APPLICATIONS OF A NEW THERMALLY AND CHEMICALLY STABLE LEWIS-ACID DEACTIVATED REVERSED-PHASE ZIRCONIA STATIONARY PHASE FOR LC/MS

Eastern Analytical Symposium 2004

CLAYTON V. MCNEFF¹, BINGWEN YAN¹, DAVID BELL², RICHARD HENRY²

¹ZirChrom Separations, Inc., 617 Pierce Street, Anoka, MN 55303
²Supelco, 595 N Harrison Rd., Bellefonte, PA 16823
Abstract

We report here the development of a novel zirconia-based reversed-phase high performance liquid chromatographic (HPLC) stationary phase achieved by covalently crosslinking a metal chelator into a polybutadiene coated zirconia in order to make a thermally and chemically stable Lewis acid “deactivated” reversed-phase zirconia stationary phase. We also demonstrated the feasibility of doing separations on this new stationary phase using no special strong Lewis base additives (acetate, fluoride, or phosphate), which are absolutely essential for the separation of low MW hard Lewis bases on other “unprotected” commercial reversed-phase zirconia-based HPLC columns. This new stationary phase is stable over a wide pH and temperature range, namely pH 1 to 10 and at temperatures as high as 80 degrees Celsius. The selectivity for neutral compounds is close to that of bonded phase C-18 silica, while the basic and acidic samples have significantly different chromatographic selectivity as compared by retention data via $\kappa$–$\kappa$ plots. Elution data for a number of probe solutes, including many pharmaceuticals, under volatile LC/MS-compatible conditions is also shown.
Comparison of Element Electronic Structures for Silica, Titania and Zirconia

- Silicon (element 14; 2.3 g/cc; Si$^{4+}$ ionic radius 0.400 Å)
  - Ne$^{3s^23p^2}$
- Titanium (element 22; 4.5 g/cc; Ti$^{4+}$ ionic radius 0.605 Å)
  - Ar$^{3d^24s^2}$
- Zirconium (element 40; 6.5 g/cc; Zr$^{4+}$ ionic radius 0.720 Å)
  - Kr$^{4d^25s^2}$

All have four valence electrons so some chemistry is similar, but presence of d orbitals and very electropositive nature allow Ti and Zr (metals) to form strong electron donor-acceptor complexes (coordination chemistry).
Surface Chemistry of Zirconia-Based Supports for HPLC

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

Weak Brönsted Base:

Strong Lewis Acid:

SCX mode
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.
Selectivity: The Key to Resolution

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

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

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

- Selectivity ($\alpha$) has the greatest impact on improving resolution.
Selectivity Comparison of 55 Pharmaceuticals

<table>
<thead>
<tr>
<th>1</th>
<th>cotinine</th>
<th>20</th>
<th>bretyllium</th>
<th>39</th>
<th>pindolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>piroxicam</td>
<td>21</td>
<td>labetalol</td>
<td>40</td>
<td>oxyphenonium</td>
</tr>
<tr>
<td>3</td>
<td>progesterone</td>
<td>22</td>
<td>tryptophan</td>
<td>41</td>
<td>metoprolol</td>
</tr>
<tr>
<td>4</td>
<td>enalopril</td>
<td>23</td>
<td>simvastatin</td>
<td>42</td>
<td>sildenafil</td>
</tr>
<tr>
<td>5</td>
<td>hydrocortisone acetate</td>
<td>24</td>
<td>lidocaine</td>
<td>43</td>
<td>diphenhydramine</td>
</tr>
<tr>
<td>6</td>
<td>nitrazepam</td>
<td>25</td>
<td>scopolamine</td>
<td>44</td>
<td>ritalin</td>
</tr>
<tr>
<td>7</td>
<td>cortisone acetate</td>
<td>26</td>
<td>isopropramidide</td>
<td>45</td>
<td>chlorpheniramine</td>
</tr>
<tr>
<td>8</td>
<td>tadalafil</td>
<td>27</td>
<td>morphine</td>
<td>46</td>
<td>tripolidine</td>
</tr>
<tr>
<td>9</td>
<td>warfarin</td>
<td>28</td>
<td>naltrexone</td>
<td>47</td>
<td>hydroxyzine</td>
</tr>
<tr>
<td>10</td>
<td>diclofenac</td>
<td>29</td>
<td>acebutolol</td>
<td>48</td>
<td>brompheniramine</td>
</tr>
<tr>
<td>11</td>
<td>nicotine</td>
<td>30</td>
<td>berberine</td>
<td>49</td>
<td>meclizine</td>
</tr>
<tr>
<td>12</td>
<td>atenolol</td>
<td>31</td>
<td>fentanyl</td>
<td>50</td>
<td>amitriptyline</td>
</tr>
<tr>
<td>13</td>
<td>chlordiazepoxide</td>
<td>32</td>
<td>tramadol</td>
<td>51</td>
<td>fluoxetine</td>
</tr>
<tr>
<td>14</td>
<td>prednisone</td>
<td>33</td>
<td>deprenyl</td>
<td>52</td>
<td>alpenolol</td>
</tr>
<tr>
<td>15</td>
<td>methylscopolamine</td>
<td>34</td>
<td>mepenzolate</td>
<td>53</td>
<td>hydroxypropranolol (blue)</td>
</tr>
<tr>
<td>16</td>
<td>ipratropium</td>
<td>35</td>
<td>methoxyverapamil</td>
<td>54</td>
<td>propranolol</td>
</tr>
<tr>
<td>17</td>
<td>cimetidine</td>
<td>36</td>
<td>verapamil</td>
<td>55</td>
<td>terbutaline</td>
</tr>
<tr>
<td>18</td>
<td>lovastatin</td>
<td>37</td>
<td>codeine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>hydroxymetoprolol</td>
<td>38</td>
<td>vardenafil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: number indicates elution order on the ZirChrom-MS column.
**k-k Plot for 55 Ionizable Compounds**

**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, ZirChrom®-MS, 50 x 4.6 mm i.d. (3um particles), S/N:MS020204T; Silica-C18 150 x 4.6 mm i.d.,(3.5 um particles).

ZirChrom®-MS and C18 Silica have very different chromatographic selectivity for ionic drugs.

\[ R^2 = 0.00 \]

N = 55
Comparison of Retention of Basic Pharmaceuticals for ODS and ZirChrom®-MS

Solutes

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 ºC; Detection at 254 nm; Columns, ZirChrom®-MS, 50 x 4.6 mm i.d. (3um particles), S/N:MS020204T; Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).
Reversed-Phase Characteristics

\[
\log k'_{RP} = \log k_w - S \phi
\]

<table>
<thead>
<tr>
<th></th>
<th>Toluene</th>
<th>Biphenyl</th>
<th>Phenanthrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>logkw</td>
<td>2.06</td>
<td>2.67</td>
<td>2.75</td>
</tr>
<tr>
<td>S*</td>
<td>3.41</td>
<td>3.86</td>
<td>3.71</td>
</tr>
<tr>
<td>R²</td>
<td>0.980</td>
<td>0.990</td>
<td>0.990</td>
</tr>
</tbody>
</table>


ZirChrom®-MS has very similar RP behavior to Silica C18.

LC Conditions: Mobile phase, indicated composition of ACN/Water; Flow rate, 2.0 ml/min.; Temperature, 35 °C; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom®-MS.
pH 1 and 10 Stability Testing

ZirChrom®-MS, S/N: MS0082903X; Mobile phase, 15/85 ACN/pH=1 nitric acid, Temperature: 30 °C; Injection volume: 5 µl; UV, 254 nm.

ZirChrom®-MS, S/N: MS0082903X; Mobile phase, 15/85 ACN/pH=10 with tetramethylammonia hydroxide, Temperature: 30 °C; Injection volume: 5 µl; UV, 254 nm.
LC-MS of Basic Pharmaceuticals

**LC Conditions:** Column, ZirChrom®-MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v), Linear gradient 5% D to 100% D in 6 minutes, hold 100% 6-7.4 min, 100 to 5% D 7.4-8.1min, hold 5% D 8.1-13.0 min. Temperature, 35°C. Waters/Micromass ZQ single quadrupole interfaced with the LC using an electrospray ionization (ESI) interface. Positive ion mode (XIC) from full scan acquisitions from m/z 120-700. Solute concentrations = 10µg/mL, 2µL injections.

---

Propranolol

Hydroxypropranolol
HPLC-MS of Beta-Blockers*

*pH 5

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Value</th>
<th>1: Scan ES+ 249 1.29e8</th>
<th>Value</th>
<th>1: Scan ES+ 267 9.65e7</th>
<th>Value</th>
<th>1: Scan ES+ 337 1.65e8</th>
<th>Value</th>
<th>1: Scan ES+ 250 2.02e8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td></td>
<td></td>
<td>13.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Pindolol
- Atenolol
- Acebutolol
- Alprenolol

*HPLC Conditions are the same as the receding slide.
Conditions that Favor Optimum Retention of Ionizable Analytes

- **Hydrophobic or Reversed Phase Mode**
  - Smaller organic mole fraction, $\Phi_{\text{org}} = 0 - 0.30$
  - Neutral analyte: $\text{pH} << \text{pK}_a$ (acids); $\text{pH} >> \text{pK}_a$ (bases)

- **Hydrophilic or Normal Phase Mode**
  - Larger organic mole fraction, $\Phi_{\text{org}} = 0.70 – 1.0$
  - Neutral analyte$^1$: $\text{pH} << \text{pK}_a$ (acids); $\text{pH} >> \text{pK}_a$ (bases)

- **Ionic or Ion Exchange Mode** (packing and analyte oppositely charged)
  - Organic mole fraction not very important
  - Lower ionic strength (favors LC-MS solute ionization)
  - Ionic analyte: $\text{pH} >> \text{pK}_a$ (acids); $\text{pH} << \text{pK}_a$ (bases)
Conclusions

• The ZirChrom®-MS phase is a novel zirconia-based RP column designed for use with MS.
• The ZirChrom®-MS phase is Lewis acid site deactivated.
• The ZirChrom®-MS phase has very different selectivity compared to silica C18 for pharmaceuticals due to IEX character.
• ZirChrom®-MS is chemically stable from pH 1-10.
• ZirChrom®-MS has similar reversed-phase behavior to silica C18 for small neutral organic compounds.
Acknowledgements

- The author would like to thank both ZirChrom and Supelco for assistance in generating experimental data and supporting this presentation. All LC-MS data was provided by Dr. David Bell of Supelco.

- For additional zirconia information, contact the companies at ZirChrom.com or Sial.com. The author may be reached at claytonmcneff@zirchrom.com.
For More Information, Visit Us at Booth #220

For more information and web access to the free Buffer Wizard: www.zirchrom.com