

Efficiently analyze your most  
challenging compounds.  
Deliver high-quality  
results faster.

## **Agilent ZORBAX Column Selection Guide for HPLC**



Agilent Technologies



## Here it is – your single source for LC column selection.

- How do we increase productivity while our resources remain constant?
- How can we meet tight deadlines in the face of complex samples and heavy workloads?
- Which columns ensure method ruggedness for transfer to our manufacturing site?
- How can we stop losing time troubleshooting experiments?

Find the answers you need right here!


Agilent's ZORBAX Column Selection Guide for HPLC is the only chromatography guide that combines a broad offering of LC columns with expert advice that can help you achieve reliable results faster. All in a concise, easy-to-use format.

Inside, you will find columns for analytical, high-throughput, and bioanalytical HPLC... method development guidelines... and tips from industry veterans. And you will find proof that – at any pH level – ZORBAX columns deliver superior reproducibility and high resolution.



## Maximize your productivity!

Our easy-to-use guide cuts the column selection process down to four simple steps:

1. Narrow the Search – use the flow charts on pages 6-9 to choose the right column for your needs.
2. Follow the  for tips on everything from custom columns to technical support.  
Most tips can also be found at [www.agilent.com/chem/lccolumntips](http://www.agilent.com/chem/lccolumntips).
3. Prepare your order – using the part numbers and purchasing guides located near the descriptions of each column.  
You can also order online at [www.agilent.com/chem/store](http://www.agilent.com/chem/store).

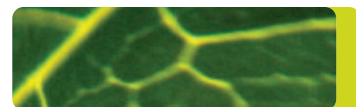
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## New columns deliver on the promise of greater productivity and superior peak shape for acids, bases, and neutrals.

Whether you are performing conventional or ultra-fast chromatography, separating biomolecules, or analyzing complex basic compounds, you can trust Agilent for the industry's highest-performing columns that deliver the fast, reproducible results you need. All engineered with Agilent's unparalleled quality and reliability.

Now, Agilent has launched two exciting new column lines that will help you meet the challenges of faster turnaround times, unrelenting workloads, and fewer staff members:

- **NEW ZORBAX Eclipse Plus LC columns** — are proven to deliver reproducible peak symmetry for basic compounds. Eclipse Plus columns stand up to — even outperform — the competition, and are designed to help you achieve excellent peak shape and resolution.
- **NEW 600 bar ZORBAX Rapid Resolution High Throughput (RRHT) 1.8  $\mu$ m LC columns** — allow you to increase the speed of your separations without compromising the quality of your resolution and results.

And remember, when you choose ZORBAX Eclipse LC columns, you get more than just a dependable product. You also get over 40 years of expertise — along with unmatched technical support — from the world's largest chromatography supplier. On the web, by phone or in person, Agilent helps you solve the problems that can slow you down and get in the way of your results.



## Run samples up to 20 times faster than conventional LC with the complete Agilent 1200 Series Rapid Resolution System.

Agilent's NEW 1200 Series HPLC is designed with the same attention to detail, quality and superior performance you expect from Agilent instruments. And it is backed by over 40 years of chromatography experience.

Together with Agilent's NEW ZORBAX RRHT 1.8  $\mu$ m LC columns, the Agilent 1200 Series can help you produce your numbers more efficiently than ever before, so you can meet your most difficult deadlines.

To learn more, visit [www.agilent.com/chem/1200RR](http://www.agilent.com/chem/1200RR)

- 60% more resolving power than traditional LC
- A peak capacity greater than 600 bar
- 30-second cycle time
- Efficiencies above 60,000 plates
- The ability to run your current methods
- Flexible, comprehensive services to ensure peak performance
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### Put the full productivity-boosting power of the 1200 Series Rapid Resolution System to work for you!

ZORBAX RRHT columns deliver very high efficiencies in both short and long column lengths. To take full advantage of this efficiency, however, you should optimize your 1200 Series HPLC with Agilent's long-life lamp and low-volume flow cells equipped with RFID tags for unambiguous data traceability.

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## Agilent's high-performance ZORBAX Eclipse LC column family

Eliminate the hassles of trial and error —  
while increasing separation speed,  
reproducibility and method scalability.

From simple analysis to complex method development. ZORBAX Eclipse LC columns can enhance your productivity, while generating reproducible results across a wide range of applications and conditions.

Eclipse LC columns feature double-endcapping and a unique bonding process. This ensures lot-to-lot consistency, extends column life, and allows you to retain methods for the long run. Additionally, Eclipse LC columns are based on durable particle technology for superior long-term chromatographic reliability and column lifetime.

Eclipse LC columns are the only scalable column family  
(from 1.8  $\mu\text{m}$  to 7  $\mu\text{m}$ ) that lets you...

- Minimize tailing and maximize peak symmetry for bases, acids and neutrals.
- Develop and transfer methods without re-validation.
- Deliver reproducible results across a wide pH range and under varied separation conditions.
- Scale from analytical to prep — or from conventional to ultra-fast methods — with particle sizes from 1.8  $\mu\text{m}$  to 7  $\mu\text{m}$ .
- Process samples up to 20 times faster — and improve resolution up to 60% — with 600 bar ZORBAX Rapid Resolution High Throughput columns.

# ZORBAX HPLC Column Selection Guidelines

## HPLC column selection and method development strategies

To make it easier to select the right column for your specific application, we have included the following reference material. So you can maximize your most valuable resource — time.

### HPLC Column Selection Flow Chart

Here, you will find step-by-step instructions on choosing an initial column for method development, based on measurable factors such as analyte and mobile phase. We will then point you to the page that has the exact column you need.

### Simple Guidelines for Choosing a Reversed Phase HPLC Column

We will show you how to select the best column for small molecule and protein/peptide analysis, based on factors such as bonded phase and column configuration.

### Quick Guide to USP Categories For HPLC Columns

This section clearly and concisely lists the U.S. Pharmacopeia categories for Agilent's ZORBAX column family — including our new Rapid Resolution HT and Eclipse Plus columns with small particle sizes. So you can more easily select the columns that conform to USP standards and monographs.

### Reversed-Phase HPLC Method Development

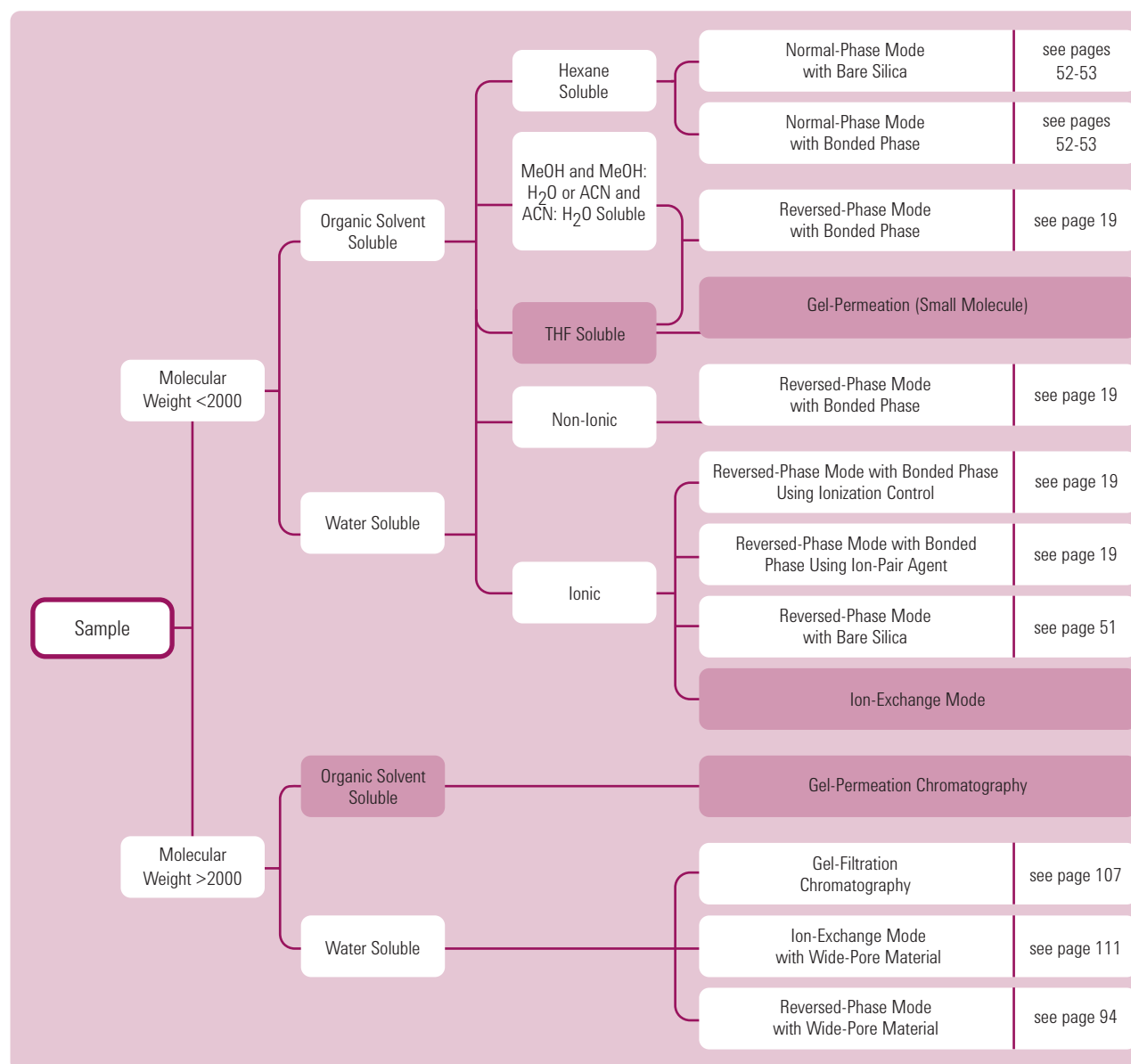
Chromatographic resolution between two or more peaks depends upon column efficiency, selectivity, and retention — all factors that are affected by pH. This section describes a method development strategy based on changing the pH level of the mobile phase.



# Column Selection

To use the column selection guide diagram below, simply follow the path for your analyte and mobile phase. At the far right, follow your final column selection to the pages indicated.

Please see the *2007-2008 Essential Chromatography and Spectroscopy Catalog* for a complete listing of LC columns and supplies.



Adapted with permission from "Practical HPLC Methodology and Applications," Brian A. Bidlingmeyer, John Wiley & Sons, Inc., New York, p. 109



**Quick Guide to ZORBAX Reversed-Phase Bonded Phases**

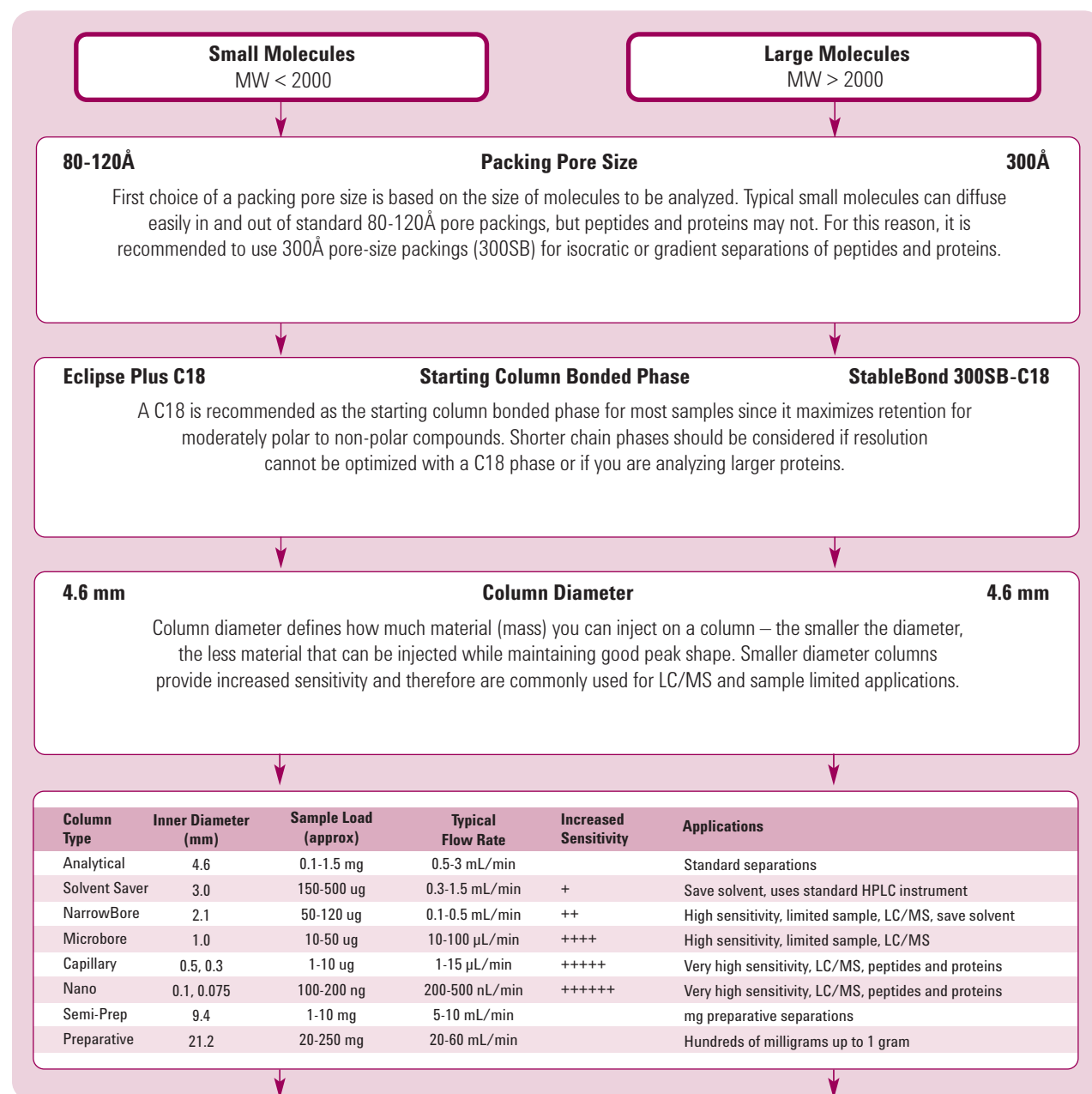
<b>Modern ZORBAX RP-HPLC Columns Recommended Uses and Applications</b>		<b>Page No.</b>
Eclipse Plus	<ul style="list-style-type: none"> <li>• Excellent first choice for method development</li> <li>• Long life from pH 2-9 for reliable separations of basic, acidic and neutral compounds</li> <li>• Superior peak shape with basic compounds</li> <li>• High resolution and efficiency with 1.8, 3.5 and 5 <math>\mu</math>m columns</li> <li>• Rigorous QA/QC testing for greater long-term reproducibility</li> </ul>	20
Eclipse XDB	<ul style="list-style-type: none"> <li>• Four selectivity choices for flexible method development</li> <li>• High performance over a wide pH range, pH 2-9</li> <li>• Good peak shape for acids, bases and neutrals</li> <li>• Long lifetime with extra dense bonding and double endcapping</li> <li>• Fast, ultra-fast, and high resolution separations using 1.8 and 3.5 <math>\mu</math>m columns</li> <li>• Choices from capillary to prep</li> </ul>	26
StableBond (SB)	<ul style="list-style-type: none"> <li>• Basic, acidic, neutral compounds</li> <li>• Exceptional stability at low pH</li> <li>• Use of high temperature (up to 90°C for C18, 80°C for C8, C3, Phenyl, CN, and Aq) and low pH as an added selectivity tool</li> <li>• Widest selection of bonded phases for different selectivity (C18, C8, C3, CN, Phenyl, Aq)</li> <li>• Uses mobile phases for LC/MS with formic acid, acetic acid, or TFA</li> <li>• Uses mobile phases with TFA for peptide and protein separation</li> <li>• Rapid separations using 1.8 and 3.5 <math>\mu</math>m columns</li> </ul>	33
ZORBAX Rx	<ul style="list-style-type: none"> <li>• General separation of basic, acidic and neutral compounds at low pH with different selectivity than SB columns</li> <li>• Rx-C8 is the same as SB-C8</li> </ul>	39
Bonus-RP	<ul style="list-style-type: none"> <li>• Separating basic compounds in higher aqueous mobile phases</li> <li>• General separation of basic, neutral, acidic compounds at mid-range pH or low pH; especially stable at low pH</li> <li>• Separating peptides for different selectivity</li> <li>• Rapid separations using 3.5 <math>\mu</math>m columns</li> </ul>	45
Extend-C18	<ul style="list-style-type: none"> <li>• Separating basic compounds above their pKa in free base form; separation of basic, acidic, neutral compounds at high pH; up to pH 11.5</li> <li>• Uses ammonium hydroxide as mobile phase additive with LC/MS with small molecules or peptides</li> <li>• Separating at high, mid-range and low pH for selectivity changes</li> <li>• Rapid separations using 3.5 <math>\mu</math>m columns</li> </ul>	41
<b>Original ZORBAX Columns</b>		<b>Page No.</b>
ZORBAX	<ul style="list-style-type: none"> <li>• General separation of basic, acidic, neutral compounds at low pH with different selectivity than SB columns; higher number of active silanols than SB</li> <li>• "Mixed mode" separation at more neutral pH values</li> </ul>	51
ZORBAX ODS Classic (non-end capped)	<ul style="list-style-type: none"> <li>• General separation of basic, acidic, neutral compounds at mid-range to low pH with different selectivity than SB or XDB columns</li> </ul>	51

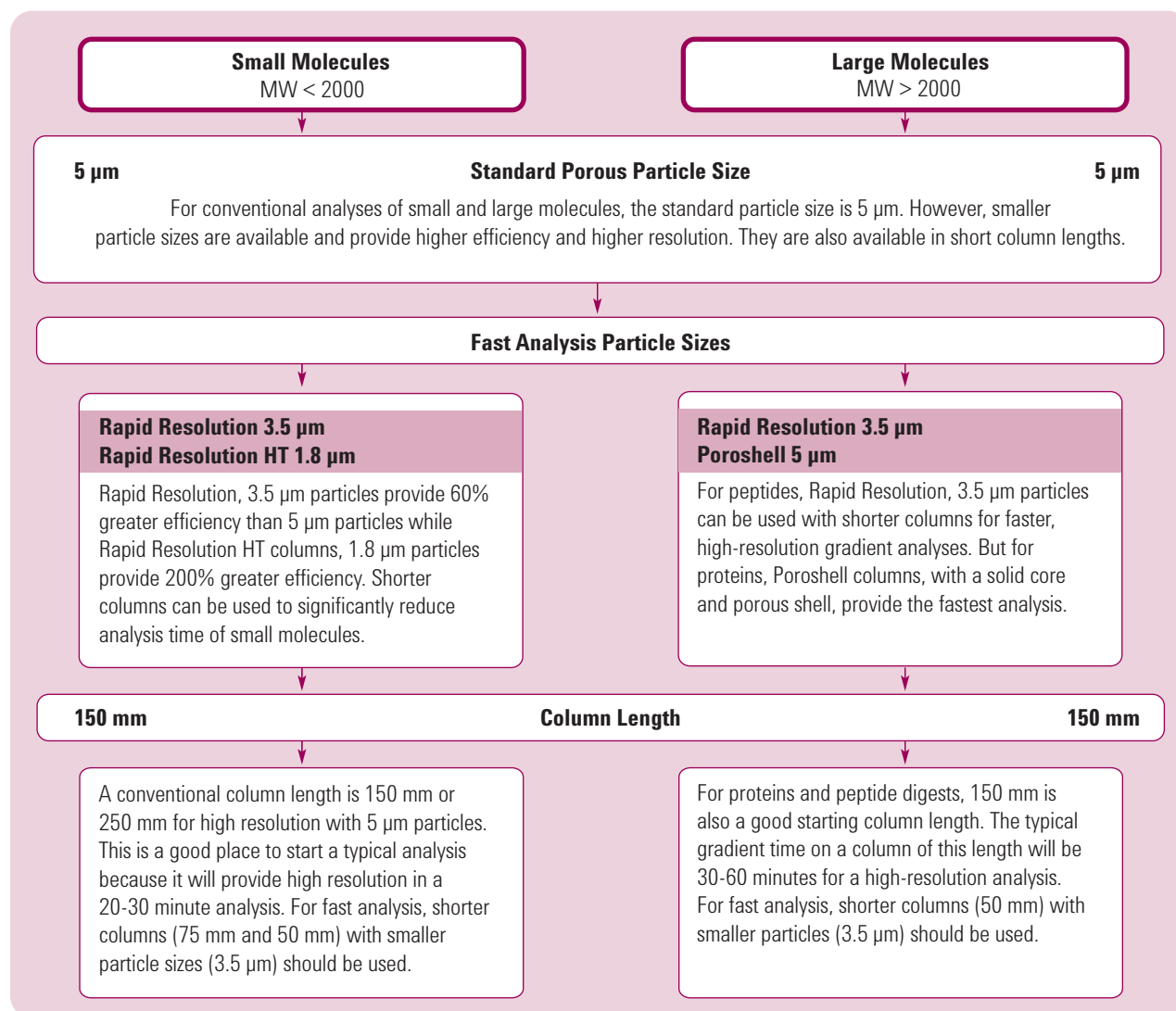
# ZORBAX Reversed-Phase HPLC Column Selection Flow Chart

## For small and large molecules

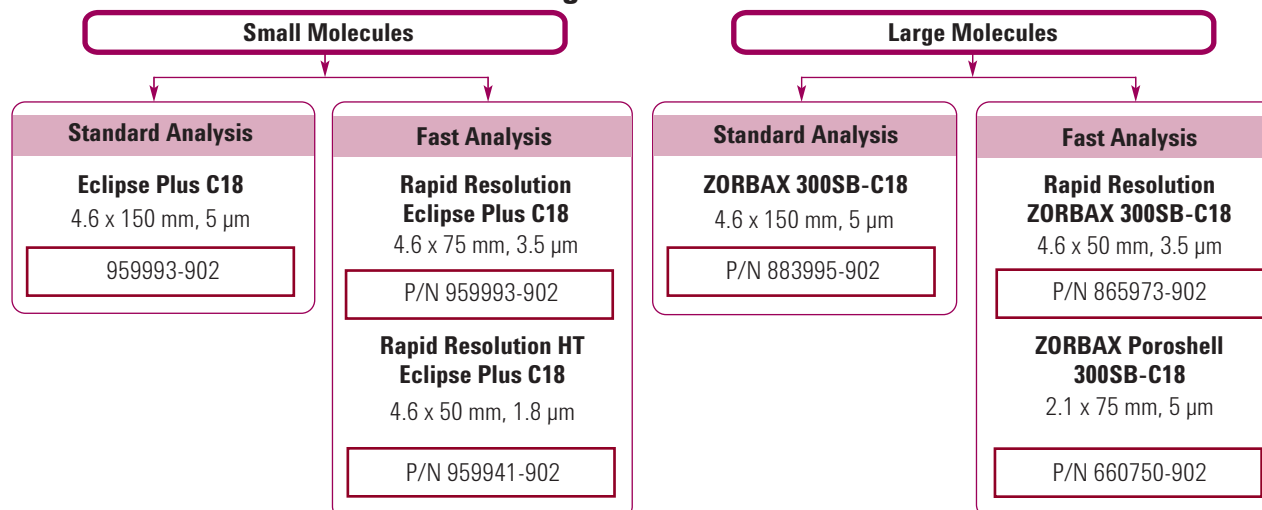
Most chromatographers use reversed-phase HPLC as one of their key analysis techniques. Reversed-phase HPLC can be used to analyze ionic and nonionic analytes. Therefore this ZORBAX Column Selection Flow Chart will focus on reversed-phase columns. To more easily select a reversed-phase column for method development of small and large molecules, follow the outline on these pages.

This flow chart provides information on choosing an initial column for method development of small molecule and protein and peptide samples, and includes decisions on bonded phase and column configuration.





### Starting Column Choices





## Column and Mobile Phase Guidelines: Reversed Phase

HPLC columns consist of two parts, the column chemistry and hardware. Consider first the molecular weight of your analyte to select pore size and then a bonded phase. Consult the sections on Analytical and Bioanalytical columns for the best bonded phases for small and large molecules. For choosing column hardware and particle sizes, consult the section on column sizes and rapid separations, including Rapid Resolution and Rapid Resolution HT columns, as well as Solvent Saver and Capillary columns and new PrepHT columns.

### Pore Size Selection

Choose a column packing with small pore (60-100Å) if the solute molecular weight is less than about 5000. Otherwise, use column packing with the 300Å pore size.

### Particle Size Selection

The standard particle size for HPLC columns is 5 µm with 3.5 µm increasing in use. If high-speed analyses or higher resolution analyses are required, packing with 1.8 µm and 3.5 µm particles can be used. Shorter columns with these particles can produce faster high-resolution separations, with the 1.8 µm particle size in Rapid Resolution HT columns, providing the highest efficiency. The 3.5 µm particle size operates at a routine operating pressure and can be used on all LC's. Short (50 mm and shorter) 1.8 µm RRHT columns can be used on optimized standard LC's, while the longer columns may require a higher pressure LC (one supporting pressures greater than 400 bar).

### Column Configuration

The column sizes most often recommended for analytical method development are 4.6 x 150 mm or 4.6 x 75 mm. If more resolution is needed, use a longer column, 4.6 x 250 mm or the same size column with a smaller particle size. During method development, choose the column internal diameter (e.g., 2.1, 3.0 mm) to accommodate additional application objectives (e.g., sensitivity, solvent usage) or compatibility with certain instrument types (capillary, nano, or prep).

### Silica Type and Bonded Phase

#### Silica Type

ZORBAX reversed phase columns use two different types of porous silica microspheres, the original ZORBAX SIL and ZORBAX Rx-SIL. ZORBAX Rx-SIL is a highly purified, less acidic silica than the original ZORBAX SIL. Less acidic silica means less potential for interaction between the analyte and silanol groups on the silica surface, especially if the solutes are basic, and contributes to improved peak shape. For new method development, we strongly recommend using reversed-phase products based on ZORBAX Rx-SIL (Eclipse, StableBond etc.). However, many excellent methods have been developed on reversed phase columns based on ZORBAX SIL and we continue to manufacture these high quality, reliable products.



## Bonded Phase

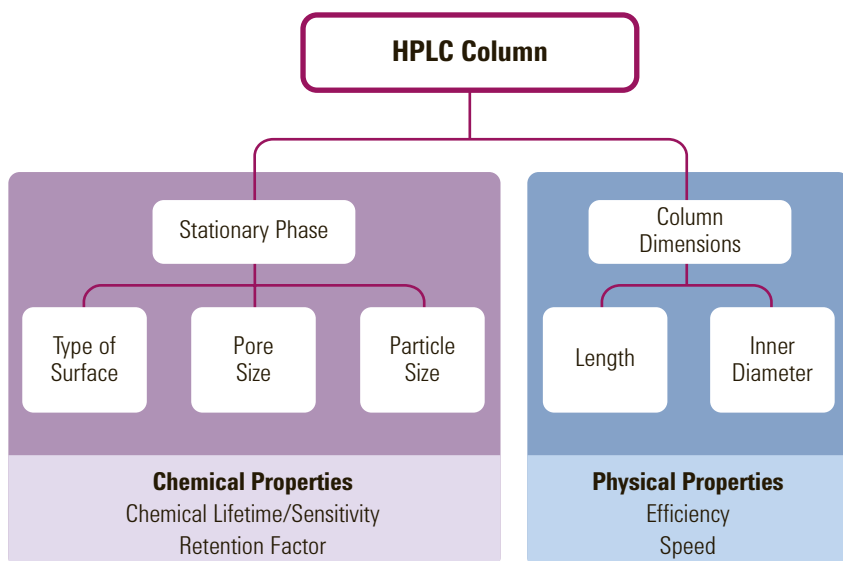
A good first choice for bonded phase is C18 or C8. If the sample solutes of interest are not adequately separated on these columns, CN and Phenyl columns may offer significant differences in selectivity from the straight-chain alkyl phases to effect the separation.

In general, larger solutes, such as proteins, are best separated on short-chain reversed-phase columns (C3, CN) and peptides and small molecules are separated on longer-chain columns (C8, C18). There are many cases, however, where this conventional wisdom does not apply. For example, peptides can also be effectively separated using short-chain columns, and hydrophobic peptides can show better recovery on longer-chain phases. Therefore, it is best to initially select a phase in the middle of the hydrophobic spectrum (e.g., C8), then change to a more hydrophobic phase or more hydrophilic phase depending on initial results and solubility properties of your sample.

## pH and Mobile Phase

The choice of mobile phase for a reversed-phase system starts with selecting the organic modifier. Selectivity differences and sample retention will vary significantly among mobile phases containing acetonitrile, methanol, and tetrahydrofuran (THF). Sample solubility is likely to differ in such solvents and dictate use of a specific solvent or solvents. UV detection at certain wavelengths is not possible with certain modifiers (e.g., methanol at 200 nm).

Both pH and ionic strength of the aqueous portion of mobile phases are important parameters in developing rugged methods that are not sensitive to small variations in conditions. With ionic compounds, retention of typical species shows significant changes with pH. It is very important to control pH in such reversed-phase systems to stabilize retention and band spacing. A pH set between 2 and 4 generally provides the most stable conditions for retention vs. small changes in pH and this pH is recommended for starting method development for most samples, including basic compounds and typical weak acids.



## USP Designations

The US Pharmacopeia (USP) is a standard source for many pharmaceutical methods. The USP specifies columns by packing materials rather than by manufacturer. The USP has updated its L1 definitions. Listed below you will see the most recent definitions and columns that apply and can be found in this column selection guide. Rapid Resolution High Throughput (RRHT) columns are now choices in the L1, L7, and L11 categories.

USP Designations	USP Packing Materials	Columns	Particle Size (µm)	Pore Size (Å)
L1	Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter	ZORBAX Eclipse Plus C18 ZORBAX Eclipse Plus XDB-C18 ZORBAX StableBond SB-C18 ZORBAX Extend-C18 ZORBAX Rx-C18 ZORBAX ODS ZORBAX ODS Classic	1.8, 3.5, 5, 7    3, 5 5	95 80 80 80 70 70
L3	Porous silica particles, 5 to 10 µm in diameter	ZORBAX Sil ZORBAX Rx-Sil	5 5	70 80
L7	Octyl silane chemically bonded to totally porous microsilica particles, 1.5 to 10 µm in diameter	ZORBAX Eclipse Plus C8 ZORBAX Eclipse XDB-C8 ZORBAX SB-C8 ZORBAX Rx-C8 ZORBAX C8	1.8, 3.5, 5, 7   3.5, 5 3, 5	95 80 80 80 70
L8	An essentially monomolecular layer of aminopropyl-silane chemically bonded to totally porous silica gel support, 10 µm in diameter	ZORBAX NH2	5	70
L10	Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter	ZORBAX Eclipse XDB-CN ZORBAX SB-CN ZORBAX CN	3.5, 5 3.5, 5 3, 5	80 80 70
L11	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter	ZORBAX Eclipse XDB-Phenyl ZORBAX SB-Phenyl ZORBAX Phenyl	3.5, 5 1.8, 3.5 5	80 80 70
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter	ZORBAX TMS	5	70
L14	Silica gel 10 µm in diameter with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating	ZORBAX SAX	5	70
L20	Dihydroxypropane groups chemically bonded to porous silica particles, 3 to 10 µm in diameter	ZORBAX GF-250	4	150
L33	Packing having the capacity to separate proteins by molecular size over a range of 4,000 to 400,000 da. It is spherical, silica-based, and processed to provide pH stability	ZORBAX GF-250	4	150
L35	A zirconium-stabilizes spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase	ZORBAX GF-250	4 6	150
L56	Propyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter	ZORBAX SB-C3	3.5, 5	80
L57	A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about 5 µm in diameter, with a pore size of 120 angstroms.	Ultron ES-OVM	5	120

## Method Development from pH 1-12

Chromatographic resolution between two or more peaks depends upon three factors – column efficiency, selectivity, and retention. With ionizable analytes – bases and acids – all of these factors change dramatically with pH. For example, retention can be improved by changing the separation pH, so that analytes are separated in their non-ionized form. Changes in mobile phase pH also improve column efficiency because the ionization of the analyte and the residual silanols can both be altered. This minimizes secondary interactions between analytes and the silica surface that cause poor peak shape. Achieving optimum resolution can also require changing the mobile phase pH. The following method development strategy explains how this is done with superior column lifetime.

Low, mid, and high pH are the three general regions for chromatographic separations as defined in Figure 1. This figure highlights the benefits of performing separations of ionizable analytes in each pH region. Method development proceeds by investigating chromatographic separations first at low pH and then at higher pH until optimum results are achieved. The ideal column is available for each pH region.

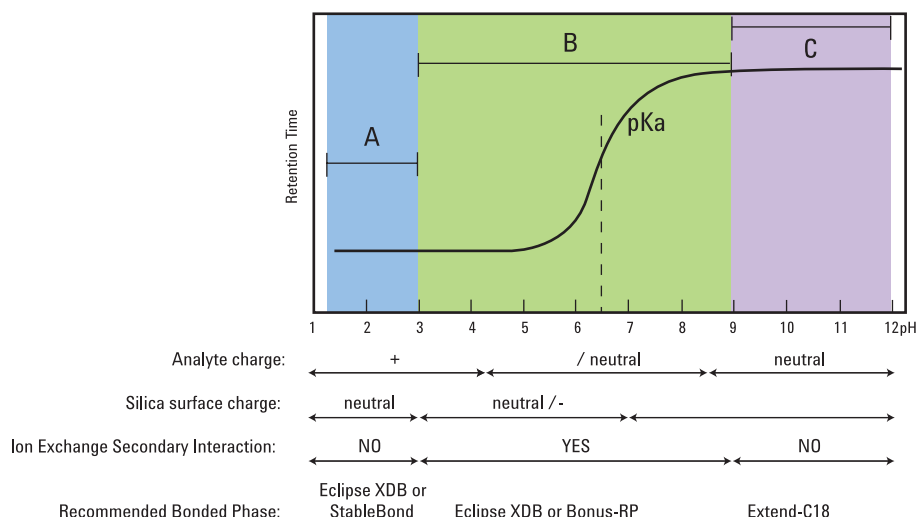


Figure 1: Three pH Regions for HPLC Separations of Basic Compounds

This figure represents the retention behavior of one basic analyte with respect to pKa and pH. Analyte pKa is 6.5

Low pH < 3 – Region A	Mid pH 7 – Region B	High pH > 9 – Region C
<ul style="list-style-type: none"> <li>Start method development at low pH, where silanols on a RP-HPLC column are protonated. This minimizes peak tailing by eliminating silanol/base interactions.</li> <li>At low pH, basic compounds are positively charged and their retention may be reduced.</li> <li>Acidic compounds may be protonated and have increased retention.</li> <li>Retention times are usually stable with small changes in pH, producing a robust method.</li> <li>Volatile mobile phase additives, such as formic acid or trifluoroacetic acid (TFA), are often used at low pH with LC/MS.</li> </ul>	<ul style="list-style-type: none"> <li>Develop methods at pHs at least 1 pH unit above or below the pKa to minimize changes in retention with small changes in pH.</li> <li>Some silica surface SiOH groups become SiO<sup>-</sup> above pH 4 to 5; tailing interactions may be possible.</li> <li>Minimize interactions by selecting a well designed and endcapped column, using additives such as TEA (triethylamine) (less desirable) or using "polar-linked" bonded phases.</li> <li>Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SIL.</li> </ul>	<ul style="list-style-type: none"> <li>In this region, basic compounds may be in their free base form.</li> <li>Increased retention and resolution of basic compounds are likely.</li> <li>Retention changes little in this region, thus robust methods can be developed.</li> <li>Silica breakdown is prevented by innovative bidentate column chemistry, heavy endcapping, use of Rx-SIL, and optimum mobile phase.</li> <li>Ammonium hydroxide is an excellent volatile mobile phase modifier at high pH.</li> </ul>



## Start method development at low pH (pH 2-3)

With so many column choices available, how do you know where to start your method development? The recommended starting point for method development is using a buffered low pH mobile phase — around pH 2-3. Using a low pH mobile phase most often results in the best peak shape for basic compounds on silica-based columns. At low pH, the silanols on the silica are fully protonated so positively charged basic compounds do not interact strongly. The result is good peak shape. Many acidic compounds are non-charged, maximizing their retention at low pH. These observations are key advantages to method development at low pH.

For standard analytical work, start method development with acetonitrile as the mobile phase organic modifier and 20-50 mM phosphate buffer (pH 2-3) as the aqueous component for non LC/MS applications. These conditions provide good pH control, necessary for the most reproducible analyses of ionizable compounds. For LC/MS applications formic acid or TFA are good mobile phase additives for low pH.

## Choose ZORBAX Eclipse Plus first for best peak shape

Select ZORBAX Eclipse Plus C18 or C8 columns first for method development at low pH. Eclipse Plus columns are the newest addition to the Eclipse family and use improved silica and bonding technologies to provide good peak shape for basic compounds. Eclipse Plus columns can be used from pH 2-9 providing method development flexibility. They are stable down to pH 2 making them an ideal choice for initial method development.



## Optimize solvents and bonded phases at low pH

The initial method development steps may lead very quickly to a satisfactory separation. But if more optimization is needed, acetonitrile can be replaced by methanol or tetrahydrofuran and the separation re-optimized. This step may lead to a satisfactory solution, but if still more selectivity optimization is needed, the column bonded phase can be changed.

At low pH there are many bonded phase choices available for optimization. These include the Eclipse Plus phases as well as the Eclipse XDB family with C18, C8, Phenyl and CN. Alternate choices include six different StableBond bonded phases: SB-C18, SB-C8, SB-Phenyl, SB-CN, SB-C3, and SB-Aq.

It may be necessary at low pH to improve the retention of acidic compounds. For these situations, lower the pH even further, down to pH 1-2, and use StableBond columns. These columns provide the greatest stability at very low pH and provide many selectivity options for achieving the highest resolution separations.





## Method development at mid pH (4-9) ZORBAX Eclipse Plus

There are some samples that may not be resolved at low pH or may have better solubility and stability at mid pH. While still using the Eclipse Plus C18 column, the mid pH range can be used for method development. The Eclipse Plus column is stable up to pH 9 so it is equally reliable at mid pH. These double endcapped columns have two key advantages – good peak shape at low and mid pH, as well as sufficient bonded phase density to protect the column from silica degradation from pH 6-9.

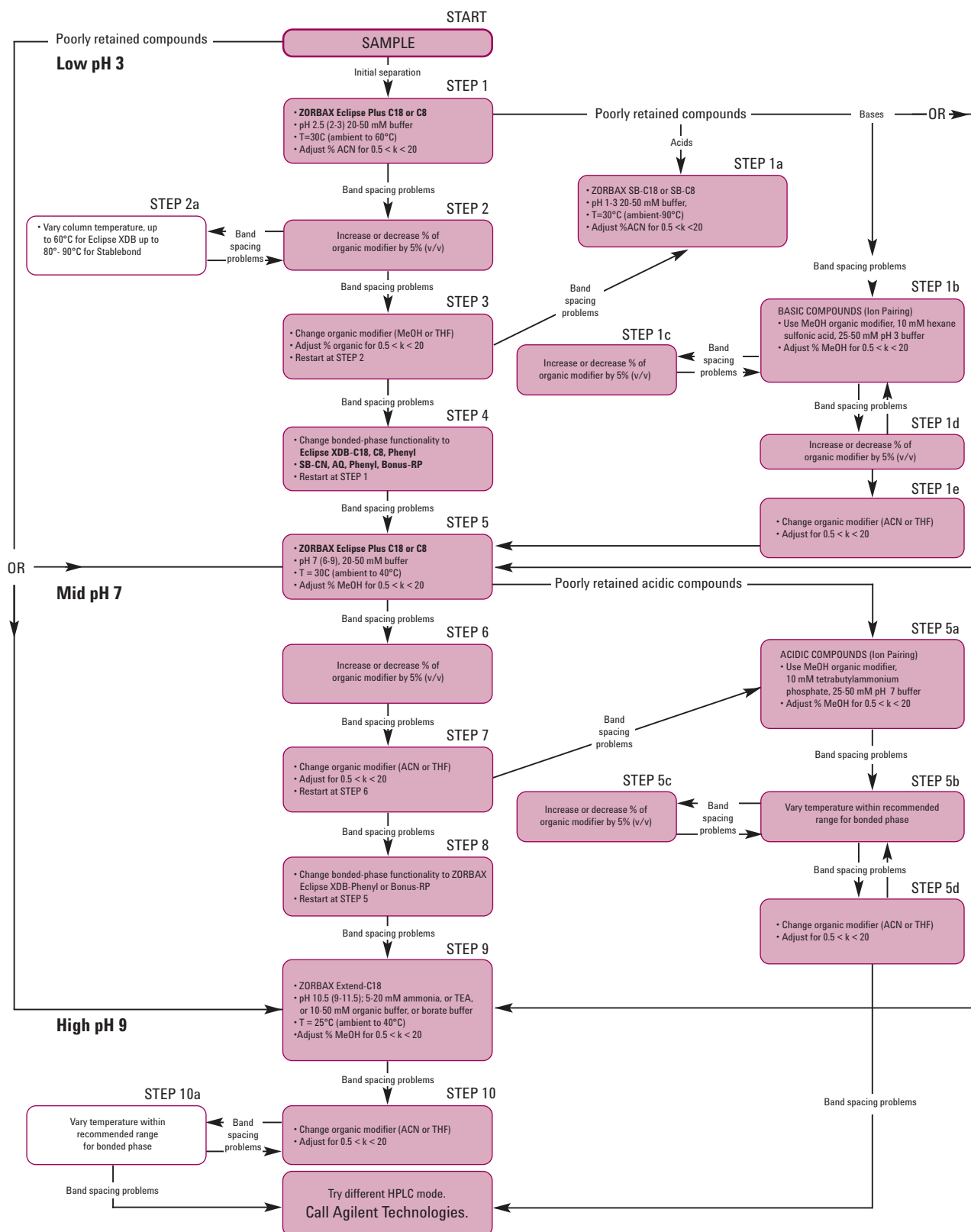
At mid pH, basic compounds (e.g., amines) may still have a positive charge and the silanols on the silica surface may have a negative charge. Therefore covering as many silanols as possible leads to the best peak shape at mid pH. This makes the Eclipse Plus C18 the best starting choice for a column at mid pH. Phosphate buffer is usually the first choice for mobile phase modifier at pH 7 because its buffer range is pH 6.1-8.1. A second choice for mid pH is acetate buffer since it buffers from pH 3.8-5.8 and its volatility makes it a good choice for LC/MS compatibility.

## Alternate selectivities – ZORBAX Eclipse XDB-Phenyl, CN and Bonus-RP

The method development process at mid pH mimics the process at low pH with optimization of the organic modifier and selecting an alternate bonded phase if resolution is not achieved after that step. The alternate bonded phases at mid pH are the Eclipse XDB-Phenyl, Eclipse XDB-CN and Bonus-RP. They provide very different selectivities for many samples and the method development process is followed again. The Bonus-RP column has a polar embedded amide group that provides different selectivity for many samples, provides good peak shape for basic compounds and allows the column to be used with up to 100% aqueous mobile phases.



## Method Development Guidelines from Low to High pH








## Cartridge Column Systems

Agilent offers a variety of popular HPLC packing material in economical, easy-to-use cartridge configurations.

Look for these icons on subsequent pages to help you select the proper guard cartridges and columns.

### Cartridge Selection Guide

Icon	Type of Cartridge	Features	Benefits
	Agilent HPLC Cartridge	Can reverse collets in the end fitting to add guard cartridges	Inexpensive Extends column lifetime Permits rapid column changes Can use 2, 3, 4 and 4.6 mm cartridges
		Cartridges have a unique filter and sieve at each end	Helps prevent blockage
	ZORBAX Guard Cartridge: Stand alone system	High efficiency, stand-alone, low dead volume cartridge	Seals up to 400 bar
		Polymeric cartridge designed for leak-tight seals against metal surfaces	No gaskets required More solvent-resistant than PEEK
		Reusable fittings	Adapt for connections to 1/16 in. LC fittings
	ZORBAX Rapid Resolution and Rapid Resolution HT Cartridge Columns: 3.5 µm and 1.8 µm packings, stand alone system	For high throughput LC/MS, LC/MS/MS and combinatorial separations	
		Packed with Eclipse XDB for pH use from 2-9 Packed with StableBond for low pH use	For all analyte types Low bleed
		Sold individually or as three-packs	
	ZORBAX Semi-Preparative Guard HPLC Hardware Kit: Stand alone system	Easy low-dead-volume assembly	Seals up to 2000 psi (135 bar, 13.5 MPa)
		Tubing (polyphenylene sulfone) designed for leak-tight seals against metal surfaces	No gaskets required
		Reusable fittings	Adapt for connections to 1/16 in. LC fittings
	ZORBAX and Agilent Prep Preparative Cartridge Column and Guard HPLC System: Stand alone and integral hardware options	Easy low-dead-volume assembly	Extends column lifetime
		Reusable fittings	Permits rapid column changes
		Hardware options for integral and external guards	Can use with 21.2 and 30 mm ID columns





## ZORBAX Columns for Analytical HPLC

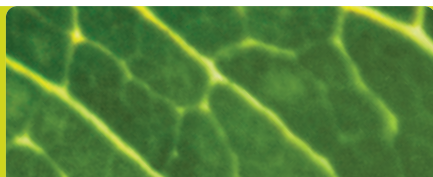
Achieve excellent peak shape and resolution — and eliminate “false starts.”

Good news for analysts who do not have time to “make columns work” for a particular application: ZORBAX columns let you choose the right column based on your sample and mobile phase — eliminating any guesswork.

Additionally, Agilent’s ZORBAX silica is manufactured by Agilent — not purchased from outside suppliers. And that means we control every step of the manufacturing process, ensuring lot-to-lot consistency, superior performance, and long-term, reliable results.

In this section, you will find a diverse range of ZORBAX columns designed for optimum resolution over a wide pH range, including:

- **ZORBAX Eclipse Plus HPLC columns** — designed to reliably produce superior peak shape for basic compounds.
- **ZORBAX Eclipse XDB HPLC columns** — a trusted choice for analytical and regulatory methods.
- **ZORBAX StableBond HPLC columns** — the industry leader for low-pH applications.
- **ZORBAX Rx HPLC columns** — provide excellent stability up to pH 9.
- **ZORBAX Extend-C18 HPLC columns** — feature bidentate bonding that allows you to develop high-resolution separations at high pH levels.
- **ZORBAX Bonus-RP HPLC columns** — an alkyl-amide column that offers excellent peak shape for bases, along with an alternative selectivity.
- **ZORBAX method development kits** — contain three columns for the price of two! Each has a different bonded phase for optimal selectivity.
- **ZORBAX method validation kits** — choose as many columns as you need (or as few) to make method validation easier and less expensive.
- **Original ZORBAX HPLC columns** — are made with Type A silica and are used in many established separation methods.



## No LC column has ever reliably produced such symmetrical peaks for basic compounds – *until now.*

Introducing ZORBAX Eclipse Plus LC columns... the proof is in the performance!

New Eclipse Plus columns provide the ultimate in performance and productivity for silica-based columns.

Like all Eclipse columns, Eclipse Plus columns achieve their superior performance through eXtra Dense Bonding, combined with a precise double-endcapping process. But Eclipse Plus columns also feature enhanced high-performance silica, new endcapping reagents, and a unique bonding process that is optimized for the improved silica.

Eclipse Plus columns are available in 1.8, 3.5, and 5  $\mu\text{m}$  particle sizes for all your analytical, high-resolution, and fast LC analyses. From nano, to analytical, to prep, Eclipse columns allow you to efficiently scale methods from 1.8  $\mu\text{m}$  to 7  $\mu\text{m}$  – and to transfer methods anywhere in the world without worrying about reproducibility. So the methods you create today will not have to be re-validated tomorrow.

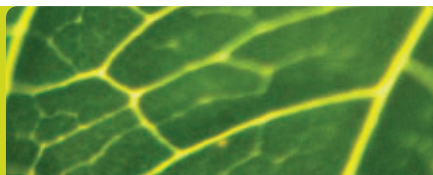


- Achieve excellent peak shape for acids, bases, and neutrals – for greater resolution and accuracy.
- Significantly reduce tailing, so you can confidently resolve difficult analytes.
- Perform ultra-fast, fast, or conventional LC under a range of temperatures, pressures, and pH conditions.
- Spend more time running samples, performing analyses, and meeting deadlines, and less time re-evaluating new column chemistries or revalidating protocols.
- Develop reliable HPLC methods under demanding deadlines.
- Choose Eclipse Plus Rapid Resolution High Throughput columns with a 1.8  $\mu\text{m}$  particle size for ultra-fast separations and superior method flexibility.

**Column Specifications**

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse Plus C18	95Å	160 m <sup>2</sup> /g	60°C	2.0-9.0	Double	8%
ZORBAX Eclipse Plus C8	95Å	160 m <sup>2</sup> /g	60°C	2.0-8.0	Double	8%

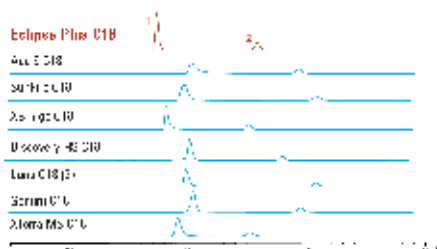




### Eclipse Plus: Best Peak Shape in the Industry Without Tailing

**Column:** Eclipse Plus C18  
959996-902  
4.6 x 100mm, 5µm

Mobile Phase: A: 60% Water  
B: 40% Acetonitrile  
Flow Rate: 1.0 mL/min  
Temperature: Ambient  
Detector: UV 254 nm  
Publication: 5989-4934EN  
Sample: Pyridine, Phenol

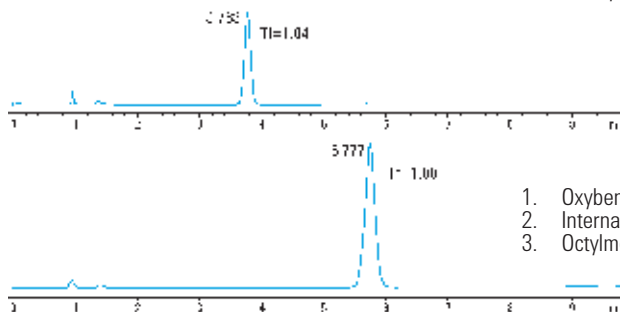


1. Pyridine
2. Phenol

### Eclipse Plus C18 vs. C8 and RRHT vs. RR

**Column A:** Eclipse Plus C18  
959941-902  
4.6 x 50mm, 1.8µm  
**Column B:** Eclipse Plus C8  
959943-906  
4.6 x 50mm, 3.5µm

Mobile Phase: Water: Acetonitrile (30:70)  
Flow Rate: 2.0 mL/min  
Temperature: 30°C  
Detector: UV 230nm  
Sample: Lip balm extract in ACN  
(melted at 100°C ACN, cooled  
and 0.45 µm filtered)



1. Oxybenzone
2. Internal Std.
3. Octylmethoxycinnamate

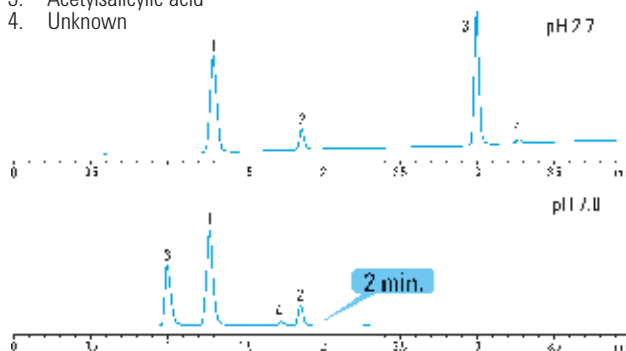
Less retention can save significant time — the C8 is a good choice here.  
The RRHT column is delivering the efficiency and resolution expected,  
but the C8 bonded phase may be the best choice.

## Rapid Analysis of an Analgesic Tablet, Selectivity Differences at pH 2 and pH 7

**Column:** Eclipse Plus C8  
959946-906  
4.6 x 50mm, 5µm

**Gradient:** 10-60% B/3 min  
pH 2.7: A: 0.1% formic acid B:  
0.1% fa in ACN  
pH 7.0: A: 20 mM Na phosphate  
B: ACN  
**Sample:** generic Excedrin tablet

1. Acetaminophen
2. Caffeine
3. Acetylsalicylic acid
4. Unknown

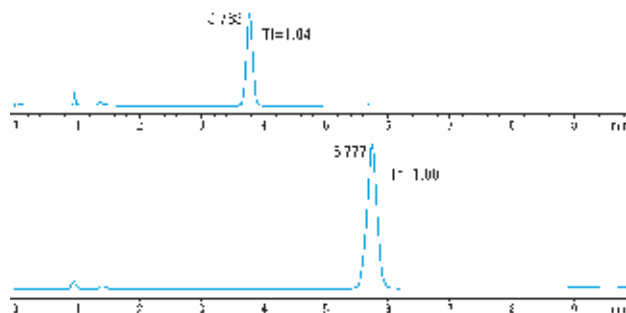


Both Eclipse Plus C18 and C8 can be used over a wide pH range to optimize selectivity or analysis time.

## Eclipse Plus C8 is Less Retentive than Eclipse Plus C18

**Column A:** Eclipse Plus C8  
959996-906  
4.6 x 100mm, 5µm  
**Column B:** Eclipse Plus C18  
959996-902  
4.6 x 100mm, 5µm

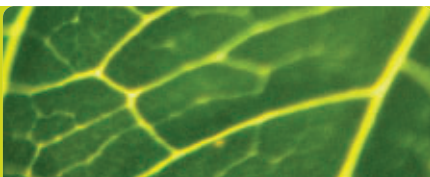
**Mobile Phase:** 80% Methanol 8mM (total)  
**Phase:** K<sub>2</sub>HPO<sub>4</sub> pH 7  
**Flow Rate:** 1.0mL/min  
**Detector:** UV 215nm  
**Sample:** Amitriptyline 0.05 µg/µL (0.5 µL injection)



A C8 column is typically selected because it will retain less than a C18 column, reducing analysis time. The Eclipse Plus C8 column shows the same behavior with excellent peak shape on difficult basic compounds.



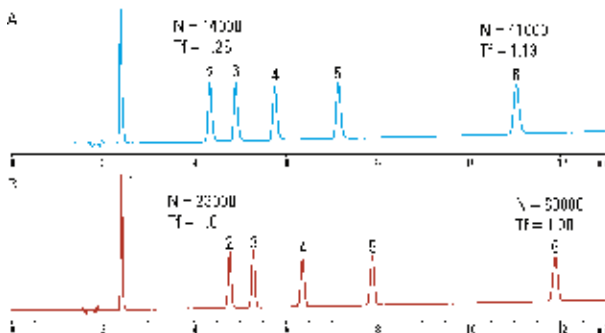




## Peak Shape and Efficiency are Better with ZORBAX Eclipse Plus

**Column A:** XBridge C18, 4.6 x 150mm, 5µm

**Column B:** Eclipse Plus C18  
959993-902  
4.6 x 150mm, 5µm



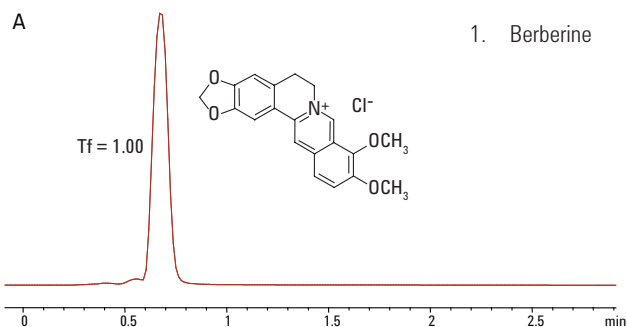
Mobile: A: 0.1% formic acid  
Phase: B: 0.1% formic acid in ACN  
Flow Rate: 1.0 mL/min  
Gradient: 0.0 min 10% B  
15 min 30% B  
Temperature: 40°C  
Detector: UV 254 nm  
Publication: 5989-4934EN  
Sample: Sulfonamides

1. Sulfanilamide
2. Sulfadiazine
3. Sulfathiazole
4. Sulfamerazine
5. Sulfmethazine
6. Sulfamethoxazole

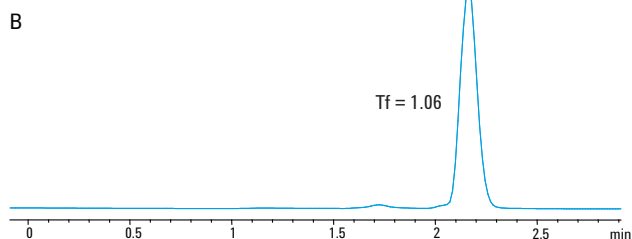
## Fast and Ultra-Fast Analysis of Basic Compounds on Eclipse Plus

**Column A:** Eclipse Plus C18  
959941-902  
4.6 x 50mm, 1.8µm

Mobile: A: 50% 8 mM K2HPO4, pH 7  
Phase: B: 50% ACN  
Flow Rate: 1.0 mL/min  
Temperature: Ambient  
Detector: UV 254 nm  
Publication: 5989-4934EN  
Sample: Berberine, 0.4 mg/mL, 2 µL

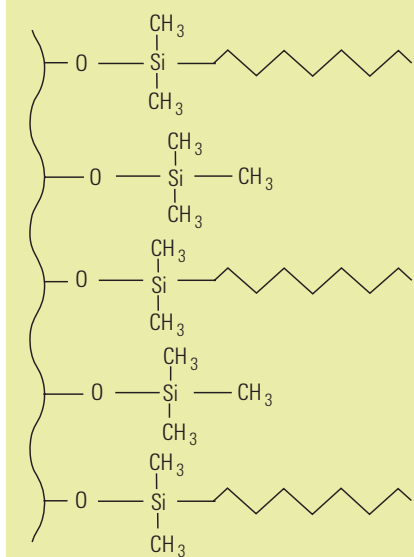
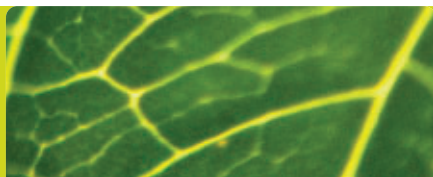


**Column B:** Eclipse Plus C18  
959993-902  
4.6 x 150mm, 5µm



**ZORBAX Eclipse Plus**

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7
	Analytical	4.6 x 250	5	959990-902	959990-906
	Analytical	4.6 x 150	5	959993-902	959993-906
	Analytical	4.6 x 100	5	959996-902	959996-906
	Analytical	4.6 x 50	5	959946-902	959946-906
	Rapid Resolution	4.6 x 150	3.5	959963-902	959963-906
	Rapid Resolution	4.6 x 100	3.5	959961-902	959961-906
	Rapid Resolution	4.6 x 75	3.5	959933-902	959933-906
	Rapid Resolution	4.6 x 50	3.5	959943-902	959943-906
	Rapid Resolution	4.6 x 30	3.5	959936-902	959936-906
	Rapid Resolution HT, 600 bar	4.6 x 150	1.8	959994-902	
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	959964-902	959964-906
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	959941-902	959941-906
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	959931-902	959931-906
	Solvent Saver	3.0 x 150	5	959993-302	959993-306
	Solvent Saver Plus	3.0 x 150	3.5	959963-302	959963-306
	Solvent Saver Plus	3.0 x 100	3.5	959961-302	959961-306
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	959964-302	959964-306
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	959941-302	959941-306
	Narrow Bore	2.1 x 150	5	959701-902	959701-906
	Narrow Bore RR	2.1 x 50	5	959746-902	959746-906
	Narrow Bore RR	2.1 x 150	3.5	959763-902	959763-906
	Narrow Bore RR	2.1 x 100	3.5	959793-902	959793-906
	Narrow Bore RR	2.1 x 50	3.5	959743-902	959743-906
	Narrow Bore RR	2.1 x 30	3.5	959733-902	959733-906
	Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	959764-902	959764-906
	Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	959741-902	959741-906
	Narrow Bore RRHT, 600 bar	2.1 x 30	1.8	959731-902	959731-906
<b>ZGC</b>	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-936	820950-937
<b>ZGC</b>	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-936	821125-937
<b>ZGC</b>	Guard Hardware Kit			820888-901	820888-901



Extra Densely Bonded and Double Endcapped Eclipse XDB Bonded Phase

## ZORBAX Eclipse XDB

- Four selectivity choices for method development optimization
- Good peak shape for basic, acidic and neutral compounds
- High performance over a wide pH range – pH 2-9
- Particle sizes from 1.8  $\mu\text{m}$  to 7  $\mu\text{m}$
- Long lifetime with extra dense bonding and double endcapping

The Agilent ZORBAX Eclipse XDB columns – C18, C8, Phenyl and CN – provide four bonded phase choices for method development optimization. These columns provide good peak shape over a wide pH range (2-9) for additional method development flexibility with one family of columns. Eclipse XDB columns can be used for method development at low pH (2-3) and the same column can be used for method development in the mid pH (6-8) region. In the mid pH region residual silanols are more active and tailing interactions are more likely. To overcome these interactions, Eclipse XDB columns are eXtra Densely Bonded and double endcapped through a proprietary process to cover as many active silanols as possible. The result is superior peak shape of basic compounds from pH 2-9. Eclipse XDB columns are available in 1.8, 3.5, 5 and 7  $\mu\text{m}$  particle sizes for high speed, high resolution, analytical and prep scale separations.



### Tips & Tools

Method development is easier and more predictable when you have several bonded phases available on the same silica. In addition to the popular C18 columns, Agilent also offers selectivities that simplify method development.

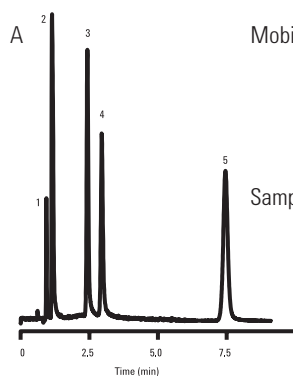
### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse XDB-C18	80Å	180 m <sup>2</sup> /g	60°C	2.0-9.0	Double	10%
ZORBAX Eclipse XDB-C8	80Å	180 m <sup>2</sup> /g	60°C	2.0-9.0	Double	7.6%
ZORBAX Eclipse XDB-Phenyl	80Å	180 m <sup>2</sup> /g	60°C	2.0-9.0	Double	7.2%
ZORBAX Eclipse XDB-CN	80Å	180 m <sup>2</sup> /g	60°C	2.0-8.0	Double	4.3%

### Good Peak Shape Over a Wide pH Range with ZORBAX Eclipse XDB

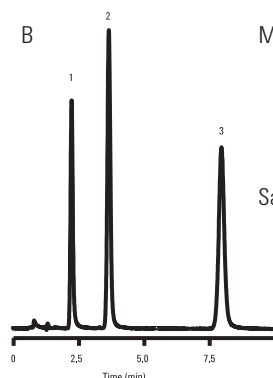
**Column:** Eclipse XDB-C8  
993967-906  
4.6 x 150mm, 5µm

Flow Rate: 1.5 mL/min  
Temperature: 40°C



Mobile Phase: pH 3.0 75% 25 mM  
phosphate buffer  
pH 3.0: 25% ACN

Sample: 1. Maleate  
2. Doxylamine  
3. Chlorpheniramine  
4. Triprolidine  
5. Diphenhydramine



Mobile Phase: pH 7.0 90% 20 mM  
phosphate pH 7.0  
10% ACN

Sample: 1. Procainamide  
2. N-acetylprocainamide  
3. N-propionylprocainamide

ZORBAX Eclipse XDB columns provide good peak shape over a wide pH range and are an excellent choice for method development from pH 2-9.

### Column Stability Testing at pH 3 and 60°C

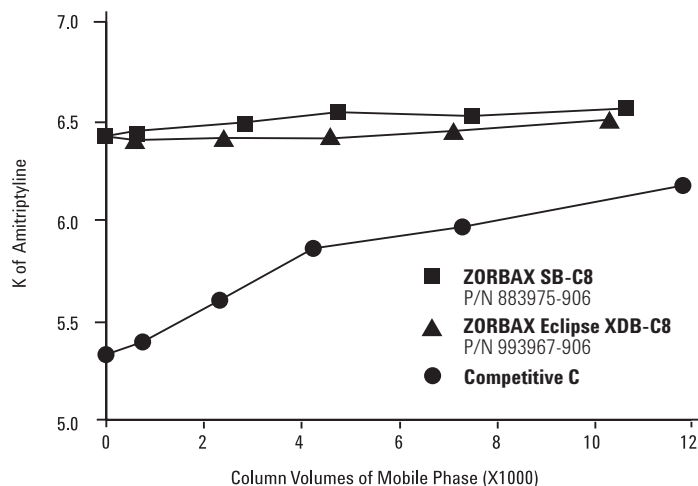
**Column:** ZORBAX SB-C8  
883975-906  
4.6 x 150mm, 5µm  
**Column:** Eclipse XDB-C8  
993967-906  
4.6 x 150mm, 5µm

Mobile Phase: Purge Conditions:  
70% 50 mM NaAc-HCl, pH 3.0  
30% ACN

Retention Test Conditions:  
65% Methanol  
35% Water

Flow Rate: 1.0 mL/min  
Temperature: 60°C

Sample: Tricyclic Antidepressants



Eclipse XDB columns are stable over a wide pH range. At low pH, Eclipse, an end capped column is extremely stable and shows equivalent stability to a non-endcapped column, SB-C8, at pH 3. The columns were purged with a pH 3 mobile phase at 60°C. Then they were tested with a strongly basic compound to determine if the endcapping or bonded phase had been hydrolyzed from the silica surface. The Eclipse XDB column was very stable, as shown by the consistency of the retention of amitriptyline over the 12,000 column volumes of the test. Another endcapped column shows less stability under these same conditions.

## Column Stability Testing at pH 7.0

**Column:** Eclipse XDB-C8

993967-906

4.6 x 150mm, 5µm

Mobile Phase: 60% ACN

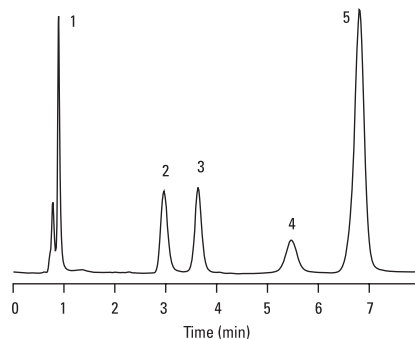
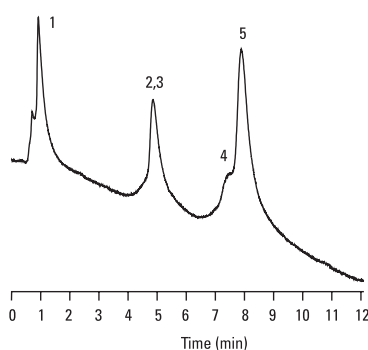
40% 250 mM Phosphate Buffer, pH 7.0

Flow Rate: 1.5 mL/min

Temperature: 60°C

Sample: Tricyclic Antidepressants

1. Uracil
2. Nortriptyline
3. Doxepin
4. Amitriptyline
5. Trimipramine



Double endcapping, dense bonding and the durable Rx-Sil particles (sol-type) combine to provide long lifetime at pH 7 when compared to single endcapped sil-gel columns used here. The conditions used for this test – high temperature (60°C) and high salt concentration (250 mM), accelerate the dissolution of silica, causing premature failure of the sil-gel type column.

## Selectivity Changes for Basic Compounds with Eclipse XDB and Stable Bond

**Column A:** Eclipse XDB-C8

966967-906

4.6 x 75mm, 3.5µm

**Column B:** ZORBAX Rx/SB-C8

866953-906

4.6 x 75mm, 3.5µm

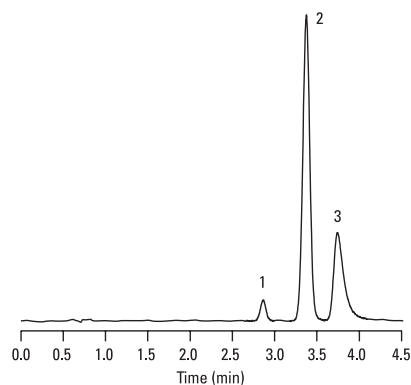
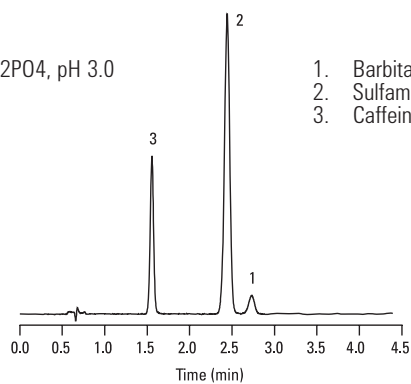
Mobile Phase: 70% 25 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 3.0

30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35°C

1. Barbitol
2. Sulfamethoxazole
3. Caffeine



Eclipse XDB and StableBond columns are based on the same silica but have different bonding and endcapping. Therefore, they can have very different selectivity for the same sample under the same conditions, as this example shows.

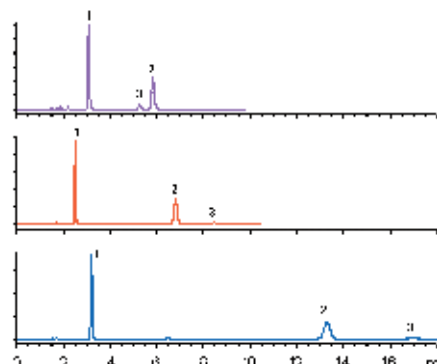


## Optimize Separations with Eclipse XDB Selectivity Options – Analysis of Sunscreens

**Column A:** Eclipse XDB-Phenyl  
963967-912  
4.6 x 150mm, 3.5µm

**Column B:** Eclipse XDB-C8  
963967-906  
4.6 x 150mm, 3.5µm

**Column C:** Eclipse XDB-C18  
963967-902  
4.6 x 150mm, 3.5µm



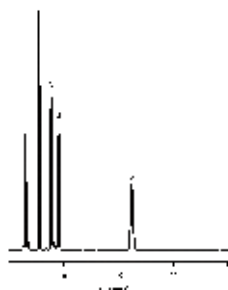
Mobile Phase: 15% H<sub>2</sub>O: 85% MeOH  
Flow Rate: 1.0 mL/min  
Temperature: 35°C  
Sample: Sunscreens

1. Oxybensone
2. Padimate-O
3. Ethylhexylsalicylate

This separation of sunscreens on all three Eclipse XDB bonded phases – C18, C8 and Phenyl – shows that different bonded phases can be used to optimize a separation. While all three bonded phases provide an adequate separation, the Eclipse XDB-Phenyl provides a different peak elution order and a much shorter overall analysis time. All three bonded phases also provide excellent peak shape with no mobile phase additives.

## Separation of Cephalosporins on Eclipse XDB-C8

**Column:** Eclipse XDB-C8  
993967-906  
4.6 x 150mm, 5µm



Mobile Phase: 85% 25 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7: 15% ACN  
Flow Rate: 1.0 mL/min  
Temperature: 35°C  
Sample: Cephalosporins

1. Ceftazidime
2. Cefachlor
3. Cefoxatime
4. Cefoxitin
5. Cephalothin

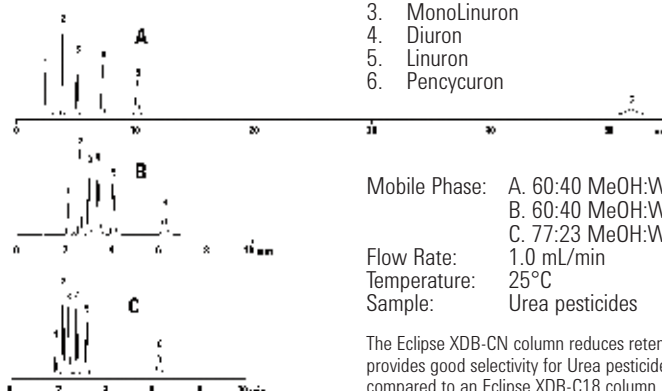
Cephalosporins are a type of antibiotic and many compounds in this family are well separated on the Eclipse XDB-C8 column.

## Selectivity for Urea Pesticides

**Column A:** Eclipse XDB-C18  
993967-902  
4.6 x 150mm, 5µm

**Column B:** Eclipse XDB-CN  
993967-905  
4.6 x 150mm, 5µm

**Column C:** Eclipse XDB-C18  
993967-902  
4.6 x 150mm, 5µm






1. Fenuron
2. Monuron
3. MonoLinuron
4. Diuron
5. Linuron
6. Pencycuron

Mobile Phase: A. 60:40 MeOH:Water  
B. 60:40 MeOH:Water  
C. 77:23 MeOH:Water  
Flow Rate: 1.0 mL/min  
Temperature: 25°C  
Sample: Urea pesticides

The Eclipse XDB-CN column reduces retention time and provides good selectivity for Urea pesticides when compared to an Eclipse XDB-C18 column.









## ZORBAX Eclipse XDB





Hardware	Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11	XDB-CN USP L10
<b>Standard Columns (no special hardware required, 400 bar)</b>							
	Semi-Preparative	9.4 x 250	5	990967-202	990967-206		
	Analytical	4.6 x 250	5	990967-902	990967-906	990967-912	990967-905
	Analytical	4.6 x 150	5	993967-902	993967-906	993967-912	993967-905
	Analytical	4.6 x 50	5	946975-902	946975-906		
	Rapid Resolution	4.6 x 150	3.5	963967-902	963967-906	963967-912	963967-905
	Rapid Resolution	4.6 x 100	3.5	961967-902	961967-906		961967-905
	Rapid Resolution	4.6 x 75	3.5	966967-902	966967-906	966967-912	966967-905
	Rapid Resolution	4.6 x 50	3.5	935967-902	935967-906	935967-912	
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	928975-902	928975-906		
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	927975-902	927975-906		
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	924975-902	924975-906		
	Rapid Resolution HT, 600 bar	4.6 x 20	1.8	926975-902	926975-906		
	Solvent Saver	3.0 x 250	5	990967-302	990967-306	990967-312	990967-305
	Solvent Saver	3.0 x 150	5	993967-302	993967-306	993967-312	993967-305
	Solvent Saver Plus	3.0 x 150	3.5	963954-302	963954-306	963954-312	963954-305
	Solvent Saver Plus	3.0 x 100	3.5	961967-302	961967-306	961967-312	
	Solvent Saver Plus	3.0 x 75	3.5	966954-302			
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	928975-302	928975-306		
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	927975-302	927975-306		
	Solvent Saver HT, 600 bar	3.0 x 30	1.8	924975-302	924975-306		
	Solvent Saver HT, 600 bar	3.0 x 20	1.8	926975-302	926975-306		
	Narrow Bore	2.1 x 150	5	993700-902	993700-906	993700-912	993700-905
	Narrow Bore	2.1 x 50	5	960967-902	960967-906	960967-912	960967-905
	Narrow Bore RR*	2.1 x 150	3.5	930990-902	930990-906		
	Narrow Bore RR*	2.1 x 100	3.5	961753-902	961753-906		961753-905
	Narrow Bore RR*	2.1 x 75	3.5	966735-902			
	Narrow Bore RR*	2.1 x 50	3.5	971700-902	971700-906		
	Narrow Bore RRHT, 600 bar**	2.1 x 100	1.8	928700-902	928700-906		
	Narrow Bore RRHT, 600 bar**	2.1 x 50	1.8	927700-902	927700-906		
	Narrow Bore RRHT, 600 bar**	2.1 x 30	1.8	924700-902	924700-906		
	Narrow Bore RRHT, 600 bar**	2.1 x 20	1.8	926700-902	926700-906		
	MicroBore RR*	1.0 x 150	3.5	963600-902	963600-906		
	MicroBore RR*	1.0 x 50	3.5	965600-902	965600-906		
	MicroBore RR*	1.0 x 30	3.5	961600-902	961600-906		
	MicroBore Guard Cartridges, 3/pk	1.0 x 17	5	5185-5921	5185-5921		
	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-925	820950-926	820950-927	820950-935
	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-926	821125-926	821125-926	821125-935
	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901

\*RR: Rapid Resolution 3.5 µm

\*\*RRHT: Rapid Resolution HT 1.8 µm

**ZORBAX Eclipse XDB (Continued)**

Hardware	Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11	XDB-CN USP L10
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>							
	PrepHT Cartridge	21.2 x 250	7	977250-102	977250-106		
	PrepHT Cartridge	21.2 x 150	7	977150-102	977150-106		
	PrepHT Cartridge	21.2 x 150	5	970150-902	970150-906		
	PrepHT Cartridge	21.2 x 100	5	970100-902	970100-906		
	PrepHT Cartridge	21.2 x 50	5	970050-902	970050-906		
	PrepHT Guard Cartridge	17 x 7.5	5	820212-925	820212-926		
	Guard Cartridge Hardware			820444-901	820444-901		
	PrepHT Endfittings, 2/pk			820400-901	820400-901		

Hardware	Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7
<b>Agilent Cartridge Columns (require hardware kit 5021-1845)</b>					
	Analytical	4.6 x 250	5	7995118-585	7995108-585
	Analytical	4.6 x 150	5	7995118-595	7995108-595
	Rapid Resolution	4.6 x 75	3.5	7995118-344	7995108-344
	Solvent Saver Plus	3.0 x 75	3.5	7995230-344	
	Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504
	Cartridge Holder			5021-1845	5021-1845
<b>Standard Columns (no special hardware required, 400 bar)</b>					
	Rapid Resolution HT	4.6 x 50	1.8	922975-902	922975-906
	Rapid Resolution HT, 3/pk	4.6 x 50	1.8	922975-932	
	Narrow Bore RRHT	2.1 x 50	1.8	922700-902	
	Narrow Bore RRHT, 3/pk	2.1 x 50	1.8	922700-932	



## ZORBAX Eclipse XDB (Continued)

Hardware	Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7
<b>Rapid Resolution HT Cartridges (require hardware kit 820555-901)</b>					
RR	Rapid Resolution Cartridge	4.6 x 30	3.5	933975-902	933975-906
RR	Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	933975-932	933975-936
RR	Rapid Resolution Cartridge	4.6 x 15	3.5	931975-902	931975-906
RR	Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	931975-932	931975-936
RR	Rapid Resolution Cartridge	2.1 x 30	3.5	973700-902	973700-906
RR	Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	973700-932	973700-936
RR	Rapid Resolution Cartridge	2.1 x 15	3.5	975700-902	975700-906
RR	Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	975700-932	975700-936
RR	Rapid Resolution HT Cartridge	4.6 x 50	1.8	925975-902	
RR	Rapid Resolution HT Cartridge, 3/pk	4.6 x 50	1.8	925975-932	
RR	Rapid Resolution HT Cartridge	4.6 x 30	1.8	923975-902	
RR	Rapid Resolution HT Cartridge, 3/pk	4.6 x 30	1.8	923975-932	
RR	Rapid Resolution HT Cartridge	4.6 x 15	1.8	921975-902	
RR	Rapid Resolution HT Cartridge, 3/pk	4.6 x 15	1.8	921975-932	
RR	Rapid Resolution HT Cartridge	2.1 x 50	1.8	925700-902	
RR	Rapid Resolution HT Cartridge, 3/pk	2.1 x 50	1.8	925700-932	
RR	Rapid Resolution HT Cartridge	2.1 x 30	1.8	923700-902	
RR	Rapid Resolution HT Cartridge, 3/pk	2.1 x 30	1.8	923700-932	
RR	Rapid Resolution HT Cartridge	2.1 x 15	1.8	921700-902	
RR	Rapid Resolution HT Cartridge, 3/pk	2.1 x 15	1.8	921700-932	
RR	Hardware Kit for RR and RRHT Cartridges			820555-901	820555-901
<b>Capillary Glass-lined Columns</b>					
	Capillary	0.5 x 250	5	5064-8286	
	Capillary	0.5 x 150	5	5064-8287	
	Capillary RR	0.5 x 150	3.5	5064-8288	
	Capillary RR	0.5 x 35	3.5	5064-8298	
	Capillary	0.3 x 250	5	5064-8269	
	Capillary	0.3 x 150	5	5064-8291	
	Capillary RR	0.3 x 150	3.5	5064-8271	
	Capillary	0.5 x 35	5	5064-8296	
	Capillary	0.3 x 35	5	5064-8297	

## ZORBAX 80Å StableBond

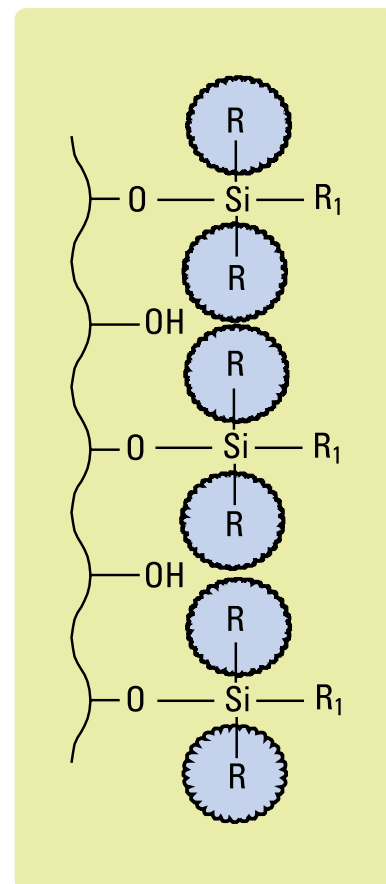
- Longest column lifetime and best reproducibility for low pH separations – down to pH 1
- Patented stable column chemistry allows use at high temperature and low pH without degradation
- Six different bonded phases provide broad selectivity – SB-C18, SB-C8, SB-CN, SB-Phenyl, SB-C3, SB-Aq
- High purity (Type B) silica for good peak shape

Agilent ZORBAX StableBond columns use patented, unique, nonfunctional silanes with bulky diisobutyl (SB-C18) or diisopropyl (SB-C8, SB-C3, SB-Phenyl, SB-CN, and SB-Aq) side chain groups that sterically protect the key siloxane bond to the silica surface from hydrolytic attack at low pH. StableBond packing materials are not endcapped in order to provide exceptional stability and to maximize lifetime and reproducibility under acidic mobile phase conditions. The high purity, low acidity silica provides excellent peak shape with acidic, basic and neutral compounds so that StableBond columns are an excellent choice for low pH method development. ZORBAX StableBond columns are compatible with all common mobile phases, including very high aqueous mobile phases.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range*	Endcapped	Carbon Load
ZORBAX SB-C18	80Å	180 m <sup>2</sup> /g	90°C	1.0-8.0	No	10%
ZORBAX SB-C8	80Å	180 m <sup>2</sup> /g	80°C	1.0-8.0	No	5.5%
ZORBAX SB-C3	80Å	180 m <sup>2</sup> /g	80°C	1.0-8.0	No	4%
ZORBAX SB-Phenyl	80Å	180 m <sup>2</sup> /g	80°C	1.0-8.0	No	5.5%
ZORBAX SB-CN	80Å	180 m <sup>2</sup> /g	80°C	1.0-8.0	No	4%
ZORBAX SB-Aq	80Å	180 m <sup>2</sup> /g	80°C	1.0-8.0	No	proprietary

\*StableBond columns are designed for optimal use at low pH. At pH 6-8, highest column stability for all silica-based columns is obtained by operating at temperatures <40°C and using lower buffer concentrations in the range of 0.01-0.02M. At mid-range pH, Eclipse XDB and Bonus-RP are recommended.



Sterically Protected StableBond Bonded Phase





### StableBond SB-C18 Shows Excellent Stability at Low pH and High Temperature (pH 0.8, 90°C)

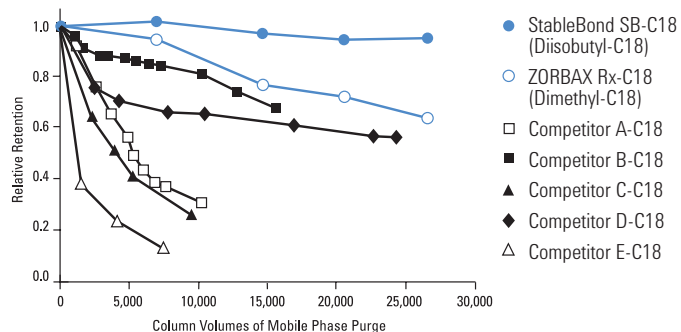
**Column:** ZORBAX SB-C18  
883975-902  
4.6 x 150mm, 5µm

**Column:** ZORBAX Rx-C18  
883967-902  
4.6 x 150mm, 5µm

Mobile Phase: 50% Methanol/50% Water  
with 1.0% TFA

Test Solute: Toluene

Temperature: 90°C



As an indicator of column breakdown, retention time of toluene was measured after purging the column with mobile phase. Only the StableBond SB-C18 is unchanged after three working months of use under these very low pH (0.8) and high temperature (90°C) conditions. ZORBAX Rx-C18 also provides a stable matrix, and can be used as an alternative selectivity to StableBond SB-C18.

### Shorter Chain ZORBAX SB-CN is also Stable at Low pH (pH 2.0, 50°C)

**Column:** ZORBAX SB-CN  
883975-905  
4.6 x 150mm, 5µm

Mobile Phase:

Flow Rate:

Gradient:

Temperature:

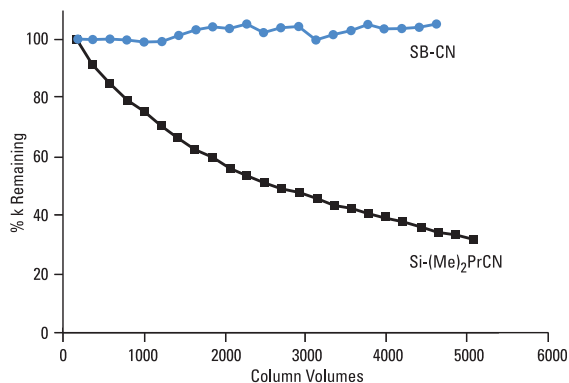
Detector:

MS Conditions:

Publication:

Sample:

ZORBAX StableBond SB-CN and the other short chain StableBond bonded phases are also exceptionally stable at low pH. Conventional dimethyl CN and similar bonded phases lack this stability.



### SB-CN Optimizes Retention and Resolution

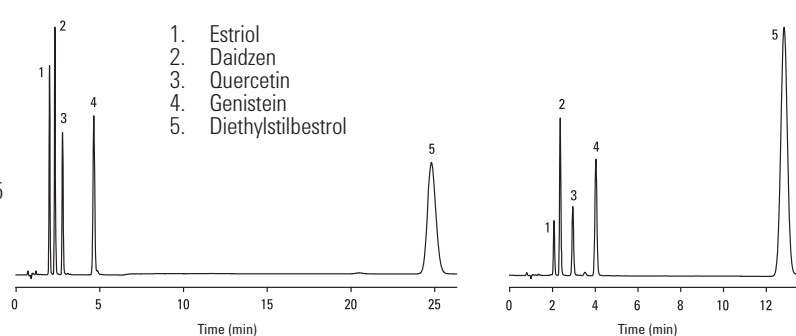
**Column A:** ZORBAX SB-C18  
866953-902  
4.6 x 75mm, 3.5µm

**Column B:** ZORBAX SB-CN  
866953-905  
4.6 x 75mm, 3.5µm

Mobile Phase: 30% ACN  
70% 25mM NaH<sub>2</sub>PO<sub>4</sub>, pH 2.5

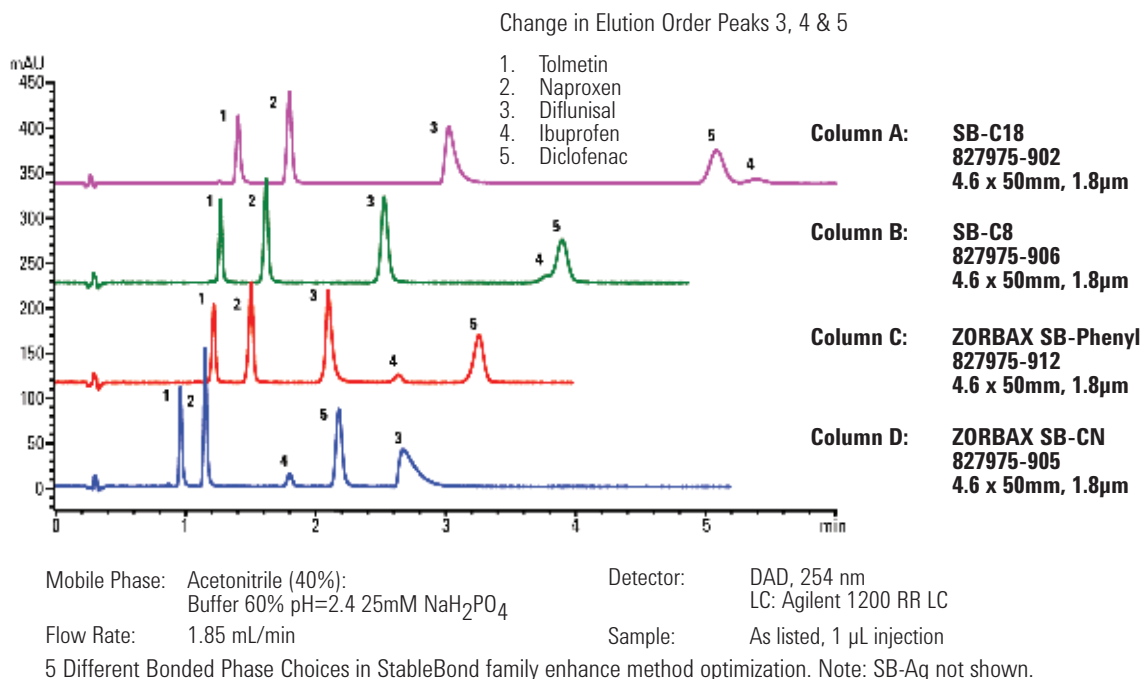
Flow Rate: 1.0 mL/min

Temperature: 35°C

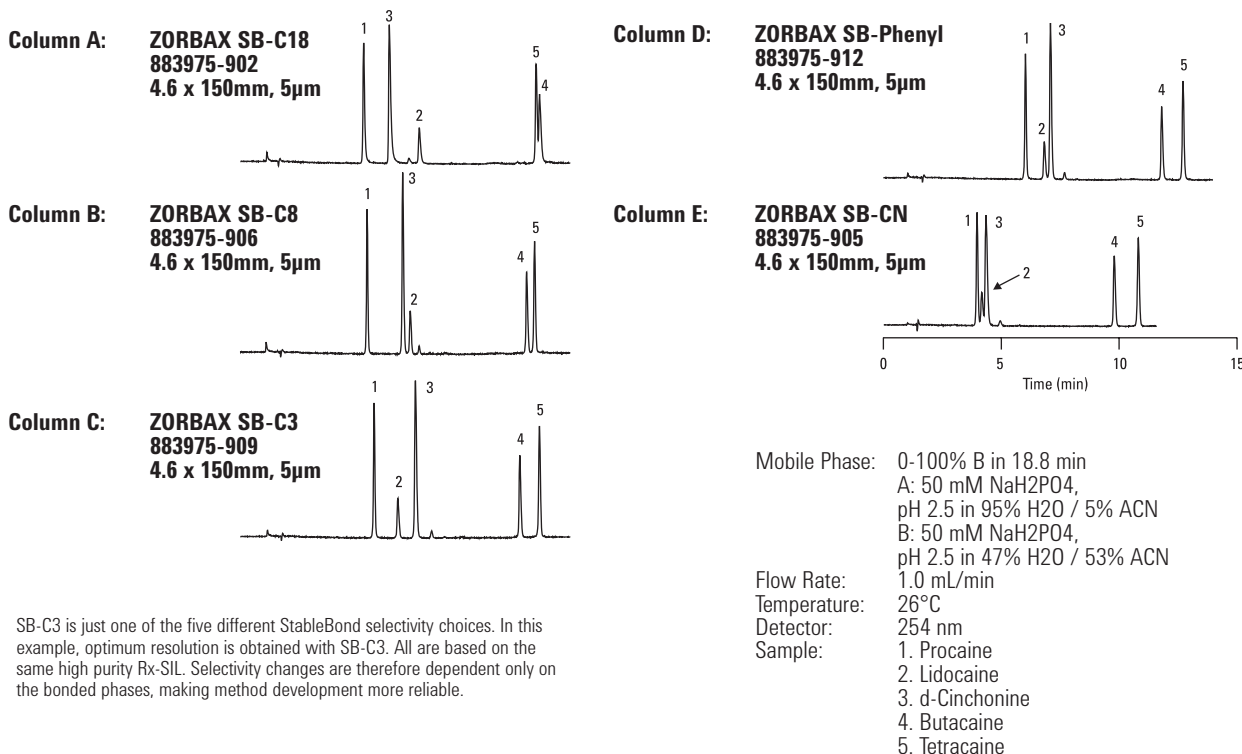


The SB-CN column is used here to reduce analysis time by 50%. The retention of the most hydrophobic analyte is cut in half. At the same time retention of the more polar, early eluting peaks increases slightly.

### More RRHT Bonded Phase Choices Allow for Rapid Method Development Optimization



### Five Different Bonded Phases Provide Selectivity Options








## ZORBAX 80Å StableBond

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-C3 USP L56	SB-Phenyl USP L11	SB-Aq
<b>Standard Columns (no special hardware required, 400 bar)</b>									
	Semi-Preparative	9.4 x 250	5	880975-202	880967-201	880975-205	880975-209	880975-212	
	Semi-Preparative	9.4 x 150	5	883975-202					
	Semi-Preparative	9.4 x 100	5	884975-202					
	Semi-Preparative	9.4 x 50	5	846975-202					
	Analytical	4.6 x 250	5	880975-902	880975-906	880975-905	880975-909	880975-912	880975-914
	Analytical	4.6 x 150	5	883975-902	883975-906	883975-905	883975-909	883975-912	883975-914
	Analytical	4.6 x 50	5	846975-902	846975-906				846975-914
	Rapid Resolution	4.6 x 250	3.5	884950-567					
	Rapid Resolution	4.6 x 150	3.5	863953-902	863953-906	863953-905		863953-912	863953-914
	Rapid Resolution	4.6 x 100	3.5	861953-902	861953-906	861953-905		861953-912	861953-914
	Rapid Resolution	4.6 x 75	3.5	866953-902	866953-906	866953-905		866953-912	866953-914
	Rapid Resolution	4.6 x 50	3.5	835975-902	835975-906	835975-905		835975-912	835975-914
	Rapid Resolution HT, 600 bar	4.6 x 150	1.8	829975-902	829975-906	829975-905		829975-912	
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	828975-902	828975-906	828975-905		828975-912	828975-914
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	827975-902	827975-906	827975-905		827975-912	827975-914
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	824975-902	824975-906	824975-905		824975-912	824975-914
	Rapid Resolution HT, 600 bar	4.6 x 20	1.8	826975-902	826975-906				
	Solvent Saver	3.0 x 250	5	880975-302	880975-306	880975-305	880975-309	880975-312	880975-314
	Solvent Saver	3.0 x 150	5	883975-302	883975-306	883975-305	883975-309	883975-312	883975-314
	Solvent Saver Plus	3.0 x 150	3.5	863954-302	863954-306	863954-305		863954-312	863954-314
	Solvent Saver Plus	3.0 x 100	3.5	861954-302	861954-306	861954-305	861954-309	861954-312	861954-314
	Solvent Saver HT, 600 bar	3.0 x 150	1.8	829975-302	829975-306	829975-305		829975-312	
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	828975-302	828975-306	828975-305		828975-312	828975-314
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	827975-302	827975-306	827975-305		827975-312	827975-314
	Solvent Saver HT, 600 bar	3.0 x 30	1.8	824975-302	824975-306	824975-305			
	Solvent Saver HT, 600 bar	3.0 x 20	1.8	826975-302	826975-306				
	Narrow Bore	2.1 x 150	5	883700-922	883700-906	883700-905	883700-909	883700-912	
	Narrow Bore	2.1 x 50	5	860975-902	860975-906	860975-905	860975-909	860975-912	860975-914
	Narrow Bore RR*	2.1 x 150	3.5	830990-902	830990-906				830990-914
	Narrow Bore RR*	2.1 x 100	3.5	861753-902	861753-906	861753-905		861753-912	861753-914
	Narrow Bore RR*	2.1 x 75	3.5	866735-902					
	Narrow Bore RR*	2.1 x 50	3.5	871700-902	871700-906				871700-914







\*RR: Rapid Resolution 3.5 µm

\*\*RRHT: Rapid Resolution HT 1.8 µm

**ZORBAX 80Å StableBond (Continued)**






Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-C3 USP L56	SB-Phenyl USP L11	SB-Aq
	Narrow Bore RRHT, 2.1 x 150 600 bar**		1.8	820700-902	820700-906	820700-905		820700-912	
	Narrow Bore RRHT, 2.1 x 100 600 bar**		1.8	828700-902	828700-906	828700-905		828700-912	828700-914
	Narrow Bore RRHT, 2.1 x 50 600 bar**		1.8	827700-902	827700-906	827700-905		827700-912	827700-914
	Narrow Bore RRHT, 2.1 x 30 600 bar**		1.8	824700-902	824700-906	824700-905		824700-914	824700-914
	Narrow Bore RRHT, 2.1 x 20 600 bar**		1.8	826700-902	826700-906				
	MicroBore RR*	1.0 x 150	3.5	863600-902	863600-906	863600-905			
	MicroBore RR*	1.0 x 50	3.5	865600-902	865600-906				
	MicroBore RR*	1.0 x 30	3.5	861600-902	861600-906				
	MicroBore Guard Cartridges, 3/pk	1.0 x 17	5	5185-5920	5185-5920				
	Guard Cartridge, 2/pk	9.4 x 15	7	820675-115	820675-115	820675-124		820675-115	
	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-920	820950-915	820950-916	820950-922	820950-917	820950-933
	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-915	821125-915	821125-924	821125-924	821125-915	821125-933
	Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901	840140-901	
	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901	820888-901	820888-901

**PrepHT Cartridge Columns (require endfittings kit 820400-901)**






















	PrepHT Cartridge	21.2 x 250	7	877250-102	877250-106	877250-105		877250-112	877250-114
	PrepHT Cartridge	21.2 x 150	7	877150-102	877150-106				877150-114
	PrepHT Cartridge	21.2 x 150	5	870150-902	870150-906				870150-914
	PrepHT Cartridge	21.2 x 100	5	870100-902	870100-906				870100-914
	PrepHT Cartridge	21.2 x 50	5	870050-902	870050-906				870050-914
	PrepHT Guard Cartridge, 2/pk	17 x 7.5	5	820212-920	820212-915	820212-915		820212-915	820212-933
	Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901	820444-901	820444-901
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901	820400-901	820400-901

\*RR: Rapid Resolution 3.5 µm

\*\*RRHT: Rapid Resolution HT 1.8 µm

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7
	Analytical	4.6 x 250	5	7995218-585	7995208-585
	Analytical	4.6 x 150	5	7995218-595	7995208-595
	Rapid Resolution	4.6 x 75	3.5	7995218-344	7995208-344
	Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504
	Cartridge Holder			5021-1845	5021-1845

## ZORBAX 80Å StableBond (Continued)

HardwareDescription	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7
<b>Standard Columns (no special hardware required, 400 bar)</b>				
Rapid Resolution HT	4.6 x 50	1.8	822975-902	822975-906
Rapid Resolution HT, 3/pk	4.6 x 50	1.8	822975-932	
Narrow Bore RRHT	2.1 x 50	1.8	822700-902	
Narrow Bore RRHT, 3/pk	2.1 x 50	1.8	822700-932	
<b>Rapid Resolution HT Cartridges (require hardware kit 820555-901)</b>				
 Rapid Resolution Cartridge	4.6 x 30	3.5	833975-902	833975-906
 Rapid Resolution Cartridge, 3/pk	4.6 x 30	3.5	833975-932	833975-936
 Rapid Resolution Cartridge	4.6 x 15	3.5	831975-902	831975-906
 Rapid Resolution Cartridge, 3/pk	4.6 x 15	3.5	831975-932	831975-936
 Rapid Resolution Cartridge	2.1 x 30	3.5	873700-902	873700-906
 Rapid Resolution Cartridge, 3/pk	2.1 x 30	3.5	873700-932	873700-936
 Rapid Resolution Cartridge	2.1 x 15	3.5	875700-902	875700-906
 Rapid Resolution Cartridge, 3/pk	2.1 x 15	3.5	875700-932	875700-936
 Rapid Resolution HT Cartridge	4.6 x 50	1.8	825975-902	
 Rapid Resolution HT Cartridge, 3/pk	4.6 x 50	1.8	825975-932	
 Rapid Resolution HT Cartridge	4.6 x 30	1.8	823975-902	
 Rapid Resolution HT Cartridge, 3/pk	4.6 x 30	1.8	823975-932	
 Rapid Resolution HT Cartridge	4.6 x 15	1.8	821975-902	
 Rapid Resolution HT Cartridge, 3/pk	4.6 x 15	1.8	821975-932	
 Rapid Resolution HT Cartridge	2.1 x 50	1.8	825700-902	
 Rapid Resolution HT Cartridge, 3/pk	2.1 x 50	1.8	825700-932	
 Rapid Resolution HT Cartridge	2.1 x 30	1.8	823700-902	
 Rapid Resolution HT Cartridge, 3/pk	2.1 x 30	1.8	823700-932	
 Rapid Resolution HT Cartridge	2.1 x 15	1.8	821700-902	
 Rapid Resolution HT Cartridge, 3/pk	2.1 x 15	1.8	821700-932	
 Hardware Kit for RR and RRHT Cartridges			820555-901	820555-901
<b>Capillary Glass-lined Columns</b>				
Capillary	0.5 x 250	5	5064-8258	
Capillary	0.5 x 150	5	5064-8256	
Capillary	0.5 x 35	5	5064-8254	
Capillary RR*	0.5 x 150	3.5	5064-8262	
Capillary RR	0.5 x 35	3.5	5064-8260	
Capillary	0.3 x 250	5	5064-8257	
Capillary	0.3 x 150	5	5064-8255	
Capillary	0.3 x 35	5	5064-8253	
Capillary RR	0.3 x 150	3.5	5064-8261	

\*RR: Rapid Resolution 3.5 µm

\*\*RRHT: Rapid Resolution HT 1.8 µm



## ZORBAX Rx

- Rx-C18 is recommended for alternate selectivity at low pH relative to Eclipse XDB-C18 and StableBond SB-C18; for higher temperature applications, StableBond is recommended. This column has a higher carbon load than SB-C18 columns (12% vs. 10%)
- Rx-C18 offers high stability and good peak shape for low pH applications
- Rx-C18 is manufactured using dimethyloctadecylsilane, is non-encapped and provides excellent stability up to pH 9.
- Rx-C8 is the same product as SB-C8.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Rx-C18	80Å	180 m <sup>2</sup> /g	60°C	2.0-9.0	No	12%
ZORBAX Rx-C8	80Å	180 m <sup>2</sup> /g	80°C	1.0-8.0	No	5.5%

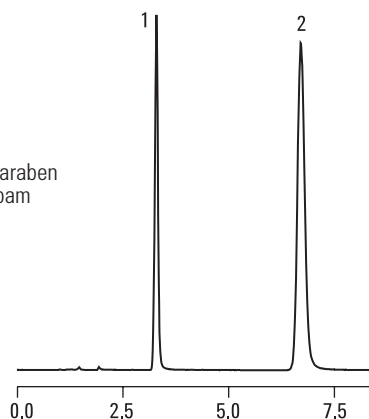
### Analysis of Diazepam on Rx-C18

**Column:** ZORBAX Rx-C18  
880967-302  
3.0 x 250mm, 5µm














Mobile Phase: 35% H<sub>2</sub>O: 65% MeOH  
Flow Rate: 0.5 mL/min

1. Ethylparaben
2. Diazepam

An Rx-C18 column is used for this USP analysis of diazepam and the internal standard ethylparaben. The Solvent Saver 3.0 mm i.d. Rx-C18 column reduces solvent usage by 60% over what would be used if the analysis were done on a 4.6 x 250 mm column.



## ZORBAX Rx

Hardware	Description	Size (mm)	Particle Size (µm)	Rx-C18 USP L1	Rx-C8 USP L7**
	Semi-Preparative	9.4 x 250	5	880967-202	880967-201
	Analytical	4.6 x 250	5	880967-902	880967-901
	Analytical	4.6 x 150	5	883967-902	883967-901
	Rapid Resolution	4.6 x 150	3.5	863967-902	863953-906
	Rapid Resolution	4.6 x 100	3.5	861967-902	861953-906
	Rapid Resolution	4.6 x 75	3.5	866967-902	866953-906
	Solvent Saver	3.0 x 250	5	880967-302	880975-306
	Solvent Saver	3.0 x 150	5	883967-302	883975-306
	Solvent Saver Plus	3.0 x 150	3.5	863967-302	863954-306
	Solvent Saver Plus	3.0 x 100	3.5	861967-302	861954-306
	Narrow Bore	2.1 x 150	5	883700-902	883700-906
	Narrow Bore RR*	2.1 x 100	3.5	861767-902	861753-906
	Guard Cartridge, 2/pk	9.4 x 15	7	820675-115	820675-115
	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-914	820950-913
	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-915	821125-915
	Guard Hardware Kit	9.4 x 15		840140-901	840140-901
	Guard Hardware Kit			820888-901	820888-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>					
	PrepHT Cartridge	21.2 x 250	7	877967-102	877250-106
	PrepHT Cartridge	21.2 x 150	7		877150-106
	PrepHT Cartridge	21.2 x 150	5		870150-906
	PrepHT Cartridge	21.2 x 100	5		870100-906
	PrepHT Cartridge	21.2 x 50	5		870050-906
	PrepHT Guard Cartridge, 2/pk			820212-914	820212-915
	Guard Cartridge Hardware			820444-901	820444-901
	PrepHT Endfittings, 2/pk			820400-901	820400-901

\*RR: Rapid Resolution 3.5 µm

\*\*Rx-C8 is the same product as SB-C8

## ZORBAX 80Å Extend-C18

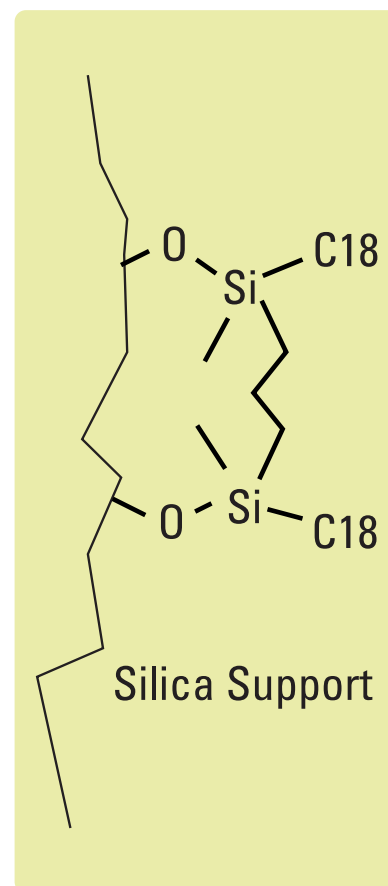
- High efficiency and long life at high pH – up to pH 11.5
- Unique bidentate bonding and double endcapping provides high pH stability
- More efficiency and better peak shape than polymer-based columns
- Improve retention, resolution and peak shape of basic compounds
- High sensitivity for LC/MS separations of peptides

The Agilent ZORBAX Extend-C18 column uses a novel bidentate C18-C18 bonding technology to make it possible to develop high-resolution separations at high pH with a silica-based column. At high pH non-charged basic compounds will not interact with the underlying silica. The result is high efficiency separations with superior peak shape and improved resolution. High pH separations are also the best choice for compounds that are more stable or more soluble in high pH solutions. Some of the mobile phase buffer options for high pH include triethylamine, pyrrolidine, glycine, borate and ammonium hydroxide. Ammonium hydroxide at pH 10.5 is an excellent mobile phase modifier for the LC/MS of peptides and small molecules with improved sensitivity compared with TFA containing mobile phase at low pH. The Extend-C18 column is stable from pH 2-11.5 with good peak shape for all types of compounds. Extend-C18 columns also provide an additional selectivity choice at low pH.

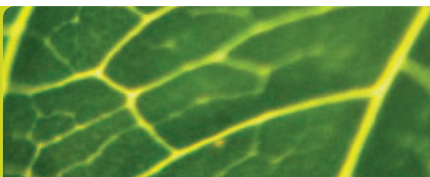
### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range	Endcapped	Carbon Load
ZORBAX Extend-C18	80Å	180 m <sup>2</sup> /g	60°C	2.0-11.5	Double	12.5%

\*Temperature limits are 60°C up to pH 8, 40°C from pH 8-11.5.



Novel Bidentate C18-C18 Bonding for Extend C-18 Bonded Phase



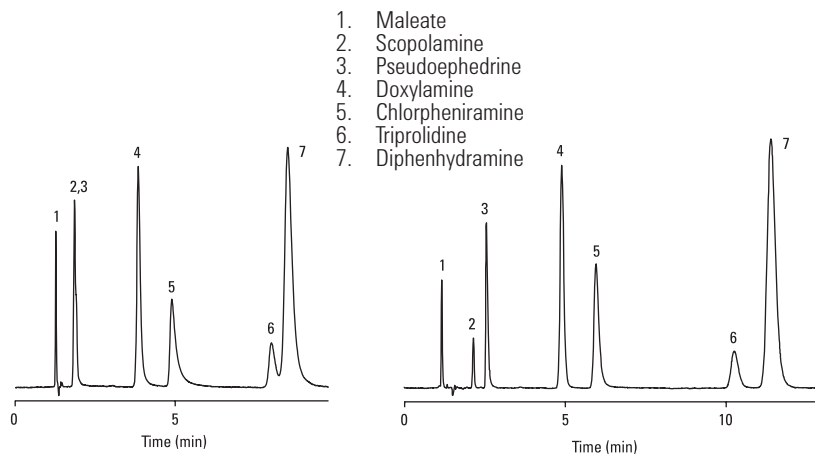
### Basic Antihistamines on Extend-C18 at High pH

**Column:** ZORBAX Extend-C18  
773450-902  
4.6 x 150mm, 5µm

**Mobile Phase:** pH 7:  
30% 20 mM NaH<sub>2</sub>PO<sub>4</sub>  
70% MeOH  
pH 11:  
30% 20 mM TEA  
70% MeOH

**Flow Rate:** 1.0 mL/min  
**Temperature:** Ambient  
**Detector:** 254 nm  
**Sample:** Antihistamines

Pseudoephedrine and scopolamine are difficult to retain at low and mid pH. Pseudoephedrine is often analyzed by ion exchange methods. The Extend-C18 column retains these compounds in a noncharged form at high pH and improves resolution.



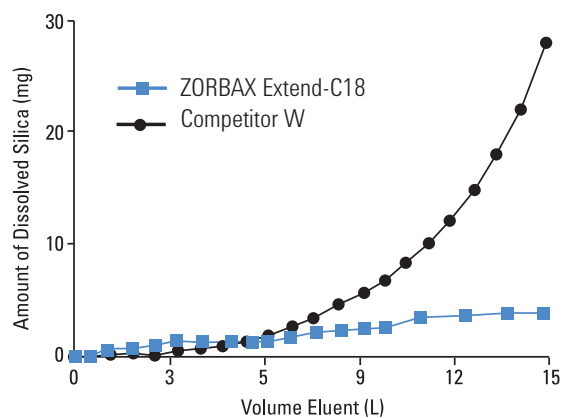
### Long Life at High pH with Extend-C18

**Column:** ZORBAX Extend-C18  
773450-902  
4.6 x 150mm, 5µm

**Mobile Phase:** 20% Methanol  
80% 0.1 M Carbonate Buffer, pH 10.0

**Flow Rate:** 1.0 mL/min  
**Temperature:** Ambient

At high pH, columns will fail due to silica dissolution. The example here shows extended lifetime of ZORBAX Extend-C18 at high pH in comparison to competitor W. This was measured by the amount of dissolved silica.



**Extend-C18 Provides Good Peak Shape at Low pH**

**Column:** ZORBAX Extend-C18  
773450-902  
4.6 x 150mm, 5µm

Mobile Phase: 80% 25 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 3.0  
20% Methanol

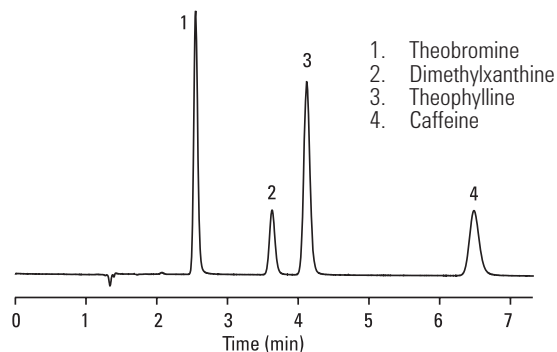
Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: 254 nm










Sample: Basic Compounds

These basic compounds are separated on the Extend-C18 at low pH with excellent peak shape. The Extend-C18 column can be used at high and low pH.

**ZORBAX 80Å Extend-C18**

Hardware	Description	Particle		
		Size (mm)	Size (µm)	Part No.
Standard Columns (no special hardware required, 400 bar)				
	Analytical	4.6 x 250	5	770450-902
	Analytical	4.6 x 150	5	773450-902
	Analytical	4.6 x 50	5	746450-902
	Rapid Resolution	4.6 x 150	3.5	763953-902
	Rapid Resolution	4.6 x 100	3.5	764953-902
	Rapid Resolution	4.6 x 75	3.5	766953-902
	Rapid Resolution	4.6 x 50	3.5	735953-902
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	728975-902
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	727975-902
	Rapid Resolution HT, 400 bar	4.6 x 50	1.8	722975-902
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	724975-902
	Rapid Resolution HT, 600 bar	4.6 x 20	1.8	726975-902
	Solvent Saver	3.0 x 250	5	770450-302
	Solvent Saver	3.0 x 150	5	773450-302
	Solvent Saver Plus	3.0 x 150	3.5	763954-302
	Solvent Saver Plus	3.0 x 100	3.5	764953-302
	Solvent Saver Plus	3.0 x 50	3.5	735954-302
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	728975-302
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	727975-302
	Solvent Saver HT, 600 bar	3.0 x 30	1.8	724975-302
	Solvent Saver HT, 600 bar	3.0 x 20	1.8	726975-302

## ZORBAX 80Å Extend-C18 (Continued)

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	Narrow Bore	2.1 x 150	5	773700-902
	Narrow Bore	2.1 x 50	5	760450-902
	Narrow Bore RR*	2.1 x 100	3.5	761753-902
	Narrow Bore RR*	2.1 x 50	3.5	735700-902
	Narrow Bore RRHT, 600 bar**	2.1 x 100	1.8	728700-902
	Narrow Bore RRHT, 600 bar**	2.1 x 50	1.8	727700-902
	Narrow Bore RRHT, 600 bar**	2.1 x 30	1.8	724700-902
	Narrow Bore RRHT, 600 bar**	2.1 x 20	1.8	726700-902
	MicroBore RR*	1.0 x 150	3.5	763600-902
	MicroBore RR*	1.0 x 50	3.5	765600-902
	MicroBore RR*	1.0 x 30	3.5	761600-902
	MicroBore Guard Cartridge, 3/pk	1.0 x 17	5	5185-5923
	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-930
	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-930
	Guard Hardware Kit			820888-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>				
	PrepHT Cartridge	21.2 x 150	5	770150-902
	PrepHT	21.2 x 100	5	770100-902
	PrepHT	21.2 x 50	5	770050-902
	PrepHT Endfittings, 2/pk			820400-901
	PrepHT Guard Cartridge, 2/pk	17 x 7.5	5	820212-930
	Guard Cartridge Hardware			820444-901

\*RR: Rapid Resolution 3.5 µm

\*\*RRHT: Rapid Resolution HT 1.8 µm



## ZORBAX Bonus-RP

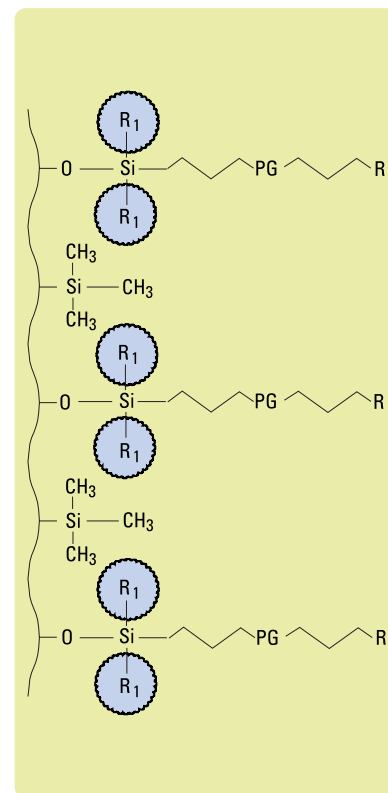
- Excellent peak shape for challenging basic compounds at low and mid pH
- Unique reversed-phase selectivity
- Novel bonding technology with embedded polar group and steric protection
- Usable in 100% aqueous mobile phases

The Agilent ZORBAX Bonus-RP column has a polar amide group embedded in a long alkyl chain. This novel bonding reduces interactions between basic compounds and the silica support, improving peak shape for the most difficult basic compounds. Peak shape and column lifetime are further improved by triple endcapping. In addition, diisopropyl side groups provide steric protection against acid hydrolysis for good lifetime at low pH. The Bonus-RP column provides an alternate selectivity to C18 and C8 alkyl bonded phases.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range	Endcapped	Carbon Load
ZORBAX Bonus-RP	80Å	180 m <sup>2</sup> /g	60°C	2.0-9.0	Triple	9.5%

\*Temperature limits are 60°C up to pH 8, 40°C from pH 8-9.



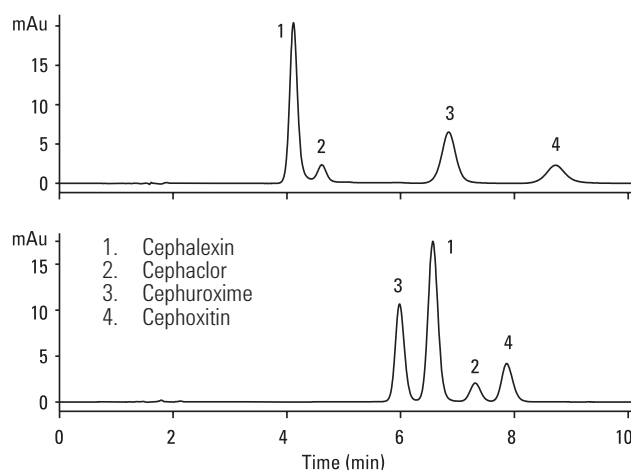
Unique, Polar Alkyl Bonus-RP Bonded Phase

### ZORBAX Bonus-RP Provides Unique Selectivity

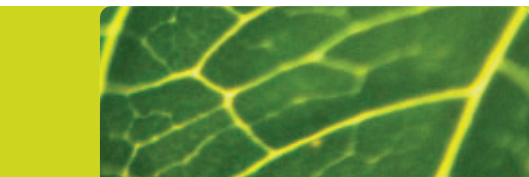
**Column A:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150mm, 5µm

**Column B:** Eclipse XDB-C8  
993967-906  
4.6 x 150mm, 5µm

Mobile Phase: 75% 25 mM Na Citrate, pH 6  
25% MeOH  
Flow Rate: 1.0 mL/min  
Temperature: Ambient  
Detector: 254 nm  
Sample: 3 µL  
Cephalosporins



Peak elution order can change dramatically when using Bonus-RP. In this example, the elution order of the first three peaks change.



### Improved Peak Shape of Basic Compounds Using Bonus-RP

**Column:** Alkyl-C8  
4.6 x 150 mm, 5 µm

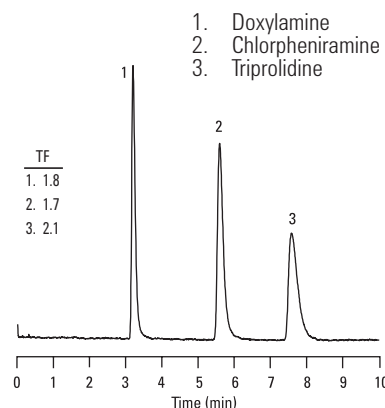
Mobile Phase: 75% 25 mM NH<sub>4</sub>OAc, pH 5.5  
25% ACN

Flow Rate: 1.5 mL/min

Temperature: 40°C

Detector: 254 nm

Bonus-RP eliminates peak tailing of these basic compounds in comparison to a typical alkyl C8 bonded phase. In the mid-pH region, residual silanols can interact more strongly with basic compounds to cause peak tailing. The polar group in the Bonus-RP bonded phase eliminates peak tailing of these basic compounds by reducing interactions with residual silanols.



### Improved Peak Shape of Basic Compounds Using Bonus-RP

**Column:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150mm, 5µm

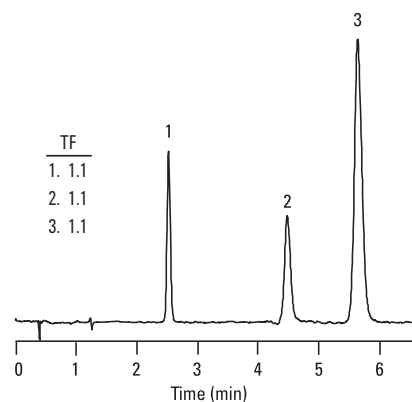
Mobile Phase: 80% 25 mM NH<sub>4</sub>OAc, pH 5.5  
20% ACN

Flow Rate: 1.5 mL/min

Temperature: 40°C

Detector: 254 nm

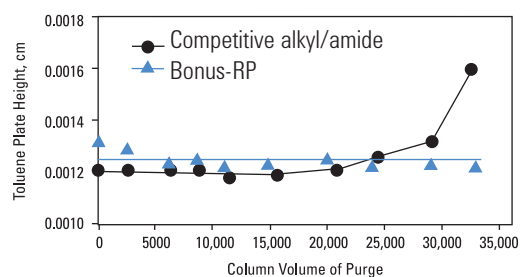
1. Doxylamine
2. Chlorpheniramine
3. Triprolidine



**ZORBAX Bonus-RP is Stable at Low and Mid pH**

**Column:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150mm, 5µm

Mobile Phase: 60% 25 mM  
Phosphate Buffer,  
pH 7.0: 40% ACN  
Flow Rate: 1.5 mL/min  
Temperature: 23°C

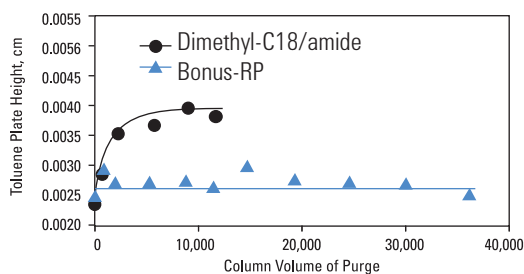


Triple endcapping of Bonus-RP enhances stability at pH 7. Each 10,000 column volumes is equivalent to approximately one working month.

**Dimethyl-C18/amide, Bonus-RP**












**Column:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150mm, 5µm

Mobile Phase: Aging:  
50% MeOH  
50% 0.1% TFA  
Test:  
80% MeOH  
20% H<sub>2</sub>O  
Flow Rate: 1.0 mL/min  
Temperature: Aging:  
60°C  
Test:  
23°C



Sterically protecting side groups provide good low pH stability and longer column lifetime than similar polar alkyl bonded phases.

## ZORBAX Bonus-RP

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
<b>Standard Columns (no special hardware required, 400 bar)</b>				
	Analytical	4.6 x 250	5	880668-901
	Analytical	4.6 x 150	5	883668-901
	Rapid Resolution	4.6 x 150	3.5	863668-901
	Rapid Resolution	4.6 x 100	3.5	864668-901
	Rapid Resolution	4.6 x 75	3.5	866668-901
	Solvent Saver	3.0 x 250	5	880668-301
	Solvent Saver	3.0 x 150	5	883668-301
	Solvent Saver Plus	3.0 x 150	3.5	863668-301
	Solvent Saver Plus	3.0 x 100	3.5	864668-301
	Narrow Bore	2.1 x 150	5	883725-901
	Narrow Bore	2.1 x 50	5	861971-901
	Narrow Bore RR*	2.1 x 150	3.5	863700-901
	Narrow Bore RR*	2.1 x 100	3.5	861768-901
	Narrow Bore RR*	2.1 x 50	3.5	861700-901
	MicroBore RR*	1.0 x 150	3.5	863608-901
	MicroBore RR*	1.0 x 50	3.5	865608-901
	MicroBore RR*	1.0 x 30	3.5	861608-901
	MicroBore Guard Cartridge, 3/pk	1.0 x 17	5	5185-5922
	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-928
	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-928
	Guard Hardware Kit			820888-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>				
	PrepHT Cartridge	21.2 x 250	7	878250-101
	PrepHT Cartridge	21.2 x 150	7	878150-101
	PrepHT Cartridge	21.2 x 150	5	868150-901
	PrepHT Cartridge	21.2 x 100	5	868100-901
	PrepHT Cartridge	21.2 x 50	5	868050-901
	PrepHT Endfittings, 2/pk			820400-901
	PrepHT Guard Cartridge, 2/pk	17 x 7.5	5	820212-928
	Guard Cartridge Hardware			820444-901

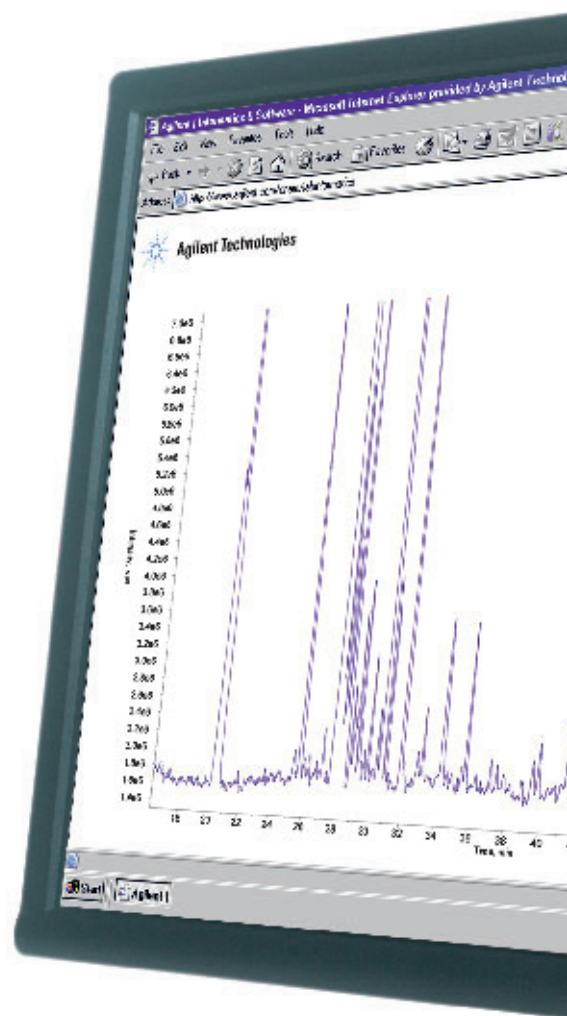
\*RR: Rapid Resolution 3.5 µm

## ZORBAX Method Development Kits

Agilent offers a series of kits that allow for fast method development at an attractive price. Each kit contains three columns. To study the effect of a change in selectivity on your separation under a given set of conditions, try either the Eclipse kits for applications in the pH range 2-9 or StableBond kits for additional choices at low pH. Try the pH kits if you want to study the effect of pH on your separation over a wide range of pH (1-11.5). The aqueous kits provide a wide range of selectivities with a set of columns that can operate under high aqueous conditions to retain highly polar analytes.

### ZORBAX Method Development Kits

Description	Part No.
StableBond Method Development Kit Includes 4.6 x 150 mm, 5 $\mu$ m columns; one each: SB-C18, SB-CN and SB-Phenyl phases	5183-4624
Fast StableBond Method Development Kit Includes 4.6 x 75 mm, 3.5 $\mu$ m columns; one each: SB-C18, SB-CN and SB-Phenyl phases	5183-4625
Eclipse XDB Method Development Kit Includes 4.6 x 150 mm, 5 $\mu$ m columns; one each: XDB-C18, XDB-C8, XDB-Phenyl phases	5183-4626
Fast Eclipse XDB Method Development Kit Includes 4.6 x 75 mm, 3.5 $\mu$ m columns; one each: XDB-C18, XDB-C8 and XDB-Phenyl phases	5183-4627
pH Method Development Kit Includes 4.6 x 150 mm, 5 $\mu$ m columns; one each: SB-C18, XDB-C18 and Extend-C18 phases	5185-5807
Fast pH Method Development Kit Includes 4.6 x 75 mm, 3.5 $\mu$ m columns; one each: SB-C18, XDB-C18 and Extend-C18 phases	5185-5808
Aqueous Method Development Kit Includes 4.6 x 150 mm, 5 $\mu$ m columns; one each: SB-Aq, Bonus RP and SB-C18	5185-5809
Fast Aqueous Method Development Kit Includes 4.6 x 75 mm, 3.5 $\mu$ m columns; one each: SB-Aq, Bonus RP and SB-C18	5185-5810





## ZORBAX Method Validation Kits

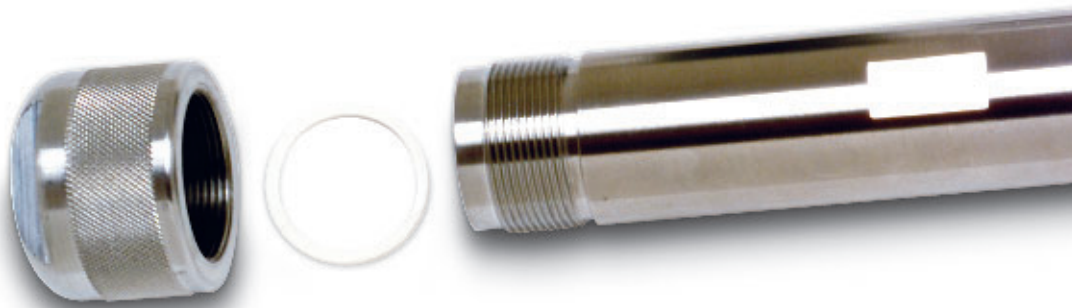


### Tips & Tools

Need to order a custom column? Go to **[www.agilent.com/chem/lccustom](http://www.agilent.com/chem/lccustom)** and fill out the custom column request form. You will receive a quote from your Customer Service Agent within 1-2 business days.

ZORBAX Method Validation Kits are supplied to customers who need the same HPLC column type (bonded phase, particle size, configuration) but from different manufacturing lots. To request columns from different lots, contact Agilent Technologies or your local distributor using the following procedure.

1. Request Validation Kits (columns from different lots) by using Part Number 899999-888
2. Indicate the Part Number of the current column you are using
3. Indicate the Lot Number of the current column you are using
4. Indicate the number of additional columns needed from different lots (example: you have a current column and may need two additional lots)
5. Please fax your request to (302) 993-5354 or email to **[custom\\_columns@agilent.com](mailto:custom_columns@agilent.com)**. You will receive a quote from your Customer Service Agent within 1-2 business days. Delivery for your custom column is usually 3 weeks or less from the time your order is placed, depending on lot availability.





## ZORBAX Original Reversed Phase Columns

Original ZORBAX columns are made with Type A silica and are useful for many applications of acidic or neutral compounds. These columns have a higher activity level and are therefore useful for separating isomers (e.g. cis-trans, geometric) or other compounds where silanol activity enhances selectivity. These columns are used in many established methods.

Hardware	Description	Size (mm)	Particle Size (µm)	ODS (C18) USP L1	C8 USP L7	Phenyl USP L11	CN USP L10	TMS USP L13
<b>Standard Columns (no special hardware required, 400 bar)</b>								
	Semi-Preparative	9.4 x 250	5	880952-202	880952-206			
	Analytical (Endcapped)	4.6 x 250	5	880952-702	880952-706	880952-712	884950-507	880952-710
	Analytical (Non-endcapped)	4.6 x 250	5	884950-543				
	Analytical	4.6 x 150	5	883952-702	883952-706	883952-712	884950-526	883952-710
	Solvent Saver	3.0 x 250	5	880952-302				
	Solvent Saver	3.0 x 150	5	883952-302				
<b>Guard Columns (hardware required)</b>								
<b>P</b>	Guard Cartridge, 2/pk	9.4 x 15	7	820675-115	820675-115	820675-115	820675-124	
<b>ZGC</b>	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-902	820950-906	820950-912	820950-905	820950-924
<b>P</b>	Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901	840140-901
<b>ZGC</b>	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901	820888-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>								
<b>PI</b>	PrepHT Cartridge	21.2 x 250	7	877952-102	877952-106		877952-105	
<b>PI</b>	PrepHT Endfittings, 2/pk			820400-901	820400-901		820400-901	





## ZORBAX Normal Phase Columns

For normal-phase chromatography, the ZORBAX product line offers a choice of bonded and non-bonded silica packings.

### ZORBAX Rx-SIL

- Made from highly pure (>99.995%) porous silica microspheres (pore size is the space between the solid silica microparticles)
- Stronger than other silica types
- Less acidic than ZORBAX-SIL, lower metal content
- Low acidity and low metal content make ZORBAX Rx-SIL ideal for normal-phase separation of polar compounds that exhibit poor peak symmetry on more acidic silica
- Useful for very hydrophilic compounds with high organic mobile phases in HILIC mode

### ZORBAX Eclipse XDB-CN

- Made from highly pure Rx-SIL
- Excellent choice for normal phase applications with basic compounds
- Equilibrates more rapidly than ZORBAX Rx-SIL and is used for many of the same normal-phase applications

### ZORBAX CN

- Cyanopropyltrimethylsilane monolayer bonded to ZORBAX SIL
- Equilibrates more rapidly than ZORBAX SIL, and used for many of the same normal-phase applications
- Less prone to fouling and less water sensitive than silica

### ZORBAX NH2

- Amino-propyl silane phase bonded to ZORBAX SIL
- Used for normal phase and weak anion-exchange, and reversed-phase HPLC of polar compounds
- Vitamins A and D are separated in the normal-phase mode
- Carbohydrates and sugars are separated in the reversed-phase mode



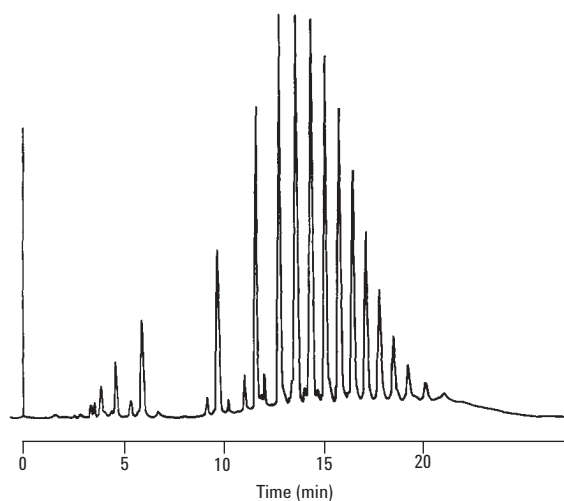
**Column Specifications**

Bonded Phase	Pore Size	Surface Area	pH Range	Endcapped	Carbon Load
ZORBAX Rx-SIL	80Å	180 m <sup>2</sup> /g	0-8.0	No	
ZORBAX Eclipse XDB-CN	80Å	180 m <sup>2</sup> /g	2.0-8.0	Yes	4.3%
ZORBAX SIL	70Å	300 m <sup>2</sup> /g	0-8.0	No	
ZORBAX CN	70Å	300 m <sup>2</sup> /g	2.0-7.0	Yes	7%
ZORBAX NH <sub>2</sub>	70Å	300 m <sup>2</sup> /g	2.0-7.0	Yes	4%











**High Resolution Normal Phase Separation of Octylphenoxy Ethanol Surfactant on ZORBAX CN**

**Column:** ZORBAX CN  
880952-705  
4.6 x 250mm, 5µm

Mobile Phase: Primary: Heptane  
Secondary: 2-Methoxyethanol/Isopropanol (50/50)  
Flow Rate: 2 mL/min  
Gradient: 2-20% Secondary in 10 min., Linear Hold at 20%  
Temperature: 50°C  
Detector: 278 nm  
Sample: Octylphenoxy (polyethylene oxy)  
Ethanol Surfactant (n= 10)



### ZORBAX Normal Phase Columns

Hardware	Description	Size (mm)	Particle Size (µm)	Rx-SIL	SIL USP L3	CN USP L10	NH2 USP L8	Carbohydrate Analysis	XDB-CN USP L10
<b>Standard Columns (no special hardware required, 400 bar)</b>									
	Semi-Prep	9.4 x 250	5	880975-201	880952-201	880952-205	880952-208		
	Analytical	4.6 x 250	5	880975-901	880952-701	880952-705	880952-708	840300-908	990967-905*
	Analytical	4.6 x 150	5	883975-901	883952-701	883952-705	883952-708	843300-908	993967-905*
	Narrow Bore	2.1 x 150	5	883700-901					993700-905*
	Narrow Bore	2.1 x 50	5				860700-708		
<b>Guard Columns (hardware required)</b>									
	Guard Cartridge, 2/pk	9.4 x 15	5	820675-119	820675-119	820675-111	820675-111		
	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-919	820950-901	820950-905	820950-908	820950-908	820950-935
	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-919					821125-935
	Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901		
	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901	820888-901	820888-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>									
	PrepHT Cartridge	21.2 x 250	7	877250-101	877952-101				
	PrepHT Cartridge	21.2 x 250	7			877952-105	877952-108		
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901		
	PrepHT Guard Cartridge, 2/pk	17 x 7.5	5	820212-919					
	Guard Cartridge Hardware			820444-901					

\*These columns ship containing reversed phase solvents. Flush with isopropanol before using normal phase solvents.



## HPLC Columns for Special Applications


Reproducible results  
from high-throughput, to capillary, to prep.

No matter how many samples you have — or how complex they may be — you need to feel confident that you can achieve reproducible results without wasting valuable time testing different columns and configurations.

The following column families deliver industry-leading performance for specific measurement and purification challenges:

- **EXPANDED! ZORBAX Rapid Resolution (3.5  $\mu$ m) and Rapid Resolution High Throughput (1.8  $\mu$ m) columns** — enhance your productivity by delivering fast analysis without compromising resolution. Available in Eclipse XDB, StableBond, and NEW Eclipse Plus bonded phases.
- **ZORBAX Solvent Saver columns** — reduce solvent usage by 50% over 4.6 mm ID columns, and are compatible with most conventional HPLC instruments and LC/MS detectors. Ideal for cost-effective analysis.
- **ZORBAX MicroBore HPLC columns** — are a good choice when sample sizes are limited. They can also improve detection limits 5 times over 2.1 mm ID columns when the same sample mass is used.
- **ZORBAX capillary and nano columns** — are best for limited-sample and proteomics applications, because they enhance sensitivity by reducing on-column sample dilution. Now available in a wide variety of phases, pore sizes, and dimensions.
- **Agilent and ZORBAX preparative columns, preparative cartridge columns, and guards** — are engineered for high purity, recovery, and throughput. They feature a broad range of selectivities, and are available in a variety of bonded phases for exceptional method development flexibility.
- **Ultron chiral columns with two complementary protein-based chiral stationary phases** — are an excellent choice for enantiomeric separations. Ideal for many pharmacological applications.





## Agilent ZORBAX Rapid Resolution High Throughput (RRHT) and Rapid Resolution (RR) HPLC columns

Enhance your lab's productivity without sacrificing performance, reliability, or convenience.

Chances are, you are under increased pressure to generate conclusive data under demanding time constraints.

That is why Agilent has expanded our ZORBAX Rapid Resolution High Throughput line to include columns that can be used at pressures of 600 bar for faster, higher-resolution separations. Together with the Agilent 1200 Series Rapid Resolution System, RRHT columns can reduce your analysis time by up to 95%!

With their 1.8  $\mu\text{m}$  particle size, Agilent RRHT columns allow you to push flow rates to the limit without compromising the efficiency or quality of your separations. And if you have already developed conventional LC methods on Agilent's ZORBAX LC columns, you can easily and securely transfer these methods to Agilent's RRHT columns. So you can analyze complex separations on shorter columns – and re-evaluate current methods on fast LC without changing separation conditions



### TIPS & TOOLS

Use **Rapid Resolution (RR)** columns when you want speed and resolution under lower pressures.

Use **Rapid Resolution High Throughput (RRHT)** columns when you need high speed and superior resolution under high pressures.

### Together with the Agilent 1200 Series Rapid Resolution System, ZORBAX RRHT 1.8 $\mu\text{m}$ HPLC columns can help you...

- Process samples up to 20 times faster.
- Confidently move methods from your lab to any lab in the world.
- Perform conventional, fast, and ultra-fast separations on the same unit.
- Choose from over 100 Rapid Resolution and Rapid Resolution High Throughput configurations.
- Increase resolution by 30-40% over conventional HPLC.
- Significantly reduce operator training time.
- Minimize switching costs.



## ZORBAX Rapid Resolution High Throughput 1.8 $\mu\text{m}$

- New high pressure (600 bar) columns for ultra high speed or maximum resolution analyses with Rapid Resolution HT columns packed with totally porous, 1.8  $\mu\text{m}$  packings
- Carefully engineered particles deliver maximum resolution at 25% less pressure than other sub 2-micron materials
- Reduce analysis time by up to 95%
- Develop HPLC methods more quickly
- Securely transfer conventional methods with over 80 RRHT column choices
- Analyze complex samples on shorter columns faster and maximize peak capacity
- Perform faster analyses and use less solvent
- Short (50 mm long and less) column can be used on some conventional LCs

ZORBAX Rapid Resolution HT (1.8  $\mu\text{m}$ ) columns use a totally porous, 1.8  $\mu\text{m}$  particle to provide maximum resolution in fast, ultra-fast and high resolution analyses. You can reduce analysis time by up to 95% in comparison to 250 mm length columns. With more than 80 RRHT column choices, including the new high performance ZORBAX Eclipse Plus and many other ZORBAX column choices (Eclipse XDB, StableBond, Extend), methods can be developed quickly or securely transferred to a smaller particle size column with no loss in resolution. The small particle size provides double the efficiency of a 3.5  $\mu\text{m}$  column in the same column length providing the highest efficiency and resolution possible. This permits the analysis of complex samples on shorter column with the highest resolution and peak capacity. The 1.8  $\mu\text{m}$  Rapid Resolution HT columns take high-speed, high-resolution HPLC to a new level. The 600 bar columns can be used with the new Agilent 1200 Rapid Resolution LC up to this high pressure limit. In addition, the shorter columns can be used on many other LC's, including the Agilent 1100 by using the RRHT-1100 conversion kits to maximize performance.





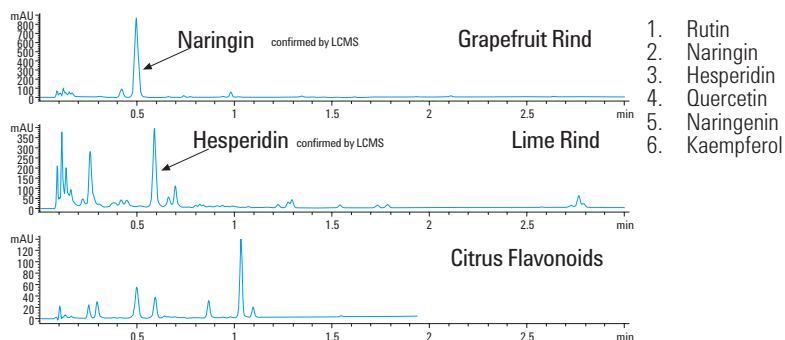
## 1100 Series Conversion Kits for Fast LC

These kits make it easy to convert your Agilent 1100 system with a binary pump to a lower-volume system for RRHT LC columns. Each kit contains all capillaries, a flow cell, starter columns, and detailed instructions for system conversion. Note: you will still be able to use your converted 1100 for standard methods and columns.

Kit Selection	Description	Part No.
For Variable Wavelength Detectors (VWD)	Columns: 4.6 x 50 mm, 1.8 $\mu$ m (3) Flow Cell for VWD, 5 $\mu$ l capillaries, $\mu$ -LC inline filter	5188-5323
For Diode Array Detectors (DAD & DAD SL) and Multiple Wavelength Detectors (MWD)	Columns: 4.6 x 50 mm, 1.8 $\mu$ m (2) Flow Cell for DAD, 5 $\mu$ l capillaries, $\mu$ -LC inline filter	5188-5324
For Diode Array Detector and Mass Spec	Columns: 2.1 x 50 mm, 1.8 $\mu$ m (2) Flow Cell for DAD, 1.7 $\mu$ l capillaries, ZDV union	5188-5328

### RRHT – Flavanoids, Fast Analysis of Citrus Rinds, Confirmation by LC/MS

**Column:** Eclipse XDB-C18  
924975-302  
3.0 x 30mm, 1.8 $\mu$ m  
**Mobile Phase:** A: 0.1% FA, B: 0.075% FA in ACN  
**Gradient:** 18 to 65% B/2 min  
65-88% B/3 min  
**Detector:** 276 nm  
**Temperature:** 50°C  
**Sample:** 2g ground fresh peel  
and (10 mL MeOH + 10  $\mu$ L KOH)  
Ultrasonicate 10 min and 0.45  $\mu$ m filter  
Inject 1  $\mu$ L



This is an analysis with potentially 10-20 analytes. It can still be done quickly for improved productivity.

## Rapid Resolution HT – Up to 20X Faster

**Column:** ZORBAX SB-C18  
883975-902

**4.6 x 150mm, 5µm**

827700-902, 827700-902

Mobile Phase:

A: H<sub>2</sub>O

B: ACN

Flow Rate:

1.2 mL/min, 1.0 mL/min, 2.4 mL/min

Gradient:

0.0 min 50% B

11/1.2/0.4 min 100% B

Temperature:

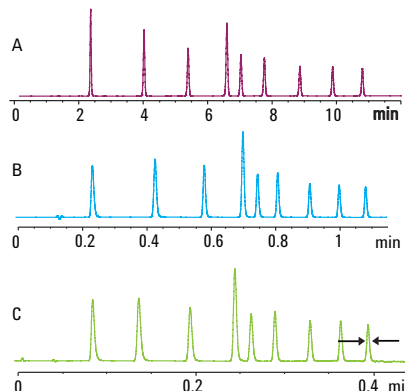
40°C, 40°C, 95°C

Detector:

UV 254 nm

Sample:

Alkylphenones

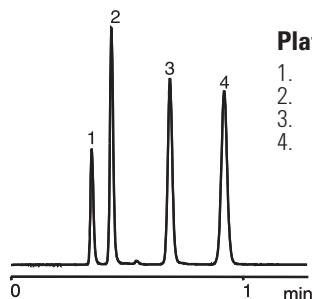


1. C3-Alkylphenone
2. C4-Alkylphenone
3. C5-Alkylphenone
4. C6-Alkylphenone
5. C7-Alkylphenone
6. C8-Alkylphenone
7. C9-Alkylphenone
8. C10-Alkylphenone
9. C12-Alkylphenone

## Rapid Resolution HT Provides Double the Efficiency of Rapid Resolution Columns

**Column:** ZORBAX SB-C18  
835975-902

**4.6 x 50mm, 3.5µm**



## Plates (N)

1. 3476
2. 4585
3. 5673
4. 6180

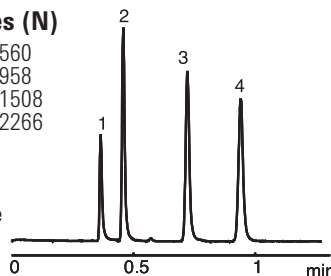
1. Uracil
2. Phenol
3. 4-Cl-Nitrobenzene
4. Toluene

**Column:** ZORBAX SB-C18  
835975-902

**4.6 x 50mm, 3.5µm**

## Plates (N)

1. 6560
2. 8958
3. 11508
4. 12266



Mobile Phase: 25% Water, 75% MeOH

Phase:

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: 254 nm

This figure shows that Rapid Resolution HT columns can provide double the efficiency of a 3.5 µm column in the same column length. This high efficiency can be used for very high-resolution, high throughput analyses.

## Reduce Analysis Time Dramatically with Rapid Resolution HT Columns

**Column A:** Eclipse XDB-C18  
990967-902  
4.6 x 250mm, 5µm

**Column B:** Eclipse XDB-C18  
963967-902  
4.6 x 150mm, 3.5µm

**Column C:** Eclipse XDB-C18  
966967-902  
4.6 x 75mm, 3.5µm

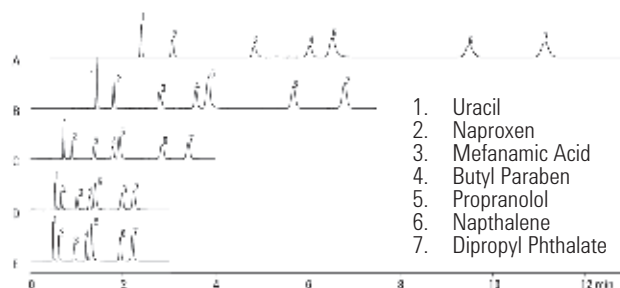
**Column D:** Eclipse XDB-C18  
925975-902  
4.6 x 50mm, 1.8µm

Mobile Phase: 73% MeOH: 27% 20 mM  
Phosphate Buffer, pH 7.0

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: 254 nm



This figure shows the dramatic reduction in analysis time possible by using Rapid Resolution HT columns. Chromatogram A shows a separation that takes 11.5 minutes on a 25 cm, 5 µm column. Rapid Resolution (3.5 µm) columns, shown in chromatogram B and C, reduce analysis time substantially, but with a slight compromise in resolution. The Rapid Resolution HT column reduces analysis time to 2.2 minutes, an 80% reduction, while still maintaining baseline resolution.

## Increase Peak Capacity with RRHT Columns

**Column A:** Eclipse XDB-C8  
928700-906  
2.1 x 100mm, 1.8µm

**Column B:** Eclipse XDB-C18  
961753-902  
2.1 x 100mm, 3.5µm

Mobile Phase: A: H<sub>2</sub>O  
B: ACN

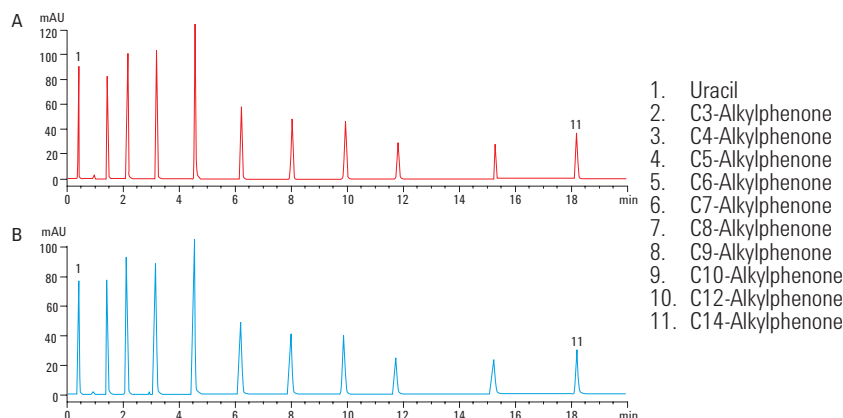
Flow Rate: 0.5 mL/min

Gradient: 0.0 min 50% B  
20.0 min 100% B

Temperature: 40°C

Detector: UV 254 nm

Sample: Alkylphenones



## Long Lifetime of RRHT Columns at Elevated Temp.

**Column:** SB-C18  
827700-902  
2.1 x 50mm, 1.8µm

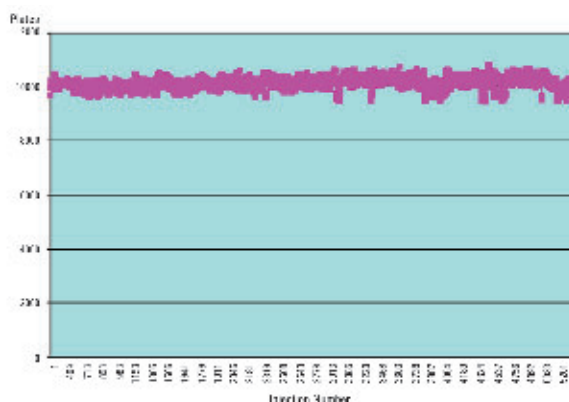
Mobile Phase: A: 60% H<sub>2</sub>O  
B: 40% ACN

Flow Rate: 1 mL/min

Temperature: 80°C

Detector: UV 254 nm

Sample: QC Test Mix



**Comparison of Efficiencies – RRHT (1.8µm)  
and Rapid Resolution (3.5µm) Columns**

Column Length	Resolving Power N (3.5 µm)*	Resolving Power N (1.8 µm)
<b>High Resolution</b>		
150	21,000	32,500
100	14,000	24,000
75	10,500	17,000**
<b>Ultra Fast</b>		
50	7000	12,000
30	4200	6000
20	—	3500
15	2100	2500

Resolution  $\propto N^{1/2}$

\*5 µm HPLC columns of the same length have 40% fewer plates (N-value); 4.6 mm ID

\*\*Available as a custom column

**ZORBAX Rapid Resolution HT Columns for High Pressure Use (Maximum Pressure: 600 bar, 9000 psi)**

Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Extend-C18 USP L1
Rapid Resolution HT, 600 bar	4.6 x 150	1.8	959994-902				
Rapid Resolution HT, 600 bar	4.6 x 100	1.8	959964-902	959964-906	928975-902	928975-906	728975-902
Rapid Resolution HT, 600 bar	4.6 x 50	1.8	959941-902	959941-906	927975-902	927975-906	727975-902
Rapid Resolution HT, 600 bar	4.6 x 30	1.8	959931-902	959931-906	924975-902	924975-906	724975-902
Rapid Resolution HT, 600 bar	4.6 x 20	1.8			926975-902	926975-906	726975-902
Solvent Saver HT, 600 bar	3.0 x 100	1.8	959964-302	959964-306	928975-302	928975-306	728975-302
Solvent Saver HT, 600 bar	3.0 x 50	1.8	959941-302	959941-306	927975-302	927975-306	727975-302
Solvent Saver HT, 600 bar	3.0 x 30	1.8			924975-302	924975-306	724975-302
Solvent Saver HT, 600 bar	3.0 x 20	1.8			926975-302	926975-306	726975-302
Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	959764-902	959764-906	928700-902	928700-906	728700-902
Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	959741-902	959741-906	927700-902	927700-906	727700-902
Narrow Bore RRHT, 600 bar	2.1 x 30	1.8	959731-902	959731-906	924700-902	924700-906	724700-902
Narrow Bore RRHT, 600 bar	2.1 x 20	1.8			926700-902	926700-906	726700-902
MicroBore RRHT, 600 bar	1.0 x 100	1.8			928600-902	928600-906	728600-902
MicroBore RRHT, 600 bar	1.0 x 50	1.8			922600-902	922600-906	722600-902



**ZORBAX Rapid Resolution HT Columns for High Pressure Use (Maximum Pressure: 600 bar, 9000 psi)**

Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-Phenyl USP L11	SB-CN USP L10	SB-Aq
Rapid Resolution HT, 600 bar	4.6 x 150	1.8	829975-902	829975-906	829975-912	829975-905	
Rapid Resolution HT, 600 bar	4.6 x 100	1.8	828975-902	828975-906	828975-912	828975-905	828975-914
Rapid Resolution HT, 600 bar	4.6 x 50	1.8	827975-902	827975-906	827975-912	827975-905	828975-914
Rapid Resolution HT, 600 bar	4.6 x 30	1.8	824975-902	824975-906	824975-912	824975-905	824975-914
Rapid Resolution HT, 600 bar	4.6 x 20	1.8	826975-902	826975-906			
Solvent Saver HT, 600 bar	3.0 x 150	1.8	829975-302	829975-306	829975-312	829975-305	
Solvent Saver HT, 600 bar	3.0 x 100	1.8	828975-302	828975-306	828975-312	828975-305	828975-314
Solvent Saver HT, 600 bar	3.0 x 50	1.8	827975-302	827975-306	827975-312	827975-305	827975-314
Solvent Saver HT, 600 bar	3.0 x 30	1.8	824975-302	824975-306		824975-305	
Solvent Saver HT, 600 bar	3.0 x 20	1.8	826975-302	826975-306			
Narrow Bore RRHT, 600 bar	2.1 x 150	1.8	820700-902	820700-906	820700-912	820700-905	
Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	828700-902	828700-906	828700-912	828700-905	828700-914
Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	827700-902	827700-906	827700-912	827700-905	827700-914
Narrow Bore RRHT, 600 bar	2.1 x 30	1.8	824700-902	824700-906	824700-912	824700-905	824700-914
Narrow Bore RRHT, 600 bar	2.1 x 20	1.8	826700-902	826700-906			
MicroBore RRHT, 600 bar	1.0 x 100	1.8	828600-902	828600-906		828600-905	


















**ZORBAX Rapid Resolution HT Columns and Cartridges (Maximum Pressure: 400 bar, 6000 psi)**

Hardware	Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	SB-C18 USP L1	SB-C8 USP L7	Extend-C18 USP L1
	Rapid Resolution HT	4.6 x 50	1.8	922975-902	922975-906	822975-902	822975-906	722975-902
	Rapid Resolution HT, 3/pk	4.6 x 50	1.8	922975-932		822975-932		
	Narrow Bore RRHT	2.1 x 50	1.8	922700-902		822700-902		
	Narrow Bore RRHT, 3/pk	2.1 x 50	1.8	922700-932		822700-932		

**Rapid Resolution HT Cartridges (require hardware kit 820555-901)**

	Rapid Resolution HT Cartridge	4.6 x 50	1.8	925975-902		825975-902		
	Rapid Resolution HT Cartridge, 3/pk	4.6 x 50	1.8	925975-932		825975-932		
	Rapid Resolution HT Cartridge	2.1 x 50	1.8	925700-902		825700-902		
	Rapid Resolution HT Cartridge, 3/pk	2.1 x 50	1.8	925700-932		825700-932		
	Rapid Resolution HT Cartridge	4.6 x 30	1.8	923975-902		823975-902		
	Rapid Resolution HT Cartridge, 3/pk	4.6 x 30	1.8	923975-932		823975-932		
	Rapid Resolution HT Cartridge	2.1 x 30	1.8	923700-902		823700-902		
	Rapid Resolution HT Cartridge, 3/pk	2.1 x 30	1.8	923700-932		823700-932		
	Rapid Resolution HT Cartridge	4.6 x 15	1.8	921975-902		821975-902		
	Rapid Resolution HT Cartridge, 3/pk	4.6 x 15	1.8	921975-932		821975-932		
	Rapid Resolution HT Cartridge	2.1 x 15	1.8	921700-902		821700-902		
	Rapid Resolution HT Cartridge, 3/pk	2.1 x 15	1.8	921700-932		821700-932		
	Hardware Kit for RR and RRHT Cartridges			820555-901		820555-901		



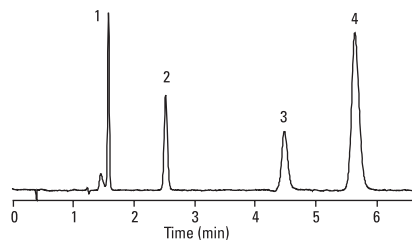
## ZORBAX Rapid Resolution 3.5 $\mu\text{m}$ Columns

- Reduce analysis time and solvent usage and increase sample throughput
- High efficiency in short and ultra-short column lengths
- Available in analytical (4.6 mm) and narrow-bore (2.1 mm) ID
- Comparable lifetime to 5  $\mu\text{m}$  columns

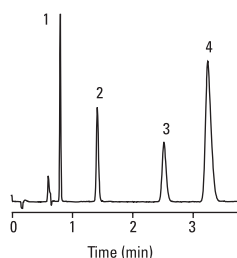
Agilent ZORBAX Rapid Resolution columns, with a 3.5  $\mu\text{m}$  particle size, reduce analysis time and increase sample throughput for any application when compared to 5  $\mu\text{m}$  columns. Rapid Resolution columns are available from 15-150 mm, in 1-4.6 mm IDs, so the best configurations are available for high throughput, LC/MS, combinatorial chemistry and rapid analytical applications. Rapid Resolution 3.5  $\mu\text{m}$  particles have superior mechanical strength, so every column has an extremely stable packed bed and provides a comparable lifetime to 5  $\mu\text{m}$  columns. These are available in most ZORBAX bonded phases.

### Rapid Resolution Columns Reduce Analysis Time While Maintaining Resolution

**Column A:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150mm, 5 $\mu\text{m}$

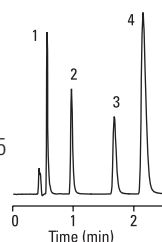


**Column B:** ZORBAX Bonus-RP  
866668-901  
4.6 x 75mm, 3.5 $\mu\text{m}$



**Column C:** ZORBAX Bonus-RP  
861700-901  
2.1 x 50mm, 3.5 $\mu\text{m}$

Mobile Phase: 80% 25 mM NH<sub>4</sub>Ac, pH 5.5  
20% ACN  
Flow Rate: 1.5 mL/min  
1.5 mL/min  
0.3 mL/min  
Temperature: 40°C  
Detector: 254 nm



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

Rapid Resolution 3.5  $\mu\text{m}$  columns are available in narrow bore configurations for great compatibility with LC/MS.

## Run Method Development Chromatograms on Rapid Resolution Columns

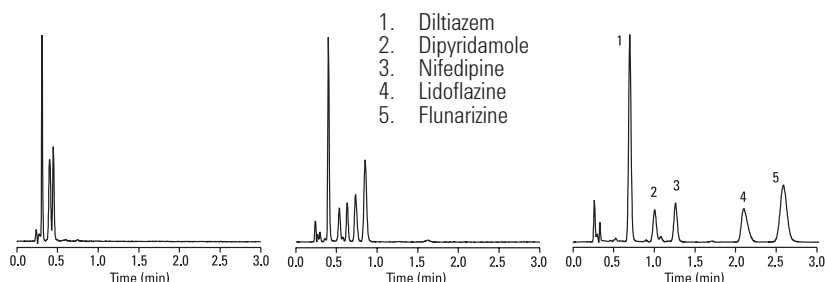
**Column:** ZORBAX SB-C18  
866953-902  
4.6 x 75mm, 3.5µm

**Mobile Phase:** A: 25 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 3.0  
B: MeOH

**Flow Rate:** 2.0 mL/min

**Temperature:** 35°C

**Detector:** 254 nm






Rapid Resolution 4.6 x 75 mm, 3.5 µm columns are a good choice for initial method development because they allow you to quickly determine if a given column and mobile phase combination can produce the desired separation.

## ZORBAX Rapid Resolution 3.5 µm Eclipse Columns

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Eclipse XDB-Phenyl USP L11	Eclipse XDB-CN USP L10
	Rapid Resolution	4.6 x 150	3.5	959963-902	959963-906	963967-902	963967-906	963967-912	963967-905
	Rapid Resolution	4.6 x 100	3.5	959961-902	959961-906	961967-902	961967-906		961967-905
	Rapid Resolution	4.6 x 75	3.5	959933-902	959933-906	966967-902	966967-906	966967-912	966967-905
	Rapid Resolution	4.6 x 50	3.5	959943-902	959943-906	935967-902	935967-906	935967-912	
	Rapid Resolution	4.6 x 30	3.5	959936-902	959936-906	934967-902	934967-906		
	Rapid Resolution	4.6 x 20	3.5			932967-902	932967-906		
	Solvent Saver Plus	3.0 x 150	3.5	959963-302	959963-306	963954-302	963954-306	963954-312	963954-305
	Solvent Saver Plus	3.0 x 100	3.5	959961-302	959961-306	961967-302	961967-306	961967-312	
	Solvent Saver Plus	3.0 x 75	3.5			966954-302			
	Narrow Bore RR*	2.1 x 150	3.5	959763-902	959763-902	930990-902	930990-906		
	Narrow Bore RR*	2.1 x 100	3.5	959793-902	959793-906	961753-902	961753-906		961753-905
	Narrow Bore RR*	2.1 x 75	3.5			966735-902			
	Narrow Bore RR*	2.1 x 50	3.5	959743-902	959743-906	971700-902	971700-906		
	Narrow Bore RR*	2.1 x 30	3.5	959733-902	959733-906	974700-902	974700-906		
	Narrow Bore RR*	2.1 x 20	3.5			972700-902	972700-906		
	MicroBore RR*	1.0 x 150	3.5			963600-902	963600-906		
	MicroBore RR*	1.0 x 50	3.5			965600-902	965600-906		
	MicroBore RR*	1.0 x 30	3.5			961600-902	961600-906		
	MicroBore Guard Cartridges, 3/pk	1.0 x 17	5			5185-5921	5185-5921		
ZGC	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-936	820950-937	820950-925	820950-926	820950-927	820950-935
ZGC	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-936	821125-937	821125-926	821125-926	821125-926	821125-935
ZGC	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901	820888-901	820888-901

\*RR: Rapid Resolution 3.5 µm

## ZORBAX Rapid Resolution 3.5 µm StableBond Columns

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-Phenyl USP L11	SB-C3 USP L56	SB-Aq
	Rapid Resolution	4.6 x 150	3.5	863953-902	863953-906	863953-905	863953-912		863953-914
	Rapid Resolution	4.6 x 100	3.5	861953-902	861953-906	861953-905	861953-912		861953-914
	Rapid Resolution	4.6 x 75	3.5	866953-902	866953-906	866953-905	866953-912		866953-914
	Rapid Resolution	4.6 x 50	3.5	835975-902	835975-906	835975-905	835975-912		835975-914
	Rapid Resolution	4.6 x 30	3.5	834975-902	834975-906				
	Rapid Resolution	4.6 x 20	3.5	832975-902	832975-906				
	Solvent Saver Plus	3.0 x 150	3.5	863954-302	863954-306	863954-305	863954-312		863954-314
	Solvent Saver Plus	3.0 x 100	3.5	861954-302	861954-306	861954-305	861954-312	861954-309	861954-314
	Narrow Bore RR*	2.1 x 150	3.5	830990-902	830990-906				830990-914
	Narrow Bore RR*	2.1 x 100	3.5	861753-902	861753-906	861753-905	861753-912		861753-914
	Narrow Bore RR*	2.1 x 75	3.5	866735-902					
	Narrow Bore RR*	2.1 x 50	3.5	871700-902	871700-906				871700-914
	Narrow Bore RR*	2.1 x 30	3.5	874700-902	874700-906				
	Narrow Bore RR*	2.1 x 20	3.5	872700-902	872700-906				
	MicroBore RR*	1.0 x 150	3.5	863600-902	863600-906				
	MicroBore RR*	1.0 x 50	3.5	865600-902	865600-906				
	MicroBore RR*	1.0 x 30	3.5	861600-902	861600-906				
	MicroBore Guard Cartridges, 3/pk	1.0 x 17	5	5185-5920	5185-5920				
	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-920	820950-915	820950-916	820950-917	820950-922	820950-933
	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-926	821125-926	821125-924	821125-926	821125-924	821125-933
	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901	820888-901	820888-901

\*RR: Rapid Resolution 3.5 µm

**ZORBAX Rapid Resolution 3.5  $\mu$ m Rx, Bonus-RP, and Extend-C18 Columns**

Hardware	Description	Size (mm)	Particle Size ( $\mu$ m)	Rx-C18 USP L1	Rx-C8 USP L7	Bonus-RP	Extend-C18
	Rapid Resolution	4.6 x 150	3.5	863967-902	863953-906	863668-901	763953-902
	Rapid Resolution	4.6 x 100	3.5	861967-902	861953-906	864668-901	764953-902
	Rapid Resolution	4.6 x 75	3.5	866967-902	866953-906	866668-901	766953-902
	Rapid Resolution	4.6 x 50	3.5				735953-902
	Solvent Saver Plus	3.0 x 150	3.5	863967-302	863954-306	863668-301	763954-302
	Solvent Saver Plus	3.0 x 100	3.5	861967-302	861954-306	864668-301	764953-302
	Narrow Bore RR	2.1 x 150	3.5		830990-906	863700-901	
	Narrow Bore RR*	2.1 x 100	3.5	861767-902	861753-906	861768-901	761753-902
	Narrow Bore RR*	2.1 x 50	3.5			861700-901	735700-902
	MicroBore RR*	1.0 x 150	3.5			863608-901	763600-902
	MicroBore RR*	1.0 x 50	3.5			865608-901	765600-902
	MicroBore RR*	1.0 x 30	3.5			861608-901	761600-902
	MicroBore Guard Cartridge, 3/pk	1.0 x 17	5			5185-5922	5185-5923
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-914	820950-913	820950-928	820950-930
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-915	821125-915	821125-928	821125-930
ZGC	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901

\*RR: Rapid Resolution 3.5  $\mu$ m



## ZORBAX Solvent Saver

- Provide 60% reduction in mobile phase usage and waste generation compared to a 4.6 mm ID column
- Provide 2- to 3-fold signal-to-noise (S/N ratio) improvement
- Deliver optimal LC/MS performance at intermediate flow rates
- Can be used with most conventional LC instrument configurations without modification

ZORBAX Solvent Saver columns have a 3.0 mm internal diameter. This is ideal for reducing solvent usage by 50% over 4.6 mm ID columns. Solvent Saver columns are also ideal for LC/MS. With a typical flow rate of 0.5 ml/min, these columns are compatible with electrospray, atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) MS interfaces. These columns also improve sensitivity 2 to 3 times over 4.6 mm ID columns. Solvent Saver columns can be used with conventional HPLC instruments and are a good choice for cost effective analyses.

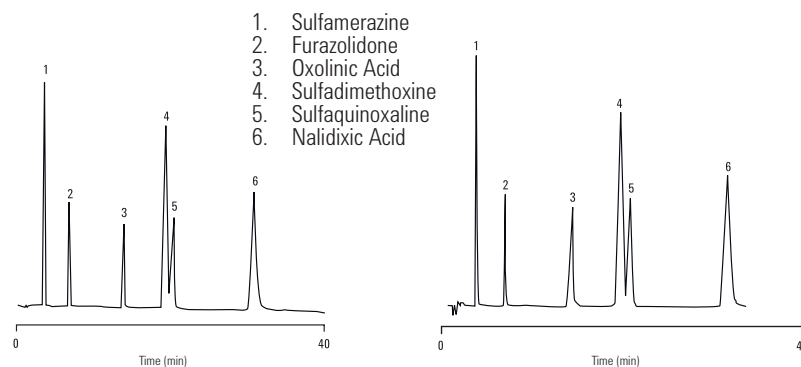
### Solvent Saver Columns Provide up to 60% Reduction in Solvent Use and Waste

**Column A:** ZORBAX SB-C18  
883975-902

**Column B:** ZORBAX SB-C18  
883975-302  
3.0 x 150mm, 5µm

Mobile Phase: 20% ACN: 80% 0.2 M Na<sub>2</sub>HPO<sub>4</sub>  
+ 0.1 M Citric Acid, pH 2.6

Temperature: Ambient  
Sample: Antibacterials



This separation of antibacterials on a 4.6 and 3.0 mm ID columns, shows that solvent use is reduced by 50% just by changing to the Solvent Saver column with no change in the chromatography. This reduces the cost of analyses dramatically.



## Solvent Saver Columns Increase Sensitivity

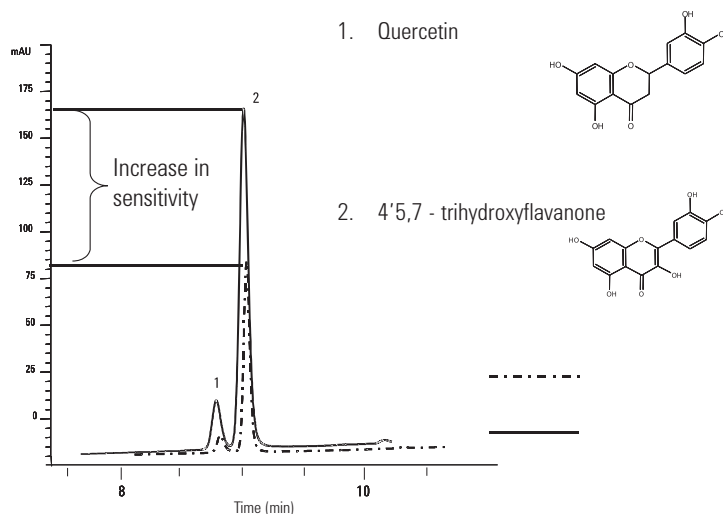
**Column A:** ZORBAX SB-C18  
863953-902  
4.6 x 150mm, 3.5µm

**Column B:** ZORBAX SB-C18  
863954-302  
3.0 x 150mm, 3.5µm

Mobile Phase: 25% Methanol:  
75% 0.4% Formic Acid

Detector: 254 nm

This figure shows sensitivity is increased 2-3 times with Solvent Saver columns compared to 4.6 mm ID columns when the same mass sample is injected. No change in the HPLC instrumentation is required to see the sensitivity improvements.



## Solvent Saver Columns are Ideal for LC/MS

**Column:** ZORBAX SB-C18  
861954-302  
3.0 x 100mm, 3.5µm

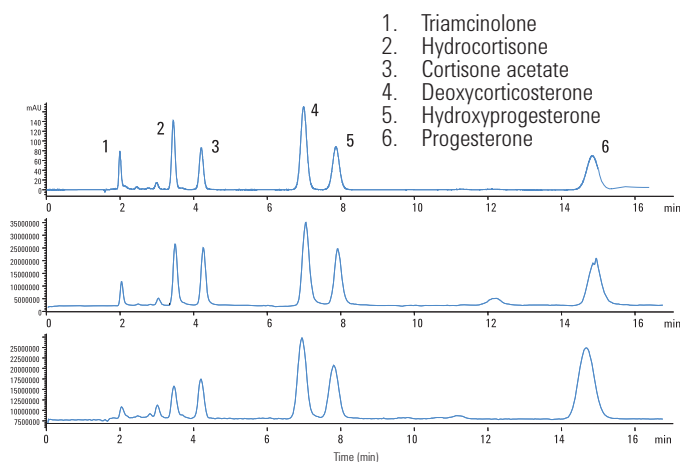
Mobile Phase: A: 70% Methanol+0.4% Formic Acid  
B: 30% Water+0.4% Formic Acid

Flow Rate: 0.425 mL/min

Detector: A: UV 254 nm  
B: Positive Ion APCI  
C: Positive Ion Electrospray

Sample: Steroids

Solvent Saver columns are ideal for LC/MS because the typical 0.5 mL/min flow rate allows samples to be evaluated and analyzed without changing columns when the MS interface is changed from electrospray to APCI.





### **ZORBAX 80Å StableBond**

Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-C3 USP L56	SB-Phenyl USP L11	SB-Aq
Solvent Saver	3.0 x 250	5	880975-302	880975-306	880975-305	880975-309	880975-312	880975-314
Solvent Saver	3.0 x 150	5	883975-302	883975-306	883975-305	883975-309	883975-312	883975-314
Solvent Saver Plus	3.0 x 150	3.5	863954-302	863954-306	863954-305	863954-309	863954-312	863954-314
Solvent Saver Plus	3.0 x 100	3.5	861954-302	861954-306	861954-305	861954-309	861954-312	861954-314

### **ZORBAX 300Å StableBond**

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
Solvent Saver Plus	3.0 x 150	3.5	863974-302	863974-306	863974-309	863974-309
Solvent Saver Plus	3.0 x 100	3.5		861973-306		

### **ZORBAX 80Å Eclipse XDB**

Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	XDB-Phenyl USP L11	XDB-CN USP L10
Solvent Saver	3.0 x 250	5	990967-302	990967-306	990967-312	990967-305
Solvent Saver	3.0 x 150	5	993967-302	993967-306	993967-312	993967-905
Solvent Saver Plus	3.0 x 150	3.5	963954-302	963954-306	963954-312	963954-305
Solvent Saver Plus	3.0 x 100	3.5	961967-302	961967-306	961967-312	
Solvent Saver Plus	3.0 x 75	3.5	966954-302			

### **ZORBAX 80Å Bonus-RP, and Rx**

Description	Size (mm)	Particle Size (µm)	Bonus-RP	Rx-C18 USP L1	Rx-C8 USP L7
Solvent Saver	3.0 x 250	5	880668-301	880967-302	880975-306
Solvent Saver	3.0 x 150	5	883668-301	883967-302	883975-306
Solvent Saver Plus	3.0 x 150	3.5	863668-301	863967-302	863954-306
Solvent Saver Plus	3.0 x 100	3.5	864668-301	861967-302	861954-306

### **ZORBAX 80Å Extend-C18**

Description	Size (mm)	Particle Size (µm)	Extend-C18
Solvent Saver	3.0 x 250	5	770450-302
Solvent Saver Plus	3.0 x 150	3.5	763954-302
Solvent Saver	3.0 x 150	5	773450-302
Solvent Saver Plus	3.0 x 100	3.5	764953-302
Solvent Saver Plus	3.0 x 50	3.5	735954-302

## ZORBAX MicroBore (1.0 mm ID)

- High sensitivity for small sample sizes
- Compatible with LC/MS interfaces
- Wide variety of bonded phases

MicroBore (1 mm ID) columns are often a good choice when sample sizes are limited. They can improve detection limits 5 times over 2.1 mm ID columns when the same sample mass is used. This increase in sensitivity can be critical. MicroBore columns use low flow rates (typically ~ 50  $\mu$ L/min). Therefore, these columns are ideal for use with detectors requiring low flow rates such as some mass spectrometers and with capillary LC systems.

MicroBore columns perform optimally with HPLC systems purchased or modified for microbore use. A wide variety of bonded phases are available for use up to 400 bar including StableBond SB-C18, SB-C8, 300SB-C18; Eclipse XDB-C18 and XDB-C8; Bonus RP, Extend C-18; and Poroshell columns. Guard columns are also now available with an adjustable tube stop depth to provide a perfect zero dead volume connection every time. A selection of bonded phases are available in 1.8  $\mu$ m and a higher pressure format. See the product listings for more detail.

### Separation of a Tryptic Digest on ZORBAX MicroBore 300SB-C18

**Column:** ZORBAX 300SB-C18  
863630-902  
1.0 x 150mm, 3.5 $\mu$ m

**Mobile Phase:** Gradient: 2-60% B in 60 Min.  
A: 0.1% TFA  
B: 0.075% TFA/80% ACN

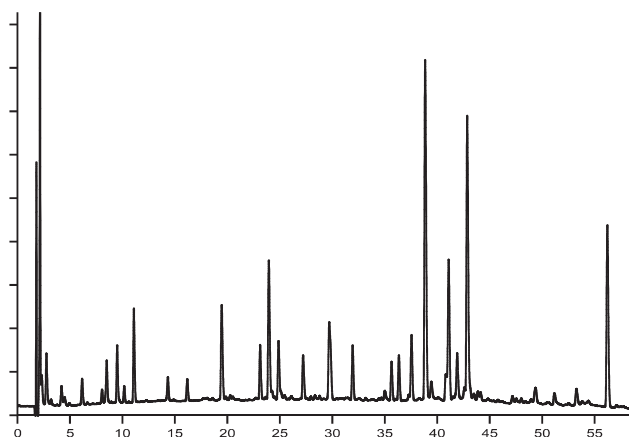
**Flow Rate:** 50  $\mu$ L/min

**Temperature:** 50°C

**Detector:** 215 nm

**Sample:** 2  $\mu$ L  
Tryptic Digest of rhGH

This example of a tryptic digest separated on a MicroBore column demonstrates the high sensitivity and resolution possible with 1.0 mm ID columns.



### ZORBAX MicroBore (1.0 mm ID)

Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	300SB-C18 USP L1	300SB-C8 USP L7	SB-CN USP L10
MicroBore	1.0 x 250	5			861630-902		
MicroBore RR*	1.0 x 150	3.5	863600-902	863600-906	863630-902	863630-906	
MicroBore RR*	1.0 x 50	3.5	865600-902	865600-906	865630-902	865630-906	
MicroBore RR*	1.0 x 30	3.5	861600-902	861600-906			
MicroBore RRHT**	1.0 x 50	1.8	822600-902	822600-906			822600-905
MicroBore Guard Cartridges, 3/pk	1.0 x 17	5	5185-5920	5185-5920	5185-5920	5185-5920	
Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7	Bonus-RP	Extend-C18	
MicroBore RR*	1.0 x 150	3.5	963600-902	963600-906	863608-901	763600-902	
MicroBore RR*	1.0 x 50	3.5	965600-902	965600-906	865608-901	765600-902	
MicroBore RR*	1.0 x 30	3.5	961600-902	961600-906	861608-901	761600-902	
MicroBore RRHT, 600 bar**	1.0 x 100	1.8	928600-902	928600-906		728600-902	
MicroBore Guard Cartridges, 3pk	1.0 x 17	5	5185-5921	5185-5921	5185-5922	5185-5923	
Description	Size (mm)	Particle Size (µm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18	
MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902	
MicroBore Guard Cartridge, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968		

\*RR: Rapid Resolution 3.5 µm

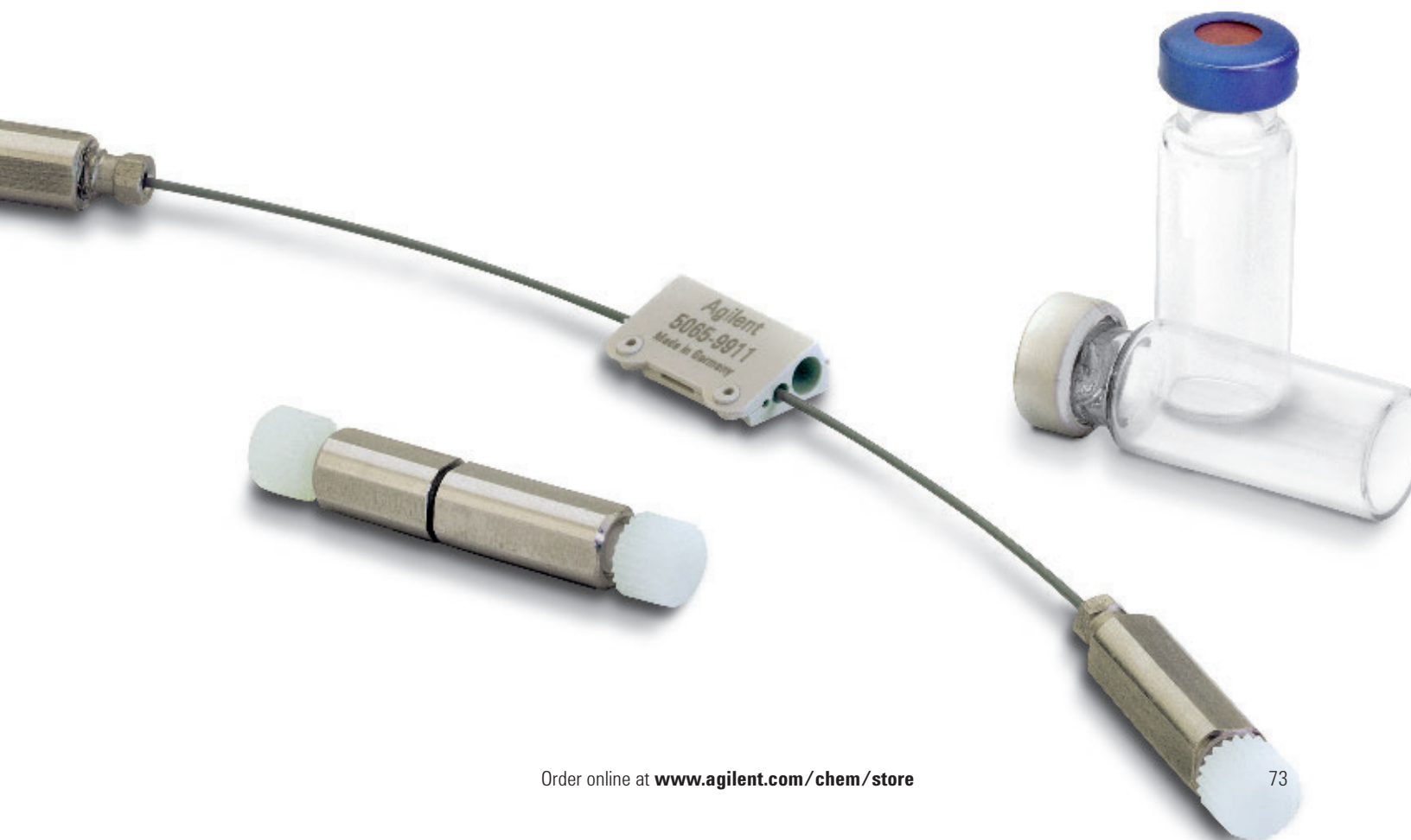
\*\*RRHT: Rapid Resolution HT 1.8 µm

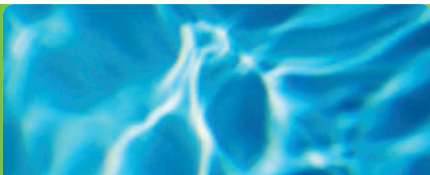


## ZORBAX Capillary and Nano

- Highest sensitivity for your smallest sample sizes
- Compatible with all LC/MS interfaces
- Internal diameters of 0.5, 0.3, 0.1, and 0.075 mm
- Packings/phases for both small and large molecules (80Å and 300Å pore sizes, respectively)
- Ideal for 1-D and 2-D (proteomics) applications

Agilent ZORBAX capillary (0.5, 0.3 mm ID) and nano (0.1, 0.075 mm ID) columns are now available in a wide variety of phases, pore sizes, and dimensions. These columns are ideal for very sample-limited applications because they provide enhanced sensitivity by reducing on-column sample dilution. This high sensitivity can be provided with exceptional reproducibility using Agilent columns and low dispersion HPLC instruments. The fastest growing application for capillary and nano columns is 2-D LC/MS for complex proteomics samples. Agilent provides all the columns needed for the 2-D separation – the SCX columns for the first dimension, the reversed-phase trapping column, and the reversed-phase column for the second dimension.





## Separation of Peptides on Capillary Columns

**Column A:** ZORBAX 300SB-C8  
5065-4460

**Column B:** ZORBAX Eclipse XDB-C18  
5064-8291

**Column C:** ZORBAX Eclipse XDB-C18  
5064-8291

**Column D:** ZORBAX SB-C18  
5064-8255

**Column E:** ZORBAX 300SB-C18  
5064-8267

**Column F:** ZORBAX 300Extend-C18  
5065-4464

**Mobile Phase:** Water + 0.05% TFA, pH = 2.2 = A  
Acetonitrile + 0.045% TFA = B  
Gradient 0.5%B/min: at 0 min = 1%B,  
at 60 min = 31%B, at 70 min = 50%B,  
at 75 min = 85%B,  
at 80 min = 85%B, at 81 min = 1%B,  
at 110 min = 1%B

**Flow Rate:** 5.5 µl/min

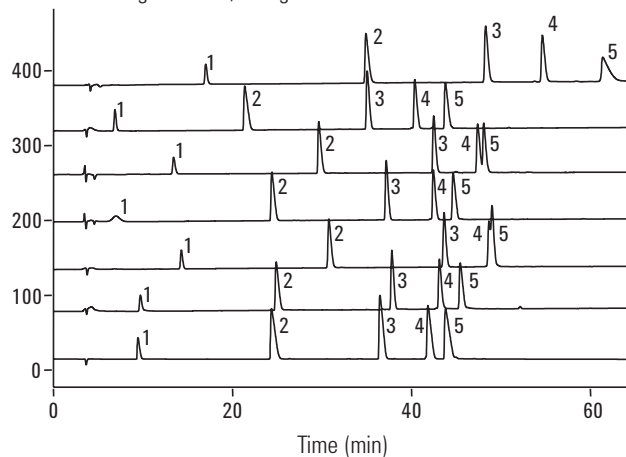
**Low Solvent Consumption:**  
200-500 µl/min

**Temperature:** 30°C

**Detector:** 206/10 nm, ref 450/80 nm

**Sample:** 0.1 µl, automatic delay volume reduction was  
activated Peptides

1. Gly-Tyr, 5 ng/100 nl
2. Val-Tyr-Val, 20 ng/100 nl
3. Met Enkephalin, 28 ng/100 nl
4. Low Enkephalin, 20 ng/100 nl
5. Angiotensin II, 20 ng/100 nl



This example shows a peptide standard mixture separated on a variety of the ZORBAX capillary columns. These chromatograms demonstrate the wide range of selectivities available, which can be used to optimize your specific separation.

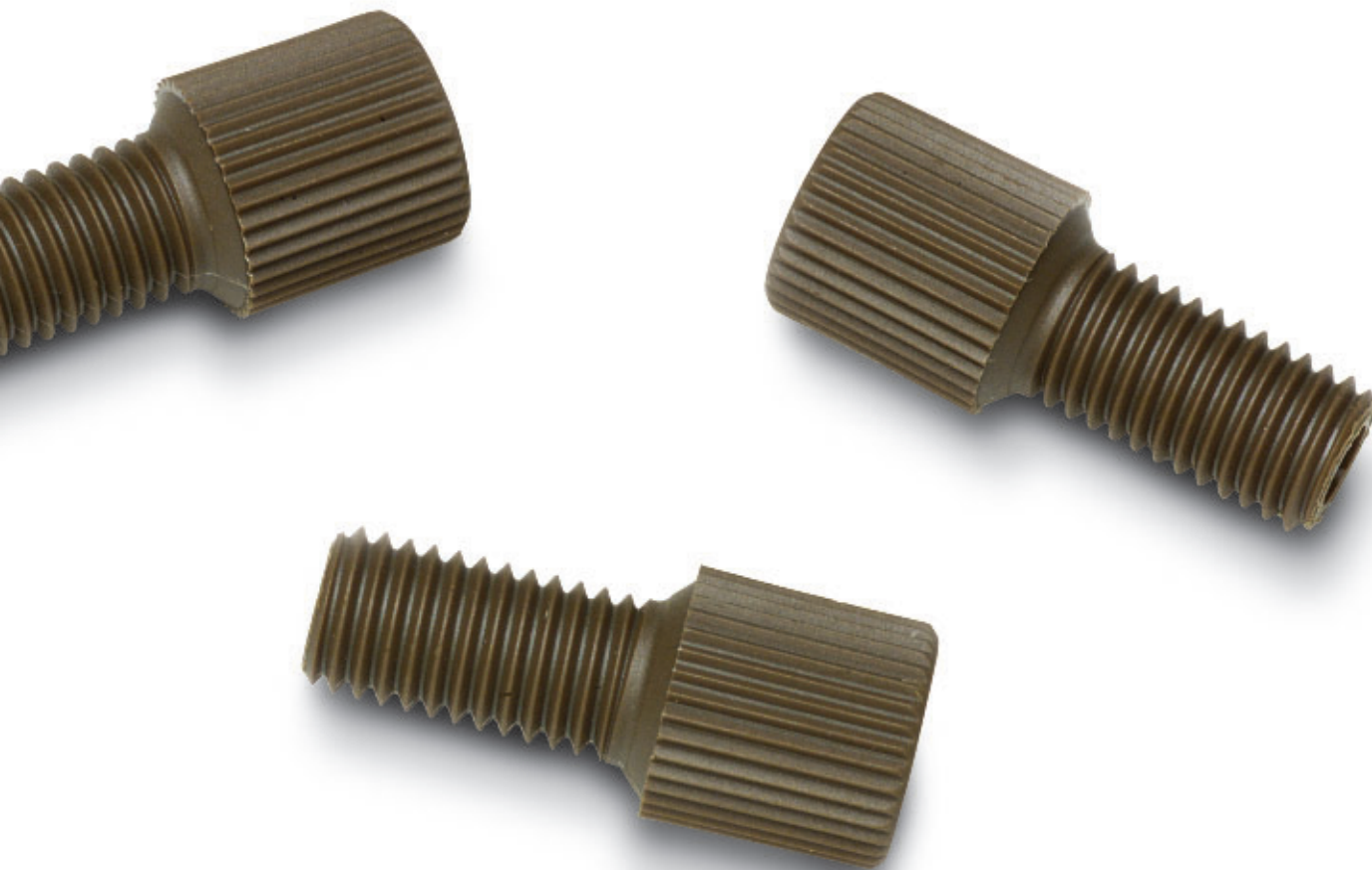
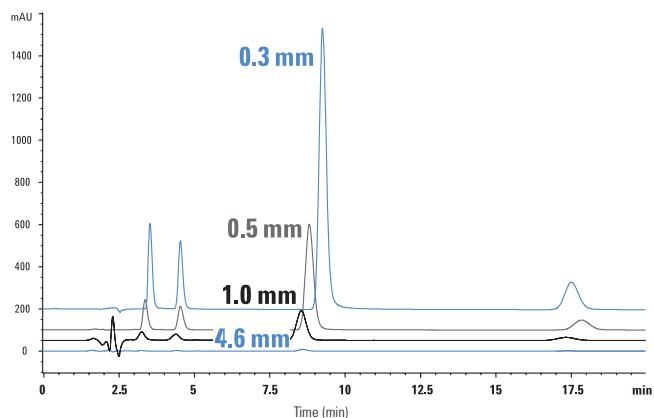


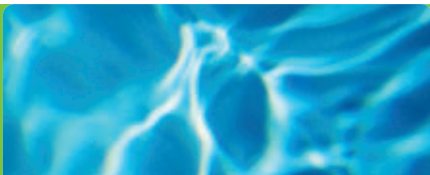


## High Sensitivity with Capillary Columns

**Column:** ZORBAX SB-C18  
 5064-8255  
 0.3 x 150mm, 5 $\mu$ m  
**Column:** ZORBAX SB-C18  
 5064-8256  
 0.5 x 150mm, 5 $\mu$ m  
**Column:** ZORBAX SB-C18  
 863600-902  
 1.0 x 150mm, 3.5 $\mu$ m  
**Column:** ZORBAX SB-C18  
 883975-902  
 4.6 x 150mm, 5 $\mu$ m  
**Sample:** 200 ng Biphenyl

Sample-limited applications require capillary column dimensions to minimize on-column sample dilution and to enhance sensitivity. The 0.3 mm capillary in this example provides 100 times more sensitivity than the standard 4.6 mm column. Nanobore (0.1 mm-0.075 mm ID) columns can provide up to 2000 times more sensitivity for your most limited sample applications.





### Excellent Column-to-Column Reproducibility with Agilent Capillary Columns

**Column:** ZORBAX SB-C18

**5064-8256**

**0.5 x 150mm, 5µm**

Mobile Phase: A: 75% H<sub>2</sub>O with 0.4% formic acid  
B: 25% MeOH with 0.4% formic acid

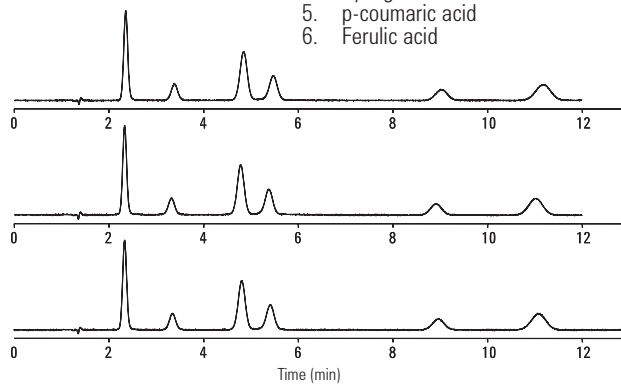
Flow Rate: 20 µL/min

Temperature: 25°C

Sample: 0.1 µL

Polar organic acids

1. Protocatechuic acid
2. Chlorogenic acid
3. Caffeic acid
4. Syringic acid
5. p-coumaric acid
6. Ferulic acid

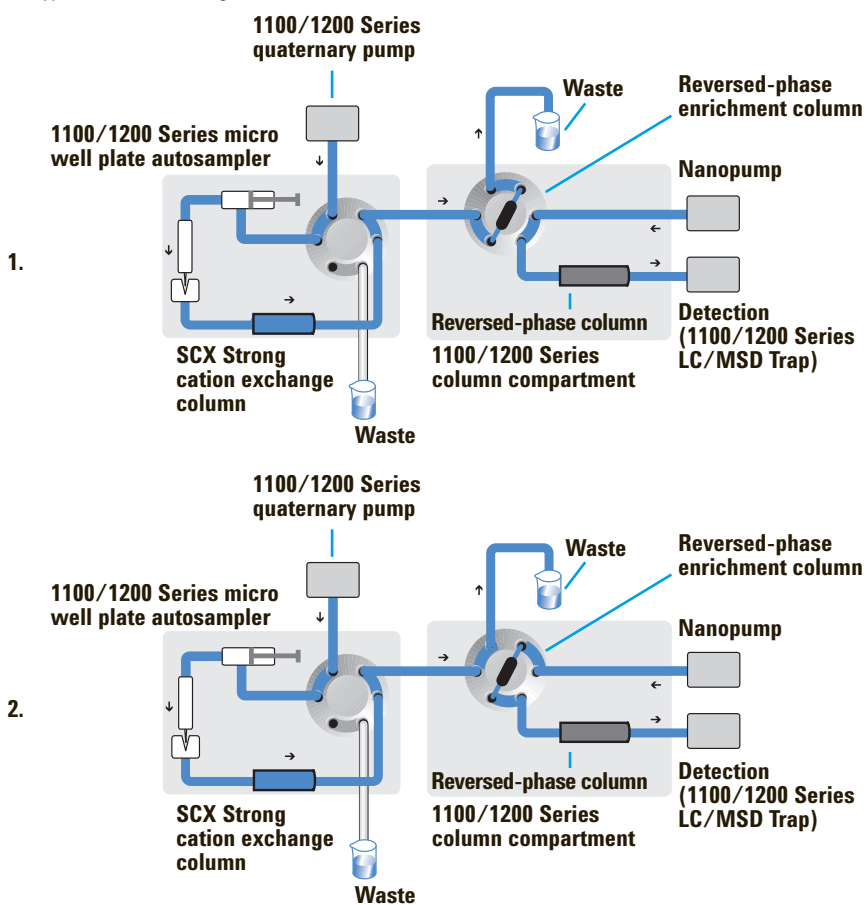


Excellent reproducibility is seen for a separation of polar organic acids on three different StableBond-C18, 0.5 x 150 mm, 5 µm columns. Retention (k) varied less than 0.8% RSD and selectivity (α) varied less than 0.4% RSD.



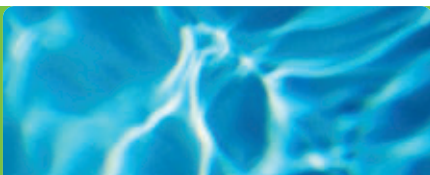
## 2D LC/MS Analyses Using ZORBAX Capillary and Nano LC Columns

Typical Column Configuration for 2D HPLC



Flow path of the Agilent nanoflow proteomic solution system

1. Sample loading, elution from SCX and trapping on enrichment column
2. Valve switch in column compartment, elution from enrichment column, separation on RP and MS-analysis



## Proteins in a Complex Sample by 2-D HPLC with Nano HPLC Columns

**Column:** ZORBAX 300SB-C18  
5065-9913

**Column:** ZORBAX 300SB-C18  
5065-9911  
0.075 x 150mm, 3.5µm

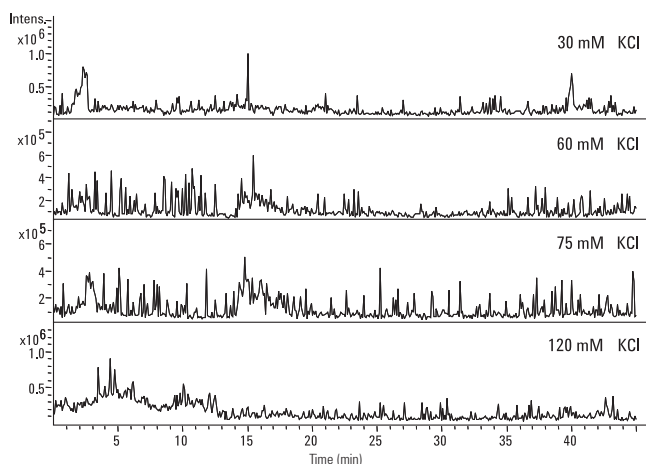
**Mobile Phase:** Quaternary Pump: 3% Acetonitrile/0.1% Formic Acid  
Nanopump: A = Water, 0.1% Formic Acid, B = ACN, 0.1% Formic Acid

**Flow Rate:** Quaternary Pump: 30 µL/min  
Nanopump: 300 nL/min

**Gradient:** Quaternary Pump: Isocratic  
Nanopump:  
6 min = 3%B, 120 min = 60%B,  
125 min = 80%B,  
130 min = 80% B, 131 min = 3%B,  
140 min = 3% B

**MS Conditions:** Source: Nano ESI, drying gas flow: 5L/min, drying gas temp.: 225°C.  
Ion Trap: Skim: 1:35 V, cap exit offset: 115 V, octopole 1:12 V, octopole 2:3.5 V, trap drive: 80 V. ICC: on, averages: 4, max accu time: 150 ms; target 60.000, ion mode positive, MS/MS mode.

**Sample:** Tryptic Digest of bovine serum albumin  
Volume: 1 to 8 µL  
Salt Step Elution: 8ml of 10 mM-100 mM KCl (10 mM increments), 125 mM, 150 mM, 200 mM, 300 mM, 500 mM, 1M.



Tryptic digest of bovine serum albumin (BSA). The base peak chromatograms show a selection of fractions from a 2-dimensional HPLC separation. Single chromatograms represent peptides from BSA eluting at a given salt concentration followed by enrichment and reversed phase chromatography.



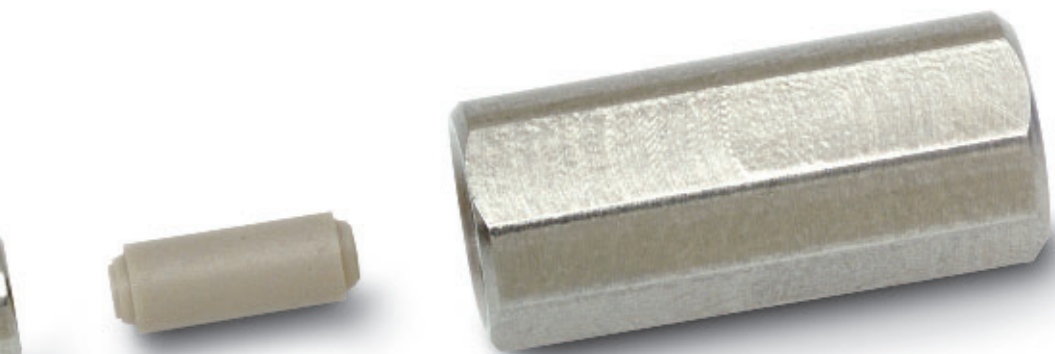
**ZORBAX Capillary and Nano**

Description	Size (mm)	Particle Size (µm)	SB-C18	Eclipse XDB-C18	300SB-C18	300SB-C8	Poroshell 300SB-C8	300Extend C18	Bio-SCX Series II
Capillary	0.8 x 50	3.5							5065-9942
Capillary	0.5 x 250	5	5064-8258	5064-8286	5064-8266				
Capillary	0.5 x 150	5	5064-8256	5064-8287	5064-8264				
Capillary	0.5 x 75	5					5065-4468		
Capillary	0.5 x 35	5	5064-8254	5064-8296	5064-8294				
Capillary RR*	0.5 x 35	3.5	5064-8260	5064-8298	5065-4459				
Capillary	0.3 x 250	5	5064-8257	5064-8269	5064-8265				
Capillary	0.3 x 150	5	5064-8255	5064-8291	5064-8263				
Capillary	0.3 x 35	5	5064-8253	5064-8297	5064-8295				
Capillary	0.3 x 35	3.5							5065-9912
Capillary RR*	0.3 x 150	3.5	5064-8261	5064-8271	5064-8267	5065-4460		5065-4464	
Capillary RR*	0.3 x 100	3.5			5064-8259	5065-4461		5065-4465	
Capillary RR*	0.3 x 75	3.5			5064-8270	5065-4462		5065-4466	
Capillary RR*	0.3 x 50	3.5			5064-8300	5065-4463		5065-4467	
Replacement Screens, 10/pk			5065-4427	5065-4427	5065-4427	5065-4427	5065-4427	5065-4427	

\*RR: Rapid Resolution 3.5 µm

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7
Nano RR*	0.1 x 150	3.5	5065-9910	
Nano RR*	0.075 x 150	3.5	5065-9911	
Nano RR*	0.075 x 50	3.5	5065-9924	5065-9923
Trap/Guard, 5/pk	0.3 x 5	5	5065-9913	5065-9914
Trap/Guard Hardware kit			5065-9915	5065-9915

\*RR: Rapid Resolution 3.5 µm







## ZORBAX PrepHT

- Easy scale-up from analytical to preparative scale with ZORBAX phases
- Fast preparative separations, up to 2000 mg
- 5 to 7  $\mu\text{m}$  particles for high efficiency and high yield
- Easy to install finger tight connections seal up to 5000 psi/350 bar

High purity, high recovery and high throughput can be easily achieved with Agilent ZORBAX PrepHT columns. These are available in a variety of bonded phases – Eclipse XDB, StableBond, Bonus-RP, and Extend-C18 – for optimized resolution and loadability under any conditions.

ZORBAX PrepHT columns are packed with 5 and 7  $\mu\text{m}$  particle sizes for very high resolution. The high resolution allows high loadability, high yield, and high purity of compounds. The larger diameter columns and mechanically stronger ZORBAX particles allow for flow rates up to 100 ml/min, thus increasing throughput.

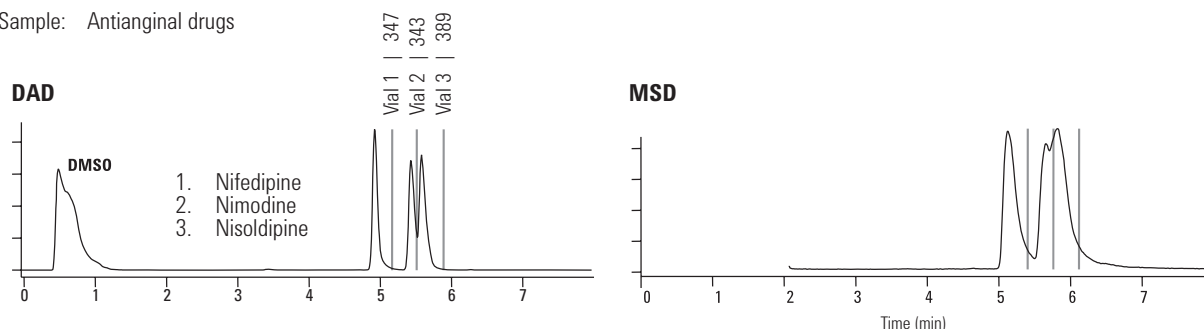
ZORBAX PrepHT columns are designed for rapid scale-up from analytical to preparative scale without losing resolution. For complex separations on larger columns (21.2 mm ID, 150 mm length and longer), Agilent has carefully chosen the 7  $\mu\text{m}$  particle size to achieve a balance between high efficiency and high loadability.





### High Purity and High Recovery with ZORBAX PrepHT Columns

Sample: Antianginal drugs

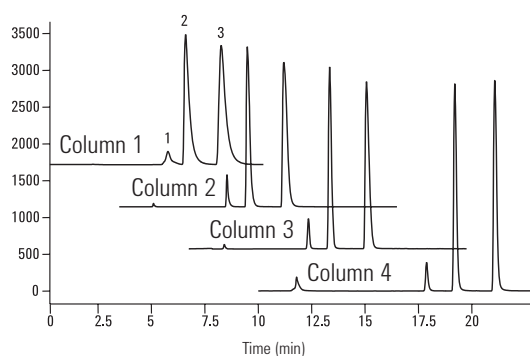


Mass-based fraction collection using ZORBAX SB-C18 column shows high purity and high recovery of each compound (Application Note publication number 5988-7113EN). The separation of the three antianginal drugs was successfully done in a single run with high recovery and >90% purity. Separations up to 2000 mg are possible depending on the complexity of separation.

ZORBAX PrepHT columns are designed for rapid scale-up from analytical to preparative scale without losing resolution. For complex separations for larger columns (21.2 mm ID and higher), 150 mm length and higher), Agilent has carefully chosen the 7  $\mu$ m particle size to achieve a balance between high efficiency and high loadability.

### Scale-Up from Analytical to Prep ZORBAX SB-C18 Columns Using the Same Pump

Column	Size	Flow (mL/min)	Injection ( $\mu$ L)	Detector Cell	Part No.
Column 1	50 x 150 mm	100	2200	0.3 mm quartz	Custom Column
Column 2	21.2 x 150 mm	18	400	0.3 mm quartz	877150-102
Column 3	9.4 x 150 mm	3.5	80	0.3 mm quartz	883975-202
Column 4	4.6 x 150 mm	0.85	2.0	3 mm SST	883975-902









Using the same 1100 pump, a scale-up from 4.6 mm to 50 mm ID was possible without any loss of resolution. This increase throughput by reducing the time required for redeveloping and adjusting the method.







1. Theobromine
2. Theophylline
3. Caffeine

### ZORBAX PrepHT StableBond

#### ZORBAX PrepHT 80ÅStableBond

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-C3 USP L56	SB-Phenyl USP L11
	PrepHT Cartridge*	21.2 x 250	7	877250-102	877250-106	877250-114	877250-105	877250-112
		21.2 x 150	7	877150-102	877150-106	877150-114		
		21.2 x 150	5	870150-902	870150-906	870150-914		
		21.2 x 100	5	870100-902	870100-906	870100-914		
		21.2 x 50	5	870050-902	870050-906	870050-914		
	PrepHT Guard Cartridge, 2/pk**	17 x 7.5	5	820212-920	820212-915	820212-933	820212-933	820212-915







#### ZORBAX PrepHT 300ÅStableBond

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
	PrepHT Cartridge*	21.2 x 250	7	897250-102	897250-106	897250-109	897250-105
		21.2 x 150	7	897150-102	897150-106	897150-109	
		21.2 x 150	5	895150-902	895150-906	895150-909	
		21.2 x 100	5	895100-902	895100-906	895100-909	
		21.2 x 50	5	895050-902	895050-906	895050-909	
	PrepHT Guard Cartridge, 2/pk**	17 x 7.5	5	820212-921	820212-918	820212-924	820212-924

\*Requires PrepHT endfittings 820400-901

\*\*Requires Guard hardware kit (820444-901) which contains guard column fitting, seal insertion tool, and 1 polymeric seal







### ZORBAX PrepHT Eclipse XDB

Hardware	Description	Size (mm)	Particle Size (µm)	XDB-C18 USP L1	XDB-C8 USP L7
	PrepHT Cartridge*	21.2 x 250	7	977250-102	977250-106
		21.2 x 150	7	977150-102	977150-106
		21.2 x 150	5	970150-902	970150-906
		21.2 x 100	5	970100-902	970100-906
		21.2 x 50	5	970050-902	970050-906
	PrepHT Guard Cartridge, 2/pk**	17 x 7.5	5	820212-925	820212-926

\*Requires PrepHT endfittings 820400-901

\*\*Requires Guard hardware kit (820444-901) which contains guard column fitting, seal insertion tool, and 1 polymeric seal


**ZORBAX PrepHT Bonus-RP and Extend-C18**

Hardware	Description	Size (mm)	Particle Size (µm)	Bonus-RP	Extend-C18
	PrepHT Cartridge*	21.2 x 250	7	878250-101	
		21.2 x 150	7	878150-101	
		21.2 x 150	5	868150-901	770150-902
		21.2 x 100	5	868100-901	770100-902
		21.2 x 50	5	868050-901	770050-902
	PrepHT Guard Cartridge, 2/pk**	17 x 7.5	5	820212-928	820212-930

\*Requires PrepHT endfittings 820400-901




\*\*Requires Guard hardware kit (820444-901) which contains guard column fitting, seal insertion tool, and 1 polymeric seal

**ZORBAX PrepHT Original**

Hardware	Description	Size (mm)	Particle Size (µm)	ODS (C18) USP L1	C8 USP L7	CN USP L10	NH2 USP L8	SIL USP L3
	PrepHT Cartridge*	21.2 x 250	7	877952-102	877952-106	877952-105	877952-108	877952-101

\*Requires PrepHT endfittings 820400-901




**ZORBAX PrepHT Rx-SIL**

Hardware	Description	Size (mm)	Particle Size (µm)	SIL USP L3	Rx-C18 USP L1
	PrepHT Cartridge*	21.2 x 250	7	877250-101	
		21.2 x 250	7		877967-102
	PrepHT Guard Cartridge, 2/pk**	17 x 7.5	5	820212-919	820212-914

\*Requires PrepHT endfittings 820400-901

\*\*Requires Guard hardware kit (820444-901) which contains guard column fitting, seal insertion tool, and 1 polymeric seal

**ZORBAX PrepHT Accessories**

Hardware	Description	Part No.
	Guard Cartridge Hardware	820444-901
	PrepHT Endfittings, 2/pk	820400-901
	Replacement Seals	820385-901



## Agilent Prep LC Columns



- High loadability for maximum sample purification
- Easy scalability from 4.6 mm ID up to 50 mm ID for rapid method development
- High throughput 21.2 mm ID cartridges for fast purification
- Exceptional column stability and loadability up to pH 10

Agilent Prep LC columns are designed for high loadability to purify milligram to gram quantities of products. Preparative sized columns are available in 21.2, 30, and 50 mm internal diameters with lengths ranging from 50-250 mm. Columns are available in 5 and 10 µm particle sizes with very high efficiency in every dimension. These column choices accommodate almost every preparative sample.

Agilent Prep 21.2 mm ID columns are available with Agilent's Preparative Cartridge Hardware. This reliable cartridge hardware makes it simple to use columns with different lengths to increase sample load. Guard columns are easily integrated onto these columns providing superior protection of the analysis column. Analytical sized 4.6 mm ID scalar columns are available for method development and optimization prior to scaling up to larger columns. Bulk material is also available.

Agilent Prep columns are available in a C18 bonded phase suitable for purification of a wide variety of non-polar and polar compounds. Unbonded silica columns are also available.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
C18	100Å	400 m <sup>2</sup> /g	60°C*	2.0-10.0	Single	24%
Silica	100Å	400 m <sup>2</sup> /g	**	1.0-8.0	N/A	N/A

\*Temperature limits are 60°C up to pH 8, 40°C from pH 8-10.

\*\*Temperature limits for bare silica are determined by the pH of the mobile phase.

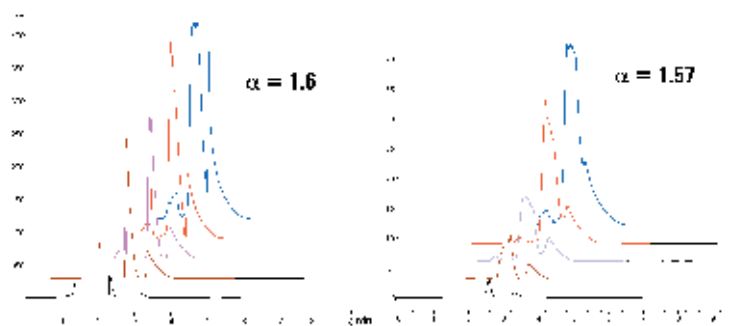
## Superior Loadability on Agilent Prep C18 with Basic Compounds

**Column:** Agilent Prep-C18  
443905-902  
4.6 x 150mm, 5µm

**Mobile Phase:** 50% 0.1%TFA:  
50% ACN

**Flow Rate:** 1 mL/min

**Sample:** 10 µL  
Doxepin/Amitriptyline  
0.5–50 mg/mL



Agilent Prep Columns show better resolution and loadability than competitor columns.

## Steroids: Easy Scalability Using Agilent Prep Columns

**Column A:** Agilent Prep-C18  
443905-902  
4.6 x 150mm, 5µm

**Column B:** 443905-102  
21.2 x 150mm, 5µm

**Column C:** 413910-302  
30 x 150mm, 10µm

**Column D:** 413910-502  
50 x 150mm, 10µm

**Mobile Phase:** 55% Water:45% ACN

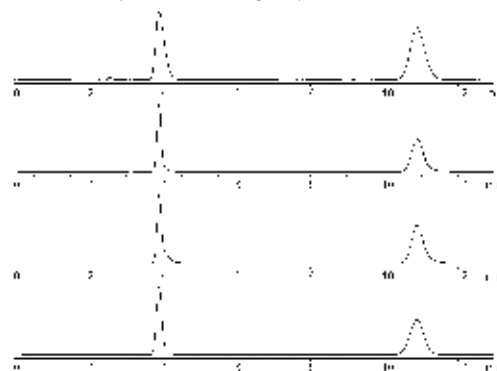
**Flow Rate:** 0.7 mL/min  
14.87 mL/min  
29.77 mL/min  
85.37 mL/min

**Temperature:** Ambient

**Detector:** 240 nm

**Sample:** 2 µL  
42.4 µL  
170 µL  
488 µL











1. Hydrocortisone
2. Testosterone (in MeOH @ 1mg/mL)



Agilent Prep-C18 shows excellent scalability making method transfer simple and predictable.

## HPLC Columns for Special Applications

### Agilent Prep LC Columns

Hardware	Description	Size (mm)	Particle Size (µm)	C18	Silica
<b>Standard Columns (no special hardware required, 400 bar)</b>					
	Scalar	4.6 x 250	5	440905-902	440905-901
	Scalar	4.6 x 150	5	443905-902	443905-901
	Scalar	4.6 x 100	5	449905-902	449905-901
	Scalar	4.6 x 50	5	446905-902	446905-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)*</b>					
	PrepHT	21.2 x 250	10	410910-102	410910-101
	PrepHT	21.2 x 150	10	413910-102	413910-101
	PrepHT	21.2 x 50	10	446910-102	
	PrepHT	21.2 x 150	5	443905-102	443905-101
	PrepHT	21.2 x 100	5	449905-102	449905-101
	PrepHT	21.2 x 50	5	446905-102	446905-101
	PrepHT Endfittings, 2/pk			820400-901	820400-901
<b>Standard Columns (no special hardware required, 400 bar)</b>					
	Prep 30	30 x 250	10	410910-302	410910-301
	Prep 30	30 x 150	10	413910-302	413910-301
	Prep 30	30 x 100	10	419910-302	419910-301
	Prep 30	30 x 100	5	449905-302	449905-301
	Prep 30	30 x 50	5	446905-302	446905-301
	Prep 50	50 x 250	10	410910-502	410910-501
	Prep 50	50 x 150	10	413910-502	413910-501
	Prep 50	50 x 100	10	419910-502	419910-501
	Prep 50	50 x 100	5	449905-502	449905-501
	Prep 50	50 x 50	5	446905-502	446905-501
<b>Guard Columns (hardware required)</b>					
	PrepHT Guard Cartridges, 2/pk	21.2 x 10	10	420212-902	420212-901
	Guard Cartridge Hardware			820444-901	820444-901
	PrepHT External Guard Hardware Kit			420420-901	420420-901
	Bulk Packing (1kg)		10	420910-902	420910-901

\*All PrepHT cartridge columns require hardware kit 820400-901. If a guard column is desired for the 21.2 mm ID columns then the PrepHT Guard Hardware Kit, 820444-901, is also required. If the guard column is used on a 30 mm ID column then the external guard column hardware kit, 420420-901, is required.





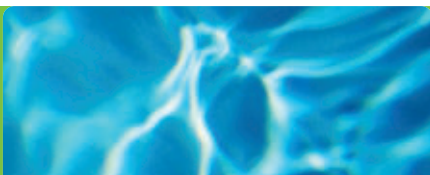
## Ultron Chiral columns

Separate a wide range of chiral compounds and develop chiral methods more quickly.

Ultron Chiral columns are immobilized protein columns that feature numerous chiral recognition sites for enantiomeric separations of dozens of chiral compounds. They are engineered with two complementary protein-based chiral stationary phases, making them an excellent choice for the HPLC separation of enantiomers without derivatization – including a growing number of drug substances of interest.

- Broad compatibility and long lifetime with aqueous, buffered, and organic mobile phases.
- Recognize ionic, polar, hydrogen-bonding, and hydrophobic groups – as well as three-dimensional molecular structures.
- Method development guidelines are included in each column box, saving you time and eliminating guesswork.





### Separation of Enantiomers of Fluoxetine (Prozac)

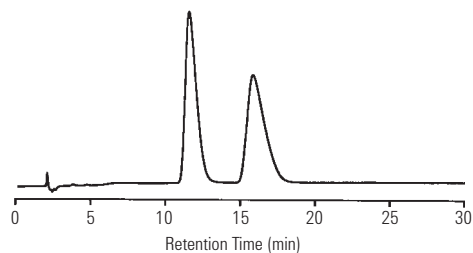
**Column:** **Ultron ES-OVM Chiral**  
**702111651**  
**4.6 x 150mm, 5µm**

Mobile Phase: 25:75 (v/v) EtOH / 20 mM KH<sub>2</sub>PO<sub>4</sub>, pH 5.5  
(adjusted with NaOH)

Temperature: Ambient

Detector: UV (225 nm)

Sample: Mixture Fluoxetine (Prozac) enantiomers



*Courtesy of D. S. Risley and V. S. Sharp of Lilly Research Laboratories, Eli Lilly and Co.*

### Separation of Ethiazide (diuretic drug) on ULTRON ES-OVM Column

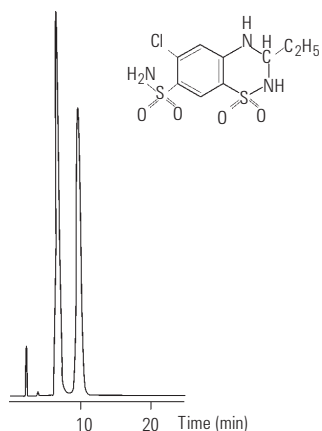
**Column:** **Ultron ES-OVM Chiral**  
**702111651**  
**4.6 x 150mm, 5µm**

Mobile Phase: 20 mM KH<sub>2</sub>PO<sub>4</sub> (pH 4.6)

Flow Rate: 1 mL/min

Temperature: 25°C

Detector: 220 nm



Description	Particle		ES-OVM	ES-Pepsin
	Size (mm)	Size (µm)		
Semi-Prep	10 x 150	5	722111723	
Analytical	4.6 x 250	10	724111653	
Analytical	4.6 x 150	5	702111651	822111651
Analytical, with Guard	4.6 x 150	5	702111651A	822111631A
Narrow Bore	2.0 x 150	5	702111610	
Guard Column	4.0 x 10	5	712111630	832111630





## ZORBAX Columns for Bioanalytical Chromatography

Is your lab ready for the ever-increasing number — and variety — of bioanalytical HPLC applications?

Basic peptide separations. High-sensitivity, high-resolution amino acid analyses. Fast-size exclusion separations of antibodies. The number of bioanalytical HPLC applications continues to grow at an unprecedented rate. And Agilent's durable — and reproducible — line of ZORBAX columns can help you meet your lab's evolving needs for performance and speed.

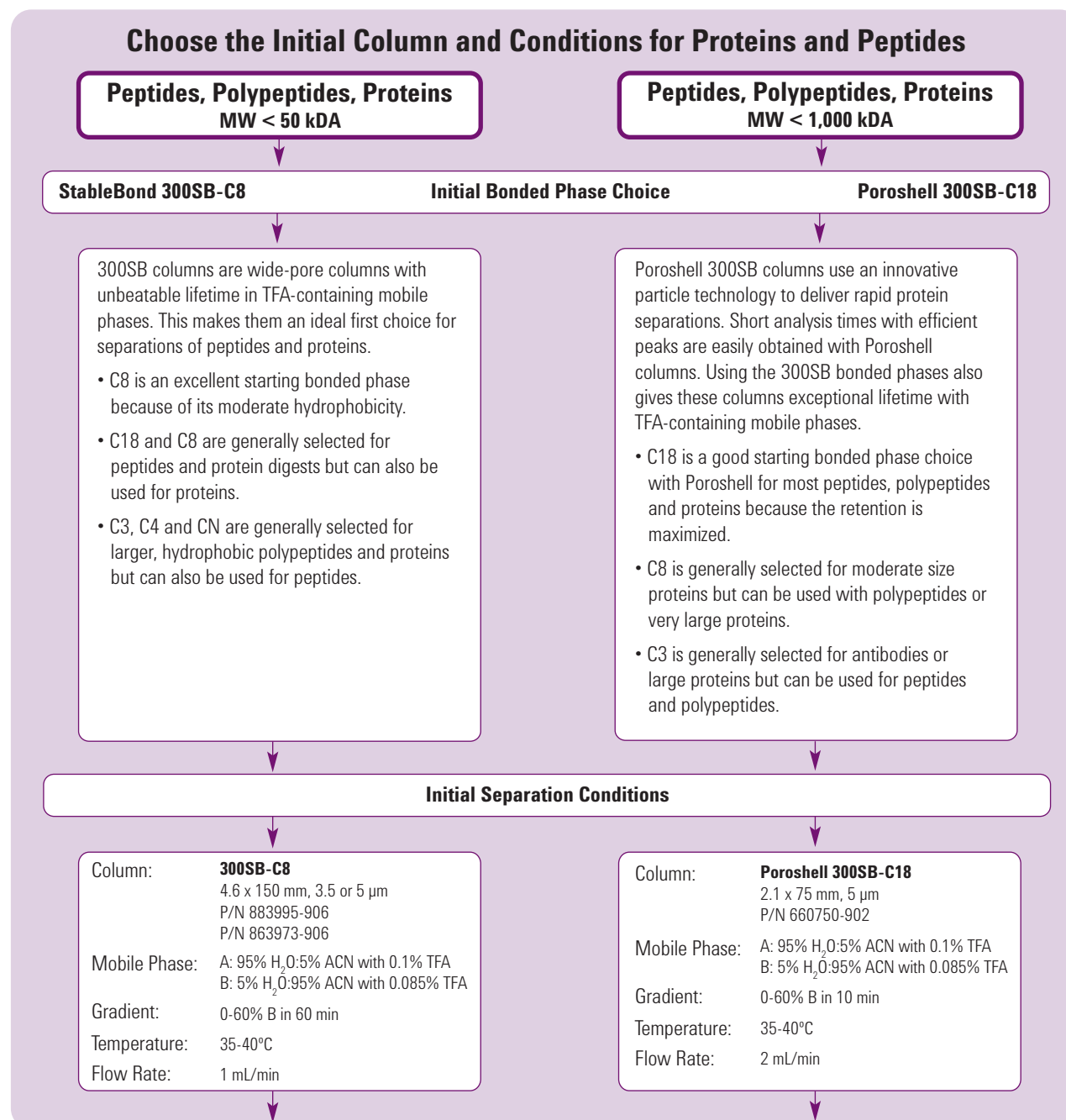
On the following pages, you will find key facts and specifications about columns for reversed-phase HPLC, size exclusion chromatography, ion-exchange chromatography, and hydrophobic interaction chromatography, including:

- **ZORBAX wide-pore 300StableBond columns** — deliver long lifetime, high resolution, and good peak shape for peptides and proteins. And they are compatible with common mobile phases containing TFA.
- **ZORBAX wide-pore 300Extend columns** — ideal for LC/MS of proteins and peptides at pH 10.
- **ZORBAX Poroshell HPLC columns** — feature a unique, powerful particle for fast, high-resolution separations of proteins, polypeptides, and antibodies.
- **ZORBAX Eclipse Amino Acid Analysis (AAA) HPLC columns** — a high-efficiency choice for rapid amino acid separation! These columns make it possible to reduce your injection-to-injection analysis time to 14 minutes (7.5 cm column length) and 24 minutes (15 cm column length).
- **ZORBAX GF-250/450 Size Exclusion HPLC columns** — a rugged, reproducible column specially designed for size separations of proteins. Size exclusion columns are compatible with organic modifiers and mobile phase denaturants, so you can eliminate protein aggregation for proper size determination. And they have a separation range of 4,000 - 900,000 for globular proteins when using a GF-250 and GF-450 column in series.
- **ZORBAX Ion Exchange columns** — available as Strong Anion Exchange (SAX), Strong Cation Exchange (SCX), and Bio-SCX.



# ZORBAX Strategy for Reversed-Phase Method Development of Proteins and Peptides

This ZORBAX Column Selection Strategy for Proteins and Peptides provides some critical details on method development for proteins or polypeptides. For small peptides, Molecular Weight < 2000, please follow the method development strategy for small and large molecules in the reference section of this guide. For efficient separations of large molecules, columns with a wide-pore size (300Å) are required. For method development of larger peptides and proteins, review the suggested guidelines outlined below. Wide-pore column choices are described in the following section of this Column Selection Guide.



### Start at low pH with simple aqueous/organic gradient

Typically a Water/Acetonitrile with 0.1% TFA gradient is used to elute all components of interest. A typical high-resolution gradient on a 300Å pore size column requires 30-60 min. A Poroshell column requires a shorter analysis time and a higher flow rate and still provides exceptional resolution. Then to improve resolution, increase the gradient time, decrease column length, or increase flow rate.

### Optimize sample solubility

For best peak shape and recovery at any pH, it is important to solubilize a sample completely. Highly acidic or neutral solvents can be used with ZORBAX 300StableBond and Poroshell 300SB while neutral solvents and dilute bases can be used with ZORBAX 300Extend-C18.

#### Solvent Choices to Solubilize Proteins and Peptides

Water/Phosphate Buffer  
Dilute Acid (TFA, Acetic Acid or HCl)  
Neutral pH, 6-8 M Guanidine-HCl or Isothiocyanate  
5% HOAc/6 M Urea  
Dilute Acid + Aqueous/Organic Solvents (ACN, MeOH, THF)  
Dilute Base (Ammonium Hydroxide)  
DMSO or 0.1%-1% TFA in DMSO  
Formamide.

Weakest



Strongest

### Raise the Temperature

Separations of proteins and peptides are influenced by temperature and higher column temperature can dramatically improve both resolution and recovery of proteins and hydrophobic and aggregating peptides.

StableBond 300SB — up to 80°C

Poroshell 300SB — up to 80°C

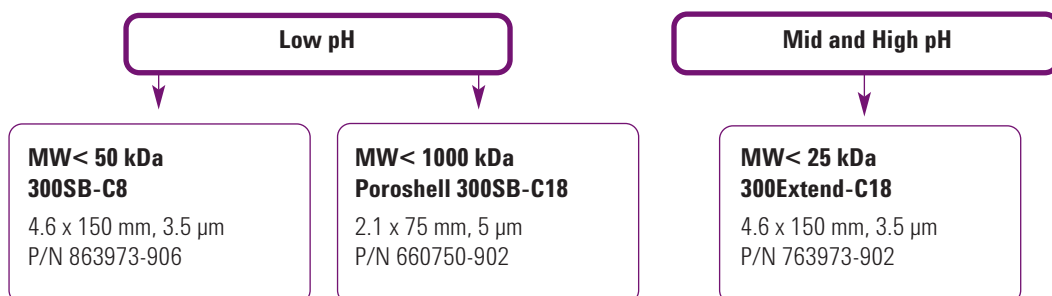
### Optimize Mobile Phase pH

#### Try mid and high pH if low pH does not work

If an optimized low pH method does not provide an ideal separation, then mid or high pH mobile phase can be used. At high pH selectivity is often very different because acidic amino acids become negatively charged and some basic amino acids may lose their charge. ZORBAX 300Extend-C18 is an excellent choice for mid to high pH separation.

Column:	<b>300Extend-C18</b> 4.6 x 150 mm, 5 µm P/N 773995-902
Mobile Phase:	A: 20 mM NH <sub>4</sub> OH in H <sub>2</sub> O B: 20 mM NH <sub>4</sub> OH in 80% ACN
Flow Rate:	1 mL/min
Temperature:	25-30°C (< 60°C)
Gradient:	5-60% B in 30 minutes

## Starting Column Choices for Analytical Separations of Peptides, Polypeptides, and Proteins

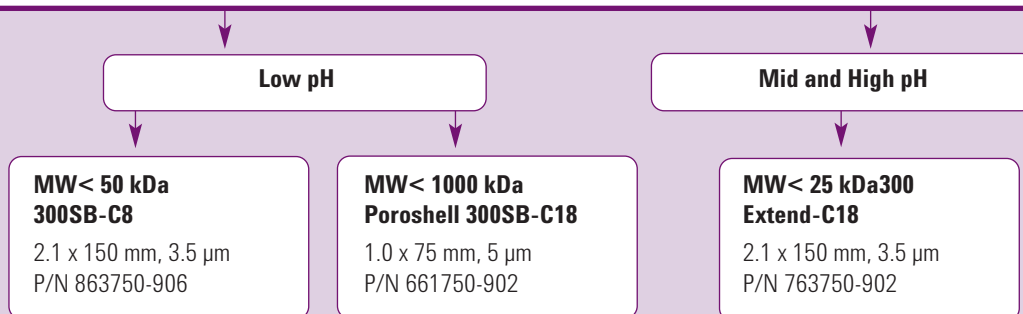


## Separations of Proteins and Peptides Using Reversed-Phase LC/MS Methods

LC/MS of proteins and peptides is used to provide information for protein characterization, to accurately identify post-translational modifications of proteins, and to determine the molecular weight of synthetic and natural peptides. LC/MS is used to provide protein identification in 2-D separations for proteomics applications. Therefore, LC/MS of proteins and peptides is a critical separation area, which requires some special column and mobile phase recommendations. In general, smaller column sizes are used for LC/MS and TFA is generally not used in mobile phase because of reduced sensitivity in the MS with this mobile phase additive.

### Initial Column Choices for LC/MS Separations of Proteins and Polypeptides

**Analytical LC/MS Applications – 2.1 mm i.d. columns will provide good sensitivity when sample size is not limited. With Poroshell columns, smaller column i.d.'s are used.**

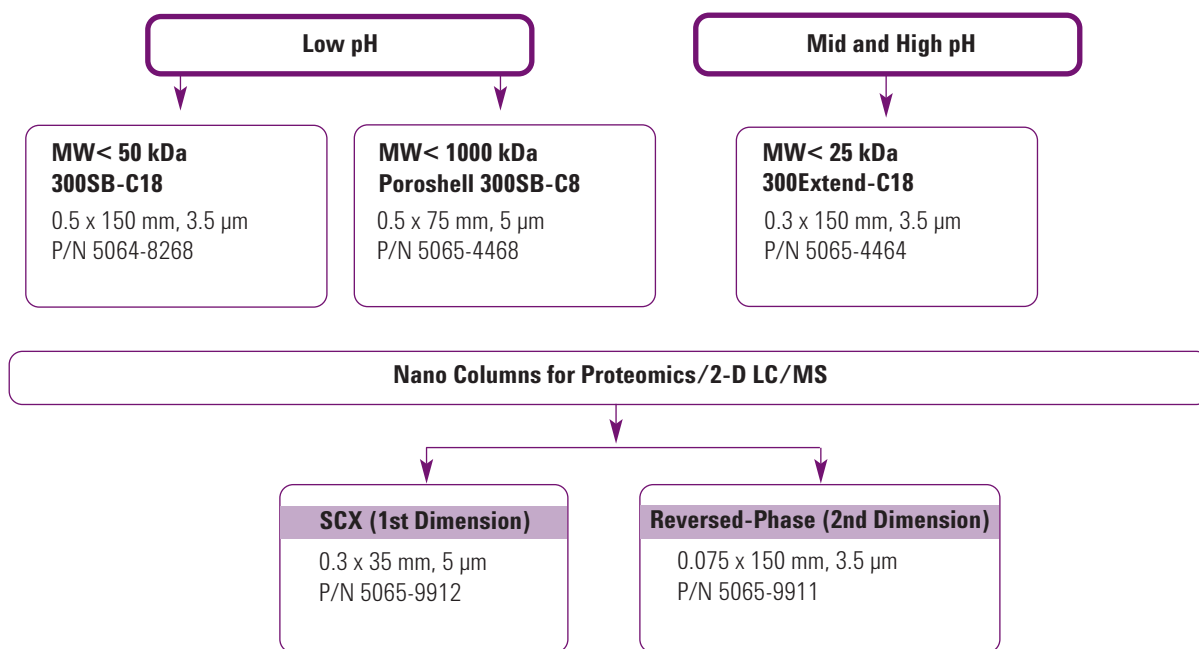


#### High Sensitivity/Proteomics Applications

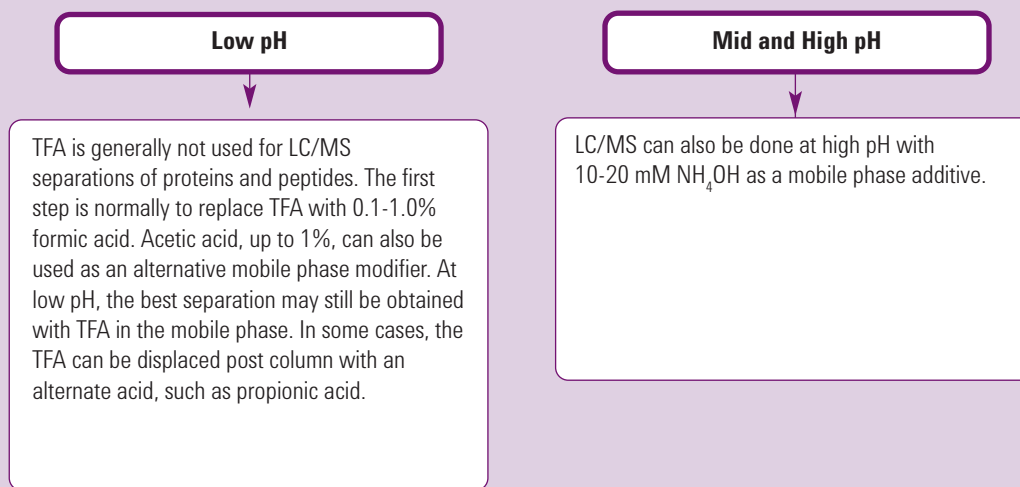
Capillary columns are used for high sensitivity protein and peptide applications. The 0.5 mm i.d. columns are used for protein and protein digest separations while the 0.3 mm i.d. columns are most often used for protein digests. These can be analyzed at high pH with an ammonium hydroxide mobile phase. Nano columns (0.1 and 0.075 mm i.d.) are often used in 2-D LC/MS systems for proteomics and the initial choice is a C18 bonded phase.

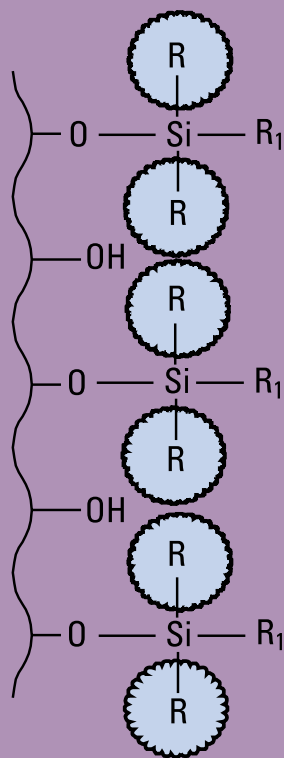


## High Sensitivity Capillary Columns



## Mobile Phase Considerations





Sterically Protected 300StableBond Bonded Phase



### Tips & Tools

Typical mobile phases for protein and peptide separations combine a very low pH with TFA (or other acids) to solubilize proteins. StableBond columns have extremely long lifetimes under these conditions. They are available in 300Å pore size for proteins up to 100-500kDa, or 80Å pore size for peptides below 4000Da.

## ZORBAX 300Å StableBond

ZORBAX 300StableBond columns are an ideal choice for the reproducible separations of proteins and peptides for two key reasons. First, wide-pore, 300Å columns are necessary for an efficient separation of proteins and peptides, or other large molecules, in order to allow these analytes to completely access the bonded phase. Second, 300StableBond columns are unmatched in their durability at low pH, such as with the TFA containing mobile phases typically used for protein and peptide separations. For LC/MS separations at low pH, 300StableBond columns can also be used with formic acid and acetic acid mobile phase modifiers. These columns are available in four different bonded phases (C18, C8, C3, and CN) for selectivity and recovery optimization of proteins and polypeptides. To further increase sample recovery and improve efficiency for difficult proteins, 300StableBond columns can be used up to 80-90°C. 300SB-C18 and 300SB-C8 columns are an ideal choice for complex protein and protein digest separations. These columns are available in capillary (0.3, 0.5 mm ID) and nano (0.075 and 0.10 mm ID) dimensions for reversed-phase LC/MS separations of these protein digests. These capillary and nano columns can be used for either 1-D or 2-D proteomics separations.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range*	Endcapped	Carbon Load
ZORBAX 300SB-C18	300Å	45 m <sup>2</sup> /g	90°C	1.0-8.0	No	2.8%
ZORBAX 300SB-C8	300Å	45 m <sup>2</sup> /g	80°C	1.0-8.0	No	1.5%
ZORBAX 300SB-C3	300Å	45 m <sup>2</sup> /g	80°C	1.0-8.0	No	1.1%
ZORBAX 300SB-CN	300Å	45 m <sup>2</sup> /g	80°C	1.0-8.0	No	1.2%

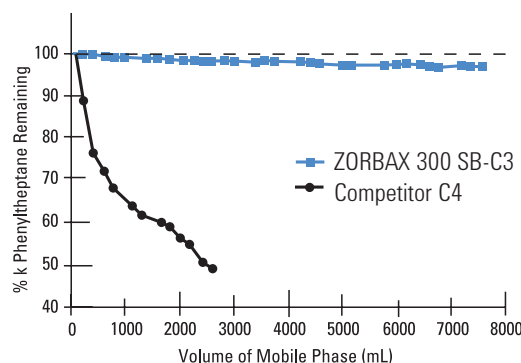
\*300 StableBond columns are designed for optimal use at low pH. At pH 6-8, highest column stability for all silica-based columns is obtained by operating at temperatures <40°C and using low buffer concentrations in the range of 0.01-0.02 M. At mid or high pH, 300Extend-C18 is recommended.

## Short-Chain ZORBAX 300SB-C3 Is Stable at Low pH, High Temperature

**Column:** ZORBAX 300SB-C3  
883995-909  
4.6 x 150mm, 5µm

**Mobile Phase:** Gradients 0-100% B in 80 min  
A: 0.5% TFA in Water  
B: 0.5% TFA in Acetonitrile  
**Isocratic Retention Test Conditions:**  
1-phenylheptane 50% A, 50% B

**Flow Rate:** 1.0 mL/min  
**Temperature:** 60°C



## Four Different 300SB Bonded Phases Optimize Separation of Large Polypeptides

**Column A:** ZORBAX 300SB-C18  
883995-902  
4.6 x 150mm, 5µm

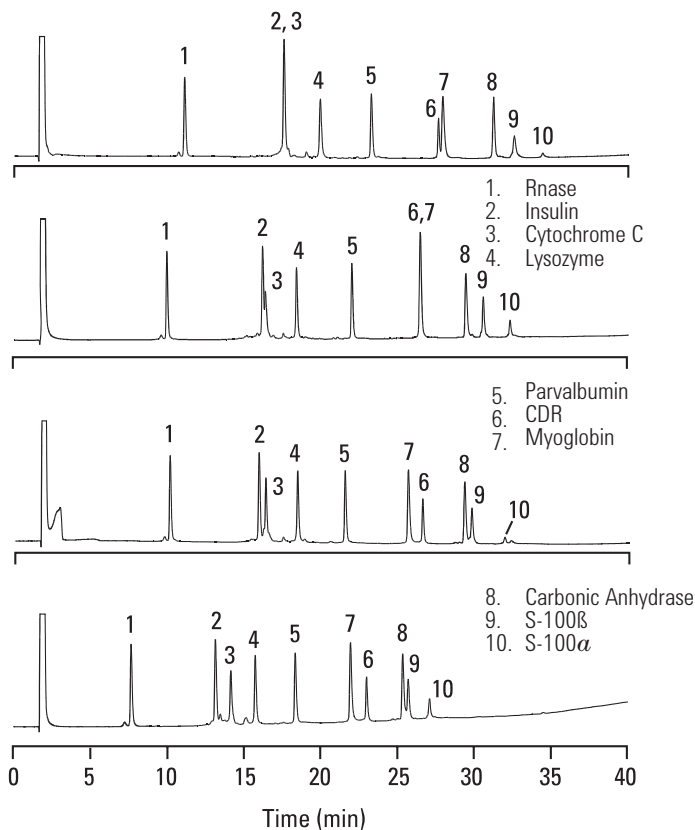
**Column B:** ZORBAX 300SB-C8  
883995-906  
4.6 x 150mm, 5µm

**Column C:** ZORBAX 300SB-C3  
883995-909  
4.6 x 150mm, 5µm

**Column D:** ZORBAX 300SB-CN  
883995-905  
4.6 x 150mm, 5µm

**Mobile Phase:** Linear Gradient, 25 - 70% B in 40 min  
A: 0.1% TFA in Water  
B: 0.09% TFA in 80%  
Acetonitrile/20% Water

**Flow Rate:** 1.0 mL/min  
**Temperature:** 60°C  
**Sample:** 3 µg each protein



The 300SB-C18, C8, C3, and CN bonded phases all provide a different separation of this group of polypeptides. This adds an important parameter for quickly optimizing protein separations. The 300SB-CN column offers unique selectivity for more hydrophilic polypeptides.

## Capillary Columns for HPLC Analyses with UV and MS Detection

**Column:** ZORBAX 300SB-C18  
5064-8263  
0.3 x 150mm, 5µm

**Mobile Phase:** 5-55% B in 50 min, to 85% B from 55-57 min

A: 0.1% Formic Acid in Water

B: 0.1% Formic Acid in ACN

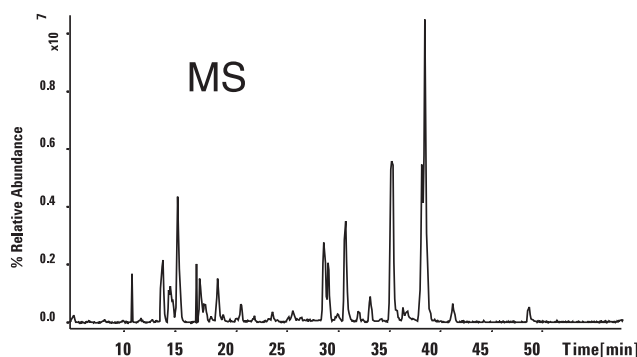
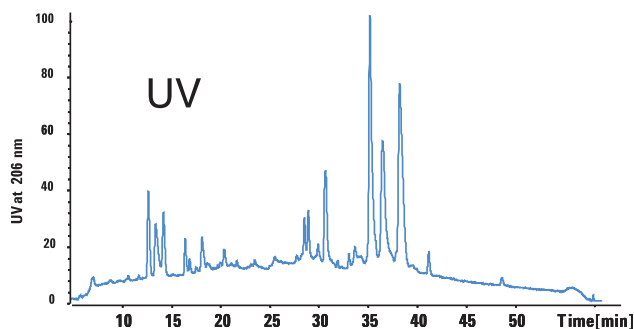
**Flow Rate:** 5.5 µL/min

**Detector:** 206 nm

**MS Conditions:** LC/MS: Pos. Ion ESI with  
LC/MSD trap-Vcap 4000 V  
Drying Gas Flow: 7 L/min  
Drying Gas Temperature: 250°C  
Nebulizer: 15 psi  
Capillary Exit Volt: 50 V  
Max Accum Time: 300 ms  
Total Averages: 3  
Isolation Width: 3 m/z  
Frag Amplitude: 1.0 V  
100 nL

**Sample:** Beta Casein Digest (4 pmol)

A ZORBAX 300SB-C18 capillary column (0.3 mm ID) is used for the separation of the protein digest. Detection is by both UV and Electro spray MS. MS detection can be used for identification of peptide fragments.



## ZORBAX Nano Columns For High Sensitivity Protein Digest Analysis by LC/MS

**Column:** ZORBAX 300SB-C18  
5065-9911  
0.075 x 150mm, 3.5µm

**Mobile Phase:** A: Water + 0.1% Formic Acid,

B: ACN + 0.1% Formic Acid

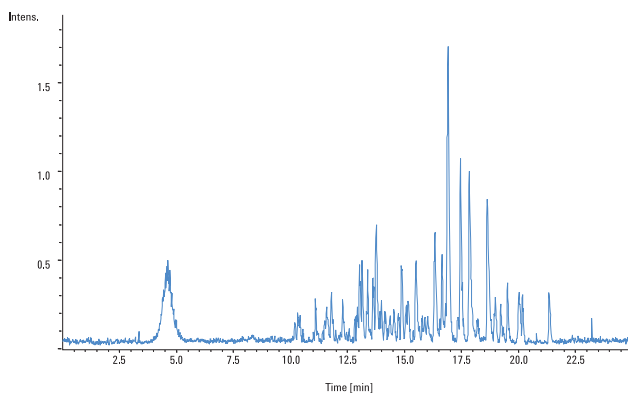
**Flow Rate:** 600 nL/min

**Gradient:** 2% B to 52% B in 25 min














**Detector:** Positive Ion Nano Electrospray MS

**Sample:** 100 fm (1 µL) Digest of 8 Proteins

A ZORBAX nano HPLC column, 0.075 mm ID, is used for high sensitivity LC/MS analysis of a protein digest sample.



**ZORBAX 300Å StableBond**

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
<b>Standard Columns (no special hardware required, 400 bar)</b>							
	Semi-Preparative	9.4 x 250	5	880995-202	880995-206	880995-205	880995-209
	Analytical	4.6 x 250	5	880995-902	880995-906	880995-905	880995-909
	Analytical	4.6 x 150	5	883995-902	883995-906	883995-905	883995-909
	Analytical	4.6 x 50	5	860950-902	860950-906	860950-905	860950-909
	Rapid Resolution	4.6 x 150	3.5	863973-902	863973-906	863973-905	863973-909
	Rapid Resolution	4.6 x 100	3.5	861973-902	861973-906		
	Rapid Resolution	4.6 x 50	3.5	865973-902	865973-906	865973-905	865973-909
	Solvent Saver Plus	3.0 x 150	3.5	863974-302	863974-306		863974-309
	Solvent Saver Plus	3.0 x 100	3.5		861973-306		
	Narrow Bore	2.1 x 250	5	881750-902			
	Narrow Bore	2.1 x 150	5	883750-902	883750-906	883750-905	883750-909
	Narrow Bore RR*	2.1 x 150	5		863750-906		
	Narrow Bore RR*	2.1 x 100	3.5	861775-902	861775-906		
	Narrow Bore RR*	2.1 x 50	3.5	865750-902	865750-906		
	MicroBore	1.0 x 250	5	861630-902			
	MicroBore RR*	1.0 x 150	3.5	863630-902	863630-906		
	MicroBore RR*	1.0 x 50	3.5	865630-902	865630-906		
	MicroBore Guard Cartridges, 3/pk	1.0 x 17	5	5185-5920	5185-5920		
	Guard Cartridge, 2/pk	9.4 x 15	7	820675-124	820675-124	820675-124	820675-124
	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-921	820950-918	820950-923	820950-924
	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-918	821125-918	821125-924	821125-924
	Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	840140-901
	Guard Hardware Kit			820888-901	820888-901	820888-901	820888-901
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>							
	PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109
	PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106		897150-109
	PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906		895150-909
	PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906		895100-909
	PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906		895050-909
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901
	PrepHT Guard Cartridge, 2/pk	17 x 7.5	5	820212-921	820212-918	820212-924	820212-924
	Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901

## ZORBAX 300Å StableBond (Continued)

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
<b>Capillary Glass-lined Columns</b>							
	Capillary	0.5 x 250	5	5064-8266			
	Capillary	0.5 x 150	5	5064-8264			
	Capillary	0.5 x 35	5	5064-8294			
	Capillary RR*	0.5 x 150	3.5	5064-8268			
	Capillary RR*	0.5 x 35	3.5	5065-4459			
	Capillary	0.3 x 250	5	5064-8265			
	Capillary	0.3 x 150	5	5064-8263			
	Capillary	0.3 x 35	5	5064-8295			
	Capillary RR*	0.3 x 150	3.5	5064-8267	5065-4460		
	Capillary RR*	0.3 x 100	3.5	5064-8259	5065-4461		
	Capillary RR*	0.3 x 35	3.5	5064-8270	5065-4462		
	Capillary RR*	0.3 x 50	3.5	5064-8300	5065-4463		
<b>Nano Columns (PEEK fused silica)</b>							
	Nano RR*	0.1 x 150	3.5	5065-9910			
	Nano RR*	0.075 x 150	3.5	5065-9911			
	Nano RR*	0.075 x 50	3.5	5065-9924	5065-9923		
	Trap/Guard, 5/pk	0.3 x 5	5	5065-9913	5065-9914		
	Trap/Guard Hardware kit			5065-9915	5065-9915		

\*RR: Rapid Resolution 3.5 µm





## ZORBAX 300Å Extend-C18

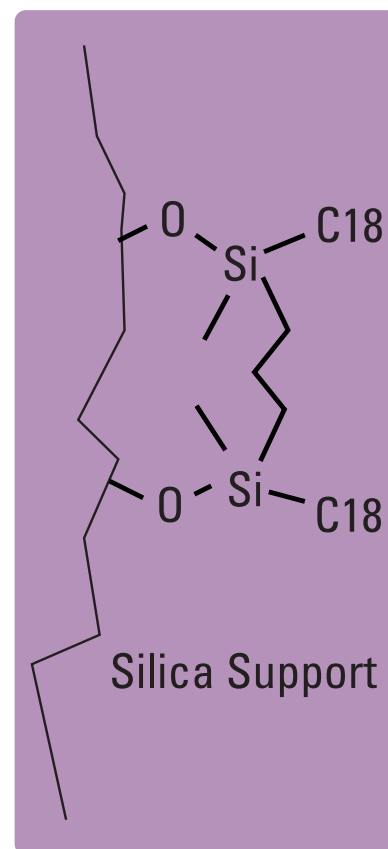
- Rugged, high and low pH separations of polypeptides and peptides from pH 2-11.5
- Different selectivity possible at high and low pH
- High efficiency and good recovery of hydrophobic peptides at high pH
- Ideal for LC/MS with ammonium hydroxide-modified mobile phase

ZORBAX 300Extend C-18 is a wide-pore HPLC column for high efficiency separations of peptides from pH 2-11.5. The unique, bidentate bonded phase provides excellent lifetime and reproducibility at high and low pH. At high pH, retention and selectivity of peptides and polypeptides can change dramatically as a result of changes in charge on molecules. Excellent recoveries of hydrophobic polypeptides have been achieved at room temperature and high pH. LC/MS sensitivity of peptides and polypeptides can also be improved at high pH using a simple ammonium hydroxide-containing mobile phase.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range	Endcapped	Carbon Load
ZORBAX 300Extend-C18	80Å	180 m <sup>2</sup> /g	60°C	2.0-11.5	Double	4%

\*Temperature limits are 60°C up to pH 8, 40°C from pH 8-11.5.

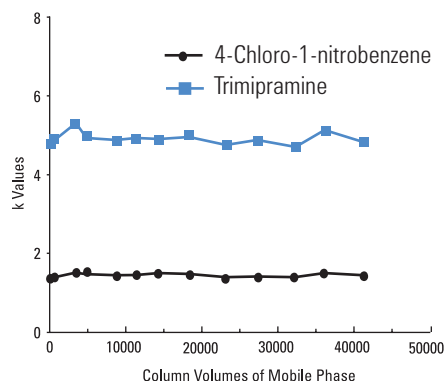


Novel Bidentate C18-C18 Bonding for Extend-C18 Bonded Phase

### Long Life at High pH with 300Extend-C18

**Column:** ZORBAX Extend-C18  
773450-902  
4.6 x 150mm, 5µm

Mobile Phase: 20% 20 mM NH<sub>4</sub>OH,  
pH 10.5  
80% Methanol  
Flow Rate: 1.5 mL/min  
Temperature: Aging 24°C  
Tests 40°C



Each 10,000 column volume is approximately one working month.

## Extend-C18 and StableBond SB-C18 Are Stable at Low pH

**Column A:** ZORBAX SB-C18  
883975-902  
4.6 x 150mm, 5µm

**Column B:** ZORBAX Extend-C18  
773450-902  
4.6 x 150mm, 5µm

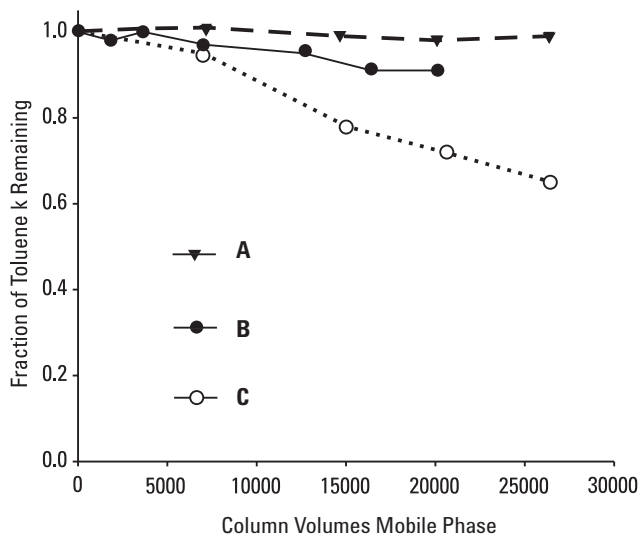
**Column C:** ZORBAX Rx-C18  
883967-902  
4.6 x 150mm, 5µm

Mobile Phase: Column Aging:  
50% Methanol : 50% Water + 1% TFA

Column Test:  
60% Methanol : 40% Water  
Test Solute: Toluene

Flow Rate: 1.5 mL/min, continuous

Temperature: Aging:  
90°C  
Test:  
Ambient



The 300Extend-C18 column can be used at high and low pH – from pH 2-11.5. This chart shows that the 300Extend-C18 has the needed stability at low pH for long-term reproducible separations. Therefore, one wide-pore column can be used for selectivity optimization at low and high pH with both TFA and ammonium hydroxide mobile phases.

## LC/MS Analysis of Angiotensin on Extend-C18

**Column:** ZORBAX Extend-C18  
773700-902  
2.1 x 150mm, 5µm

Mobile Phase: Acidic Conditions: A: 0.1% TFA in water B: 0.085% TFA in 80% acetonitrile (ACN)  
Basic Conditions: A: 10 mM NH<sub>4</sub>OH in water B: 10 mM NH<sub>4</sub>OH in 80% ACN

Flow Rate: 0.2 mL/min  
Gradient: 15-50% B in 15 min

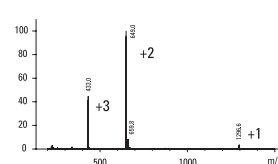
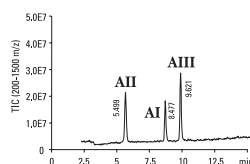
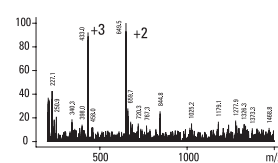
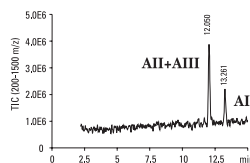
Temperature: 35°C

MS Conditions: Pos. Ion ESI- V<sub>f</sub> 70V, V<sub>cap</sub> 4.5 kV,

N<sub>2</sub>- 35 psi, 12 L/min., 325°C

Sample: 2.5 µL sample (50 pmol each)  
Angiotensin I, II, III

Reference: B.E. Boyes. Separation and Analysis of Peptides at High pH Using RP-HPLC/ESI-MS, 4th WCBP, San Francisco, CA Jan 2000.



Both small and large peptides demonstrate selectivity changes at high and low pH. At high pH, due to a change in charge, all three Angiotensins can be resolved. In addition, the spectral clarity of Angiotensin I is dramatically improved at high pH with the ammonium hydroxide mobile phase. The Extend-C18 column can be used for the analysis of small peptides at high pH as well.

**ZORBAX 300Å Extend-C18**

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	Analytical	4.6 x 250	5	770995-902
	Analytical	4.6 x 150	5	773995-902
	Rapid Resolution	4.6 x 150	3.5	763973-902
	Rapid Resolution	4.6 x 100	3.5	761973-902
	Rapid Resolution	4.6 x 50	3.5	765973-902
	Narrow Bore RR*	2.1 x 150	3.5	763750-902
	Narrow Bore RR*	2.1 x 100	3.5	761775-902
	Narrow Bore RR*	2.1 x 50	3.5	765750-902
<b>ZGC</b>	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-932
<b>ZGC</b>	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-932
<b>ZGC</b>	Guard Hardware Kit			820888-901

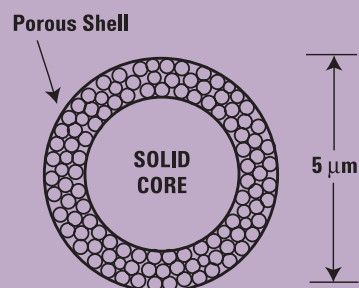
**Capillary Glass-lined Columns**

	Capillary RR*	0.3 x 150	3.5	5065-4464
	Capillary RR*	0.3 x 100	3.5	5065-4465
	Capillary RR*	0.3 x 75	3.5	5065-4466
	Capillary RR*	0.3 x 50	3.5	5065-4467

\*RR: Rapid Resolution 3.5 µm



### Innovative Poroshell Particles Are Ideal for Ultra-Fast Protein Separations



The unique Poroshell particle design consists of a solid core of high-purity silica surrounded by a thin layer of porous, high-purity silica. Pores in the thin, outer layer measure 300Å in diameter and are bonded with sterically protected ligands (SB-C18, SB-C8 or SB-C3) for maximum column life with low pH (i.e., TFA and formic acid) mobile phases. During separation, proteins diffuse rapidly in and out of the porous shell – allowing for the use of high flow velocities – thereby eluting as sharp peaks in just seconds.



### Tips & Tools

Reversed-phase HPLC is a key technique for separating peptides and proteins because of its high achievable resolution. Only Agilent offers reversed-phase Poroshell columns for fast, high-resolution protein separations.

## ZORBAX Poroshell

- High-resolution separations of biomolecules with unique particle design
- High efficiency and recovery with proteins (up to 1,000 kDa) and monoclonal antibodies
- Achieve long lifetime at low pH with Poroshell 300SB; at high pH with 300Extend-C18
- Optimize recovery and selectivity with four different bonded phases – 300SB-C18, 300SB-C8, 300SB-C3, and 300Extend-C18

ZORBAX Poroshell columns are ideal for fast separations of proteins and peptides because the unique particle allows for fast flow rates to be used while maintaining sharp, efficient peaks. Peptides and proteins are typically separated slowly to reduce the potential peak broadening of these slow diffusing analytes. But Poroshell columns use a unique particle made with a thin layer of porous silica on a solid core of silica. This reduces the diffusion distance for proteins making practical rapid HPLC separations of peptides and proteins up to 500-1,000 kDa. Poroshell columns bonded with StableBond bonded phases provide excellent stability and selectivity choices with TFA and formic acid mobile phases. The Poroshell 300Extend-C18 column can be used from pH 2-10 for unique separations. These columns can be used for analytical protein separations as well as LC/MS separations.

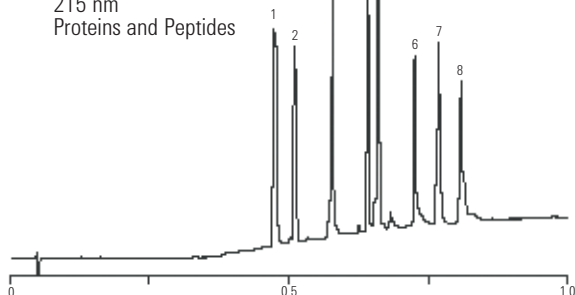


## Poroshell Columns Can Separate Proteins and Peptides in Seconds

**Column:** ZORBAX Poroshell 300SB-C18  
660750-902  
2.1 x 75mm, 5µm

Mobile Phase: A: 0.1% TFA in H<sub>2</sub>O  
B: 0.07% TFA in ACN  
Flow Rate: 3.0 mL/min  
Gradient: 5-100% B in 1.0 min  
Temperature: 70°C, 260 bar pressure  
Detector: 215 nm  
Sample: Proteins and Peptides

1. Angiotensin II
2. Neurotensin
3. Rnase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic Anhydrase
8. Ovabumin

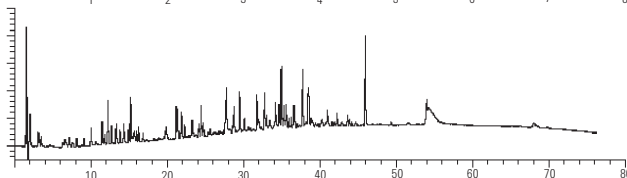
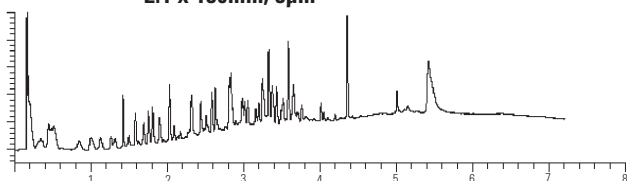


This separation of eight polypeptides and proteins is completed in less than 60 seconds. Each peak is sharp and efficient.

## Reduce Peptide Map Analysis Time by 90% with ZORBAX Poroshell 300SB

**Column A:** ZORBAX Poroshell 300SB-C18  
660750-902  
2.1 x 75mm, 5µm

**Column B:** ZORBAX 300SB-C18  
883750-902  
2.1 x 150mm, 5µm



Mobile Phase: A: 95% H<sub>2</sub>O, 5% ACN,  
0.1% TFA  
B: 5% H<sub>2</sub>O, 95% ACN,  
0.07% TFA

Flow Rate: 1 mL/min  
0.208 mL/min  
Gradient: 0-100%B = 12 min  
0-100%B = 120 min

Temperature: 70°C  
Sample: 20 µL (0.22 µg/1 µL)  
BSA Tryptic Digest  
(15 hours, 70 pmol)

A single chromatographic run of a protein tryptic digest can require an hour or more to complete. With ZORBAX Poroshell columns, the same complex separation can be completed in 1/10th the time.

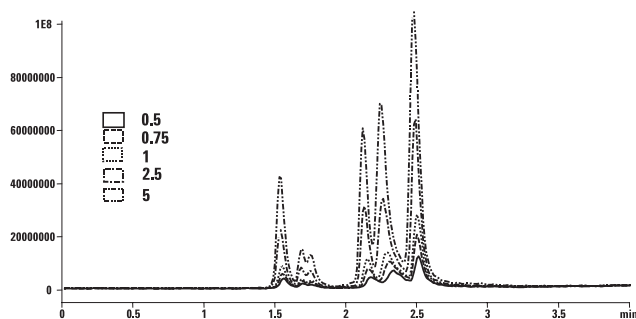




## MicroBore ZORBAX Poroshell Columns Provide Maximum Sensitivity for LC/MS

**Column:** ZORBAX Poroshell 300SB-C18  
661750-902  
1.0 x 75mm, 5µm

Mobile Phase: A: Water + 0.1% Formic Acid  
B: ACN + 0.1% Formic Acid  
Flow Rate: 600 µL/min  
Gradient: 20-100% B in 5.5 min  
Temperature: 80°C  
MS Conditions: LC/MS: Pos. Ion ESI – Vcap 6000 V  
Drying Gas Flow: 12 Liters/min  
Drying Gas Temperature: 350°C  
Nebulizer: 45 psi  
Fragmentor Volatage: 140 V  
Scan: 600-2500  
Stepsize: 0.15 amu  
Peakwidth: 0.06 min  
Sample: 1 µL



With narrow bore diameters like 2.1 mm, 1.0 mm, and 0.5 mm, ZORBAX Poroshell columns make an ideal LC/MS partner. When the sample is very limited, the 1.0 mm or 0.5 mm ID Poroshell columns are an excellent choice for high sensitivity LC/MS analyses. Sensitive MS molecular weight determinations are possible with as little as 0.5 to 5 pmole of protein on Poroshell columns. Poroshell columns have also been used for rapid MS identification of intact proteins even in the presence of stabilizers and tissue culture media.

## ZORBAX Poroshell

Hardware	Description	Size (mm)	Particle Size (µm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18
	Narrow Bore	2.1 x 75	5	660750-902	660750-906	660750-909	670750-902
	MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902
	Capillary	0.5 x 75	5		5065-4468		
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821075-920	821075-918	821075-924	
ZGC	Guard Hardware Kit			820888-901	820888-901	820888-901	
	MicroBore Guard Cartridge, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	5185-5968



## ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

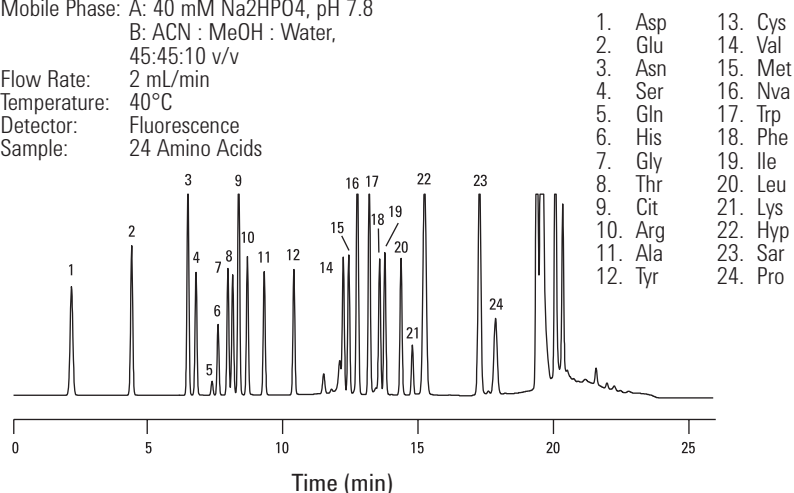
- High resolution and rapid analysis of 24 amino acids
- Use tested for amino acid analysis
- Uses well known OPA and FMOC precolumn derivatization chemistry
- Easily automated using a detailed online, derivatization protocol available for use with Agilent 1100/1200 Autosampler

The ZORBAX Eclipse AAA high efficiency column rapidly separates amino acids following an updated and improved protocol. Total analysis from injection to injection can be achieved in as little as 14 min. (9 min. analysis time) on shorter, 7.5 cm length columns and 24 min. (18 min. analysis time) on the 15 cm column length. Exceptional sensitivity (5-50 pmol with DAD, FLD) and reliability are achieved using both OPA and FMOC derivatization chemistries in one fully automated procedure using the Agilent 1100/1200 HPLC instrument.

### High Resolution of 24 Amino Acids Using ZORBAX Eclipse-AAA Protocol

**Column:** ZORBAX Eclipse AAA  
963400-902  
4.6 x 150mm, 3.5µm

Mobile Phase: A: 40 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.8  
B: ACN : MeOH : Water,  
45:45:10 v/v  
Flow Rate: 2 mL/min  
Temperature: 40°C  
Detector: Fluorescence  
Sample: 24 Amino Acids



This high resolution separation of 24 amino acids is done in 18 minutes. If the Rapid Resolution 4.6 x 75 mm Eclipse AAA column is selected, these amino acids are resolved in 9 minutes.





### ***ZORBAX Eclipse Amino Acid Analysis (AAA) Columns***

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	Analytical routine sensitivity	4.6 x 150	5	993400-902
	Analytical routine sensitivity, high-resolution using FLD	4.6 x 150	3.5	963400-902
	Analytical routine sensitivity, high-throughput	4.6 x 75	3.5	966400-902
	Solvent Saver high sensitivity, high resolution	3.0 x 150	3.5	961400-302
<b>ZGC</b>	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-931
<b>ZGC</b>	Guard Hardware Kit			820888-901

### **Amino Acid Standards**

Each amino acid standards contains the following amino acids:

- Glycine
- L-cystine
- L-histidine
- L-tyrosine
- L-leucine
- L-methionine
- L-serine
- L-alanine
- L-phenylalanine
- L-glutamic acid
- L-proline
- L-isoleucine
- L-arginine
- L-threonine
- L-valine
- L-lysine
- L-aspartic acid

### ***Amino Acid Standards, 10 x 1 ml ampoules\****

Description	Part No.
1 nmol/µl	5061-3330
250 pmol/µl	5061-3331
100 pmol/µl	5061-3332
25 pmol/µl	5061-3333
10 pmol/µl	5061-3334
Amino acids supplement kit Includes 1 g each of norvaline, sarcosine, asparagine, glutamine, tryptophan, and 4-hydroxyproline	5062-2478

\*Consider shelf-life and buy limited quantities, 5062-2478 ships as 1 g vials

### ***Amino Acid Separations Reagents***

Description	Part No.
OPA reagent, 10 mg/ml each in 0.4 M borate buffer o-phthalaldehyde (OPA) and 3-mercaptopropionic acid, 6 x 1 ml ampoules	5061-3335
FMOC reagent, 2.5 mg/ml in acetonitrile, 9-fluorenylmethylchloroformate, 1 ml, 10 ampoules	5061-3337
Borate buffer, 100 ml	5061-3339
DTDPA (Dithiodipropionic) reagent, for analysis of cysteine, 5 g	5062-2479

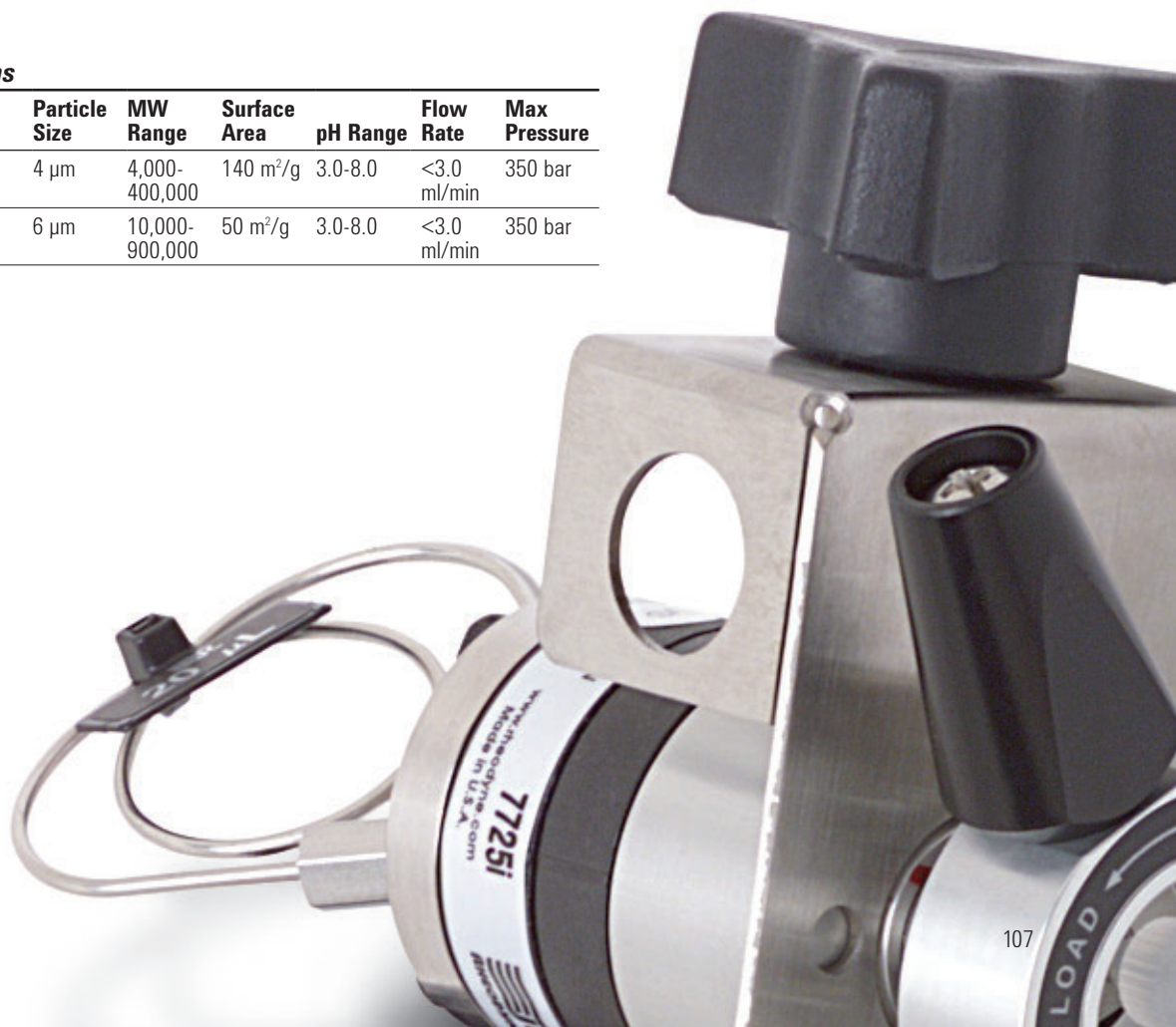
## ZORBAX GF-250 and GF-450 Gel Filtration Columns

- High efficiency and reproducibility with short analysis time
- Hydrophilic diol bonded phase for good protein recovery
- Compatible with organic modifiers and denaturants
- Wide usable pH range (pH 3-8)

ZORBAX GF-250 and GF-450 size exclusion (gel filtration) columns are ideal for the size separations of proteins and other biomolecules. The separation range is 4,000-900,000 for globular proteins when using GF-250 and GF-450 columns in series. The GF-250/GF-450 size exclusion columns have a hydrophilic diol bonded phase for high recovery of proteins (typically >90%) and a unique zirconia modification of the silica to extend the pH range from 3-8. The GF-250 and GF-450 columns are packed with precisely sized porous silica microspheres with narrow pore size and particle size distributions. The result is a highly efficient, rugged and reproducible size exclusion column for separations of proteins with flow rates of up to 3 ml/min. These columns are compatible with organic modifiers (<25%) and denaturants in the mobile phase to eliminate protein aggregation for proper size determination. Some common applications include separations of protein monomers, dimers and aggregates, desalting, protein molecular weight estimation and separations of modified proteins.

### Column Specifications

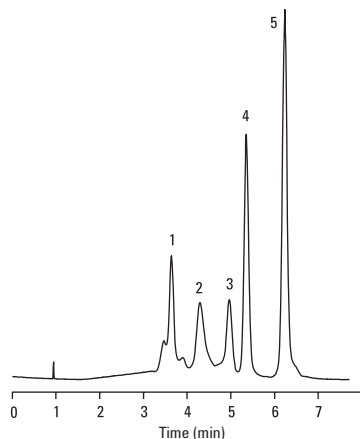
Bonded Phase	Pore Size	Particle Size	MW Range	Surface Area	pH Range	Flow Rate	Max Pressure
ZORBAX GF-250	150Å	4 µm	4,000-400,000	140 m <sup>2</sup> /g	3.0-8.0	<3.0 ml/min	350 bar
ZORBAX GF-450	300Å	6 µm	10,000-900,000	50 m <sup>2</sup> /g	3.0-8.0	<3.0 ml/min	350 bar



## Separation of Protein Standards on the ZORBAX GF-250 SEC Column

**Column:** ZORBAX GF-250  
884973-901  
9.4 x 250mm, 4µm

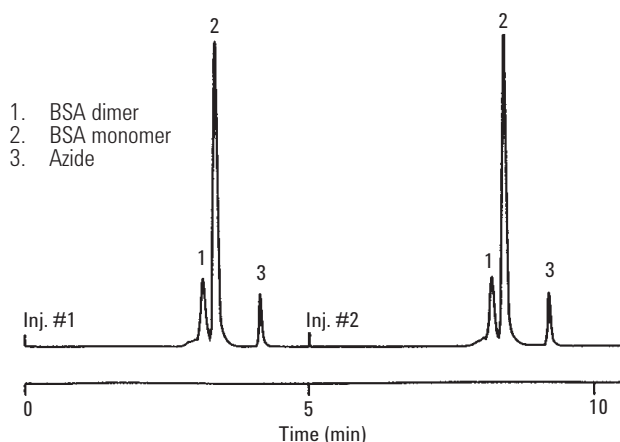
Mobile Phase: 200 mM Sodium Phosphate, pH 7.0  
Flow Rate: 2 mL/min  
Temperature: Ambient  
Detector: 254 nm  
Sample: BioRad Gel Filtration Standards for Size Exclusion  
1. Thyroglobulin 670,000 Da  
2. Bovine Gamma Globulin 158,000 Da  
3. Chicken Ovalbumin 44,000 Da  
4. Equine Myoglobin 17,000 Da  
5. Vitamin B-12 1,350 Da



The protein standards separated here are a commonly selected set of standards. The ZORBAX GF-250 column shows excellent resolution for this sample. Additional resolution of the thyroglobulin can be obtained by adding the GF-450 column in series.

## High-Speed Size Exclusion Separations

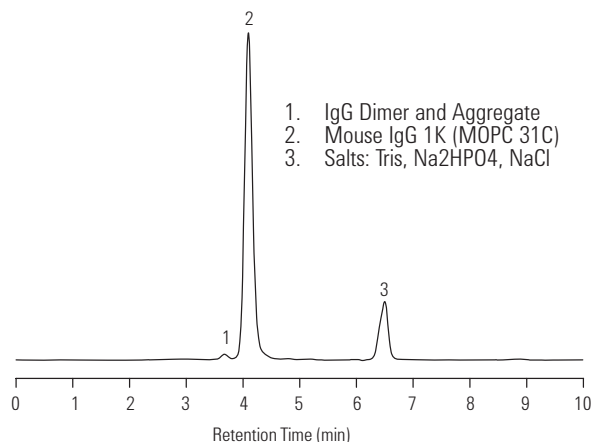
A: BSA and BSA Dimers (duplicate injections)



**Column:** ZORBAX GF-450  
884973-902  
9.4 x 250mm, 6µm

Mobile Phase: PBS (phosphate buffered saline), pH 7.4  
Flow Rate: 3 mL/min  
Temperature: Ambient  
Detector: 220 nm  
Sample: BSA and BSA Dimers

B: Antibody Separation



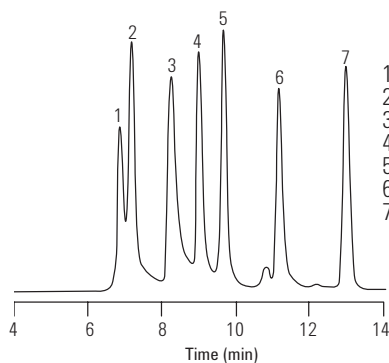
**Column:** ZORBAX GF-450  
884973-902  
9.4 x 250mm, 6µm

Mobile Phase: 200 mM Na Phosphate Monobasic pH 7.0/0.1% Azide  
Flow Rate: 2 mL/min  
Detector: 225 nm  
Sample: 10 µg in 50 mM Sodium Phosphate pH 7.0

## Separation of a Protein Mixture on the 9.4 x 250 mm ZORBAX GF-250 Column

**Column:** ZORBAX GF-250  
884973-701  
4.6 x 250mm, 4µm

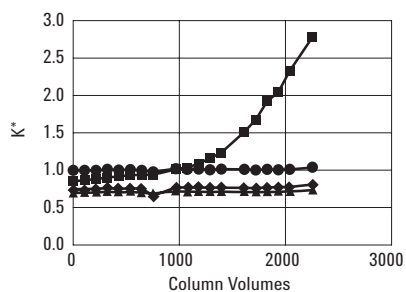
Mobile Phase: 130 mM NaCl/20 mM KCl/  
50 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.0  
Flow Rate: 1 mL/min  
Detector: 210 nm  
Sample: Protein mixture



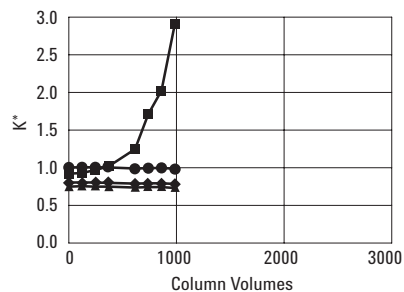
1. Mouse IgM 900,000 Da
2. Bovine Thyroglobulin 670,000 Da
3. Sweet Potato  $\beta$ -Amylase 200,000 Da
4. Bovine Serum Albumin 67,000 Da
5. Chicken Albumin 45,000 Da
6. Bovine RNase 13,700 Da
7. Azide 65 Da

## ZORBAX GF-250 Shows Extended Column Lifetime

Stability of GF-250:  $K^*$  of Standard Proteins



Stability of Competitor Column:  $K^*$  of Standard Proteins





## ZORBAX GF-250 and GF-450 Gel Filtration Columns

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	GF-250, 150Å	9.4 x 250	4	884973-901
	GF-250, 150Å	4.6 x 250	4	884973-701
	GF-450, 300Å	9.4 x 250	6	884973-902

### Guard Columns (hardware required)

	GF-250 Diol, Guard Cartridge, 2/pk	9.4 x 15	6	820675-111
	GF-250 Diol, Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-911
	GF-450 Diol, Guard Cartridge, 2/pk	9.4 x 15	6	820675-111
	GF-250 Diol, Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-911
	Guard Hardware Kit	9.4 x 15		840140-901
	Guard Hardware Kit			820888-901

### PrepHT Columns

	PrepHT GF-250, 150Å	21.2 x 250	6	877974-901
	PrepHT GF-450, 300Å	21.2 x 250	6	877974-910
	PrepHT Endfittings, 2/pk			820400-901
	PrepHT GF-250, Guard Cartridge, 2/pk	17 x 7.5	6	820212-911
	PrepHT GF-450, Guard Cartridge, 2/pk	17 x 7.5	6	820212-911
	Guard Cartridge Hardware			820444-901





## ZORBAX Ion Exchange Columns – SAX and SCX

- ZORBAX SAX and 300SCX columns are based on rugged Zorbax silica
- Stable from pH 2-7
- Provide high efficiency, rapid separations
- Compatible with organic mobile phase modifiers
- ZORBAX Bio-SCX Series II for 2-D separations

Zorbax strong ion-exchange columns are available as both Strong Anion Exchange (SAX) and Strong Cation Exchange (300SCX) columns. Each column is packed with bonded, 5 µm, spherical silica particles for optimum efficiency.

Zorbax also has Bio-SCX Series II columns designed for optimized 2-D separations of peptides and proteins using LC/MS. This packing is based on ultra-pure 3.5 µm Zorbax silica particles, bonded with a bio-friendly polymer that is functionalized with sulfonic acid groups. This gives strong retention and good peak shape in the ion exchange step of 2-D analysis of peptides and proteins.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	Functionality	Max Pressure
ZORBAX SAX	70Å	300 m <sup>2</sup> /g	2.0-7.0	Quaternary amine	350 bar
ZORBAX 300SCX	300Å	50 m <sup>2</sup> /g	2.0-7.0	Sulfonic acid	350 bar
ZORBAX Bio-SCX Series II	300Å	90 m <sup>2</sup> /g	2.5-8.5	Sulfonic acid	350 bar

### ZORBAX Ion Exchange Columns – SAX and SCX

Description	Size (mm)	Particle Size (µm)	SAX	300SCX	Bio-SCX Series II
Semi-preparative	9.4 x 250	5	880952-203	880952-204	
Analytical	4.6 x 250	5	880952-703	880952-704	
Analytical	4.6 x 150	5	883952-703	883952-704	
Analytical	4.6 x 50	5		846952-704	
Solvent Saver	3.0 x 50	5		860700-304	
Narrow Bore	2.1 x 150	5		883700-704	
Narrow Bore	2.1 x 50	5		860700-704	
Capillary	0.3 x 35	3.5			5065-9912
Capillary	0.8 x 50	3.5			5065-9942
Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-903	820950-904	
Guard Hardware Kit			820888-901	820888-901	

## Technical Support at Work for You



### Tech Support

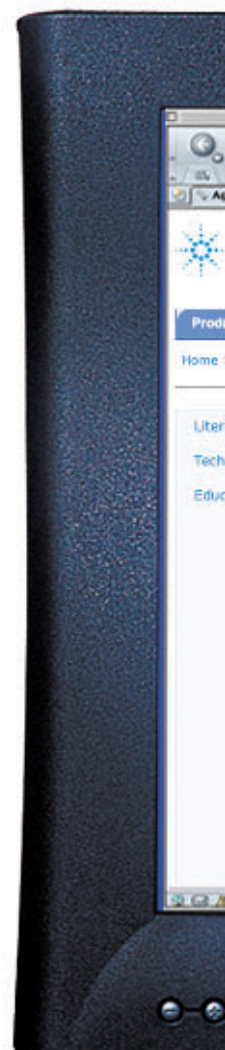
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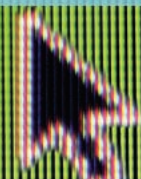
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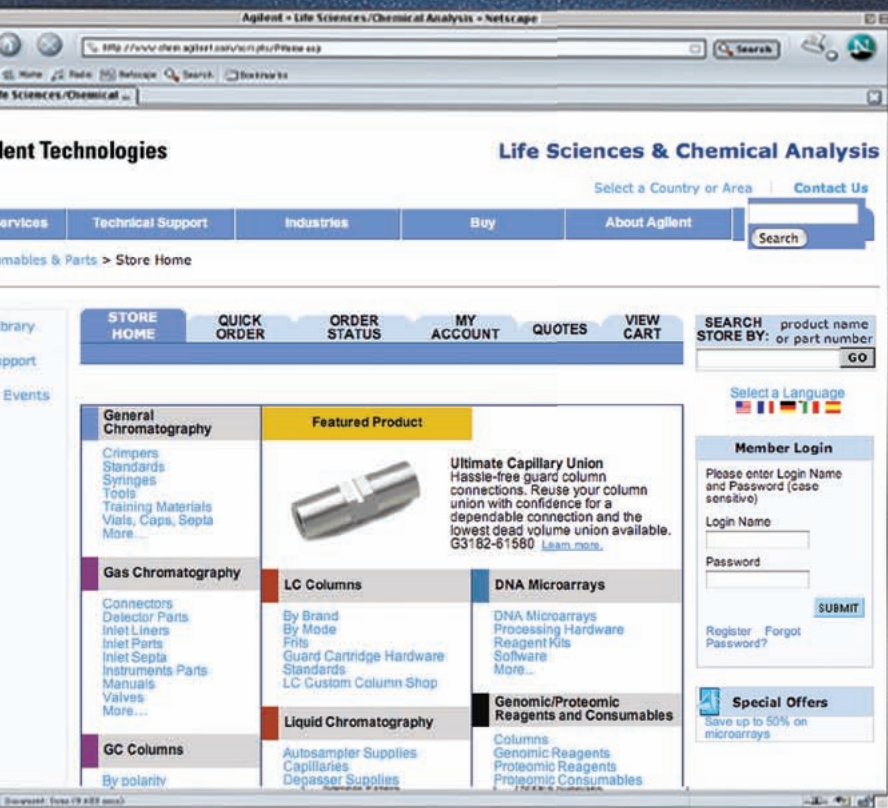
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