

Silica-Based Columns for Reversed-Phase HPLC Separations at Temperature and pH Extremes

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The Incentive for Silica

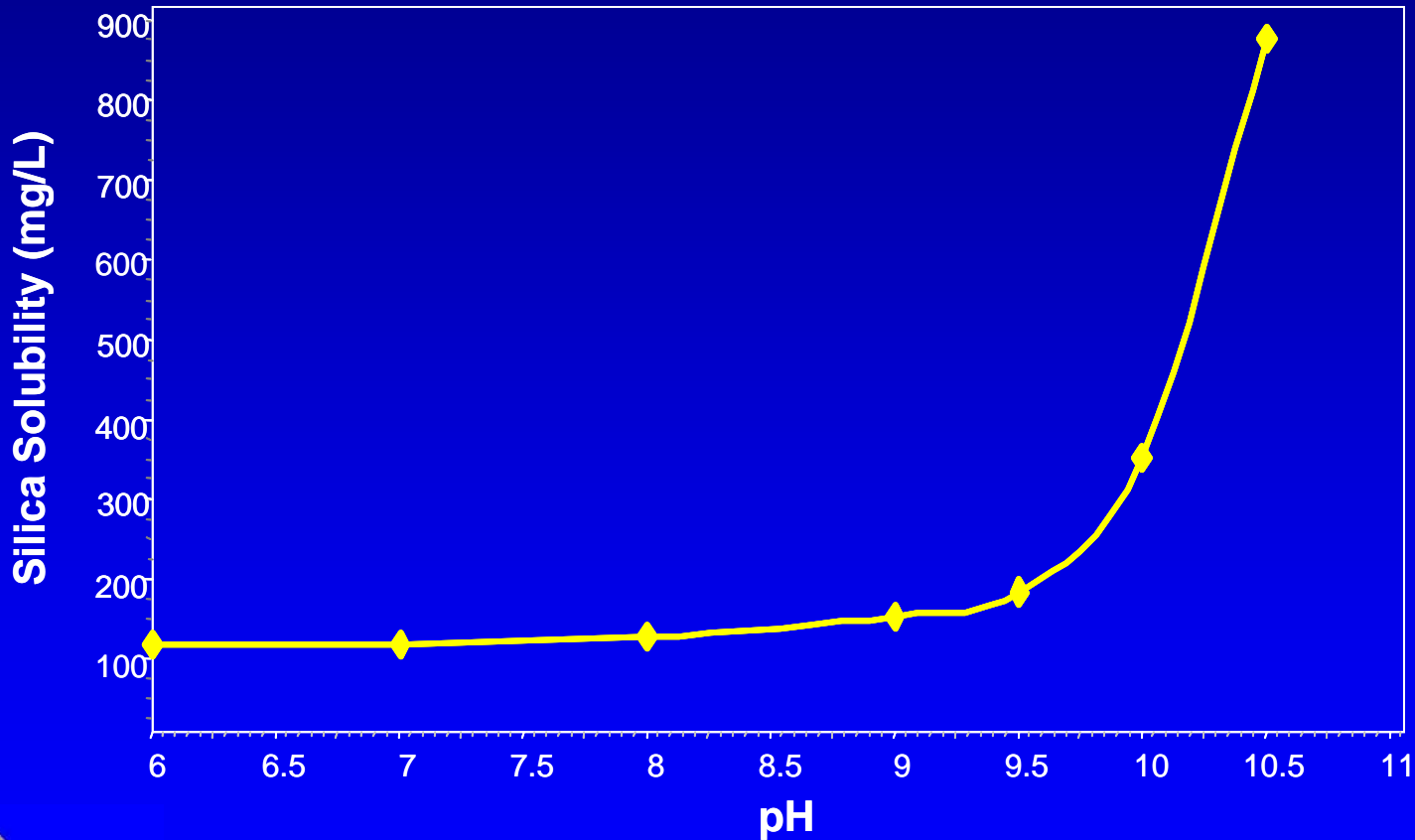
- Selectivity is widely accepted and predictable
- High efficiency
- Available in a wide range of dimensions
 - Particle size
 - Pore size
 - Surface area
- High particle strength



**High temperature and pH
extremes stress
columns in similar ways**

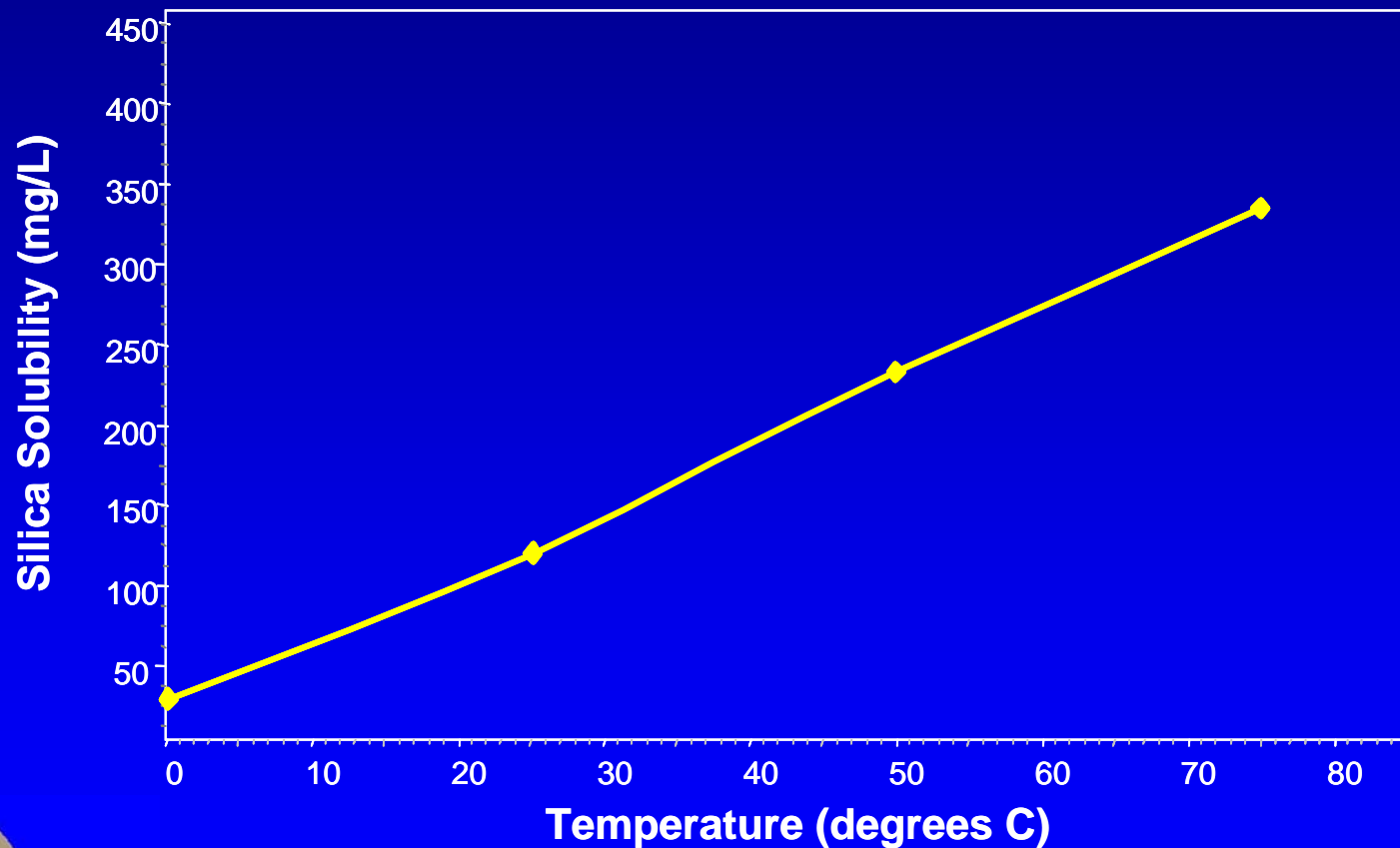


Silica Solubility in Water vs. pH



--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 Precipitation from Geothermal Water



- The Blue Lagoon, Iceland



Silica Dissolution Rate

- More than just bulk solubility in the fluid
- Kinetics play a role
 - Affected by surface area exposed
 - Typically $>150 \text{ m}^2/\text{gm}$ for chromatographic silica
 - Affected by fluid flow across the surface
- Water concentration and pH influence corrosivity



Benefits of Stable Columns

- High thermal stability
 - Selectivity tuning
 - Faster analysis
 - Less organic modifier required
 - Wider temperature programming range
 - Extended column lifetimes under more moderate conditions



Benefits of Stable Columns

- Wide pH range
 - Ion suppression for acids
 - Ion suppression for amines
 - Column regeneration by elution of contaminants at pH extremes

For example: Separating basic analytes at high pH gives:

- Increased loading
- Increased retention
- Increased resolution



Factors Affecting Retention That Are Influenced by Temperature

- Hydrogen bonding
- Solvation sphere around analytes
- Hydration extent of column surface
- Functional group interaction
- Ordering, shape and hydration transitions
 - Conformation changes
- Dielectric constant of mobile phase



HPLC Columns Stable at High Temperatures

- Polymeric DVB
 - Stable to 150°C and pH 0-14 (requires SS frits)
- Carbon (Hypercarb™)
 - Stable to 200 °C (requires SS frits)
- Polydentate modified silica (Selerity/Restek Blaze™ series)
 - Newest phase stable to 200 °C and pH 1-12
- Sterically hindered silane modified silica (Agilent StableBond™)
 - Useful for low pH and moderately high temperatures
- Hybrid organically modified silica (Waters Xterra™)
 - Phase loss and silica backbone breakdone under reversed phase conditions at high temperatures
- Zirconia
 - Alternative Selectivity: Not recommended for temperature programmed conditions because of excessive bleed

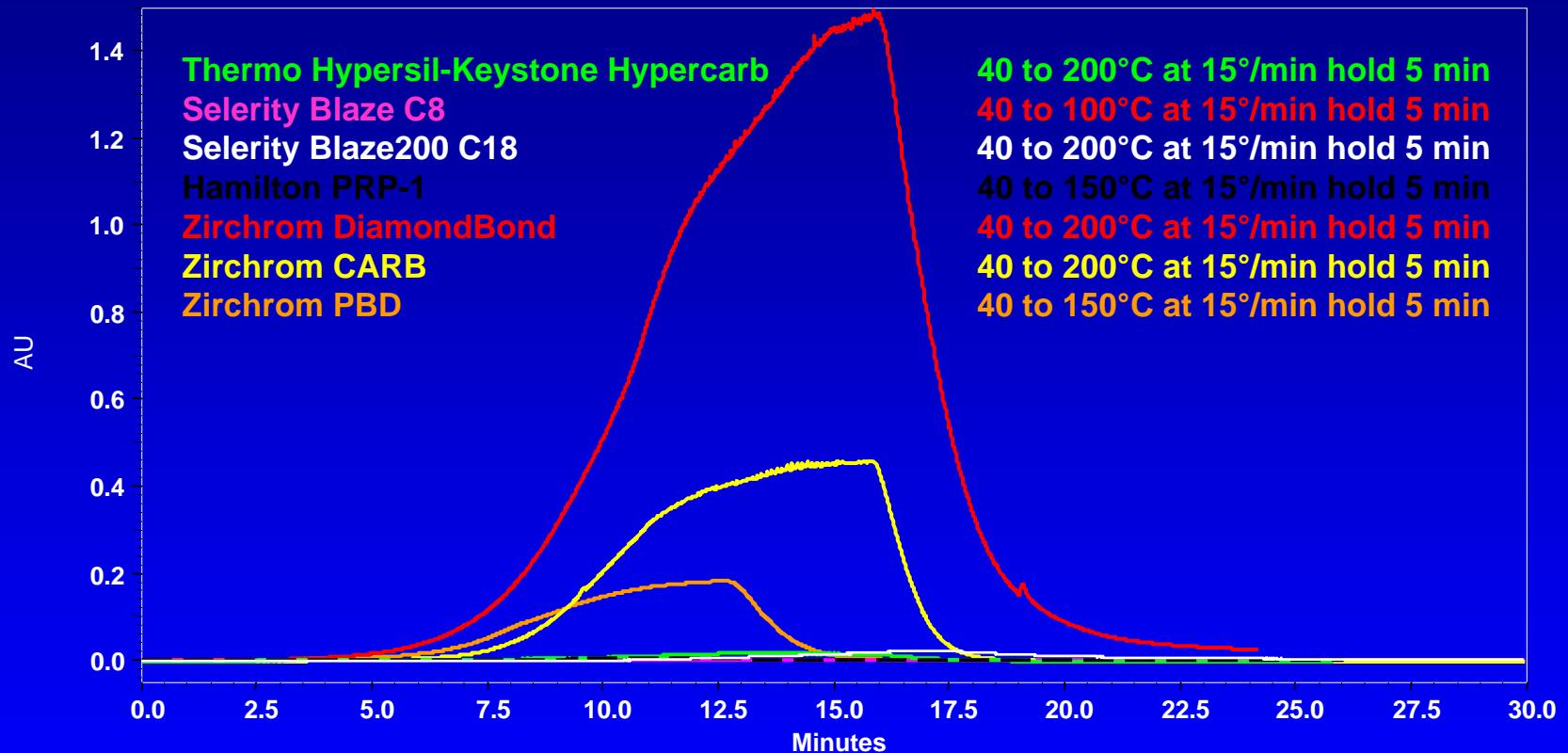


Selerity Polaratherm™ Series 9000 Total Temperature Controller

- Used in 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



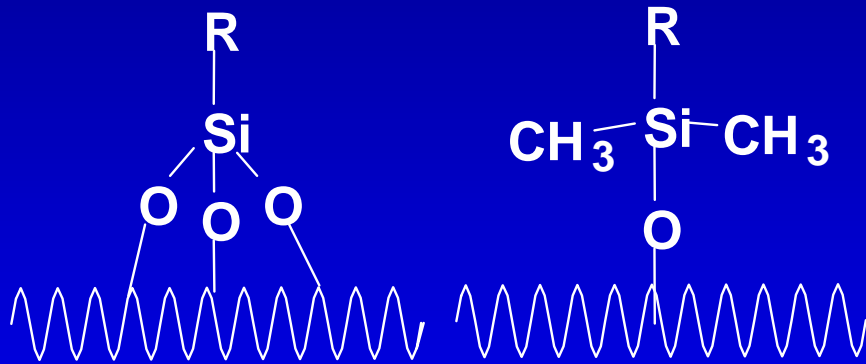
Column Bleed Comparison During Thermal Programming



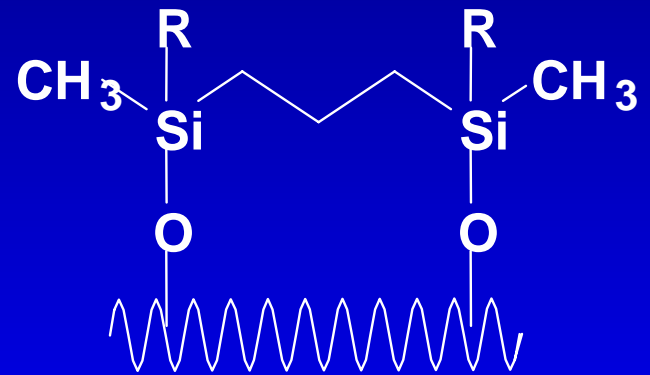
50:50 Acetonitrile:Water at 254 nm



Early Silane Bonding



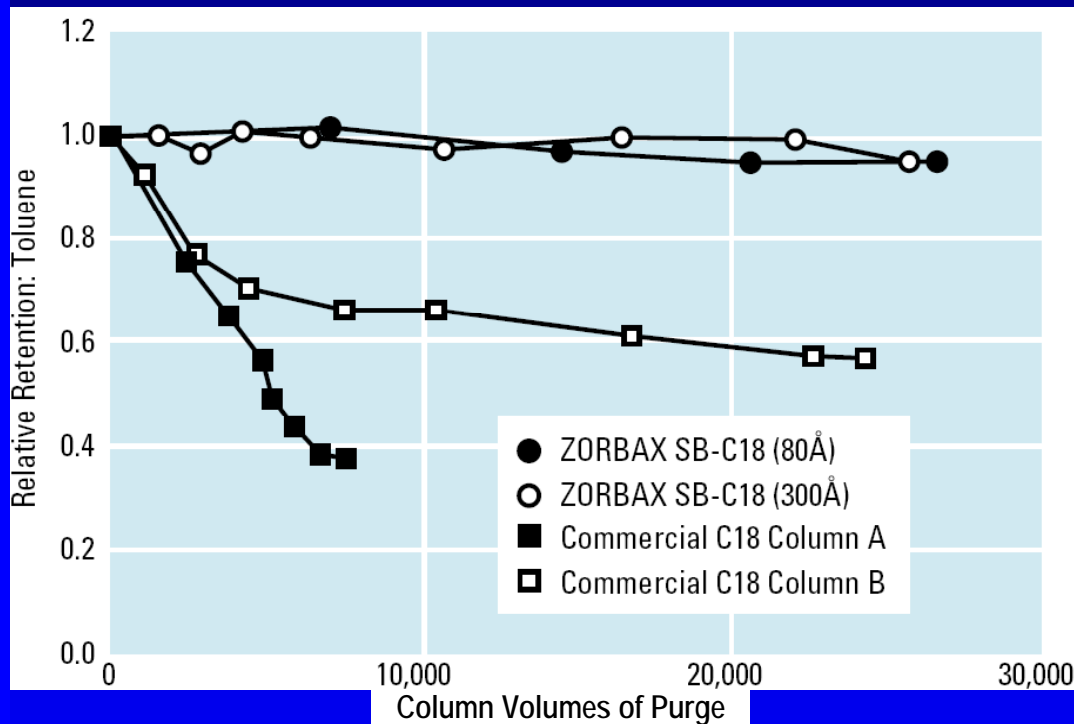
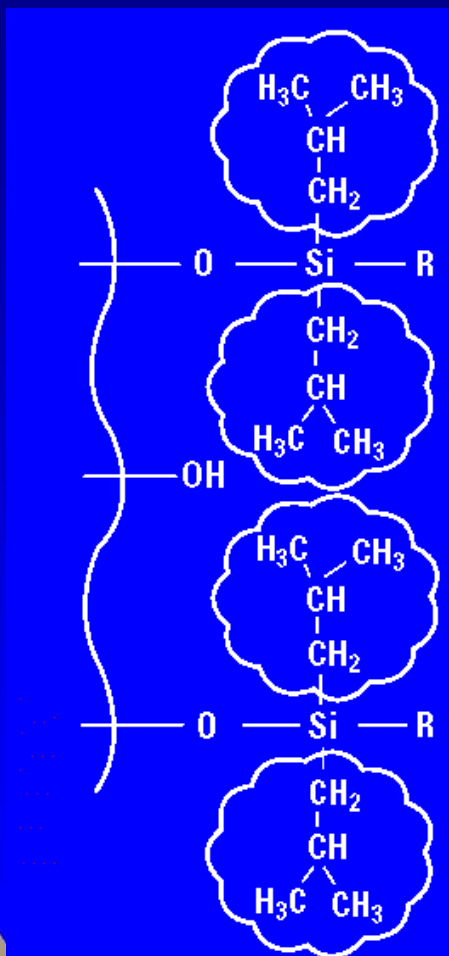
Monodentate



Bidentate



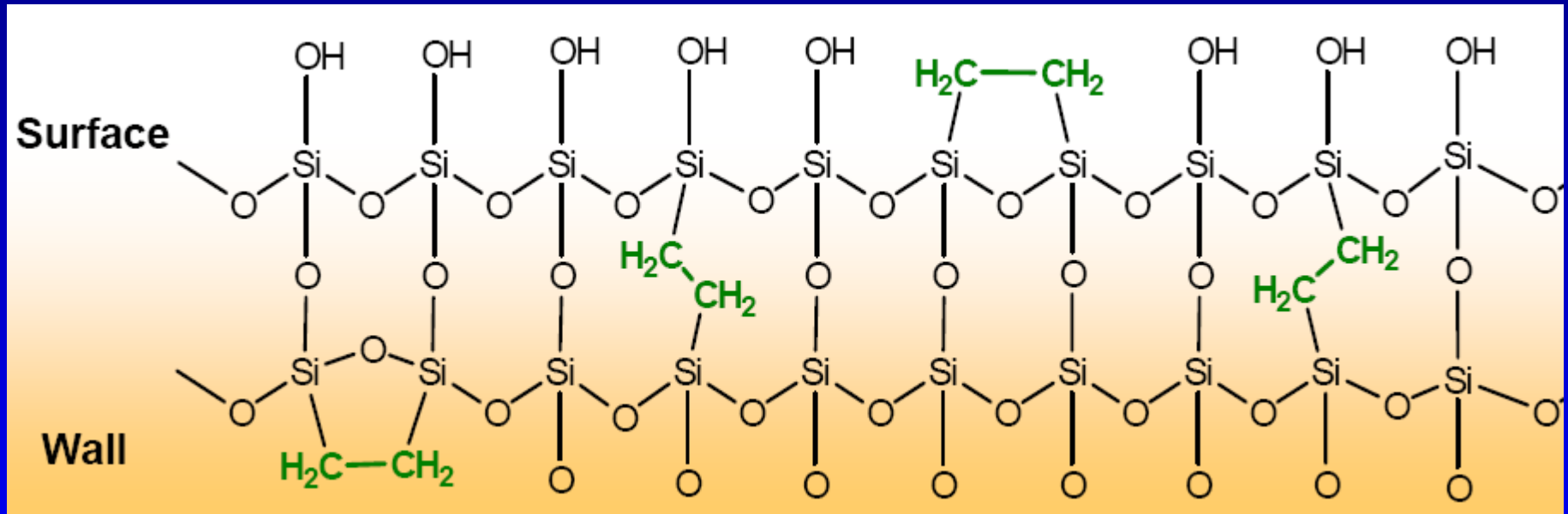
Agilent Stablebond™ Sterically Hindered Phases



Purge solvent: 50%MeOH/50% Water
with 1.0% TFA, pH 0.8, 90°C



Waters 2nd Generation Hybrid

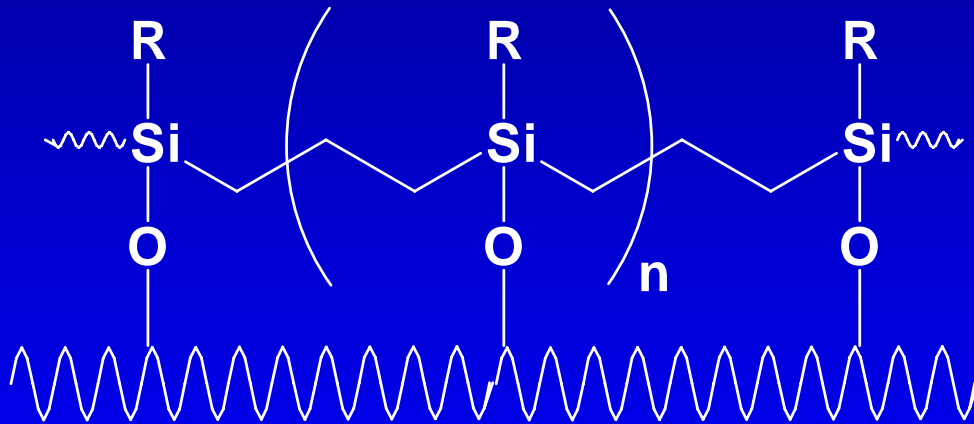


Bridged Hybrid



--Drawing from Waters Marketing Literature

Selerity Blaze™ Multiple Point Bonding



**Polycarbosilane-
Branching
Added**

Gen. 1 Polydentate

Gen. 2 Polydentate



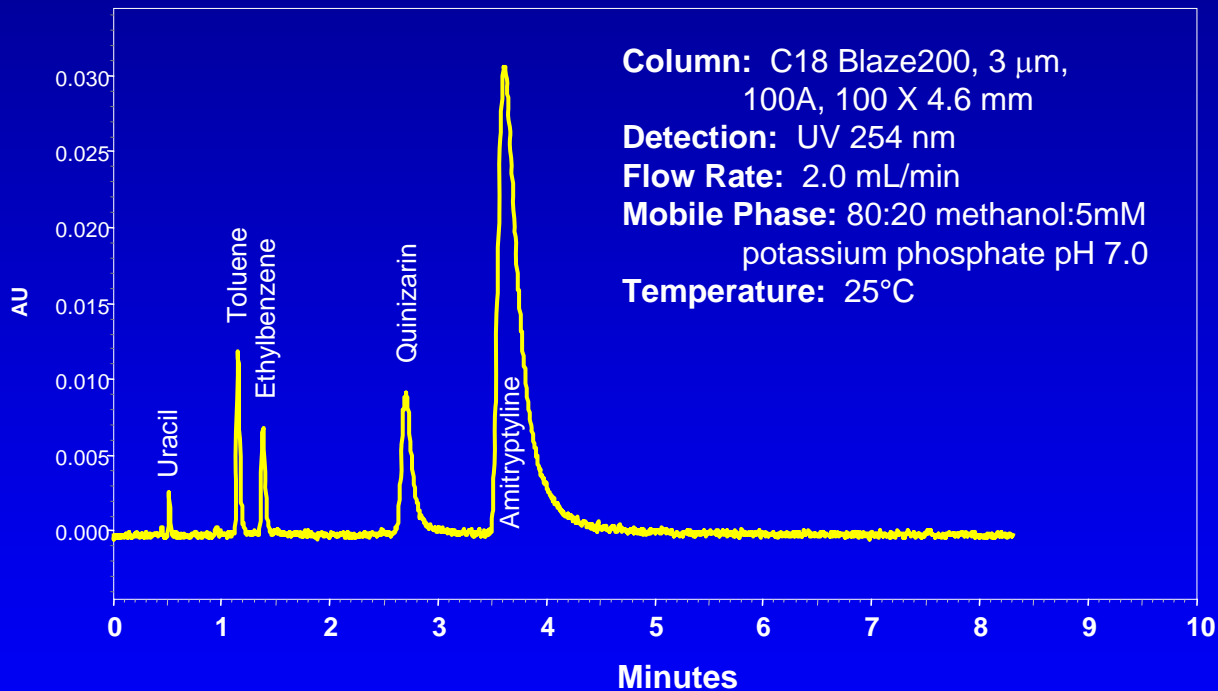
US and International Patents Pending

Comparison Between Durable Chemistries

- Waters
 - Each particle type must be optimized
 - Particle strength partially compromised
 - Surface organic groups reduce low pH stability
- Agilent
 - Sterically hindered phases give good stability with low pH and elevated temperatures
- Selerity/Restek
 - Simple surface coating
 - Works on any silica
 - Underlying particle structure undisturbed
 - Stability at low and high pH and high temperature



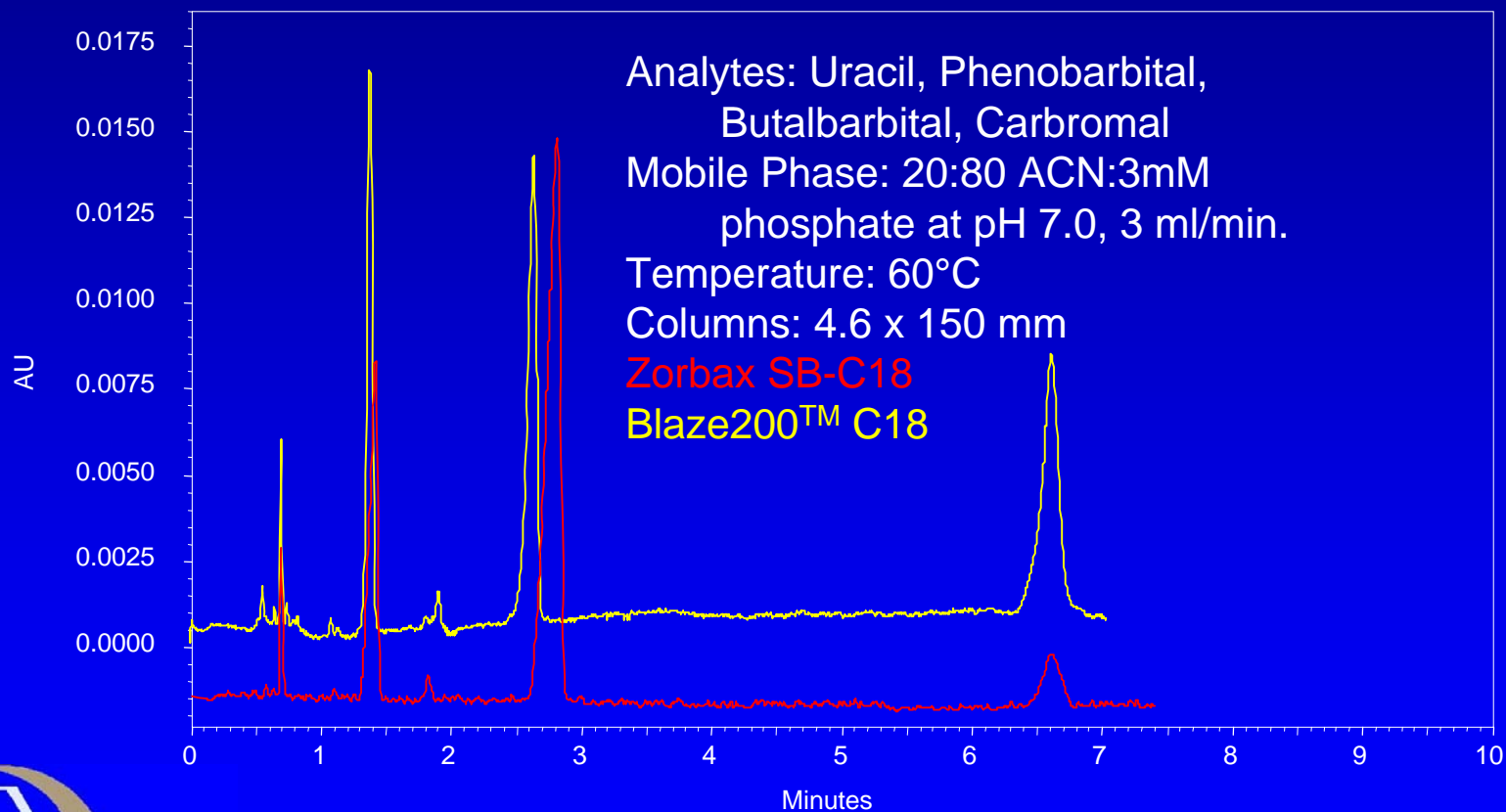
Selectivity Determination Blaze200™



SRM 870 test conditions were followed, and indicate typical C18 silica column selectivity. Tailing and asymmetry for amitriptyline indicate some silanol interaction. Peak shape and elution of quinizarin indicate low activity toward metal chelating agents.



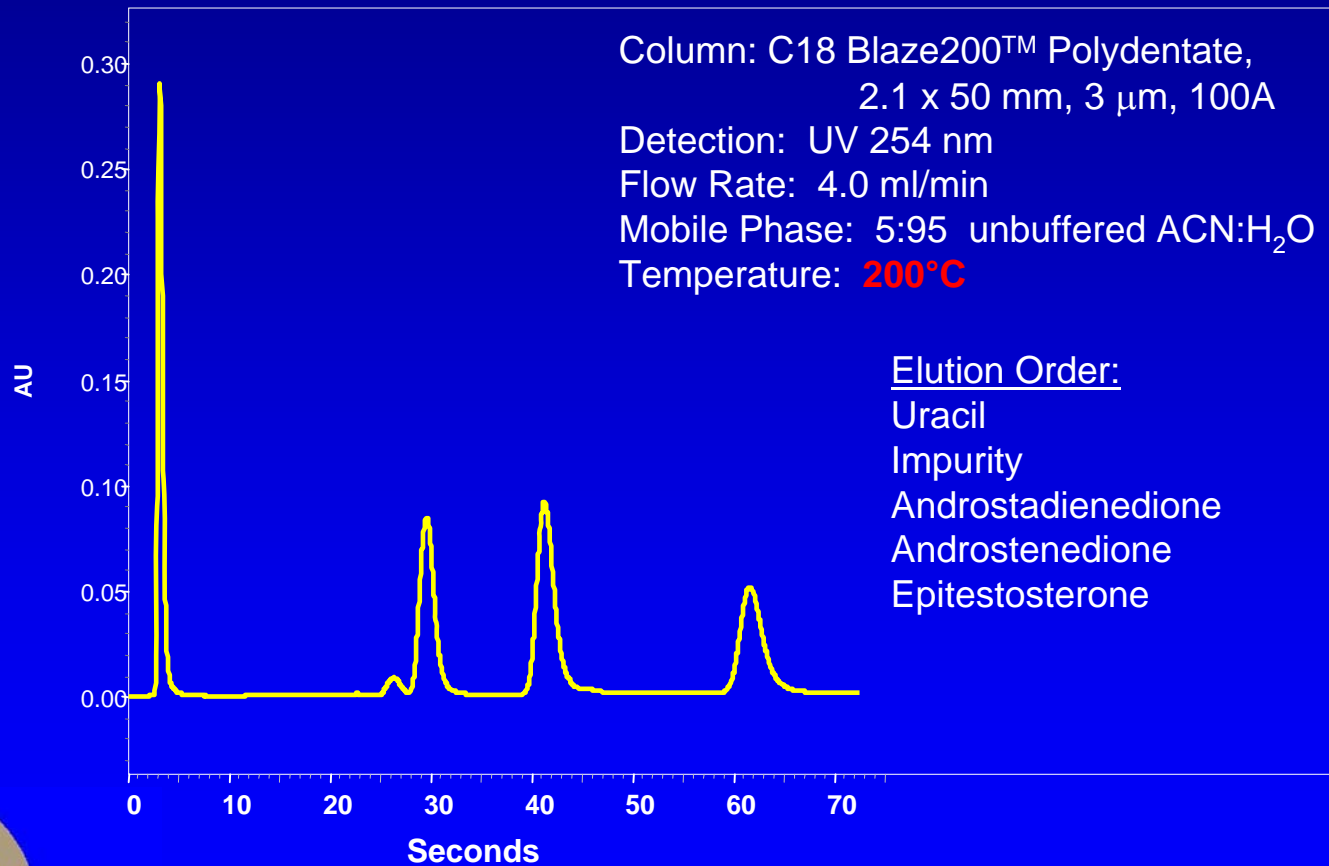
Selectivity Comparison Blaze200™ C18 / Zorbax SB-C18



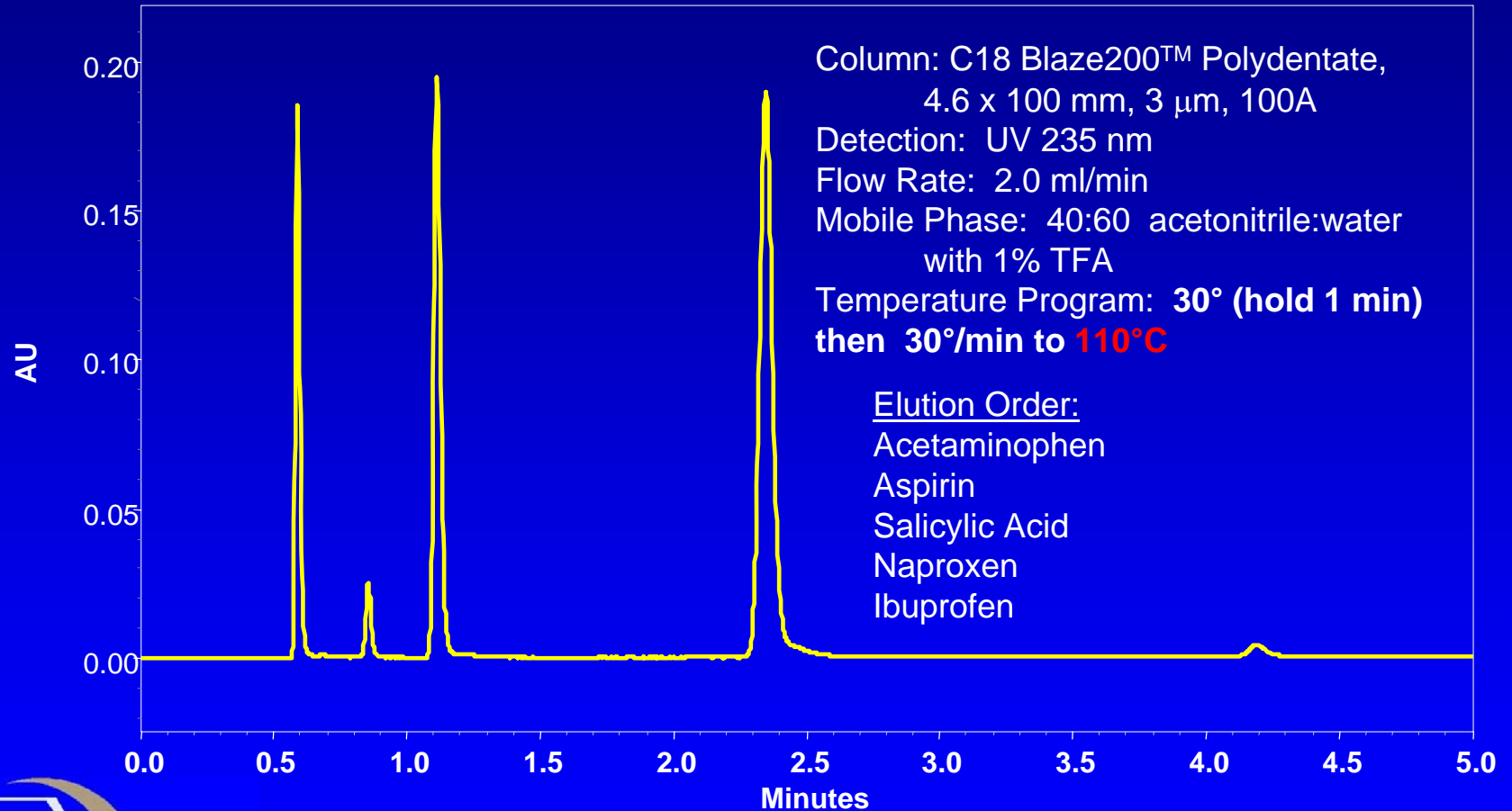
Selerity Caloratherm™ Mobile Phase Temperature Controller



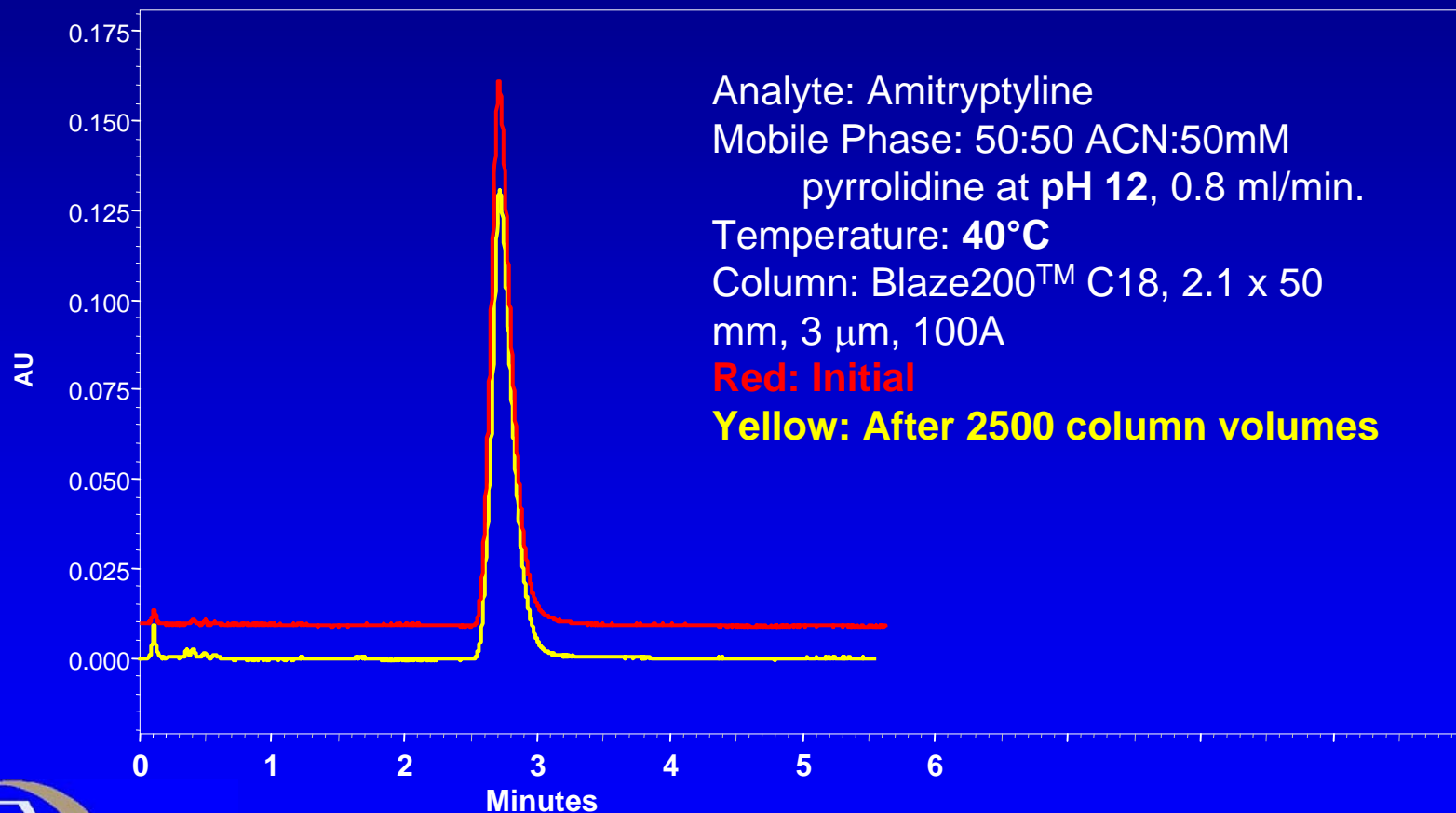
High Water Content Mobile Phase Separation at 200°C



Separation of Analgesics at pH 1



High pH Stability Evaluation



Conclusions

- A polydentate bonded phase for silica particles with unprecedented hydrolytic and thermal stability has been demonstrated.
- Reversed-phase operation with silica column selectivity can be performed at temperatures to at least 200°C and a pH range including 1-12.



 **Turn up the Heat!** 

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Partnering with Restek Corporation

