

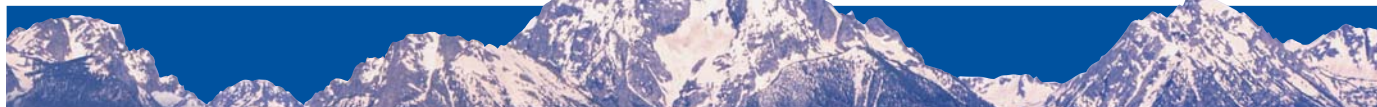


# HPLC Column Product Guide

2009-2010

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

- Microbore
- Analytical
- Semi-Preparative
- Preparative



## Table of Contents



### Introduction

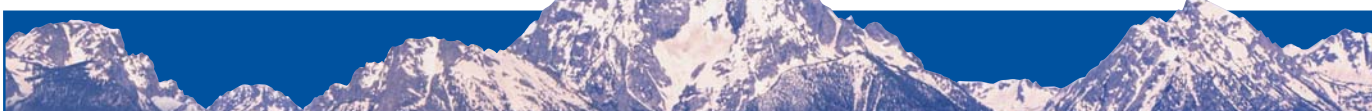
Company History . . . . .	2
Technology Information . . . . .	2

### Product Specific Information

Reversed-Phase Chromatography . . . . .	3
Reversed-Phase Selection Guide . . . . .	4
Stability Data . . . . .	5
ZirChrom®-PBD . . . . .	6-7
ZirChrom®-PS . . . . .	8
DiamondBond®-C18 . . . . .	9
ZirChrom®-CARB . . . . .	10
ZirChrom®-EZ . . . . .	11-12
ZirChrom®-MS . . . . .	11-12
Sachtopore® Titania-Based HPLC Columns . . . . .	13
Ion Exchange Chromatography . . . . .	14
Ion Exchange Chromatography - Applications . . . . .	15
ZirChrom®-SHAX . . . . .	16
ZirChrom®-WAX . . . . .	16
ZirChrom®-WCX . . . . .	16
ZirChrom®-PEZ . . . . .	16
ZirChrom®-SAX . . . . .	16
ZirChrom®-Chiral . . . . .	17-18
Rhinophase®-AB . . . . .	19
ProTain® . . . . .	20
Sample Preparation (Phosphopeptide Enrichment) . . . . .	21
Reference Information . . . . .	22-23
Other Products and Services . . . . .	24
Ordering Information . . . . .	25

*Specialists in Ultra-Durable,  
High-Efficiency HPLC Phases*





## GENERAL INFORMATION

### 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 ( $ZrO_2$ ), 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  $\mu m$  (for analytical work) up to 25  $\mu m$  (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.

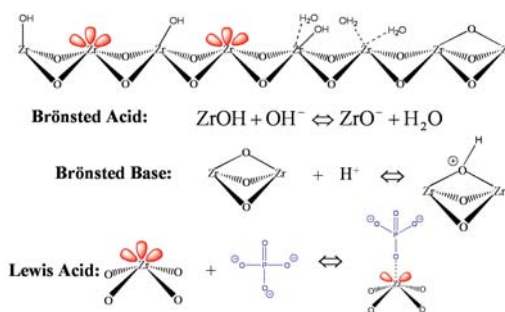
- Don't worry about the column stability—  
Use the optimal conditions to separate your analytes
- Methods at extreme conditions are robust,  
with long column life

- 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) Brönsted acid, b) Brönsted base and c) Lewis acid, which are shown below:



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



- Widest Range of RPC Selectivities at High Efficiency
- Excellent General Purpose Phases
- Specific Columns for Acids and Bases
- Superior Columns for LC/MS
- Ideal Columns for Diastereomers/Geometric Isomers
- Specialized Column for Highly Aqueous Mobile Phase

### 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.

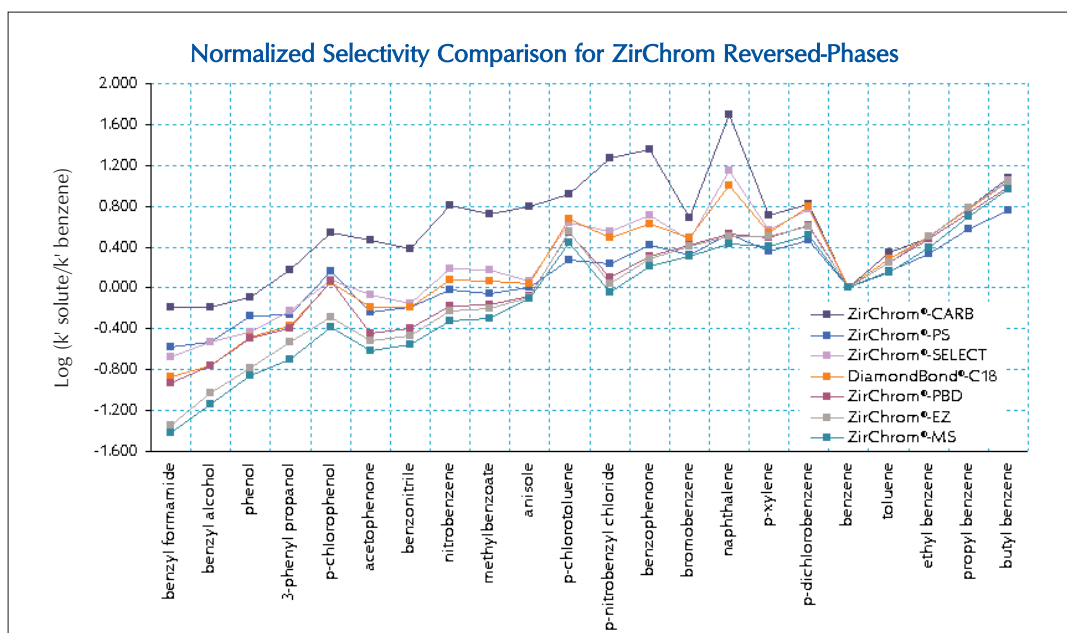
#### Traditional ZirChrom® Reversed-Phases

- 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

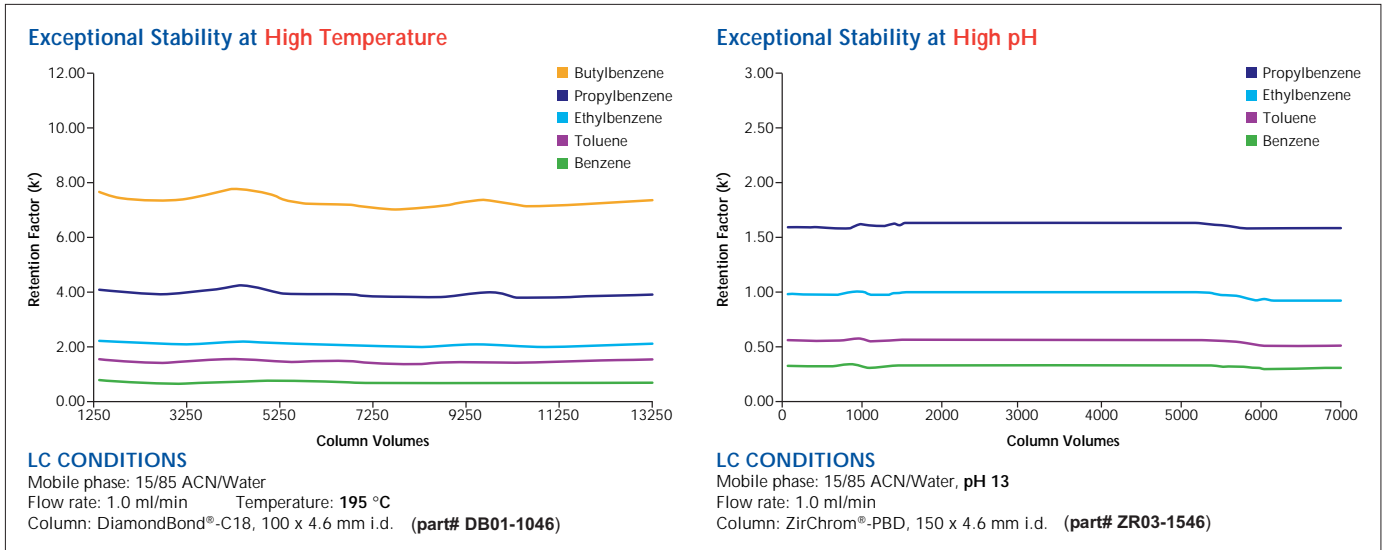
Current Problem/Concern	Column	Suggested Conditions
<b>Improved Selectivity</b>		
<b>Need improved selectivity for nonelectrolytes, isomers, diastereomers.</b> Currently using carbon, cyano, phenyl or fluoro phases. ZirChrom®-SELECT	DiamondBond®-C18, ZirChrom®-CARB or ZirChrom®-CARB set temperature to at least 50 °C.	Use an acetonitrile and/or THF eluent. Add octylamine to improve peak shape. If using DiamondBond®-C18
<b>Need improved selectivity for bases.</b>	ZirChrom®-MS, ZirChrom®-PBD	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 ( $\leq 14$ ).
<b>Need improved selectivity for acids.</b>	ZirChrom®-EZ, ZirChrom®-MS	Use buffer of choice in a pH range of 1-10. Try low pH first. 5 - 25 mM phosphate may improve peak shape.
<b>Change Retention</b>		
<b>Need more retention for very polar (hydrophilic) nonelectrolytes.</b> Currently using nearly 100% water eluent or polar embedded phase.	DiamondBond®-C18, ZirChrom®-CARB ZirChrom®-SELECT	Can use in high water mobile phase.
<b>Need more retention for very polar bases.</b> Currently using nearly 100% water eluent or polar embedded phase or sulfonic acid paired ion reagent.	ZirChrom®-MS	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.
<b>Need more retention for very polar acids.</b> Currently using nearly 100% water eluent or polar embedded phase or quaternary amine paired ion reagent.	ZirChrom®-EZ, ZirChrom®-CARB, DiamondBond®-C18	Use buffer of choice in a pH range of 1-10. Try low pH first. For tough separations, decrease pH below pKa ( $\geq 1$ ). 5 - 25 mM phosphate may improve peak shape.
<b>Need less retention with any solute type.</b>	ZirChrom®-PS	Least hydrophobic phase. Can be used with 100% water eluent.
<b>Improve Efficiency/Productivity</b>		
<b>Inadequate stability and selectivity.</b> Having trouble with silica-based phases, changed to alumina or polymer column and analytes were still not sufficiently resolved.	All ZirChrom® Reversed-Phase (RP) Columns	Zirconia phases exhibit excellent pH and temperature stability. ZirChrom® RPs give higher efficiency and better peak shape than alumina or polymer columns.
<b>Poor column reproducibility.</b> Experiencing retention changes at low or high pH, at elevated temperature or when using phosphate or carbonate buffer.	All ZirChrom® RP Columns	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.
<b>Separations taking too long.</b>	All ZirChrom® RP Columns	Increase temperature up to max. operating range for LC and/or analyte. Increase flow rate. Easily improves speed 2-3 fold.
<b>Column overloaded too easily with basic solutes.</b>	ZirChrom®-MS, ZirChrom®-PBD	The mixed-mode (reversed-phase / cation exchange) retention mechanism enables enhanced loadability.
<b>Improve Detection Sensitivity</b>		
<b>Need to go to shorter wavelength to enhance sensitivity in UV.</b> Solute does not have long wavelength absorption or is very dilute.	ZirChrom®-PS	Use a high water or pure water eluent and go deep into UV.
<b>Need LC/MS detection of Lewis base analytes at low pH.</b>	ZirChrom®-EZ, ZirChrom®-MS	Use buffer of choice (in pH range 1-10) with these Lewis acid site deactivated phases.
<b>Need to decrease bleed in LC/MS.</b>	All ZirChrom® RP Columns	All ZirChrom® columns are extremely low bleed. The ZirChrom®-MS column was designed for LC/MS.

Also refer to [www.zirchrom.com](http://www.zirchrom.com) for zirconia publication numbers 16, 17, 23, 51, 65, 71, 77, and 87.

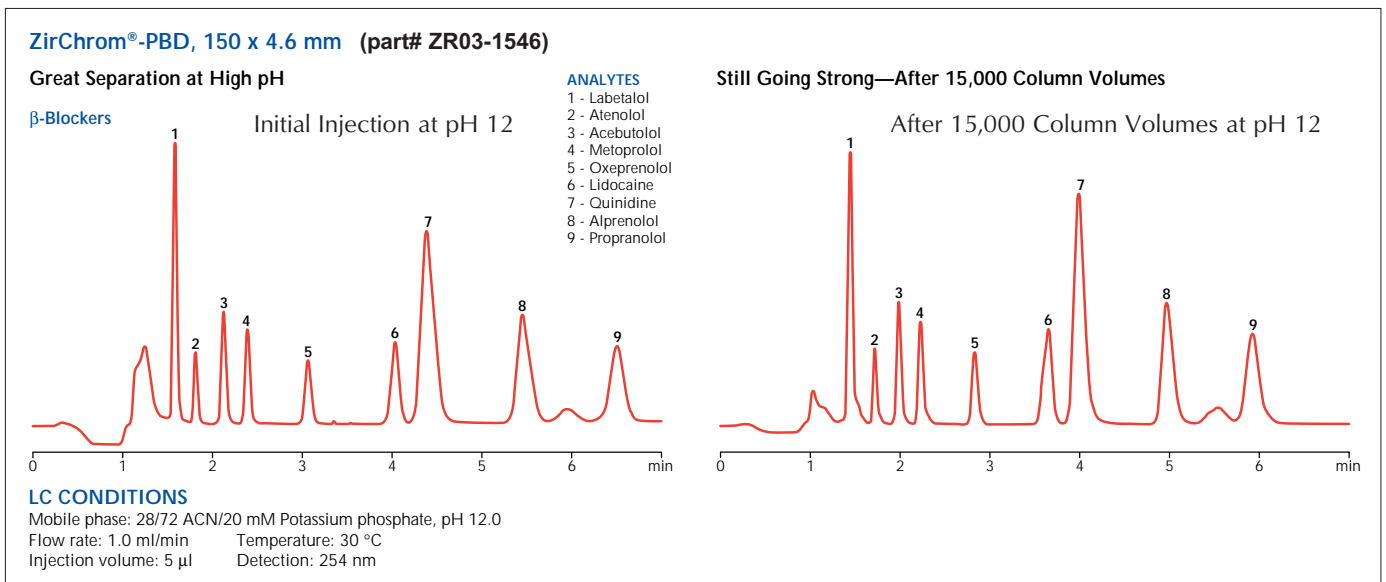


# ZirChrom's traditional reversed-phase columns stand up to your most challenging applications!

## Chemical and Thermal Stability



## “Real World” Example at High pH

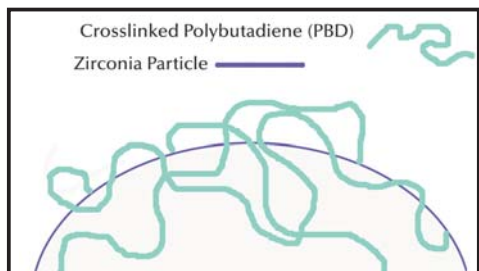


The top figures show typical stability data for our products at extreme pH and temperature. Below is a typical example of a robust separation at high pH.





## ZIRCHROM®-PBD



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

### Method Development with ZirChrom®-PBD (USP L49)

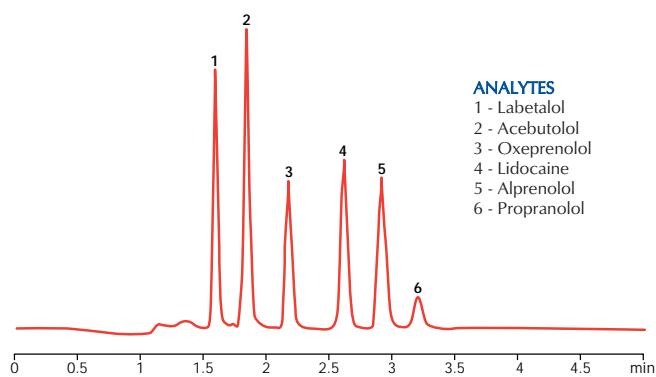
ZirChrom®-PBD is produced by coating ultra-stable zirconia particles with an equally stable extremely thin layer of crosslinked polybutadiene. The chemical selectivity of ZirChrom®-PBD columns is similar to that of a traditional C8 or C18 silica based column for non-ionic analytes. In the case of ionizable 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.

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.*

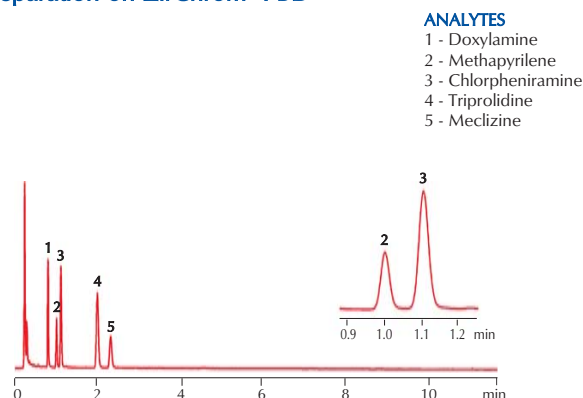
#### ZirChrom®-PBD Gives Excellent Peak Shapes for Basic Drugs at High pH



##### LC CONDITIONS

Mobile phase: 35/65 ACN/20 mM Potassium phosphate, pH 12.2  
Flow rate: 1.0 ml/min      Temperature: 30 °C  
Injection volume: 5 µl      Detection: 254 nm  
Column: ZirChrom®-PBD, 150 x 4.6 mm i.d. (part# ZR03-1546)

#### Fast, High Temperature Antihistamines Separation on ZirChrom®-PBD



##### LC CONDITIONS

Mobile phase: 28/72 Acetonitrile/50mM tetramethylammonium hydroxide (pH 12.2)  
Flow rate: 3.0 ml/min      Temperature: 80 °C  
Injection volume: 0.2 µl      Detection: 254 nm  
Pressure drop: 195 bar  
Column: ZirChrom®-PBD, 100 x 4.6 mm i.d. (part# ZR03-1046)



## 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).

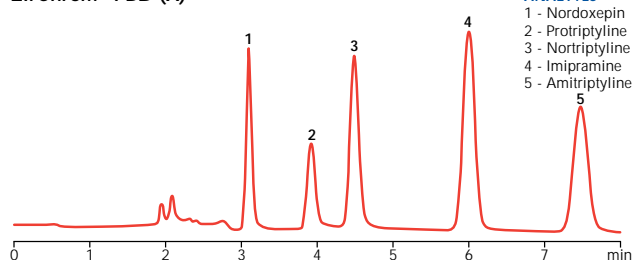
## ZirChrom®-PBD for Azithromycin

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.

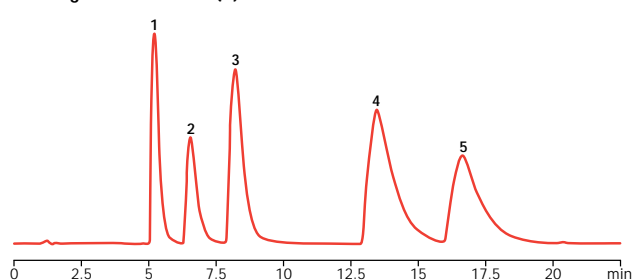
PACKING	MODE	PART
ZirChrom®-PBD	Reversed-Phase	ZR03
Microbore, Semi-Prep and Prep Formats Available—see Page 24		

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

#### ZirChrom®-PBD (A)



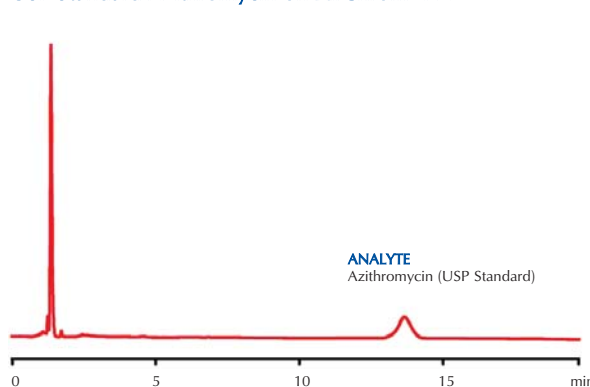
#### Leading Silica Column (B)



#### LC CONDITIONS

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

### USP Standard Azithromycin on ZirChrom®-PBD

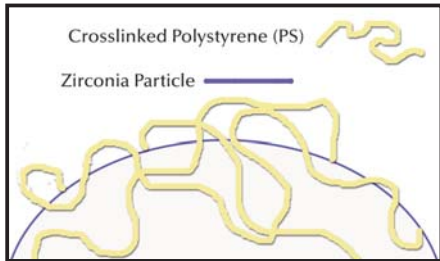


#### LC CONDITIONS

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  
Flow rate: 1.0 ml/min Temperature: 30 °C  
Injection volume: 5 µl (1mg/mL) Detection: 215 nm  
Column: ZirChrom®-PBD, 150 x 4.6 mm i.d. (part# ZR03-1546)



**ZIRCHROM®-PS**



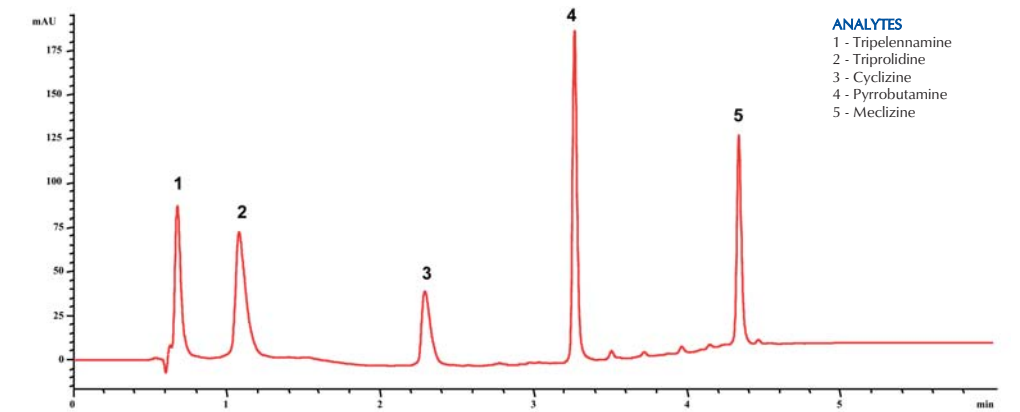
- pH Stable from 1 up to 13
- Excellent Thermal Stability
- Superior Selectivity for Poly Aromatic Compounds

**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.

PACKING	MODE	PART
ZirChrom®-PS	Reversed-Phase	ZR09
Microbore, Semi-Prep and Prep Formats Available—see Page 24		

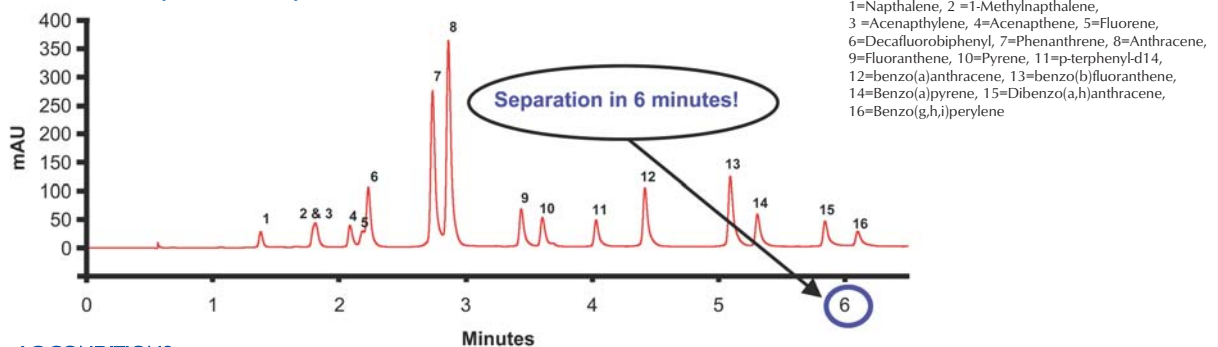
**Antihistamines on ZirChrom®-PS**



**LC CONDITIONS**

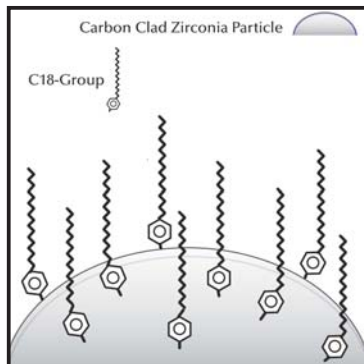
Mobile phase: [A] 25 mM HCl, pH 1.9  
[B] Acetonitrile  
Gradient Elution: 5 - 15 % B over 0 - 1 minutes, 15 - 60 % B over 1 - 4 minutes  
Flow rate: 1.0 ml/min Temperature: 40 °C  
Injection volume: 0.5 µl Detection: 254 nm  
Column: ZirChrom®-PS, 50 x 4.6 mm i.d. (part# ZR09-0546)

**Sixteen Polyaromatic Hydrocarbons in Six Minutes**



**LC CONDITIONS**

Mobile phase: [A] Water  
[B] Acetonitrile  
Gradient Elution: 10 - 50 % B over 0 - 7 minutes  
Flow rate: 3.0 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)



# DIAMONDBOND®-C18

- Ideal for Separating Steroids and Analogues
- Excellent Selectivity for Acidic Compounds
- pH Stable from 1 to 14 for Robust Methods
- Excellent Thermal Stability for Fast Separations

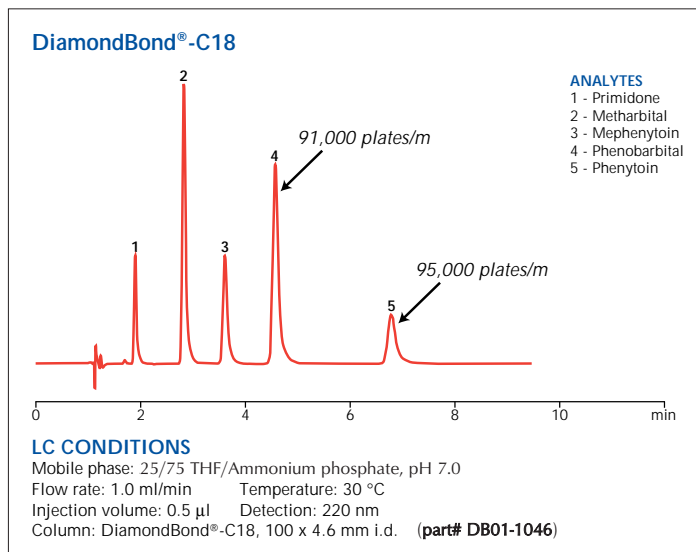
## Method Development with DiamondBond®-C18

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,

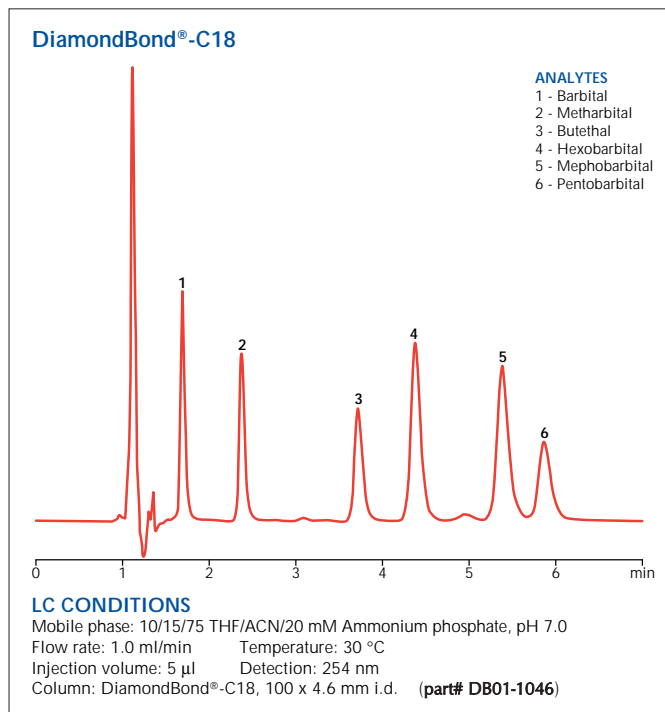
and unique selectivity compared to silica phases. Like for all traditional reversed-phase zirconia products, proper buffer selection helps to ensure the best peak shapes and band spacing. DiamondBond®-C18 is stable up to 200 °C.

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

### Anticonvulsants

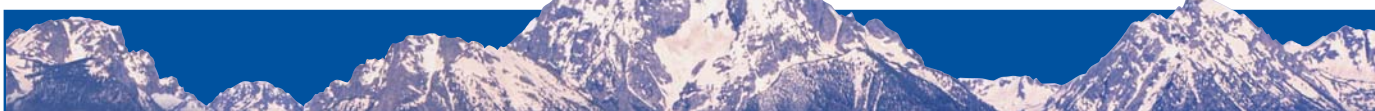


### Barbiturates

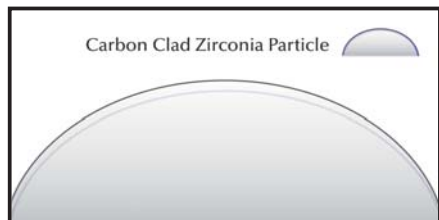


PACKING	MODE	PART
DiamondBond®-C18	Reversed-Phase	DB01
Microbore, Semi-Prep and Prep Formats Available—see Page 24		





## ZIRCHROM®-CARB



- Ideal for Separating Steroids and Analogues
- Excellent Selectivity for Acidic Compounds
- pH Stable from 1 to 14 for Robust Methods
- Excellent Thermal Stability for Fast Separations

## Method Development with ZirChrom®-CARB

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.

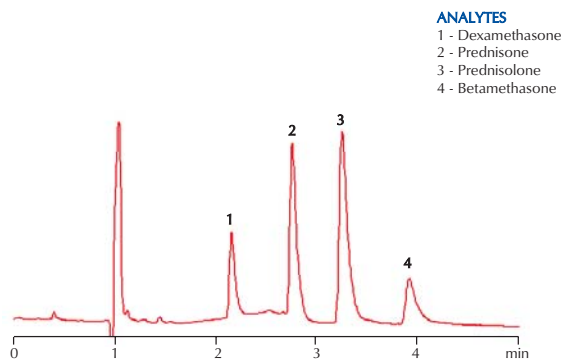
ZirChrom®-CARB is great for geometric isomer separations, and is superb for separating diastereomers. The selectivity of ZirChrom®-CARB is more different from ODS than phenyl or cyano phases. This makes it an excellent choice for orthogonal screening in drug discovery

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.*

PACKING	MODE	PART
ZirChrom®-CARB	Reversed-Phase	ZR01
Microbore, Semi-Prep and Prep Formats Available—see Page 24		

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

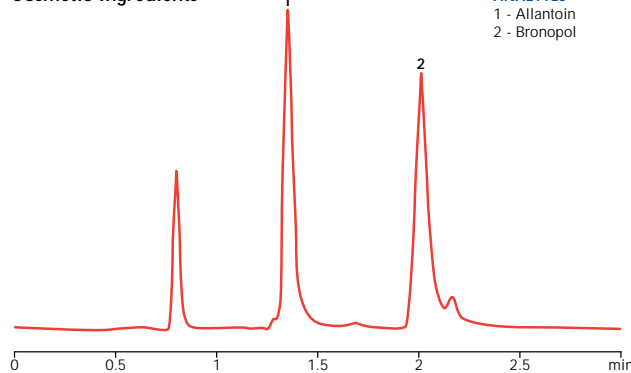


#### LC CONDITIONS

Mobile phase: 60/10/30 Acetonitrile/MTBE/Water  
Flow rate: 1.5 ml/min Temperature: 80 °C  
Injection vol.: 15 µl Detection: 215 nm  
Column: ZirChrom®-CARB, 150 x 4.6 mm i.d. (part# ZR01-1546)

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

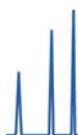
#### Cosmetic Ingredients



#### 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. (part# ZR01-1046)

\*NOTE: These compounds are not retained on ODS in pure water



ZIRCHROM®-EZ / ZIRCHROM®-MS



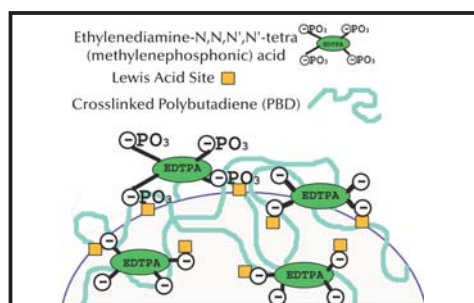
- Easy-to-Use (No Special Additives Required)
- Designed for LC/MS
- Unique Selectivity for Basic Compounds
- High Efficiency (>120,000 plates/meter)
- pH Stable from 1 up to 10

### 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

that zirconia-based columns are known to have compared to traditional bonded silica phases.

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

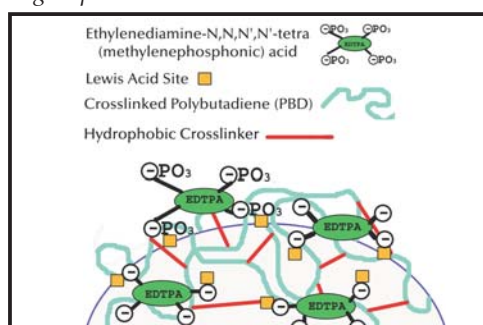


### Method Development with ZirChrom®-MS (Lewis Acid “Deactivated”)

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.

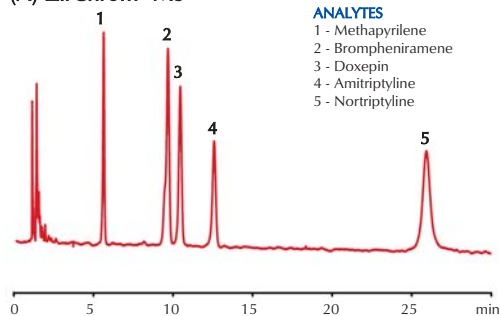
For more detailed guidelines, consult our new “Deactivated” Column Method Development Guide. Or, contact our technical support group at 1-866-STABLE-1.



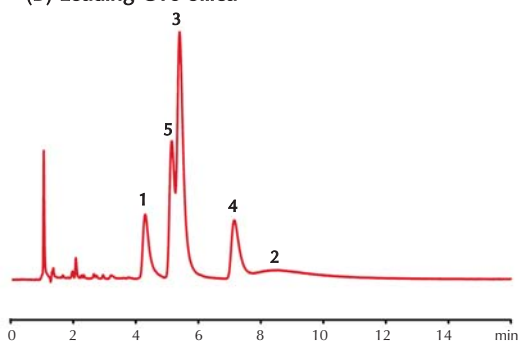


### ZirChrom®-MS Versus C18 Silica for Basic Drugs

(A) ZirChrom®-MS



(B) Leading C18 Silica



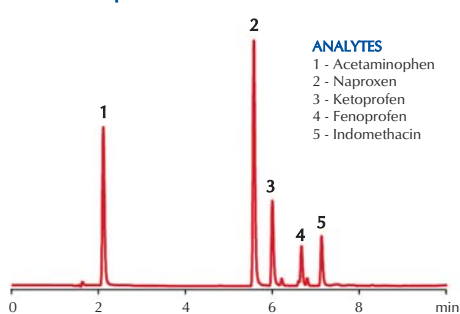
#### LC CONDITIONS

Mobile phase: Machine mixed 80/20 ACN/10 mM ammonium acetate, pH 6.7 without pH adjustment  
Flow rate: 1.0 ml/min Temperature: 35 °C  
Injection volume: 0.1 µl Detection: 254 nm  
Column A: ZirChrom®-MS, 150 x 4.6 mm i.d. (3 micron) (part# MS01-1546)  
Column B: Leading C18 Silica, 150 x 4.6 mm i.d. (3.5 micron)

PACKING	MODE	PART
ZirChrom®-EZ	Reversed-Phase	EZ01
ZirChrom®-MS	Reversed-Phase	MS01
ZirChrom®-SELECT	Reversed-Phase	ST01

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

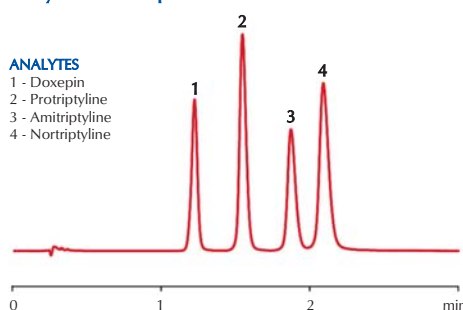
### NSAIDs Separation on ZirChrom®-EZ



#### LC CONDITIONS

Mobile phase: (A) 100 ACN  
(B) 20 mM ammonium acetate, pH 5.0  
Gradient elution: 10 - 90% A over 0 - 10 min  
Flow rate: 1.0 ml/min Temperature: 35 °C  
Injection volume: 10 µl Detection: 254 nm  
Column: ZirChrom®-EZ, 150 x 4.6 mm i.d. (part# EZ01-1546)

### Tricyclic Antidepressants on ZirChrom®-EZ



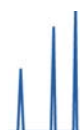
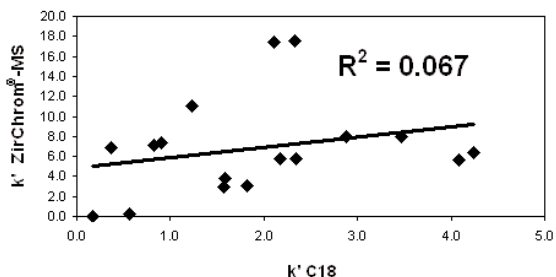
#### LC CONDITIONS

Mobile phase: 65/35 ACN/20mM ammonium acetate, pH 6.0  
Flow rate: 2.0 ml/min Temperature: 35 °C  
Injection volume: 5 µl Detection: 254 nm  
Column: ZirChrom®-EZ, 50 x 4.6 mm i.d. (part# EZ01-0546)

## The Analysis of Basic Compounds Using Neutral pH Conditions: A Column Comparison Study

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).

### Selectivity Comparison of ZirChrom®-MS Versus a Leading Bonded Phase C18 Silica for Basic Compounds



SACHTOPORE®-RP / SACHTOPORE®-NP

- Highly Stable Titania-Based Particles
- Widest Range of Particle/Pore Sizes
- Reversed-Phase and Normal Phase Medias
- Ideal for Preparative Scale Separations

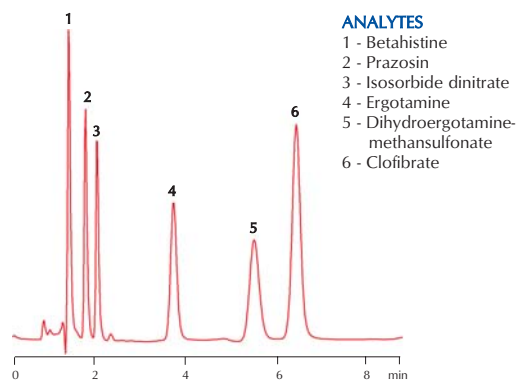


Method Development with Sachtopore®-RP and Sachtopore®-NP

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.

PACKING	MODE	PART
Sachtopore®-RP	Reversed-Phase	TI01
Sachtopore®-NP	Normal Phase	TI02
Microbore, Semi-Prep and Prep Formats Available—see Page 24		

Antihypertensives Separation at pH 9.5

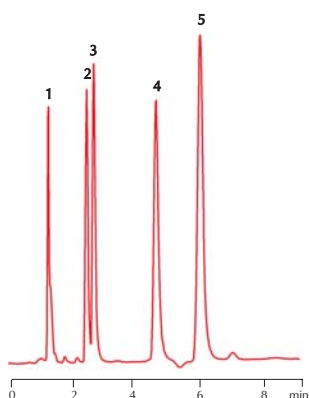


- ANALYTES**
- 1 - Betahistine
  - 2 - Prazosin
  - 3 - Isosorbide dinitrate
  - 4 - Ergotamine
  - 5 - Dihydroergotamine-methansulfonate
  - 6 - Clofibrate

LC CONDITIONS

Mobile phase: 70/30 40 mM NH<sub>3</sub> + 10 mM K<sub>2</sub>HPO<sub>4</sub> + 10 mM Na<sub>2</sub>CO<sub>3</sub> / 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)

Antihistamines Separation at pH 10

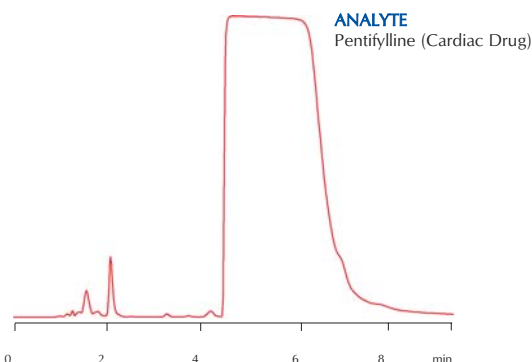


- ANALYTES**
- 1 - Maleic Acid
  - 2 - Carbinoxamine
  - 3 - Chlorphenamine
  - 4 - Diphenylpyraline
  - 5 - Phenyltoloxamine

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)

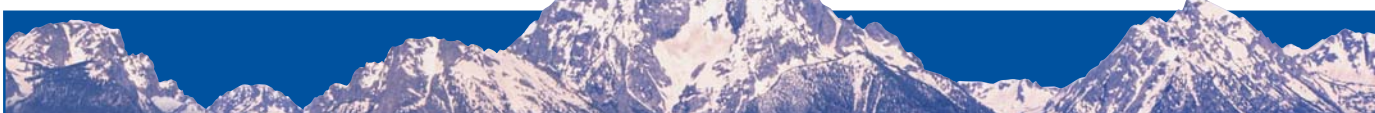
Preparative Scale Loadability on Sachtopore-RP



- ANALYTE**  
Pentifylline (Cardiac Drug)

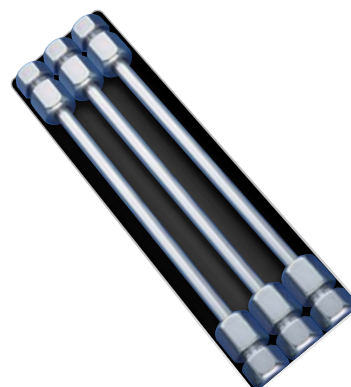
LC CONDITIONS

Mobile phase: 81/19 10 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> + 1 mM H<sub>3</sub>BO<sub>3</sub> / 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)



## ZIRCHROM ION EXCHANGERS

- 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



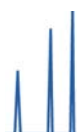
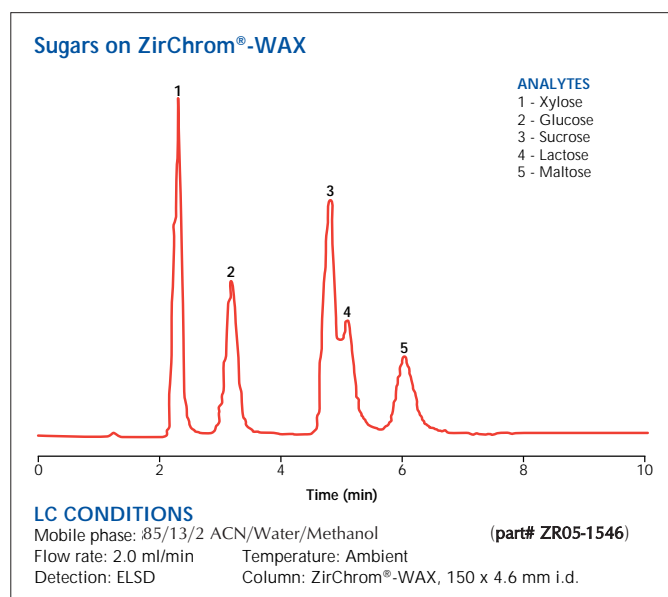
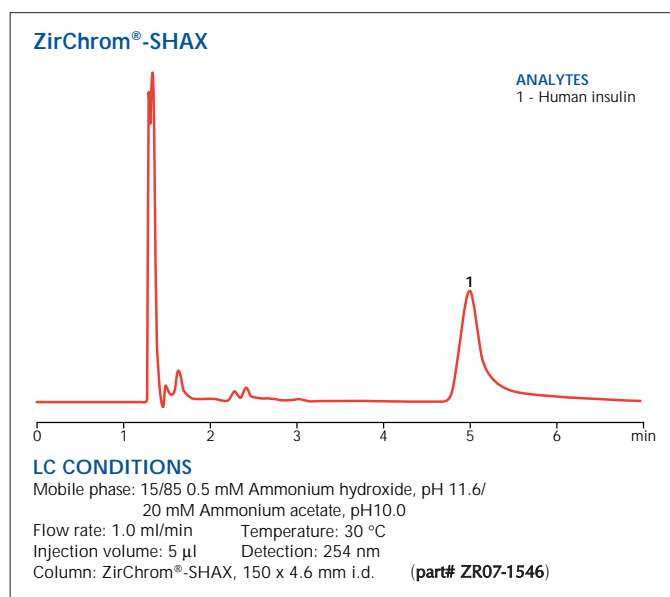
## Method Development with ZirChrom's Ion Exchange Phases

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.

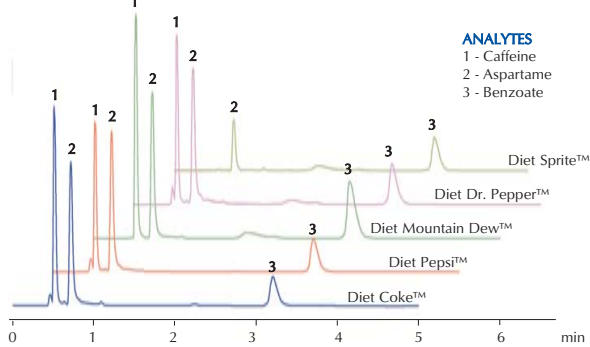
If desired, mixed-mode separation modes may be exploited to optimize separations, including Lewis acid-base interactions, hydrophobic interactions and ion-exchange interactions. These modes may be attenuated by adjusting the strong Lewis base content, organic content and ionic strength of the mobile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



ION EXCHANGE CHROMATOGRAPHY

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



**ANALYTES**  
1 - Caffeine  
2 - Aspartame  
3 - Benzoate

**LC CONDITIONS**

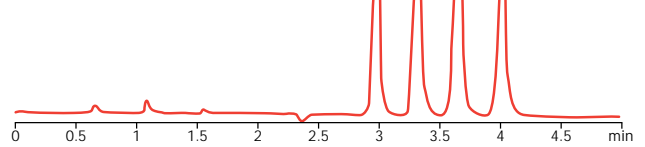
Mobile phase: 10mM Ammonium phosphate, 5mM Ammonium carbonate, pH 6.6  
Flow rate: 1.0 ml/min Temperature: 50 °C  
Injection volume: 5 µl Detection: 210 nm  
Column: ZirChrom®-SAX, 100 x 3.0 mm i.d. (part# ZR06-1030)

**Organic Acids**

ZirChrom®-SAX

**ANALYTES**

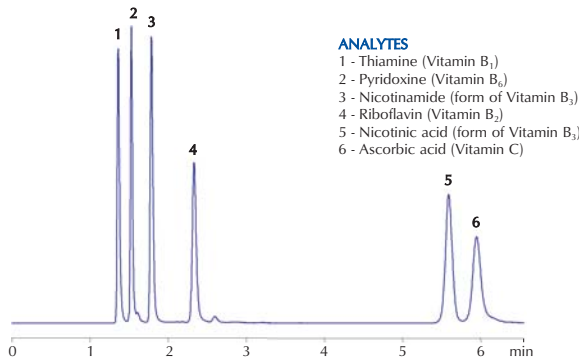
- 1 - Acetic acid
- 2 - Propionic acid
- 3 - Formic acid
- 4 - Butyric acid



**LC CONDITIONS**

Mobile phase: 5 mM Ammonium phosphate, pH 6.0 Pressure drop: 140 bar  
Flow rate: 1.5 ml/min Temperature: 50 °C Column: ZirChrom®-SAX,  
Injection volume: 5 µl Detection: 210 nm 150 x 4.6 mm i.d.  
(part# ZR06-1546)

Water Soluble Vitamins on ZirChrom®-SAX



**ANALYTES**

- 1 - Thiamine (Vitamin B<sub>1</sub>)
- 2 - Pyridoxine (Vitamin B<sub>6</sub>)
- 3 - Nicotinamide (form of Vitamin B<sub>3</sub>)
- 4 - Riboflavin (Vitamin B<sub>2</sub>)
- 5 - Nicotinic acid (form of Vitamin B<sub>3</sub>)
- 6 - Ascorbic acid (Vitamin C)

**LC CONDITIONS**

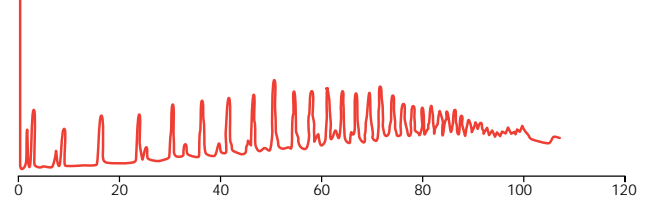
Mobile phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5  
Flow rate: 1.0 ml/min Temperature: 30 °C  
Injection volume: 5 µl Detection: 254 nm  
Column: ZirChrom®-SAX, 150 x 4.6 mm i.d. (part# ZR06-1546)

**Oligonucleotides**

ZirChrom®-SAX

**ANALYTES**

Partial Poly (G) hydrolysate



**LC CONDITIONS**

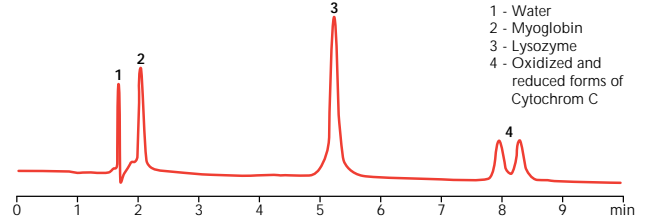
Mobile phase: (A) 20 mM Potassium phosphate dibasic at pH 7.0,  
200 mM Sodium chloride  
(B) 200 mM Potassium phosphate dibasic at pH 7.0,  
2.0 M Sodium chloride; Gradient: 10 to 90% B over 180 min.  
Flow rate: 1.0 ml/min Temperature: 100 °C Column: ZirChrom®-SAX,  
Injection volume: 25 µl Detection: 260 nm 150 x 4.6 mm i.d.  
(part# ZR06-1546)

**Protein Separations**

ZirChrom®-PEZ

**ANALYTES**

- 1 - Water
- 2 - Myoglobin
- 3 - Lysozyme
- 4 - Oxidized and reduced forms of Cytochrom C



**LC CONDITIONS**

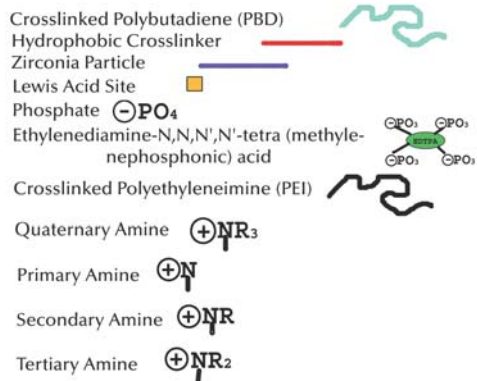
Mobile phase: Gradient elution with 25 to 100% B over 10 min. where A is  
2 mM EDTPA, 50 mM NaCl, 20 mM MES, pH 5.5, and B is  
2 mM EDTPA, 1.0 M NaCl, 20 mM MES, pH 5.5  
Flow rate: 1.0 ml/min Temperature: 30 °C Column: ZirChrom®-PEZ,  
Injection Volume: 10 µl Detection 280 nm 150 x 4.6 mm i.d.  
(part# ZR08-1546)

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

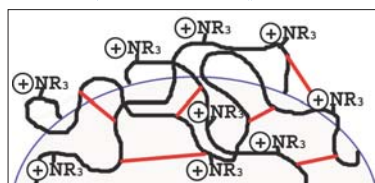


## ION EXCHANGE CHROMATOGRAPHY

### Legend for Surface Chemistry Diagrams

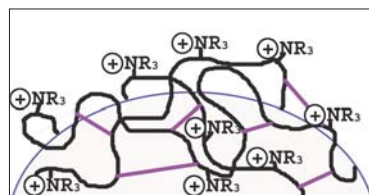


### ZirChrom®-SAX (Stable from pH 1-12, and to 80 °C)



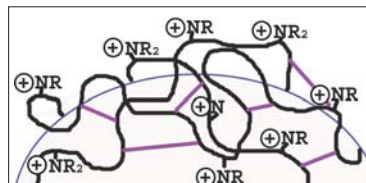
- Cross-linked polyethyleneimine-coated zirconia for strong anion-exchange
- Useful for inorganic and organic anions
- Ideal for the separation of water-soluble vitamins
- Useful for the separation of bio-molecules such as nucleotides, nucleosides, oligonucleotides, oligodeoxynucleotides, amino acids,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

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



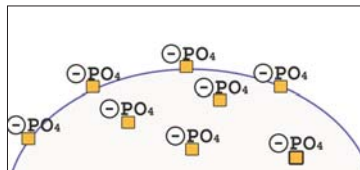
- Quaternized polyethyleneimine-coated zirconia for strong hydrophilic anion-exchange.
- ZirChrom-SHAX has all the advantages of ZirChrom-SAX except that the surface is much more hydrophilic making it useful for anion-exchange of proteins

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



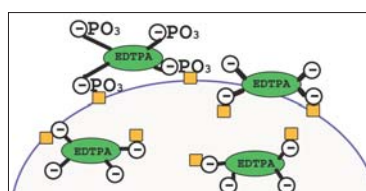
- Cross-linked polyethyleneimine-coated zirconia for weak anion-exchange
- Efficient weak anion-exchanger useful for inorganic and organic anions
- Useful for the separation of bio-molecules such as nucleotides, nucleosides, oligonucleotides, oligodeoxynucleotides, amino acids, peptides, and proteins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

PACKING	MODE	PART
ZirChrom®-WCX	Weak Cation-Exchange	ZR04
ZirChrom®-WAX	Weak Anion-Exchange	ZR05
ZirChrom®-SAX	Strong Anion-Exchange	ZR06
ZirChrom®-SHAX	Strong Hydrophilic Anion-Exchange	ZR07
ZirChrom®-PEZ	Cation-Exchange for Proteins	ZR08

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



## Method Development with ZirChrom®-Chiral

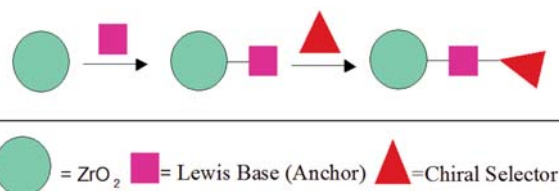
ZirChrom Separations, Inc. is pleased to announce the arrival of a full line of zirconia-based chiral HPLC phases, ZirChrom®-Chiral. These new patent-pending chiral stationary phases incorporate the unsurpassed chemical and mechanical stability of zirconia with the flexibility of Lewis acid/base anchored 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.

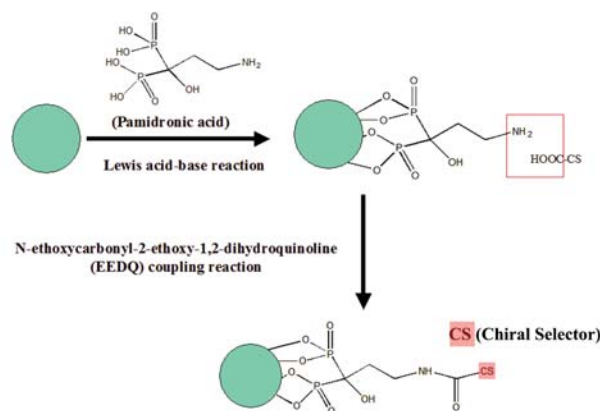
This novel approach to the production of chiral stationary phases on zirconia that has been developed that offers significant method development advantages over other platforms. Zirconium atoms on the surface of zirconia (zirconium dioxide) particles act as strong Lewis acid sites that allow for facile 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.



### Phase I Reaction Scheme



Typical two-step chemical reaction involving the addition of a reactive chelator (pamidronic acid) followed by EEDQ amide bond formation with a chiral carboxylic acid reagent.



Example of a typical chemical reaction used to tether a chiral selector to the zirconia surface.

PACKING	USE	PART
ZirChrom®-Chiral(S)LEU	Pirkle Chiral Phase Pi Acceptor	ZRC01
ZirChrom®-Chiral(R)NESA	Pirkle Chiral Phase Pi Donor	ZRC02
ZirChrom®-Chiral(S)NESA	Pirkle Chiral Phase Pi Donor	ZRC03
ZirChrom®-Chiral(S)PG	Pirkle Chiral Phase Pi Acceptor	ZRC04
ZirChrom®-Chiral(R)PG	Pirkle Chiral Phase Pi Acceptor	ZRC05
ZirChrom®-CelluloZe	Carbohydrate Chiral Phase	ZRC06

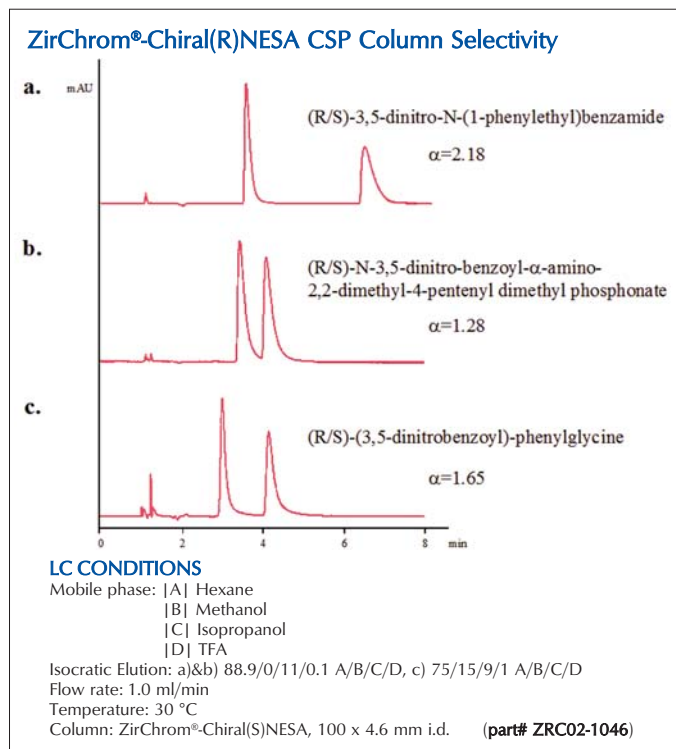
Microbore, Semi-Prep and Prep Formats Available—see Page 24



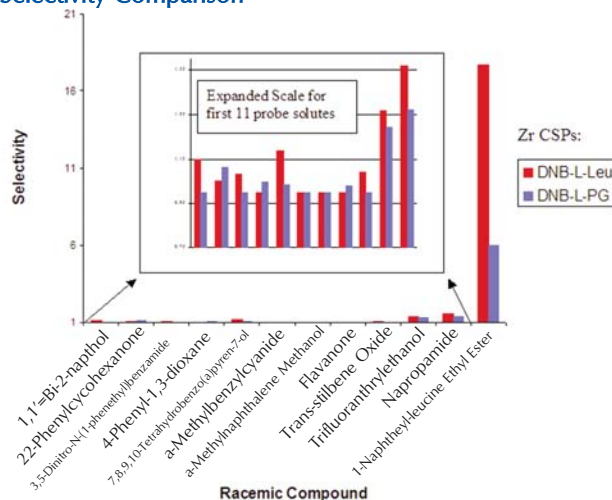


## ZIRCHROM®-CHIRAL

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.

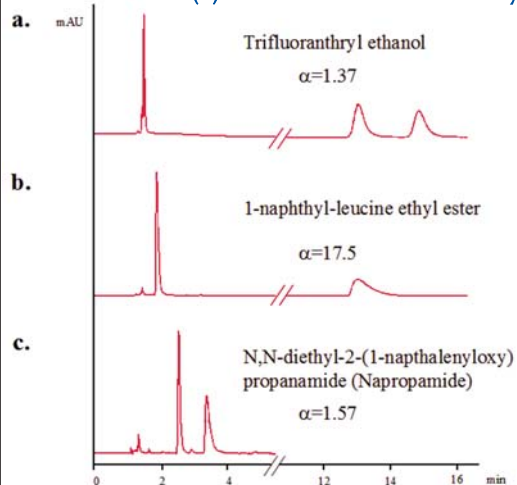
(part# ZRC01-1046)

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 CSP and zirconia (S)-dinitrobenzoyl-L-phenylglycine CSP. 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.

### ZirChrom®-Chiral(S)LEU CSP Column Selectivity



#### LC CONDITIONS

Mobile phase: |A| Hexane

                  |B| Isopropanol

Isocratic Elution: 99/1 A/B

Flow rate: 1.0 ml/min

Temperature: 30 °C

Column: ZirChrom®-Chiral(S)LEU, 100 x 4.6 mm i.d.

(part# ZRC01-1046)

## Antibody Purification with Rhinophase<sup>®</sup>-AB

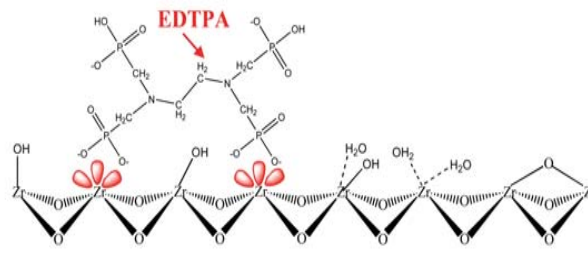
ZirChrom introduces a next generation chromatographic media enabling the fast, reliable purification of monoclonal antibodies. Rhinophase<sup>®</sup>-AB is a biocompatible stationary phase useful for both small and large-scale purifications. Rhinophase<sup>®</sup>-AB may be packed into columns or SPE 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<sup>®</sup>-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<sup>®</sup>-AB Research Kit (right; **part# AB01-RES, \$178.00 list price**) and 2) the Rhinophase<sup>®</sup>-AB Production Kit (not shown; **part# AB01-HTP, \$2,619.00 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.

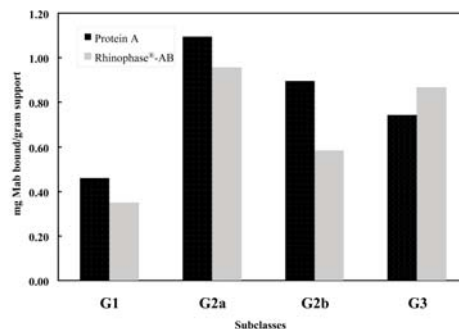


ZirChrom's Rhinophase<sup>®</sup>-AB Research Kit, part# AB01-RES  
(just add water!)

### Surface Chemistry of Rhinophase<sup>®</sup>-AB

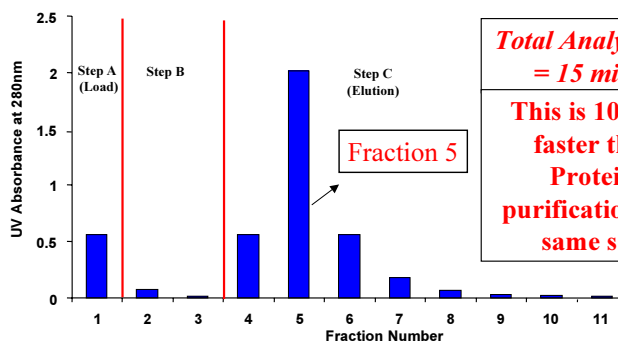


### Binding Capacity of Rhinophase<sup>®</sup>-AB



Rhinophase<sup>®</sup>-AB had high binding capacity for a variety of different Mab subclasses and is comparable to Protein A media.

### Rapid Results with Rhinophase<sup>®</sup>-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 EDTPA, 50 mM NaCl @ pH 4.0, Step B = 20 mM MES buffer, 4 mM EDTPA, 50 mM NaCl @ pH 4.0, Step C = 20 mM MES buffer, 4 mM EDTPA, 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<sup>®</sup>-AB in tube = 10 grams.

PACKING	USE	PART
Rhinophase <sup>®</sup> -AB	Antibody Purification	AB01
Microbore, Semi-Prep and Prep Formats Available—see Page 24		



**PROTAIN®**

## 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.



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.

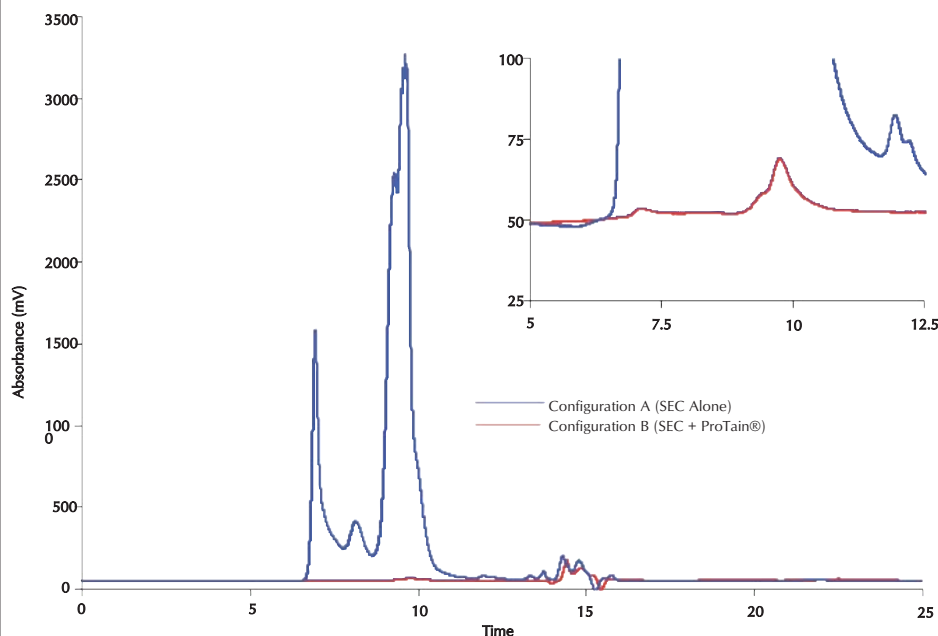
PACKING	USE	PART
ProTain®	In-Line Protein Removal	PT01
Custom Sizes Available Upon Request		

**PRICING INFORMATION**

ProTain® Holders: **part# 850-00-2** (for 4.6 mm i.d. inserts)  
**\$78.00 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)  
**\$313.00 list price** **part# PT01-0221** (20 x 2.1 mm i.d.; set of 3)

### Elution of BSA with and without ZirChrom's ProTain® system



**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

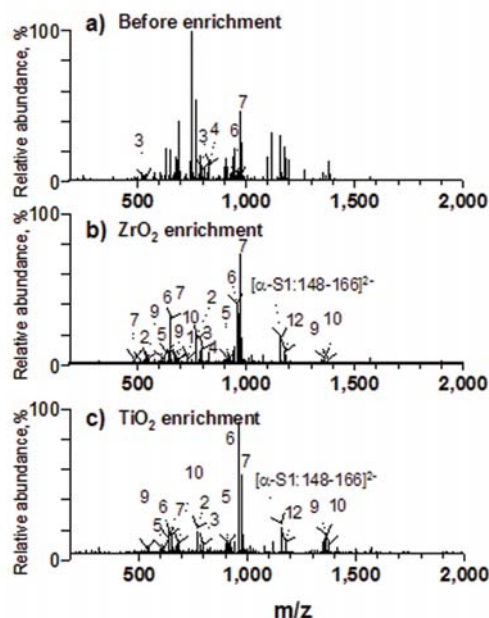
## Phosphopeptide Enrichment

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

*Sachtopore is a registered trademark of Sachtleben, Duisburg, Germany*

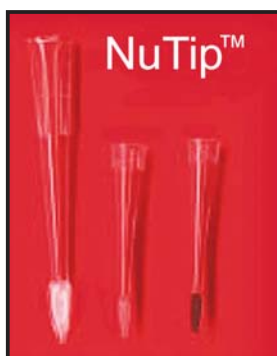


Negative mode ESI FT-ICR mass spectra (8 scans) of a tryptic digest of a-casein obtained; (a) prior to enrichment, (b) after ZrO<sub>2</sub> enrichment, and (c) after TiO<sub>2</sub> enrichment. Excerpt with author permission from work published in: Kweon, H.K.; Hakansson, K; Analytical Chemistry, 78, 1743-1749 (2006).

## 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

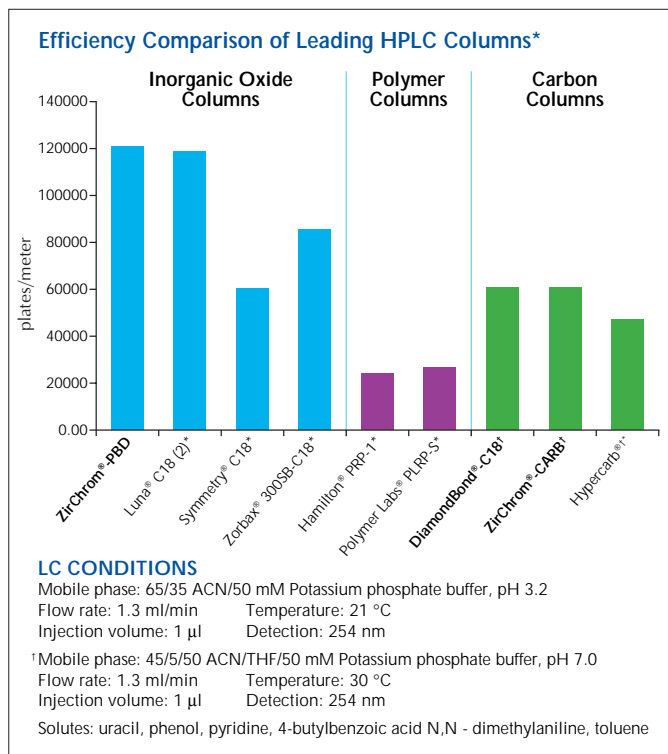
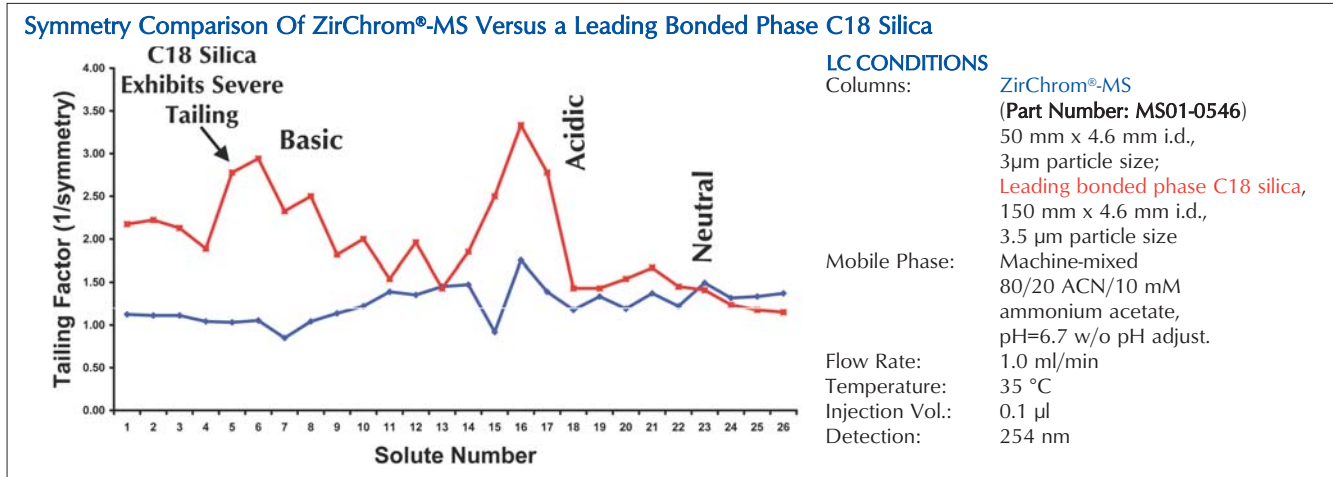
PACKING	USE	PART
TopTip™	Phosphopeptide Enrichment	TT
NuTip™	Phosphopeptide Enrichment	NT

All ZirChrom®, DiamondBond® and Sachtopore® stationary phases are available in SPE pipette tip formats upon request—see Page 24

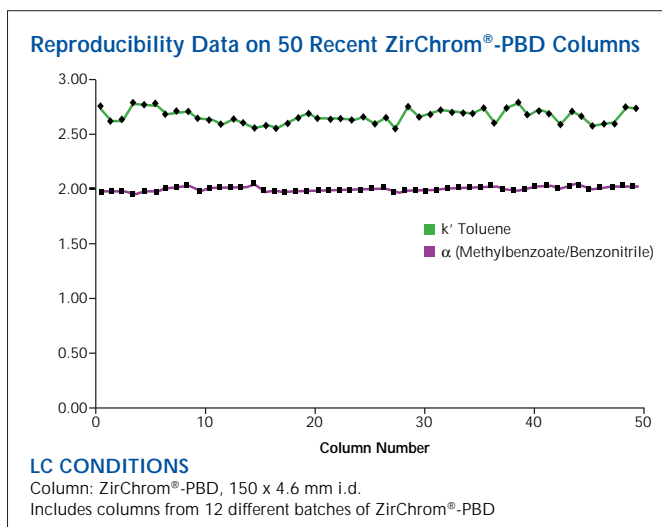
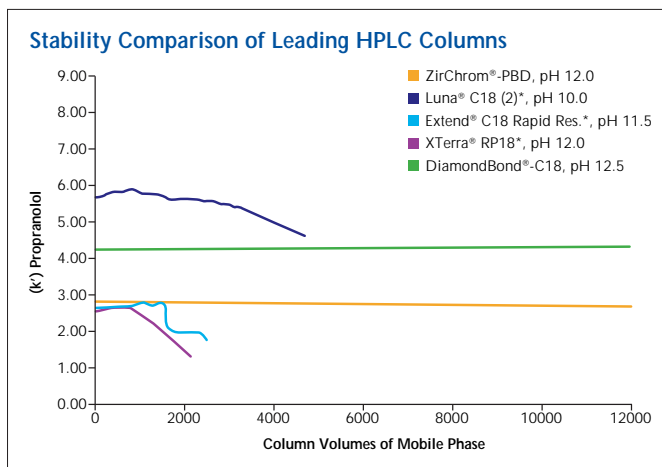


## REFERENCE INFORMATION

### ZirChrom® HPLC Columns are Efficient, Stable and Reproducible...



\*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





Representative Application Notes Not Shown in Product Guide

Bulletin #	Column	Compound Class	Analytes
198	ZirChrom®-PBD	Opioids	Aminophylline, Morphine, Phenacetin, Codeine, Hydrocodone, Ethylmorphine, Oxycodone, Papaverine
199	ZirChrom®-PBD	Anticonvulsants	Phenobarbital, Primidone, Methsuximide, Diazepam
210	ZirChrom®-PBD	Emamectin	Emamectin B1a, Emamectin B1b; Separation and Scale-up
261	ZirChrom®-PBD	Benzodiazepines	7-aminoflunitrazepam, Clonazepam, Alprazolam, Nordiazepam, Diazepam, Medazepam
262	ZirChrom®-PBD	Benzodiazepines	Flunitrazepam, Clordiazepoxide, Triazolam, Desalkylflurazepam, Midazolam
264	ZirChrom®-PBD	Steroids	Boldenone, Nandrolone, Testosterone
269	ZirChrom®-PBD	Antihistamines	Doxylamine, Methapyrilene, Chlorpheniramine, Triprolidine, Meclizine
272	ZirChrom®-PBD	Sedative	Clozapine
276	ZirChrom®-PBD	Antihypoglycemic	Glyburide
277	ZirChrom®-PBD	Immunosuppressant	Rapamycin (Sirolimus)
292	ZirChrom®-PBD	Polymers	Mono and Di-esters from BPA(EO)30DMA Methacrylated Polyol
300	ZirChrom®-PBD	Basic Pharmaceuticals	Fentanyl, Norfentanyl
301	ZirChrom®-PBD	Basic Pharmaceuticals	Carfentanil, Naltrexone
302	ZirChrom®-PBD	Basic Pharmaceuticals	STI-571, N-Desmethyl-STI
318	ZirChrom®-PBD	Herbicide	Simazine, Cyanazine, Simetryn, Atrazine, Prometon, Propazine, Ametryn, Terbutylazine, Prometryn, Terbutryn
134	ZirChrom®-CARB	Isomers	Ethylbenzene, m-Xylene, p-Xylene, O-Xylene
244	ZirChrom®-CARB	Steroids	Proprietary Sulfated Steroids
266	ZirChrom®-CARB	Steroids	Dihydroestradiol Sulfate, Dihydroequilin Sulfate, Equilin Sulfate, Estrone Sulfate, Equilenin Sulfate, Dihydroequilenin Sulfate
273	ZirChrom®-CARB	Steroids	Testosterone and Estradiol
281	ZirChrom®-CARB	Monomer	Acrylamide, Acrylic Acid
223	DiamondBond®-C18	PTH-Amino Acids	Serine, Glycine, Alanine, Aminoisobutyric Acid, Aminobutyric Acid, Valine, Norvaline
224	DiamondBond®-C18	NSAID's	Acetaminaphen, Ketoprofen, Ibuprofen, Naproxen
231	DiamondBond®-C18	Opioids	Naloxone, Codeine, Ethylmorphine, Oxycodone
235	DiamondBond®-C18	Parabens and Phenols	Uracil, Phenol, Methyl Paraben, Ethyl Paraben, Propyl Paraben, Butyl Paraben, Heptyl Paraben
236	DiamondBond®-C18	Beta-Blockers	Atenolol, Metoprolol, Oxprenolol, Alprenolol
238	DiamondBond®-C18	Steroids	Prednisolone, Hydrocortisone
250	DiamondBond®-C18	Antihistamines	Pheniramine, Doxylamine, Chlorpheniramine, Methapyrilene, Tripelennamine, Cyclizine
284	DiamondBond®-C18	Steroids	Epietiocholanolone, Etiocholanolone, Androsterone, Epiandrosterone
295	DiamondBond®-C18	Food/Cosmetics	N-nitrosamines
282	ZirChrom®-EZ	Organic Acids	4-hydroxybenzoic Acid, 4-ethoxybenzoic Acid, 4-propoxybenzoic Acid, 4-butoxybenzoic Acid
283	ZirChrom®-EZ	Opioids	Morphine, Hydroxymorphone, Codeiene, Hydroxycodone
286	ZirChrom®-EZ	Benzodiazepines	Flunitrazepam, Lorazepam, Temazepam, Diazepam
287	ZirChrom®-EZ	Benzodiazepines	Triazolam, Hydroxytriazolam
288	ZirChrom®-EZ	Drugs of Abuse	Cocaine, Benzoylcoognine
297	ZirChrom®-MS	NSAIDs	Acetaminophen, Ketoprofen, Naproxen, Ibuprofen, & Impurity
298	ZirChrom®-MS	Beta-Blockers	Lidocaine, Atenolol, Metoprolol, Oxprenolol, Alprenolol
299	ZirChrom®-MS	Basic Pharmaceuticals	Methapyrilene, Pyrilamine, Tripelennamine, Brompheniramine, Desipramine, Nortryptiline, Doxepin, Amitriptyline
166	ZirChrom®-SAX	Oligonucleotides	Poly (A)-hydrolysate
214	ZirChrom®-SAX	Iodide	2M Nitrate Sample Matrix, Iodide (Trace)
274	ZirChrom®-SAX	Inorganic sulfate	Inorganic Sulfate
278	ZirChrom®-SAX	Food/Cosmetics	Measuring Caffeine, Aspartame and Benzoate in Soda
307	ZirChrom®-SAX	NSAIDs	Acetaminophen, Ibuprofen, Naproxen, Impurity, Ketoprofen
320	ZirChrom®-SAX	Glyphosate	Isopropylamine, Glyphosate, peak D isomers, peak C Impurity
136	ZirChrom®-WAX	Inorganic Anions	Bromate, Nitrite, Nitrate, Iodide
218	ZirChrom®-WAX	Natural Flavoring Agent	Saponins in the Yucca Shidigera Flavoring Extract
138	ZirChrom®-WCX	Proteins	Myoglobin, Lysozyme, $\alpha$ -chymotrypsin, Ribonuclease A, Cytochrome c
253	ZirChrom®-PEZ	Pyridines	2-hydroxypyridine, Ethylpyridine, 4-dimethylaminopyridine, 2,6-diaminopyridine
142	ZirChrom®-PHASE	Steroids	Cortisone, Hydrocortisone
143	ZirChrom®-PHASE	Plant Genins	Sarsasapogenin, Tigogenin, Hecogenin
291	ProTain®	Proteins	Effect of Buffer Type and pH on Capacity
243	Rhinophase®-AB	Antibodies	User Recommendations for Rhinophase®-AB

For a complete listing of application notes, visit our website at [www.zirchrom.com](http://www.zirchrom.com). For access to new application note distributions mail, contact ZirChrom technical support ([support@zirchrom.com](mailto:support@zirchrom.com)) and request "Z-APPS".





## OTHER PRODUCTS AND SERVICES

### 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)

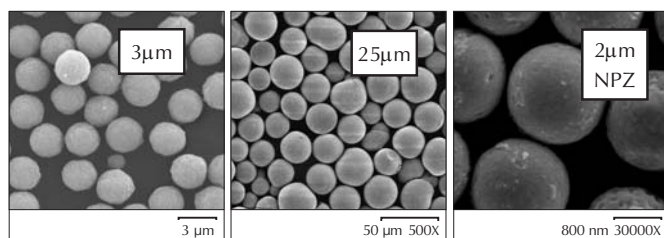


<b>MK01</b>	Ion Exchange Method Development Kit #1 (contains 1 each of ZirChrom®-SAX, ZirChrom®-SHAX, and ZirChrom®-WAX)	* 3 $\mu\text{m}$ 5 $\mu\text{m}$	<b>\$1359</b> <b>\$1254</b>
<b>MK02</b>	Ion Exchange Method Development Kit #2 (contains 1 each of ZirChrom®-SAX, ZirChrom®-WCX, and ZirChrom®-PEZ)	* 3 $\mu\text{m}$ 5 $\mu\text{m}$	<b>\$1359</b> <b>\$1254</b>
<b>MK03</b>	Reversed-Phase Method Development Kit #1 (contains 1 each of ZirChrom®-PBD, ZirChrom®-CARB, and DiamondBond®-C18)	* 3 $\mu\text{m}$ 5 $\mu\text{m}$	<b>\$1359</b> <b>\$1254</b>
<b>MK04</b>	Reversed-Phase Method Development Kit #2 (contains 1 each of ZirChrom®-PBD, ZirChrom®-CARB, and ZirChrom®-EZ (or ZirChrom®-MS))	* 3 $\mu\text{m}$ 5 $\mu\text{m}$	<b>\$1359</b> <b>\$1254</b>

\*All columns will be packed with 3  $\mu\text{m}$  particles unless 5  $\mu\text{m}$  particles are specifically requested.

### Particle Sizes

ZirChrom's analytical columns are packed with 3  $\mu\text{m}$  particles. On request, we can pack columns with 5, 7, 10, or 25  $\mu\text{m}$  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  $\mu\text{m}$  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# TI02**) 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](http://www.zirchrom.com)).

An advanced version of the Buffer Wizard with 50 buffer systems is available for sale (**part# BW01; \$105.00 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.**



ORDERING INFORMATION

### Ordering Information

This catalog contains information regarding ZirChrom's line of analytical columns for HPLC. Current pricing information is contained on the back of this catalog. Further information regarding preparative-scale separation media is available upon request. Pricing information about custom column formats is available upon request. Call 1-866-STABLE-1 or visit us at [www.zirchrom.com](http://www.zirchrom.com).

### Discounts

ZirChrom will provide special quotations for high volume purchases of columns. Please call for details.

### Terms and Conditions

Terms of payment are NET 30 days, F.O.B. Anoka, Minnesota, U.S.A. A 1.5% per month (18% A.P.R.) service charge will be added to delinquent accounts. All prices are subject to change without notice. Orders will be shipped via UPS or common carrier unless otherwise instructed.

### Return Privilege and Policy

Please contact our Customer Service/Ordering Department to assist you, should any problem or concern arise about your order. Prior to returning any items, contact our Customer Service/Ordering Department for a Return Authorization Number and shipping instructions. A return authorization will ensure proper handling of the product and enable us to expedite processing of your order to your satisfaction. Returns must be made within 90 days of original shipment.

### Liability

Our products are intended for use in the laboratory. ZirChrom Separations, Inc. assumes no responsibility if these products are misused in any way. The information published in this catalog is correct and accurate to the best of our knowledge. ZirChrom assumes no responsibility with respect thereto.

### 90-Day Risk-Free Warranty

*ZirChrom Separations, Inc. is dedicated to the production of zirconia-based separation media for High Performance Liquid Chromatography. To assure a reproducible and robust final product, only the highest quality standards are used.*



**ZirChrom®**  
...For Peak Performance

## HPLC Column Ordering Information

Phase Number	Phase Name	Mode of Use
DB01	DiamondBond®-C18	Reversed-Phase
EZ01	ZirChrom®-EZ	Reversed-Phase
MS01	ZirChrom®-MS	Reversed-Phase
ST01	ZirChrom®-SELECT	Reversed-Phase
TI01	Sachtopore®-RP	Reversed-Phase
TI02	Sachtopore®-NP	Normal Phase and SEC
ZR01	ZirChrom®-CARB	Reversed-Phase
ZR02	ZirChrom®-PHASE	Normal Phase and SEC
ZR03	ZirChrom®-PBD (USP L49)	Reversed-Phase
ZR04	ZirChrom®-WCX	Weak Cation-Exchange
ZR05	ZirChrom®-WAX	Weak Anion-Exchange
ZR06	ZirChrom®-SAX	Strong Anion-Exchange
ZR07	ZirChrom®-SHAX	Strong Anion-Exchange
ZR08	ZirChrom®-PEZ	Cation-Exchange (Proteins)
ZR09	ZirChrom®-PS	Reversed-Phase
ZRC01	ZirChrom®-Chiral(S)LEU	Brush-Type, pi-acceptor
ZRC02	ZirChrom®-Chiral(R)NESA	Brush-Type, pi-donor
ZRC03	ZirChrom®-Chiral(S)NESA	Brush-Type, pi-donor
ZRC04	ZirChrom®-Chiral(S)PG	Brush-Type, pi-acceptor
ZRC05	ZirChrom®-Chiral(R)PG	Brush-Type, pi-acceptor
ZRC06	ZirChrom®-CelluloZe	Carbohydrate

A column part number is constructed as follows: PPPP-XXYY where PPPP is the phase number, XX is the column length, and YY is the column diameter. For example, MS01-1546 specifies a 15 cm by 4.6 mm column containing ZirChrom®-MS. The table below has the prices for ZirChrom's products in different column formats; for Guard Columns, PPPP is the phase number, then add XXX for column diameter; G20 for 2.1 mm column diameter or G40 for 4.6 mm diameter.

Column Format	Phase Number			
	Specialty Phases DB01, ST01, ZR01, ZRC01 thru ZRC05	Polymer Reversed-Phases EZ01, MS01, TI01 ZR03, ZR09	Normal/Ion Exch. Phases TI02, ZR02, ZR04, ZR05, ZR06, ZR07, ZR08	Carbohydrate Phase ZRC06
5 cm Columns 0521 (5 cm x 2.1 mm) 0546 (5 cm x 4.6 mm)	3 µm \$708* 5 µm \$656	3 µm \$603* 5 µm \$551	3 µm \$603* 5 µm \$551	3 µm \$1,206* 5 µm \$1,102
10 cm Columns 1021 (10 cm x 2.1 mm) 1046 (10 cm x 4.6 mm)	3 µm \$708* 5 µm \$656	3 µm \$603* 5 µm \$551	3 µm \$603* 5 µm \$551	3 µm \$1,206* 5 µm \$1,102
15 cm Columns 1521 (15 cm x 2.1 mm) 1546 (15 cm x 4.6 mm)	3 µm \$729* 5 µm \$677	3 µm \$624* 5 µm \$572	3 µm \$624* 5 µm \$572	3 µm \$1,248 5 µm \$1,144
1 cm Guard Columns G20 (use with 2.1 mm) G40 (use with 4.6 mm)	Set of 3 inserts \$229	Set of 3 inserts \$208	Set of 3 inserts \$208	Set of 3 inserts \$416
Guard Column Holders 852-00 (use with 2.1 mm) 850-00 (use with 4.6 mm)	\$78	\$78	\$78	\$78

\*All columns will be packed with 3 µm particles unless 5 µm particles are specifically requested. PEEK hardware and custom column formats (including microbore columns) are available by request.



**ZirChrom®**

*Specialists in Ultra-Durable,  
High Efficiency HPLC Phases*

**ZirChrom Separations, Inc.**

617 Pierce Street  
Anoka, MN 55303

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Email: [support@zirchrom.com](mailto:support@zirchrom.com)



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