



Modified Zirconia Stationary Phases for the Analysis of Small Molecules in LC

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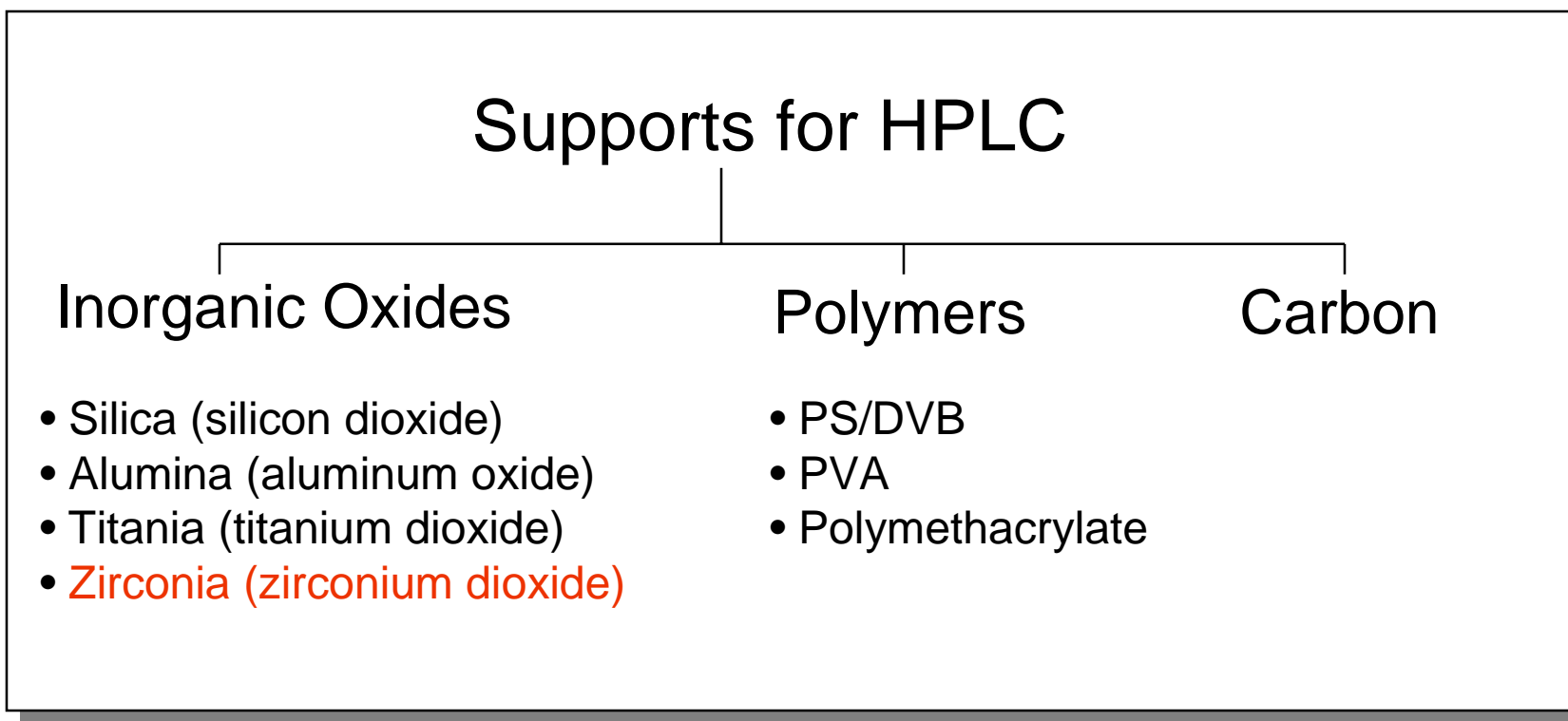


Introduction

- **The primary advantage of zirconia over either silica or polymeric stationary phases is its extreme chemical and thermal stability. Silica-based columns will typically exhibit short lifetimes if used at pH less than 2 or greater than 8 with inorganic buffers.**
- **Modified zirconia supports exhibit excellent stability over the entire pH range of 1-14 and temperatures up to 200°C, with very high mechanical strength, and high efficiencies.**
- **Four zirconia based phases are discussed. We demonstrate the utility of performing HPLC separations at pH extremes using these zirconia based columns. These columns operate based on both ion-exchange and hydrophobic mechanisms. This offers the analyst more possibilities in affecting selectivity and retention.**

What is Zirconia?

An alternate HPLC support to silica, polymers, and carbon.





Why Zirconia?

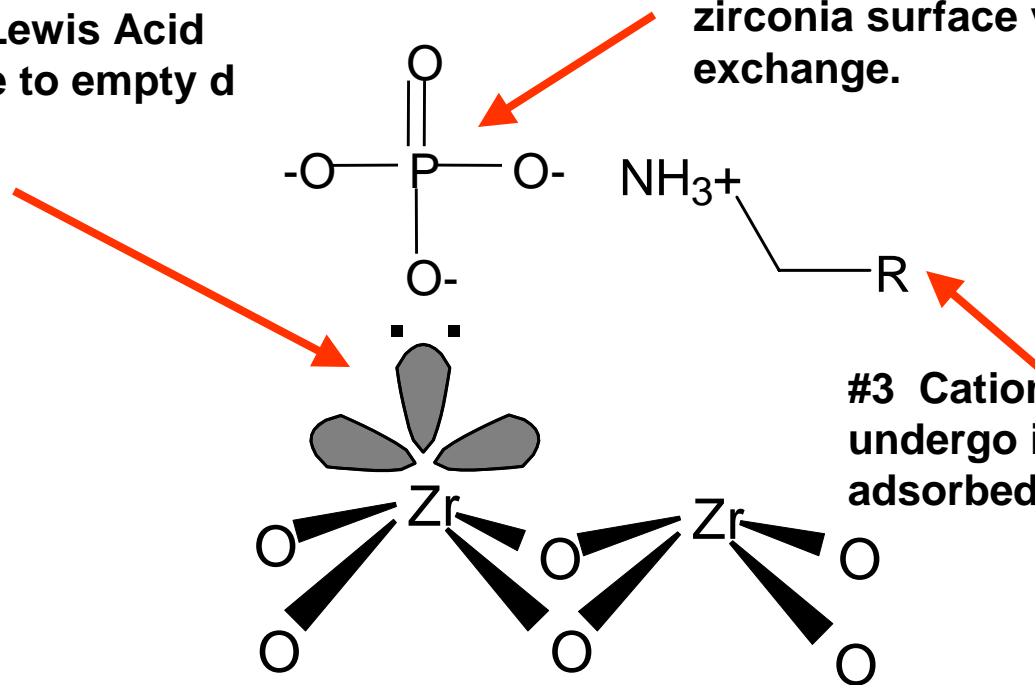
Four reasons:

1. Allows temperatures up to 200°C to reduce analysis time and/or decrease the amount of organic modifier.
2. Has selectivity and retention of ion-exchange in addition to reversed-phase.
3. Carbon clad Zirconia phases will exhibit shape selectivity towards geometric isomers.
4. Allows the full use of pH to alter selectivity and retention.

Zirconia Substrate Surface Chemistry

#1 Zr atom acts as Lewis Acid (e-pair acceptor) due to empty d orbitals.

#2 Lewis Base (e.g. phosphate) from mobile phase interacts with zirconia surface via ligand exchange.



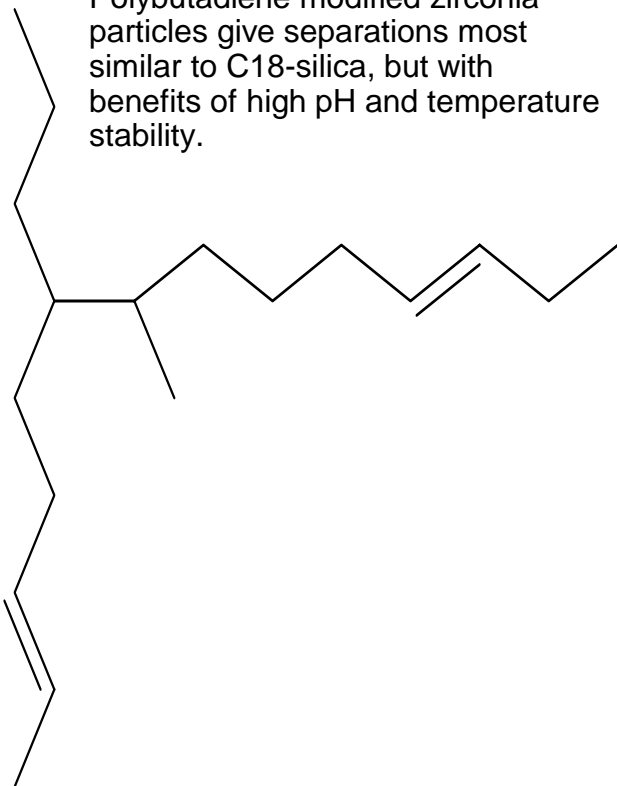
#3 Cationic analytes can undergo ion-exchange with adsorbed Lewis Base ions.

Polymer-Coated Discovery Zr Packings

Discovery Zr-PBD

Polybutadiene modified zirconia particles give separations most similar to C18-silica, but with benefits of high pH and temperature stability.

Zr Surface



Discovery Zr-PS

Polystyrene modified zirconia particles are ideal for separations of aromatic compounds and amines, with selectivity similar to Phenyl-silica or polymers based on polystyrene-DVB.

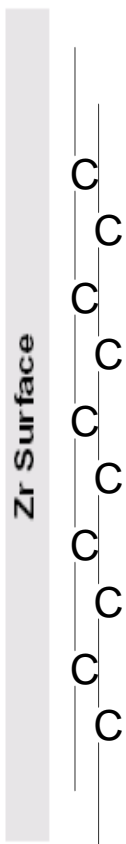


Stable from pH 1 – 13, and up to 150°C

Carbon-Clad Discovery Zr Packings

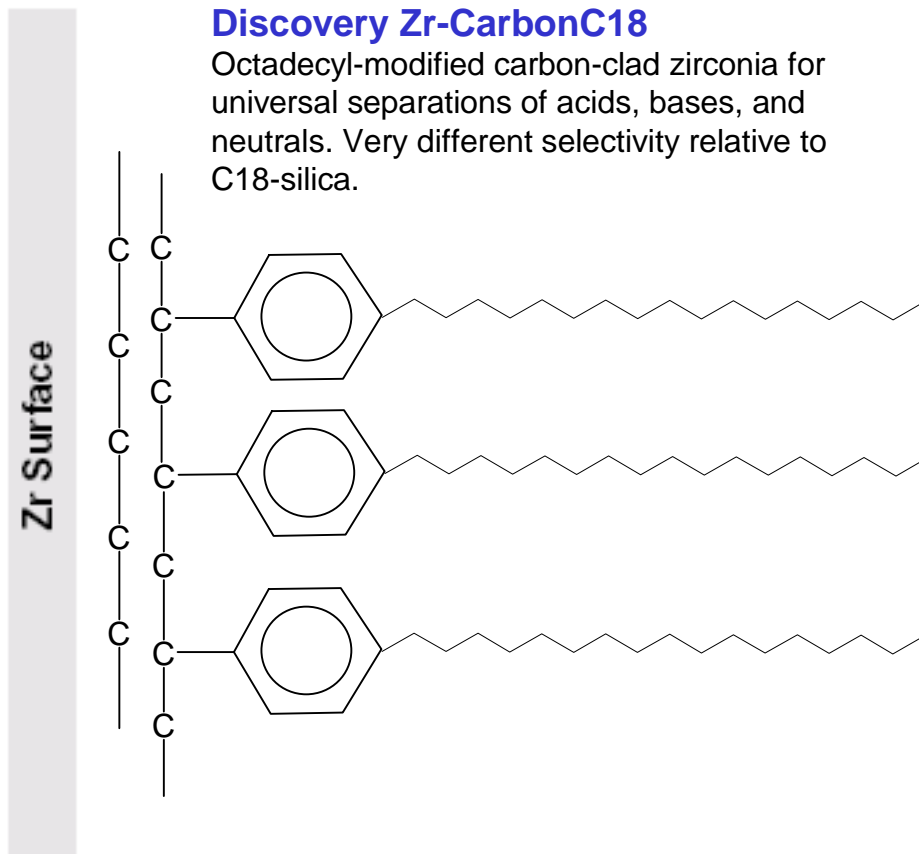
Discovery Zr-Carbon

Carbon-clad zirconia for separating geometric isomers and diastereomers.



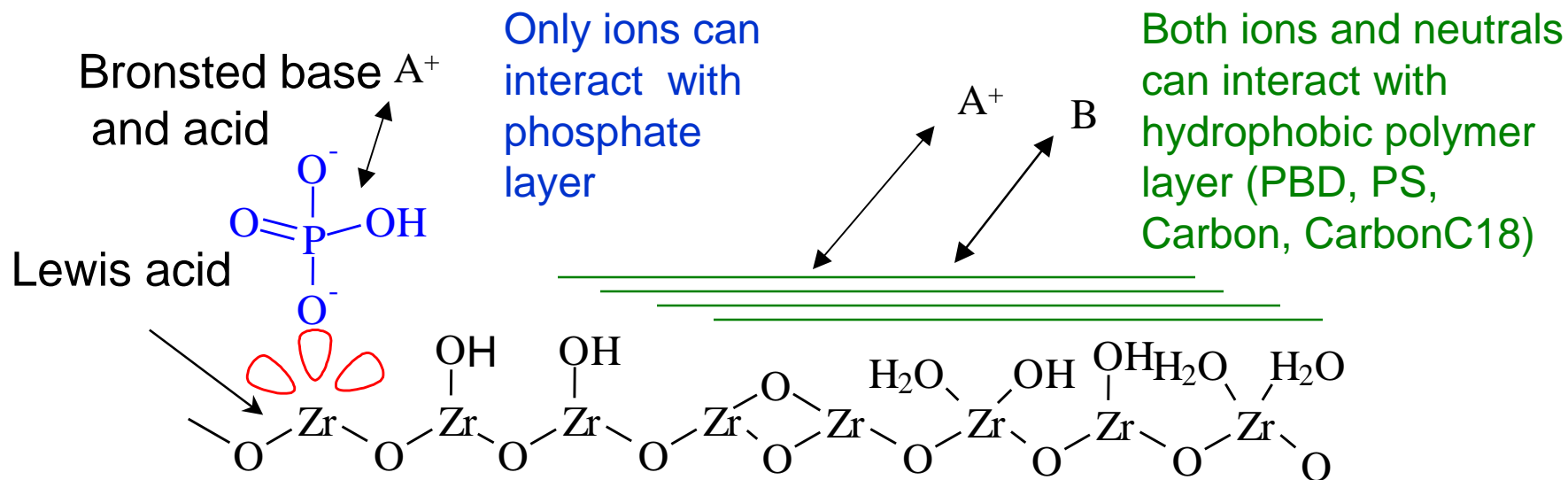
Discovery Zr-CarbonC18

Octadecyl-modified carbon-clad zirconia for universal separations of acids, bases, and neutrals. Very different selectivity relative to C18-silica.



Stable from pH 1 – 14, and up to 200°C

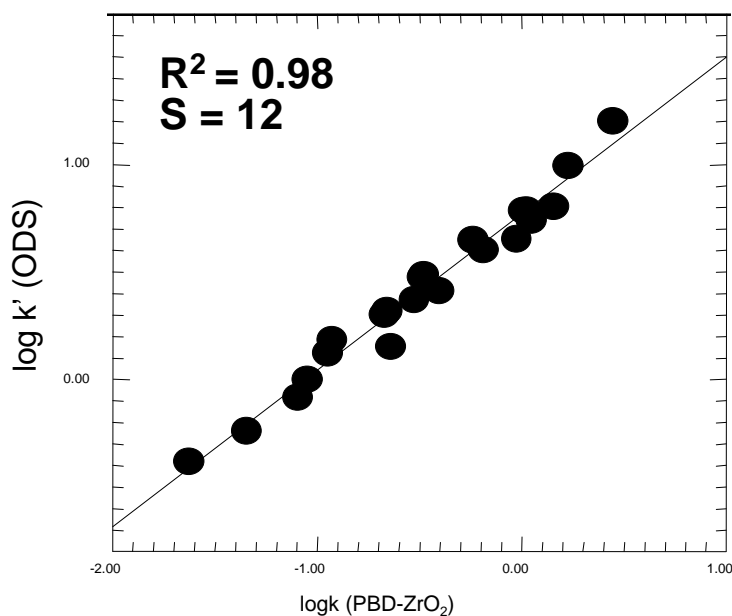
Origins of Unique Selectivity on Zirconia



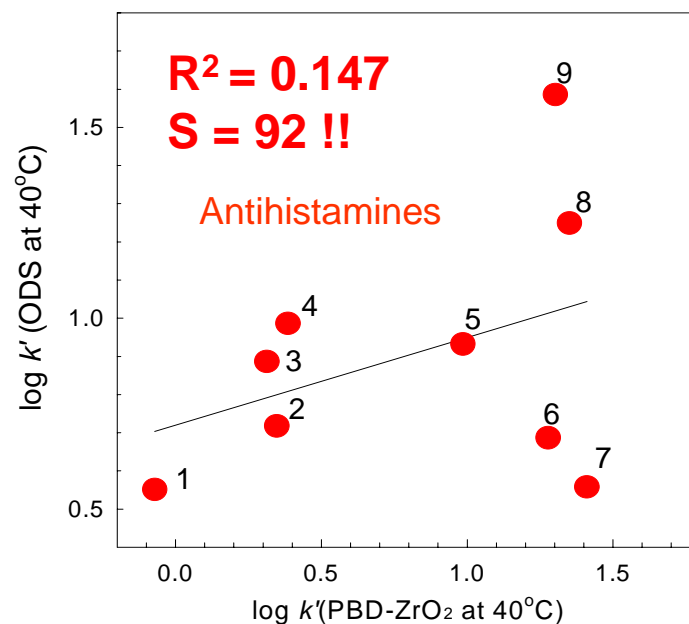
- Zirconia, as a transition metal oxide, has very rich, reproducible surface chemistry.
- Coated zirconia (Carbon and PBD) has mixed-mode surface properties (RPC and IEC) which allow simultaneous nonpolar and polar interaction.
- The retention of various basic and acidic analytes can be fine-tuned by changing pH and buffer or salt concentration; selectivity is also strongly affected by chemical nature of the mobile phase additive.

Impact of Mixed-Mode on Sample Types

Discovery Zr-PBD vs. C18-silica



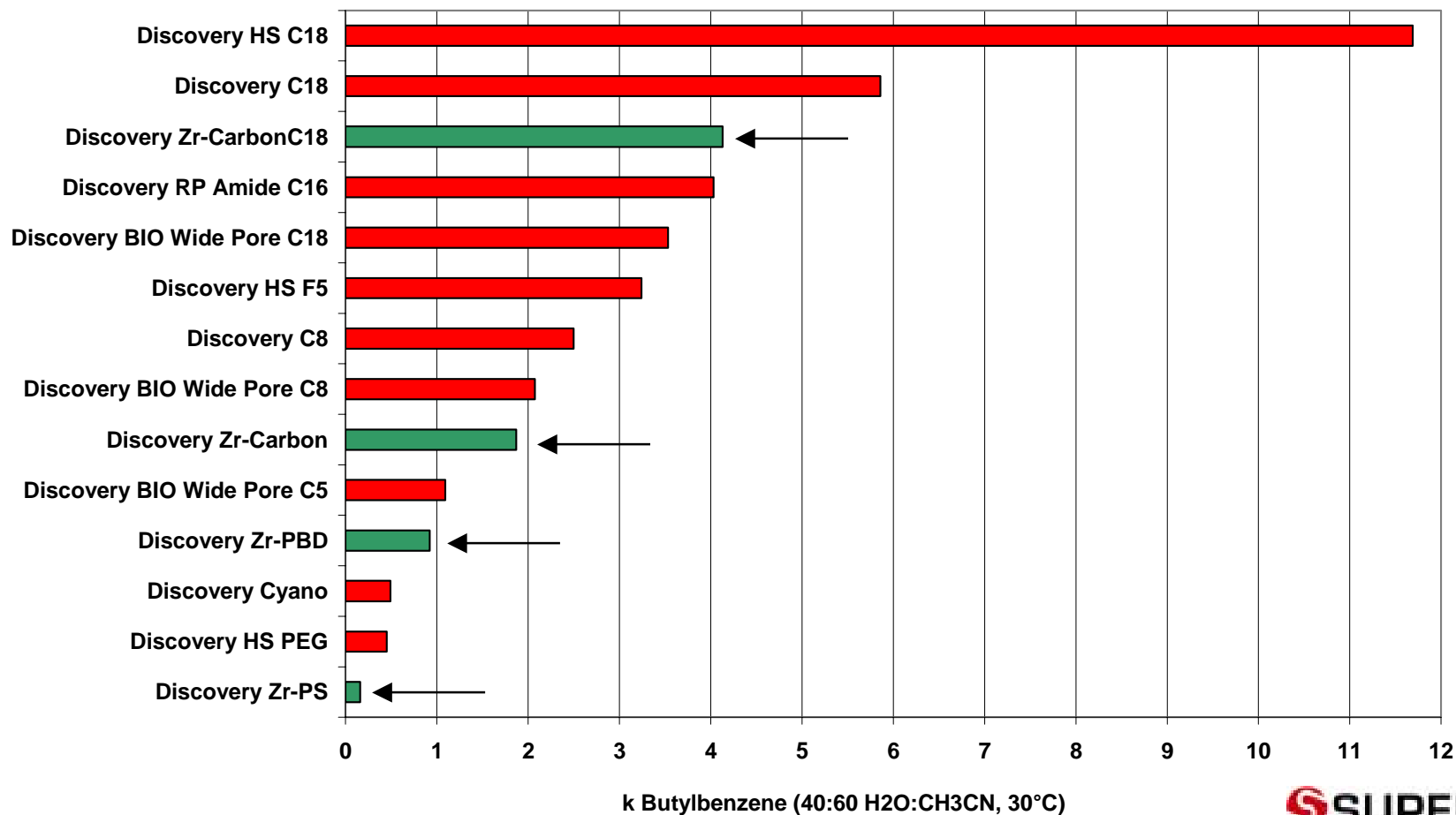
Nonionic solutes:
Columns are very similar



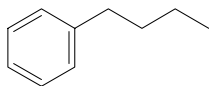
Ionic solutes:
Columns are very different

Hydrophobicity of Discovery RP Columns

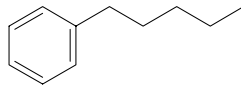
Discovery Zr phases are different from each other and from other Discovery RP columns.



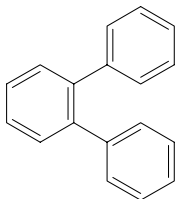
Hydrophobic Selectivity



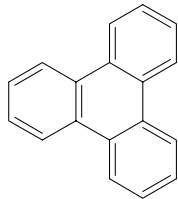
Butylbenzene



Pentylbenzene



o-Terphenyl

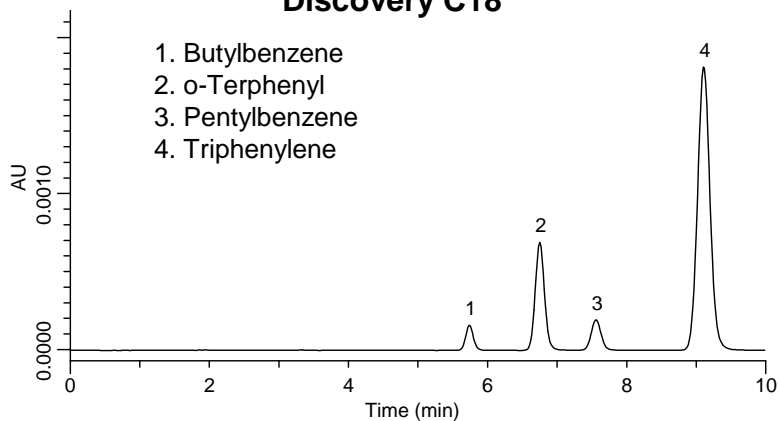


Triphenylene

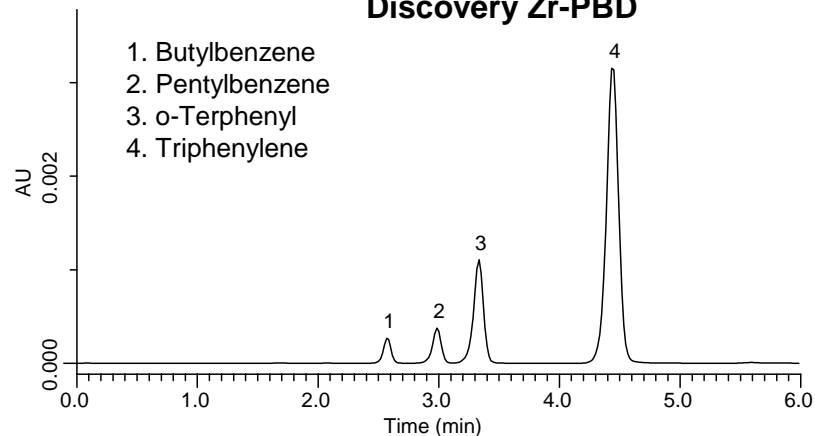
Conditions:

Column: 15cm x 4.6mm ID, 5 μ m
Mobile Phase: 20:80, water:methanol
Flow Rate: 1.0mL/min
Temperature: 40°C
Injection Volume: 5 μ L
Detection: UV, 254nm

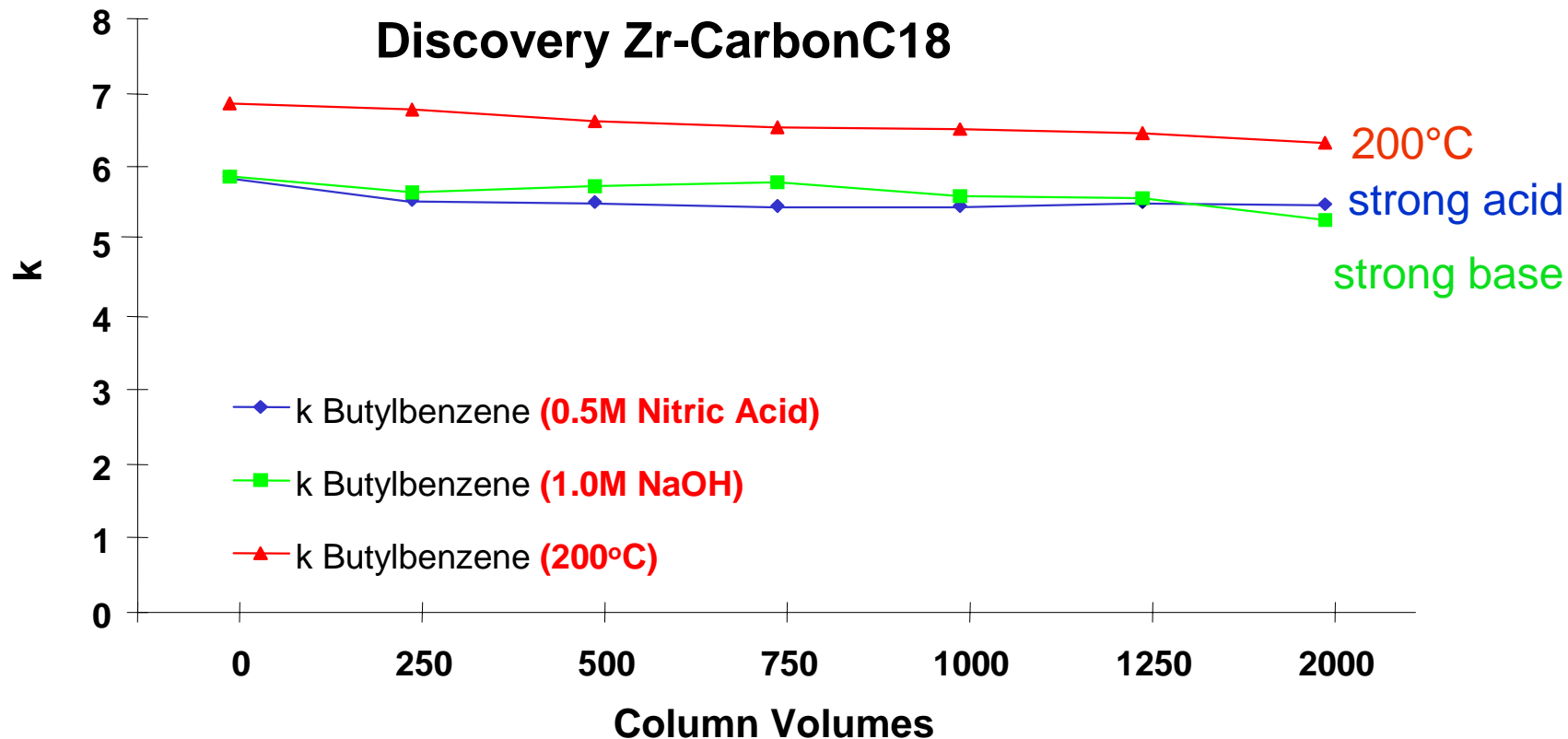
Discovery C18



Discovery Zr-PBD



Discovery Zr Column Stability Data-Extreme Temperature and pH



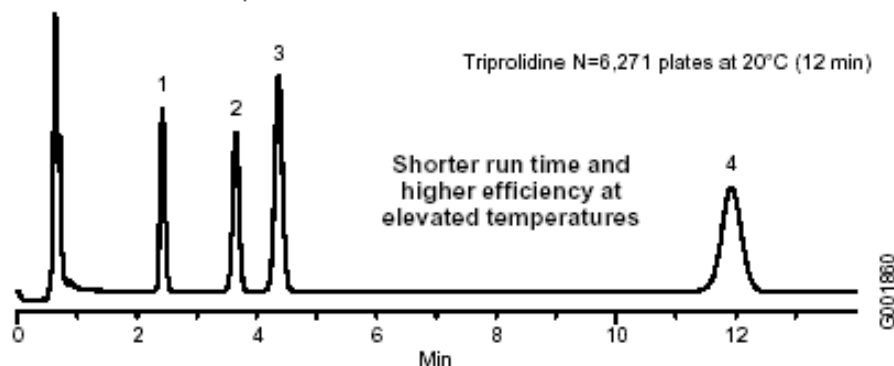
Conditions: Discovery Zr-CarbonC18, 5cm x 4.6mm ID, 5 μ m; 50% CH₃CN in water; 30°C; 1mL/min

Discovery Zr-PBD Application: Amines

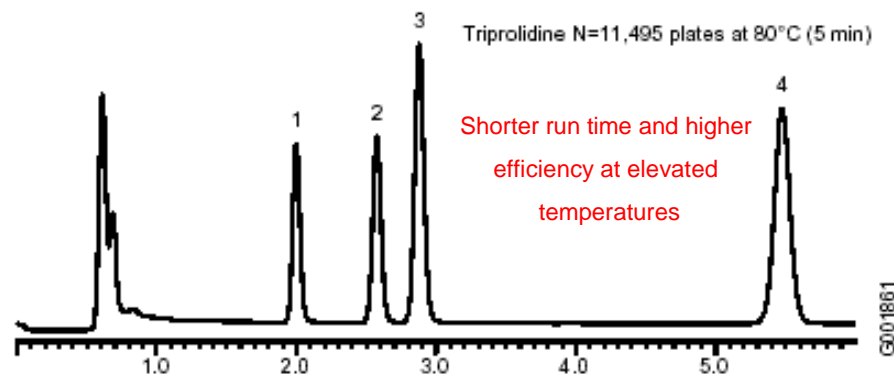
Column: Discovery Zr-PBD, 7.5cm x 4.6mm, 3 μ m
Cat. No.: 65717-U
Mobile Phase: (75:25) 50mM triethylammonium hydroxide (pH 12.6):CH₃CN
Flow Rate: 1mL/min
Det.: UV, 254nm
Temp.: 20°C or 80°C
Pressure: 130bar at 20°C
Inj.: 1 μ L
Sample: Doxylamine, methapyrilene, chlorpheniramine (1 μ g/mL),
triprolidine (2 μ g/mL)

1. Doxylamine
2. Methapyrilene
3. Chlorpheniramine
4. Triprolidine

pH 12.6
20°C



pH 12.6
80°C

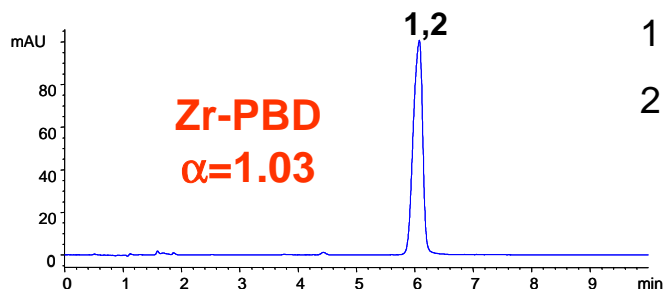


Discovery Zr Carbon is Shape-Selective

..... for nonionic solutes

Discovery Zr-PBD, 10cm x 4.6mm ID

Mobile Phase: 35/65 A/B
A: ACN
B: Water
Flow Rate: 1.0mL/min
Temperature: 30°C
Injection Volume: 5µL
Detection: 254nm

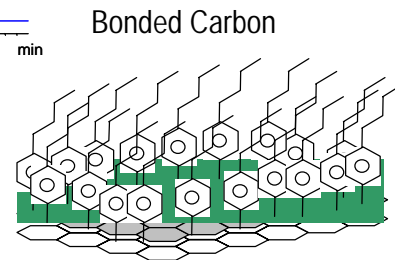
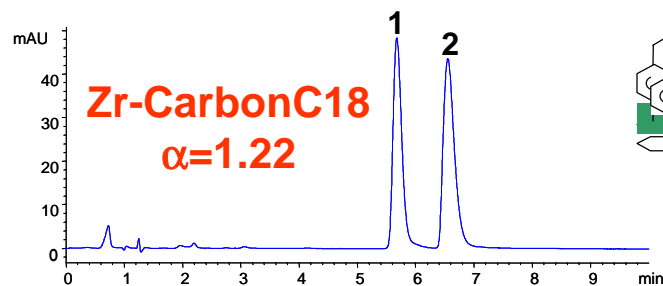


Isomers:

1. Ethylbenzene
2. p-Xylene

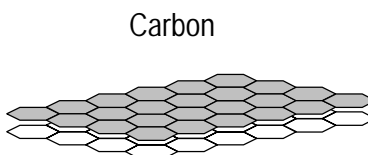
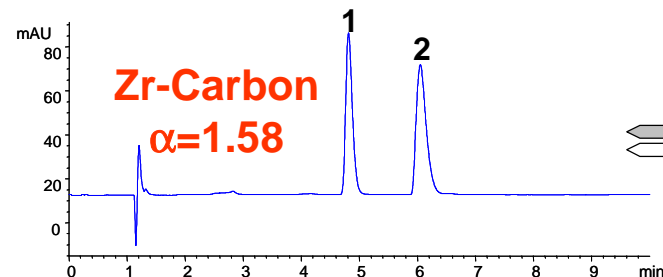
Discovery Zr-CarbonC18, 10cm x 4.6mm ID

Mobile Phase: 37.5/5/57.5 A/B/C
A: ACN
B: THF
C: Water
Flow Rate: 1.0mL/min
Temperature: 60°C
Injection Volume: 5µL
Detection: 254nm



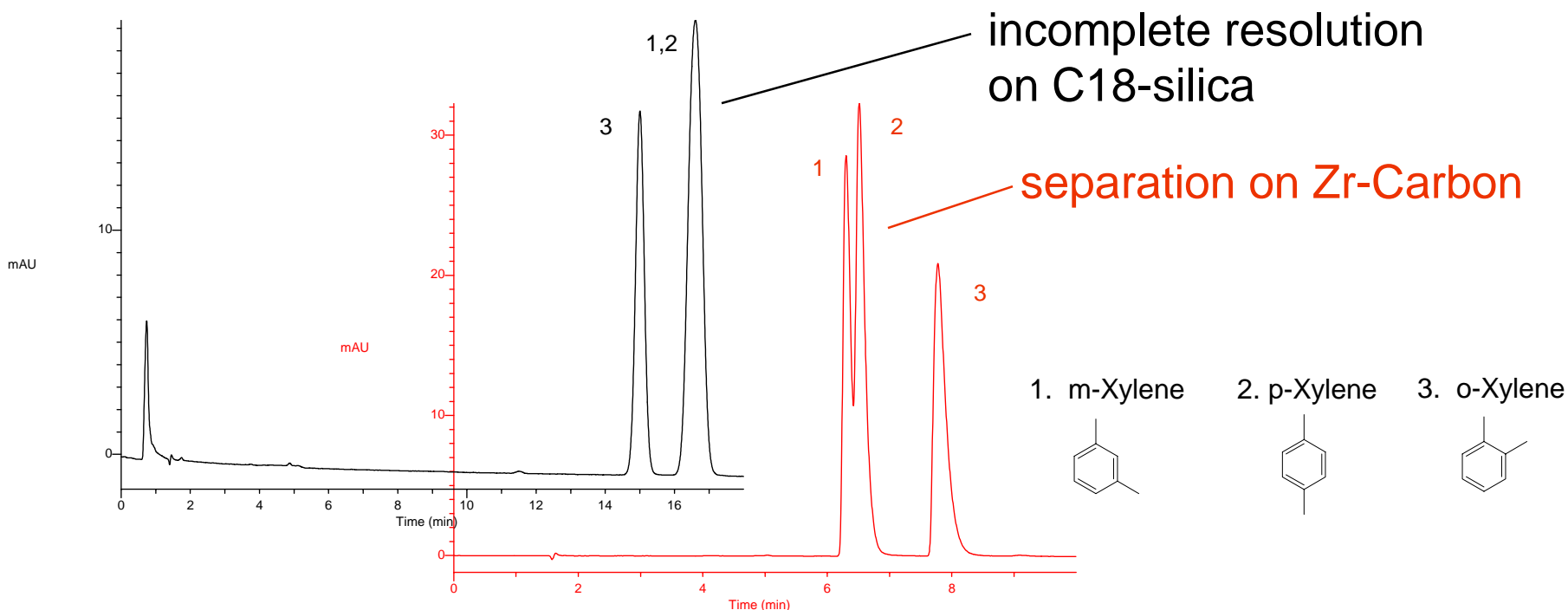
Discovery Zr-Carbon, 10cm x 4.6mm ID

Mobile Phase: 32.5/67.5 A/B
A: ACN
B: Water
Flow Rate: 1.0mL/min
Temperature: 60°C
Injection Volume: 5µL
Detection: 254nm



Structurally Similar Compounds

Unlike a C18-silica, the rigid Zr-Carbon surface can distinguish compounds with the same hydrophobicity, but different molecular shape.

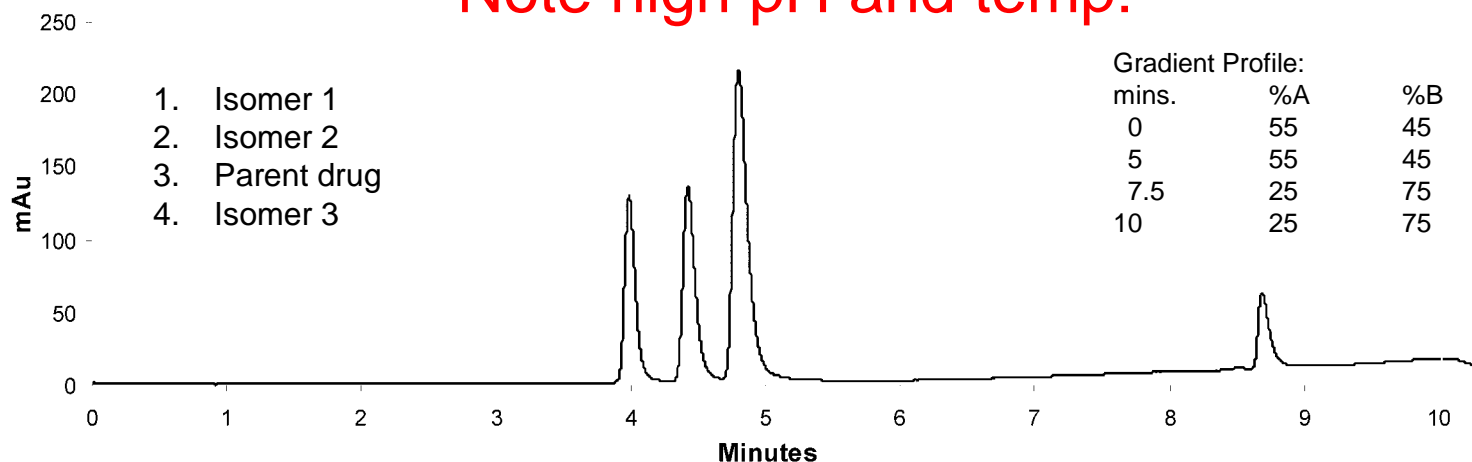


Conditions: Discovery Zr-PBD or Zr-Carbon, 15cm x 4.6mm ID, 3 μ m particles; 80% CH₃CN in water; 60°C, 0.5mL/min; UV 254nm; 10 μ L each compound 870 μ g/mL.

Positional Isomers of Sulfonamide Drug

Shape-selective surface of Zr-CarbonC18 allows separation of positional isomers of a proprietary sulfonamide drug.

Note high pH and temp:



Conditions: Discovery Zr-CarbonC18, 15cm x 4.6mm ID, 3 μ m particles; (A) 10mM diethylamine (pH 10.8); (B) CH₃CN; 80°C, 1.5mL/min; UV 240nm; 5 μ L injection.



Conclusion

- 1. Zirconia based phases offer different selectivity and retention compared to silica.**
- 2. Retention is based on the Lewis Acid/Base concept in addition to hydrophobic interactions.**
- 3. Zirconia based phases can be used at pH and temperature extremes. Materials are stable and durable.**
- 4. Zr-Carbon exhibits shape selectivity.**
- 5. Zr-Carbon C18 combines shape selectivity of carbon surface with hydrophobic character of C18.**