



Comparison of Zirconia- and Silica-Based Stationary Phases for the Retention and Selectivity of Pharmaceutically Relevant Analytes

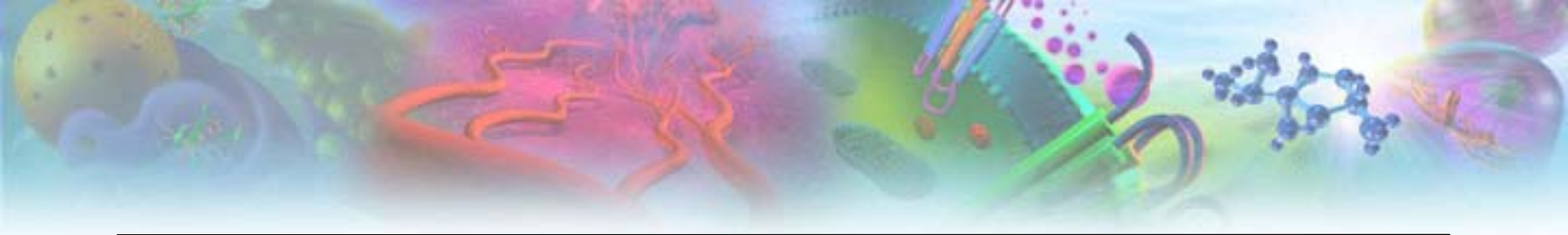
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Abstract

Silica-based stationary phases remain the workhorse for high-performance liquid chromatography (HPLC) analyses; however, systems based on modified zirconia phases are fast becoming a popular alternative. The interest in zirconia columns stems from their ability to withstand extreme pH and temperature conditions as well as for their offering of unique selectivity and retention for various classes of compounds. Several modified zirconia phases are commercially available including polybutadiene (PBD), polystyrene (PS), carbon and C18-modified carbon (carbonC18).



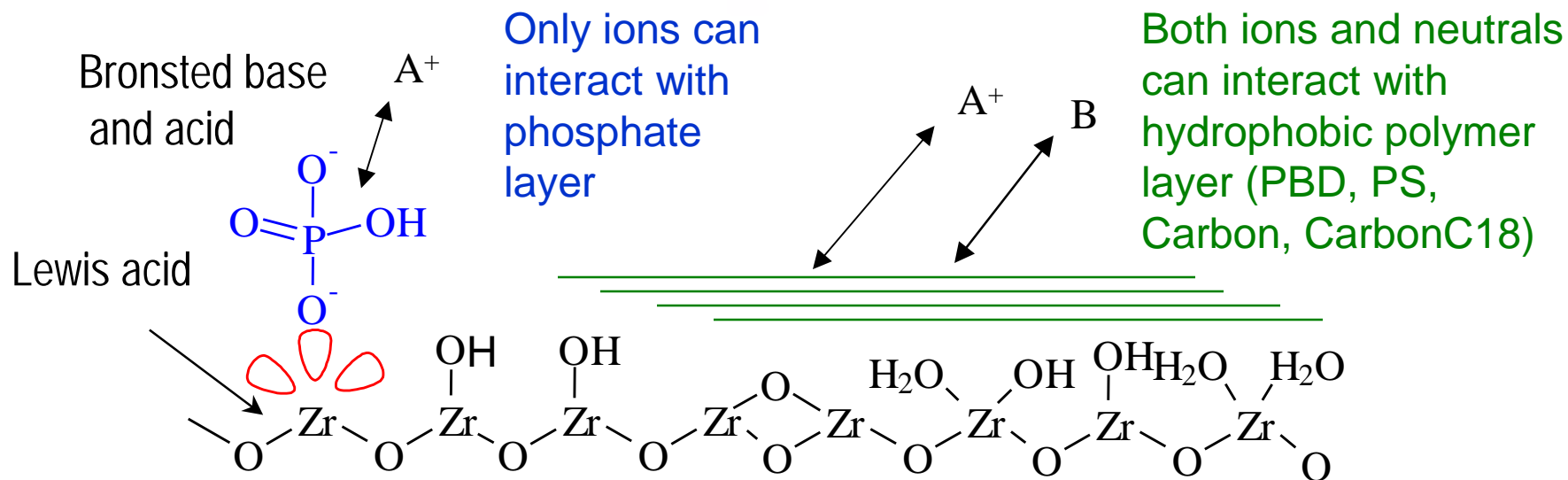
This study aims to assess the growing array of zirconia-based HPLC columns through comparative analysis with traditional silica-based stationary phases. The analytes chosen for this comparison represent several classes of pharmaceutically relevant analytes including acids, bases and neutrals. The advantages and disadvantages of utilizing zirconia-based phases will be discussed in terms of retention and selectivity. In addition, recommendations for method development strategies for the various classes of analytes will be introduced.



Introduction

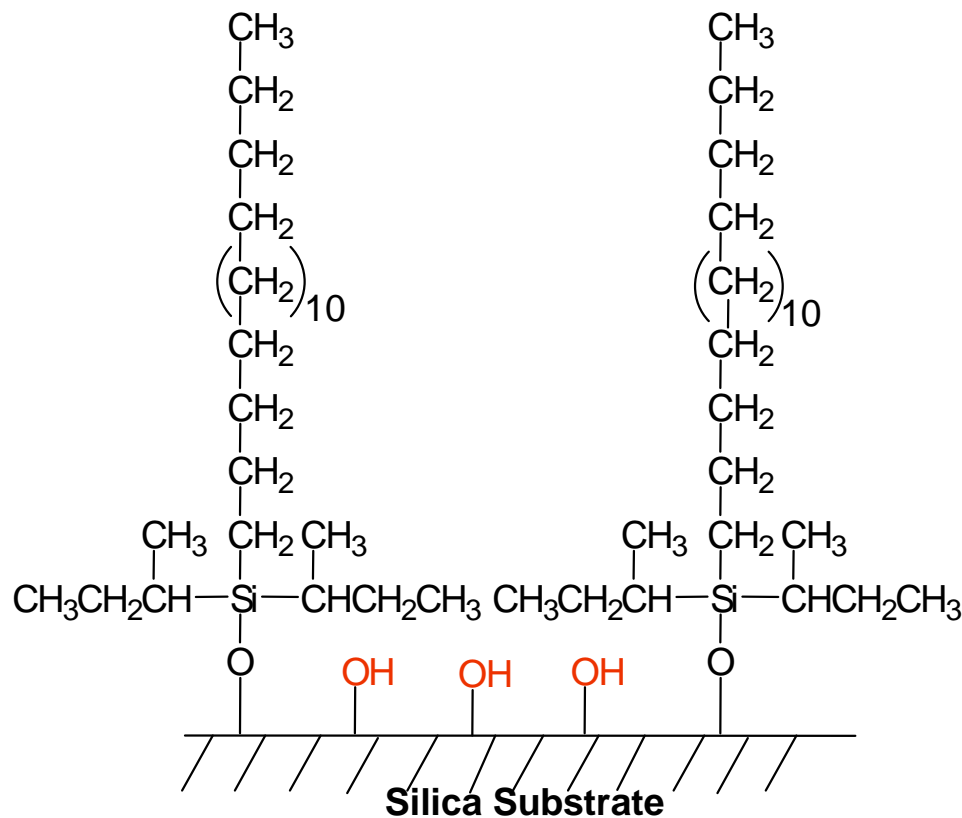
- **Methods based on modified zirconia phases are fast becoming a popular alternative to silica-based columns in traditional high-performance liquid chromatography (HPLC)**
- **In order to fully utilize these Zr phases for analyses, a firm understanding of the underlying interactions is paramount**
- **This study was aimed at:**
 - Comparing the retention and selectivity for traditional C18 columns and a Zirconia-based polybutadiene (Discovery Zr-PBD)
 - Assessing the advantages and disadvantages of the Zr phase compared to C18
 - Developing usable scenarios for method development on Zr phases

Origins of Unique Selectivity on Zirconia



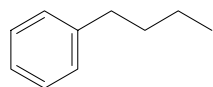
- Zirconia, as a transition metal oxide, has very rich, reproducible surface chemistry.
- Coated zirconia (Carbon and PBD) has mixed-mode surface properties (RPC and IEC) which allow simultaneous nonpolar and polar interaction.
- The retention of various basic and acidic analytes can be fine-tuned by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

Origins of Unique Selectivity on Zirconia

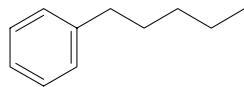


- Strong efforts have been made by the silica-based column manufacturers to mask the effects of silanols.
- There is an increasing need for columns which have different selectivity than silica-based bonded phases.

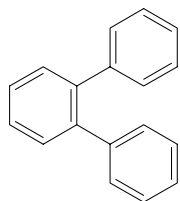
Experimental Neutral Analyte Selectivity



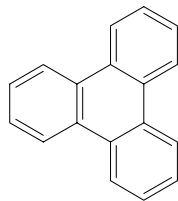
Butylbenzene



Pentylbenzene



o-Terphenyl

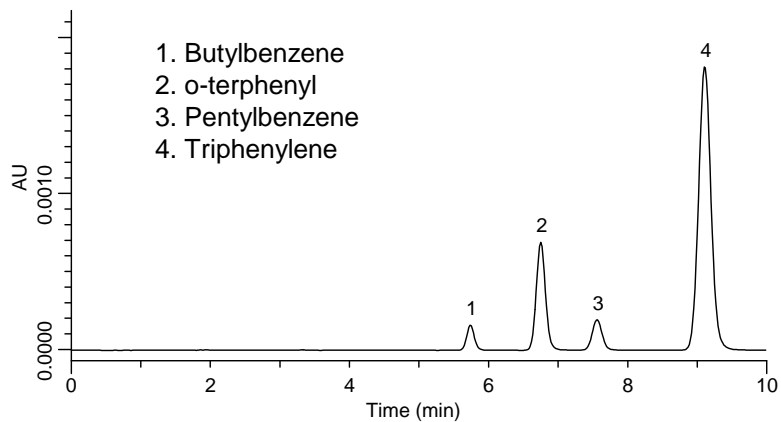


Triphenylene

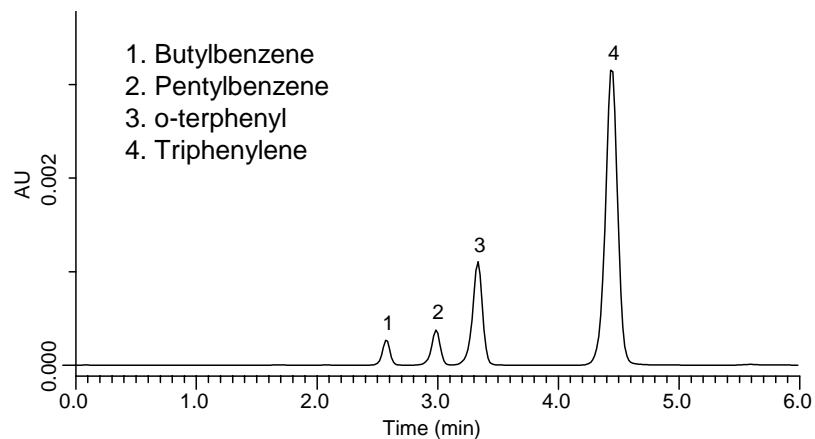
Conditions:

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

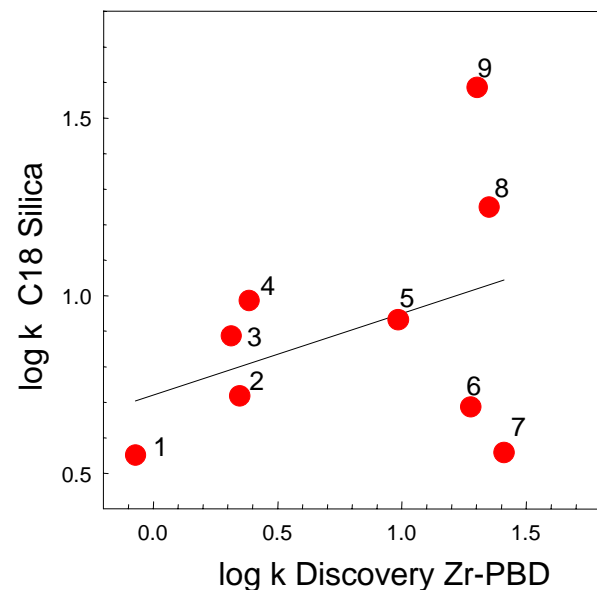
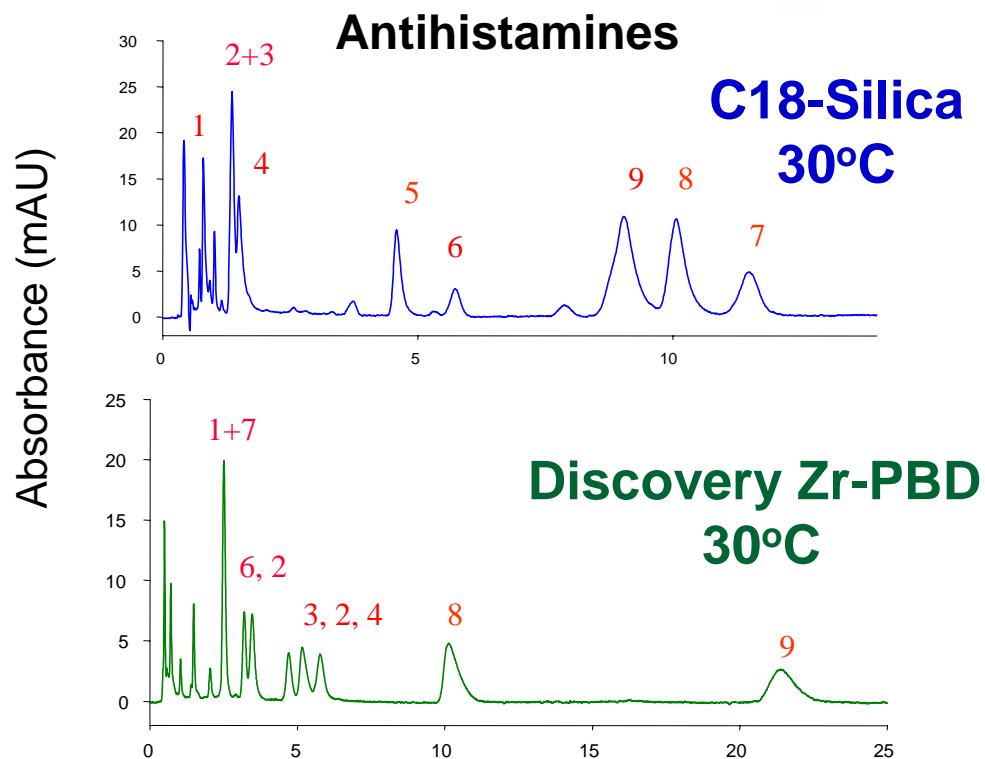
Discovery C18



Discovery Zr-PBD



Ionic Solute Selectivity

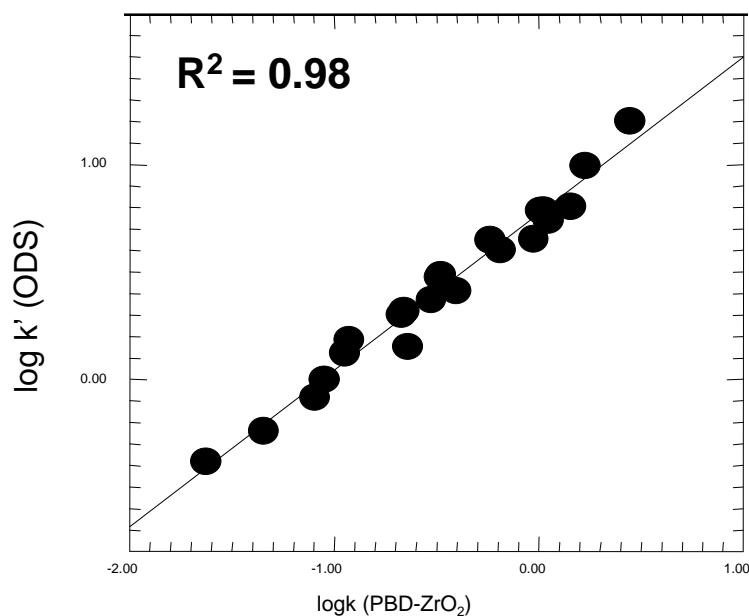


Mobile Phase: 40% CH₃CN in 25 mM phosphate (pH 7)

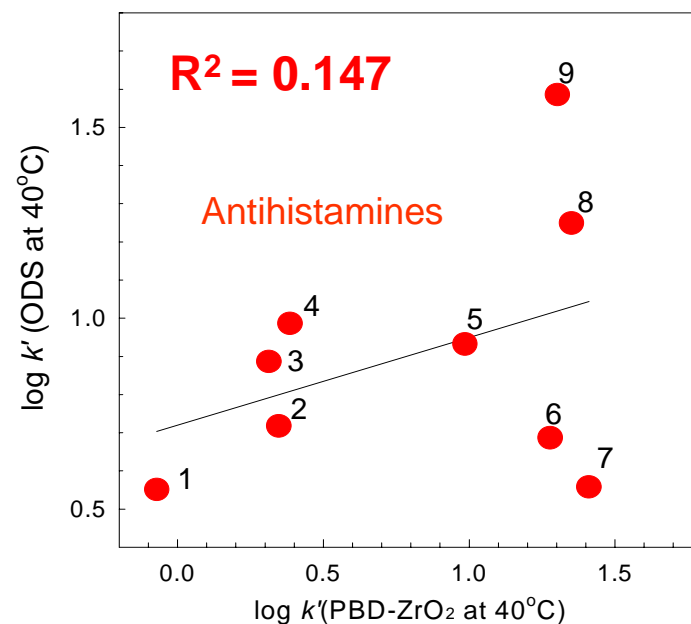
The selectivity of zirconia-based RP columns towards **ionizable** compounds becomes very different from that of traditional silica RP columns. Buffer type and pH have a large effect on Discovery Zr's **mixed-mode retention** (RP and IEX).

Impact of Mixed-Mode on Sample Types

Discovery Zr-PBD vs. C18-silica



Nonionic solutes:
Columns are very similar

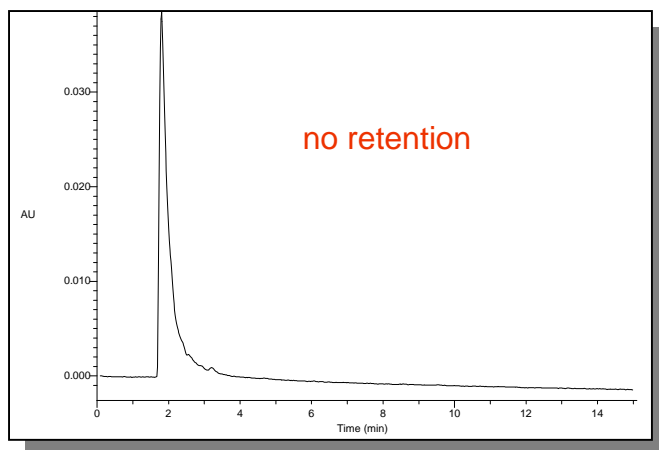


Ionic solutes:
Columns are very different

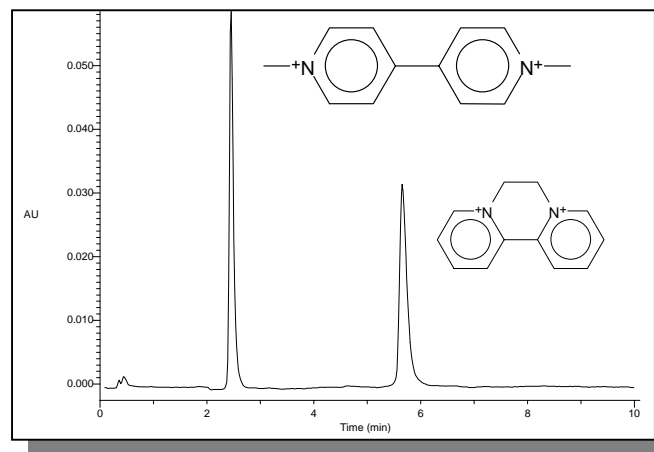
Utilization of Ion-Exchange on Zirconia

Quaternary amines paraquat and diquat are retained and resolved on Zr-PS because of the mixed-mode RP and ion-exchange.

C18-silica:
reversed-phase only



Zirconia:
reversed-phase and ion-exchange

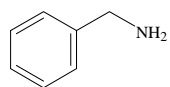


Separation of paraquat and diquat on C18-silica vs. Discovery Zr-PS

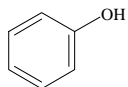
C18-silica conditions: Discovery C18, 15cm x 4.6mm ID, 3 μ m particles; 5% CH₃CN in 25mM H₃PO₄ (to pH 7 with NH₄OH); 35°C, 1mL/min, UV 290nm.

Zr-PS conditions: Discovery Zr-PS, 7.5cm x 4.6mm ID, 3 μ m particles; 50% CH₃CN in 25mM H₃PO₄, 25mM NH₄F, (to pH 8 with NH₄OH); 65°C, 3mL/min, UV 290nm.

Complex Sample Selectivity



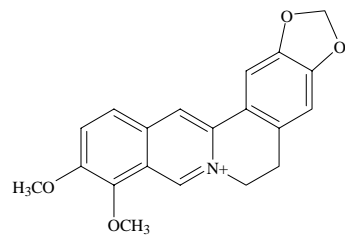
Benzylamine



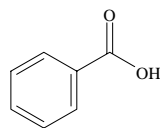
Phenol



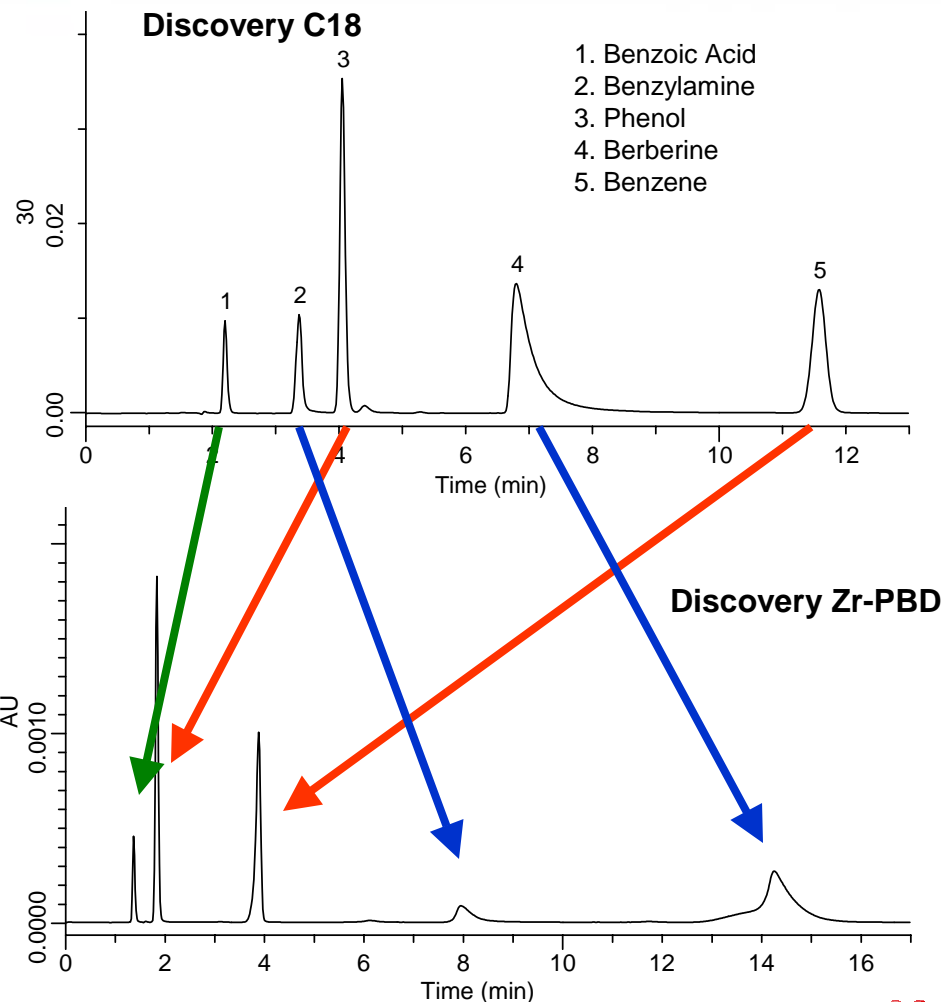
Benzene



Berberine



Benzoic Acid



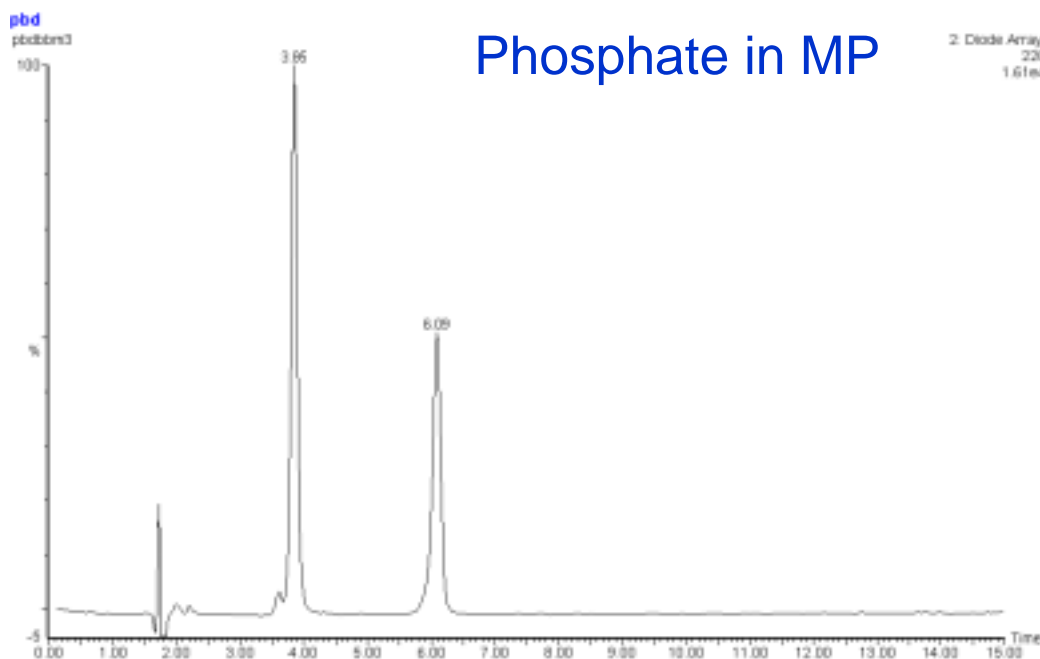
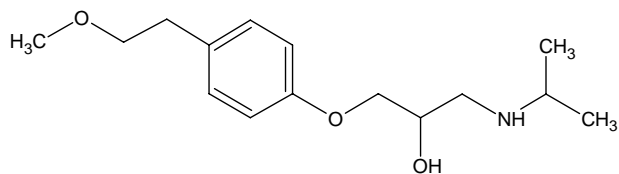
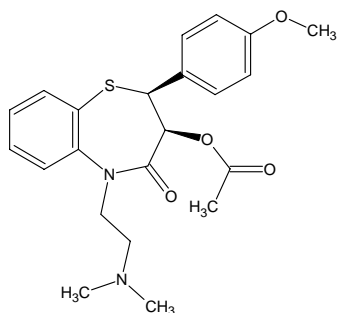
Column: 15cm x 4.6mm ID, 5 μ m
Mobile Phase: 60:40 water (20mM K₂HPO₄, pH 7.6 with H₃PO₄):methanol
Flow Rate: 1.0mL/min
Temperature: 40°C
Injection Volume: 5 μ L
Detection: UV, 254nm

Effect of Phosphate Buffer on Discovery Zr-PBD Retention of Basic Analytes

Conditions:

Column: Discovery Zr-PBD, 15cm x 4.6mm ID, 5 μ m particles
Mobile Phase: (70:30) 25mM potassium phosphate, pH 3.0 :CH₃CN
Flow Rate: 1.0mL/min
Det.: UV, 220nm
Temp.: 35°C
Inj.: 10 μ L
Sample: 25 μ g/mL diltiazem, metoprolol in (50:50)
25mM potassium phosphate,
pH 3.0 ;acetonitrile

- Good peak shape, selectivity and retention

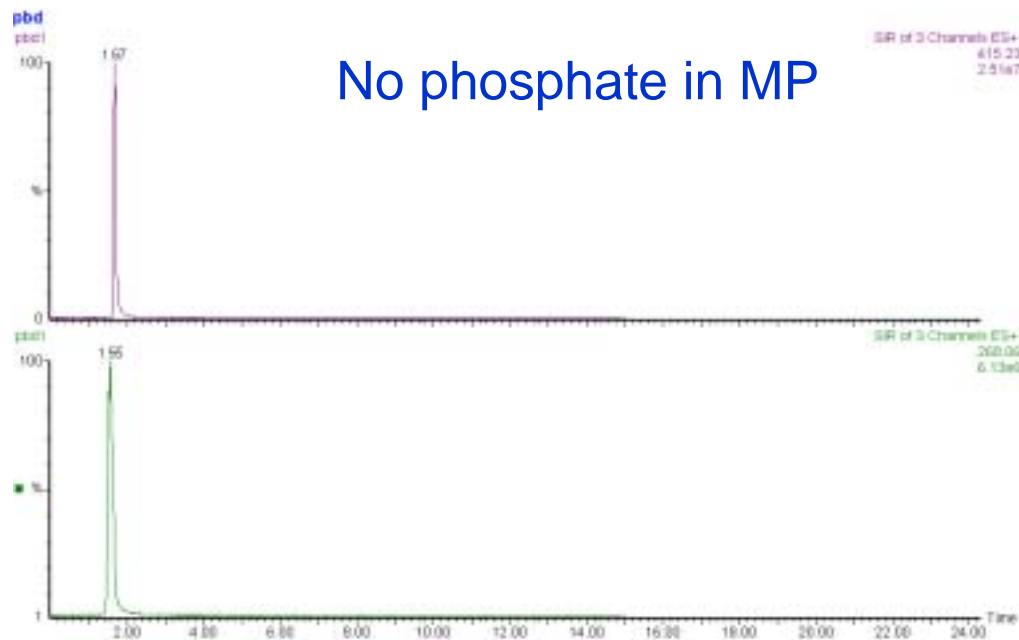
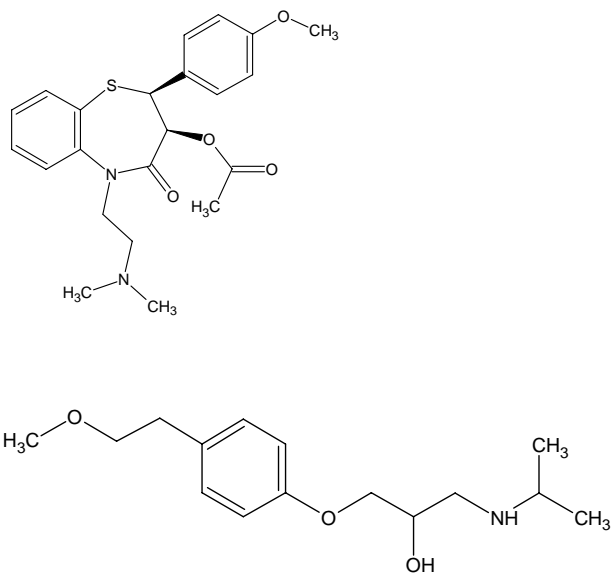


Effect of Phosphate Buffer on Discovery Zr-PBD Retention of Basic Analytes

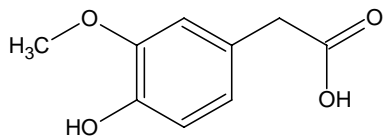
Conditions:

Column: Discovery Zr-PBD, 5cm x 2.1mm ID, 3 μ m particles
Mobile Phase: (60:40) 10mM ammonium acetate, unadjusted :CH₃CN
Flow Rate: 0.2mL/min
Det.: ms, esi (+)
Temp.: 40°C
Inj.: 5 μ L
Sample: 1 μ g/mL diltiazem, metoprolol in (60:40) water;acetonitrile

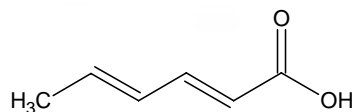
•Lack of retention



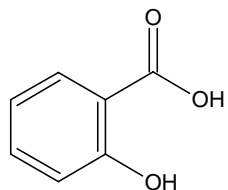
Acidic Compounds on Discovery Zr-PBD



homovanillic acid



sorbic acid

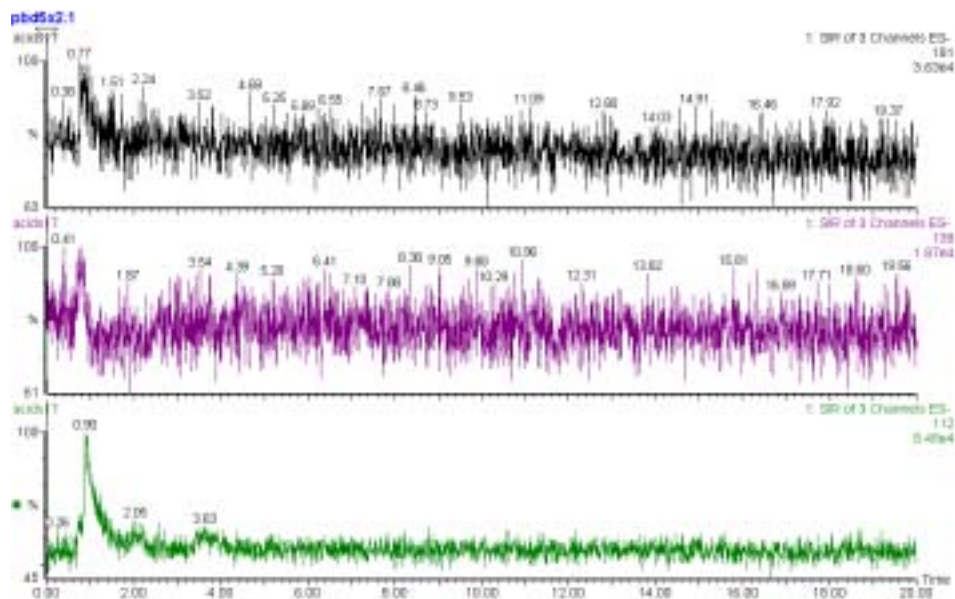


salicylic acid

- No elution – Lewis base activity

Conditions:

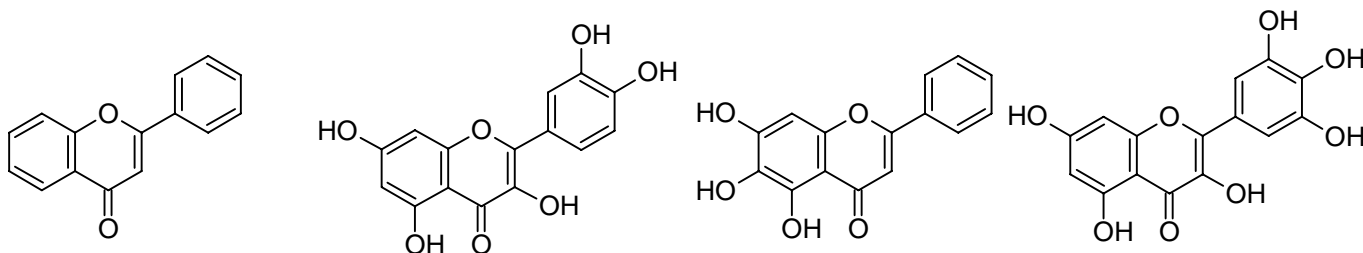
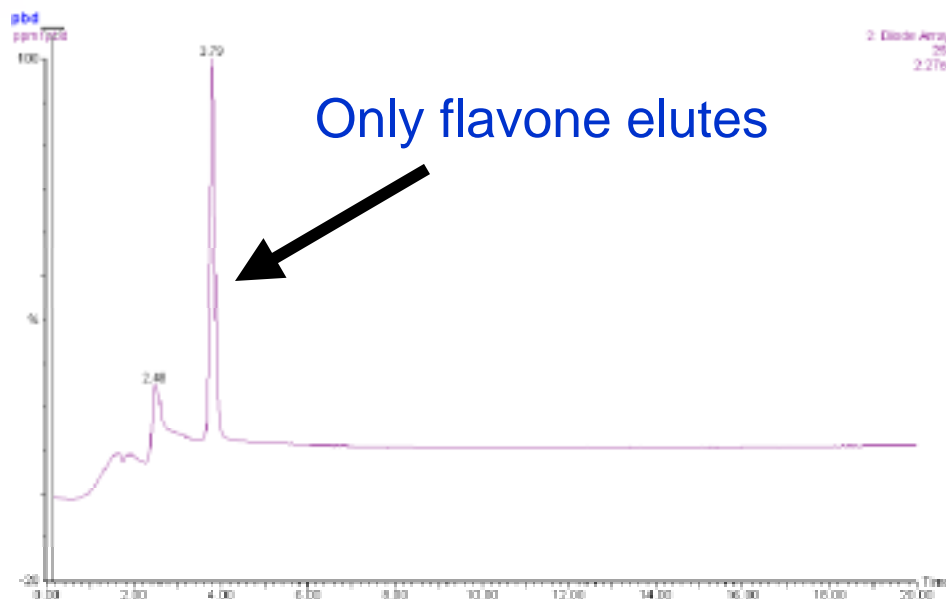
Column: Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
Mobile Phase: (95:5) 10mM ammonium formate, pH 3.50 :CH3CN
Flow Rate: 0.2mL/min
Det.: ms, esi (-)
Temp.: 40°C
Inj.: 10µL
Sample: 1µg/mL each in (50:50) water;methanol



Chelators on Discovery Zr-PBD

Conditions:

Column: Discovery Zr-PBD, 15cm x 4.6mm ID,
5µm particles
Cat. No.: 65723-U
Mobile Phase: 45:55, 0.1% formic acid in water: 0.1% formic acid in CH₃OH
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10µL
Sample: as indicated below (25µg/mL in 0.1% formic acid in water)
Flavone
Quercetin
Baicalein
Myricetin
Luteolin





Discussion

- **Zirconia-based stationary phases provide unique selectivity toward acidic, basic and neutral analytes**
- **The unique selectivity arises from alternative analyte-stationary phase interactions**
- **The combination of ion-exchange and partition mechanisms results in very different chromatography**



Discussion (cont'd.)

- **Significant Lewis acid character exists on zirconia phases**
 - Analytes that are Lewis bases (carboxylic acids, for example) tend to interact strongly with the Lewis acid support
 - Buffers play an additional role in the chromatography compared to silica-based systems
 - Phosphate is responsible for providing the negative charge on the Zr surface resulting in significant ion-exchange capabilities
 - Retention of bases is shown to be minimal without the addition of phosphate
 - Phosphate can act as a Lewis acid/base mediator for acidic analytes
 - Chelators also tend to strongly interact with the Zr surface



Summary

- Typical RP partition interactions are observed for neutral analytes
 - **Alternative selectivity is often observed**
 - **No special attention to MP buffer component is required**
- Ion-exchange and partition interactions are observed for basic analytes
 - **Provides significant selectivity differences**
 - **Is dependent on the chosen buffer system**
 - **Phosphate buffers induce ionic interactions with basic analytes and can also mask any unwanted Lewis acid/base interactions**
- Acidic analytes, due to their Lewis base character, interact strongly with the Lewis acid character of the Zr support
 - **Lewis base mobile phase modifiers are therefore required**