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SUPELCO

595 North Harrison Road
Bellefonte, PA 16823-0048 USA
Telephone 800-247-6628 • 814-359-3441
Fax 800-447-3044 • 814-359-3044
email: supelco@sial.com
sigma-aldrich.com/supelco

Bulletin 931

Discovery Zr: High pH and High Temperature HPLC

Reversed-phase, zirconia-based particles expand your HPLC method development options by leveraging the unique selectivity and retention provided by pH and temperature extremes.

Use Discovery Zr phases when:

1. Low or high pH is desirable to control the ionization state of your analyte
2. You would like a significant reduction in analysis time
3. Silica-based phases cannot give the resolution you require

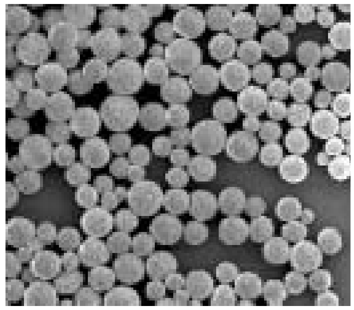
Discovery Zr comprises four phase chemistries bonded to porous, spherical, 3 and 5 micron zirconia particles. Zirconia particles have exceptional pH and thermal stability compared to silica and alumina particles. Compared to polymer particles, zirconia does not shrink or swell with changes in temperature, ionic strength, or organic concentration, and has exceptional mechanical strength. The presence of controlled, predictable reversed-phase and ion-exchange retention modes combined with thermal and pH stability open up your method development options. Four different Discovery Zr bonded phase chemistries, Carbon, CarbonC18, PS, and PBD, give you choices in bonded phase selectivity.

Why use Zirconia Particles over Conventional Silica or Polymer Particles for HPLC?

Zirconia = zirconium dioxide or ZrO_2

Since the beginning of the science of chromatography, many different support particle chemistries have been employed. Inorganic oxides, including silicon dioxide (silica) and aluminum dioxide (alumina), and organic polymers and copolymers, including graphite carbon, polymethacrylate and polystyrene-divinylbenzene, comprise the vast majority of commercially-available HPLC supports. Each of these have limitations that fuel the search for the ideal HPLC particle candidate; one that has the physical attributes that give rise to efficient and stable packed column beds, can be functionalized, and are chemically immutable under a wide range of mobile phase and operating conditions. Recent developments in the science behind manufacturing spherical microparticulate zirconium dioxide (zirconia) have given rise to particles that have the physical and chemical characteristics approaching the ideal support particle for HPLC.

Zr Particles



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Discovery Zr particles are uniform spheres for high efficiency and column stability. Although they look like silica particles, they have pH stability that silica does not.

It all reduces to chemistry:

- The chemistry of zirconia that gives pH and thermal stability,
- Lewis acid-base chemistry that provides ion-exchange character, and allows you to adjust selectivity by the type of buffer used,
- The chemistry of our four unique bonded phases that gives diverse selectivities from each other and compared to silica-based phases.

The Members of the Discovery Zr Family

Discovery Zr-PBD

Polybutadiene-modified zirconia particles give separations most similar to C18-silica, but with benefits of high pH and temperature stability.

Discovery Zr-PS

Polystyrene modified zirconia particles are ideal for separations of hydrophobic compounds and amines.

Discovery Zr-CarbonC18

Octadecyl-modified carbon-clad zirconia for universal separations of acids, bases, and neutrals. Very different selectivity relative to C18-silica.

Discovery Zr-Carbon

Carbon-clad zirconia for separations of geometric isomers and diastereomers.

The Power of pH

Use Discovery Zr at High and Low pH

Unlike siloxane bonds (Si-O-Si), the Zr-O-Zr bonds that form the zirconia particle structure are not susceptible to chemical attack at high pH. Also unlike silica, Zr bonded phases are not susceptible to chemical attack at low pH.

Why Run an HPLC Method at pH Extremes?

pH is a powerful tool to adjust selectivity and retention in HPLC separations of ionizable compounds. The ionization state of a compound is influenced by the pH of the mobile phase until well above or below its pK_a. In purely reversed-phase separations, compounds exhibit better retention when they are not ionized. However, when working with silica-based reversed-phase packings, if the pH needed to suppress ionization for adequate retention is outside the allowable pH limits (usually pH 2 – 8), oppositely charged ion-pair agents are required to obtain adequate retention.

However, by using an HPLC material that allows for unrestricted pH, you can control the ionization state of even very basic or acidic analytes. If the HPLC material also has ion-exchange character, then you have the added dimension of an ion-exchange mechanism contributing to retention and selectivity.

Discovery Zr zirconia particles are not susceptible to acidic or basic hydrolysis and therefore do not have the pH limitation of silica. Discovery Zr particles also have ion-exchange character via the adsorbed Lewis base buffer ions. Table 1 shows the effect of pH on hydrophobicity (reversed-phase character) and ionization (ion-exchange character) of basic and acidic analytes, and the zirconia surface. Figure A shows the stability of Discovery Zr phases at high pH, compared to purportedly pH-stable C18-silica particles.

Table 1: Summary of Effect of pH on Ionization and Hydrophobicity of Analytes and Zr Surface

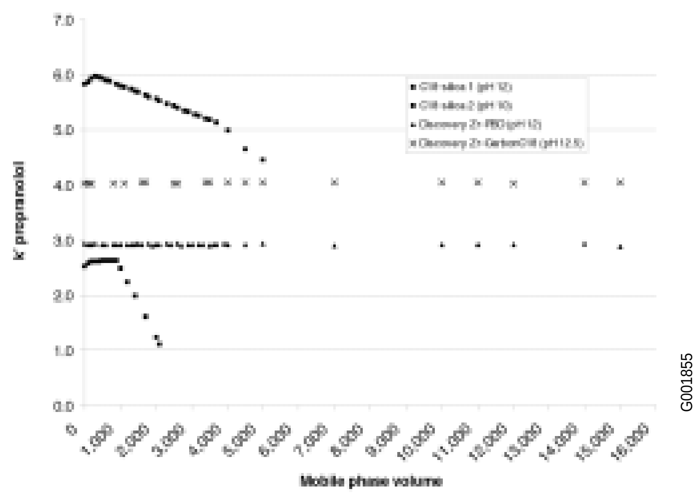
	Ionization	Hydrophobicity
Acidic Analytes	Increases with increasing pH	Decreases with increasing pH
Basic Analytes	Decreases with increasing pH	Increases with increasing pH
Zirconia (Zr) Surface	Positively charged at low pH Negatively charged at high pH	No effect

Figure A: Effect of Exposure to High pH on Silica Particles vs. Zirconia Particles

Stress Conditions
Mobile Phase: CH_3CN in 50mM potassium phosphate (pH as indicated in Figure)
Temp.: 30°C

Test Conditions
Mobile phase: CH_3CN (or THF) in 50mM potassium phosphate (pH as indicated in Figure)
Flow Rate: 1mL/min
Det.: UV, 254nm
Temp.: 30°C
Inj: 5µL
Sample: Propranolol, 10µg/mL

Discovery Zr particles do not dissolve at high pH like silica particles do.



Silica particles are not stable at high pH. Exposure to basic conditions will dissolve the particles and destroy the column.

The Power of Temperature

Use Discovery Zr up to 100°C in Conventional Hardware and 200°C in Special Hardware

The same chemistry that gives zirconia particles pH stability also gives it excellent thermal stability.

Why Run at High Temperatures?

Increasing the temperature of a separation has many desirable effects, including:

1. sorption kinetics are increased, decreasing retention time and peak width
2. mobile phase viscosity is reduced, allowing for higher flow rates and higher efficiency
3. decrease in retention allows use of lower organic modifier concentration, reducing hazardous waste
4. lower mobile phase viscosity reduces wear-and-tear on pumps

The primary requirement of utilizing elevated temperatures is the stability of the stationary phase. Typical silica-based HPLC particles will quickly deteriorate at elevated temperatures, especially at the elevated pH values necessary to be above the pK_a of most basic pharmaceutical compounds. Discovery Zr zirconia particles exhibit the necessary thermal and chemical stability to operate at elevated temperatures and extreme pH values. The most significant effect of increased temperature is decreased run time. Figure B shows the separation of five alkaloids on Discovery Zr-PBD columns at 30°C and 65°C at constant pressure.

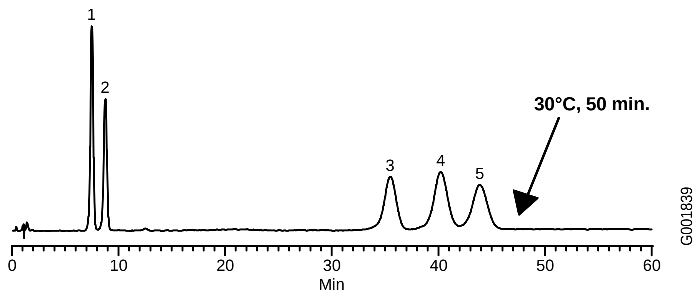
An Extreme Example

The benefits of extreme pH and temperature stability of Discovery Zr are clearly demonstrated in the separation of ß-blockers in Figure C. The high pH gives excellent resolution, and the high temperature gives short analysis time.

Figure B: Temperature Effect on Analysis Time: Alkaloids at 30°C and 65°C

Column: **Discovery Zr-PBD, 15cm x 4.6mm, 3µm**
Cat. No.: **65718-U**
Mobile Phase: (90:10) 20mM potassium phosphate (pH 12) : CH_3CN
Flow Rate: 1mL/min at 30°C; 2.35mL/min at 65°C
Det.: UV, 220nm
Temp.: 30°C or 65°C
Inj: 10µL
Sample: codeine, strychnine, papaverine, quinine, quinidine, each compound 50µg/mL

1. Codeine
2. Strychnine
3. Papaverine
4. Quinine
5. Quinidine



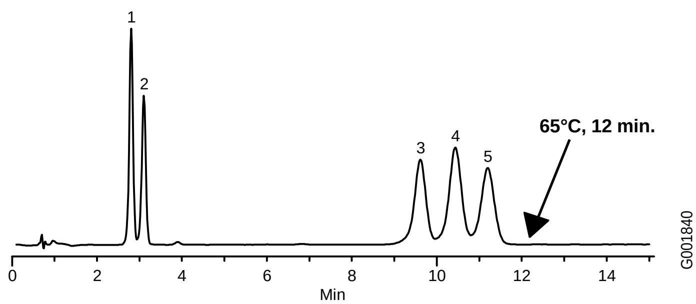
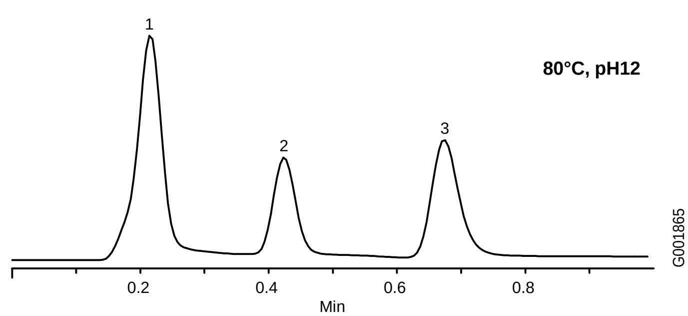


Figure C: Extreme Temperature and pH Gives Rapid Separation of ß-Blockers on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 5cm x 4.6mm, 3µm**
Cat. No.: **65704-U**
Mobile Phase: (55:45) 20mM potassium phosphate (pH 12):CH_3CN
Flow Rate: 3mL/min
Det.: UV, 210nm
Temp.: 80°C
Pressure: 99bar
Inj.: 5µL
Sample: Labetolol (500µg/mL), metoprolol (250µg/mL), alprenolol (250µg/mL)

1. Labetolol
2. Metoprolol
3. Alprenolol



Summary of Benefits of Zirconia Over Other Chromatography Particles

	Discovery Zr Particles	Silica Particles	Polymer Particles	Carbon Particles
Stability at high pH (>11)	yes	no	yes	yes
Stability at low pH (<2)	yes	no	yes	yes
Thermal stability (>60°C)	yes	no	some	yes
No limits to organic solvents	yes	yes	no	yes
High efficiency	yes	yes	no	no
Good mass transfer into and out of pores	yes	yes	no	?
Tunable selectivity for amines	yes	no	no	no
Low backpressure	yes	yes	no	yes
Predictable mixed-mode operation	yes	no	no	no

Choosing and Using Discovery Zr

Developing Methods on Discovery Zr

Discovery Zr uses all the reversed-phase method development tools you use for developing methods on silica. However, Discovery Zr gives you four new tools that silica does not allow:

1. The full power of pH: to alter the retention of acids and bases
2. The power of temperature: to decrease analysis time
3. The power of ionic strength: to alter selectivity, efficiency, and retention
4. The power of Lewis acid-base interactions: to give unique selectivity over silica for ionic compounds

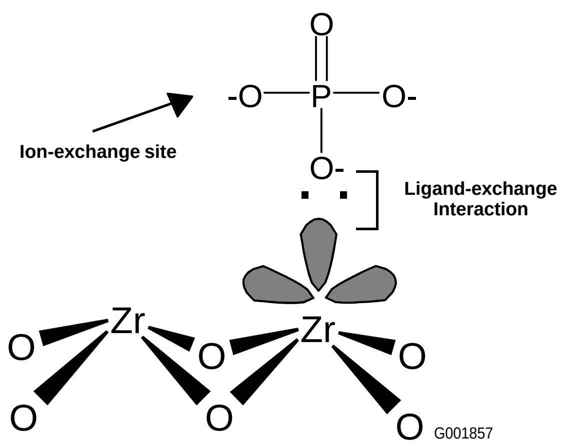
Discovery Zr Phases Feature Unique Lewis Acid-Base Chemistry

Although predominantly reversed-phase, Discovery Zr phases have secondary ionic interactions – called Lewis Acid-Base interactions – that give an added dimension to method development of ionic compounds.

To successfully develop separations of ionic compounds on Discovery Zr, it is important to understand the role of Lewis acid-base chemistry on zirconia. The Lewis electron theory states that an acid is an electron-pair acceptor, and a base is an electron-pair donor. The zirconium atom in zirconia is a strong Lewis acid site and plays a significant role in retention of ionic analytes. The Lewis acid zirconia surface attracts Lewis base buffer ions – like phosphate - via ligand-exchange. This adsorbed buffer ion then acts as an ion-exchange site (Figure D). If the pH is below the pK_a of the basic analyte, it will cation-exchange with the adsorbed buffer anion. The result is a significant portion of retention due to ion-exchange interactions. An added benefit is that different buffer ions give very different selectivity.

Understanding and utilizing the ion-exchange character of zirconia is important to getting the most out of your Discovery Zr column.

Figure D: Discovery Zr Particles Have Strong Lewis Acid Sites That Can Undergo Ligand-exchange Interactions with Lewis Bases



Zirconia particles possess strong Lewis acid sites that can form predictable, controllable ligand-exchange interactions. Control is via the use of strong Lewis base buffer ions, like fluoride, phosphate, and acetate.

Choosing a Discovery Zr Phase

Method development first begins by choosing the Discovery Zr phase right for the analyte and conditions. The most important things to consider:

- All Discovery Zr phases operate by reversed-phase mechanisms
- Each of the four Discovery Zr phases are different from each other and have their own unique selectivity – just like silica bonded phases are different from each other
- Ionic compounds will also interact with ion-exchange mechanism
- You are not limited by pH or temperature (up to 200°C)

Figure E: Choosing a Discovery Zr Phase Based on Analyte and Conditions

Discovery Zr-PS high aqueous mobile phases, an alternative to ODS selectivity	**Discovery Zr-Carbon** diastereomers, geometric isomers, greatest difference from a C18-silica
Discovery Zr-PBD perfect general-purpose phase, great for bases, most similar to C18-silica for non-electrolytes	**Discovery Zr-CarbonC18** unique selectivity for acidic compounds, exhibits both RP and shape selectivity

Discovery Zr-PBD

Polybutadiene-modified zirconia particles give separations most similar to C18-silica, but with benefits of high pH and temperature stability.

Discovery Zr-PBD comprises spherical, porous zirconia particles with a durable coating of polybutadiene. It operates via a reversed-phase mechanism, but is less hydrophobic, so less organic solvent is required for elution. Discovery Zr-PBD complements the selectivity offering of the other zirconia-, silica-, and polymer-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 13.

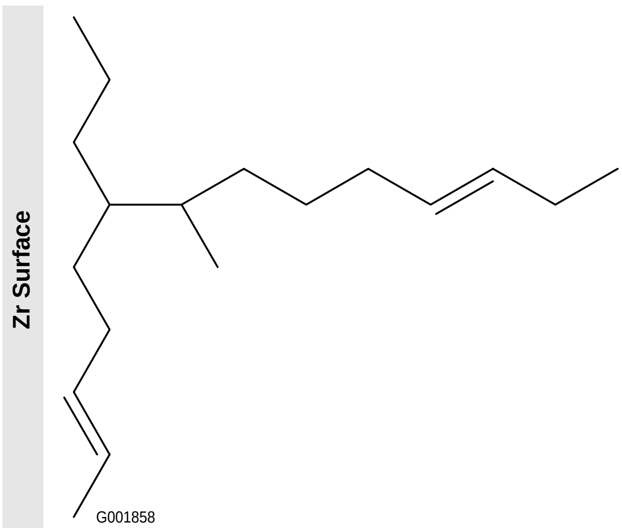
Discovery Zr-PBD Characteristics

Discovery Zr-PBD - polybutadiene (PBD)-coated zirconia

Particle Size:	3 and 5 micron
Surface Area (m^2/g):	30m^2/g
Pore Size:	300Å
pH Range:	1 - 13
Temperature Range*:	< 100°C

*Special column hardware for operations between 100°C and 150°C is available.

Structure of Discovery Zr-PBD:



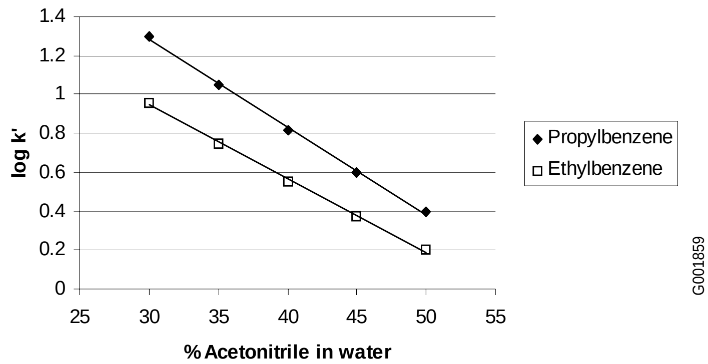
Features of Discovery Zr-PBD:

- Good for bases, amines
- Similar to ODS-silica
- pH stable from 1-13
- Thermally stable up to 100°C (up to 150°C in special hardware)

Discovery Zr-PBD is Similar to C18-silica, But with Added Selectivity and pH and Thermal Stability

Discovery Zr-PBD columns have selectivity similar to C18-silica for non-ionic compounds. Figure F shows that Discovery Zr-PBD operates via a predictable, reversed-phase mechanism.

Figure F: Linear Relationship Between Log k' and %CH_3CN Demonstrates a Reversed-phase Mechanism on Discovery Zr-PBD



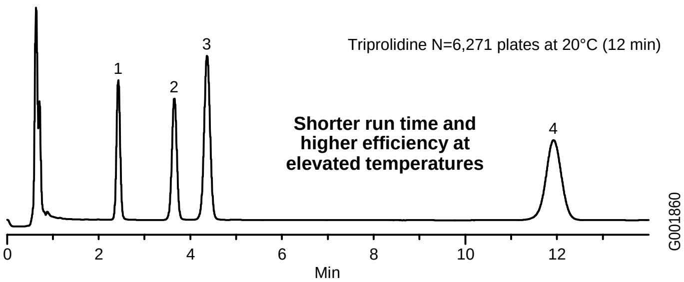
However, for ionic compounds, especially bases, the secondary Lewis acid-base interactions give significant added selectivity to separations on Discovery Zr-PBD. The Lewis acid zirconia surface attracts Lewis base buffer ions – like phosphate. If the pH is below the pK_a of the basic analyte, it will cation-exchange with the buffer anion. The result is a significant portion of retention due to ionic interactions. An added benefit is that different buffer ions give very different selectivity. Above the pK_a of the base, there are no ionic interactions and retention is due solely to reversed-phase interactions with the polybutadiene bonded phase.

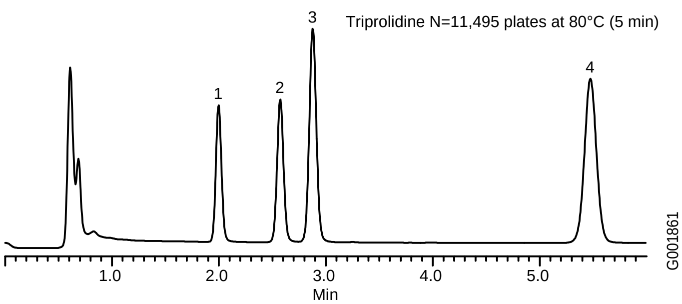
Another significant difference between Discovery Zr-PBD and C18-silica is that it can be used with basic pH mobile phases and elevated temperatures where basic analytes have better peak shape and higher efficiency. This is demonstrated in the separation of basic antihistamine compounds in Figure G.

Figure G: Example of Fast, High pH Separation of Amines on Discovery Zr-PBD Columns

Column: **Discovery Zr-PBD, 7.5cm x 4.6mm, 3µm**
Cat. No.: **65717-U**
Mobile Phase: (75:25) 50mM triethylammonium hydroxide (pH 12.6):CH_3CN
Flow Rate: 1mL/min
Det.: UV, 254nm
Temp.: 20°C or 80°C
Pressure: 130bar at 20°C
Inj.: 1µL
Sample: Doxylamine, methapyrilene, chlorpheniramine (1µg/mL), triprolidine (2µg/mL)

1. Doxylamine
2. Methapyrilene
3. Chlorpheniramine
4. Triprolidine



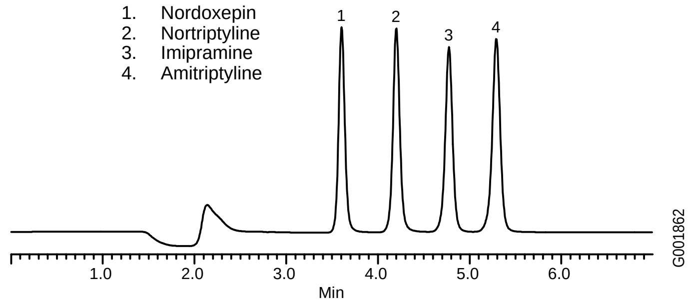


Another example of the utility of Discovery Zr-PBD for basic compounds is shown in the separation of tricyclic antidepressants in Figures H.

Figure H: Tricyclic Antidepressants at pH 12 on Discovery Zr-PBD

Column: **Discovery Zr-PBD, 15cm x 4.6mm, 3µm**
Cat. No.: **65718-U**
Mobile Phase: (40:60) 20mM potassium phosphate (pH 12.0):CH_3CN
Flow Rate: 0.5mL/min
Det.: UV, 254nm
Temp.: 35°C
Inj.: 1µL
Sample: Nordoxepin, nortriptyline, amitriptyline (250µg/mL), imipramine (150µg/mL)

1. Nordoxepin
2. Nortriptyline
3. Imipramine
4. Amitriptyline



Discovery Zr-CarbonC18

Octadecyl-modified carbon-clad zirconia combines partitioning mechanism with shape selectivity.

Discovery Zr-CarbonC18 comprises spherical, porous carbon-clad zirconia particles covalently modified with octadecyl (C18) groups. It complements the selectivity offering of the other zirconia, silica-, and polymer-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 14.

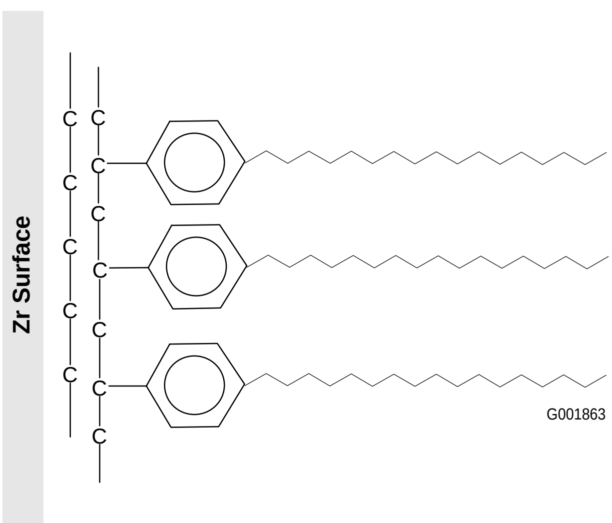
Discovery Zr-CarbonC18 Characteristics

Discovery Zr-CarbonC18 - carbon-clad zirconia with covalently-bonded octadecyl groups

Particle Size:	3 and 5 micron
Surface Area (m^2/g):	$30m^2/g$
Pore Size:	300Å
pH Range:	1 – 14
Temperature Range *:	< 100°C

*Special column hardware for operations between 100°C and 200°C is available.

Structure of Discovery Zr-CarbonC18:



Features of Discovery Zr-CarbonC18:

- Partitioning mechanism
- Shape selectivity
- Resistant to phase hydrolysis
- pH stable from 1-14
- Thermally stable up to 100°C (up to 200° in special hardware)

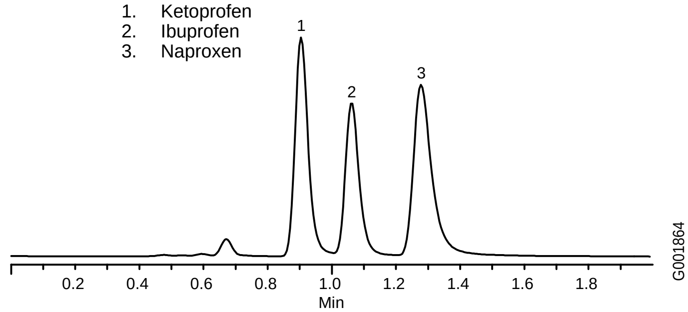
Discovery Zr-CarbonC18 Combines Partitioning Mechanism with pH and Temperature Stability.

Octadecyl (C18) is by far the most common member among the population of reversed-phased functional groups. The C18 reagent is relatively common and synthesis is straightforward and controllable. It has nearly universal application since the majority of organic compounds are hydrophobic enough to interact with C18 chains to some degree. The partitioning interactions between it and analytes are understood and therefore predictable. Indeed, the major limitations of C18 are due to the substrate it is bonded to, which is most often silica. In general, silica's limited pH range restricts the application of C18 phases bonded to it to between pH 2 and 8. Temperatures above 60°C can also damage bonded silicas. Discovery Zr-CarbonC18 overcomes the limitations of silica by covalently bonding C18 chains to a chemically and thermally inert carbon surface. The resultant phase has the partitioning mechanism of C18, but because it is bonded to a highly inert, carbonaceous support, it is immune to pH and temperature extremes. The example of the acidic non-steroidal anti-inflammatory compounds in Figure I run at pH 1.75 and 80°C on Discovery Zr-CarbonC18 demonstrates the extreme applicability of this phase.

Figure I: Rapid Separation of NSAIDS on Discovery Zr-CarbonC18

Column:	**Discovery Zr-CarbonC18, 15cm x 4.6mm, 3µm**
Cat. No.:	**65706-U**
Mobile Phase:	(50:50) 50mM H_3PO_4 (pH 1.75) : CH_3CN
Flow Rate:	4mL/min
Det.:	UV, 254nm
Temp.:	80°C
Pressure:	260bar
Inj.:	1µL
Sample:	Ketoprofen, ibuprofen, naproxen, each 1mg/mL

1. Ketoprofen
2. Ibuprofen
3. Naproxen



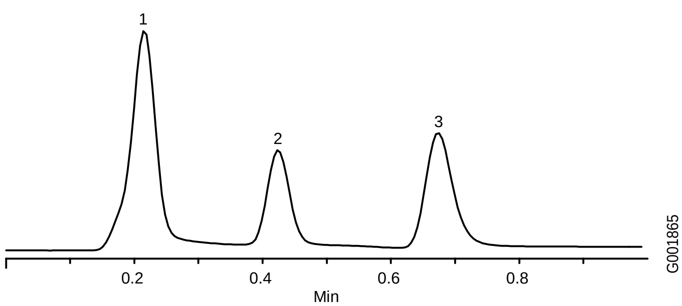
For Rapid Analysis, Consider Discovery Zr-CarbonC18 in Short Columns Run at High Temperatures.

Increasing the temperature can greatly reduce the analysis time. The thermal stability of all Discovery Zr phases allows temperatures up to 100°C and higher with special hardware. The separation of ß-blockers on Discovery Zr-CarbonC18 at 80°C in less than 1 minute is shown in Figure J.

Figure J: Extreme Temperature and pH Give Rapid Separation of ß-blockers on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 5cm x 4.6mm, 3µm**
Cat. No.: **65704-U**
Mobile Phase: (55:45) 20mM potassium phosphate (pH 12):CH_3CN
Flow Rate: 3mL/min
Det.: UV, 210nm
Temp.: 80°C
Pressure: 99bar
Inj.: 5µL
Sample: Labetolol (500µg/mL), metoprolol (250µg/mL), alprenolol (250µg/mL)

1. Labetolol
2. Metoprolol
3. Alprenolol



The Underlying Carbon Surface Confers a Degree of Shape Selectivity on Discovery Zr-CarbonC18.

One of the benefits of carbon particles as a HPLC support is its ability to distinguish between molecular shapes. Unlike C18 chains that can conform to the shape molecule, the rigid carbon surface cannot. Molecules that have the same overall hydrophobicity but different shapes, like geometric isomers, are not separable on C18 phases. However, because these molecules have a different hydrophobic footprint, they can be separated on rigid supports. One of the downsides to carbon supports is that they are often too hydrophobic. Discovery Zr-CarbonC18 combines a partitioning mechanism of C18 with the shape selective ability of carbon. The result is separation of positional isomers in less time with lower percent organic. The separation of positional isomers of a proprietary sulfonamide drug is shown in Figure K. Here the parent compound is easily distinguished between its three corresponding positional isomers.

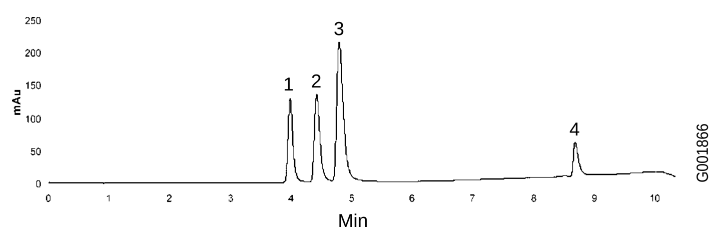
Figure K: Separation of Positional Isomers of a Sulfonamide Drug on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 15cm x 4.6mm, 3µm**
Cat. No.: **65706-U**
Mobile Phase: (A) 10mM diethylamine, pH 10.8
(B) CH_3CN
Flow Rate: 1.5mL/min
Det.: UV, 240nm
Temp.: 80°C
Inj.: 5µL

1. Isomer 1
2. Isomer 2
3. Parent drug
4. Isomer 3

Gradient:

Time (mins)	%A	%B
0.0	55	45
5.0	55	45
7.5	25	75
10.0	25	75



Discovery Zr-PS

Polystyrene-modified zirconia particles are ideal for separations of hydrophobic compounds and amines.

Discovery Zr-PS comprises spherical, porous zirconia particles modified with cross-linked polystyrene. It operates via a reversed-phase mechanism, but is less retentive. It has unique selectivity especially for aromatic compounds. Discovery Zr-PS complements the selectivity offering of the other zirconia-, silica, and polymer-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 13.

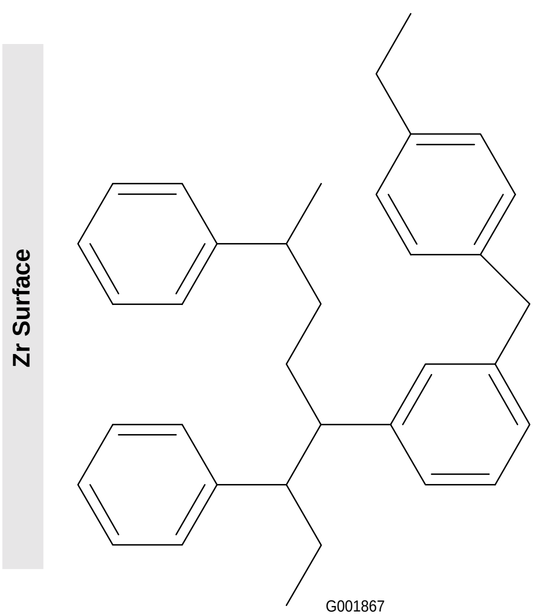
Discovery Zr-PS Characteristics

Discovery Zr-PS - cross-linked polystyrene on zirconia

Particle Size:	3 and 5 micron
Surface Area (m^2/g):	$30m^2/g$
Pore Size:	300Å
pH Range:	1 – 13
Temperature Range*:	< 100°C

*Special column hardware for operations between 100°C and 150°C is available.

Structure of Discovery Zr-PS:



Features of Discovery Zr-PS:

- Good for very hydrophobic compounds
- Good for basic compounds and amines
- pH stable from 1-13
- Thermally stable up to 100°C (up to 150°C in special hardware)

Discovery Zr-PS Gives Short Retention of Hydrophobic Amines with Excellent Peak Shape.

The relatively polar surface of Discovery Zr-PS permits rapid analysis of hydrophobic compounds. Because of the stability of the underlying zirconia surface, analyses can be run at low and high pH, and temperatures up to 150°C. Figure L shows a rapid gradient of acetonitrile in pH 1.8 buffer that effectively resolved three aromatic, hydrophobic amine drugs.

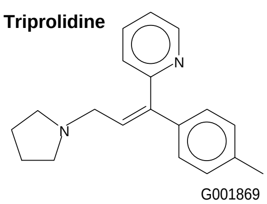
Figure L: Rapid Gradient Resolution of Hydrophobic Amines at Low pH on Discovery Zr-PS

Column:	**Discovery Zr-PS, 5cm x 4.6mm, 3µm**
Cat. No.:	**65740-U**
Mobile Phase:	(A) 25mM HCl, pH 1.8
	(B) CH_3CN
Flow Rate:	1mL/min
Det.:	UV, 254nm
Temp.:	40°C
Inj.:	1µL
Sample:	Tripreleneamine, triprolidine (1mg/mL), meclizine (3mg/mL)

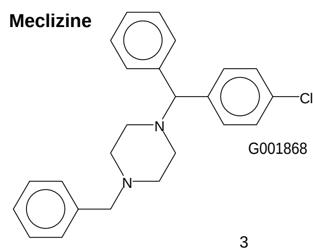
1. Tripreleneamine
2. Triprolidine
3. Meclizine

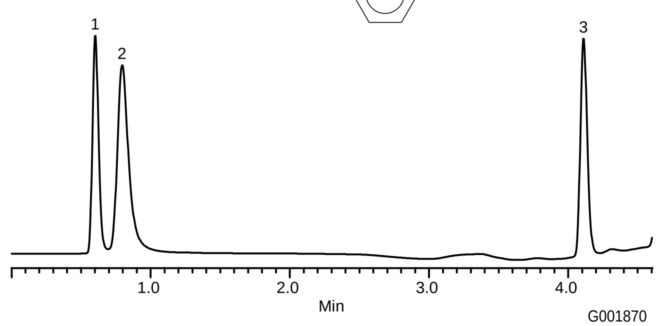
Gradient: Time (mins)	%A	%B
0	100	0
1	98	2
4	40	60

Triprolidine



Meclizine





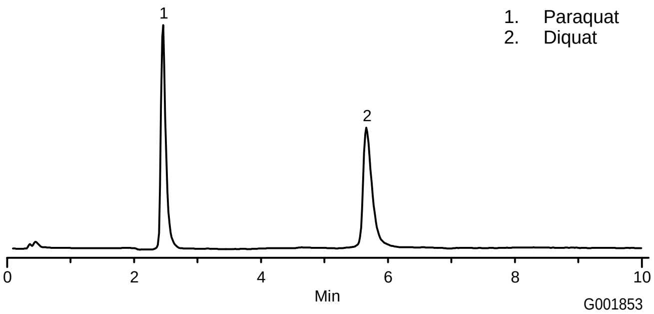
Quaternary Amines can be Analyzed on Discovery Zr-PS at High pH Without Ion-pairing

Basic compounds, especially quaternary amines, often suffer from lack of hydrophobic retention on C18-silica phase. To remedy this, ion-pairing is employed. However, ion-pair agents have well-known disadvantages. By running at high pH, the hydrophobicity of the amine is increased and ion-pair agents are not required. Discovery Zr-PS is stable at high pH. Figure M shows the separation of paraquat and diquat, two quaternary amines, on Discovery Zr-PS and C18-silica. Note that ion-pairing is not needed to have retention on the Discovery Zr-PS. Retention is due to both hydrophobicity and the presence of ion-exchange with the adsorbed Lewis base mobile phase buffer ion (phosphate).

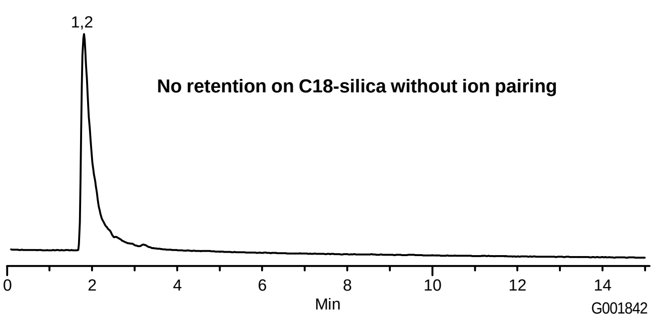
Figure M: Paraquat and Diquat on Discovery Zr-PS vs. C18-silica

Discovery Zr-PS
Column: **Discovery Zr-PS, 7.5cm x 4.6mm, 3µm**
Cat. No.: **65741-U**
Mobile Phase: (50:50) 25mM H_3PO_4, 25mM NH_4F (pH 8 with NH_4OH):CH_3CN
Flow Rate: 3mL/min
Det.: UV, 290nm
Temp.: 65°C
Inj.: 10µL
Sample: Paraquat, diquat (50µg/mL)

1. Paraquat
2. Diquat



C18-silica
Column: C18-silica, 15cm x 4.6mm, 3µm
Mobile Phase: (95:5) 25mM H_3PO_4 (pH 7 with NH_4OH):CH_3CN
Flow Rate: 1mL/min
Det.: UV, 290nm
Temp.: 35°C
Inj.: 10µL
Sample: Paraquat, diquat (50µg/mL)



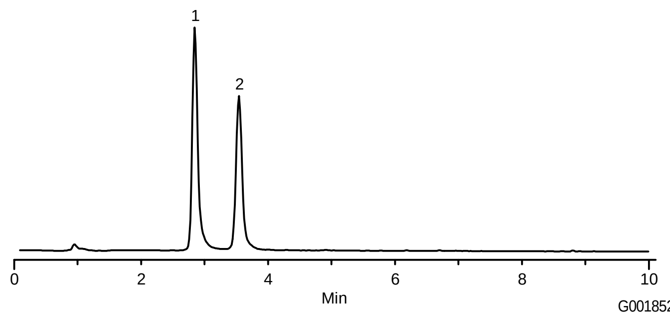
Difficult Basic Compounds Exhibit Symmetrical Peaks on Discovery Zr-PS at High pH

Another problem with basic compounds on silica is their tendency to tail because of silanol interactions. This can be avoided by running at high pH where the charge on the base is neutralized. However, silica is typically limited to below pH 8. Figure N shows a difficult pair of bases on Discovery Zr-PS at pH 12. The symmetrical peaks are testimony to the lack of undesirable secondary interactions.

Figure N: Fluoxetines on Discovery Zr-PS vs. C18-silica

Column: **Discovery Zr-PS, 7.5cm x 4.6mm, 3µm**
Cat. No.: **65741-U**
Mobile Phase: (70:30) 25mM potassium phosphate (pH 12):CH_3CN
Flow Rate: 1mL/min
Det.: UV, 230nm
Temp.: 35°C
Inj.: 10µL
Sample: Norfluoxetine, fluoxetine (50µg/mL)

1. Norfluoxetine
2. Fluoxetine



Discovery Zr-Carbon

Carbon-clad zirconia for separations geometric isomers and diastereomers and enhanced retention of polar compounds.

Discovery Zr-Carbon comprises spherical, porous carbon-coated zirconia particles. It is ideal for the reversed-phase separation of positional isomers and diastereomers. It complements the selectivity offering of the other zirconia-, silica-, and polymer-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 14. It is a great alternative when C18 does not work.

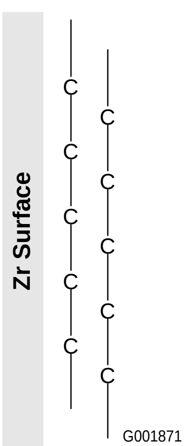
Discovery Zr-Carbon Characteristics

Discovery Zr-Carbon - zirconia coated with permanent layer of carbon

Particle Size:	3 and 5 micron
Surface Area (m^2/g):	30m^2/g
Pore Size:	300Å
pH Range:	1 – 14
Temperature Range *:	< 100°C

*Special column hardware for operations between 100°C and 200°C is available.

Structure of Discovery Zr-Carbon:



Features of Discovery Zr-Carbon:

- Excellent separation of geometric isomers and diastereomers
- Very hydrophobic surface
- Most different retention compared to other Discovery Zr phases for non-ionic compounds
- Similar to porous graphitic carbon, but with added ion-exchange interactions
- pH stable from 1-14
- Thermally stable up to 100°C (up to 150°C in special hardware)
- Avoid fused-ring aromatics as they are too strongly absorbed by Discovery Zr-Carbon

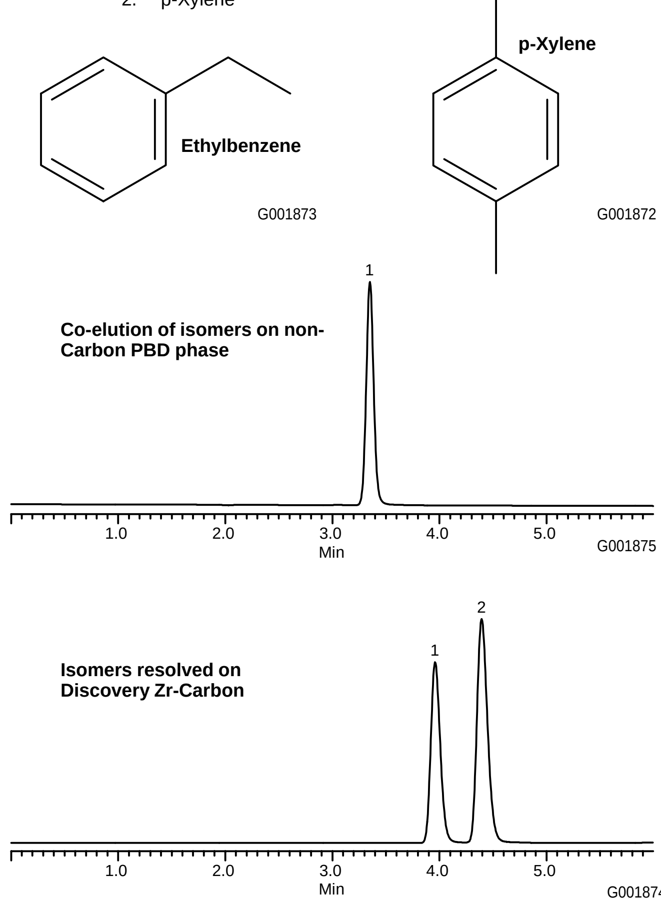
The Rigid Surface of Discovery Zr-Carbon Permits the Separation of Structurally Similar Compounds.

Carbon-based packings have found a niche within the population of HPLC supports. The main benefits of carbon over silica are enhanced chemical and thermal stability, and the ability to separate positional isomers. Compounds that have the same hydrophobicity, but different molecular shape, can be separated on the rigid carbon surface but not on phases that comprise flexible ligands, like C18-bonded silica. In Figure O, the isomers ethylbenzene and p-xylene co-elute on the non-carbon Discovey Zr-PBD phase, but are resolved on Discovery Zr-Carbon.

Figure O: Separation of Structurally Similar Compounds on Discovery Zr-Carbon vs. Non-Carbon Phase

Columns:	**Discovery Zr-PBD, 15cm x 4.6mm, 3µm**
Cat. No.:	**65718-U**
	Discovery Zr-Carbon, 15cm x 4.6mm, 3µm
Cat. No.:	**65730-U**
Mobile Phase:	(20:80) water : CH_3CN
Flow Rate:	0.5mL/min
Det.:	UV, 254nm
Temp.:	60°C
Inj.:	10µL
Sample:	Ethylbenzene, p-xylene (870µg/mL)

1. Ethylbenzene
2. p-Xylene



Positional Isomers are Easily Resolved on Discovery Zr-Carbon

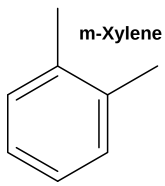
The ability of Discovery Zr to distinguish positional isomers is demonstrated in Figure P below. The isomers co-elute on a C18-silica column, but are resolved on the Discovery Zr-Carbon column.

Figure P: Separation of Positional Isomers on Discovery Zr-Carbon vs. Non-Carbon Phase

Columns:	**Discovery Zr-Carbon, 15cm x 4.6mm, 3µm**
Cat. No.:	**65730-U**
	C18-silica, 15cm x 4.6mm, 3µm
Mobile Phase:	(50:50) water:CH_3CN
Flow Rate:	1mL/min
Det.:	UV, 254nm
Temp.:	30°C
Inj.:	1µL
Sample:	o-xylene, m-xylene, p-xylene (290µg/mL)

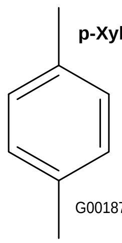
1. m-Xylene
2. p-Xylene
3. o-Xylene

m-Xylene



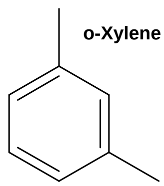
G001876

p-Xylene

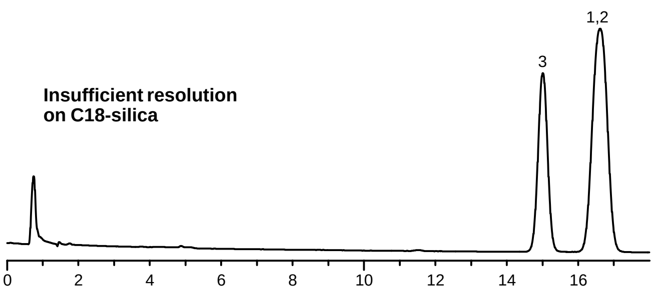


G001877

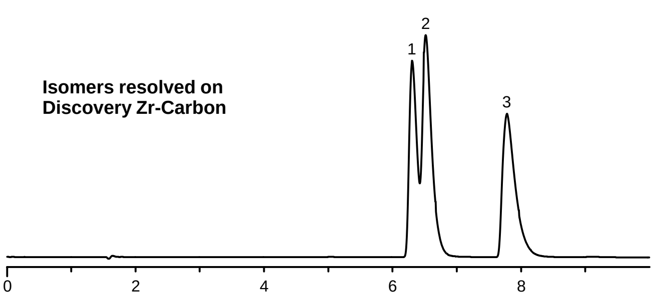
o-Xylene



G001878



G001879

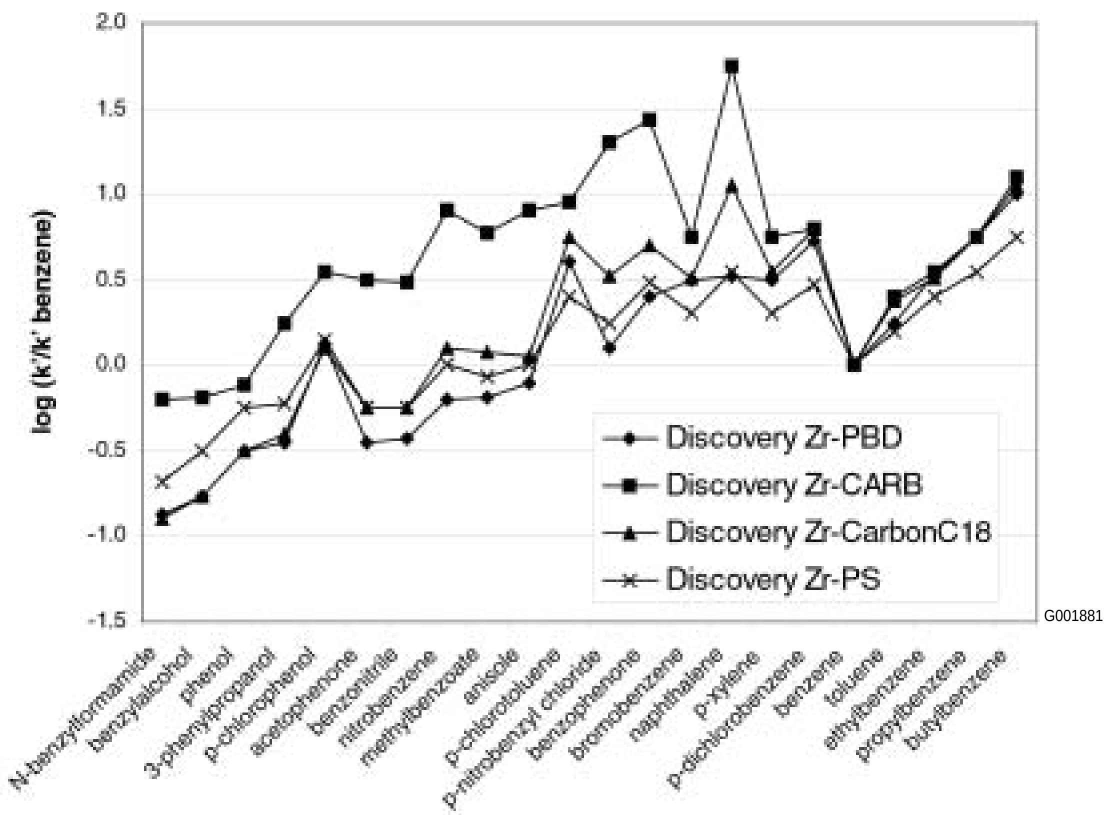


G001880

Discovery Zr-Carbon Has the Most Unique Selectivity Within the Discovery Zr Family.

Figure Q shows a selection of twenty three different non-ionic probes. Each was run on the four Discovery Zr phases. Retention relative to benzene was plotted. For these compounds, the Discovery Zr-Carbon has the most unique selectivity.

Figure Q: Comparison of Selectivity Among Discovery Zr Phases



NEW PRODUCTS

Discovery Zr - Unique retention and selectivity for HPLC method development at pH and temperature extremes.

Expand your method development options with the new Discovery Zr phases. Because they are based on zirconia particle chemistry, Discovery Zr phases are stable from pH 1 to 14 and up to 100°C in conventional hardware, and higher in special hardware. Discovery Zr combines the power of pH to alter selectivity and the power of temperature to decrease analysis time to give better and faster separations.

Discovery Zr-PBD

Polybutadiene-modified zirconia particles give separations most similar to C18-silica, but with benefits of high pH and temperature stability.

Discovery Zr-PS

Polystyrene modified zirconia particles are ideal for separations of hydrophobic compounds and amines.

Discovery Zr-CarbonC18

Octadecyl-modified carbon-clad zirconia combines partitioning mechanism and shape selectivity.

Discovery Zr-Carbon

Carbon-clad zirconia for separations geometric isomers and diastereomers.

Description	Cat. No.
Discovery Zr-PBD	
3 micron	
5cm x 2.1mm	**65713-U**
7.5cm x 2.1mm	**65714-U**
15cm x 2.1mm	**65715-U**
5cm x 4.6mm	**65716-U**
7.5cm x 4.6mm	**65717-U**
15cm x 4.6mm	**65718-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65811-U**
1cm x 4mm Supelguard Cartridge Kit	**65813-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65812-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65814-U**
5 micron	
5cm x 2.1mm	**65719-U**
15cm x 2.1mm	**65720-U**
5cm x 4.6mm	**65722-U**
15cm x 4.6mm	**65723-U**
25cm x 4.6mm	**65724-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65815-U**
1cm x 4mm Supelguard Cartridge Kit	**65817-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65816-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65818-U**
Discovery Zr-PS	
3 micron	
5cm x 2.1mm	**65737-U**
7.5cm x 2.1mm	**65738-U**
15cm x 2.1mm	**65739-U**
5cm x 4.6mm	**65740-U**
7.5cm x 4.6mm	**65741-U**
15cm x 4.6mm	**65742-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65841-U**
1cm x 4mm Supelguard Cartridge Kit	**65843-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65842-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65844-U**
5 micron	
5cm x 2.1mm	**65743-U**
15cm x 2.1mm	**65744-U**
5cm x 4.6mm	**65746-U**
15cm x 4.6mm	**65747-U**
25cm x 4.6mm	**65748-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65845-U**
1cm x 4mm Supelguard Cartridge Kit	**65847-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65846-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65848-U**

Description	Cat. No.
Discovery Zr-CarbonC18	
3 micron	
5cm x 2.1mm	**65701-U**
7.5cm x 2.1mm	**65702-U**
15cm x 2.1mm	**65703-U**
5cm x 4.6mm	**65704-U**
7.5cm x 4.6mm	**65705-U**
15cm x 4.6mm	**65706-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65801-U**
1cm x 4mm Supelguard Cartridge Kit	**65803-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65802-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65804-U**
5 micron	
5cm x 2.1mm	**65707-U**
15cm x 2.1mm	**65708-U**
5cm x 4.6mm	**65710-U**
15cm x 4.6mm	**65711-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65805-U**
1cm x 4mm Supelguard Cartridge Kit	**65807-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65806-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65808-U**
Discovery Zr-Carbon	
3 micron	
5cm x 2.1mm	**65725-U**
7.5cm x 2.1mm	**65726-U**
15cm x 2.1mm	**65727-U**
5cm x 4.6mm	**65728-U**
7.5cm x 4.6mm	**65729-U**
15cm x 4.6mm	**65730-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65821-U**
1cm x 4mm Supelguard Cartridge Kit	**65823-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65822-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65824-U**
5 micron	
5cm x 2.1mm	**65731-U**
15cm x 2.1mm	**65732-U**
5cm x 4.6mm	**65734-U**
15cm x 4.6mm	**65735-U**
1cm x 2.1mm Supelguard Cartridge Kit	**65826-U**
1cm x 4mm Supelguard Cartridge Kit	**65828-U**
1cm x 2.1mm Supelguard Cartridges, pk. of 2	**65827-U**
1cm x 4mm Supelguard Cartridges, pk. of 2	**65829-U**

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