

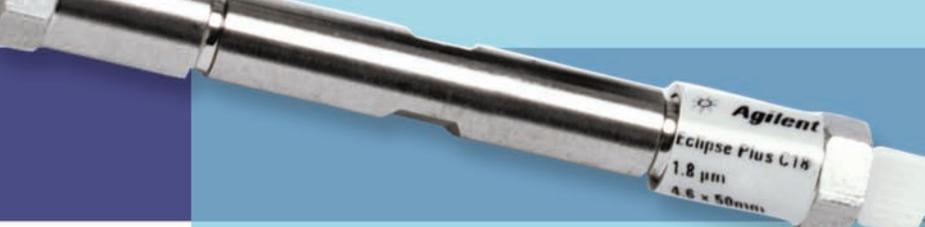


Efficiently analyze your most
challenging compounds.
Deliver high-quality
results faster.

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!

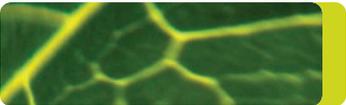
Our easy-to-use guide cuts the column selection process down to three simple steps:

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.
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.

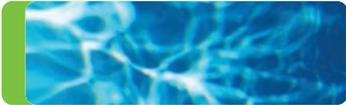

Featured Products2

ZORBAX HPLC Column Selection Guidelines5

| | |
|--|----|
| HPLC Column Selection Flow Chart..... | 6 |
| Quick Guide to ZORBAX Reversed-Phase Bonded Phases..... | 7 |
| ZORBAX Reversed-Phase HPLC Column Selection Flow Chart..... | 8 |
| Simple Guidelines for Choosing a Reversed Phase HPLC Column..... | 10 |
| Quick Guide to USP Categories For HPLC Columns..... | 12 |
| Reversed-Phase HPLC Method Development..... | 13 |
| Cartridge Selection Guide..... | 18 |


ZORBAX Columns for Analytical HPLC19

| | |
|---|----|
| ZORBAX Eclipse Plus..... | 20 |
| ZORBAX Eclipse XDB..... | 26 |
| ZORBAX 80Å StableBond..... | 33 |
| ZORBAX Rx..... | 39 |
| ZORBAX 80Å Extend-C18..... | 41 |
| ZORBAX Bonus-RP..... | 45 |
| ZORBAX Method Development Kits..... | 49 |
| ZORBAX Method Validation Kits..... | 50 |
| ZORBAX Original Reversed Phase Columns..... | 51 |
| ZORBAX Normal Phase Columns..... | 52 |


HPLC Columns for Special Applications55

| | |
|---|----|
| ZORBAX Rapid Resolution High Throughput 1.8 µm..... | 57 |
| ZORBAX Rapid Resolution 3.5 µm Columns..... | 64 |
| ZORBAX Solvent Saver..... | 68 |
| ZORBAX MicroBore (1.0 mm ID)..... | 71 |
| ZORBAX Capillary and Nano..... | 73 |
| ZORBAX PrepHT..... | 80 |
| Agilent Prep LC Columns..... | 84 |
| Ultron Chiral columns..... | 87 |


ZORBAX Columns for Bioanalytical Chromatography89

| | |
|---|-----|
| ZORBAX 300Å StableBond..... | 94 |
| ZORBAX 300Å Extend-C18..... | 99 |
| ZORBAX Poroshell..... | 102 |
| ZORBAX Eclipse Amino Acid Analysis (AAA) Columns..... | 105 |
| ZORBAX GF-250 and GF-450 Gel Filtration Columns..... | 107 |
| ZORBAX Ion Exchange Columns – SAX and SCX..... | 111 |



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 μ 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 μm 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

- 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
- 24x7 technical support by phone or Web
- Access application notes, specifications, chromatogram libraries, and more at www.agilent.com/chem

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.

Order online at www.agilent.com/chem/store





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 μm to 7 μm) 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 μm to 7 μm .
- 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

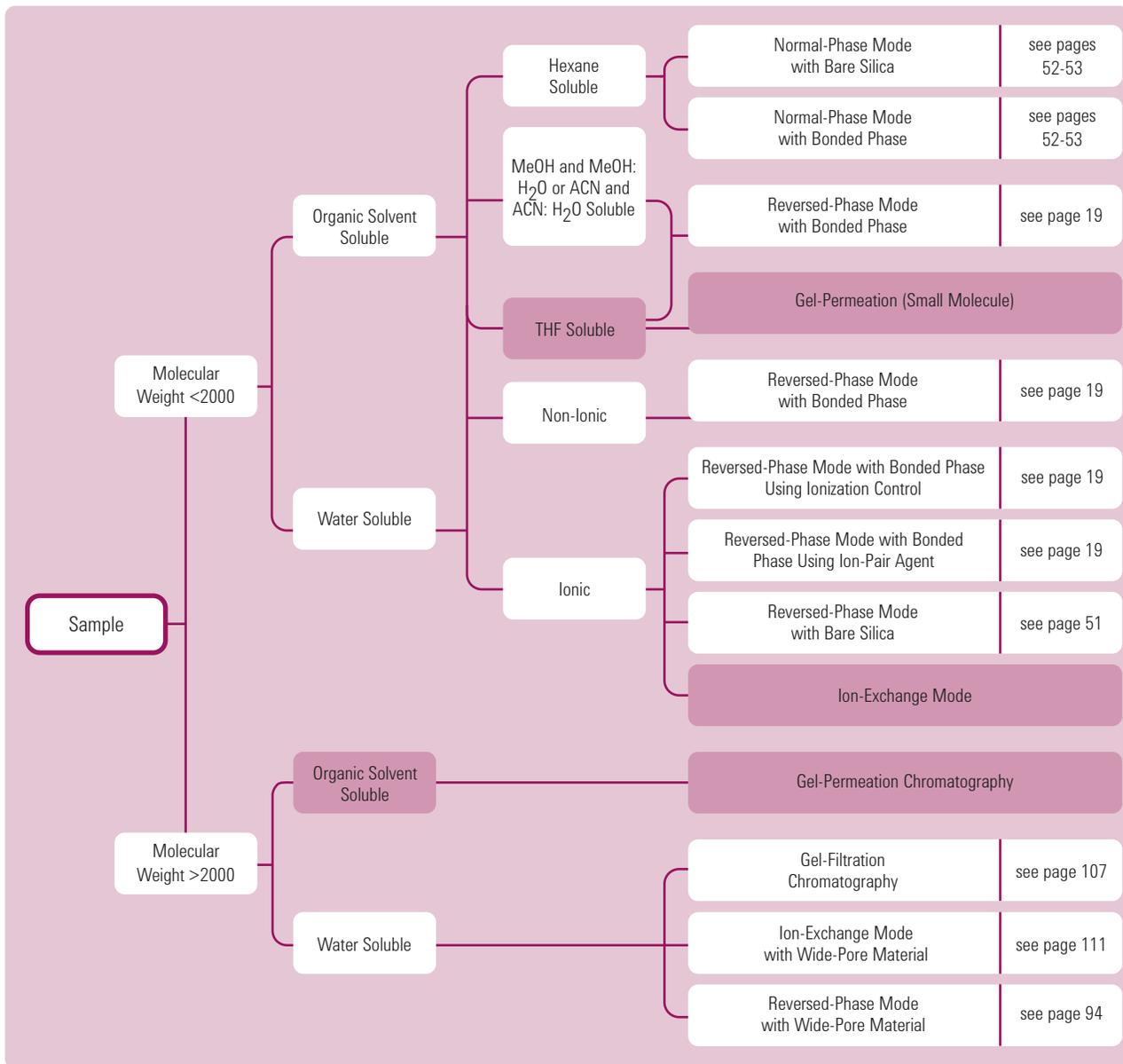
Chromatographic resolution between two or more peaks depends upon column efficiency, selectivity, and retention – all factors that are affected by pH. This section describes a method development strategy based on changing the pH level of the mobile phase.



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

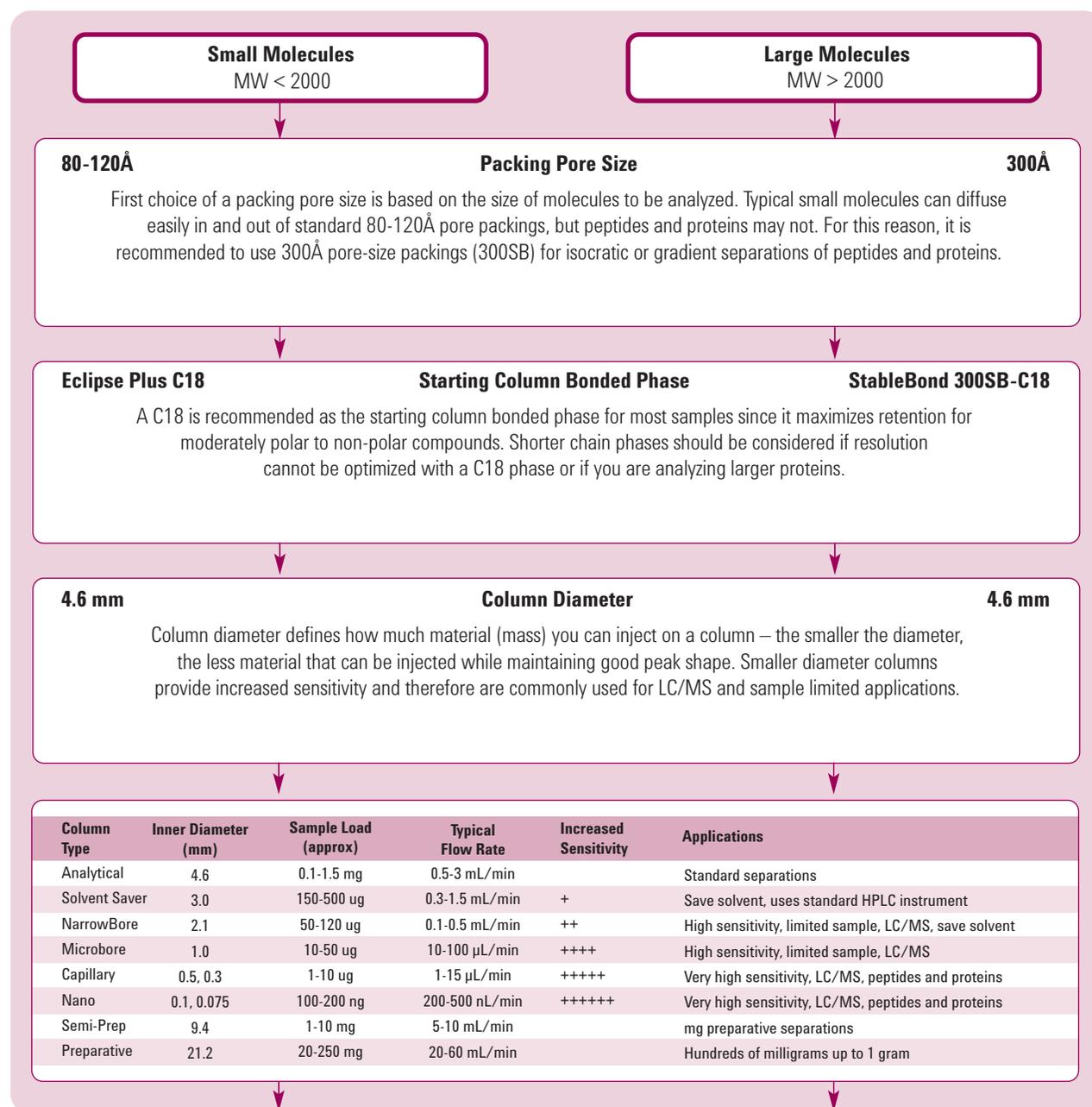
| Modern ZORBAX RP-HPLC Columns Recommended Uses and Applications | | Page No. |
|--|---|-----------------|
| Eclipse Plus | <ul style="list-style-type: none"> • Excellent first choice for method development • Long life from pH 2-9 for reliable separations of basic, acidic and neutral compounds • Superior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

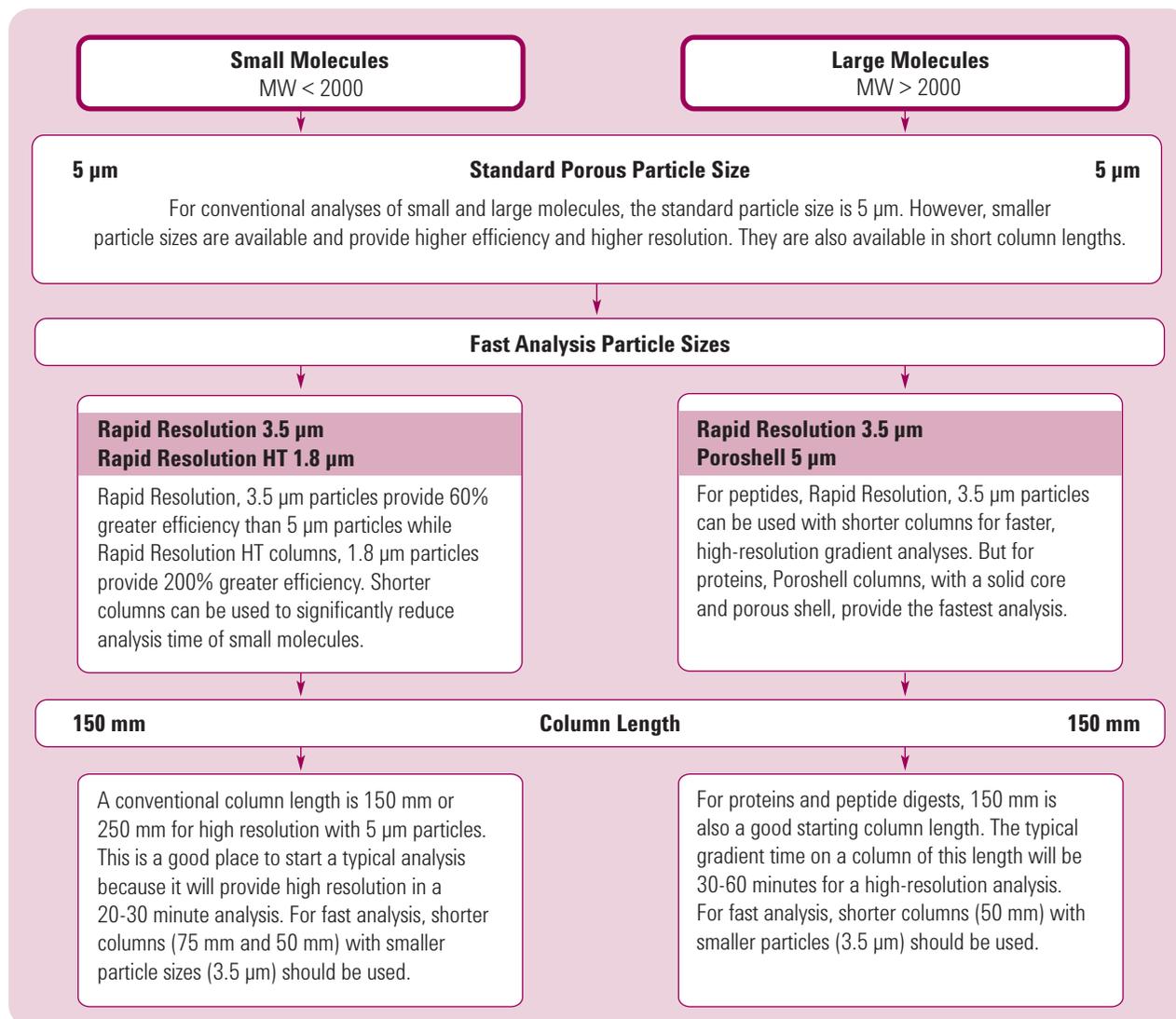
ZORBAX Reversed-Phase HPLC Column Selection Flow Chart

For small and large molecules

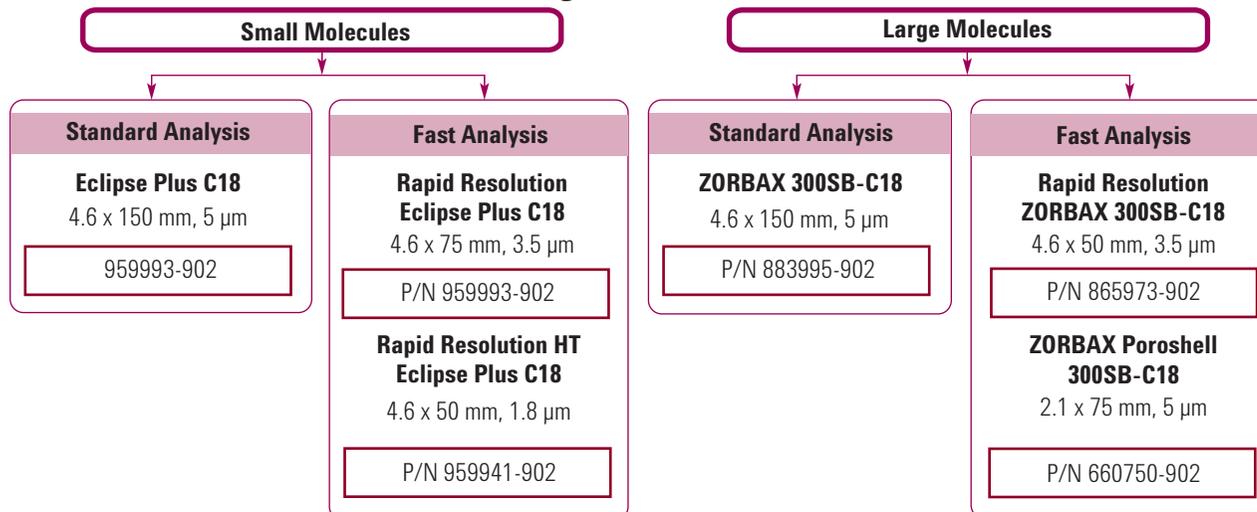
Most chromatographers use reversed-phase HPLC as one of their key analysis techniques. Reversed-phase HPLC can be used to analyze ionic and nonionic analytes. Therefore this ZORBAX Column Selection Flow Chart will focus on reversed-phase columns. To more easily select a reversed-phase column for method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

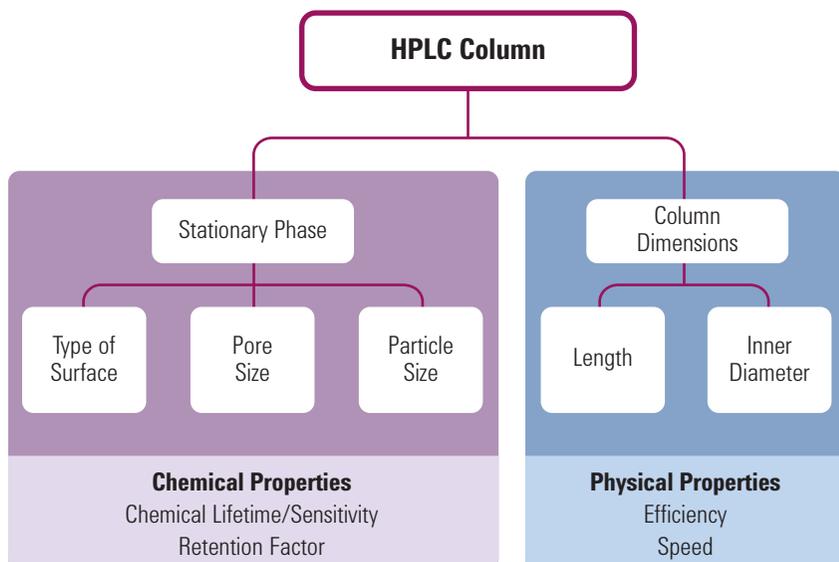
A good first choice for bonded phase is C18 or C8. If the sample solutes of interest are not adequately separated on these columns, CN and Phenyl columns may offer significant differences in selectivity from the straight-chain alkyl phases to effect the separation.

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 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.

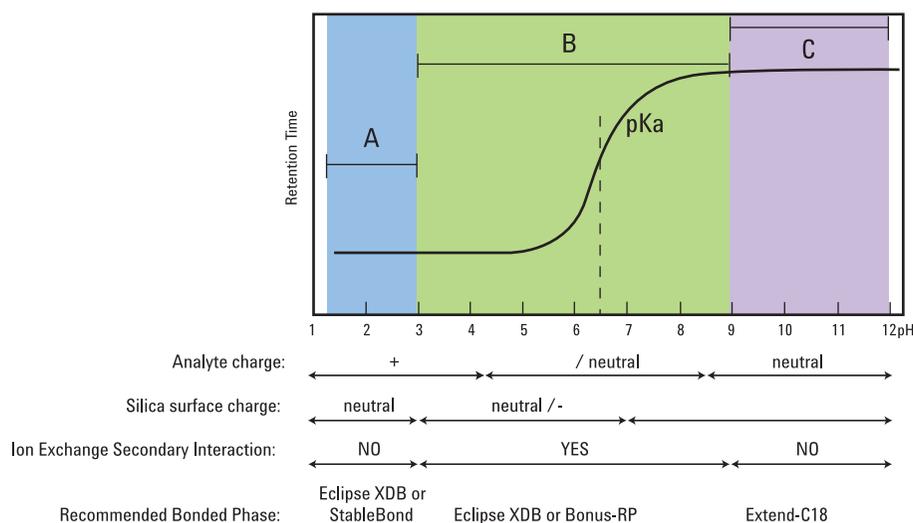


Figure 1: Three pH Regions for HPLC Separations of Basic Compounds

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

- Start method development at low pH, where silanols on a RP-HPLC column are protonated. This minimizes peak tailing by eliminating silanol/base interactions.
- At low pH, basic compounds are positively charged and their retention may be reduced.
- Acidic compounds may be protonated and have increased retention.
- Retention times are usually stable with small changes in pH, producing a robust method.
- Volatile mobile phase additives, such as formic acid or trifluoroacetic acid (TFA), are often used at low pH with LC/MS.

Mid pH 7 – Region B

- Develop methods at pHs at least 1 pH unit above or below the pKa to minimize changes in retention with small changes in pH.
- Some silica surface SiOH groups become SiO⁻ above pH 4 to 5; tailing interactions may be possible.
- Minimize interactions by selecting a well designed and endcapped column, using additives such as TEA (triethylamine) (less desirable) or using "polar-linked" bonded phases.
- Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SiL.

High pH > 9 – Region C

- In this region, basic compounds may be in their free base form.
- Increased retention and resolution of basic compounds are likely.
- Retention changes little in this region, thus robust methods can be developed.
- Silica breakdown is prevented by innovative bidentate column chemistry, heavy endcapping, use of Rx-SiL, and optimum mobile phase.
- Ammonium hydroxide is an excellent volatile mobile phase modifier at high pH.



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.

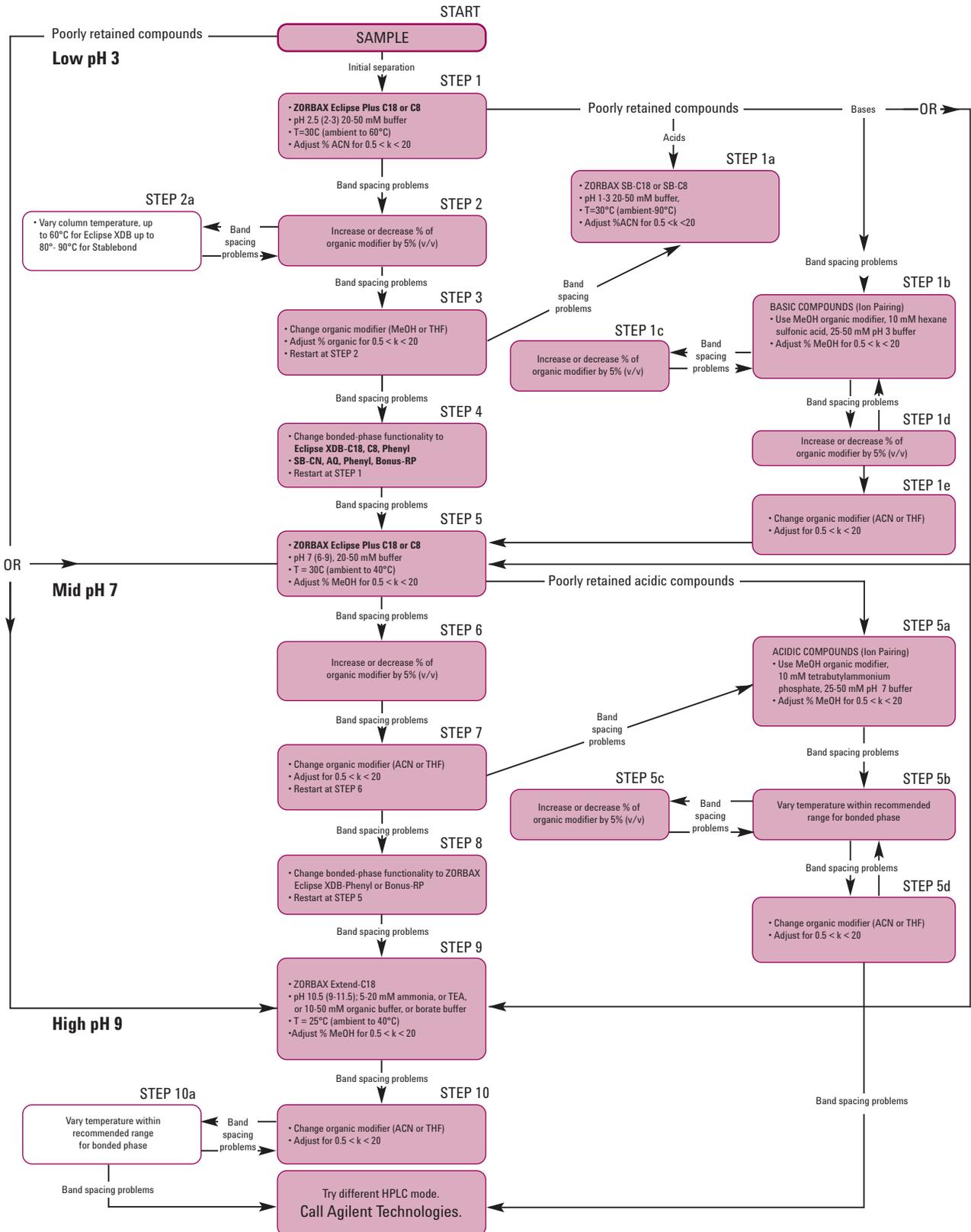
At mid pH, basic compounds (e.g., amines) may still have a positive charge and the silanols on the silica surface may have a negative charge. Therefore covering as many silanols as possible leads to the best peak shape at mid pH. This makes the Eclipse Plus C18 the best starting choice for a column at 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 |
|------|--|--|---|
| AC | 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 |
| ZGC | 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 |
| RR | 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 | |
| P | 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 |
| PI | 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 μm 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 μm to 7 μm – 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.

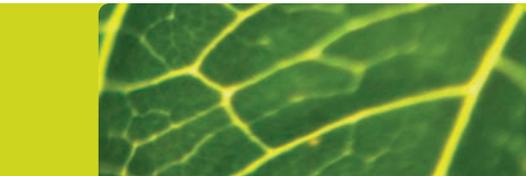


- Achieve excellent peak shape for acids, bases, and neutrals – for greater resolution and accuracy.
- Significantly reduce tailing, so you can confidently resolve difficult analytes.
- Perform ultra-fast, fast, or conventional LC under a range of temperatures, pressures, and pH conditions.
- Spend more 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 μm 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 ² /g | 60°C | 2.0-9.0 | Double | 8% |
| ZORBAX Eclipse Plus C8 | 95Å | 160 m ² /g | 60°C | 2.0-8.0 | Double | 8% |

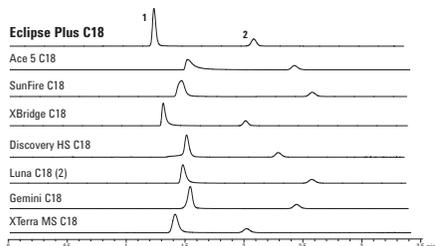




Eclipse Plus: Best Peak Shape in the Industry Without Tailing

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



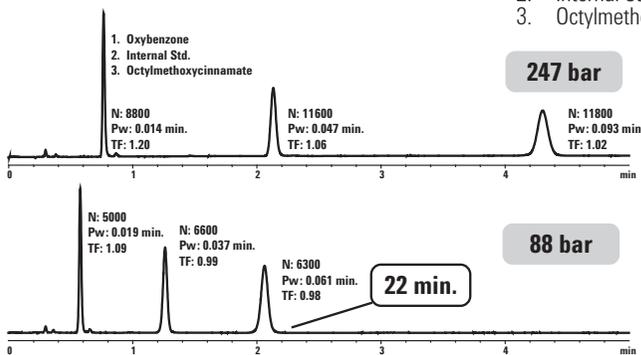
1. Pyridine
2. Phenol

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



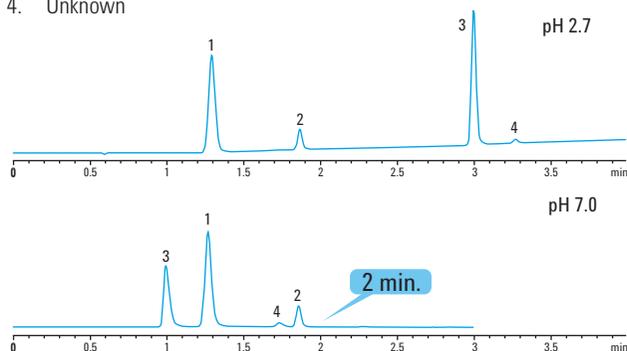
Less retention can save significant time – the C8 is a good choice here. The RRHT column is delivering the efficiency and resolution expected, but the C8 bonded phase may be the best choice.

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

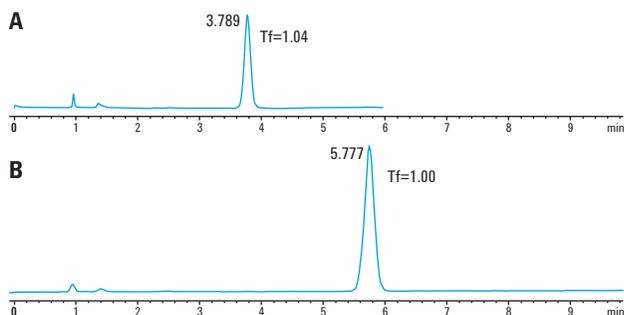


Both Eclipse Plus C18 and C8 can be used over a wide pH range to optimize selectivity or analysis time.

Eclipse Plus C8 is Less Retentive than Eclipse Plus C18

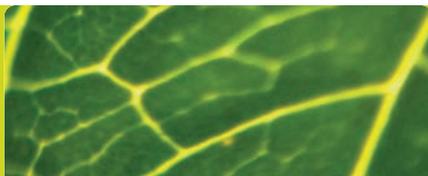
Column A: Eclipse Plus C8
959996-906
4.6 x 100mm, 5µm
Column B: Eclipse Plus C18
959996-902
4.6 x 100mm, 5µm

Mobile Phase: 80% Methanol 8mM (total)
 K_2HPO_4 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.

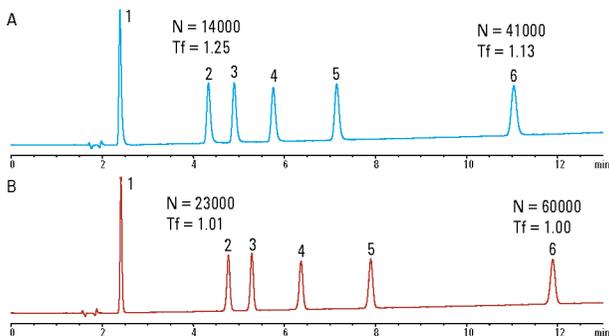




Peak Shape and Efficiency are Better with ZORBAX Eclipse Plus

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

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



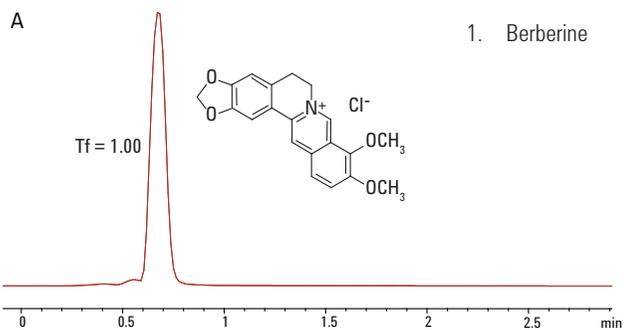
Mobile: A: 0.1% formic acid
 Phase: B: 0.1% formic acid in ACN
 Flow Rate: 1.0 mL/min
 Gradient: 0.0 min 10% B
 15 min 30% B
 Temperature: 40°C
 Detector: UV 254 nm
 Publication: 5989-4934EN
 Sample: Sulfonamides

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

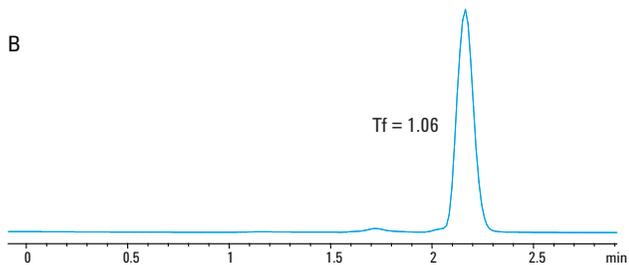
Fast and Ultra-Fast Analysis of Basic Compounds on Eclipse Plus

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

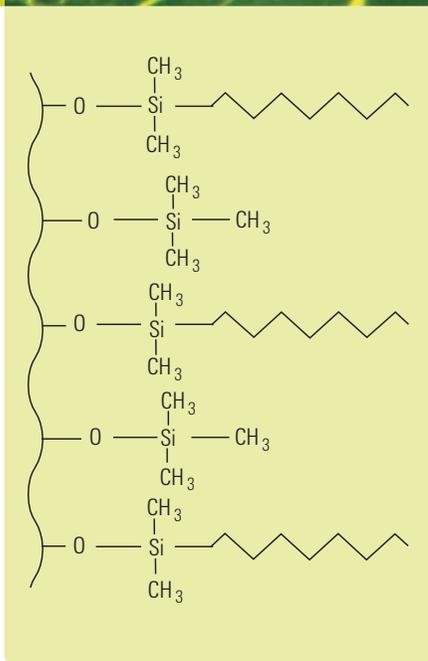


Column B: Eclipse Plus C18 959993-902 4.6 x 150mm, 5µm



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 |
| ZGC | Guard Cartridges, 4/pk | 4.6 x 12.5 | 5 | 820950-936 | 820950-937 |
| ZGC | Guard Cartridges, 4/pk | 2.1 x 12.5 | 5 | 821125-936 | 821125-937 |
| ZGC | 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 μm to 7 μm
- 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 μm 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.

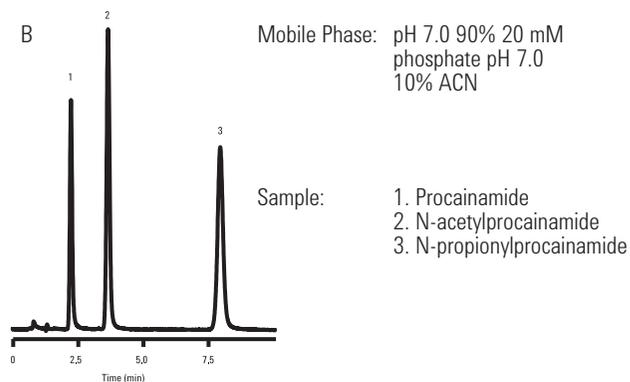
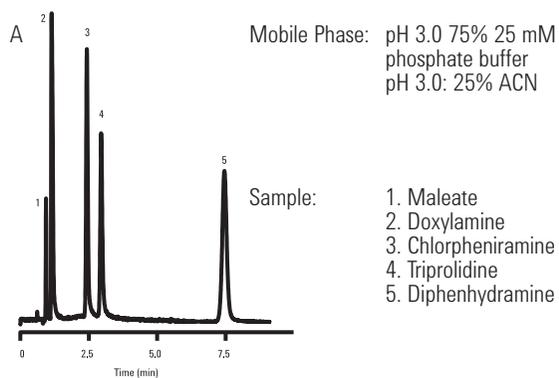
Column Specifications

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



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

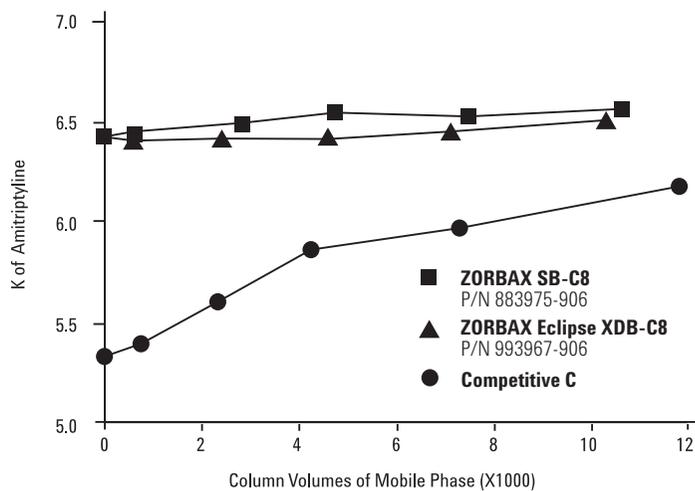
Column: Eclipse XDB-C8
993967-906
4.6 x 150mm, 5µm

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

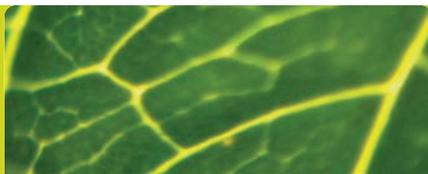
Retention Test Conditions:
65% Methanol
35% Water

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

Sample: Tricyclic Antidepressants



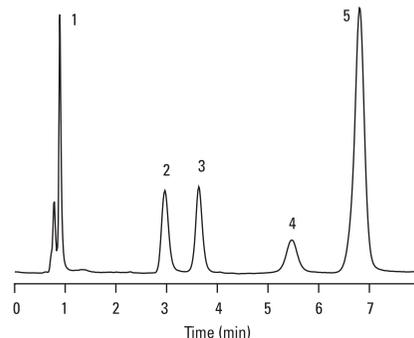
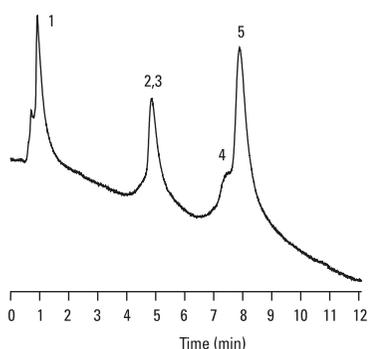
Eclipse XDB columns are stable over a wide pH range. At low pH, Eclipse, an end capped column is extremely stable and shows equivalent stability to a non-endcapped column, SB-C8, at pH 3. The columns were purged with a pH 3 mobile phase at 60°C. Then they were tested with a strongly basic compound to 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.

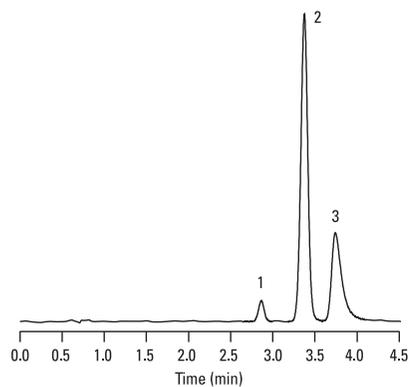
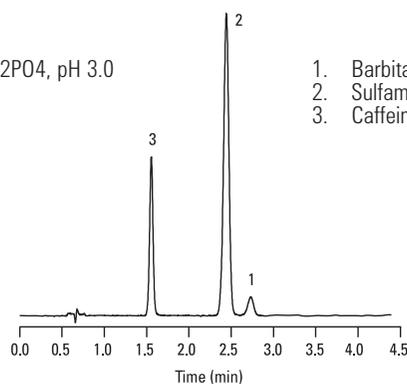
Selectivity Changes for Basic Compounds with Eclipse XDB and Stable Bond

Column A: Eclipse XDB-C8
966967-906
4.6 x 75mm, 3.5µm

Mobile Phase: 70% 25 mM NaH₂PO₄, pH 3.0
30% Methanol
Flow Rate: 1.0 mL/min
Temperature: 35°C

Column B: ZORBAX Rx/SB-C8
866953-906
4.6 x 75mm, 3.5µm

1. Barbitol
2. Sulfamethoxazole
3. Caffeine



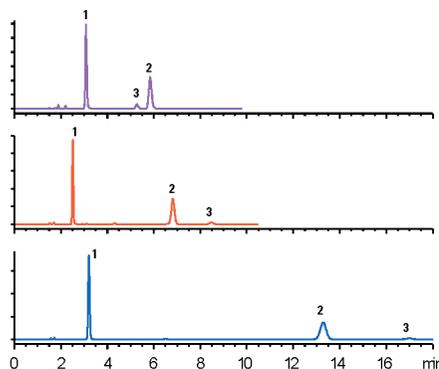
Eclipse XDB and StableBond columns are based on the same silica but have different bonding and endcapping. Therefore, they can have very different selectivity for the same sample under the same conditions, as this example shows.

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



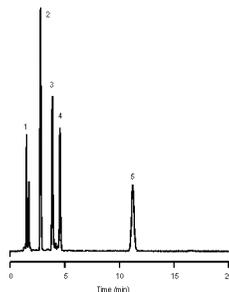
Mobile Phase: 15% H2O: 85% MeOH
Flow Rate: 1.0 mL/min
Temperature: 35°C
Sample: Sunscreens

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 Na2HPO4 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

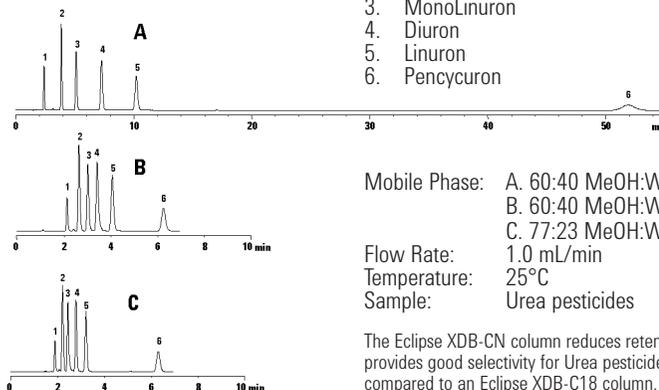
Cephalosporins are a type of antibiotic and many compounds in this family are well separated on the Eclipse XDB-C8 column.

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 |
|--|------------|--------------------|----------------|---------------|--------------------|----------------|
| Standard Columns (no special hardware required, 400 bar) | | | | | | |
| 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 |
|--|------------|--------------------|----------------|---------------|--------------------|----------------|
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | | | |
|  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 |
|--|-----------|--------------------|----------------|---------------|
| Agilent Cartridge Columns (require hardware kit 5021-1845) | | | | |
|  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 |
| Standard Columns (no special hardware required, 400 bar) | | | | |
| 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 |
|---|---|-----------|--------------------|----------------|---------------|
| Rapid Resolution HT Cartridges (require hardware kit 820555-901) | | | | | |
| 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 |
| Capillary Glass-lined Columns | | | | | |
| | 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

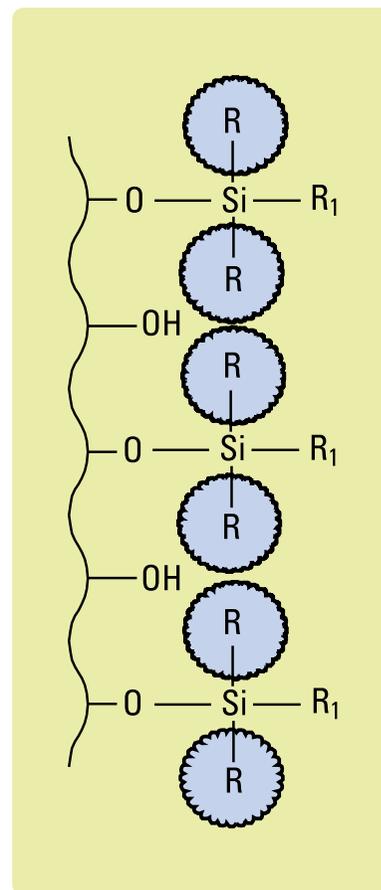
- Longest column lifetime and best reproducibility for low pH separations – down to pH 1
- Patented stable column chemistry allows use at high temperature and low pH without degradation
- Six different bonded phases provide broad selectivity – SB-C18, SB-C8, SB-CN, SB-Phenyl, SB-C3, SB-Aq
- High purity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

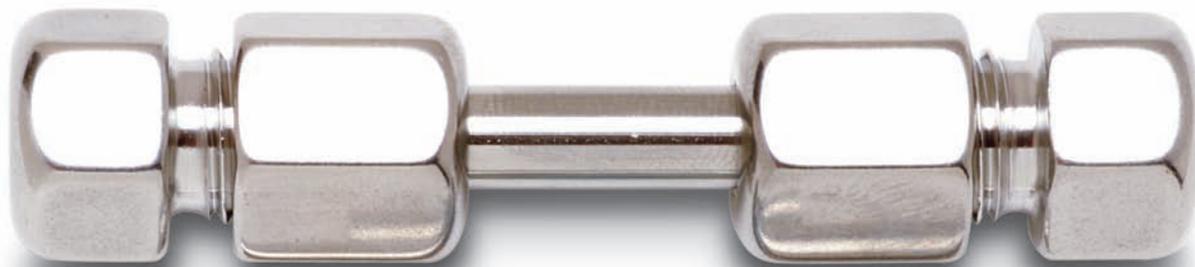
Column Specifications

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

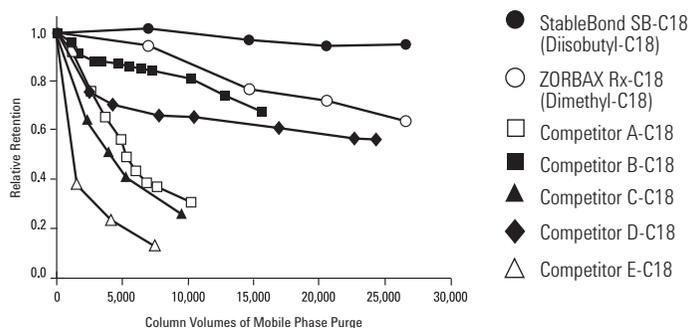
Column: ZORBAX SB-C18
883975-902
4.6 x 150mm, 5µm

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

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

Test Solute: Toluene

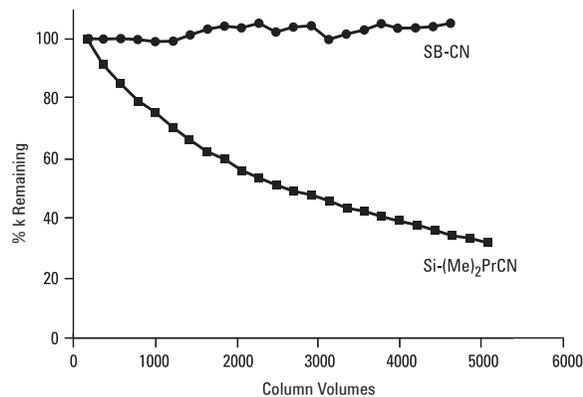
Temperature: 90°C



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



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

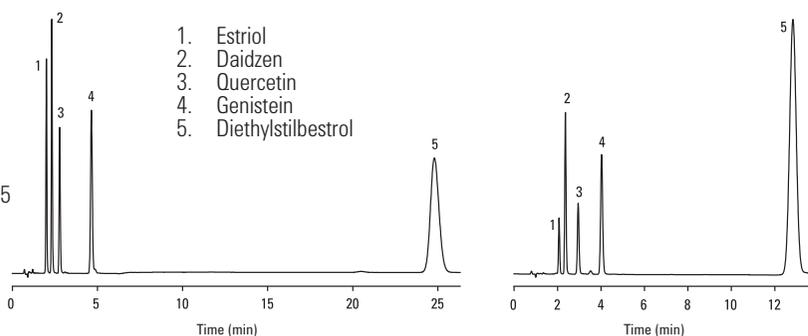
Column A: ZORBAX SB-C18
866953-902
4.6 x 75mm, 3.5µm

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

Mobile Phase: 30% ACN
70% 25mM NaH₂PO₄, pH 2.5

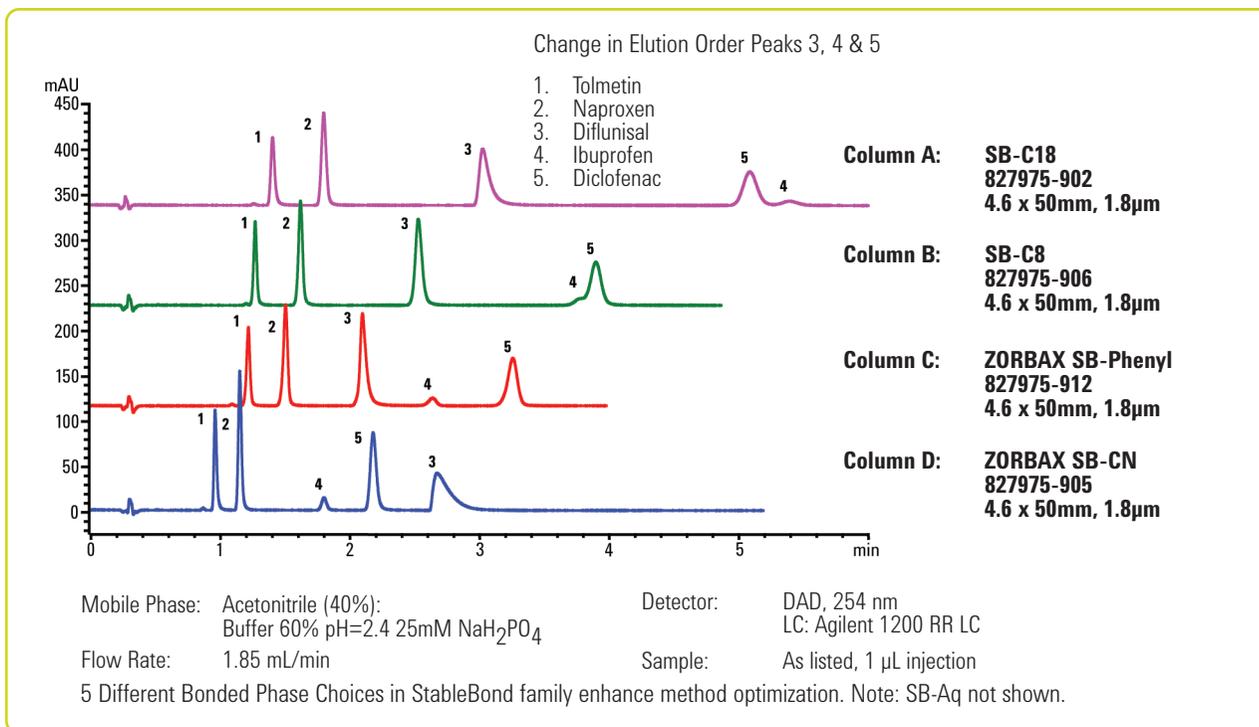
Flow Rate: 1.0 mL/min

Temperature: 35°C

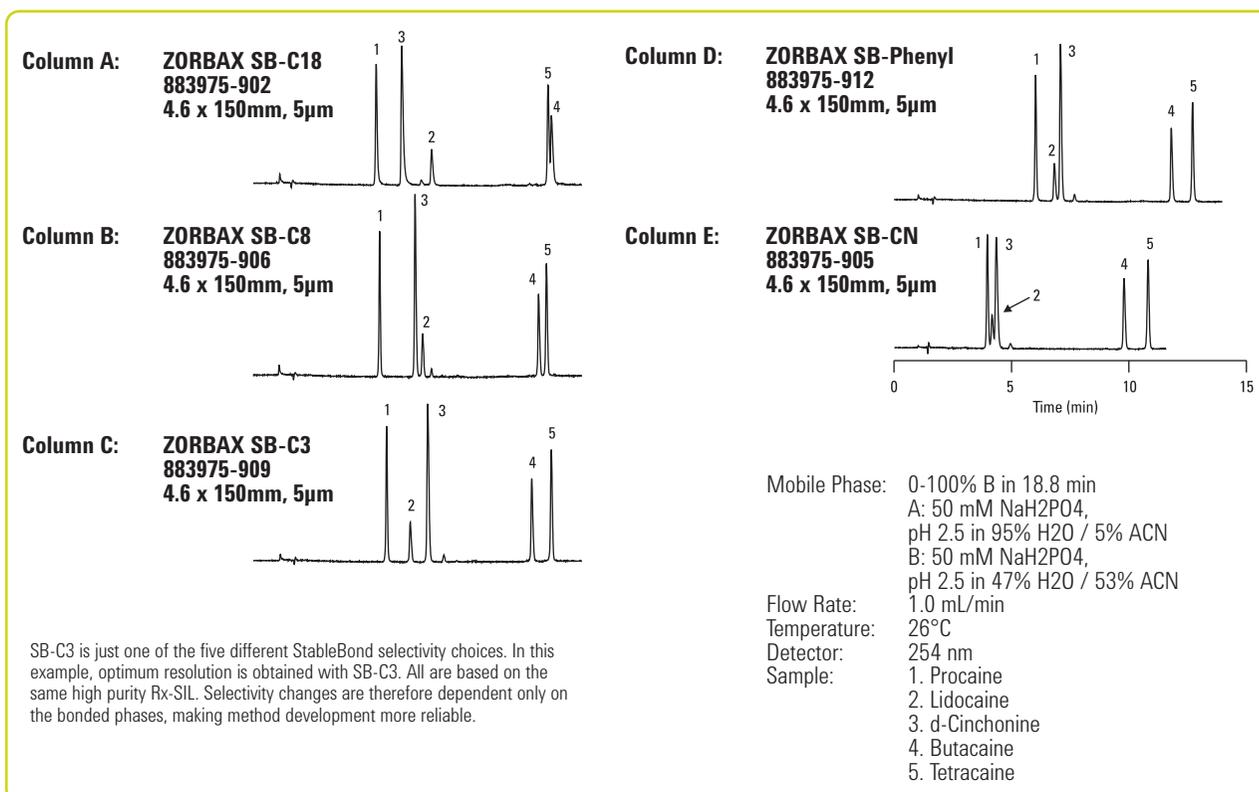


The SB-CN column is used here to reduce analysis time by 50%. The retention of the most hydrophobic analyte is cut in half. At the same time retention of the more polar, early eluting peaks increases slightly.

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 |
|---|------------------------------|-----------|--------------------|---------------|--------------|---------------|---------------|-------------------|------------|
| Standard Columns (no special hardware required, 400 bar) | | | | | | | | | |
| | 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, 600 bar** | 2.1 x 150 | 1.8 | 820700-902 | 820700-906 | 820700-905 | | 820700-912 | |
| | Narrow Bore RRHT, 600 bar** | 2.1 x 100 | 1.8 | 828700-902 | 828700-906 | 828700-905 | | 828700-912 | 828700-914 |
| | Narrow Bore RRHT, 600 bar** | 2.1 x 50 | 1.8 | 827700-902 | 827700-906 | 827700-905 | | 827700-912 | 827700-914 |
| | Narrow Bore RRHT, 600 bar** | 2.1 x 30 | 1.8 | 824700-902 | 824700-906 | 824700-905 | | 824700-912 | 824700-914 |
| | Narrow Bore RRHT, 600 bar** | 2.1 x 20 | 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 | | | | |
| P | Guard Cartridge, 2/pk | 9.4 x 15 | 7 | 820675-115 | 820675-115 | 820675-124 | | 820675-115 | |
| ZGC | Guard Cartridge, 4/pk | 4.6 x 12.5 | 5 | 820950-920 | 820950-915 | 820950-916 | 820950-922 | 820950-917 | 820950-933 |
| ZGC | Guard Cartridge, 4/pk | 2.1 x 12.5 | 5 | 821125-915 | 821125-915 | 821125-924 | 821125-924 | 821125-915 | 821125-933 |
| P | Guard Hardware Kit | 9.4 x 15 | | 840140-901 | 840140-901 | 840140-901 | 840140-901 | 840140-901 | |
| ZGC | Guard Hardware Kit | | | 820888-901 | 820888-901 | 820888-901 | 820888-901 | 820888-901 | 820888-901 |

PrepHT Cartridge Columns (require endfittings kit 820400-901)

| | | | | | | | | | |
|-----------|------------------------------|------------|---|------------|------------|------------|------------|------------|------------|
| PI | PrepHT Cartridge | 21.2 x 250 | 7 | 877250-102 | 877250-106 | 877250-105 | | 877250-112 | 877250-114 |
| PI | PrepHT Cartridge | 21.2 x 150 | 7 | 877150-102 | 877150-106 | | | | 877150-114 |
| PI | PrepHT Cartridge | 21.2 x 150 | 5 | 870150-902 | 870150-906 | | | | 870150-914 |
| PI | PrepHT Cartridge | 21.2 x 100 | 5 | 870100-902 | 870100-906 | | | | 870100-914 |
| PI | PrepHT Cartridge | 21.2 x 50 | 5 | 870050-902 | 870050-906 | | | | 870050-914 |
| PI | 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 |
|---|-------------------------|-----------|--------------------|---------------|--------------|
| Agilent Cartridge Columns (require hardware kit 5021-1845) | | | | | |
| AC | Analytical | 4.6 x 250 | 5 | 7995218-585 | 7995208-585 |
| AC | Analytical | 4.6 x 150 | 5 | 7995218-595 | 7995208-595 |
| AC | Rapid Resolution | 4.6 x 75 | 3.5 | 7995218-344 | 7995208-344 |
| AC | Guard Cartridges, 10/pk | 4.0 x 4 | 5 | 7995118-504 | 7995118-504 |
| AC | Cartridge Holder | | | 5021-1845 | 5021-1845 |

ZORBAX 80Å StableBond (Continued)

| Hardware | Description | Size (mm) | Particle Size (µm) | SB-C18 USP L1 | SB-C8 USP L7 |
|---|---|-----------|--------------------|---------------|--------------|
| Standard Columns (no special hardware required, 400 bar) | | | | | |
| | 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 | |
| Rapid Resolution HT Cartridges (require hardware kit 820555-901) | | | | | |
| RR | Rapid Resolution Cartridge | 4.6 x 30 | 3.5 | 833975-902 | 833975-906 |
| RR | Rapid Resolution Cartridge, 3/pk | 4.6 x 30 | 3.5 | 833975-932 | 833975-936 |
| RR | Rapid Resolution Cartridge | 4.6 x 15 | 3.5 | 831975-902 | 831975-906 |
| RR | Rapid Resolution Cartridge, 3/pk | 4.6 x 15 | 3.5 | 831975-932 | 831975-936 |
| RR | Rapid Resolution Cartridge | 2.1 x 30 | 3.5 | 873700-902 | 873700-906 |
| RR | Rapid Resolution Cartridge, 3/pk | 2.1 x 30 | 3.5 | 873700-932 | 873700-936 |
| RR | Rapid Resolution Cartridge | 2.1 x 15 | 3.5 | 875700-902 | 875700-906 |
| RR | Rapid Resolution Cartridge, 3/pk | 2.1 x 15 | 3.5 | 875700-932 | 875700-936 |
| RR | Rapid Resolution HT Cartridge | 4.6 x 50 | 1.8 | 825975-902 | |
| RR | Rapid Resolution HT Cartridge, 3/pk | 4.6 x 50 | 1.8 | 825975-932 | |
| RR | Rapid Resolution HT Cartridge | 4.6 x 30 | 1.8 | 823975-902 | |
| RR | Rapid Resolution HT Cartridge, 3/pk | 4.6 x 30 | 1.8 | 823975-932 | |
| RR | Rapid Resolution HT Cartridge | 4.6 x 15 | 1.8 | 821975-902 | |
| RR | Rapid Resolution HT Cartridge, 3/pk | 4.6 x 15 | 1.8 | 821975-932 | |
| RR | Rapid Resolution HT Cartridge | 2.1 x 50 | 1.8 | 825700-902 | |
| RR | Rapid Resolution HT Cartridge, 3/pk | 2.1 x 50 | 1.8 | 825700-932 | |
| RR | Rapid Resolution HT Cartridge | 2.1 x 30 | 1.8 | 823700-902 | |
| RR | Rapid Resolution HT Cartridge, 3/pk | 2.1 x 30 | 1.8 | 823700-932 | |
| RR | Rapid Resolution HT Cartridge | 2.1 x 15 | 1.8 | 821700-902 | |
| RR | Rapid Resolution HT Cartridge, 3/pk | 2.1 x 15 | 1.8 | 821700-932 | |
| RR | Hardware Kit for RR and RRHT Cartridges | | | 820555-901 | 820555-901 |
| Capillary Glass-lined Columns | | | | | |
| | 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 ² /g | 60°C | 2.0-9.0 | No | 12% |
| ZORBAX Rx-C8 | 80Å | 180 m ² /g | 80°C | 1.0-8.0 | No | 5.5% |

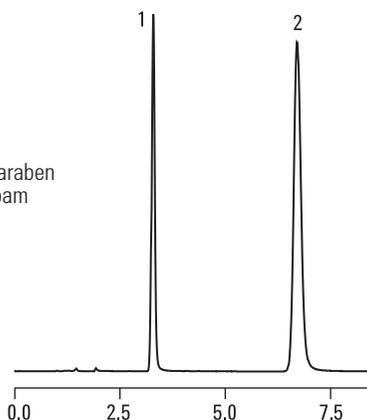
Analysis of Diazepam on Rx-C18

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

Mobile Phase: 35% H₂O: 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 |
| P | Guard Cartridge, 2/pk | 9.4 x 15 | 7 | 820675-115 | 820675-115 |
| ZGC | Guard Cartridge, 4/pk | 4.6 x 12.5 | 5 | 820950-914 | 820950-913 |
| ZGC | Guard Cartridge, 4/pk | 2.1 x 12.5 | 5 | 821125-915 | 821125-915 |
| P | Guard Hardware Kit | 9.4 x 15 | | 840140-901 | 840140-901 |
| ZGC | Guard Hardware Kit | | | 820888-901 | 820888-901 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | | |
| PI | PrepHT Cartridge | 21.2 x 250 | 7 | 877967-102 | 877250-106 |
| PI | PrepHT Cartridge | 21.2 x 150 | 7 | | 877150-106 |
| PI | PrepHT Cartridge | 21.2 x 150 | 5 | | 870150-906 |
| PI | PrepHT Cartridge | 21.2 x 100 | 5 | | 870100-906 |
| PI | PrepHT Cartridge | 21.2 x 50 | 5 | | 870050-906 |
| PI | PrepHT Guard Cartridge, 2/pk | | | 820212-914 | 820212-915 |
| PI | Guard Cartridge Hardware | | | 820444-901 | 820444-901 |
| PI | 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

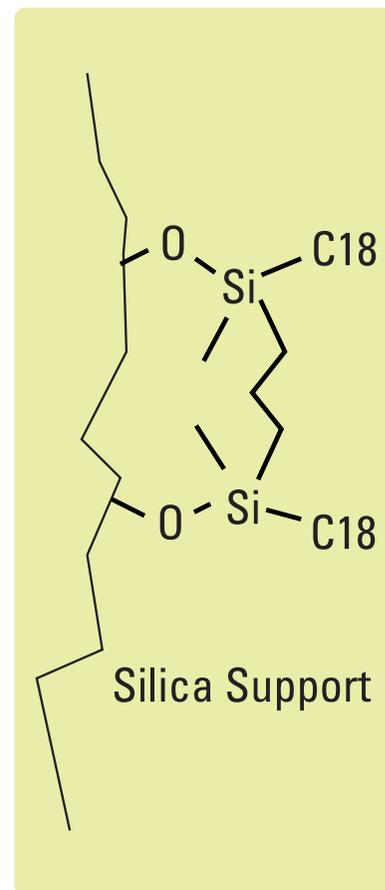
- High efficiency and long life at high pH – up to pH 11.5
- Unique bidentate bonding and double endcapping provides high pH stability
- More efficiency and better peak shape than polymer-based columns
- Improve retention, resolution and peak shape of basic compounds
- High sensitivity for LC/MS separations 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 ² /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



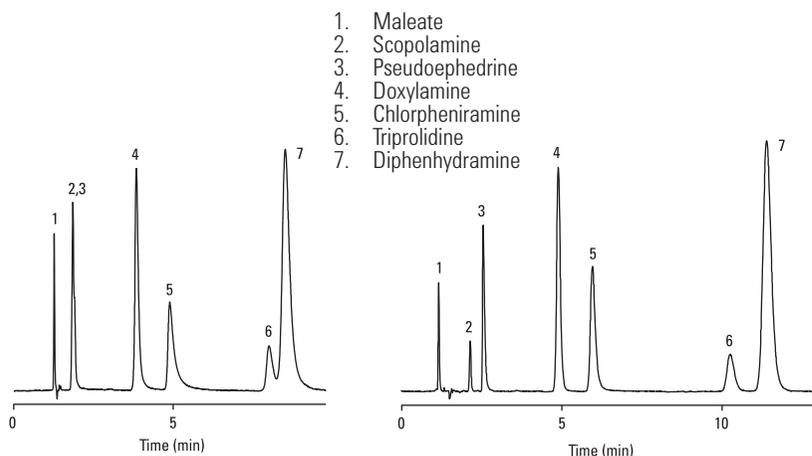
Basic Antihistamines on Extend-C18 at High pH

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

Mobile Phase: pH 7:
30% 20 mM Na₂HPO₄
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.



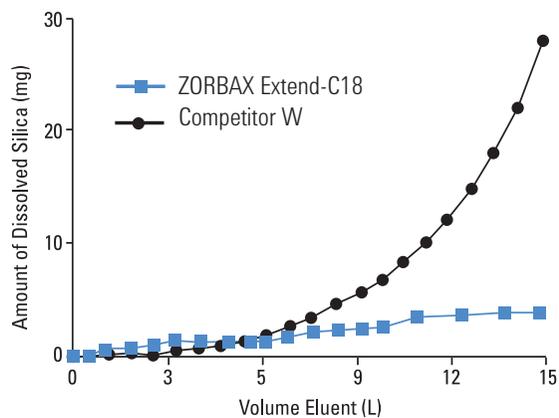
Long Life at High pH with Extend-C18

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 NaH₂PO₄, pH 3.0
20% Methanol

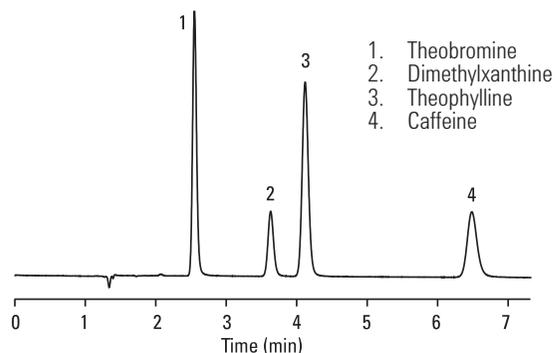
Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: 254 nm

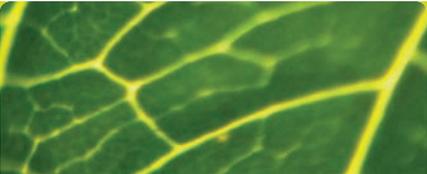
Sample: Basic Compounds

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 | Size (mm) | Particle 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 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | |
|  | 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

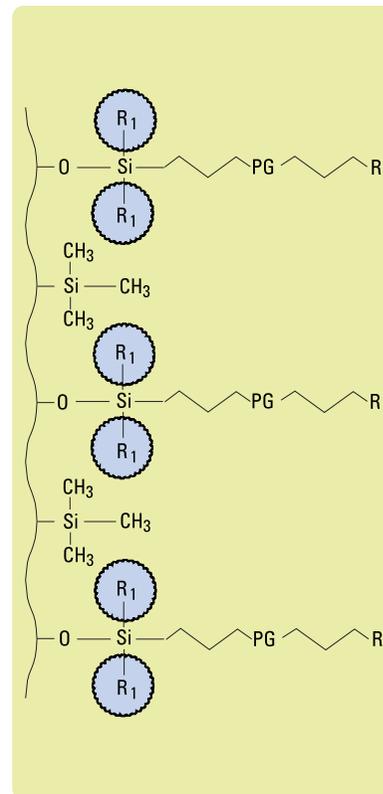
- Excellent peak shape for challenging basic compounds at low and mid pH
- Unique reversed-phase selectivity
- Novel bonding technology with embedded polar group and steric protection
- Usable in 100% aqueous mobile phases

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 ² /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.



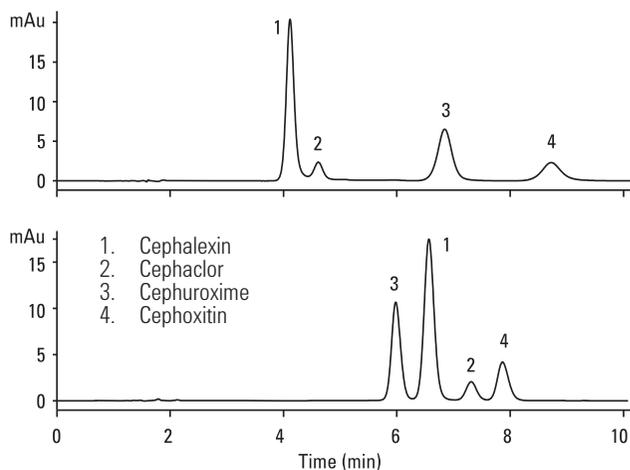
Unique, Polar Alkyl Bonus-RP Bonded Phase

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

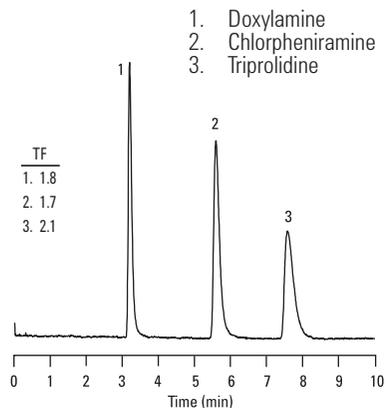


Peak elution order can change dramatically when using Bonus-RP. In this example, the elution order of the first three peaks change.



Improved Peak Shape of Basic Compounds Using Bonus-RP

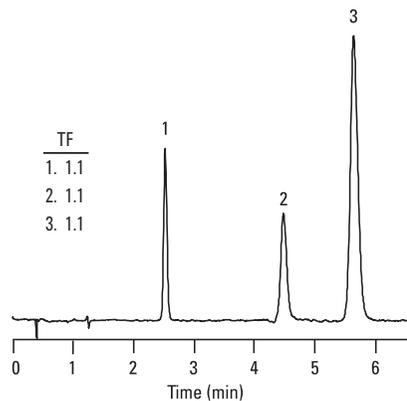
Column: Alkyl-C8
4.6 x 150 mm, 5 µm
Mobile Phase: 75% 25 mM NH₄OAc, pH 5.5
25% ACN
Flow Rate: 1.5 mL/min
Temperature: 40°C
Detector: 254 nm



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

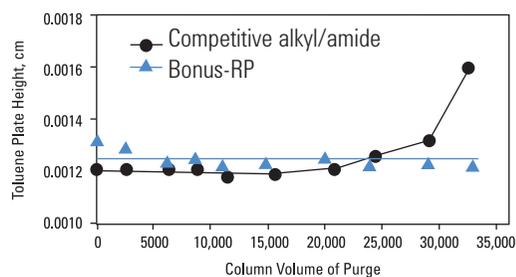
Column: ZORBAX Bonus-RP
883668-901
4.6 x 150mm, 5µm
Mobile Phase: 80% 25 mM NH₄OAc, pH 5.5
20% ACN
Flow Rate: 1.5 mL/min
Temperature: 40°C
Detector: 254 nm



ZORBAX Bonus-RP is Stable at Low and Mid pH

Column: ZORBAX Bonus-RP
883668-901
4.6 x 150mm, 5 μ m

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

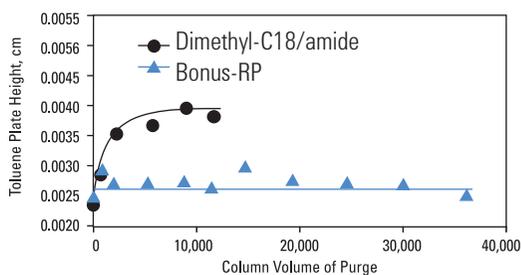


Triple endcapping of Bonus-RP enhances stability at pH 7. Each 10,000 column volumes is equivalent to approximately one working month.

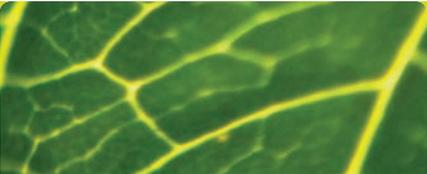
Dimethyl-C18/amide, Bonus-RP

Column: ZORBAX Bonus-RP
883668-901
4.6 x 150mm, 5 μ m

Mobile Phase: Aging:
50% MeOH
50% 0.1% TFA
Test:
80% MeOH
20% H₂O
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. |
|---|---------------------------------|------------|--------------------|------------|
| Standard Columns (no special hardware required, 400 bar) | | | | |
| | 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 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | |
|  | 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 |

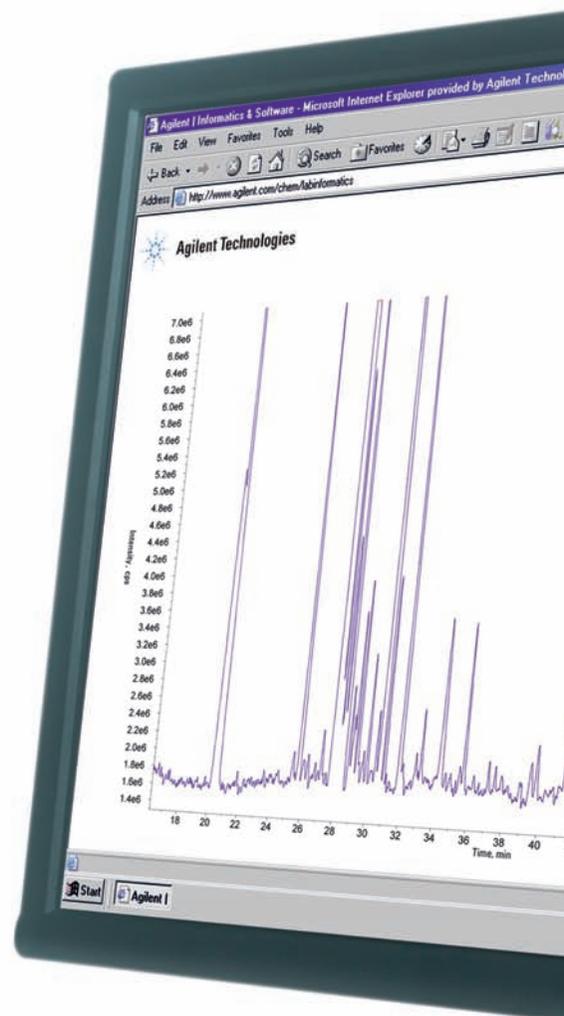
*RR: Rapid Resolution 3.5 µm

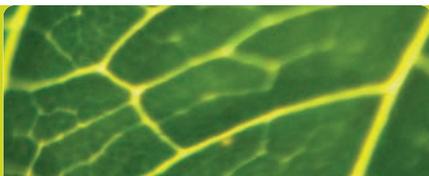
ZORBAX Method Development Kits

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 µm 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 µm 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 µm 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 µm columns; one each: XDB-C18, XDB-C8 and XDB-Phenyl phases | 5183-4627 |
| pH Method Development Kit Includes 4.6 x 150 mm, 5 µm 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 µm columns; one each: SB-C18, XDB-C18 and Extend-C18 phases | 5185-5808 |
| Aqueous Method Development Kit Includes 4.6 x 150 mm, 5 µm 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 µm columns; one each: SB-Aq, Bonus RP and SB-C18 | 5185-5810 |





ZORBAX Method Validation Kits

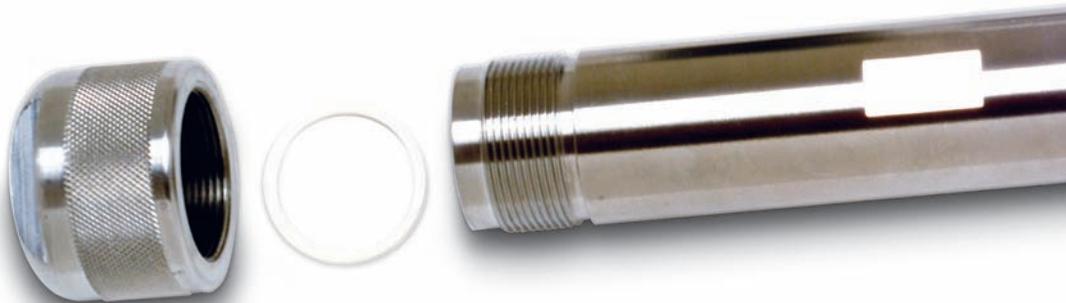


Tips & Tools

Need to order a custom column? Go to www.agilent.com/chem/lccustom 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. 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.

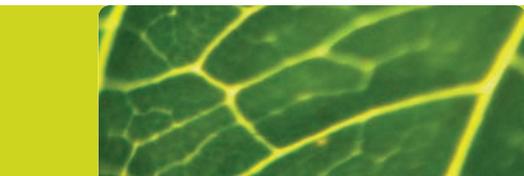


ZORBAX Original Reversed Phase Columns

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 |
|--|----------------------------|------------|--------------------|------------------|------------|----------------|------------|-------------|
| Standard Columns (no special hardware required, 400 bar) | | | | | | | | |
| | 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 | | | | |
| Guard Columns (hardware required) | | | | | | | | |
| P | Guard Cartridge, 2/pk | 9.4 x 15 | 7 | 820675-115 | 820675-115 | 820675-115 | 820675-124 | |
| ZGC | Guard Cartridge, 4/pk | 4.6 x 12.5 | 5 | 820950-902 | 820950-906 | 820950-912 | 820950-905 | 820950-924 |
| P | Guard Hardware Kit | 9.4 x 15 | | 840140-901 | 840140-901 | 840140-901 | 840140-901 | 840140-901 |
| ZGC | Guard Hardware Kit | | | 820888-901 | 820888-901 | 820888-901 | 820888-901 | 820888-901 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | | | | | |
| PI | PrepHT Cartridge | 21.2 x 250 | 7 | 877952-102 | 877952-106 | | 877952-105 | |
| PI | 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



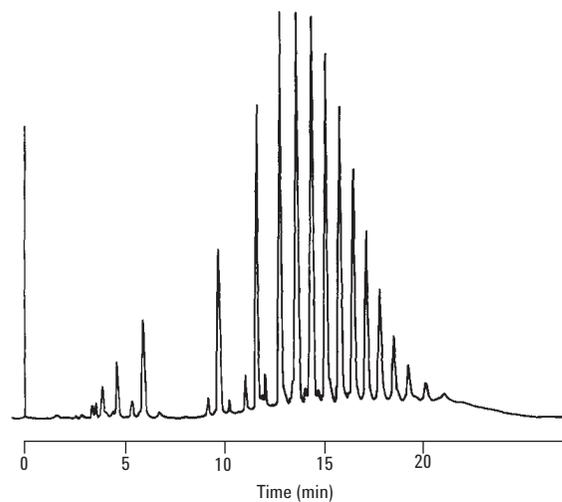
Column Specifications

| Bonded Phase | Pore Size | Surface Area | pH Range | Endcapped | Carbon Load |
|------------------------|-----------|-----------------------|----------|-----------|-------------|
| ZORBAX Rx-SIL | 80Å | 180 m ² /g | 0-8.0 | No | |
| ZORBAX Eclipse XDB-CN | 80Å | 180 m ² /g | 2.0-8.0 | Yes | 4.3% |
| ZORBAX SIL | 70Å | 300 m ² /g | 0-8.0 | No | |
| ZORBAX CN | 70Å | 300 m ² /g | 2.0-7.0 | Yes | 7% |
| ZORBAX NH ₂ | 70Å | 300 m ² /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 |
|---|------------------------------|------------|--------------------|------------|------------|------------|------------|-----------------------|----------------|
| Standard Columns (no special hardware required, 400 bar) | | | | | | | | | |
| | 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 | | |
| Guard Columns (hardware required) | | | | | | | | | |
|  | 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 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | | | | | | |
|  | 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 μm) and Rapid Resolution High Throughput (1.8 μm) 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 μm 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

Together with the Agilent 1200 Series Rapid Resolution System, ZORBAX RRHT 1.8 μm 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.



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.

ZORBAX Rapid Resolution High Throughput 1.8 μm

- 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 μm 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 μm) columns use a totally porous, 1.8 μm 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 μm 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 μm 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 μ m (3) Flow Cell for VWD, 5 μ l capillaries, μ -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 μ m (2) Flow Cell for DAD, 5 μ l capillaries, μ -LC inline filter | 5188-5324 |
| For Diode Array Detector and Mass Spec | Columns: 2.1 x 50 mm, 1.8 μ m (2) Flow Cell for DAD, 1.7 μ 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 μ m

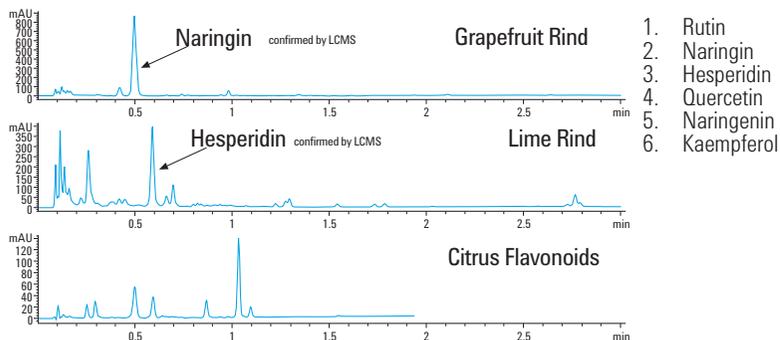
Mobile Phase: A: 0.1% FA, B: 0.075% FA in ACN

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 μ L KOH)
Ultrasonicate 10 min and 0.45 μ m filter
Inject 1 μ 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:H2O
B: ACN

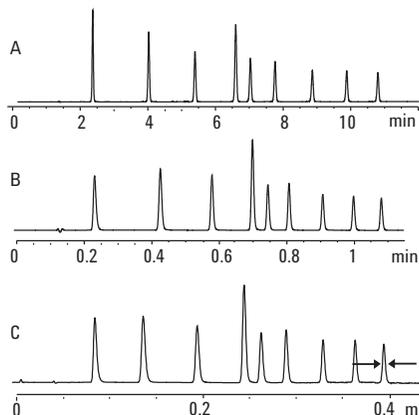
Flow Rate: 1.2 mL/min, 1.0 mL/min, 2.4 mL/min

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

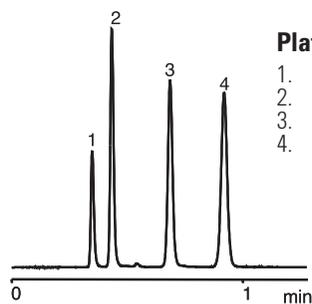
Sample: Alkylphenones



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)

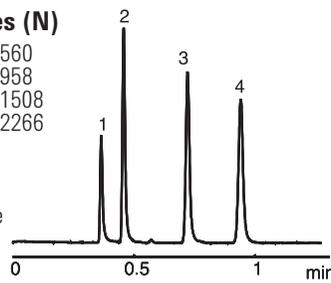
1. 3476
2. 4585
3. 5673
4. 6180

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

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

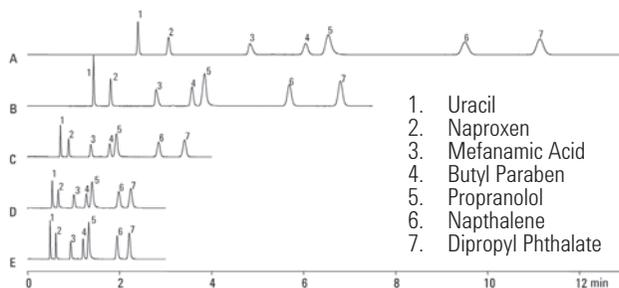
Column D: Eclipse XDB-C18
925975-902
4.6 x 50mm, 1.8µm

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

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: 254 nm



1. Uracil
2. Naproxen
3. Mefenamic Acid
4. Butyl Paraben
5. Propranolol
6. Napthalene
7. Dipropyl Phthalate

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:H2O
B: ACN

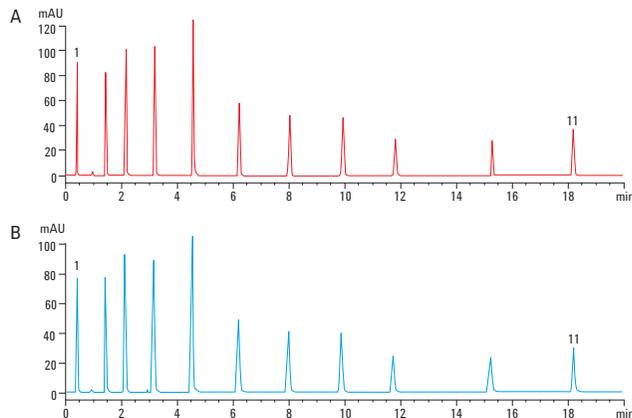
Flow Rate: 0.5 mL/min

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

Temperature: 40°C

Detector: UV 254 nm

Sample: Alkylphenones



1. Uracil
2. C3-Alkylphenone
3. C4-Alkylphenone
4. C5-Alkylphenone
5. C6-Alkylphenone
6. C7-Alkylphenone
7. C8-Alkylphenone
8. C9-Alkylphenone
9. C10-Alkylphenone
10. C12-Alkylphenone
11. C14-Alkylphenone

Long Lifetime of RRHT Columns at Elevated Temp.

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

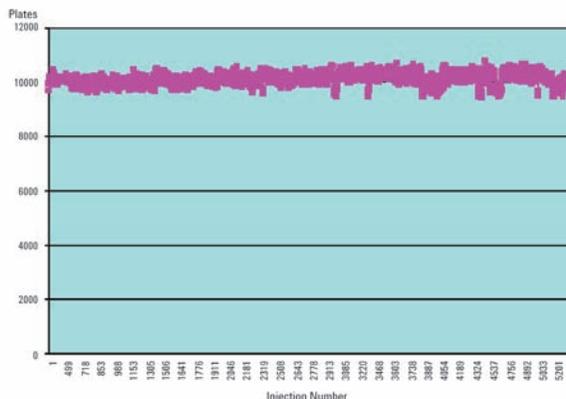
Mobile Phase: A: 60% H2O
B: 40% ACN

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) |
|------------------------|--------------------------------|-------------------------------|
| High Resolution | | |
| 150 | 21,000 | 32,500 |
| 100 | 14,000 | 24,000 |
| 75 | 10,500 | 17,000** |
| Ultra Fast | | |
| 50 | 7000 | 12,000 |
| 30 | 4200 | 6000 |
| 20 | – | 3500 |
| 15 | 2100 | 2500 |

*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 | | |



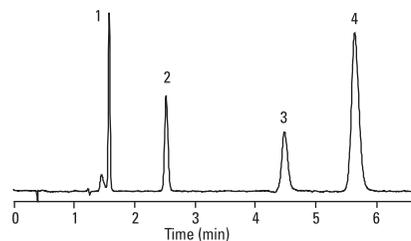
ZORBAX Rapid Resolution 3.5 μm Columns

- 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 μm columns

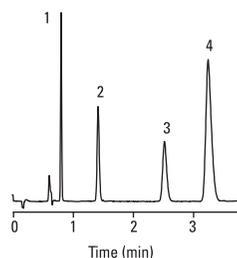
Agilent ZORBAX Rapid Resolution columns, with a 3.5 μm particle size, reduce analysis time and increase sample throughput for any application when compared to 5 μm columns. Rapid Resolution columns are available from 15-150 mm, in 1-4.6 mm IDs, so the best configurations are available for high throughput,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

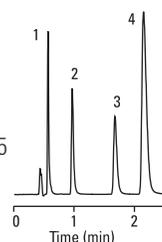


Column B: ZORBAX Bonus-RP
866668-901
4.6 x 75mm, 3.5 μm



Column C: ZORBAX Bonus-RP
861700-901
2.1 x 50mm, 3.5 μm

Mobile Phase: 80% 25 mM NH₄Ac, 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 μm columns are available in narrow bore configurations for great compatibility with LC/MS.

Run Method Development Chromatograms on Rapid Resolution Columns

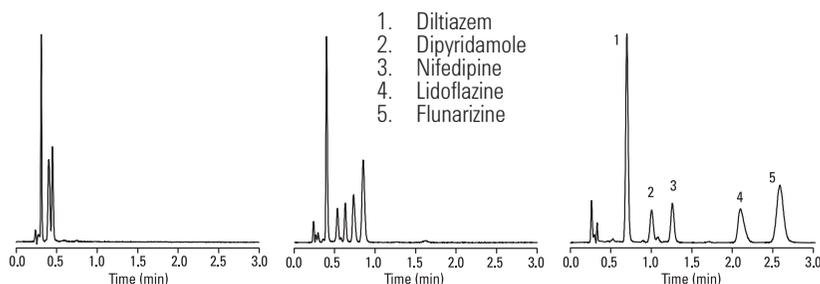
Column: ZORBAX SB-C18
866953-902
4.6 x 75mm, 3.5µm

Mobile Phase: A: 25 mM NaH₂PO₄, 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 | | | | |
| ZGC | Guard Cartridge, 4/pk | 4.6 x 12.5 | 5 | 820950-920 | 820950-915 | 820950-916 | 820950-917 | 820950-922 | 820950-933 |
| ZGC | Guard Cartridges, 4/pk | 2.1 x 12.5 | 5 | 821125-926 | 821125-926 | 821125-924 | 821125-926 | 821125-924 | 821125-933 |
| 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 μ m Rx, Bonus-RP, and Extend-C18 Columns

| Hardware | Description | Size (mm) | Particle Size (μ 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 μ 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

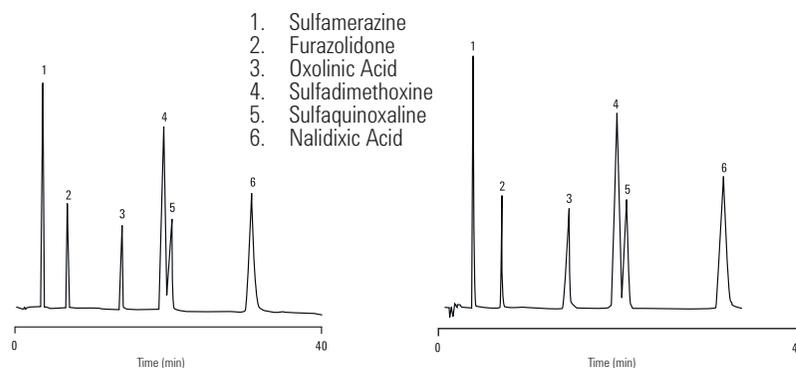
Column A: ZORBAX SB-C18
883975-902
4.6 x 150mm, 5µm

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

Mobile Phase: 20% ACN: 80% 0.2 M Na₂HPO₄
+ 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

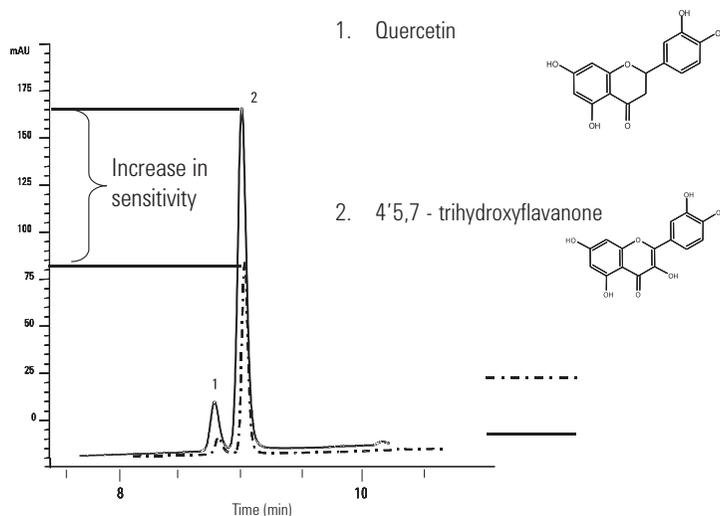
Column A: ZORBAX SB-C18
863953-902
4.6 x 150mm, 3.5µm

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

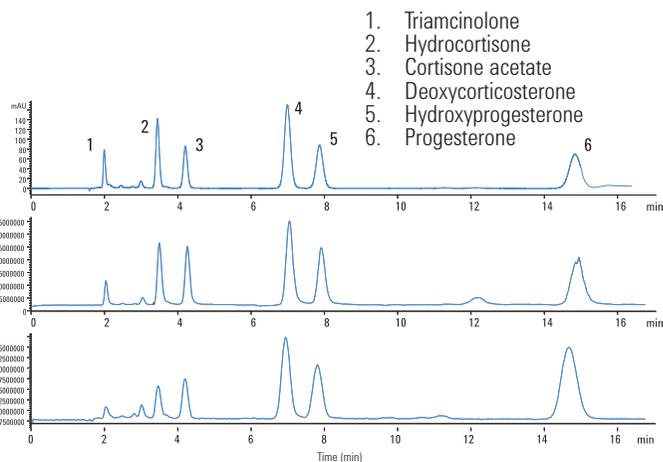
Mobile Phase: A: 70% Methanol+0.4% Formic Acid
B: 30% Water+0.4% Formic Acid

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 $\mu\text{L}/\text{min}$). Therefore, these columns are ideal for use with detectors requiring low flow rates such as some mass spectrometers and with capillary LC systems.

MicoBore 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 μm 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 μm

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

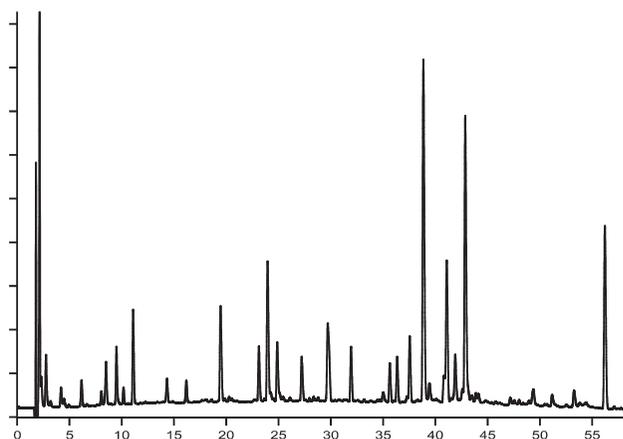
Flow Rate: 50 $\mu\text{L}/\text{min}$

Temperature: 50°C

Detector: 215 nm

Sample: 2 μL
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, 3/pk | 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

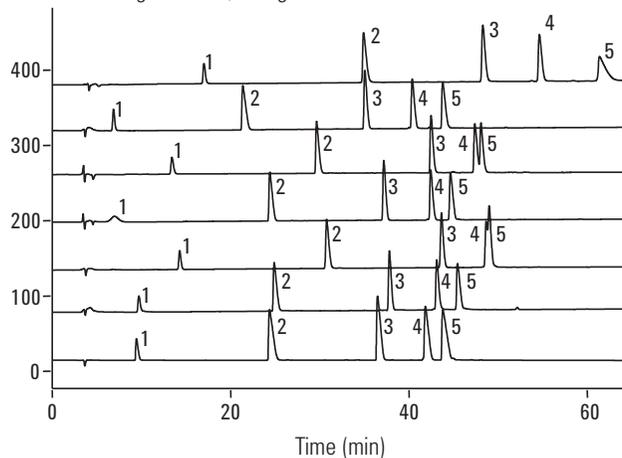
Low Solvent Consumption:
200-500 µl/min

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



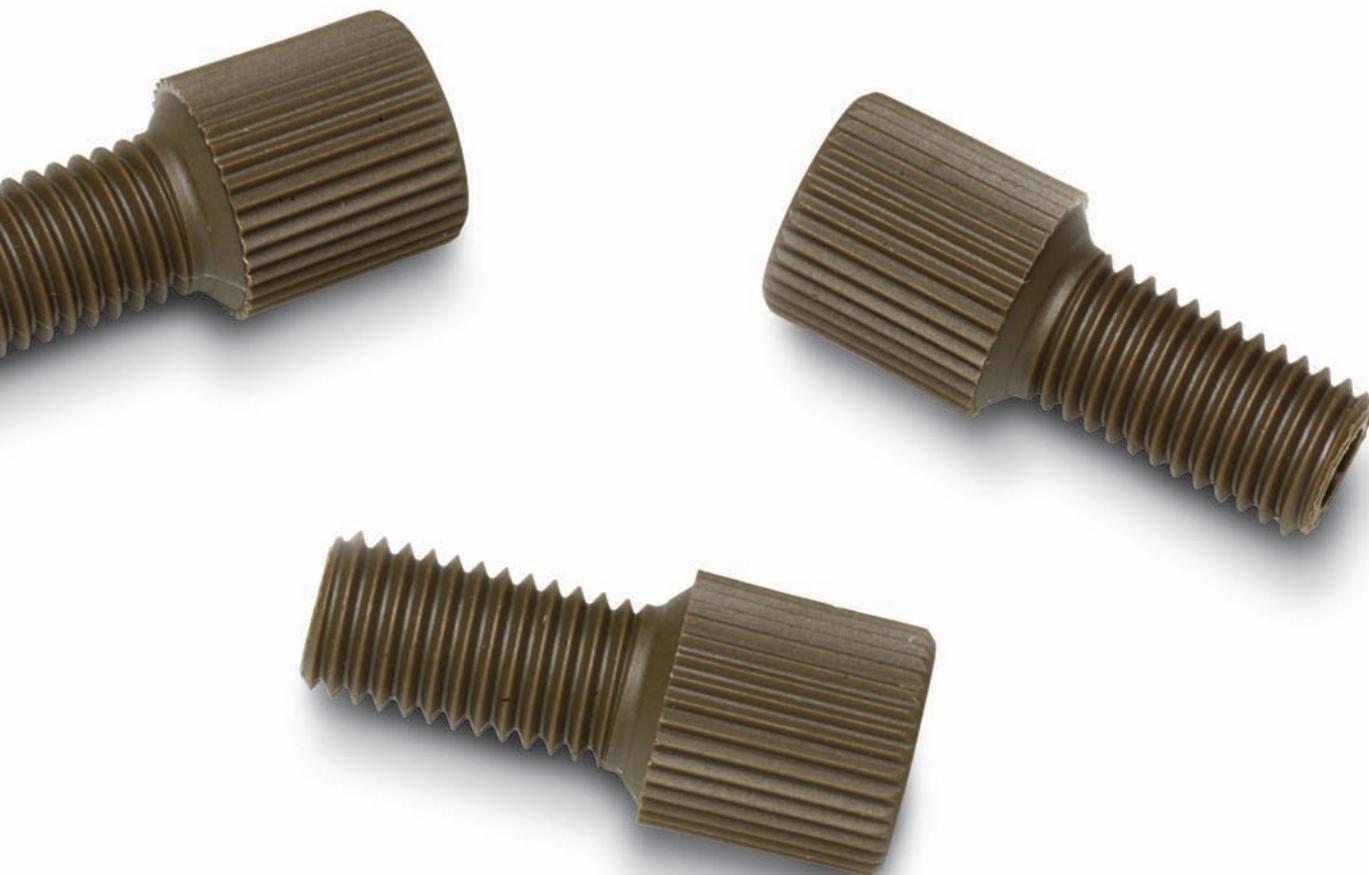
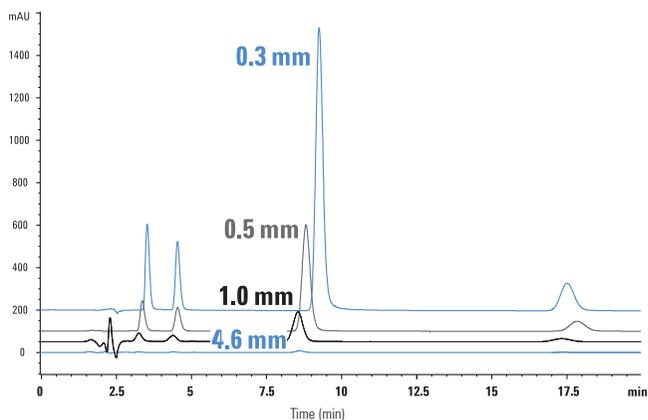
This example shows a peptide standard mixture separated on a variety of the ZORBAX capillary columns. These chromatograms demonstrate the wide range of selectivities available, which can be used to optimize your specific separation.



High Sensitivity with Capillary Columns

Column: ZORBAX SB-C18
5064-8255
0.3 x 150mm, 5 μ m
Column: ZORBAX SB-C18
5064-8256
0.5 x 150mm, 5 μ m
Column: ZORBAX SB-C18
863600-902
1.0 x 150mm, 3.5 μ m
Column: ZORBAX SB-C18
883975-902
4.6 x 150mm, 5 μ 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

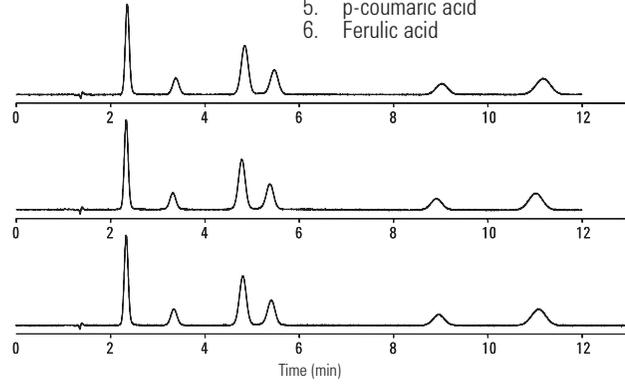
Mobile Phase: A: 75% H₂O with 0.4% formic acid
B: 25% MeOH with 0.4% formic acid

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

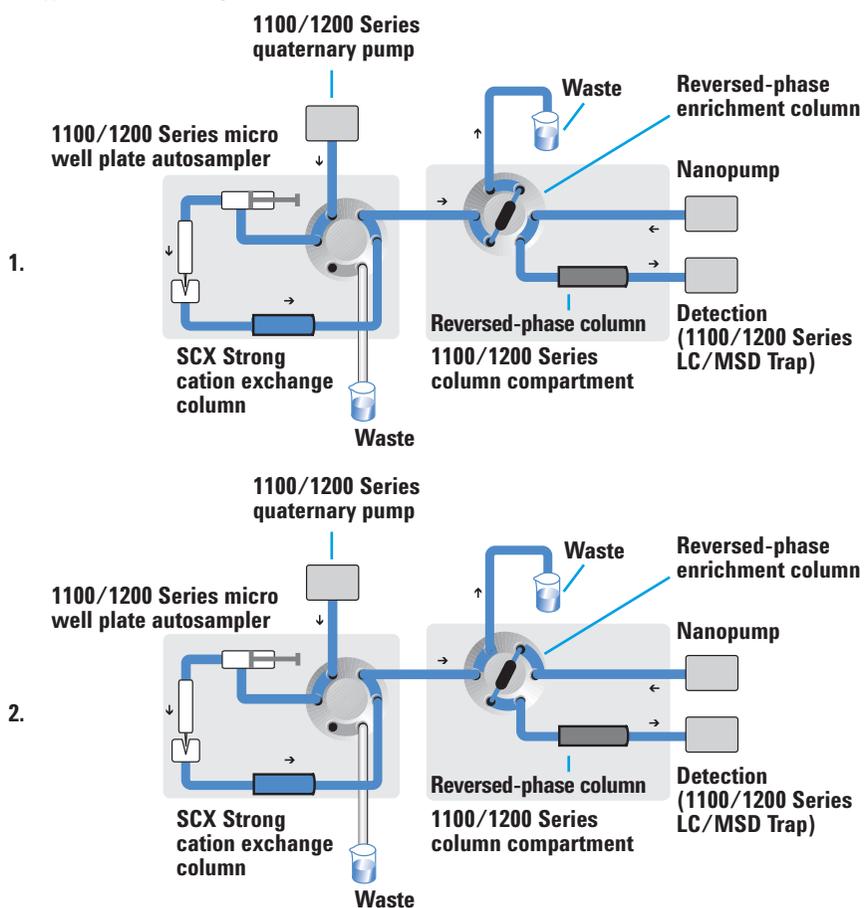


Excellent reproducibility is seen for a separation of polar organic acids on three different StableBond-C18, 0.5 x 150 mm, 5 µm columns. Retention (k) varied less than 0.8% RSD and selectivity (α) varied less than 0.4% RSD.



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

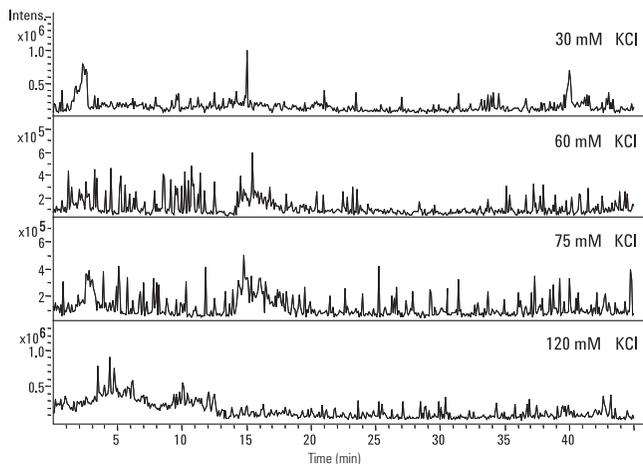
Mobile Phase: Quaternary Pump: 3% Acetonitrile/0.1% Formic Acid
Nanopump: A = Water, 0.1% Formic Acid, B = ACN, 0.1% Formic Acid

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 μm 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.

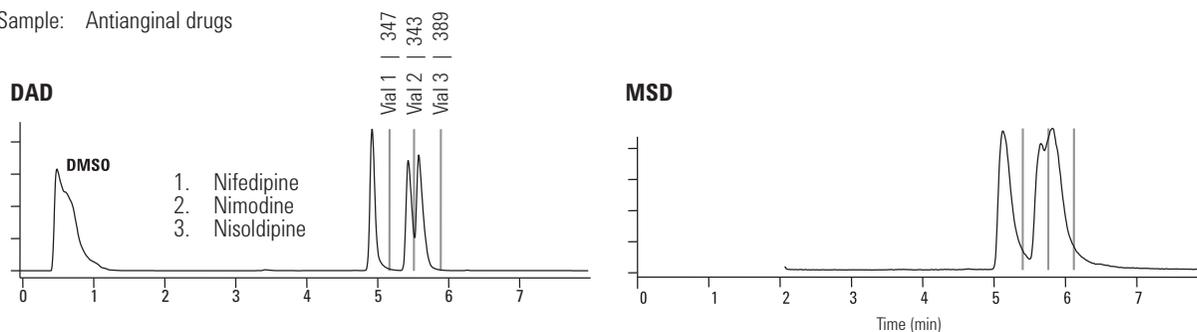
ZORBAX PrepHT columns are packed with 5 and 7 μm particle sizes for very high resolution. The high resolution allows high loadability, high yield, and high purity of compounds. The larger diameter columns and mechanically stronger ZORBAX particles allow for flow rates up to 100 ml/min, thus increasing 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 μm particle size to achieve a balance between high efficiency and high loadability.



High Purity and High Recovery with ZORBAX PrepHT Columns

Sample: Antianginal drugs

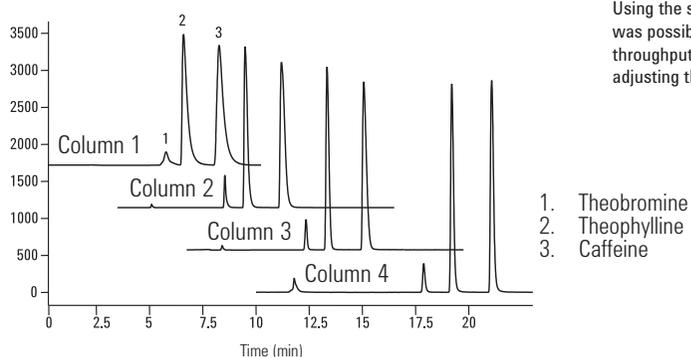


Mass-based fraction collection using ZORBAX SB-C18 column shows high purity and high recovery of each compound (Application Note publication number 5988-7113EN). The separation of the three antianginal drugs was successfully done in a single run with high recovery and >90% purity. Separations up to 2000 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 μ 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 (μ 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 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 |
|---|--------------------------------|------------|--------------------|---------------|--------------|---------------|---------------|-------------------|
|  | 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 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 |
|---|--------------------------------|------------|--------------------|------------------|-----------------|------------------|------------------|
|  | 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 | | Extend-C18 |
|---|-----------------------------------|------------|-----------|-----------|------------|
| | | | Size (mm) | Size (µm) | |
|  | PrepHT Cartridge* | 21.2 x 250 | 7 | Bonus-RP | 878250-101 |
|  | | 21.2 x 150 | 7 | Bonus-RP | 878150-101 |
|  | | 21.2 x 150 | 5 | Bonus-RP | 770150-902 |
|  | | 21.2 x 100 | 5 | Bonus-RP | 770100-902 |
|  | | 21.2 x 50 | 5 | Bonus-RP | 770050-902 |
|  | PrepHT Guard Cartridge, 2/pk** | 17 x 7.5 | 5 | Bonus-RP | 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) | C8 | CN | NH2 | SIL |
|---|-------------------|------------|-----------------------|------------|------------|------------|------------|------------|
| | | | | USP L1 | USP L7 | USP L10 | USP L8 | 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 | Rx-C18 |
|---|-----------------------------------|------------|-----------------------|------------|------------|
| | | | | USP L3 | 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 ² /g | 60°C* | 2.0-10.0 | Single | 24% |
| Silica | 100Å | 400 m ² /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.

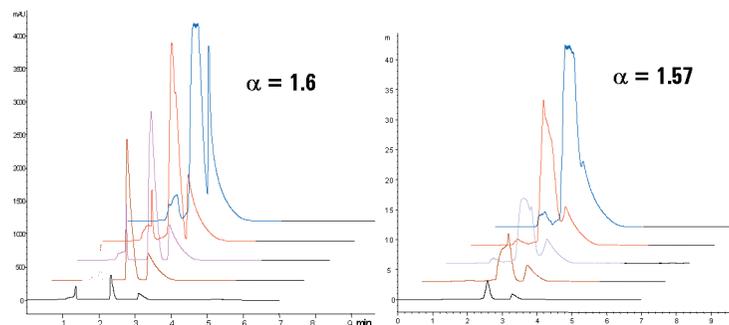
Superior Loadability on Agilent Prep C18 with Basic Compounds

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

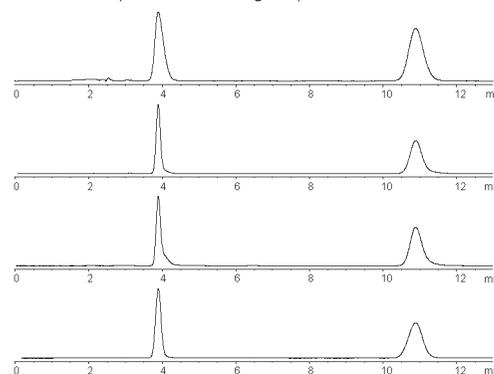
Flow Rate: 0.7 mL/min
14.87 mL/min
29.77 mL/min
85.37 mL/min

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.

Agilent Prep LC Columns

| Hardware | Description | Size (mm) | Particle | | C18 | Silica |
|---|------------------------------------|------------|-----------|--|------------|------------|
| | | | Size (µm) | | | |
| Standard Columns (no special hardware required, 400 bar) | | | | | | |
| | Scalar | 4.6 x 250 | 10 | | 440910-902 | 440910-901 |
| | Scalar | 4.6 x 150 | 10 | | 443910-902 | 443910-901 |
| | Scalar | 4.6 x 100 | 10 | | 449910-902 | |
| | 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 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901)* | | | | | | |
|  | 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 |
| Standard Columns (no special hardware required, 400 bar) | | | | | | |
| | 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 |
| Guard Columns (hardware required) | | | | | | |
|  | 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.

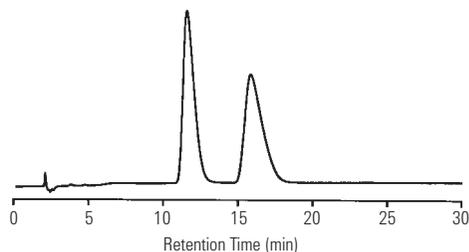
- Broad compatibility and long lifetime with aqueous, buffered, and organic mobile phases.
- Recognize ionic, polar, hydrogen-bonding, and hydrophobic groups – as well as three-dimensional molecular structures.
- Method development guidelines are included in each column box, saving you time and eliminating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





Separation of Enantiomers of Fluoxetine (Prozac)

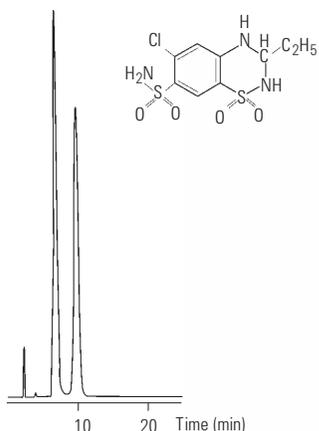
Column: **Ultron ES-OVM Chiral**
702111651
4.6 x 150mm, 5µm
Mobile Phase: 25:75 (v/v) EtOH / 20 mM KH₂PO₄, pH 5.5
(adjusted with NaOH)
Temperature: Ambient
Detector: UV (225 nm)
Sample: Mixture Fluoxetine (Prozac) enantiomers

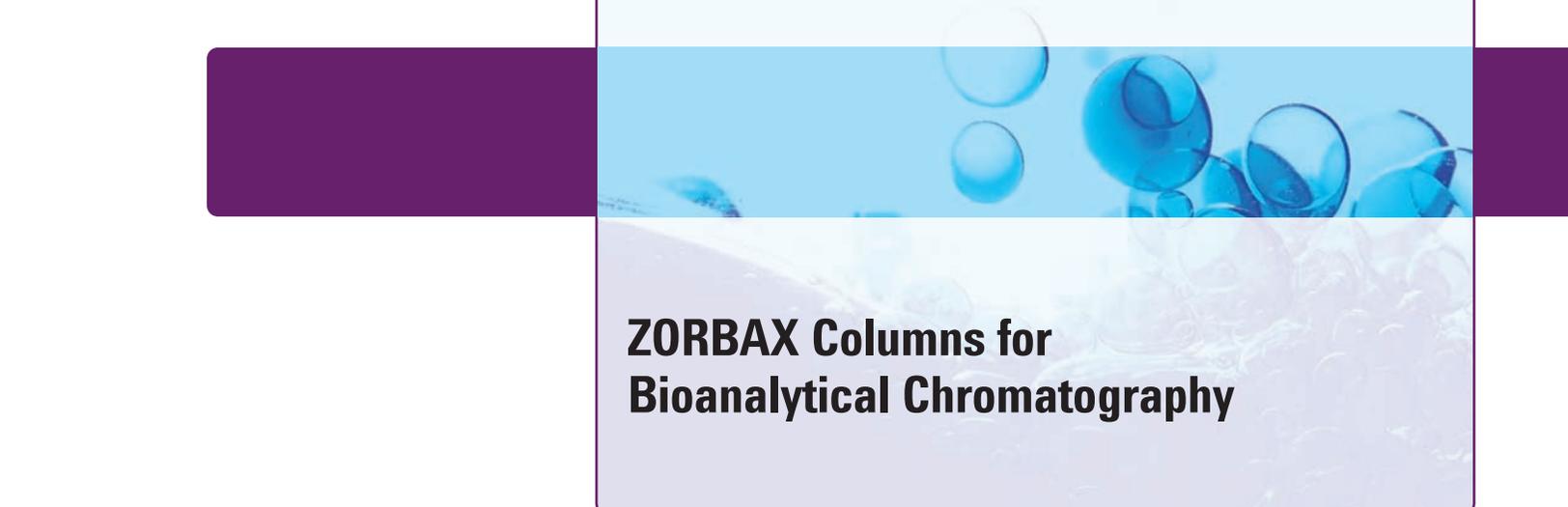


Courtesy of D. S. Risley and V. S. Sharp of Lilly Research Laboratories, Eli Lilly and Co.

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 KH₂PO₄ (pH 4.6)
Flow Rate: 1 mL/min
Temperature: 25°C
Detector: 220 nm





ZORBAX Columns for Bioanalytical Chromatography

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

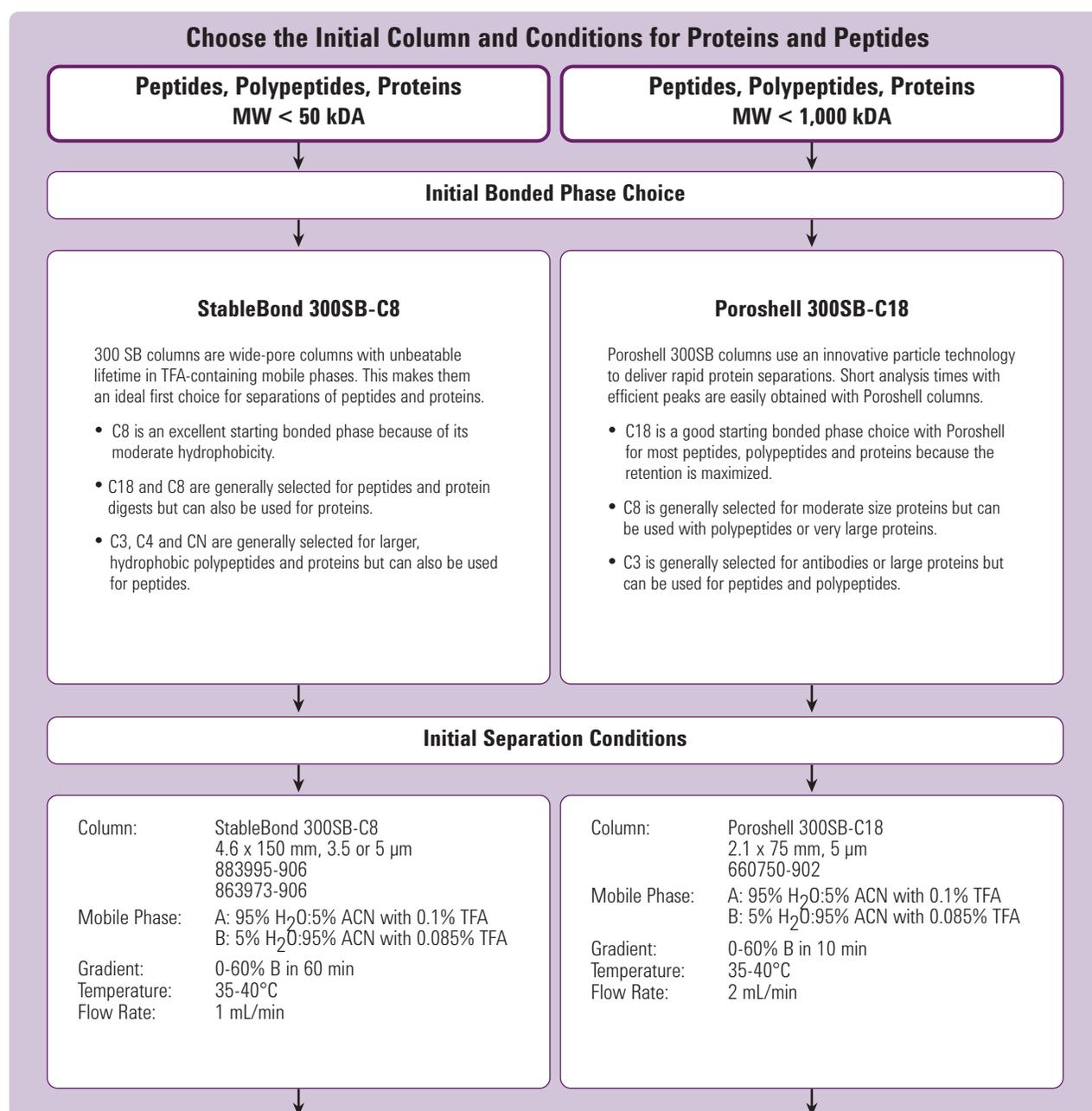
Basic peptide separations. High-sensitivity, high-resolution amino acid analyses. Fast-size exclusion separations of antibodies. The number of bioanalytical HPLC applications continues to grow at an unprecedented rate. And Agilent's durable – and reproducible – line of ZORBAX columns can help you meet 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 30-50 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 (ACE, 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: **300Extend-C18**
4.6 x 150 mm, 5 µm
773995-902

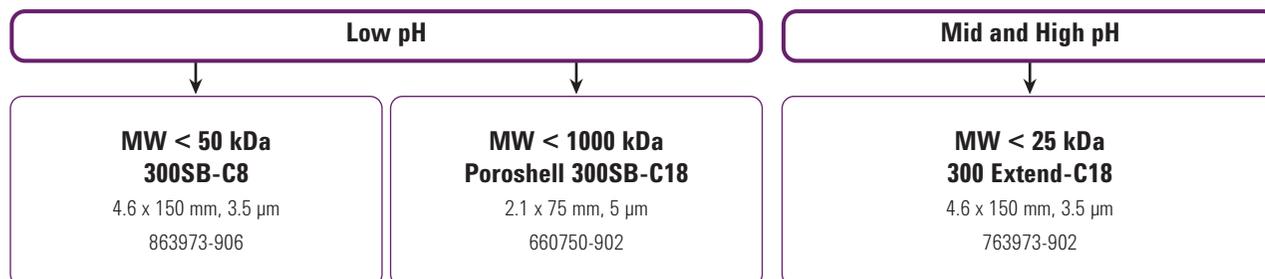
Mobile Phase: A: 20 mM NH₄OH in H₂O
 B: 20 mM NH₄OH in 80% ACN

Gradient: 5-60% B in 30 minutes

Temperature: 25-30°C (<60°C)

Flow Rate: 1 mL/min

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

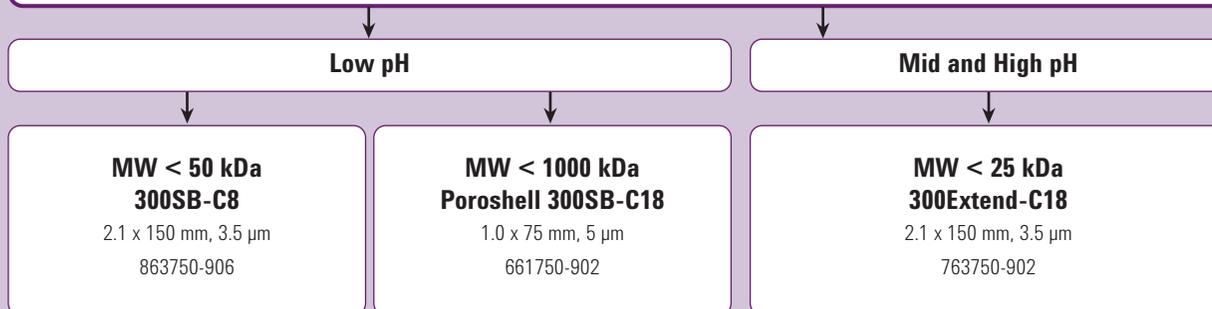


Separations of Proteins and Peptides Using Reversed-Phase LC/MS Methods

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

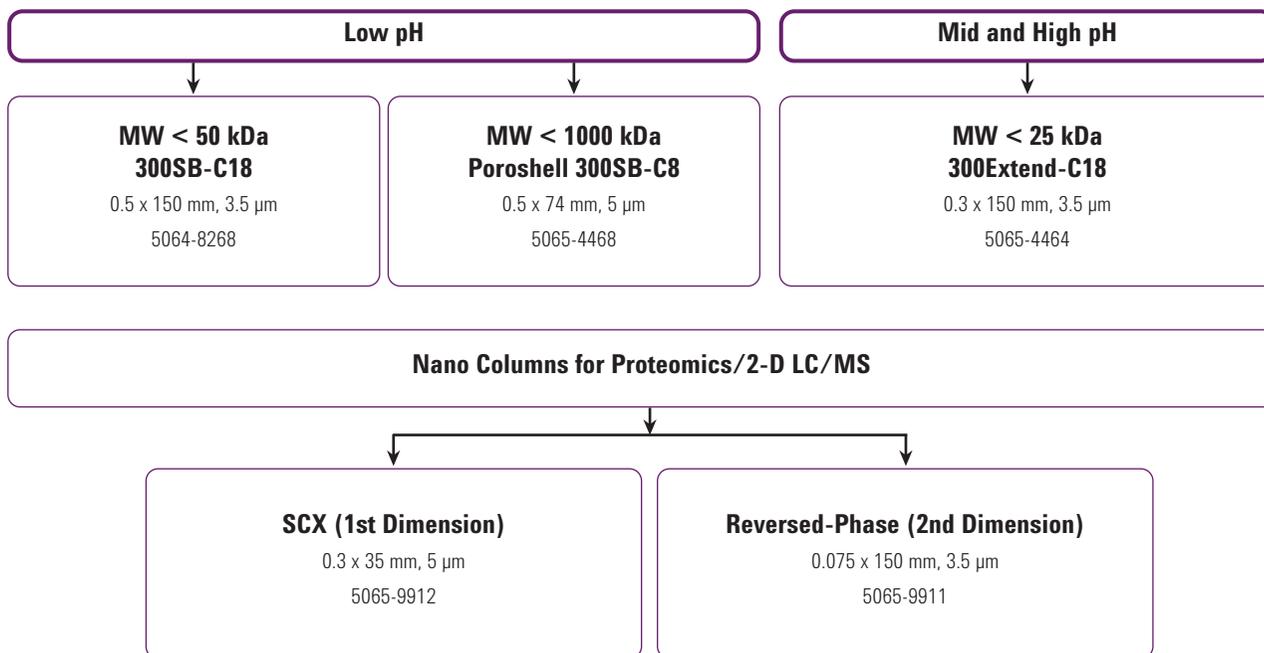
Analytical LC/MS Applications – 2.1 mm I.D. columns will provide good sensitivity when sample size is not limited.
With Poroshell columns, smaller column I.D.s are used.



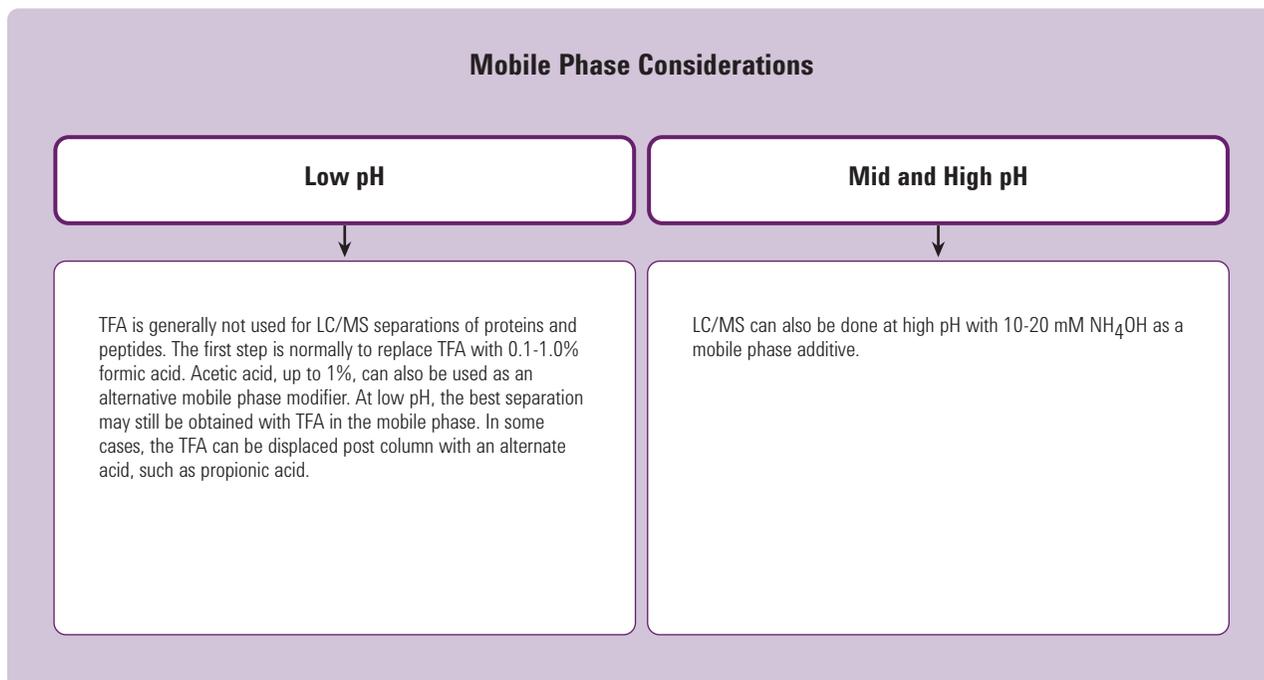
High Sensitivity/Proteomics Applications

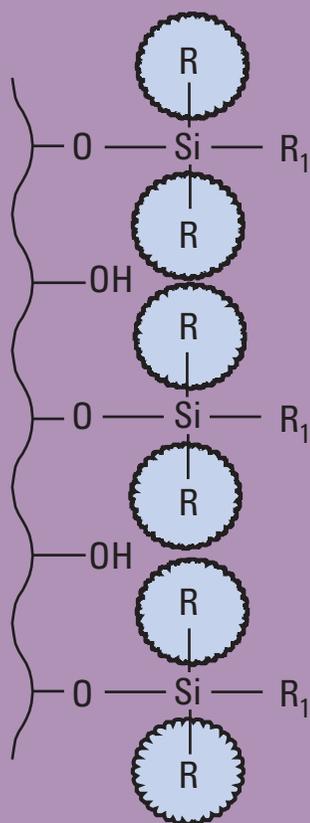
Capillary columns are used for high sensitivity protein and peptide applications. The 0.5 mm I.D. columns are used for protein and protein digest separations while the 0.3 mm I.D. columns are most often used for protein digests. These can be analyzed at high pH with an ammonium hydroxide mobile phase. 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

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 ² /g | 90°C | 1.0-8.0 | No | 2.8% |
| ZORBAX 300SB-C8 | 300Å | 45 m ² /g | 80°C | 1.0-8.0 | No | 1.5% |
| ZORBAX 300SB-C3 | 300Å | 45 m ² /g | 80°C | 1.0-8.0 | No | 1.1% |
| ZORBAX 300SB-CN | 300Å | 45 m ² /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.



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.

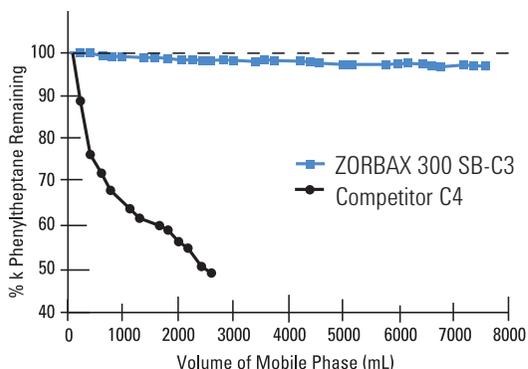
Short-Chain ZORBAX 300SB-C3 Is Stable at Low pH, High Temperature

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

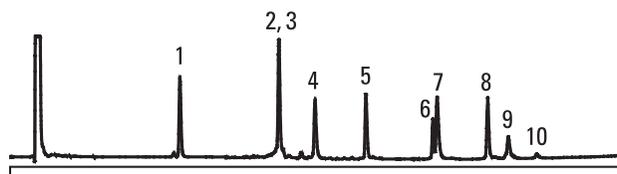
Isocratic Retention Test Conditions:
1-phenylheptane 50% A, 50% B

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

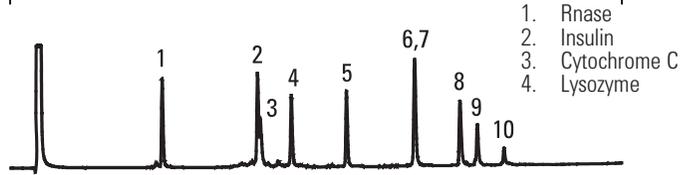


Four Different 300SB Bonded Phases Optimize Separation of Large Polypeptides

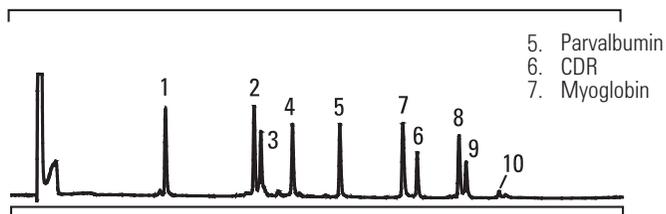
Column A: ZORBAX 300SB-C18
883995-902
4.6 x 150mm, 5µm



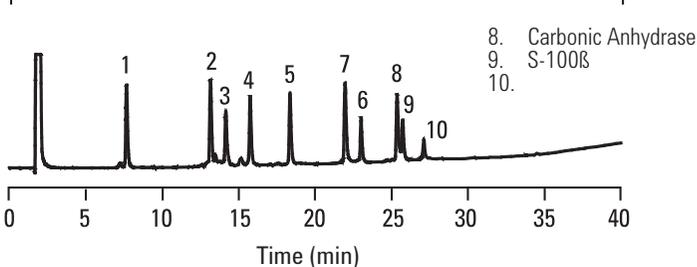
Column B: ZORBAX 300SB-C8
883995-906
4.6 x 150mm, 5µm



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

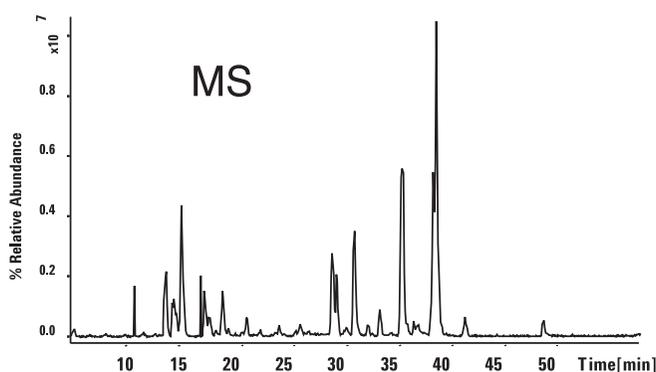
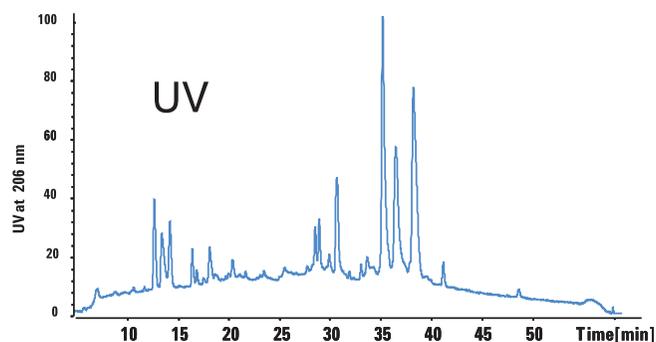
Flow Rate: 5.5 µL/min

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

Sample: 100 nL
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 Electrospray 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

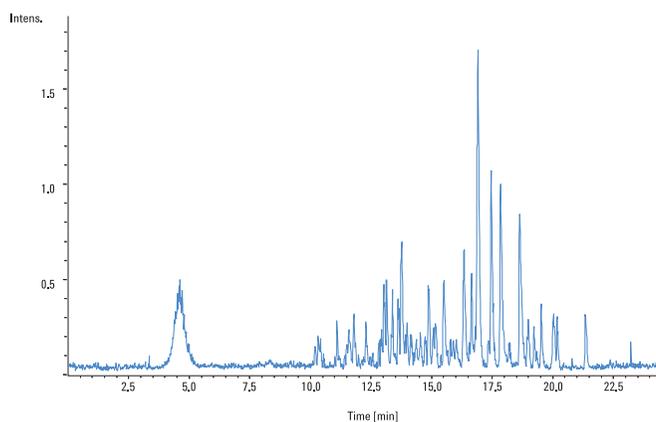
Flow Rate: 600 nL/min

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 |
|--|----------------------------------|------------|--------------------|---------------------|--------------------|---------------------|---------------------|
| Standard Columns (no special hardware required, 400 bar) | | | | | | | |
| | 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 | | |
| P | Guard Cartridge, 2/pk | 9.4 x 15 | 7 | 820675-124 | 820675-124 | 820675-124 | 820675-124 |
| ZGC | Guard Cartridge, 4/pk | 4.6 x 12.5 | 5 | 820950-921 | 820950-918 | 820950-923 | 820950-924 |
| ZGC | Guard Cartridge, 4/pk | 2.1 x 12.5 | 5 | 821125-918 | 821125-918 | 821125-924 | 821125-924 |
| P | Guard Hardware Kit | 9.4 x 15 | | 840140-901 | 840140-901 | 840140-901 | 840140-901 |
| ZGC | Guard Hardware Kit | | | 820888-901 | 820888-901 | 820888-901 | 820888-901 |
| PrepHT Cartridge Columns (require endfittings kit 820400-901) | | | | | | | |
| PI | PrepHT Cartridge | 21.2 x 250 | 7 | 897250-102 | 897250-106 | 897250-105 | 897250-109 |
| PI | PrepHT Cartridge | 21.2 x 150 | 7 | 897150-102 | 897150-106 | | 897150-109 |
| PI | PrepHT Cartridge | 21.2 x 150 | 5 | 895150-902 | 895150-906 | | 895150-909 |
| PI | PrepHT Cartridge | 21.2 x 100 | 5 | 895100-902 | 895100-906 | | 895100-909 |
| PI | PrepHT Cartridge | 21.2 x 50 | 5 | 895050-902 | 895050-906 | | 895050-909 |
| PI | PrepHT Endfittings, 2/pk | | | 820400-901 | 820400-901 | 820400-901 | 820400-901 |
| PI | PrepHT Guard Cartridge, 2/pk | 17 x 7.5 | 5 | 820212-921 | 820212-918 | 820212-924 | 820212-924 |
| PI | 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 |
|---|-------------------------|-------------|--------------------|------------------|-----------------|------------------|------------------|
| Capillary Glass-lined Columns | | | | | | | |
| | 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 | | |
| Nano Columns (PEEK fused silica) | | | | | | | |
| | 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

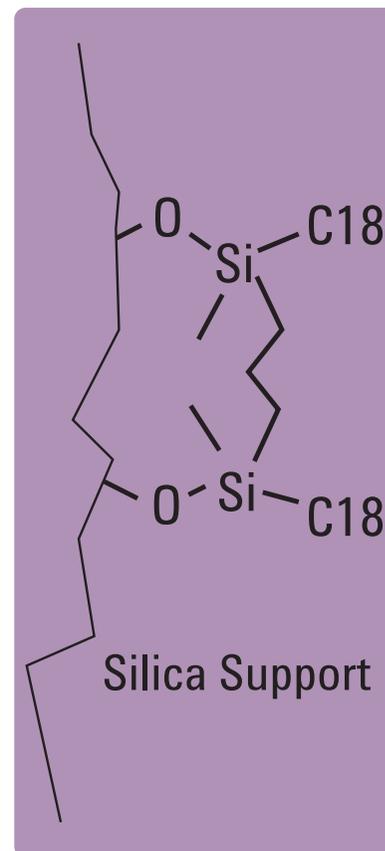
- Rugged, high and low pH separations of polypeptides and peptides from pH 2-11.5
- Different selectivity possible at high and low pH
- High efficiency and good recovery of hydrophobic peptides at high pH
- Ideal for LC/MS with ammonium hydroxide-modified mobile phase

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 ² /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.



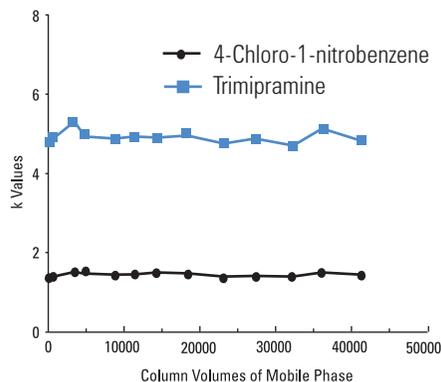
Novel Bidentate C18-C18 Bonding for Extend-C18 Bonded Phase

Long Life at High pH with 300Extend-C18

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

Mobile Phase: 20% 20 mM NH₄OH,
pH 10.5

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

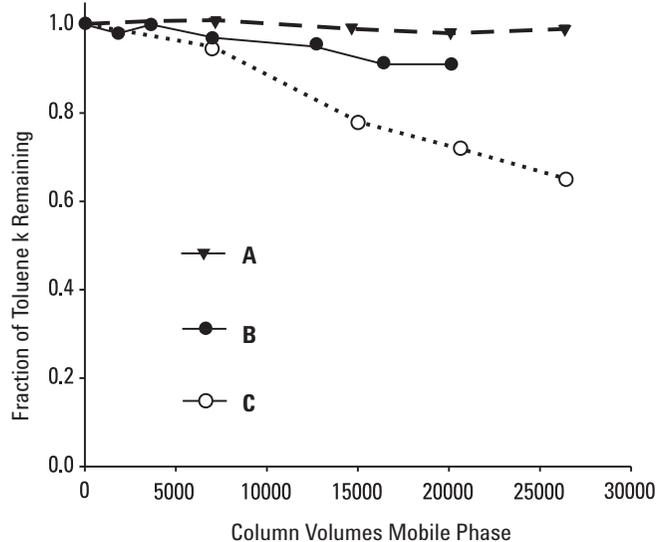
Column C: ZORBAX Rx-C18
883967-902
4.6 x 150mm, 5µm

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

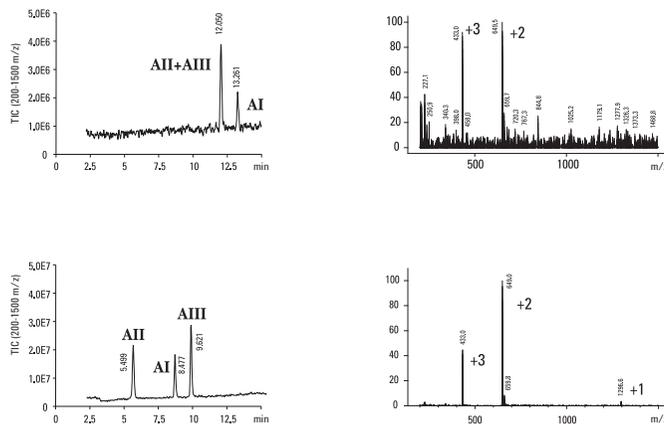
Mobile Phase: Acidic Conditions: A: 0.1% TFA in water B: 0.085% TFA in 80% acetonitrile (ACN)
Basic Conditions: A: 10 mM NH₄OH in water B: 10 mM NH₄OH in 80% ACN

Flow Rate: 0.2 mL/min
Gradient: 15-50% B in 15 min
Temperature: 35°C

MS Conditions: Pos. Ion ESI- V_f 70V, V_{cap} 4.5 kV,
N₂- 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

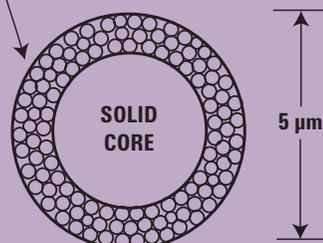
| Hardware | Description | Particle | | Part No. |
|---|-----------------------|------------|-----------|------------|
| | | Size (mm) | Size (µm) | |
| | Analytical | 4.6 x 250 | 5 | 770995-902 |
| | Analytical | 4.6 x 150 | 5 | 773995-902 |
| | Rapid Resolution | 4.6 x 150 | 3.5 | 763973-902 |
| | 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 |
|  | Guard Cartridge, 4/pk | 4.6 x 12.5 | 5 | 820950-932 |
|  | Guard Cartridge, 4/pk | 2.1 x 12.5 | 5 | 821125-932 |
|  | 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

Porous Shell



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.

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.



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.

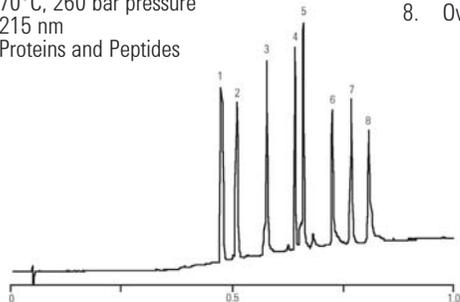


Poroshell Columns Can Separate Proteins and Peptides in Seconds

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

Mobile Phase: A: 0.1% TFA in H₂O
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. Pnase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic Anhydrase
8. Ovabumin

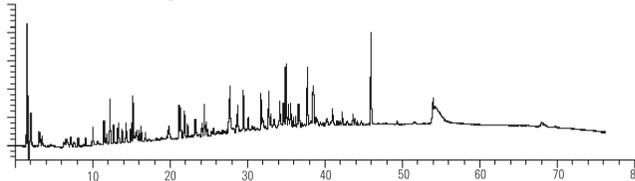
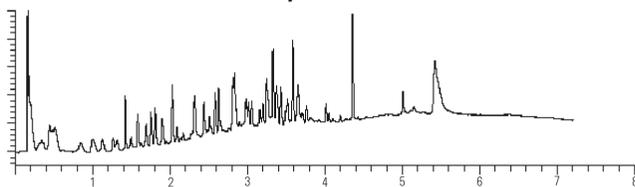


This separation of eight polypeptides and proteins is completed in less than 60 seconds. Each peak is sharp and efficient.

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% H₂O, 5% ACN,
0.1% TFA
B: 5% H₂O, 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

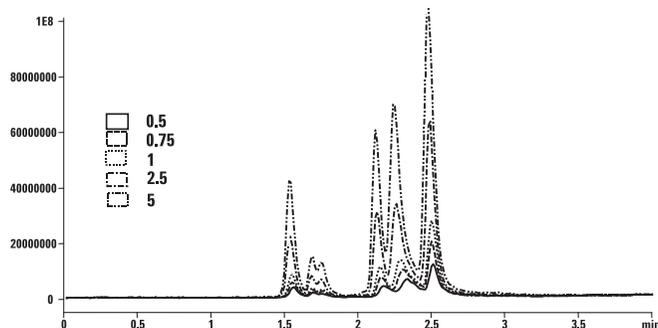
Temperature: 70°C
Sample: 20 µL (0.22 µg/1 µL)
BSA Tryptic Digest
(15 hours, 70 pmol)

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 | | |
|  | Guard Cartridge, 4/pk | 2.1 x 12.5 | 5 | 821075-920 | 821075-918 | 821075-924 | |
|  | 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 Na₂HPO₄, pH 7.8
B: ACN : MeOH : Water,
45:45:10 v/v

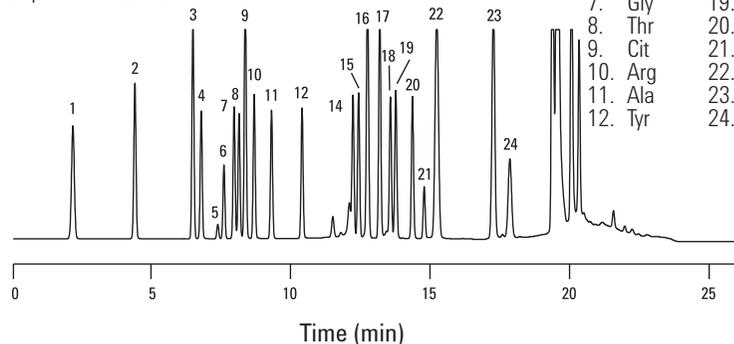
Flow Rate: 2 mL/min

Temperature: 40°C

Detector: Fluorescence

Sample: 24 Amino Acids

| | |
|---------|---------|
| 1. Asp | 13. Cys |
| 2. Glu | 14. Val |
| 3. Asn | 15. Met |
| 4. Ser | 16. Nva |
| 5. Gln | 17. Trp |
| 6. His | 18. Phe |
| 7. Gly | 19. Ile |
| 8. Thr | 20. Leu |
| 9. Cit | 21. Lys |
| 10. Arg | 22. Hyp |
| 11. Ala | 23. Sar |
| 12. Tyr | 24. Pro |



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 |
| ZGC | Guard Cartridges, 4/pk | 4.6 x 12.5 | 5 | 820950-931 |
| ZGC | 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 ² /g | 3.0-8.0 | <3.0 ml/min | 350 bar |
| ZORBAX GF-450 | 300Å | 6 µm | 10,000-900,000 | 50 m ² /g | 3.0-8.0 | <3.0 ml/min | 350 bar |

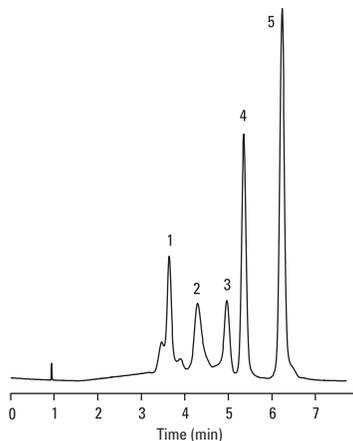


Separation of Protein Standards on the ZORBAX GF-250 SEC Column

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

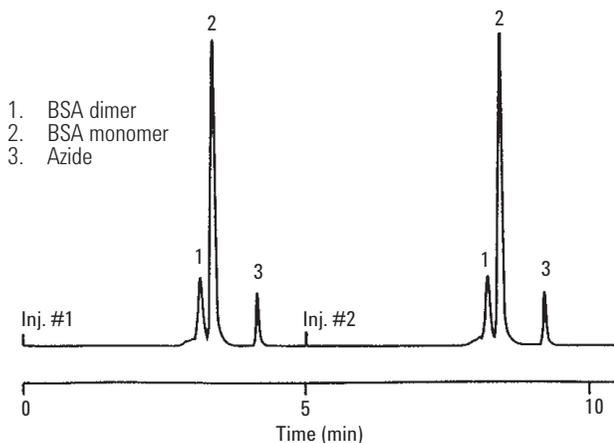
Mobile Phase: 200 mM Sodium Phosphate, pH 7.0
Flow Rate: 2 mL/min
Temperature: Ambient
Detector: 254 nm
Sample: BioRad Gel Filtration Standards
for Size Exclusion
1. Thyroglobulin 670,000 Da
2. Bovine Gamma Globulin 158,000 Da
3. Chicken Ovalbumin 44,000 Da
4. Equine Myoglobin 17,000 Da
5. Vitamin B-12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

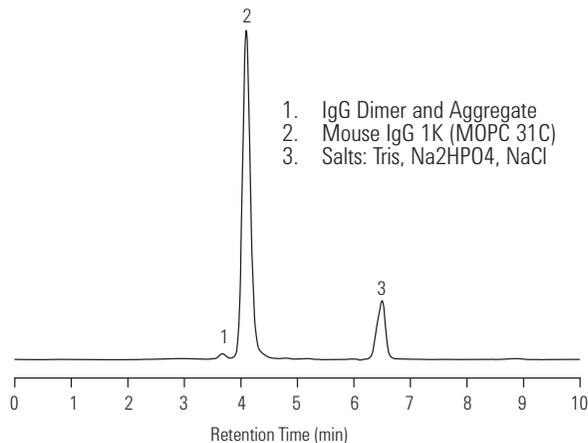
A. BSA and BSA Dimers (duplicate injections)



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



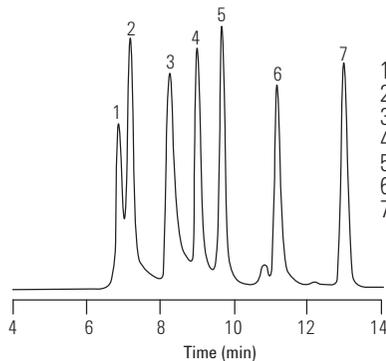
Column: ZORBAX GF-450
884973-902
9.4 x 250mm, 6µm

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, 4 μ m

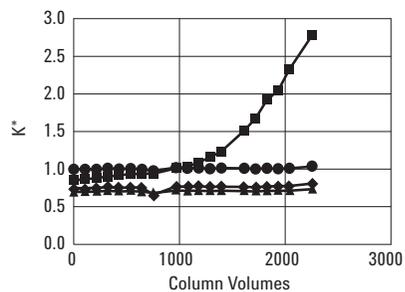
Mobile Phase: 130 mM NaCl/20 mM KCl/
50 mM Na₂HPO₄, pH 7.0
Flow Rate: 1 mL/min
Detector: 210 nm
Sample: Protein mixture



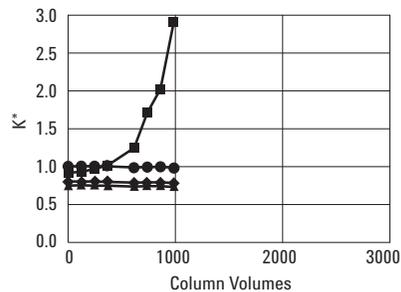
1. Mouse IgM 900,000 Da
2. Bovine Thyroglobulin 670,000 Da
3. Sweet Potato β -Amylase 200,000 Da
4. Bovine Serum Albumin 67,000 Da
5. Chicken Albumin 45,000 Da
6. Bovine RNase 13,700 Da
7. Azide 65 Da

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 ² /g | 2.0-7.0 | Quaternary amine | 350 bar |
| ZORBAX 300SCX | 300Å | 50 m ² /g | 2.0-7.0 | Sulfonic acid | 350 bar |
| ZORBAX Bio-SCX Series II | 300Å | 90 m ² /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 | |



Tech Support

Find HPLC course descriptions and seminars online at www.agilent.com/chem/Education.

Learn from Agilent HPLC experts through classroom training courses, custom on-site training, and free e-seminars. HPLC courses cover instrument and column selection, separation optimization, HPLC maintenance and troubleshooting, Chemstation and more.

Technical Support at Work for You

Have a hardware, software, application, or troubleshooting question? Agilent's technical experts are available to answer your questions. With years of laboratory experience, our technical support specialists can provide in-depth knowledge and experience.

For questions pertaining to columns found in this catalog, call your local Agilent sales office or **1-800-227-9770** in the U.S. or Canada. You can also visit www.agilent.com/chem/techsupport for a wealth of knowledge, tips, and insight.

- Frequently Asked Questions
- Downloads and Utilities
- Installation and Maintenance Videos
- Interactive Troubleshooter
- Warranty Information
- Technical Support Contact Information





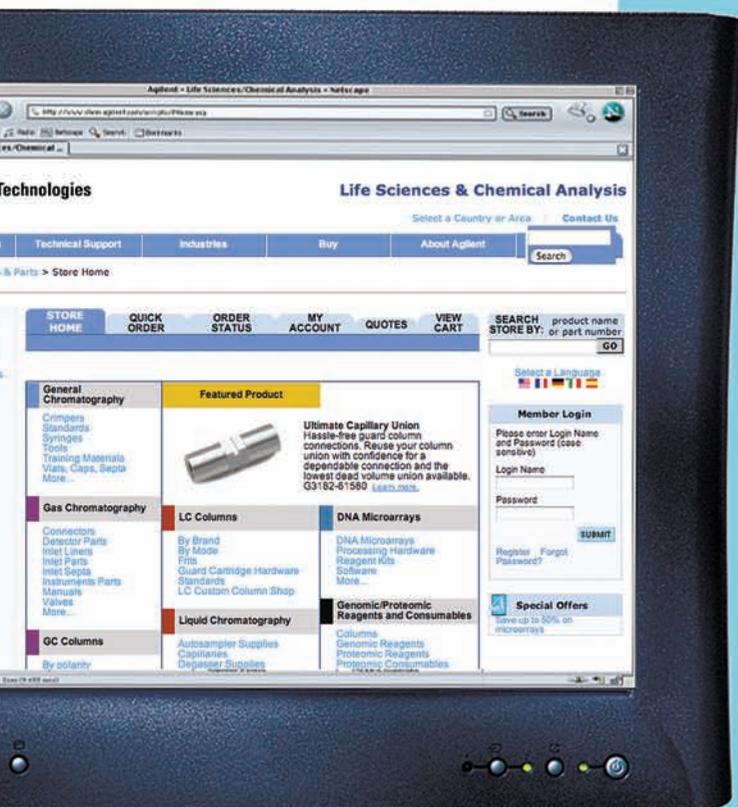
Peak productivity starts here: www.agilent.com/chem/store

Welcome to Agilent's Life Sciences & Chemical Analysis online store!

Whether you want to increase sample throughput – or scale and transfer methods – you will find all the columns and supplies you need for your day-to-day operations. So you can spend less time searching for products, and more time achieving your best results.

Our online store also adapts to your company's purchasing policies with features like Online Purchase Approval. After you fill your cart, you can simply e-mail a secure, single-use ID to your purchasing agent, who can then access your cart, make any changes, and place the order.

And of course, if you prefer to speak with our experts directly, or if you have additional questions, you can contact your local Agilent office or authorized distributor.



- View local list prices and see prices specific to your organization (once your account is activated).
- Submit your order in seconds using our "Quick Buy" feature.
- Instantly see when Agilent can ship an item to you.
- Configure products and receive a price quote for purchase approval or to buy online.
- Choose from five languages – including French, Italian, German, Spanish, and English.
- Create a personal catalog of items that you request frequently. So you can select these items more quickly the next time you order.
- Track your order from OUR door to YOURS.
- View your ordering history with just a few clicks of your mouse.

How to contact Agilent

For the latest information on the complete line of Agilent Technologies columns and supplies:

- Visit our Web site: www.agilent.com/chem
- Contact your local Agilent sales office
- Contact your local Authorized Agilent Distributor
- In the U.S. and Canada call **1 800 227 9770**
Sales — Option 1, then 1
Technical Support — Option 4, then 2

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc. 2008
Printed in the USA September 26, 2008
5989-5992EN

