New Stable Chemically Bonded Carbon Stationary Phases for HPLC

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DIAMOND BOND™ HPLC Columns

Outline

- Advantages of Stable Phases
- Development of a New Type of Reversed Phase Column: DiamondBond®-C18
- Selectivity Comparison of DiamondBond®-C18 and Other DiamondBond Phases with ODS Silica
- Applications
- Summary

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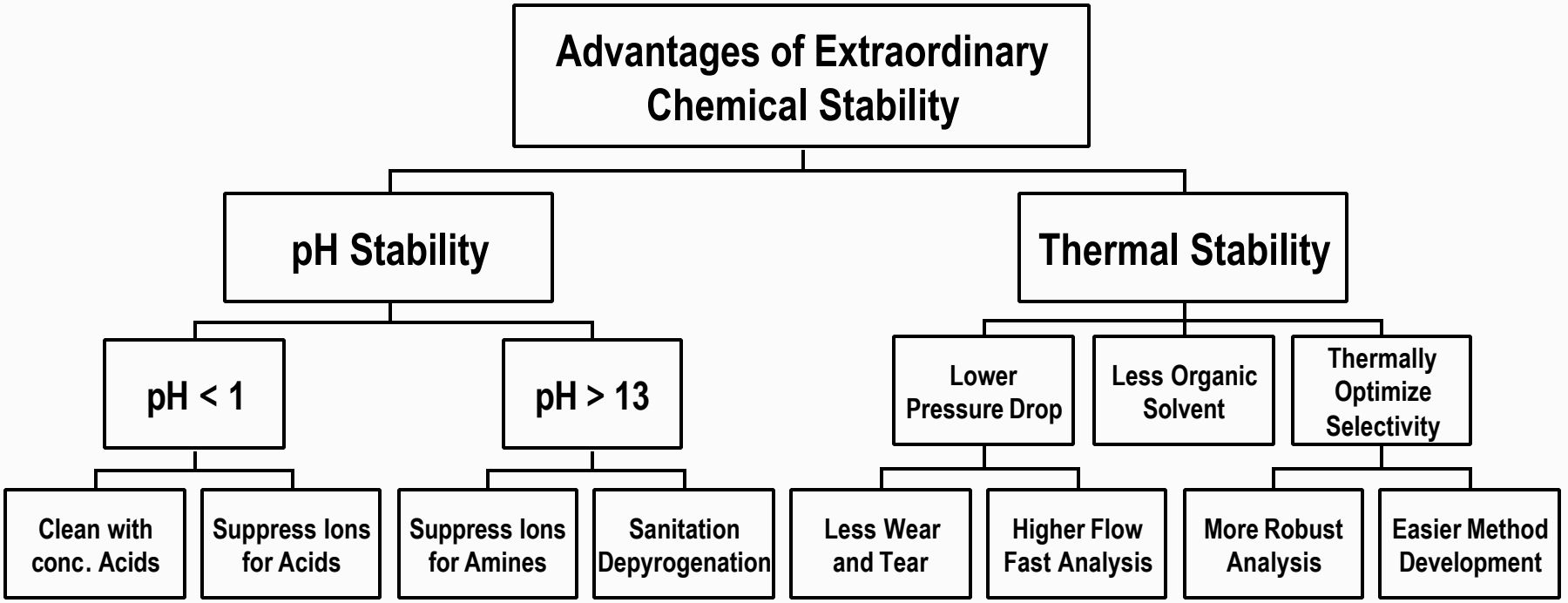
Why Stable Phases?



Stable Stationary Phases have advantages in terms of Selectivity, Column Lifetime, and Analysis Time

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Improving the Stability of HPLC Phases: History

- Pure organic polymers
- Silica “hardened” by coating with alumina or zirconia
- Pure carbon
- Silica improvements
  - Sterically bulk & bidentate ligand
  - Polymer coated silica
  - Hybrid organic-inorganic silicaceous composite phase
- Polymer coated porous alumina and zirconia
- Carbon coated zirconia
- **Chemically bonded carbon-coated zirconia**

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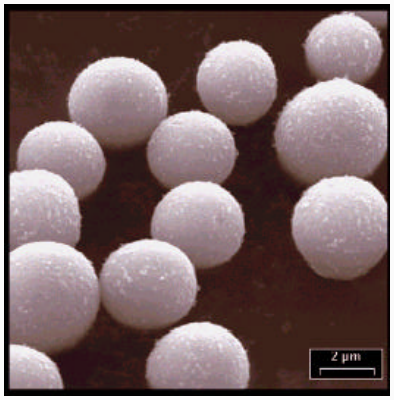
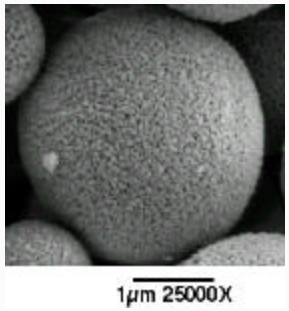
ZirChrom® Particle Properties



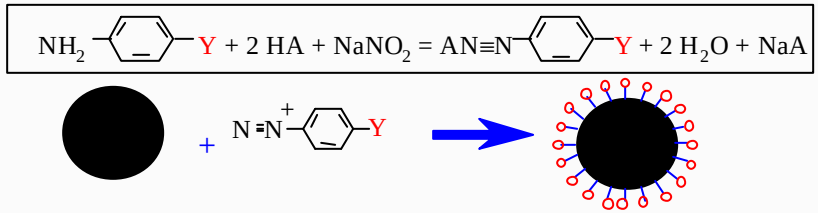


Characteristic	Property
Surface Area (m^2/g)	22
Pore Volume (cc/g)	0.13
Pore Diameter (Å)	250-300
Porosity	0.45
Density (g/cc)	5.8 (2.5x silica)
Particle Diameter (µ)	3.0

ZirChrom®-Carb particles are prepared by coating base zirconia particles with a thin layer of carbon using a chemical vapor deposition process

DIAMOND BOND - C18

Bonding Reaction on Carbon Clad Zirconia



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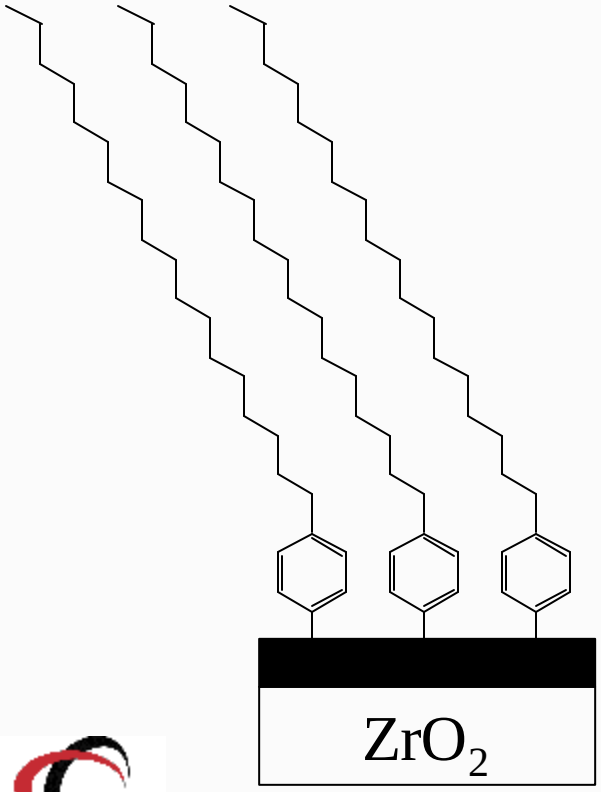
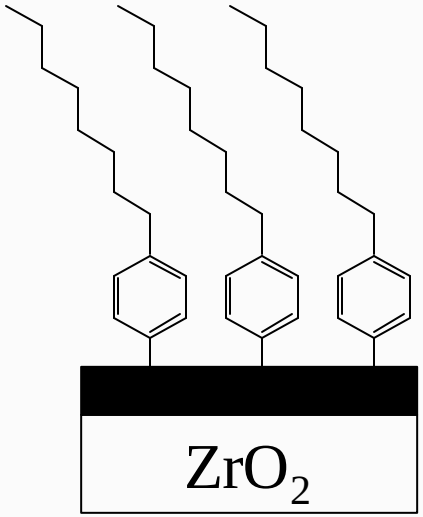
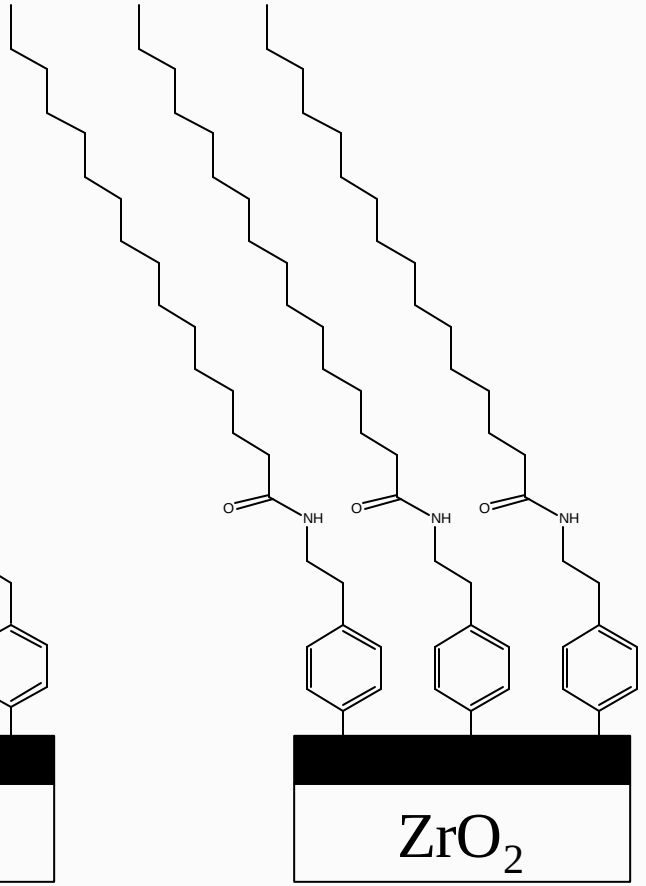
DiamondBond®: A New Family of Stable Phases



DB-C18



DB-C8



DB-Amide18

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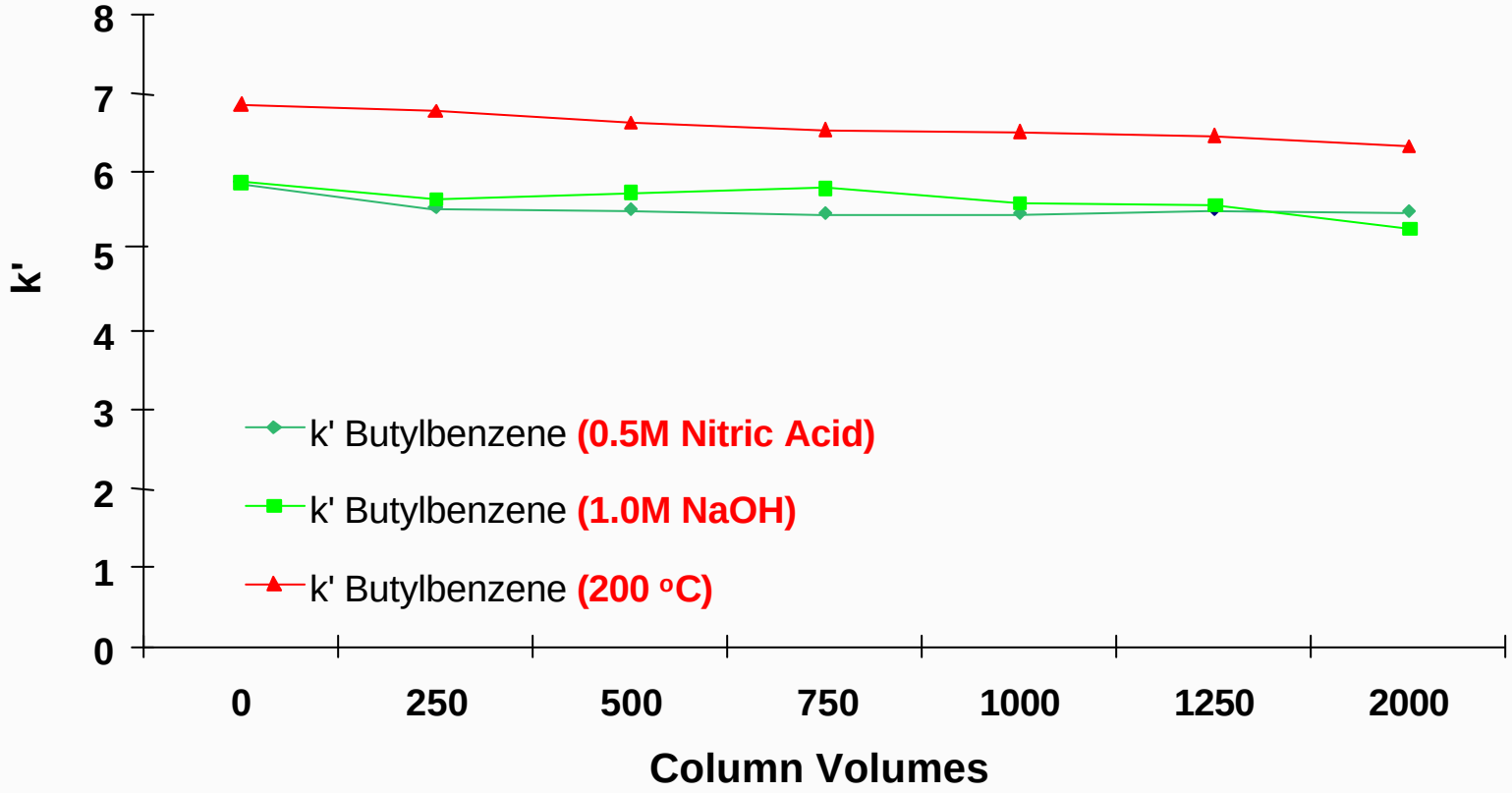
HPLC Columns

DiamondBond-C18 Stability



LC Conditions:

Base Stability—DiamondBond™ Phase A, 30 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.

Acid Stability—DiamondBond™ Phase A, 50 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.

Temperature Stability-- DiamondBond™ Phase B, 50 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.

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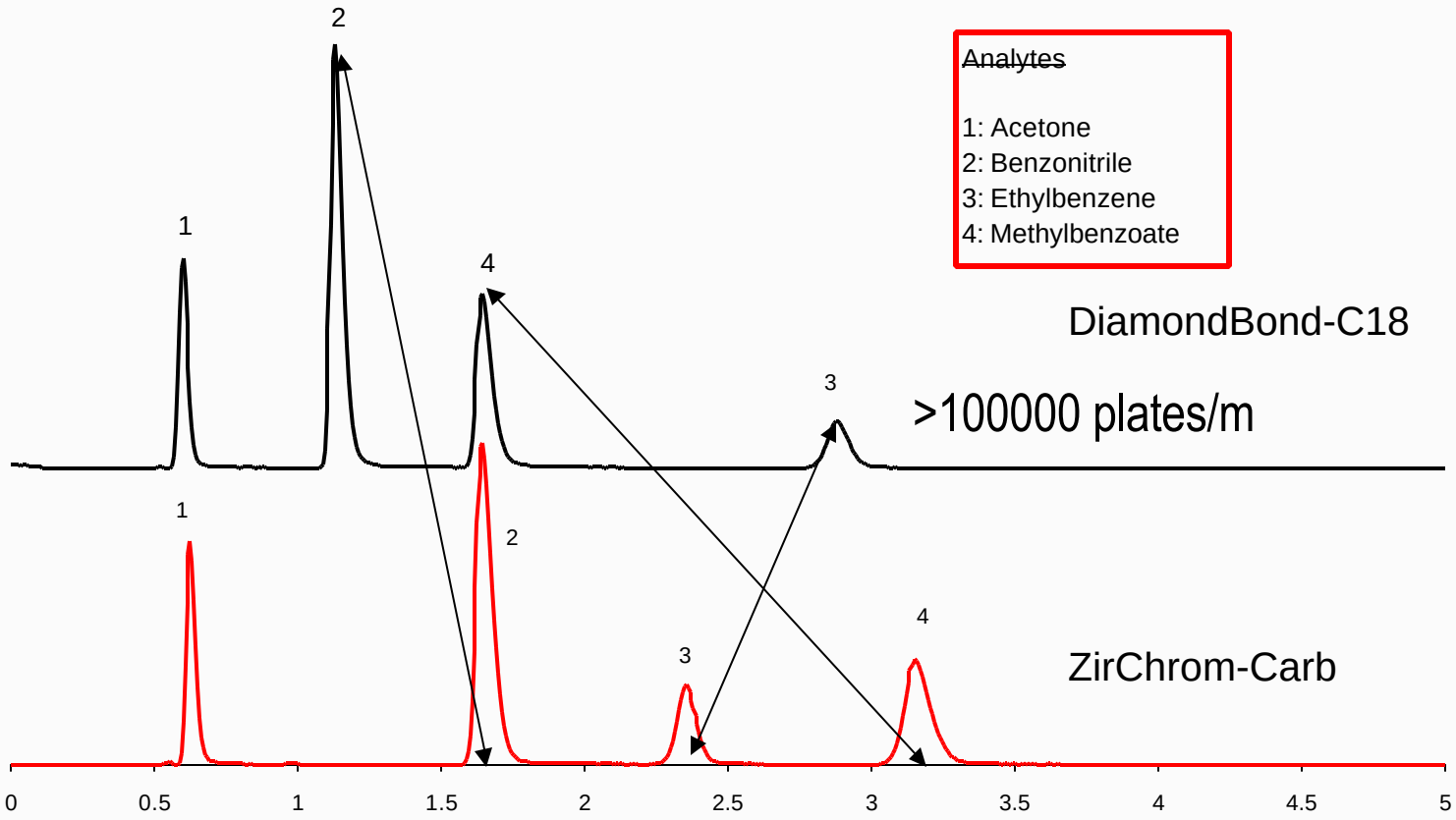
Test Chromatogram on DB-C18



LC Conditions: Column dimension, 50 x 4.6 mm id.; Mobile phase, 37.5/5/57.5 ACN/THF/Water; Temperature, 60 °C; Flow rate, 1.0 ml/min.; Injection volume, 5 µl; Detection at 254 nm.

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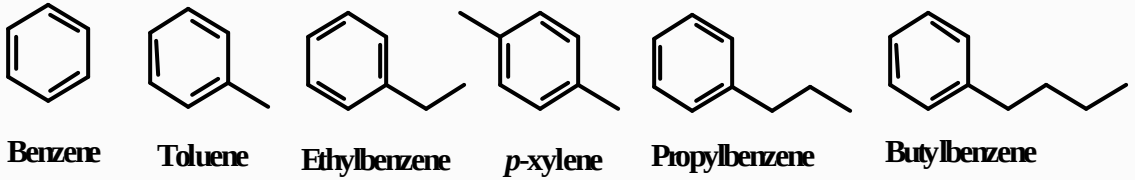
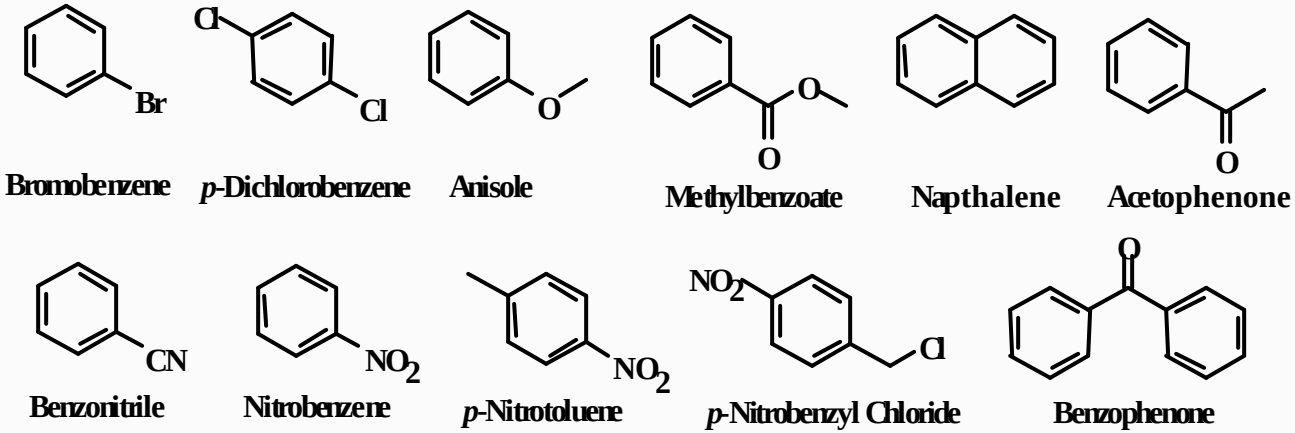
22 Non-Ionizable Solutes

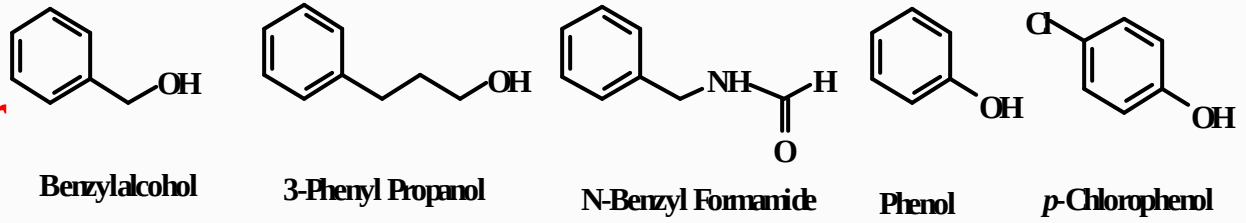
Nonpolar



Polar



HB Donor



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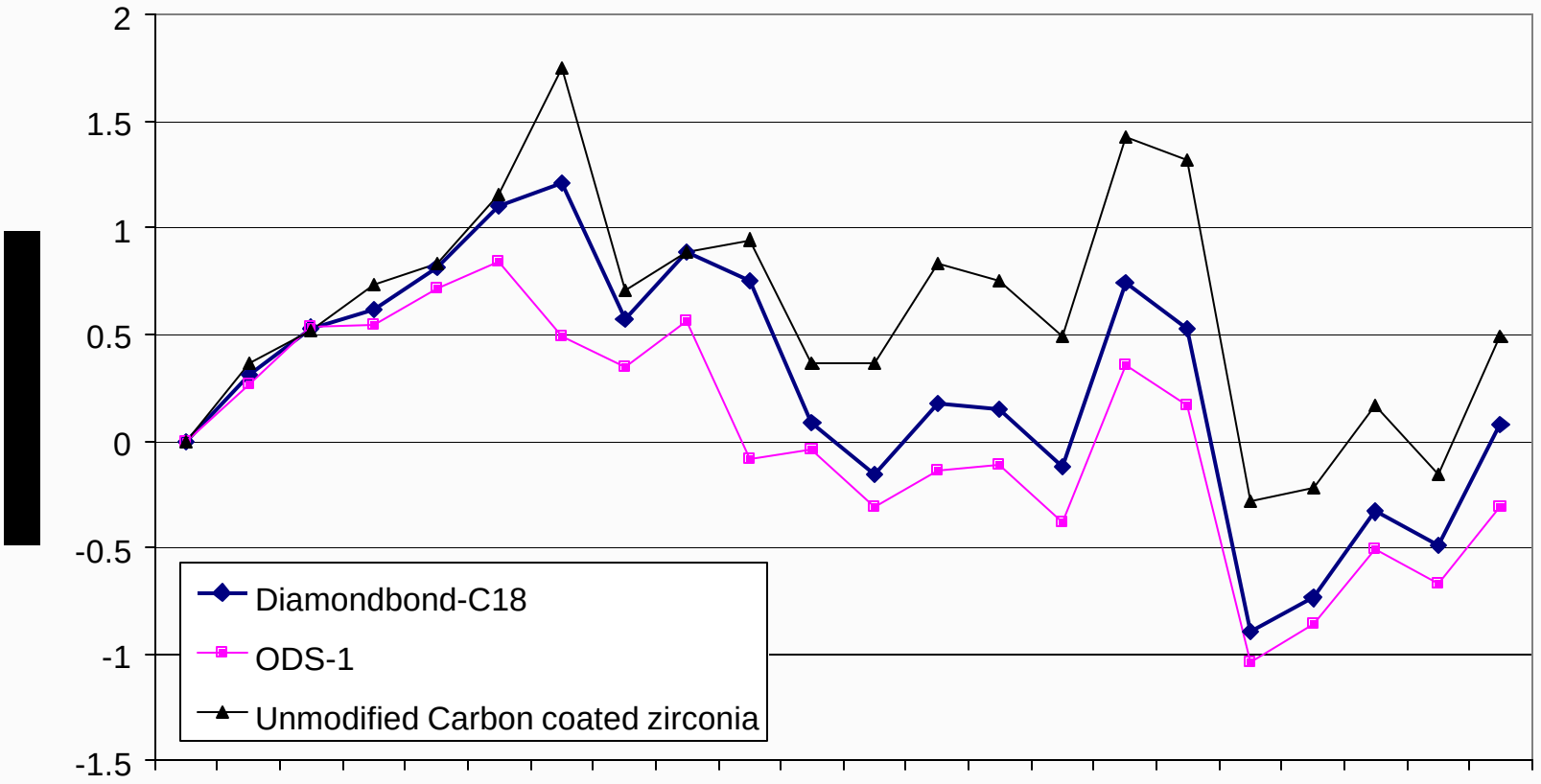
Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Detection at 254nm; 5 ul Injection volume.

Selectivity Comparison



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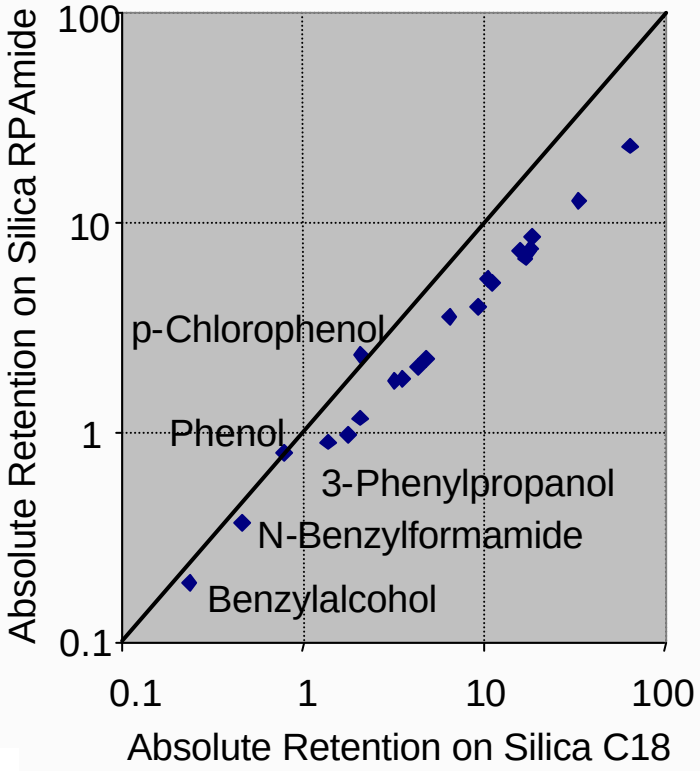
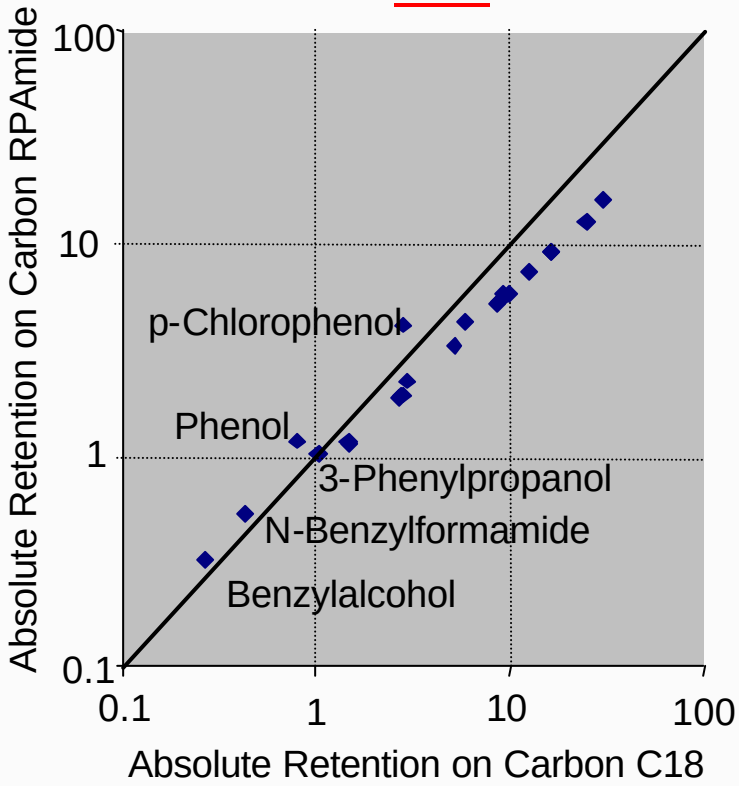
Effect of Polar Embedded Amide

Silica Amide vs. C18



Carbon Amide vs. C18



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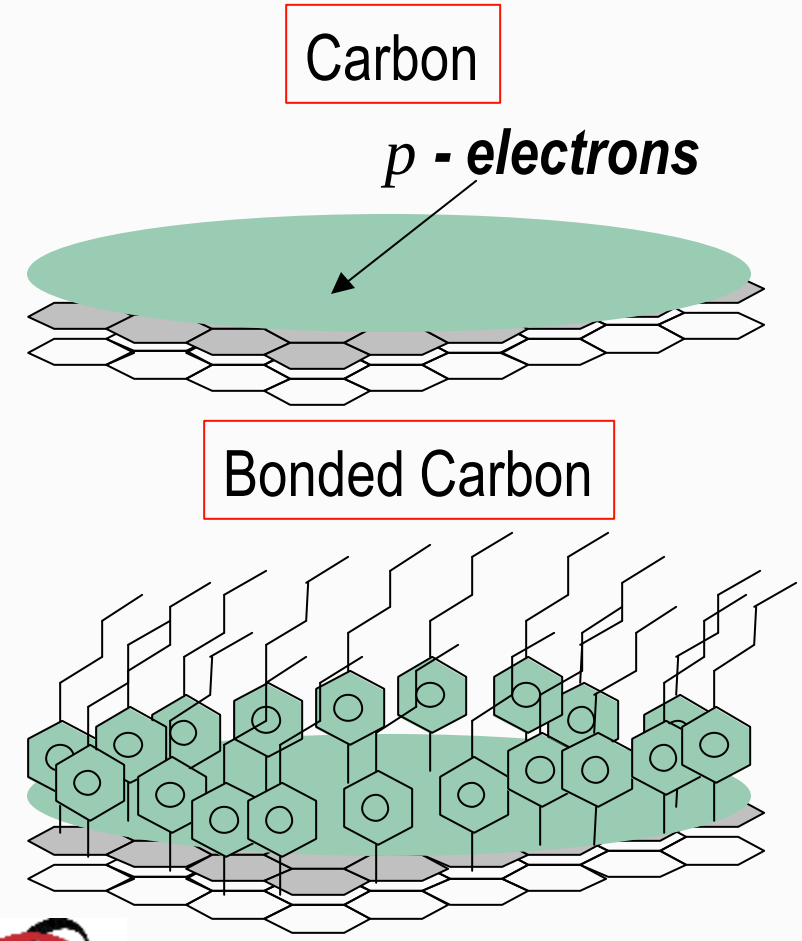
RPAmide shows increased retention of HB Donors on silica and carbon-based phases

Why are Bonded Carbons Unique?



- Carbon surfaces have π-electrons which increase retention of certain types of analytes:
  - fused polyaromatics (e.g. naphthalene, etc)
  - polar molecules (e.g. amides, ketones, alcohols, etc)
- Surface modified carbon surfaces combine some of these interactions with interactions specific to the bonded surface groups.
- Bonded Carbons maintain the high pH and thermal stability that is inherent in the C-C bond.

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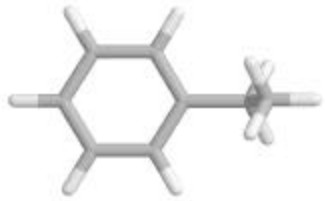
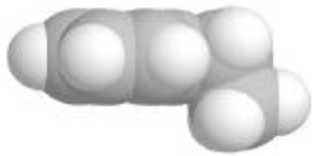
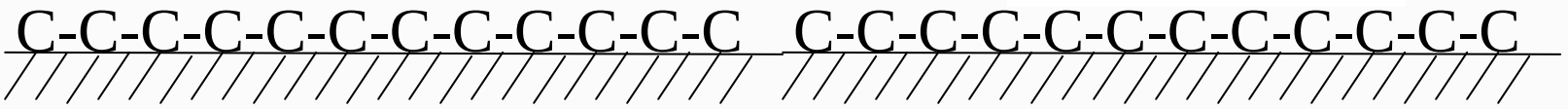
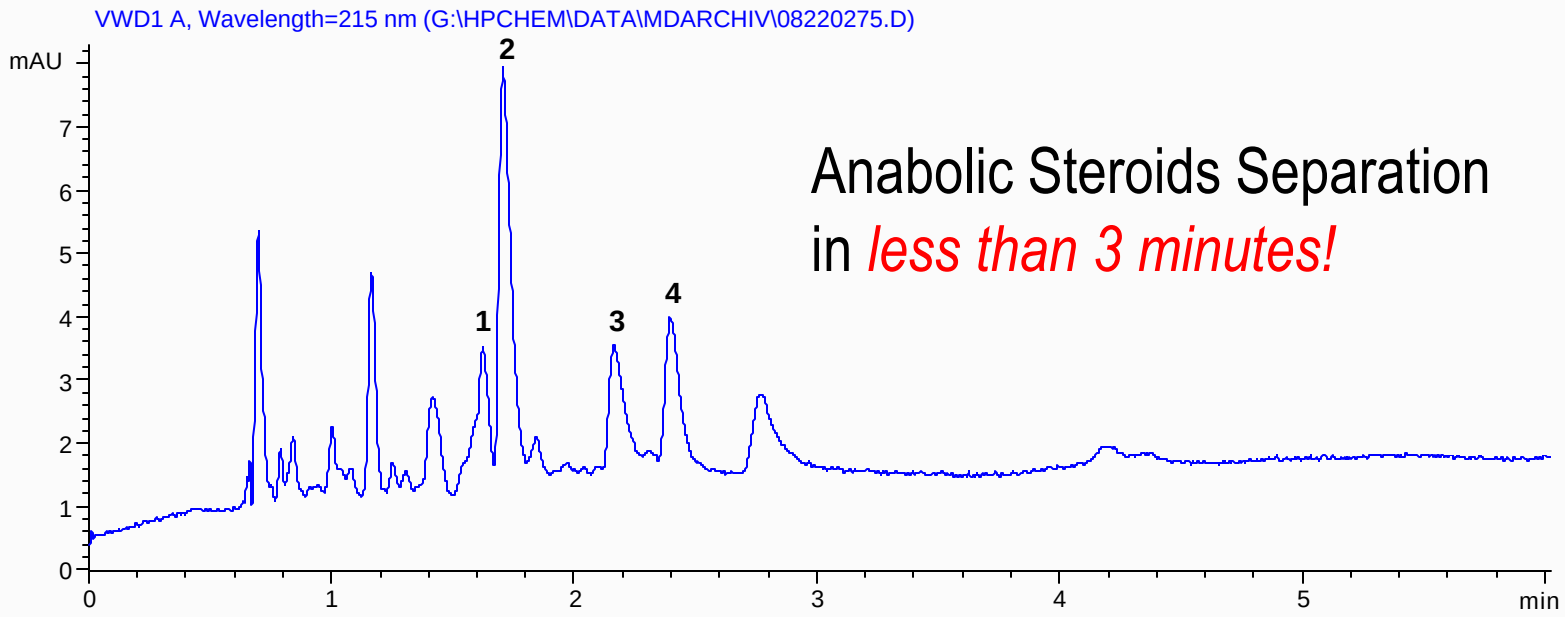
Shape Makes a Difference

p-xylene		ethylbenzene
	$a_{ODS}=1.03$	
	$a_{CARB}=1.58$	
	$a_{DB-C18}=1.22$	



Shape Selectivity: Anabolic Steroids

LC Conditions: Column, 150 x 4.6 DiamondBond-C18; Mobile phase, 60/40 ACN/Water; Flow rate, 2.0 ml/min.; Temperature, 100 ^{o}C; Injection volume, 10ul; Detection at 215nm; Solutes: 1=Epietiocholanolone, 2=Etiocholanolone, 3=Androsterone, 4=Epiandrosterone



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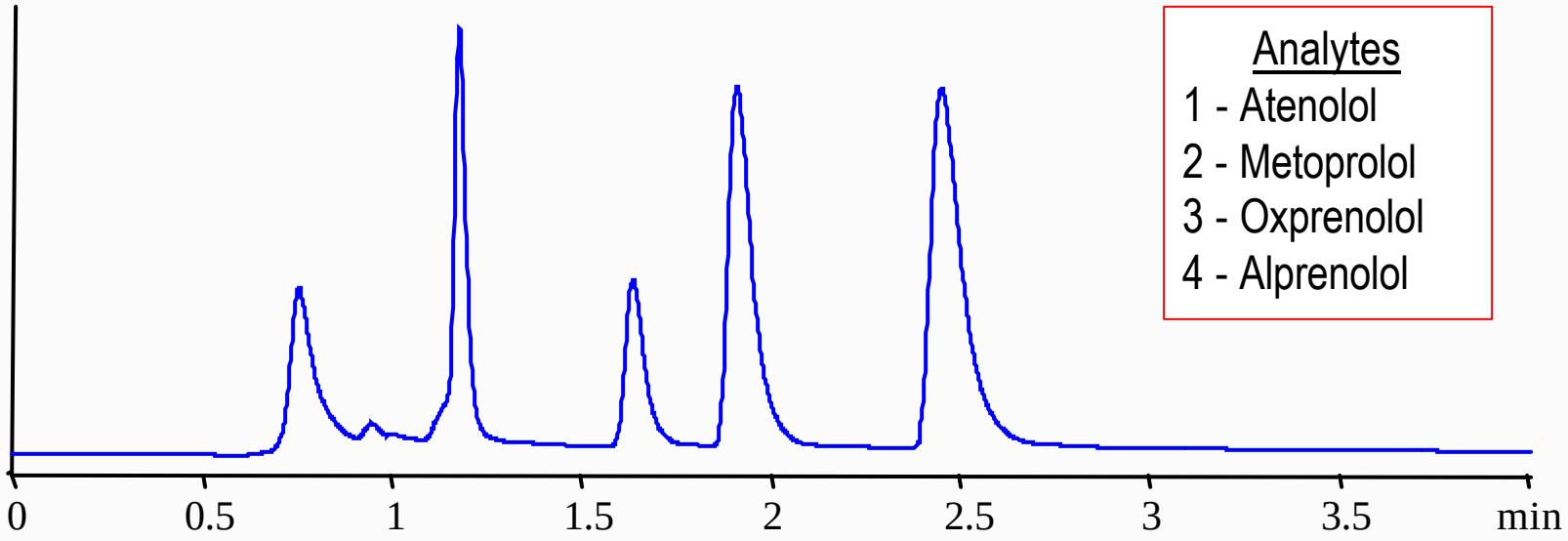
High pH Stability - Beta Blockers



LC Conditions:

20/20/60 ACN/THF/200 mM TMAH and 200 mM NaCl, **pH 13.3**

Flow Rate: 1 ml/min. Temperature: 75 °C. Injection Volume: 5 ul

Detection: 254 nm.

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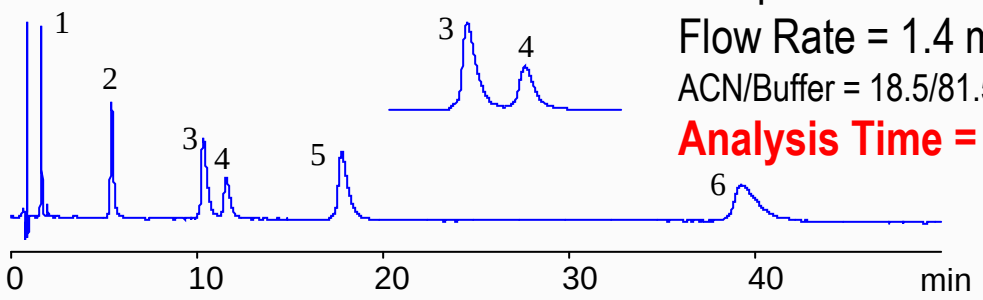
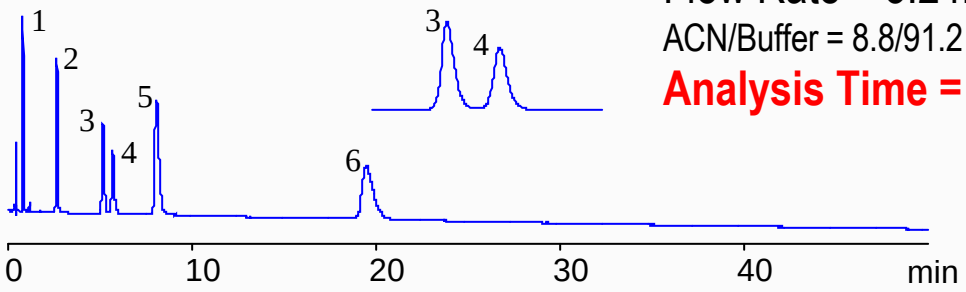
High Temperature Stability - Speed



Resolution (min; 3,4) =2.2
Temperature = 21 °C
Flow Rate = 1.4 ml/min.
ACN/Buffer = 18.5/81.5
Analysis Time = 43 min.



Resolution (min; 3,4) = 2.2
Temperature = 80 °C
Flow Rate = 3.2 ml/min.
ACN/Buffer = 8.8/91.2
Analysis Time = 21 min.

Analytes:
1 = Barbital
2 = Butabarbital
3 = Pentobarbital
4 = Carbromal
5 = Secobarbital
6 = Methohexital

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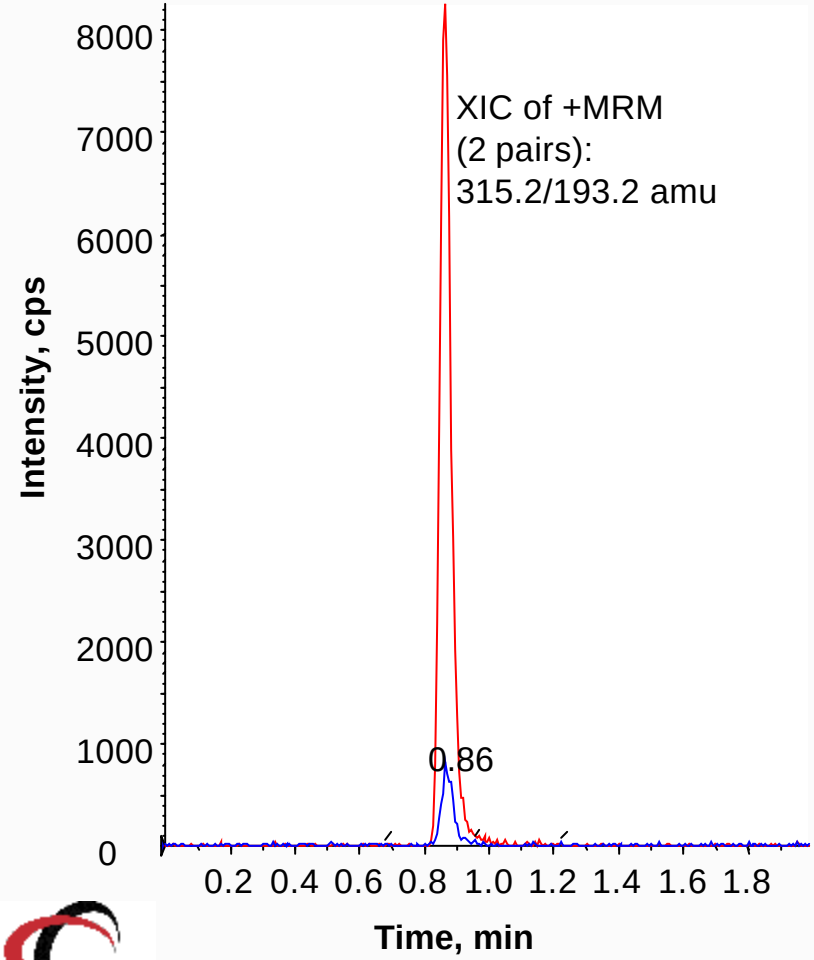
Mobile Phase: ACN/5mM Ammonium phosphate, pH 7.0

Pressure drop =195 bar

Application in LC/MS/MS



THC in Saliva by LC/MS/MS†

- Blue – THC (tetrahydrocannabinol parent drug)
- Red – D3 THC (Internal Standard)
- Column – 50mm X 4.6mm DBC18
  - 80º C @ 1.5 mL/min
  - Solvent A – 20mM $NH_4CH_3CO_2$ in 70% Acetonitrile, 30% aqueous (0.1% acetic acid, pH 4.5)
  - Solvent B – Acetonitrile
- Isocratic 35% A, 65%B – 25 ul injection

† Data Courtesy R. Clouette - Clinical Reference Laboratories

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Summary

- The effect of ligand type on retention for bonded carbon phases is similar to that for bonded silicas
  - Unique surface chemistry enables unique separations
- The carbon-carbon attachment bond is extremely stable
  - Low pH and High pH applications
  - High Temperature / Fast HPLC
- Both “normal” and high pH, high temperature applications are possible on these new materials
  - LC/MS pharmaceutical applications enabled by this technology

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