

Alternatives to Sub-2 μm UHPLC Columns

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Why Use Sub-2 μ m Particles?

- **Smaller particles improve efficiency allowing faster separations**
 - Efficiency is directly proportional to the reduction in particle size $N \sim (1/d_p)$
 - High efficiency in short columns
 - Fast method development
 - Short run times
 - Improved productivity
 - Less solvent usage
 - Sharper peaks for more sensitivity

Are Sub-2 μm **SPP** Needed When Separating Small Molecules?

- SPP shown to have unusually high efficiency
 - 2.6 – 2.7 μm SPP have efficiency of sub-2 μm TPP
- Theory predicts efficiency advantages of smaller SPP particles
- Sub-2 μm SPP already available
- General consensus is “Yes”

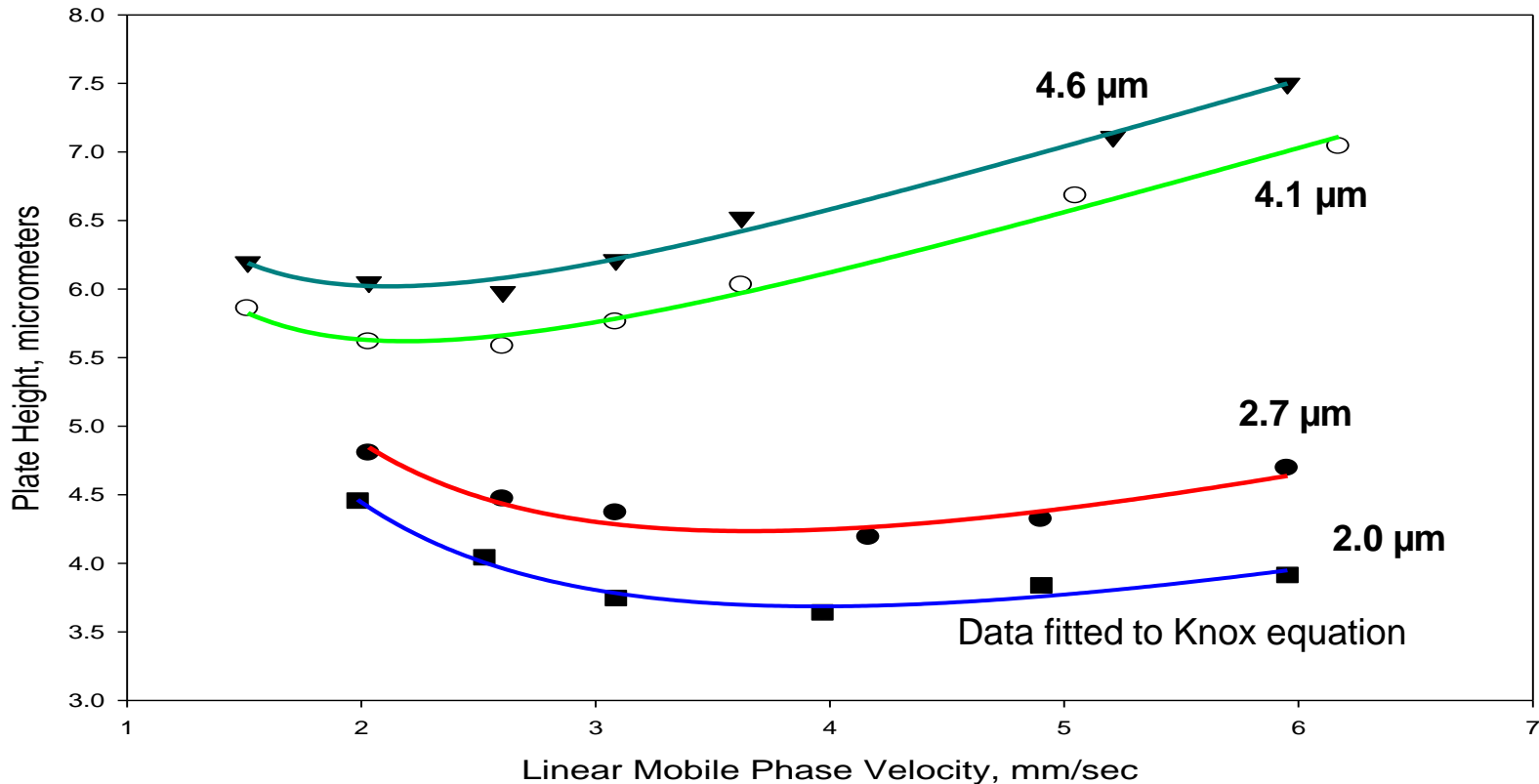
Downside of Using Sub-2 μ m Particles

- Pressure goes up as the square of the reduction in particle size $P \sim (1/d_p)^2$
- Specially designed (expensive) instruments required for optimum use
 - 400 – 600 bar often insufficient for optimum flow
 - Low-dispersion design required to minimize extra-column effects for highest efficiency
 - Small ID tubing and flow cells significantly add to operational pressure
 - Maintenance is expensive and often not user-friendly

Downside of Using Sub-2 μ m Particles

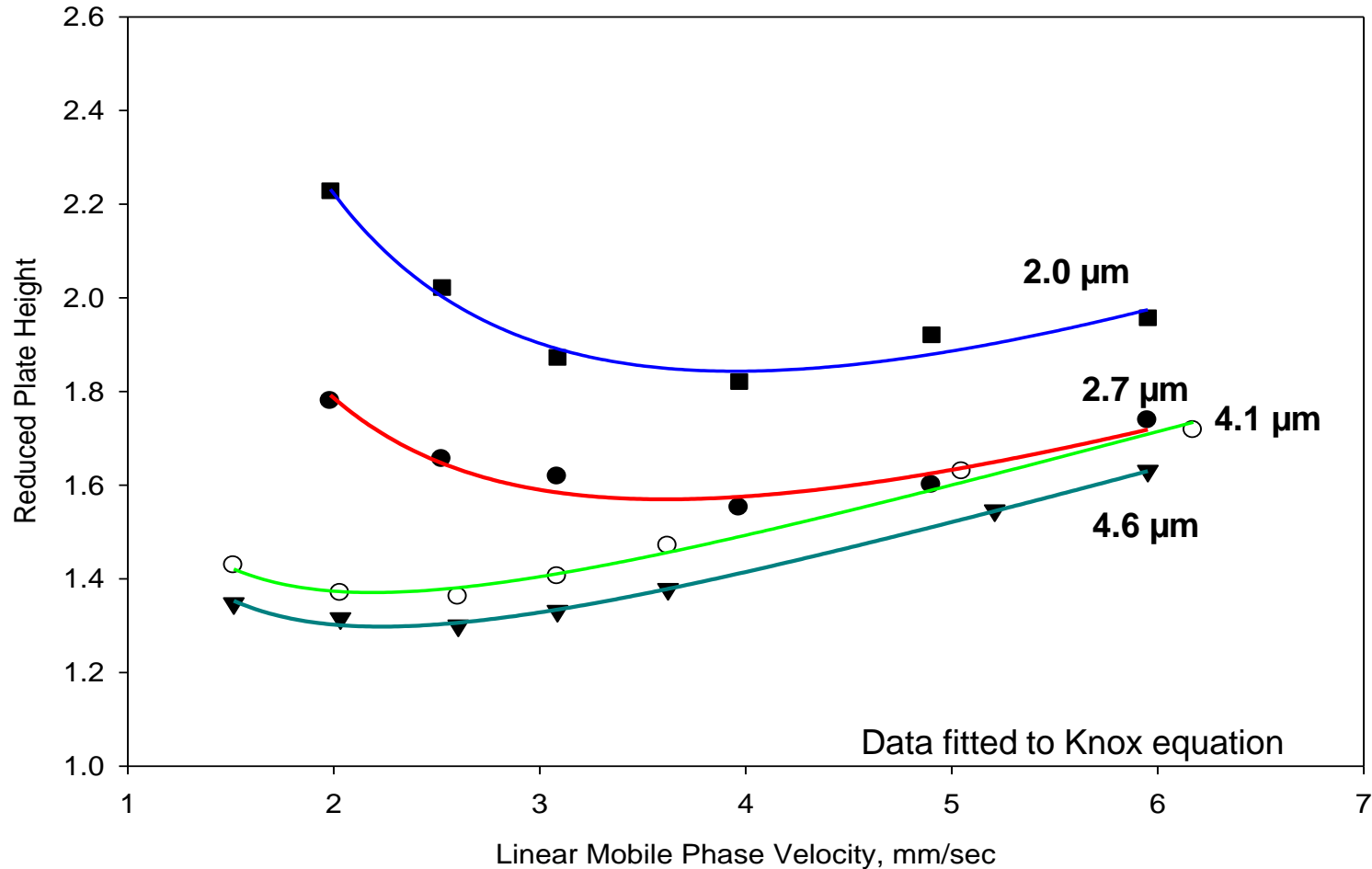
- Column frits with small pores (0.2 – 0.5 μ m) required to retain particles in columns
 - More subject to plugging than 2 μ m frits
 - Additional efforts needed to avoid particulate fouling (filter samples and mobile phases)
- Frictional heating of columns
 - More pronounced as d_p is reduced
 - Can result in band-broadening and changes in retention
 - ≤ 3 mm i.d. columns required to minimize frictional heating effects
- Columns may not exhibit expected efficiency or stability
 - Small particles harder to pack into homogeneous beds for highest efficiency

Effect of Particle Size on H vs v Plots



The Plate Heights of columns packed with SPP particles of different sizes, as expected, get smaller as the particle size gets smaller.

Effect of Particle Size on h vs v Plots



Reduced Plate Heights ($h = H/d_p$) get smaller as the particle size is increased, indicating less homogeneity in packed beds for the smaller particles.

For Superficially Porous Particles (SPP)

(assumes $h = 1.6$)

<u>particle size</u>	<u>column length</u>	<u>theoretical plates</u>	<u>pressure</u>	<u>time</u>
5 microns SPP	250 mm	31,200	200 bar	1.0

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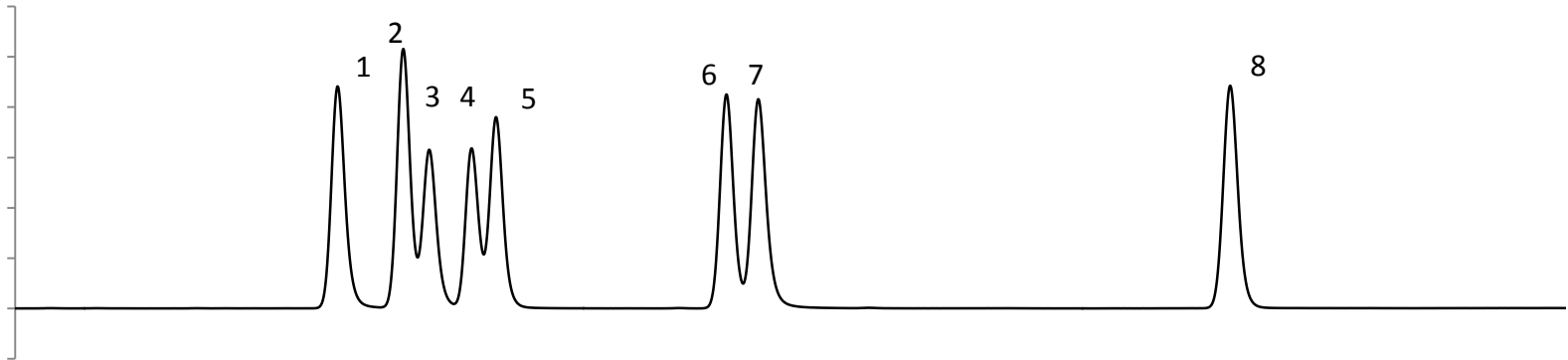
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1.3 microns SPP	75 mm	36,000	900 bar	0.3

What are the Alternatives to
Operating at High Pressures?

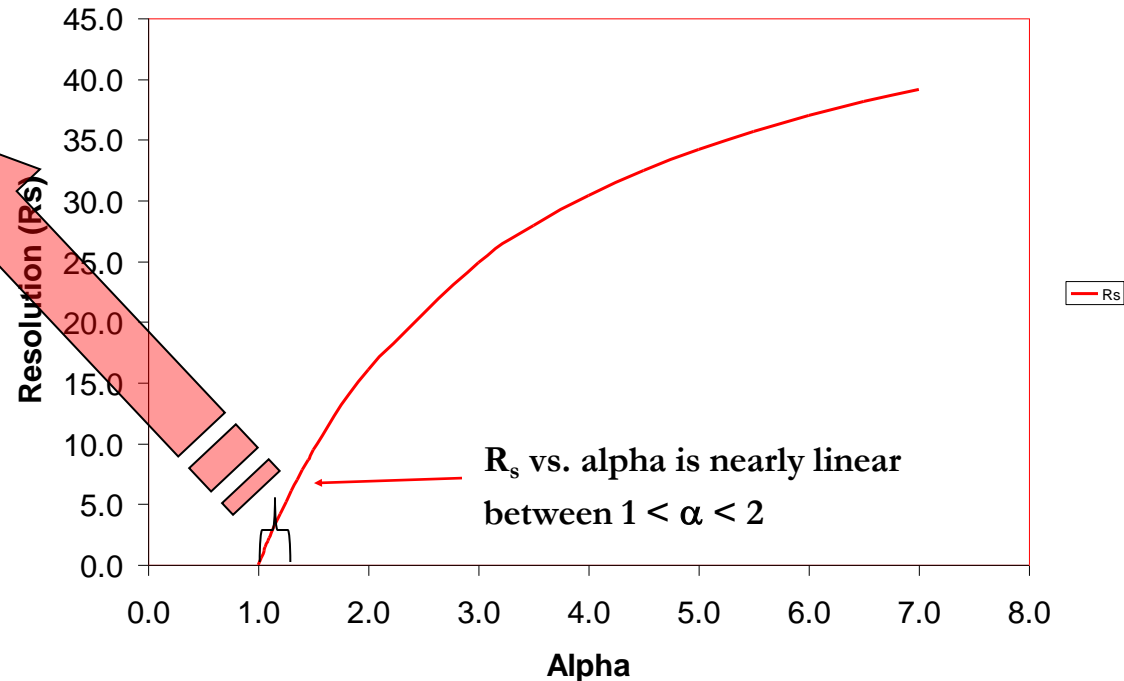
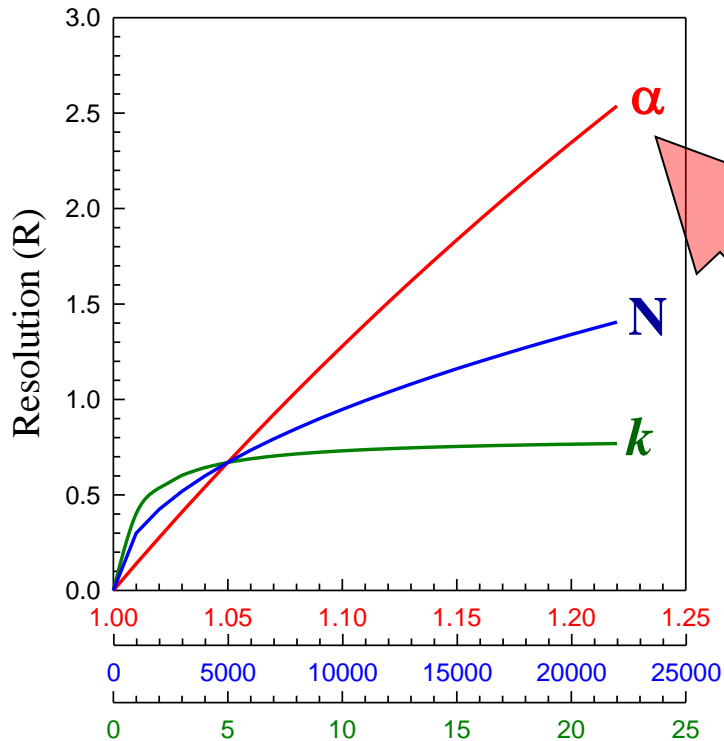
Method Development Needed When Peaks of Interest are not Fully Separated



$$R_s = \left(\frac{1}{4} \right) \sqrt{N} \left[\frac{(\alpha - 1)}{\alpha} \right] \left(\frac{k_2}{(1 + \bar{k})} \right)$$

Resolution Equation Shows that Selectivity is More Effective Parameter to Change

$$R_s = \left(\frac{1}{4} \right) \sqrt{N} \left[\frac{(\alpha - 1)}{\alpha} \right] \left(\frac{k_2}{(1 + \bar{k})} \right)$$



Most Effective Parameters to Change Selectivity

The analysis condition parameters that most affect selectivity, α are¹:

Column type (C18, phenyl, amide, etc.)

++

more effective

B-solvent (acetonitrile, methanol, etc.)

++

Mobile phase pH

++

Ion-pair concentration

++

%B solvent/gradient steepness

+

Column temperature

+

Buffer concentration

+

less effective

¹adapted from "Introduction to Modern Liquid Chromatography", 3rd Edition, L. R. Snyder, J. J. Kirkland, J. W. Dolan; p. 29, 2010, John Wiley & Sons, Inc.

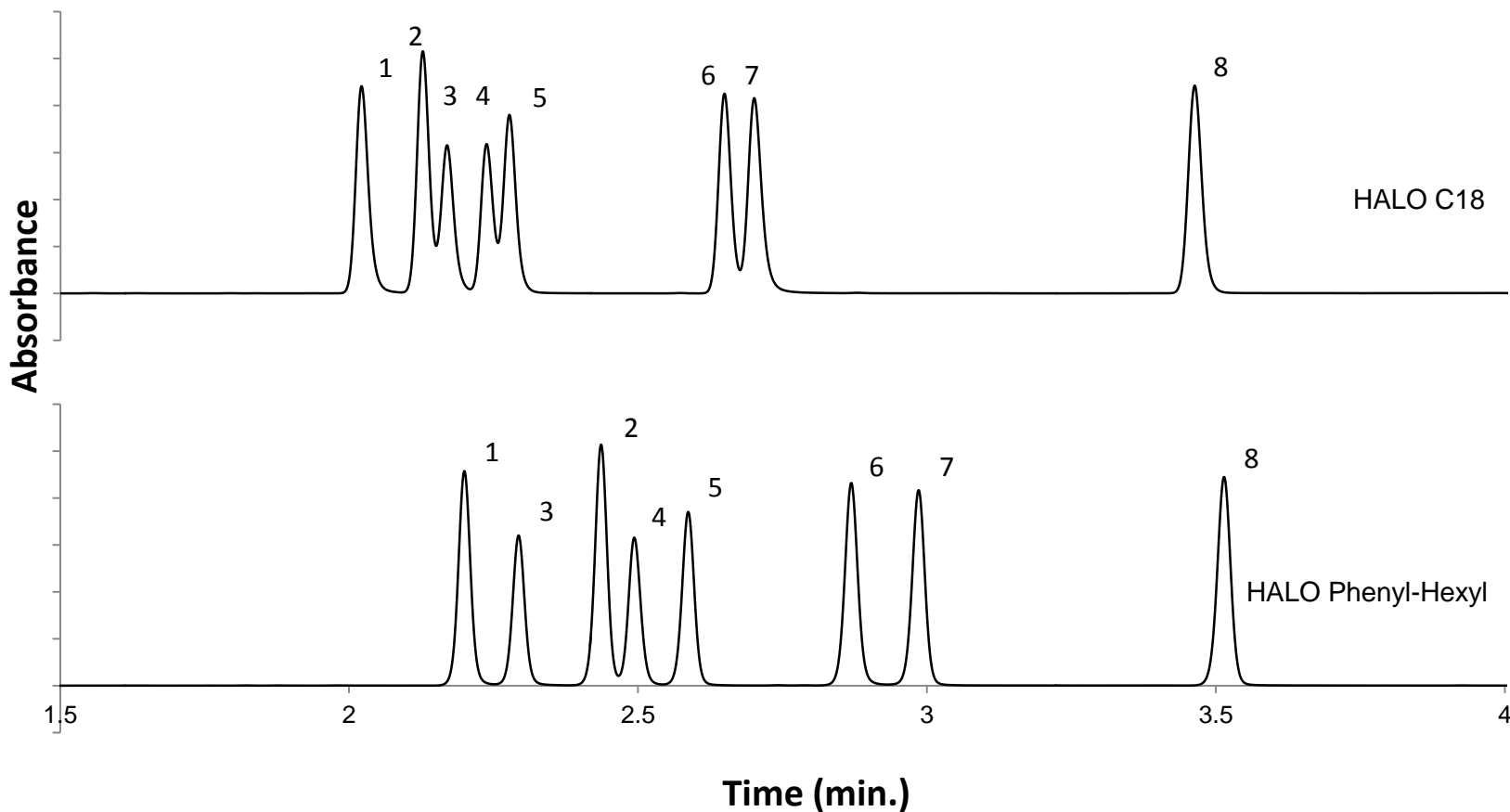
Change Bonded Phase to Vary Selectivity

Test Conditions:

Column size: 4.6 x 50 mm
A = 25 mM Ammonium Acetate
B = Acetonitrile
Flow rate = 1.5 mL/min.
Gradient = 34–63 %B in 3.5 min.
Pressure = 200 bar
Temperature = 35 °C
UV=254 nm, 1 µL Injection

Peak Identities:

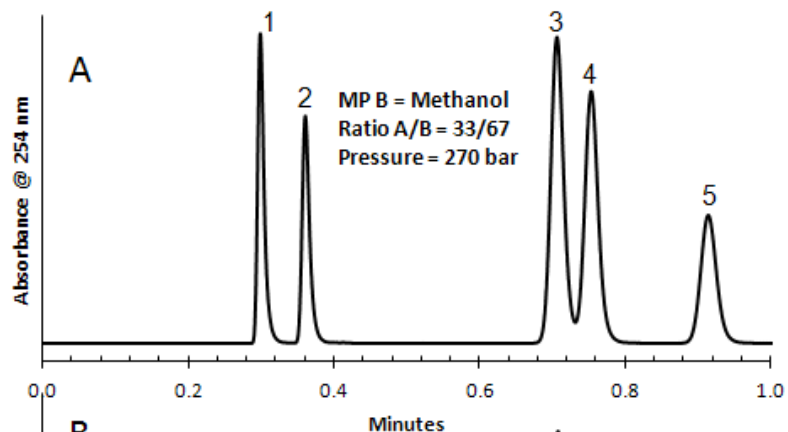
- | | |
|---------------|------------------|
| 1. Oxazepam | 5. Clonazepam |
| 2. Lorazepam | 6. Temazepam |
| 3. Nitrazepam | 7. Flunitrazepam |
| 4. Alprazolam | 8. Diazepam |



Change Organic Modifier to Vary Selectivity

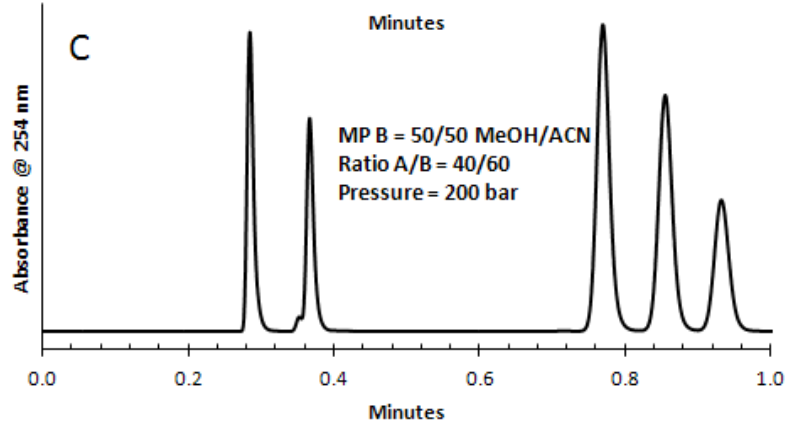
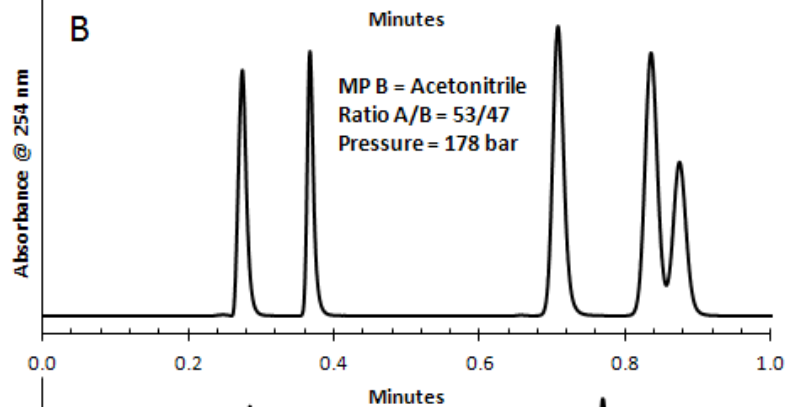
Test Conditions:

Column size: 4.6 x 50 mm HALO C18
A = 0.02 M Potassium Phosphate, pH 3
B = as indicated
Mobile Phase Composition = as indicated
Flow rate = 1.8 mL/min.
Pressure = as indicated
Temperature = 30 °C
UV=254 nm, 0.5 µL Injection



Peak Identities

1. Acetaminophen
2. Aspirin
3. Tolmetin
4. Ketoprofen
5. Naproxen

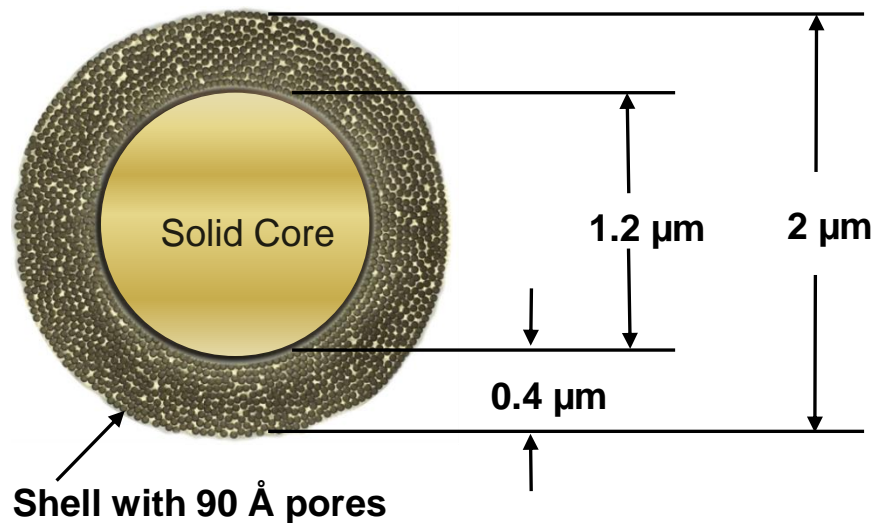
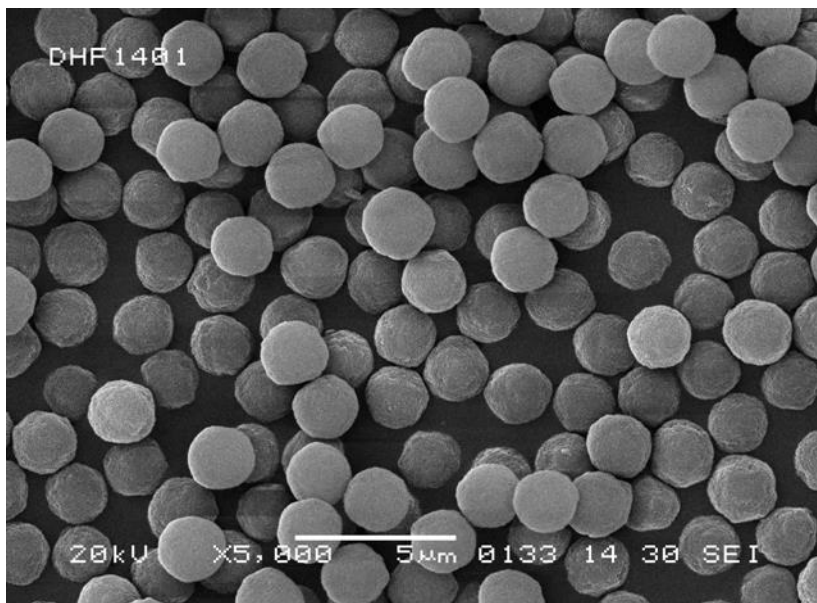


Method Development Recommendations

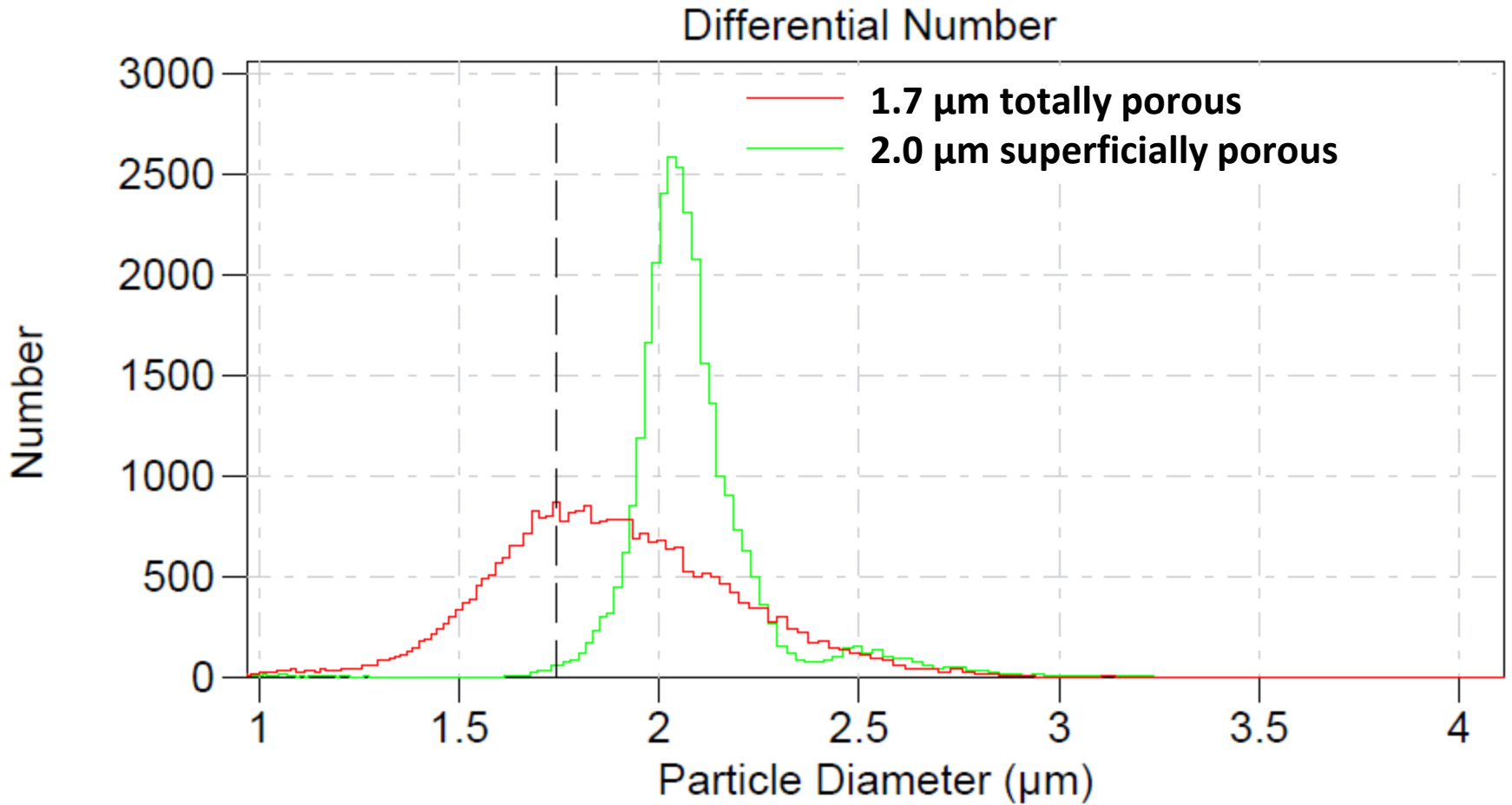
- 1. Use short, high-efficiency 2.7 μm SPP C18 columns to initially evaluate separation**
 - 2.7 μm gives high efficiency at moderate pressures
 - Short columns give short run times for rapid method development
 - SPP shown to have ~40% efficiency advantage over totally porous particles of same size
 - C18 phases are rugged and effective for RPLC
- 2. Increase efficiency (longer column, smaller particles) if close to adequate resolution, if not:**
- 3. Change Selectivity of the separation**
 - Modify mobile phase (type of organic modifier, pH, etc.)
 - Change C18 phase to other type bonded phase

An Alternative to Sub- $2\mu\text{m}$ Particle Columns

$2\mu\text{m}$ SPP



Particle Size Distributions



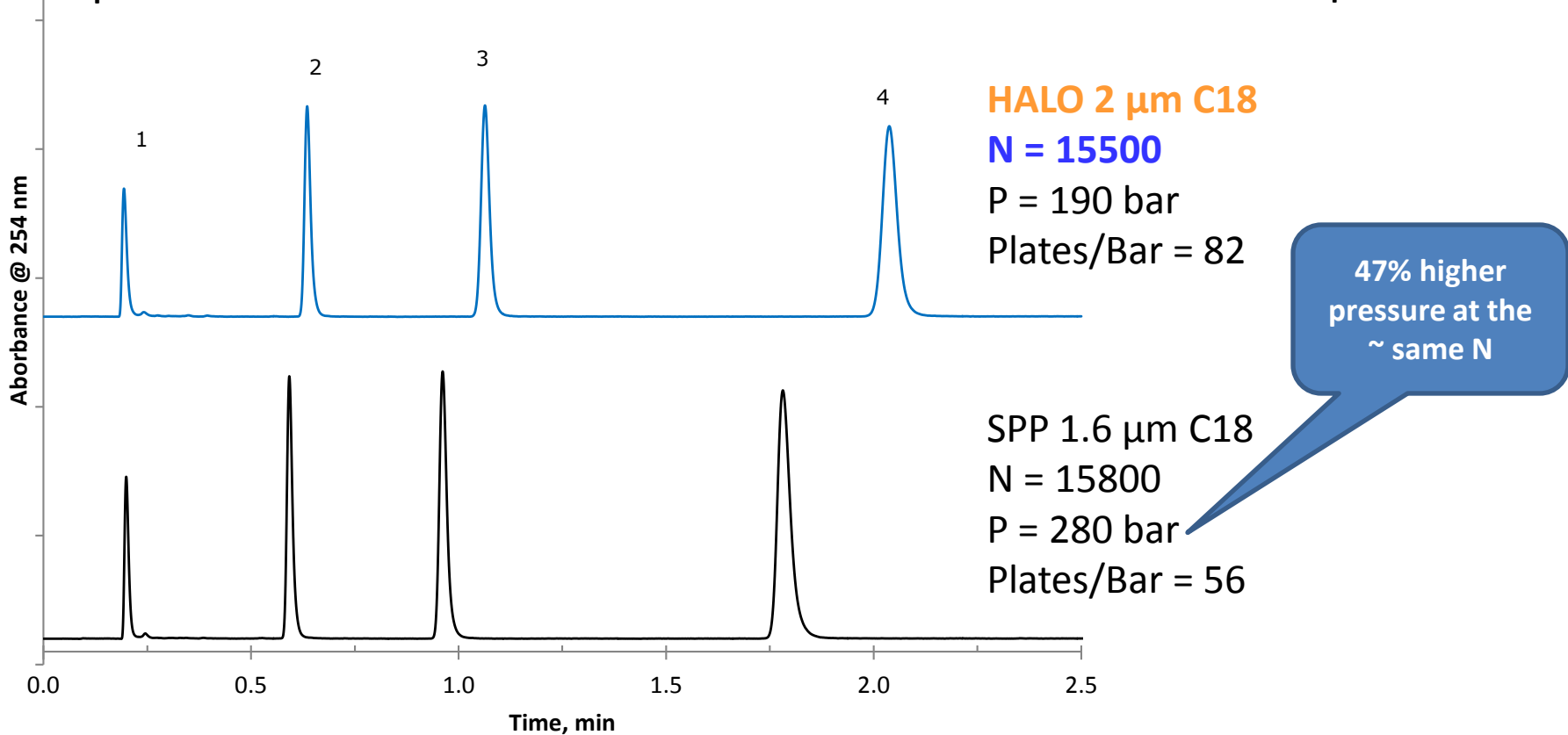
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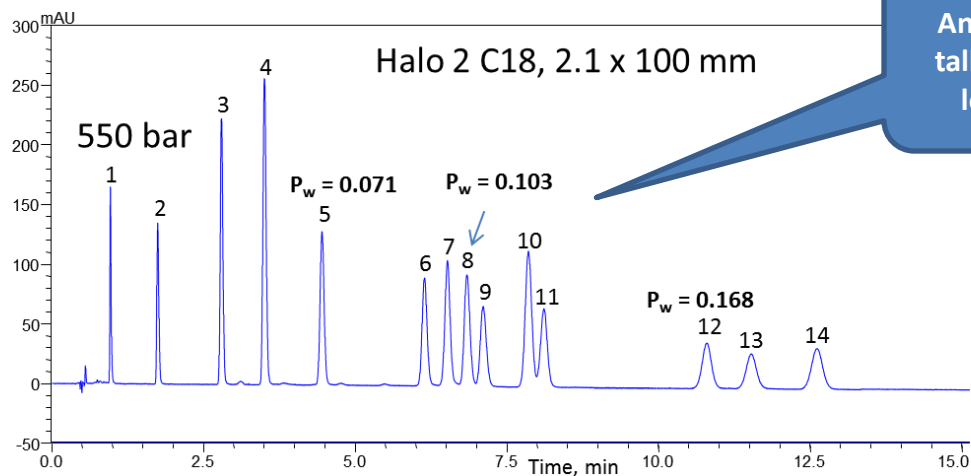
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HALO 2 C18 vs. solid-core sub-2- μm column

Columns:	2.1 x 50 mm	Mobile Phase A:	water	Peak Identities:
Instrument:	Shimadzu Nexera	Mobile Phase B:	acetonitrile	1. Uracil
Injection Volume:	0.2 μL	Ratio A/B:	15/85	2. Pyrene
Detection:	254 nm	Flow rate:	0.5 mL/min	3. Decanophenone
Temperature:	25°C			4. Dodecanophenone



Explosives: HALO 2 C18 vs. Non-Core Sub-2- μm

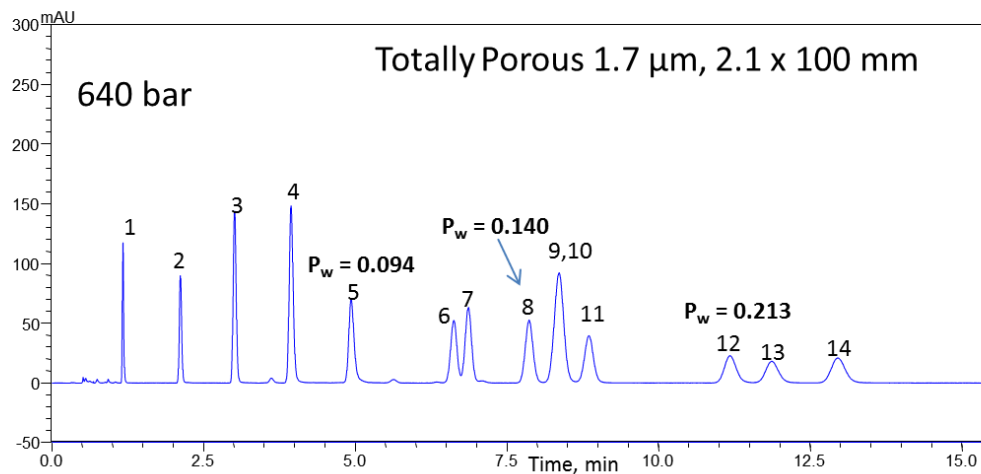


An average of 57% taller peaks at 15% lower pressure

Temperature: 42 °C
Flow rate: 0.4 mL/min
Mobile Phase A: Water
Mobile Phase B: Methanol
Ratio A/B: 72/28
Detection: PDA @ 254 nm

Peak Identities:

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2,4,6-Trinitrotoluene
8. 2-Amino-4,6-Dinitrotoluene
9. 4-amino-2,6-dinitrotoluene
10. 2,4-Dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene



An Alternative to Sub-2 μ m – 2 μ m SPP

- **2 μ m SPP keeps pressure within a comfort zone and retains most of advantages of sub-2 μ m columns**
 - **Higher efficiencies than sub-2 μ m TPP columns**
 - **Lower pressure than sub-2 μ m columns (TPP or SPP)**
 - **Short columns exhibit the high efficiencies wanted for fast method development**
- **Minimizes disadvantages of sub-2 μ m columns**
 - **Greater efficiencies than sub-2 μ m TPP with lower pressure requirements**
 - **Similar efficiencies as sub-2 μ m SPP with lower pressure requirements**
 - **Uses 1-micron frits that are less prone to plugging**
 - **Reduced frictional heating**

Are Sub-2 μ m SPP Needed for Small Molecules?

- **Our conclusion: not necessary**
 - **Advantages of very small particles are not sufficient to overcome the disadvantages for most small molecule applications**
 - **Selectivity manipulations via bonded-phase or mobile phase are more effective at improving resolution than is increasing efficiency**
 - **Conclusion may be different for large molecules. Large molecules may require shorter diffusion paths of small particle size SPP for adequate mass transfer**

Conclusions

- **Sub-2 μm SPP not needed for most routine small molecule applications**
- **Larger SPP are less problematic for high throughput operation (e.g., QC Labs)**
- **Columns of 2- μm SPP appear to be a good compromise of speed and efficiency with superior advantages for small molecule applications**

Acknowledgements

Thanks go to Robert Moran who supplied much of the data with 2- μm particles for this presentation