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

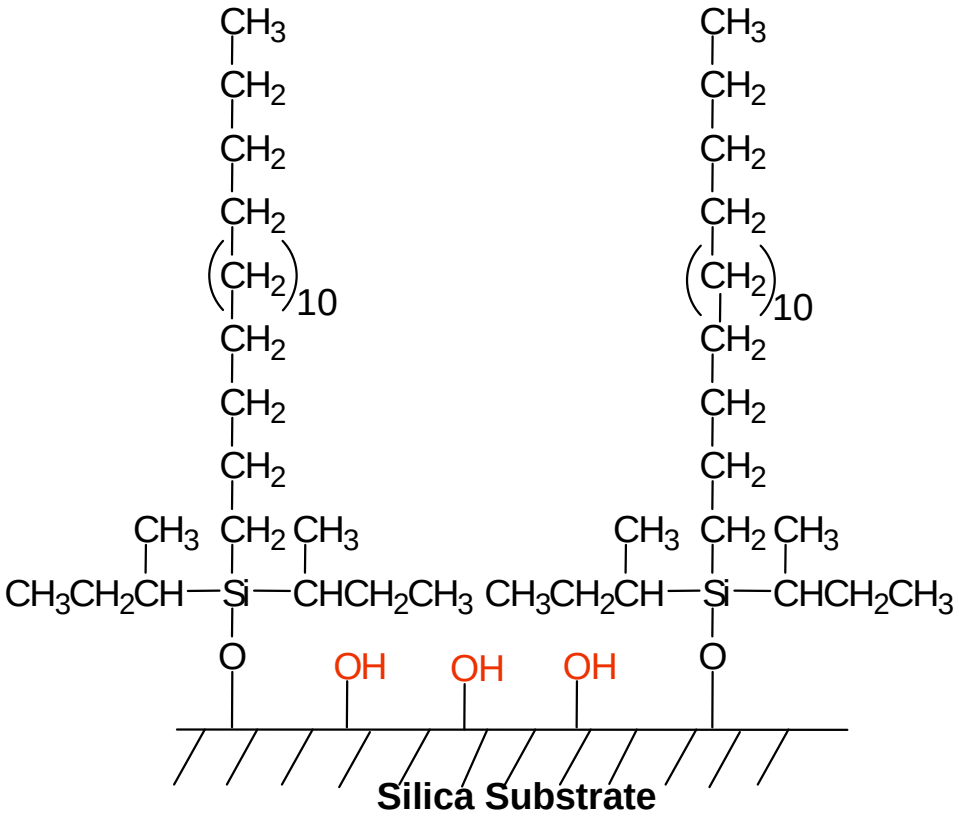
- **Several modified zirconia phases are commercially available including polybutadiene (PBD), polystyrene (PS), carbon and C18-modified carbon (carbonC18).**

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**

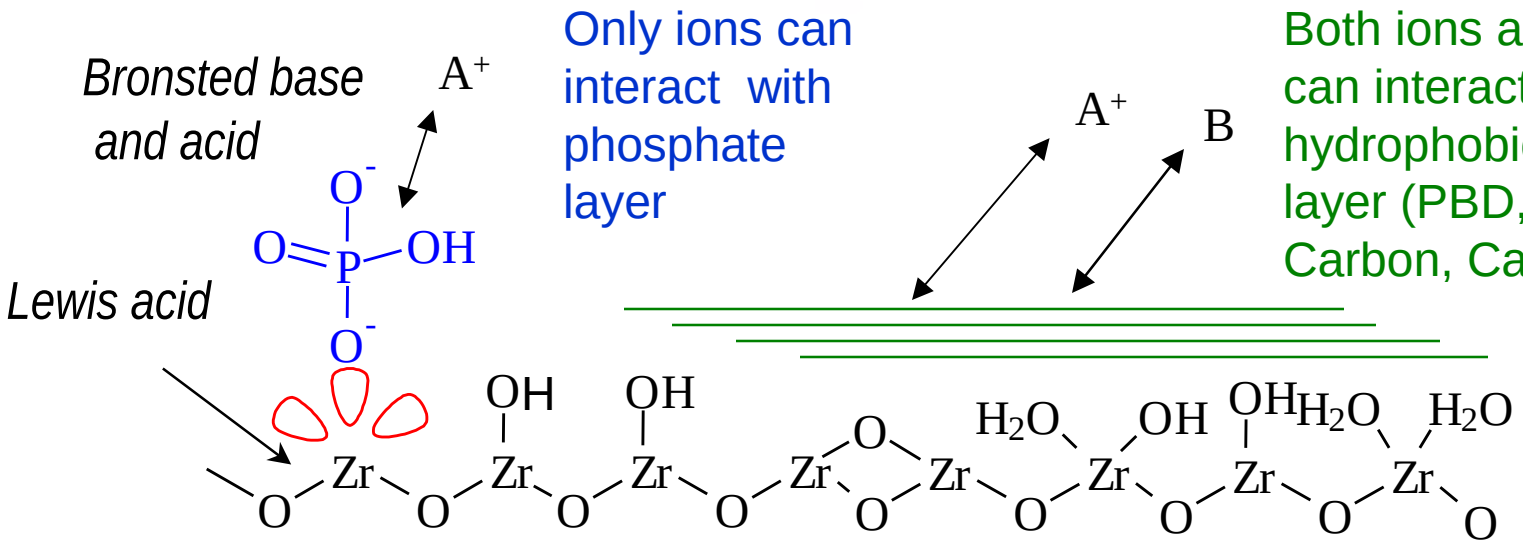
- **This study was aimed at:**
  - Assessing the need for phosphate in various systems
  - Determining if existing Zr phases could be LC/MS compatible
  - Investigating Lewis acid endcapping as a viable solution

Silica C18 Structure



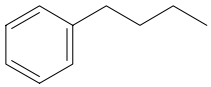
- **Strong efforts have been made by the silica-based column manufacturers to mask the effects of silanols, causing these columns to become more similar to each other.**
- **There is an increasing need for columns which have different selectivity than silica-based bonded phases.**

Origins of Unique Selectivity on Zirconia

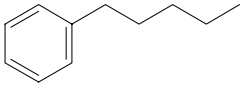


- Zirconia, as a transition metal oxide, has very rich, reproducible surface chemistry.
- Coated zirconia (Carbon and PBD) has mixed-mode surface properties (RPC and IEC) which allow simultaneous nonpolar and polar interactions.
- The retention of various basic and acidic analytes can be fine-tuned by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

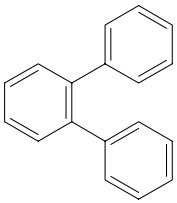
Neutral Analyte Comparison



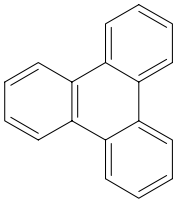
Butylbenzene



Pentylbenzene



o-Terphenyl

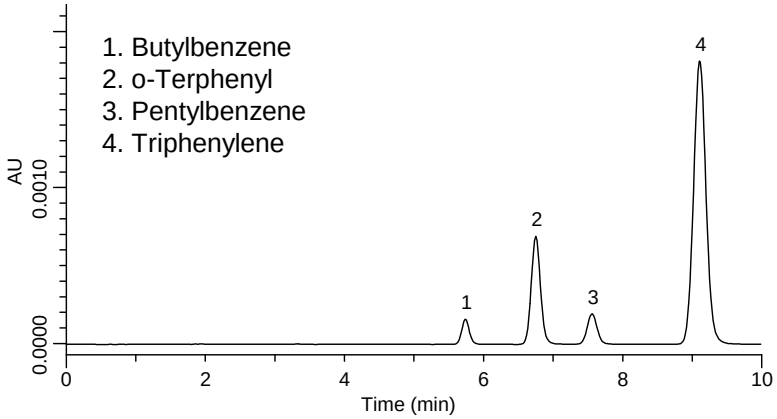


Triphenylene

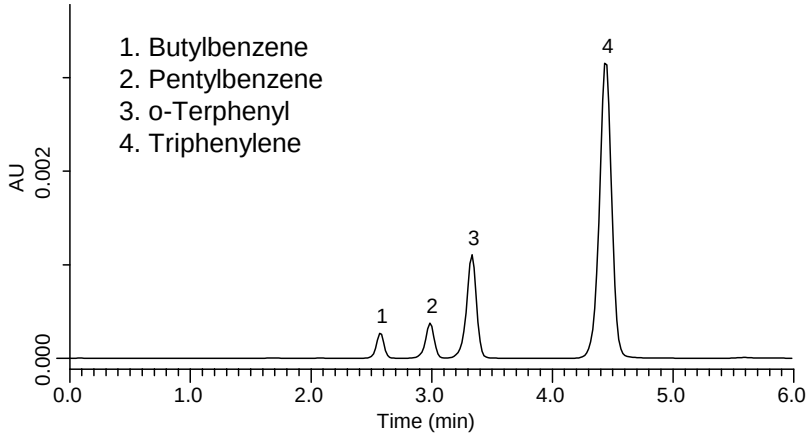
Conditions:

Columns:	15cm x 4.6mm ID, 5µm
Mobile Phase:	20:80, water:methanol
Flow Rate:	1.0mL/min
Temperature:	40ºC
Injection Volume:	5µL
Detection:	UV, 254nm

Discovery C18



Discovery Zr-PBD



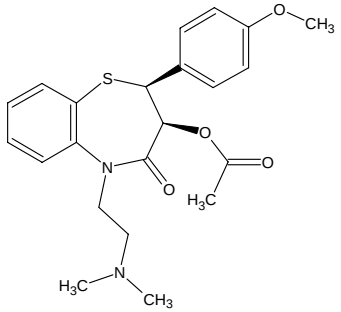
Bases on Discovery Zr-PBD with Phosphate

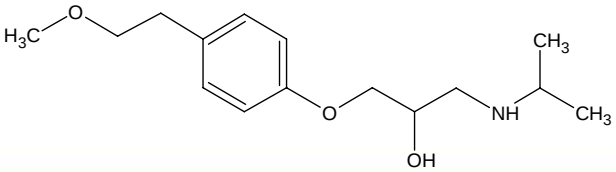
Conditions:

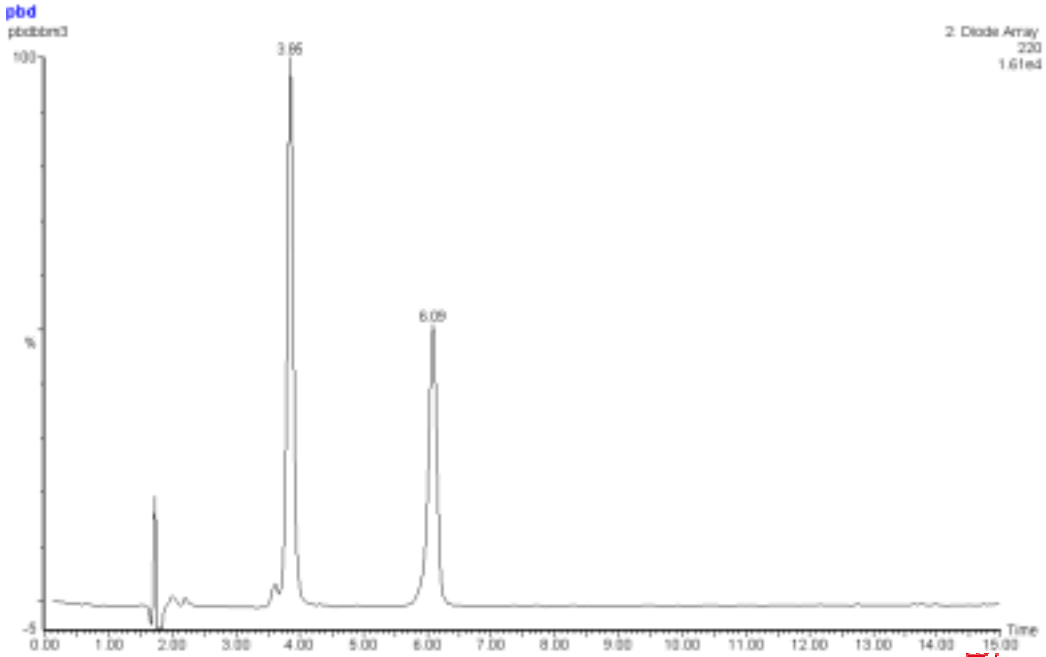
Column:	Discovery Zr-PBD, 15cm x 4.6mm ID, 5µm particles
Mobile Phase:	(70:30) 25mM potassium phosphate, pH 3.0 :CH_3CN
Flow Rate:	1.0mL/min
Det.:	UV, 220nm
Temp.:	35°C
Inj.:	10µL
Sample:	25µg/mL diltiazem, metoprolol in (50:50) 25mM potassium phosphate, pH 3.0:acetonitrile

- Good peak shape, selectivity and retention

With phosphate in MP





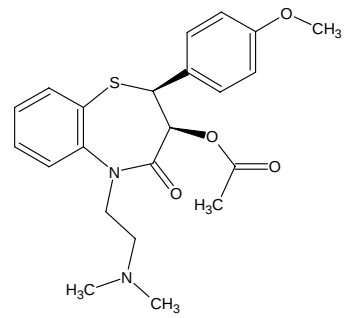


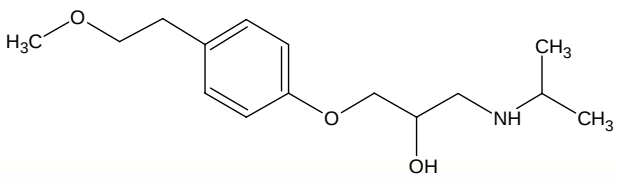
Bases on Discovery Zr-PBD Without Phosphate

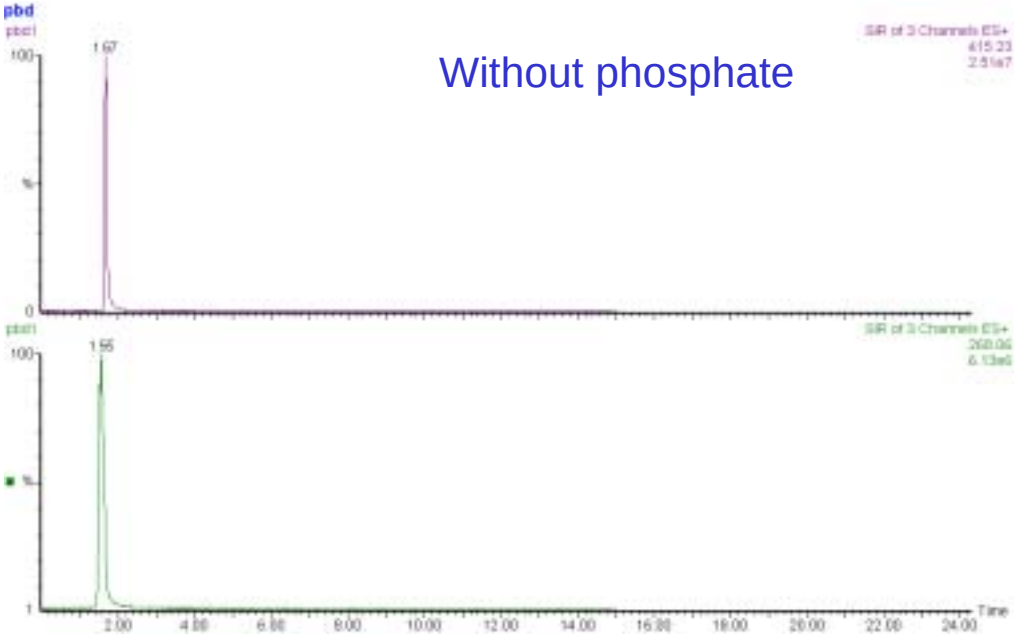
Conditions:

Column:	Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
Mobile Phase:	(60:40) 10mM ammonium acetate, unadjusted :CH_3CN
Flow Rate:	0.2mL/min
Det.:	ms, esi (+)
Temp.:	40°C
Inj.:	5µL
Sample:	1µg/mL diltiazem, metoprolol in (60:40) water:acetonitrile

- Lack of retention





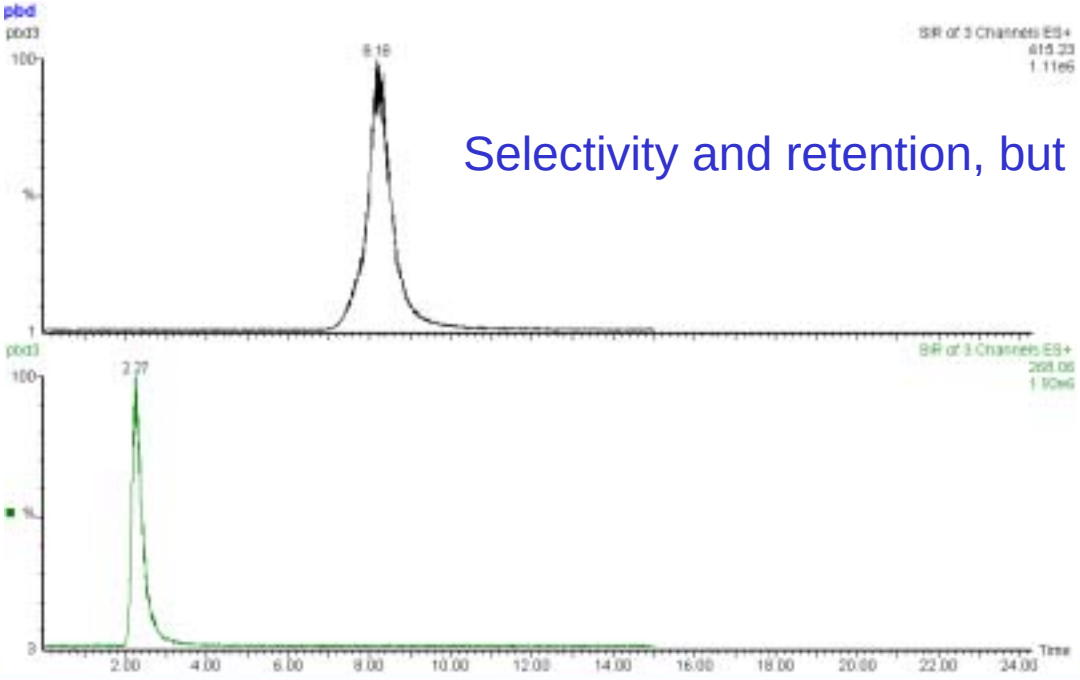


Without phosphate

Bases on Discovery Zr-PBD – Lowered Organic Percentage

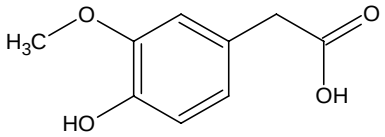
Conditions:

Column:	Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
Mobile Phase:	(80:20) 10mM ammonium acetate, unadjusted :CH_3CN
Flow Rate:	0.2mL/min
Det.:	ms, esi (+)
Temp.:	40ºC
Inj.:	5µL
Sample:	1µg/mL diltiazem, metoprolol in (60:40) water:acetonitrile

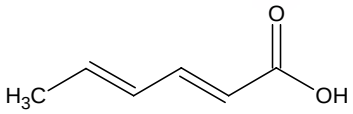


Selectivity and retention, but poor peak shape

Acidic Compounds on Discovery Zr-PBD

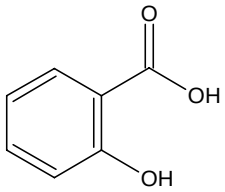


homovanillic acid



sorbic acid

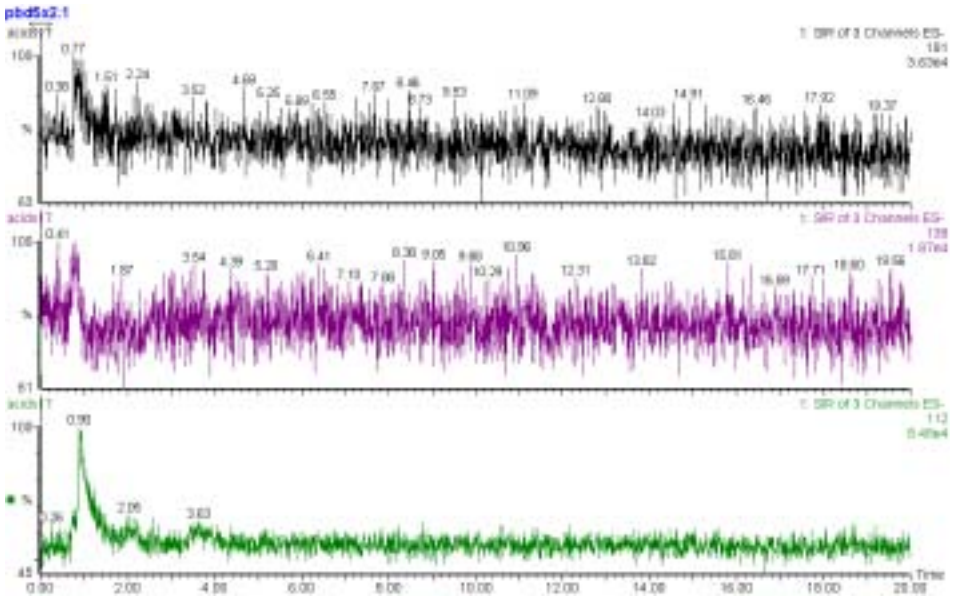
No elution – Lewis base activity



salicylic acid

Conditions:

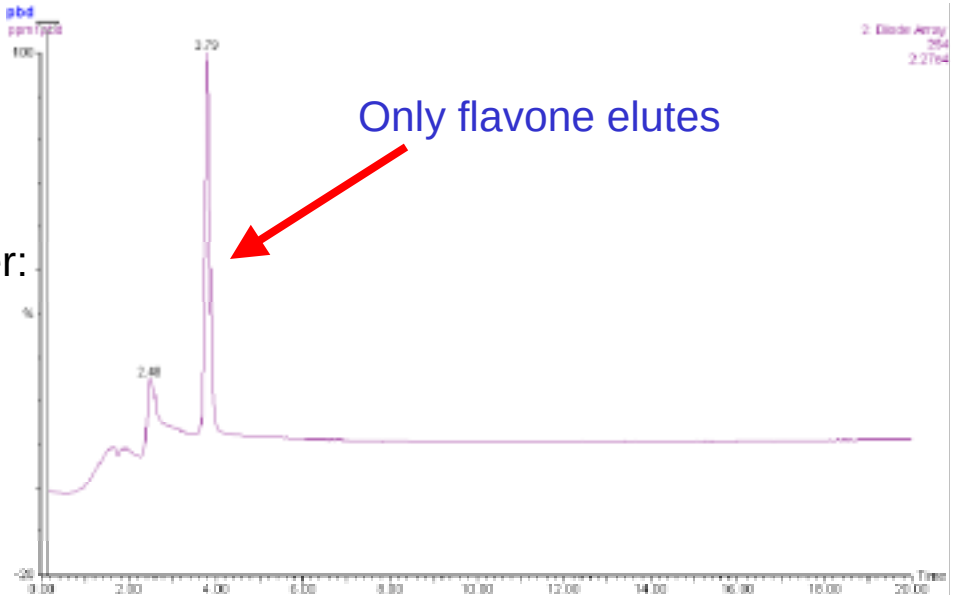
Column:	Discovery Zr-PBD, 5cm x 2.1mm ID, 3µm particles
Mobile Phase:	(95:5) 10mM ammonium formate, pH 3.50 :CH_3CN
Flow Rate:	0.2 mL/min
Det.:	ms, esi (-)
Temp.:	40°C
Inj.:	10µL
Sample:	1µg/mL each in (50:50) water:methanol

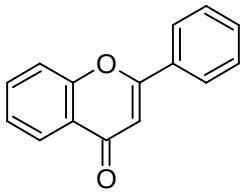


Chelators on Discovery Zr-PBD

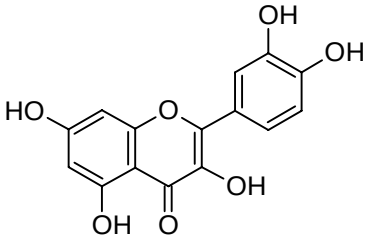
Conditions:

Column:	Discovery Zr-PBD, 15cm x 4.6mm, 5µm particles
Cat. No.:	65723-U
Mobile Phase:	45:55, 0.1% formic acid in water: 0.1% formic acid in CH_3OH
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)

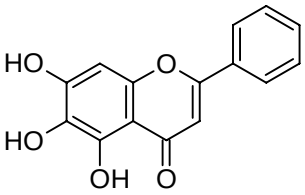




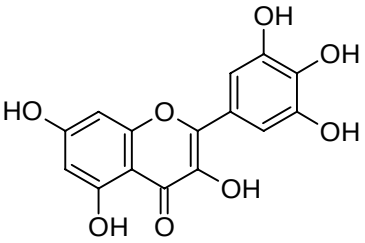
Flavone



Quercetin



Baicalein

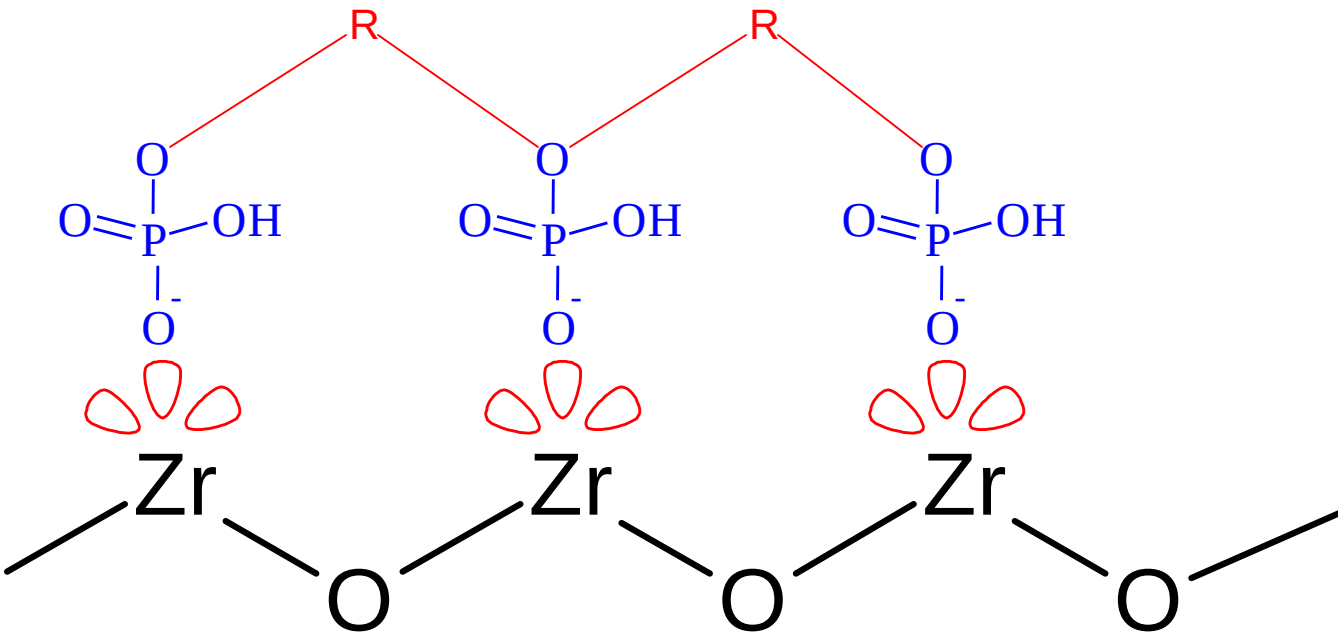


Myricetin

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



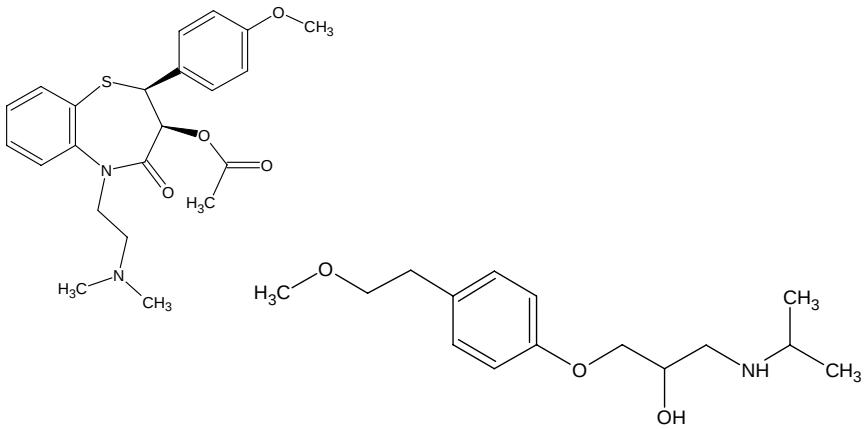
Add reagent with multiple Lewis base sites to:

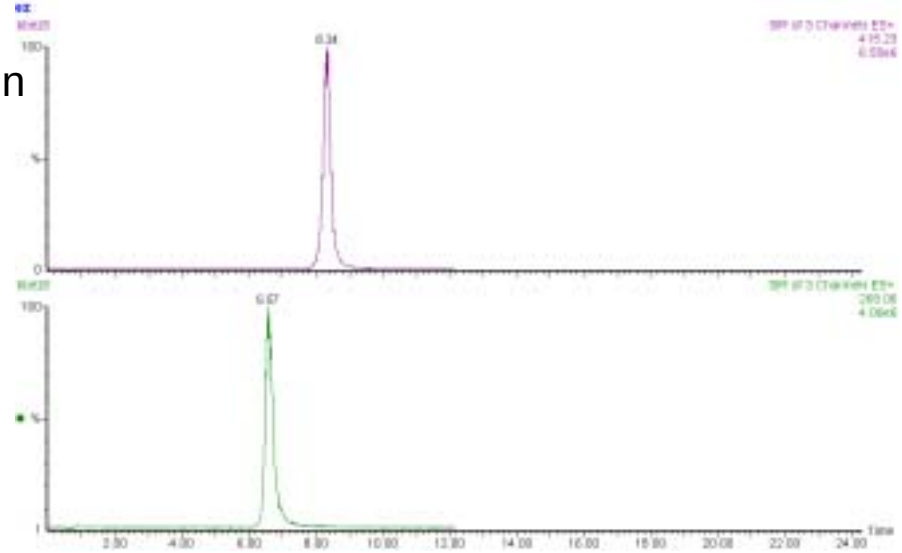
1. Mask Lewis acid character
2. Provide negative charge for IEX

Solution for LC/MS of Ionic Species on Zr

Conditions:

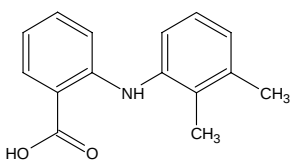
Column:	Modified Zr-PBD, 5cm x 2.1mm ID, 3µm particles
Mobile Phase:	(60:40) 10mM ammonium acetate, unadjusted :CH_3CN
Flow Rate:	0.2mL/min
Det.:	MS, ESI (+)
Temp.:	40ºC
Inj.:	5µL
Sample:	1µg/mL diltiazem, metoprolol in (60:40) water:acetonitrile



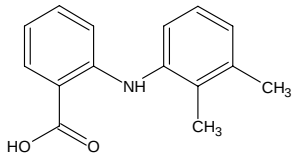


- Good peak shape, selectivity and retention on modified Zr-PBD

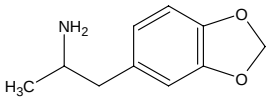
Amphetamines on C18 vs. Modified Zr-PBD



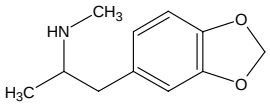
amphetamine



methamphetamine

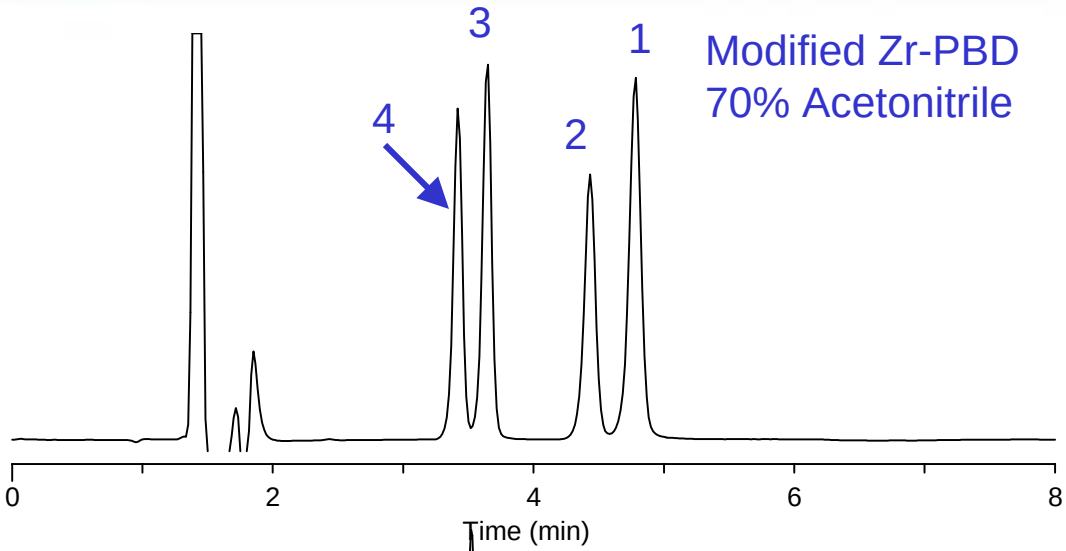


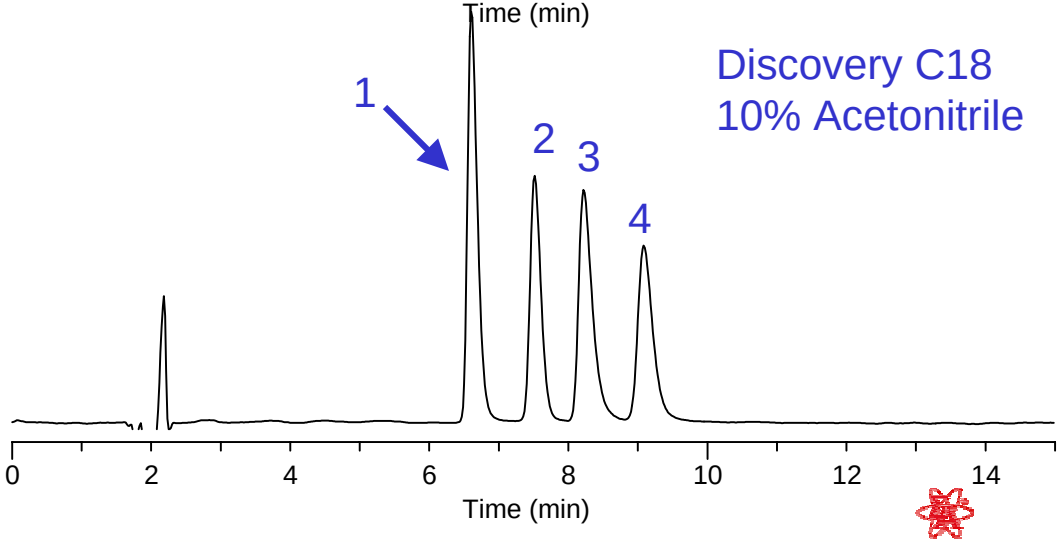
MDA



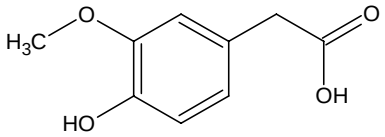
MDMA

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

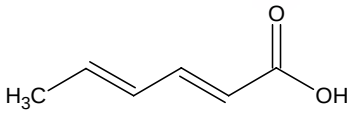




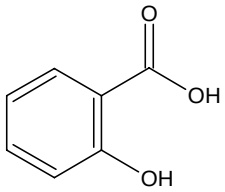
Acidic Analytes on Modified Zr-PBD



homovanillic acid



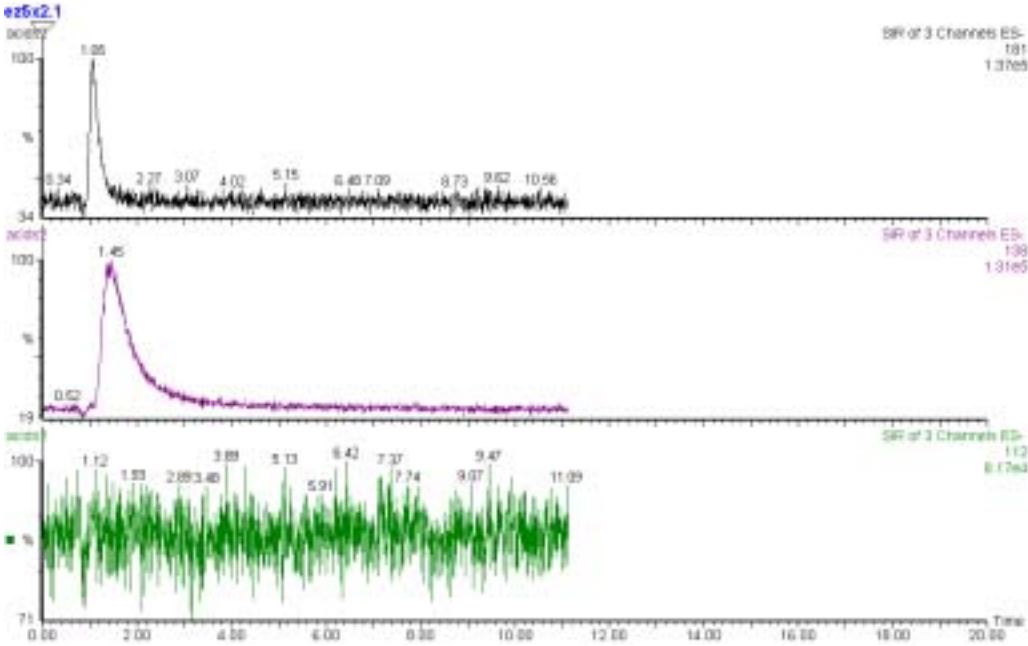
sorbic acid



salicylic acid

Conditions:

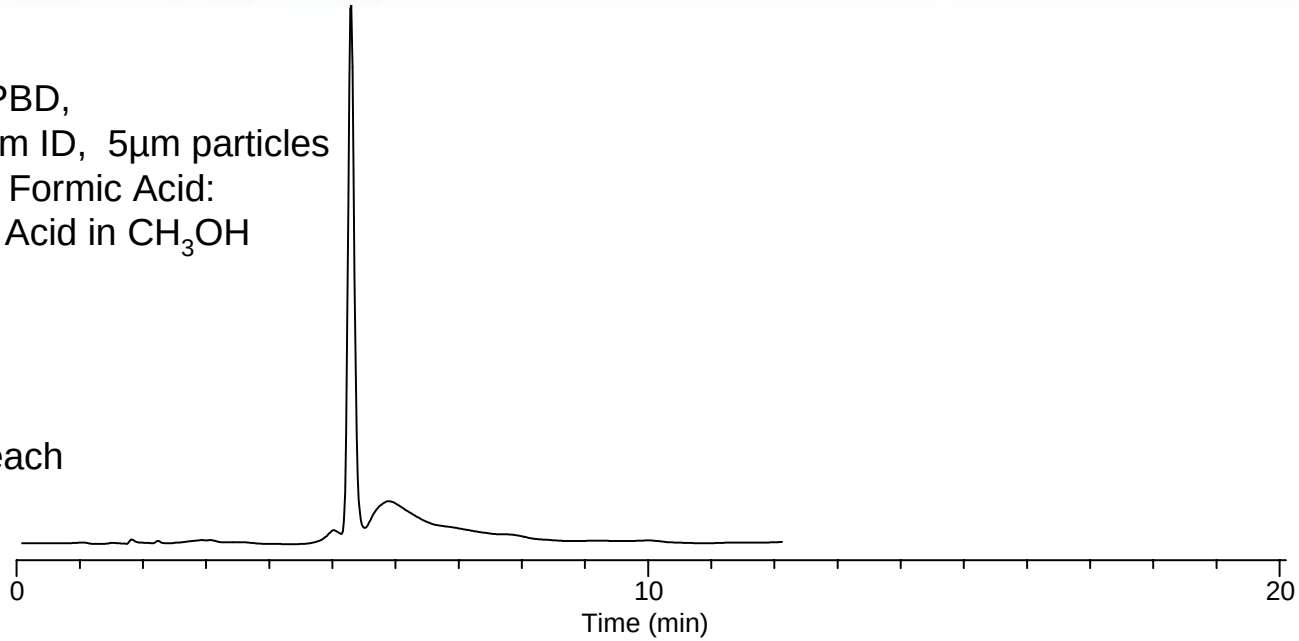
Column:	Modified Zr-PBD, 5cm x 2.1mm ID, 3µm
Mobile Phase:	10mM ammonium formate, pH 3.5:CH_3OH
Flow Rate:	0.2mL/min
Det.:	MS, ESI (-)
Temp.:	40°C
Injection:	10mL
Sample:	1mg/mL each in (50:50) water:CH_3OH

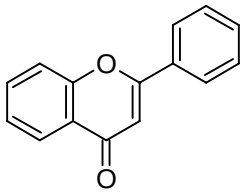


Chelators on Modified Zr-PBD

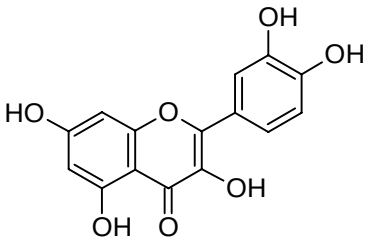
Conditions:

Column:	Modified Zr-PBD, 15cm x 4.6mm ID, 5µm particles
Mobile Phase:	(45:55) 0.1% Formic Acid: 0.1% Formic Acid in CH_3OH
Flow Rate:	1mL/min
Det.:	UV, 254nm
Temp.:	35C
Inj.:	10µL
Sample:	25µg/mL of each

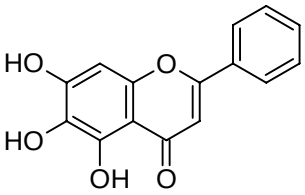




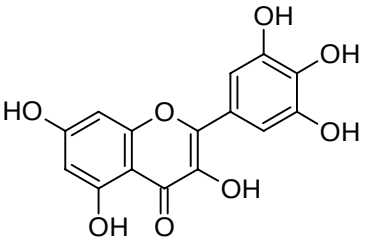
Flavone



Quercetin



Baicalein



Myricetin

LC/MS Bleed Studies

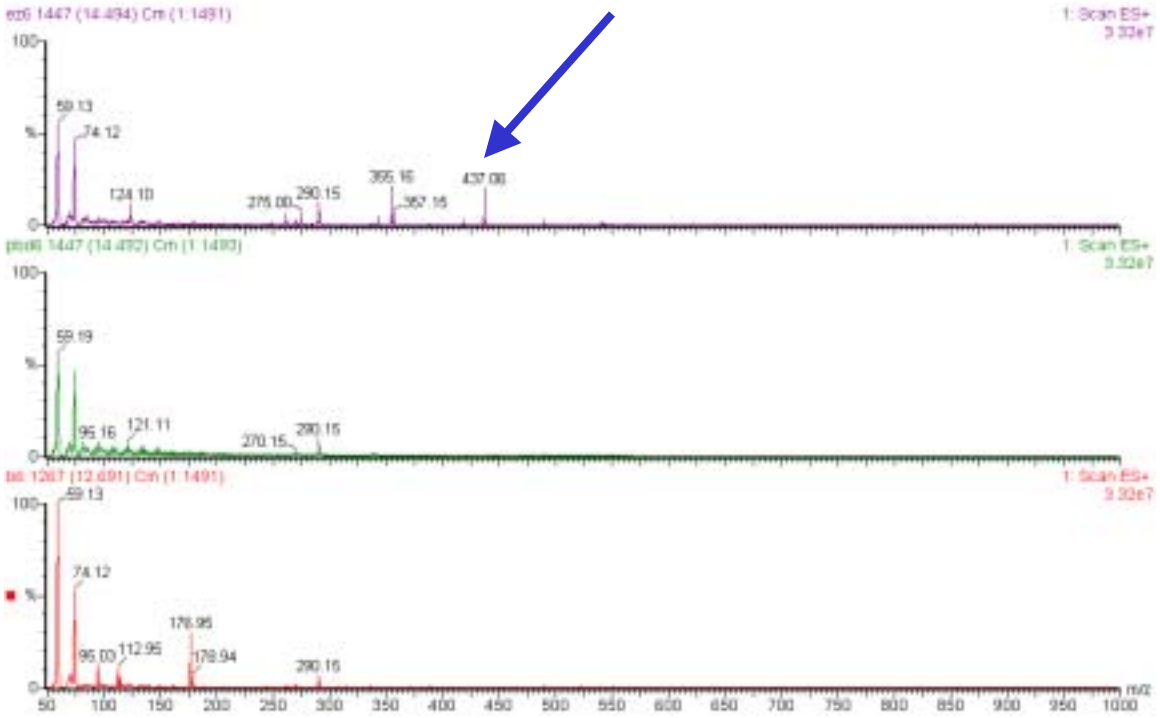
Method:

- **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 ºC**
- **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)