Characteristics and Advantages of Zirconia-Based Stationary Phases for Use in Multi-Dimensional HPLC

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Outline

1. Review of theory and requirements – Why bother with multi-dimensional chromatography?

2. Why use zirconia for two-dimensional chromatography?

3. ZirChrom®-CARB and DiamondBond-C18™—Very unique phases for RPLC

4. ZirChrom®-PBD, ZirChrom®-EZ and ZirChrom®-MS—Phases with mixed mode retention characteristics for ionizable analytes

5. Selectivity comparisons using ZirChrom®-CARB

6. Selectivity comparisons using ZirChrom®-PBD

7. An example two-dimensional HPLC separation of ten triazine herbicides using ZirChrom®-PBD and ZirChrom®-CARB

8. Conclusions
A Common Problem in HPLC

Sample composed of 20 components with randomly distributed k’ values

150 mm x 4.6 mm i.d. column

Even with state-of-the-art HPLC, only 50% of the components in this sample can be resolved !!!

N = 10,000 plates/column
n_c = 38, 12 unresolved components

N = 25,000 plates/column
n_c = 60, 9 unresolved components
Two conditions must be met for the technique to be considered “two-dimensional”

1. Orthogonality of separation mechanisms – This is a requirement imposed on the stationary phase chemistry
2. Separation gained in one dimension cannot be diminished by separation in the other dimension

If these two conditions are satisfied, the maximum total peak capacity of the two-dimensional system is:

\[ n_{cTotal} = n_{c1} \times n_{c2} \]

What Can We Expect From a Two-Dimensional Separation Based on Known One-Dimensional Data?

Condition A’ is the same as Condition A except that the retention has been varied randomly by 5%

Condition B assumes no relationship to Condition A

This scenario is ineffective in two-dimensional HPLC

This scenario has a higher probability of success in two-dimensional HPLC
Comparison of Variables Affecting Selectivity

Stationary phase type can have a very large effect on selectivity.
Why Zirconia-Based Phases - Advantages for Multi-Dimensional RPLC

1. Stability - Enables the use of otherwise extreme conditions for adjustment of selectivity

2. Stationary phase chemistry – Allows the user to explore a wide range of chemistry to obtain the largest changes in selectivity
   - A. Carbon-clad zirconia phases
   - B. Polymer coated phases with mixed mode characteristics
     - I. Reversed-phase
     - II. Ion-exchange

3. Speed – Thermal stability allows for faster multi-dimensional separations
<table>
<thead>
<tr>
<th>Part #</th>
<th>Packing</th>
<th>Mode</th>
</tr>
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<tbody>
<tr>
<td>DB01</td>
<td>DiamondBond®-C18</td>
<td>Reversed-Phase</td>
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<tr>
<td>EZ01</td>
<td>ZirChrom®-EZ</td>
<td>Reversed-Phase (Lewis Acid Deactivated)</td>
</tr>
<tr>
<td>MS01</td>
<td>ZirChrom®-MS</td>
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<td>ZirChrom®-CARB</td>
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<td>ZR02</td>
<td>ZirChrom®-PHASE</td>
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<td>Weak Cation-Exchanger</td>
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<td>ZR05</td>
<td>ZirChrom®-WAX</td>
<td>Weak Anion-Exchanger and Sugar Analysis</td>
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<td>Strong Anion-Exchanger</td>
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<td>ZirChrom®-PEZ</td>
<td>Cation-Exchanger for Proteins</td>
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ZirChrom®-CARB and DiamondBond®-C18

ZirChrom®-CARB

Zirconia Substrate

DiamondBond-C18™
Retention of Different Solutes on ODS, ZirChrom®-PBD and ZirChrom®-CARB

| 5. p-chlorophenol | 10. Anisole | 15. Benzophenone | 20. p-dichlorobenzene |
Selectivity and Shape: Isomeric Analytes

\[ \alpha_{\text{ODS}} = 1.03 \]
\[ \alpha_{\text{C-Zr}} = 1.58 \]

p-xylene

ethylbenzene
Selectivity of ZirChrom®-CARB and SB-C18 for 18 Substituted Phenols

LC Conditions: Mobile phase, 45/55 ACN/10mM phosphoric acid, pH 2.4; Flow rate, 2.0 ml/min.; Temperature, 40 °C – Data courtesy of Adam Schellinger
1. Coat bare zirconia with polybutadiene (PBD)¹
2. Crosslink PBD chains together using dicumyl peroxide as initiator
3. Reflux PBD-ZrO₂ in Ethylenediamine-N,N,N’,N’-tetra(methylene phosphonic) acid (EDTPA) solution
4. Wash to remove residual EDTPA

1 Chemisorb Ethylenediamine N,N,N’,N’-tetra(methyleneephosphonic)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 Study of Eleven Antidepressants

Selectivity for Antidepressant Compounds on ODS Brand A vs. Brand B

\[ R^2 = 0.84 \]
\[ \text{Std. Dev.} = 0.15 \]

**LC Conditions:** Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6.0; Flow rate, 1.0 mL/min; Temperature, 35 °C; UV detection at 254 nm.
Selectivity for Antidepressant Compounds on ZirChrom®-PBD vs. ODS

**LC Conditions:** Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6.0; Flow rate, 1.0 mL/min; Temperature, 35 °C; UV detection at 254 nm.

$R^2 = 0.09$
Std. Dev. = 0.66
Significantly Higher Ion-Exchange Retention of Amines on ZirChrom®-PBD Leads To Selectivity Differences

\[ k'_{IEX} = k' - k'_{RP} \]
Retrieval of Basic Analytes on ZirChrom®-PBD

- **PBD Coating — Reversed-Phase (RP) Moieties**
- **Lewis Base Anions — Ion-Exchange (IEX) Sites**

\[
\begin{align*}
\text{Zr-}{L^-} : X^+ + A^+ & = \text{Zr-}{L^-} : A^+ + X^+ \\
\text{Zr-}{O^-} : X^+ + A^+ & = \text{Zr-}{O^-} : A^+ + X^+
\end{align*}
\]

A\(^+\): analyte cation, X\(^+\): counterion, L\(^-\): adsorbed Lewis base anion.
ZirChrom®-PBD is Very Different Compared to All ODS Phases

The very large s.d. for ZirChrom®-PBD vs. all other phases indicates a dramatic difference in selectivity from ODS (Antidepressant solute set).
ZirChrom®-MS Compared to ODS

**LC Conditions:** Mobile Phase, 72/28 MeOH/25mM Ammonium phosphate, pH 6.0; Flow Rate, 1.0 ml/min.; Temperature, 35 °C; Injection Volume, 5 μl; Detection by UV at 254 nm; Solutes from left to right: Methapyrilene, Pyrilamine, Tripelennamine, Brompheniramine, Desipramine, Nortryptyline, Doxepin, and Amitryptyline.

Basic Compounds are much more retained on ZirChrom-MS than on Silica C18 and have very different chromatographic selectivity.
An Example 2DLC Separation - Ten Triazine Herbicides
2DLC Separation of Ten Triazine Herbicides

**1\textsuperscript{st Dimension Conditions}:** Column, 50 mm x 2.1 mm i.d. ZirChrom\textsuperscript{®}-PBD; Mobile phase, 20/80 ACN/Water; Flow rate, 0.08 ml/min.; Injection volume, 20 \( \mu \text{l} \); Temperature, 40 \( ^\circ \text{C} \)

**2\textsuperscript{nd Dimension Conditions}:** Column, 50 mm x 2.1 mm i.d. ZirChrom\textsuperscript{®}-CARB; Mobile phase, 20/80 ACN/Water; Flow rate, 7.0 ml/min.; Injection volume, 15 \( \mu \text{l} \); Temperature, 150 \( ^\circ \text{C} \); 1\textsuperscript{st} dimension sampling frequency, 0.1 Hz
Selectivity Comparison of 55 Pharmaceuticals

<table>
<thead>
<tr>
<th></th>
<th>cotinine</th>
<th>20 piroxicam</th>
<th>39 bretyllium</th>
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<tr>
<td>2</td>
<td>piroxicam</td>
<td>21 labetalol</td>
<td>40 oxyphenonium</td>
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<tr>
<td>3</td>
<td>progesterone</td>
<td>22 tryptophan</td>
<td>41 metoprolol</td>
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<td>4</td>
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<td>23 simvastatin</td>
<td>42 sildenafil</td>
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<td>5</td>
<td>hydrocortisone acetate</td>
<td>24 lidocaine</td>
<td>43 diphenhydramine</td>
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<td>6</td>
<td>nitrazepam</td>
<td>25 scopolamine</td>
<td>44 ritalin</td>
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<tr>
<td>7</td>
<td>cortisone acetate</td>
<td>26 isopropramide</td>
<td>45 chlorpheniramine</td>
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<tr>
<td>8</td>
<td>tadalafil</td>
<td>27 morphine</td>
<td>46 tripolidine</td>
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<td>9</td>
<td>warfarin</td>
<td>28 naltrexone</td>
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<td>diclofenac</td>
<td>29 acebutolol</td>
<td>48 brompheniramine</td>
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<tr>
<td>11</td>
<td>nicotine</td>
<td>30 berberine</td>
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<td>12</td>
<td>atenolol</td>
<td>31 fentanyl</td>
<td>50 amitriptyline</td>
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<td>13</td>
<td>chlordiazepoxide</td>
<td>32 tramadol</td>
<td>51 fluoxetine</td>
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<td>prednisone</td>
<td>33 deprenyl</td>
<td>52 alprenolol</td>
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<td>34 mepenzolate</td>
<td>53 hydroxypropranolol (blue)</td>
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<td>35 methoxyverapamil</td>
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<tr>
<td>19</td>
<td>hydroxymetoprolol</td>
<td>38 vardenafil</td>
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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.

R² = 0.00
N = 55
LC Conditions: Column, ZirChrom® MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2 mL/min, Mobile phases channel C=10 mM ammonium acetate at pH 5, channel D=10 mM 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 7.4-8.1 min, 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 = 10 μg/mL, 2 μL injections.
HPLC-MS of Beta-Blockers*

*HPLC Conditions are the same as the receding slide.
Conclusions

1. The importance of differences in selectivity between conditions selected for different dimensions in multi-dimensional chromatography cannot be emphasized enough.

2. The most dramatic changes in selectivity are most easily brought about by changing the stationary phase.

3. Zirconia-based reversed phases (there are 5 of them) offer dramatically different selectivity relative to conventional silica-based phases for several classes of analytes.
Acknowledgements

Supelco - Dr. David Bell