

Novel LC/MS-Compatible Stationary Phase with Polar Selectivity

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Introduction

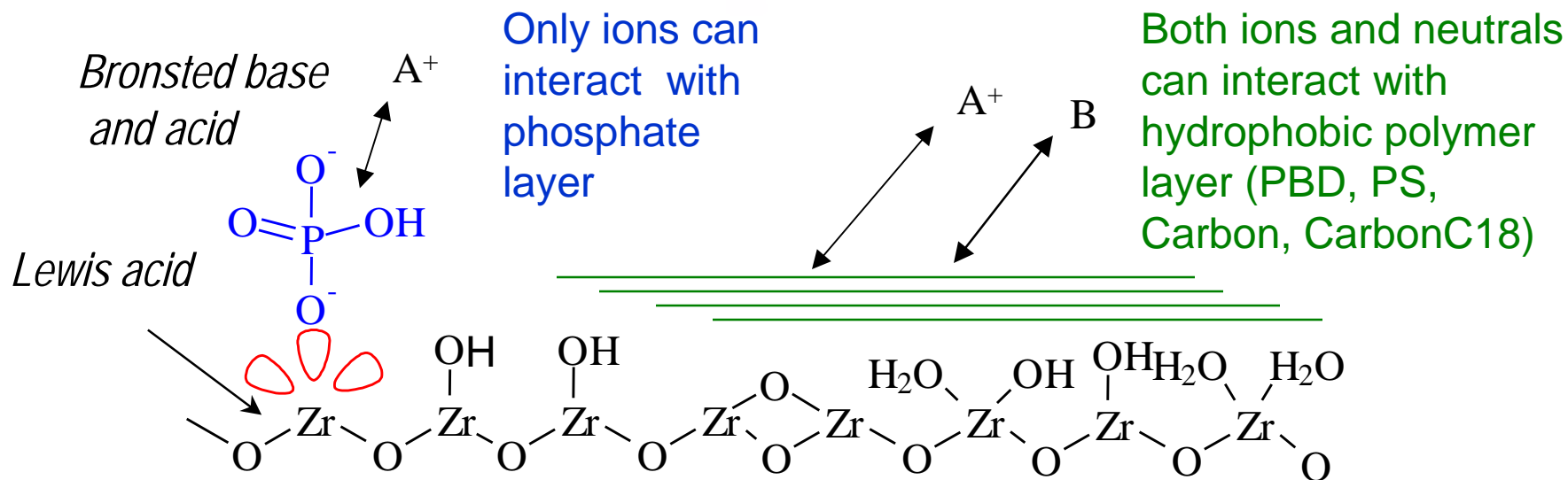
- **Stationary phases based on silica supports remain the workhorse for liquid chromatography/mass spectrometry (LC/MS) analyses**
- **Zirconia phases are becoming a popular alternative in UV analyses**
- **The interest in zirconia columns stems from:**
 - their ability to withstand extreme pH and temperature conditions
 - 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).**



Introduction

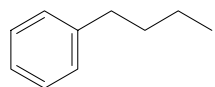
- **Many of the applications developed on such phases are not LC/MS compatible due to high ionic strength mobile phases and the use of phosphate buffers**
- **This study was aimed at:**
 - Assessing the need for phosphate in various systems
 - Determining if existing Zr phases could be LC/MS compatible
 - Investigating Lewis acid endcapping as a viable solution

Origins of Unique Selectivity on Zirconia

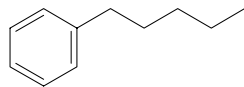


- Zirconia, as a transition metal oxide, has very rich, reproducible surface chemistry.
- Coated zirconia (Carbon and PBD) has mixed-mode surface properties (RPC and IEC) which allow simultaneous nonpolar and polar interactions.
- The retention of various basic and acidic analytes can be fine-tuned by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

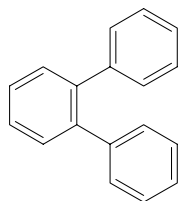
Neutral Analyte Comparison



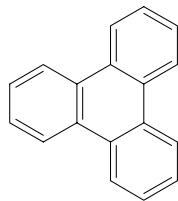
Butylbenzene



Pentylbenzene



o-Terphenyl

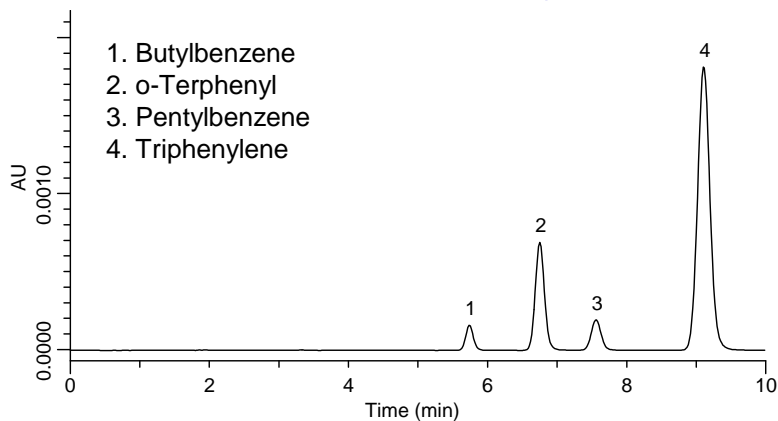


Triphenylene

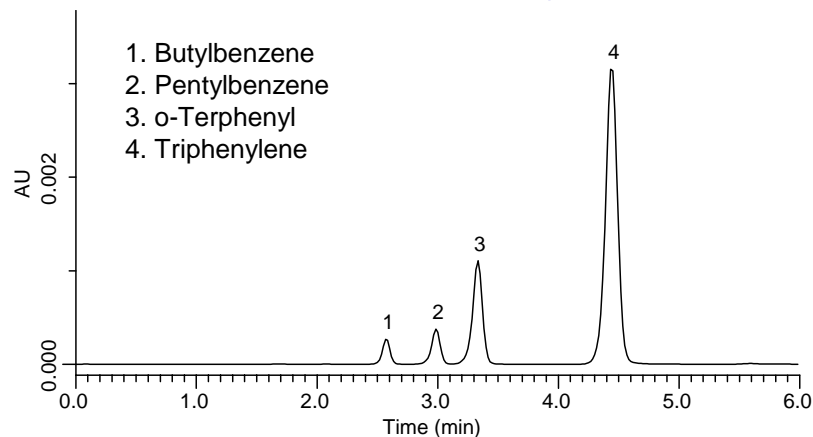
Conditions:

Columns: 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



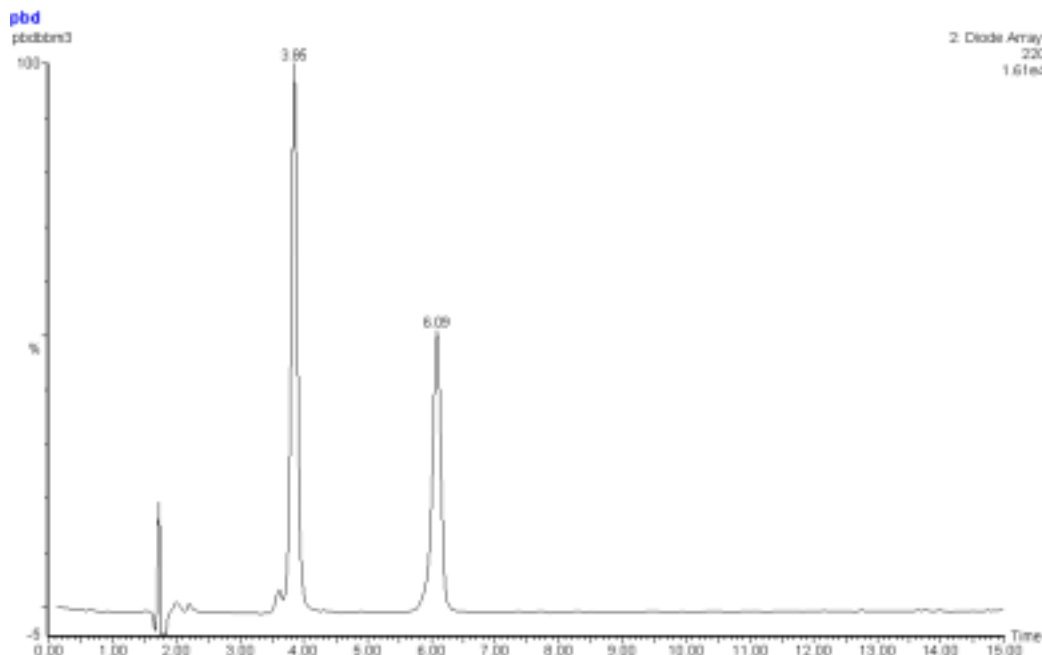
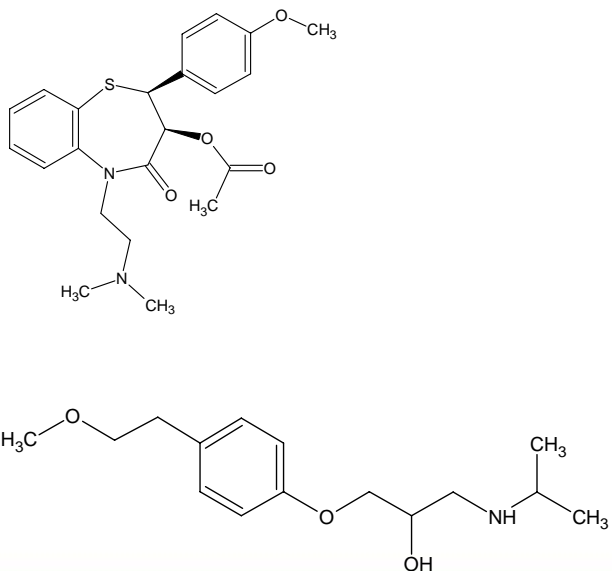
Bases on Discovery Zr-PBD with Phosphate

Conditions:

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

•Good peak shape, selectivity and retention

With phosphate in MP

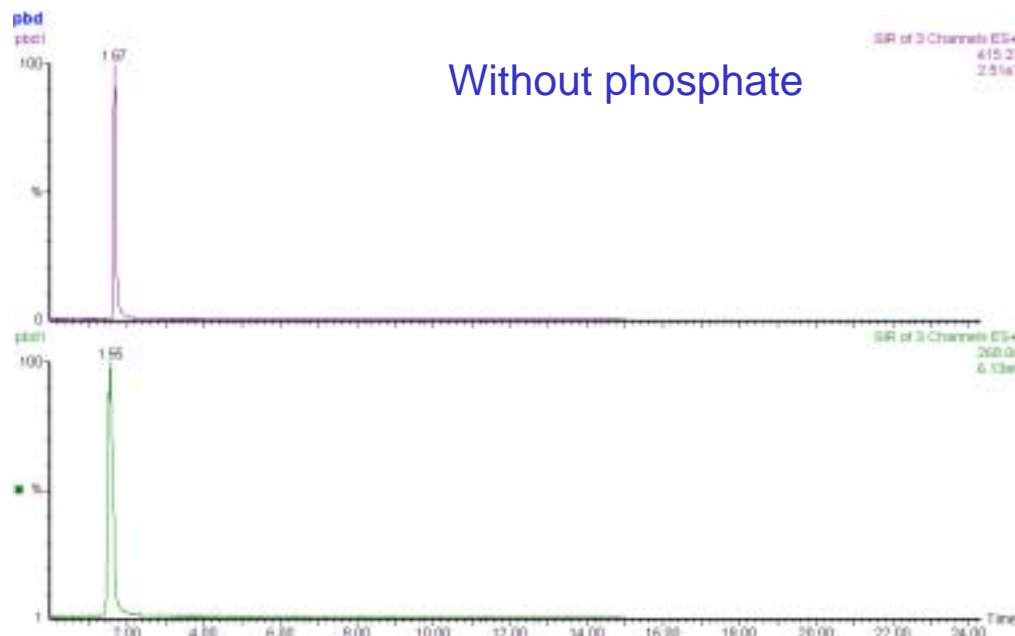
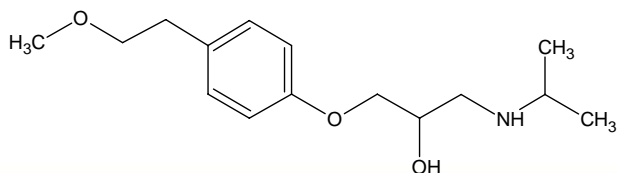
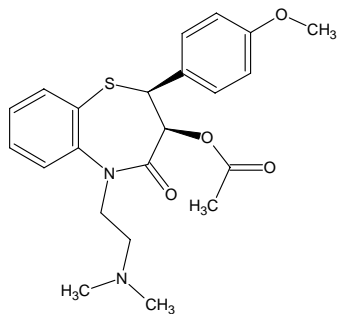


Bases on Discovery Zr-PBD Without Phosphate

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

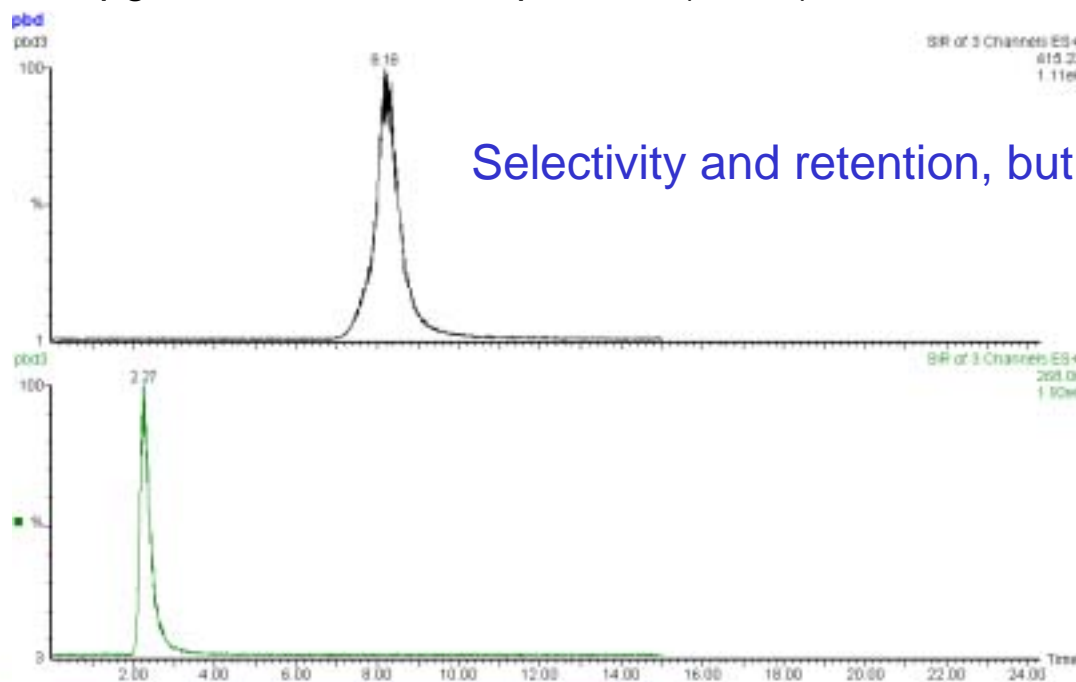
•Lack of retention



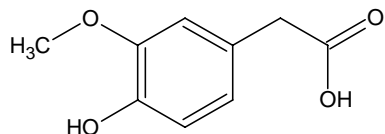
Bases on Discovery Zr-PBD – Lowered Organic Percentage

Conditions:

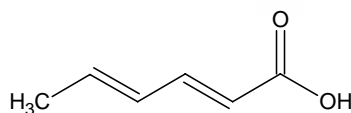
Column: Discovery Zr-PBD, 5cm x 2.1mm ID, 3 μ m particles
Mobile Phase: (80:20) 10mM ammonium acetate, unadjusted :CH₃CN
Flow Rate: 0.2mL/min
Det.: ms, esi (+)
Temp.: 40°C
Inj.: 5 μ L
Sample: 1 μ g/mL diltiazem, metoprolol in (60:40) water:acetonitrile



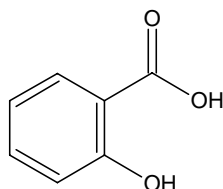
Acidic Compounds on Discovery Zr-PBD



homovanillic acid

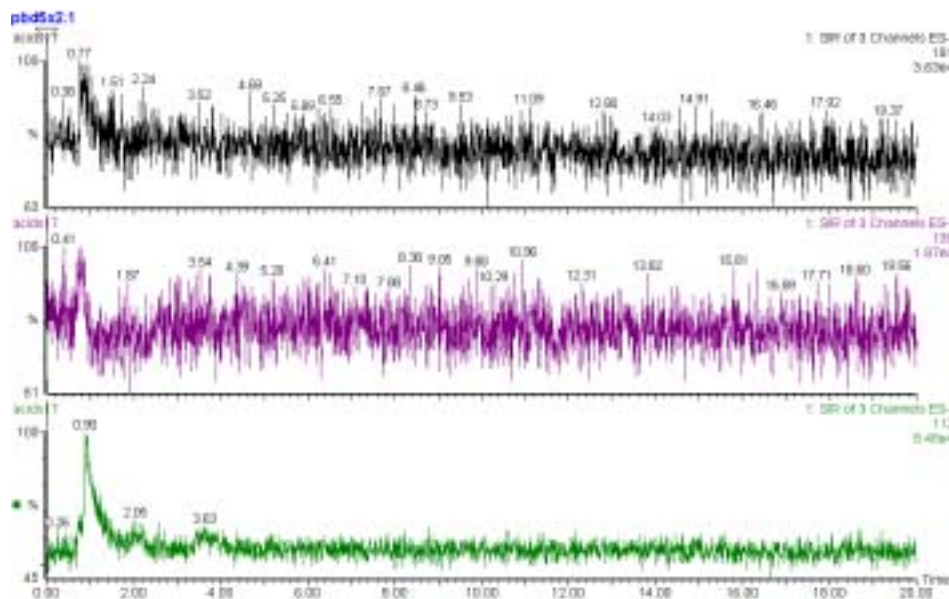


sorbic acid



salicylic acid

No elution – Lewis base activity



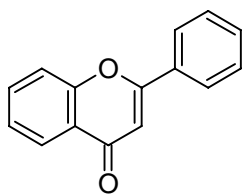
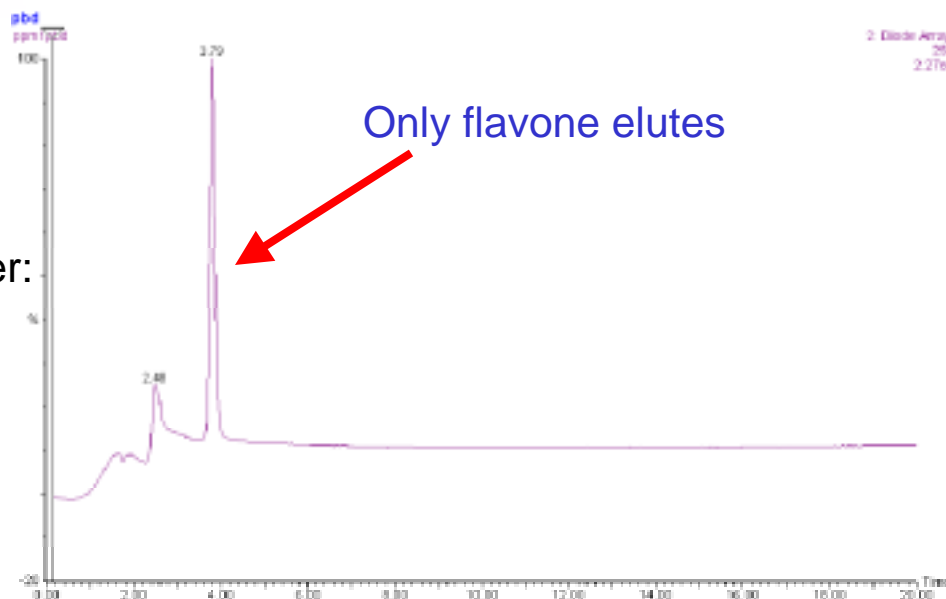
Conditions:

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

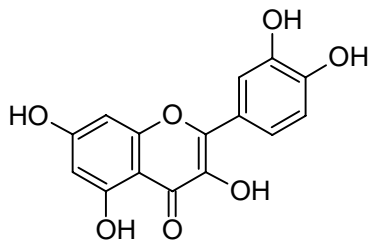
Chelators on Discovery Zr-PBD

Conditions:

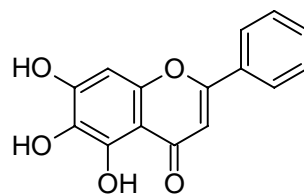
Column: Discovery Zr-PBD,
15cm x 4.6mm, 5 μ m particles
Cat. No.: 65723-U
Mobile Phase: 45:55, 0.1% formic acid in water:
0.1% formic acid in CH₃OH
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10 μ L
Sample: as indicated below (25 μ g/mL in
0.1% formic acid in water)



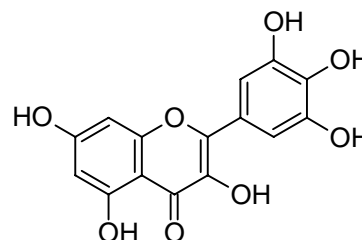
Flavone



Quercetin



Baicalein



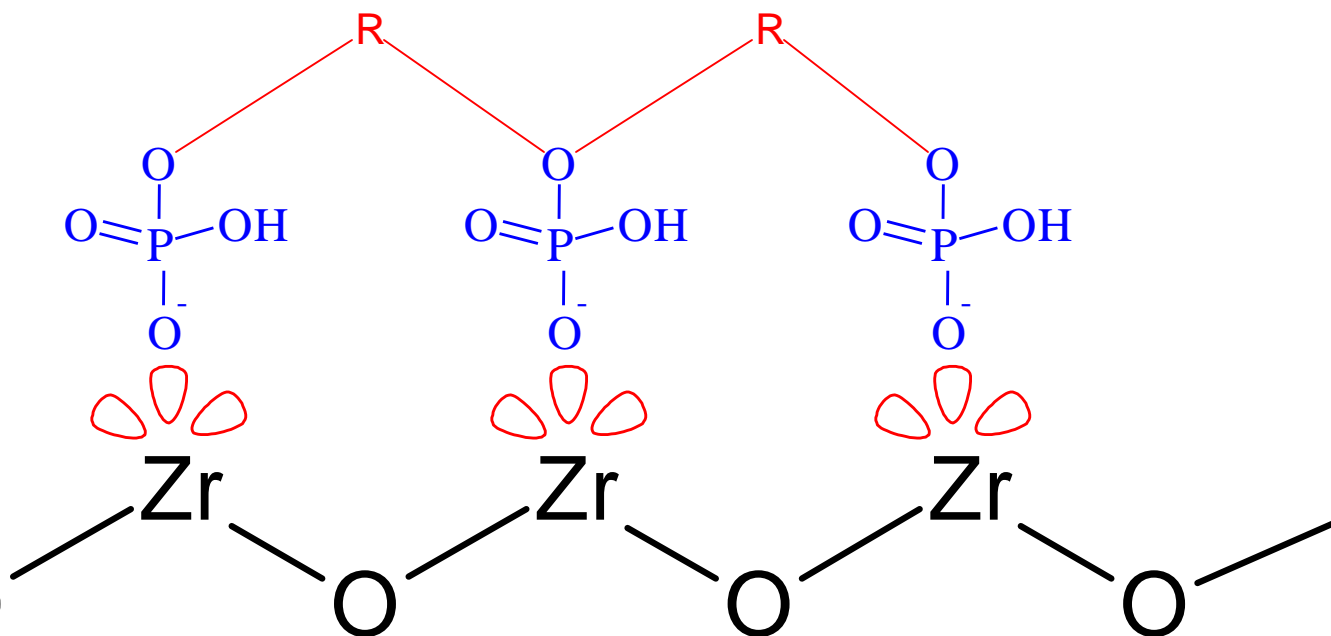
Myricetin



Solution for LC/MS of Ionic Species on Zr

- **Issues arise for ionic analytes due to the Lewis acid character of Zr:**
 - need for Lewis base additives (phosphate)
 - Moderate strong Lewis acid/base interactions
 - Moderate chelation activity
 - Impart negative charge to surface to generate IEX
- **A permanent “endcapping” agent with ionic character may solve these issues**
- **Current research is showing promising results**

Lewis Acid Endcapping



Add reagent with multiple Lewis base sites to:

1. Mask Lewis acid character
2. Provide negative charge for IEX

Solution for LC/MS of Ionic Species on Zr

Conditions:

Column: Modified 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

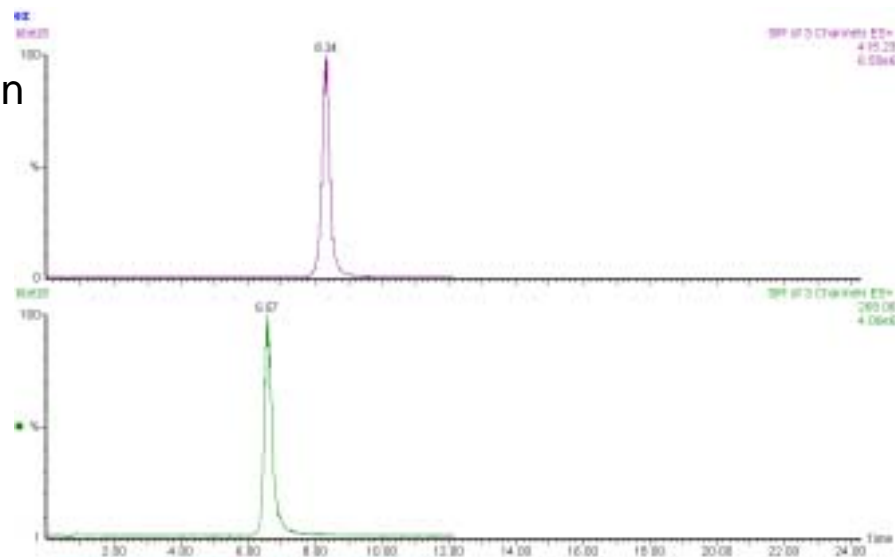
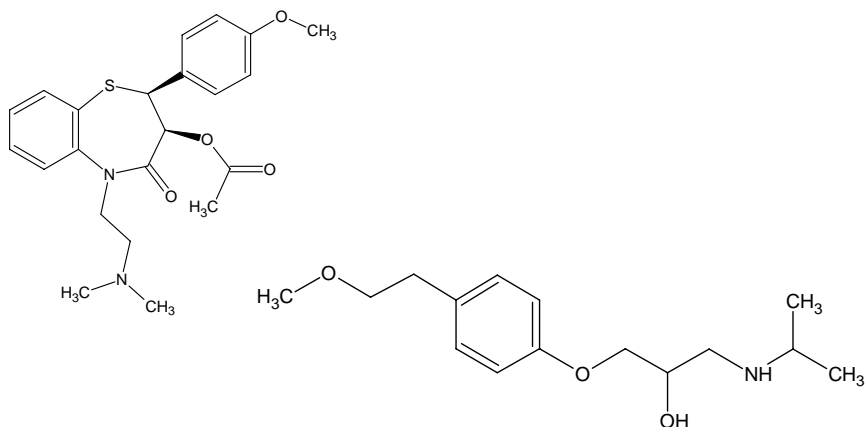
Det.: MS, ESI (+)

Temp.: 40°C

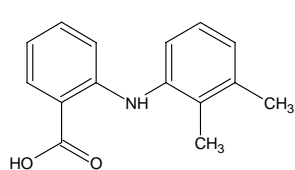
Inj.: 5 μ L

Sample: 1 μ g/mL diltiazem, metoprolol in (60:40) water:acetonitrile

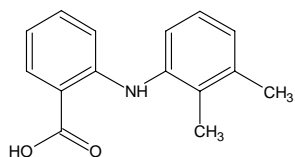
• Good peak shape, selectivity and retention on modified Zr-PBD



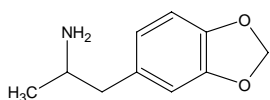
Amphetamines on C18 vs. Modified Zr-PBD



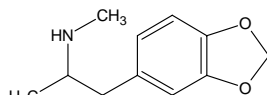
amphetamine



methamphetamine

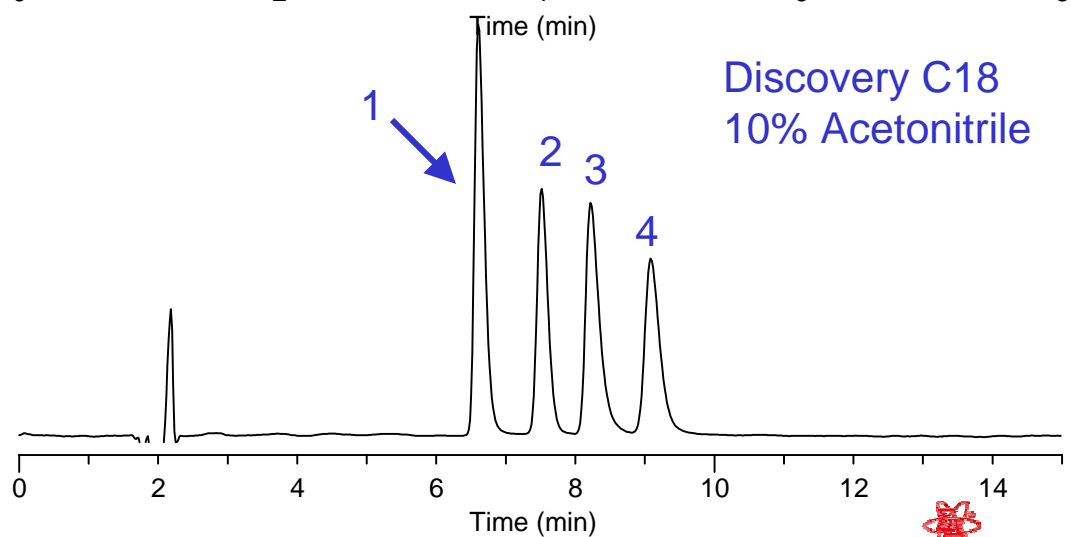
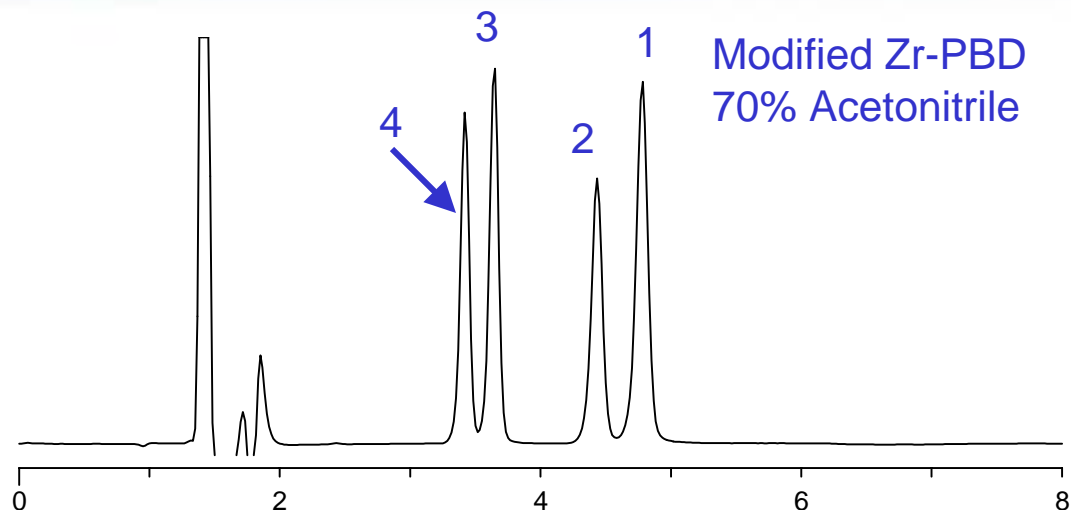


MDA

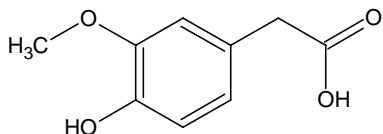


MDMA

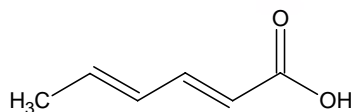
1. Amphetamine
2. MDA
3. Methamphetamine
4. MDMA



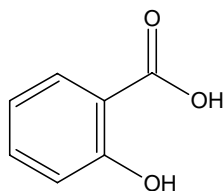
Acidic Analytes on Modified Zr-PBD



homovanillic acid



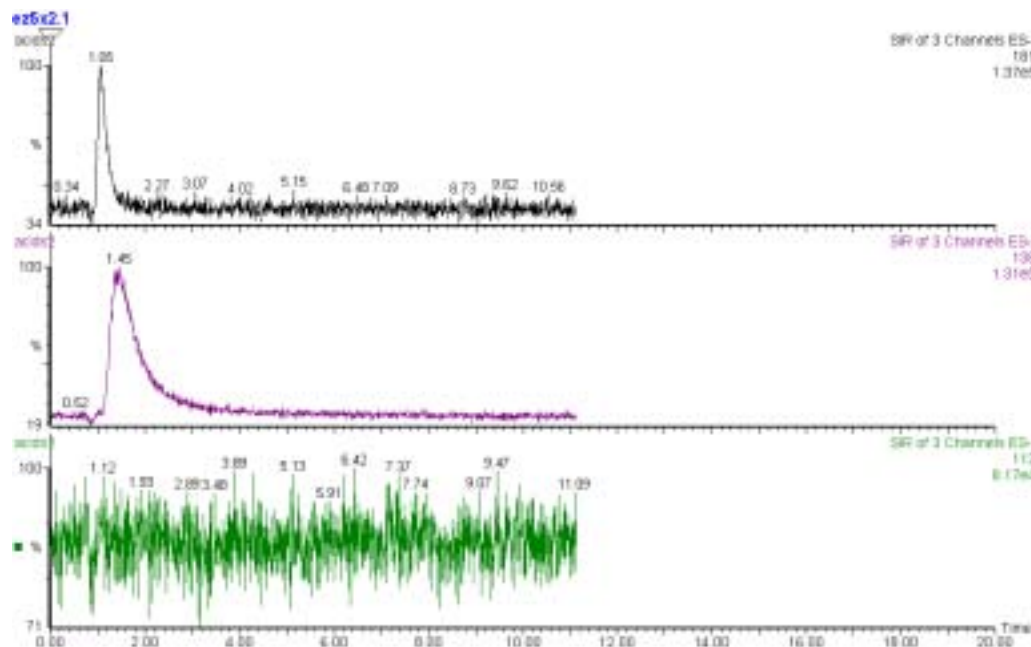
sorbic acid



salicylic acid

Conditions:

Column: Modified Zr-PBD,
5cm x 2.1mm ID, 3 μ m
Mobile Phase: 10mM ammonium formate,
pH 3.5:CH₃OH
Flow Rate: 0.2mL/min
Det.: MS, ESI (-)
Temp.: 40°C
Injection: 10mL
Sample: 1mg/mL each in (50:50) water:CH₃OH



Chelators on Modified Zr-PBD

Conditions:

Column: Modified Zr-PBD,
15cm x 4.6mm ID, 5 μ m particles

Mobile Phase: (45:55) 0.1% Formic Acid:
0.1% Formic Acid in CH₃OH

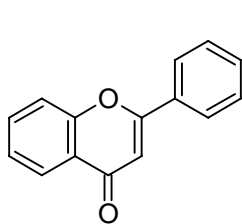
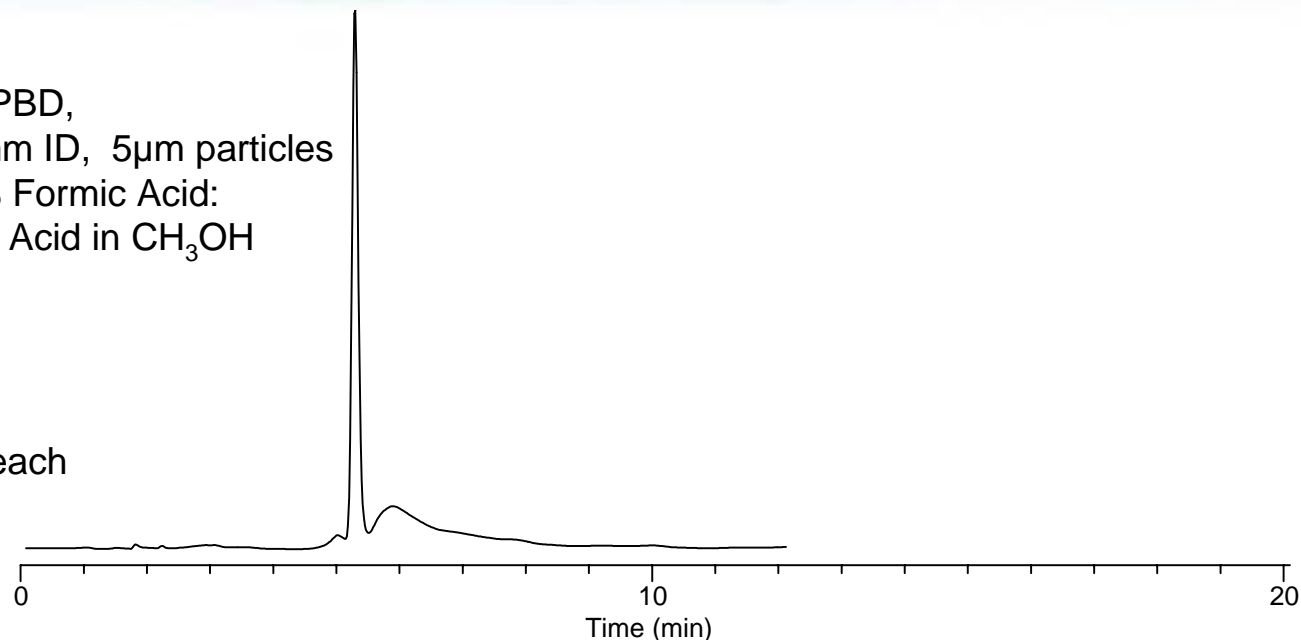
Flow Rate: 1mL/min

Det.: UV, 254nm

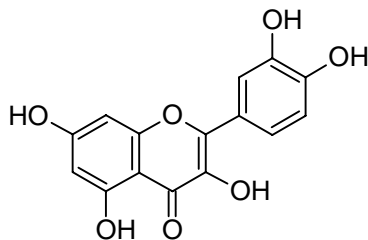
Temp.: 35C

Inj.: 10 μ L

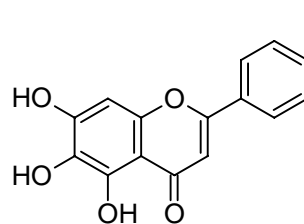
Sample: 25 μ g/mL of each



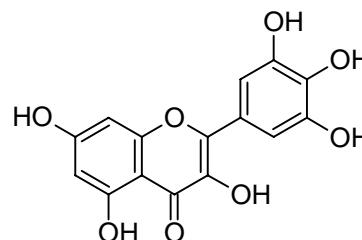
Flavone



Quercetin



Baicalein



Myricetin



LC/MS Bleed Studies

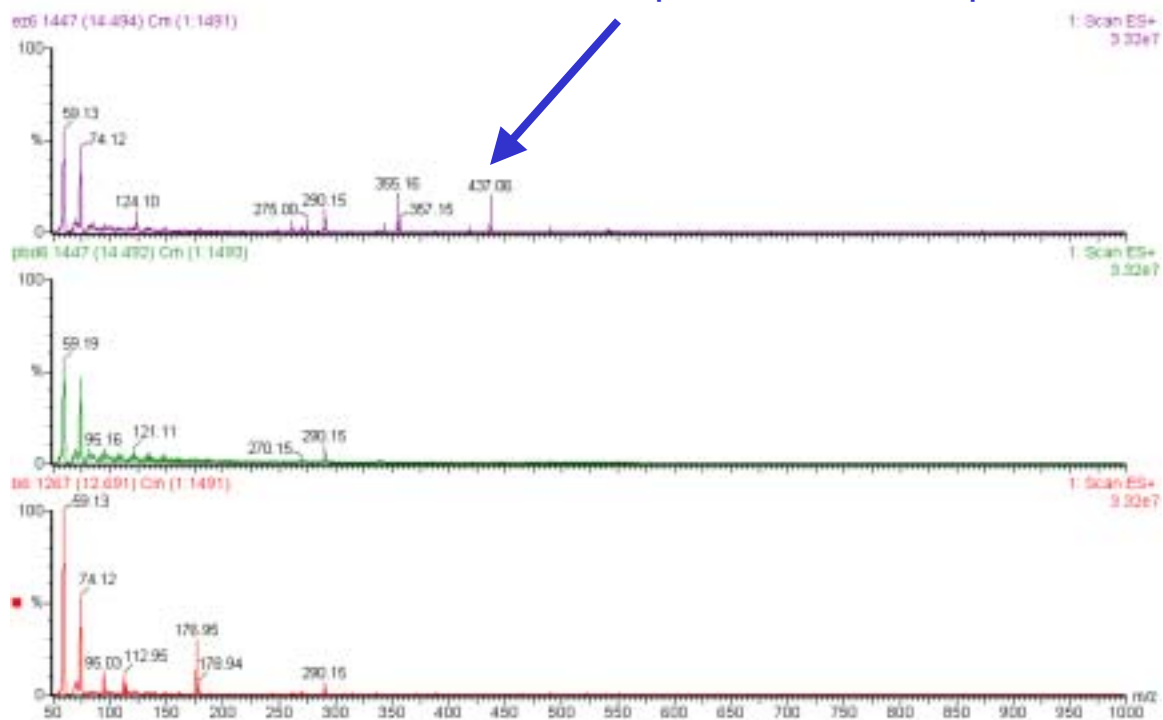
Method:

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

LC/MS Bleed Studies

Bleed was only observed at High pH (>10)

Expected mass response





Summary

- **Discovery Zr phases: possible retention modes.**
 - **Typical RP partition interaction depending on phase and solute chemical and physical structures.**
 - **Highly LC/MS compatible for neutral molecules**
 - **Secondary interactions do not pose any restrictions**
 - **Ion-exchange with adsorbed Lewis base buffer anions.**
 - **Ion-exchange due to presence of phosphate – not LC/MS compatible**
 - **Removal of phosphate results in:**
 - **Lower retention of basic analytes**
 - **Lewis base interactions when dealing with organic acids**
 - **Chelation**



Summary

- **Research on Lewis “endcapped” Zr phase has shown promising results for LC/MS compatibility using ionic analytes**
 - Excellent retention of basic analytes under LC/MS compatible conditions
 - Alternative selectivity to silica-based C18
 - No “endcapping” reagent bleed except at high pH
 - Still issues with acidic and chelating analytes
- **Continued research is underway to produce LC/MS-compatible Zr phases that take advantage of the unique selectivity**



Acknowledgements

- **Carmen Santasania and Shawn R. Wyatt (Supelco)**
- **Tracy Ascah-Ross (Penn State)**
- **Dwight Stoll (University of Minnesota)**