Synthesis of a Novel C18 Polar-Embedded Reversed-Phase Zirconia For HPLC

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Outline

• Project Goal – First *Stable* Bonded Phase C18 Zirconia Stationary Phase.
• Zirconia Surface Chemistry
• Synthesis of C18 Bonded Phases
• Chromatographic Data
  • *Selectivity* Comparison between Silica-C18 and Zr-C18
  • *Chemical Stability* Testing
  • *Example Applications*
• **Conclusion** – The new Zr-C18 column is stable from pH 1 to 9 and has *very different chromatographic selectivity for basic compounds compared to Silica-C18*. The column also performs well under MS-compatible conditions.
Project Goal

To produce a first of its kind bonded phase C18 Reversed-Phase Zirconia Stationary Phase that has unique selectivity and good peak shape for basic drugs.
Zirconia -
The difference is the surface chemistry.
Surface Chemistry of Zirconia

**Brönsted Acid:** \( \text{ZrOH} + \text{-OH} \rightleftharpoons \text{ZrO}^- + \text{H}_2\text{O} \)

**Brönsted Base:** \( \text{Zr} \begin{array}{c} \text{O} \end{array} \text{Zr} + \text{H}^+ \rightleftharpoons \text{Zr} \begin{array}{c} \text{O} \end{array} \text{Zr} \)

**Lewis Acid:** \( \text{Zr}^{4+}: \text{H}_2\text{O} + \text{R-COO}^- \rightleftharpoons \text{Zr}^{4+}:\text{OOC-R} + \text{H}_2\text{O} \)

**RPO}_3^{2-} or Catechol**
Zr-C18 Synthesis

titania or zirconia substrate

Pamidronic Acid (PDA)

R = -(CH₂)$_{16}$CH₃

EEDQ
Selectivity: The Key to Success

Efficiency Retention Selectivity

$$R = \frac{\sqrt{N}}{4} \cdot \frac{k'}{k' + 1} \cdot \frac{\alpha - 1}{\alpha}$$

$$\alpha = \frac{k_j'}{k_i'}$$

➤ Selectivity ($\alpha$) has the greatest impact on improving resolution.
Selectivity Comparison Solutes

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<tr>
<td>Benzene</td>
<td>Toluene</td>
<td>Ethylbenzene</td>
<td>p-xylene</td>
<td>Propylbenzene</td>
<td>Butylbenzene</td>
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<tr>
<td>Bromobenzene</td>
<td>p-Dichlorobenzene</td>
<td>Anisole</td>
<td>Methylbenzoate</td>
<td>Napthalene</td>
<td>Acetophenone</td>
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<tr>
<td>Benzyalcohol</td>
<td>3-Phenyl Propanol</td>
<td>N-Benzyl Formamide</td>
<td>Phenol</td>
<td>p-Chlorophenol</td>
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Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Detection at 254nm; 5μl Injection volume.
Comparison of Selectivity for Zr-C18 and Silica-C18 for Neutral Solutes

Nonelectrolytes are less retained on Zr-C18 compared to Silica-C18.

- Benzyl formamide
- Benzyl alcohol
- Phenol
- 3-phenyl propanol
- p-chlorophenol
- Acetophenone
- Benzonitrile
- Nitrobenzene
- Methylbenzoate
- Anisole
- Benzene
- p-chlorotoluene
- p-nitrobenzyl chloride
- Toluene
- Benzophenone
- Bromobenzene
- Naphthalene
- Ethyl benzene
- p-xylene
- p-dichlorobenzene
- Propyl benzene
- Butyl benzene

Log k'

Solute Number

Silica-C18
Zr-C18
K–K Plot for 22 Solutes on Zr-C18 and Silica-C18

Zr-C18 has similar selectivity to Silica-C18 for nonelectrolytes.

Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Detection at 254nm; 5μl Injection volume.
Selectivity Study: Acidic Solutes

1. Benzoic acid
2. 4-hydroxybenzoic acid
3. 4-cyanobenzoic acid
4. 4-methoxybenzoic acid
5. 4-fluorobenzoic acid
6. 4-nitrobenzoic acid
7. 4-chlorobenzoic acid
8. 4-bromobenzoic acid
9. 4-iodobenzoic acid
Comparison Between Zr-C18 and Silica-C18

(1) Benzoic acids and phenols on Zr-C18 have much less retention than on silica.
(2) There is no selectivity difference between Zr-C18 and Silica-C18 for acidic compounds.

**LC Conditions:** Machine-mixed 74/26 ACN/25 mM phosphoric acid (H₃PO₄) pH=2.08 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 1 μl; Temperature, 35 °C; Detection at 254 nm; Columns, ZR-C18, 50 x 4.6 mm i.d. (3μm particles); Silica-C18 50 x 4.6 mm i.d.
Comparison of Retention of Basic Pharmaceuticals for Zr-C18 and Silica-C18

Basic drugs have much higher retained on Zr-C18 than on Silica-C18.

**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 μl; Temperature, 35 °C; Detection at 254 nm; Columns, Zr-C18, 50 x 4.6 mm i.d. (3um particles); Silica-C18 150 x 4.6 mm i.d., (3 um particles).
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 µl; Temperature, 35 ºC; Detection at 254 nm; Columns, Zr-C18, 50 x 4.6 mm i.d. (3µm particles); Silica-C18 150 x 4.6 mm i.d., (3 um particles).
**Chemical Stability**

**Exposure and Evaluation Conditions:** Mobile phase, 15/85 ACN/0.1M Nitric acid, pH 1.0, or 20 mM Ammonium hydrogen carbonate, pH 9.0; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 μl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. Zr-C18.
Phenols Separation on Zr-C18 and Silica-C18

LC Conditions: Machine-mixed 26/74 ACN/25 mM H₃PO₄ pH=2.08 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 °C; Detection at 254 nm; Solutes: (1) phenol, (2) 4-fluorophenol, (3) 4-nitrophenol, (4) 4-chlorophenol
Benzoic Acids Separation on Zr-C18 and Silica-C18

LC Conditions: Machine-mixed 26/74 ACN/25 mM H₃PO₄ pH=2.08 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 ul; Temperature, 35 °C; Detection at 254 nm; Solutes: (1) benzoic acid, (2) 4-nitrobenzoic acid, (3) 4-chlorobenzoic acid, (4) 4-bromobenzoic acid, (5) 4-iodobenzoic acid
Separation Comparison of Basic Pharmaceuticals on Zr-C18 and Silica C18

Compounds elute according to IEX, not RP interactions at near neutral pHs.

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, 5 ul; 35 °C; UV @ 254 nm; Solutes: (1) Methapyrilene, (2) Brompheniramine, (3) Doxepin, (4) Amitriptyline, (5) Nortryptiline.
LC-MS of UV Degraded Nifedipine on Zr-C18 and Silica C18

Mobile Phase:
(A) 0.1% ammonium acetate
(B) ACN
Flow rate: 1ml/min
Detection: MS, ESI (+)

Gradient program

<table>
<thead>
<tr>
<th>Time</th>
<th>A%</th>
<th>B%</th>
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<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
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<td>5</td>
</tr>
<tr>
<td>20</td>
<td>95</td>
<td>5</td>
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</tbody>
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Peak Identification
1. m/z 329
2. m/z 345
3. m/z 329
4. Nifedipine m/z 347
5. m/z 315
6. m/z 410
Conclusions

• The Zr-C18 phase is *a first of its kind* stable bonded phase *compatible with MS detection.*

• The Zr-C18 phase is *Lewis acid site deactivated.*

• The Zr-C18 phase has *similar selectivity and RP behavior* to Silica-C18 *for neutral and acidic compounds.*

• Zr-C18 *has very different selectivity* than Silica-C18 *for basic compounds.*

• Zr-C18 *is chemically stable from pH 1-9.*
Thanks very much for listening!

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