Zirconia-Based Phases as a Powerful Complement to Silica-Based Phases for LC and LC-MS under Non-extreme Mobile Phase Conditions

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Abstract

Highly stable zirconia-based HPLC stationary phases have important advantages over other HPLC phases when column operation at elevated temperature or extreme pH is desired. The unique selectivity of polymer-coated zirconia phases may also be exploited in certain cases at near-ambient temperature with similar mobile phase pH and composition to that employed for popular silica phases.

This paper will briefly describe the origins of unique selectivity for Discovery® Zr phases and compare performance to Discovery® silica phases for a selection of compounds under non-extreme operating conditions.

Importance of LC/MS

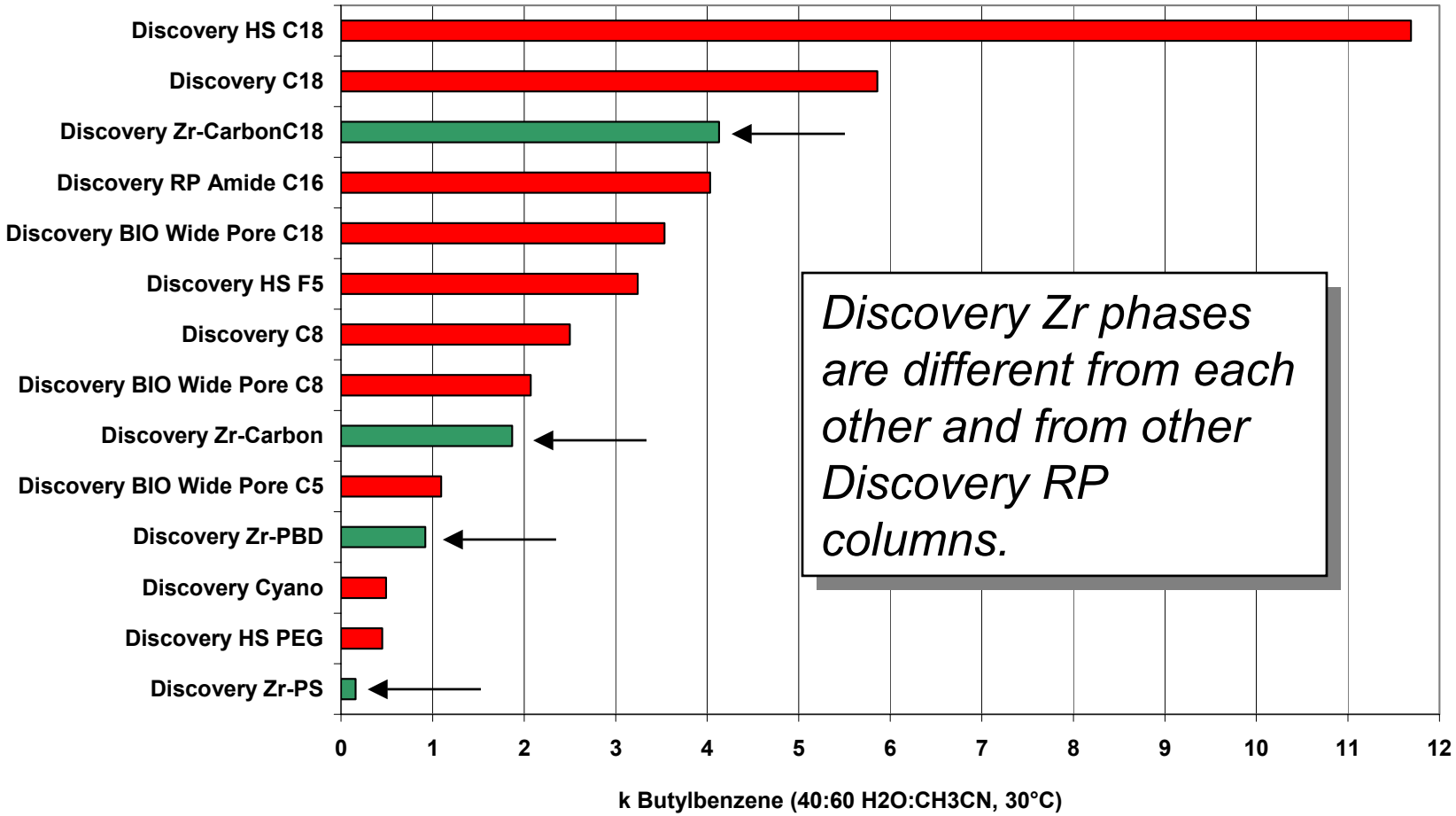
LC-MS with C8 and C18 silica-based columns has become extremely popular; however, there is still a need for stable, efficient columns that show different selectivity than silica packings with pure alkyl stationary phases.

While silica packings with polar phases fulfill some of the need for different selectivity, they can show excessive bleed and high background ionization in LC-ESI-MS even under non-extreme mobile phase conditions. A study of the utility of several zirconia phases for LC-ESI-MS under normal (non-extreme) operating conditions will be included.

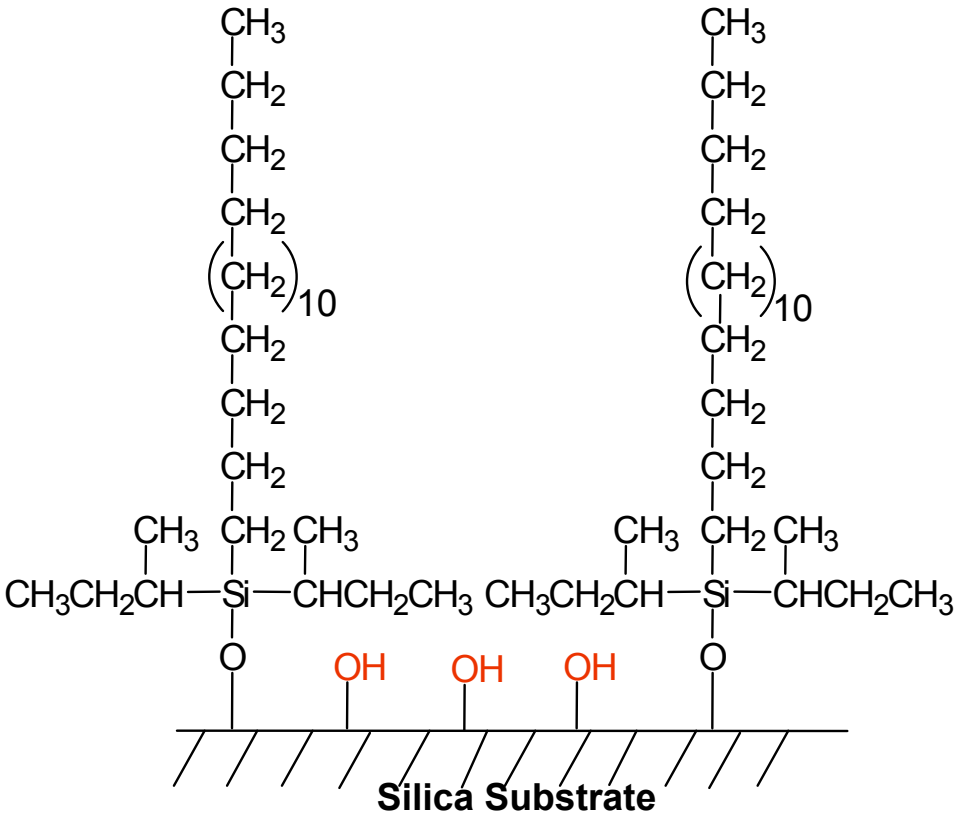
Background

- Stationary phases based on silica supports remain the workhorse for liquid chromatography (LC) and LC/MS.
- Zirconia phases with phosphate buffers are becoming a popular alternative to silica for LC analyses which employ UV detection. Important advantages include:
  - ability to withstand extreme pH and temperature conditions
  - unique selectivity and retention for various classes of compounds (focus of this paper)
- There is still a need for stable, selective alternatives to the current silica phases for LC/MS, where volatile mobile phases are required.

Hydrophobicity of Discovery® RP Columns

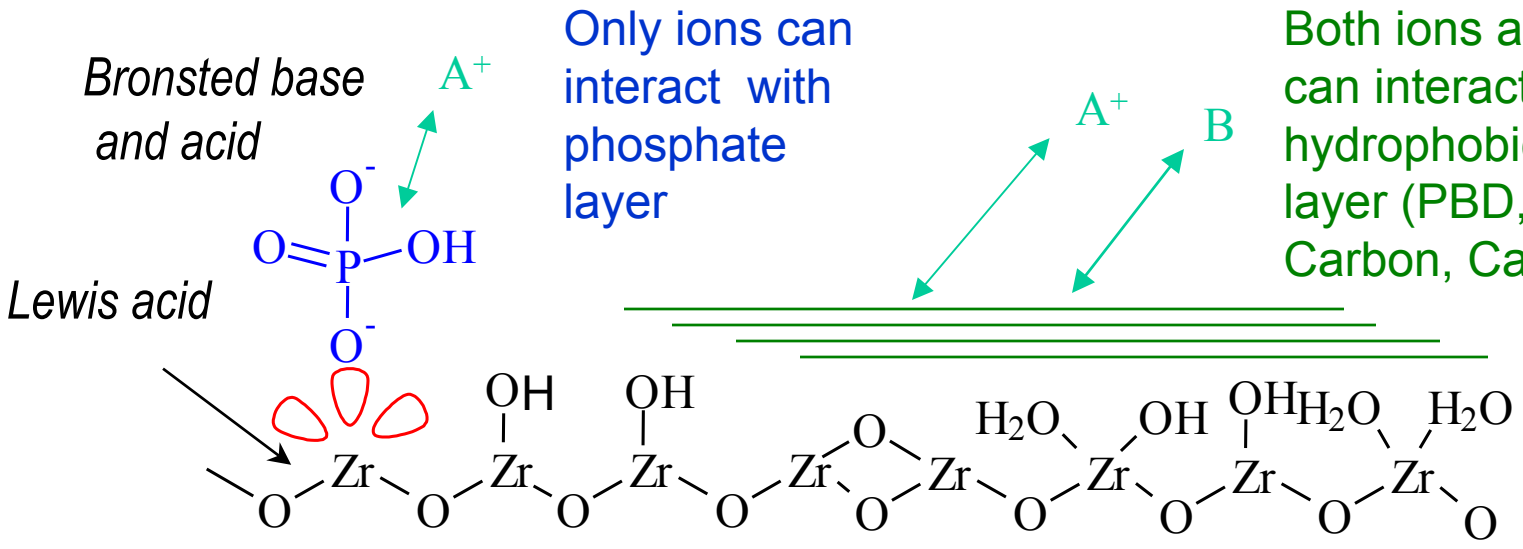


Silica C18 Structure



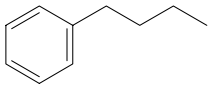
- **Strong efforts have been made by the silica-based column manufacturers to mask silanol effects, causing columns having the same phase functional groups to become increasingly similar to each other.**
- **There is still a strong need for stable columns which have different selectivity than silica-based bonded phases.**

Origins of Unique Selectivity on Zirconia

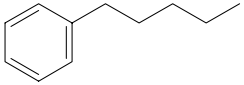


- **Zirconia, a transition metal oxide, has very rich surface chemistry.**
- **Coated zirconia (Carbon and PBD) exhibits mixed-mode surface properties (RPC and IEC) which allow simultaneous nonpolar and polar interaction with solutes.**
- **Retention of various basic and acidic analytes can be fine-tuned by changing pH, buffer and buffer concentration.**

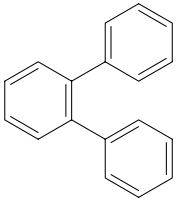
Neutral Analyte Selectivity: Zr-PBD Similar to Silica-C18 (No Phosphate Required)



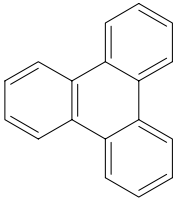
Butylbenzene



Pentylbenzene



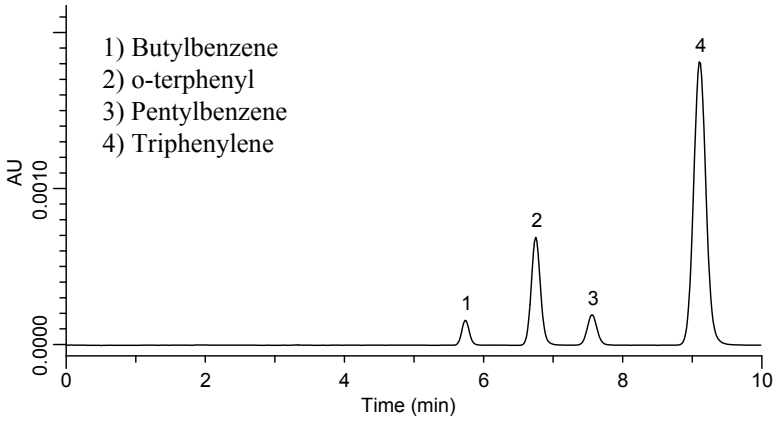
o-Terphenyl



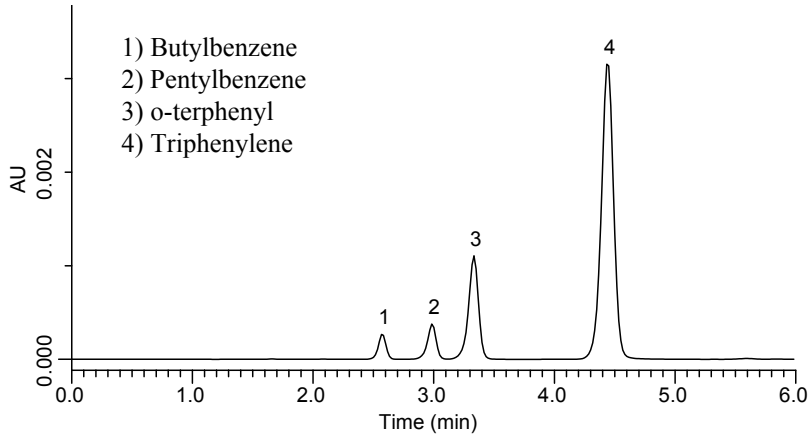
Triphenylene

Column:	150 x 4.6mm, 5 µm
Mobile Phase:	20:80 water:methanol
Flow Rate:	1.0mL/min
Temperature:	40ºC
Injection Volume:	5µL
Detection:	UV, 254nm

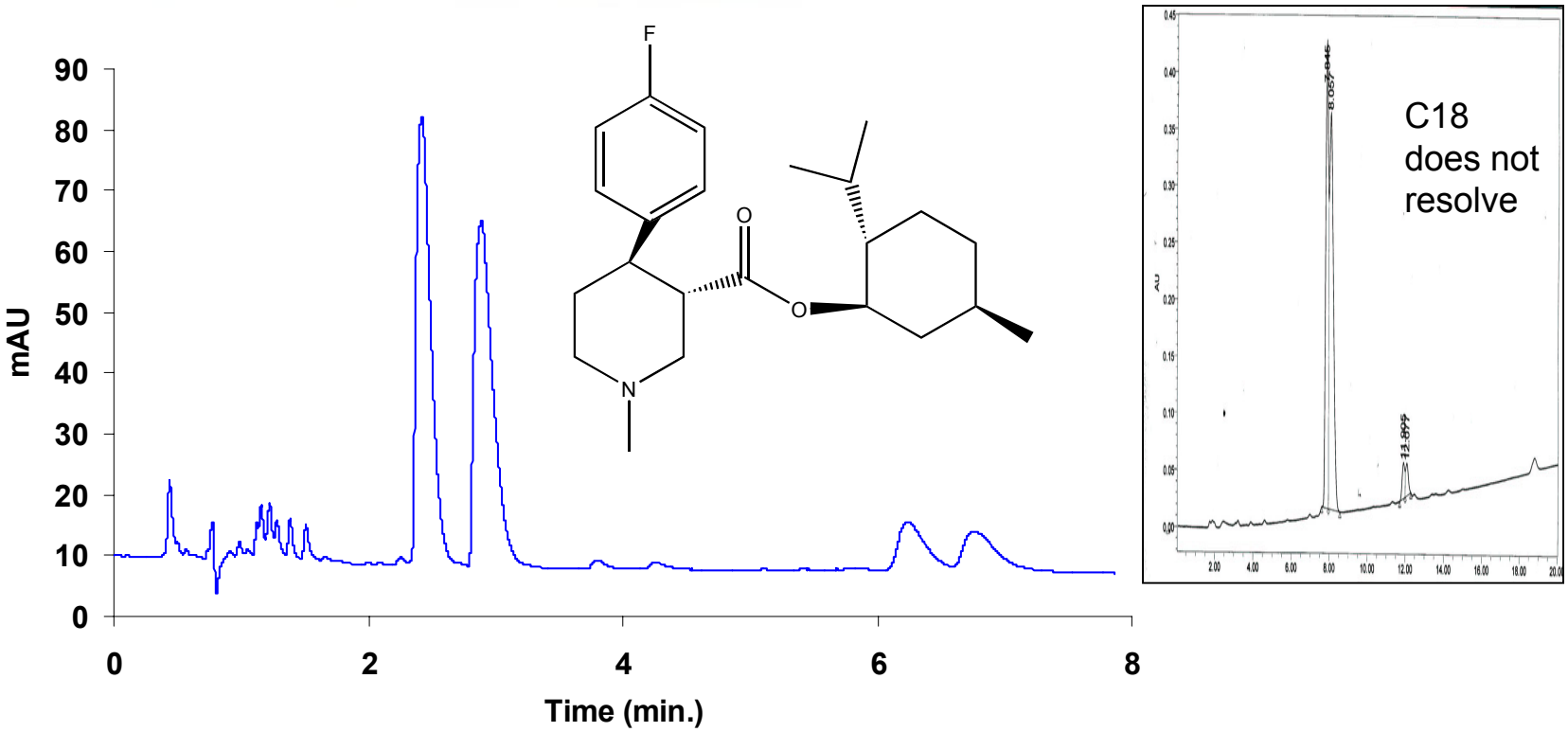
Discovery C18



Discovery Zr-PBD

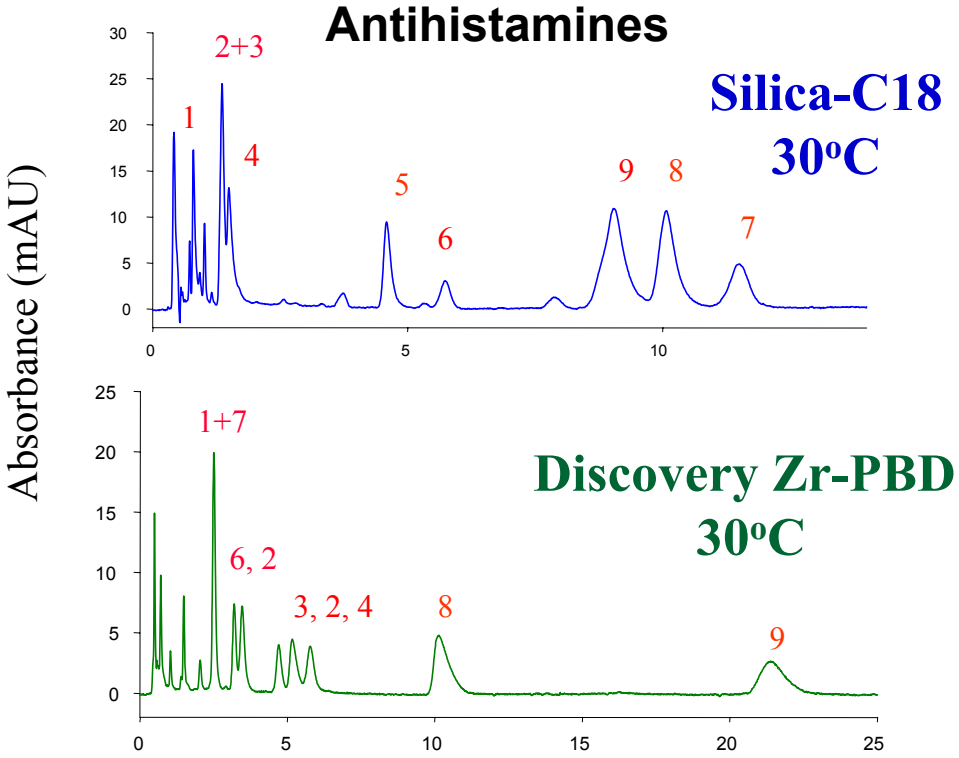


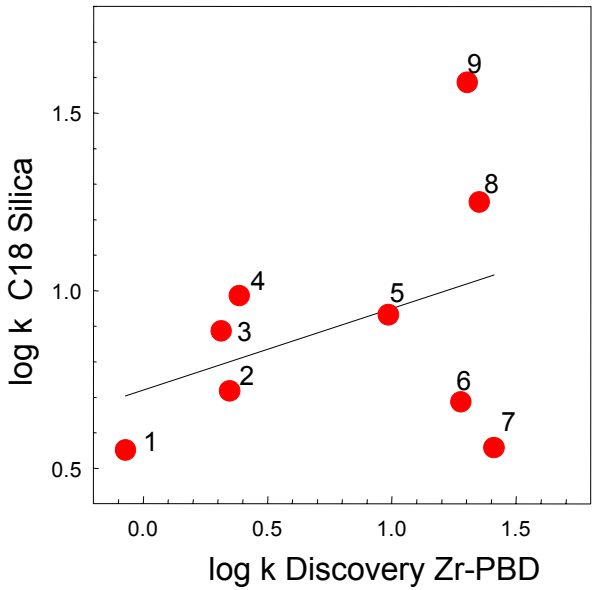
Neutral Diastereomers on Discovery® Zr-CARB (No Phosphate Required @ High pH)



LC Conditions: Column, 150 mm x 4.6 mm i.d. ZirChrom-CARB; Mobile phase, 35/35/30 ACN/Butanol/10mM Diethylamine, pH 11.2; Flow rate, 2.00 ml/min.; Temperature, 80 °C; Injection volume, 5 µl; Detection at 265 nm.

Ionic Solute Selectivity- Very Different



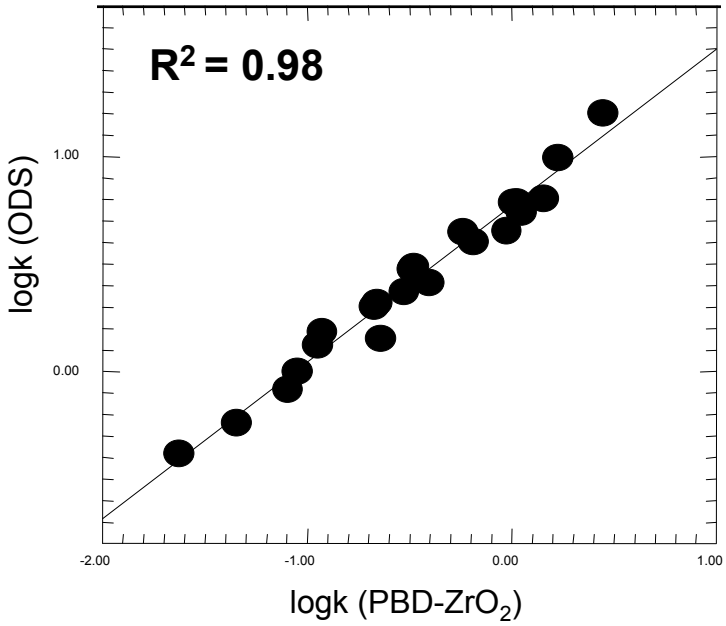


Mobile Phase: 40% CH_3CN in 25 mM phosphate (pH 7)

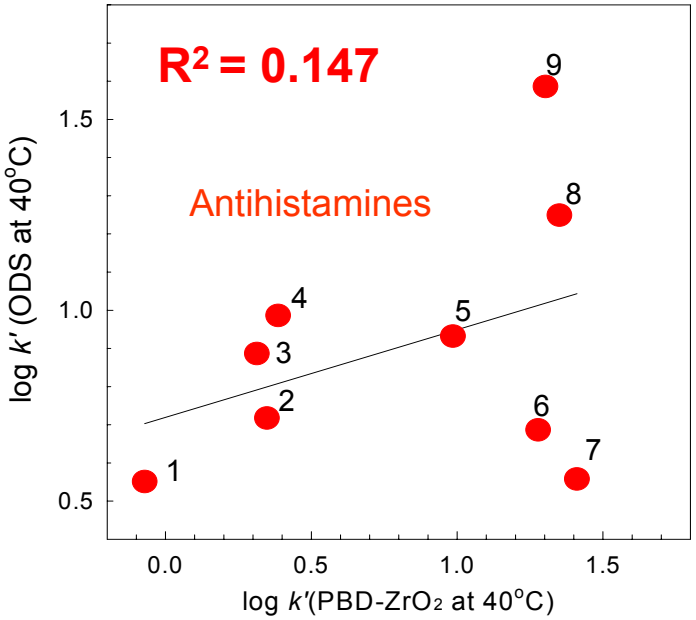
The selectivity of zirconia-based RP columns towards basic compounds becomes very different from that of traditional silica-based RP columns when phosphate is employed in the mobile phase under acidic and neutral conditions, and the Zr-PBD column becomes comparable in retention to a typical Silica-C18.

Impact of Sample Types on Selectivity

Discovery® Zr-PBD *vs.* Silica-C18



Nonionic solutes: Columns are very similar due to retention by RP mode only, but Zr-PBD is less retentive



Ionic solutes: Columns are very different due to retention by combined RP and IEC modes

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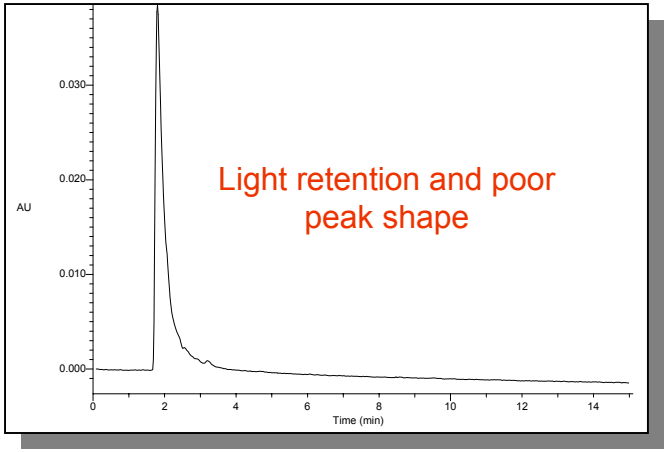
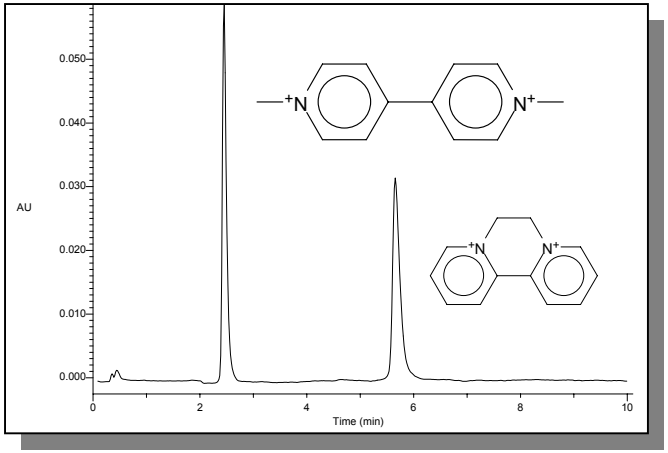
Advantage of Ion-Exchange on Zirconia

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

Silica-C18:
reversed-phase only



Zirconia-PS:
reversed-phase and ion-exchange



C18-silica conditions: Discovery C18, 15cm x 4.6mm, 3µm particles; 5% CH_3CN in 25mM H_3PO_4 (to pH 7 with NH_4OH); 35°C, 1mL/min, UV 290nm
Zr-PS conditions: Discovery Zr-PS, 7.5cm x 4.6mm, 3µm particles; 50% CH_3CN in 25mM H_3PO_4, 25mM NH_4F, (to pH 8 with NH_4OH); 65°C, 3mL/min, UV 290nm

Discovery® Zr for LC/MS

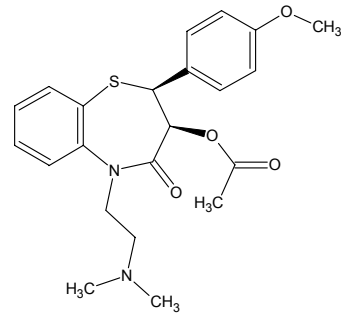
- Many applications for neutral compounds on Discovery^{R} Zr phases do not require phosphate and are LC/MS compatible; however, guards and regular washes/regenerations are recommended.
- Most applications for ionic compounds are not LC/MS compatible due to the requirement of high ionic strength phosphate buffers, especially under desired acidic conditions for optimum sample detection by ESI+.
- This study was aimed at:
  - Assessing the need for phosphate in various systems
  - Determining if Discovery^{R} Zr phases can be made LC/MS compatible for ionic samples
  - Investigating Lewis acid endcapping as a viable solution

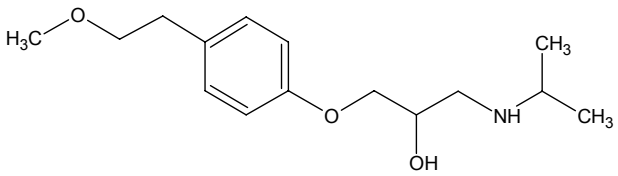
Bases on Discovery® Zr-PBD with Phosphate

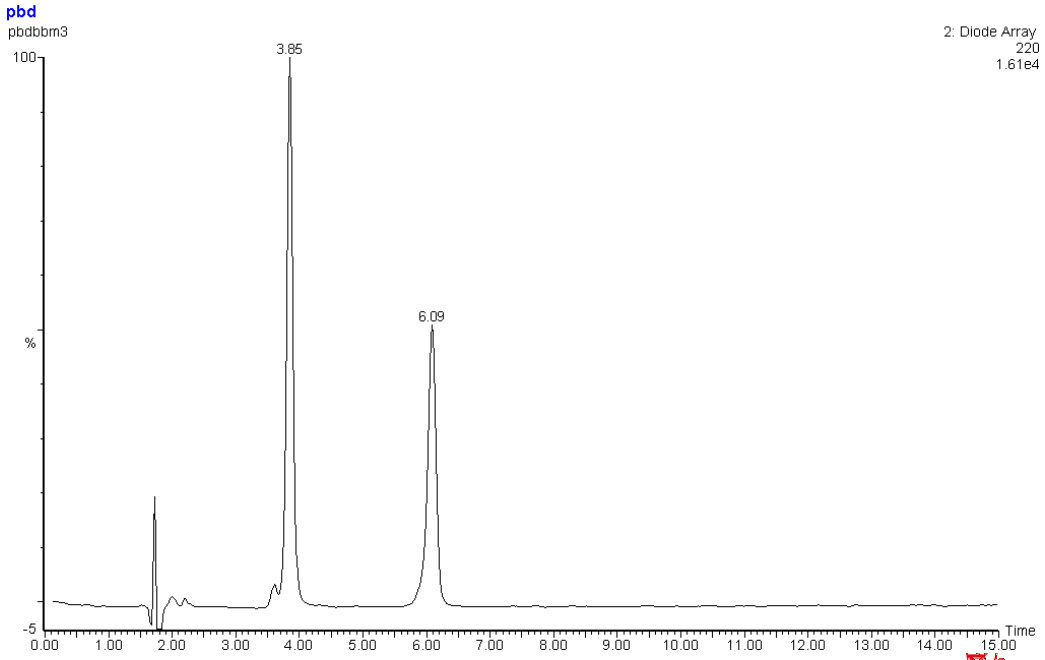
Discovery Zr-PBD
15cm x 4.6mm ID, 5µm particles
(70:30) 25mM potassium phosphate, pH 3.0: acetonitrile
1.0 mL/min
UV, 220nm
35° C
25µg/mL diltiazem, metoprolol in (50:50) 25mM potassium phosphate, pH 3.0:acetonitrile

Not MS Compatible

Good peak shape, selectivity and retention with phosphate mobile phase







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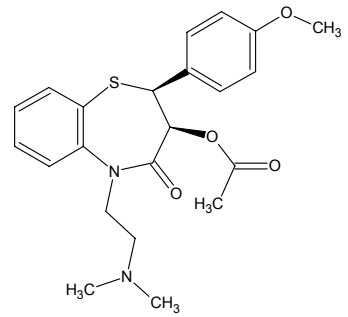
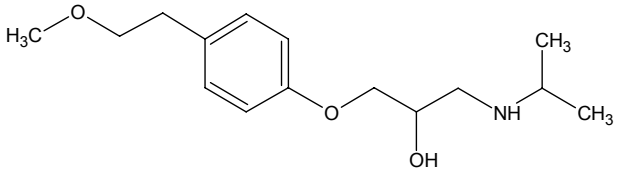
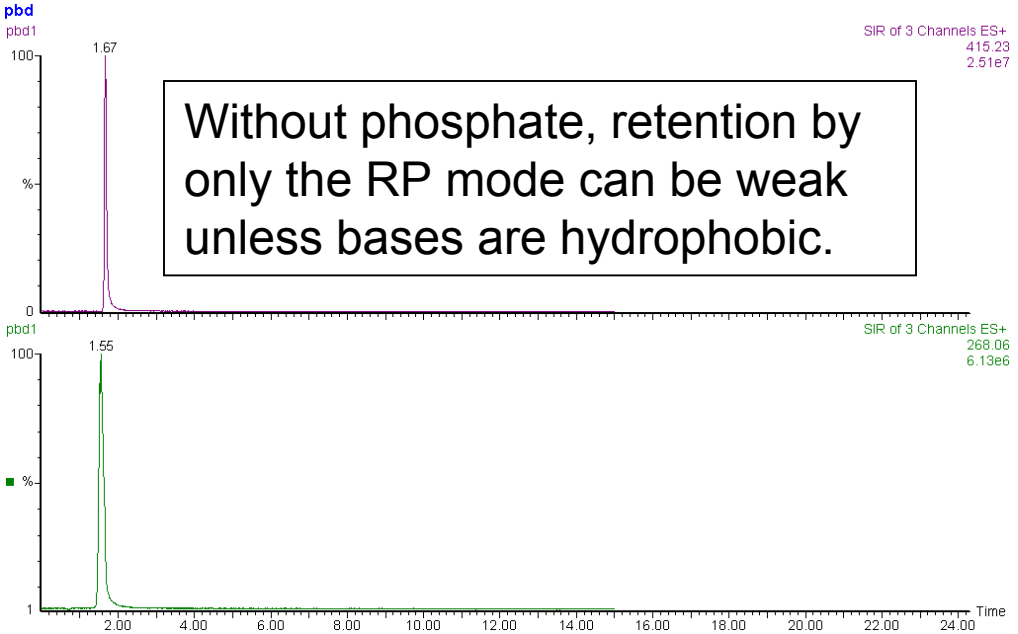
Bases without Phosphate

Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
(60:40) 10mM ammonium acetate, unadjusted :CH_3CN
0.2 mL/min
ms, esi (+)
40° C
1µg/mL diltiazem, metoprolol in (60:40) water:acetonitrile

Poor Retention





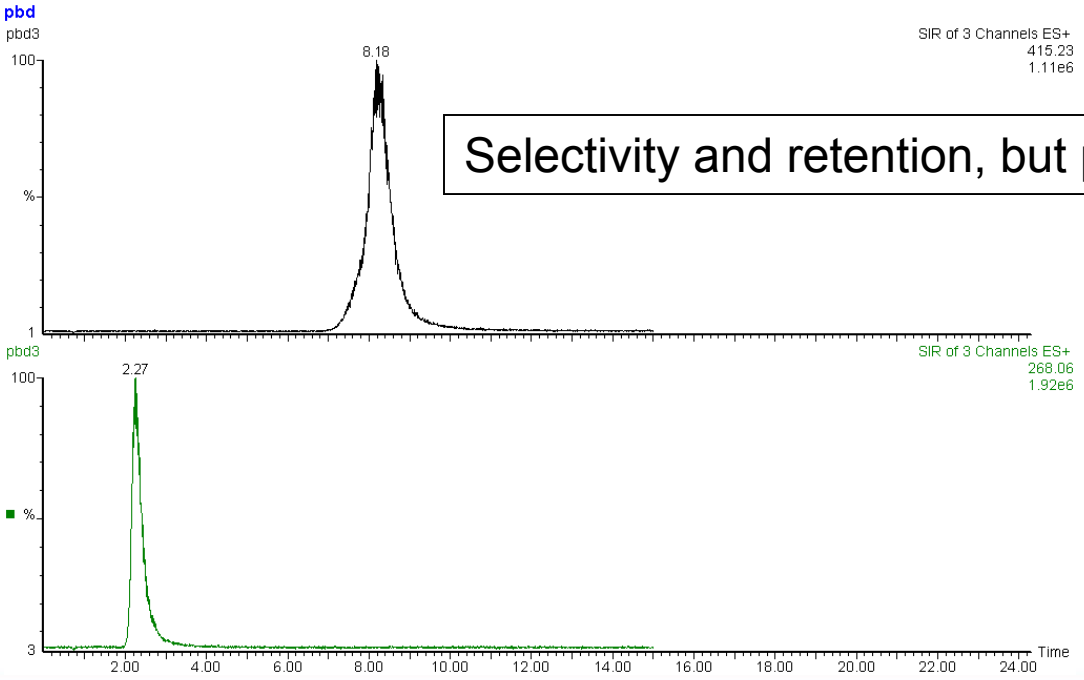


Without phosphate, retention by only the RP mode can be weak unless bases are hydrophobic.

Bases without Phosphate

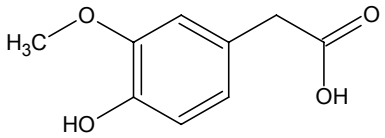
Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
(80:20) 10mM ammonium acetate, unadjusted :CH_3CN
0.2 mL/min
ms, esi (+)
40° C
1µg/mL diltiazem, metoprolol in (60:40) water:acetonitrile

Reducing Organic Percentage Isn't the Answer

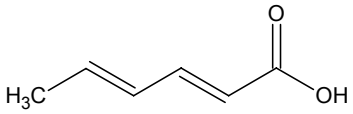


Selectivity and retention, but poor peak shape

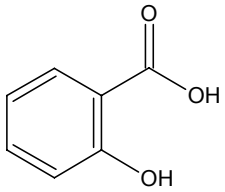
Acids without Phosphate



homovanillic acid



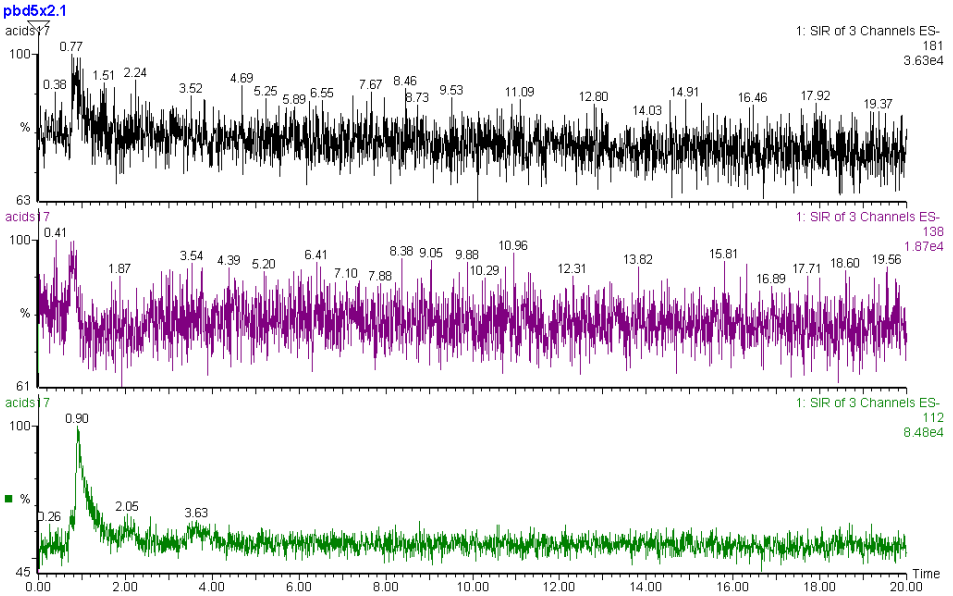
sorbic acid



salicylic acid

Retention Too Strong

No elution due to strong Lewis acid-base interaction



Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
(95:5) 10mM ammonium formate, pH 3.5 :CH_3CN
0.2 mL/min
ms, esi (-)
40° C
1µg/mL each in (50:50) water:methanol

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Chelators without Phosphate

Retention Too Strong

Discovery Zr-PBD, 15cm x 4.6mm, 5µm

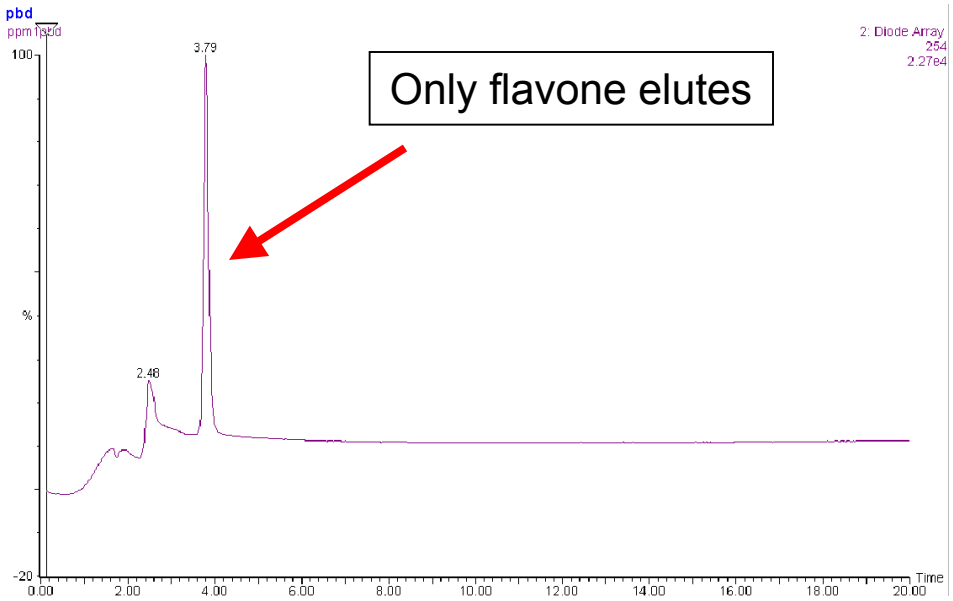
(45:55) 0.1% formic acid in water: 0.1% formic acid in CH3OH

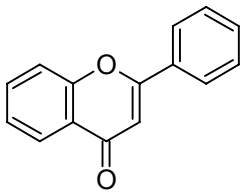
1 mL/min

35°C

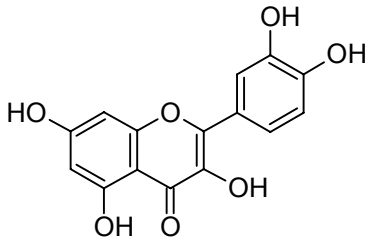
UV at 254nm

25 µg/mL in 0.1% formic acid in water

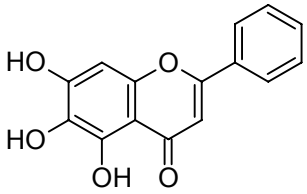




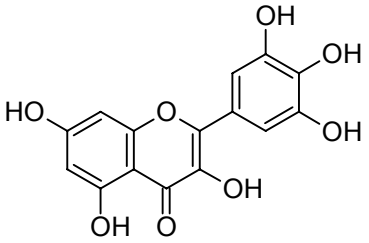
Flavone



Quercetin



Baicalein



Myricetin

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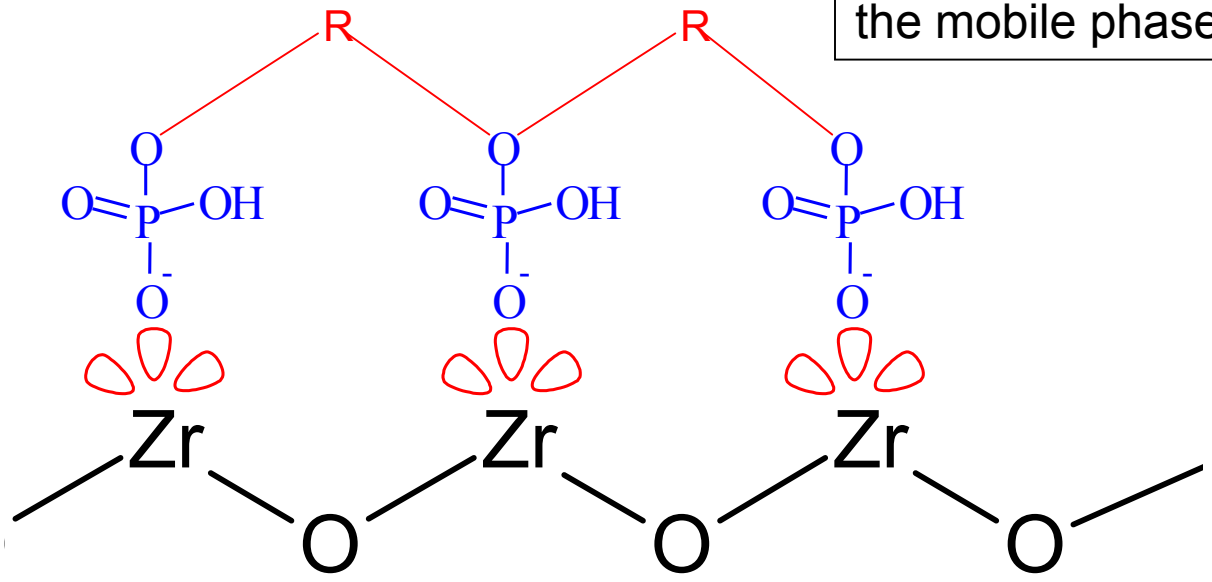
Possible Discovery® Zr Solution for LC/MS

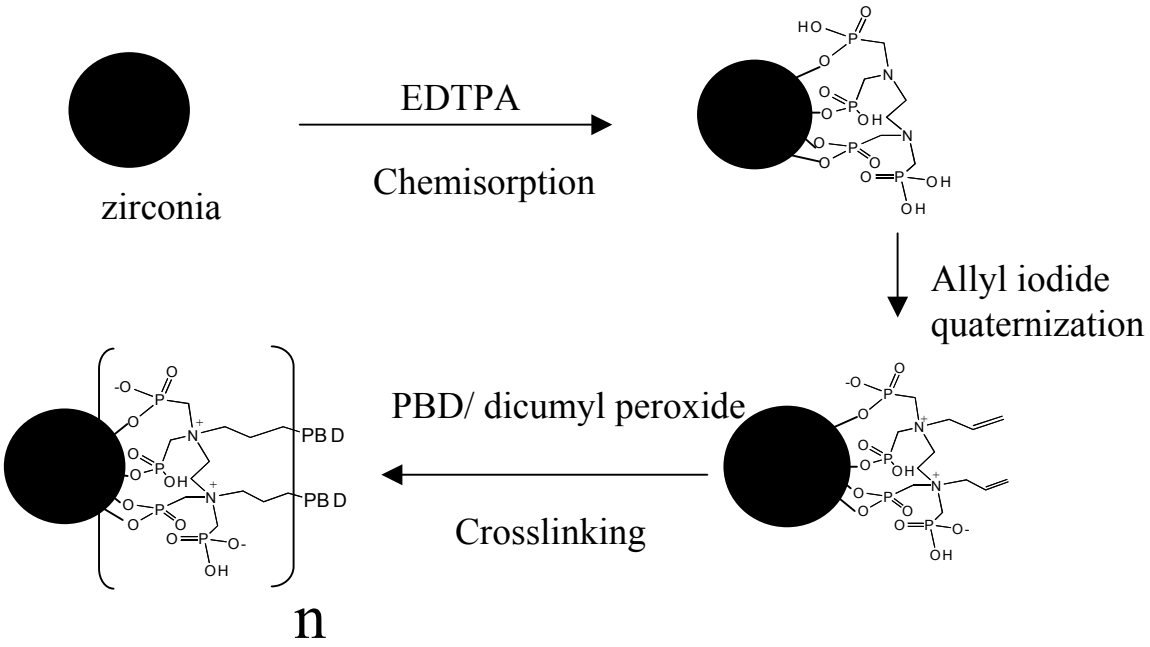
- Without phosphate or another strong Lewis base in the mobile phase
  - there is no negative character imparted to the surface to generate valuable cation exchange properties
  - bases are not well retained under desired acidic conditions and may exhibit poor peak shape
  - acids and chelators can interact strongly and may not elute
- A permanent “endcapping” agent with Lewis base character may solve these issues
- Current research has shown promising results

Proposed Lewis Acid Modification

A reagent with phosphate groups could provide the ion-exchange properties and mask the Lewis acid sites without being a component of the mobile phase



A New Stationary Phase Strategy for LC/MS



1. **Chemisorb Ethylenediamine N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) to the zirconia surface.**
2. **Quaternize amines on the zirconia surface with allyl iodide.**
3. **Coat polybutadiene (PBD) on the chelator-modified zirconia surface and crosslink PBD with allyl group and PBD itself using dicumyl peroxide as initiator.**

LC/MS on Modified Zr-PBD

Modified Zr-PBD, 5cm x 2.1mm ID, 3µm particles

(60:40) 10mM ammonium acetate, unadjusted :CH_3CN

0.2 mL/min

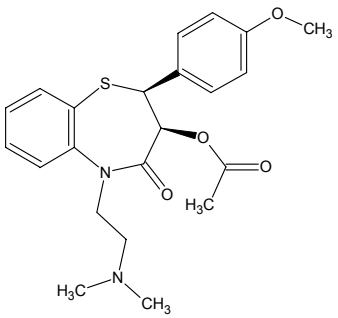
MS, ESI (+)

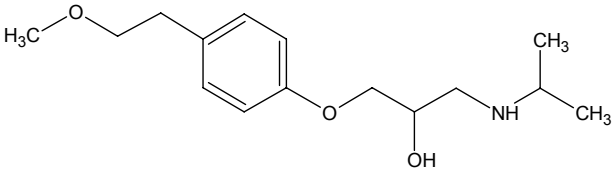
40° C

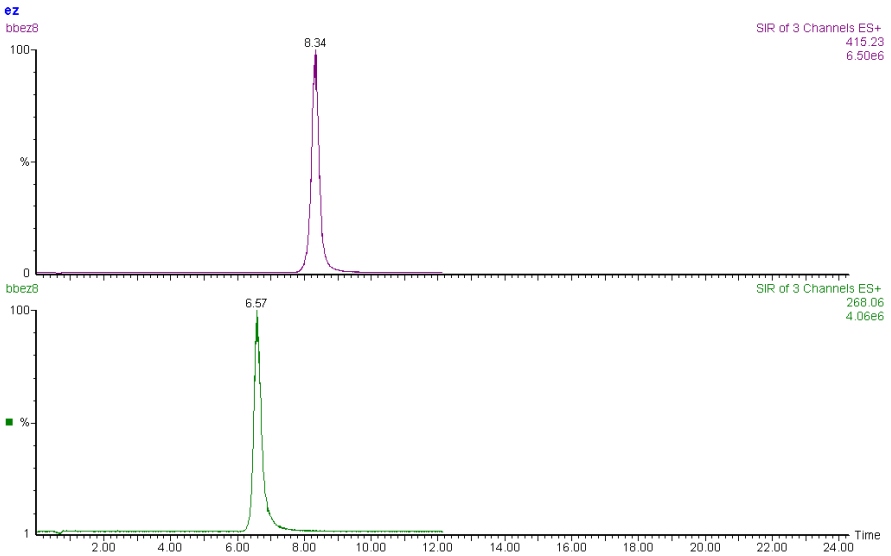
1µg/mL diltiazem, metoprolol in (60:40) water:acetonitrile

Without Phosphate in Mobile Phase

Good peak shape, selectivity and retention on modified Zr-PBD







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Same Bases on Discovery^{R} Zr-PBD

Discovery Zr-PBD, 15cm x 4.6mm ID, 5µm

(70:30) 25mM potassium phosphate, pH 3.0: acetonitrile

1.0 mL/min

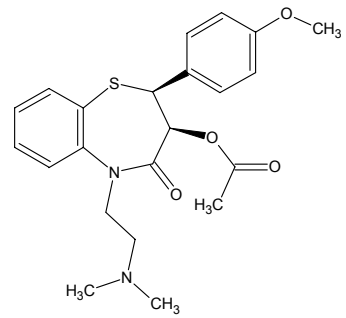
UV, 220nm

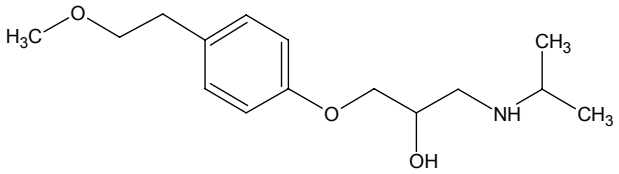
35° C

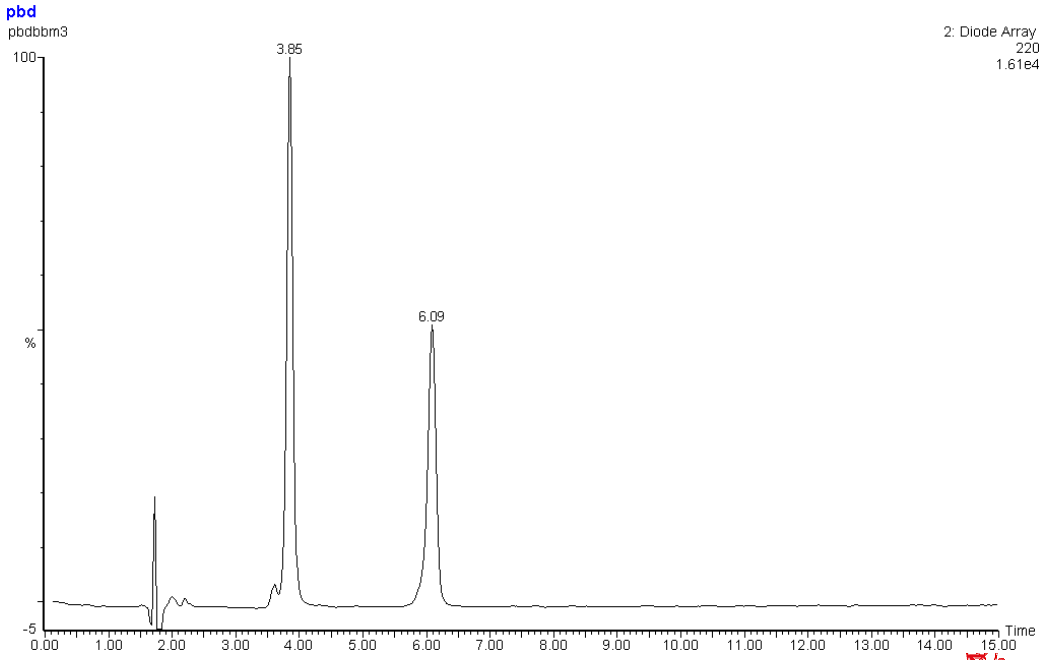
25µg/mL diltiazem, metoprolol in (50:50) 25mM potassium phosphate, pH 3.0:acetonitrile

Not MS Compatible

Good peak shape, selectivity and retention with phosphate mobile phase







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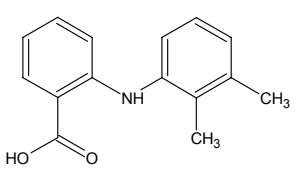
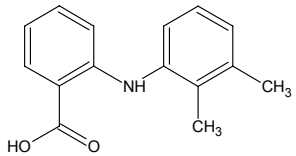
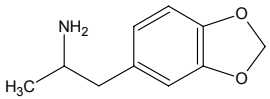
Amphetamines on Modified Zr-PBD



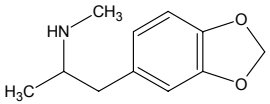
amphetamine



methamphetamine

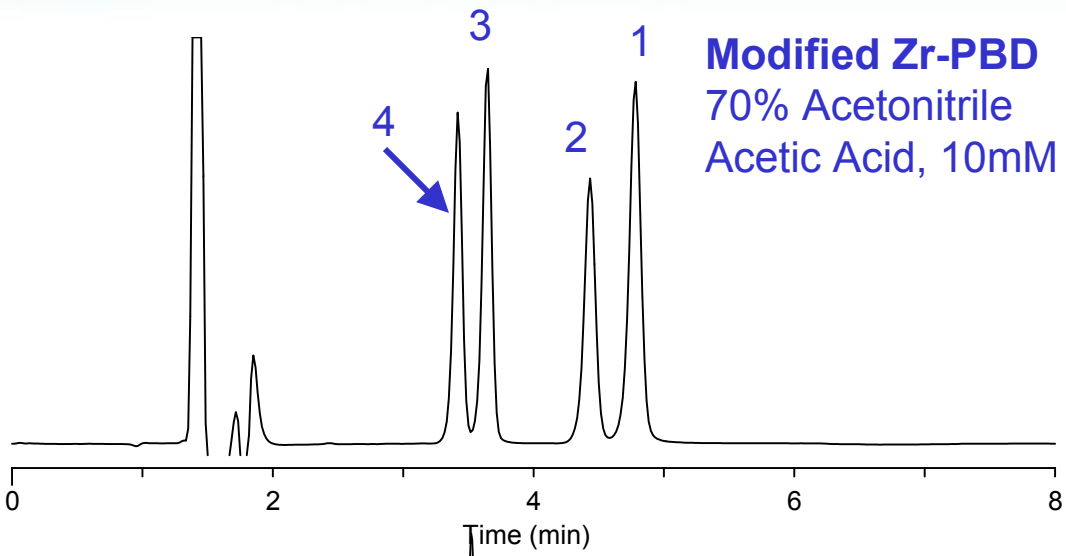


MDA

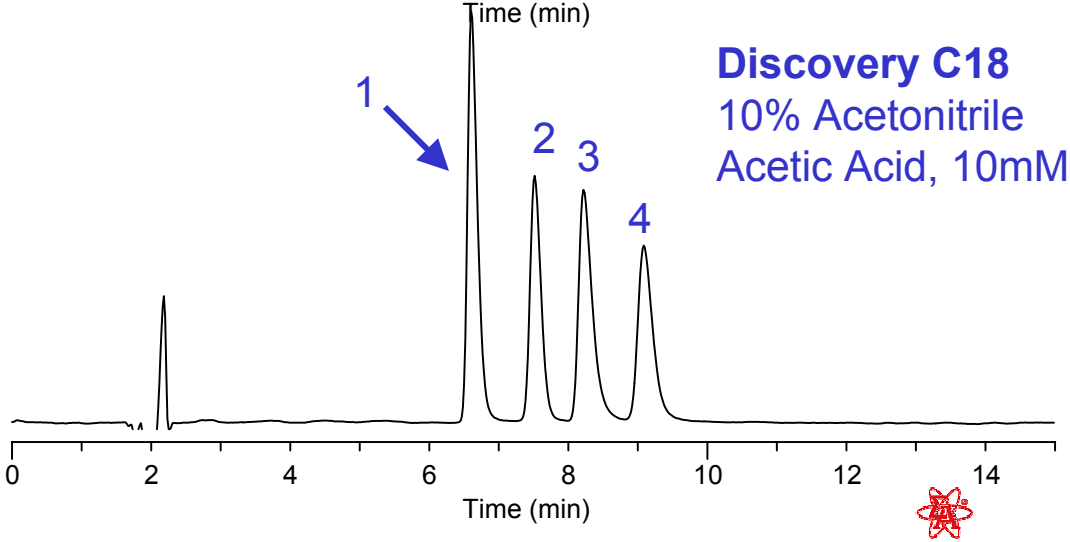


MDMA

1. Amphetamine
2. MDA
3. Methamphetamine
4. MDMA



Modified Zr-PBD
70% Acetonitrile
Acetic Acid, 10mM



Discovery C18
10% Acetonitrile
Acetic Acid, 10mM

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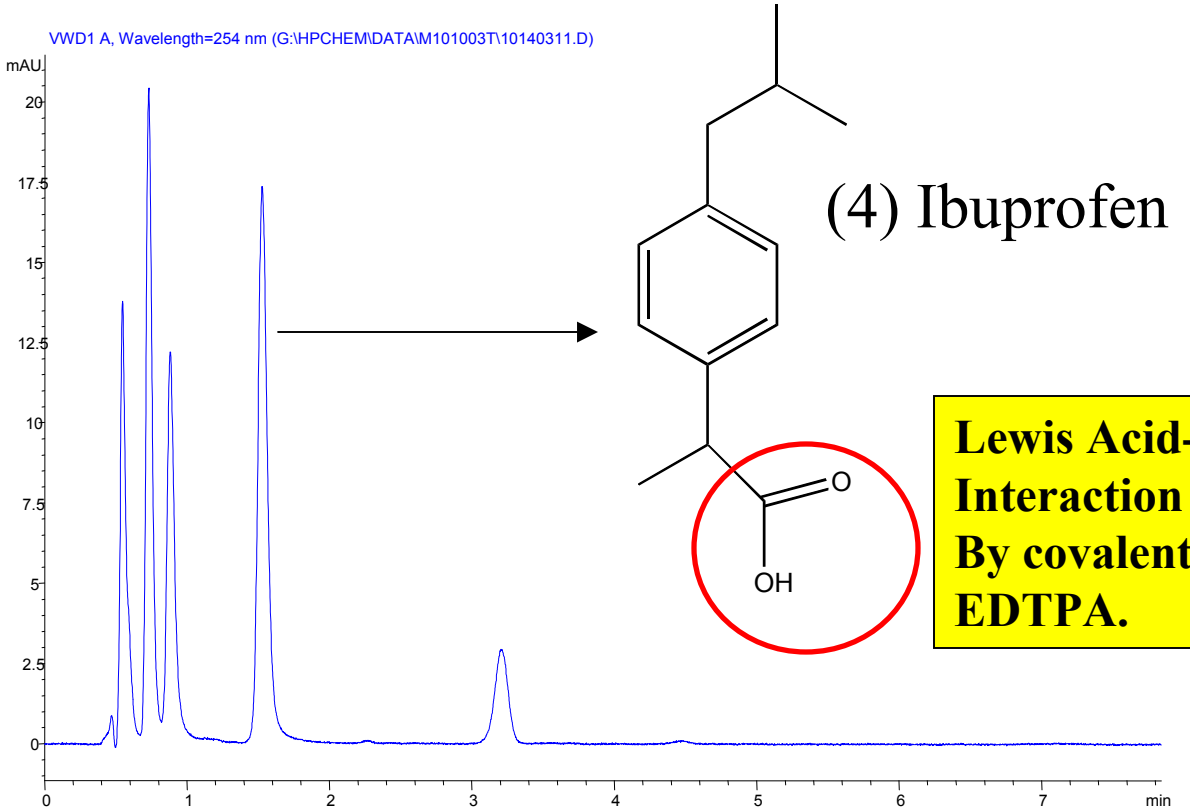
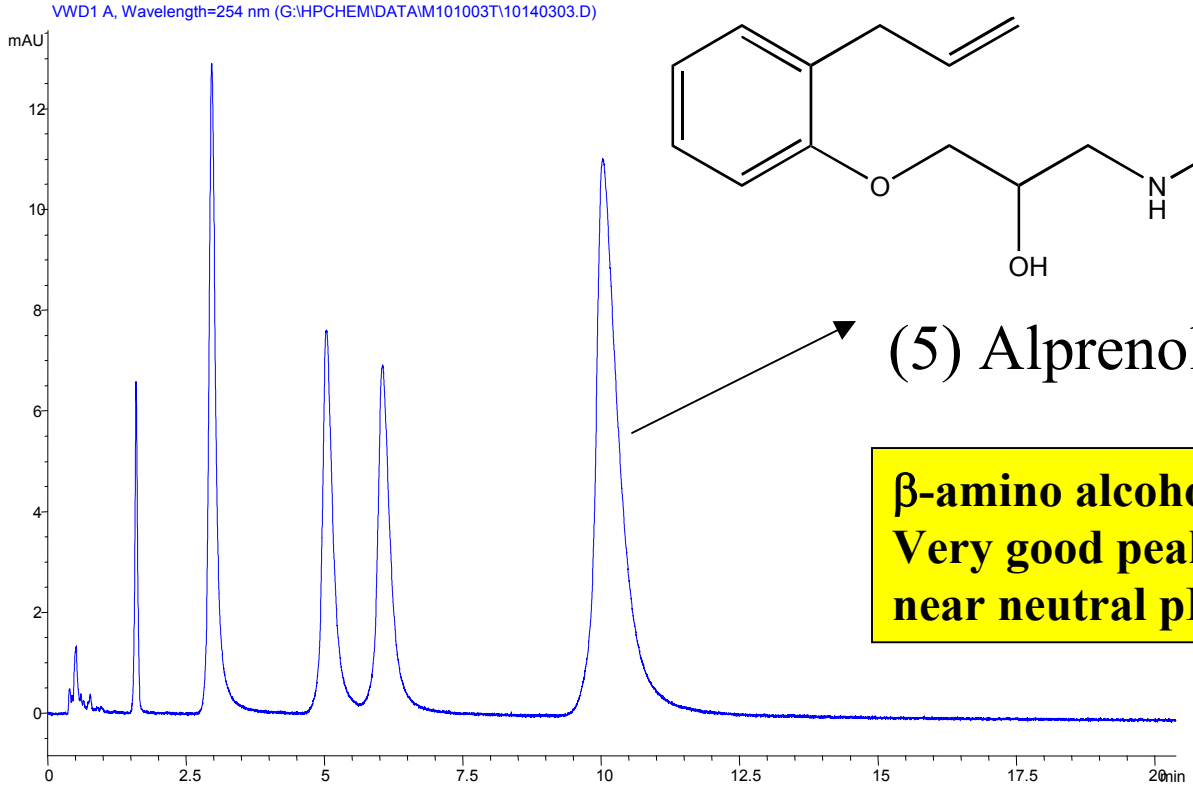
Acidic Drugs on Modifed Zr-PBD



Lewis Acid-Base Interaction "blocked" By covalently attached EDTPA.

Chromatographic Conditions: Column Dimension: 50X4.6 MS101003T; Mobile phase, Machine-mixed 40/60 ACN/10 mM ammonium acetate pH=5. Flow rate:1 ml/min, Temperature, 35° C; Injection volume: 5 µl; Solutes eluted in order, (1) Acetaminophen, (2) Ketoprofen, (3) Naproxen, (4) Ibuprofen, (5) Impurity; Detection, 254 nm. Pressure drop, 68 bar.

β-Blockers on Modified Zr-PBD



Chromatographic Conditions: Column Dimension: 50X4.6 **Modified Zr-PBD**; Mobile phase: Machine-mixed 65/35 ACN/10 mM ammonium acetate pH=5; Flow rate:1 ml/min; Temperature, 35° C; Injection volume: 5 µl. Solutes eluted in order: (1) Lidocaine, (2) Atenolol, (3) Metoprolol, (4) Oxprenolol, (5) Alprenolol Detection: 254 nm; Pressure drop, 59 bar.

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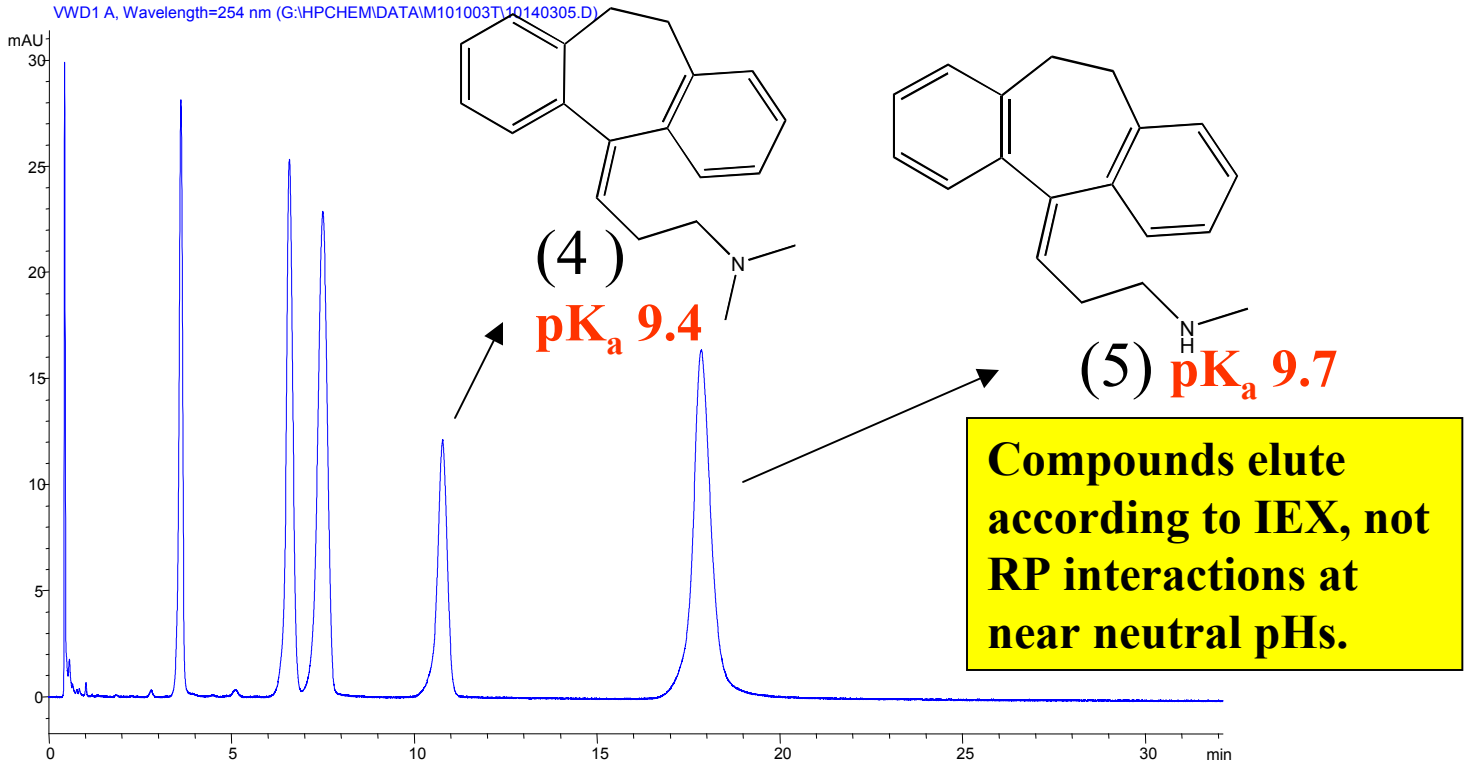
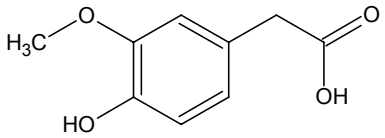
Basic Drugs on Modifed Zr-PBD

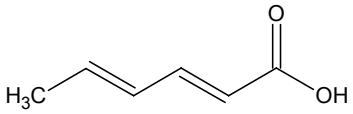


Chromatographic Conditions: Column Dimension: 50X4.6 **Modified Zr-PBD**; Mobile phase, Machine-mixed 65/35; ACN/10 mM ammonium acetate pH=5; Flow rate, 1 ml/min; Temperature, 35^{o} C; Inject volume, 1 µl; Solutes eluted in order: (1) Methapyrilene, (2) Bromphenriamine, (3) Doxpin, (4) Amtriptyline, (5) Nortryptyline; Detection, 254 nm; Pressure drop, 59 bar.

Acids on Modified Zr-PBD

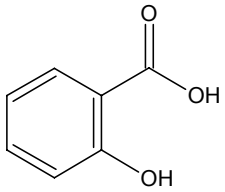


homovanillic acid



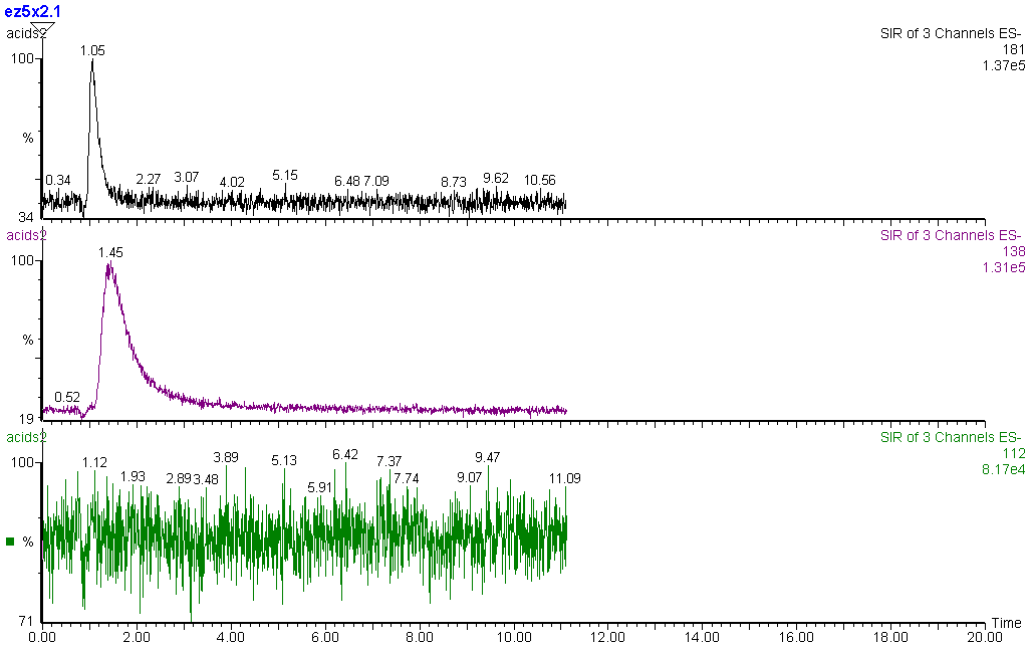
sorbic acid

Acids with strong Lewis base character or chelating properties are still retained too strongly.



salicylic acid

Modified Zr-PBD, 5 cm x 2.1 mm, 3 μm
10 mM ammonium formate, pH 3.5:CH_3OH
0.2 mL/min
MS, ESI (-)
40°C
1 μg/mL each in (50:50) water:CH_3OH



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Chelators on Modified Zr-PBD

Modified Zr-PBD, 15cm x 4.6mm ID, 5µm particles

(45:55) 0.1% Formic Acid: 0.1% Formic Acid in CH_3OH

1mL/min

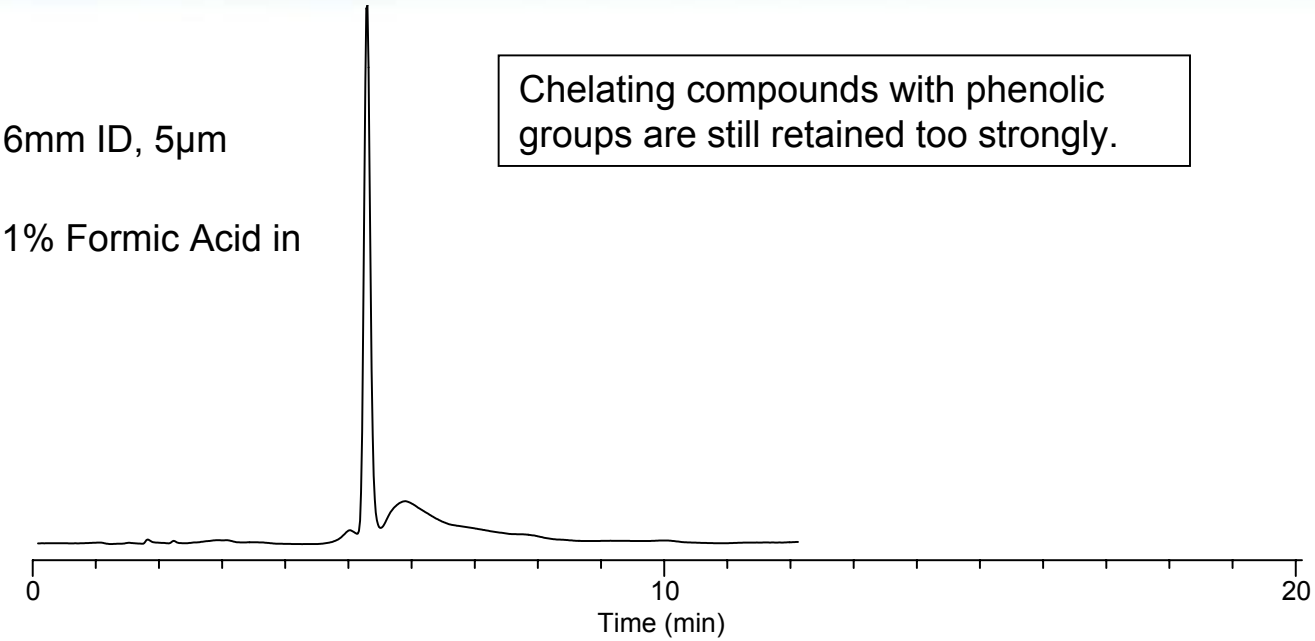
UV, 254nm

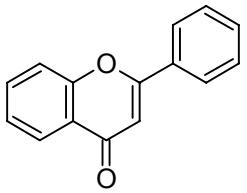
35C

10µL

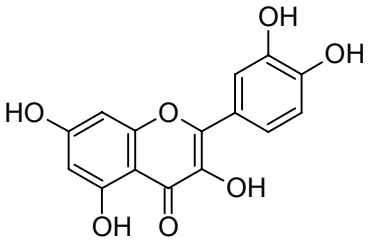
25µg/mL of each

Chelating compounds with phenolic groups are still retained too strongly.

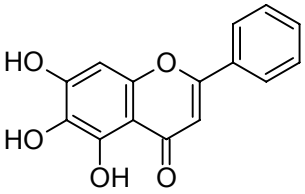




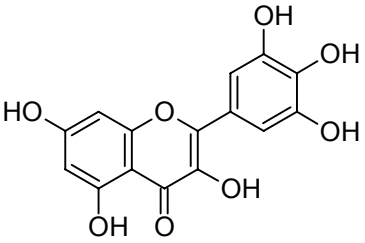
Flavone



Quercetin



Baicalein



Myricetin

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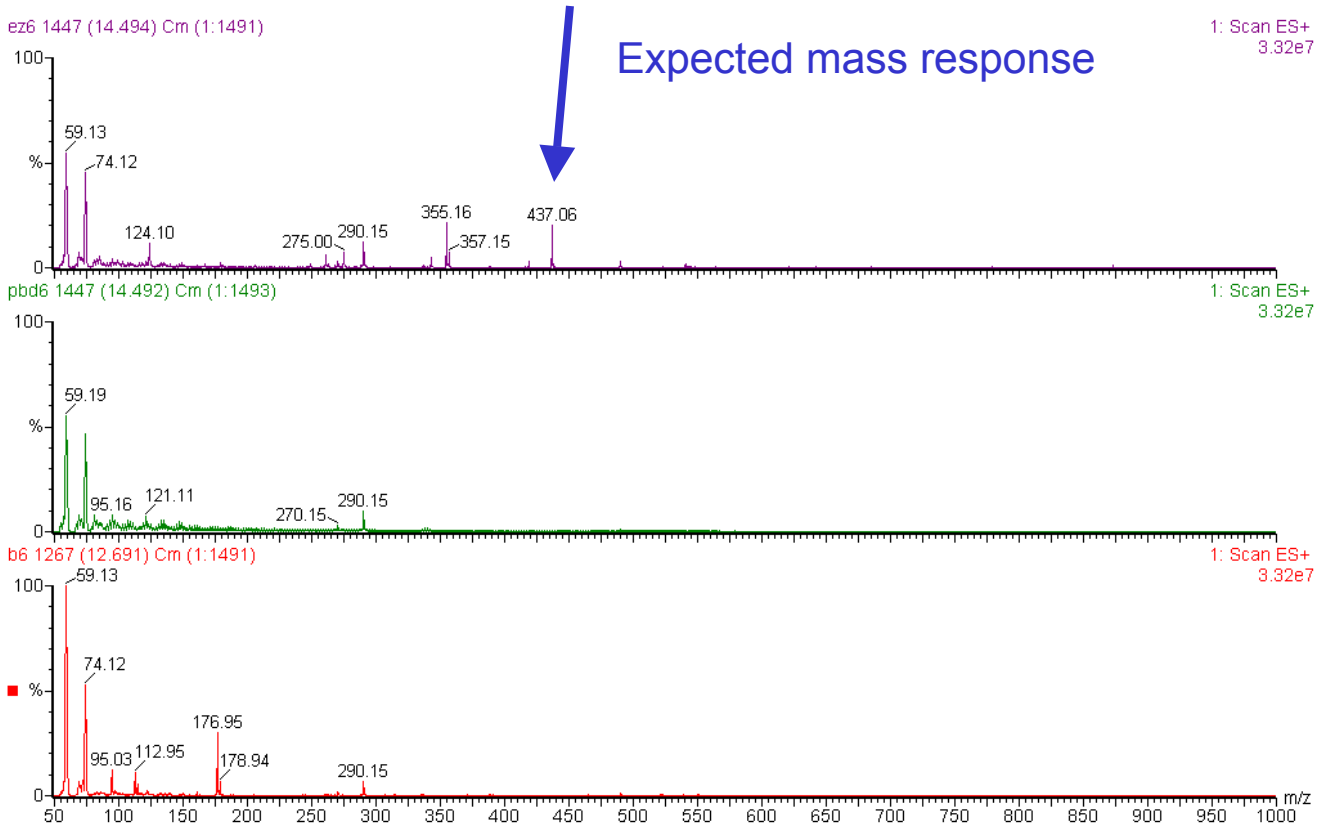
LC/MS Bleed Studies

Method:

- **Waters Micromass ZQ Mass Spectrometer coupled to a Waters 2690 Liquid Chromatograph**
- **A 2.1 mm X 15 cm Modified Zr-PBD and a 2.1 mm X 15 cm Discovery Zr-PBD**
- **Gradient elution was performed using the following buffer systems and acetonitrile**
  - 5mM Ammonium Hydroxide, pH 10.9, unadjusted
  - 10 mM Ammonium Acetate, pH 7.0, unadjusted
  - 10 mM Ammonium Acetate, pH 5.0, adjusted with acetic acid
  - 10 mM Ammonium Formate, pH 3.0, adjusted with formic acid
  - 0.1% Formic Acid, unadjusted
- **Column Temp: 35 ºC**
- **Detection: UV Diode Array and ESI MS in both (+) and (-) ion modes.**

LC/MS Bleed Results

Significant bleed was only observed at high pH (>10)



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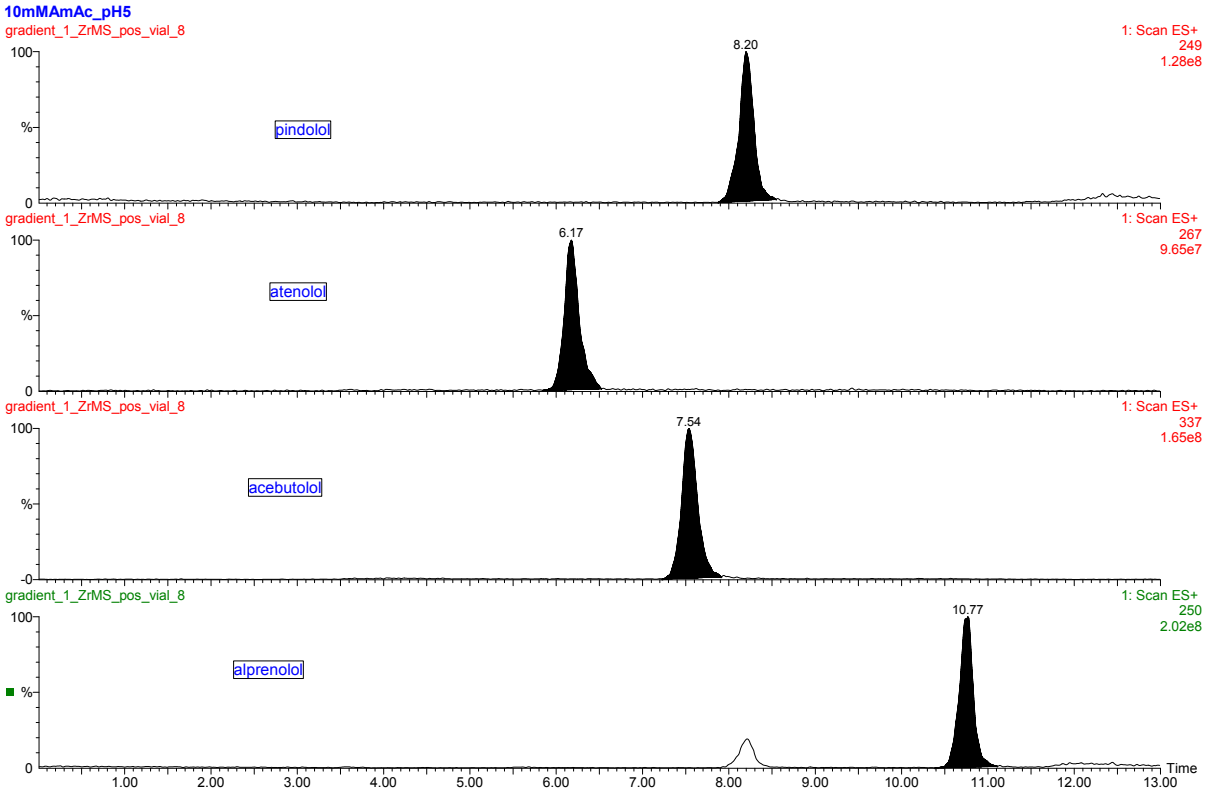
β-Blocker Drugs on Modified Zr-PBD



Modified Zr-PBD

Gradient 5-100%B

A: 10 mM NH_4Ac, pH 5

B: 10 mM NH4Ac, pH 5, 10:90 buffer:ACN

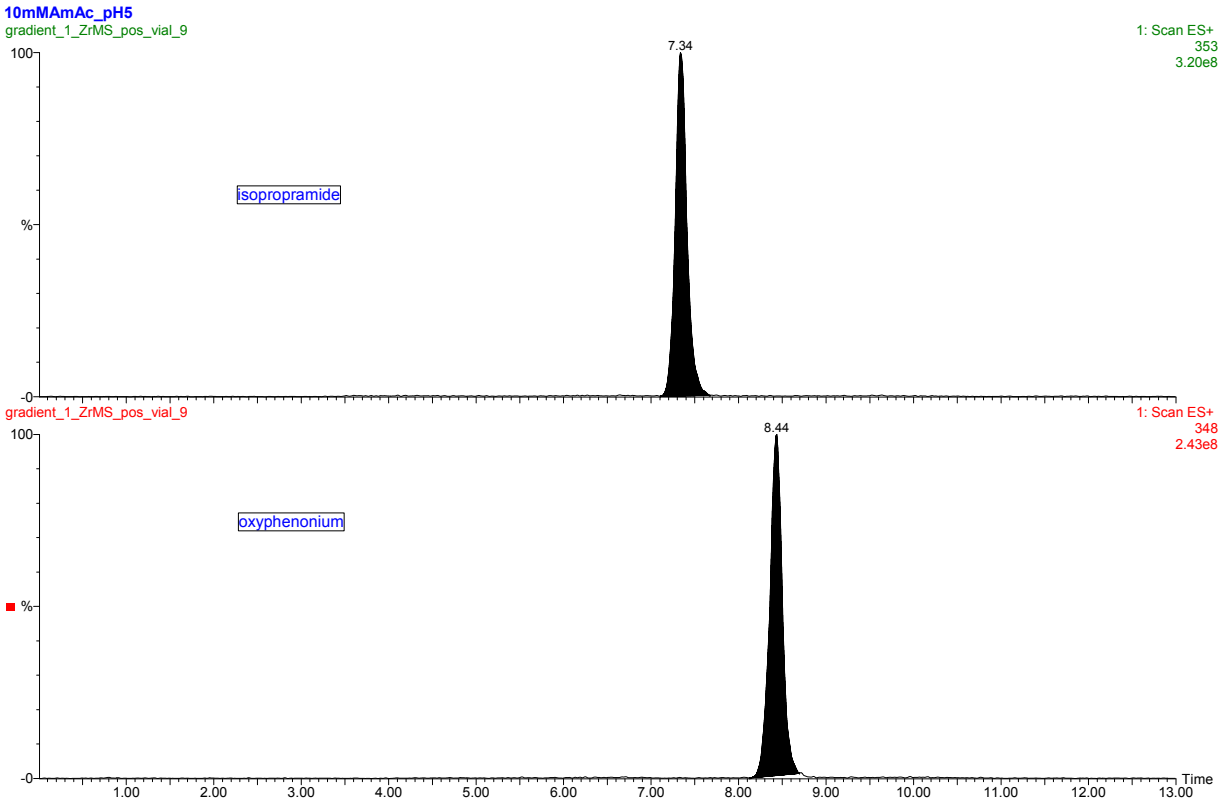
Detection: MS ESI+

Sample:

- Pindolol
- Atenolol
- Acebutolol
- Alprenolol

Retention by RP and SCX modes

Quaternary Amine Drugs on Modified Zr-PBD



Modified Zr-PBD

Gradient 5-100%B

A: 10 mM NH4Ac, pH 5

B: 10 mM NH4Ac, pH 5, 10:90 buffer: ACN

Detection: MS ESI+

Sample:

Isopropramide

Oxyphenonium

Summary

- **Research on a Lewis acid modified Zr-PBD phase has shown promising results for separating ionic analytes using LC/MS compatible mobile phases**
  - **Excellent retention of basic analytes without phosphate additives**
  - **Alternative selectivity to silica-based C18, especially for bases, due to mixed-mode retention mechanism**
  - **Minimal reagent bleed, except at high pH**
  - **Issues remain with certain acidic and chelating analytes**
- **Continued research is underway to produce LC/MS-compatible Zr phases that take advantage of its unique selectivity and stability**

References and Acknowledgements

The authors gratefully acknowledge the assistance of scientists at Supelco Division of Sigma-Aldrich, Bellefonte, PA and Zirchrom Inc., Anoka, MN.

Copies of the paper may be requested at the Sigma-Aldrich Booth Number 4379