

Applications Using Temperature Programmed HPLC and a New High Temperature Silica Column

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Introduction

The introduction of silica-based polycarbosilane bonded phases for HPLC at extreme conditions began a new era of stability and durability for columns with traditional bonded alkylsilane selectivity. Their resistance to hydrolytic degradation and shielding of the base support surface compared to materials based on siloxane is key to their performance.

We have expanded on this early work by synthesis of new branched polydentate carbosilane materials with even greater durability and shielding capability, and demonstrate



Introduction

their use under aqueous reversed-phase conditions at temperatures as high as 200 °C in ultra-fast chromatographic separations with low organic mobile phases. Thermal bleed is far lower than seen for columns with zirconia-based particles and non-bonded organic layers. The lack of bleed response allows temperature programmed operation over a wide range without baseline shifts requiring compensation.

Selectivity and durability evaluations are shown with applications previously unattainable using aggressive mobile phases at temperature and pH extremes.



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



Benefits of Stable Columns

- Wide pH range
 - Ion suppression for acids
 - Ion suppression for amines
 - Column regeneration by elution of contaminants at pH extremes
- High thermal stability
 - Selectivity tuning
 - Faster Analysis
 - Less organic modifier required
 - Wider temperature programming range

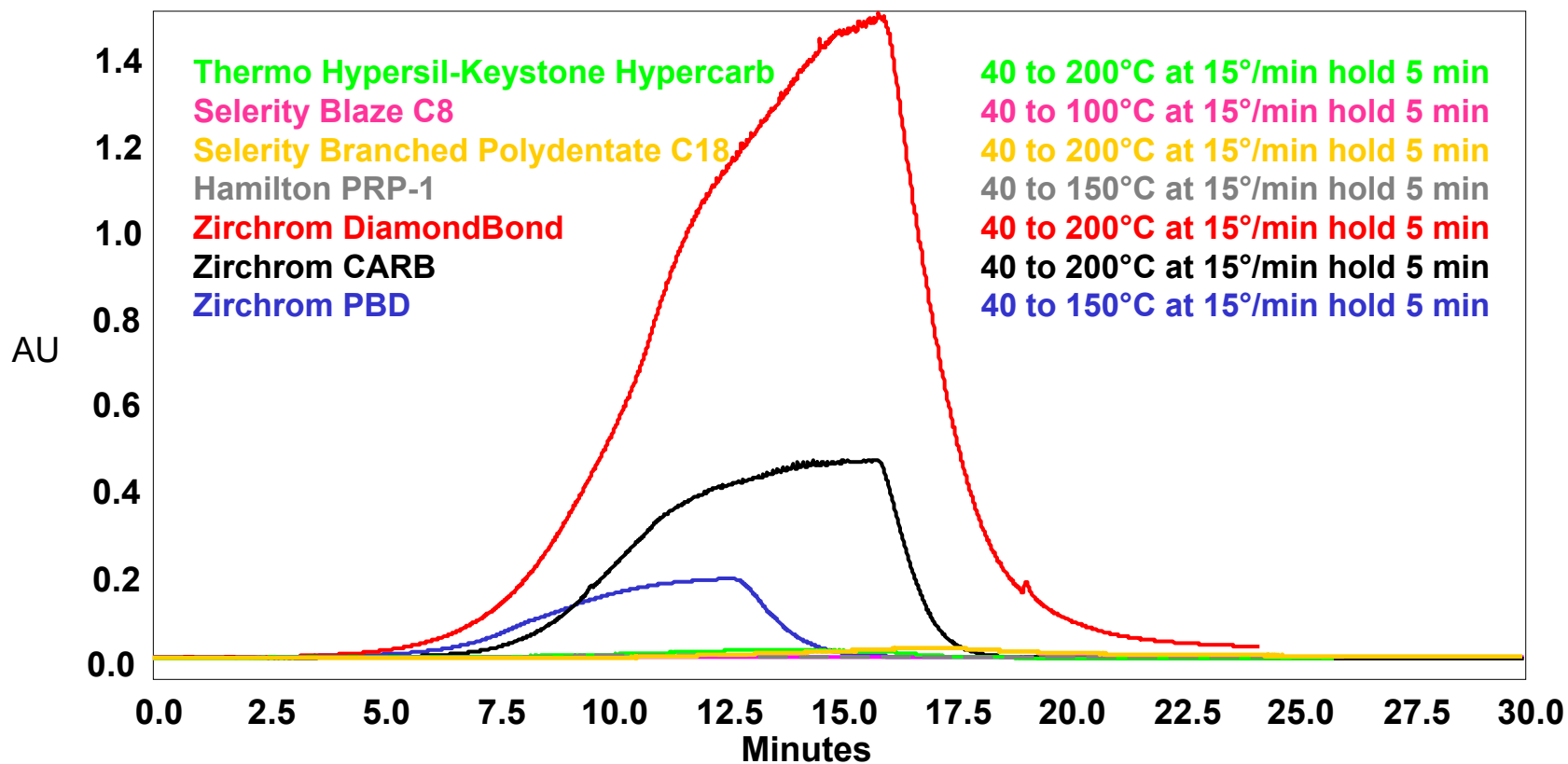


HPLC Column Stable at High Temperature

- Polymeric DVB
 - Stable to 150°C and pH 0-14
- Zirconia
 - Not recommended for temperature programming due to column bleed (see page 7)
- Carbon (Hypercarb™)
 - Stable to 200°C and pH 0-14
- Hybrid Organically Modified Silica (Waters Xterra)
- Polydentate Modified Silica (Selerity Blaze 100)
 - Stable to 100°C



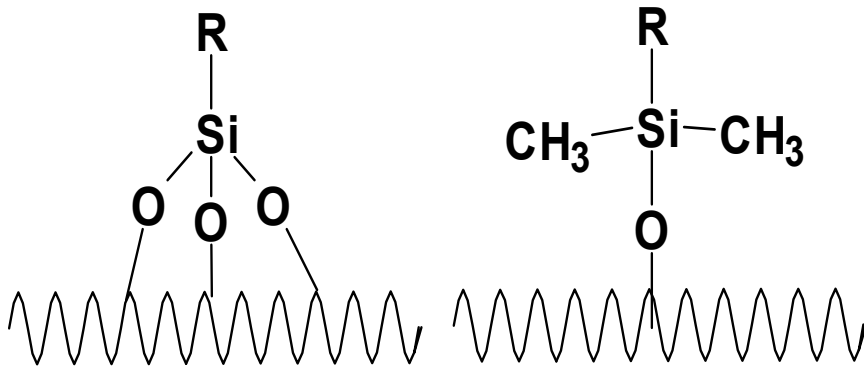
Column Bleed Comparison During Thermal Programming



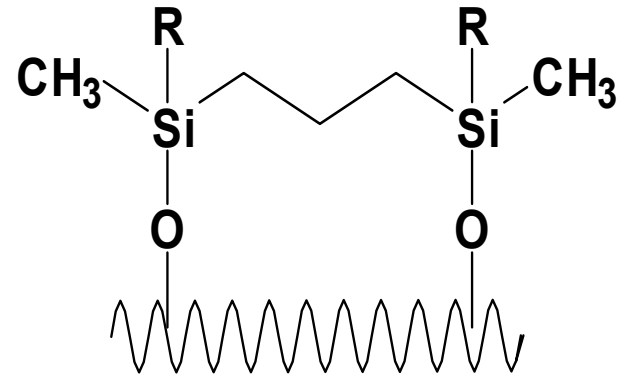
50:50 Acetonitrile:Water at 254 nm



Early Silane Bonding

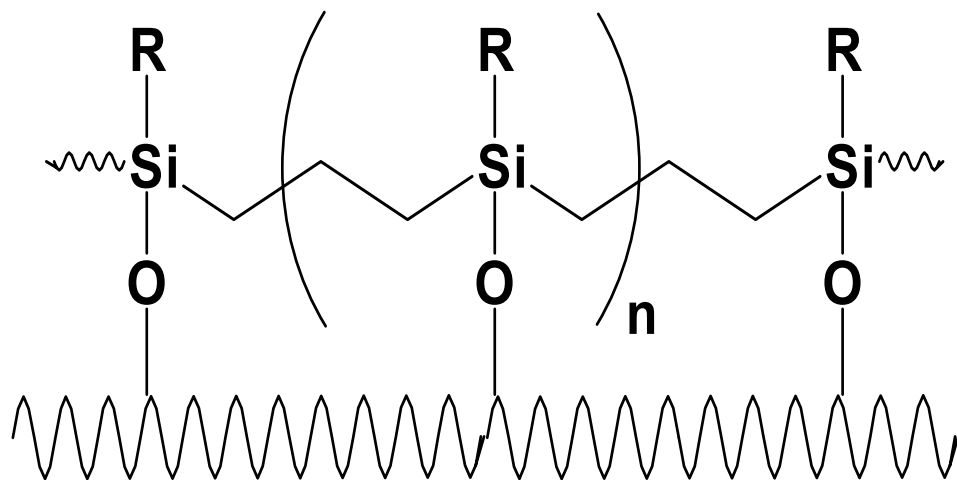


Monodentate



Bidentate

Selerity Silica Columns with Multiple Point Bonding



Gen. 1 Polydentate

**Polycarbosilane-
Branching
Added**

Gen. 2 Polydentate



Other Multiple Point Attachment Mechanisms

In Nature



In Commercial Products (Velcro)

U.S. Patent Oct. 4, 1988

4,775,310

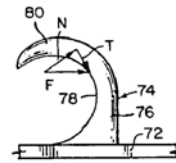


FIG. 8

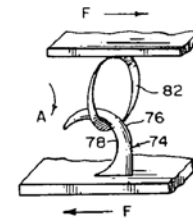


FIG. 9

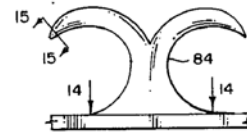


FIG. 10

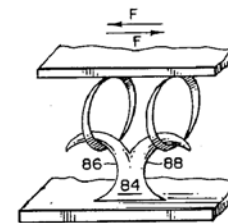
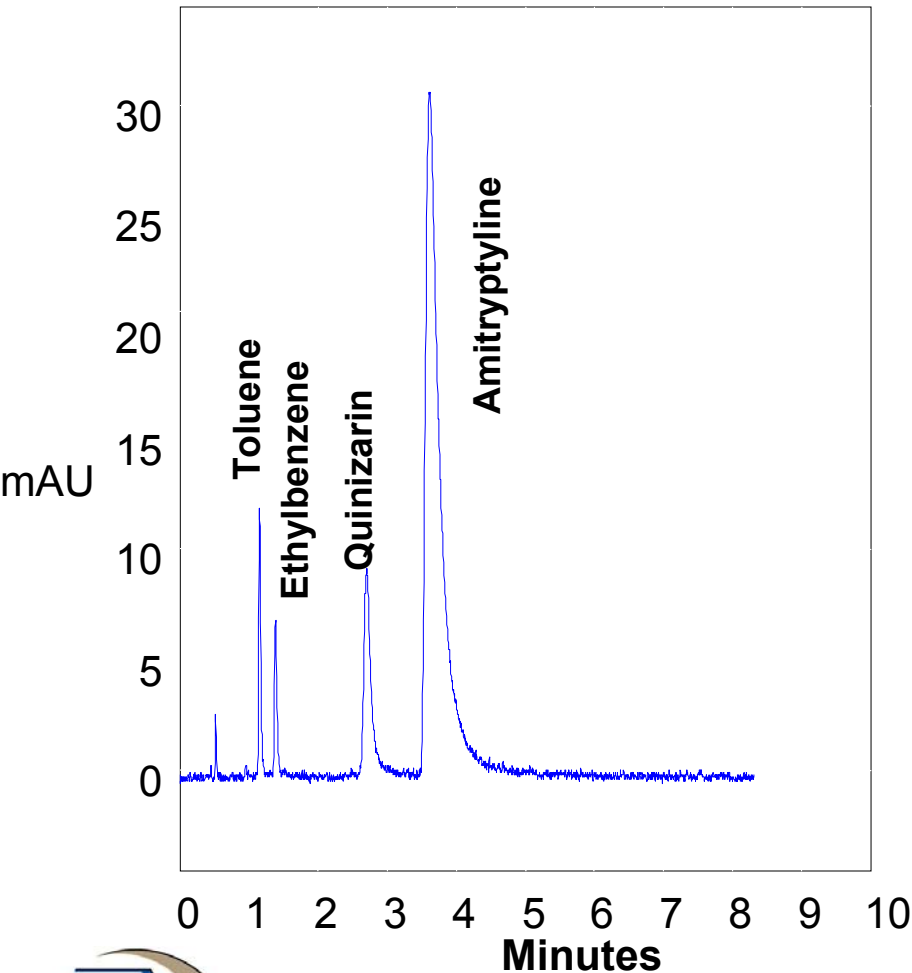


FIG. 11



Selectivity Determination – ASTM SRM 870 test Mix



Column: C18 Polydentate, 3 μm , 100A, 100 X 4.6 mm

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

Flow Rate: 2.0 mL/min

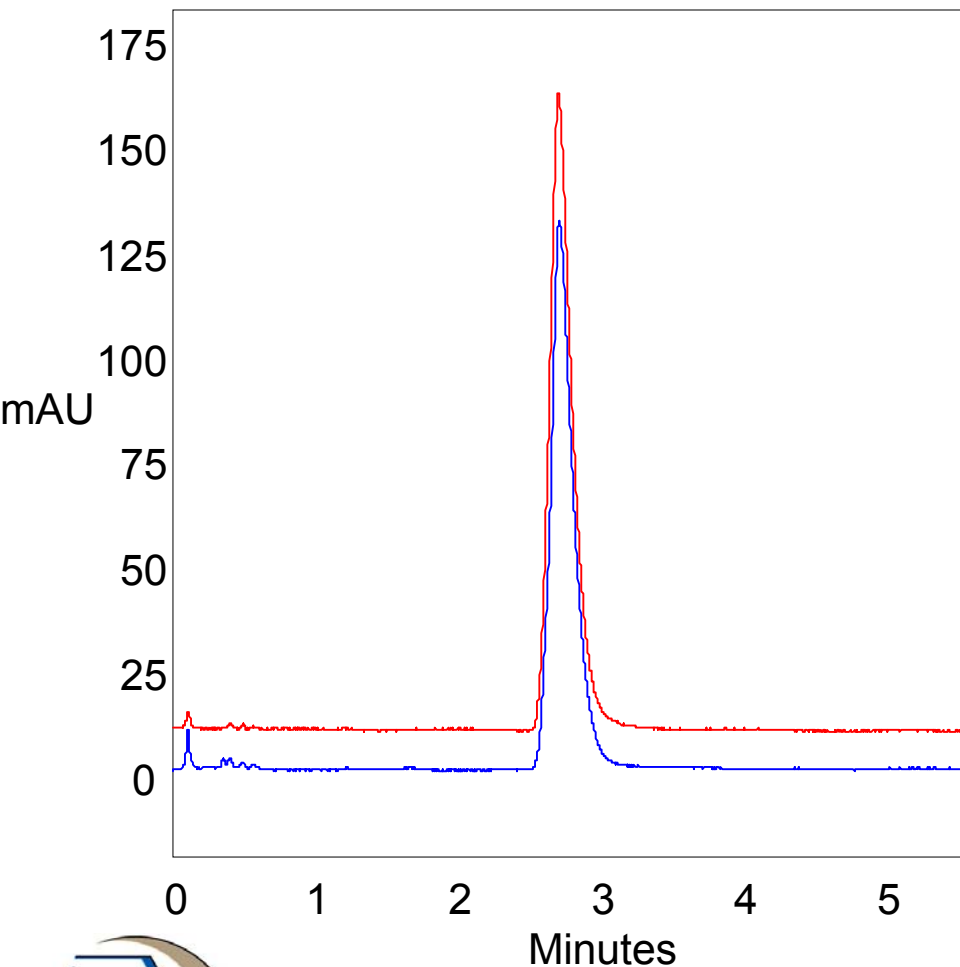
Detection: UV 254 nm

Temperature: 25°C

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.



High pH Stability Evaluation



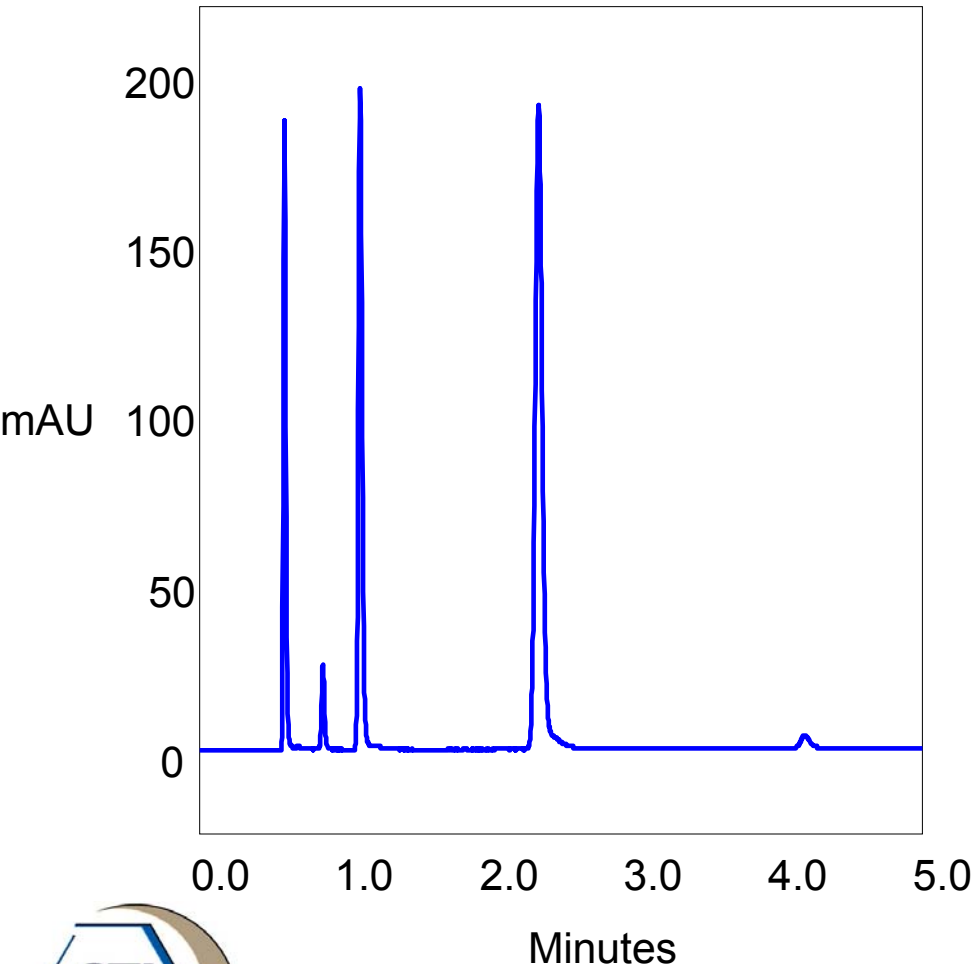
Column: C18 Branched
Polydentate, 2.1 x 50 mm, 3 μ m,
100A
Mobile Phase: 50:50 ACN:50mM
pyrrolidine at **pH 12**,
Flow Rate: 0.8 ml/min
Detection: UV at 254 nm
Temperature: 40°C
Analyte: Amitriptyline

Red: Initial

**Blue: After 2500 column volumes
at pH 12!**



Separation of Analgesics at pH 1



Column: C18 Branched Polydentate, 4.6 x 100 mm, 3 μ m, 100A

Mobile Phase: 40:60 acetonitrile:water with 1% TFA

Flow Rate: 2.0 ml/min

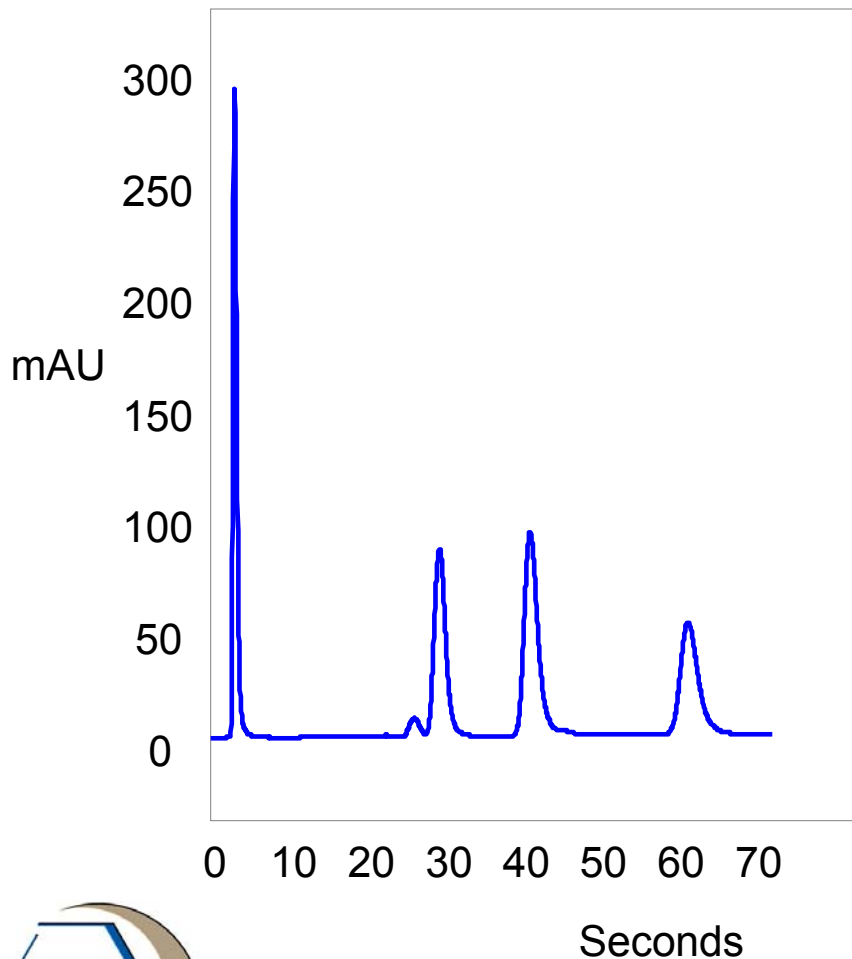
Detection: UV 235 nm

Temperature Program: 30° (hold 1 min) then 30°/min to 110°C

Elution Order:
Acetaminophen
Aspirin
Salicylic Acid
Naproxen
Ibuprofen



High Water Content Mobile Phase Separation at 200°C



Column: C18 Branched Polydentate,
2.1 x 50 mm, 3 μ m, 100A
Mobile Phase: 5:95 acetonitrile:water
Flow Rate: 4.0 ml/min **Detection:** UV
254 nm
Temperature: 200°C

Elution Order:
Uracil
Androstadienedione
Androstenedione
Epitestosterone



Conclusions

- A second generation 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 up to 200°C and a pH range including 1-12.



Acknowledgements

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were kindly provided by:

Restek Corporation

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Zirchrom Corporation

Hamilton Company



Turn up the Heat!

