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

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

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

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

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

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

Silica Solubility in Water vs. Temperature

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.”

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

For long term studies, stainless steel capillaries were used...

Selerity SFC Columns
ASTM-5186 Column Performance

Columns: 1.0 x 500 mm, 5 μm, 60Å, Petrosil silica
CO₂ at 200 atm, 40°C; Detection: FID
Hexadecane, Toluene, Tetralin, Naphthalene

Rs=15
Rs=7.4
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
SFC Test of pHidelity™C\textsubscript{18} Column

Column: 1.0 x 500 mm, 5 μm, 200Å, pHidelity™C\textsubscript{18}; CO\textsubscript{2} at 200atm, 40°C; FID

pHidelity is a Trademark of Restek Corporation

Toluene
Hexadecane
Tetralin
Naphthalene
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

Column: 1.0 x 500 mm, 5 μm, 200Å, pHidelity<sup>TM</sup> particles
Detection: UV 235 nm, Flow Rate: 0.1 ml/min
Mobile Phase: 35ACN:65 0.2% TFA
Temperature: 50°(4 min) 10°/min to 110°C

1. Acetaminophen
2. Caffeine
3. Salicylamide
4. Aspirin
5. Salicylic acid
6. Naproxen
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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