ZirChrom®

The Development and Applications of Polymer and Carbon Coated Zirconia-based Supports for Reversed Phase LC

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ZirChrom Separations, Inc.

DIAMONDBOND™ HPLC Columns

Outline

- Advantages of Stable Phases
- Selectivity Comparison of Zirconia based phases to ODS Silica
- Development of a New Type of Reversed Phase Column: DiamondBond®-C18
- Applications
- Summary

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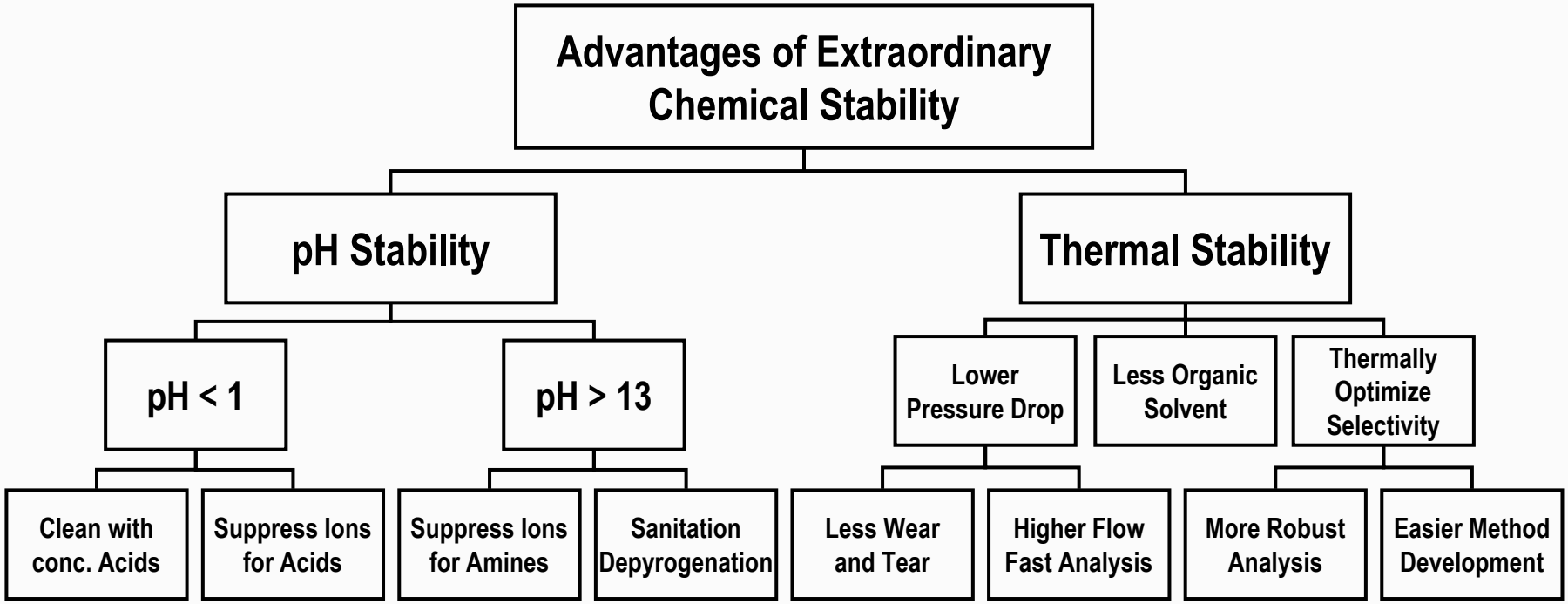
Why Stable Phases?



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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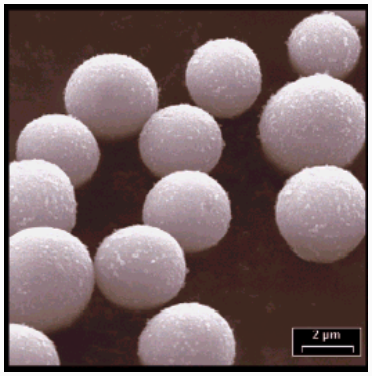
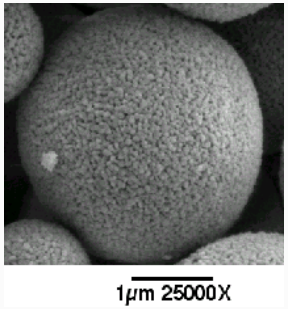
ZirChrom® Particle Properties





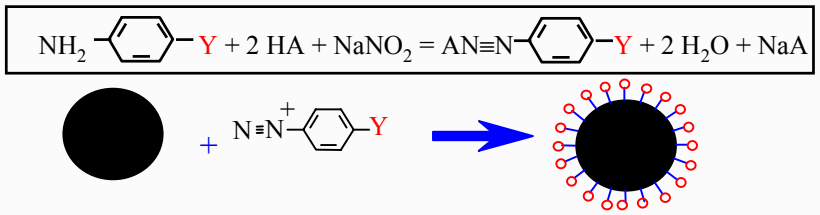
ZirChrom®-Carb particles are prepared by coating base particles with a thin layer of carbon using a chemical vapor deposition process

ZirChrom®-PBD particles are prepared by coating base with a layer of highly crosslinked polymer

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 Diameters (µ)	3.0, 5.0, 10.0

DIAMONDBOND™ - C18

Bonding Reaction on Carbon Clad Zirconia



Carbon Clad Zirconia Diazonium Salt Modified Carbon Clad Zirconia

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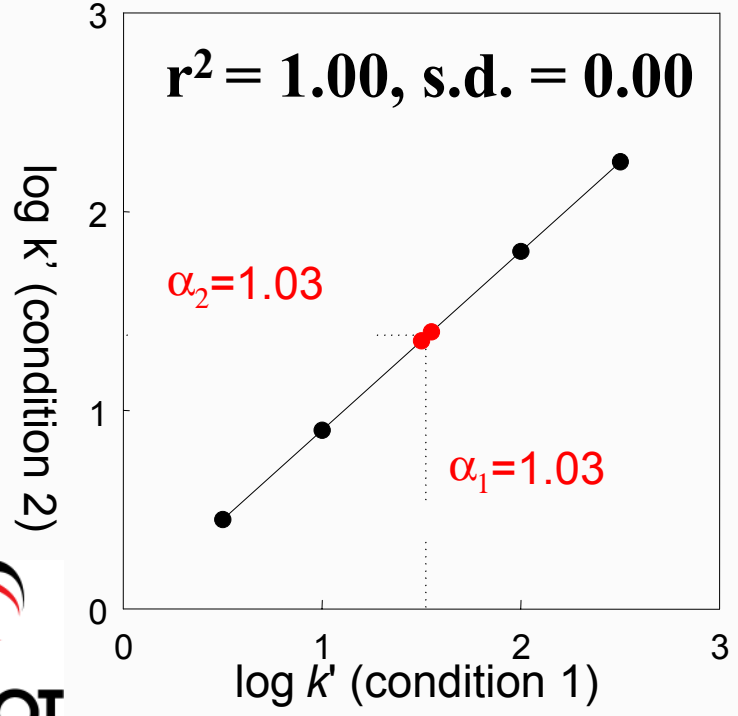
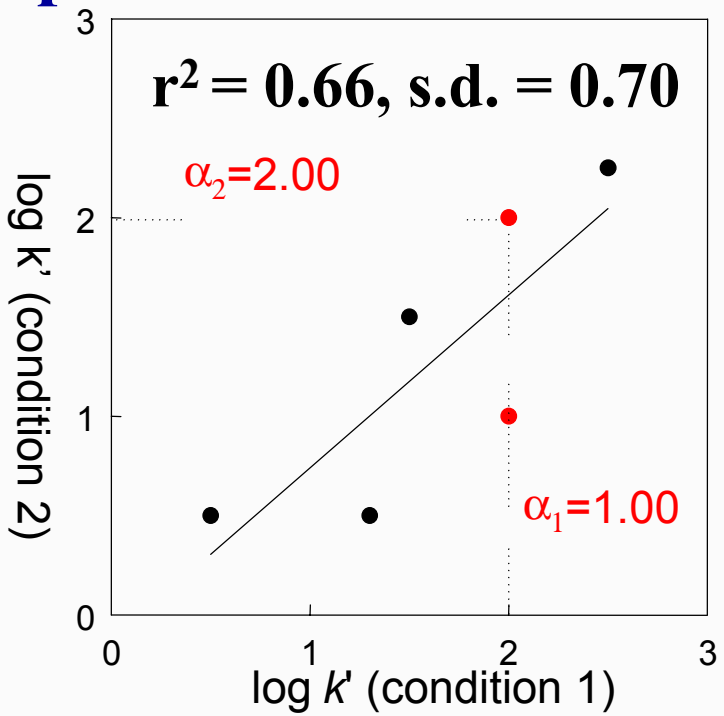
Adjusting Selectivity in HPLC

- Mobile Phase Composition (B%)
- Mobile Phase Type (ACN, MeOH, THF)
- Stationary Phase Type (C18-SiO_2, C-ZrO_2, PBD-ZrO_2)
- Temperature

➢ **Poor correlations in the κ–κ plot indicate changes in selectivity.**

κ–κ plot





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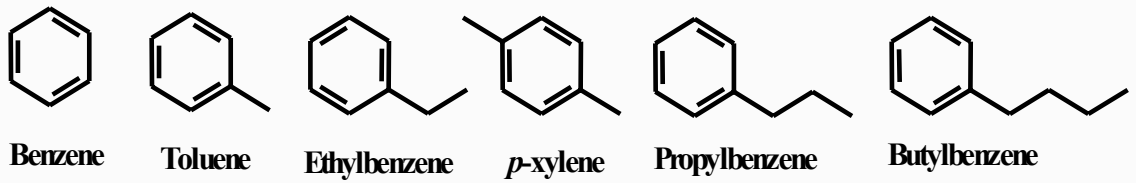
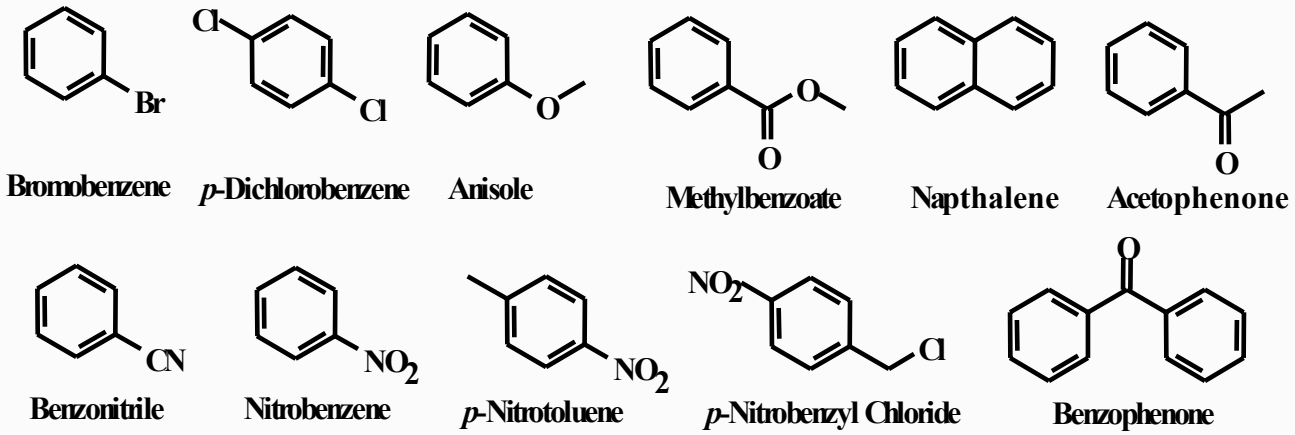
22 Non-Ionizable Solutes

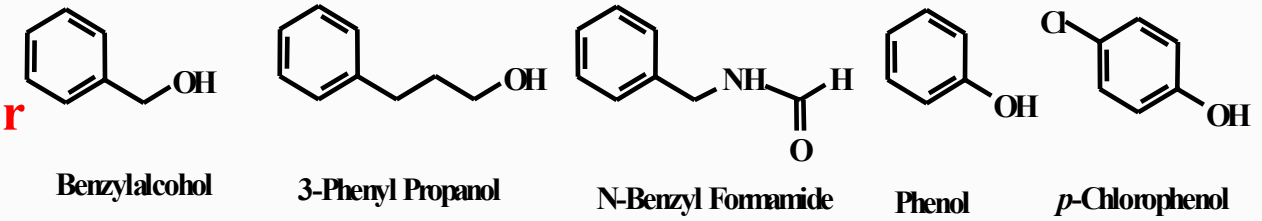
Nonpolar



Polar



HB Donor



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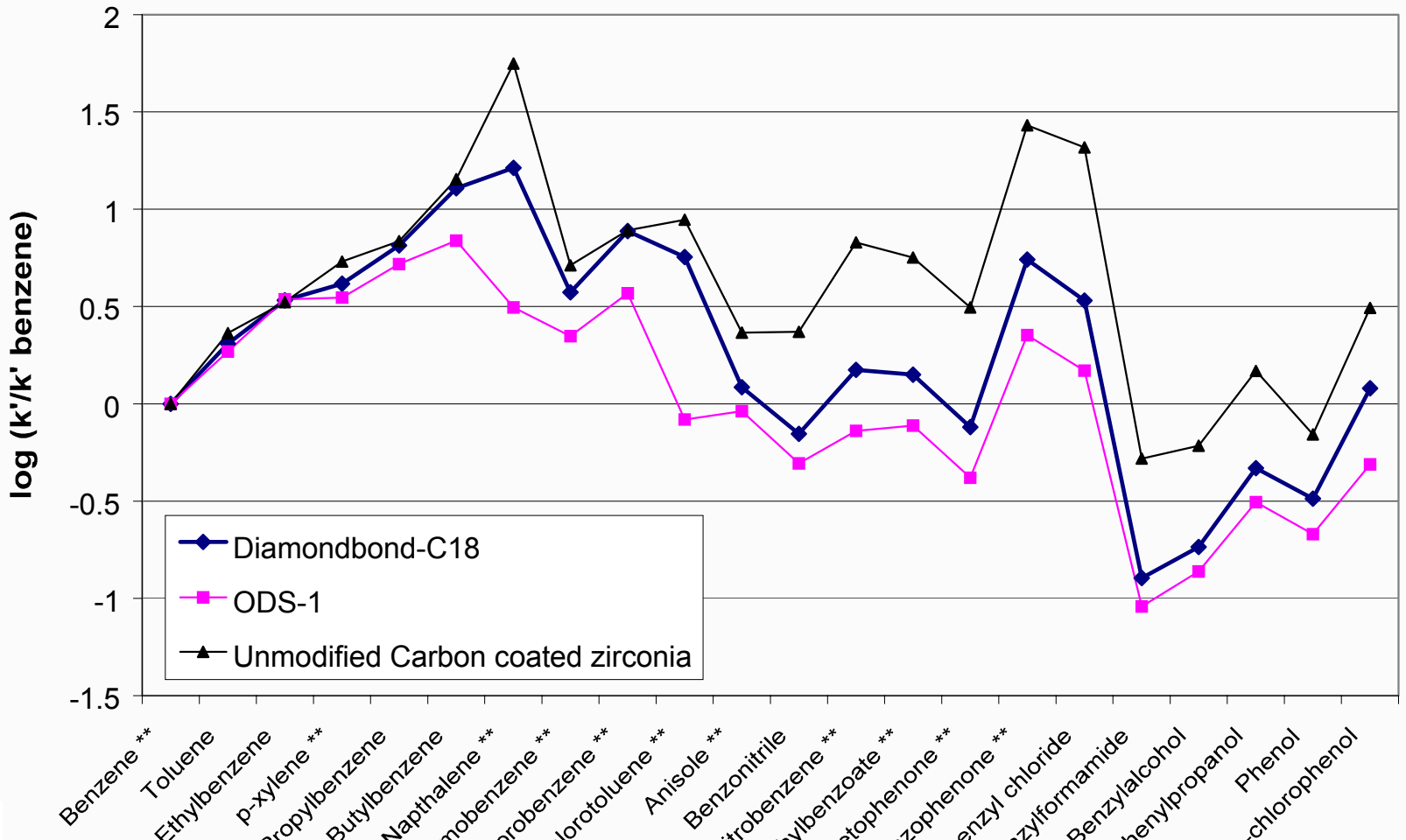
Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Detection at 254nm; 5 ul Injection volume.

Selectivity Comparison



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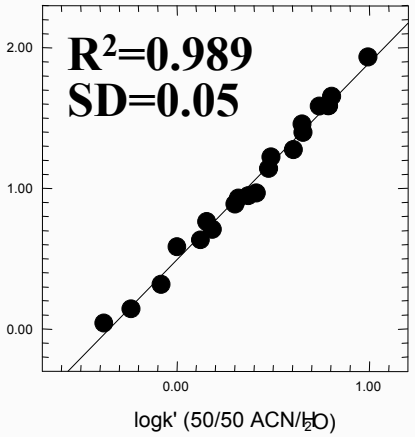
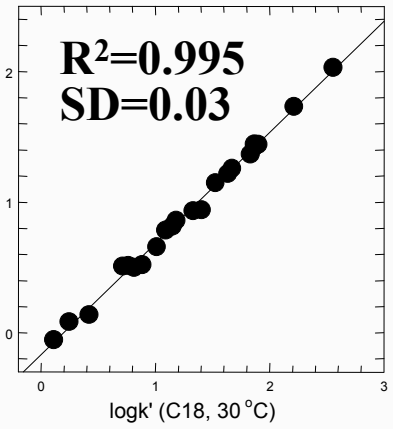
Comparison of Variables Affecting Selectivity

30% ACN vs. 50% ACN

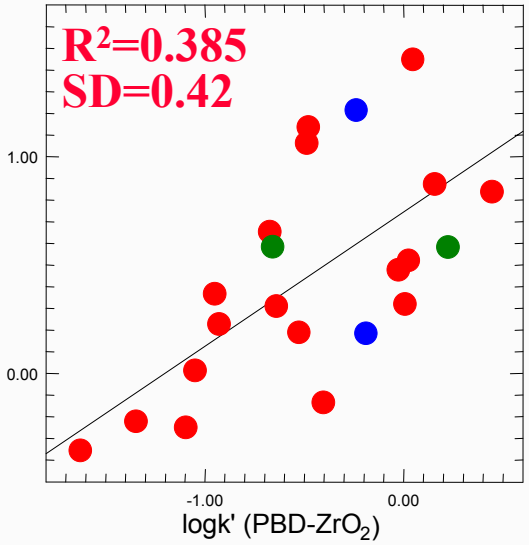


80°C vs. 30°C

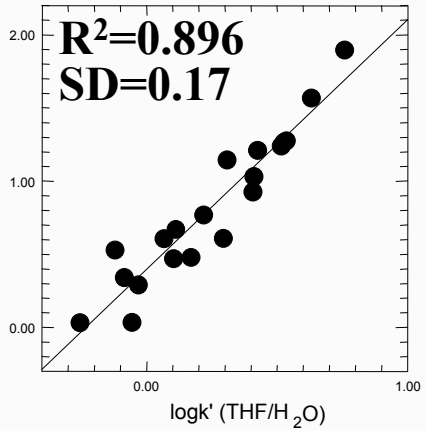


Stationary Phase Type

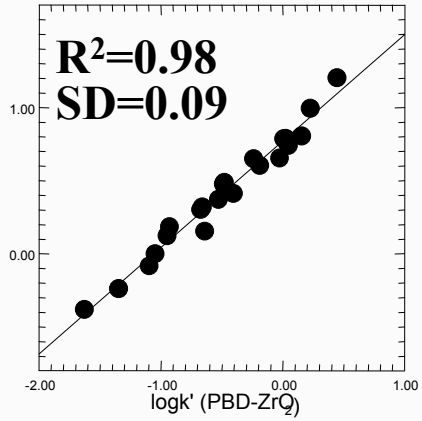
Carbon-ZrO_2 vs.
PBD-ZrO_2



MeOH vs. THF



C18-SiO_2 vs. PBD-ZrO_2



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❖ **Stationary phase type has a large effect on selectivity.**

Regression Data from log k' vs. log k' Plots vs ODS

Column	R^2	Selectivity Difference*
ZirChrom®-PBD	0.985	12
DiamondBond®-C18	0.889	33
ZirChrom®-Carb	0.549	67

- For non-ionizable solutes:
  - ZirChrom-Carb and Diamondbond-C18 columns have very different selectivities from traditional C18-Silica HPLC columns
  - ZirChrom-PBD has selectivity similar to C18-Silica
- For ionizable solutes the picture is very different

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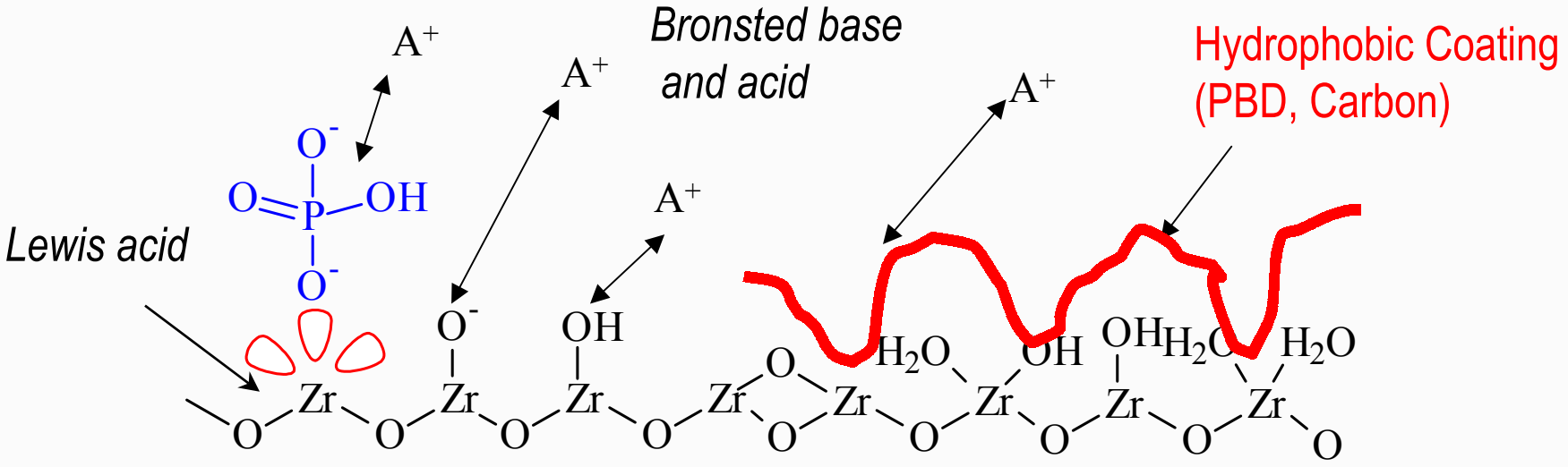
*$S=100(1-R^2)^{0.5}$, as described by U. Neue at FACSS meeting

Zirconia Has Unique Surface Chemistry



- Zirconia by itself has very rich surface chemistry
- Coated zirconia phases (Carbon and PBD) have mixed surface properties
- The retention of various basic and acidic analytes can be fine tuned by changing pH, buffer, salt concentration, in addition to mobile phase modifier concentration and type

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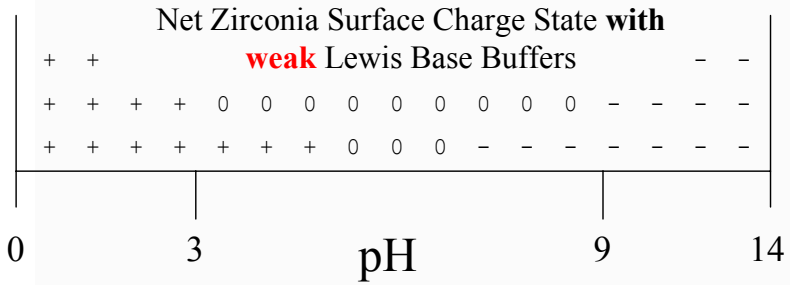
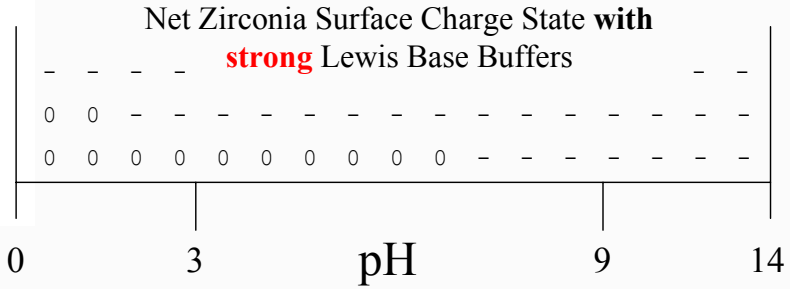
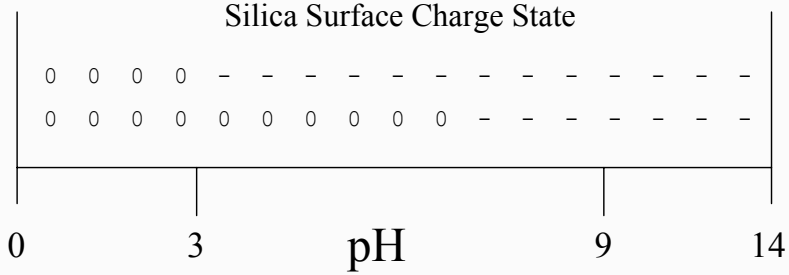
Zirconia Features Tunable Surface Properties

Interaction Strength	Lewis Base
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
Weakest	Chloride





- The choice of buffer and pH on zirconia columns affects the surface charge and the elution properties of ionizable analytes



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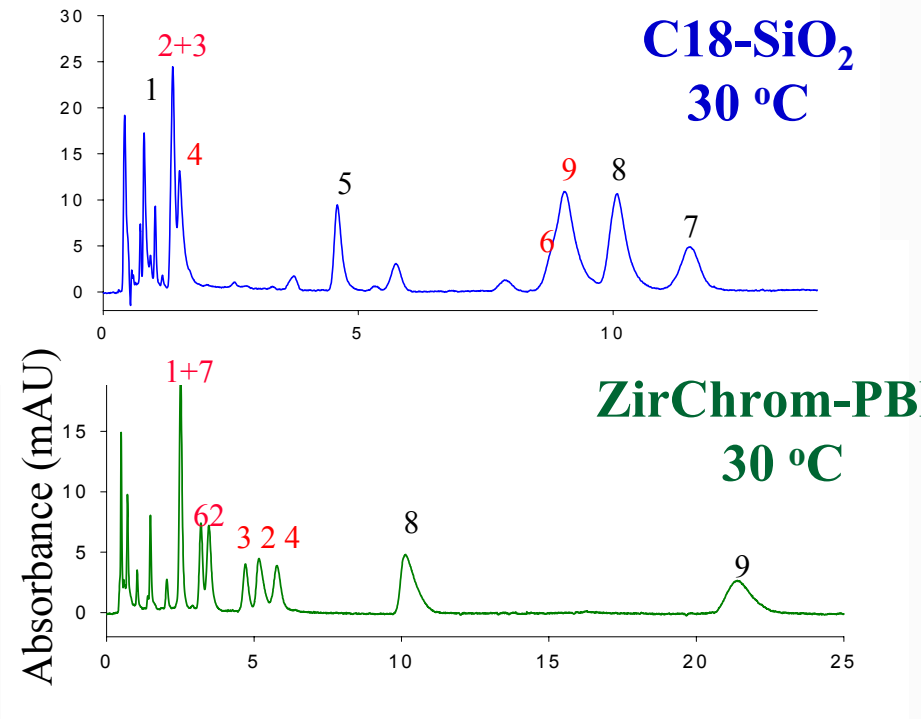
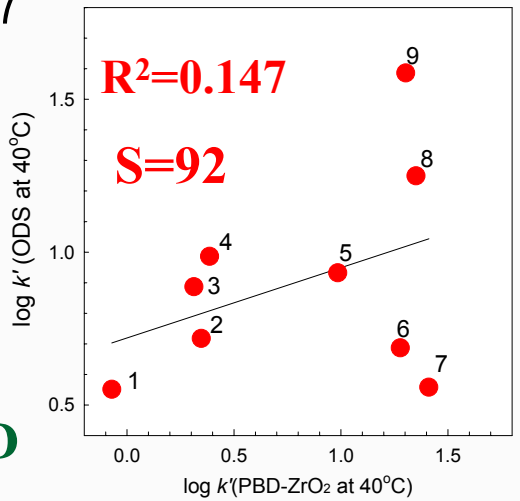
Antihistamine Drug Selectivity Comparison

Mobile Phase: 40/60 Acetonitrile/25 mM Phosphate, pH=7





- The selectivity of zirconia based columns towards ionizable compounds becomes very different from that of traditional silica columns when Lewis base buffers are used

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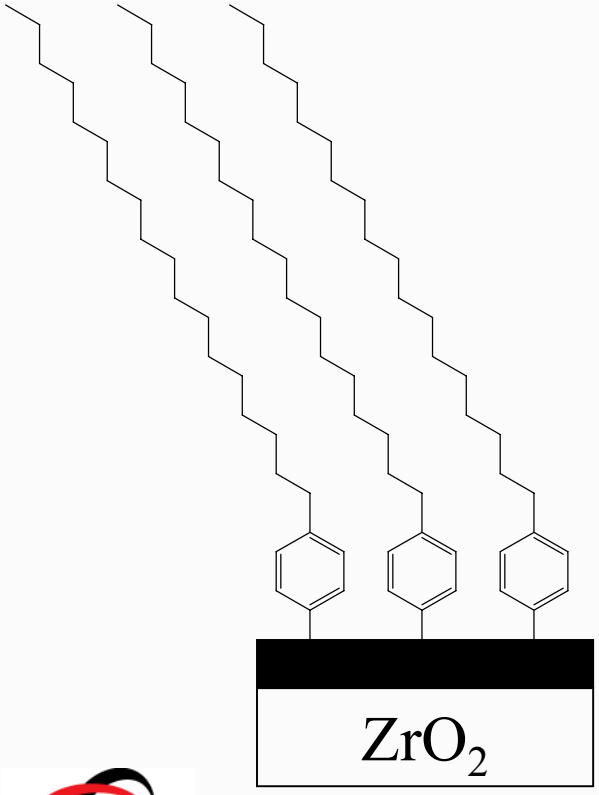
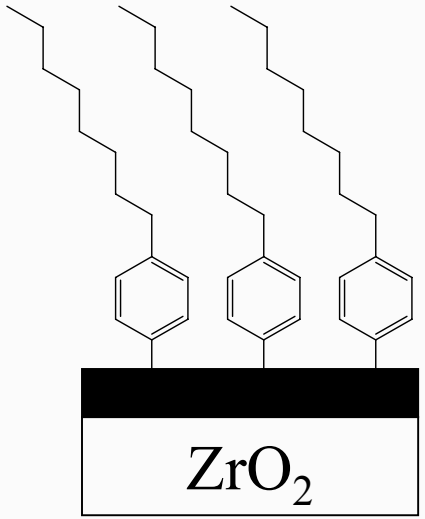
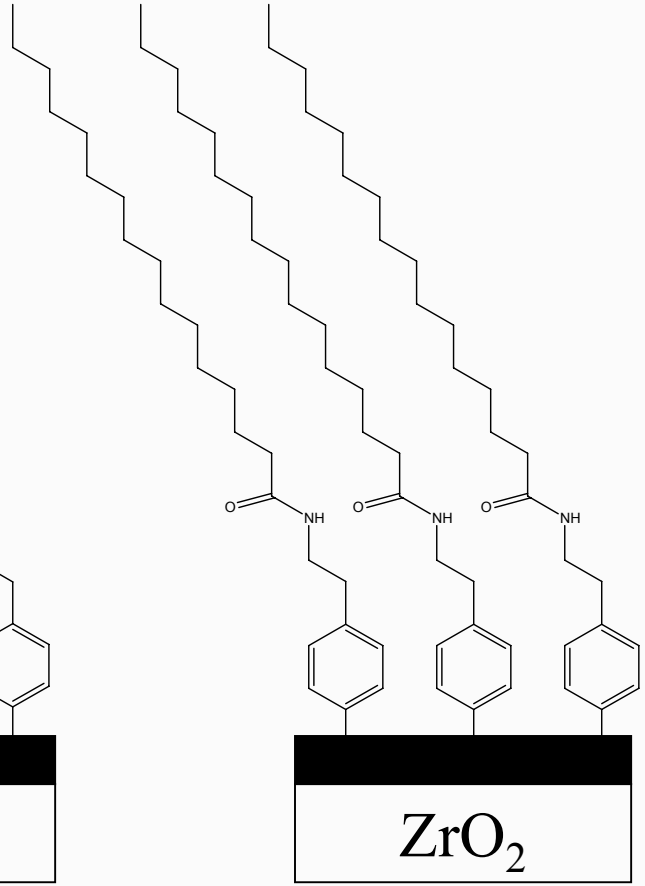
DiamondBond®: A New Family of Stable Phases



DB-C18



DB-C8



DB-Amide18

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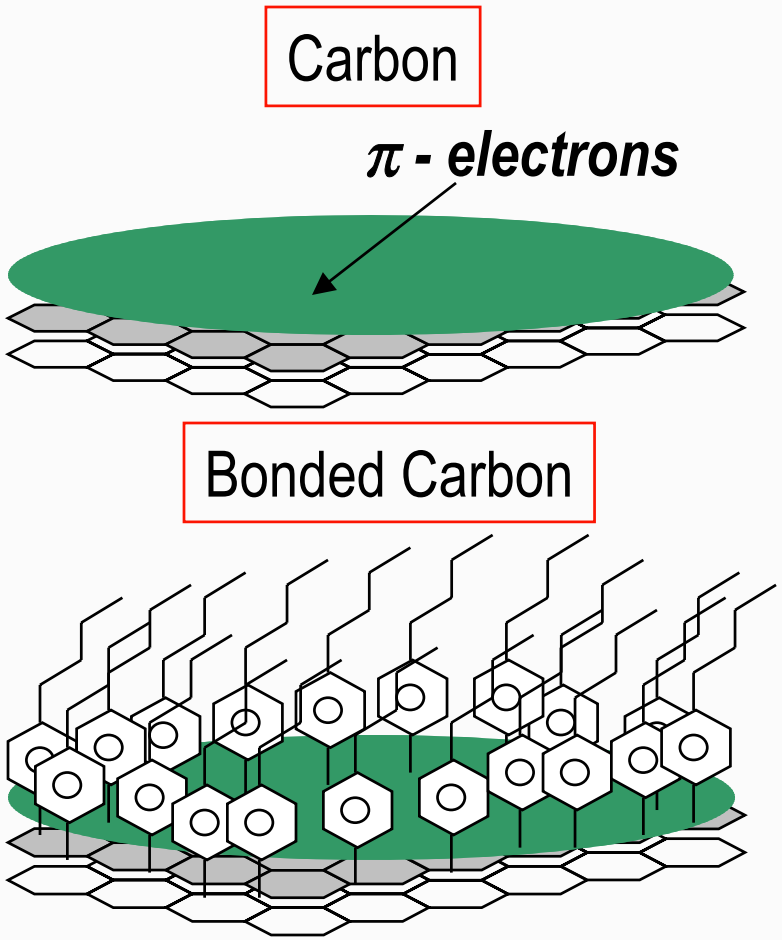
Why are Bonded Carbons Unique?



- 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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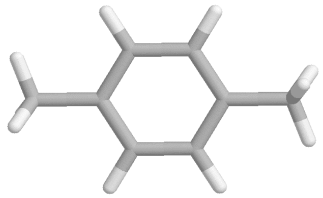
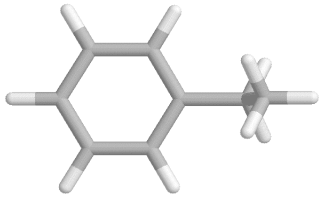
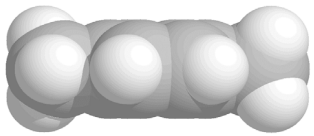
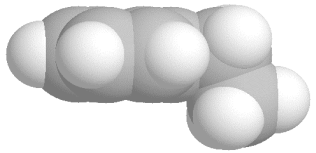
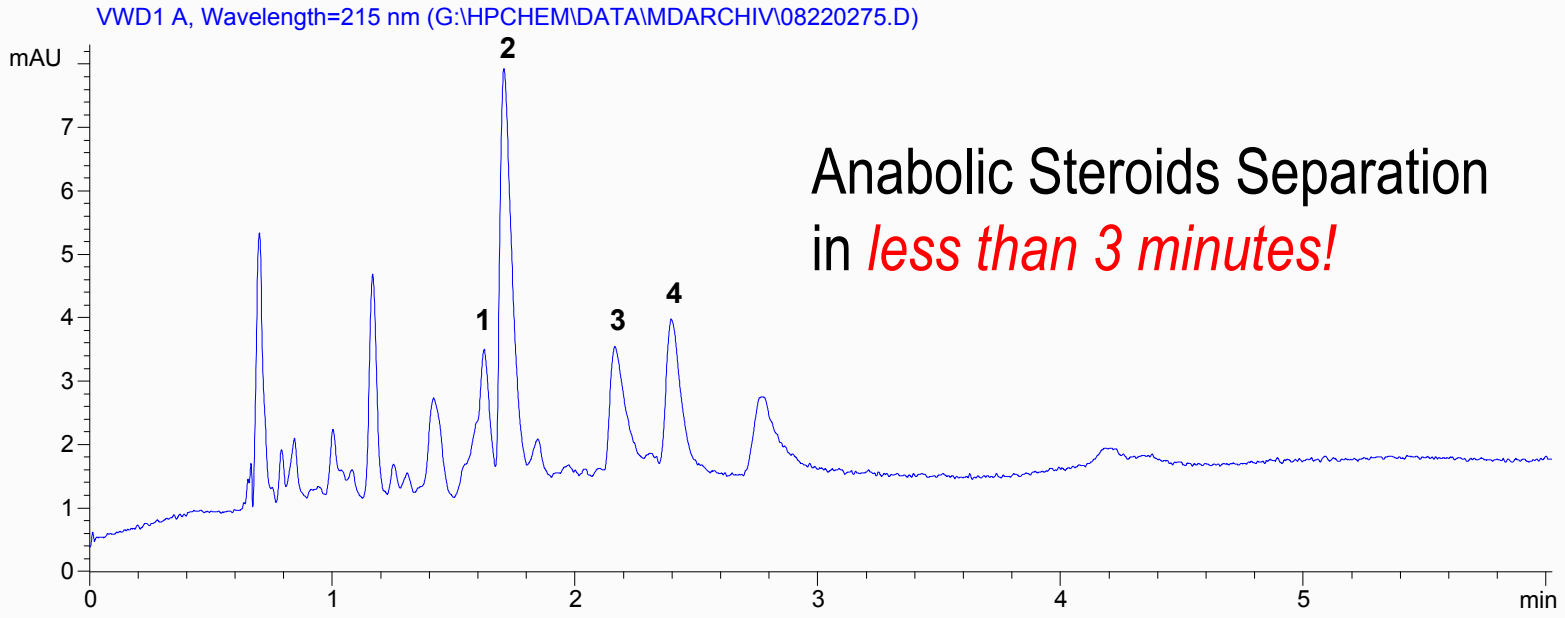
Shape Makes a Difference

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

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 °C; Injection volume, 10ul; Detection at 215nm; Solutes: 1=Epietiocholanolone, 2=Etiocholanolone, 3=Androsterone, 4=Epiandrosterone



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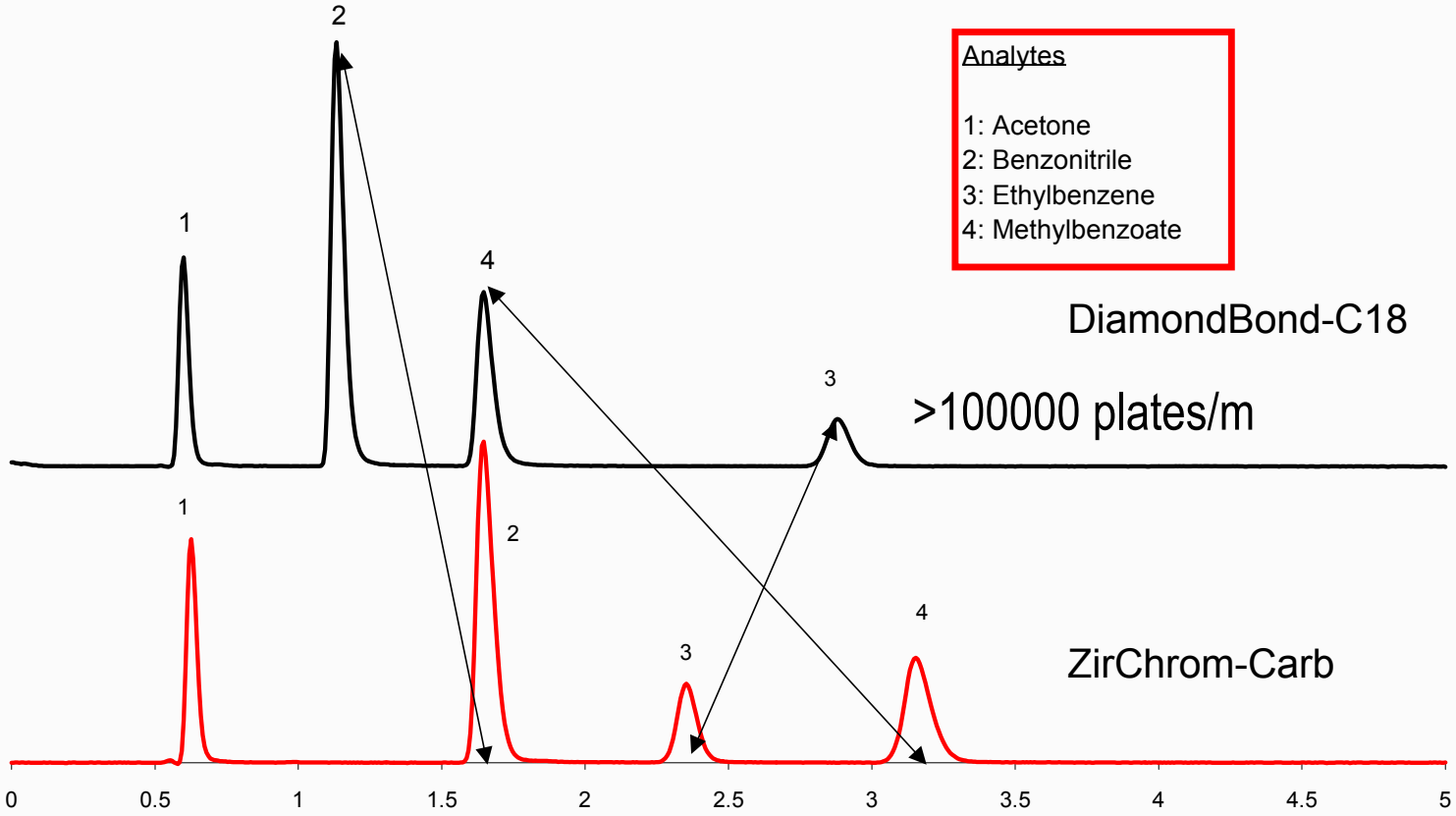
Test Chromatogram on DB-C18



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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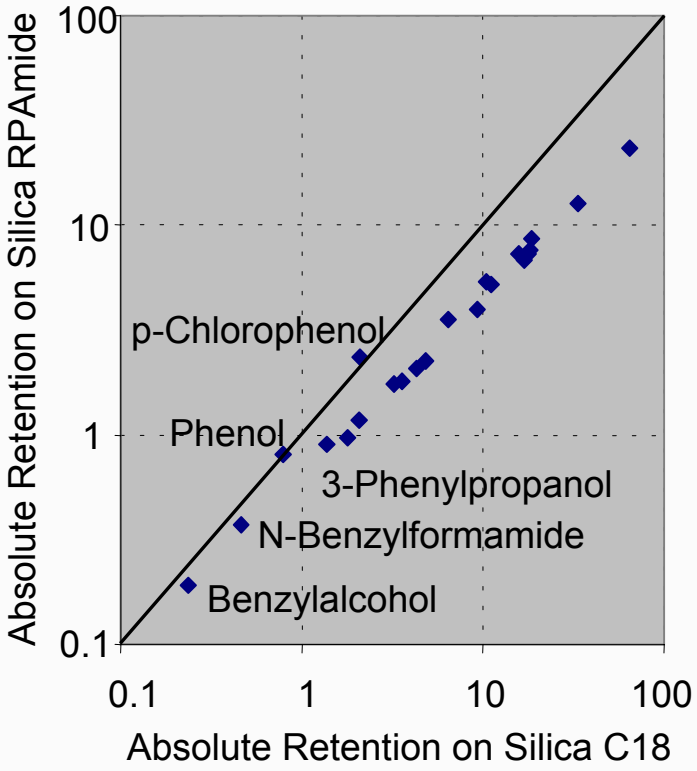
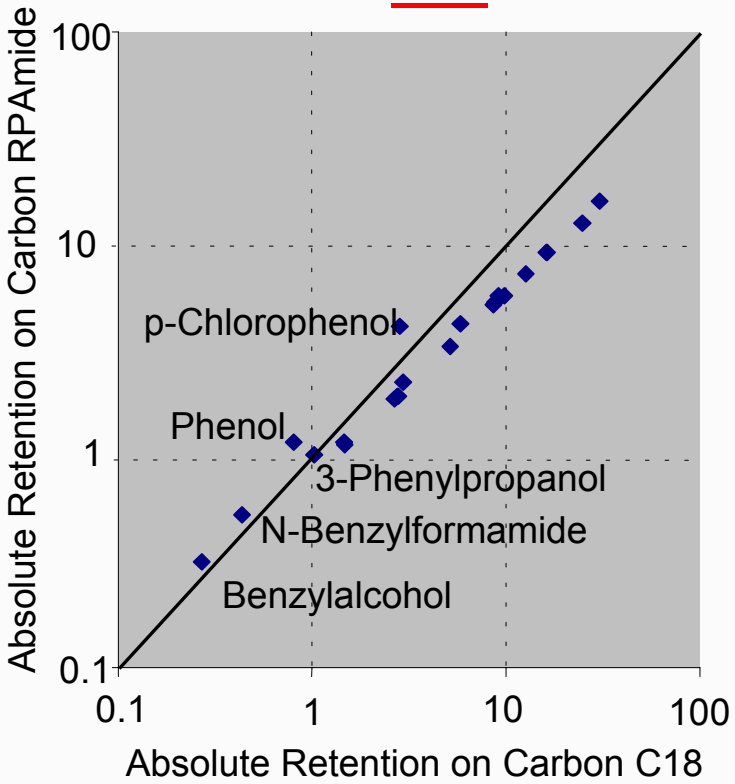
DIAMONDBOND™ HPLC Columns

Effect of Polar Embedded Amide

Silica Amide vs. C18



Carbon Amide vs. C18



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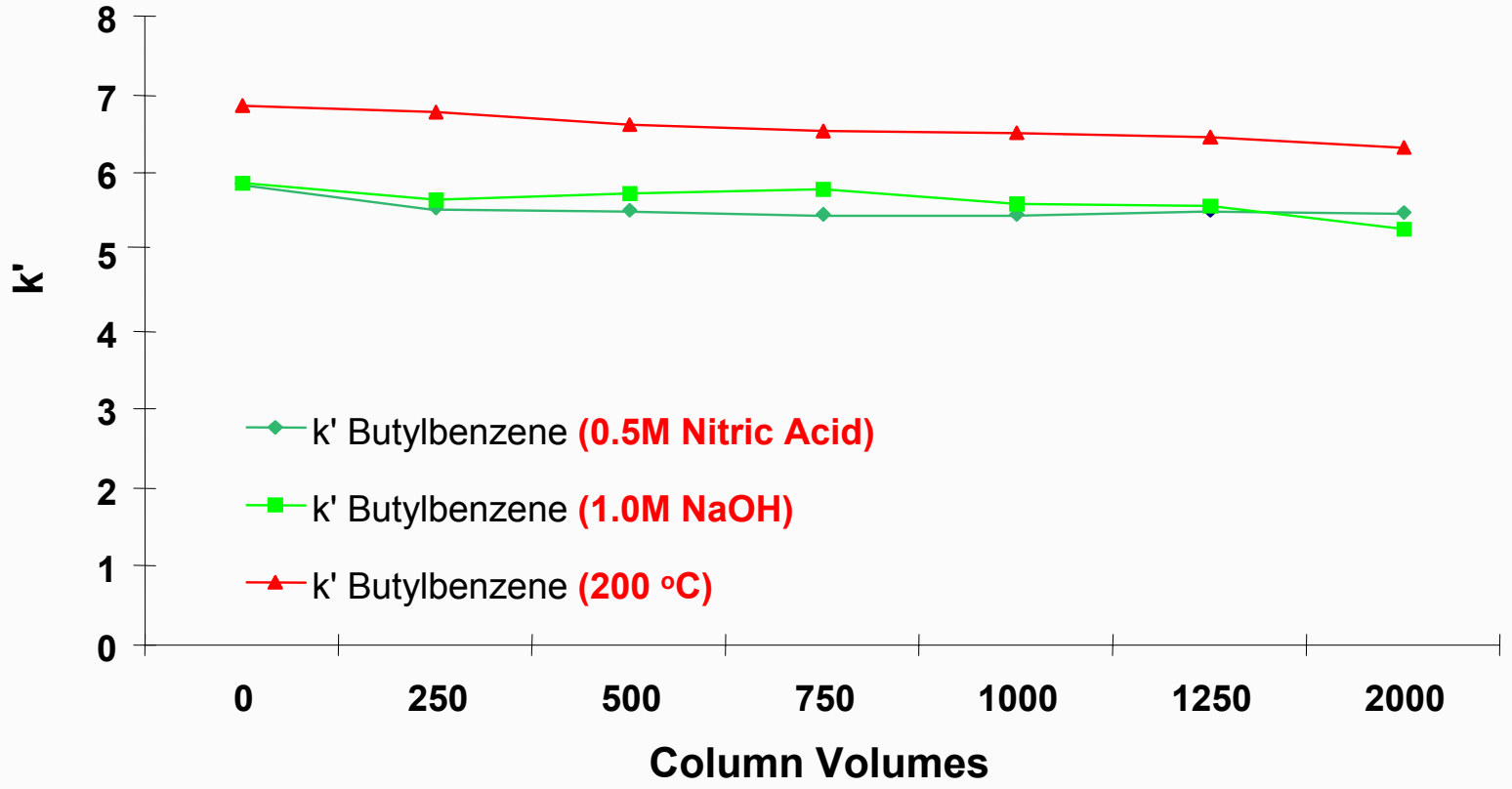
RPAmide shows increased retention of HB Donors on silica and carbon-based phases

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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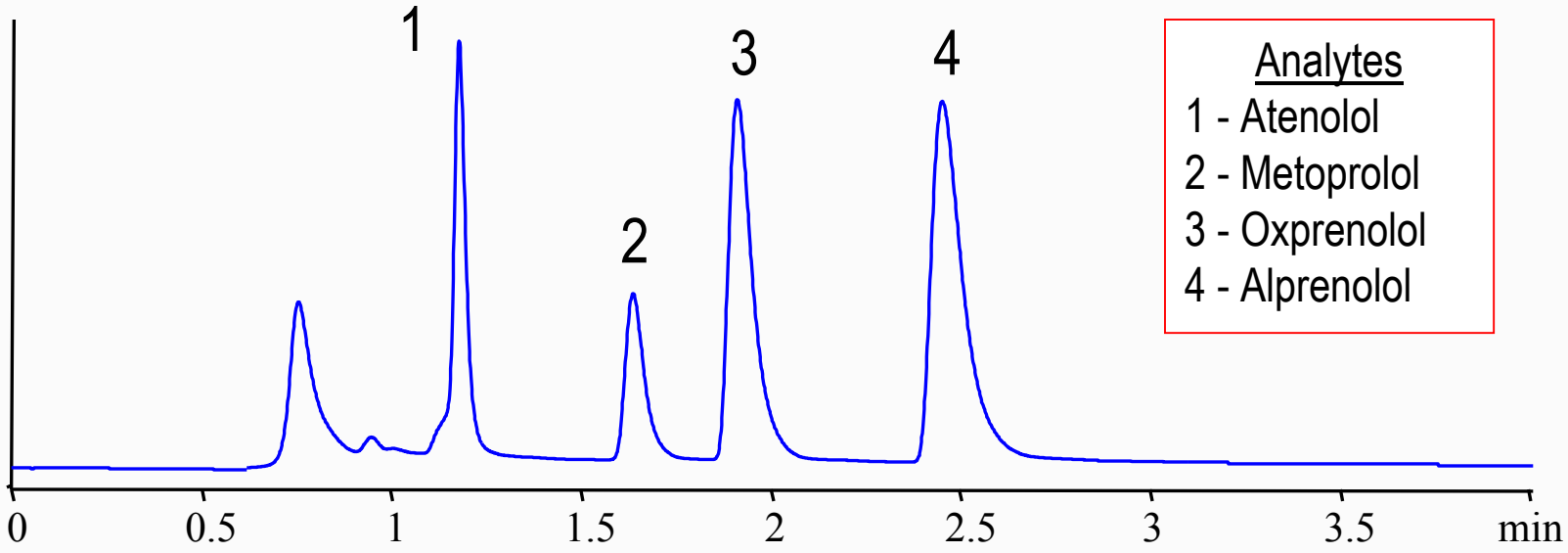
High pH Stability - Beta Blockers



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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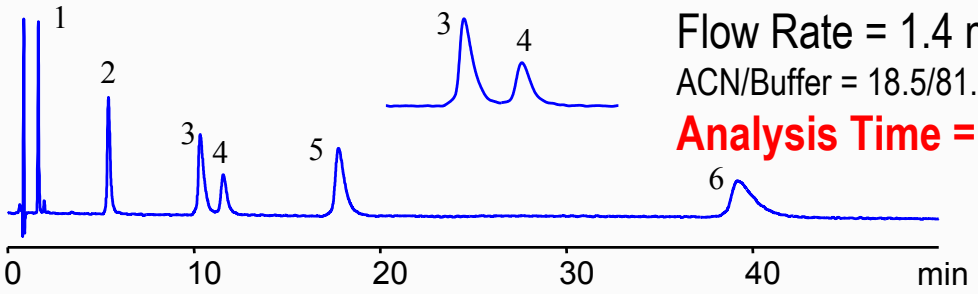
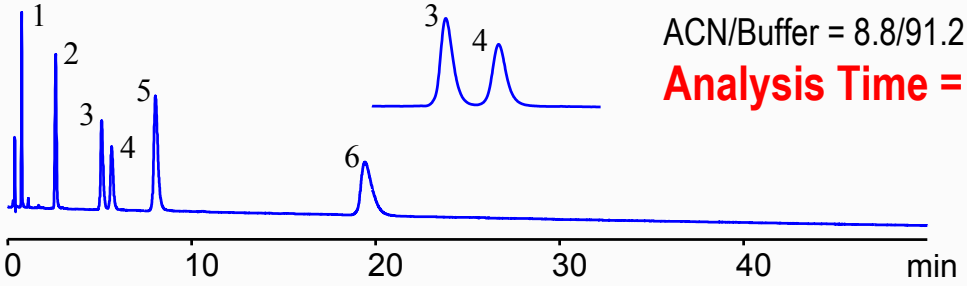
High Temperature Stability - Speed



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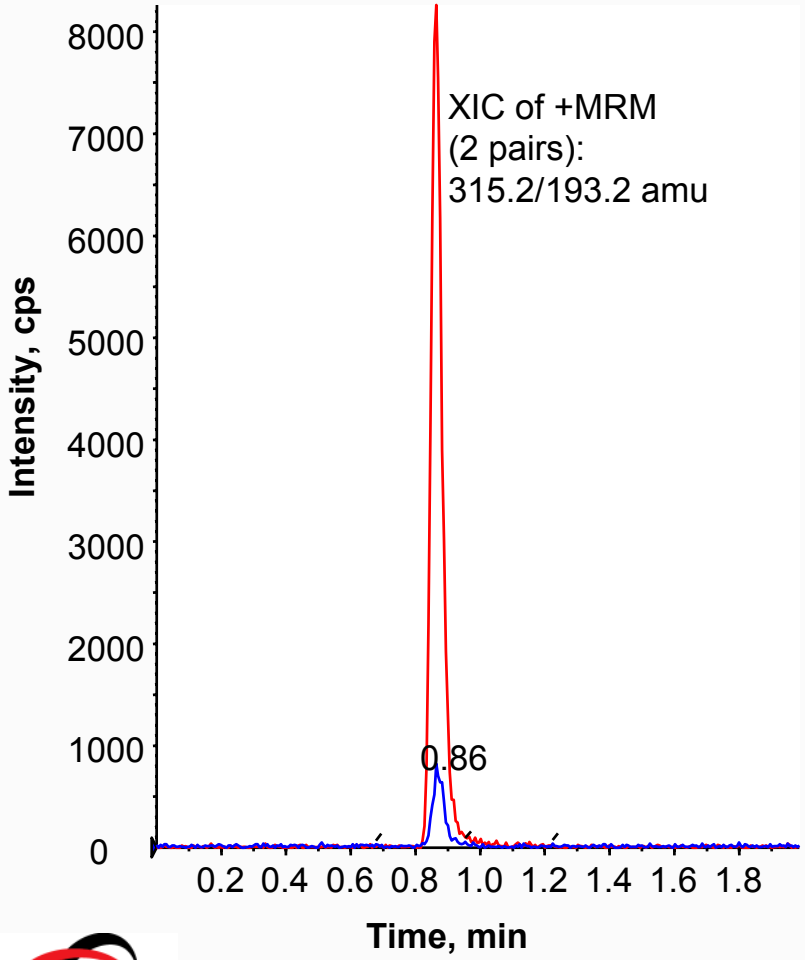
Mobile Phase: ACN/5mM Ammonium phosphate, pH 7.0

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
  - 80° 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

- All zirconia-based supports have surface chemistry that enables unique separations
- Even bonded zirconia-based phases are 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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