High Resolution Separations Using Longer Columns and Elevated Temperature

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High Resolution in HPLC

- Why is it desired
- How it can be accomplished
  - Long columns
  - Small particles
- Ability to move the mobile phase through the column is limiting
  - Raise pressure
  - Decrease viscosity

Column Pressure Drop

The column pressure drop DP depends on the mobile phase viscosity h, the mobile phase velocity u (flow rate), column length L and the square of the particle diameter of the packing material d_p:

$$\Delta P = 1000\ \eta \cdot u \cdot L / d_p^2$$

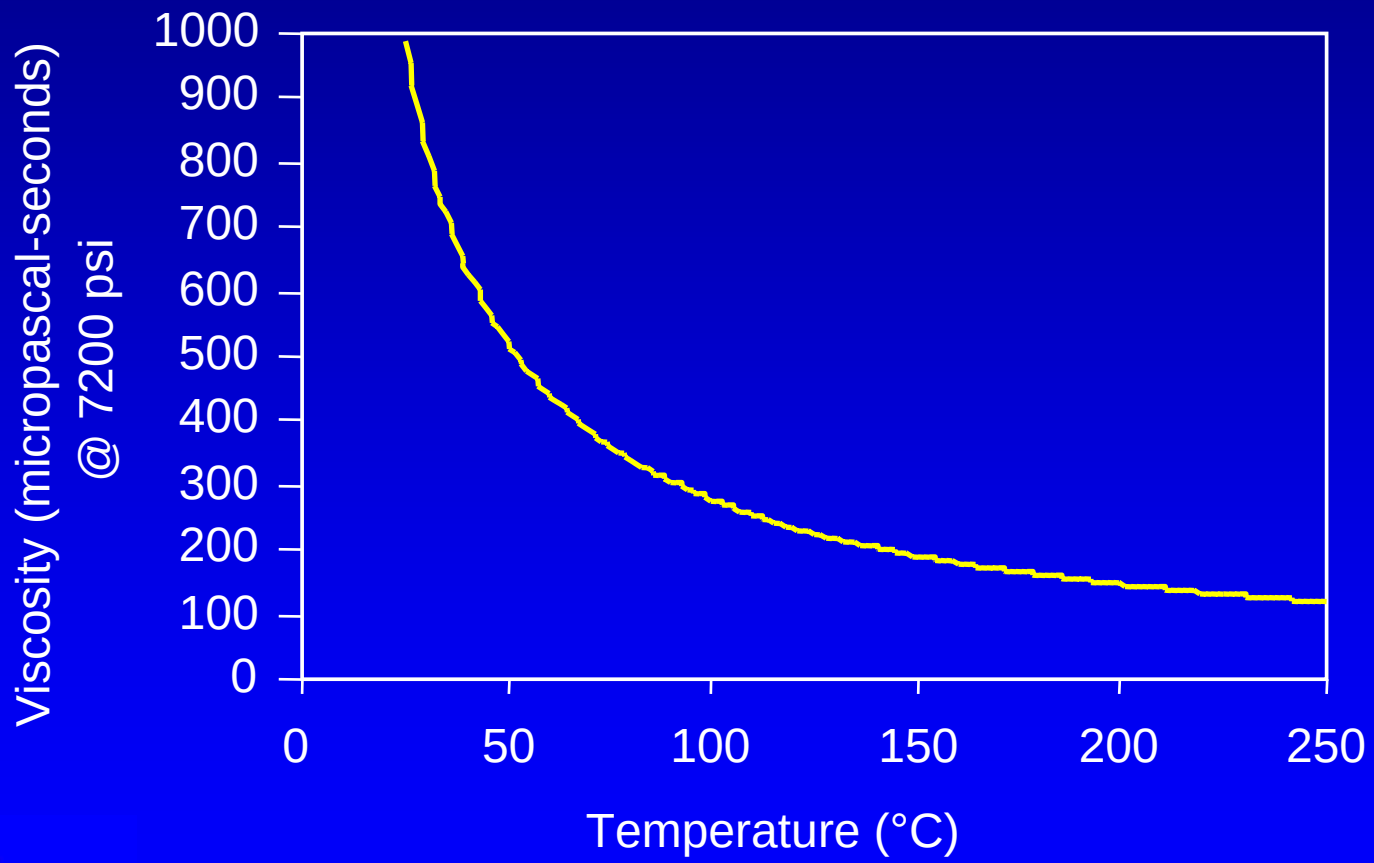
$$u = \Delta P \cdot d_p^2 / 1000\ \eta \cdot L$$

Increasing plate number in HPLC is thus primarily limited by pressure drop (both L increase and d_p decrease lead to increased pressure drop)

Temperature as a Powerful Tool in LC

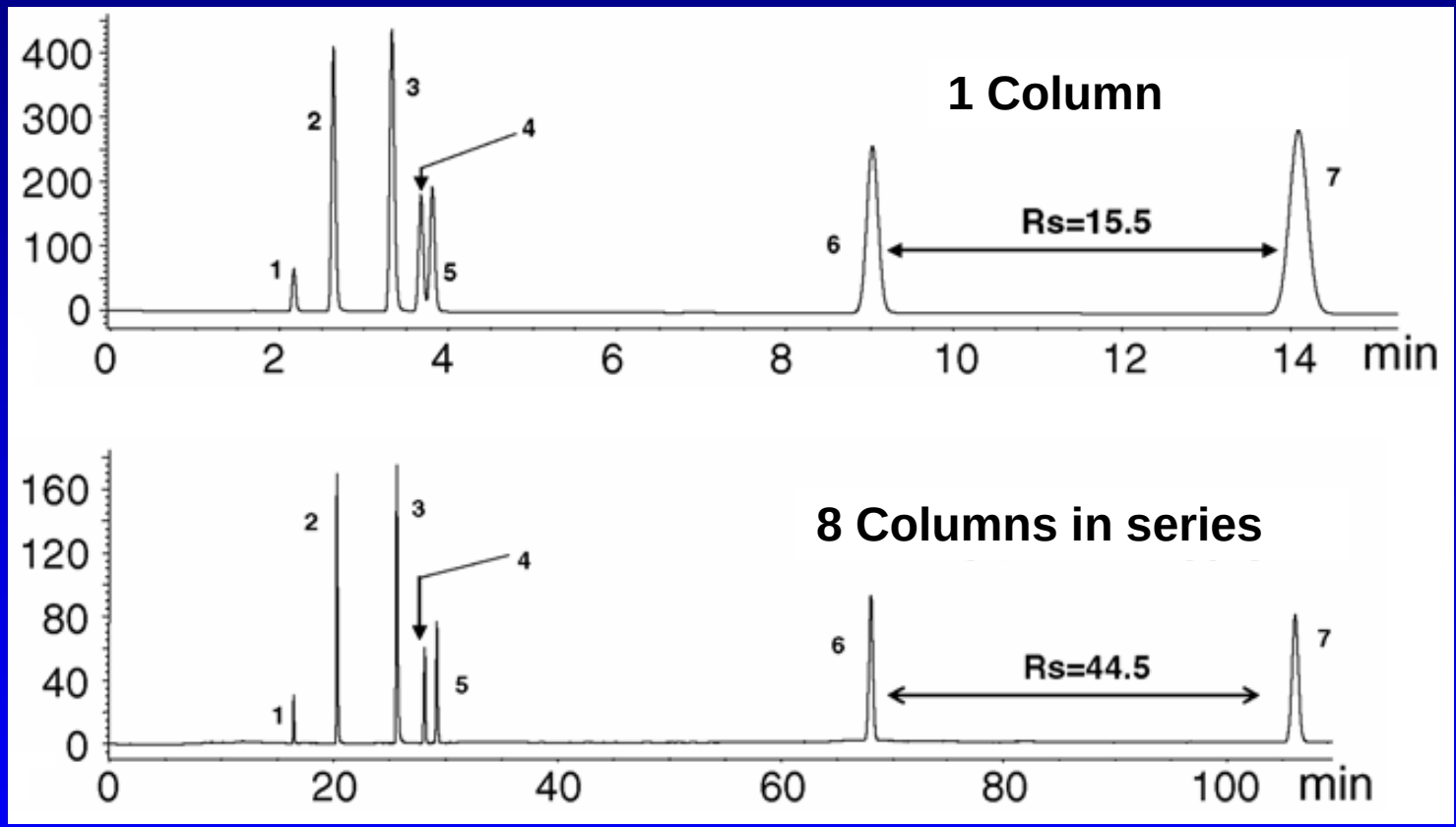
- to increase productivity - speed
- for new unique selectivities
- for higher efficiencies (lower mobile phase viscosity)
- for improved peak symmetry
- for higher sensitivities (less band-broadening)
- to reduce organic solvent usage – “green chromatography”

Viscosity of Water vs. Temperature



--T. S. Kephart and P. K. Dasgupta, *Talanta* **56** (2002) 977-987

Viscosity Reduction at Elevated Temperature Enables the Use of Long Columns



25 cm columns, 5µm particles; 80°C; flow rate 1.0 mL/min, 40%ACN. Peaks:
1. uracil; 2. caffeine; 3. pyridine; 4. phenol; 5. aniline; 6. benzene; 7. toluene

-F. Lestremau, A. Cooper, R. Szucs, F. David, P. Sandra in *J. Chromatogr. A* (2006), in press

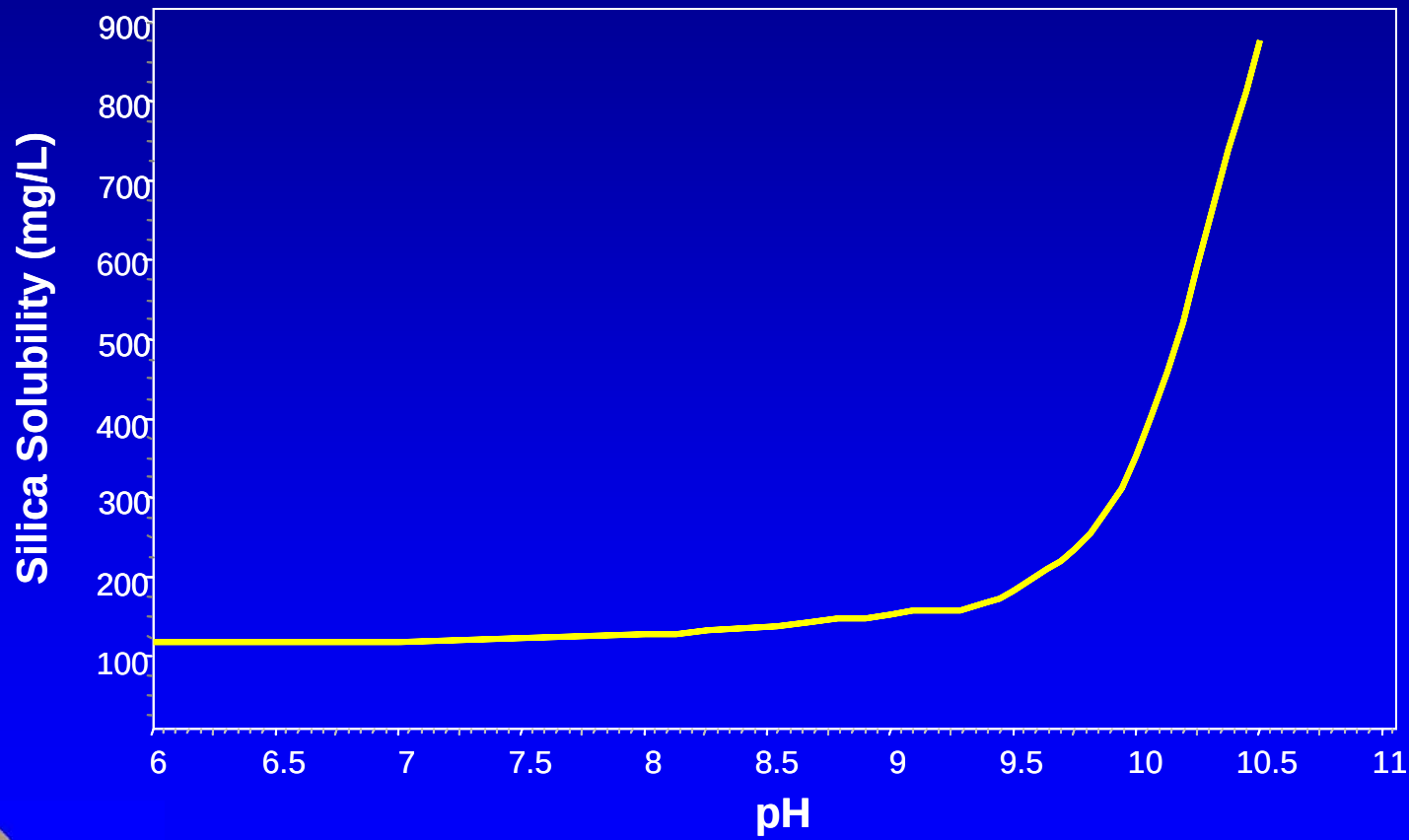
Narrow-Bore Columns

- Sensitivity
- Small samples
- Low mobile phase usage
- Detector compatibility
- Fast thermal program tracking

Historical Microbore Columns

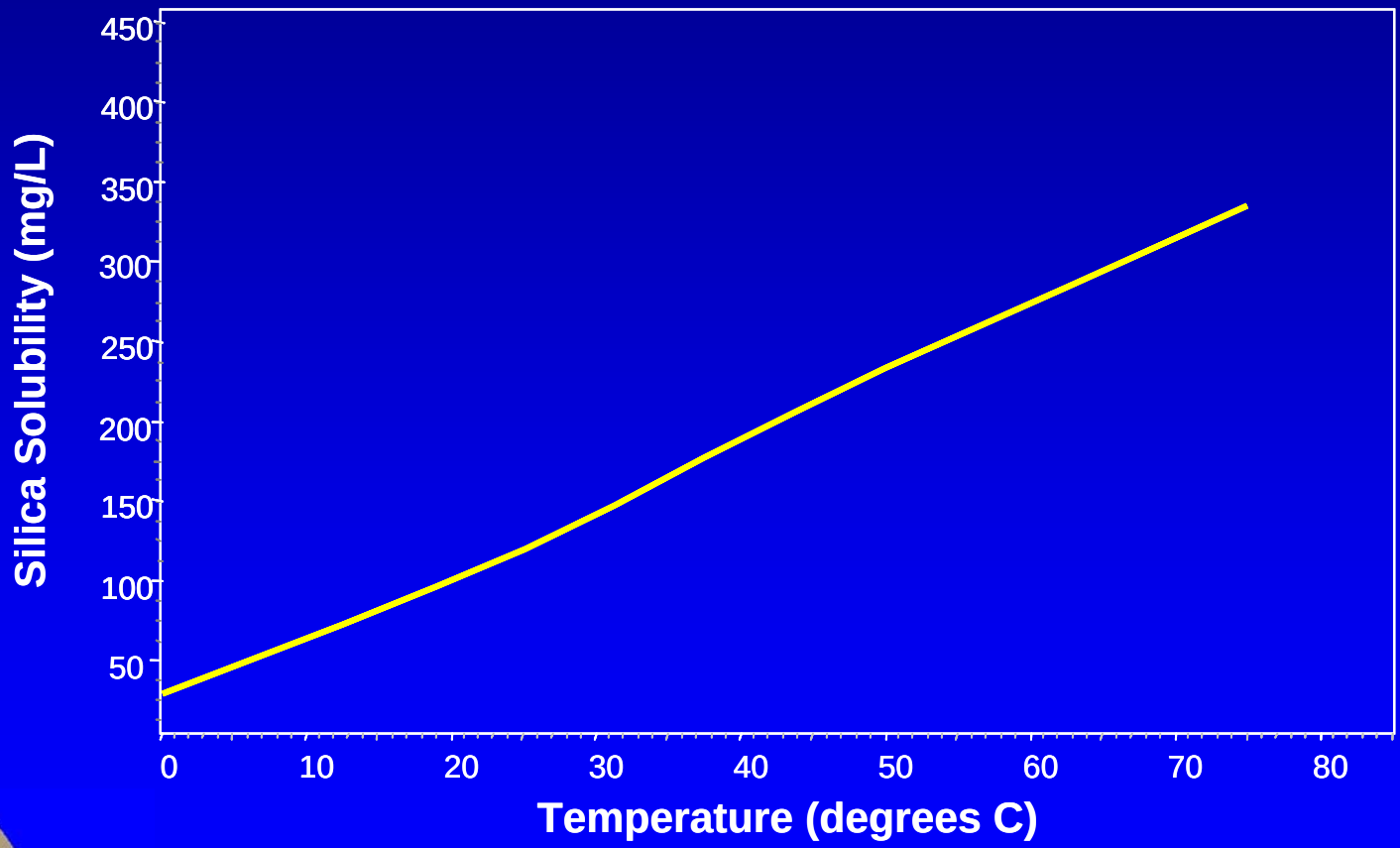
- Generally used for normal phase separations only when at elevated temp or moderate pH with reversed-phase solvents
- Packed fused silica capillaries
- PEEK encapsulated or PEEK sealing components (limits temperature to ~100°C)

Silica Solubility in Water vs. pH



--From R.K. Iler, The Chemistry of Silica, John Wiley & Sons, New York, NY, (1979)

Silica Solubility in Water vs. Temperature



--From R.K. Iler, The Chemistry of Silica, John Wiley & Sons, New York, NY, (1979)

Silica Capillary Dissolution Rate

- More than just bulk solubility in the fluid
- Affected by fluid flow across the surface
- Water concentration and pH influence corrosivity

Comments on the Use of Fused Silica Tubing Based Packed Capillaries for Reversed-Phase Separations Under Aggressive Conditions:

"A silica saturator is used to minimize the dissolution of silica from the capillary wall (not the column, the packing is not silica-based) by the superhot water."

"Overheating the pre-saturator can result in dissolved silica subsequently depositing in the injector."

-T. S. Kephart and P. K. Dasgupta, *Talanta* **56** (2002) 977-987

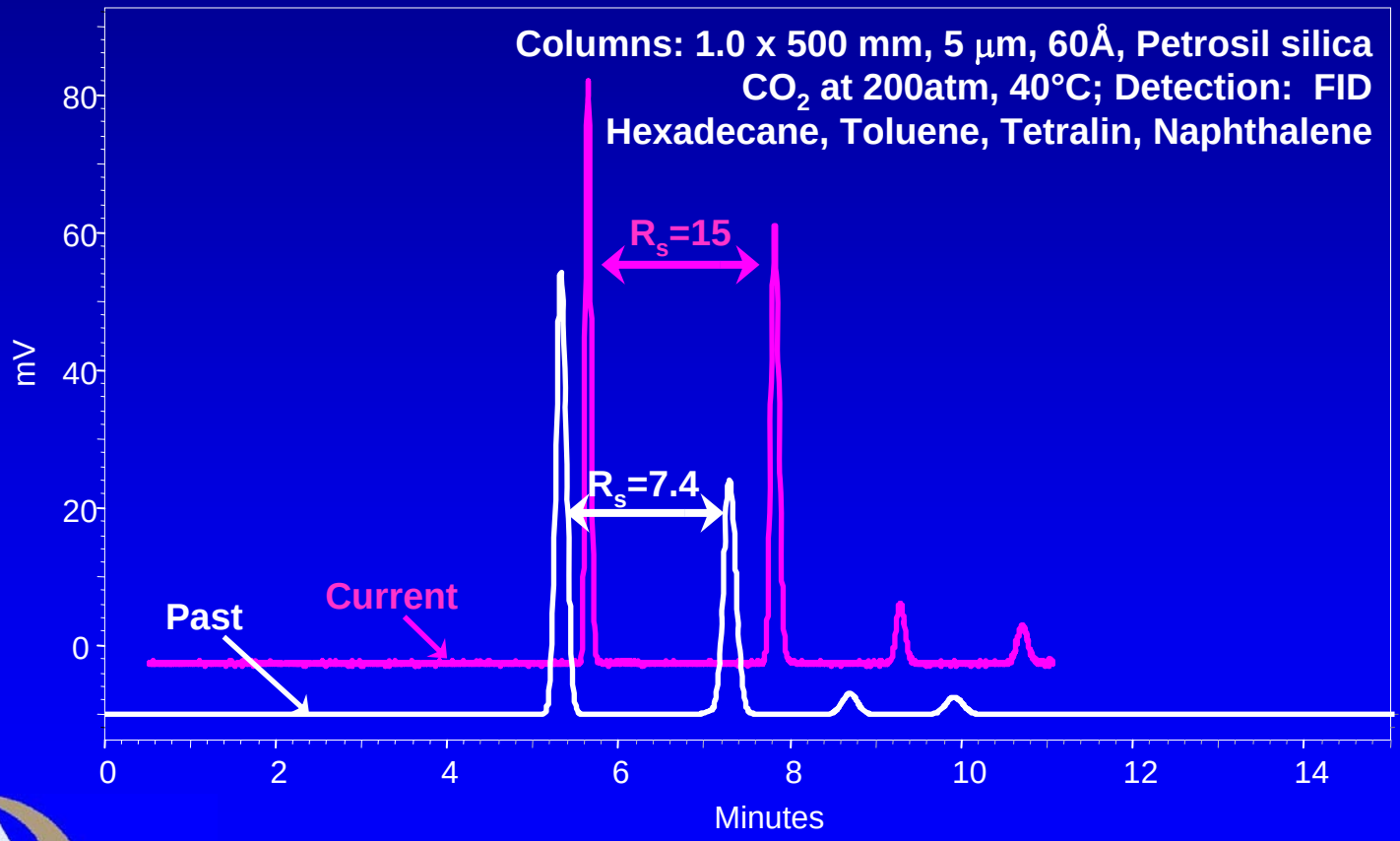
…Bursting of fused silica capillaries occurred even with a silica saturator.

For long term studies, stainless steel capillaries were used…

•T. S. Kephart and P. K. Dasgupta, *Talanta* **56** (2002) 977-987

Selerity SFC Columns

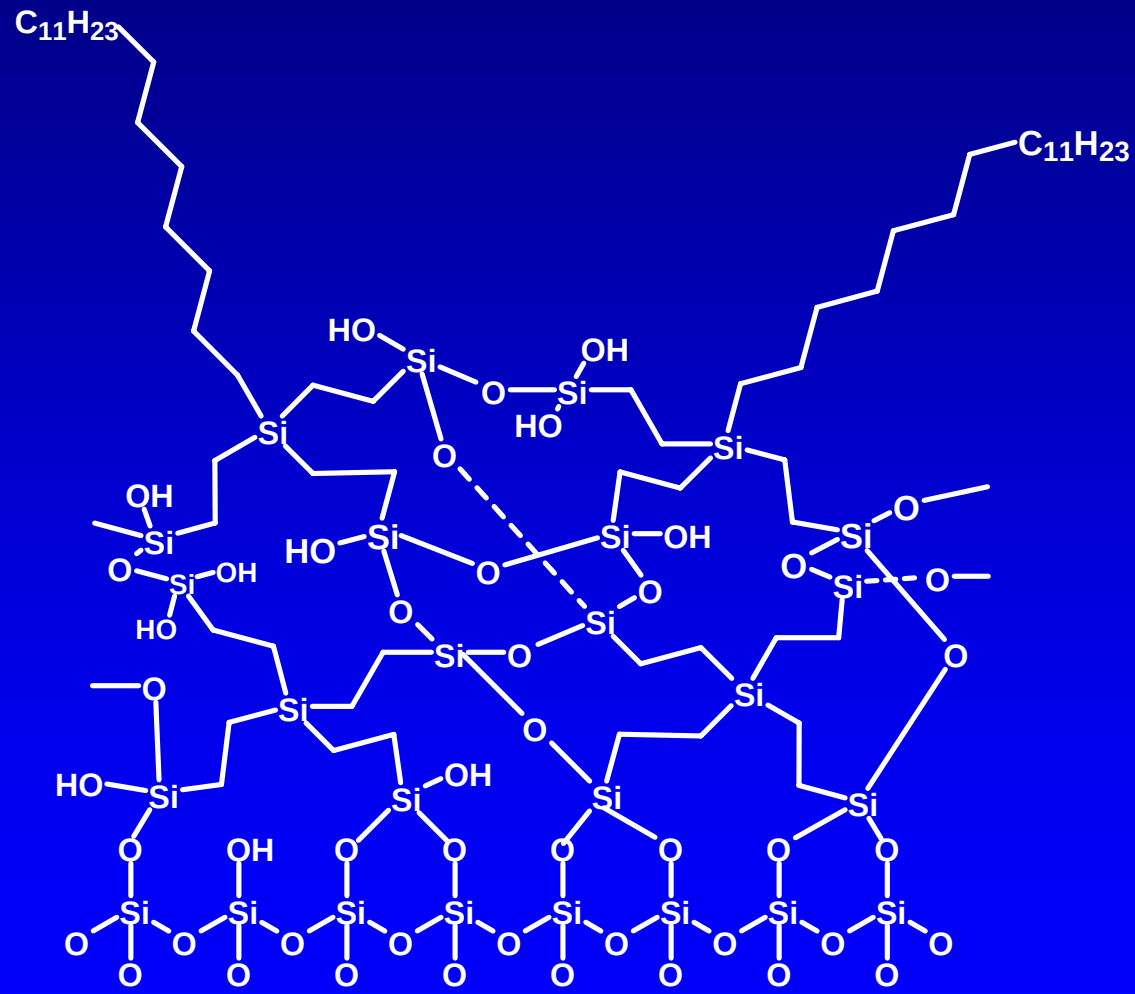
ASTM-5186 Column Performance



Keys to Improving Narrow-bore Column Performance

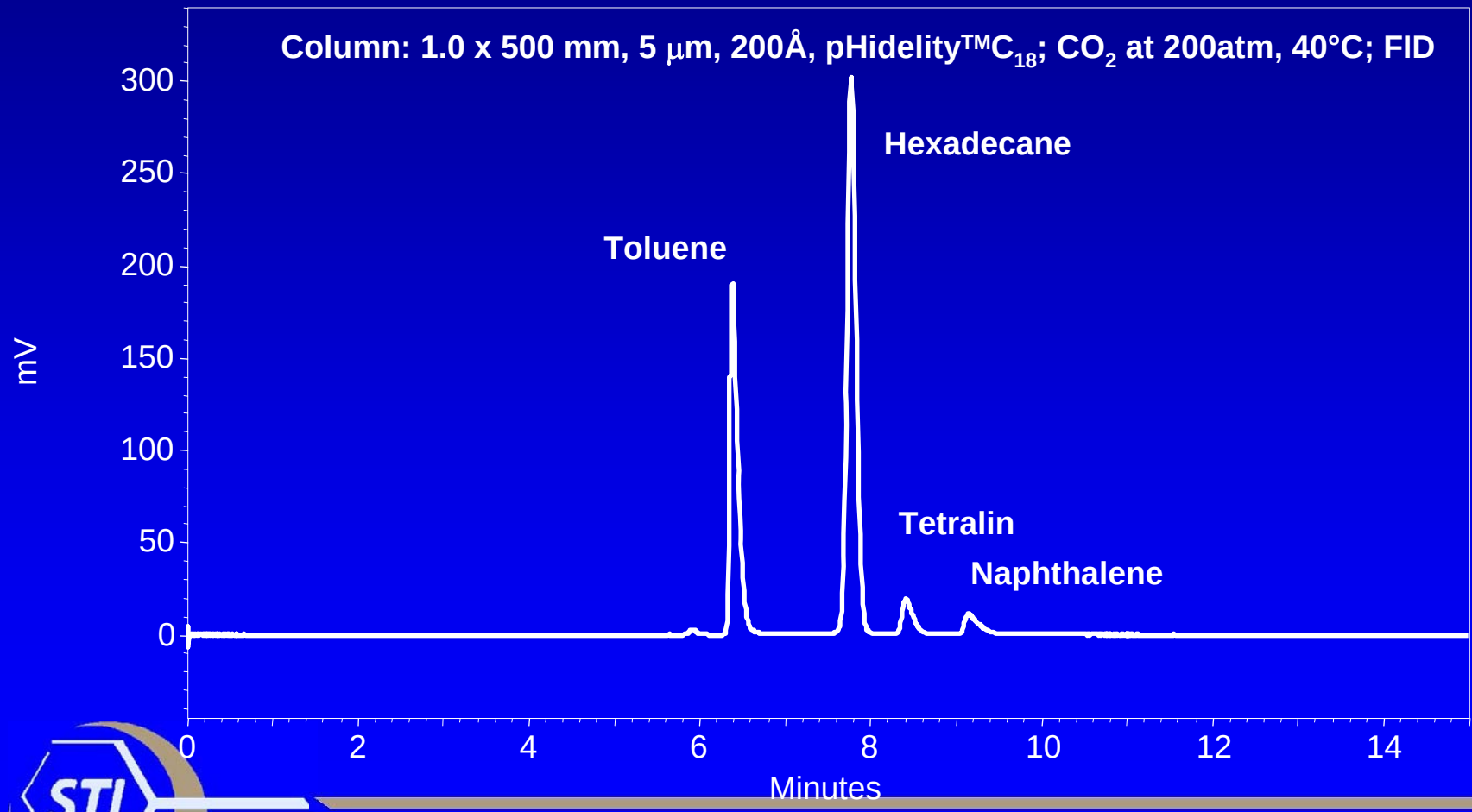
- Improvements in packing technology
- Improvements in producing long lengths of highly polished narrow bore stainless steel tubing
- Development of strong particles with traditional silica selectivity and stability in aqueous mobile phases over wide pH and temperature ranges

pHidelity™ C_{18}/Blaze$_{200}$™ Surface



US and International Patents Pending

SFC Test of pHidelity™ C_{18} Column

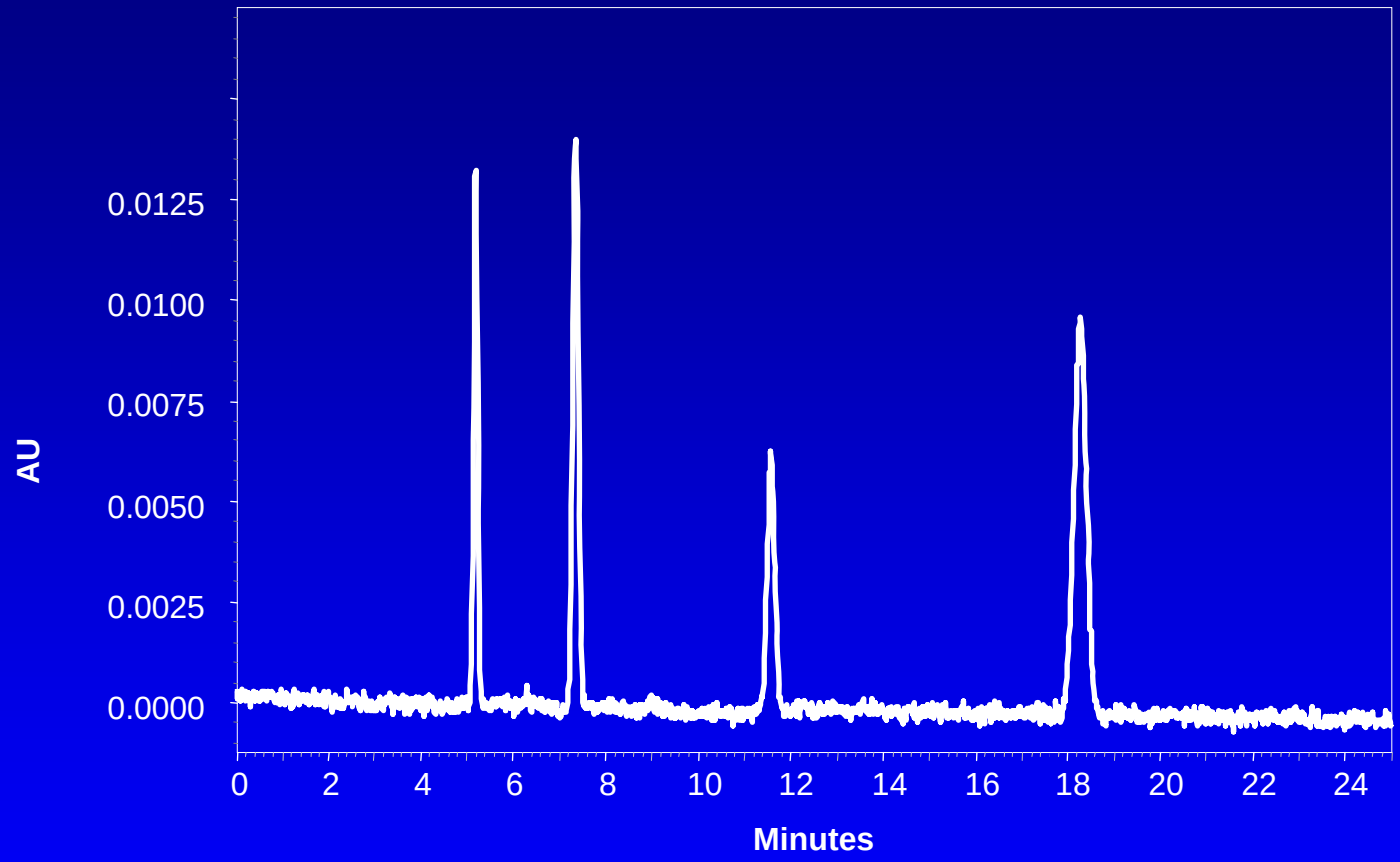


pHidelity is a Trademark of Restek Corporation

Selerity Polaratherm™ Series 9000 Total Temperature Controller

- Used in the LC portion of this study
- Forced air oven and chiller
- Isothermal and thermal gradient operation
  - Sub-zero to 200°C
  - Thermal gradients up to 30°C/min
- Mobile phase preheating and pre-cooling
- Peltier effluent temperature control
- Vapor sensor
- Compatible with any HPLC system

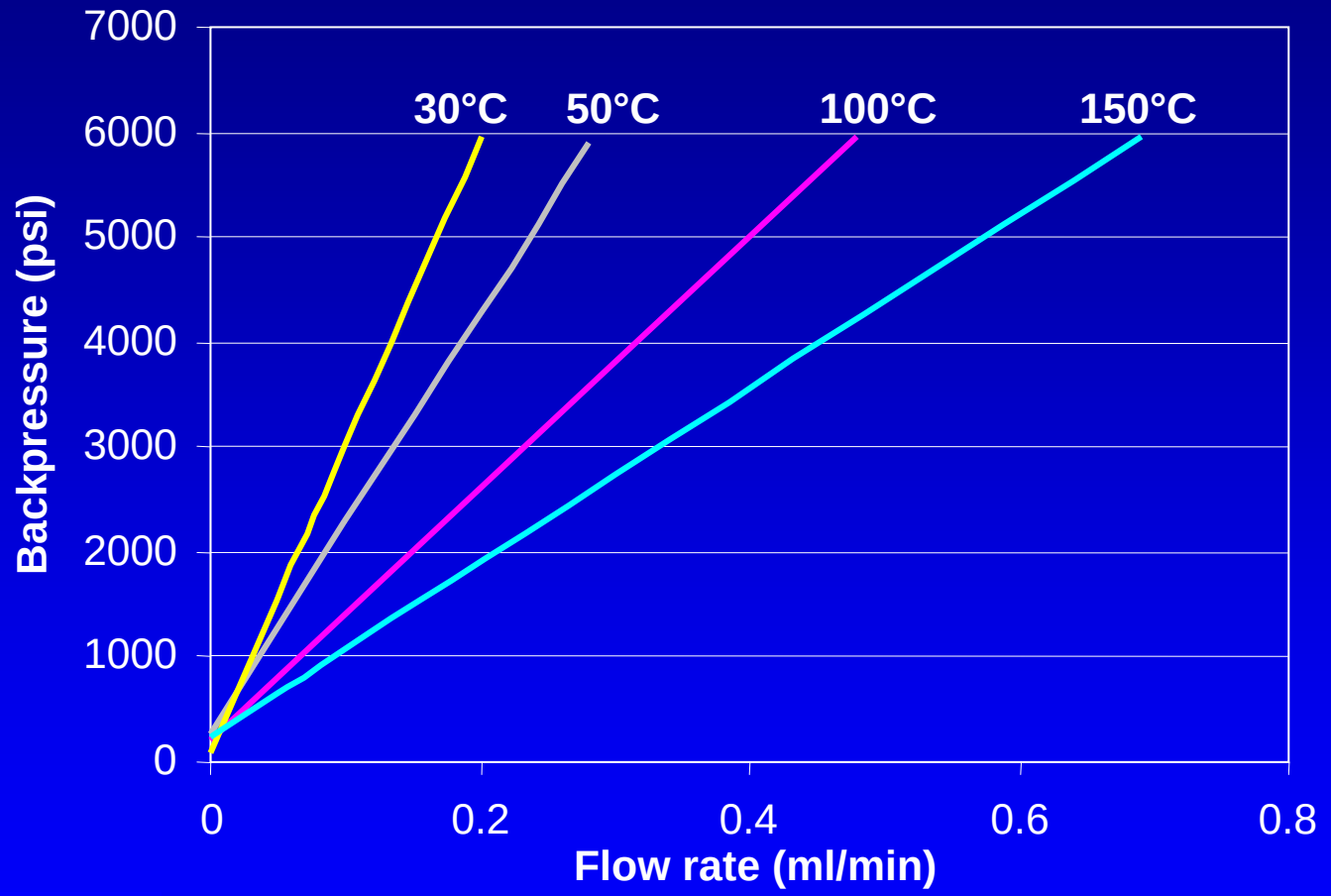
Resolution Test



Column: 1.0 x 500 mm, 5 µm, 200Å, pHidelity™ particles, 50°C
UV 254nm, 170nL cell; 60nL injection; Flow: 0.05 ml/min 50%ACN

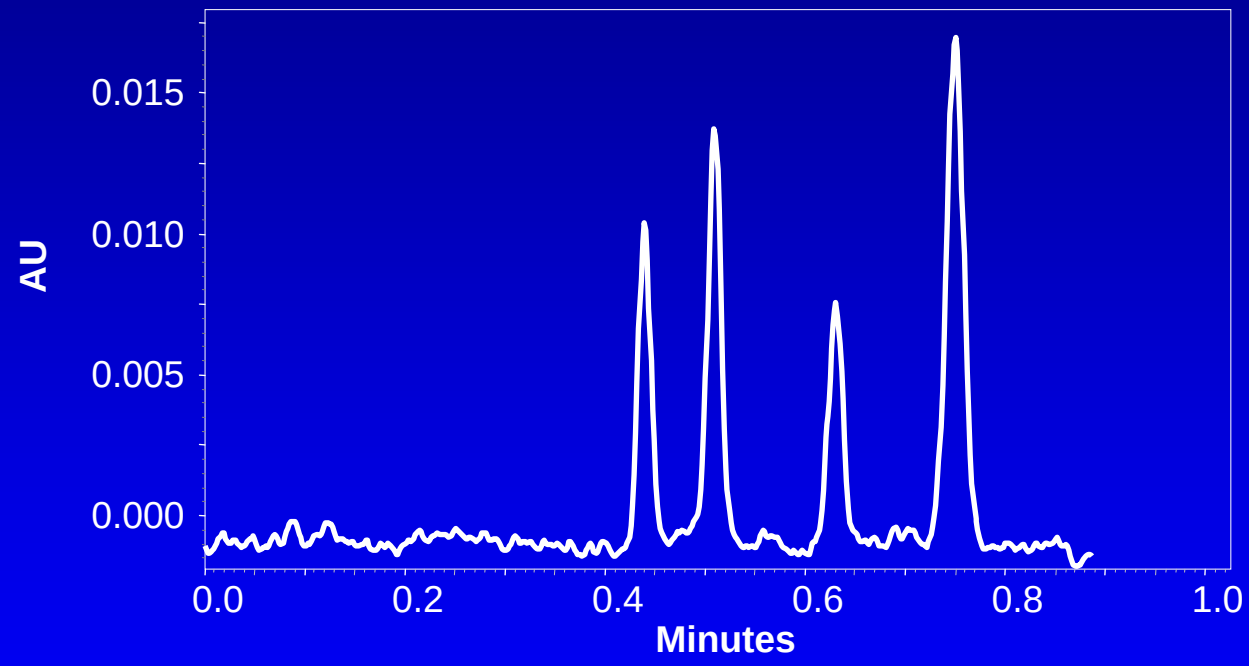
Elution: uracil, phenol, benzene, naphthalene; Reduced plate height: ~4

Pressure Drop vs. Temperature



50%ACN 1x500mm pHidelity™/Blaze™ column 5um particles

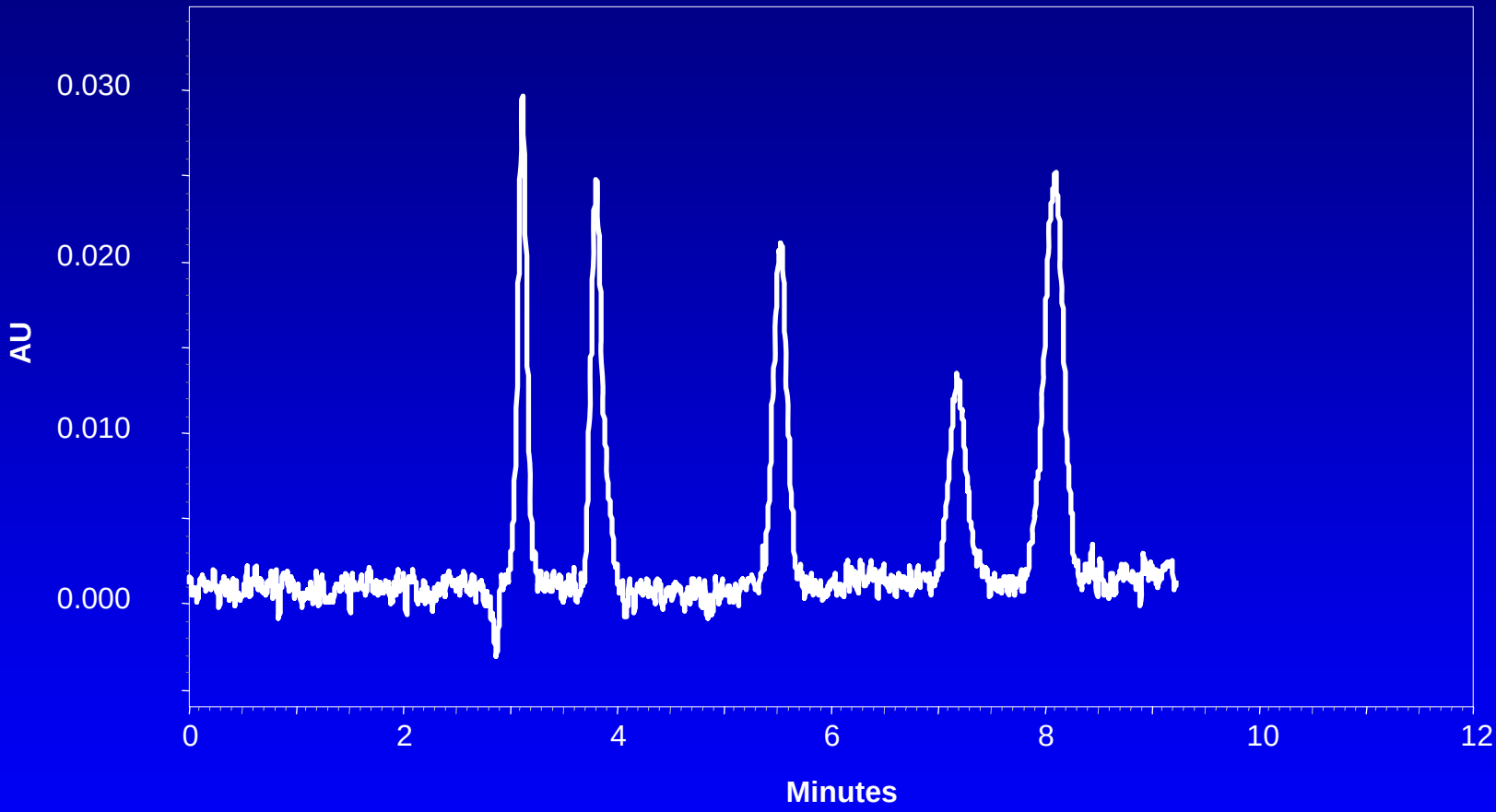
High Speed Separation with a 1 x 500 mm Column



Column: 1.0 x 500 mm, 5 µm, 200Å, pHidelity™ particles, 150°C
Detection: UV 254 nm, 170nL cell, Flow: 0.4 ml/min 50%ACN

Elution: uracil, phenol, benzene, naphthalene

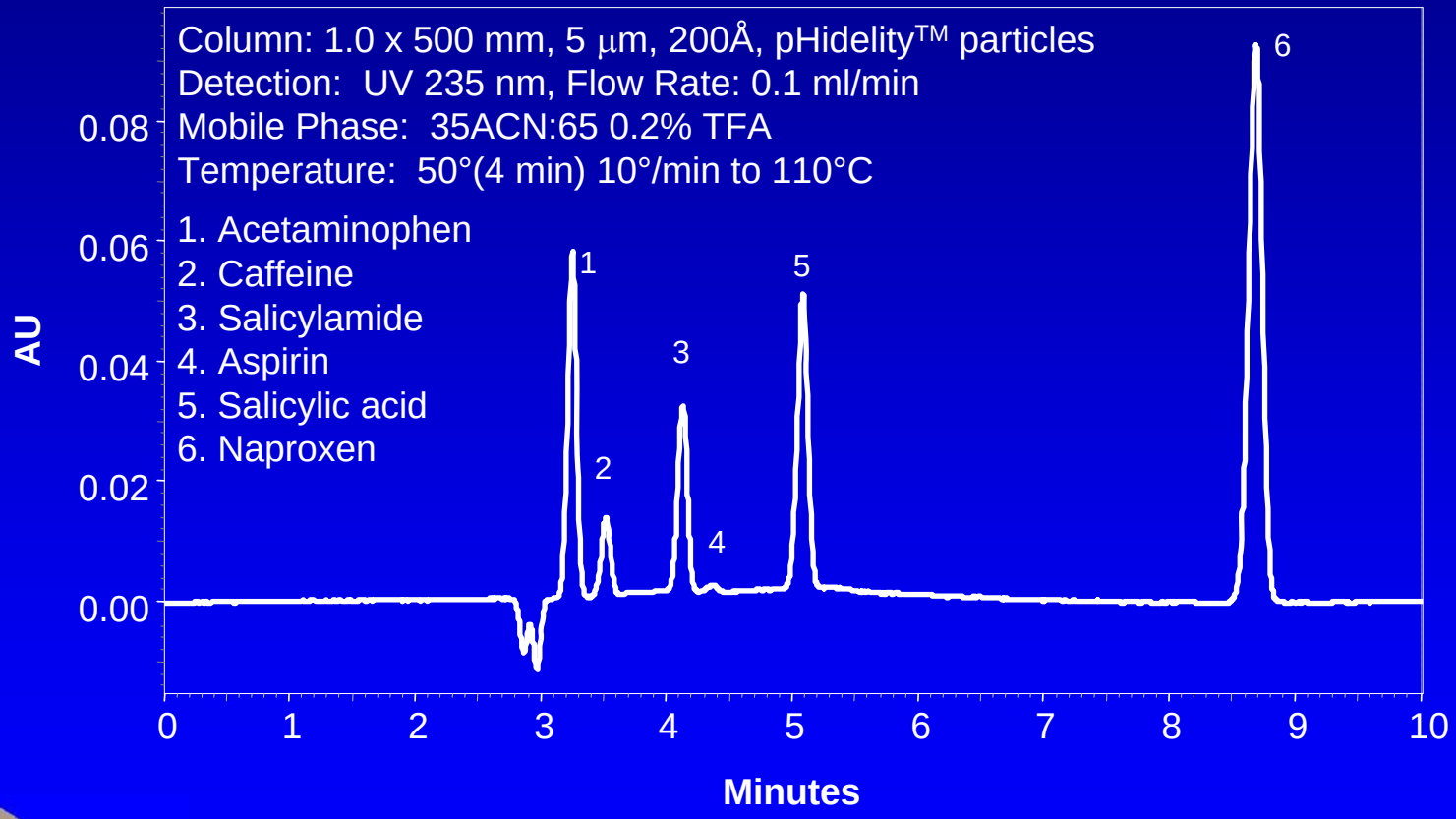
Sleep Aid Separation



Column: 1.0 x 500 mm, 5 µm, 200Å, pHidelity™C_{18} particles, 50°C; UV 254 nm
170nL cell, 60nL Injection; Flow: 0.1 ml/min 35%ACN, 0.2%TFA

Elution: doxylamine, Lunesta™, Ambien™, diphenhydramine, Sonata™

Analgesic Separation



Conclusions

- Polished stainless steel tubing is capable of producing narrow-bore columns in long lengths with good efficiency.
- Stable narrow-bore columns for reversed-phase chromatography can be used with wider pH and temperature ranges.

Turn up the Heat!

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