

Introducing Discovery Zr: HPLC Method Development at pH and Temperature Extremes

The primary advantages of zirconia over either silica- or polymer-based particles is its extreme chemical and thermal stability and its unique chromatographic selectivity. Silica-based particles will typically exhibit short lifetimes if used at pH values less than 2 or greater than 8 with inorganic buffers. Polymer-based particles can shrink or swell as a function of mobile phase organic content or ionic strength. Additionally, polymer-based particles exhibit relatively low efficiencies. Zirconia supports exhibit excellent stability over the entire pH range of 1-14 and temperatures up to 200°C (pH 1-13 and temperatures up to 150°C for polymer-coated zirconia), very high mechanical strength, and high efficiencies (150,000 plates/m for toluene). The extreme stability of zirconia columns results in a very long column lifetime. This translates to the ability to run the columns under a much wider range of chromatographic conditions, often enabling difficult separations, and reduced cost per analysis.

Discovery Zr-PBD and Zr-PS are polybutadiene- and polystyrene-coated zirconia particles that operate as reversed-phase HPLC (RP-HPLC) materials. For non-ionic analytes, the chromatographic selectivity of the Zr-PBD and Zr-PS is similar to C18 and phenyl silica based columns, respectively. For ionic compounds, there are secondary interactions (ion-exchange), which can be used to fine-tune the selectivity. This ion-exchange character, in conjunction with hydrophobic retention, gives the analyst additional means to optimize a separation not present with typical silica-based reversed phases. Because of the extreme chemical stability, these secondary interactions can be eliminated, if desired, by utilizing a mobile phase with a pH greater than the pKa of the analytes, for bases, or less than the pKa of the analytes, for acids. Under these circumstances, the retention is based solely on the hydrophobicity of the analytes. Additionally, the high thermal stability of these phases allows the analyst to take advantage of the greater efficiencies and speed of analysis at higher temperatures. Figures A and B are typical separations performed on Discovery Zr-PBD and Zr-PS. Note the elevated pH of the mobile phase.

Discovery Zr-Carbon comprises zirconia particles coated with a permanent, thin layer of elemental carbon. The resulting material yields performance characteristics very different from silica-based reversed phases. The carbon surface allows geometric isomer separations not attainable by reversed-phase or bonded normal-phase columns. Additionally, Discovery Zr-Carbon is a good choice when you need more retention or significantly different selectivity than silica-based C18 phase.

LC
issue

Figure C shows the separation of

Figure A. Tricyclic Antidepressants on Discovery Zr-PBD

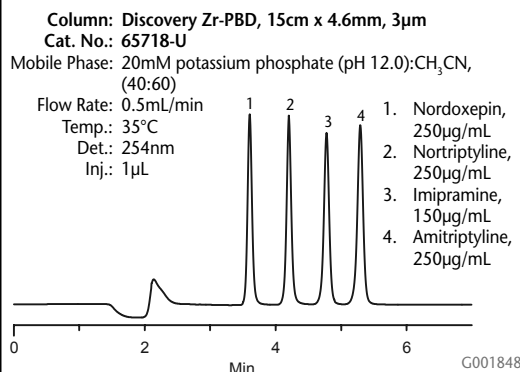


Figure B. Fluoxetine of Discovery Zr-PS

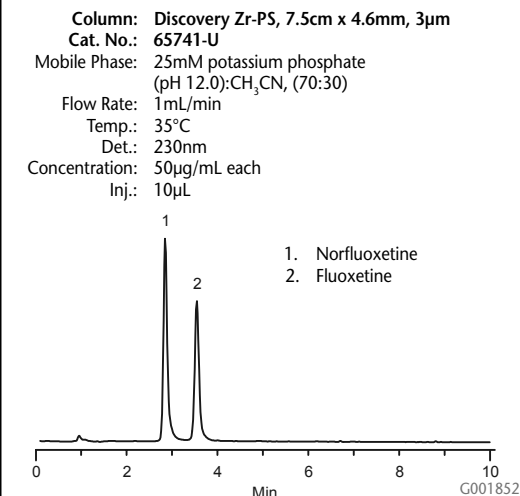
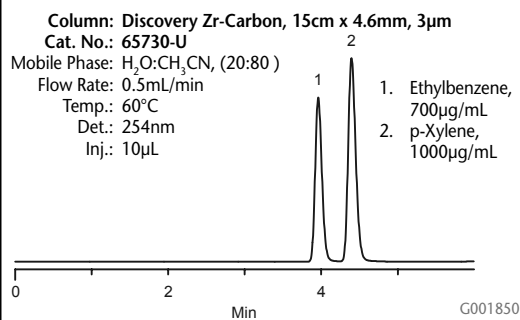


Figure C. Ethylbenzene and p-Xylene on Discovery Zr-Carbon



(continued on page 4)

CONTENTS

Page 1:

- Introducing Discovery Zr: HPLC Method Development at pH and Temperature Extremes

Page 2:

- New Products
Discovery Zr

Page 3:

- New Applications
RP-HPLC Analysis of Paraquat and Diquat Without Ion-Pairing
- Liquid Chromatography Performance Tip
Guidelines for Buffer Preparation when Using Discovery Zr Columns

Page 4:

- Introducing Discovery Zr . . . (contd.)
- Case Study 5
Why Use Elevated Temperature?

SUPELCO
a member of the Sigma-Aldrich family

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-Carbon

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

Discovery Zr-CarbonC18

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

Discovery Zr-PBD

Polybutadiene-modified zirconia particles give separations most similar to C18-silica for non-ionic compounds, but with benefits of high pH and temperature stability. Unique selectivity compared to C18 for ionic compounds.

Discovery Zr-PS

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

Description	Cat. No.	Description	Cat. No.
Discovery Zr-Carbon		Discovery Zr-PBD	
3 micron		3 micron	
5cm x 2.1mm	65725-U	5cm x 2.1mm	65713-U
7.5cm x 2.1mm	65726-U	7.5cm x 2.1mm	65714-U
15cm x 2.1mm	65727-U	15cm x 2.1mm	65715-U
5cm x 4.6mm	65728-U	5cm x 4.6mm	65716-U
7.5cm x 4.6mm	65729-U	7.5cm x 4.6mm	65717-U
15cm x 4.6mm	65730-U	15cm x 4.6mm	65718-U
1cm x 2.1mm Supelguard Cartridge Kit	65821-U	1cm x 2.1mm Supelguard Cartridge Kit	65811-U
1cm x 4mm Supelguard Cartridge Kit	65823-U	1cm x 4mm Supelguard Cartridge Kit	65813-U
1cm x 2.1mm Supelguard Cartridges, pk. of 2	65822-U	1cm x 2.1mm Supelguard Cartridges, pk. of 2	65812-U
1cm x 4mm Supelguard Cartridges, pk. of 2	65824-U	1cm x 4mm Supelguard Cartridges, pk. of 2	65814-U
5 micron		5 micron	
5cm x 2.1mm	65731-U	5cm x 2.1mm	65719-U
15cm x 2.1mm	65732-U	15cm x 2.1mm	65720-U
5cm x 4.6mm	65734-U	5cm x 4.6mm	65722-U
15cm x 4.6mm	65735-U	15cm x 4.6mm	65723-U
1cm x 2.1mm Supelguard Cartridge Kit	65826-U	25cm x 4.6mm	65724-U
1cm x 4mm Supelguard Cartridge Kit	65828-U	1cm x 2.1mm Supelguard Cartridge Kit	65815-U
1cm x 2.1mm Supelguard Cartridges, pk. of 2	65827-U	1cm x 4mm Supelguard Cartridge Kit	65817-U
1cm x 4mm Supelguard Cartridges, pk. of 2	65829-U	1cm x 2.1mm Supelguard Cartridges, pk. of 2	65816-U
		1cm x 4mm Supelguard Cartridges, pk. of 2	65818-U
Discovery Zr-CarbonC18		Discovery Zr-PS	
3 micron		3 micron	
5cm x 2.1mm	65701-U	5cm x 2.1mm	65737-U
7.5cm x 2.1mm	65702-U	7.5cm x 2.1mm	65738-U
15cm x 2.1mm	65703-U	15cm x 2.1mm	65739-U
5cm x 4.6mm	65704-U	5cm x 4.6mm	65740-U
7.5cm x 4.6mm	65705-U	7.5cm x 4.6mm	65741-U
15cm x 4.6mm	65706-U	15cm x 4.6mm	65742-U
1cm x 2.1mm Supelguard Cartridge Kit	65801-U	1cm x 2.1mm Supelguard Cartridge Kit	65841-U
1cm x 4mm Supelguard Cartridge Kit	65803-U	1cm x 4mm Supelguard Cartridge Kit	65843-U
1cm x 2.1mm Supelguard Cartridges, pk. of 2	65802-U	1cm x 2.1mm Supelguard Cartridges, pk. of 2	65842-U
1cm x 4mm Supelguard Cartridges, pk. of 2	65804-U	1cm x 4mm Supelguard Cartridges, pk. of 2	65844-U
5 micron		5 micron	
5cm x 2.1mm	65707-U	5cm x 2.1mm	65743-U
15cm x 2.1mm	65708-U	15cm x 2.1mm	65744-U
5cm x 4.6mm	65710-U	5cm x 4.6mm	65746-U
15cm x 4.6mm	65711-U	15cm x 4.6mm	65747-U
1cm x 2.1mm Supelguard Cartridge Kit	65805-U	1cm x 2.1mm Supelguard Cartridge Kit	65748-U
1cm x 4mm Supelguard Cartridge Kit	65807-U	25cm x 4.6mm	65845-U
1cm x 2.1mm Supelguard Cartridges, pk. of 2	65806-U	1cm x 4mm Supelguard Cartridge Kit	65847-U
1cm x 4mm Supelguard Cartridges, pk. of 2	65808-U	1cm x 2.1mm Supelguard Cartridges, pk. of 2	65846-U
		1cm x 4mm Supelguard Cartridges, pk. of 2	65848-U

All literature mentioned in this issue can be obtained from the website, sigma-aldrich.com/TheReporter, by completing the Literature Request section on the reply card, or by calling our Technical Service Dept.

NEW APPLICATIONS

RP-HPLC Analysis of Paraquat and Diquat Without Ion-Pairing

Paraquat and diquat are quaternary amines in the bipyridylum class of herbicides.

The determination of paraquat and diquat is usually performed by ion-pair HPLC. Baseline resolution is not typically obtained and quantitation is performed spectrally. Although it is an accepted practice (US EPA Method 549.2), errors may arise because incomplete chromatographic resolution allows for significant error due to the fact that there is a small, but significant, absorbance from both compounds at the recommended wavelengths for quantitation. Therefore, an ideal HPLC method for the determination of these herbicides would allow for baseline resolution of the two species, with the use of a simple mobile phase (isocratic, binary, no ion-pairing reagents).

Figure G is a chromatogram showing the separation of paraquat and diquat on a Discovery Zr-PS column, without the use of ion-pairing reagents. Note the excellent retention, efficiencies, peak shapes, and resolution of the compounds. Figure H is a chromatogram showing the separation of the compounds on a typical C18 column without the use of ion-pairing reagents. The compounds coelute, are not significantly retained, and tail excessively.

Even with the use of ion-pairing reagents, quantitation of paraquat and diquat on silica-based reversed phase columns is not ideal because the typically poor peak shapes and resolution fall short of good chromatography. The use of Discovery Zr-PS will allow the chromatographer to confidently perform the determination of paraquat and diquat, utilizing an easy and robust HPLC method.

For more information, request T102931 and T402075.

Figure G. Paraquat and Diquat on Discovery Zr-PS

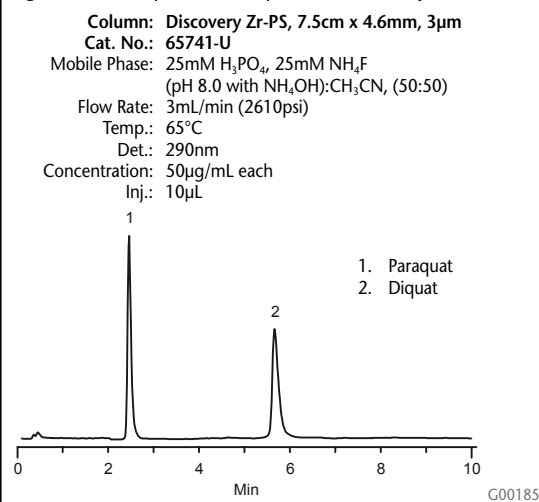
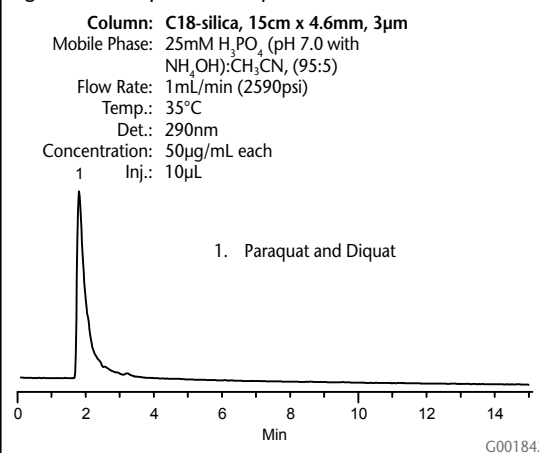


Figure H. Paraquat and Diquat on a C18



LIQUID CHROMATOGRAPHY PERFORMANCE TIP

Guidelines for Buffer Preparation when Using Discovery Zr Columns

Buffers are commonly used with Discovery Zr columns when charged analytes are being separated, much as buffers would be used with silica based columns. With this in mind, we thought it would be beneficial to review a few practical points on buffer preparation and use.

1. Good buffer to use with Discovery Zr are strong Lewis Bases (fluoride phosphate, citrate, sulfate acetate).
2. In preparing the buffer, dissolve the solid in ~95% of the final volume desired. After the solution is adjusted to the pH desired, bring the solution to volume.
3. Always calibrate the pH meter either at the final adjusted pH (the final adjusted pH must match the pH of a standard) or calibrate the pH meter at values above and below the final adjusted pH. A measurement will not be reliable outside the range for which the meter was calibrated.
4. For consistent results, pH adjustments to the aqueous solution should be made before addition of or-

ganic. The actual pH will change after addition of the organic, but there is no good method for reliable measurement of pH after such addition. This will ensure good lab practice.

5. All buffer solutions should be filtered before use. This removes particulates that may be in the water or introduced by the solid buffer when dissolved. A hydrophilic 0.45µm filter is recommended. Filtration should be a last step before use as is or before mixing with organic.
6. Pre-check buffer solubility with the highest amount of organic modifier to be used. The buffer cation effects solubility with sodium salts being the least soluble.
7. 25mM is a reasonable starting concentration. Most inorganic buffers are more soluble in methanol than acetonitrile or THF.

For more information, request T401012 and T102931.

Introducing the Discovery Zr (continued from page 1)

ethylbenzene and p-xylene on Discovery Zr-Carbon. These compounds have similar hydrophobicity and therefore co-elute on C18-silica phases. However, they are resolved on Discovery Zr-Carbon because it can distinguish between molecules of the same hydrophobicity but different molecular shape.

Discovery Zr-CarbonC18 is made by covalently bonding C18 ligands to the surface of the carbon-coated zirconia particles of Discovery Zr-Carbon. Because the surface below the C18 phase is carbon rather than silica, the retention and selectivity of Discovery Zr-CarbonC18 is quite different from a C18 phase bonded to silica. Figure D shows a fast separation of non-steroidal anti-inflammatory drugs on Discovery Zr-CarbonC18.

For more information, request T102931 and T402075.

CASE STUDY 5

Why Use Elevated Temperature?

Increasing the temperature of a separation increases the efficiency and speed of a separation in three ways:

1. diffusivity increases, thereby decreasing the resistance to mobile phase mass transfer
2. sorption kinetics are accelerated
3. mobile phase viscosity is reduced, allowing higher flow rates

The primary requirement of utilizing elevated temperatures is the stability of the stationary phase. To simplify the expected effect of temperature, it is desirable to employ a mobile phase with a pH greater than the pKa of the analyte (for basic compounds) to eliminate ion exchange effects that may complicate the separation by creating a significant change in selectivity with a variation in temperature. Typical silica-based HPLC packings will quickly deteriorate at elevated temperatures, particularly at the elevated pH values necessary to be above the pKa of most basic pharmaceutical compounds. Discovery Zr-PBD exhibits the necessary thermal and chemical stability to operate at elevated temperatures and extreme pH.

Figures E and F show a separation of alkaloids at two different temperatures and flow rates, while maintaining a constant pressure. Alkaloids are naturally occurring bases with complex multi-ring structures. They are often difficult candidates for RP-HPLC separation, since they are prone to polar and ionic, in addition to hydrophobic, interactions. By performing the separation at pH 12, ionic interactions can be eliminated. The difficulty of this separation is to maintain adequate retention ($k' \geq 2$) and resolution of codeine and strychnine, while allowing for timely isocratic elution and resolution of papaverine, quinine, and quinidine. Although the Discovery Zr-PBD column is stable to 100°C (150°C with custom hardware), a temperature of 65°C was selected because it is attainable with most column heaters.

Analysis time was reduced from 46 minutes to 12 minutes with only a slight decrease in resolution.

For more information, request T102931 and T402075.

Figure D. Fast Separation of Non-steroidal Anti-inflammatory Drugs on Discovery Zr-CarbonC18

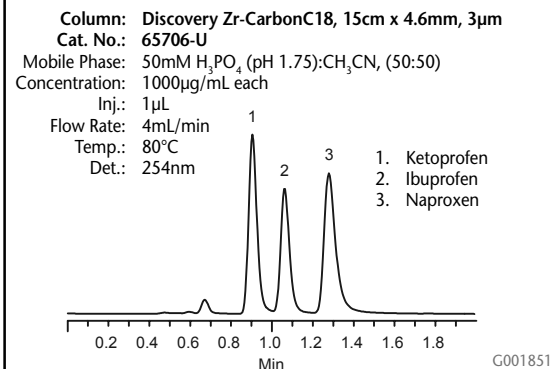


Figure E. Separation of Alkaloids at 30°C

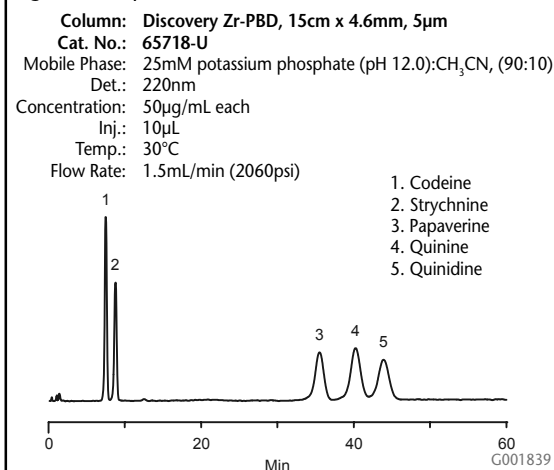
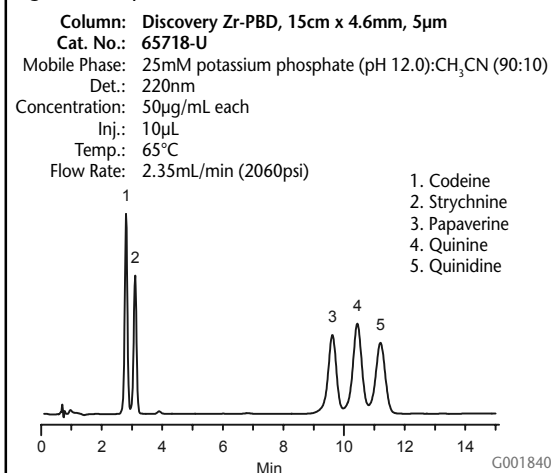


Figure F. Separation of Alkaloids at 65°C



sigma-aldrich.com/supelco

Order/Customer Service 800-247-6628, 800-325-3010 • Fax 800-325-5052 • E-mail supelco@sial.com
Technical Service 800-359-3041, 814-359-3041 • Fax 800-359-3044, 814-359-5468 • E-mail techservice@sial.com
SUPELCO • Supelco Park, 595 North Harrison Road, Bellefonte, PA 16823-0048 • 814-359-3441

ISO 9001
REGISTERED

We are committed to the success of our Customers, Employees and Shareholders through leadership in Life Science, High Technology and Service.

The SIGMA-ALDRICH Family



T202009
ENW

© 2002 Sigma-Aldrich Co. Printed in USA. Supelco brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.