Alternatives to Sub-2 µm UHPLC Columns

J. J. DeStefano, B. E. Boyes, S. Schuster,
W. L. Miles, and J. J. Kirkland
Advanced Materials Technology, Inc.
3521 Silverside Rd., Quillen Bld., Ste. 1-K
Wilmington, DE 19810 USA

Why Use Sub-2µm 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-2µm SPP Needed When Separating Small Molecules?

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

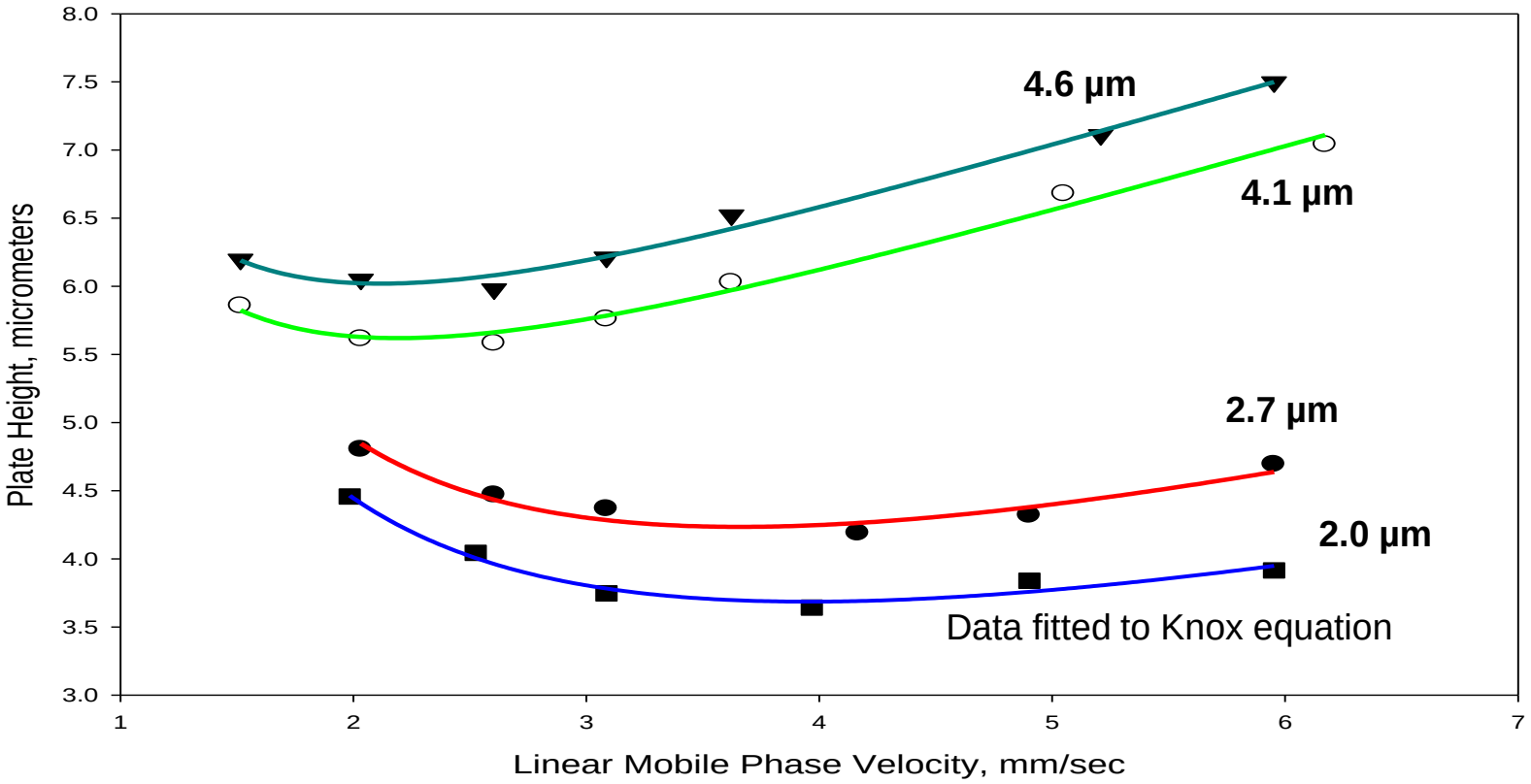
Downside of Using Sub-2µm Particles

- **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-2µm Particles

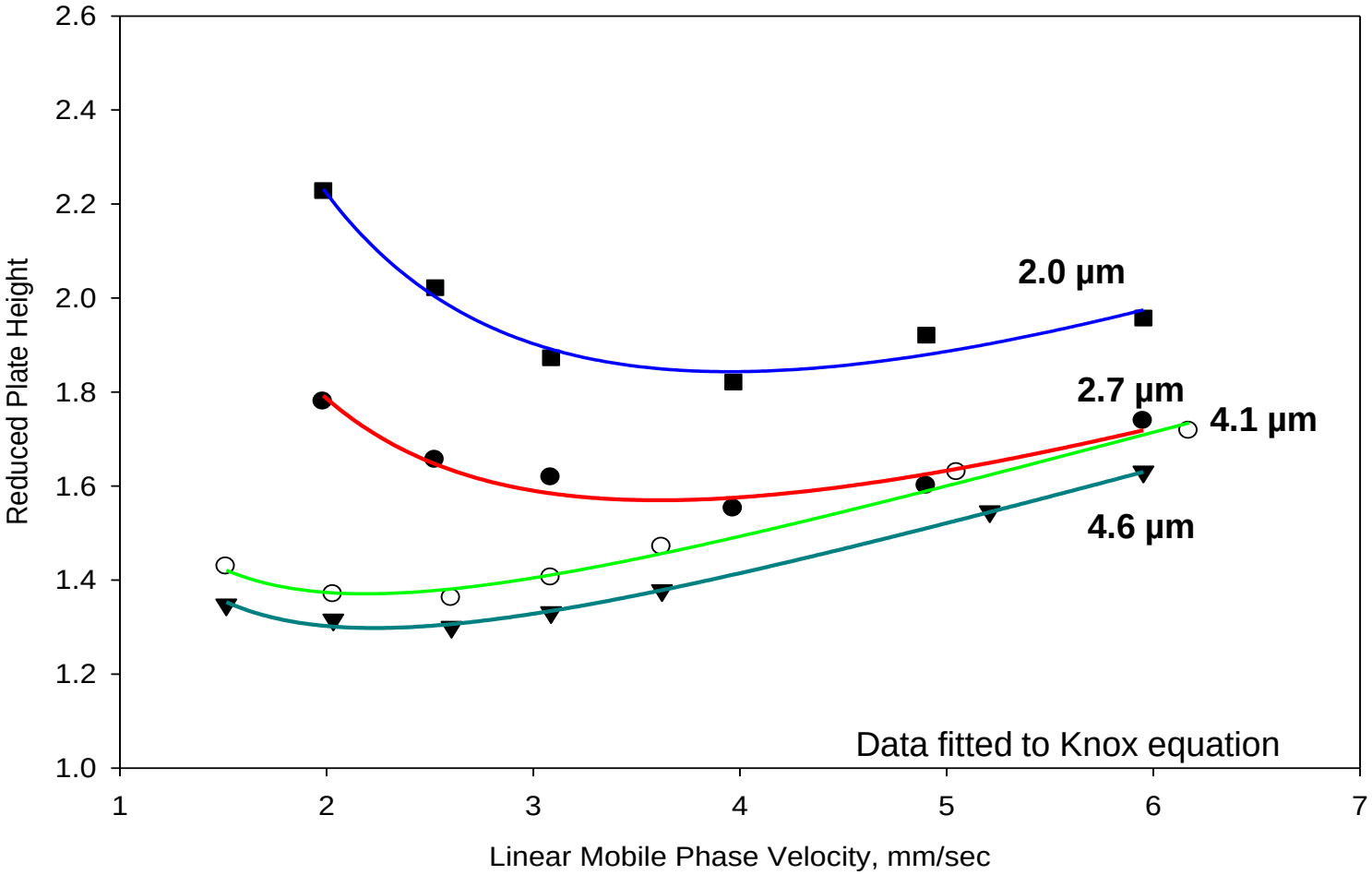
- Column frits with small pores (0.2 – 0.5µm) required to retain particles in columns
  - More subject to plugging than 2µm frits
  - Additional efforts needed to avoid particulate fouling (filter samples and mobile phases)
- Frictional heating of columns
  - More pronounced as d_p is reduced
  - Can 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 <u>increased</u>, indicating less homogeneity in packed beds for the smaller particles.

For Superficially Porous Particles (SPP)

(assumes h = 1.6)

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

For Superficially Porous Particles (SPP)

(assumes h = 1.6)

particle size	column length	theoretical plates	pressure	time
5 microns SPP	250 mm	31,200	200 bar	1.0
2.7 microns SPP	150 mm	34,700	410 bar	0.6

For Superficially Porous Particles (SPP)

(assumes h = 1.6)

particle size	column length	theoretical plates	pressure	time
5 microns SPP	250 mm	31,200	200 bar	1.0
2.7 microns SPP	150 mm	34,700	410 bar	0.6
1.7 microns SPP	100 mm	36,700	700 bar	0.4

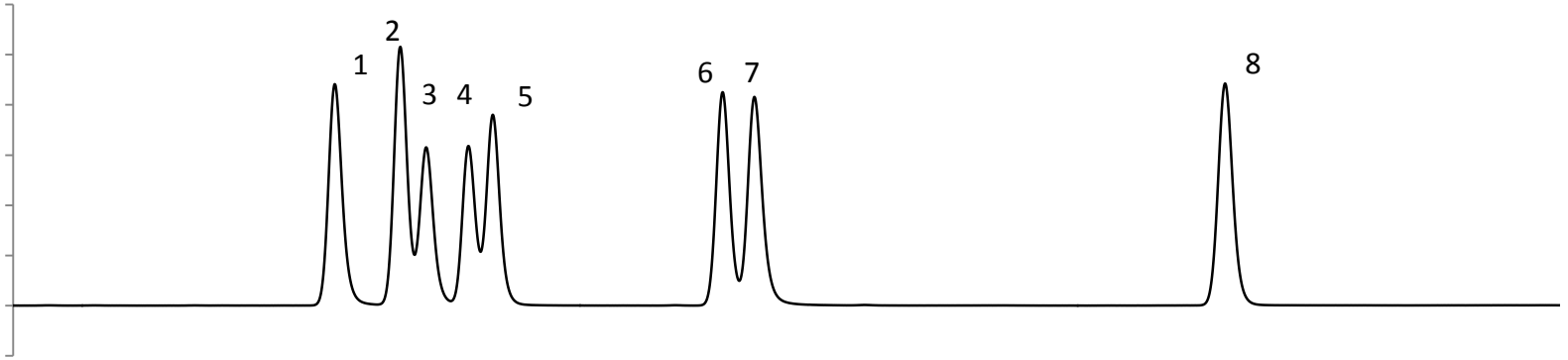
For Superficially Porous Particles (SPP)

(assumes h = 1.6)

particle size	column length	theoretical plates	pressure	time
5 microns SPP	250 mm	31,200	200 bar	1.0
2.7 microns SPP	150 mm	34,700	410 bar	0.6
1.7 microns SPP	100 mm	36,700	700 bar	0.4
1.3 microns SPP	75 mm	36,000	900 bar	0.3

What are the Alternatives to Operating at High Pressures?

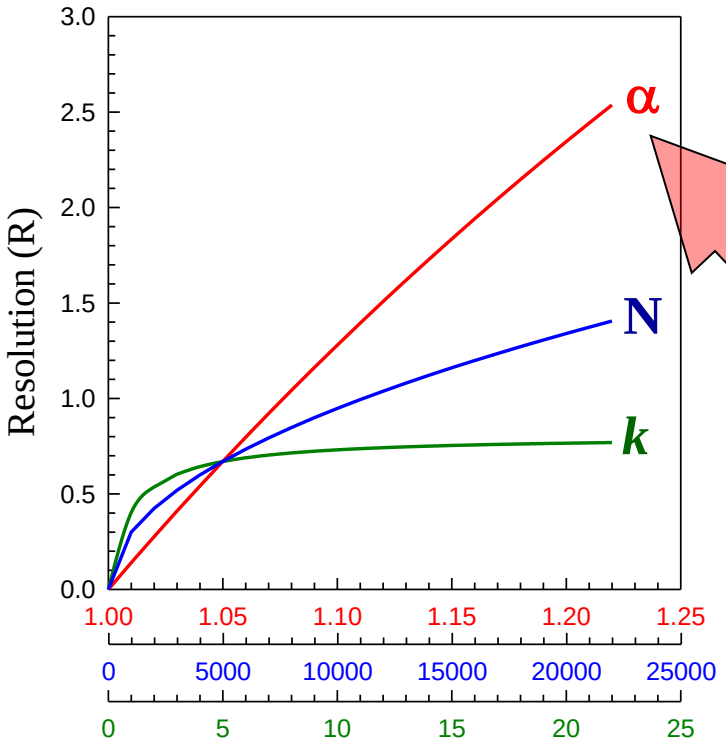
Method Development Needed When Peaks of Interest are not Fully Separated

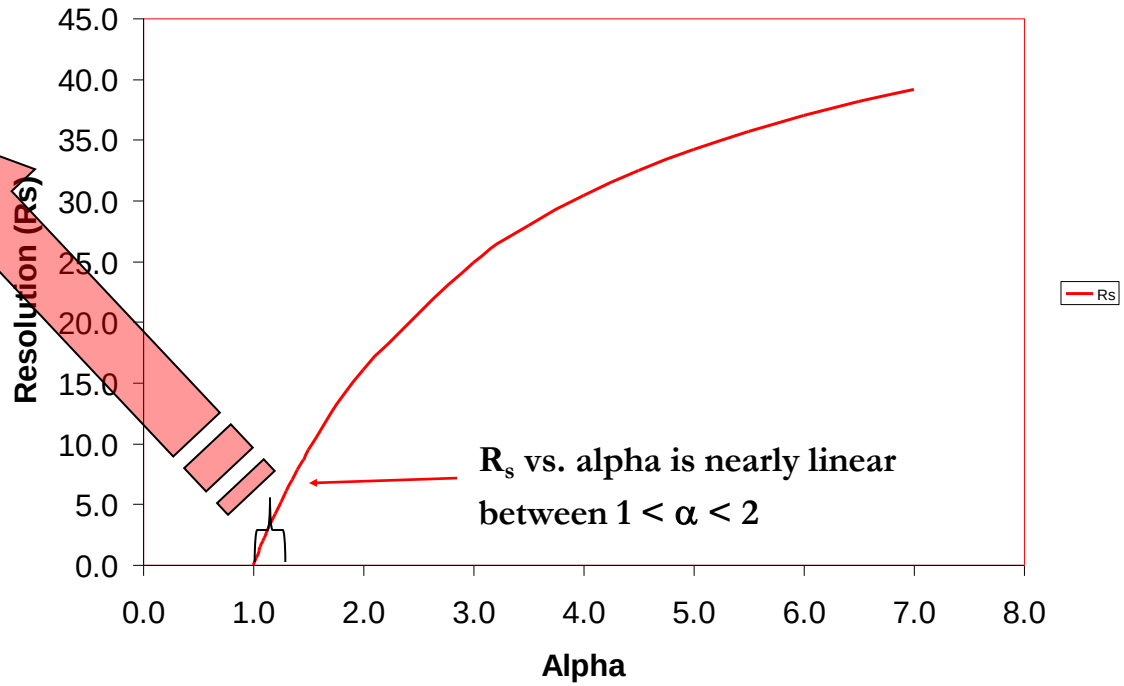


$$R_s = \left(\frac{1}{4} \right) \sqrt{N} \left[\frac{(\alpha - 1)}{\alpha} \right] \left(\frac{k_2}{(1 + \overline{k})} \right)$$

Resolution Equation Shows that Selectivity is More Effective Parameter to Change

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



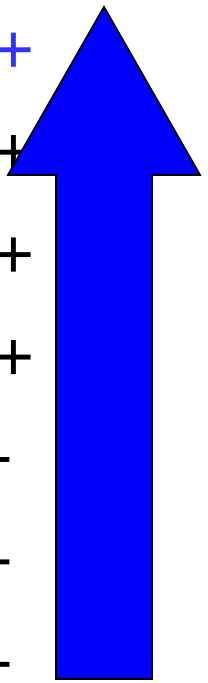


Source: Jun Mao, PhD Thesis with Professor Peter Carr, U. of Minnesota, 2001

Most Effective Parameters to Change Selectivity

The analysis condition parameters that most affect selectivity, α are[1]:

Column type (C18, phenyl, amide, etc.)	++
B-solvent (acetonitrile, methanol, etc.)	++
Mobile phase pH	++
Ion-pair concentration	++
%B solvent/gradient steepness	+
Column temperature	+
Buffer concentration	+



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

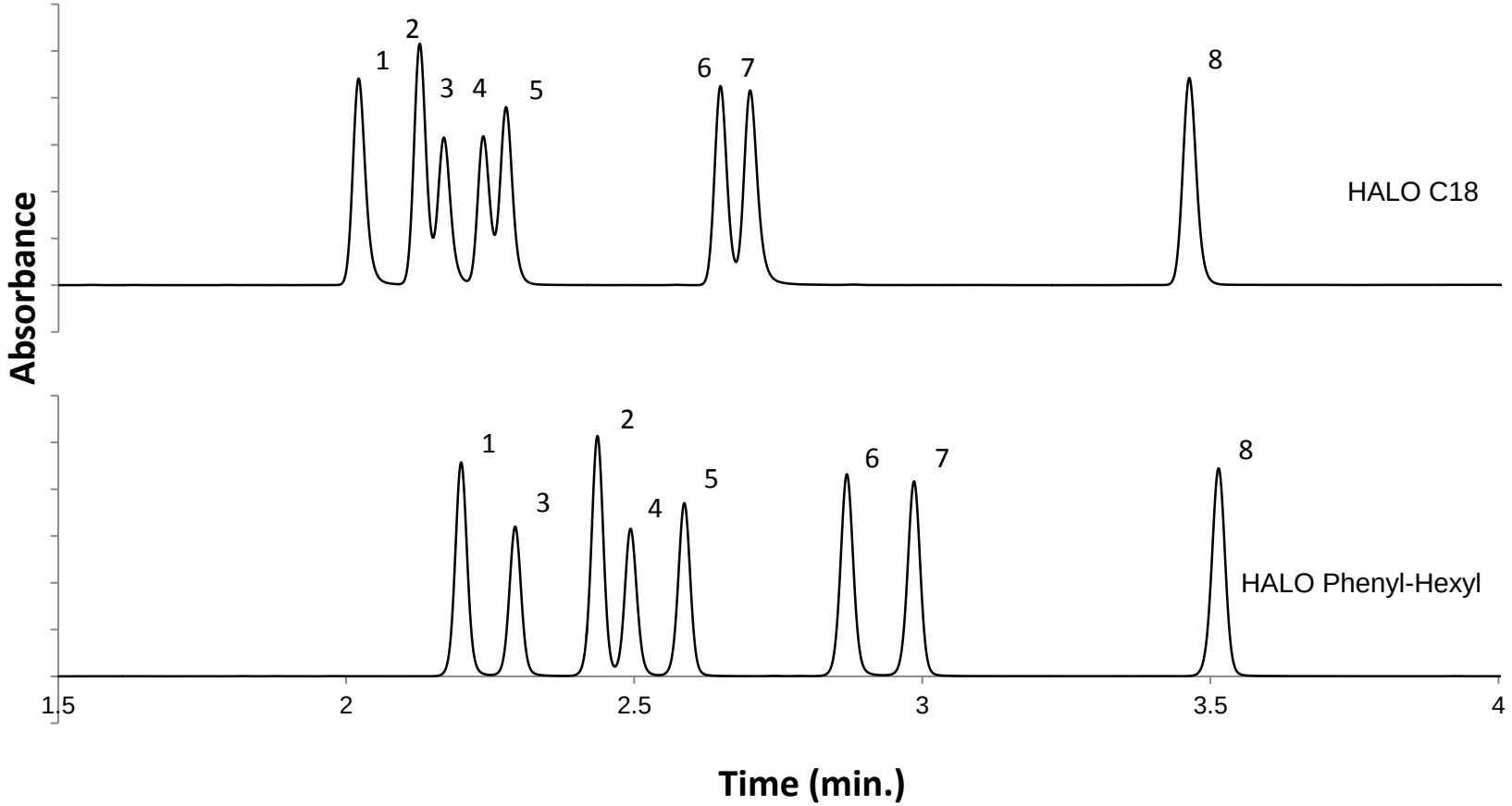
Change Bonded Phase to Vary Selectivity

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

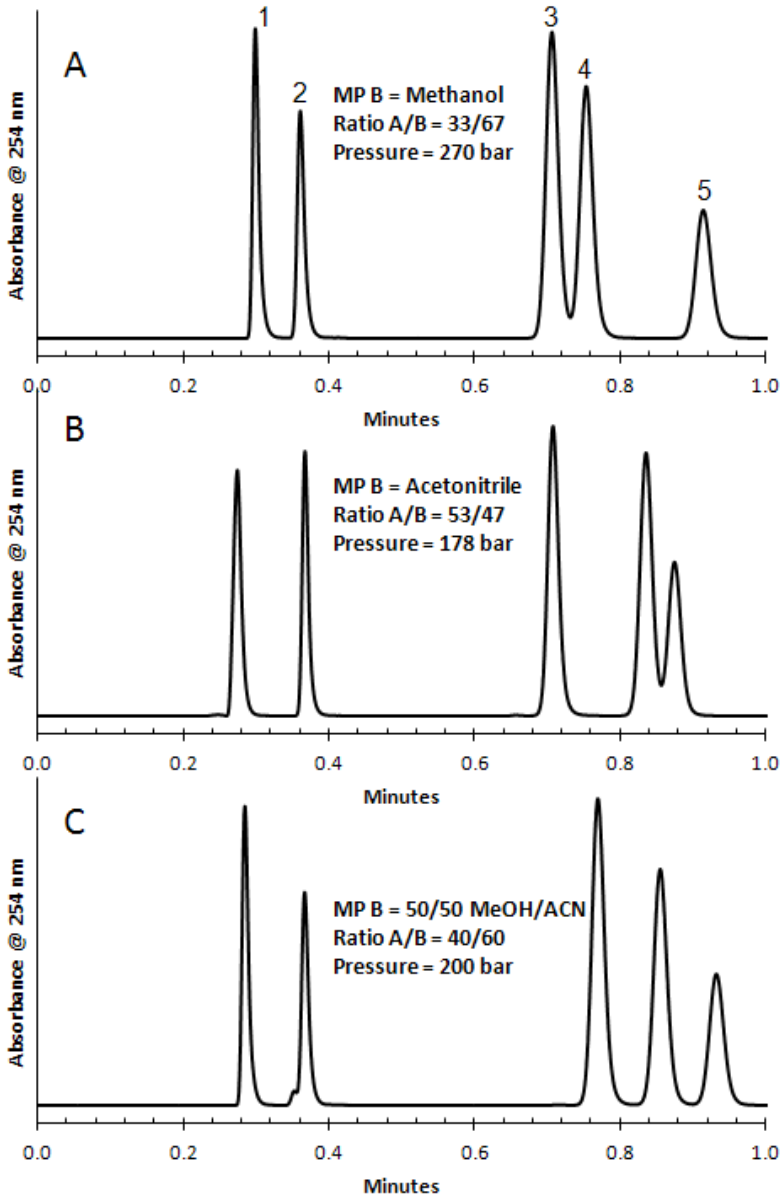
Peak Identities:

1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Alprazolam
5. Clonazepam
6. Temazepam
7. Flunitrazepam
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

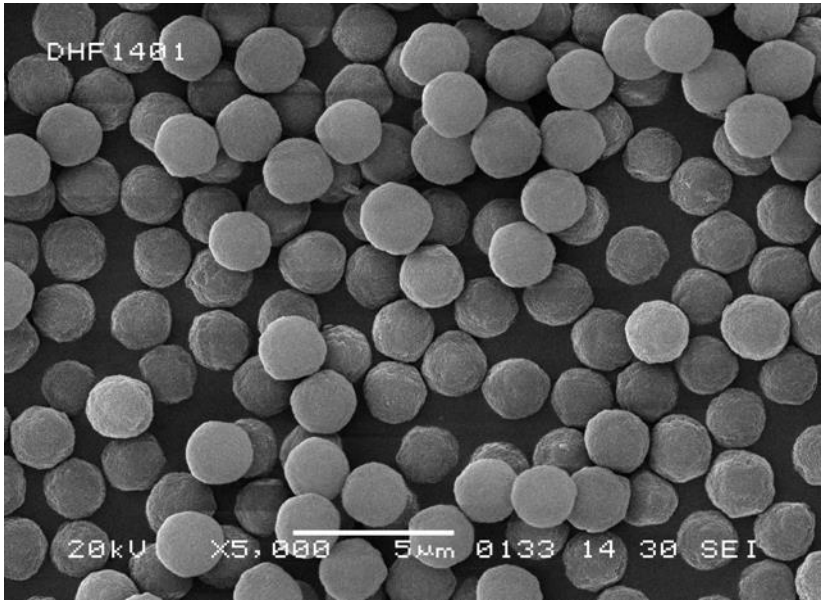
1. Use short, high-efficiency 2.7 µm SPP C18 columns to initially evaluate separation
   - 2.7 µm gives high efficiency at moderate pressures
   - Short columns give short run times for rapid method development
   - SPP shown to have ~40% efficiency advantage over totally porous particles of same size
   - C18 phases are rugged and effective for RPLC

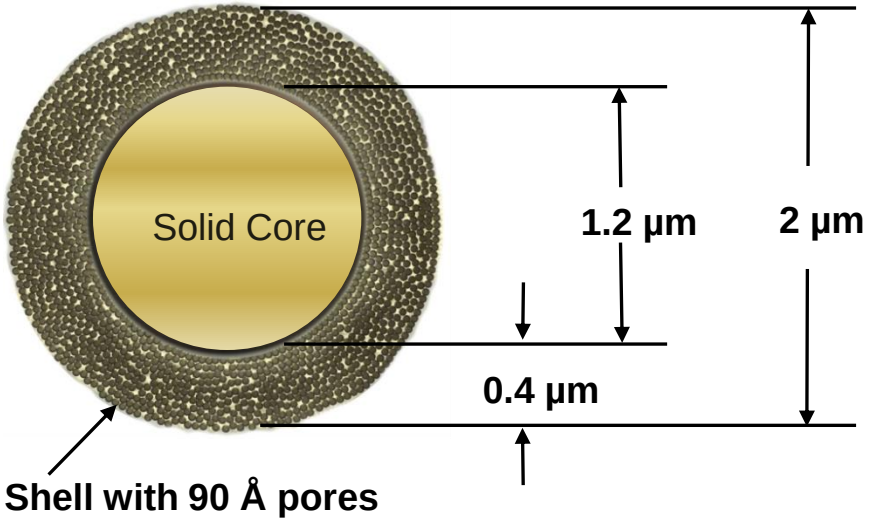
2. Increase efficiency (longer column, smaller particles) if close to adequate resolution, if not:

3. Change Selectivity of the separation
   - Modify mobile phase (type of organic modifier, pH, etc.)
   - Change C18 phase to other type bonded phase

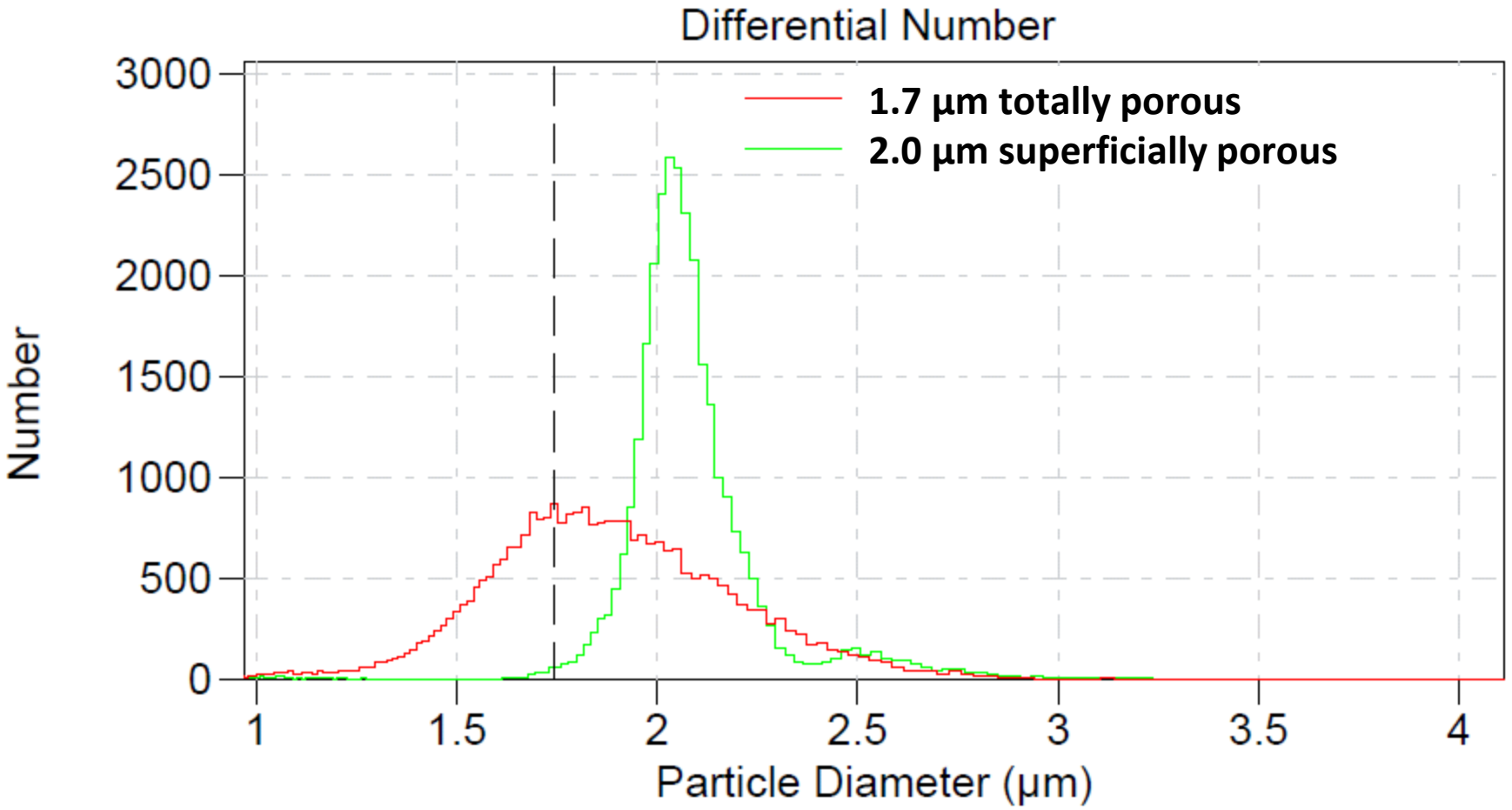
An Alternative to Sub-2µm Particle Columns

2µm SPP





Particle Size Distributions



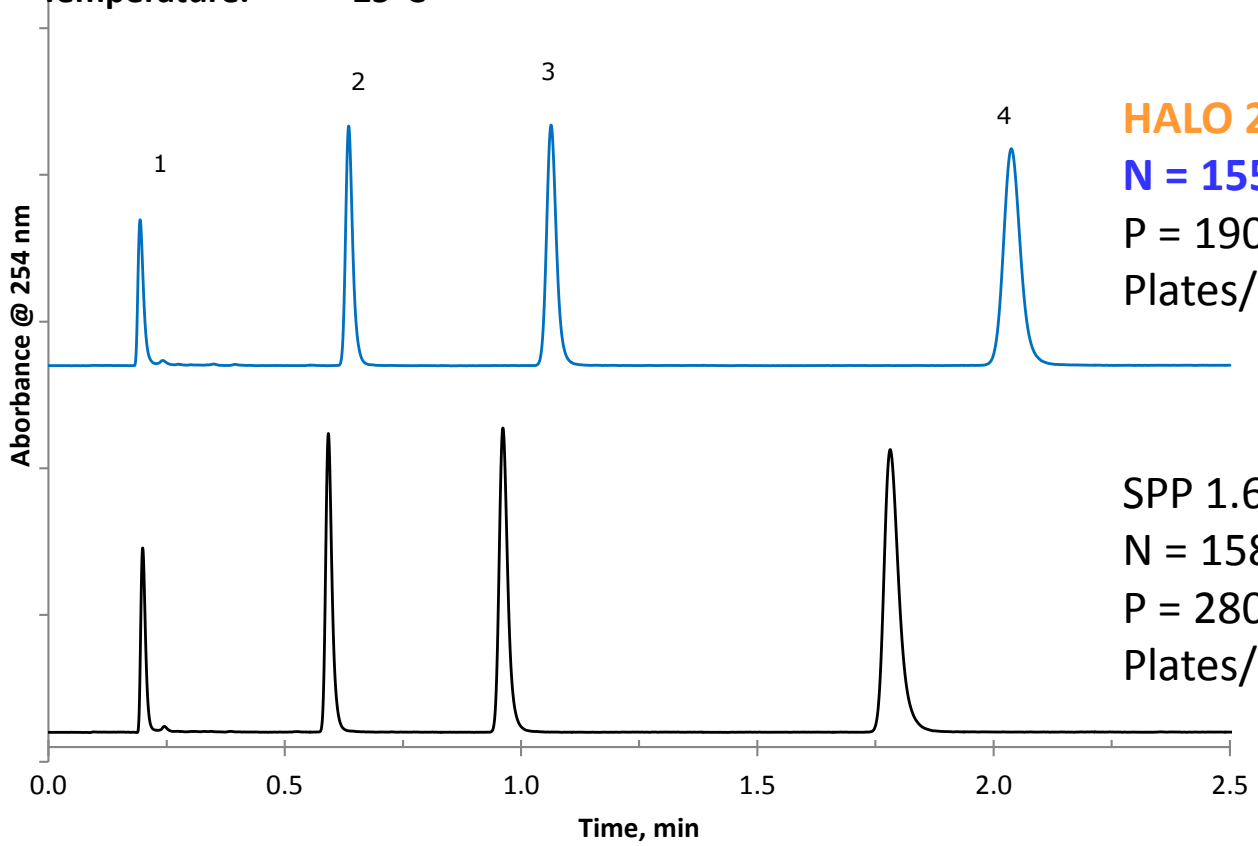
For Superficially Porous Particles (SPP)

(assumes h = 1.6)

particle size	column length	theoretical plates	pressure	time
5 microns SPP	250 mm	31,200	200 bar	1.0
2.7 microns SPP	150 mm	34,700	410 bar	0.6
2 microns SPP	100 mm	31,200	500 bar	0.4
1.7 microns SPP	100 mm	36,700	700 bar	0.4
1.3 microns SPP	75 mm	36,000	900 bar	0.3

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**

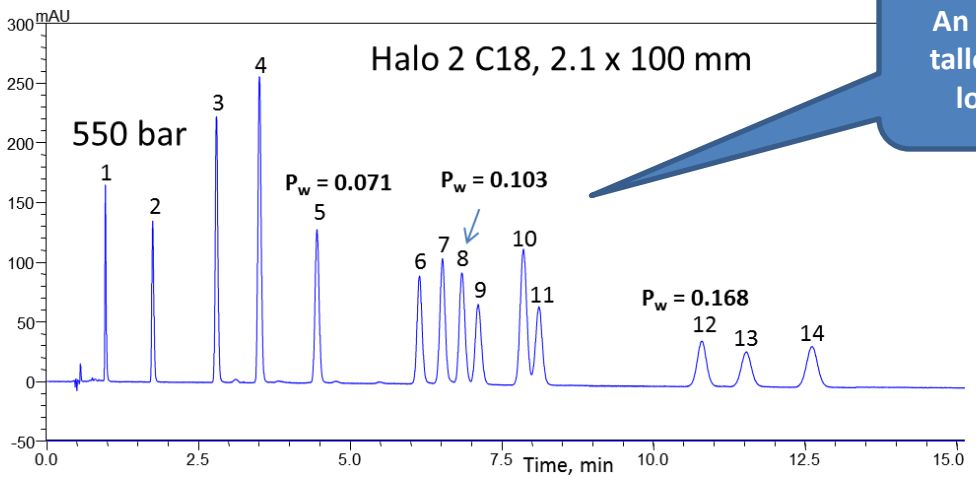


HALO 2 µm C18
N = 15500
P = 190 bar
Plates/Bar = 82

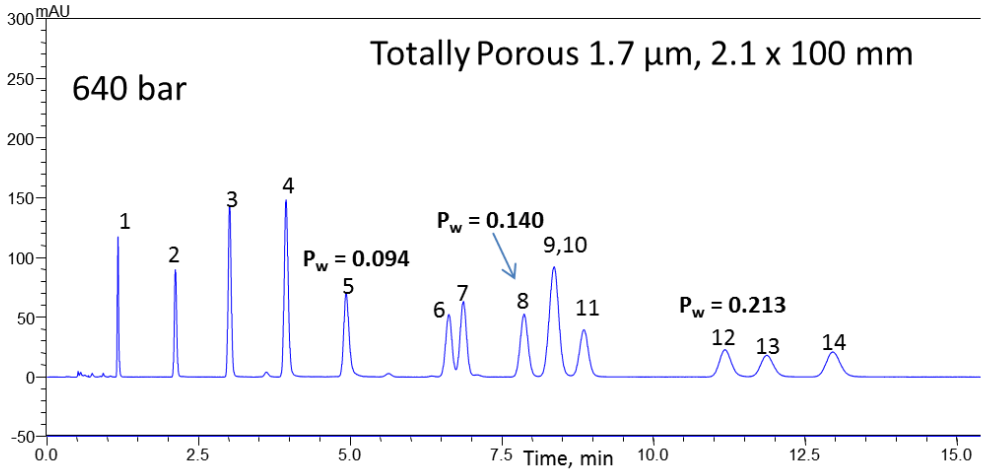
SPP 1.6 µm C18
N = 15800
P = 280 bar
Plates/Bar = 56

47% higher pressure at the ~ same N

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-2µm – 2µm SPP

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

Are Sub-2µm 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