Fast Screening of Chiral Stationary Phases for Chiral Separations on Zirconia

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Specialists in High Efficiency, Ultra-Stable Phases for HPLC.
Goal—To Make Zirconia Based Chiral Stationary Phases for Fast Chiral Selector Screening

• Why Zirconia?
• Synthetic Approach
• Zirconia-based vs Silica-based CSPs
• Chromatographic Comparison of Different Anchors
• Stability Study
• Example Separations on Zirconia Based CSPs
• Use for Fast Chiral Selector Screening

• Conclusions – Zirconia Based CSPs Have Comparable Chromatographic Performance Compared to Silica Based CSPs. Fast Chiral Selector Screening is Possible.
Surface Chemistry of Zirconia

**Brönsted Acid:**

\[ \text{Zr OH} + \cdot \text{OH} \rightleftharpoons \text{Zr O} + \text{H}_2\text{O} \]

**Brönsted Base:**

\[ \text{Zr} \cdot \text{O} \text{Zr} + \text{H}^+ \rightleftharpoons \text{Zr} \text{H}^+ \text{O} \text{Zr} \]

**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
New Way to Attach Chiral Selectors to Zirconia Surface

= ZrO₂ = Lewis Base (Anchor)  △ = Chiral Selector
Three Anchors Studied

Anchors should have two function groups: (1) A group anchoring to zirconia surface, and (2) A group bonding to Chiral selector.

1) APPA (Aminopropylphosphonic acid)

2) DHNP (3,4-Dihydroxynorephedrine)

3) ASPA (Aspartic acid)
Chiral Selectors in This Study

- **DNB-LEU** (3,5-dinitrobenzoylLeucine)
- **DNB-PG** (3,5-dinitrobenzoylphenylglycine)
- **DNB-PRO** (3,5-dinitrobenzoylproline)
- **NAP-VAL** (Naphthoylvaline)
- **2-NAP-LEU** (2-Naphthoylleucine)
- **NAP** (naproxen)
Example of Lewis Acid-Base Modified Zirconia CSPs

Lewis acid-base reaction

Steric Site

H-donor

π-acceptor

EEDQ coupling reaction

(APP A)
List of Zirconia and Silica CSPs Studied

<table>
<thead>
<tr>
<th>Column</th>
<th>CSP</th>
<th>Anchor</th>
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<tbody>
<tr>
<td>Z1</td>
<td>DNB-Leu</td>
<td>APPA</td>
</tr>
<tr>
<td>Z2</td>
<td>DNB-Leu</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Z3</td>
<td>DNB-Leu</td>
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<tr>
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<tr>
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<td>Naproxen</td>
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<tr>
<td>R1</td>
<td>DNB-PG</td>
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</tr>
<tr>
<td>R2</td>
<td>DNB-Leu</td>
<td>--</td>
</tr>
</tbody>
</table>

Z1-Z10 zirconia based CSPs, R1, R2-commercialized silica based CSPs
Direct Comparison of DNB-L-LEU Zirconia and Silica Based CSPs

Much better separations for napropamide and 1-naphthyl leucine ester are obtained on zirconia-based CSPs.

1 trans-stilbene oxide
2 1,1’-bi-2-naphthol
3 trifluoranthyl ethanol
4 napropamide
5 1-naphthyl leucine ester
Chromatographic Comparison of Differently Anchored Zirconia-based DNB-L-LEU

Different anchors show different selectivity.
Stability Comparison of Differently Anchored Zirconia-Based DNB-L-LEU

Flush Solvent: 100% MeOH

Flush Solvent: 99/1/0.1 Hexane/IPA/TFA

Test solute: trifluoranthryl ethanol. Note that the retention factor ratio is for the less retained isomer.
Novel Chiral Selector Screening Method

- Attached a CSP to a bare zirconia column in-situ.
- Flush the column to remove unbound CSP.
- Screen chiral target compound.
- Strip off CSP using basic conditions.
- Repeat Cycle until desired resolution is achieved using different CSPs.
Example Attachment and Detachment Cycle

- Pass a solution of 20 mM N-(4-nitrobenzoyl)-L-glutamic acid (CSP) in tetrahydrofuran for 10 minutes at a column temperature of 60°C and a flow rate of 1 mL/min.
- Flushed column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemate solution of \((\pm)-2,2,2\text{-trifluoro-1-(9-anthyl)}\)ethanol.
- Strip the CSP by flushing the column with a 50 mM solution of tetramethylammonium hydroxide solution (pH 12) for 20 minutes at 60°C using a flow rate of 1 mL/min.
- Repeat procedure using the same CSP.
Comparison between the initial and final separation of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle. Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 °C, solute concentration = 1mg/mL, 5 microliter injection.
Conclusions

• **Flexible** attachment chemistry.
• APPA is the best anchor in terms of column stability.
• Zirconia based CSPs have *comparable chromatographic performance compared to the commercial silica* based CSPs for a wide range of chiral compounds.
• Phase II testing will involve *new anchors and different CSPs* with more optimization of screening techniques.
• Acknowledgement: National Institutes of Health Grant (Phase I) R43 HL070334-01.
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