Applications of Sub-2µm Zirconia-PBD Columns at Elevated pH and Temperature

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Multi-Mode Behavior of Zirconia

- Zirconia substrate exhibits polar and ionic solute interaction: especially cation-exchange.
- With stable organic coatings, reproducible reversed-phase behavior can be added.
- Extreme resistance to temperature, pH and mechanical stress are unique advantages.
Addition of RP Behavior with Coated Zirconia Phases

- Retention (and selectivity) of ionic analytes modulated by pH, buffer/salt type and concentrations, and temperature.
- Retention of neutral solutes modulated by organic solvent.
C18 and Zr-PBD are Orthogonal for Basic Drugs

C18 (RP) columns separate mainly by hydrophobic forces and Zr-PBD columns separate by a combination of ionic and hydrophobic forces.

Zr-PBD and Si-C18 have very different selectivity for ionic drugs (especially in phosphate) due to the SCX ZrO₂ component.

Solutes
1. Chlordiazepoxide
2. Hydroxyzine
3. Buclizine
4. Thiothixene
5. Doxepin
6. Amitriptyline
7. Imipramine
8. Perphenazine
9. Nortriptyline
10. Desipramine
11. Thioridazine

Data provided by Sigma-Supelco
Cation Retention Observed for Zr-PBD Even in High Organic

Basic Pharmaceutical retention in 80% ACN

Excess k due mainly to IE mode

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-PBD, 50 x 4.6 mm i.d. (3 µm particles); Silica-C18 150 x 4.6 mm i.d., (3.5 µm particles).
Difficult Compounds for Silica Often Separate on Zirconia

Quaternary amines paraquat and diquat are retained and resolved on Zr-PS (also Zr-PBD or bare ZrO₂) due to the cation exchange mechanism; 50% ACN is useful to suppress or regulate retention by RP mode.

**Silica-C18:**
reversed-phase (silanol tailing)

- Column: Discovery® C18, 15 cm x 4.6 mm I.D., 3µm
- Mobile phase: 5% acetonitrile in 25 mM phosphate (pH 7)
- Flow rate: 1 mL/min.
- Temp.: 35 °C
- Det.: UV 290 nm

Light retention and poor peak shape even in low organic (5% ACN)

**Zirconia-PS:**
primarily ion-exchange

- Column: Discovery® Zr-PS, 7.5 cm x 4.6 mm, 3µm
- Mobile phase: 50% acetonitrile in 25 mM phosphate (pH 7)
- Flow rate: 3 mL/min.
- Temp.: 65 °C
- Det.: UV 290 nm

Data provided by Sigma-Supelco
Analytical Diameter Porous Zirconia Particles

- Particles produced by a sol-gel process with 1000Å sol
- Pore diameter 250-300Å
- Density: 2.6 g/cc (2.5X silica)
- Surface area: 25 m²/g
- Particle diameters: 3µm and sub-2µm
- Totally porous (porosity: 0.45)

1µm 25000X
Idealized van Deemter Plots

\[ H = A + \frac{B}{v} + Cv \]

Data plots move lower and become flatter for small particles due to combined effects of the equation terms (esp. A and C).

- The B term is important at low flows but drops out at high flows.

Plate Height, \( H \) (\( \mu \)m)

Flow velocity, \( v \) (mm/sec)

Elements of the drawing provided by Sigma-Supelco
Flow Studies on 3\(\mu\)m Zr-PBD: Alkylbenzenes

Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 °C, Mobile phase: 55/45 ACN/water, Column: ZirChrom®-PBD, 50 x 4.6mm, Agilent 1100/UV with micro cell (0.007”i.d. tubing).

Plate Height Vs. Linear Velocity for a PBD Column

2 mL/min test conditions
Flow Studies on Sub-2μm Zr-PBD: Alkylbenzenes

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV Micro Cell/0.007” i.d. tubing.
Sub-2µm Pressure Drop at Different Temperatures*

Mobile phase:
50/50 ACN/water
50x4.6mm column

*3µm particles show about half the pressure drop
Optimization and Configuration for Elevated Temperature Operation
# Background Pressure Drop Across Agilent 1100 at High Flow Rate

<table>
<thead>
<tr>
<th>100% H₂O at 30 °C</th>
<th>100% H₂O at 75 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow (mL/min)</strong></td>
<td><strong>BP (bar)</strong></td>
</tr>
<tr>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
</tr>
</tbody>
</table>

* Reference point: Waters Acquity (0.005” ID inlet/0.0025” ID outlet), 60/40 ACN/water, 0.5 mL/min, background pressure = 1700 psi (113 bar).
Flow Studies on Sub-2μm Zr-PBD: Factory Instrument

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Standard Cell and 0.007” i.d. tubing.

EAS 2009
Flow Studies on Sub-2μm Zr-PBD: Factory + Micro Cell Only

Plate Height Vs. Linear Velocity for a PBD Column

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.007” i.d. tubing.

EAS 2009
Flow Studies on Sub-2µm Zr-PBD: Micro Cell + Optimized Tubing

Plate Height Vs. Linear Velocity for a PBD Column

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and optimized 0.005” i.d. tubing.

EAS 2009
Flow Studies on Sub-2μm Zr-PBD: Heat Exchanger + Fitting + μCell

Plate Height Vs. Linear Velocity for a PBD Column

Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell, high pressure fitting and passing through heat exchanger.

EAS 2009
Flow Studies on Sub-2μm Zr-PBD: Factory Instrument at Ambient

Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Standard Cell and 0.007” i.d. tubing.

\[ N = \frac{L}{H} \]
Flow Studies on Sub-2µm Zr-PBD: Micro Cell

Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.007” i.d. tubing.
Flow Studies on Sub-2μm Zr-PBD: Micro Cell + Tubing

Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell and 0.005” i.d. tubing.
Flow Studies on Sub-2μm Zr-PBD: Heat Exchanger + Fitting + μCell

Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k’ in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell, high pressure fitting and passing through heat exchanger.
Flow Studies on 3μm Zr-PBD: Factory Instrument

Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 ºC, Mobile phase: 50/50 ACN/water, Column: ZirChrom®-PBD, 50 x 4.6mm, Agilent 1100/UV standard cell (0.007” i.d. tubing).
Flow Studies on 3μm Zr-PBD: Factory + Micro Cell

Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 °C, Mobile phase: 50/50 ACN/water, Column: ZirChrom®-PBD, 50 x 4.6mm, Agilent 1100/UV micro cell (0.007” i.d. tubing).
Alkylbenzylamine Separation on sub-2μm Zr-PBD, 50 °C

LC Conditions:  Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2μm Mobile Phase: 21/79 ACN/20 mM K₃PO₄ at pH=12; Flow rate: 1.5 mL/min; Temperature: 50 °C; Injection Vol.: 3.0 μL; Detection: UV at 254 nm
Antihistamine Separation on sub-2μm Zr-PBD, 80 °C

LC Conditions: Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2μm Mobile Phase: 28/72 ACN/50 mM TMA-OH at pH=12.2; Flow rate: 2.5 mL/min; Temperature: 80 °C; Injection Vol.: 2.0 μL; Detection: UV at 254 nm

260 bar
β-blockers on ZirChrom®-PBD
sub-2μm, High Temp

Analytes
1=Labetalol
2=Atenolol
3=Acebutolol
4=Metoprolol
5=Oxprenolol
6=Lidocaine
7=Quinidine
8=Alprenolol
9=Propranolol

246 bar (3660 psi)
Background pressure: ca. 25 bar
Detector Response Time (0.5s)

163,000 plates/m
**β-blockers on ZirChrom®-PBD**  
sub-2μm, High Temp, Faster sampling

**Analytes**  
1=Labetalol  
2=Atenolol  
3=Acebutolol  
4=Metoprolol  
5=Oxprenolol  
6=Lidocaine  
7=Quinidine  
8=Alprenolol  
9=Propranolol

ZirChrom®-PBD  
50mm x 4.6mm, sub-2μm  
21/79 ACN/20mM K₃PO₄ at pH=12  
F=2.5 mL/min  
UV=254nm  
T=75 °C, faster sampling rate

**246 bar (3660 psi)**  
**Background pressure: ca. 25 bar**  
Detector Response Time (<0.12s)

**185,000 plates/m**
Plans for Further Development

• Extend the range of ultra-high speed applications using sub-2µm Zr-PBD, especially at high pH and temperature (“extreme conditions for silica”); develop generic conditions for LC-MS.

• Develop sub-2µm Zr-CARB and compare performance to Zr-PBD under ambient and extreme temperature conditions.

• Study additional advantages of optimizing the IBW of an Agilent Model 1100 HPLC instrument using a high performance (Model 1200) heat exchanger.
Conclusions

- Multi-mode HPLC columns have become popular for difficult applications where compounds have ionic character and vary widely in chemical nature. Several ZirChrom® phases are ideal and popular for multi-mode applications and are stable over a much wider range of pH and temperature than any silica-based phase.
- Zirconia 3µm HPLC columns are currently available in a wide range of stable coatings and produce efficiencies in excess of 100,000 N/M.
- New sub-2µm zirconia UHPLC columns with very high efficiency in excess of 200,000 N/M with a PBD polymer-coated phase permit higher speed separations with shorter residence time at elevated temperatures.
References and Acknowledgements


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