

Temperature and pH Stability of a New High Temperature C18 Polydentate Silica Column

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Outline

- Benefits of durable silica columns
- Durable column chemistries
- Blaze 200 chemistry
- Selectivity comparison
- Blaze 200 evaluation



Benefits of pH Stable Columns

- Wide pH range
 - Ion suppression for acids
 - Ion suppression for amines
 - Column regeneration by elution of contaminants at pH extremes
- Separating basic analytes at high pH gives:
 - Increased loading
 - Increased retention
 - Increased resolution



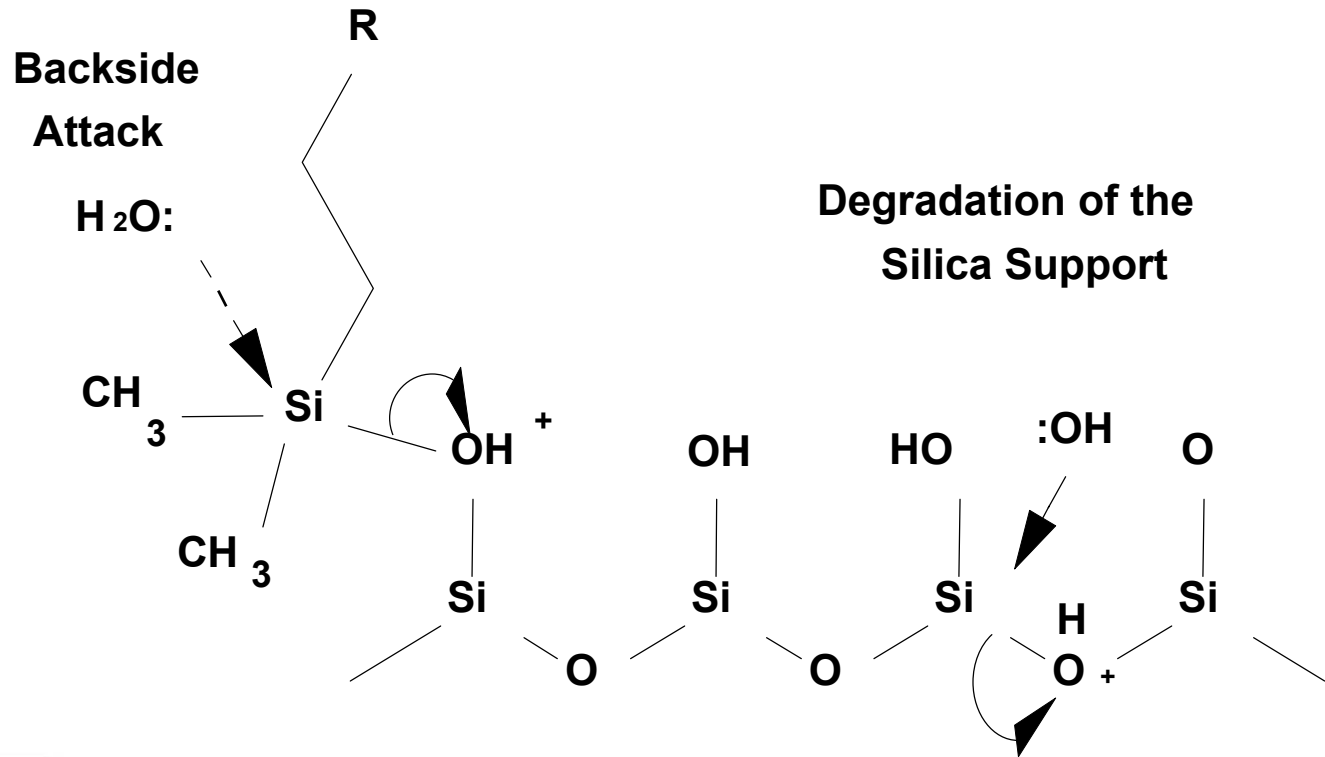
Benefits of Temperature Stable Columns

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



Silica Columns are Subject to Hydrolysis and Degradation

Water attacks siloxane bond or behind point of phase attachment



Durable Non-Silica Column Chemistries

Columns stable at high temperatures

- Zirconia columns
- Polymeric PSDVB or DVB columns
 - Hamilton PRP-1 Jordi DVB
 - Stable to 150°C
 - pH 0 to 14
- Graphitic Carbon
 - Thermo Hypercarb
 - Stable to 200°C
 - pH 0 to 14



Comparison Between Durable Silica Based Chemistries

- Sterically Hindered Phases
 - Agilent Stablebond
 - give good stability only with low pH at elevated temperatures
- Organic/inorganic hybrid family
 - Waters Xterra
 - Carbon/silica hybrid particle
 - Stable chemistry, apparently usable at high pH and elevated temperatures
 - Surface organic groups reduce low pH stability



Comparison Between Durable Silica Based Chemistries

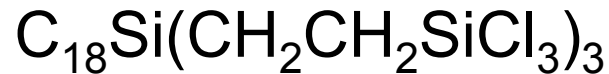
- Selerity Blaze₂₀₀TM
 - Simple surface coating with dendrimeric reagents that can be readily purified
 - Works on any silica and has traditional silica selectivity
 - Underlying particle structure undisturbed, resulting in the highest strength and efficiency
 - One column has stability at low and high pH and high temperature



Blaze₂₀₀TM Bonding Chemistry

Generation 1 ethyl-bridged carbosilane dendrimers with multiple attachment points as barrier layers and functional layers

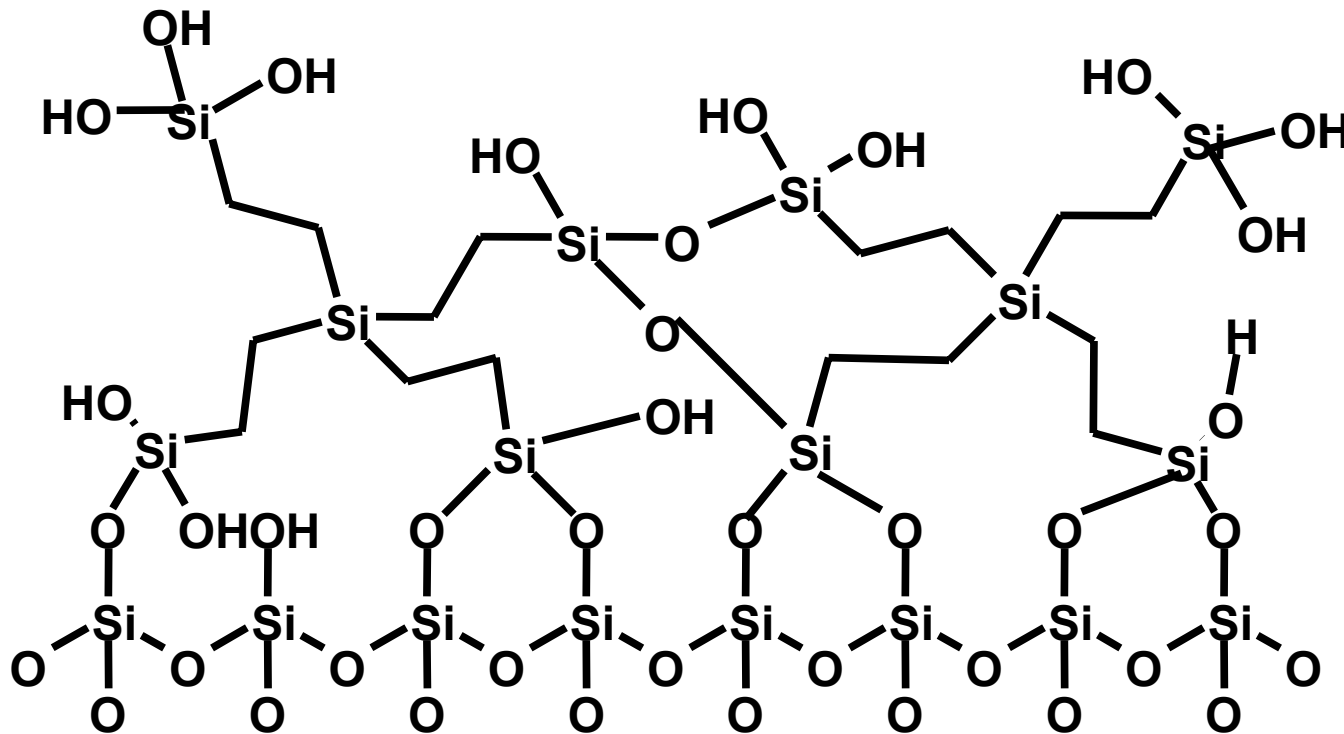
Representative reagents:



US Patent 6,794,044, and additional US and International Patents Pending



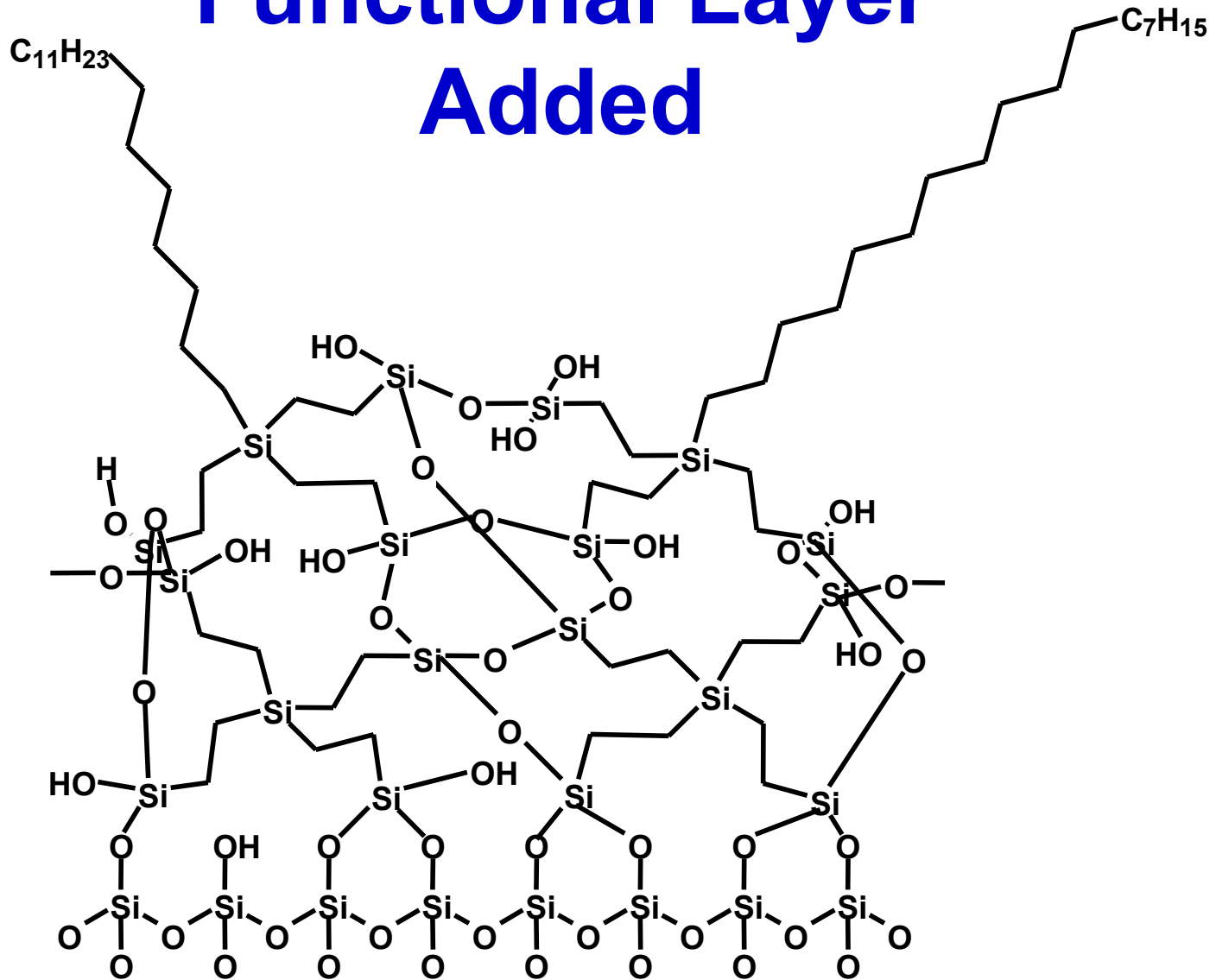
Selerity Blaze₂₀₀TM Multiple Point Bonding



Silica Surface & Barrier Layer

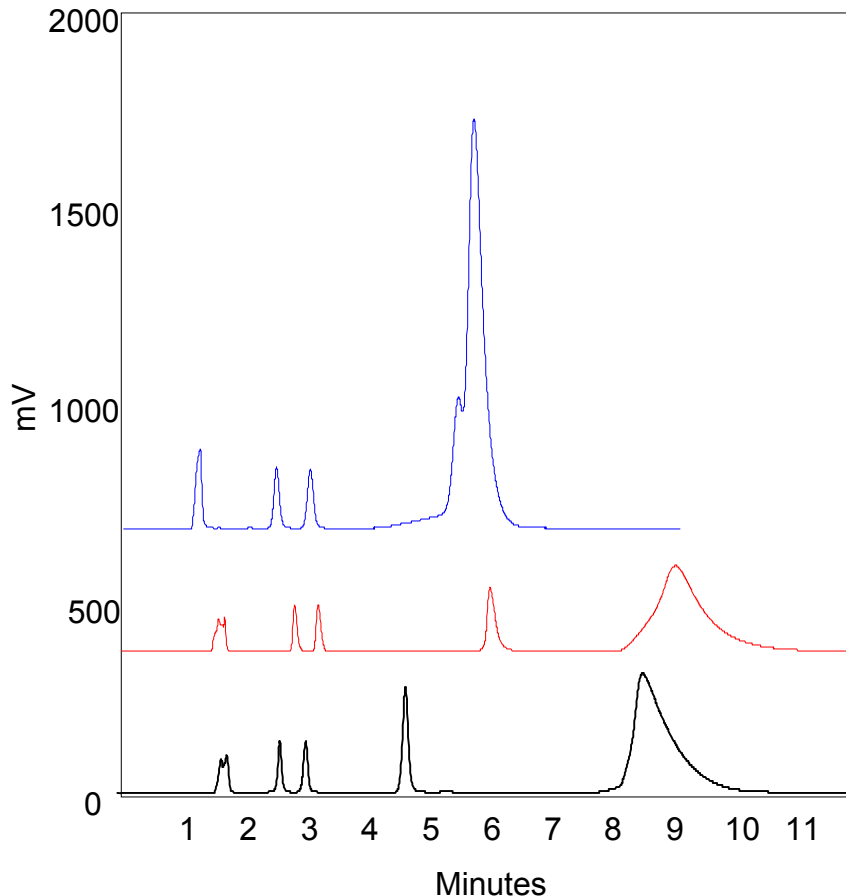


Functional Layer Added



Selectivity Comparison

Agilent SB, Xbridge and Blaze₂₀₀TM



SRM 870 test conditions were followed, and indicate typical C18 silica column selectivity. Uracil measures void volume. Toluene and ethylbenzene measure overall retention. Peak shape and elution of quinizarin indicate activity toward metal chelating agents. Tailing and asymmetry for amitriptyline indicates silanol interaction.

Detection: UV 254 nm

Mobile Phase: 80:20 methanol:5mM potassium phosphate pH 7.0

Temperature: 23°C

Waters Xbridge (150 x 3.5 mm)

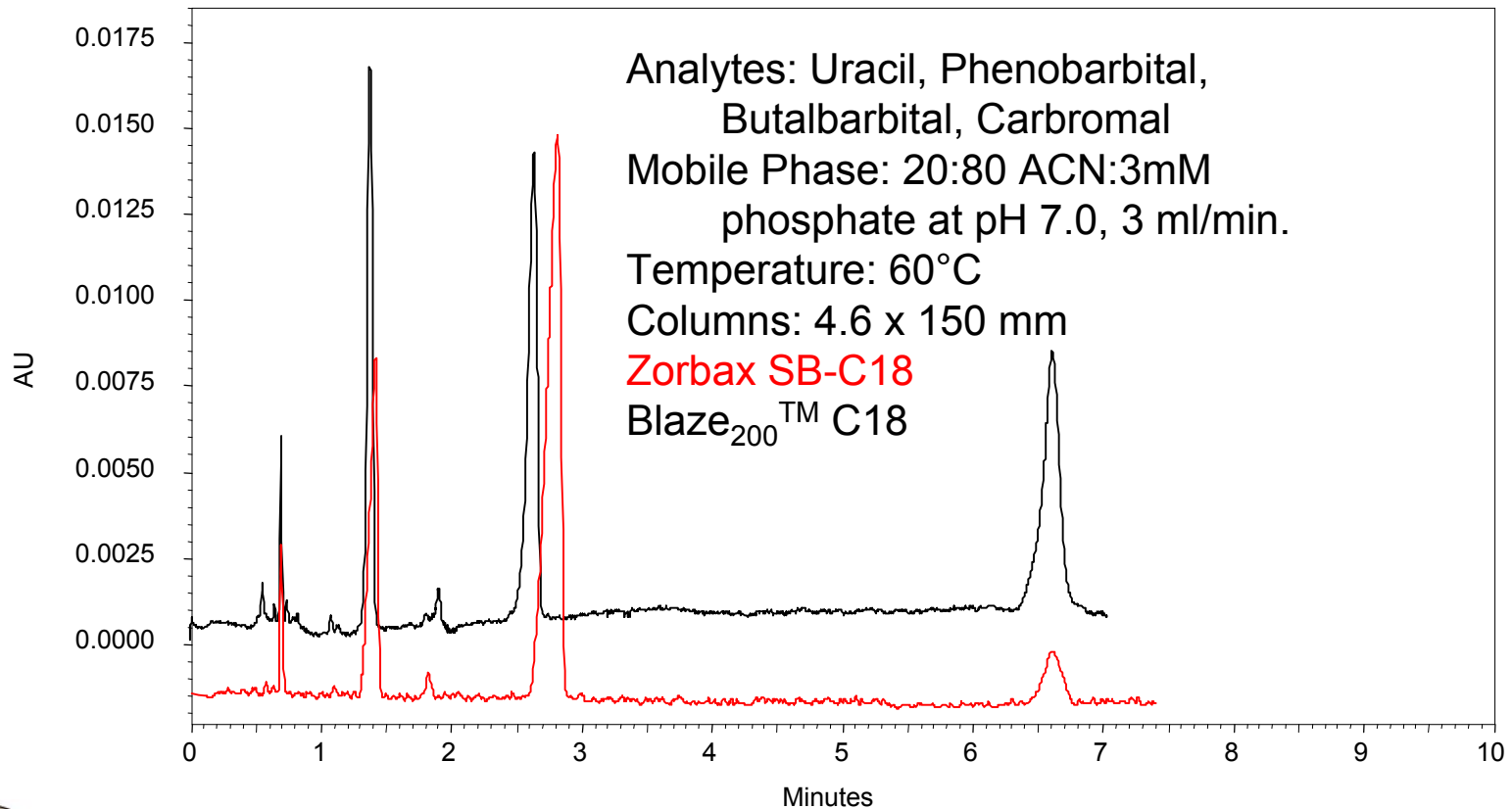
Blaze 200 (150 x 4.6 mm)

Agilent SB C18 (150 x 4.6 mm)

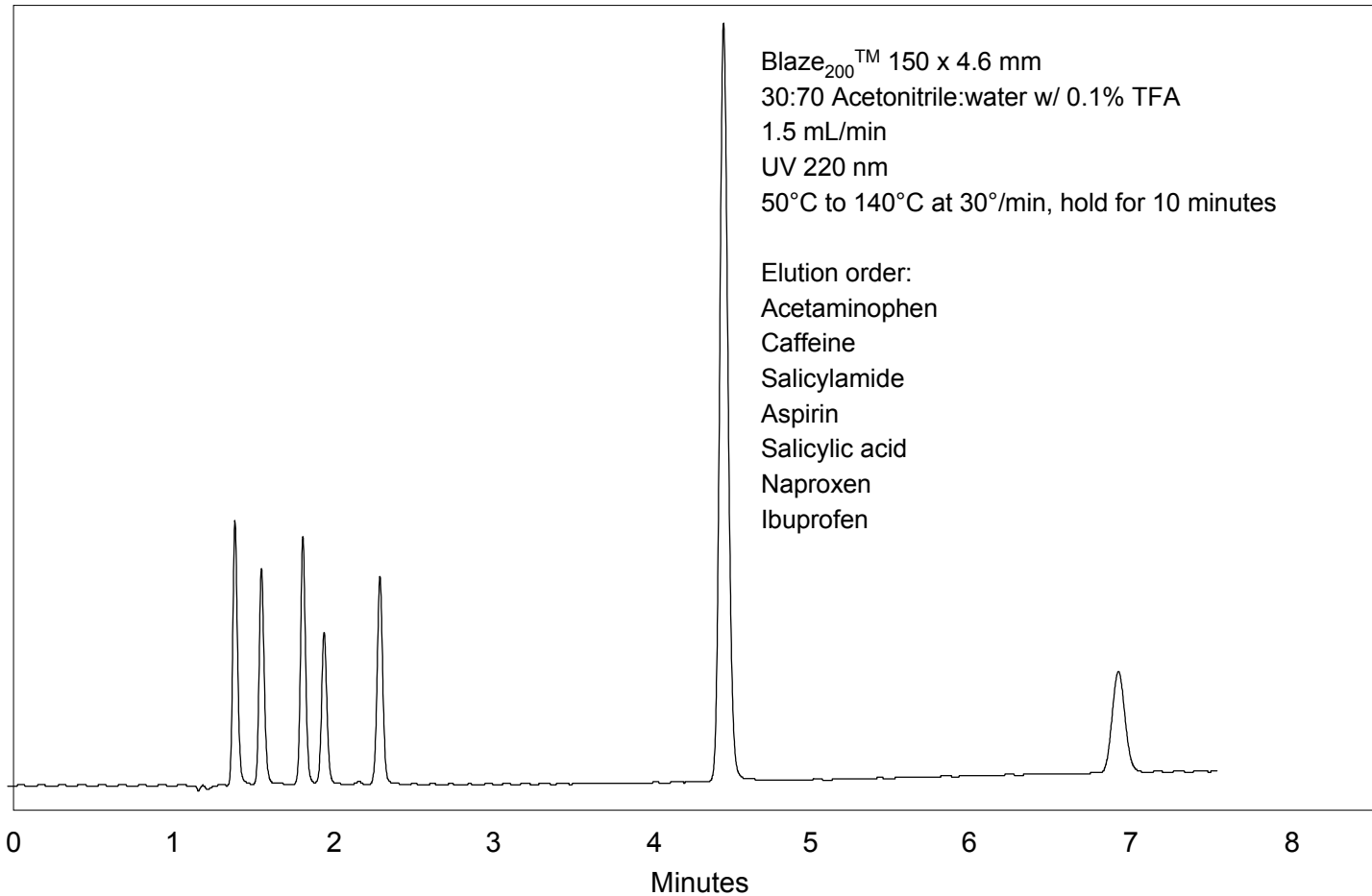


Selectivity Comparison

Blaze₂₀₀TM C18 / Zorbax SB-C18

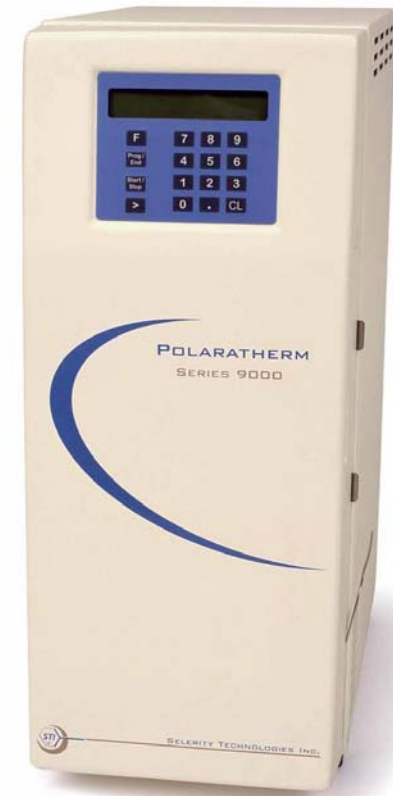


Analgesics Blaze₂₀₀TM C18

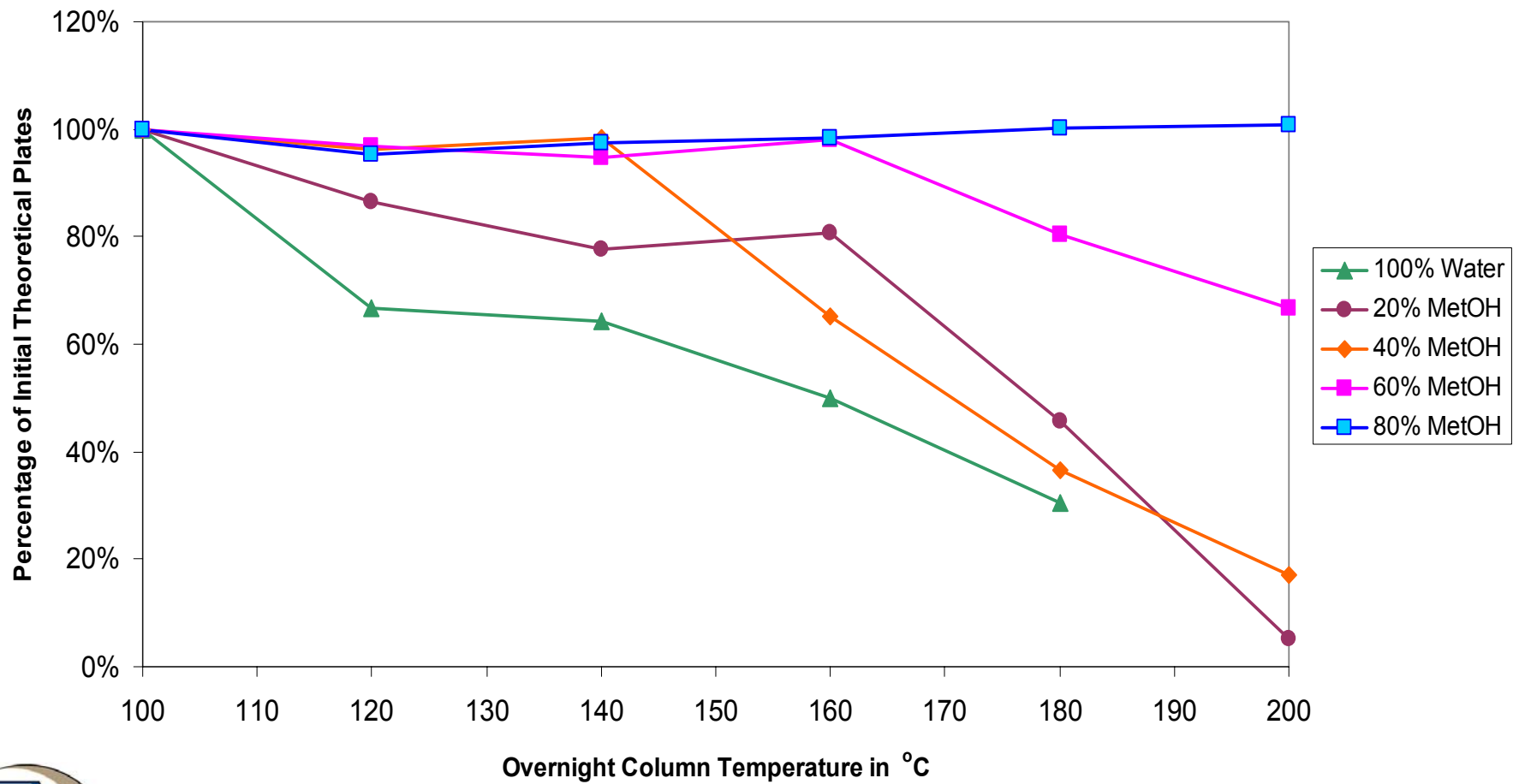


Selerity Polaratherm™ Series 9000 Total Temperature Controller

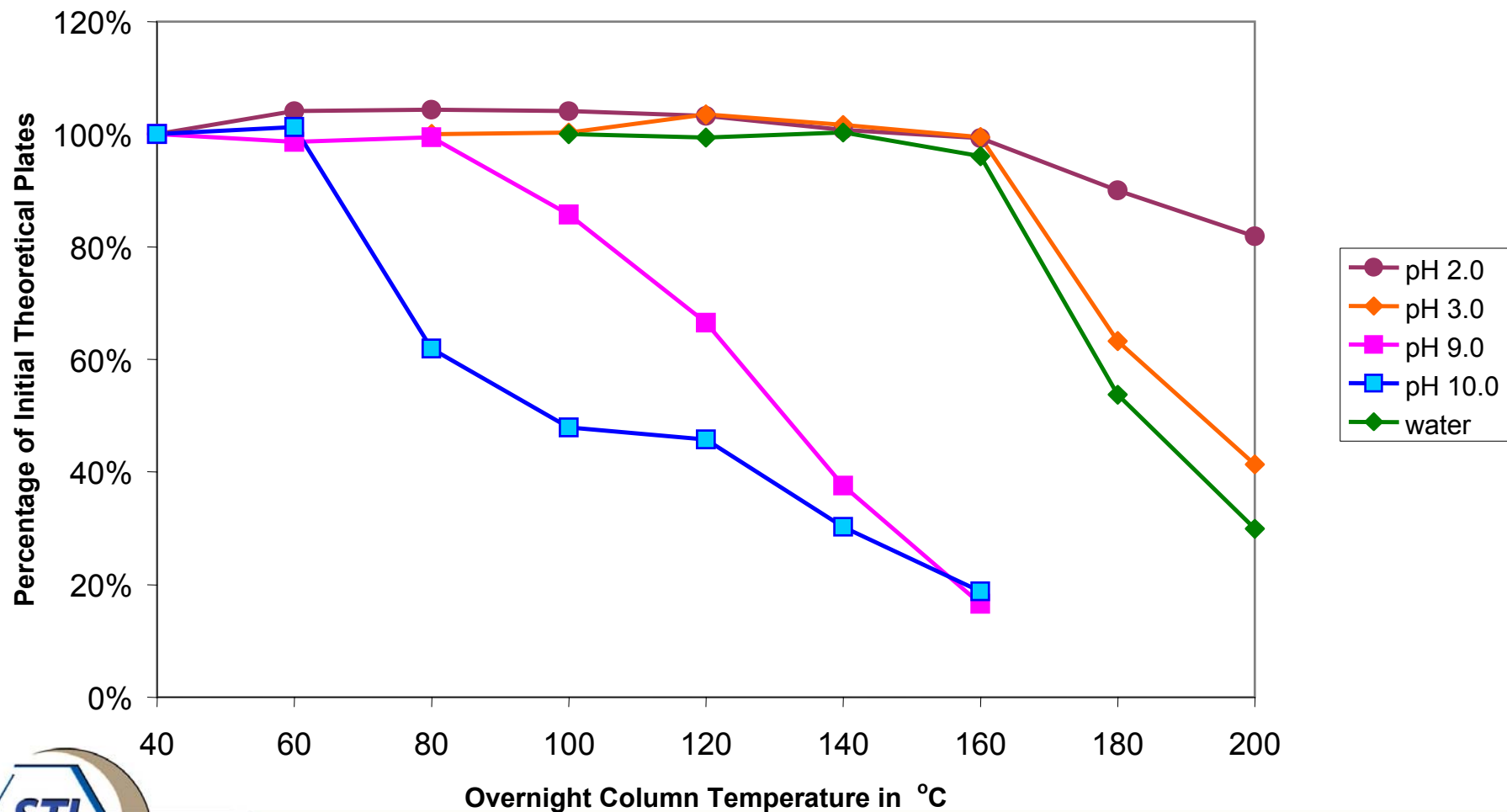
- Used in this study except where noted
- Forced air oven and chiller
- Isothermal and thermal gradient operation
 - Sub-zero to 200°C
 - Thermal gradients up to 30°C/min
- Mobile phase preheating and pre-cooling
- Peltier effluent temperature control
- Vapor sensor
- Compatible with any HPLC system
- Integrated software control with Waters, Agilent, and EZChrom



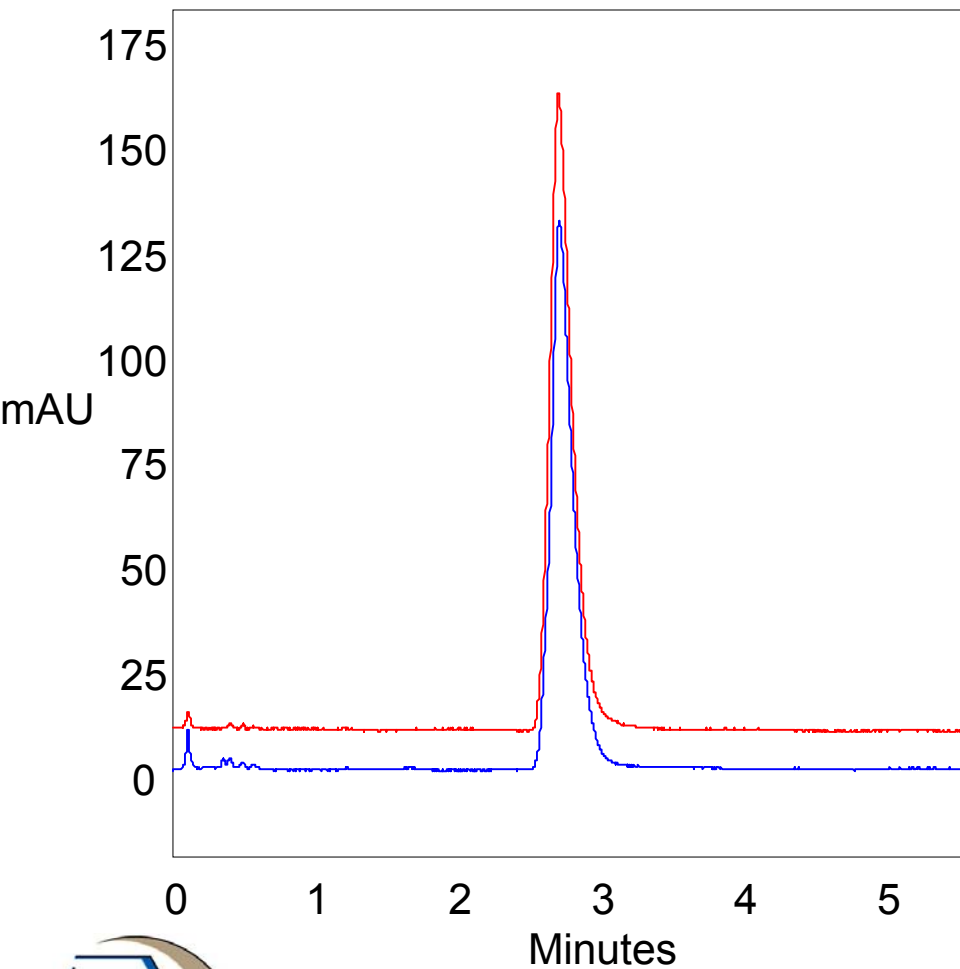
Effects of Temperature on the Theoretical Plates of Acetophenone Peak Methanol:Water



Effects of Temperature and pH on the Theoretical Plates of Acetophenone Peak 60% Acetonitrile



High pH Stability Evaluation



Column: Blaze 200 C18 , 2.1 x 50 mm, 3 μ m, 100A

Mobile Phase: 50:50 ACN:50mM pyrrolidine at **pH 12**,

Flow Rate: 0.8 ml/min

Detection: UV at 254 nm

Temperature: 40°C

Analyte: Amitriptyline

Red: Initial

Blue: After 2500 column volumes at pH 12!



Availability of Columns

- High temperature, Blaze₂₀₀TM - Selerity, RIC
- pH extreme, pHidelityTM – Through Restek Corporation under license from Selerity
- Future work:
 - Pore sizes, particle sizes, expanded chemistries



Conclusions

- Highly durable coating and protective layer
- Silica columns with traditional selectivity stable at extended pH and temperature ranges



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