

A C18 Silica Column With Exceptional Temperature and pH Stability

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Abstract

New instrumentation has made it possible to separate materials via liquid chromatography at temperatures up to 200°C, but most traditional silica-based stationary phases can only be used to about 60°C. In this paper, we report the development of a polycarbosilane bonding chemistry that is hydrolytically stable against breakdown in aqueous environments up to 200°C. An underlying barrier layer composed of polyfunctional silanes provides the base for the octadecylsilane functional layer, without compromising the traditional selectivity seen with silica-based columns. This new stationary phase was subjected to different mobile phase compositions at temperatures up to 200°C, and evaluated for signs of stationary phase degradation or collapse. The underlying silica support is also protected by the bonded phase against dissolution and remains intact at pH extremes as well.



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



Benefits of Stable Columns

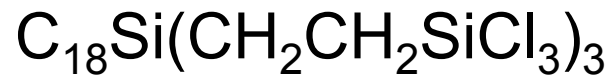
- High thermal stability
 - Selectivity tuning
 - Faster analysis
 - Less organic modifier required
 - Wider temperature programming range
 - Extended column lifetimes under more moderate conditions



Blaze₂₀₀TM Bonding Chemistry

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

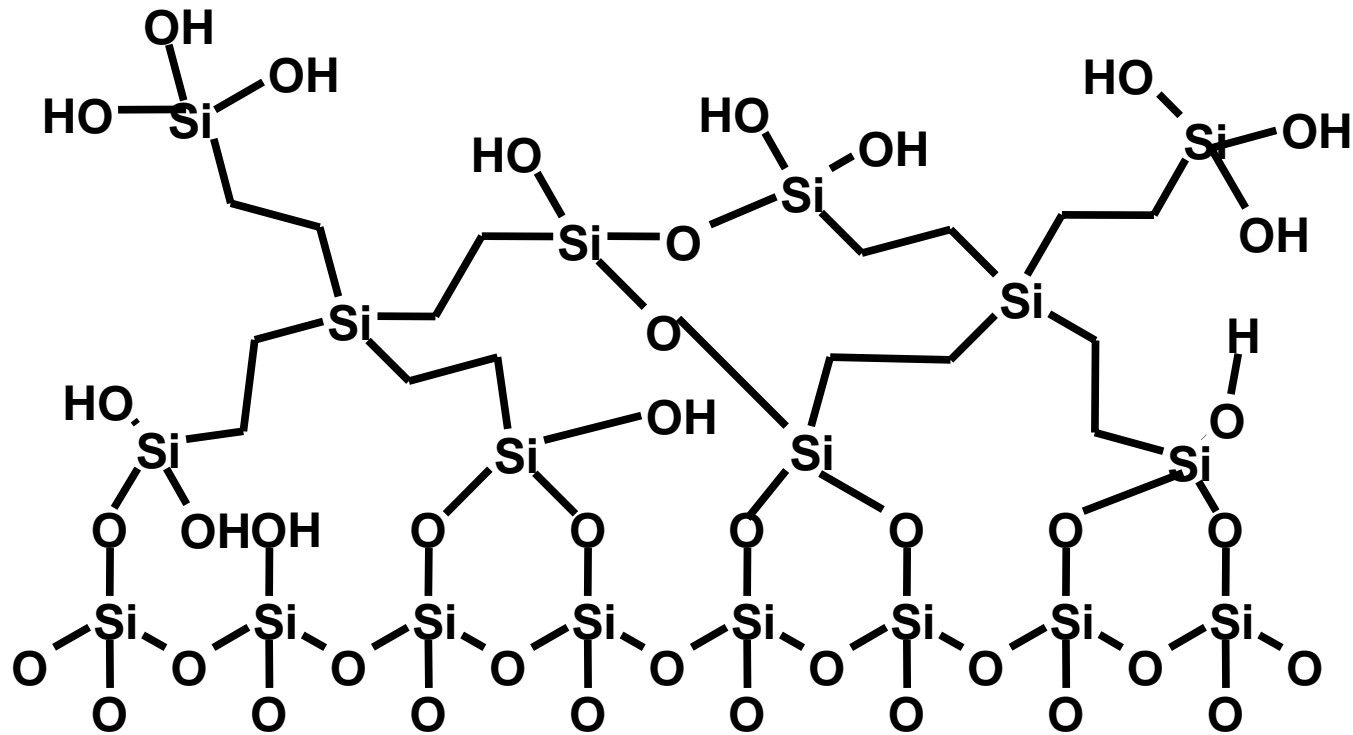
Representative reagents:



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

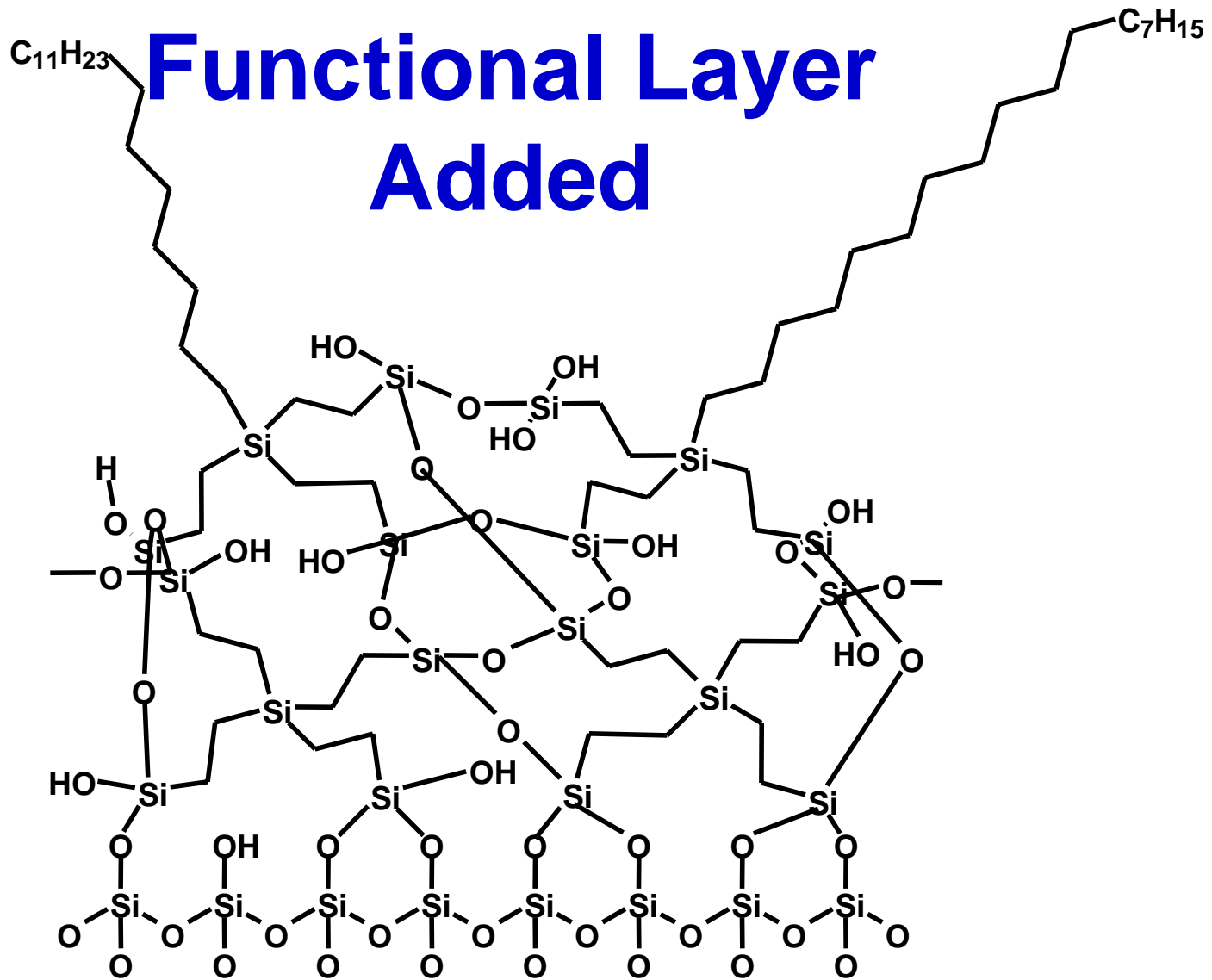


Selerity Blaze₂₀₀TM Multiple Point Bonding



Silica Surface & Barrier Layer





Comparison Between Durable Chemistries

- Waters organic/inorganic hybrid family
 - Stable chemistry, apparently usable at high pH and elevated temperatures
 - Each particle type must be optimized
 - Particle strength partially compromised,
 - *Anal. Chem.* **75** (2003) 6781-6788.
 - Surface organic groups reduce low pH stability
 - US Patent 6,528,167, assigned to Waters Corporation
- Agilent StableBond
 - Sterically hindered phases give good stability only with low pH at elevated temperatures
- Selerity Blaze₂₀₀TM
 - Simple surface coating with readily purifiable dendrimeric reagents
 - 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

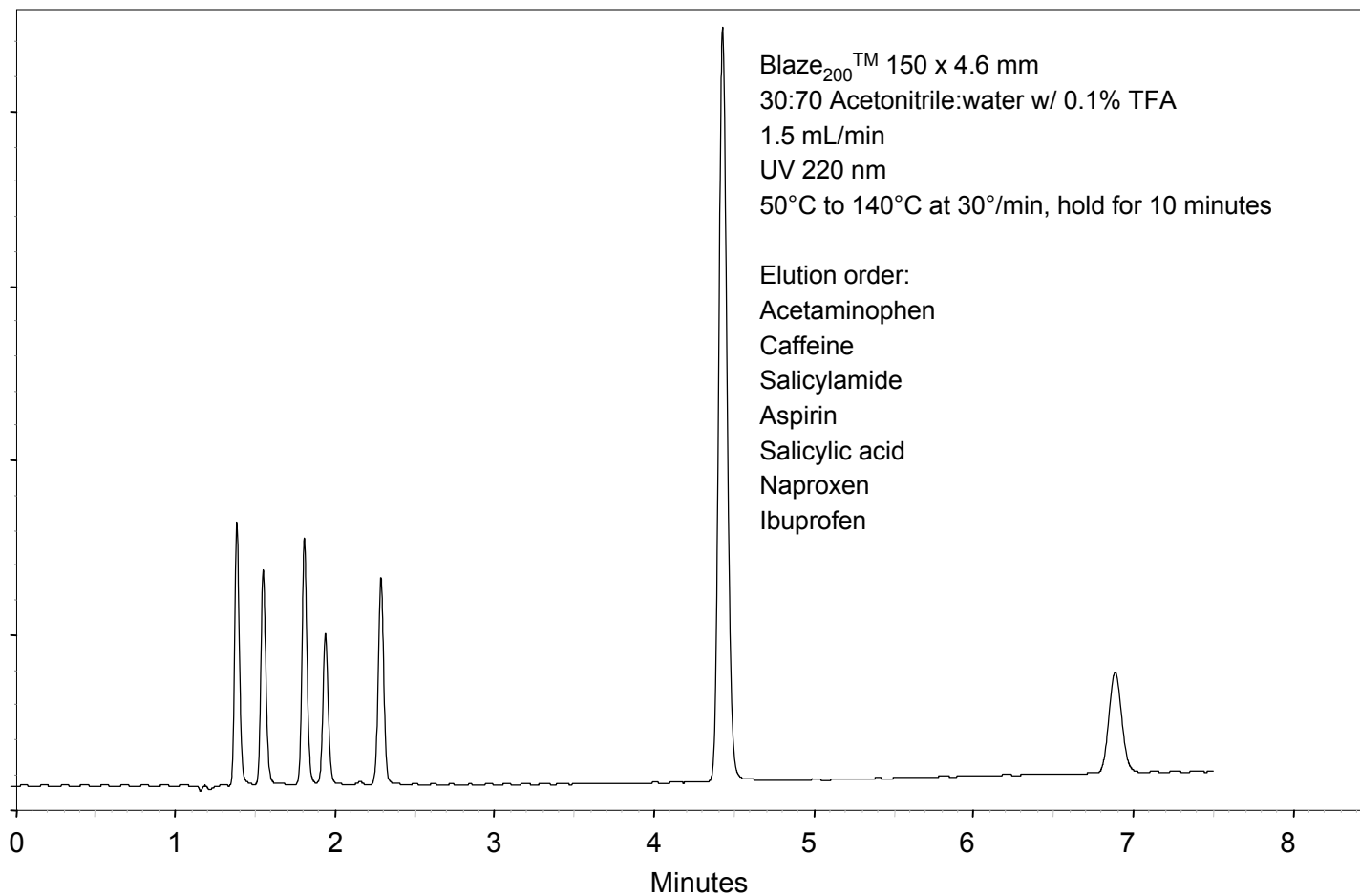


Selerity Polaratherm™ Series 9000 Total Temperature Controller

- 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

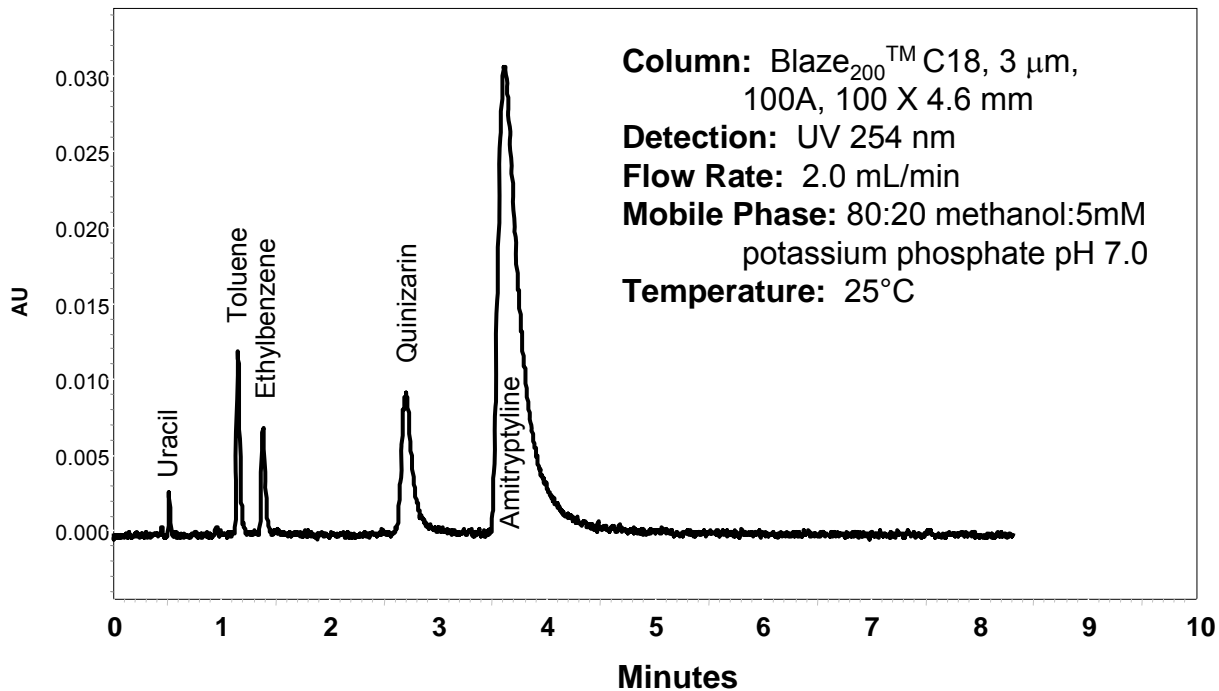


Analgesics Blaze₂₀₀TM C18



Selectivity Determination

Blaze₂₀₀TM

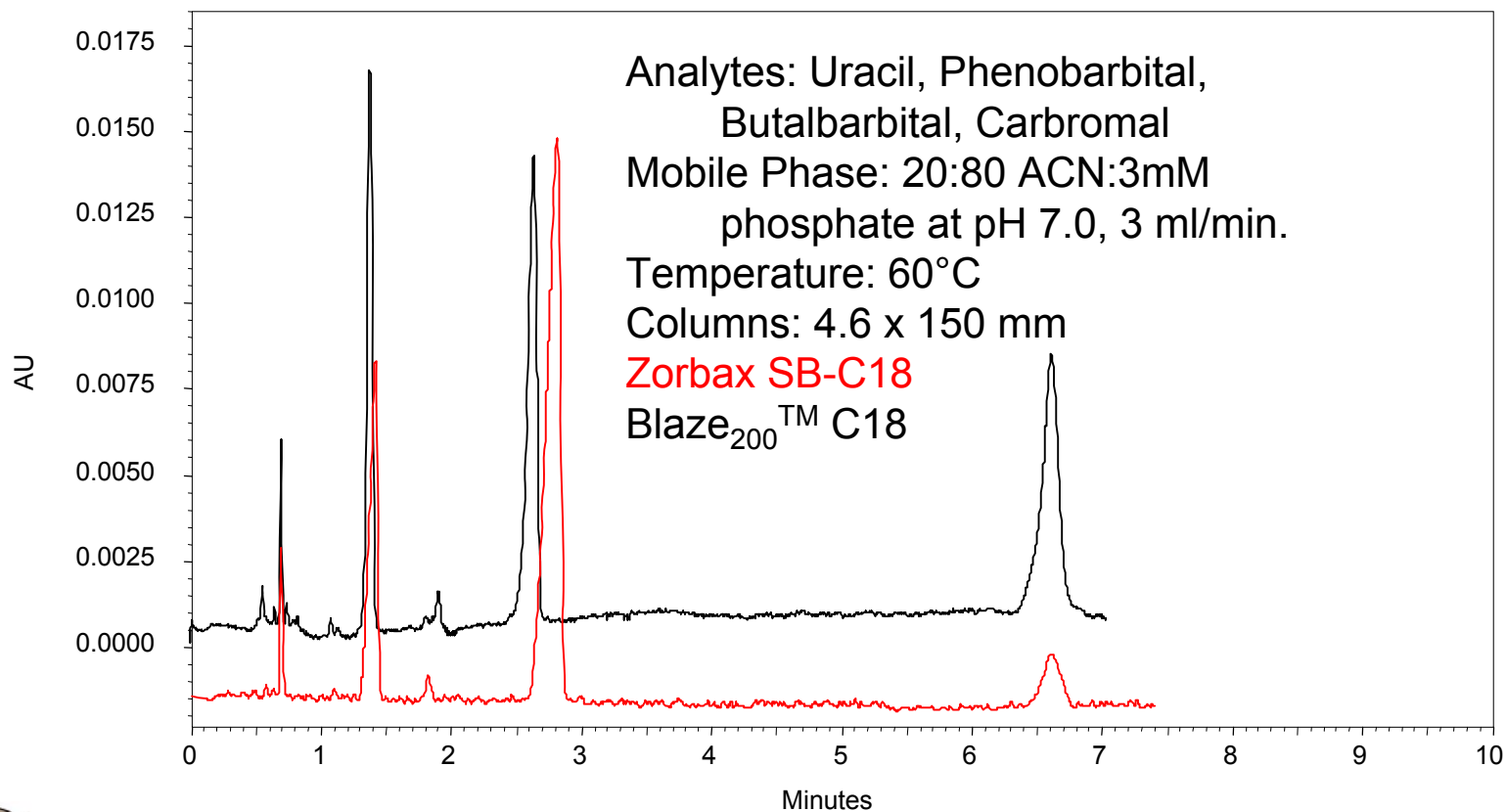


SRM 870 test conditions were followed, and indicate typical C18 silica column selectivity. Tailing and asymmetry for amitriptyline indicate some silanol interaction. Peak shape and elution of quinizarin indicate low activity toward metal chelating agents.



Selectivity Comparison

Blaze₂₀₀TM C18 / Zorbax SB-C18



Column degradation studies were performed with an LC Module I (Waters Corporation, Milford, Massachusetts, USA) equipped with an integrated auto sampler and an internal UV/Vis variable wavelength detector. The HPLC instrument was coupled to a Series 8000 column oven (Selerity). All separations were carried out with prototype Blaze₂₀₀TM C18 columns (2.1 x 100mm) packed with 3 µm particles.

The stability of the columns was evaluated by increasing the temperature using various mobile phase and pH conditions. All columns were tested by flushing with 1000 column volumes of 40:60 organic to water mobile phase at 50°C prior to being exposed to the experimental conditions. This allowed the determination of loss of chromatographic performance during the column evaluation. A test mixture containing uracil, phenol, benzene, and acetophenone in 35:65 ACN:water was used.

The baseline data for mobile phases containing less than 50% organic solvent was taken at 100°C. Separation of the test mixture could not be achieved at organic mobile phase concentrations greater than 50% at 100°C. Therefore, all mobile phase treatment conditions involving compositions with organic modifier greater than 50% were either evaluated at a base line of or switched to a 40% organic isocratic solution during the column evaluation.

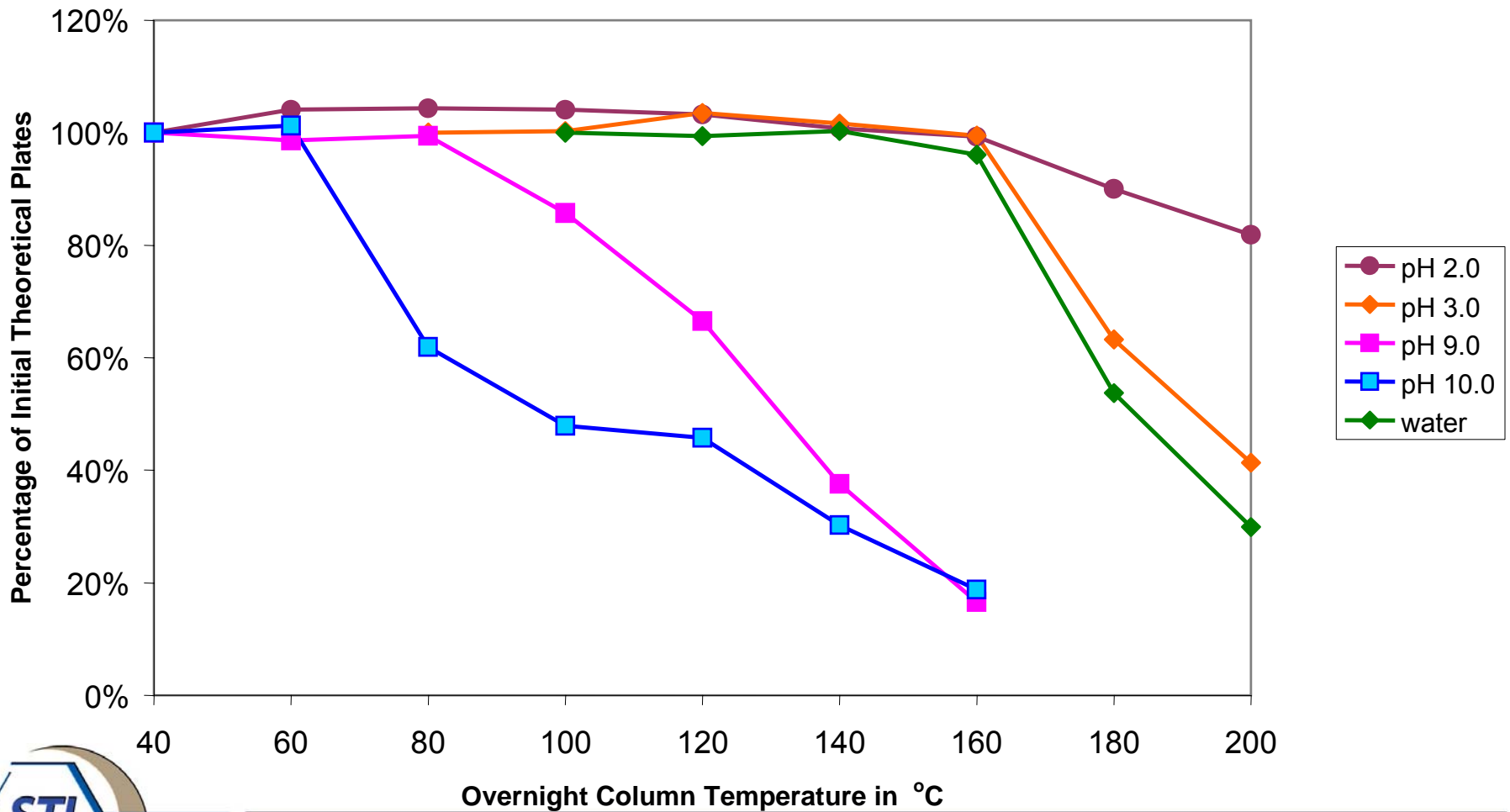


The column temperature was increased in 20 degree increments. After flushing with 1000 column volumes at elevated temperature, the temperature effects on the column were evaluated by switching the column temperature back to the baseline conditions and injecting the test sample.

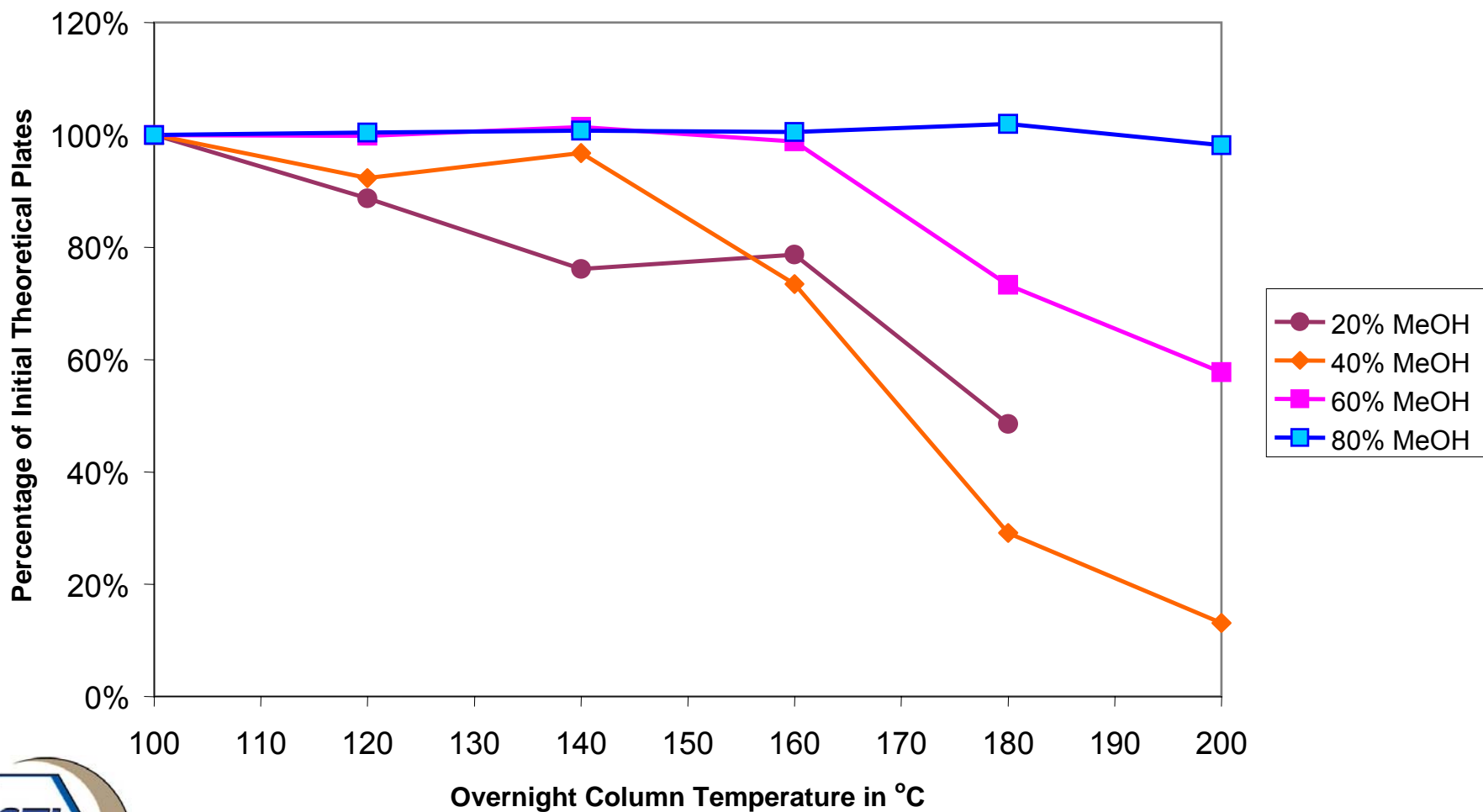
For the pH treatment studies, the columns were subjected to either 60% or 20% ACN isocratic compositions at each pH. The buffers used were: ammonium acetate for pH 9 and 10, piperidine for pH 12, and monobasic sodium phosphate for pH 2 and 3. All buffers were prepared in HPLC grade water and then filtered through a 0.22 μm (name of) filter membrane (company and location). After the overnight 1000 column volume treatment at each pH and temperature, the pumps were primed and the column was flushed for at least 15 min with ACN and water at a 40:60 ratio to remove all buffer from the system. Each column temperature treatment was evaluated by running the diluted test mix at the baseline temperature of 50°C with the 40:60 ACN:water mobile phase. The system was then switched back to the buffer:ACN mobile phase for the next temperature increment.



Effects of Temperature and pH on the Efficiency of Acetophenone for 60% ACN



Temperature Effects on the Theoretical Plates of Benzene for Various Mobile Phases (Blaze₂₀₀TM)



Availability of Columns

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

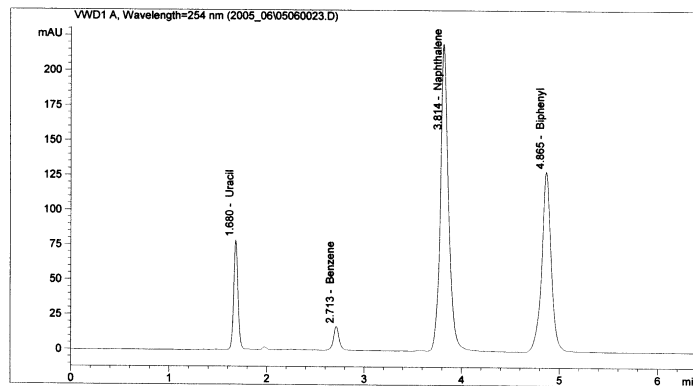




Quality Assurance Report

Packing Material: BLAZE 200 C18
Catalog Number : 557961
Serial Number : 05060023P
Batch Number : 013-137A1

Column Length : 150 mm
Inside Diameter: 4.6 mm
Particle Size : 3 μm
Pore Size : 140 \AA



Mobile Phase: Methanol 75% Water 25%
Flow Rate : 1.00 ml/min Wavelength : 254 nm
Sample : RP Mix Sample Amount: 5.0 μl
Pressure : 207 Bar Temperature : Ambient

Performance results for peak #4:

Retention time : 4.87 min
k' : 1.90
Width at 1/2 height: 0.09 min
Symmetry at 10% : 1.00
Plates/column : 15054
Plates/meter : 100358

#	Compound Name	Ret. Time	Area	Area %	Width
1	Uracil	1.680	236	9.5	0.046
2	Benzene	2.713	70	2.8	0.061
3	Naphthalene	3.814	1288	52.0	0.085
4	Biphenyl	4.865	883	35.7	0.101

Analyst: LP Tested: 6/2/2005

Restek Corporation 110 Benner Circle, Bellefonte, PA 16823-8812
814-353-1300 • 800-356-1688 • Fax: 814-353-1309 • www.restekcorp.com



Conclusions

- Highly durable coating and protective layer
- Silica columns with traditional selectivity usable in new realms of pH and temperature



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