A new generation of HPLC columns for more powerful method development and ...peak performance

- Microbore
- Analytical
- Semi-Preparative
- Preparative
Introducing ZirChrom® UHPLC Sub 2 Micron HPLC Columns

A New Class of Small Particle Chromatography Products

ZirChrom Separations, Inc. is pleased to announce the arrival of a full line of zirconia-based sub 2 micron HPLC phases, ZirChrom® UHPLC. These new small particle stationary phases incorporate the unsurpassed chemical and mechanical stability of zirconia with the efficiency benefits of sub 2 micron particles.

High Temperature Analysis of Nine Pharmaceuticals using Sub 2-μm ZirChrom®-PBD

<table>
<thead>
<tr>
<th>ANALYTES</th>
<th>1 - Labetalol</th>
<th>2 - Atenolol</th>
<th>3 - Acebutolol</th>
<th>4 - Metoprolol</th>
<th>5 - Oxprenolol</th>
<th>6 - Lidocaine</th>
<th>7 - Quinidine</th>
<th>8 - Alprenolol</th>
<th>9 - Propranolol</th>
</tr>
</thead>
</table>

**LC CONDITIONS**
- Mobile phase: 22/78 Acetonitrile/20mM Potassium Phosphate, pH 12.0
- Flow rate: 2.5 mL/min
- Injection volume: 2 μL
- Temperature: 75 °C
- Pressure drop: 246 bar
- Column: ZirChrom®-PBD, 50 x 4.6 mm i.d.
- Detection: 254 nm

**PACKING**

<table>
<thead>
<tr>
<th>PACKING</th>
<th>PART</th>
<th>PRICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZirChrom®-CARB</td>
<td>ZR01-0546-1.9</td>
<td>$853.00</td>
</tr>
<tr>
<td>ZirChrom®-PHASE</td>
<td>ZR02-0546-1.9</td>
<td>$743.00</td>
</tr>
<tr>
<td>ZirChrom®-PBD</td>
<td>ZR03-0546-1.9</td>
<td>$743.00</td>
</tr>
<tr>
<td>ZirChrom®-MS</td>
<td>MS01-0546-1.9</td>
<td>$743.00</td>
</tr>
<tr>
<td>ZirChrom®-SAX</td>
<td>ZR06-0546-1.9</td>
<td>$743.00</td>
</tr>
</tbody>
</table>
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Specialists in Ultra-Durable, High-Efficiency HPLC Phases
Company History

ZirChrom Separations, Inc. was founded in 1995 in Anoka, Minnesota. ZirChrom manufactures a full line of zirconia-based high performance chromatographic materials for the analysis of compounds primarily by high performance liquid chromatography (HPLC). The combination of zirconia’s unequaled stability, high efficiency and its unique chromatographic selectivity allows for optimized separation conditions which are not possible using any other HPLC support materials.

Technology Information

Porous Zirconia

Zirconia, or zirconium dioxide (ZrO₂), is a metal oxide that can exist in a number of crystalline and amorphous forms. The primary advantage of zirconia relative to conventional silica-based phases is its unique chromatographic selectivity combined with extreme chemical and thermal stability. Unlike silica, zirconia is completely stable over the entire pH range and at column temperatures as high as 200 °C. Unlike polymeric phases, zirconia does not shrink or swell as a function of mobile phase organic content or ionic strength. Additionally, it is very efficient and mechanically stable.

The extreme stability of zirconia and its unique chromatographic selectivity allows for the optimization of separation conditions, which are unachievable by other types of supports. Zirconia supports allow for faster, more selective separations, with high efficiencies.

Particles are available in diameters of 3 μm (for analytical work) up to 25 μm (for preparative scale chromatography). These particles have narrow particle size distributions, and excellent porosity.

The Benefits of Stable HPLC Phases

ZirChrom’s products all have outstanding chemical and thermal stability. Many can be used across the entire pH range and at column temperatures as high as 200 °C.

- Less retention drift, more stable methods
- Excellent temperature stability allows much faster analyses by routine use of elevated temperature as necessary

Unique Surface Chemistry

Inherent differences exist between the surface chemistries of zirconia and conventional silica gels. It is important for a chromatographer to be aware of these differences and to know how to overcome the unique challenges that result from them during method development.

The Lewis acid-base chemistry of zirconia has been extensively studied. The three types of chromatographically important sites on zirconia are a) Brønsted acid, b) Brønsted base and c) Lewis acid, which are shown below:

![Lewis Acid/Base Sites](image)

The large population of Lewis acid sites typically dominate the surface chemistry of zirconia due to the ease with which these sites can adsorb constituents from the mobile phase to form a chemisorbed layer on the zirconia surface. The worst case scenario from the perspective of method development is that Lewis base moieties in analyte molecules can adsorb to these Lewis acid sites causing irreversible adsorption of the analyte.

Deactivation Technology

New research performed at ZirChrom has simplified the surface chemistry considerations associated with using zirconia-based media. The next generation of zirconia-based RPLC columns is modified with a metal chelator group that effectively “deactivates” the Lewis acid sites, and is chemically stable from pH 1 to 10.
Reversed-Phase Chromatography (RPC)

Reversed-Phase Chromatography (RPC) is the most commonly used mode of HPLC employed, accounting for about 80% of all HPLC separations. All of ZirChrom’s RPC supports are based on ultra-stable, highly efficient zirconia particles. ZirChrom offers seven types of reversed-phase columns, giving chromatographers a wide range of selectivity options.

Traditional ZirChrom® Reversed-Phases

- ZirChrom®-PBD—great for general purposes and ideal for basic compounds, similar to ODS for non-electrolytes
- ZirChrom®-PS—ideal for highly aqueous mobile phases, an alternative to ODS selectivity
- ZirChrom®-CARB—ideal for diastereomers/geometric isomers, greatest difference in selectivity versus ODS
- DiamondBond®-C18—great for acidic compounds, very different selectivity compared to ODS

New “Deactivated” ZirChrom® Reversed-Phases

- ZirChrom®-MS—easy-to-use with unique selectivity compared to ODS, ideal for LC/MS of basic compounds
- ZirChrom®-EZ—easy-to-use general purpose “deactivated” reversed-phase, great for acids and bases
- ZirChrom®-SELECT—the first “deactivated” carbon phase, orthogonal selectivity to ODS with improved shape
How to Select a ZirChrom Reversed-Phase

There are several ways to select the most appropriate ZirChrom reversed-phase for your application:

1. Use the general selectivity descriptions on the previous page to choose the column that seems best for your application or class of compounds,
2. Use the table below to select a column based on your specific problem or application need, or
3. Call our Technical Support group at 1-866-STABLE-1 (or e-mail us at support@zirchrom.com). We will be happy to help you select the ZirChrom reversed-phase column that is most likely to solve your separation problem.

Reversed-Phase Column Selection Guide

<table>
<thead>
<tr>
<th>Current Problem/Concern</th>
<th>Column</th>
<th>Suggested Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved Selectivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need improved selectivity for nonelectrolytes, isomers, diastereomers. Currently using carbon, cyanopropyl, phenyl or fluoro phases.</td>
<td>DiamondBond®-C18, ZirChrom®-CARB or ZirChrom®-CARB select</td>
<td>Use an acetonitrile and/or THF eluent. Add octylamine to improve peak shape. If using DiamondBond®-C18, set temperature to at least 50 °C.</td>
</tr>
<tr>
<td>Need improved selectivity for bases.</td>
<td>ZirChrom®-MS, ZirChrom®-PBD</td>
<td>Use buffer of choice in a pH range of 1-10. If required, 5 - 25 mM phosphate may improve peak shape. If using ZirChrom®-PBD, increase pH above pKa (≥14).</td>
</tr>
<tr>
<td>Need improved selectivity for acids.</td>
<td>ZirChrom®-EZ, ZirChrom®-MS</td>
<td>Use buffer of choice in a pH range of 1-10. Try low pH first. 5 - 25 mM phosphate may improve peak shape.</td>
</tr>
<tr>
<td>Change Retention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need more retention for very polar (hydrophilic) nonelectrolytes. Currently using nearly 100% water eluent or polar embedded phase.</td>
<td>DiamondBond®-C18, ZirChrom®-CARB or ZirChrom®-SELECT</td>
<td>Can be used in high water mobile phase.</td>
</tr>
<tr>
<td>Need more retention for very polar bases. Currently using nearly 100% water eluent or polar embedded phase or sulfonic acid paired ion reagent.</td>
<td>ZirChrom®-MS</td>
<td>Use buffer of choice in a pH range of 1-10. If required, 5 - 25 mM phosphate may improve peak shape. High water mobile phases are no problem.</td>
</tr>
<tr>
<td>Need more retention for very polar acids. Currently using nearly 100% water eluent or polar embedded phase or quaternary amine paired ion reagent.</td>
<td>ZirChrom®-EZ, ZirChrom®-MS, DiamondBond®-C18</td>
<td>Use buffer of choice in a pH range of 1-10. Try low pH first. For tough separations, decrease pH below pKa (≥1). 5 - 25 mM phosphate may improve peak shape.</td>
</tr>
<tr>
<td>Need less retention with any solute type.</td>
<td>ZirChrom®-PS</td>
<td>Least hydrophobic phase. Can be used with 100% water eluent.</td>
</tr>
<tr>
<td>Improve Efficiency/Productivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate stability and selectivity. Having trouble with silica-based phases, changed to alumina or polymer column and analytes were still not sufficiently resolved.</td>
<td>All ZirChrom® Reversed-Phase (RP) Columns</td>
<td>Zirconia phases exhibit excellent pH and temperature stability. ZirChrom® RPs give higher efficiency and better peak shape than alumina or polymer columns.</td>
</tr>
<tr>
<td>Poor column reproducibility. Experiencing retention changes at low or high pH, at elevated temperature or when using phosphate or carbonate buffer.</td>
<td>All ZirChrom® RP Columns</td>
<td>Zirconia phases are very reproducible from batch-to-batch, column-to-column and run-to-run. Every ZirChrom® column is tested prior to shipment to insure optimal performance.</td>
</tr>
<tr>
<td>Separations taking too long.</td>
<td>All ZirChrom® RP Columns</td>
<td>Increase temperature up to max, operating range for LC and/or analyte. Increase flow rate. Easily improves speed 2-3 fold.</td>
</tr>
<tr>
<td>Column overloaded too easily with basic solutes.</td>
<td>ZirChrom®-MS, ZirChrom®-PBD</td>
<td>The mixed-mode (reversed-phase/cation exchange) retention mechanism enables enhanced loadability.</td>
</tr>
<tr>
<td>Improve Detection Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need to go to shorter wavelength to enhance sensitivity in UV. Solute does not have long wavelength absorption or is very dilute.</td>
<td>ZirChrom®-PS</td>
<td>Use a high water or pure water eluent and go deep into UV.</td>
</tr>
<tr>
<td>Need LC/MS detection of Lewis base analytes at low pH.</td>
<td>ZirChrom®-EZ, ZirChrom®-MS</td>
<td>Use buffer of choice in a pH range of 1-10 with these Lewis acid site deactivated phases.</td>
</tr>
<tr>
<td>Need to decrease bleed in LC/MS.</td>
<td>All ZirChrom® RP Columns</td>
<td>All ZirChrom® columns are extremely low bleed. The ZirChrom®-MS column was designed for LC/MS.</td>
</tr>
</tbody>
</table>

Also refer to www.zirchrom.com for zirconia publication numbers 16, 17, 23, 51, 65, 71, 77, and 87.
ZirChrom’s traditional reversed-phase columns stand up to your most challenging applications!

Chemical and Thermal Stability

**Exceptional Stability at High Temperature**

<table>
<thead>
<tr>
<th>Retention Factor (k')</th>
<th>Column Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butylenzene</td>
<td>12.00</td>
</tr>
<tr>
<td>Propybenzene</td>
<td>10.00</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>8.00</td>
</tr>
<tr>
<td>Toluene</td>
<td>6.00</td>
</tr>
<tr>
<td>Benzene</td>
<td>4.00</td>
</tr>
</tbody>
</table>

**Mobile phase:** 15/85 ACN/Water  
**Flow rate:** 1.0 ml/min  
**Column:** DiamondBond®-C18, 100 x 4.6 mm i.d.  
**Temperature:** 195 °C

**Exceptional Stability at High pH**

<table>
<thead>
<tr>
<th>Retention Factor (k')</th>
<th>Column Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propybenzene</td>
<td>3.00</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>2.50</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.00</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.50</td>
</tr>
<tr>
<td>Butylenzene</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Mobile phase:** 15/85 ACN/Water, pH 13  
**Flow rate:** 1.0 ml/min  
**Column:** ZirChrom®-PBD, 150 x 4.6 mm i.d.

**“Real World” Example at High pH**

**ZirChrom®-PBD, 150 x 4.6 mm (part# ZR03-1546)**

**Great Separation at High pH**

**ANALYTES**
1 - Labetalol  
2 - Atenolol  
3 - Acebutolol  
4 - Metoprolol  
5 - Oxprenanol  
6 - Lidocaine  
7 - Quinidine  
8 - Alprenolol  
9 - Propranolol  

**Initial Injection at pH 12**

**Still Going Strong—After 15,000 Column Volumes**

**LC CONDITIONS**
Mobile phase: 28/72 ACN/20 mM Potassium phosphate, pH 12.0  
Flow rate: 1.0 ml/min  
Temperature: 30 °C  
Injection volume: 5 μl  
Detection: 254 nm

The top figures show typical stability data for our products at extreme pH and temperature. Below is a typical example of a robust separation at high pH.
Consider using ZirChrom®-PBD with a phosphate buffer if either the tailing of amines or their selectivities are problematic on C18 silica, and explore the full pH range (pH 1-14) to optimize your separation. For example, a 20 mM phosphate buffer will produce good peak shapes for many ionizable compounds.

For more detailed guidelines, consult our new Method Development Guide. Or, contact our technical support group at 1-866-STABLE-1.

Method Development with ZirChrom®-PBD (USP L49)

ZirChrom®-PBD is produced by coating ultra-stable zirconia particles with an equally stable extremely thin layer of crosslinked polybutadiene. The chemical selectivity of ZirChrom®-PBD columns is similar to that of a traditional C8 or C18 silica based column for non-ionic analytes. In the case of ionizable analytes there are secondary interactions which can be used to fine tune the chromatographic selectivity (band spacing).

The surface chemistry of zirconia is very rich and may be used to change chemical selectivity of the column simply through the addition of mobile phase additives.

\begin{itemize}
\item Great Peak Shapes for Basic Compounds
\item Easy Method Transfer from ODS Silica
\item pH Stable from 1 up to 14
\item Excellent Thermal Stability
\item High Efficiency (>120,000 plates/meter)
\end{itemize}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{zirchrom_pbd_method_development.png}
\caption{ZirChrom®-PBD Gives Excellent Peak Shapes for Basic Drugs at High pH}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{antihistamines_separation.png}
\caption{Fast, High Temperature Antihistamines Separation on ZirChrom®-PBD}
\end{figure}
ZirChrom®-PBD vs. C-18 Silica and Polymers

ZirChrom®-PBD columns combine the high stability of polymer columns with the high efficiency of silica columns. An ultra-stable reversed-phase alternative to bonded phases, ZirChrom®-PBD is a conventional reversed-phase support with the selectivity and column efficiency of C18, allowing for easy method transfer. But, ZirChrom®-PBD is vastly more stable than any silica phase—both chemically and thermally (pH 1-14, up to 150 °C). This makes ZirChrom®-PBD far superior to silica-based phases for the separation of basic compounds.

ZirChrom®-PBD is also a superior reversed-phase alternative to polymer (PRP) phases. Like PRP, ZirChrom®-PBD offers extreme chemical and thermal stability. Unlike PRP, ZirChrom®-PBD has high efficiency (generally 2-3 times the plates/meter of polymer phases).

ZirChrom®-PBD for Fast HPLC

The extraordinary thermal stability of ZirChrom®-PBD allows for fast separations at elevated temperature. By raising the temperature to 50 °C, many separations are twice as fast. At 80 °C, separations up to 3-5 times faster are possible. Unlike silica-based columns, ZirChrom®-PBD has superior column life at these temperatures.

ZirChrom®-PBD for Amines

ZirChrom®-PBD has excellent selectivity for amines, and also very high loadability. Often, 50% more compound can be loaded on ZirChrom®-PBD compared to C18 silica (see technical bulletin #200 for more details).

ZirChrom®-PBD for Azithromycin

ZirChrom®-PBD has been designated by the USP as L49 and can be used for the analysis of azithromycin (see technical bulletin #311 for more details). The high pH necessary for the analysis of azithromycin prohibits the use of traditional silica based substrates and necessitates the use of the pH stable zirconia-based ZirChrom®-PBD.

**ZirChrom®-PBD Versus ODS-Silica for Basic Drugs**

<table>
<thead>
<tr>
<th>ANALYTES</th>
<th>ZirChrom®-PBD (A)</th>
<th>Leading Silica Column (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Nordoxepin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - Protriptyline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - Nortriptyline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - Imipramine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - Amitriptyline</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LC CONDITIONS</th>
<th>Mobile phase: (A) 45/55 ACN/20 mM Potassium phosphate, pH 12.0 (B) 50/50 ACN/20 mM Potassium phosphate, pH 7.0</th>
<th>Flow rate: 1.0 ml/min</th>
<th>Temperature: 30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume</td>
<td>5 μl</td>
<td>Detection: 254 nm</td>
<td></td>
</tr>
<tr>
<td>Column: ZirChrom®-PBD, 150 x 4.6 mm i.d.</td>
<td>(part# ZR03-1546)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**USP Standard Azithromycin on ZirChrom®-PBD**

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>Azithromycin (USP Standard)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>LC CONDITIONS</th>
<th>Mobile phase: 5.8 g monobasic potassium phosphate in 2130 ml of water, added to 870 ml of acetonitrile adjusted to pH 11.0 with potassium hydroxide</th>
<th>Flow rate: 1.0 ml/min</th>
<th>Temperature: 30 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume</td>
<td>5 μl (1mg/μl)</td>
<td>Detection: 215 nm</td>
<td></td>
</tr>
<tr>
<td>Column: ZirChrom®-PBD, 150 x 4.6 mm i.d.</td>
<td>(part# ZR03-1546)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
METHOD DEVELOPMENT WITH ZIRCHROM®-PS

ZirChrom®-PS uses an extremely thin layer of polystyrene, instead of the polybutadiene coating used in ZirChrom®-PBD. This gives ZirChrom®-PS an alternative selectivity and less retention, making it ideal for non-polar analytes, or where highly aqueous mobile phases are necessary.

- pH Stable from 1 up to 13
- Excellent Thermal Stability
- Superior Selectivity for Poly Aromatic Compounds

LC CONDITIONS
Mobile phase: [A] 25 mM HCl, pH 1.9
[B] Acetonitrile
Gradient Elution: 5 - 15% B over 0 - 1 minutes, 15 - 60% B over 1 - 4 minutes
Flow rate: 1.0 ml/min Temperature: 40°C
Injection volume: 0.5 μl Detection: 254 nm
Column: ZirChrom®-PS, 50 x 4.6 mm i.d. (part# ZR09-0546)

ANALYTES
1 = Naphtalene, 2 = 1-Methylnaphtalene, 3 = Acenaphthylene, 4 = Acenaphthene, 5 = Fluorene, 6 = Decafluorobiphenyl, 7 = Phenanthrene, 8 = Anthracene, 9 = Fluoranthene, 10 = Pyrene, 11 = p-terphenyl-d14, 12 = benzo(a)anthracene, 13 = benzo(b)fluoranthene, 14 = Benzo(a)pyrene, 15 = Dibenzo(a,h)anthracene, 16 = Benzo(g,h,i)perylene

LC CONDITIONS
Mobile phase: [A] Water
[B] Acetonitrile
Gradient Elution: 10 - 50 % B over 0 - 7 minutes
Flow rate: 1.0 ml/min Temperature: 125°C
Injection volume: 2 μl Detection: 254 nm
Column: ZirChrom®-PS, 150 x 4.6 mm i.d. (part# ZR09-1546)

ANALYTES
1 = Tripelennamine, 2 = Triprolidine, 3 = Cyclizine, 4 = Pyrrobutamine, 5 = Meclizine

ZirChrom® Separations, Inc., 617 Pierce Street, Anoka, MN 55303
Method Development with DiamondBond®-C18
DiamondBond®-C18 is made by covalently bonding C18 ligands to the surface of carbon-clad zirconia. This creates the first truly bonded carbon phases in the industry. Because the surface below the C18 ligands is carbon and not silica, DiamondBond®-C18 has different selectivity from other phases (see the graph on page 3). DiamondBond®-C18 has better peak shapes than unmodified carbon phases, and unique selectivity compared to silica phases. Like for all traditional reversed-phase zirconia products, proper buffer selection helps to ensure the best peak shapes and band spacing. DiamondBond®-C18 is stable up to 200 °C.

For more detailed guidelines, consult our new Method Development Guide. Or, contact our technical support group at 1-866-STABLE-1.

Anticonvulsants

Barbiturates

<table>
<thead>
<tr>
<th>PACKING</th>
<th>MODE</th>
<th>PART</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiamondBond®-C18</td>
<td>Reversed-Phase</td>
<td>DB01</td>
</tr>
<tr>
<td>Microbore, Semi-Prep and Prep Formats Available—see Page 24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ZirChrom®-CARB is produced by coating our zirconia particle with an extremely thin layer of elemental carbon. The resulting phase gives ZirChrom®-CARB very different selectivity than any bonded or polymeric phase, and represents an excellent alternative when bonded phases do not provide the required selectivity.

ZirChrom®-CARB is great for geometric isomer separations, and is superb for separating diastereomers. The selectivity of ZirChrom®-CARB is more different from ODS than phenyl or cyano phases. This makes it an excellent choice for orthogonal screening in drug discovery and impurity profiling. Like all of our traditional zirconia-based products, proper buffer selection helps to ensure the best peak shapes and band spacing. ZirChrom®-CARB is stable from pH 1-14, and up to 200 °C.

For more detailed guidelines, consult our new Method Development Guide. Or, contact our technical support group at 1-866-STABLE-1.

<table>
<thead>
<tr>
<th>PACKING</th>
<th>MODE</th>
<th>PART</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZirChrom®-CARB</td>
<td>Reversed-Phase</td>
<td>ZR01-1546</td>
</tr>
</tbody>
</table>

**Fast Separation of Corticosteroids on ZirChrom®-CARB**

<table>
<thead>
<tr>
<th>ANALYTES</th>
<th>1 - Dexamethasone</th>
<th>2 - Prednisone</th>
<th>3 - Prednisolone</th>
<th>4 - Betamethasone</th>
</tr>
</thead>
</table>

**LC CONDITIONS**
- Mobile phase: 60/10/30 Acetonitrile/MTBE/Water
- Flow rate: 1.5 ml/min
- Temperature: 80 °C
- Injection vol.: 15 μl
- Detection: 215 nm

**Separation of Highly Polar Analytes on ZirChrom®-CARB**

**Cosmetic Ingredients**
- ANALYTES: 1 - Allantoin, 2 - Bronopol

**LC CONDITIONS**
- Mobile phase: 20/80 ACN/Water
- Flow rate: 1.0 ml/min
- Temperature: 25 °C
- Injection volume: 5 μl
- Detection: 200 nm
- Column: ZirChrom®-CARB, 100 x 4.6 mm i.d.

*NOTE: These compounds are unretained on ODS in pure water*
Method Development with ZirChrom®-EZ (Lewis Acid “Deactivated”)

ZirChrom®-EZ is a first of its kind Lewis acid deactivated zirconia-based reversed-phase HPLC column. Compared to traditional zirconia-based reversed phases, ZirChrom®-EZ is easier to use because it is less prone to problems caused by solute interactions with the strong Lewis acid sites on the zirconia surface. The deactivation of Lewis acid sites on the surface of the ZirChrom®-EZ particle allows for the chromatography of Lewis base analytes (like carboxylates or phosphates) using traditional mobile phase additives of the user’s choice including conventional LC/MS compatible buffers (such as acetate and formate) throughout the pH range of 1-10, and up to 50 °C. This new column still maintains the very different chromatographic selectivity for basic pharmaceuticals.

Method Development with ZirChrom®-MS (Lewis Acid “Deactivated”)

ZirChrom®-MS is revolutionary new zirconia-based reversed-phase column for HPLC. This new column was designed from the ground up to be used in conjunction with MS detection. It uses the same type of Lewis acid deactivation chemistry as the ZirChrom®-EZ column but with a covalent attachment for low bleed. ZirChrom®-MS also has about 2.5 times as much retention for simple reversed-phase compounds than the ZirChrom®-EZ column, which is beneficial for MS detection. The deactivation of Lewis acid sites on the zirconia surface allows for the chromatography of Lewis base analytes (like carboxylates or phosphates) using volatile mobile phase additives of the user’s choice including conventional LC/MS compatible buffers (such as acetate and formate) throughout the pH range of 1-10, and up to 50 °C. Due to a mixed-mode separation mechanism the ZirChrom®-MS column offers unique selectivity for pharmaceutical method development.

For more detailed guidelines, consult our new “Deactivated” Column Method Development Guide. Or, contact our technical support group at 1-866-STABLE-1.
A column comparison study using pharmaceutically relevant compounds was performed to demonstrate the unique selectivity of ZirChrom®-MS relative to a leading bonded phase C18 silica (see left). The LC/MS compatible operating conditions that were used included a volatile, near neutral pH mobile phase with an ammonium acetate buffer. Results of the column comparison study indicate that ZirChrom®-MS exhibits enhanced retention, improved peak shape and greater efficiency (versus C18 silica) for basic pharmaceutical compounds under LC/MS compatible operating conditions (see technical bulletin #303 for more details).
• Highly Stable Titania-Based Particles  
• Widest Range of Particle/Pore Sizes  
• Reversed-Phase and Normal Phase Medias  
• Ideal for Preparative Scale Separations

Method Development with Sachtopore®-RP and Sachtopore®-NP

Sachtopore®-RP (reversed-phase) and Sachtopore®-NP (normal phase), offered by Sachtleben Chemie GmbH, are titania-based HPLC columns that can be used over the entire pH range. These next generation columns are offered in both analytical and preparative formats, containing a wide selection of particle (3, 5, 10, 20, 40 and 80 micron) and pore (60, 100, 300, 1000 and 2000 angstrom) sizes. Sachtleben Chemie brings a long-standing tradition of excellence to the chromatography market as well as the capability to produce large quantities of particles very reproducibly. These new titania-based packings offer the chromatographer a very rugged alternative to silica for both analytical and preparative-scale separations.

Antihistamines Separation at pH 10

**ANALYTES**
1. Maleic Acid  
2. Carbinoxamine  
3. Chlorphenamine  
4. Diphenylpyraline  
5. Phenyltoloxamine

**LC CONDITIONS**
- Mobile phase: 70/30 10 mM Borax + 10 mM Soda / Acetonitrile  
- Flow rate: 1.0 ml/min  
- Temperature: 35 °C  
- Injection volume: 5 μl  
- Detection: 230 nm  
- Column: Sachtopore®-RP, 150 x 4.6 mm i.d. (3 micron)  
- (part# TI01-1546)

Antihypertensives Separation at pH 9.5

**ANALYTES**
1. Betahistine  
2. Prazosin  
3. Isosorbide dinitrate  
4. Ergotamine  
5. Dihydroergotamine-methanesulfonate  
6. Clofibrate

**LC CONDITIONS**
- Mobile phase: 70/30 40 mM NH₃ + 10 mM K₂HPO₄ + 10 mM Na₂CO₃ / Acetonitrile, pH 9.5  
- Flow rate: 1.0 ml/min  
- Temperature: 35 °C  
- Injection volume: 1 μl  
- Detection: 230 nm  
- Column: Sachtopore®-RP, 150 x 4.6 mm i.d. (5 micron)  
- (part# TI01-1546-5)

Preparative Scale Loadability on Sachtopore-RP

**ANALYTE**
Pentifylline (Cardiac Drug)

**LC CONDITIONS**
- Mobile phase: 81/19 10 mM Na₂B₄O₇ + 1 mM H₃BO₃ / Acetonitrile, pH 8.8  
- Flow rate: 1.0 ml/min  
- Temperature: 35 °C  
- Injection volume: 20 μl  
- Detection: 280 nm  
- Column: Sachtopore®-RP, 150 x 4.6 mm i.d. (3 micron)  
- (part# TI01-1546)
• Phases for Sugars and Proteins
• Wide Range of Ion Exchange Selectivity
• No Shrinking or Swelling — Use Any Organic Solvent
• Significantly Higher Efficiency than Polymeric Phases

Method Development with ZirChrom's Ion Exchange Phases

Each ZirChrom ion exchange phase is produced by coating ultra-stable zirconia particles with an extremely thin layer of an ionic polymer. This method creates phases with much higher efficiency and, oftentimes, higher capacity than pure polymeric phases. Also, ZirChrom’s ion exchangers do not shrink or swell as a function of ionic strength or organic modifier content of the mobile phase.

ZirChrom’s SAX and SHAX phases are thermally stable up to 80 °C, which causes different selectivity, allowing high speed separations with lower ionic strength mobile phases. This is very important in the preparation of RNA and DNA samples.

If desired, mixed-mode separation modes may be exploited to optimize separations, including Lewis acid-base interactions, hydrophobic interactions and ion-exchange interactions. These modes may be attenuated by adjusting the strong Lewis base content, organic content and ionic strength of the mobile phase, respectively.

For more detailed guidelines, consult our Technical Bulletins for examples of the use of these phases. Or, contact our technical support group at 1-866-STABLE-1.
**HPLC COLUMN**

**PRODUCT GUIDE 2011-2012**

**ION EXCHANGE CHROMATOGRAPHY**

---

### Protein Separations

**ZirChrom®-PEZ**

**ANALYTES**

1. Water
2. Myoglobin
3. Lysozyme
4. Oxidized and reduced forms of Cytochrome C

**LC CONDITIONS**

Mobile phase: Gradient elution with 25 to 100% B over 10 min, where A is 2 mM EDTPA, 50 mM NaCl, 20 mM MES, pH 5.5, and B is 2 mM EDTPA, 1.0 M NaCl, 20 mM MES, pH 5.5

Flow rate: 1.0 ml/min

Temperature: 30 °C

Injection Volume: 10 μl

Detection: 280 nm

Column: ZirChrom®-PEZ, 150 x 4.6 mm i.d.

(part# ZR08-1546)

---

### Organic Acids

**ZirChrom®-SAX**

**ANALYTES**

1. Acetic acid
2. Propionic acid
3. Formic acid
4. Butyric acid

**LC CONDITIONS**

Mobile phase: 5 mM Ammonium phosphate, pH 6.0

Flow rate: 1.5 ml/min

Temperature: 50 °C

Injection volume: 5 μl

Detection: 210 nm

Pressure drop: 140 bar

Column: ZirChrom®-SAX, 150 x 4.6 mm i.d.

(part# ZR06-1546)

---

### Oligonucleotides

**ZirChrom®-SAX**

**ANALYTES**

Partial Poly (G) hydrolysate

**LC CONDITIONS**

Mobile phase: (A) 20 mM Potassium phosphate dibasic at pH 7.0, 200 mM Sodium chloride

(B) 200 mM Potassium phosphate dibasic at pH 7.0, 2.0 M Sodium chloride; Gradient: 10 to 90% B over 180 min.

Flow rate: 1.0 ml/min

Temperature: 100 °C

Injection volume: 25 μl

Detection: 260 nm

Column: ZirChrom®-SAX, 150 x 4.6 mm i.d.

(part# ZR06-1546)

---

### Water Soluble Vitamins on ZirChrom®-SAX

**ANALYTES**

1. Thiamine (Vitamin B1)
2. Pyridoxine (Vitamin B6)
3. Nicotinamide (form of Vitamin B3)
4. Riboflavin (Vitamin B2)
5. Nicotinic acid (form of Vitamin B3)
6. Ascorbic acid (Vitamin C)

**LC CONDITIONS**

Mobile phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5

Flow rate: 1.0 ml/min

Temperature: 30 °C

Injection volume: 5 μl

Detection: 254 nm

Column: ZirChrom®-SAX, 150 x 4.6 mm i.d.

(part# ZR06-1546)

---

### "Green" Analysis of Diet Soft Drinks on ZirChrom®-SAX

**ANALYTES**

1. Caffeine
2. Aspartame
3. Benzoate

**Diet Sprite™**, **Diet Dr. Pepper™**, **Diet Mountain Dew™**, **Diet Pepsi™**, **Diet Coke™**

**LC CONDITIONS**

Mobile phase: 10mM Ammonium phosphate, 5mM Ammonium carbonate, pH 6.6

Flow rate: 1.0 ml/min

Temperature: 50 °C

Injection volume: 5 μl

Detection: 210 nm

(part# ZR06-1030)

---

*NOTE: The high temperature specification for ZirChrom®-SAX is 80 °C. Routine column use above the high temperature specification will significantly shorten column lifetime.*
**Legend for Surface Chemistry Diagrams**

- Crosslinked Polybutadiene (PBD)
- Hydrophobic Crosslinker
- Zirconia Particle
- Lewis Acid Site
- Phosphate \( \text{PO}_4 \)
- Ethylenediamine-\( \text{N}_2\text{N}_2\text{N}_2\text{N}_2 \)tetra (methylenephosphonic acid)
- Crosslinked Polyethyleneimine (PEI)

**ZirChrom®-SAX (Stable from pH 1-12, and to 80 °C)**

- Cross-linked polyethyleneimine-coated zirconia for strong anion-exchange
- Useful for inorganic and organic anions
- Ideal for the separation of water-soluble vitamins
- Useful for the separation of bio-molecules such as nucleotides, nucleosides, oligonucleotides, oligodeoxynucleotides, amino acids, and peptides

**ZirChrom®-SHAX (Stable from pH 1-12, and to 80 °C)**

- Quaternized polyethyleneimine-coated zirconia for strong hydrophilic anion-exchange.
- ZirChrom-SHAX has all the advantages of ZirChrom-SAX except that the surface is much more hydrophilic making it useful for anion-exchange of proteins

**ZirChrom®-WAX (Stable from pH 3-9, and to 50 °C)**

- Cross-linked polyethyleneimine-coated zirconia for weak anion-exchange
- Efficient weak anion-exchanger useful for inorganic and organic anions
- Useful for the separation of bio-molecules such as nucleotides, nucleosides, oligonucleotides, oligodeoxynucleotides, amino acids, peptides, and proteins
- Extremely stable amino phase for normal phase separation of carbohydrates

**ZirChrom®-WCX (Stable from pH 1-10, and to 50 °C)**

- Phosphate-coated zirconia for weak cation-exchange
- Useful for protein chromatography in the cation-exchange mode

**ZirChrom®-PEZ (Stable from pH 1-10, and to 50 °C with mobile phase additive)**

- EDTPA-coated zirconia for cation-exchange
- Useful for protein chromatography in the cation-exchange mode
- Excellent phase for monoclonal antibody separations

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<td>ZR04</td>
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<tr>
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<td>Weak Anion-Exchange</td>
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<td>ZirChrom®-SAX</td>
<td>Strong Anion-Exchange</td>
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<td>ZirChrom®-SHAX</td>
<td>Strong Hydrophilic Anion-Exchange</td>
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<td>Cation-Exchange for Proteins</td>
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Method Development with ZirChrom®-Chiral

ZirChrom Separations, Inc. is pleased to announce the arrival of a full line of zirconia-based chiral HPLC phases, ZirChrom®-Chiral. These new patent-pending chiral stationary phases incorporate the unsurpassed chemical and mechanical stability of zirconia with the flexibility of Lewis acid/base anchored chiral selectors. This combination creates a CSP that is reproducible, durable and can be regenerated.

The surface chemistry of zirconia is very different from silica gel due to the presence of a high population of strong Lewis acid (Zr+4) sites. The synthesis of the ZirChrom®-Chiral phases capitalizes on the presence of Lewis acid sites on the surface of the zirconia to provide a more robust and chemically flexible platform for CSP design.

This novel approach to the production of chiral stationary phases on zirconia that has been developed that offers significant method development advantages over other platforms. Zirconium atoms on the surface of zirconia (zirconium dioxide) particles act as strong Lewis acid sites that allow for facile attachment of chiral stationary phases by a tethering group having strong electron donor chelating properties. ZirChrom®-Chiral phases are synthesized using a simple two-step approach: 1) attach an appropriate tethering group to the zirconia surface through a Lewis acid-base reaction, and 2) covalently attach the desired CSP to the tethering group using amide bond formation chemistry.

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<tr>
<th>PACKING</th>
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<th>PART</th>
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<td>Pirkle Chiral Phase Pi Acceptor</td>
<td>ZRC01</td>
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<tr>
<td>ZirChrom®-Chiral(R)NESA</td>
<td>Pirkle Chiral Phase Pi Donor</td>
<td>ZRC02</td>
</tr>
<tr>
<td>ZirChrom®-Chiral(S)NESA</td>
<td>Pirkle Chiral Phase Pi Donor</td>
<td>ZRC03</td>
</tr>
<tr>
<td>ZirChrom®-Chiral(S)PG</td>
<td>Pirkle Chiral Phase Pi Acceptor</td>
<td>ZRC04</td>
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<tr>
<td>ZirChrom®-Chiral(R)PG</td>
<td>Pirkle Chiral Phase Pi Acceptor</td>
<td>ZRC05</td>
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<tr>
<td>ZirChrom®-CelluloZe</td>
<td>Carbohydrate Chiral Phase</td>
<td>ZRC06</td>
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</table>

Microbore, Semi-Prep and Prep Formats Available—see Page 24

Example of a typical chemical reaction used to tether a chiral selector to the zirconia surface.
ZirChrom®-Chiral columns were compared to silica columns having analogous chiral selectors and found to have similar resolving power for the selected probe enantiomers. Most importantly, the chemisorbed chiral selectors on ZirChrom®-Chiral were found to be stable enough for extended routine use; however, they could be completely removed by washing with a high pH (>pH 12) aqueous solution and could be easily regenerated.

**ZirChrom®-Chiral(S)LEU & ZirChrom®-Chiral(S)PG Selectivity Comparison**

**LC CONDITIONS**
- Mobile phase: |A| Hexane, |B| Isopropanol
- Isocratic Elution: 99/1 A/B
- Flow rate: 1.0 ml/min
- Temperature: 30 °C

**Columns:**
- ZirChrom®-Chiral(S)LEU, 100 x 4.6 mm i.d. (part# ZRC01-1046)
- ZirChrom®-Chiral(S)PG, 100 x 4.6 mm i.d. (part# ZRC04-1046)

Selectivity is compared for 12 probe solute enantiomers on zirconia (S)-dinitrobenzoyl-L-leucine and zirconia (S)-dinitrobenzoyl-L-phenylglycine CSPs. As expected, changing the chiral selector had a significant effect on the resolution of enantiomers. This ability to change chiral selectors on the same column can reduce the influence of other column factors and allow the focus to be placed on choosing the best chiral selector during method development.
Antibody Purification with Rhinophase®-AB

ZirChrom introduces a next generation chromatographic media enabling the fast, reliable purification of monoclonal antibodies. Rhinophase®-AB is a biocompatible stationary phase useful for both small and large-scale purifications. Rhinophase®-AB may be packed into columns or SPE tubes and run at very high mobile phase linear velocities compared to the mechanically soft affinity gels such as Protein A* and Protein G*, which results in a dramatic increase in purification throughput. Furthermore, Rhinophase®-AB can effectively purify a wide range of Mab subclasses, as well as polyclonal hIgG, IgA and IgM, providing an almost universal antibody purification media.

ZirChrom now offers two complete solutions for monoclonal antibody purification; 1) the Rhinophase®-AB Research Kit (right; part# AB01-RES, $187.00 list price) and 2) the Rhinophase®-AB Production Kit (not shown; part# AB01-HTP, $2,750.00 list price). Each kit contains the items necessary to perform laboratory scale purifications (just add water!). The Research Kit is designed for small scale jobs. The Production Kit employs a vacuum filtration apparatus for high throughput applications. Refer to technical bulletin # 243 for further details.

ZirChrom’s Rhinophase®-AB Research Kit, part# AB01-RES (just add water!)

![Surface Chemistry of Rhinophase®-AB](image1)

**Rapid Results with Rhinophase®-AB**

**Total Analysis Time = 15 minutes**

This is 100 times faster than a Protein G purification of the same scale!

**Step A** = 20 mM MES buffer, 4 mM EDTA, 50 mM NaCl @ pH 4.0, **Step B** = 20 mM MES buffer, 4 mM EDTA, 50 mM NaCl @ pH 4.0, **Step C** = 20 mM MES buffer, 4 mM EDTA, 2.0 M NaCl @ pH 4.0. Flow Rate = 60 mL/min, Injection size = 31.6 mL serum-free cell culture supernatant diluted 4-times with loading buffer, (3.98 mg of Mab), Amount of Rhinophase®-AB in tube = 10 grams.

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<tr>
<th>PACKING</th>
<th>USE</th>
<th>PART</th>
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</thead>
<tbody>
<tr>
<td>Rhinophase®-AB</td>
<td>Antibody Purification</td>
<td>AB01-RES</td>
</tr>
</tbody>
</table>

![Binding Capacity of Rhinophase®-AB](image2)

![Fraction Number](image3)

![UV Absorbance at 280nm](image4)
ProTain® In-Line Protein Removal System

The HPLC analysis of small molecules in matrices containing proteins of variable origin is often problematic because of poor resolution between the analyte of interest and the matrix constituents and potential fouling of the analytical column by matrix proteins and debris. The incorporation of ZirChrom’s new ProTain® in-line protein removal system upstream of any type of analytical column offers a selective, cost effective and simple method of reducing matrix interferences for the HPLC analysis of small molecules in bio-samples.

The type of buffer, specifically its strength as a Lewis base, and the pH of the mobile phase play a significant role in determining the actual protein binding capacity of the ProTain® system. Refer to technical bulletin # 291 for further details.

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<tr>
<td>Custom Sizes Available Upon Request</td>
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</table>

ZirChrom’s ProTain® in-line protein removal system is comprised of one ProTain® system insert and one ProTain® system holder. A section of capillary tubing and all of the necessary nuts and ferrules are included with the holder.

**PRICING INFORMATION**

- **ProTain® Holders:**
  - part # 850-00-2 (for 4.6 mm i.d. inserts) $82.00 list price
  - part # 852-00-2 (for 2.1 mm i.d. inserts)

- **ProTain® Inserts:**
  - part # PT01-0246 (20 x 4.6 mm i.d.; set of 3) $329.00 list price
  - part # PT01-0221 (20 x 2.1 mm i.d.; set of 3)

**LC CONDITIONS**

Samples of Bovine Serum Albumin (BSA) were prepared in a phosphate buffer at pH 6.8 and injected onto two column configurations:

A: TSK G3000 Size Exclusion Column

B: ZirChrom’s ProTain® in-line protein removal system (Holder - part # 850-00-2; Set of three inserts- part #PT01-0246) installed in front of the TSK G3000 SEC column

UV detection: 215 nm
Phosphopeptide Enrichment

Recent research highlights the unparalleled selectivity of our titanium dioxide and zirconium dioxide particles (in bulk or in Glygen Lab-in-a-tip™ SPE tips) for phosphopeptide enrichment. Titanium dioxide enrichment is fast becoming the new standard for enrichment of phosphorylated peptides for MS analysis. This trend is demonstrated by the overwhelming number of recent publications employing titanium dioxide in their enrichment procedure.

ZirChrom® currently offers the following phosphopeptide solutions:

- Glygen Lab-in-a-tip™
- ZirChrom® Bulk Material
- ZirChrom® Guard, Analytical, & Prep Columns
- Sachtopore® Bulk Material
- Sachtopore® Guard, Analytical, & Prep Columns

Zirconia and Titania Lab-in-a-Tip™ SPE Pipette Tips

NuTip™

A revolutionary new SPE cartridge in which chromatography material is embedded in the inner surface of a pipette tip. This maximizes the surface area in contact with the sample. The lack of polymers or glue for embedding the material, avoids potential problems with contamination or permeability.

- Faster sample preparation with minimal sample loss
- No contamination from the supporting matrix
- Sample volumes as small as 0.1ul
- Available in volumes of: 0.1-10ul and 10-200ul

TopTip™

This is a unique concept in solid phase extraction (SPE). Top Tip is a pipette tip with a fine slit at the bottom (slit width: 1-2 μm which permits liquid to pass through but retains the chromatographic material (20-30 μm) in the tip. This also eliminates the need for a filter and, thus, dead volume. Top Tip contains just your desired chromatography material and nothing else and is excellent for working with small samples.

Revolutionary SPE Micropipette Tips:

- Can also be used as spin column
- Faster sample preparation with minimal sample loss
- No contamination from the supporting matrix
- Sample volumes as small as 0.1μl
- Available in volumes of: 0.1-10μl and 10-200μl

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<th>PACKING</th>
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<td>Phosphopeptide Enrichment</td>
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<tr>
<td>NuTip™</td>
<td>Phosphopeptide Enrichment</td>
<td>NT</td>
</tr>
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All ZirChrom®, DiamondBond®, and Sachtopore® stationary phases are available in SPE pipette tip formats upon request.
ZirChrom® HPLC Columns are Efficient, Stable and Reproducible...

Symmetry Comparison Of ZirChrom®-MS Versus a Leading Bonded Phase C18 Silica

LC CONDITIONS
Columns: ZirChrom®-MS
(Part Number: MS01-0546)
50 mm x 4.6 mm i.d.,
3μm particle size;
Leading bonded phase C18 silica,
150 mm x 4.6 mm i.d.,
3.5 μm particle size

Mobile Phase: Machine-mixed
80/20 ACN/10 mM ammonium acetate,
ph=6.7 w/o pH adjust.
Flow Rate: 1.0 ml/min
Temperature: 35 °C
Injection Vol.: 0.1 μl
Detection: 254 nm

Efficiency Comparison of Leading HPLC Columns*

Stability Comparison of Leading HPLC Columns

Reproducibility Data on 50 Recent ZirChrom®-PBD Columns

*Column names are registered trademarks of their respective manufacturers:
Luna is a registered trademark of Phenomenex
Xterra and Symmetry are registered trademarks of Waters
Hypercarb is a registered trademark of Thermo-Hypersil-Keystone
Extend and Zorbax are registered trademarks of Agilent Technologies

LC CONDITIONS
Mobile phase: 65/35 ACN/50 mM Potassium phosphate buffer, pH 3.2
Flow rate: 1.3 ml/min    Temperature: 21 °C
Injection volume: 1 μl   Detection: 254 nm

Mobile phase: 45/50 ACN/THF/50 mM Potassium phosphate buffer, pH 7.0
Flow rate: 1.3 ml/min    Temperature: 30 °C
Injection volume: 1 μl   Detection: 254 nm

Solutes: uracil, phenol, pyridine, 4-butylbenzoic acid, N,N-dimethylaniline, toluene

Inorganic Oxide Columns
Polymer Columns
Carbon Columns

ZirChrom®-PBD, pH 12.0
Luna® C18 (2), pH 10.0
Xterra® RP18*, pH 11.5
XTerra® RP18, pH 12.0
DiamondBond®-C18, pH 12.5

Column Volumes of Mobile Phase

Column Number

k’ Toluene
k’ (Methylbenzoate/Benzonitrile)
## Representative Application Notes Not Shown in Product Guide

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<th>Class</th>
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For a complete listing of application notes, visit our website at www.zirchrom.com. For access to new application note distributions via e-mail, contact ZirChrom technical support (support@zirchrom.com) and request “Z-APPS".
**Method Development Kits**

Not sure which column is best for your application? Try one of ZirChrom’s method development kits. Each kit contains 3 columns with different selectivity for faster screening (columns are 50 x 4.6 mm i.d.; additional sizes available by request; phase substitutions allowed)

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<th>Kit Number</th>
<th>Kit Description</th>
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<td>(contains 1 each of ZirChrom®-SAX, ZirChrom®-SHAX, and ZirChrom®-WAX)</td>
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<td>(contains 1 each of ZirChrom®-PBD, ZirChrom®-CARB, and ZirChrom®-EZ (or ZirChrom®-MS))</td>
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*All columns will be packed with 3 μm particles unless 5 μm particles are specifically requested.

**Particle Sizes**

ZirChrom’s analytical columns are packed with 3 μm particles. On request, we can pack columns with 1.9, 5, 7, 10, or 25 μm particles, or larger, with identical chemistry, making scale-up using ZirChrom’s products fast and easy.

**Non-Porous Zirconia**

ZirChrom has available non-porous particles for ultra-fast chromatography. These particles are available in sizes of 0.5, 1.0, 1.5, 2.0 and 3.0 μm and in all of our normal phase and reversed phase chemistries. Ion exchange versions can be custom made. Specify NPZ on your order.

**Prep/Semi-Prep Formats**

All of our phases are available in semi-prep and prep formats. See our Technical Bulletin #196 for more information on easy scale-up to prep formats.

**Microbore and LC/MS Formats**

All of our phases are available in microbore (0.3, 0.5 & 1.0 mm i.d.) and LC/MS formats upon request. Our ZirChrom®-MS phase was designed for LC/MS use.

**Normal Phase LC**

Unmodified zirconia and titania particles make an excellent support for normal phase LC and phosphopeptide enrichment. ZirChrom®-PHASE (part# ZR02) and Sachtopore®-NP (part# T102) are packed into the same column formats as our reversed phase and ion exchange supports. Bulk pricing available upon request.

**Technical Support**

ZirChrom’s products are often used in cutting edge separations where silica supports fail. Our technical support group has extensive experience, particularly in pharmaceutical and environmental HPLC. We are happy to assist you with your difficult separations. Call us at 1-866-STABLE-1, (763) 421-5264 or e-mail our technical support group at support@zirchrom.com.

**Buffer Wizard**

The ZirChrom BUFFER WIZARD is a web-based laboratory consultant designed to do the calculations needed to prepare buffers specifically for use in HPLC. In addition to doing the calculations it provides many helpful hints as to the proper choice of buffer and issues messages when the buffer capacity is too low to do the job or when too high or too low pH might damage conventional stationary phases.

To access the Buffer Wizard and other HPLC relevant data, visit the ZirChrom Home Page (www.zirchrom.com). An advanced version of the Buffer Wizard with 50 buffer systems is available for sale (part# BW01; $110.25 list price).

**International Inquiries/Orders**

International customers can reach ZirChrom directly at +1 (763) 421-5264. Our Fax number is +1 (763) 421-2319. You may also e-mail our technical support group at support@zirchrom.com.