Ion-Exchange Separations on Zirconia-based Stationary Phases

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Specialists in High Efficiency, Ultra-Stable Phases for HPLC
Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid:  \[ \text{Zr(IV): } \text{H}_2\text{O} + \text{RPO}_3^{2-} \rightleftharpoons \text{Zr(IV): } \text{RPO}_3^{2-} + \text{H}_2\text{O} \]

Other Lewis base examples:  \( \text{PO}_4^{3-}, \text{RCO}_2^{-}, \text{Catechol} \)
Interaction Strength of Lewis Bases with Zirconia

<table>
<thead>
<tr>
<th>Interaction Strength</th>
<th>Lewis Base (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strongest</strong></td>
<td>Hydroxide</td>
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<tr>
<td></td>
<td>Phosphate</td>
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<tr>
<td></td>
<td>Fluoride</td>
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<tr>
<td></td>
<td>Citrate</td>
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<tr>
<td></td>
<td>Sulfate</td>
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<tr>
<td></td>
<td>Acetate</td>
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<td></td>
<td>Formate</td>
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<tr>
<td></td>
<td>Nitrate</td>
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<td></td>
<td>Chloride</td>
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<tr>
<td></td>
<td>Water</td>
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<tr>
<td><strong>Weakest</strong></td>
<td>Small Lewis bases with high electron density and low polarizability interact more strongly with Zr atoms.</td>
</tr>
</tbody>
</table>

Retention of Basic Analytes on ZirChrom®-PBD and ZirChrom®-PS

- **PBD, PS Coating — Reversed-Phase (RP) Moieties**

- **Lewis Base Anions — Ion-Exchange (IEX) Sites**

\[
\text{Zr}-L^- : X^+ + A^+ = \text{Zr}-L^- : A^+ + X^+
\]

\[
\text{Zr}-O^- : X^+ + A^+ = \text{Zr}-O^- : A^+ + X^+
\]

A\(^+\): analyte cation, X\(^+\): counterion, L\(^-\): adsorbed Lewis base anion.
Retention Comparison: Si-C18 vs Zr-PBD

LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 °C; Detection at 254 nm; Columns, ZirChrom®-PBD, 50 x 4.6 mm i.d. (3 um particles); Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).
Effect of Ionic Strength on the Separation of Quaternary Amines

**Column:** Discovery® Zr-PS, 7.5 cm x 2.1 mm ID, 3µ particles

**Mobile Phase 1:** 50:50, (20 mM H₃PO₄, 40 mM NH₄HCO₃, pH 7.0 w/ NH₄OH) : Acetonitrile

**Mobile Phase 2:** 50:50, (20 mM H₃PO₄, 100 mM NH₄HCO₃, pH 7.0 w/ NH₄OH) : Acetonitrile

**Flow:** 0.2 mL/min

**Temp:** as indicated

**Det:** UV at 250nm & 305nm

**Inj:** 1 µL

**Sample:** diquat and paraquat in water; 50 mg/L ea.

- k values for diquat are 25-30 at low ionic strength in 50% ACN.

- k values for diquat decrease to about 5 at high ionic strength without changing %ACN.

- The classic method for reducing k in IE mode is to increase ionic strength, confirming IE mode.

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4 Data used by permission of Supelco
Effect of Reversed Phase Character on the Separation of Quaternary Amines

Column: Discovery® Zr-PS, 150 cm x 2.1 mm ID, 3µm particles
Mobile Phase 1: 50: 20 : 30, (20 mM H₃PO₄, 100 mM NH₄HCO₃, pH 7.0 w/ NH₄OH) : Water : Acetonitrile
Mobile Phase 2: 50: 40 : 10, (20 mM H₃PO₄, 100 mM NH₄HCO₃, pH 7.0 w/ NH₄OH) : Water : Acetonitrile
Flow: 0.3 mL/min
Temp: 50° C
Det: UV at 250nm & 305nm
Inj: 1 µL
Sample: diquat & paraquat in water; 100 mg/L ea.

- At 30% ACN, the polymer coating adds very little to retention or selectivity for these ionic compounds.
- When nonionic compounds are present, changes in organic solvent strength will have a greater impact and can be used for optimizing resolution.

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**LC Conditions**

Discovery® Zr-PBD 100mm x 2.1mm i.d., 3 μm  
Mobile Phase A: 50:50 [20 mM H₃PO₄, pH 7.0 w/ NH₄OH]:water  
Mobile Phase B: 50:30:20 [20 mM H₃PO₄, pH 7.0 w/ NH₄OH]:water:ACN  
Gradient 90:10 to 0:100 A:B over 18 minutes  
Temp = 80 °C, Flow = 0.3 mL/min, Inj vol = 2 μL,  
UV 225 nm, sample in ~60:40 Mobile phase A:MeOH

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1. Pipenzolate (20 mg/L)  
2. Scopolamine (100 mg/L)  
3. Ipratropium (100 mg/L)  
4. Methscopolamine (100 mg/L)  
5. Propantheline (20 mg/L)  
6. Oxyphenonium (100 mg/L)

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Data used by permission of Supelco
ZirChrom-PBD Separation of Catecholamines: sub-2μm vs 3μm, T = 30 °C

**Conditions**
- Column: ZirChrom-PBD 50mm x 4.6mm
- Mobile Phase: 85/15 ACN/30mM NH₄OAc, 10mM NH₄H₂PO₄ adjusted to pH=3.4 w/ HCl
- Flow rate = 1.5 mL/min
- Temperature = 30°C
- Inj Vol = 5 μL
- Elution order: 1=Tyramine, 2=Epinephrine, 3=Dopamine, 4=3,4-dihydroxynorephedrine

- Smaller particle gives increased efficiency
- Good reproducibility
- Fast separation times
1. Chemisorb Ethylenediamine N,N,N’,N’-tetra(methylene phosphonic) 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.
ZirChrom®-MS Multi-Mode Character

- Multi-mode retention on ZirChrom®-MS (MS01-0546)
- Addition of fluoride blocks Lewis acid sites, decreasing the ligand exchange between zirconia and maleic acid.

25/75 ACN/25mM NH₄OAc pH=5, T=30 °C, F=1mL/min, UV=210

25/75 ACN/25mM NH₄OAc, 2mM NH₄F pH=5, T=30 °C, F=1mL/min, UV=210

1, Maleic acid  2, Benzonitrile
3, Benzyl amine

HO₂C\(\equiv\)CO₂H
\[\text{phenyl} \quad \text{CN}\]

\[\text{ phenyl} \quad \text{NH}_2\]
Beta Blockers on ZirChrom®-MS

**LC Conditions:** 65/35 ACN/10 mM ammonium acetate pH=5.0; Flow rate, 1.0 mL/min.; Injection volume 5.0 μL; Temperature, 35 °C; Detection at 254 nm; Columns, ZirChrom®-MS, 50 x 4.6 mm i.d. (3 um particles)

- Multi-mode retention creates high k’ for bases even in high organic.
- Bases are charged at pH 5 and can interact strongly with negative charges on phosphonate groups of EDTPA.
- High organic mobile phase with ammonium acetate is ideal for LC-MS.
HPLC Analysis of Cattle Plasma Using ZirChrom®-MS

- Analysis of pipecolic acid and lysine levels in cattle plasma ZirChrom®-MS (MS01-1546).
- IEX and RP modes contribute to performing this difficult separation.
- Organic solvent, pH and ionic strength are all important variables with ZirChrom-MS.

• Analysis of pipecolic acid and lysine levels in cattle plasma ZirChrom®-MS (MS01-1546).

5 Data used by permission of SarTec Corporation (Anoka, MN)
Surface Chemistry and Retention Mechanisms of QPEI-Zirconia

- Anion-exchange
- Hydrophobic interactions
- Lewis acid-base interactions
Water-Soluble Vitamin Analysis on ZirChrom®-SAX

1 - Thiamine (Vit. B₁)
2 - Pyridoxine (Vit. B₆)
3 - Nicotinamide (form of Vit. B₃)
4 - Riboflavin (Vit. B₂)
5 - Nicotinic acid (form of Vit. B₃)
6 - Ascorbic acid (Vit. C)

Vitamin C is strongly retained on ZirChrom®-SAX

LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm i.d. (part number: ZR06-1546), Mobile Phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5, Flow rate: 1.0 mL/min. Temperature: 30 °C, Injection Vol.: 5.0 μL, Detection: UV at 254 nm
• Mixed-mode applications have become popular for difficult applications where compounds vary widely in chemical nature.
• Several ZirChrom® phases, including Zr-PBD, Zr-PS, Zr-MS and Zr-SAX, are ideal for mixed-mode applications and show unique selectivity.
• ZirChrom® phases are stable and reproducible over a wider range of pH and temperature than silica-based phases.

For more information contact ZirChrom support at www.zirchrom.com or stop by Booth 746.

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