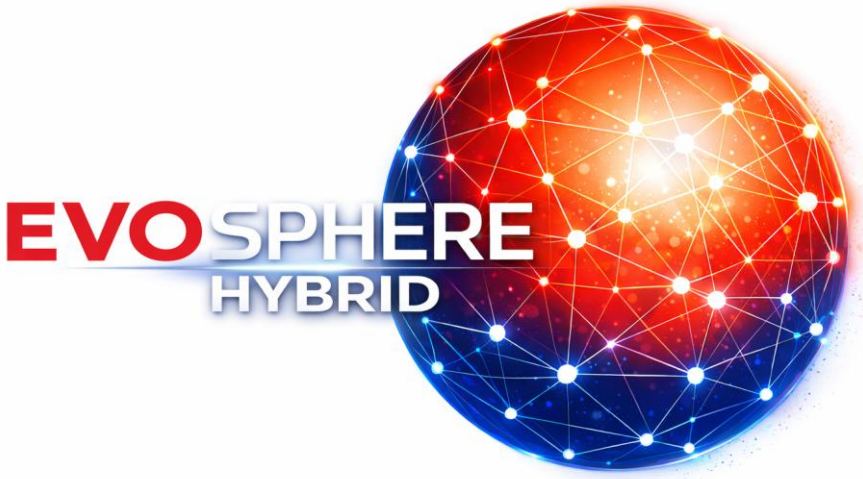
Launching at HPLC 2026
Evosphere Hybrid Monodisperse C18!



- pH Range – 1-12
- Temperature Range – 40ºC - 90ºC
- Available in:
  - All analytical sizes (1.7, 3.0, and 5 µm)
  - Preparative Sizes (7.8, 10.0, 21.2 mm, 30.0 mm, and 50 mm ID)
  - Inert Coated Hardware

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The Evolution of HPLC Columns

Analytical and Preparative Sizes readily Available!

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Conclusion

- Monodisperse Particle Columns lead to lower Reduced Plate Heights in the same standard UHPLC and HPLC formats
- Leveraging higher N and surprising polar retention can lead to useful tools for the chromatographers toolbox

Acknowledgements

- All Industry and Academic Collaborators
- All MM collaborators including Edward Faden and Alex Nasseh
- All Fortis Technologies Collaborators including Mark Woodruff, and Ken Butchart

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